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New Synthetic Siderophores and Their β-Lactam Conjugates Based on Diamino Acids and Dipeptides

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Abstract—Linking of siderophores to antibiotics improves the penetration and therefore increases the antibacterial activity of the antibiotics. We synthesized the acylated catecholates and hydroxamates as siderophore components for antibiotic conjugates to reduce side effects of unprotected catecholate and hydroxamate moieties. In this paper, we report on bis- and tris-catecholates and mixed catecholate hydroxamates based on diamino acids or dipeptides. These compounds were active as siderophores in a growth promotion assay under iron limitation. Most of the conjugates with β-lactams showed high in vitro activity against Gram-negative bacteria especially *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens* and *Stenotrophomonas maltophilia*. The compounds with enhanced antibacterial activity use active iron uptake routes to penetrate the bacterial outer membrane barrier, demonstrated by assays with mutants deficient in components of the iron transport system. Correlation between chemical structure and biological activity was studied. © 2002 Published by Elsevier Science Ltd.

Introduction

Siderophores linked to β-lactam antibiotics can improve their antibacterial activity significantly. Siderophore antibiotic conjugates were used as a Trojan Horse strategy to overcome penetration mediated bacterial antibiotic resistance.[1,2] Siderophores are bacterial iron chelators mostly containing catecholate or hydroxamate groups as chelating ligands. They are expressed under iron starvation conditions and sequester extracellular ferric ions.[3] Specific bacterial outer membrane receptors recognize the iron complexed siderophores and initiate the active transport into the cell. Some siderophores contain amino acids or dipeptides as scaffolds.[4] Examples are enterobactin, a trimer of *N*-(2,3-dihydroxybenzoyl)-serine,[5,6] found in *Escherichia coli,* or N^2,N^6-bis-(2,3-dihydroxybenzoyl)-L-lysine[7,8] and *N*-(2,3-dihydroxybenzoyl)-glycine,[9] siderophores from *Acotobacter vinelandii* and *Bacillus subtilis*, respectively. Published synthetic analogues of these siderophores are *N*-(2,3-dihydroxybenzoyl)-L-threonine[10] and N^2-[$N^{2'}$,$N^{6'}$-bis-(2,3-dihydroxybenzoyl)-L-lysyl]-N^6-(2,3-dihydroxybenzoyl)-L-lysine.[11] We synthesized a series of mono-, bis-, and tris-catecholates of amino acids, among which the triscatecholates demonstrated the highest siderophore activity due to their optimal iron chelation properties.[12] β-lactam conjugates with monocatecholates as siderophore components were active as antibacterials.[13] So far these siderophore antibiotic conjugates were not used as therapeutics probably due to side effects of the free catechole moiety. Carbacephalosporins with bis-catecholates based on L-lysine and with mixed catecholates and hydroxamate ligands based on spermidine and L-lysine were synthesized, but their antibacterial activity was not significantly increased.[8,14,15] In these compounds the catecholate or hydroxamate moieties are unprotected. We used the acylated catecholates or hydroxamates as protected siderophore components to facilitate the synthesis especially for sulfur containing β-lactams. For iron chelating these protected siderophores obviously have to be cleaved enzymatically to free catecholates or hydroxamates. By this process free catecholates may become available only slowly which possibly reduces their pharmacological side effects. The feasibility of using protected siderophores was confirmed by recently prepared 8-acyloxy-1,3-benzoxazine-

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2,4-diones or 2,3-diacetoxybenzoyl derivatives and their antibiotic conjugates.[16–18] In this paper we report on the synthesis and biological activity of new acylated 4- and 6-ligand catecholates or mixed catecholate hydroxamates as siderophores based on amino acids and on dipeptides as well as of their conjugates with antibiotics.

Results

To overcome penetration mediated antibiotic resistance by linking of synthetic siderophores to β-lactams we synthesized new acylated bis- and triscatecholates and mixed biscatecholate hydroxamates based on diamino acids (L- and D-ornithine, L-lysine) or corresponding dipeptides. Catecholic moieties were attached in form of 2,3-diacetoxybenzoyl or heterocyclic 8-acyloxy-2,4-dioxo-1,3-benzoxazine units. As acylated hydroxamates we used *O*-benzoyl derivatives of dicarboxylic acid monohydroxamates. In these hydroxamates the NOH and CO groups are exchanged (reversed hydroxamates),[19] compared with natural hydroxamates[4] based on *N*-hydroxyamino acids such as N^5-hydroxyornithine.[20] These synthetic acylated chelators were active as siderophores for Gram-negative bacteria in a growth promotion assay under iron deficient conditions. The new siderophores were coupled with the β-lactam antibiotics ampicillin (Ap), amoxicillin (Ax), cephalexin (Ce) and cefaclor (Cf). Most of the conjugates were highly active against Gram-negative bacteria especially against strains of *Pseudomonas aeruginosa* and *Strenotrophomonas maltophilia*.

Chemistry

In this work we describe the synthesis of acylated bis- and triscatecholates as well as biscatecholate hydroxamates with a diamino acid or dipeptide scaffold. As acylated catecholate moieties, we used 2,3-diacetoxybenzoyl and 8-methoxycarbonyloxy-2,4-dioxo-benzoxazine units. As acylated hydroxamates we used their benzoyl derivatives.

Biscatecholates (Scheme 1)

The acetylated biscatecholates **3a**–**d** were synthesized from the diamino acids **1a**–**c** and 2,3-diacetoxybenzoyl chloride **2a** or its 5-bromo derivative **2b.** The benzoxazine compounds **8a**–**c** were prepared from **1a**–**c** and 2,3-di-methoxycarbonyloxybenzoyl chloride **2c**. Moreover, the modification of the scaffold was accomplished by attachment of the amino acids L-phenylalanine and L-tryptophan which resulted in biscatecholates **10a**, **b**.

Triscatecholates (Schemes 2 and 3)

Triscatecholates and biscatecholate hydroxamates offer optimal conditions for hexagonal iron chelating. In the case of biscatecholates two molecules should be necessary for iron complexing. The acetylated triscatecholates **13a,b** and **16** were prepared from N^5-L-ornithyl-L-ornithine **12a**, N^6-L-lysyl-L-lysine **12b** and N^2-L-lysyl-L-lysine **15**, respectively, by reacting with acid chloride **2a**. Reaction of N^2-L-lysyl-L-lysine benzylester tritosylate with acid chloride **2c** gave after hydrogenolysis the tris-(8-methoxycarbonyloxy-2,4-dioxo-benzoxazine) derivative **18** (Scheme 2). As a further tris-(8-methoxycarbonyloxy-2,4-dioxo-benzoxazine) derivative we synthesized compound **21** with a more extensive and possibly more flexible backbone. Synthesis started from N^6-[$N^{2'}$,$N^{6'}$-di-Z-L-lysyl]-L-lysine **20**, that was reacted in succession with ε-*Z*-aminocaproic acid, hydrogen and finally with acid chloride **2c** to form compound **21** (Scheme 3).

Biscatecholate hydroxamates (Schemes 3 and 4)

We synthesized two types of biscatecholate hydroxamates derived from compounds **3a** or **3b** by linking the hydroxamate unit via a L-glutamic or glutaric acid spacer. Subsequent reaction of **3a** or **3b** with 1-benzyl L-glutamate, *N*-benzoyloxy-*N*-methyl-amine and catalytic hydrogenation with Pd/C led to the biscatecholate hydroxamates **24a** and **b**, respectively (Scheme 3). The corresponding *N*-cyclohexyl derivatives **27a** and **b**, respectively, were synthesized from 1-benzyl *Z*-L-glutamate, which was reacted in succession with *N*-benzoyloxy-*N*-cyclohexyl-amine, hydrogen and finally **3a** or **3b**. The biscatecholate hydroxamates **31a** and **b** were synthesized as follows. *N*-substituted *N*-benzoyloxy-glutaric amides **29a** and **b**, prepared before from glutaric anhydride and the corresponding *N*-substituted *N*-benzoyloxyamines, were reacted with N^6-Z-L-lysine. Following hydrogenolysis gave derivatives **30a** and **30b**, which were acylated with **3a** to the biscatecholate hydroxamates **31a** and **b**, respectively (Scheme 4).

β-Lactame conjugates (Schemes 1–5)

The siderophore analogues **3a**–**d**, **8a**–**c**, **10a**,**b**, **13a**,**b**, **16**, **18**, **21**, **23a**,**b**, **27a**,**b** and **31a**,**b** were linked to the free amino group of the antibiotics via mixed anhydride method forming the ampicillin conjugates (X = Ap) **4a**–**d**, **9a**–**c**, **11a**,**b**, **14a**,**b**, **17a**, **19a**, **22**, **25a**,**b**, **28a**,**b**, **32a**,**b**, the amoxicillin conjugates (X = Ax) **5a**,**b**, **17b** and **19b**, the cephalexin conjugates (X = Ce) **6a**,**b** and the cefaclor conjugates (X = Cf) **7a**,**b** and **19c**. To obtain water soluble compounds sodium salts were formed from the conjugates by reacting the acids with sodium 2-ethylhexanoate.

Biology

Siderophore activity

Most of the compounds were active in a growth promotion assay to study the siderophore activity (Table 1). The biscatecholates **3b**, **3c** based on D- and L-ornithine were comparable active in growth promotion of all strains used, but the 5-bromocatecholate **3d** was inactive in *Salmonella typhimurium* enb7. The biscatecholate **3a**, based on L-lysine, was active using all test strains comparable to **3b** and **3c**. All other compounds promoted growth of all test strains. The bis- and tris-

benzoxazine derivatives **8a–c** and **18** were active as siderophores in contrast to results obtained with monobenzoxazine derivatives, which can only act as siderophore components in β-lactam antibiotic conjugates.[16] Especially high was the activity of the trisbenzoxazine derivative **16** and the triscatecholate **13a**. The triscatecholate **13b** was inactive in *S. typhimurium* enb7.

