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12- to 22-Membered Bridged β-Lactams as Potential Penicillin-Binding Protein Inhibitors

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Abstract: As potential inhibitors of penicillin-binding proteins (PBPs), we focused our research on the synthesis of non-traditional 1,3-bridged β-lactams embedded into macrocycles. We synthesized 12- to 22-membered bicyclic β-lactams by the ring-closing metathesis (RCM) of bis-ω-alkenyl-3(*S*)-aminoazetidinone precursors. The reactivity of 1,3-bridged β-lactams was estimated by the determination of the energy barrier of a concerted nucleophilic attack and lactam ring-opening process by using ab initio calculations. The results predicted that 16-membered cycles should be more reactive. Biochemical evaluations against R39 DD-peptidase and two resistant PBPs, namely, PBP2a and PBP5, revealed the inhibition effect of compound **4d**, which featured a 16-membered bridge and the N-*tert*-butyloxycarbonyl chain at the C3 position of the β-lactam ring. Surprisingly, the corresponding bicycle, **12d**, with the $PhOCH_2CO$ side chain at C3 was inactive. Reaction models of the R39 active site gave a new insight into the geometric requirements of the conformation of potential ligands and their steric hindrance; this could help in the design of new compounds.

Keywords: ab initio calculations · enzymes · inhibitors · macrocycles · metathesis

Introduction

The introduction of penicillins to the therapeutic arsenal, in the early 1940s, was the starting point for the antibiotic era, which saved millions of people from potentially fatal infectious diseases. However, since the initial use of penicillins as chemotherapeutic agents, the phenomenon of bacterial resistance has been reported.[1] Owing to antibiotic pressure, the occurrence of resistant bacteria is increasing and leading to a worrisome situation with regards to antibiotics efficiency, so worrying, in fact, that the World Health Organization decided to dedicate World Health Day in 2011 to microbial resistance.

Despite the discovery of several other classes of antibiotics, the β-lactam class (exemplified nowadays by cephalosporins, carbapenems, and penems)[2] still remains that most prescribed. Initially the activity of β-lactam antibiotics (i.e., penicillins) was attributed to strain in the four-membered ring and the twisted amide bond of the 2-azetidinone functionality. Therefore, the search for new β-lactam antibiotics has been focused on strained 1,4-fused bicyclic structures, which are intended to improve the so-called "acylating power" of the β-lactam ring versus target serine proteases (i.e., bacterial DD-peptidases).[3] Tebipenem, tomopenem, or razupenem (Scheme 1), which are three carbapenems currently in phase II clinical studies, represent examples of this strategy.[4]

However, this traditional model of reactivity seems to be overevaluated because there is still no clear relationship be-

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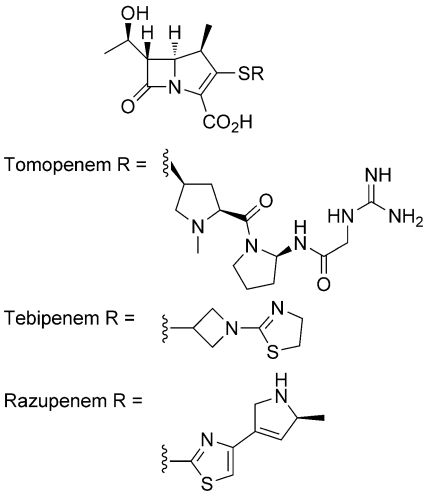
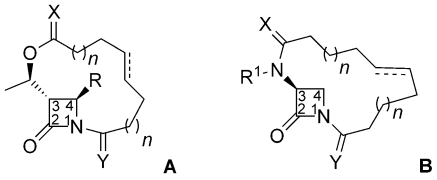
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/asia.201100732. It includes structural data for compounds **2a–c**, **2e**, **2f**, **3b**, **3c**, **3e**, **3f**, **4b**, **4c**, **4e**, **4f**, **10a–c**, **11b**, **11c**, **12b**, and **12c**, as well as ^{13}C NMR spectra of all new compounds.



