Highly Antibacterial Active Aminoacyl Penicillin Conjugates with Acylated Bis-Catecholate Siderophores Based on Secondary Diamino Acids and Related Compounds

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Received December 3, 2001

New acylated bis-catecholates and 1,3-benzoxazine-2,4-dione derivatives based on secondary diamino acids (*N*-(aminoalkyl)glycines, *N*-aminopropyl-alanine, and *N*-aminopropyl-4-aminovaleric acid), on *N*-(aminoalkyl)aminomethyl benzoic acids, on *N*-(aminoalkyl)aminomethyl phenoxyacetic acids, or on 3,5-diaminobenzoic acid were synthesized as artificial siderophores. The corresponding diamino acids were obtained from the diamines and oxocarboxylic acids by catalytic hydrogenation. The acylated bis-catecholates and 1,3-benzoxazine-2,4-diones were coupled with ampicillin or amoxicillin to new siderophore aminoacylpenicillin conjugates. These conjugates exhibited very strong antibacterial activity in vitro against Gram-negative bacterial pathogens including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Serratia marcescens*. The ampicillin derivative **7b** (HKI 9924154) and the corresponding amoxicillin derivative **8** (HKI 9924155) represent the most active compounds. The conjugates can use bacterial iron siderophore uptake routes to penetrate the Gram-negative outer membrane permeability barrier. This was demonstrated by assays with mutants deficient in components of the iron transport systems. New siderophore penicillin V conjugates with the siderophore component attached to the phenyl ring of penicillin V are inactive against these Gram-negative bacteria.

Introduction

The outer membrane permeability barrier of bacteria is one important reason for β-lactam antibiotic resistance of Gram-negative bacterial pathogens. This applies especially to *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* strains. To overcome this membrane-mediated resistance, siderophore structures covalently bound to the antibiotic can function as a shuttle for active transport into the bacterial cell. Siderophores are microbial iron chelators excreted to sequester extracellular ferric ions under iron starvation conditions such as found in infected tissues. Specific outer membrane receptors recognize the siderophore iron complex and initiate its active transport into the cell. Many natural siderophores contain two or three catecholate groups as chelating ligands based on di- or triamines.[1] Examples are bis-catecholate derivatives of spermidine[2] or of diamino acids such as *N*,*N*′-bis-(2,3-dihydroxybenzoyl)-L-lysine.[3] Artificial siderophores of these structures based on spermidine or norspermidine were published.[4−6] Recently, we synthesized artificial siderophores based on amino acids or dipeptides[7] and mono-1,3-benzoxazine-2,4-dione derivatives as masked catecholates. These compounds can act as siderophore components in β-lactam conjugates.[8] Examples of siderophore antibiotic conjugates with one catecholate moiety and enhanced in vitro antibacterial activities have been published.[9,10] Ampicillin conjugates with natural pyoverdins were specifically active only against that *Pseudomonas* strain producing the corresponding pyoverdin.[11] Pyoverdin−quinolone adducts were synthesized, too.[12] β-Lactam conjugates with a series of different noncatecholate siderophore structures seem to act independently of iron transport systems and can reach activities near that of the cephalosporin moiety.[13]

In this paper, we report on the synthesis of *N*-(aminoalkyl)amino acids from diamines and oxocarboxylic acids by catalytic hydrogenation, on the acylation of these compounds or of 3,5-diaminobenzoic acid to acylated bis-catecholate and 1,3-benzoxazine-2,4-dione derivatives as new artificial siderophores and on their condensation with aminoacyl penicillins and on the investigation of the biological activities of the siderophores and their conjugates with ampicillin and amoxicillin. We used acylated catecholates and the corresponding benzoxazine derivatives as siderophore components to facilitate the synthesis and to decrease pharmacological side effects in comparison to free catecholates.

Results and Discussion

Synthesis of the Siderophores. As the backbone for new artificial siderophores, we synthesized *N*-(aminoalkyl)amino acids **3a**−**d**,**o**,**p**, *N*-(aminoalkyl)-aminomethyl benzoic acids **3k**,**l**, and *N*-(aminoalkyl)-aminomethyl phenoxyacetic acids **3m**,**n** from the diamines **1a**−**e** and oxocarboxylic acids **2a**−**f** by hydro-

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10.1021/jm010546b CCC: $22.00 © 2002 American Chemical Society
Published on Web 06/06/2002

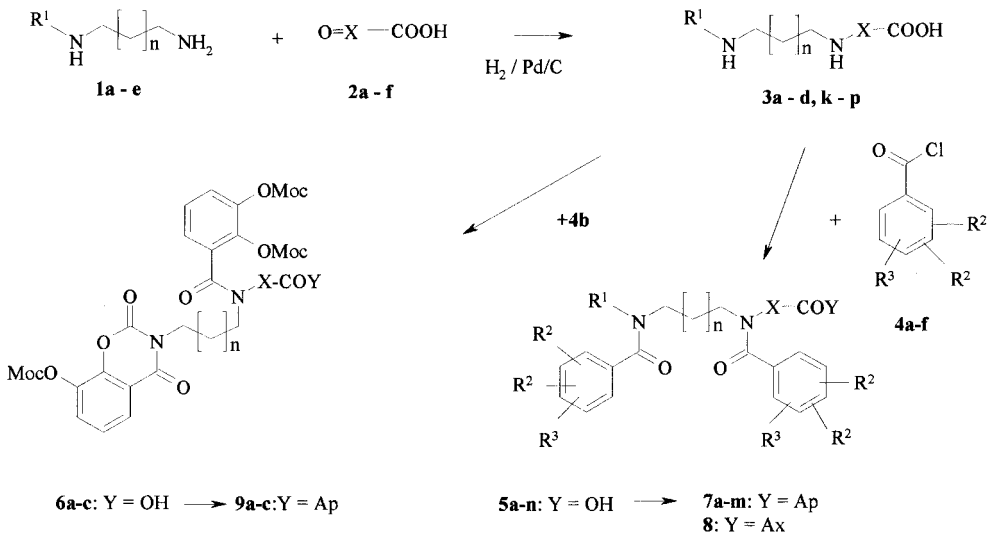

Figure 1. Syntheses of the siderophore components **5** and **6** and of the antibiotic conjugates **7**−**9**; Ac = $COCH_3$; Moc = $COOCH_3$; **1a**: $n = 0$, R^1 = H; **1b**: $n = 1$, R^1 = H; **1c**: $n = 1$, $R^1 = CH_3$; **1d**: $n = 2$, R^1 = H; **1e**: $n = 3$, R^1 = H; **2a**: X = CH; **2b**: X = C-CH_3; **2c**: X = C-CH_3-$(CH_2)_2$-; **2d**: X = CH-oC_6H_4; **2e**: X = CH-pC_6H_4-OCH_2; **2f**: X = CH-oC_6H_4-OCH_2; **4a**: R^2 = 2,3-OAc, R^3 = H; **4b**: R^2 = 2,3-OMoc, R^3 = H; **4c**: R^2 = 2,3-OMoc, R^3 = 5-Cl; **4d**: R^2 = 2,3-OMoc, R^3 = 5-Br; **4e**: R^2 = 2,3-OMoc, R^3 = 5,6-di-Cl; **4f**: R^2 = 3,4-OAc, R^3 = H; **6a**, **9a**: $n = 2$, X = CH_2; **6b**, **9b**: $n = 1$, X = $CHCH_3$; **6c**, **9c**: $n = 1$, X = $CH(CH_3)CH_2CH_2$; other substituents, see Table 1.

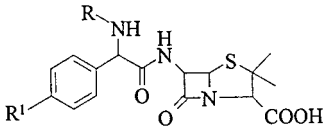

Figure 2. Structure of the aminoacyl penicillin components; R = siderophore components of Figures 1 and 4; R^1 = H: ampicillin derivatives, Ap; R^1 = OH: amoxicillin derivative, Ax.

genation catalyzed by palladium on active carbon. The synthesis of *N*-(aminoethyl)glycine **3a** by this procedure was known.[14] The intermediate in this reaction is possibly an *N*-heterocyclic acid like hexahydro-pyrimidine-2-carboxylic acid as a derivative of 1,3-diamino-*n*-propane[15] and not the corresponding azomethine, because no CH=N signal was found in ^{1}H nuclear magnetic resonance (NMR) spectra. Compounds **3** were purified by codistillation with toluene to separate unreacted diamines or via the synthesis of CBZ derivatives with benzyl chloroformate, separation from impurities by preparative high-performance liquid chromatography (HPLC), and hydrogenolysis with Pd/C. The diamino acids **3a**−**n** were acylated with diacyloxybenzoyl chlorides **4a**−**f** in aqueous sodium hydrogen carbonate solution to the bis-catecholate compounds **5a**−**n** (Figure 1). The 4,5-dichloro-2,3-di-methoxycarbonyloxy-benzoyl chloride **4e** was prepared analogously to **4b** [13] from 4,5-dichloro-2,3-dihydroxybenzoic acid[16] and methyl chloroformate by reaction of the obtained 4,5-dichloro-2,3-di-methoxycarbonyloxy-benzoic acid with PCl_5. Compounds **5a**−**c**,**l**−**n** and the corresponding β-lactam conjugates **7a**−**c**,**l**,**m**, **8**, and **10a**,**b** obviously exist in two rotameric forms because two triplets for CONH were found in the ^{1}H NMR spectra, which change to singlets at 360 K studied on compounds **5b**,**m** and **5b** and **10a**, respectively.

