Synthesis and antibacterial activity of new antibiotics arising from cephalosporin-monobactam coupling

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This paper is dedicated to Professor Krohn on the occasion of 60th birthday
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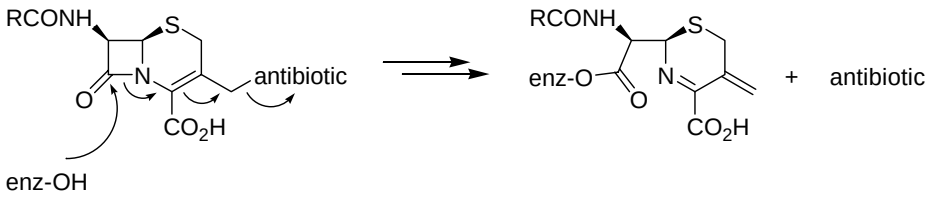
Abstract
New β-lactam antibiotics were obtained by coupling the cephalosporin Cefotaxime with monobactams in order to assess the possibility to enhance the cephalosporin activity through a synergistic dual-action mechanism. The activities were tested, *in vitro*, against a panel of selected bacteria. Preliminary results showed a light change in antibacterial activity when compared with that of the starting cephem counterpart.

Keywords: Dual-action, antibiotic, monobactam, cephalosporin

Introduction

The dual-action mechanism exploited by cephalosporins coupled with other antibiotics has been described.[1,2] It consists of a primary interaction between the β-lactam ring of the cephem counterpart with penicillin binding proteins or β-lactamases, that results in the release of the other antibiotic moiety in position 3, following a chemical displacement mechanism (Scheme 1).



Scheme 1. Dual action mechanism of a generic cephalosporin coupled with another antibiotic.

So far, with cephem-quinolones[3–5] (Figure 1), the dual-action mechanism has involved two active moieties inhibiting different molecular targets: *i.e.* cephalosporin exploiting a cell wall activity,[6,7] quinolone acting inside the cytoplasmic membrane at the DNA level.[8,9]

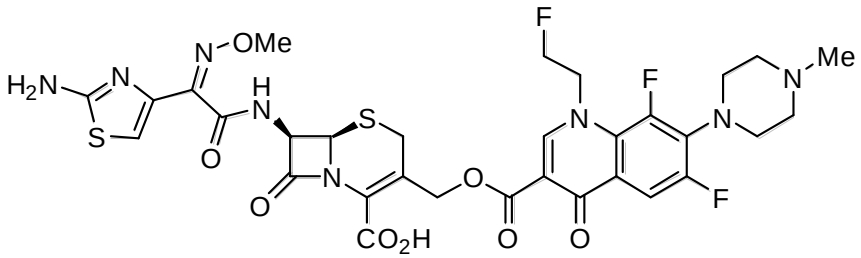


Figure 1. A cephem-quinolone dual action antibiotic.

To the best of our knowledge, no dual action products arising from the coupling between two β-lactam moieties have been reported, so far. In the present paper we describe the synthesis and the antibacterial activity of new compounds deriving from the above reported chemical linkage. A cephem and a monobactam moiety, both acting against bacterial cell wall targets, were used as parent compounds.

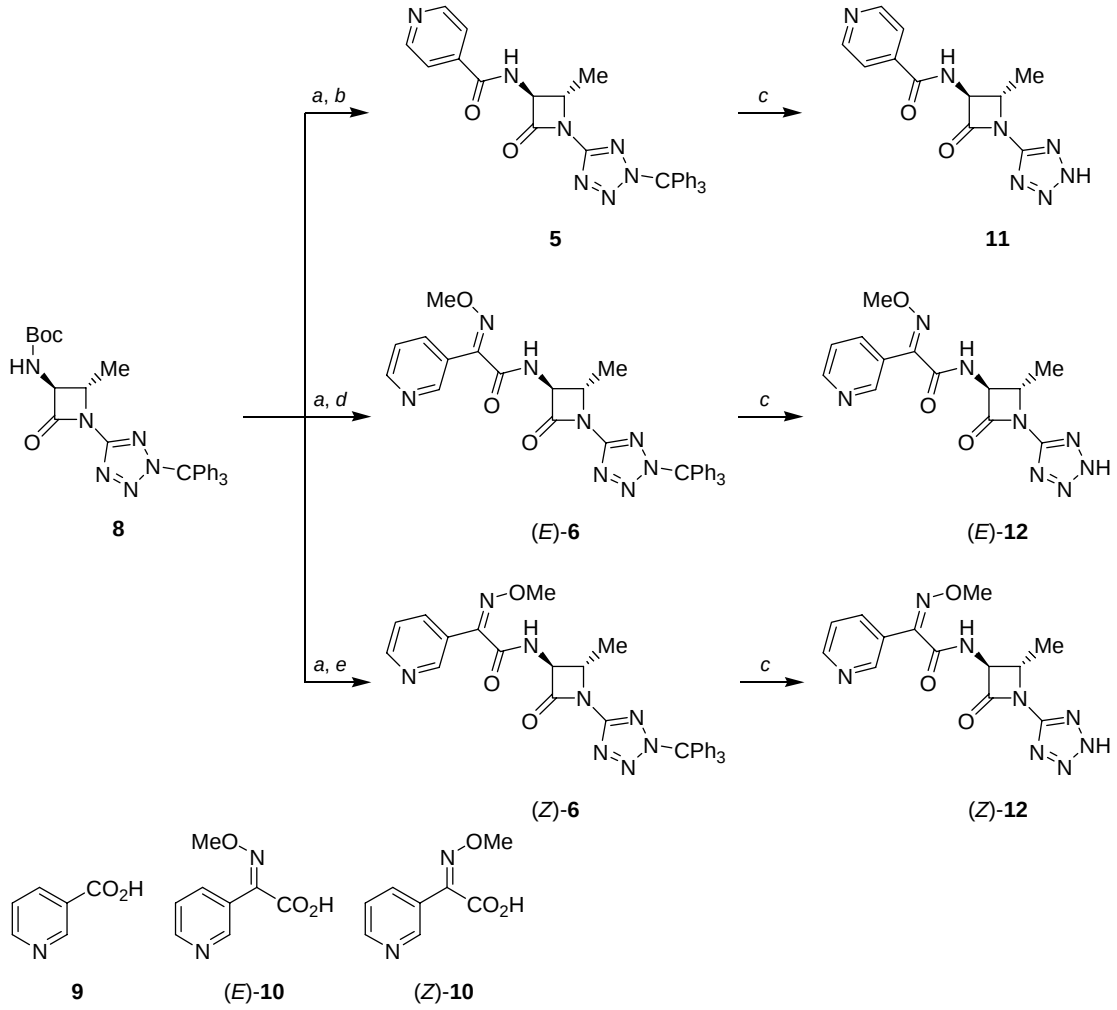
The expected dual action of the cephem-monobactam molecule and the monobactam, when released after enzymatic displacement, was based on the possible synergism resulting from a simultaneous action of these two moieties against different penicillin binding proteins (PBPs).[10]

