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SAR and Structural Analysis of Siderophore-Conjugated Monocarbam Inhibitors of *Pseudomonas aeruginosa* PBP3

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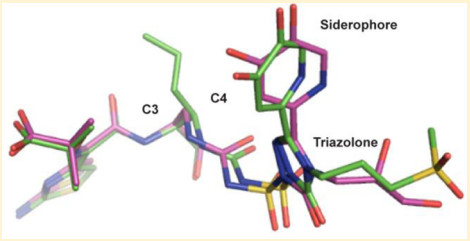
Supporting Information

ABSTRACT: A main challenge in the development of new agents for the treatment of *Pseudomonas aeruginosa* infections is the identification of chemotypes that efficiently penetrate the cell envelope and are not susceptible to established resistance mechanisms. Siderophore-conjugated monocarbams are attractive because of their ability to hijack the bacteria's iron uptake machinery for transport into the periplasm and their inherent stability to metallo-β-lactamases. Through development of the SAR we identified a number of modifications to the scaffold that afforded active anti-*P. aeruginosa* agents with good physicochemical properties. Through crystallographic efforts we gained a better understanding into how these compounds bind to the target penicillin binding protein PBP3 and factors to consider for future design.



KEYWORDS: *Pseudomonas aeruginosa, β-lactam, penicillin binding protein, siderophore, monocarbam, structure-guided design*

One of the largest challenges in the discovery of new Gram-negative antibacterial agents is the identification of chemotypes that can penetrate the cell envelope.[1] The pathogens, such as *Pseudomonas aeruginosa* (*P. aeruginosa*), have evolved complex cell architectures including an outer membrane composed of charged lipopolysaccharides, a thin peptidogylcan layer, and an inner membrane made up of phospholipids.[2] Identifying molecules with the necessary features to enable permeation has been a challenge.

β-Lactams are one chemotype that have been successful in the treatment of *P. aeruginosa*.[3] Since the discovery of penicillin, β-lactams have been used in the clinic and are still widely prescribed. One of the most effective classes of β-lactams for the treatment of *P. aeruginosa* infections are the carbapenems (e.g., meropenem, **1**, Figure 1).[4] Their effectiveness stems from their ability to acylate their penicillin-binding protein (PBP) targets, aided by high permeability into *P. aeruginosa* through the outer membrane porins.[5] However, they are currently being challenged in the clinic by the increasing emergence of broad-spectrum serine β-lactamases and metallo-β-lactamases which hydrolyze most β-lactams and render them ineffective.[6] There is one class of β-lactams that is stable to the metallo-β-lactamases: the monocyclic β-lactams represented by aztreonam (**2**). Aztreonam however has limited effectiveness against *P. aeruginosa* presumably due to its poor permeation of the outer membrane, β-lactamase susceptibility, and high propensity for efflux (*P. aeruginosa* $MIC_{90} \geq 1024$ μg/mL, $n = 20$ panel).[3,7]

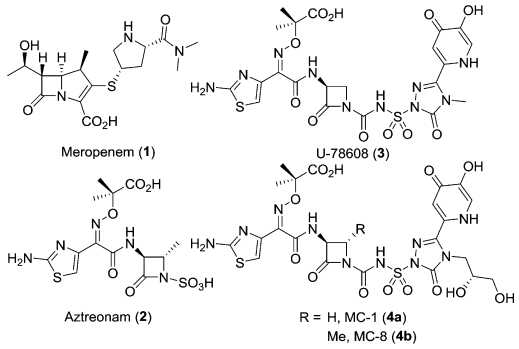


Figure 1. Gram-negative active β-lactams.

To address the poor activity of **2**, we focused on improving permeation by introducing siderophore mimics to promote uptake.[8−10] Siderophores are small Fe-chelating molecules synthesized and secreted by bacteria to scavenge iron from the host.[9,11] The iron-complexed siderophores are then brought into the cell via the iron uptake machinery, enabling the bacteria to access the much needed nutrient. It has been shown that introduction of iron-chelating groups to a monocyclic β-lactam scaffold can produce highly potent *P. aeruginosa* agents that are stable to metallo-β-lactamases.[7,12−16]