The 1,3-benzoxazine-2,4-dione derivatives **6a**−**c** were synthesized from the diamino acids **3b**,**o**, or **p** and 2,3-dimethoxycarbonyloxybenzoyl chloride **4b** (Figure 1). Bis-2,3-di-(methoxycarbonyloxy)-benzoyl intermediates

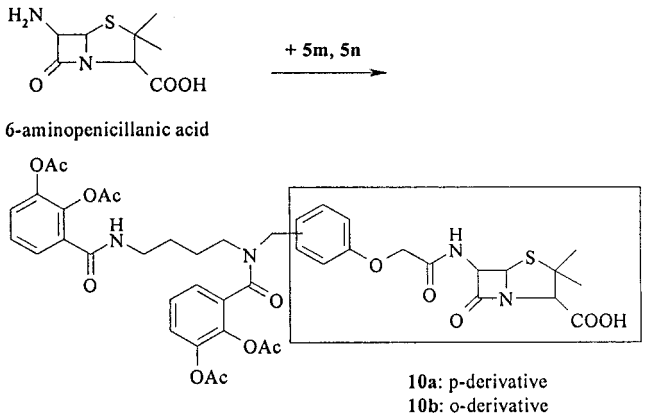

Figure 3. Penicillin V−derivatives **10a**,**b**, Ac = $COCH_3$.

according to structure **5** were cyclized to structure **6** by reaction in acetonitrile. These compounds represent a heterocyclic acylated form of catecholates, which can be transformed enzymatically into the free catecholates. β-Lactam conjugates with one benzoxazindione moiety showed high antibacterial activity.[8] As a further diamino acid, we used 3,5-diaminobenzoic acid as the backbone for siderophores, which were acylated with **4a** to compound **11** and with **4b**, with [8-(methoxycarbonyloxy)-1,3-benzoxazine-2,4-dione-3-yl]acetyl chloride **4g** and the corresponding propionyl chloride **4h**,[8] respectively, to the compounds **13a**−**c** (Figure 4).

Synthesis of the β-Lactam Conjugates. The compounds **5a**−**m**, **6a**−**c**, **11**, and **13a**−**c** were coupled with ampicillin or amoxicillin via their mixed anhydrides with isobutyl chloroformate to the ampicillin conjugates **7a**−**m**, **9a**−**c**, **12**, and **14a**−**c** and to the amoxicillin conjugates **8** and **15c** (Figure 2). The bis-catecholates **5m**,**n** were also coupled with 6-aminopenicillanic acid to give derivatives of penicillin V **10a** (p-derivative) and **10b** (o-derivative) (Figure 3, the structure of penicillin V is marked). In these compounds, the siderophore component is placed on the phenyl of the phenoxy-

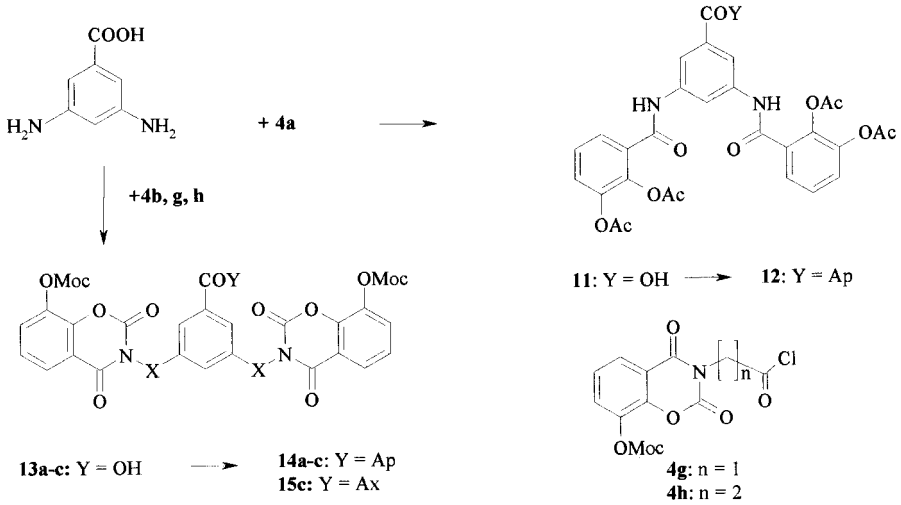

Figure 4. Synthesis of 3,5-diaminobenzoic acid derivatives **11**−**15**, Ac = $COCH_3$, Moc = $COOCH_3$; **13a** and **14a**: X = direct bond; **13b** and **14b**: X = CH_2CONH; **13c**, **14c**, and **15c**: X = CH_2CH_2CONH-.

Table 1. Substituent Pattern of Compounds **3**, **5**, **7**, **8**, Ac = $COCH_3$, and Moc = $COOCH_3$

compd	n	R^1	R^2	R^3	X
3a	0	H			CH_2
5a	0	H	2,3-OAc	H	CH_2
7a	0	H	2,3-OAc	H	CH_2
3b	2	H			CH_2
5b	2	H	2,3-OAc	H	CH_2
7b	2	H	2,3-OAc	H	CH_2
8	2	H	2,3-OAc	H	CH_2
3c	3	H			CH_2
5c	3	H	2,3-OAc	H	CH_2
7c	3	H	2,3-OAc	H	CH_2
3d	1	CH_3			CH_2
5d	1	CH_3	2,3-OAc	H	CH_2
7d	1	CH_3	2,3-OAc	H	CH_2
5e	1	CH_3	2,3-OMoc	H	CH_2
7e	1	CH_3	2,3-OMoc	H	CH_2
5f	1	CH_3	2,3-OMoc	5-Cl	CH_2
7f	1	CH_3	2,3-OMoc	5-Cl	CH_2
5 g	1	CH_3	2,3-OMoc	5-Br	CH_2
7 g	1	CH_3	2,3-OMoc	5-Br	CH_2
5h	1	CH_3	2,3-OMoc	5,6-di-Cl	CH_2
7h	1	CH_3	2,3-OMoc	5,6-di-Cl	CH_2
5i	2	H	3,4-OAc	H	CH_2
7i	2	H	3,4-OAc	H	CH_2
3k	1	CH_3			CH_2-(o-C_6H_4)
5k	1	CH_3	2,3-OAc	H	CH_2-(o-C_6H_4)
7k	1	CH_3	2,3-OAc	H	CH_2-(o-C_6H_4)
3l	2	H			CH_2-(o-C_6H_4)
5l	2	H	2,3-OAc	H	CH_2-(o-C_6H_4)
7l	2	H	2,3-OAc	H	CH_2-(o-C_6H_4)
3m	2	H			CH_2-(p-C_6H_4)-OCH_2
5m	2	H	2,3-OAc	H	CH_2-(p-C_6H_4)-OCH_2
7m	2	H	2,3-OAc	H	CH_2-(p-C_6H_4)-OCH_2
3n	2	H			CH_2-(o-C_6H_4)-OCH_2
5n	2	H	2,3-OAc	H	CH_2-(o-C_6H_4)-OCH_2
3o	1	H			CH_3CH
3p	1	H			CH_3CH-$(CH_2)_2$

acetylpenicillanic acid representing a new type of siderophore β-lactam conjugate.

Siderophore Activity. Siderophore activity of the synthesized compounds was determined by the assay of their growth-promoting activity in Gram-negative bacteria under iron-limited conditions.

According to Table 2, the bis-catecholates of *N*-(aminoalkyl)glycines **5b**−**d**,**f**−**h** and **6a**,**b** efficiently act as siderophores and promote the growth of the bacterial test strains. The growth promotion activities of compounds **5e**,**i**, **6c**, **11**, and **13b** exhibited decreased activity for some of the strains. The acylated catecholates active as siderophores obviously were transformed to free catecholates in bacterial cultures.

Table 2. Growth Promotion of the Bis-catecholate Derivatives **5a**−**n**, **6a**−**c**, **11**, and **13a**−**c** of Gram-Negative Bacteria under Iron Limitation (5 μL of a 2 mM Solution Was Applicated on a 6 mm Paper Disk) and the Results of CAS Assay[a]

	P. aeruginosa					*E. coli*	
compd	ATCC 27853	SG 137	NCTC 10662	ATCC 9027	K799/ WT	ATCC 25922	CAS assay
5b	20	26	25	20	25	28	++
5c	30	28	30	20	25	27	+++
5d	19	15	23	18	22	20	+++
5e	15	9	18	13	25	20	+
5f	25	26	25	24	25	30	+
5g	22	25	27	20	27	32	+
5h	25	30	30	25	25	30	++
5i	25	10	14	19	20	21	++
5k	20	19	18	nt	17	27	+
5l	22	21	23	nt	15	34	+
5m	22	19	23	25	12	27	+
5n	18	nt	27	26	25	26	+
6a	18	27	24	nt	23	30	+
6b	15	19	25	nt	22	22	++
6c	13	15	20	nt	14	15	+
11	0	20	17	10	nt	27	++
13a	15	25	18	20	nt	20	+
13b	15	20	20	20	nt	15	(+)
13c	18	23	20	20	nt	20	(+)
desferal	35	30	30	35	36	38	

[a] nt = not tested.

Iron-Complexing Capacity. All bis-catecholates showed a positive chromazurol-S (CAS) reaction (Table 2), which is associated with iron chelation, a basic requirement for siderophore activity.

Antibacterial Activity. Compounds **7**−**10**, **12**, **14**, and **15c** were tested for their antibacterial activity in vitro against the Gram-negative bacteria *P. aeruginosa* SG 137 and ATCC 27853, *S. maltophilia* GN 12873, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Serratia marcescens* SG 621, and against the Gram-positive strain *Staphylococcus aureus* SG 511 (Table 3). The compounds **7a**−**h**, **8**, and **9a** exhibited extremely high antibacterial activity against the Gram-negative test strains, by far exceeding the activity of the ampicillin moiety, azlocillin, and partly also the activity of meropenem, especially against *S. maltophilia*.