Scheme 1. Examples of carbapenems under development.

tween structural characteristics and biological activity. An alternative model of reactivity was proposed by our group some years ago, based on 1,3-bridged, planar β-lactam motifs embedded into macrocycles. The aim was to possibly decrease the activation barrier for cleavage of the 2-azetidinone N–C(O) bond by increasing the conformational adaptability involved in the atom reorganization during the formation of an acyl–enzyme intermediate.[5] An initial series of compounds **A**, structurally related to carbapenems, were previously reported and evaluated against bacterial enzymes with mitigated success attributed to an inadequate configuration of the C3 chiral center. To further explore our hypothesis, we decided to synthesize the series of compounds **B** structurally related to cephalosporins and penicillins, with an amino substituent and inversion of configuration at C3. Because nucleophilic attack by serine enzymes occurs normally on the α face of the β-lactam, the macrocycle in **B** that surrounds the β face would not hamper the serine enzyme processing.

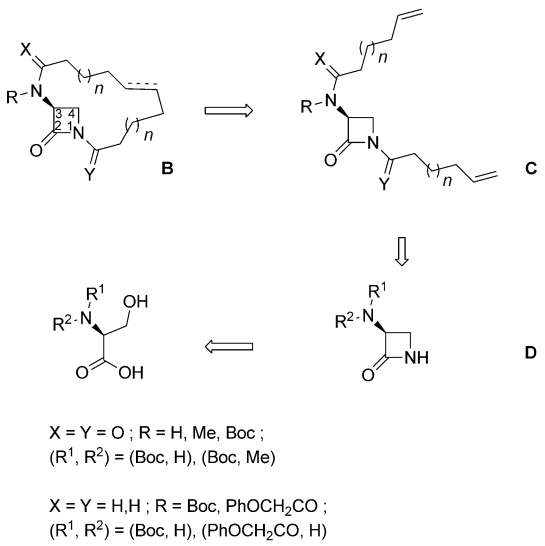


R = H, *n*Pr, OAc
R^1 = H, Me, COR^2
X = Y = O ; X = Y = H,H ; X = O and Y = H,H

The ring-closing metathesis (RCM) reaction was chosen as the key step for the formation of the bridging macrocycles. The precursors were chiral azetidin-2-ones **C** with ω-alkenoyl or ω-alkenyl chains on the N1 and C3–N positions. The starting chirons **D**, which were derivatives of (*S*)-3-aminoazetidin-2-one, came from L-serine.

First, we tried to synthesize the bicyclic family **B** with bis-acylated chains (X = Y = O). Unexpectedly, when applying the RCM reaction to the bis-acylated precursors **C** (X = Y = O, Scheme 2), we recovered exclusively cyclodimers, except in one case (R = *tert*-butyloxycarbonyl (Boc); $n = 2$).[6] Due to the presence of amide and imide functionalities in the precursors **C**, the conformers leading to the desired cyclizations are strongly disfavored. This should not be the case for the bis-alkylated precursors (X = Y = H,H). Therefore, we kept the same retrosynthetic strategy to synthesize the 1,3-bridged bicyclic β-lactam compounds **B** with bis-alkylated chains (X = Y = H,H).

Herein, we describe the successful synthesis of target molecules **B** in the bis-alkylated family (X = Y = H,H). All of these compounds were investigated from a theoretical point of view and their reactivity in a model of a penicillin-binding protein (PBP) cavity was studied. Theoretical predictions could be experimentally confirmed by in vitro evaluations against R39 DD-peptidase,[7] which is the commonly



X = Y = O ; R = H, Me, Boc ;
(R^1, R^2) = (Boc, H), (Boc, Me)

X = Y = H,H ; R = Boc, $PhOCH_2CO$;
(R^1, R^2) = (Boc, H), ($PhOCH_2CO$, H)

Scheme 2. Retrosynthetic strategy for the synthesis of series **B** compounds.

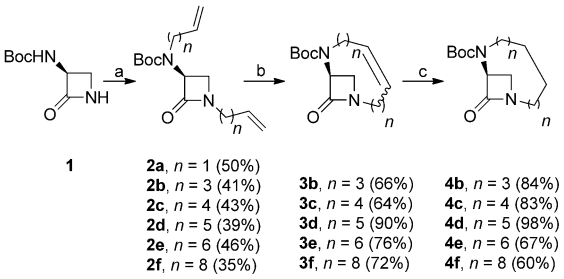
used model for bacterial enzymes, and against two resistant PBPs, namely, PBP2a from *Staphylococcus aureus*[8] and PBP5 from *Enterococcus faecium*.[9]

Results and Discussion

Synthesis

The Boc family was the first series of bis-alkylated bicycles **B** synthesized (X = Y = H,H; R = Boc). The starting material was (*S*)-3-(*tert*-butyloxycarbonyl)amino-2-azetidinone (**1**), which was readily prepared from commercially available Boc-L-serine, as previously described.[6]