The chosen β-lactam moieties have been Cefotaxime[11] (see Table 2), well known for its antibacterial activity, and monobactams **11,** (*E*)-**12** and (*Z*)-**12**. Monobactam (*Z*)-**7** is the commercially available antibiotic drug Aztreonam®,[12] especially used against Gram-negative aerobic organisms. The structures of unknown monobactams **11,** (*E*)-**12** and (*Z*)-**12** were designed on the basis of the following considerations: (1) The 3-amidic and 4-methyl substituents in *trans* geometry mimic the structure of Aztreonam®.[12] (2) The tetrazole ring, successfully used as a β-lactam ring activating group,[13,14] was preferred over the sulfonic acid anion, present at the N-1 position of the Aztreonam®. This neutral group, in fact, did not introduce further charges in the cephem-monobactam molecule, charges that could make difficult its penetration through the bacterial cell wall. (3) A pyridyl group at the 3-C substituent has been already used in *N*-(2*H*-tetrazol-5-yl)-azetidin-2-ones[14] obtaining monobactam with antibacterial activity. Furthermore, its introduction in the skeleton of monobactam has allowed linking the monobactam to the cephem as 3' quaternary ammonium salts. Compounds of this type have been shown to be a "third generation" antibacterial agents with excellent activity against a wide variety of Gram-positive and Gram-negative pathogens.[15]

Results and Discussion

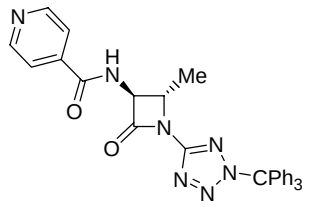
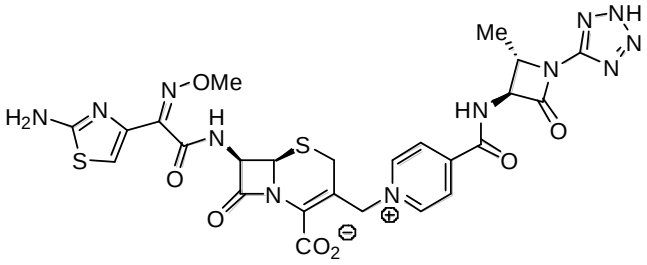
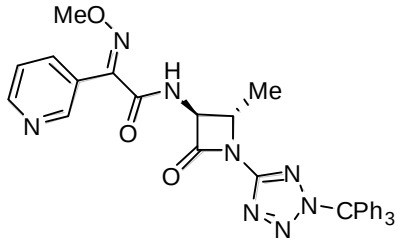
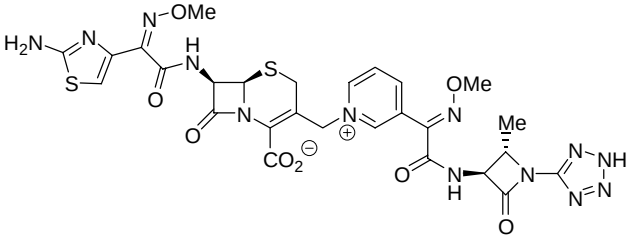
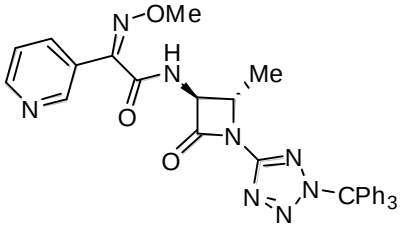
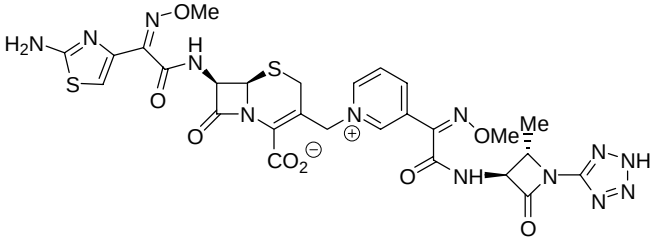
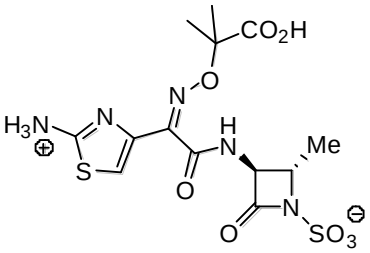
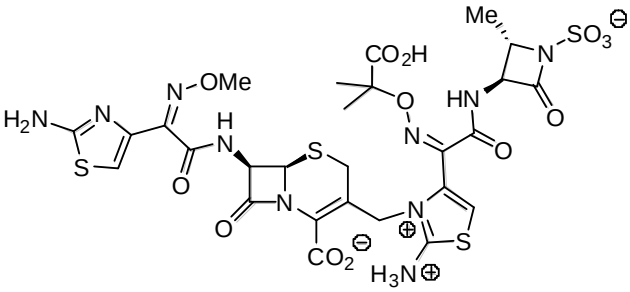
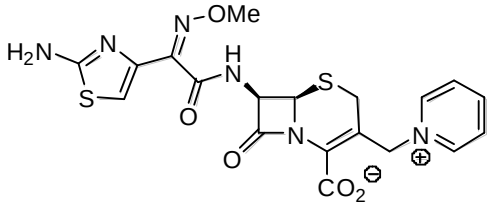
The synthesis of a series of coupling products (*Z*)-**1,** (*Z*,*E*)-**2**, (*Z*,*Z*)-**2**, (*Z*,*Z*)-**3**, derived from Cefotaxime and monobactams, **11,** (*E*)-**12** and (*Z*)-**12**, (*Z*)-**7**, was carried out (see Scheme 4, Table 1). Compound (*Z*)-**4** deriving from Cefotaxime and pyridine was synthesised for comparison. The activity of compounds (*Z*)-**1,** (*Z*,*E*)-**2**, (*Z*,*Z*)-**2**, (*Z*,*Z*)-**3** and (*Z*)-**4** against a panel of selected bacteria was tested.

