



Published in final edited form as:

*J Antibiot (Tokyo)*. 2017 March ; 70(3): 292–296. doi:10.1038/ja.2016.149.

## Studies at the Ionizable Position of Cephalosporins and Penicillins: Hydroxamates as Substitutes for the Traditional Carboxylate Group

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### Abstract

Classically,  $\beta$ -lactams need an ionizable group to potentiate antibacterial activity. Sets of cephalosporins and penicillins featuring different substituted hydroxamates in place of the traditional carboxylate group have been synthesized and tested for antibiotic activity. Many of the compounds exhibited anti-bacterial activities with notable MIC values in the range of 6–0.2  $\mu$ M.

### Keywords

Cephalosporin; Penicillin; Ionizable Group; Antibiotic resistance

### Introduction

Among the various classes of antibiotics, the  $\beta$ -lactams continue to stand out as a hallmark of human accomplishment. This broad antibiotic group has saved countless numbers of lives and still makes up a majority of the antibiotic market today. The penicillins and cephalosporins represent the first two  $\beta$ -lactam scaffolds used clinically. These cores require an ionizable group to potentiate activity. For bicyclic  $\beta$ -lactams (e.g. penams, carbapenams, cephalosporins), the ionizable group is a carboxylate, while the monocyclic  $\beta$ -lactams (monobactams) typically utilize a sulfonate ( $-\text{SO}_3$ ) on the  $\beta$ -lactam ring nitrogen (Figure 1).<sup>1,2</sup> Herein we report the incorporation of hydroxamates ( $\text{pK}_a$  9–10)<sup>3</sup> or a hydroxamic acid ( $\text{pK}_a$  ~6–7)<sup>3</sup> at the ionizable position of representative penicillins and cephalosporins to determine whether they could act as alternate ionizable groups and promote anti-bacterial activity.

Over the last 80 years, a substantial number of person years have gone into modifying the peripheral functionality around the  $\beta$ -lactam core. These modifications often involved variations of the penicillin C-6 and cephalosporin C-7 acylamino sidechains and especially

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Supporting Information.

Synthetic procedures, compound characterization data, and assay procedures are available online. This material is available free of charge via the Internet.

functionality at the C-3' position of cephalosporins (see Figure 1 for numbering). However, few reports have focused on modifying the ionizable position of penicillins and cephalosporins. Reports of penicillins and cephalosporins bearing hydroxamate or hydroxamic acid functionalities are very scarce in the literature. Brown reported the syntheses of penicillin G and ampicillin derivatives bearing amide and hydroxamic acid functionality in place of the traditional carboxylate, with several of the compounds exhibiting notable activity against Gram-positive bacteria.<sup>4</sup> Further, Godfrey reported on the syntheses of a library of penicillin hydroxamates.<sup>5</sup> Some of these reported compounds are illustrated in Figure 2. However, only select activities of these compounds were reported, with no broad antibacterial assessments and comparisons included.

Related variations of cephalosporins included a library of 7-phenylacetyl and 7-thiophene-2-acetyl amino cephalosporins bearing various carboxamides patented by Lewis.<sup>6</sup> A number of these compounds, shown in Figure 3, were quite active, especially against *S. aureus* and *B. cereus*, exhibiting MIC values in the range of 6.25 to 0.003 µg/mL. However, again this report did not provide broad anti-bacterial screenings and comparisons to classic penicillin and cephalosporin scaffolds. With these reports in mind, we were interested in expanding upon the studies of β-lactams bearing different hydroxamate functionality at the ionizable position. Additionally, broad biological assessments and structure activity relationships among the different series are also presented, including consequences of removal of the acidic hydroxamate proton by alkylation.

## Results and Discussion

Our initial syntheses of cephalosporins are illustrated in Scheme 1. Commercially available 7-ACA was first acylated with phenylacetyl chloride to give **1** which was then coupled to both benzyl and *O*-TBS hydroxylamine to give **2a-b** in good yields. Since removal of the benzyl group would likely be challenging in the presence of the divalent sulfur,<sup>7</sup> deprotection of intermediate **2a** to give **3** was not studied. Rather, intermediate **2b** was deprotected with TBAF/AcOH to give **3** in 42% yield.

Efforts then focused on the syntheses of cephalosporins that featured the more complex 2-(2-amino-4-thiazolyl)-2(*Z*)-(methoxyimino)acetamido (ATMO)<sup>8</sup> side chain that is often associated with a broader spectrum of antibiotic activity.<sup>8</sup> Thus, cefotaxime sodium was coupled to both *O*-benzylhydroxylamine hydrochloride (OBHA•HCl) and *O*-allylhydroxylamine hydrochloride (OAHA•HCl) to give cephalosporins **4a-b** (Scheme 2).