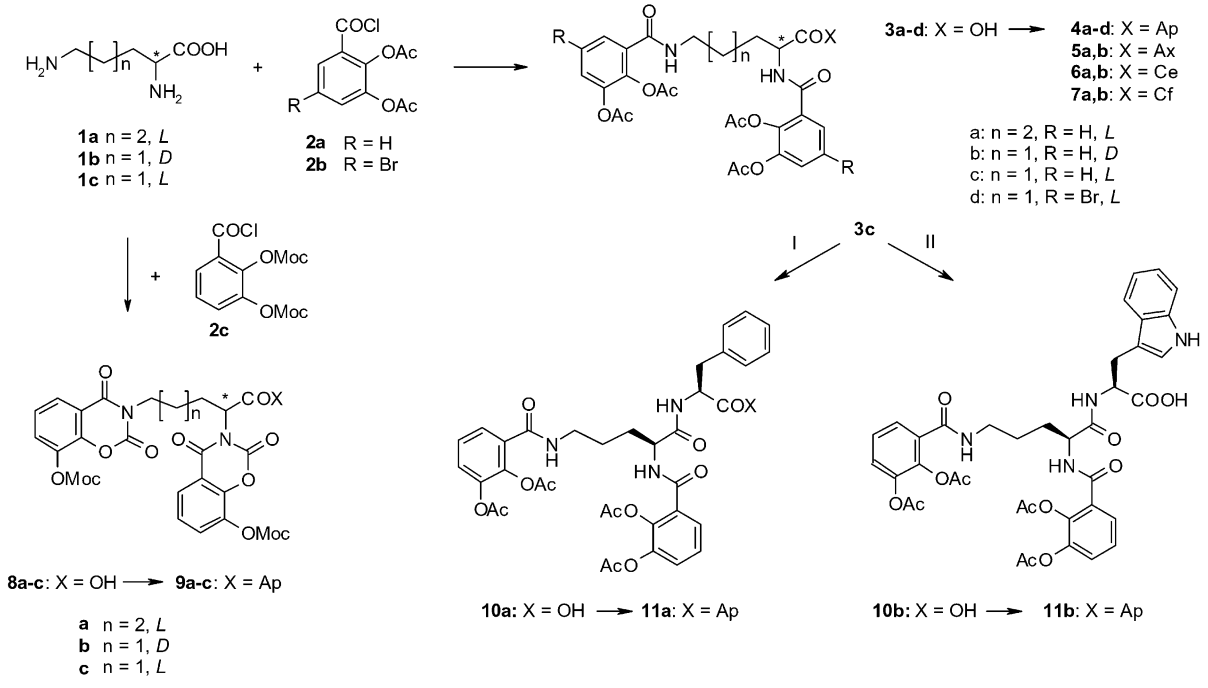
Antibacterial activity

In a first approach all siderophores and siderophore antibiotic conjugates were screened for antibacterial activity in an agar diffusion assay. Siderophores alone were generally inactive in a concentration of 100 mg/L and an application volume of 50 μL per agar well. Conjugates were all active in different levels and minimal inhibitory concentrations (MIC) were determined consequently (Table 2). Most of the conjugates were highly active against Gram-negative bacteria, but exhibited only low activity against the Gram-positive strain *S. aureus* SG 511. Generally ampicillin conjugates were superior in activity compared to amoxicillin, cephalexin and cefaclor conjugates. Amongst the biscatecholates the acetylated derivatives, especially the bromo derivative **4d**, showed the highest activity. The phenylalanine moiety in **11a** increased the activity against *E. coli* ATCC 25922 compared to the basic compound **4c**, while the more polar tryptophan derivative **11b** showed no difference to **4c**. Highly active were also the triscatecholate **17a** and the biscatecholate hydroxamates **25a**,**b** and **28b.** The compounds **28a**,**b** and **32b** were highly active against *Klebsiella pneumoniae*. The compounds **4d**, **11a**, **25a**,**b** and **28b** showed the strongest activity of all tested compounds. Beside the high activity against *P. aeruginosa* and *K. pneumoniae, S. maltophilia,* which was resistant against the tested reference β-lactams, was inhibited by the conjugates with MIC values below 0.05 mg/L. Activity against Gram-negative pathogens exceeded that of ampicillin, azlocillin, cefaclor and partly that of meropenem.

Studies of the mechanism of action of the conjugates using *E. coli* strains deficient in siderophore receptors showed the following results. Compared with the wild type *E. coli* strain H 1443 there was no decrease in activity of the conjugates against the *fepA* mutant H873. Thus, activity does not depend on the enterobactin receptor *fepA*. Against the mutants H 1875 and H 1877 missing one of the receptors *cir* and *fiu* (for uptake of the breakdown products of enterobactin[28]) there were only minor changes in activity. In contrast there was a strong decrease in activity against the *cir* and *fiu* double mutant H 1876 for most of the compounds, indicating that entry of the compounds into the bacterial cells was hindered. All tested compounds exhibited high activity against *E. coli* AB2847 (*tonB* +) and reduced or no activity against the *tonB* negative mutant BR158, indicating a dependence of activity on active iron transport mechanisms. On the other hand azlocillin, ampicillin or cefaclor were not affected by the presence or the absence of siderophore receptors or active iron transport systems. This demonstrates that the new conjugates, but not ampicillin, cefaclor or azlocillin, reached their targets via iron transport uptake routes.

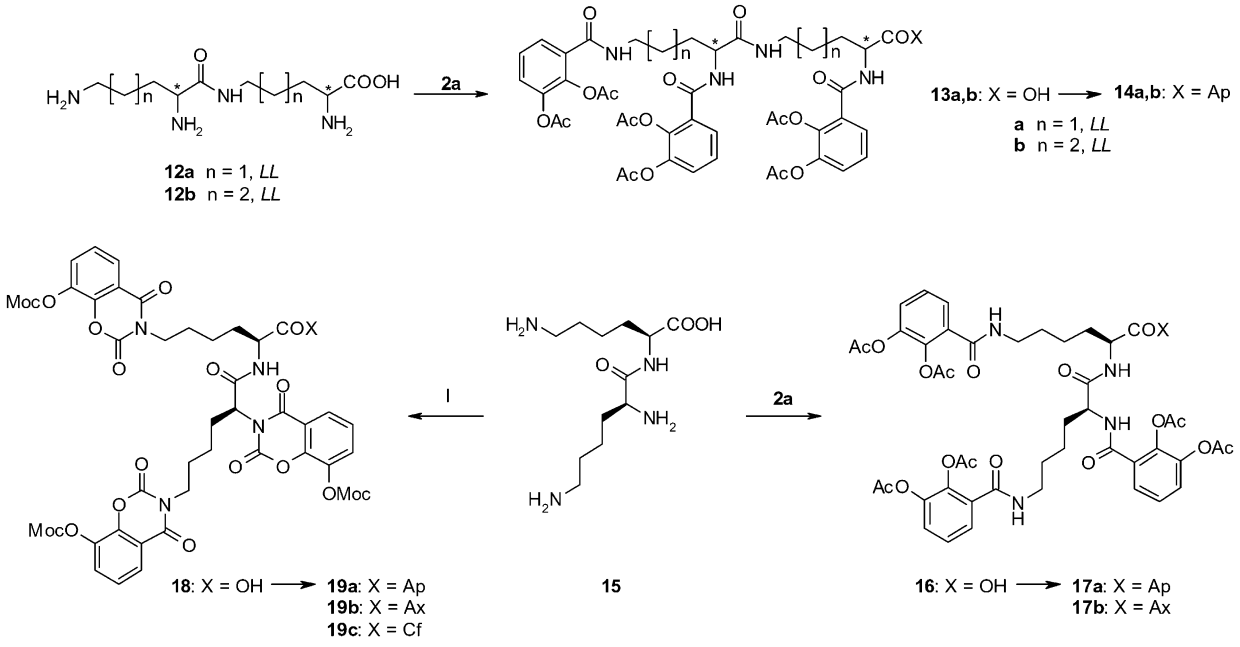
Conclusions

Acylated bis- or triscatecholates and mixed biscatecholate hydroxamates were synthesized. The compounds showed high siderophore activity in a growth promotion