The preparation of the unknown β-lactams **5**, (*E*)-**6** and (*Z*)-**6 ,** employed as 3'-cephalosporin substituents (see Table 1), was performed by coupling the (2*S**,3*S**)-[2-methyl-4-oxo-1-(2-trityl-2*H*-tetrazol-5-yl)-azetidin-3-yl]carbamic acid *tert*-butyl ester **8** [obtained from (d,l)-threonine by slight modification of the known procedure[14]] with the appropriate acid (Scheme 2). Deprotection from the trityl protecting group gave the NH-monobactams **11**, (*E*)-**12** and (*Z*)-**12**.



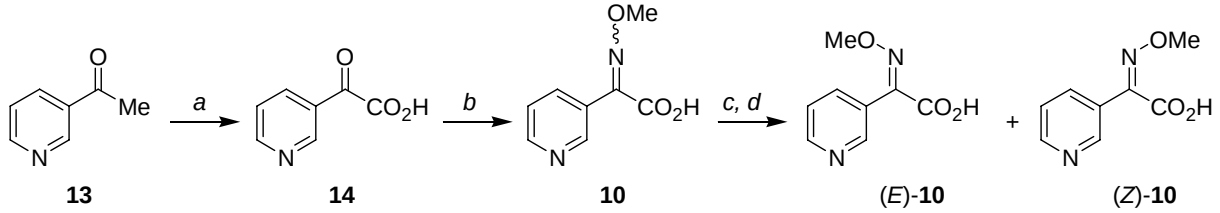
Scheme 2. *Reagents and conditions*: *a*: CF_3CO_2H; *b*: MeCN, TEA, EDC,HCl HOBT, **9**; c: HCO_2H (80%), acetone; *d*: MeCN, TEA, EDC,HCl HOBT, (*E*)-**10**; *e*: MeCN, TEA, EDC,HCl HOBT, (*Z*)-**10**.

Table 1. Monobactam moieties and coupling products

Monobactam derivatives	Coupling product[a,b]
 **5**	 (*Z*)-**1**
 (*E*)-**6**	 (*Z*,*E*)-**2**
 (*Z*)-**6**	 (*Z*,*Z*)-**2**
 (*Z*)-**7** **Aztreonam**	 (*Z*,*Z*)-**3**
	 (*Z*)-**4**

[a] Products **1,2** are diasteromeric mixtures. [b] All products **1**–**4** gave ^{1}H-NMR and IR spectra consistent with the structure shown.

The *anti* and the *syn* isomers of the methoxyiminopyridin-3-ylacetic acids (*E*)-**10** and (*Z*)-**10** were obtained by treating oxopyridin-3-ylacetic acid **14** (synthesized by oxidation of 3-acetylpyridine **13** as reported in the literature[16]) with *O*-methylhydroxylamine (Scheme 3). After esterification with diazomethane the two isomers were separated by flash chromatography and were subsequently hydrolyzed to the free acids (*E*)-**10** and (*Z*)-**10**: The configuration was assigned on the basis of the relative rates of methyl ester hydrolysis. In fact, for a series of α-alkoxyimino esters it has been demonstrated that the *Z* isomers (*syn*) hydrolyze much more slowly than the corresponding *E* forms (*anti*).[17,18]



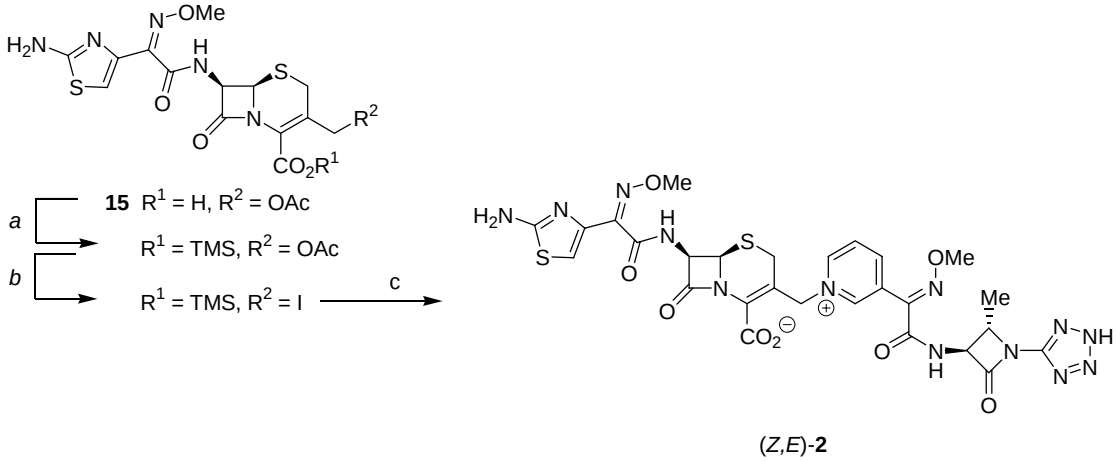
13 **14** **10** (*E*)-**10** (*Z*)-**10**

Scheme 3. *Reagents and conditions*: *a*: SeO_2, pyridine (40%); b: $MeONH_2 \cdot HCl$, $NaHCO_3$ (5% aqueous solution); *c*: CH_2N_2, separation of methyl esters by flash chromatography; *d*: NaOH, MeOH.