Attention then shifted to the syntheses of cephalosporins in which the hydroxamate proton was replaced with a methyl group. Syntheses of the necessary *N*-methyl-*O*-alkyl hydroxylamines<sup>9-10</sup> and their coupling to cephalosporins are illustrated in Schemes 3 and 4. Starting with either OBHA•HCl or OAHA•HCl (**5a-b**), reaction with Boc anhydride, followed by reaction with NaH, then methyl iodide gave intermediates **6a-b** in good yields. Removal of the Boc group was accomplished with concentrated HCl to give desired salts **7a-b** that were then neutralized and coupled to both cefotaxime sodium and cephalosporin **1** to give compounds **8-10**.

Penicillin carboxylates were also converted to the corresponding hydroxamates. Attempted EDC-mediated coupling of *O*-*tert*-butyl(dimethylsilyl)hydroxylamine to cloxicillin sodium under organic conditions (Scheme 5) produced the desired product (**11**) in low yield with the major product being the rearranged *N*-acyl urea (**12**), produced during carbodiimide activation of the carboxyl group. We hypothesized that the combination of both a sterically bulky hydroxylamine and steric hindrance from the C-2 methyl groups of cloxicillin allowed the active *O*-acylisourea intermediate to rearrange faster than it was attacked by the hydroxylamine. A less sterically bulky hydroxylamine, *O*-allylhydroxylamine hydrochloride (OAHA•HCl), was employed and the coupling reaction was run under aqueous buffered conditions. As shown in Scheme 6, this change worked well and penams **13–14** were obtained in improved yield (Scheme 6). Next, penicillins in which the free hydroxamate proton was replaced with a methyl group were made (Scheme 7). Thus, *N*-Methyl-*O*-allyl hydroxylamine hydrochloride (**7a**, from Scheme 3) was coupled to both cloxicillin and carbenicillin sodium to give *N*-methylated hydroxamates **15** and **16**.

All cephalosporins were tested for antibacterial activity using the agar diffusion assay, with minimum inhibitory concentrations (MICs) determined for select compounds.<sup>11–12</sup> As shown in Table 1, many compounds exhibited notable activity against Gram-positive bacteria, albeit less than their parent counterparts (i.e. featuring free carboxylate). Highlighted among the compounds was the activity of cephalosporin **2b**, with MIC values of 0.2  $\mu$ M, 3  $\mu$ M, and 3  $\mu$ M against *B. subtilis*, *S. aureus*, and *M. luteus*, respectively. Further, compound **3**, featuring a free hydroxamic acid exhibited good activity, with MIC values of 0.4  $\mu$ M and 1.6  $\mu$ M against *B. subtilis* and *S. aureus*, respectively. Although most of the compounds were not very active against Gram-negative bacteria, cephalosporin **4a**, featuring the ATMO sidechain, showed good activity against *Pseudomonas aeruginosa* (K799/61) and both strains of *Escherichia coli* tested. With this impressive display of broad spectrum activity, MIC values were calculated for **4a** and are listed in Table 1. When analyzing how *N*-substitution affected activity, cephalosporins bearing *N*-methyl substituted hydroxamates exhibited, in general, a decrease in both potency and/or broadness of antibacterial activity. This is illustrated by comparing the activities of **4b** with **9** and **2a** and **10**.

Similarly, penicillins were tested via diffusion assays, with MIC values determined for select compounds (Table 2). Hydroxamate containing penicillins were quite active against Gram-positive microbes as well, although generally not as active as the parent compounds. Notably, compounds **11** and **13** demonstrated significant activity against *S. aureus*, with MIC values of 0.4  $\mu$ M and 1.6  $\mu$ M, respectively. Interestingly, the rearranged urea, **12**, had good activity against Gram-positive rods and activity against *E. coli* DC2 with a zone of inhibition of 26 mm. Removal of the acidic proton by *N*-methylation only slightly affected the activity of cloxicillin derivatives (compounds **13** and **15**) but had a substantial effect on the carbenicillins, as replacing both carboxylates with hydroxamates gave a compound (i.e. *N*-methyl hydroxamate **16**) that was completely inactive.

As shown in Table 3, select compounds were also screened against three *S. aureus* strains (SG511, ISR-001 and ISR-002). All compounds tested showed notable zones of inhibition, ranging from 22–32 mm. With these encouraging results, MIC values were also determined. Of special interest were the values for compounds **11** and **13**, with MICs as low as 0.4  $\mu$ M

for both compounds. Lastly, carbenicillin analogue **14** exhibited MICs in the range of 1.6–6  $\mu$ M against all three strains while the parent compound was seen to be inactive against strains ISR-001 and ISR-002).