Table 3. Antibacterial Activities In Vitro of the Siderophore−β-Lactam Conjugates **7**−**15** (MIC in mg/L)

	P. aeruginosa		*E. coli*	*K. pneumoniae*	*S. maltophilia*	*S. marcescens*	*S. aureus*
compd	SG 137	ATCC 27853	ATCC 25922	ATCC 10031	GN 12873	SG 621	SG 511
7a	0.4	0.78	0.78	0.1	0.78	0.78	3.12
7b	<0.005	0.05	<0.005	<0.005	0.005	<0.005	5
7c	0.04	0.16	0.16	<0.005	0.02	0.04	2.5
7d	0.005	0.08	0.02	<0.005	0.02	0.05	3.12
7e	0.04	0.31	0.02	0.005	0.08	0.02	5
7f	<0.005	0.1	0.05	<0.005	0.02	0.02	12.5
7g	<0.005	0.05	<0.005	<0.005	0.02	0.02	6.25
7h	0.05	0.1	0.02	<0.005	0.05	0.05	3.12
7i	0.2	0.78	0.78	0.4	50	3.12	0.78
7k	0.1	0.4	1.56	0.2	<0.05	0.4	0.78
7l	0.04	0.2	0.4	<0.05	<0.05	0.24	6.25
7m	0.2	6.25	0.78	<0.05	0.2	6.25	1.56
8	0.01	0.16	0.01	<0.005	0.005	<0.005	5
9a	0.04	0.156	0.01	<0.005	0.04	<0.005	2.5
9b	0.1	3.12	0.4	<0.05	0.4	0.2	1.56
9c	0.1	3.12	1.56	0.2	0.4	0.1	1.56
10a	50	50	nt	nt	25	nt	1.56
10b	25	50	100	100	25	50	3.12
12	0.4	3.12	100	3.12	1.56	nt	25
14a	0.2	1.56	25	6.25	0.78	12.5	25
14b	0.05	1.56	25	1.56	3.12	12.5	25
14c	0.05	0.2	6.25	0.2	1.56	3.12	6.25
15c	0.2	1.56	1.56	0.2	6.25	0.4	6.25
azlocillin	6.25	6.25	6.25	6.25	25	50	0.4
ampicillin	>100	>100	6.25	6.25	>100	25	0.4
meropenem	0.2	0.4	0.04	0.04	>100	0.06	0.1

As known from literature, the increase in activity against Gram-negative bacteria is more or less at the expense of activity against Gram-positive *S. aureus*.[17,18] An exception is the derivative **7k** based on 2-[*N*-(aminobutyl)aminomethyl]benzoic acid, which inhibited both Gram-positive and Gram-negative strains. The 3,5-diaminobenzoic acid derivatives **12**, **14a**−**c**, and **15c** generally had a lower antibacterial activity than the derivatives **7**−**9** based on *N*-(aminoalkyl)amino acids. The most active antibacterial compounds from this series were the 2,3-diacyloxybenzoyl derivatives based on *N*-(aminobutyl)glycine, the ampicillin derivatives **7b**,**d**,**f**−**h** and the amoxicillin derivative **8**. The corresponding 3,4-diacyloxy derivative **7i** was less active.

In contrast to the ampicillin and amoxicillin conjugates, derivatives of penicillin V **10a**,**b** were nearly inactive against the Gram-negative strains. From these results, we can deduce that the distance of the siderophore component to the β-lactam ring affects the activity of the conjugates. The siderophore moiety is closer to the β-lactam ring if attached to the NH group of aminoacyl penicillins than if attached to the phenyl ring of penicillin V derivatives. Possibly, the β-lactam structure is involved in the iron complexation.

Mechanism of Action. The mechanism of action of the highly active conjugates was studied by agar diffusion assays using *E. coli*−porin and iron transport mutants. The outer membrane porin proteins OmpF and OmpC allow the diffusion of small hydrophilic molecules such as β-lactam antibiotics. The TonB protein is essential for energy transfer in the active siderophore iron transport. FepA, Cir, and Fiu are outer membrane receptors for catecholate type siderophores.[19] Table 4 demonstrates the inhibition of *E. coli* mutants either deficient in OmpF or OmpC or overexpressing OmpC as well as the inhibition of a TonB mutant by ampicillin, azlocillin, and the siderophore conjugates. Activity of ampicillin alone but not of the acylamino penicillin conjugates is strongly influenced by the presence of the outer membrane porins. In contrast, activity of the siderophore acylamino penicillin conjugates, but not of ampicillin or azlocillin, essentially depends on the presence of TonB and thus on the function of active iron transport mechanisms. This was investigated in more detail using catecholate siderophore receptor mutants of *E. coli*. As shown in Table 5, the activities of the new conjugates are drastically decreased against strain H1876, which is deficient in all three catecholate siderophore receptors. In contrast, activity against strain H873, deficient only in the enterobactin receptor

Table 4. Influence of Porins and TonB on Antibacterial Activity in *E. Coli* (Diameter of Inhibition Zones in mm, 50 μL of a 0.2 mM Solution Was Filled in 9 mm Agar Wells)[a]

compd	KB4	KB5	PLB268	AB2847	BR158
OmpF	−	+	+	+	+
OmpC	+	−	+++	+	+
TonB	+	+	+	+	−
azlocillin	16	15	32	20	20
ampicillin	10.5	17	25	20	21
7a	20	20	25	20.5	12
7b	31.5	31	34	29.5	12.5
7c	26	25	30	24	11.5
7d	29.5	29	32.5	28	13
7e	30.5	28.5	33	31.5	14.5
7f	31	30	33	30	13
7g	30.5	31	33	29.5	12.5
7h	30.5	29	32.5	29	14.5
7i	23	19	25	21	16
7k	25	19	27	24	14
7l	25	20	27	25	12
8	31.5	29.5	33	30	11.5
9a	31	29.5	33.5	32	15.5
9b	24	20	29	22.5	13
9c	22	19	25.5	20.5	10
12	15.5	11	18.5	nt	nt
14a	15.5	13	19	16	0
14b	20	18	23.5	22.5	0
14c	19.5	22	24	21	0
15c	19.5	22.5	22	23.5	0

[a] nt = not tested.

Table 5. Influence of Outer Membrane Siderophore Receptors on Antibacterial Activity in *E. Coli* (Diameter of Inhibition Zones in mm, 50 μL of a 0.2 mM Solution Was Filled in 9 mm Agar Wells)

	H1443	H1876	H873	H1877	H1875
FepA	+	−	−	−	−
Cir	+	−	+	+	−
Fiu	+	−	+	−	+
ampicillin	20.5	20	19.5	20	20.5
azlocillin	16	16	17	16	16
7a	22	11	22	21	20
7b	34	12	33	30.5	27
7c	29	0	28.5	25	23
7d	32	11	31.5	29.5	27
7e	30	12.5	30	27.5	26
7f	30	12	29.5	28	26.5
7g	34	12	32.5	32.5	29
7h	31	16	32	31	28.5
7i	24.5	13	24	22	23
7k	25	0	25	24	21
7l	25	0	25.5	24	22
8	32	11	32	29	26
9a	32	14	31	28	24
9b	24.5	11.5	25	22.5	22
9c	22.5	0	23	19.5	20.5
12	18	0	17	0	16
14a	16.5	0	18.5	13	18
14b	21	0	21	18.5	20
14c	20.5	0	23	18	21.5
15c	22.5	0	22	19	21.5

FepA, is the same as against the parent strain H1443 where the complete set of catecholate receptors is available. Thus, activity of the conjugates depends on the receptors Cir or Fiu for the building blocks or breakdown products of enterobactin, namely, mono-, di-, and trimers of 2,3-dihydroxybenzoic acid, not on the receptor FepA. There is evidence for a coordinating function of Cir and Fiu as there is only a minor or no decrease in activity of the conjugates against the mutants H1875 and H1877, deficient only in one of the receptors Cir or Fiu. Cir may play a more dominant role for uptake of the conjugates **7a**−**l** and **8** (acetylated catecholates based on *N*-(aminoalkyl)amino acids and Fiu for the conjugates **12**, **14a**−**c**, and **15c** (diaminobenzoic acid derivatives). The activity of ampicillin and azlocillin is independent of siderophore receptors. There is no marked change in activity against the parent strain H1443 and the different receptor mutants. These results confirm the importance of iron transport for the high antibacterial activities of the new conjugates **7**−**9**, **12**, **14**, and **15**.

Conclusion

New *N*-(aminoalkyl)amino acids, *N*-(aminoalkyl)aminomethyl benzoic acids or *N*-(aminoalkyl)aminomethyl phenoxyacetic acids were prepared from oxocarboxylic acids and diamines by catalytic hydrogenation. These compounds and 3,5-diaminobenzoic acid are useful scaffolds for new acylated bis-catecholates. The acylated catecholates were synthesized as 2,3-di-acetoxybenzoyl derivatives and in heterocyclic form as 2,4-dioxo-1,3-benzoxazine derivatives. These acylated bis-catecholates can function as artificial siderophores for Gram-negative bacteria as demonstrated for *P. aeruginosa* and *E. coli*. The acylated bis-catecholates were coupled with ampicillin or amoxicillin to new siderophore−antibiotic conjugates, which were able to use siderophore iron uptake routes to penetrate the bacterial outer membrane. This results in strongly increased activity against Gram-negative bacteria including strains of *P. aeruginosa*, *S. maltophilia*, *E. coli*, *K. pneumoniae*, and *S. marcescens*. The bis-catecholates of *N*-(aminoalkyl)aminomethyl phenoxyacetic acids were linked to 6-aminopenicillanic acid to form a new type of siderophore−β-lactam conjugate of penicillin V in which the siderophore moiety is attached to the phenyl ring of aminoacyl penicillins. This constellation, in which the siderophore part is more distant to the β-lactam part than in conjugates with the siderophore moiety linked to the NH group of aminoacyl penicillins, resulted in inactive compounds. We can assume that the β-lactam moiety is involved in the iron complexation in highly active conjugates.