The coupling products (*Z*)-**1,** (*Z,E*)-**2**, (*Z,Z*)-**2,** (*Z,Z*)-**3** and (*Z*)-**4** are quaternary cephalosporin derivatives and were prepared by modification of the general method of Bonjouklian and Phillips (in Scheme 4 the synthesis of (*Z,E*)-**2** is reported as a typical example).[15,19] Silylation of Cefotaxime **15** in acetonitrile with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) was followed by in situ formation of the 3'-iodide derivative with trimethylsilyliodide (TMSI). Excess of trimethylsilyliodide was destroyed by addition of tetrahydrofuran. The 3'-iodide was then replaced by the pyridin-containing monobactam (*E*)-**6** in acetonitrile. The silylated cephem was hydrolyzed by treatment with water, and concomitant detritylation of the tetrazole ring on the monobactam moiety occurred.

Cefotaxime-monobactam derivatives (*Z*)-**1,** (*Z,E*)-**2**, (*Z,Z*)-**2,** (*Z,Z*)-**3** and (*Z*)-**4** were tested for their *in vitro* activity in comparison with Cefotaxime, with compound (Z)-**7** (Aztreonam)® and with the free monobactam partners **11,** (*E*)-**12** and (*Z*)-**12**. Despite the lack of any *in vitro* activity of monobactams **11,** (*E*)-**12** and (*Z*)-**12** (MIC>128μg/mL) the coupling compounds (*Z*)-**1,** (*Z,E*)-**2**, (*Z,Z*)-**2** were tested against some selected bacteria. As a matter of fact, the inactivity *in vitro* of the monobactam counterpart, against the whole bacterial cell, might have been ascribed to difficulty of penetration through the bacterial cell wall. In contrast, the coupling compounds (*Z*)-**1,** (*Z,E*)-**2**, (*Z,Z*)-**2** could have been able to exploit their potential intrinsic activity on their PBP targets, once entered the bacterial cell wall. Further studies are planned to understand whether the lack of activity of monobactam (*E*)-**12** and (*Z*)-**12** has to be ascribed to a lack of intrinsic activity or a difficult penetration through the Gram-negative outer membrane. Compounds (*Z*)-**1,** (*Z,E*)-**2**, (*Z,Z*)-**2,** (*Z,Z*)-**3** showed better activity with respect to compound (*Z*)-**4** , which is a poor antibacterial agent. However, although maintaining some antibacterial activity, compound (*Z*)-**1**

was significantly less active than Cefotaxime. By contrast, the antimicrobial activity of compounds (*Z,E*)**-2**, (*Z,Z*)-**2** against most of bacterial strains matched that of Cefotaxime (Table 2), and compound (*Z,Z*)-**2** was even somewhat more active than Cefotaxime against one strain of *E. coli* and one strain of *K. pneumoniae*. Since compounds (*Z,E*)**-2**, (*Z,Z*)-**2** differ only in the geometry of the monobactam moiety's methoxy-imine, their chemophysical properties have to be very similar. Thus, if the activity of these compounds had to be ascribed only to the cephem nature without any concomitant dual action, it would be hard to understand the differences between the antibacterial activity of (*Z,E*)**-2**, (*Z,Z*)-**2**. Otherwise, stating the occurrence of the dual action mechanism, these differences can be ascribed to the release of an inactive monobactam moiety in the case of the compound (*Z,E*)**-2** and of an active one in the case of the compound (*Z,Z*)-**2**. Since this dual-action mechanism should depend on the PBP's and/or β-lactamases, it could happen that it is exploited to a different extent in different bacterial species and strains. In contrast, compound (*Z,Z*)-**3**, derived from coupling of Cefotaxime with Aztreonam (*Z*)-**7**, was less active than either Cefotaxime or Aztreonam alone. This poor activity could be caused by a difficult cell wall penetration due to the total negative charge. Furthermore, the comparison of its activity with that of Aztreonam, that is better against Gram- positive organisms but worst against Gram-negative, may suggest that compound (*Z,Z*)-**3** maintains a cephalosporin-type spectrum of activity without significantly releasing the monobactam counterpart, as a consequence of a possible preferred primary interaction between the monobactam-ring and its target PBPs in Gram negative bacteria.



Scheme 4. *Reagents and conditions*: *a*: MSTFA, MeCN; *b*: TMSI, THF; *c*: (*E*)-**6**, MeCN followed by H_2O.

These results strongly suggest that the expected dual-action mechanism is at least partially exploited. Work is in progress in order to assess the possibility of exploiting a dual action mechanism by coupling different β-lactam-antibiotics.