To close, we have synthesized a focused set of cephalosporins and penicillins with hydroxamate replacement of the classical carboxyl groups. Preliminary biological assessments indicated that several of the hydroxamates retained broad spectrum activity against Gram-positive (including select MRSA strains) and Gram-negative pathogens. Further, removal of the acidic proton of the hydroxamate portion diminished both level of and the spectrum of activity. Thus, further functionalization of the ionizable position of these important antibiotics might be of value in development of much needed alternative forms of antibiotics.

## Supplementary Material

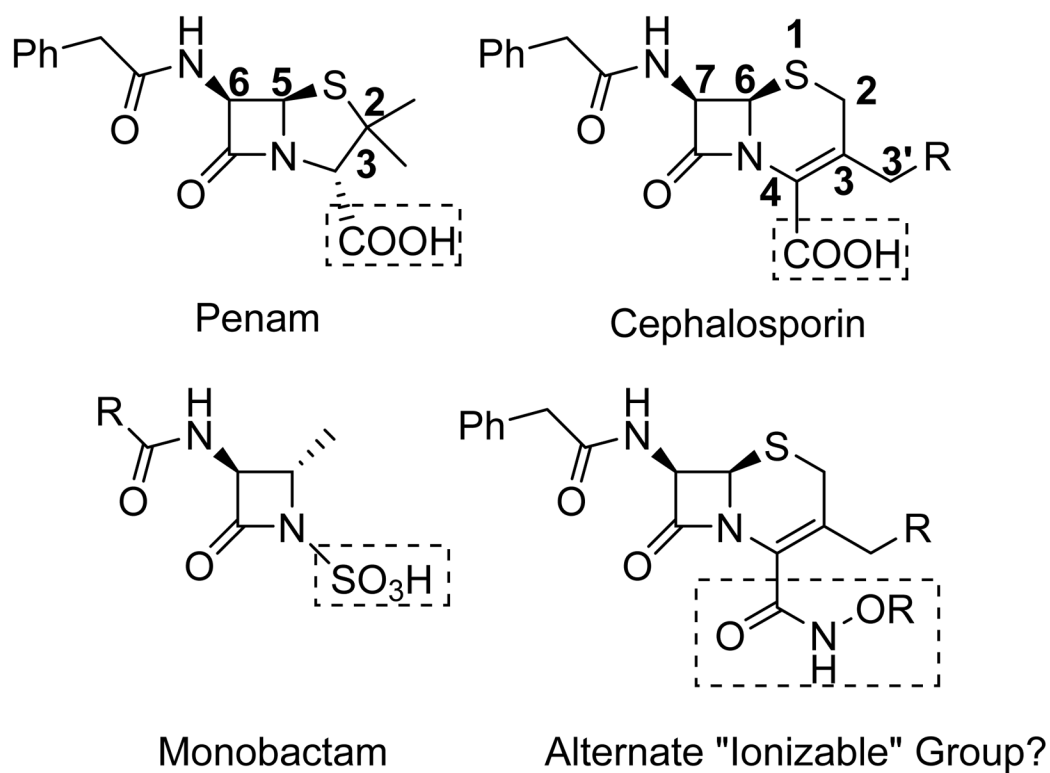
Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We acknowledge the University of Notre Dame, and partial funding from the NIH (NIH-2R01-AI054193-05A2) for support of this work. We acknowledge Nonka Sevova (Mass Spectrometry and Proteomics Facility, UND) for mass spectroscopic analyses and Viktor Krchnak (UND) for LC/MS analysis. M.W.M acknowledges an ECK Global Health Fellowship (2014-2015, UND).