The ampicillin and amoxicillin derivatives of *N*,*N*′-(2,3-diacetoxybenzoyl)-*N*-aminobutyl-glycine **7b** (HKI 9924154) and **8** (HKI 9924155) were also highly active in vivo in a murine septicaemic model and exhibited low acute toxicity (data will be presented in a following paper). Accompanied with low synthetic effort, these results favor compounds **7b** and **8** as promising candidates for further preclinical and clinical investigations.

Experimental Section

General. ^{1}H NMR spectra were recorded on a 300 MHz Bruker NMR spectrometer. Chemical shifts (δ) are given in ppm related to tetramethylsilane as internal standard. Coupling constants (*J*) are reported in Hz. Mass spectrometry (MS) and high-resolution MS (HRMS) spectra were obtained by a Finnigan MAT 95 XV high-resolution mass spectrometer with electron-spray ionization (ESI) technique. Purification of the compounds by preparative HPLC was performed on an ABIMED GILSON apparatus equipped with an 115 UV detector (254 nm) and a KNAUER VERTEX reversed phase column (250 mm × 32 mm or 50 mm × 20 mm) packed with Eurosper 100-C18 (7 μm). Eluents used were acetonitrile and water, beginning with ratio 30:70 (v/v) and achieving 100:0 (v/v) after a period of 20 min (flux rate, 20 or 10 mL/min). Column chromatography was performed using silica gel (Merck 60, 0.040−0.063 mm). The purity of all compounds was controlled by ^{1}H NMR spectra and by thin-layer chromatography (TLC) using precoated silica plates (Merck 60 F254) and *n*-butyl acetate/acetic acid (4:1) or *n*-butyl acetate/methanol/formic acid/water (32.5:15:2.5:5) as solvents.

The following compounds were prepared according to published procedures: 2,3-Di-acetoxy-benzoyl chlorides **4a**,[20] 2,3-di(methoxycarbonyloxy)benzoyl chloride **4b**,[8] 5-chloro-2,3-di-(methoxycarbonyloxy)benzoyl chloride **4c**,[8] 5-bromo-2,3-di-(methoxycarbonyloxy)benzoyl chloride **4d**,[8] and 3,4-diacetoxybenzoyl chloride **4f**.[21] 2,3-Dichloro-5,6-dimethoxycarbonyloxybenzoyl chloride **4e** was synthesized analogously to compound **4b** as follows: 2,3-dichloro-5,6-dihydroxybenzoic acid [16] and methyl chloroformate in sodium hydroxide solution gave 2,3-dichloro-5,6-di-(methoxycarbonyloxy)benzoic acid, yield 60%. ^{1}H NMR (DMSO-d_6): δ 8.06 (s, 1H), 3,85 (s, 6H). MS: ESI 337.0 [M − H]$^-$. Subsequent reaction with PCl_5 in tetrachloro methane resulted in **4e** as a yellowish oil (yield 89%).

General Procedure for the Preparation of Secondary Diamino Acids 3b−d,o,p, *N*-(Aminoalkyl)aminomethyl Benzoic Acids 3k,l, and *N*-(Aminoalkyl)aminomethyl Phenoxyacetic Acids 3m,n According to the Synthesis of 3a.[14] In an ice bath, a solution of 0.1 mol of the respective oxocarboxylic acid **2a**−**f** in methanol (20 mL) was dropped with stirring into a solution of 0.12 mol of the respective diamine **1a**−**e** in methanol (30 mL), and then, 1.0 g Pd/C (10%) was added under nitrogen. The mixture was hydrogenated at ambient temperature and atmospheric pressure. Then, the reaction mixture was filtered by Celite and the solvent was evaporated. Three times toluene (20 mL) was added to the residue and distilled off at 120 °C. The residue was dried in a vacuum to give a light yellow or colorless oil of **3**. In most cases,

the product was pure enough for the following reaction. The product can be further purified by reaction with benzyl chloroformate to obtain the di-*Z*-derivative, separating from impurities by preparative HPLC and following hydrogenolysis with Pd/C (10%). **3b**: *N*-(Amino-*n*-butyl)glycine, from **1d** and **2a**, yield 93%. ^{1}H NMR (D_2O): δ 3.21 (s, 2H), 2.58−2.93 (m, 4H), 1.53−1.62 (m,4H). **3c**: *N*-(Amino-*n*-pentyl)glycine, from **1e** and **2a**, yield 90%. ^{1}H NMR (DMSO-d_6): δ 3.30 (s, 2H), 2.71−2.74 and 2.77−2.80 (2× t, 4H), 1.40−1.64 (t, 2H), 1.59−1.64 (m, 4H). **3d**: *N*-(Methylaminopropyl)glycine, from **1c** and **2a**, yield 80%. ^{1}H NMR (DMSO-d_6): δ 3.01 (s, 2H), 2.71 (t, 2H), 2.57 (t, 2H), 2.32 (s, 3H), 1.63 (t, 2H). MS: 146.9 [M + H]$^+$. **3k**: 2-[*N*-(Methylamino-*n*-propyl)aminomethyl]benzoic acid, from **1c** and **2d**, yield 60%. ^{1}H NMR (D_2O): δ 7.32−7.55 (m, 4H), 3.96 (d, J = 8.2, 2H), 2.70−2.76 (m, 4H), 1.50−1.59 (m, 4H). **3l**: 2-[*N*-(Aminobutyl)aminomethyl]benzoic acid, from **1d** and **2d**, yield 95%. ^{1}H NMR (D_2O): δ 7.32−7.55 (m, 4H), 3.96 (d, J = 8.2, 2H), 2.70−2.76 (m, 4H), 1.50−1.59 (m, 4H). **3m**: 4-[*N*-(Aminobutyl)aminomethyl]phenoxyacetic acid, from **1d** and **2e**, yield 90%. ^{1}H NMR (DMSO-d_6): δ 7.23−7.28 (m, 2H), 6.89−6.91 (m, 2H), 4.40−4.44 (m, 2H), 3.75 (s, 2H), 2.75−2.80 (m, 2H), 2.62−2.68 (m, 2H), 1.54−1.55 (m, 4H). **3n**: 2-[*N*-(Aminobutyl)aminomethyl]phenoxyacetic acid**,** from **1d** and **2f**, yield 60%. MS: 275.0 [M + Na]$^+$. **3o**: *N*-(Amino-*n*-propyl)-alanine), from **1b** and **2b**, yield 65%. MS: 147.0 [M + H]$^+$. **3p**: *N*-(Aminopropyl)-4-aminovaleric acid, from **1b** and **2c**, yield 70%. MS: 175.2 [M + H]$^+$.