Table 2. *In Vitro* antibacterial activity (MIC[a], μg/mL) of the most representative compounds

Organism	Ref. Compd Cefotaxime	Ref. Compd. **4**	Ref. Compd. Aztreonam **7**	(*Z,E*)-**2**	(*Z,Z*)-**2**	(*Z,Z*)-**3**
S.enteriditis[b] UAA11RX	0.125	4	0.125	0.5	1	1
E.coli[b] K12	0.125	1	0.125	0.25	0.5	1
E.coli[b] TEM2	0.125	16	0.5	8	2	4
E coli[b] TEM4	128	>128	32	>128	64	>128
E coli[b] Cl. Is.	32	>128	128	64	16	>128
K.pneumoniae[b] Cl. Is.	128	>128	>128	128	8	>128
P.vilgaris[b] ATCC 881	0.125	1	0.125	1	0.25	0.25
P.mirabilis[b] Cl. Is.	0.125	1	0.125	0.25	<0.125	0.25
P.aeruginosa[b] ATCC 10145	16	32	16	128	128	64
E.cloacae[b] DER	>128	>128	128	n.t.	n.t.	>128
S.aureus[c] β-lactamase producer	16	16	>128	32	16	32
S.aureus[c] Smith	8	16	>128	8	8	32
S.aureus[c] Met-R Cl. Is.	128	128	>128	>128	>128	>128
S.pyrogenes[c] C203	0.125	0.25	2	<0.125	0.25	0.125

n.t. = not tested.
[a] MIC = Minimum inhibitory concentration. [b] gram-negatives. [c]gram-positives.

Experimental Section

General Procedures. All starting compounds, unless otherwise stated, were purchased. Reactions were run under an atmosphere of dry nitrogen or argon. FT-IR Spectra were recorded on a Perkin-Elmer infrared spectrometer, mass spectra at 70 eV, using the electron impact mode were obtained on Finnigan MAT GCQ instrument, NMR spectra on spectrometers Varian VXR 200, Varian Gemini 300, or Varian Mercury 400 MHz using the residual signal of the solvent as internal standard. HPLC analysis were carried out using a HP 1100 instrument, column Hibar Lichrospher 100 RP-18 (5μm), eluting with a gradient from KH_2PO_4 buffer (0.01N, pH 3.2) to MeCN/ KH_2PO_4 buffer 85/15.

(*E*) and (*Z*)-2-(Methoxyimino)-2-(3-pyrid-3-yl)acetic acid [(*E*)-10] and [(*Z*)-10]. *O*-Methyl-hydroxylamine hydrochloride (8.3g, 98 mmol, 32.5 mL of a 25% solution in water) was added at room temperature and stirring to oxopyridin-3-ylacetic acid **14** (3.7g, 24 mmol). The pH 5 was adjusted by adding a saturated solution of $NaHCO_3$. The resulting mixture was stirred over night affording a homogeneous solution (pH 5.2). 1N HCl was added to adjust pH 4, and the water was removed in vacuo to afford a white solid. This product was dissolved in methanol and heated to 60 °C under stirring. The precipitate was separated by decantation and was a mixture of (*E*)-**10** and (*Z*)-**10**, which was trearted diazomethane. Separation of the corresponding methyl esters by flash chromatography (cyclohexane/ethyl acetate 7:3) followed by hydrolysis with NaOH/MeOH, and subsequent addition of HCl (aq) to adjust pH 4.2) afforded the acids (*E*)-**10** (2.54 g, 58%) and (*Z*)-**10** (0.52g, 12%)**.**

(*E*)-**10.** Colorless crystals, mp 115–120 °C. IR (nujol): 1642, 1445 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 13.40 (bs, 1H), 8.56 (m, 2H), 7.81 (m, 1H), 7.45 (m, 1H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6, 400MHz): δ 164.4, 150.5, 149.9, 147.9, 137.4, 126.9, 123.8, 63.9. MS: m/e (%): 180 (10), 149(45), 135, 120(75), 104(100), 77(90). Anal. Calcd. For $C_8H_8N_2O_3$ (180.05): C, 53.33; H, 4.48; N, 15.55. Found: C, 53.14; H, 4.50; N, 15.47.

(*Z*)-**10.** Colorless crystals, mp 125-130 °C**.** IR (nujol): 1643, 1598 cm^{-1}; ^1H NMR (DMSO-d_6, 400MHz): δ 8.53 (s, 1H), 8.45 (d, J = 4.6 Hz, 1H), 7.75 (d, J = 7.4 Hz, 1H), 7.35 (dd, J = 7.4, 4.6 Hz, 1H), 3.76 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 165.7, 155.1, 149.2, 137.0, 129.7, 123.4, 123.3, 62.2. MS: m/e (%): 180 (10), 149(45), 135, 120(75), 104(100), 77(90). Anal. Calcd. For $C_8H_8N_2O_3$ (180.05): C, 53.33; H, 4.48; N, 15.55. Found: C, 53.34; H, 4.55; N, 15.42.

(*Z*)-*N*1-[(2*R,3*R**)-2-Methyl-4-oxo-1-(2-trityl-2*H*-1,2,3,4-tetrazol-5-yl)azetidin-3-yl]-2-(methoxyimino) -2-(3-pyridyl)acetamide [(*Z*)-6]. Typical procedure for the preparation of β-lactams 5, 6**

A cooled (0 °C) solution of *N*-[(2*S**,3*S**)-2-methyl-4-oxo-1-(2-trityl-2*H*-tetrazol-5-yl)azetidin-3-yl]carbamic acid *tert*-butyl ester **8** (227 mg, 0.45 mmol) in trifluoroacetic acid (99%, 3 mL) was allowed to warm to room temperature within 10 min under a stream of nitrogen. The trifluoroacetic acid was then removed in vacuo at room temperature affording a yellow oil. This product was dissolved under nitrogen in dry acetonitrile (15 mL), and, at 0 °C triethylamine (1.575 mL, 1.13 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (94 mg, 0.5 mmol), 1-hydroxybenzotriazole (78 mg, 0.59 mmol) and (*Z*)-2-(methoxyimino)-2-(3-pyrid-3-yl)acetic acid (200 mg, 0,39 mmol) (*Z*)-**10** were added. The reaction mixture was stirred at room temperature overnight, was then poured into an aqueous solution of sodium carbonate (5 mL, 5% solution) and extracted with dichloromethane (3 x 15mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under vacuo. (*Z*)-**6** was isolated as pure product by flash chromatography (cyclohexane/ethyl acetate 3/7).