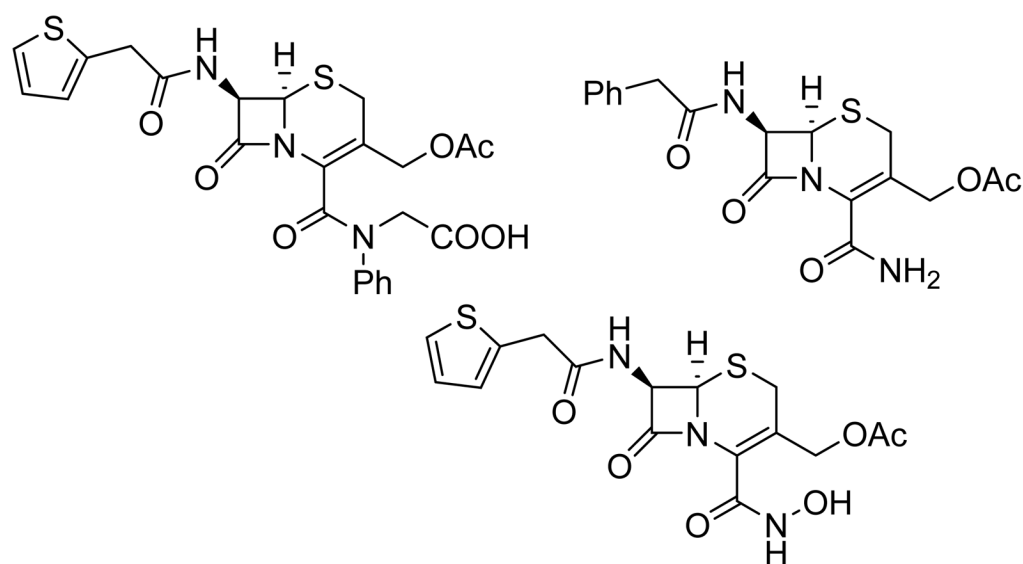
## References

1. Page MI, Jaws AP. The chemical reactivity of  $\beta$ -lactams,  $\beta$ -sultams and  $\beta$ -phospholactams. *Tetrahedron*. 2000; 56:5631–5638.
2. Varetto L, Frere JM, Ghuysen JM. The importance of the negative charge of  $\beta$ -lactam compounds for the inactivation of the active-site serine DD-peptidase of *Streptomyces* R61. *FEBS*. 1987; 225:218–222.
3. Miller MJ, DeBons FE, Loudon GM. The chemistry of a method for the determination of carboxyl-terminal residues in peptides. *J Org Chem*. 1977; 42:1750–1762. [PubMed: 853324]
4. Brown LD, Zygmunt WA, Stavely HE. Active derivatives of penicillin. *App Microbiol*. 1969; 17:339–343.
5. Godfrey, JC. Bristol-Myers Co. *N*-Hydroxypenicillinamides. US. 3301811/19670131..
6. Lewis, BA., Sassiver, LM., Shepherd, RG. American Cyanamid Company. 7- (Phenylacetyl amino) Cephalosporin Carboxamides and 7-(Thiophene-2-Acetyl amino) Cephalosporin Carboxamides. US. 3459747A/19690805..
7. Desikan P, Amberg CH. Catalytic hydrodesulfurization of thiophene. V. Hydrothiophenes. Selective poisoning and acidity of the catalyst surface. *Can J Chem*. 1964; 42:843–850.
8. Woulfe SR, Miller MJ. Synthesis and biological activity of substituted [[3(*S*)- (acylamino)-2-oxo-1-azetidiny] oxy]acetic acids. A new class of heteroatom-activated  $\beta$ -lactam antibiotics. *J Med Chem*. 1985; 28:1447–1453. [PubMed: 4045920]
9. Ramasamy K, Olsen RK, Emery T. *N*-Methylation of *O*-benzyl- $\alpha$ -*N*-(alkoxycarbonyl)- $\alpha$ -amino acid hydroxamate derivatives. *J Org Chem*. 1981; 46:5438–5441.
10. Sharma SK, Miller MJ, Payne SM. Spermaxin and spermaxol: new synthetic spermidine-based siderophore analogues. *J Med Chem*. 1989; 32:357–367. [PubMed: 2521517]
11. Afonin S, et al. 4-fluorophenylglycine as a label for  $^{19}\text{F}$  NMR structure analysis of membrane-associated peptides. *Chem Bio Chem*. 2003; 4:1151–1163.
12. Murray, PR., Baron, EJ., Pfaller, MA., Tenover, FC., Tenover, RH. *Manual of Clinical Microbiology*. American Society for Microbiology; Washington, DC: 1999.