General Procedure for the Preparation of Acylated Bis-catecholates 5a−n and 11 and of the Benzoxazine-2,4-dione Derivatives 13b,c. In an ultrasonic bath, a solution of 20 mmol of the respective compounds **4a**−**h** in absolute tetrahydrofuran (20 mL) was added dropwise at 0−5 °C with stirring to a solution of 10 mmol of compounds **3a**−**d,k**−**n** or 3,5-diaminobenzoic acid, respectively, in a 0.5 M aqueous sodium hydrogen carbonate solution (60 mL, 30 mmol). The mixture was stirred for 1 h at 0−5 °C, and then, the tetrahydrofuran was removed in a vacuum. The obtained aqueous solution was cooled to 0 °C, acidified to pH 2 using 6 N HCl, and extracted with ethyl acetate. The organic layer was separated, washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and evaporated. The residue was dried in a vacuum to give compounds **5a**−**n** and **13b**,**c**, respectively, as colorless to yellow solid foams. The compounds could be purified by preparative HPLC. **5a**: *N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminoethyl)glycine, from **3a**[14] and **4a**, yield 35%. ^{1}H NMR (DMSO-d_6): δ 8.30, 8.40 (2× t, 1H), 7.11−7.78 (m, 6H), 3.28−3.41 (m, 4H), 2.14−2.29 (m, 12H). **5b**: *N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)glycine, from **3b** and **4a**, yield 60%. ^{1}H NMR (DMSO-d_6): δ 8.25, 8.35 (2× t, at 360 K changed to s, 1H), 6.94−7.42 (m, 6H), 3.95 (m, 2H), 3.22 (m, 2H), 2.77 (s, 3H), 2.27 (s, 6H), 2.20 (s, 6H), 1.40−1.60 (m, 4H). MS: 609.2 [M + Na]$^+$. **5c**: *N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminopentyl)glycine, from **3c** and **4a**, yield 70%. ^{1}H NMR (DMSO-d_6): δ 8.22, 8.29 (2× t, 1H), 7.09−7.80 (m, 6H), 4.00−4.03 (m, 4H), 3.16−3.28 (m, 4H), 2.16−2.49 (m, 12H), 1.14−1.49 (m, 6H). MS: (ESI) 623.2, [M + Na]$^+$, 599.4 [M − H]$^-$. **5d**: *N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(methylaminopropyl)-glycine, from **3d** and **4a**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 6.94−7.39 (m, 6H), 3.98 (m, 2H), 2.87−3.15 (m, 2H), 2.77 (s, 3H), 2.27 (s, 6H), 2.20 (s, 6H), 1.75 (m, 2H). MS: 587.2 [M + Na]$^+$. **5e**: *N*,*N*′-Bis-(2,3-dimethoxycarbonyloxy-benzoyl)-*N*-(methylamino-*n*-propyl)glycine, from **3d** and **4b**, yield 89%. ^{1}H NMR (DMSO-d_6): δ 7.2−7.6 (m, 6H), 4.00 (s, 2H), 3.80 (m, 12H), 2.96−3.40 (m, 4H), 1.75 (m, 2H). MS: 673.0 [M + Na]$^+$. **5f**: *N*,*N*′-Bis-(2,3-dimethoxycarbonyloxy-5-chloro-benzoyl)-*N*-(methylamino-*n*-propyl)glycine, from **3d** and **4c,** yield 30%. ^{1}H NMR (DMSO-d_6): δ 7.80−7.20 (m, 4H), 4.00 (m, 2H), 3.81 (m, 12H), 2.96−3.40 (m, 4H), 2.78 (s, 3H), 1.75 (m, 2H). **5g**: *N*,*N*′-Bis-(2,3-dimethoxycarbonyloxy-5-bromo-benzoyl)-*N*-(methylaminopropyl)glycine, from **3d** and **4d**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 7.35−7.90 (m, 4H), 4.00 (m, 2H), 3.81 (m, 12H), 2.96−3.40 (m, 4H), 2.78 (s, 3H), 1.75 (m, 2H). **5h**: *N*,*N*′-Bis-(2,3-dimethoxycarbonyloxy-5,6-dichloro-benzoyl)-*N*-(methylaminopropyl)glycine, from **3d** and **4e**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 8.04 (m, 2H), 4.00 (m, 2H), 3.81 (m, 12H), 2.96−3.40 (m, 4H), 2.78 (s, 3H), 1.80 (m, 2H). **5i**: *N*,*N*′-Bis-(3,4-diacetoxy-benzoyl)-*N*-(aminobutyl)glycine, from **3b** and **4f**. The crude product was purified by column chromatography on silicagel using ethyl acetate/methanol (3:1) as eluent, yield 17%. ^{1}H NMR (DMSO-d_6): δ 8.10, 8.20 (2× t, 1H), 6.69−7.80 (m, 6H), 3.52−3.68 (m, 2H), 3.13−3.30 (m, 4 H), 2.26−2.28 (m, 12H), 1.19−1.56 (m, 4H). MS: 609.5 [M + Na]$^+$. **5k**: 2-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(methylamino-*n*-propyl)-aminomethyl]benzoic acid, from **3k** and **4a**, yield 34%. ^{1}H NMR (DMSO-d_6): δ 6.83−7.92 (m, 10H), 4.75−5.12 (m, 2H), 2.70−3.29 (m, 4H), 2.53 (s, 3H), 2.10−2.28 (m, 12H), 1.73−1.91 (m, 2H). MS: 661.8 [M − H]$^-$. **5l**: 2-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)aminomethyl]benzoic acid, from **3l** and **4a**, yield 15%. ^{1}H NMR (DMSO-d_6): δ 8.20, 8.29 (2× t, 1H), 7.07−7.90 (m, 10H), 4.71−5.04 (m, 2H), 2.99−3.29 (m, 4 H), 2.15−2.27 (m, 12H), 1.22−1.56 (m, 4H). MS: 661.3 [M − H]$^-$. **5m**: 4-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)aminomethyl]phenoxyacetic acid, from **3m** and **4a**, yield 47%. ^{1}H NMR (DMSO-d_6): δ 13.01 (s, 1H), 8.20, 8.28 (2× t, at 360 K changed to s, 1H), 6.88−7.36 (m, 10H), 4.65 (s, 2H), 2.90−3.08 (m, 4H), 1.97−2.49 (m, 14H), 1.42−1.55 (m, 4H). MS: 715.2 [M + Na]$^+$, 691.3 [M − H]$^+$. **5n**: 2-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)aminomethyl]phenoxyacetic acid, from **3n** and **4a**, yield 50%. ^{1}H NMR (DMSO-d_6): δ 8.18, 8.30 (2× t, 1H); 6.85−7.39 (m, 12H); 4.65, 4.73 (s, 2 × 2H); 3.01−3.29 (m, 4H); 2.05−2.26 (m, 12 H); 1.49−1.54 (m, 4H). MS: 715.3 [M + Na]$^+$, 691.3 [M − H]$^-$. **11**: 3,5-Di-[(2,3-diacetoxybenzoyl)amino]benzoic acid, from 3,5-diaminobenzoic acid and **4a**, mp 197−200 °C (ethyl acetate), yield 55%. ^{1}H NMR (DMSO-d_6): δ 10.65 (s, 2H), 8.33 (m, 1H), 8.06 (d, J = 1.9, 2H), 7.59 (dd, J_1=5.5, J_2=1.9, 2H), 7.56 (s, 2H), 7.44 (d, J = 1.9, 2H), 2.30 (s, 3H), 2.22 (s, 3H). MS: 591.3 [M − H]$^-$. **13b**: 3,5-[Bis-(8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)acetylamino]benzoic acid, from 3,5-diaminobenzoic acid and **4g** (8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)-acetylchloride, yield 53%, mp 190−195 °C. ^{1}H NMR (DMSO-d_6): δ 10.48 (s, 2H), 8.14 (m, 1H), 7.96−7.86 (m, 6H), 7.49 (t, J = 7.9 Hz, 2H), 4.69 (s, 4H), 3.91 (s, 6H). MS: 705.1 [M − H]$^-$. **13c**: 3,5-Bis-[3-(8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)propionyl-amino]benzoic acid, from 3,5-diaminobenzoic acid and **4h** (8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)propionyl chloride, yield 51%, mp 160−165 °C. ^{1}H NMR (DMSO-d_6): δ 10.23 (s, 2H), 8.08 (m, 1H), 7.94−7.80 (m, 6H), 7.46 (t, J = 7.9, 2H), 4.16 (t, J = 8,0, 4H), 3.90 (s, 6H), 2.70 (t, J = 8,0, 4H). MS: 733.0 [M − H]$^-$.

General Procedure for the Preparation of the 1,3-benzoxazine-2,4-dione Derivatives 6a−c or 13a. Analogously to the procedure for compound **5**, a solution of 2 mmol of 2,3-di-(methoxycarbonyloxy)benzoyl chloride **4b** in tetrahydrofuran was added to a solution of 1 mmol of compound **3b,o**,**p** or 3,5- diaminobenzoic acid, respectively, in 0.5 M aqueous sodium hydroxide solution at 0−5 °C in an ultrasonic bath. After it was stirred for 1 h, the tetrahydrofuran was removed and the residual aqueous solution was acidified using 6 N HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was stirred in acetonitrile for 3 h at ambient temperature. The solvent was then evaporated, and the residue was purified by preparative HPLC. Acetonitrile was evaporated in a vacuum from the fraction containing the target product, and the residual aqueous solution was dried by lyophilization to give **6a**−**c** and **13a**, respectively, as colorless solids. **6a**: 7-(8-Methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)-3-(2,3-dimethoxy-carbonyl-oxybenzoyl)-3-aza-heptanoic acid, from **3b** and **4b**, yield 50%. ^{1}H NMR (DMSO-d_6): δ 7.25−7.60 (m, 6H), 4.15 (s, 2H), 3.72−3.94 (m, 9H), 3.65 (t, 2H), 3.10 (t, 2H), 1.35−1.65 (m, 4H). MS: 641.0 [M + Na]$^+$. **6b**: 6-(8-Methoxycarbonyloxy-1,3-benzoxazine-dione-3-yl)-3-(2,3-dimethoxy-carbonyloxybenzoyl)-2-methyl-3-aza-hexanoic acid, from **3o** and **4b**. ^{1}H NMR (DMSO-d_6): δ 3.75 (t, 2H), 7.28−7.89 (m, 6H), 4.15 (s, H), 3.78−3.91 (m, 9H), 3.20 (t, 2H), 1.80−1.95 (m, 2H), 1.32−1.40 (m, 3H). MS: 641.2 [M + Na]$^+$. **6c**: 8-(8-Methoxycarbonyloxy-1,3-benzoxazine-2,4-

dione-3-yl)-5-(2,3-dimethoxy-carbonyloxybenzoyl)-5-aza-4-methyl-octanoic acid, from **3p a**nd **4b**, yield 60%. ^{1}H NMR (DMSO-d_6): δ 7.20−7.90 (m, 6H), 3.85−4.05 (m, 9H), 3.40−3.85 (m, 4H), 3.05 (q, 1H), 1.9−2.2 (m, 4H), 1.75 (m, 2H), 1.15 (d, 3H). **13a**: 3,5-Bis-(8-methoxycarbonyloxy-1,3-benzoxazin-2,4-dione-3-yl)benzoic acid, yield 64%, mp 164−166 °C. ^{1}H NMR (DMSO-d_6): δ 8.20 (d, J = 1.9, 2H), 7.92 (dd, J_1=7.8, J_2=1.5, 2H), 7.86 (dd, J_1=7.8, J_2=1.5, 2H), 7.78 (t, J = 1.8, 1H), 7.50 (t, J = 8,0, 2H), 3.92 (s, 6H). MS: 591.4 [M − H]$^-$.

General Procedure for the Preparation of the Aminoacylpenicillin Conjugates 7a−m, 8, 9a−c, 12, 14a−c, and 15c. To a solution of 1 mmol of compounds **5a−m**, **6a−c**, **11**, or **13a−c**, respectively, and *N*-methylmorpholine (112 μL) in absolute tetrahydrofuran (10 mL), methylchloroformate (131 μL) was added at − 20 °C with stirring. The mixture was stirred for 1 h at −10 °C, and then, a solution of ampicillin trihydrate (445 mg, 1.1 mmol) or amoxicillin trihydrate (461 mg), respectively, and triethylamine (140 μL, 1 mmol) in tetrahydrofuran (4 mL) and water (1 mL) was added at −20 °C. The mixture was stirred for 1 h at −10 °C and 1 h at ambient temperature and then evaporated. Ethyl acetate and water were added to the residue. The mixture was acidified to pH 3 using diluted HCl with shaking. The organic phase was separated, washed with brine, dried with Na_2SO_4, filtered, and evaporated. The obtained residue was purified by preparative HPLC. The fraction containing the respective conjugate was evaporated, and the residual aqueous solution was dried by lyophilization to give **7a−m**, **8 or 9a−c**, **12**, **14a−c**, or **15c**, respectively, as a colorless solid.