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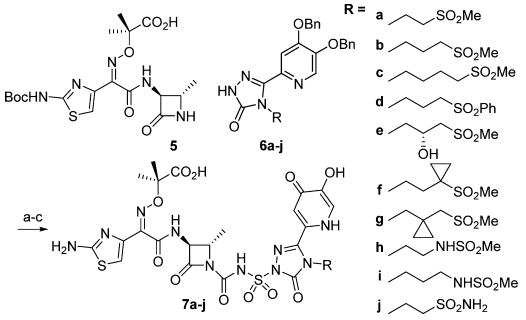
Known examples of monocyclic β-lactams incorporating iron-chelating groups are monocarbams U-78608 (**3**, Figure 1),[16] MC-1 (**4a**), and MC-8 (**4b**).[14] These compounds have been shown to be potent inhibitors of *P. aeruginosa* PBP3 and resistant to hydrolysis by β-lactamases.[17] Some limitations to this class have been shown to be poor hydrolytic stability and high human plasma protein binding (PPB).[14] Here we present our efforts to further develop the SAR of the monocarbams, exploring previously unexplored areas of the scaffold. Through crystallography efforts we were able to offer hypotheses to explain our data.

Initial studies focused on varying the side chain of the triazolone, attempting to introduce polar functionalities that could enable additional interactions with the target PBP and improve the activity. These analogs were synthesized utilizing a known three-step coupling procedure[14] (Scheme 1) from β-lactam **5**[14,18] and a substituted triazolone (**6a–j**). A subsequent two-step deprotection provided the target molecules.

Scheme 1. Synthesis of Side Chain Analogs[a]



[a](a) (i) **6a–j**, MSTFA, THF; (ii) **5**, CSI, DCM, 0 °C; (iii) THF, 0 °C to rt, 38–78%; (b) Pd black, H_2, EtOH, 28–77%; (c) TFA, DCM, 0 °C, 29–90%.

As can be seen in Table 1, all the analogs (**7a–j**) demonstrated excellent *P. aeruginosa* MICs and PBP3 acylation rate constants equivalent to **2**. These examples also demonstrated good hydrolytic stability; however, the free fraction did not increase, with observed values between 3% and 9% free.

Next, modifications to the iron-chelating group (siderophore) were explored to determine if there was any effect on activity or physicochemical properties. The syntheses of 1,3-dihydroxypyridin-4-one monocarbam derivatives have never been reported in the literature. However, the catechol-isostere has been shown to be optimal for other classes of monocyclic β-lactams.[15,19] Synthesis of these analogs was achieved via peroxide oxidation of the protected 3-hydroxypyrid-4-one to provide the *N*-oxides **8a** and **8b**. The addition of acid was required for the oxidation because of the poor solubility of the triazolone intermediate. Subsequent coupling of the triazolones to the core **5** and deprotection (Scheme 2) provided the target molecules.

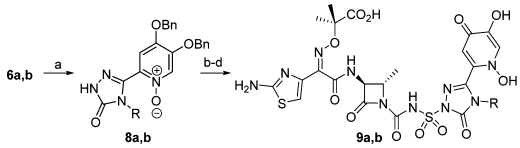
As can be seen in Table 1 for both **9a** and **9b**, no change was observed in target affinity as compared to the des-hydroxy analogs **7a** and **7b**. Whereas with the 3-hydroxypyridin-4-one analogs, the MICs were equivalent for the different triazolone substitutions, there was differing activity for the analogs with the 1,3-dihydroxypyridin-4-one. Both **9a** and **9b** afforded elevated MICs, with **9b** showing a 4-fold increase. One highlight of these compounds was a 3-fold improvement in free fraction for **9a**, unprecedented for the triazolone class of compounds.

Table 1. *P. aeruginosa* Antibacterial Activity and Physicochemical Properties of Siderophore-Conjugated Monocarbam Analogs