Colorless crystals (24%), mp 145–148 °C. IR ($CDCl_3$): 3377, 1770 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.83 (bs, 1H), 8.53 (m, 1H), 7.95 (m, 1H), 7.44 (m, 1H), 7.40-7.21 (m, 11H), 7.12 (m, 5H), 4.83 (dd, J_1 = 6.4 Hz, J_2 = 2.8 Hz, 1H), 4.36 (m, 1H), 4.11 (s, 3H), 1.70 (d, J = 6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.6, 161.1, 156.7, 149.7, 148.3, 146.8, 140.7, 130.2,

128.4, 127.9, 127.8, 127.7, 127.1, 83.7, 63.6, 58.2, 21.6, 17.0. Anal. Calcd for $C_{32}H_{28}N_8O_3$ (572.62): C, 67.12; H, 4.93; N, 19.5. Found: C, 67.29; H, 4.96; N, 19.40.

Following the same procedure and using the appropriate reagents products **(5)** and **(*E*)-6** were obtained in the yields reported in square brackets.

***N*1-[(2*R**,3*R**)-2-Methyl-4-oxo-1-(2-trityl-2*H*-1,2,3,4-tetrazol-5-yl)azetidin-3-yl]-isonicotinamide (5).** Pale yellow crystals (25%), mp 170 °C (dec.). IR (film): 1786 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, *J* = 5.9 Hz, 2H), 8.07 (d, *J* = 7.0 Hz, 1H), 7.75 (d, *J* = 5.9 Hz, 2H), 7.41–7.18 (m, 10H), 7.04 (m, 5H), 4.96 (dd, J_1 = 7.0 Hz, J_2 = 2.4 Hz, 1H), 3.94 (m, 1H), 1.59 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100MHz): δ 165.5, 162.6, 156.5, 150.5, 140.6. 139.8, 130.2, 128.5, 127.8, 121.2, 83.9, 64.0, 59.1, 17.1. MS: m/z (%): 514, 459 (10), 243 (100), 165 (50), 106, 78. Anal. Calcd for $C_{30}H_{25}N_7O_2$ (515.57): C, 69.89; H, 4.89; N, 19.02 Found: C, 70.09; H, 4.91; N, 19.00.

(*E*)-*N*1-[(2*R,3*R**)-2-Methyl-4-oxo-1-(2-trityl-2*H*-1,2,3,4-tetrazol-5-yl)azetidin-3-yl]-2-(methoxyimino)-2-(3-pyridyl)acetamide [(*E*)-6].** Pale yellow crystals (22%), mp 185 °C (dec.): IR ($CDCl_3$): 3377, 1770 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 8.67 (bs, 1H), 8.62 (bs, 1H), 7.80 (m, 1H), 7.63 (m, 1H), 7.35 (m, 11H), 7.12 (m, 5H), 4.81 (dd, J_1 = 7.04 Hz, J_2 = 3.52 Hz, 1H), 4.33 (m, 1H), 4.01 (s, 3H), 1.66 (d, *J* = 6.16 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100MHz): δ 162.3, 161.6, 157.3, 150.1, 146.2, 141.2, 140.8, 137.6, 130.9, 130.3, 128.4, 128.3, 127.8, 124.3, 60.4, 58.2, 29.2, 17.3. Anal. Calcd for $C_{32}H_{28}N_8O_3$ (572.62): C, 67.12; H, 4.93; N, 19.5. Found: C, 67.39; H, 4.95; N, 19.49.

***N*1-[(2*R**,3*R**)-2-Methyl-4-oxo-1-(-2*H*-1,2,3,4-tetrazol-5-yl)azetidin-3-yl]-isonicotinamide (11): Typical procedure for the preparation of β-lactams 11, 12.** To a suspension of **5** (60 mg, 0.12 mmol) in acetone (10 mL) was added formic acid (80%, 1 mL each) in two portions. After 6 h the solution was dried in vacuo, and the resulting solid was washed with diethyl ether to eliminate triphenylmethanol. **12** was obtained as white solid (31 mg, 95%), mp 190 °C (dec.). IR (nujol): 2923, 2853, 1766, 1680 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): δ 9.52 (d, *J* = 7.4 Hz, 1H), 8.69 (d, *J* = 5.9 Hz, 2H), 7.69 (d, *J* = 5.9, 2H), 4.85 (dd, J_1 = 2.9 Hz, J_2 = 7.4 Hz, 1H), 4.37 (m, 1H), 3.28 (bs, 1H), 1.53 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100MHz): δ 164.9, 163.1, 156.0, 150.6, 139.8, 121.2, 63.8, 56.5, 16.3. Mz (m/z): 258(30) (M^+–15), 243(35), 229, 205, 162(65), 147(100), 106, 78. Anal. Calcd for $C_{11}H_{11}N_7O_2$ (273.25): C, 48.35; H, 4.06; N, 35.88. Found: C, 48.51; H, 4.08; N, 35.75.

Following the same procedure and starting from (*E*)-**6** and (*Z*)-**6** products (*E*)-**12** and (*Z*)-12, respectively, were obtained.

(*E*)-*N*1-[(2*R,3*R**)-2-Methyl-4-oxo-1-(2*H*-1,2,3,4-tetrazol-5-yl)azetidin-3-yl]-2-(methoxyimino)-2-(3-pyridyl)acetamide [(*E*)-12].** Pale yellow crystals (85%), mp 172–175 °C (dec.): IR: 1776 (nujol) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.78 (d, *J* = 8.2 Hz, 1H), 8.71 (d, *J* = 1.2 Hz, 1H), 8.62 (dd, J_1 = 5.2 Hz, J_2 = 1.6 Hz, 1H), 7.91 (dt, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 7.47 (m, 1H), 5.02 (dd, J_1 = 8.20 Hz, J_2 = 3.2 Hz, 1H), 4.54 (m, 1H), 4.15 (s, 3H), 1.74 (d, *J* = 6.4 Hz, 3H). Anal. Calcd for $C_{13}H_{14}N_8O_3$ (330.12): C, 47.27; H, 4.27; N, 32.92. Found: C, 47.47; H, 4.30; N, 32.75.