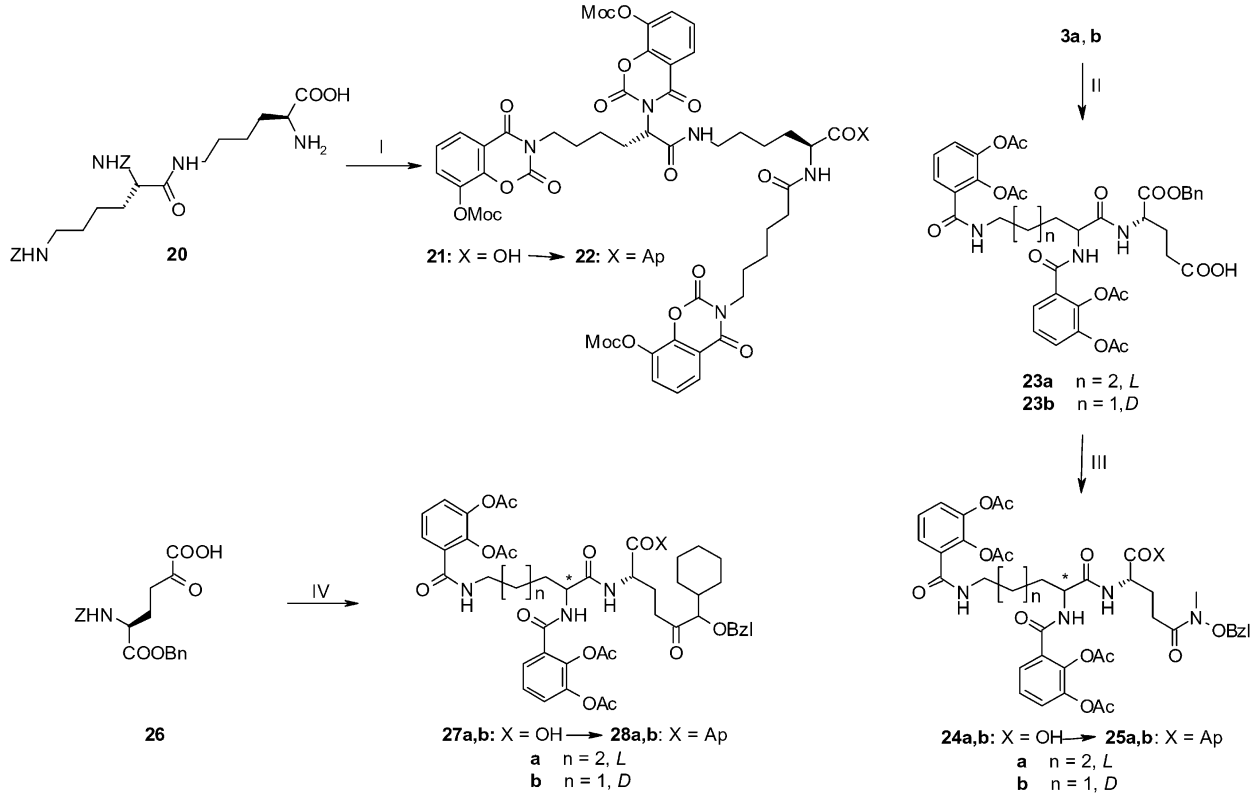


Scheme 1. Synthesis of biscatecholates. Ac = $COCH_3$, Moc = $COOCH_3$. Reagents and conditions: (I) (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) L-H-Phe–OH, Et_3N; II (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) L-H-Trp–OH, Et_3N.

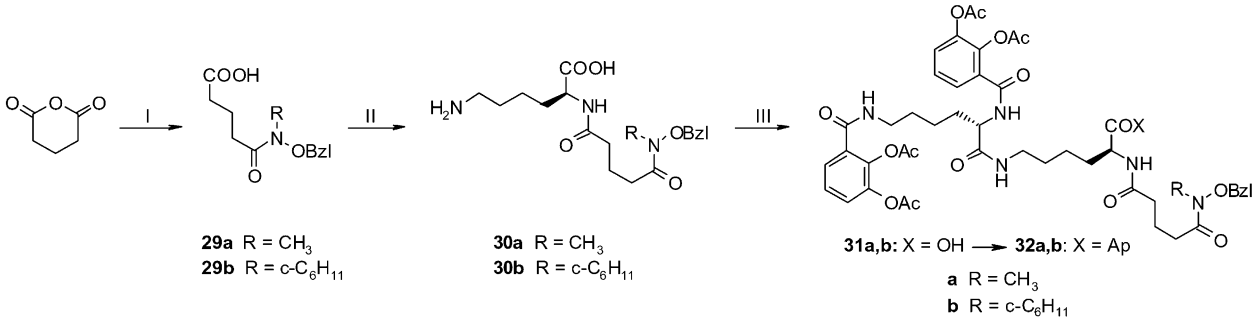
assay under iron limitation. Their conjugates with ampicillin, amoxicillin and cefaclor, exhibited strong in vitro activity against Gram-negative bacteria, especially *P. aeruginosa* and *S. maltophilia,* due to their MIC. Whereas the cephalexin derivatives were less active, the ampicillin derivatives were the most active ones. Compounds with 8-acyloxy-2,4-dioxobenzoxazines as siderophore components were less active than conjugates with acetylated siderophores. This improved antibacterial activity was due to active uptake via iron



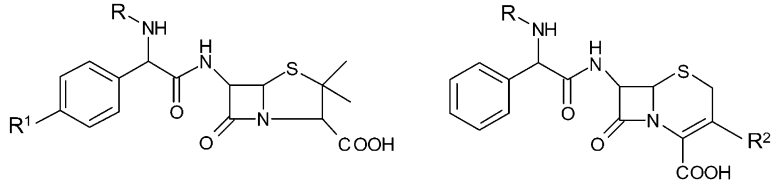
Scheme 2. Synthesis of triscatecholates. Ac = $COCH_3$, Moc = $COOCH_3$. Reagents and conditions: (I) (i) TsOH, $C_6H_5CH_2OH$, 110 °C; (ii) **2c**, $NaHCO_3$; (iii) H_2, Pd/C, rt.



Scheme 3. Synthesis of triscatecholates and biscatecholate hydroxamates. Bn = benzyl, Z = benzyloxycarbonyl, Bzl = benzoyl. Reagents: (I) (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) $NH_2(CH_2)_5COOH$, Et_3N; (iii) H_2, Pd/C 10%, rt; (iv) **2c**, $NaHCO_3$; (II) (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) H-Glu-OBn, Et_3N; (III) (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) CH_3–NHOBzl, Et_3N; (iii) H_2, Pd/C 10%, rt; (IV) (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) c-C_6H_{11}–NHOBzl, Et_3N; (iii) H_2, Pd/C 10%, rt; (iv) **3a** or **3b**, *i*Bu-OCOCl, *N*-methylmorpholine.



Scheme 4. Synthesis of biscatecholate hydroxamates. Reagents and conditions: (I) R-NHOBzl, Et_3N;. (II) (i) *i*Bu-OCOCl, *N*-methylmorpholine; (ii) L-H-Lys(Z)-OH, Et_3N; (iii) H_2, Pd/C 10%, rt; (III) **3a**, *i*Bu-OCOCl, *N*-methylmorpholine.



Scheme 5. Antibiotic components of the conjugates: R = siderophore components of Schemes 1–4; R^1 = H: **Ap** = ampicillino; R^1 = OH: **Ax** = amoxicillino; R^2 = CH_3: **Ce** = cephalexino; R^2 = Cl: **Cf** = cefacloro.

transport pathways. The triscatecholate **17a**, the biscatecholates **4d**, and **11a** and the biscatecholate hydroxamates **25a**,**b** and **28b** were the most active compounds from this series. For compound **25a** in vivo therapeutic activity in a *P. aeruginosa* septicaemic mouse model (ED_{50}: 10.4 mg/kg) could be demonstrated together with moderate toxicity in mice (LD_{50}: 125–250 mg/kg) (more data will be presented in a following paper). This compound is a promising candidate for further preclinical and clinical investigation.

Table 1. Growth promotion of Gram-negative bacteria by catecholate and catecholate hydroxamate derivatives under iron limitation

Compd	*P. aeruginosa*				*E. coli*	*S. typhimurium*
	ATCC 27853	SG137	NCTC 10662	ATCC 9027	ATCC 25922	enb7
3a	24	20	25	20	32	37
3b	20	25	27	25	28	33
3c	23	23	22	20	25	32
3d	25	28	32	27	28	0
8a	20	20	18	15	20	24
8b	20	27	27	17	25	28
8c	17	22	28	20	28	28
10a	20	20	28	22	30	32
10b	20	25	25	24	30	31
13a	23	28	30	35	30	30
13b	24	29	27	26	31	0
16	25	30	30	25	34	25
18	18	19	20	30	23	25
21	15	15	18	20	40	25
23a	23	25	30	20	30	26
23b	20	23	20	24	32	30
24a	26	31	28	22	35	29
24b	23	30	30	20	25	27
27a	25	27	25	24	33	34
27b	19	23	20	20	29	27
31a	20	22	21	17	30	32
31b	22	24	25	27	34	25
Desferal	35	30	30	35	36	38

5 μL of a 2 mM solution was applied on a 6 mm filter disc. Diameters of growth zones (mm).

Experimental

Chemistry

General. The chemicals were purchased from Bachem (amino acids and dipeptides), Aldrich-Chemie (Germany) and Fluka (Switzerland). Tetrahydrofuran (THF) was distilled over sodium/benzophenone prior to use. CCl_4 was dried over $CaCl_2$, distilled and stored over molecular sieves (4A). All other solvents were purchased as pa (pro analysis) quality. Melting points (corrected) were determined by a Boetius hot stage microscope (Rapido, Germany). ^1H NMR spectra were recorded on a 300 MHz Bruker NMR spectrometer. The chemical shifts δ are given in ppm related to tetramethyl silane as internal standard. The coupling constants *J* are reported in Hz. High resolution mass spectra were obtained by a Finnigan MAT 95 XV high resolution mass spectrometer with fast-atom bombardment (FAB) as well as positive (PI) and negative ion (NI) electron-spray ionization (ESI). Preparative HPLC was performed on a Gilson Abimed apparatus (Abimed Analysentechnik, Germany) equipped with a 115 UV detector (254 nm) and a Vertex reversed-phase column (250×32 mm I.D., Knauer, Germany), packed with Eurospher 100-C18 (7 μm). Eluents used were acetonitrile (HPLC quality, Merck, Germany) and double distilled water, beginning with a ratio of 20:80 (v/v) and achieving 80:20 (v/v) after a period of 20 min (flux rate 20 mL/min).