compd	PAO1 MIC[a] (μg/mL)	PBP3[c] k_{on} (M^{-1} s^{-1})	human PPB[d,g] (% free)	hydrolytic stability[e,g] $t_{1/2}$ (h)
1	1	4.9×10^4	NT	95 ± 2.6
2	4	2.6×10^5	62 ± 8.8	>150[f]
3	0.78[b]	1.7×10^5	5 ± 0.1	NT
4a	0.78[b]	8.1×10^4	6 ± 0.5	NT
4b	0.25	7.2×10^4	4 ± 0.9	117 ± 17
7a	0.13	3.2×10^5	6 ± 0.2	86 ± 13
7b	0.25	1.7×10^5	9 ± 2.2	93 ± 12
7c	0.25	6.2×10^5	7 ± 0.5	100 ± 8.5
7d	1	$>4.0 \times 10^5$	3 ± 0.04	>150[f]
7e	1	5.4×10^5	4 ± 0.1	87 ± 5.7
7f	0.5	4.5×10^5	4 ± 0.3	94 ± 8.4
7g	0.5	2.5×10^5	9 ± 0.2	71 ± 23
7h	0.5	4.1×10^5	8 ± 0.1	61 ± 9.1
7i	0.5	3.0×10^5	8 ± 0.2	NT
7j	<0.6	$>4.0 \times 10^5$	NT	102 ± 7.1
9a	1	2.5×10^5	20 ± 9.3	102 ± 7.1
9b	4	1.5×10^5	12 ± 1.0	82 ± 7.1
10a	0.25	5.0×10^5	27 ± 8.7	85 ± 4.7
10b	1	3.2×10^5	6 ± 1.5	118 ± 23

[a]PAO1 = *P. aeruginosa* ARC545. [b]MICs reported in μM. [c]*P. aeruginosa* PBP3. [d]n = 3, standard deviation. [e]pH = 7.4, 37 °C. [f]Not determined. [g]NT = not tested.

Scheme 2. Synthesis of 1,3-Dihydroxypyridin-4-one Derivatives[a]



[a](a) **6a**: *m*-CPBA, TFA, DCM, 69%. Or **6b**: 50% H_2O_2, AcOH/THF 1:1, 60 °C, 34%. (b) (i) MSTFA, THF; (ii) CSI, DCM, 0 °C; (iii) THF, 0 °C to rt, 24% **8a**, 48% **8b**; (c) Pd black, H_2, EtOH, 58% **8a**, 46% **8b**; (d) TFA, DCM 0 °C, 54% **9a**, 38% **9b**.

It has been established that for the monocyclic β-lactam and cephalosporin classes, the aminothiazole provides optimal interactions with the *P. aeruginosa* PBP3 enzyme; however there are known changes that are tolerated.[20−22] Using known methods, we synthesized propylsulfone thiadiazole **10a** and chlorothiazole **10b** (Figure 2) to investigate if there would be any changes in the physicochemical properties.[23] The most dramatic change observed was for thiadiazole **10a** (Table 1). The *P. aeruginosa* MIC was similar to the parent **7b**; however, the acylation rate was increased 2-fold presumably due to

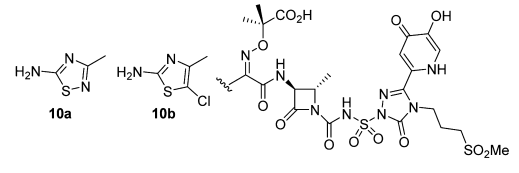


Figure 2. Modified thiazole siderophore-conjugated monocarbams.

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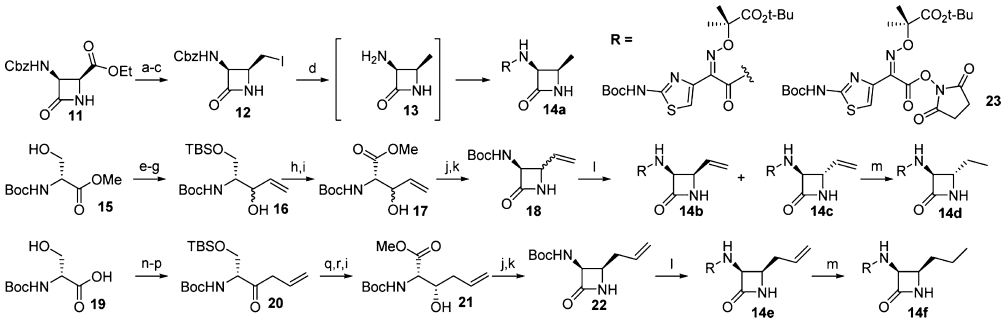
Scheme 3. Synthesis of 4-Substituted β-Lactam Cores[a]