ω-Alkenyl bromides of various lengths were used to access various sizes of bicycles. All of the ω-alkenyl bromides used were commercially available reagents. The bis-alkyl derivatives **2a–f** were synthesized in one step, with moderate yields, by using 2 equivalents of NaH and 2.2 equivalents of ω-alkenyl bromides (step a in Scheme 3). Other strong bases were tested (namely, lithium hexame-



1

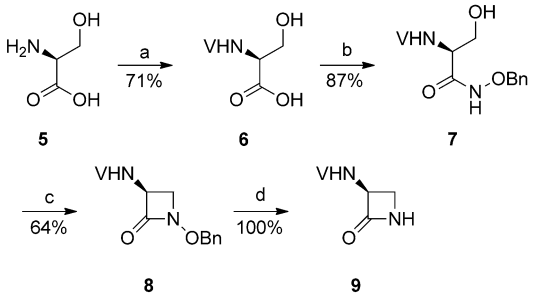
2a, *n* = 1 (50%)		
2b, *n* = 3 (41%)	**3b**, *n* = 3 (66%)	**4b**, *n* = 3 (84%)
2c, *n* = 4 (43%)	**3c**, *n* = 4 (64%)	**4c**, *n* = 4 (83%)
2d, *n* = 5 (39%)	**3d**, *n* = 5 (90%)	**4d**, *n* = 5 (98%)
2e, *n* = 6 (46%)	**3e**, *n* = 6 (76%)	**4e**, *n* = 6 (67%)
2f, *n* = 8 (35%)	**3f**, *n* = 8 (72%)	**4f**, *n* = 8 (60%)

Scheme 3. Synthesis of bis-alkylated azetidinones **2** followed by a RCM reaction: a) NaH, $Br-(CH_2)_n-CH=CH_2$, *N*,*N*-dimethylformamide (DMF), 0 to 20 °C, 12 h; b) Grubbs II catalyst (2 × 5 mol %), CH_2Cl_2, 40 °C, 12 h; c) H_2, Pd/C catalyst, MeOH, 20 °C, 3 h.

thyldisilazide (LiHMDS), KOH in the presence of Bu_4NHSO_4, and NaI), without improvement in the yield. The use of an excess of base led to racemization of the bis-alkyl derivatives **2**. The bis-alkyl derivative **2** with $n=2$ was not obtained, probably because HBr elimination occurred from 1-butenyl bromide. With these precursors in hand, we could validate the RCM strategy: using the second-generation Grubbs catalyst, macrocycles **3b–f** were readily formed and isolated in good yields after chromatographic purification (step b in Scheme 3), thereby leading to 12- to 22-membered rings. The bis-alkyl derivative **2a** did not cyclize; the expected 8-membered ring bicycle is too small. Catalytic hydrogenation led to the corresponding saturated macrocycles **4b–f** in high yields (step c in Scheme 3).

As the N-unprotected derivatives **B** (X = Y = H,H; R = H) were desirable for biological evaluation and/or further derivatization with the side chain of penicillin, we attempted to remove the Boc group from precursors **2** and bicycles **3** and **4**. Several conditions were tested (trifluoroacetic acid (TFA) in CH_2Cl_2, HCl 2 M solution, ceric ammonium nitrate (CAN) in CH_3CN at reflux,[10] or trimethylsilyl chloride (TMSCl) in the presence of NaI[11]) without success; in all cases degradation of the β-lactam ring was observed (IR and NMR spectroscopy analyses).

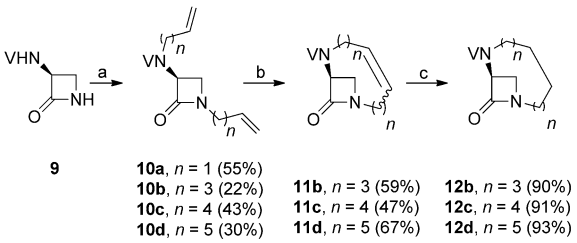
We tried to replace the Boc protecting group with the penicillin V side chain (V = $PhOCH_2CO$) in situ by direct acylation (with phenoxyacetyl chloride and NEt_3 or ethyldiisopropylamine (DIEA)) of the crude mixtures resulting from the treatment of **2**, **3**, and **4** under the above-mentioned deprotection conditions. Again, the results were disappointing with the recovery of intractable mixtures. Hence, to access the bis-alkylated bicycles **B** with the V side chain (X = Y = H,H; R = $PhOCH_2CO$) we restarted the total synthesis from L-serine (Scheme 4).