The following compounds were synthesized according to published procedures: 2,3-diacetoxybenzoyl chloride **2a**,[21] 5-bromo-2,3-diacetoxybenzoyl chloride **2b**,[21]

Table 2. Antibacterial activities of siderophore β-lactam conjugates in vitro MIC (mg/L)

Compound	*P. aeruginosa*		*E. coli* ATCC 25922	*K. pneumoniae* ATCC 10031	*S. maltophilia* GN 12873	*S. marcescens* SG 621	*S. aureus* SG 511
	SG 137	ATCC 27853					
4a	0.05	1.56	12.5	0.2	6.25	n.d.	12.5
4b	0.1	0.78	1.56	0.4	0.1	0.1	n.d.
4c	0.2	6.25	6.25	0.2	0.1	1.56	12.5
4d	<0.05	0.4	0.78	<0.05	<0.05	<0.05	25
5a	3.12	6.25	6.25	6.25	50	6.25	6.25
5b	0.78	6.25	6.25	0.4	50	6.25	n.d.
6a	12.5	25	100	0.2	0.2	100	50
6b	<0.05	3.12	>100	3.12	1.56	n.d.	12.5
7a	0.1	6.25	6.25	<0.05	0.1	n.d.	12.5
7b	<0.05	3.12	3.12	0.05	0.2	n.d.	12.5
9a	0.4	6.25	50	1.56	3.125	12.5	25
9b	1.56	12.5	6.25	0.4	1.56	0.78	0.78
9c	0.1	6.25	6.25	<0.05	0.1	n.d.	12.5
11a	<0.05	0.4	0.4	<0.05	<0.05	<0.05	12.5
11b	0.2	6.25	1.56	<0.05	0.78	<0.05	6.25
14a	1.56	25	12.5	3.12	0.1	1.56	12.5
14b	0.78	0.78	3.12	0.4	0.2	0.1	25
17a	0.78	3.12	0.1	<0.05	<0.05	<0.05	12.5
17b	0.78	3.12	1.56	0.1	0.05	1.56	25
19a	0.2	0.78	6.25	0.4	0.4	n.d.	3.12
19b	0.78	6.25	3.12	<0.05	0.1	0.78	12.5
19c	50	50	1.56	>0.05	100	25	6.25
22	6.25	12.5	3.12	0.4	1.56	0.78	3.12
25a	0.01	0.2	0.1	0.01	0.04	0.05	12.5
25b	<0.05	0.4	0.4	<0.05	<0.05	<0.05	12.5
28a	0.4	1.56	3.12	<0.005	0.05	0.1	12.5
28b	0.05	0.78	0.2	<0.005	0.01	0.005	12.5
32a	<0.05	1.56	0.78	0.1	0.2	0.78	6.25
32b	0.78	3.12	3.12	<0.005	0.2	1.56	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
Cefaclor	100	100	12.5	3.12	100	100	6.25

n.d., not determined.

2,3-dimethoxycarbonyloxybenzoyl chloride **2c**,[16] *N*-benzoyloxy-*N*-methyl-amine hydrochloride,[22] and *N*-benzoyloxy-*N*-cyclohexyl-amine hydrochloride.[23]

General methods

Method A: Attachment of catecholate moieties. In an ultrasonic bath, a solution of substituted benzoyl chloride **2** (1 mmol per amino group) in absolute THF (10 mL) was added to a solution of the amino acid or dipeptide (1 mmol) in freshly prepared 0.5 M aqueous sodium hydrogen carbonate solution (1 mmol per amino and carboxyl group) at 0–5 °C with stirring. The mixture was stirred for 1 h at 0–5 °C. Then the THF was evaporated. The residual aqueous solution was acidified to pH 2 at 0 °C with 2 M aqueous HCl, and then extracted with ethyl acetate. The organic layer was washed three times with brine, dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by preparative HPLC.

Method B: Mixed anhydrides. Isobutyl chloroformate (0.130 mL, 1 mmol) was added to a solution of carboxylic acid (1 mmol) and *N*-methylmorpholine (0.112 mL, 1 mmol) in absolute THF (10 mL) at −20 °C with stirring. The mixture was stirred for 45 min at −10 °C, and then a solution or suspension of the amino component (1 mmol) and triethylamine (1 mmol per amino and per carboxyl group) in THF (8 mL) and water (2 mL) was added. The mixture was stirred for 1 h at −10 °C and for 1 h at ambient temperature and then evaporated. The residue was dissolved in ethyl acetate and water and carefully acidified with 2 M HCl. After shaking the organic layer was separated, washed with brine, dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by preparative HPLC.

Preparation of bis- and triscatecholates

N^2,N^6-Bis-(2,3-diacetoxybenzoyl)-L-lysine (3a). Preparation according to method A from **1a** and **2a** gave compound **3a** as a white solid, yield 45%. ^{1}H NMR ($CDCl_3$) δ 1.28 (2H, m), 1.60 (2H, m,), 1.85 (1H, m), 1.99 (1H, m), 2.29 (3H s), 2.30 (6H, s), 2.34 (3H, s), 3.38 (2H, m), 4.75 (1H, m), 6.59 (1H, t, J = 5.7), 7.22–7.32 (5H, m), 7.47 (1H, dd, J = 2.3, 7.2), 7,65 (1H, dd, J = 1.9, 7.4).

N^2,N^5-Bis-(2,3-diacetoxybenzoyl)-D-ornithine (3b). Preparation according to method A from **1b** and **2a** gave compound **3b** as a white solid, yield 60%. ^{1}H NMR (DMSO-d_6) δ 1.50–1.90 (4H, m), 2.22 (6H, s), 2.27 (6H, s), 3.19 (2H,m), 4.33 (1H, m), 7.32–7.49 (6H, m), 8.34 (1H, m), 8.52 (1H, d).

N^2,N^5-Bis-(2,3-diacetoxybenzoyl)-L-ornithine (3c). Preparation according to method A from **1c** and **2a** gave compound **3c** as a white solid, yield 85%. ^{1}H NMR (DMSO-d_6) δ 1.50–1.90 (4H, m), 2.20 (6H, s), 2.27 (6H,s), 3.18 (2H, m), 4.35 (1H, m), 7.30–7.50 (6H, m), 8.35 (1H, t), 8.51 (1H, d). MS (ESI-PI) m/z 595.2 $[M+Na]^+$.

N^2,N^5-Bis-(5-brom-2,3-diacetoxybenzoyl)-L-ornithine (3d). Preparation according to method A from **1a** and **2b** gave compound **3d** as a white solid, yield 85%. ^{1}H NMR (DMSO-d_6) δ 1.50–1.90 (4H, m), 2.19 (3H, s), 2.21 (3H, s), 2.27 (6H, s), 3.19 (2H, m), 4.31 (1H, m), 7.61 (2H, dd), 7.71 (2H, dd), 8.46 (1H, t), 8.69 (1H, d). MS (ESI-PI) m/z 753.3 $[M+Na]^+$.

2L,6-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazine-3-yl)-*n*-hexanoic acid (8a). Preparation according to method A from **1a** and **2c** gave compound **8a** as a white solid, yield 80%. Mp 111–115 °C. ^{1}H NMR (DMSO-d_6) δ 1.37–1.79 (4H, m), 2.20–2.35 (2H, m), 3.95 (3H, s), 3.96 (3H, s), 3.97 (2H, m), 5.47 (1H, m), 7.31 (1H, t, $J=8.0$), 7.36 (1H, t, $J=8.0$), 7.51 (1H, dd, $J_1=8.0$, $J_2=1.4$), 7.57 (1H, dd, $J_1=8.0$, $J_2=1.4$), 7.86 (1H, dd, $J_1=8.0$, $J_2=1.4$), 7.94 (1H, dd, $J_1=8.0$, $J_2=1.4$). MS (ESI-NI) m/z 585.2 $[M-H]^-$.

2D,5-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-pentanoic acid (8b). Preparation according to method A from **1b** and **2c** gave compound **8b** as a white solid, yield 50%. ^{1}H NMR (DMSO-d_6) δ 1.70 (2H, m), 1.95–2.25 (2H, m), 3.22 (2H, m), 3.90 (6H, s), 5.35 (1H, m), 7.45–7.95 (6H, m). MS (ESI-NI) m/z 571.2 $[M-H]^-$.

2L,5-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-pentanoic acid (8c). Preparation according to method A from **1c** and **2c** gave compound **8c** as a white solid, yield 50%. ^{1}H NMR (DMSO-d_6) δ 1.69 (2H, m), 1.90–2.25 (2H, m), 3.20 (2H, m), 3.90 (6H, s), 5.34 (1H, m), 7.40–7.95 (6H, m). MS (ESI-NI) m/z 571.1 $[M-H]^-$.

N^2-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-L-ornithyl]-L-phenylalanine (10a). Reacting **3c** and L-phenylalanine according to method B gave compound **10a** as a white solid, yield 30%. ^{1}H NMR (DMSO-d_6) δ 1.90–1.50 (m, 4H), 2.22 (s, 6H), 2.27 (s, 6H), 2.99 (m, 2H), 3.17 (m, 2H), 4.45 (m, 2H), 7.50–7.15 (m, 11H), 8.15 (d, 1H), 8.20 (d, 1H), 8.30 (t, 1H). MS (FAB) m/z 720.3 $[M+1]^+$.

N^2-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-L-ornithyl]-L-tryptophan (10b). Reacting **3c** and L-tryptophan according to method B gave compound **10b** as a white solid, yield 60%. In contrast to the general procedure, citric acid was used instead of HCl. ^{1}H NMR (DMSO-d_6) δ 1.90–1.50 (m, 4H), 2.20 (s, 6H), 2.27 (s, 6H), 3.18 (m, 2H), 3.60 (m, 2H), 4.51 (m, 2H), 7.55–7.27 (m, 11H), 8.17 (d, 1H), 8.24 (d, 1H), 8.32 (t, 1H), 10.81 (s, 1H). MS (ESI-NI) m/z 757.4 $[M-H]^-$.

N^5-[$N^{2'}$,$N^{5'}$-(2,3-diacetoxybenzoyl)-L-ornithyl]-N^2-(2,3-diacetoxybenzoyl)-L-ornithine (13a). Preparation according to method A from N^5-L-ornithyl-L-ornithine **12a** and **2a** gave compound **13a** as a white solid, yield 50%. ^{1}H NMR (DMSO-d_6) δ 1.40–1.85 (8H, m), 2.19 (3H, s), 2.20 (3H, s), 2.22 (3H, s), 2.27 (9H, s), 3.10 (4H, m), 4.35 (2H, m), 7.30–7.55 (9H, m), 7.93 (1H, d), 8.24 (1H, d), 8.32 (1H, m), 8.50 (1H, d). MS (ESI-NI) m/z 905.3 $[M-H]^-$.

N^6-[$N^{2'}$,$N^{6'}$-(2,3-diacetoxybenzoyl)-L-lysyl]-N^2-(2,3-diacetoxybenzoyl)-L-lysine (13b). Preparation according to method A from N^6-L-lysyl-L-lysine **12b** and **2a** gave compound **13b** as a white solid, yield 45%. ^{1}H NMR (DMSO-d_6) δ 1.35–1.82 (12H, m), 2.22 (9H, s), 2.30 (9H,s), 3.20 (4H, m), 4.21 (1H, m), 4.45 (1H, m), 7.20–7.60 (9H, m), 8.15–8.40 (m, 4H).