(*Z*)-*N*1-[(2*R,3*R**)-2-Methyl-4-oxo-1-(2*H*-1,2,3,4-tetrazol-5-yl)azetidin-3-yl]-2-(methoxyimino)-2-(3-pyridyl)acetamide [(*Z*)-12].** Pale yellow crystals (90%), mp 183–186 °C (dec.): ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.80 (s, 1H), 8.79 (bs, 1H), 8.63 (m, 1H), 8.01 (dt, J_1 = 8.2 Hz, J_2= 2.0 Hz, 1H), 7.45, (dd, J_1 = 8.2 Hz, J_2 = 4.8 Hz, 1H), 4.99 (m, 1H), 4.64 (m, 1H), 4.01 (s, 3H), 1.75 (d, J = 6.0 Hz, 3H). Anal. Calcd for $C_{13}H_{14}N_8O_3$ (330.12): C, 47.27; H, 4.27; N, 32.92. Found: C, 47.50; H, 4.38; N, 32.75.

(*Z*)-(7*R*,7a*R*)-7-[2-(2-Amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-3-[3-[(*Z*)-*N*1-[(2*R,3*S**)-2-methyl-4-oxo-1-(2*H*-1,2,3,4-tetraazol-5-yl)-azetidin-3-yl]-2-(methoxyimino)-acetamide-2-yl]-1-pyridiniumyl]methyl-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]-thiazine-4-carboxylate [(*Z*,*Z*)-2]. Typical procedure for the preparation of quaternary cephalosporins 1–4. Ceftaxime 15** (45 mg, 0.1 mmol) was suspended in acetonitrile (4 mL) under a nitrogen atmosphere at 5–10 °C. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (49μL, 0.35 mmol) was added and the mixture was stirred at 5–15 °C for 2h. To the resulting homogeneous solution of cephem trimethylsilyl ester was added trimethylsilyliodide (49 μL, 0.35 mmol). The solution was stirred at 10 °C for 35 min and was then allowed to warm to room temperature. Excess of TMSI and the solvent were evaporated in vacuo to afford 3'-iodomethylcephem as viscous oil. This oil was dissolved in acetonitrile (4 mL), tetrahydrofuran (37μL, 0.42mmol) was added to destroy the excess of TMSI, and the solution was stirred at room temperature for 10 min. To the solution (*Z*)-**6** (63mg, 0.1 mmol) was added in one portion. The reaction was stirred at room temperature for 3 h until a precipitate separated. Water (4 μL, 0.24 mmol) was added and the precipitate was separated from the solution by decantation. Trituration with diethyl ether and acetone and drying in vacuo afforded product (*Z*,*Z*)-**2** (18.5 mg, 25%) of sufficient spectroscopic purity for biological tests. Due to the difficulty of separation of diastereomeric mixtures the products (*Z*)-**1,** (*Z*,*E*)-**2** and (*Z*,*Z*)-**2** were tested as such. Spectroscopic data, including the ^{1}H NMR are given below. The signals of the minor isomer are given in square brackets.

(*Z*,*Z*)-2. Pale yellow crystals, mp 197–200°C (dec.). IR (nujol) 1765 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.62 (d, J = 7.6Hz, 1H), 9.58 (d, J = 7.2Hz, 1H), 8.72 (bs, 1H), 8.68 (bs, 1H), 7.96 (m, 1H), 7.55 (m, 1H), 7.20 (bs, 2H), 6.74 (s, 1H), [6.73 (s)], [5.78 (dd, J_1 = 8.4 Hz, J_2 = 4.8 Hz)], [5.14 (d, J = 4.8Hz)], 5.09 (d, J = 4.4Hz, 1H), 4.98 (d, J = 13.0Hz, 1H), [4.87 (dd, J_1= 6.4 Hz, J_2 = 3.6 Hz)], 4.83 (dd, J_1 = 7.2 Hz, J_2 = 4.4 Hz, 1H), 4.67 (d, J = 13.0 Hz, 1H), [4.44 (m)], 4.37 (m, 1H), 4.05 (s, 3H), 3.98 (s, 3H), 3.52 (d, J = 18.0 Hz, 1H), 3.39 (d, J = 18.0 Hz, 1H); [1.61 (d, J = 6.0Hz)], 1.53 (d, J = 6.4Hz, 3H). Anal. Calcd for $C_{28}H_{30}N_{13}O_8S_2$ (740.75): C, 45.40; H, 4.08; N, 24.58. Found: C, 45.60; H, 4.09; N, 24.50.

The same procedure employing azetidinones (*E*)-**6** and (Z)-**7** were used for the synthesis of coupling products **1** and **2**. The relative yields are reported in square brackets.