Scheme 4. Synthesis of chiron **9**: a) phenoxyacetyl chloride, a saturated aqueous solution of $NaHCO_3$, CH_3CN, RT, 12 h; b) *N,N*-dicyclohexylcarbodiimide (DCC), NH_2OBn (Bn = benzyl), tetrahydrofuran (THF), 0 °C to RT, 12 h; c) PPh_3, CCl_4, NEt_3, CH_3CN, 0 °C to RT, 12 h; d) H_2, Raney-Ni, MeOH/EtOAc, RT, 12 h. V = $PhOCH_2CO$.

L-Serine (**5**) was acylated under Schotten–Baumann conditions to give (*S*)-3-hydroxy-2-(2-phenoxyacetamido)propanoic acid (**6**) in 71% yield. This compound was converted into the corresponding hydroxamate **7**, using *O*-benzylhydroxylamine and *N,N'*-dicyclohexylcarbodiimide (DCC), in 87% yield. Intramolecular cyclization by the method proposed by Miller et al.[12] afforded β-lactam **8** in 64% yield. Subsequent hydrogenation in the presence of Raney-Ni gave the desired chiron **9** in quantitative yield.

Similar to the Boc family, the bis-alkyl derivatives **10** were synthesized in one step, from **9** in moderate yields. Under the conditions used for the RCM reaction, precursors **10b–d** (but not **10a**) cyclized into bicycles **11b–d** in good yields, thus affording 12- to 16-membered rings. Catalytic hydrogenation led to the saturated bicycles **12b–d**. (Scheme 5)



Scheme 5. Synthesis of bis-alkylated azetidinones **10** followed by a RCM reaction: a) NaH, $Br-(CH_2)_n-CH=CH_2$, DMF, 0 °C to 20 °C, 12 h; b) Grubbs II catalyst (2 × 5 mol %), CH_2Cl_2, 40 °C, 12 h; c) H_2, Pd/C catalyst, MeOH, 20 °C, 3 h.

All precursors (**2**, **10**) and bicycles (**3–4**, **11–12**) were characterized by IR and NMR spectroscopy and MS (see the Experimental Section and the Supporting Information). In particular, MS was useful for detecting the possible occurrence of side products that resulted from (cyclo)oligomerizations and/or double-bond migrations (in precursors), leading to cyclic products with the formal extrusion of one CH_2 unit. Only the 18- and 22-membered-ring bicycles, these are, compounds **3e–f**, and consequently, **4e–f**, were contaminated with small amounts of lower homologues (17- and 21-membered-ring bicycles, respectively).

The NMR spectra confirmed our hypothesis that 1,3-bridged β-lactams embedded into large rings were endowed with certain conformational adaptability, as discussed in the next section.

Computational Chemistry: Conformational Study—Heat of Formation

Many conformers can exist for the monocyclic molecules **2** and **10**, as well as for bicyclic molecules **3**, **4**, **11**, and **12**. In solution, the coexistence of conformers for these compounds was experimentally detected by NMR spectroscopy; ^{13}C NMR spectra of the compounds were recorded in $[D_2]$1,1,2,2-tetrachloroethane at different temperatures. At 30 °C, many signals were visible because some carbon atoms gave rise to splitting of the signals. Increasing the temperature to 120 °C led to signals coalescence, and thus, allowed structural assignment. For example, for compound **12d**, the quaternary carbon atoms (Cq) at $\delta = 167.8$ (C=O), 165.3

(C=O), and 158.0 ppm (PhO) at 120 °C appeared as multiple signals at 30 °C. The methylene peak of the V side chain at $\delta =$ 67.9 ppm at 120 °C presented two distinct signals at 30 °C. The C3 carbon of the β-lactam ring at $\delta = 62.2$ ppm at 120 °C gave two very well spaced signals at 30 °C. (The ^{13}C spectra of compound **12d**, as well as **10d** and **11d**, can be found in the Supporting Information.)

The geometry of all molecules was fully optimized at the RHF level by using the minimal basis set MINI-1′.[13]

In the case of bis-alkylated precursors **2** and **10** (X = Y = H,H), the molecules are conformationally less constrained than the bis-acylated compounds (X = Y = O)[6] because the carbonyl groups are replaced with methylene groups, which can accommodate more conformations. Depending on the nature of the N substituent (Boc or V), a large number of local minima could be trapped.