[a](a) $NaBH_4$, MeOH, 0 °C, 90%; (b) MsCl, pyridine, DCM, 0 °C to rt, 71%; (c) NaI, acetone, reflux, >95%; (d) (i) H_2, 10% Pd/C, MeOH; NaOAc; (ii) **23**, MeOH, rt, 50%; (e) TBSCl, imid, DMF, quant; (f) DIBAL-H, toluene, −78 °C, 88%; (g) $CH_2CHMgBr$, DCM, −78 °C, 34%; (h) 2 N HCl, MeOH, 43%; (i) (i) 20 mol % TEMPO, $NaHCO_3$, NaClO; (ii) MeI, K_2CO_3, DMF, 0 °C, 57% **17**, 59% **21**; (j) (i) NH_2OH·HCl, KOH, MeOH, 0 °C; (ii) Ac_2O, 0 °C, 56% **18**, 28% **22**; (k) (i) DIAD, PPh_3, THF; (ii) $NaHCO_3$, MeOH/H_2O, 0 °C, 60% **18**, 30% **22**; (l) (i) TFA, DCM, 0 °C to rt ; (ii) **23**, 4-methylmorpholine, EtOH, toluene, 13% **14b**, 52% **14c**, 36% **14e**; (m) 10% Pd/C, H_2 (1 atm); EtOH, quant **14d**, quant **14f**; (n) CH_3NHOMe·HCl, EDC·HCl, 4-methylmorpholine, DCM, −15 °C, 76%; (o) TBSCl, imid, DMF, 0 °C, 76%; (p) CH_2CHCH_2MgBr, THF, −78 °C, quant; (q) $NaBH_4$, EtOH, −78 °C, 49%.; (r) TBAF, THF, 78%.

improved interactions of the thiadiazole with the PBP3 enzyme as compared to the thiazole. The free fraction increased 3-fold to 27% free, a 5-fold improvement in PPB as compared to the reference monocarbams **3**, **4a**, and **4b**, highlighting that small changes can significantly affect physicochemical properties.

Introduction of a methyl substituent to the 4-position of the β-lactam core has been shown to increase the hydrolytic stability of the monocarbams.[14,24] However, besides the *trans*-methyl **5**, no other substitutions have been investigated in the monocarbam series. Wanting to develop this SAR further, a number of substituted β-lactam cores were targeted. The synthesis of the β-lactam cores is depicted in Scheme 3.

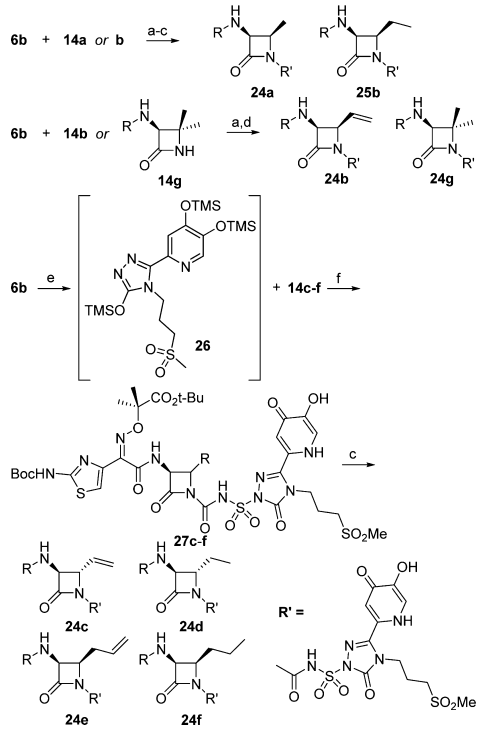
Synthesis of the *cis*-analogue (3*S*,4*R*)-3-amino-4-methylazetidin-2-one **14a** began with known chiral ester **11**[25] which was converted to iodide **12** in three steps (Scheme 3). Pd-mediated reduction with simultaneous removal of the Cbz group afforded intermediate **13**, which was treated in situ with activated ester **23** to provide the substituted β-lactam core **14a**.

Synthesis of vinyl and ethyl substituted cores began with Boc-D-serine methyl ester (**15**). Silylation of the primary alcohol with TBSCl followed by DIBAL-H reduction of the ester and vinyl Grignard addition to the resultant aldehyde produced alcohol **16** as a ∼1:1 mixture of diastereomers. Alcohol **16** was then deprotected and oxidized to provide **17**. Following procedures developed by Miller and co-workers,[18] **17** was converted to the hydroxamate and cyclized under Mitsunobu conditions to provide β-lactam **18**. The diastereomeric mixture was subsequently deprotected and condensed with activated ester **23** to afford the coupled β-lactams **14b** and **14c** which were separable by silica gel chromatography. The vinyl β-lactams could be reduced under hydrogenation conditions to provide the ethyl substituted core as demonstrated with β-lactam **14d**.