The sodium salts were prepared from the solution of 1 mmol of the compounds **7−9**, **12**, **14**, or **15c**, respectively, in ethyl acetate (5 mL) by addition of sodium ethylhexanoate (1.5 mmol) in ethyl acetate (3 mL). Precipitation was completed by adding petroleum ether. The obtained colorless solid was filtered, washed with petroleum ether, and dried in a vacuum.

7a: *N*-[*N*′,*N*″-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminoethyl)glycyl]ampicillin, from **5a**, yield 23%. ^{1}H NMR (DMSO-d_6): δ 9.10−9.20 (m, 1H), 8.72−8.74 (m, 1H), 8.25, 8.35 (2× t, 1H), 7.24−7.49 (m, 11H), 5.72 (m, 1H), 5.49−5.51 (m, 1H), 5.35−5.38 (m, 1H), 4.14 (s, 1H), 3.10−3.40 (m, 6 H), 2.05−2.27 (m, 12H), 1.40−1.52 (m, 6H). MS: 912.3 [M + Na]$^+$. **7b**: *N*-[*N*′,*N*″-Bis-(2,3-diacetoxybenzoyl)-*N*′-(aminobutyl)glycyl]ampicillin, from **5b,** yield 50%. ^{1}H NMR (DMSO-d_6): δ 8.70 (m, 2H), 8.25, 8.34 (2× t, changed to s at 360 K, 1H), 6.94−7.52 (m, 11H), 5.72 (m, 1H), 5.51 (m, 1H), 5.38 (m, 1H), 4.19 (s, 1H), 3.95 (m, 2H), 3.30 (s, 3H), 3.22 (m, 2H), 2.27 (s, 6H), 2.21 (s, 3H), 2.17 (s, 3H), 1.54 (s, 3H), 1.40 (s, 3H), 1.40−1.60 (m, 4H). MS: 940.1 [M + Na]$^+$. **7c**: *N*-[*N*′,*N*″-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminopentyl)glycyl]ampicillin, from **5c**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 9.11−9.15 (m, 1H), 8.68 (m, 1H), 8.29, 8.38 (2× t, 1H), 7.25−7.49 (m, 11H), 5.72 (m, 1H), 5.48 (m, 1H), 5.35 (m, 1H), 4.15 (m, 2H), 3.90 (m, 1H), 3.16−3.31 (m, 4H), 2.16−2.23 (m, 12H), 1.40−1.54 (m, 12H). MS: 954.6 [M + Na]$^+$, 930.2 [M − H]$^+$. **7d**: *N*-[*N*′,*N*″-Bis-(2,3-diacetoxybenzoyl)-*N*-(methylaminopropyl)glycyl]ampicillin from **5d**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 9.15 (m, 1H), 8.73 (m, 1H), 6.94−7.48 (m, 11H), 5.85 (m, 1H), 5.52 (m, 1H), 5.39 (m, 1H), 3.98 (m, 2H), 2.87−3.15 (m, 2H), 2.75 (s, 3H), 2.27 (s, 6H), 2.20 (s, 6H), 1.75 (m, 2H), 1.53 (s, 3H), 1.40 (s, 3H). **7e**: *N*-[*N*′,*N*″-Bis-(2,3-dimethoxycarbonyloxy-benzoyl)-*N*-(methylaminopropyl)glycyl]ampicillin, from **5e**, yield 45%. ^{1}H NMR (DMSO-d_6): δ 8.71, 9.15 (m, 2 × 1H), 7.25−7.60 (11H), 5.72 (q, 1H), 5.50 (m, 1H), 5.38 (m, 1H), 4.17 (s, 1H), 3.77−4.20 (m, 14H), 2.96−3.40 (m, 4H), 2.76 (s, 3H), 1.75 (m, 2H), 1.53 (s, 3H), 1.40 (s, 3H). MS: 1004.0 [M + Na]$^+$. **7f**: *N*-[*N*′,*N*″-Bis-(2,3-dimethoxycarbonyloxy-5-chloro-benzoyl)-*N*-(methylaminopropyl)glycyl]ampicillin, from **5f**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 9.19 (d, 1H), 8.75 (m, 1H), 7.75−7.20 (m, 9H), 5.74 (d, 1H), 5.48 (q, 1H), 5.38 (d, 1H), 4.18 (s, 1H), 3.93 (m, 2H), 3.85 (m, 6H), 3.81 (m, 6H), 2.96−3.40 (m, 4H), 2.78 (s 3H), 1.75 (m, 2H), 1.53 (s, 3H), 1.39 (s, 3H). MS: 1048.1 [M − H]$^-$. **7g**: *N*-[*N*′,*N*″-Bis-(2,3-dimethoxycarbonyloxy-5-chloro-benzoyl)-*N*-(methylaminopropyl)glycyl]-ampicillin, from **5g**, yield 50%. ^{1}H NMR (DMSO-d_6): δ 9.20 (d, 1H), 8.75 (m, 1H), 7.85−7.25 (m, 9H), 5.74 (d, 1H), 5.38 (d, 1H), 5.52 (q, 1H), 4.18 (s, 1H), 3.95 (m,2H), 3.85 (m,6H), 3.80 (m, 6H), 2.96−3.40 (m, 4H), 2.78 (s, 3H), 1.75 (m, 2H), 1.53 (s, 3H), 1.39 (s, 3H). MS: 1038.5 [M − H]$^-$. **7h**: *N*-[*N*′,*N*″-Bis-(2,3-dimethoxycarbonyloxy-5,6-dichloro-benzoyl)-*N*-(methylaminopropyl)glycyl]ampicillin, from **5h**, yield 30%. ^{1}H NMR (DMSO-d_6): δ 9.19 (d, 1H), 8.65 (m, 1H), 7.20−8.05 (m, 7H), 5.70 (m, 1H), 5.48 (m, 1H), 5.38 (m, 1H), 4.19 (s, 1H), 4.05 (m, 2H), 3.85 (m, 6H), 3.82 (m, 6H), 2.96−3.40 (m, 4H), 2.78 (s, 3H), 1.80 (m, 2H), 1.53 (s, 3H), 1.39 (s, 3H). MS: 1118.4 [M − H]$^-$. **7i**: *N*-[*N*′,*N*″-Bis-(3,4-diacetoxy-benzoyl)-*N*-(aminobutyl)-glycyl]ampicillin, from **5i**, yield 9%. ^{1}H NMR (DMSO-d_6): δ 8.91−9.12 (m, 2H), 8.44−8.74 (m, 1H), 7.25−7.87 (m, 11H), 5.69−5.75 (m, 1H), 5.46−5.52 (m, 1H), 5.37−5.41 (m, 1H), 4.16 (s, 1H), 3.12−3.36 (m, 6H), 2.28−2.29 (m, 12H), 1.53 (s, 3H), 1.40 (s, 3H), 1.24−1.45 (m, 4H). MS: 939.7 [M + Na]$^+$, 917.7 [M + H]$^+$. **7k**: *N*-{2-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(methylaminopropyl)aminomethyl]benzoyl}ampicillin, from **5k**, yield 20%. ^{1}H NMR (DMSO-d_6): δ 8.96−9.26 (m, 2H), 6.88−7.49 (m, 15H), 5.84−5.93 (m, 1H), 5.50−5.55 (m, 1H), 5.38−5.40 (m, 1H), 4.18 (s, 1H), 2.74−3.29 (m, 6H), 2.71 (s, 3H), 2.12−2.27 (m, 12H), 1.52 (s, 3H), 1.40 (s, 3H), 1.45−1.82 (m, 2H). MS (ESI + NH_4OAc): 1012.2 [M + NH_4]$^+$. **7l**: *N*-{2-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)aminomethyl]benzoyl}-ampicillin**,** from **5l**, yield 36%. ^{1}H NMR (DMSO-d_6):δ 8.92−9.12 (m, 2H), 8.20, 8.30 (2× t, 1H), 7.24−7.54 (m, 15H), 5.88−5.91 (m, 1H), 5.48−5.59 (m, 1H), 5.39−5.40 (m, 1H), 4.19 (s, 1H), 2.95−3.23 (m, 6H), 2.15−2.26 (m, 12H), 1.52 (s, 3H), 1.40 (s, 3H), 1.16−1.45 (m, 4H). MS: 1016. [M + Na]$^+$. **7m**: *N*-{4-[*N*′,*N*″-Bis-(2,3-diacetoxybenzoyl)-*N*′-(aminobutyl)aminomethyl]-phenoxyacetyl}ampicillin, from **5m**, yield 65%. ^{1}H NMR (DMSO-d_6): δ 9.18−9.20 (m, 1H), 8.54−8.57 (m, 1H), 8.20, 8.30 (2× t, 1H), 6.88−7.41 (m, 15H), 5.75−5.85 (m, 1H), 5.51−5.52 (m, 1H), 5.39 (d, J = 4.0, 1H), 4.63 (s, 2H), 4.18 (s, 2H), 2.90−3.15 (m, 4H), 2.16−2.27 (m, 12H), 1.39−1.53 (m, 10H). MS: 1047.6 [M + Na]$^+$. **8**: *N*-[*N*′,*N*″-Bis-(2,3-diacetoxybenzoyl)-*N*′-(aminobutyl)glycyl]amoxicillin, from **5b** and amoxicillin, yield 40%. ^{1}H NMR (DMSO-d_6): δ 9.38 (s, 1H), 9.02 (m, 1H), 8.56 (m, 1H), 8.25, 8.35 (2× t, 1H), 6.60−7.45 (m, 10H), 5.55 (m, 2H), 5.38 (m, 1H), 4.18 (s, 1H), 3.95 (m, 2H), 3.03 (m, 2H), 3.13 (m, 3H), 2.27 (s, 6H), 2.21 (s, 3H), 2.17 (s, 3H), 1.54 (s, 3H), 1.41 (s, 3H), 1.40−1.60 (m, 4H). MS: 956.6 [M + Na]$^+$. **9a**: *N*-[7-(8-Methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)-3-(2,3-dimethoxycarbonyloxy-benzoyl)-3-aza-heptanoyl]ampicillin, from **6a**, yield 55%. ^{1}H NMR (DMSO-d_6): δ 8.67−9.15, 2H), 7.25−7.90 (m, 11H), 5.67 (q, 1H), 5.51 (m, 1H), 5.38 (m, 1H), 4.19 (s, 1H), 4.15 (s, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H), 3.71 (t, 2H), 3.08 (t, 2H), 1.53 (s, 3H), 1.40 (s, 3H), 1.35−1.65 (m, 4H). MS: 980.1 [M + Na]$^+$. **9b**: *N*-[6-(8-Methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3yl)-3-(2,3-dimethoxycarbonyloxy-benzoyl)-3-aza-2-methyl-hexanoyl]ampicillin, from **6b**, yield 40%. MS: 972.4 [M + Na]$^+$, 948.6 [M − H]$^-$. **9c**: *N*-[8-(8-Methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)-5-(2,3-dimethoxycarbonyloxy-benzoyl)-5-aza-4-methyl-octanoyl]ampicillin, from **6c**, yield 73%. ^{1}H NMR (DMSO-d_6): δ 8.50−9.10 (m, 2H), 7.2−7.9 (m, 11H), 5.67 (q,1H), 5.51 (m, 1H), 5.37 (m, 1H), 4.19 (s, 1H), 3.8−4.0 (m, 9H), 3.05 (q, 1H), 1.8−2.2 (m, 4H), 1.75 (m, 2H), 1.55 (s, 3H), 1.40 (s, 3H), 1.15 (d, 3H). MS: 978.3 [M + H]$^+$. **12**: *N*-[3,5-Di-(2,3-diacetoxybenzoylamino)benzoyl]ampicillin, from **11,** yield 25%. ^{1}H NMR (DMSO-d_6): δ 10.61 (s, 2H), 9.08 (d, 1H), 8.67 (d, 1H), 8.29 (m, 1H), 7.86 (d, 2H), 7.58 (m, 2H), 7.51 (d, 2H), 7.45 (s, 2H), 7.43 (d, 2H), 7.32 (m, 3H), 5.86 (d, 1H), 5.54 (q, 1H), 5.40 (d, 1H), 4.19 (s, 1H), 2.29 (s, 6H), 2.21 (s, 6H), 1.51 (s, 3H), 1.40 (s, 3H). MS: 922.3 [M − H]$^-$. **14a**: *N*-[3,5-*N*′,*N*″-Bis-(8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)benzoyl]ampicillin, from **13a**, yield 81%. ^{1}H NMR (DMSO-d_6): δ 9,13 (d, J = 7.6, 1H), 8.94 (d, J = 7.9, 1H), 8.15 (d, J = 1.9, 2H), 7.92 (dd, J_1=7.8, J_2=1.5, 2H), 7.86 (dd, J_1=7.8, J_2=1.5, 2H), 7.72 (t, J = 1.8, 1H), 7.49 (m, 4H), 7.32 (m, 3H), 5.89 (d, J = 8,0, 1H), 5.53 (q, 1H), 5.41 (d, J = 3.8, 1H), 3.92 (s, 6H), 4.18 (s, 1H), 1.50 (s, 3H), 1.38 (s, 3H). MS: 922.2 [M − H]$^-$. **14b**: *N*-{[3,5-*N*,*N*′-Bis-(8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)acetylamino]benzoyl}ampicillin, from **13b**, yield 90%. ^{1}H NMR (DMSO-d_6): δ 10.48 (s, 2H), 9.06 (d, 1H), 8.86