For all of the compounds studied, two conformations, "i" and "ii", of the bridging cycle (of compounds **3**, **11**, **4**, and **12**) have been located with respect to the β-lactam ring. Conformation i expands the cycle to the upper-right corner of the β-lactam C4; in conformation ii, the cycle is more orientated to the carbonyl C2 of the β-lactam. For each conformation, the carbonyl of the side chain (Boc or V) can rise above the four-membered ring ("a" conformation) or below ("b" conformation) (Figure 1). For unsaturated compounds **3** and **11**, the *trans* configuration of the substituted ethylene has been considered.

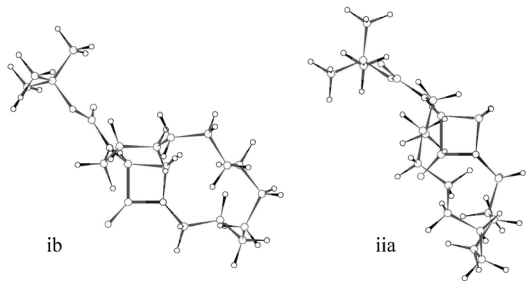


Figure 1. Conformers ib and iia of compound **4d**.

The heat of formation of **3** and **11** has been computed with respect to the open precursor with the same conformation as that of the corresponding cyclized molecule. For the Boc and V side chains, the 4 conformations lie in the same range of stability; the relative energies are less than 8 kcal mol^{-1} in the case of the ii conformations, which are often more stable than those of i type (Table 1). The size of the ring has a significant impact on the conformations for the smallest (12-membered ring) and the largest ones (22-membered ring). For compounds **3b** and **11b**, only the i conformation can be trapped. In **3f** the ring is so large that it expands on both sides of the β-lactam in a pseudo-i conformation.

Table 1. Relative energies (E) of the i and ii conformations of the precursors/bicycles and respective heats of formation (H) resulting from cyclization.

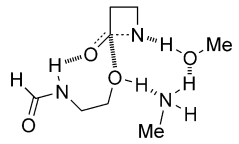
Precursor	Product	Geometry	$E_{precursors}$ [kcal mol^{-1}]		$E_{bicycles}$ [kcal mol^{-1}]		$H_{bicycles}$ [kcal mol^{-1}]	
			i	ii	i	ii	i	ii
2b	**3b**	a	1.39		0.31		6.54	
		b	0		0		7.61	
2c	**3c**	a	2.16	0.44	2.84	0.29	5.66	4.83
		b	2.17	0.00	3.23	0.00	6.05	4.98
2d	**3d**	a	2.95	0.94	2.66	0.00	8.30	7.64
		b	3.01	0.00	1.93	0.18	7.50	8.76
2e	**3e**	a	2.95	2.92	7.83	0.00	11.50	3.69
		b	2.95	0.00	7.66	0.27	11.32	6.88
2f	**3f**	a	0.01		0.33		5.67	
		b	0.00		0.00		5.37	
10b	**11b**	a	0.10		0.00		6.47	
		b	0.00		0.58		7.14	
10c	**11c**	a	2.47	0.96	1.88	1.11	4.19	4.92
		b	2.99	0.00	0.00	0.22	1.78	4.99
10d	**11d**	a	3.79	2.54	1.45	0.68	7.94	8.42
		b	3.49	0.00	0.99	0.00	7.78	10.28

As the bis-alkylated precursors **2** and **10** are highly flexible, their cyclization can easily lead to desired compounds **3** and **11**. Similar reactions were not possible for the bis-acylated precursors.[6]

Reactivity versus Serine Enzyme Models

The reactivity of the bridged molecules was studied by using a simple model of the PBP cavity (Scheme 6) at the RHF/MINI-1′ level (Table 2).

In this model, the ring opening of the β-lactam occurs through a concerted process: the nucleophilic serine is mimicked by 2-(formyl)amino-1-ethanol, interacts with methylamine, which acts as a proton relay to methanol, and transfers the proton to the β-lactam nitrogen.[14] The formamide moiety mimics oxyanion hole stabilization. At the transition state (TS), this pseudo eight-membered ring is described by the reaction coordinate associated to the negative curvature of the second-derivative energy matrix.



Scheme 6. Model of concerted nucleophilic attack on the β-lactam ring.