The synthesis of allyl substituted β-lactam **14e** began with D-serine-OH (**19**). Introduction of the allyl was achieved through allyl Grignard addition to a Weinreb amide to provide ketone **20**. Alcohol **21** was synthesized as a 9:1 mixture of diastereomers (favoring the depicted isomer) in three steps. Cyclization of **21**, employing the previously described conditions, and subsequent coupling with activated ester **23** afforded β-lactam **14e**, which could be reduced to produce propyllactam **14f**.

Assembly of the monocarbams could be achieved using one of three methods depending on the β-lactam substitution (Scheme 4). *cis*-Methyl **14a** and *cis*-vinyl **14b** were coupled utilizing the already described methods to give **24a** and **25b** after a two-step deprotection sequence (Scheme 4). For vinyl analogue **24b**, after monocarbam formation, a global BCl_3 deprotection was employed to prevent reduction of the olefin. This method was also utilized to couple known β-lactam core **14g**.[26,27] Alternatively, to avoid exposure of the fully coupled

Scheme 4. Synthesis of Monocarbams with 4-Substituted β-Lactams[a]



[a](a) (i) **14a** or **14b**, MSTFA, THF; (ii) **6b**, CSI, DCM, 0 °C; (iii) THF, 0 °C − rt; (b) Pd black, H_2, EtOH; (c) TFA, DCM 0 °C; (d) BCl_3, DCM; (e) (i) Pd black, H_2 (1 atm), THF; (ii) MSTFA, THF; (f) (i) **5**, CSI, DCM, 0 °C; (ii) THF, 0 °C − rt.

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monocarbams to hydrogenation conditions, the benzyl groups on triazolone **6b** could be removed prior followed by in situ treatment with MSTFA to afford the trisilylated intermediate **26**. This material was not isolated but directly treated with the chlorosulfonamide intermediate of **14c−f** to afford moderate yields of debenzylated coupled material (**27c−f**). Subsequent TFA deprotection produced the final targets (**24c−f**).

The data for these analogs can be seen in Table 2. For the alkyl-substituted β-lactams the observed SAR indicated the

Table 2. Antibacterial Activity and Hydrolytic Stability of C4-Substituted β-Lactam Analogs

compd		PAO1[a] MIC (μg/mL)	PBP3[b] k_{on} (M^{-1} s^{-1})	hydrolytic stability[c] $t_{1/2}$ (h)
7b	*trans*-methyl	0.5	1.7×10^5	93 ± 12
24a	*cis*-methyl	1	1.6×10^4	76 ± 6.8
24b	*cis*-vinyl	2	1.9×10^4	38 ± 4.1
25b	*cis*-ethyl	1	8.8×10^3	94 ± 13
24c	*trans*-vinyl	0.5	2.1×10^5	53 ± 2.8
24d	*trans*-ethyl	0.5	3.6×10^5	98 ± 12
24e	*cis*-allyl	8	5.9×10^3	126 ± 25
24f	*cis*-propyl	4	7.4×10^3	>150[d]
24g	dimethyl	8	1.1×10^4	>150[d]

[a]PAO1 = *P. aeruginosa* ARC545. [b]*P. aeruginosa* PBP3. [c]pH = 7.4, 37 °C. [d]Not determined.

trans-β-lactams were favored. We observed higher acylation rate constants and better MICs for all the *trans*-alkyl substituted β-lactams ($k_{on} \geq 1.7 \times 10^5$ M^{-1} s^{-1}) as compared to the *cis* (compare **7b** to **24a**, **24c** to **24b**, **24d** to **25b**). In fact, as the *cis*-substituent became larger, the acylation rate decreased. For example, the acylation rate constants for the *cis*-allyl (**24e**) and *cis*-propyl (**24f**) derivatives were 200-fold lower. The lower affinity for the enzyme is reflected in higher *P. aeruginosa* MICs for these analogs.