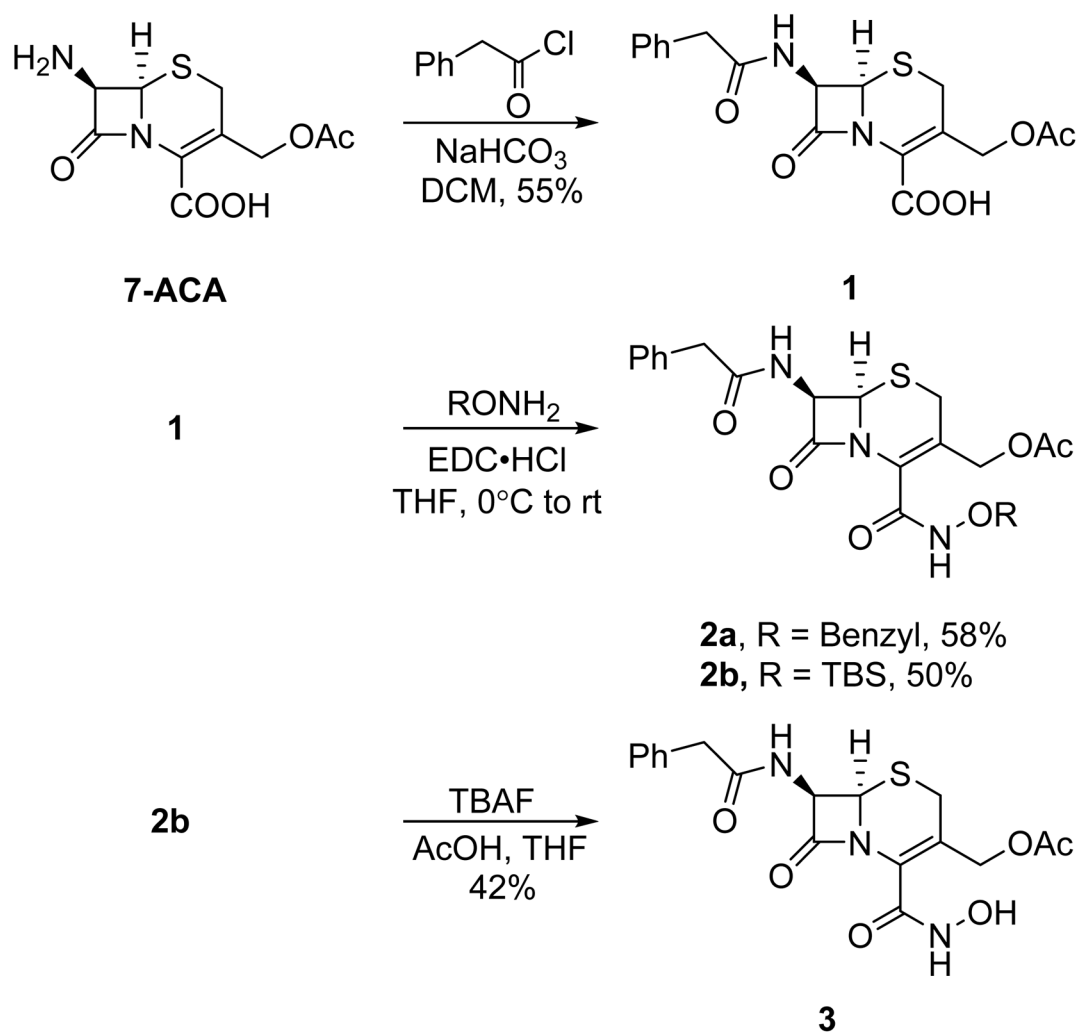


**Figure 1.**  
Classic  $\beta$ -Lactam Scaffolds and Proposed Hydroxamate Alternative



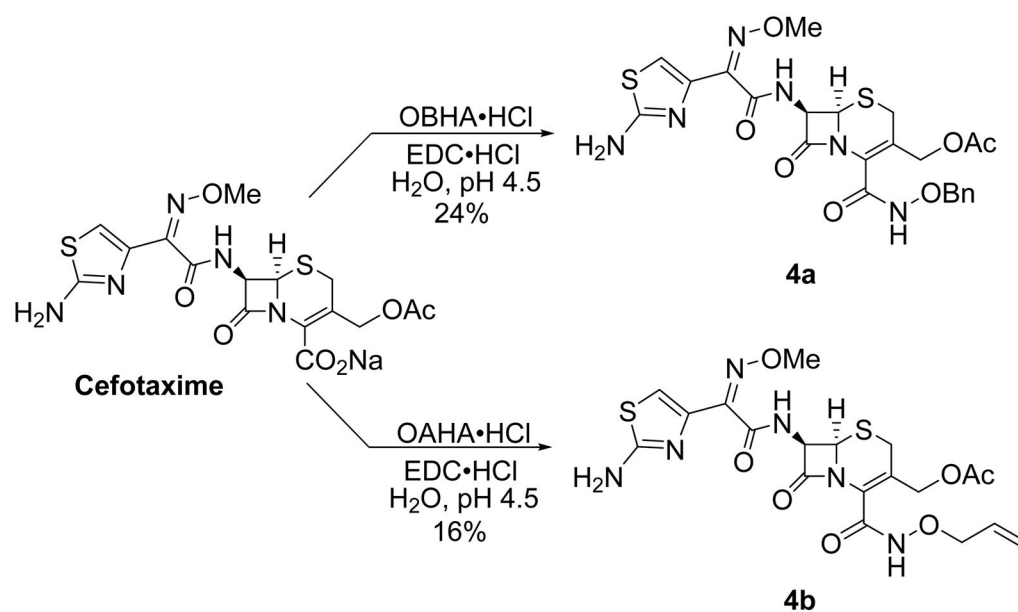