The results obtained with Boc and V side chains present some common features concerning the lowest energy barriers: the optimum size of the cycle is a 16-membered ring (i.e., **3d**–**4d** and **11d**–**12d**), while the shorter and the bigger ones enhance the energy barrier calculated with the model. In most of the cases, the activation energy is also higher for

Table 2. Activation energy of concerted nucleophilic attack (MINI-1′).

Unsaturated compound	Saturated compound	Geometry	ΔE unsaturated [kcal mol^{-1}]		ΔE saturated [kcal mol^{-1}]	
			i	ii	i	ii
3b	**4b**	a	27.86		28.37	
		b	27.18		31.78	
3c	**4c**	a	22.79	25.12	25.15	26.31
		b	20.89	23.21	21.86	25.28
3d	**4d**	a	18.90	20.47	19.48	23.76
		b	16.86	20.55	17.96	22.41
3e	**4e**	a	20.84	19.66	20.19	20.55
		b	19.13	17.71	19.17	18.43
3f	**4f**	a	28.82		28.80	
		b	28.62		28.86	
11b	**12b**	a	21.96		23.42	
		b	26.36		30.87	
11c	**12c**	a	26.87	25.35	29.36	24.53
		b	23.95	22.99	23.11	21.14
11d	**12d**	a	21.47	18.95	21.22	19.82
		b	15.53	18.95	16.52	18.38
PenG			9.33			

the saturated cycle with respect to the corresponding unsaturated one (with the *trans* configuration at the C=C bond).

Inhibition of R39, PBP2a, and PBP5

All products were evaluated for their potential inhibition effect on bacterial serine enzymes. R39 from *Actinomadura* is a model serine enzyme of low-molecular-weight DD-peptidases, which are usually considered for preliminary screening of penicillin-like compounds. R39 and the tested β-lactam (100 μM) were incubated (1 h, 25 °C). Then the enzyme residual activity (RA) was determined by observing the hydrolysis of the thioester S2d substrate[15] in the presence of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), which labeled the formed thiol, and reading at 412 nm. The results are given in Table 3 as percentages of RA. The activity in the absence of inhibitor was set at 100 %, and therefore, low values indicated a very active compound because the bacterial enzyme was inhibited by the tested compound, and consequently, could not hydrolyze its substrate. A tested compound was considered a "hit" (i.e., potential inhibitor) when RA was less than 80 %. All of the compounds were also evaluated against two high-molecular-weight DD-peptidases responsible for bacterial resistance to β-lactam antibiotics: PBP2a from methicillin-resistant *S. aureus* (MRSA) and PBP5 from *E. faecium*. The tested β-lactams (1 mM) were incubated with the PBPs (4 h, 30 °C), then fluorescein-labeled ampicillin was added to detect the residual activity. This reagent is an inhibitor that forms a stable acyl–enzyme intermediate. After denaturation and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation of the acylated enzyme from the reagent band, fluorescence was measured. The fluorescence intensity was proportional to the residual active protein, that is, protein not acylated by the tested compound.

Interestingly, the lowest RA values were found for Boc molecules with saturated (**4d**; Table 3, entry 21) and unsaturated (**3d**; Table 3, entry 13) 16-membered rings, and their open precursor (**2d**; Table 3, entry 4). Some activities on PBP2a were also observed for other compounds of the Boc family (Table 3, entries 2, 3, 11, 15). On the other hand, none of the V side-chain molecules had a significant activity on the R39 DD-peptidase or on PBP2a and PBP5. Only the saturated 16-membered ring of the Boc family (**4d**) had a high activity on the R39 DD-peptidase. To understand this phenomenon, more elaborate reactivity models of the active site were built.

Table 3. Evaluation of bis-alkylated azetidinones against R39 DD-peptidase, PBP5, and PBP2a.