The stereochemistry had no effect on the hydrolytic stability of these compounds. The data indicated the stability was driven by the size and activating ability of the substituent. The stereochemical matched pairs (**7b** to **24a**, **24c** to **24b**, **24d** to **25b**) had about equivalent stability. The least stable pair was the vinyl substituted cores possibly because the vinyl group provides additional activation of the β-lactam. As the steric congestion on the core increased, the hydrolytic half-life increased, as can be seen with *cis*-propyl **24f** and disubstituted core **24g** which did not exhibit any hydrolysis under the assay conditions. These stability trends were the same as what has been observed with monocyclic-β-lactams in relation to β-lactamase stability.[28]

To gain further insight into these SAR trends, we obtained the crystal structures of *P. aeruginosa* PBP3 with two *trans*-substituted cores, **7b** and **24c**, and the *cis*-substituted **24e** (Figure 3). All three compounds were covalently bound to residue Ser294 of PBP3. Expectedly, the binding modes of these compounds are similar (Figure 3). The aminothiazole containing left-hand side of each analog is oriented equivalently with the thiazole, oxyiminopropylcarboxylic acid, and amide substituents assuming the same orientation. As observed previously with other crystallographic structures of *P. aeruginosa* PBP3 in complex with aminothiazole containing β-lactams, the aminothiazole group forms multiple interactions with the protein.[13,29,30] In addition, the amide carbonyl of these compounds formed a hydrogen bond with residue Asn351,

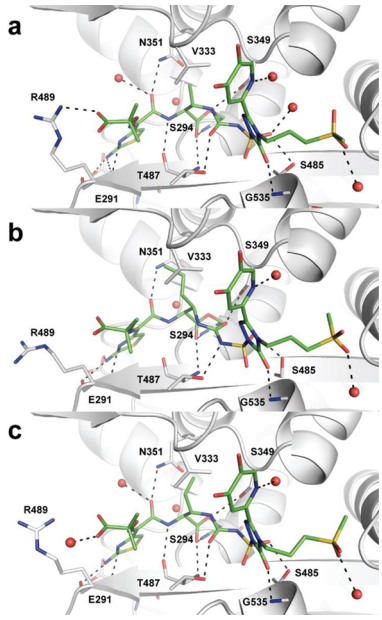

Figure 3. Binding site for liganded *P. aeruginosa* PBP3 complexes: (a) **7b**; (b) **24e**; (c) **24c**. Compounds are shown as stick models with green carbon atoms, blue nitrogen atoms, red oxygen atoms, and yellow sulfur atoms. All residues within ~3.2 Å of the covalently bound compound are shown. Water molecules that coordinate additional interactions are shown as red spheres. Hydrogen bonds are depicted as black dashes.

and an ordered water molecule. The right-hand sides of these compounds, consisting of the triazolone and siderophore moieties, also show a consistent binding mode. The triazolone carbonyl interacts with the backbone amide of residue Gly535. The propylmethylsulfone side chain is directed toward the solvent and forms limited interactions with the protein. Although the sulfone oxygens form a hydrogen bond with a conserved water molecule, no additional interactions are made with the protein. These limited interactions may explain the similar PBP3 acylation rate constants observed with analogs **7a−j**. The siderophore group is also oriented toward solvent and forms limited direct interactions with the PBP3 enzyme, which is further supported by the observation that that there is no difference in acylation rate constants between the 3-hydroxypyridin-4-ones (**7a** and **7b**) and 1,3-dihydroxypyridin-4-ones (**9a** and **9b**).

Though a number of similarities are observed among **7b**, **24c**, and **24e**, some distinct differences between the crystal structures were observed. SAR for the monocyclic β-lactams indicates the oxyiminopropylcarboxylic acid is important for *P. aeruginosa* activity, and most structures indicate an interaction with residue Arg489.[29] It is of note that for *cis*-allyl analog **24e**, the Arg is rotated away and no such interaction exists. This could be a reason for the lower affinity of the allyl analog. However, the electron density in this region is poorly defined.

Another key difference observed was in the orientation of the urea of the acylsulfonamide. *Trans*-analogs **7b** and **24c** have similar orientations with the urea carbonyl forming a hydrogen bond with the amide residue of Thr487, and the nitrogen from the β-lactam ring also makes a hydrogen bond with the hydroxyl of residue Ser349. The C4 substitutents (methyl and vinyl) are oriented in such a way as to allow van der Waals interactions with residue Val333. The allyl analog **24e** is bound with the urea portion of the molecule in a flipped orientation as

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compared to the *trans*-compounds. In this case the β-lactam nitrogen forms a hydrogen bond with residue Thr487 and the urea carbonyl is hydrogen bonded to the hydroxyl of residue Ser349. Presumably the flipped orientation is a result of preventing a steric clash between the allyl substituent and Val333.