**Figure 3.**  
Sample Cephalosporins Previous Reported



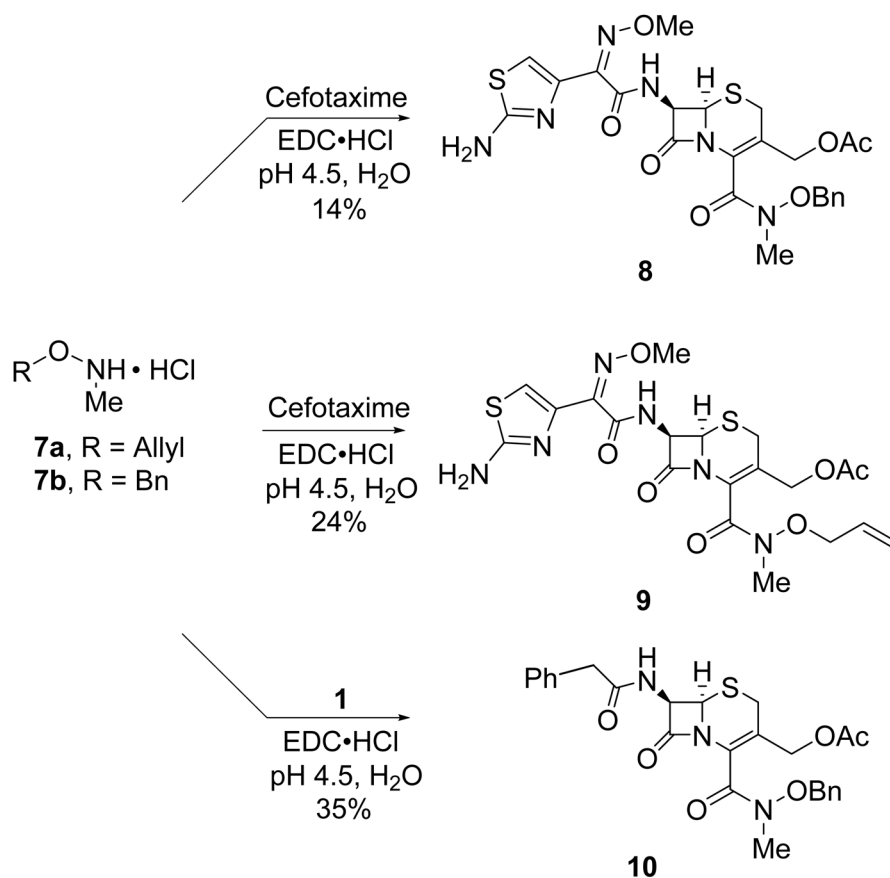
**Scheme 1.**  
Syntheses of Hydroxamate Cephalosporins 2–3