(d, 1H), 8.14 (m, 1H), 7.96−7.86 (m, 6H), 7.55−7.25 (m, 7H), 5.82 (d, 1H), 5.48 (q, 1H), 5.39 (d, 1H), 4.69 (s, 4H), 4.17 (s, 1H), 3.91 (s, 6H), 1.48 (s, 3H), 1.37 (s, 3H). MS: 1036.7 [M − H]$^-$. **14c**: *N*-{[3,5-Bis-3-(8-methoxycarbonyloxy-1,3-benzoxazine-2,4-dione-3-yl)propionylamino]benzoyl}ampicillin, from **13c**, yield 80%. ^{1}H NMR (DMSO-d_6): δ 10.23 (s, 2H), 9.11 (d, 1H), 9.01 (d, 1H), 8.07 (m, 1H), 7,89 (dd, J_1=8, J_2=1,5, 2H), 7,81 (dd, J_1=8.0, J_2=1.5, 2H), 7.66 (d, J = 1.8,0, 2H), 7.46 (m, 4H), 7.30 (m, 3H), 5.83 (d, 1H), 5.48 (q, 1H), 5.38 (d, 1H), 4.17 (s, 1H), 4.16 (t, J = 8.0, 4H), 3.91 (s, 6H), 2.69 (t, J = 8, 4H), 1.51 (s, 3H), 1.39 (s, 3H). MS: 1064.5 [M − H]$^-$. **15c**: *N*-{3,5-Bis-[3-(8-methoxycarbonyloxy-1,3-benzoxazin-2,4-dione-3-yl)-propionylamino]benzoyl}amoxicillin, from **13c**, yield 40%. ^{1}H NMR (DMSO-d_6): δ 10.18 (s, 2H), 9.46 (br s, 1H), 8.70 (d, 1H), 8.43 (d, 1H), 8.11 (s, 1H), 7.89 (d, 2H), 7.79 (d, 2H), 7.63 (br s, 2H), 7.45 (t, 2H), 7.26 (d, 2H), 6.71 (d, 2H), 5.69 (d, 1H), 5.37 (q, 1H), 5.27 (d, 1H), 4.16 (t, 4H), 3.91 (s, 6H), 3.81 (s, 1H), 2.70 (t, 4H), 1.47 (s, 3H), 1.39 (s, 3H). MS: 1080.6 [M − H]$^-$.

Procedure for the Preparation of the Penicillin V Derivatives 10a,b. Isobutyl chloroformate (65 μL, 0.5 mmol) was added with stirring at −15 °C to a solution of 0.5 mmol of **5m** or **n**, respectively, and 56 μL of *N*-methyl morpholine in absolute tetrahydrofuran (10 mL). The mixture was stirred for 1 h at −10 °C, and then, 6-aminopenicillanic acid (108 mg, 0.5 mmol) and triethylamine (70 μL) in tetrahydrofuran/water (5 mL, 4:1) were added. The mixture was stirred for 1 h at −10 °C and for 1 h at ambient temperature and then evaporated. Water was added to the residue, and the solution was then carefully acidified using dilute aqueous HCl. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by preparative HPLC. From the obtained main fraction, the acetonitrile was removed in a vacuum and the residual aqueous suspension was dried by lyophilization to afford a colorless solid of **10a** or **b**, respectively. **10a**: *N*-{4-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)aminomethyl]phenoxyacetyl}-6-aminopenicillanic acid, from **5m**, yield 65%. ^{1}H NMR (DMSO-d_6): δ 8.59−8.65 (m, 1H), 8.24, 8.32 (2× t, changed to s at 360 K, 1H), 6.90−7.50 (m, 10H), 5.50−5.65 (m, 2H), 4.50 (m, 3H), 4.25 (m, 2H), 3.05−3.25 (m, 4H), 2.15−2.25 (m, 12H), 1.40−1.60 (m, 10H). MS: 913.4 [M + Na]$^+$, 889.2 [M − H]$^+$. **10b**: *N*-{2-[*N*,*N*′-Bis-(2,3-diacetoxybenzoyl)-*N*-(aminobutyl)aminomethyl]phenoxyacetyl}-6-aminopenicillanic acid, from **5n**, yield 85%. ^{1}H NMR (DMSO-d_6): δ 8.63−8.65 (2× d, 1H), 8.21, 8.31 (2× t, 1H), 6.95−7.40 (m, 10H), 5.47−5.57 (m, 2H), 4.70 (m, 3H), 4.24−4.35 (m, 2H), 3.03−3.19 (m, 4H), 2.12−2.27 (m, 12H), 1.46−1.57 (m, 10H). MS: 913.6 [M + Na]$^+$, 889.4 [M − H]$^-$.

Determination of Biological Activities. Utilization of siderophores was determined by growth promotion assays. Bacterial strains were grown overnight on Nutrient Agar (Serva, Germany), suspended in medium VA (glycerol, 50 mL/L; NaCl, 8.5 g/L; pH 7.2), and diluted to the density of a 1 McFarland standard (approximately 1 × 10^9 cfu per mL). A 1.5 mL amount of this suspension was mixed with 99 mL of test medium prepared according to Hall and Ratledge[22] (glycerol, 20 mL/L; L-asparagin, 5 g/L; KH_2PO_4, 5 g/L in distilled water, pH 7.5). After 20 g of Al_2O_3 was added and it was sterilized at 121 °C for 15 min, the suspension was filtered and the pH was adjusted to 6.8. A 12 g amount of agar No. 1 (Unipath, Germany) was added, and the medium was sterilized at 121 °C for 15 min. Prior to inoculation, trace elements were added to the medium as a filter-sterilized solution ($ZnSO_4 \times 7H_2O$, 2.03 mg; $MnSO_4 \times 4H_2O$, 0.405 mg; $MgSO_4$, 0.2 mg; $CaCl_2 \times 2H_2O$, 1 mg; $Na_2MoO_4 \times 2H_2O$, 0.2 mg; $CuSO_4 \times 5H_2O$, 0.2 mg; $CoCl_2 \times 6H_2O$, 0.4 mg; and ethylenediamine-di(o-hydroxy-phenylacetic acid) (EDDHA), 3.6 g). Siderophore solutions were applied on assay disks (∅ 6 mm) on the surface of the inoculated test agar plates, and the zones of growth surrounding the disks were determined after incubation for 18−20 h at 37 °C.