N^2-[$N^{2'}$,$N^{6'}$-(2,3-diacetoxybenzoyl)-L-lysyl]-N^6-(2,3-diacetoxybenzoyl)-L-lysine (16). Preparation according to method A from N^2-L-lysyl-L-lysine **15** and **2a** gave compound **16** as a white solid, yield 40%. ^{1}H NMR (DMSO-d_6) δ 1.30–1.80 (12H, m), 2.21 (9H, s), 2.29 (9H, s), 3.17 (4H, m), 4.20 (1H, m), 4.46 (1H, m), 7.30–7.55 (9H, m), 8.10–8.35 (m, 4H). MS (ESI-NI) m/z 933.3 $[M-H]^-$.

2L-[2′L,6′-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoylamino]-6-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoic acid (18). Benzylalcohol (4.32 g, 40 mmol) and benzene (60 mL) were added to a mixture of N^2-L-lysyl-L-lysine **15** (dihydrochloride, 750 mg, 2.73 mmol) and *p*-toluensulfonic acid monohydrate (1.87 g, 9.84 mmol). The mixture was boiled with water separator for 4 h and then the solvent was evaporated. Diethylether was added to the residue to give N^2-L,L-lysyllysine benzylester tritosylate as a white solid (1.58 g, yield 66%). Triethylamine (1.48 mL, 10.6 mmol) was added under argon to a solution of this benzylester (1.55 g, 1.76 mmol) in absolute dimethylformamide (20 mL), followed by a solution of **2c** (1.53 g, 5.3 mmol) in absolute dimethylformamide. The mixture was stirred for 5 h at 0 °C, stored over night at ambient temperature and then the solvent was evaporated. Ethyl acetate and water were added to the residue. The solution was cooled down to 0 °C and carefully acidified with dilute hydrochloric acid. After shaking, the organic layer was separated, washed with brine and evaporated. The residue was purified by preparative HPLC (eluent acetonitrile/water 1/1 v/v with 0.5% trifluoracetic acid) to give a yellow oil of the benzylester of target compound **18** ($X=OCH_2C_6H_5$) (250 mg, yield 14%, MS (FAB) m/z 1024.6 $[M+H]^+$.

This benzylester was dissolved in ethanol (30 mL) and then hydrogenolysed with Pd/C (10%, 60 mg) at ambient temperature and atmospheric pressure to give **18** ($X=OH$) as a white solid (220 mg, yield 98%). ^{1}H NMR (DMSO-d_6) δ 1.20–1.70 (12H, m); 3.80 (4H, m), 3.83 (3H, s), 3.89 (6H, s), 4.27 (1H, m), 5.03 (1H,m), 7.43 (3H, t, $J=8.0$), 7.73- 7.90 (6H, m), 8.27 (1H, d, $J=8.1$). MS (ES-NI) m/z 933.8 $[M-H]^-$.

N^2-[ε-(8-Methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl]-N^6-[2,6-bis(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl]-L-lysine (21). According to method B, a tri-

Z-derivative was prepared from ε-*Z*-aminohexanoic acid and N^6-[$N^{2'}$,$N^{6'}$-di-*Z*-L-lysyl]-L-lysine **20** as a white solid (yield 64%). MS (ES-PI) *m*/*z* 812.4 $[M+Na]^+$.

The tri-*Z*-derivative was hydrogenolysed at ambient temperature and atmospheric hydrogen pressure over 10% Pd/C to give N^6-L-lysyl-N^2-(ε-aminocaproyl)-L-lysine as a white solid (yield 50%). MS (ES-NI) *m*/*z* 388.3 $[M+H]^+$.

This compound was reacted with acid chloride **2c** according to method A obtaining compound **21** as a white solid in 50% yield. ^{1}H NMR (DMSO-d_6) δ 1.00–2.30 (20H, m), 2.97 (2H, m), 3.80 (4H, m), 3.90 (9H, s), 4.10 (1H, m), 5.07 (1H, m), 7.39- 7.99 (11H, m). MS (ES-PI) *m*/*z* 1070.6 $[M+Na]^+$.

1-Benzyl *N*-[$N^{2'}$,$N^{6'}$-bis-(2,3-diacetoxybenzoyl)-L-lysyl] L-glutamate (23a). Preparation according to method B from **3a** and 1-benzyl L-glutamate gave compound **23a** as a white solid (Yield 30%). ^{1}H NMR (DMSO-d_6) δ 1.30–2.18 (8H, m), 2.19 (3H, s), 2.21 (3H, s), 2.27 (6H, s), 2.30 (2H, m), 3.15 (2H, m), 4.37 (2H, m), 5.11 (2H, s), 7.30–7.50 (11H, m), 8.30 (3H, m).

1-Benzyl *N*-[$N^{2'}$,$N^{5'}$-bis-(2,3-diacetoxy-benzoyl-D-ornithyl] L-glutamate (23b). Preparation according to method B from **3b** and 1-benzyl L-glutamate gave compound **23b** as a white solid (yield 40%). ^{1}H NMR (DMSO-d_6) δ 1.50–2.06 (6H, m), 2.19 (6H, s), 2.27 (6H, s), 2.34 (2H, m), 3.18 (2H, m), 4.35 (1H, m), 4.50 (1H, m), 5.11 (2H, s), 7.25–7.45 (10H, m), 7.53 (1H, dd), 8.24 (1H, d), 8.33 (1H, t), 8.42 (1H, d). MS (ESI-PI) *m*/*z* 814.2 $[M+Na]^+$.

Preparation of biscatecholate hydroxamates

***N*-[$N^{2'}$,$N^{6'}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-L-glutamic 5-(*N*-benzoyloxy-*N*-methyl)-amide (24a).** Reacting **23a** and *N*-benzoyloxy-*N*-methyl-amine according to method B gave the benzylester of compound **24a** (X = $OCH_2C_6H_5$) as a white solid (yield 40%). MS (ESI-NI) *m*/*z* 923.5 $[M-H]^+$. This benzylester was hydrogenolysed at ambient temperature and atmospheric hydrogen pressure over Pd/C (10%) to give **24a** as a white solid, in 36% overall yield as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.45–2.06 (8H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 2.39 (2H, m), 3.14 (2H, m), 3.30 (3H, m), 4.19 (1H, m), 4.37 (1H, m), 7.30–7.60 (8H, m), 7.75 (1H, t), 8.02 (2H, d); 8.12 (H, d), 8.29 (2H, m). MS (ESI-NI) *m*/*z* 847.1 $[M-H]^-$.

***N*-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-L-glutamic 5-(*N*-benzoyloxy-*N*-methyl)-amide (24b).** This was prepared from **23a** and *N*-benzoyloxy-*N*-methyl-amine analogously to compound **24a** via the corresponding benzylester in 40% overall yield as a white solid, ^{1}H NMR (DMSO-d_6) δ 1.45–2.06 (6H, m), 2.18 (3H, s), 2.20 (3H, s), 2.27 (6H, s), 2.39 (2H, m), 3.16 (2H, m), 3.30 (3H, m), 4.22 (1H, m), 4.47 (1H, m), 7.30–7.65 (8H, m), 7.75 (1H, t), 8.02 (2H, d), 8.27 (2H, m), 8.30 (1H). MS (ESI-NI) *m*/*z* 833.1 $[M-H]^-$.

***N*-($N^{2'}$,$N^{6'}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl)-L-glutamic 5-(*N*-benzoyloxy-*N*-cyclohexyl)-amide (27a).** *Z*-L-Glutamic 1-benzylester 5-(*N*-benzoyloxy-*N*-cyclohexyl)-amide was prepared from 1-benzyl *Z*-L-glutamate **26** and *N*-benzoyloxy-*N*-cyclohexyl-amine via mixed anhydride method using isobutyl chloroformate (method B). L-Glutamic 5-(*N*-benzoyloxy-*N*-cyclohexyl)-amide was formed by catalytic hydrogenolysis with Pd/C (10%) at ambient temperature and atmospheric pressure. MS (FAB) *m*/*z* 349.3 $[M+H]^+$.

Reacting the resulting amide with **3a** according to method B gave compound **27a** as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.00–2.30 (16H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 3.14 (2H, m), 4.26 (2H, m), 4.40 (1H, m), 7.31–8.05 (m, 11H), 8.18 (1H, d), 8.22 (1H, d), 8.30 (1H, m). MS (ESI-PI) *m*/*z* 939.6 $[M+Na]^+$.

***N*-($N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl)-L-glutamic 5-(*N*-benzoyloxy-*N*-cyclohexyl)-amide (27b).** This was prepared analogously to compound **27a** using biscatecholate **3b** as a white solid (Yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.00–2.30 (20H, m), 2.18 (3H, s), 2.20 (3H, s), 2.27 (6H, s), 3.15 (2H, m), 4.25 (1H, m), 4.35 (1H, m), 4.45 (1H, m), 7.32–8.05 (11H, m), 8.22 (2H, m), 8.30 (1H, d). MS (ESI-NI) *m*/*z* 901.7 $[M-H]^-$.