(*Z*)-(7*R*,7a*R*)-7-[2-(2-Amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-3-[4-[[(2*R, 3*S**)-2-methyl-4-oxo-1-(2*H*-1,2,3,4-tetraazol-5-yl)-3-azetidinyl]aminocarbonyl]-1-pyridiniumyl]methyl-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]thiazine-4-carboxylate [(*Z*)-1].** Pale yellow crystals (15%), mp 167–172°C (dec.). IR (nujol) 1778 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 10.01 (d, *J* = 7.8 Hz, 1H), 9.66 (d, *J* = 7.6 Hz, 1H), 9.21 (d, *J* = 6.6 Hz, 1H), 8.81 (d, *J* = 6.0 Hz, 1H), 8.48 (d, *J* = 6.6 Hz, 1H), 7.84 (d, *J* = 6.0 Hz, 1H), 7.10 (bs, 2H), [6.83], 6.76 (s, 1H), [5.90 (m)], 5.86 (dd, J_1= 5.0 Hz, J_2 = 7.6 Hz, 1H), 5.66 (d, *J* = 15.0 Hz, 1H), 5.56 (d, *J* = 15.0, 1H), 5.16 (d, *J* = 15.0 Hz, 1H), [5.00 (d, *J* = 6.8Hz)], 4.93 (dd, J_1 = 2.8 Hz, J_2 = 7.8 Hz, 1H), [4.49 (m)], 4.48 (m, 1H), [3.84 (s)], 3.82 (s, 3H), 3.55 (d, *J* = 16.0 Hz, 1H), 3.38 (d, *J* = 16.0 Hz, 1H), [1.62 (d, *J* = 6.0)], 1.61 (d, *J* = 6.0 Hz, 3H).

(*Z*)-(7*R*,7a*R*)-7-[2-(2-Amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-3-[3-[(*E*)-*N*1-[(2*R,3*S**)-2-methyl-4-oxo-1-(2*H*-1,2,3,4-tetrazol-5-yl)-azetidin-3-yl]-2-(methoxyimino)-acetamide-2-yl]-1-pyridiniumyl]methyl-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]thiazine-4-carboxylate [(*Z*,*E*)-2].** Pale yellow crystals (23%), mp 197–200°C (dec.). IR (nujol) 1775 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 9.59 (d, *J* = 8.0 Hz, 1H); 9.39 (d, *J* = 8.8 Hz, 1H), 9.28 (bs, 1H), 9.05 (d, *J* = 4.8 Hz, 1H), 8.72 (d, *J* = 8.4Hz, 1H), 8.27 (m, 1H), 7.26 (bs, 2H), [6.73 (s)], 6.70 (s, 1H), [5.90 (dd, J_1= 8.4, J_2 = 5.2 Hz)], 5.87 (dd, J_1 = 8.0, J_2 = 5.0 Hz, 1H), 5.62 (d, *J* = 14.8, 1H), 5.54 (d, *J* = 14.8, 1H), 5.18 (d, *J* = 5.0 Hz), [5.14 (d, *J* = 4.8)], 4.96 (m, 1H), [4.37, m], 4.42 (m, 1H), 4.06 (s, 3H) [4.05 (s)] , [3.80 (s)], 3.79 (s, 3H), 3.52 (d, *J* = 18.4 Hz, 1H), 3.39 (d, *J* = 18.4 Hz, 1H); 1.58 (d, *J* = 7.6 Hz, 3H), [1.52 (d, *J* = 5.6Hz)]. Anal. Calcd for $C_{28}H_{30}N_{13}O_8S_2$ (740.75): C, 45.40; H, 4.08; N, 24.58. Found: C, 45.65; H, 4.10; N, 24.50.

(*Z*)-(7*R*,7a*R*)-7-[2-(2-Amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-3-[3-[(2-amino)[(*Z*)-(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-azetidin-3-yl]-2-(1-carboxy-1-methylethoxy)iminoacetamide-2-yl]-1,3-thiazolinium-2-amino-3-yl]methyl-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]thiazine-4-carboxylate [(*Z*,*Z*)-3]. Aztreonam **7** was used as monobactam counterpart following the protocol above reported except for the addition of 1.1 eqiv of diisopropylethylamine for releasing Aztreonam **7** from its zwitterion. Pale yellow crystals (*Z*,*Z*)-**3** (85%), mp 208 °C (dec.). IR (nujol): 1770 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 9.58 (m, 2H), 7.21 (s, 1H), 7.20 (m, 4H), 6.69 (s, 1H), 6.67 (dd, J_1 = 7.6 Hz, J_2 = 4.4 Hz, 1H), 5.50 (m, 1H), 5.00 (m, 2H), 4.46 (dd, J_1 = 7.6 Hz, J_2 = 2.4 Hz, 1H), 3.79 (s, 3H), 3.67 (m, 1H), 3.21 (m, 2H), 1.40 (m, 9H). Anal. Calcd fo $C_{27}H_{30}N_{10}O_{13}S_4$ (830.09): C, 39.03; H, 3.64; N, 16.86. Found: C, 39.30; H, 4.36; N, 17.00.

(*Z*)-(7*R*,7a*R*)-7-[2-(2-Amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-3-[1-pyridiniumyl]ethyl-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]thiazine-4-carboxylate [(*Z*)-4]. Following the procedure reported for product **2** and using pyridine instead of monobactam, product (*Z*)-**4** was obtained as yellow crystals (84%), mp 173 °C (dec.) IR (nujol) 1774 cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz): δ 9.60 (d, *J* = 8.0 Hz, 1H), 9.02 (d, *J* = 5.4 Hz, 2H), 8.65 (m, 1H), 8.20 (dd, m, 2H), 7.23 (bs, 2H), 6.70 (s, 1H), 5.87 (dd, J_1 = 8.0 Hz, J_2 = 4.9 Hz, 1H), 5.60 (d, *J* = 14.5 Hz, 1H), 5.47 (d, *J* = 14.5 Hz, 1H), 5.19 (d, *J* = 4.9 Hz, 1H), 3.82 (s, 3H), 3.55 (d, *J* = 18.3 Hz, 1H), 3.35 (d, *J* = 1.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 200 MHz): δ 168.4, 163.9, 162.9, 162.8, 148.8, 147.0, 143.3, 145.0, 142.1, 129.3, 128.4, 119.8, 108.9, 61.9, 60.6, 59.0, 57.6. Anal.

Calcd for $C_{20}H_{21}N_6O_5S_2$ (489.55): C, 49.07; H, 4.32; N, 17.17. Found: C, 49.30; H, 4.34; N, 17.09.

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