Gram-negative bacterial strains were used as follows: *P. aeruginosa* ATCC 27853, SG 137, NCTC 10662 and *E. coli* ATCC 25922. For comparison with the synthetic siderophores, the natural siderophore ferrioxamin B (Desferal mesylate, Sigma, Germany) was used as control (Table 2).

In parallel to the growth promotion assays, the relative iron-complexing capacity of the siderophore derivatives was checked by the CAS assay according to Schwyn and Neilands (1987).[23] A positive CAS reaction is associated with iron chelation (Table 2).

The antibacterial activities of the conjugates **7**−**9**, **10**, **12**, **14**, and **15** were determined as minimal inhibitory concentrations (MIC) by the microbroth dilution method in Mueller−Hinton broth (Difco) according to the NCCLS guidelines[24] (Table 3). Test organisms were from the American Type Culture Collection (ATCC) and from the stock of the Hans Knöll institute (SG). *S. maltophilia* GN 12873 [25] was kindly provided by the Episome Institute, Gunma (Japan).

The mechanism of action, e.g., the influence of FeIII uptake routes and efflux systems on the activities of the conjugates, was studied either in agar diffusion assays in Nutrient Agar (Serva) or by determination of MIC by a microbroth dilution technique (inoculum 10^5 CFU/mL) in Nutrient Broth (Serva). The strains used were mutants of *E. coli* K-12 with alterations in outer membrane porin proteins OmpC and OmpF (deleted − or overexpressed +++), in the iron transport protein TonB (Table 4), and in the catecholate Fe^{3+} siderophore receptors Cir, Fiu, and FepA (strains were kindly provided by K. Hantke, Tübingen, Germany[19]) (Table 5). Compounds were tested with addition of clavulanic acid in a concentration of 10 μg/mL to neutralize the activity of the penicillinase integrated as a selection marker in the mutants. In the agar diffusion assay, 50 μL of a 0.2 mM solution of the compounds was filled in agar wells of 9 mm in diameter. Inhibition zones were read after overnight incubation at 37 °C.

Acknowledgment. The financial support of Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Berlin, Germany, FZ 0311232) and of Grünenthal GmbH, Aachen, Germany, is gratefully acknowledged. We thank Andrea Perner, Renate Koch, and Heike Heinecke for HRMS and NMR spectra and Antje Fritzsche, Silke Leonhardt, Roswitha Peters, Irmgard Heinemann, and Monika Golembiewski for excellent technical assistance.

Supporting Information Available: HRMS data of β-lactam conjugates. This material is available free of charge via Internet at http://pubs.acs.org.

References

(1) Drechsel, H.; Jung, G. Peptide Siderophores. *J. Pept. Sci.* **1998**, *4*, 147−181.
(2) Bergeron, R. J.; Burton, P. S.; McGovern, K. A.; Onge, E. J. St.; Streiff, R. R. Synthesis, Absorption, and Toxicity of N^1,N^8-Bis-(2,3-dihydroxybenzoyl)spermidine, a Potent Iron Chelator. *J. Med. Chem.* **1980**, *23*, 1130−1133.
(3) Corbin, J. L.; Bulen, W. A. The Isolation and Identification of 2,3-Dihydroxybenzoic Acid and 2-N,6-N-Di-(2,3-dihydroxybenzoyl)-L-lysine Formed by Iron-Deficient *Azotobacter vinelandii*. *Biochemistry* **1969**, *8*, 757−762.
(4) Bergeron, R. J.; Kline, S. J.; Stollowich, N. J.; McGovern, K. A.; Burton, P. S. Flexible Synthesis of Polyamine Catecholamides. *J. Org. Chem.* **1981**, *46*, 4524−4529.
(5) Weitl, F.; Harris, W. R.; Raymond, K. N.; Sulfonated Catecholamide Analogues of Enterobactin as Iron Sequestering Agents. *J. Med. Chem.* **1979**, *22*, 1281−1283.
(6) Buckley, G. M.; Pattenden, G.; Whiting, D. A. New Synthetic Probes of the Iron Transport System of *Paracoccus denitrificans*. *Tetrahedron* **1994**, *50*, 11781−11792.
(7) Schnabelrauch, M.; Wittmann, S.; Rahn, K.;. Reissbrodt, R.; Möllmann, U.; Heinisch, L. New synthetic catecholate-type siderophores based on amino acids and dipeptides. *BioMetals* **2000**, *13*, 333−348.
(8) Wittmann, S.; Heinisch, L.; Scherlitz-Hofmann, I.; Möllmann, U.; Ankel-Fuchs, D. 8-Acyloxy-1,3-benzoxazine-2,4-diones as siderophore components of antibiotics. *Arzneim.-Forsch./Drug Res.* **2000**, *50*, 752−757.
(9) Arisawa, M.; Sekine, Y.; Shimizu, S.; Takano, H.; Angehrn, P.; Then, R. L. In vitro and in vivo evaluation of Ro 09-1428, a new parenteral cephalosporin with high antipseudomonal activity. *Antimicrob. Agents Chemother.* **1991**, *35*, 653−659.

(10) Muñoz-Bellido, J.-L.; Garcia Rodriguez, J. A. New trends in cephalosporin developments: catechole and isoxazolidine cephalosporins. *Infect. Dis. Clin. Practice* **1996**, *5*, 232−243.
(11) Budzekiewicz, H. Siderophore-Antibiotic Conjugates Used as Trojan Horses Against *Pseudomonas aeruginosa*. *Curr. Top. Med. Chem.* **2001**, *1*, 73−82.
(12) Hennard, Ch.; Truong, Q. C.; Desnottes, J.-F.; Paris, J.-M.; Moreau, N. J.; Abdallah, M. A. Synthesis and Activities of Pyoverdin-Quinolone Adduct: A Prospective Approach to a Specific Therapy against *Pseudomonas aeruginosa*. *J. Med. Chem.* **2001**, *44*, 2139−2151.
(13) Kline, T.; Fromhold, M.; Mckennon, T. E.; Cai, S.; Treiberg, J.; Ihle, N.; Sherman, D.; Schwan, W.; Hickey, M. J.; Warrener, P.; Witte, P. R.; Brody, L. L.; Goltry, L.; Barker, L. M.; Anderson, S. U.; Tanaka, S. K.; Shawar, R. M.; Nguyen, L. Y.; Langhorne, M.; Bigelow, A.; Embuscado, L.; Naeemi, E. Antimicrobial Effects of Novel Siderophores Linked to β-Lactam Antibiotics. *Bioorg. Med. Chem.* **2000**, *8*, 73−93.
(14) Byk, G.; Gilon, Ch. Building Units for N-Backbone Cyclic Peptides. 1. Synthesis of Protected N-(ω-Aminoalkylene)amino Acids and Their Incorporation into Dipeptide Units. *J. Org. Chem.* **1992**, *57*, 5687−5692.
(15) Craig, D. C.; Doughty, A.; Kassiou, M.; MacIntosh, M. G.; Read, R. W. Reactions of Perhydro-2,2′-bipyrimidines with Carbonyl Compounds Bearing α-Carbonyl Functionality. *J. Org. Chem.* **1993**, *58*, 5753−5758.
(16) Daukshas, W. K. Electrophilic substitution in 2,3-dihydroxybenzoic acid and its derivatives. *J. Org. Chem. USSR* **1979**, *15*, 1740−1743.
(17) Basker, M. J.; Frydrych, C. H.; Harrington, F. P.; Milner, P. H. Antibacterial activity of catecholic piperacillin analogues. *J. Antibiot.* **1989**, *17*, 1328−1330.
(18) Nakagawa, S.; Sanada, M.; Matsuda, K.; Hashizume, T.; Asahi, Y.; Ushijima, R.; Ohtake, N.; Tanaka, N. In vitro and in vivo antibacterial activities of BO-1341, a new antipseudomonal cephalosporin. *Antimicrob. Agents Chemother.* **1989**, *33*, 1423−1427.
(19) Hantke, K. Dihydroxybenzoylserine—a Siderophore for *E. coli*. *FEMS Microbiol. Lett.* **1990**, *67*, 5−8.
(20) Bergeron, R. J.; McGovern, K. A.; Channing, M. A.; Burton, P. S. Synthesis of N^4-Acylated N^1,N^8-Bis(acyl)spermidines: An Approach to the synthesis of Siderophores. *J. Org. Chem.* **1980**, *45*, 1589−1592.
(21) Heinisch, L.; Ulbricht, H.; Willitzer, H.; Hanschke, K. G.; Tresselt, D.; Möllmann, U.; Eckardt, K.; Haupt, I. Synthesis and antibacterial activity of benzoylaminoacyl-penicillins and related compounds. *Arzneim.-Forsch./Drug Res.* **1992**, *42*, 6−673.
(22) Hall, M. J.; Ratledge, C. A simple method for the production of mycobactin, the lipid-soluble siderophore, from mycobacteria. *FEMS Microbiol. Lett.* **1982**, *15*, 133−136.
(23) Schwyn, B.; Neilands, J. B. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **1987**, *160*, 47−56.
(24) National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4th ed.; Villanova, Ed.; Approved Standard Document M7-A4. NCCLS, 1997.
(25) Sumita, Y.; Inoue, M.; Mitsuhashi, S. In vitro antibacterial activity and beta-lactamase stability of new carbapenem SM-7338. *Eur. J. Clin. Microbial. Infect. Dis.* **1998**, *8*, 908−916.

JM010546B