***N*^{2}-[4-(*N*-Benzoyloxy-*N*-methyl-carbamoyl)-*n*-butanoyl]-*N*^{6}-($N^{2'}$,$N^{6'}$-bis-2,3-diacetoxybenzoyl)-L-lysyl]-L-lysine (31a).** Reacting L-glutaric 5-*N*-benzoyloxy-*N*-methyl-monoamide **29a** (prepared from glutaric anhydride and *N*-benzoyloxy-*N*-methylamine hydrochloride) and N^2-*Z*-L-lysine according to method B gave N^2-[4-(*N*-benzoyloxy-*N*-methyl-carbamoyl)-*n*-butanoyl]-N^6-*Z*-L-lysine as a white solid (yield 70%, MS (ESI-PI) *m*/*z* 528.3 $[M+H]^+$). This compound was dissolved in methanol (10 mL) and hydrogenolysed with Pd/C (0.1 g, 10%) at ambient temperature and atmospheric pressure to give N^2-[4-(*N*-benzoyloxy-*N*-methyl-carbamoyl)-*n*-butanoyl]-*L*-lysine **30a** as a white solid (Yield 60%, MS (ESI-NI) *m*/*z* 394.1 $[M-H]^-$). Reacting **3a** and **30a** according to method B gave **31a** as a white solid (Yield 30%). ^{1}H NMR (DMSO-d_6) δ 1.20–2.40 (18H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 3.10 (4H, m), 4.03 (1H, m), 4.33 (1H, m), 7.30–8.05 (13H, m), 8.20 (1H, d), 8.29 (1H, m). MS (ESI-NI) *m*/*z* 959.9 $[M-H]^-$.

***N*^{2}-[4-(*N*-Benzoyloxy-*N*-cyclohexyl-carbamoyl)-*n*-butanoyl]-*N*^{6}-[$N^{2'}$,N$^{6'}$-bis-(2,3-diacetoxybenzoyl)-L-lysyl]-L-lysine (31b).** Reacting L-glutaric 5-*N*-benzoyloxy-*N*-cyclohexyl-monoamide **29b** (prepared from glutaric anhydride and *N*-benzoyloxy-*N*-cyclohexylamine) and N^2-*Z*-L-lysine according to method B gave N^2-[4-(*N*-benzoyloxy-*N*-cyclohexyl-carbamoyl)-*n*-butanoyl)-N^6-*Z*-L-lysine as a white solid (yield 70%), MS (ESI-PI) *m*/*z* 618.4 $[M+H]^+$.

This compound was dissolved in methanol (10 mL) and hydrogenolysed with Pd/C (0.1 g, 10%) at ambient temperature and atmospheric pressure to give N^2-[4-(*N*-benzoyloxy-*N*-cyclohexyl-carbamoyl)-*n*-butanoyl]-L-

lysine **30b** as a white solid (yield 60%). Reacting **3a** and **30b** according to method B gave compound **31b** as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.00–2.30 (18H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 3.03 (2H, m), 3.15 (2H, m), 4.08 (1H, m), 4.32 (2H, m) 7.30–8.06 (13H, m), 8.23 (1H, d), 8.31 (1H, m).

Antibiotic conjugates

The ampicillin conjugates (X = Ap) **4a**–**d**, **9a**–**c**, **11a**,**b**, **14a**,**b**, **17a**, **19a**, **22**, **25a**,**b**, **28a**,**b**, **32a**,**b**, the amoxicillin conjugates (X = Ax) **5a**,**b**, **17b** and **19b**, the cephalexin conjugates (X = Ce) **6a**,**b** and the cefaclor conjugates (X = Cf) **7a**,**b** and **19c** were prepared from the corresponding siderophore analogue and the antibiotic according to method B. The obtained residue was purified by preparative HPLC (Knauer Vertex B 31 Y 536, 250×32 mm, Eurospher 100 D18 7 mm, flux 20 mL/min, acetonitrile/water 37.5:62.5). The fraction containing the desired product was evaporated and the residual aqueous solution dried by lyophilization to give the conjugate.

The sodium salts were prepared from the solution of the corresponding conjugate (1 mmol) in ethyl acetate (5 mL) by addition of a solution of sodium 2-ethylhexanoate (1.5 mmol) in ethyl acetate (3 mL). Precipitation was completed by addition of petroleum ether. The precipitate was filtered, washed with petroleum ether and dried in vacuo.

***N*-[$N^{2'}$,$N^{6'}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-ampicillin (4a).** This was produced from **3a** and ampicillin hydrate as a white solid (yield 65%). ^{1}H NMR (DMSO-d_6) δ 1.32 (2H, m), 1.39 (1H, s), 1.45 (2H, m), 1.54 (1H, s), 1.70 (2H, m), 2.19 (3H, s), 2.21 (3H, s), 2.27 (3H, s), 2.28 (3H, s), 3.13 (2H, m), 3.83 (1H, s), 4.57 (1H, m), 5.24 (1H, d), 5.35 (1H, q), 5.76 (1H, d), 7.22–7.45 (9H, m), 7.51 (1H, dd), 8.29 (1H, t), 8.35 (1H, d), 8.57 and 9.00 (1H, d), MS (ESI-NI) *m*/*z* 916.3 $[M-H]^-$.

***N*-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-ampicillin (4b).** This was produced from **3b** and ampicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.45–1.90 (4H, m), 2.17 (3H, s), 2.18 (3H, s), 2.27 (6H, s), 3.18 (2H, m), 4.19 (1H, s), 4.58 (1H, m), 5.38 (1H, d), 5.52 (1H, q), 5.69 (1H, d), 7.25–7.50 (11H, m), 8.33 (1H, m), 8.38 (1H, d), 8.55 (1H, d), 9.12 (1H, d). MS (FAB) *m*/*z* 904.1 $[M+H]^+$.

***N*-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl-L-ornithyl]-ampicillin (4c).** This was produced from **3c** and ampicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, s), 1.55 (3H, s), 1.45–1.90 (m, 4H), 2.22 (3H, s), 2.23 (3H, s), 2.27 (6H, s), 3.18 (2H, m), 4.20 (1H, s), 4.65 (1H, m), 5.39 (1H, d), 5.52 (1H, q), 5.76 (1H, d), 7.20–7.60 (11H, m), 8.32 (2H, m), 8.64 (1H, d), 9.22 (1H, d). MS (ESI-NI) *m*/*z* 902.7 $[M-H]^-$.

***N*-[$N^{2'}$,$N^{5'}$-Bis-(5-bromo-2,3-diacetoxybenzoyl)-L-ornithyl]-ampicillin (4d).** This was produced from **3d** and ampicillin hydrate as a white solid (yield 40%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.45–1.90 (4H, m), 2.16 (3H, s), 2.21 (3H, s), 2.27 (6H, s), 3.17 (2H, m), 4.19 (1H,s), 4.62 (1H, m), 5.38 (1H, d), 5.52 (1H, q), 5.75 (1H, d), 7.20–7.75 (9H, m), 8.45 (1H, t), 8.52 (1H, d), 8.67 (1H, d), 9.16 (1H, d), MS (ESI-NI) *m*/*z* 1060.5 $[M-H]^-$.

***N*-[$N^{2'}$,$N^{6'}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-amoxicillin sodium salt (5a).** This was produced from **3a** and amoxicillin hydrate as a white solid (yield 46%). ^{1}H NMR (DMSO-d_6) δ 1.32 (m, 2H), 1.39 (3H, s), 1.45 (2H, m), 1.54 (3H, s), 1.70 (2H, m), 2.19 (3H, s), 2.21 (3H, s), 2.27 (3H, s), 2.28 (3H, s), 3.13 (2H, m), 4.18 (1H, s), 4.56 (1H, m), 5.38 (1H, d), 5.51 (1H, q), 5.58 (1H, d), 6.68 (2H, d), 7.19 (2H, d), 7.38 (5H, m), 7.50 (1H, dd), 8.29 (2H, m), 8.44 (1H, d), 9.01 (1H, d), 9.35 (1H, s). MS (ESI-PI) *m*/*z* 955.0 $[M+H]^+$.

***N*-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-amoxicillin (5b).** This was produced from **3b** and amoxicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.45–1.90 (4H, m), 2.17 (6H, s), 2.21 (6H, s), 3.18 (2H, m), 4.18 (1H, s), 4.55 (1H, m), 5.38 (1H, d), 5.52 (2H, m), 6.66–7.50 (10H, m), 8.34 (2H, m), 8.40 (1H, d), 8.95 (1H, d), 9.35 (1H, s). MS (FAB) *m*/*z* 920.1 $[M+H]^+$.

***N*-[$N^{2'}$,$N^{6'}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-cephalexin (6a).** This was produced from **3a** and cephalexin as a white solid (yield 45%). ^{1}H NMR (DMSO-d_6): 1.20–1.80 (6H, m), 1.96 (3H, s), 2.19 (6H, s), 2.27 (6H, s), 3.12 (2H, m), 3.41 (2H, dd), 4.56 (1H, m), 5.11 (1H, d), 5.65 (1H, d), 5.70 (1H, q), 7.24–7.60 (11H, m), 8.25 (1H, m), 8.29 (1H, d), 8.61 (1H, d), 9.30 (1H, d).

***N*-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-cephalexin (6b).** This was prepared from **3b** and cephalexin as a white solid (yield 45%). ^{1}H NMR (DMSO-d_6): 1.50–1.90 (4H, m), 1.98 (3H,s), 2.22 (6H, s), 2.27 (6H, s), 3.22 (2H, m), 3.30 (2H, dd), 4.58 (1H, m), 4.96 (1H, d), 5.63 (1H, q), 5.67 (1H, d), 7.25–7.52 (11H, m), 8.35 (1H, m), 8.40 (1H, d), 8.55 (1H, d), 9.28 (1H, d). MS (ES-) 900.3 $[M-H]^-$.

***N*-[$N^{2'}$,$N^{6'}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-cefaclor (7a).** This was prepared from **3a** and cefaclor as a white solid (yield 40%). ^{1}H NMR (DMSO-d_6) δ 1.20–1.80 (6H, m), 2.19 (6H, s), 2.21 (6H, s), 2.27 (6H, s), 3.12 (2H, m), 3.71 (2H, dd), 4.56 (1H, m), 5.10 (1H, d), 5.67 (1H, d), 5.73 (1H, q), 7.25–7.58 (11H, m), 8.31 (1H, m), 8.32 (1H, d), 8.65 (1H, d), 9.44 (1H, d).