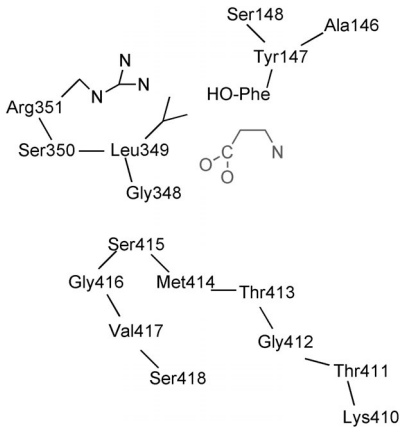
Entry		*n*	R39 RA [%]	PBP5 RA [%]	PBP2a RA [%]
1	**2a**	1	101 ± 4	81	96
2	**2b**	3	> 100	94	71
3	**2c**	4	97 ± 11	69	68
4	**2d**	5	80 ± 4	80	59
5	**2e**	6	97 ± 2	100	95
6	**2f**	8	101 ± 1	100	100
7	**10a**	1	103 ± 8	100	96
8	**10b**	3	97 ± 3	90	95
9	**10c**	4	101 ± 1	100	98
10	**10d**	5	100 ± 4	96	91
11	**3b**	3	> 100	68	53
12	**3c**	4	103 ± 9	78	96
13	**3d**	5	83 ± 4	74	58
14	**3e**	6	98 ± 1	99	96
15	**3f**	8	97 ± 3	72	69
16	**11b**	3	102 ± 4	95	89
17	**11c**	4	101 ± 3	100	97
18	**11d**	5	104 ± 4	100	93
19	**4b**	3	> 100	96	98
20	**4c**	4	96 ± 11	89	88
21	**4d**	5	52 ± 3	61	61
22	**4e**	6	95 ± 1	100	89
23	**4f**	8	96 ± 1	86	90
24	**12b**	3	101 ± 3	100	97
25	**12c**	4	97 ± 4	100	100
26	**12d**	5	105 ± 9	99	100

Building the Models

The R39 active site is composed of the three conserved motifs found in PBP and β-lactamases, as highlighted from X-ray data.[7] The first motif connects Ser49 (a nucleophilic serine) and Lys52 with Asn50 and Met51. Remarkably, the conformation of the backbone is stabilized by a hydrogen bond between the carbonyl group of the Ser49 backbone and the NH group of Lys52, thus allowing the lysine residue to extend in such a way that the amino group N_ζ lies in the vicinity of the O_γ of Ser49. The second motif is formed by

Ser298, Asn299, and Asn300. Ser298 is connected to the amino group of Lys52 residue. Owing to turns in the conformations of Asn299 and Asn300, the NH of Asn300 interacts with the ligand carbonyl side chain. The third motif is formed by Lys410, Thr411, Gly412, and Thr413. Several interactions stabilize its conformation. The carbonyl backbone of Thr413 makes a hydrogen bond with the NH ligand side chain.

The NH backbone of Thr413 interacts with the oxyanion of the ligand, while the amino group of the Lys410 side chain and the OH group of Thr411 stabilize the carboxylic group of penicillin-type antibiotics. Gly416 starts the β4 sheet with Val417 and Ser418 parallel to the β3 one. The bottom of the cavity is delimited by Gly348, Leu349, Ser350, and Arg351 on one side and by Ala146, Tyr147, and Ser148 on the other. As depicted in the 2D drawing (Scheme 7), the side chains of Arg351, Leu349, and Tyr147 could interact with the side chain of the ligand. They could also give rise to steric hindrance with part of the ligand.



Scheme 7. The bottom of the R39 cavity; the fragment in gray is part of the ligand with a side chain on C3.

Three models have been built by increasing their complexity to locate the transition structure for penicillin with a side chain limited to a formamide group (referred to as Pen). The first model contains the 49 to 52 amino acids of the first motif and methanol, which mimics Ser298 (82 atoms, 250 basis functions) in the simple model (Figure 2a). In the second model, the second motif has been added with 298 to 300 amino acids (110 atoms, 342 basis functions; Figure 2b). Finally, the inclusion of 410 to 413 amino acids of the third motif constitutes the third model formed by 168 atoms and 508 basis functions (Figure 2c; hydrogen atoms have been deleted for clarity). Model three, with hydrogen atoms and hydrogen bonds, is presented in the Supporting Information. For clarity, the point of view is also rotated around the *y* axis to show that motif three lies above the β-lactam ring.