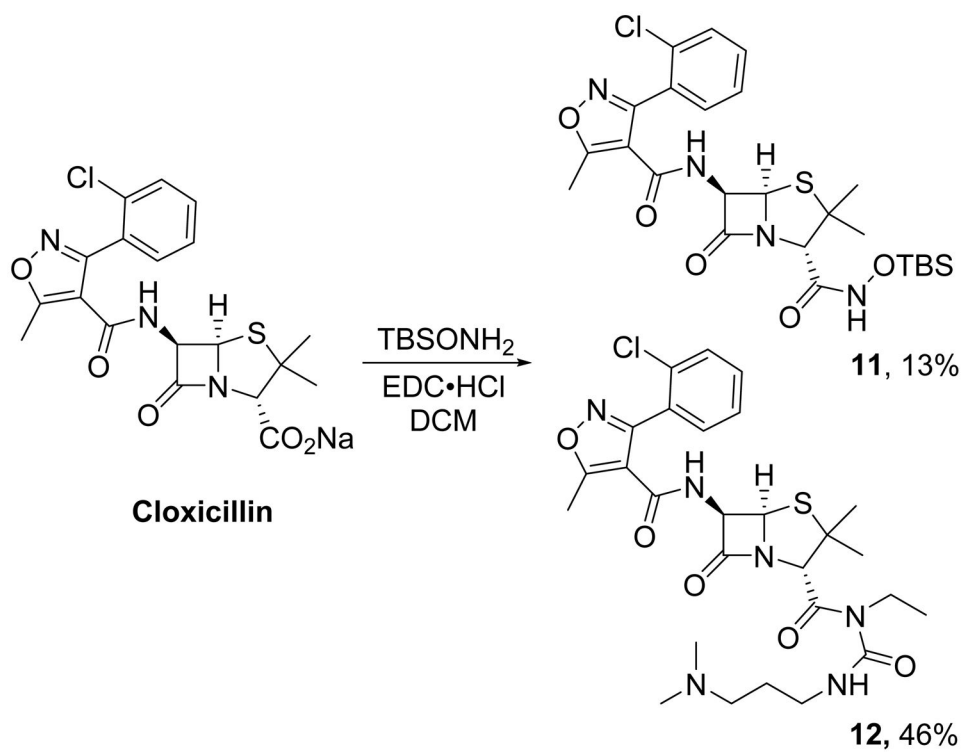
**Scheme 2.**

Syntheses of Cephalosporins with ATMO Side-Chain

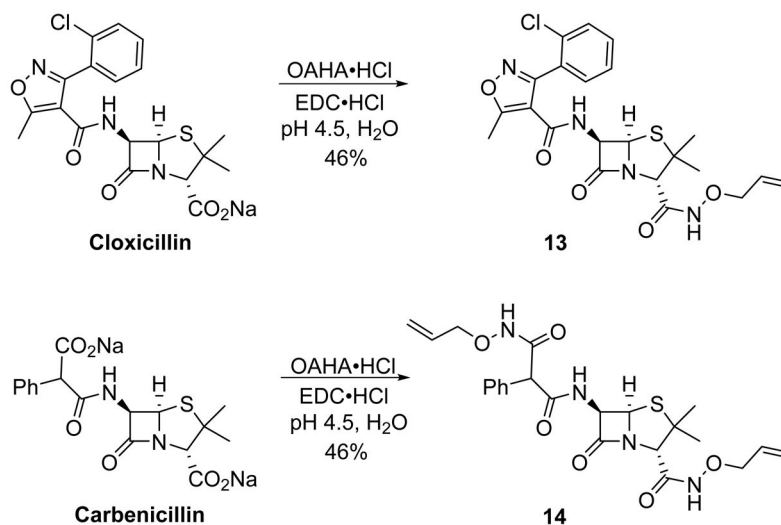




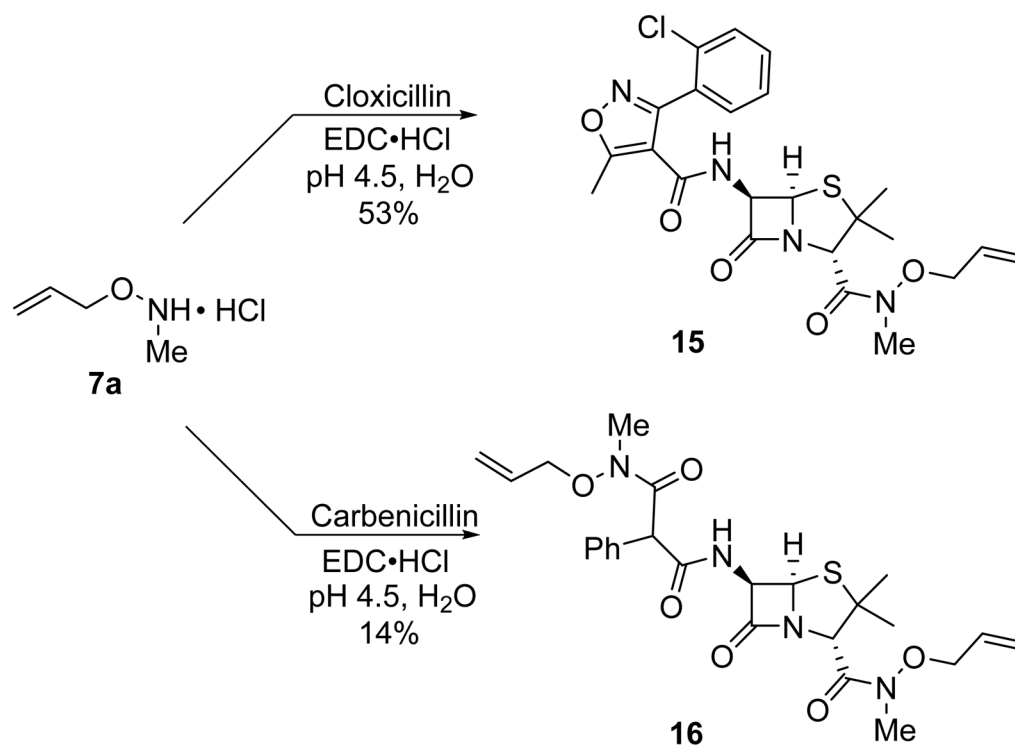
**Scheme 4.**  
Syntheses of N-Methyl Hydroxamate Cephalosporins 8–10