***N*-[$N^{2'}$,$N^{5'}$-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-cefaclor (7b).** This was produced from **3b** and cefaclor as a white solid (yield 40%). ^{1}H NMR (DMSO-d_6) δ 1.50–1.90 (m, 4H), 2.22 (6H, s), 2.27 (6H, s), 3.22 (2H, m), 3.65 (2H, dd), 4.56 (1H, m), 5.09 (1H, d), 5.62 (1H, d), 5.73 (1H, q), 7.25–7.52 (11H, m), 8.33 (1H, m), 8.37 (1H, d), 8.55 (1H, d), 9.38 (1H, d). MS (ESI-NI) *m*/*z* 919.9 $[M-H]^-$.

***N*-[2L,6-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl]-ampicillin (9a).** This was produced from **8a** and ampicillin hydrate as a white

solid, yield 34%. ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, s), 1.53 (3H, s), 1.58 (2H, m), 2.01 (1H, m), 2.28 (1H, m), 3.79 (2H, m), 3.89 (3H, s), 3.91 (3H, s), 4.19 (1H, s), 5.20 (1H, m), 5.39 (1H, d), 5.50 (1H, q), 5.76 (1H, d), 7.20–7.50 (7H, m), 7.76 (2H, m), 7.84 (2H, m), 8.79 (1H, d), 8.93 (1H, d). MS (ESI-NI) *m*/*z* 916.8 [M−H]$^-$.

***N*-[2D,5-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-pentanoyl]-ampicillin (9b).** This was produced from **8b** and ampicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.41 (3H, s), 1.54 (3H, s), 1.64 (2H, m), 1.98–2.30 (2H, m), 3.84 (2H, m), 3.90 (3H, s), 3.91 (3H, s), 4.20 (1H, s), 5.25 (1H, m), 5.41 (1H, d), 5.52 (1H, q), 5.77 (1H, d), 7.20–7.90 (11H, m), 8.83 (1H, d), 9.07 (1H, d). MS (ESI-NI) *m*/*z* 902.5 [M−H]$^-$.

***N*-[2L,5-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-pentanoyl]-ampicillin (9c).** This was produced from **8c** and ampicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, s), 1.52 (3H, s), 1.64 (2H, m), 1.98–2.30 (2H, m), 3.84 (2H, m), 3.89 (3H, s), 3.91 (3H, s), 4.19 (1H, s), 5.27 (1H, m), 5.37 (1H,d), 5.48 (1H, q), 5.74 (1H, d), 7.25–7.95 (11H, m), 8.72 (1H, d), 8.90 (1H, d). MS (ESI-NI) *m*/*z* 901.9 [M−H]$^-$.

***N*-{N^2-[Bis-$N^{2'}$,$N^{5'}$-(2,3-diacetoxybenzoyl)-L-ornithyl]-L-phenylalanyl}-ampicillin 11a.** This was prepared from **10a** and ampicillin hydrate as a white solid (Yield 30%). ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 3H), 1.55 (s, 3H), 1.90–1.50 (m, 4H), 2.18 (s, 3H), 2.19 (s, 3H), 2.27 (s, 6H), 2.99 (m, 2H), 3.17 (m, 2H), 4.18 (s, 1H), 4.45 (m, 1H), 4.82 (m, 1H), 5.37 (d, 1H), 5.52 (q, 1H), 5.78 (d, 1H), 7.50–7.10 (m, 16H), 8.10 (d, 1H), 8.21 (t, 1H), 8.30 (d, 1H), 8.85 (d, 1H), 9.22 (d, 1H). MS (ESI-NI) *m*/*z* 1049.5 [M−H]$^-$.

***N*-{N^2-[$N^{2''}$,$N^{5''}$-(Bis-(2,3-diacetoxybenzoyl)-L-ornithyl]-L-tryptophanyl}-ampicillin 11b.** This was produced from **10b** and ampicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 3H), 1.54 (s, 3H), 1.90–1.50 (m, 4H), 2.16 (s, 3H), 2.17 (s, 3H), 2.27 (s, 6H), 3.18 (m, 2H), 3.35 (m, 2H), 4.18 (s, 1H), 4.44 (m, 1H), 4.81 (m, 1H), 5.36 (d, 1H), 5.49 (q, 1H), 5.72 (d, 1H), 7.60–6.85 (m, 16H), 8.07 (d, 1H), 8.32 (m, 2H), 8.71 (d, 1H), 9.20 (d, 1H), 10.78 (s, 1H). MS (ESI-NI) *m*/*z* 1088.4 [M−H]$^-$.

***N*-{$N^{5'}$-[$N^{2''}$,$N^{5''}$-Bis-(2,3-diacetoxybenzoyl)-L-ornithyl]-$N^{2'}$-(2,3-diacetoxybenzoyl)-L-ornithyl}-ampicillin (14a).** This was produced from **13a** and ampicillin hydrate as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.54 (3H, s), 1.85–1.40 (8H, m), 2.17 (3H, s), 2.21 (6H, s), 2.27 (9H, s), 3.10 (4H, m), 4.,19 (1H, s), 4.37 (1H, m), 4.62 (1H, m), 5.37 (1H, d), 5.50 (1H, q), 5.74 (1H, d), 7.20–7.50 (14H, m), 8.22 (1H, d), 8.32 (2H, m), 8.63 (1H, d), 8.93 (1H, m), 9.19 (1H, d). MS (ESI-NI) *m*/*z* 1236.0 [M−H]$^-$.

***N*-{$N^{6'}$-[$N^{2''}$,$N^{6''}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-$N^{2'}$-(2,3-diacetoxybenzoyl)-L-lysyl}-ampicillin sodium salt (14b).** This was produced from **13b** and ampicillin hydrate as a white solid (yield 70%). ^{1}H NMR (DMSO-d_6) δ 1.37 (3H, s), 1.50 (3H, s), 1.30–1.85 (12H, m), 2.19 (3H, s), 2.20 (3H, s), 2.22 (3H, s), 2.27 (9H, s), 3.15 (4H, m), 3.81 (1H, s), 4.32 (1H, m), 4.54 (1H, m), 5.23 (1H, m), 5.34 (1H, m), 5.76 (1H, m), 7.20–7.55 (14H, m), 7.87 (1H, d), 8.22 (1H, d), 8.32 (2H, m), 8.57 (1H, d), 8.89 (1H, d). MS (ESI-PI) *m*/*z* 1288.5 [M + H]$^+$.

***N*-{$N^{2'}$-[$N^{2''}$,$N^{6''}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-$N^{6'}$-(2,3-diacetoxybenzoyl)-L-lysyl}-ampicillin sodium salt (17a).** This was produced from **16** and ampicillin hydrate as a white solid (yield 70%). ^{1}H NMR (DMSO-d_6) δ 1.38 (3H, s), 1.51 (3H, s), 1.20–1.80 (12H, m), 2.21 (9H, s), 2.29 (9H, s), 3.13 (4H, m), 3.80 (1H, s), 4.43 (2H, m), 5.23 (1H, m), 5.34 (1H, m), 5.75 (1H, m), 7.20–7.55 (14H, m), 8.00–9.00 (6H, m). MS (ESI-PI) *m*/*z* 1310.5 [M + 2Na]$^+$.

***N*-{$N^{2'}$-[$N^{2''}$,$N^{6''}$-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-$N^{6'}$-(2,3-diacetoxybenzoyl)-L-lysyl}-amoxicillin sodium salt (17b).** This was produced from **16** and amoxicillin hydrate as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.38 (3H, s), 1.50 (3H, s), 1.20–1.80 (12H, m), 2.21 (9H, s), 2.29 (9H, s), 3.13 (4H, m), 3.83 (1H, s); 4.44 (2H, m), 5.25 (1H, m), 5.34 (1H, m), 5.75 (1H, m), 6.65–7.55 (13H, m), 8.00–9.00 (6H, m). MS (ESI-PI) *m*/*z* 1304.6 [M + Na]$^+$.

***N*-{2L-[2′ L,6′-Bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoylamino]-6-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl}-ampicillin sodium salt (19a).** This was produced from **18** and ampicillin hydrate as a white solid (yield 60%). ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, s), 1.52 (3H, s), 1.20–1.70 (12H, m), 3.80 (4H, m), 3.83 (3H, s), 3.89 (6H, s), 4.18 (1H, s), 4.37 (1H, m), 5.09 (1H, m), 5.37 (1H,d), 5.47 (1H, q), 5.70 (1H, d), 7.20–7.90 (14H, m), 8.25 (1H, d), 8.93 (1H, d), 9.13 (1H, d). MS (ESI-NI) *m*/*z* 1264.5 [M–H]$^-$.

***N*-{2L-[2′L,6′-Bis-[8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoylamino]-6-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl}-amoxicillin (19b).** This was produced from **18** and amoxicillin hydrate as a white solid (Yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.50 (3H, s), 1.40 (3H, s), 1.25–1.80 (12H, m), 3.75 (4H, m), 3.80 (3H, s), 3.85 (6H, s), 4.15 (1H, s), 4.35 (1H, m), 5.00 (1H, m), 5.35 (1H, d), 5.40 (1H, q), 5.45 (1H, d), 7.15–7.85 (13H, m), 8.25 (1H, d), 8.50 (1H, d), 9.00 (1H, d), 9.35 (1H, s). MS (ESI-NI) *m*/*z* 1279.7 [M−H]$^-$.

***N*-{2L-[2L′,6′-Bis-[8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoylamino]-6-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl}-cefaclor (19c).** This was produced from **18** and cefaclor as a white solid (yield 66%). ^{1}H NMR (DMSO-d_6) δ 1.20–1.90 (14H, m), 3.70 (4H, m), 3.80 (9H, s), 4.35 (1H, m), 5.00 (1H, m), 5.10 (1H, d), 5.61 (1H, d), 5.70 (1H, d), 7.27–7.86 (14H, m), 8.25 (1H, d), 8.64 (1H, d), 9.36 (1H,d). MS (ESI-NI) *m*/*z* 1282.1 [M−H]$^-$.