It is noted that **4a** which has no C4 substituent binds in the same orientation as the *cis*-allyl analog **24e** and also forms hydrogen bonds with residues Thr487 and Ser349 (Figure 4).[29]

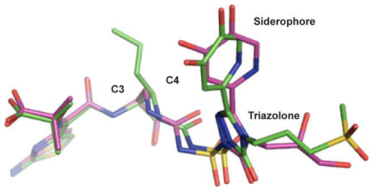

Figure 4. Overlay of *P. aeruginosa* PBP3 crystal structures of **4a** and **24e**.

However, **4a** has a 10-fold better acylation rate constant than **24e** (8.1×10^4 h versus 5.9×10^3 h). This indicates the urea orientation is not a reflection of good affinity for PBP3 but just the preferred conformation driven by the stereochemistry of the substituents on the core. Instead a contribution to the lower acylation rate constant of **24e** could be a steric clash with the protein in the initial encounter complex, prior to acylation.

Further profiling of the best compounds indicates the monocarbams demonstrate excellent pharmacokinetic (PK) properties, similar to other classes of β-lactam antibiotics (Table 3).[31]

Table 3. Intravenous Rat PK of Selected Monocarbams[a]

	compd				
	7a	**7b**	**10a**	**24c**	**24d**
rat CL (mL min^{-1} kg^{-1})	8.9	7.3	9.5	11	12
$t_{1/2}$ (h)	2.5	3.0	2.0	2.0	2.0
V_{ss} (L/kg)	0.23	0.21	0.26	0.18	0.35

[a]Han Wistar rat (male), 1 mg/kg, $n = 2$.

Compound **7a** has been shown to have a *P. aeruginosa* MIC_{90} equal to 1 μg/mL ($n = 20$) and to be efficacious against multiple *P. aeruginosa* strains in vivo.[32] Profiling of the resistance mechanisms indicates the monocarbam utilizes the siderophore receptors for uptake. Details of these studies will be reported elsewhere.

In conclusion, through our synthetic efforts we have explored and developed the SAR of the triazolone-based siderophore-conjugated monocarbams. Introduction of sulfone and sulfonamide side chains afforded highly potent and stable inhibitors of *P. aeruginosa*; however, there was no increase in free fraction. Through modifications of the siderophore and thiazole moieties, compounds with free fractions of >20% were produced. β-Lactams with substituted cores indicated *trans*-substituted β-lactam cores are favored for efficient inhibition of *P. aeruginosa* PBP3, and the hydrolytic stability of the compounds is controlled by the size and electronics of the substitution. Cocrystal structures of three analogs covalently bound to *P. aeruginosa* PBP3 were obtained. We observed some key differences between the structure of allyl analog **24e** and the structures of the methyl and vinyl analogs **7b** and **24c**, including lack of a salt bridge between Arg489 and the oxyiminopropylcarboxylic acid and a flipped conformation of the urea of the acylsulfonamide. By comparison with the structure of **4a**, we concluded the final orientation of the urea has no relation to the acylating efficiency of the molecule and instead is driven by the stereochemistry of the β-lactam substituent. These results highlight the challenges of structure-based drug design with covalent inhibitors, since the final complex does not reflect the initial encounter complex which is most likely the major factor in determining enzyme affinity.

■ ASSOCIATED CONTENT

Supporting Information

Crystallography details, *P. aeruginosa* PBP3 acylation assay, hydrolytic stability assay, microbiology assays, pharmacokinetic studies, synthesis of compounds **7a–j**, **9a**, **9b**, **10a**, **10b**, **24a–g**, **25b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PBP3, penicillin binding protein 3; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; THF, tetrahydrofuran; CSI, chlorosulfonyl isocyanate; DCM, dichloromethane; TFA, trifluoroacetic acid; *m*-CPBA, 3-chloroperbenzoic acid; MsCl, methanesulfonyl chloride; TBSCl, *tert*-butyldimethylsilyl chloride; DMF, *N*,*N*-dimethylformamide; imid, imidazole; DIBAL-H, diisobutylaluminum hydride; TEMPO, 2,2,6,6-tetramethylpiperidine 1-oxyl; DIAD, diisopropyl azodicarboxylate; EDC·HCl, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride; TBAF, tetrabutylammonium fluoride; CL, clearance; V_{ss}, volume of distribution at steady state

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