**Scheme 5.**  
Initial Coupling Attempt with Cloxicillin

**Scheme 6.**

Improved Coupling of Penams under Acidic Aqueous Conditions



**Scheme 7.**  
Coupling of 7a to Penams

## Spectrum of Activity for Cephalosporins

Table 1

Entry	Cmpd.	Zones of Growth Inhibition (mm) and MIC Values (µM, in bold)						
		<i>Bacillus subtilis</i> ATCC 6633	<i>Staphylococcus aureus</i> SG511	<i>Micrococcus luteus</i> ATCC 10240	<i>Pseudomonas aeruginosa</i> K799/wt	K799/61	DC0	<i>Escherichia coli</i> DC2
1	1 (Parent Cmpd.)	41 <b>&lt;0.1</b>	36 <b>0.2</b>	35 <b>3.13</b>	0 <b>200</b>	20 <b>50</b>	12 <b>100</b>	25
2	2a	30 <b>3.0</b>	26 <b>3.0</b>	14 <b>12.5</b>	0	0	0	0
3	2b	20 <b>0.2</b>	32 <b>3.0</b>	28 <b>3.0</b>	0	0	0	0
4	3	31 <b>0.4</b>	31 <b>1.6</b>	27 <b>6.25</b>	21	17	0	0
5	10	35 <b>1.6</b>	14 <b>50</b>	0	0	0	0	0
6	Cefotaxime (Parent Cmpd.)	36 <b>&lt;0.1</b>	35 <b>0.78</b>	51 <b>0.78</b>	36 <b>6</b>	51 <b>&lt;0.1-0.2</b>	31 <b>0.1</b>	<b>&lt;0.1</b>
7	4a	12 <b>6.25-1.6</b>	21 <b>25</b>	18 <b>25</b>	18 <b>200</b>	27 <b>6.0</b>	20 <b>6.0</b>	31 <b>1.6</b>
8	4b	17 <b>3-6</b>	24 <b>12.5</b>	14	0	15	0	22
9	8	17	11	0	0	18 <b>25</b>	14	0
10	9	0	0	0	0	22	0	0
11	10	35 <b>1.6</b>	14 <b>50</b>	0	0	20	0	0
12	Ciprofloxacin	21	21	0	18	20	nt	21

For diffusion assays, all zones represent 100% inhibition. All wells are not subtracted from reported zones. Ciprofloxacin tested at concentration of 1.66  $\mu$ g/mL. nt = not tested. DC2 and K799/61 are antibiotic hypersusceptible strains

Table 2

## Spectrum of Activity for Penicillins

Entry	Cmpd.	Zones of Growth Inhibition (mm) and MIC Values (μM, in bold)						
		<i>Bacillus subtilis</i> ATCC 6633	<i>Staphylococcus aureus</i> SG511	<i>Micrococcus luteus</i> ATCC 10240	<i>Pseudomonas aeruginosa</i> K799/wt	<i>Pseudomonas aeruginosa</i> K799/61	<i>Escherichia coli</i> DC0	<i>Escherichia coli</i> DC2
1	Cloxacillin (Parent Cmpd.)	42 0.1–0.2	40 0.2	41 6.25	nt	nt	nt	nt
2	11	nt	30 0.4	16	0	16	0	0
3	12	35 0.8	32 0.8	25 12.5	0	0	0	26 25
4	13	16	31 1.6	17	0	0	0	0
5	15	28 1.6	26 1.6	17	0	0	0	0
6	Carbenicillin (Parent Cmpd.)	0.1	0.8	0.4	nt	nt	nt	nt
7	14	24	31 1.6	19 25	0	0	0	0
8	16	0	0	0	0	0	0	0
9	Ciprofloxacin	21	21	0	18	20	nt	21

For diffusion assays, all zones represent 100% inhibition. All wells are not subtracted from reported zones. Ciprofloxacin tested at concentration of 1.66 μg/mL, nt = not tested. DC2 and K779/61 are antibiotic hypersusceptible strains



**Table 3**Spectrum of Activity for Select Compounds Against select *S. aureus* Strains

Entry	Cmpd.	Zones of Growth Inhibition (mm) and MIC ( $\mu$ M, in bold)		
		SG 511	ISR-001	ISR-002
1	1	0.2	6	6
2	2a	1.6–3	28 6.0	22 6.0
3	2b	3	28 12.5	24 12.5
4	3	1.6	25 25	23 25
5	Cloxacillin (Parent Cmpd.)	0.2	< 0.1	< 0.1
6	11	0.4–0.8	29 0.4	29 0.4–1.6
7	13	1.6	27 0.4	28 0.4–1.6
8	Carbenicillin (Parent Cmpd.)	0.8	100	100
9	14	1.6–3	28 3–6	32 6–12.5
10	Methicillin	3	6.25	6.25–25
11	Ampicillin	0.4	25	50
12	Cefaclor	6	50	50
13	Ciprofloxacin	0.2	0.471	>3.8

For diffusion assays, all zones represent 100% inhibition. All wells are not subtracted from reported zones. Ciprofloxacin tested at a concentration of 1.66  $\mu$ g/mL.