***N*-{*N*^{2}-[ε-(8-Methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl]-*N*^{6}-[2L,6-bis-(8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-1,3-benzoxazin-3-yl)-*n*-hexanoyl]-L-lysyl}-ampicillin (22).** This was produced from **21** and ampicillin hydrate as a white solid (yield 30%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.00–2.30 (20H, m), 2.97 (2H, m), 3.79 (4H, m), 3.90 (9H, s), 4.19 (1H, s), 4.25 (1H, m), 5.07 (1H, m), 5.37 (1H, d), 5.50 (1H, q), 5.65 (1H, m), 7.20–8.02 (16H, m), 8.45 (1H, m), 9.10 (1H, m). MS (ESI-NI) *m*/*z* 1377.4 $[M-H]^-$.

***N*-{2-{[*N*^{2},*N*^{6}-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-amino}-4-[(*N*-benzoyloxy-*N*-methyl)-carbamoyl]-L-*n*-butanoyl}-ampicillin sodium salt (25a).** This was produced from **24a** and ampicillin hydrate as a white solid (yield 20%). ^{1}H NMR (DMSO-d_6) δ 1.37 (3H, s), 1.50 (3H, s), 1.45–2.06 (8H, m), 2.19 (3H, s), 2.21 (3H, s), 2.27 (6H, s), 2.39 (2H, m), 3.15 (2H, m), 3.33 (3H, m), 3.81 (1H, s), 4.38 (1H, m), 4.47 (1H, m), 5.23 (1H, d), 5.33 (1H, q), 5.75 (1H, d), 7.25–7.60 (13H, m), 7.72 (1H, t), 7.98 (2H, d), 8.12 (1H, m), 8.35 (2H, m), 8.56 (1H, d), 9.02 (1H, d). MS (FAB) *m*/*z* 1224.5 $[M+H]^+$.

***N*-{2-{[*N*^{2},*N*^{5}-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-amino}-4-[(*N*-benzoyloxy-*N*-methyl)-carbamoyl]-L-*n*-butanoyl}-ampicillin (25b).** This was produced from **24b** and ampicillin hydrate as a white solid (yield 40%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.45–2.06 (6H, m), 2.18 (6H, s), 2.27 (6H, s), 2.39 (2H, m), 3.15 (2H, m), 3.26 (3H, m), 4.18 (1H, s), 4.49 (2H, m), 5.35 (1H, d), 5.49 (1H, q), 5.73 (1H, d), 7.20–7.45 (11H, m), 7.54 (2H, t), 7.75 (1H, t), 8.00 (2H, d), 8.21 (2H, d), 8.32 (1H, m), 8.65 (1H, d), 9.18 (1H, d), MS (ESI-NI) *m*/*z* 1163.7 $[M-H]^-$.

***N*-{2-[*N*^{2},*N*^{6}-Bis-(2,3-diacetoxybenzoyl)-L-lysyl]-amino}-4-[(*N*-benzoyloxy-*N*-cyclohexyl)-carbamoyl]-L-*n*-butanoyl}-ampicillin (28a).** This was produced from **27a** and ampicillin hydrate as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.00–2.30 (18H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 3.14 (2H, m), 4.18 (1H, s), 4.20 (1H, m), 4.26 (1H, m), 4.40 (1H, m), 5.36 (1H, d), 5.48 (1H, q), 5.72 (1H, d), 7.20–8.03 (16H, m), 8.08 (1H, d), 8.30 (2H, m), 8.53 (1H, d), 9.18 (1H, d). MS (ESI-PI) *m*/*z* 1270.5 $[M+Na]^+$.

***N*-{2-{[*N*^{2},*N*^{5}-Bis-(2,3-diacetoxybenzoyl)-D-ornithyl]-amino}-4-[(*N*-benzoyloxy-*N*-cyclohexyl)-carbamoyl]-L-*n*-butanoyl}-ampicillin (28b).** This was produced from **27b** and ampicillin hydrate as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.38 (3H, s, 1.53 (3H, s), 1.00–2.30 (18H, m), 2.18 (6H, s), 2.27 (6H, s), 3.15 (2H, m), 4.18 (1H, s), 4.20 (1H, m), 4.48 (2H, m), 5.35 (1H, d), 5.48 (1H, q), 5.74 (1H, d), 7.22–8.05 (16H, m), 8.22 (2H, d), 8.32 (1H, m), NH); 8.65 (1H, d), 9.19 (1H, d). MS (ESI-PI) *m*/*z* 1256.4 $[M+Na]^+$.

***N*-{*N*^{2}-[4-(*N*-Benzoyloxy-*N*-methyl)-carbamoyl)-*n*-butanoyl]-*N*^{6}-(*N*$^{2'}$,N$^{6'}$-bis-2,3-diacetoxybenzoyl)-L-lysyl]-L-lysyl}-ampicillin (32a).** This was produced from **31a** and ampicillin hydrate as a white solid (yield 30%). ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, s), 1.53 (3H, s), 1.20–2.40 (18H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 3.10 (4H, m), 4.19 (1H, s), 4.33 (2H, m), 5.37 (1H, d), 5.50 (1H, q), 5.68 (1H, d), 7.30–8.05 (18H, m), 8.20 (1H, d), 8.29 (1H, m), 8.40 (1H, m), 9.10 (1H, m). MS (ESI-NI) *m*/*z* 1290.9 $[M-H]^-$.

***N*-{*N*^{2}-[4-(*N*-Benzoyloxy-*N*-cyclohexyl)-carbamoyl)-*n*-butanoyl]-*N*^{6}-(*N*$^{2'}$,*N*$^{6'}$-bis-2,3-diacetoxybenzoyl)-L-lysyl]-L-lysyl}-ampicillin (32b).** This was produced from **31b** and ampicillin hydrate as a white solid (yield 50%). ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, s), 1.54 (3H, s), 1.00–2.30 (28H, m), 2.19 (3H, s), 2.22 (3H, s), 2.27 (6H, s), 3.03 (2H, m), 3.15 (2H, m), 4.33 (3H, m), 5.37 (1H, d), 5.51 (1H, q), 5.68 (1H, m), 7.20–7.95 (16H, m), 8.04 (2H, d), 8.29 (1H, m), 8.45 (1H, m), 9.10 (1H, m). MS (ESI-PI) *m*/*z* 1383.7 $[M+Na]^+$.

Biology

Siderophore activity. The siderophore activity of the compounds **3a**–**d**, **8a**–**c**, **10a**,**b**, **13a**,**b**, **16**, **18**, **21**, **23a**,**b**, **24a**,**b**, **27a**,**b**, and **31a**,**b** was tested under iron limitation in growth promotion assays[24,25] on agar diffusion plates using the following Gram-negative bacterial strains: *P. aeruginosa* ATCC 27853, SG 137, NCTC 10662 and ATCC 9027, *E. coli* ATCC 25922, and *S. typhimurium*

Table 3. Susceptibility of *E. coli*—mutants depending on outer membrane siderophore receptors and on *tonB*

	H1443	H1876	H873	H1877	H1875	AB2847	BR158
TonB						+	–
FepA	+	–	–	–	–		
Cir	+	–	+	+	–		
Fiu	+	–	+	–	+		
Azlocillin	16	16	17	16	16	20	20
Ampicillin	23	21	23	22	21	24	24
Cefaclor	22	21	22	22	22	21	22
4a	23	0	21.5	17	21	19	13
4b	24	0	28	19	20	23	14
4c	24	14	23	20.5	24	22.5	14
4d	24	14	22.5	19.5	23.5	23.5	13
5a	23.5	0	20.5	15	20	19.5	10
5b	21	12	22	18	18	20	14
6b	13	0	14	13	13.5	14	15
7a	21.5	14.5	21	20	19.5	19	16
7b	21.5	0	21	17.5	20.5	17.5	11
9a	17.5	0	19.5	13	18.5	19	0
9b	23.5	13.5	22.5	20.5	23	22	14
9c	20.5	12.5	20.5	17.5	20.5	20	13
11a	29.5	12	29	25	27	28	13.5
11b	26	15	25	19.5	26	23.5	14.5
14a	21	11.5	21.5	18.5	20	20	13
14b	24.5	12.5	24	21	23	23.5	14.5
17a	28	10.5	25	23	23	26.5	13
17b	27	12.5	23.5	22	22	25	14
19a	20.5	0	19	17	18.5	18	10
19b	21.5	10	20	17	19	21	10
19c	20.5	12	20	18.5	19	18.5	12
22	20	18	22.5	19	0	21	10
25a	27	12	26	20	25	24	12
25b	26.5	14	26	22.5	27	25	14.5
28b	22.5	0	22	17	22	21	14
28a	25	0	25	21	24	23	12
32a	23.5	12.5	21.5	16	21	22.5	14
32b	22.5	0	21	12	14	21	13

Application of 5 μg/9 mm agar well.
Diameter of inhibition zones (mm).

enb7 (blocked in enterobactin biosynthesis). Siderophore solutions were applied on filter discs (O 6 mm) on the surface of the inoculated test agar plates. After incubation for 18–20 h at 37 °C, the zones of growth surrounding the discs were determined. Desferal was used as a control.

Antibacterial activity. Minimal inhibititory concentrations (MIC) of the ampicillin conjugates (X = Ap) **4a**–**d**, **9a**–**c**, **11a**,**b**, **14a**,**b**, **17a**, **19a**, **22**, **25a**,**b**, **28a**,**b**, **32a**,**b**, the amoxicillin conjugates (X = Ax) **5a**,**b**, **17b** and **19b**, the cephalexin conjugates (X = Ce) **6a**,**b** and the cefaclor conjugates (X = Cf) **7a**,**b** and **19c** were determined by the micro broth dilution method in Mueller-Hinton broth (Difco) according to the NCCLS guidelines[26] (Table 2). Test organisms were from Culture Collections (ATCC, NCTC) or from the stock of the Hans-Knöll-Institute (SG). *S. maltophilia* GN 12873 was kindly provided by Prof. S. Mitsuhashi, Episome Institute, Gunma (Japan).[27]

To investigate the mode of action of the conjugates the antibacterial activity was tested in an agar diffusion assay against *E. coli* strains deficient in siderophore receptors[28] or in the periplasmic TonB protein, which is essential for energy transfer in active iron transport systems. Results were read as inhibition zone diameters (Table 3).

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