The aim of these calculations is not, at the present stage, to determine an energy barrier that could be representative of the energy involved in the enzymatic reaction with the

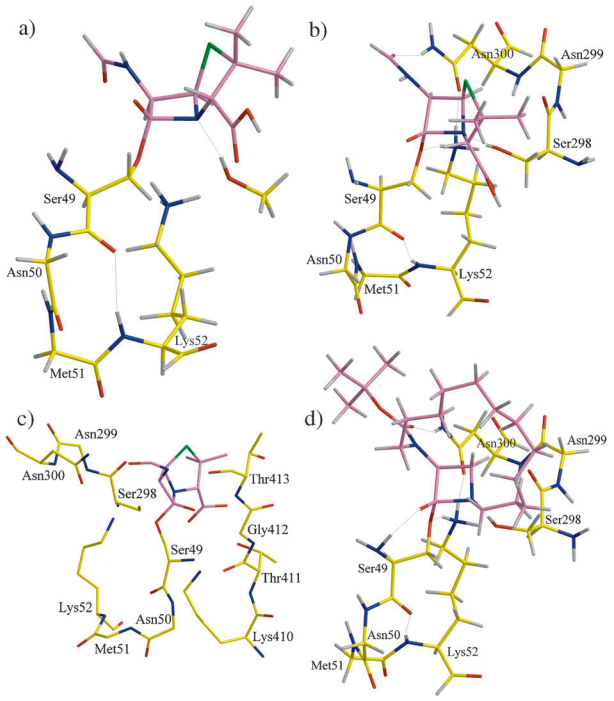


Figure 2. a) Pen in the first model. b) Pen in the second model. c) Pen in the third model. d) Compound **4d** in conformation ib in the second model. Legend: C of Pen or **4d** in purple, others in yellow; H in gray; O in red; N in blue; hydrogen bonds as thin lines.

complete protein, but to analyze the geometrical constraints resulting from the models. From a geometric point of view, the position of the thiazolidine ring of penicillin (or the tetrahydrothiazine ring of cephalosporin; results not shown) lies at the entrance of the cavity. This feature could be related to the fact that many DD-peptidases can easily accommodate large β-lactam antibiotics, such as tricyclic carbapenems.[16] A second important geometry constraint is related to the conformation of the third motif, which defines the accessible volume above the β-lactam ring.

The three TS structures have been located as first-order saddle points, for which the imaginary frequency describes the motion of hydrogen between Ser49 and Lys52, Lys52 and Ser298, and Ser298 to the nitrogen of the β-lactam ring. Remarkably, the three equilibrium structures are nearly superimposable (cf. the simple model in Scheme 6) when considering the eight-membered ring formed by the proton shuttle.

Additional calculations for **4d** were performed and the TS structures were located for the second (143 atoms, 407 basis functions) and third (201 atoms, 573 basis functions) models when using ib and iib conformations as starting geometries. It appears that conformation iib has important steric hindrance with the third motif and that only conformation ib could accommodate the geometry of the active site model (Figure 2d). The goodness of fit between this structure and that obtained with Pen could partially explain the RA value of 52% observed with **4d** on R39.

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One question remains with regards to the lack of activity of **12d**. This molecule can adopt two conformations of the phenoxy side chain, for which the ib conformation of the bridged cycle differs only by 1.52 kcal. Both conformations have been superimposed on conformation ib of **4d** (Figure 3). In one conformation, the phenoxy group has steric hindrance with Tyr147 phenol; in the other, the phenoxy group is in contact with Leu349 and Arg351 side chains at a distance that is too short. This geometric feature, occurring in both conformations, could be related to the biological results because **12d** cannot accommodate the active-site model geometry, whereas **4d** can.

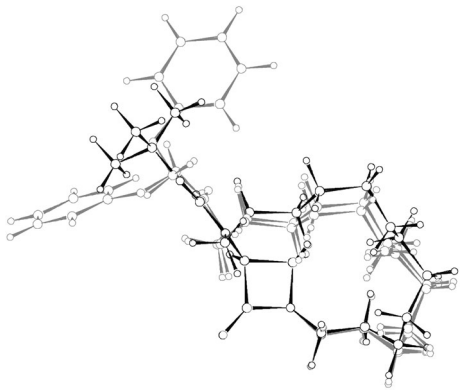

Figure 3. Compounds **4d** (black) and **12d** (gray) in conformation ib.

Conclusion

In the bis-acylated family previously reported (X=Y=O; Scheme 2), cyclodimers were obtained when applying the RCM reaction as a key step for macrocyclization. Herein, this synthetic strategy was successful and led to cyclomonomers of the bis-alkylated family (X=Y=H,H) owing to the high conformational adaptability of the bis-alkylated precursors. We synthesized 12- to 22-membered, 1,3-bridged β-lactams for the Boc series and 12- to 16-membered bicyclic β-lactams for the V side-chain series.

The reactivity of the 1,3-bridged β-lactams in the active site of a serine enzyme depends greatly on the ligand conformation and explains why compounds with a V side chain do not fit into the geometry of the catalytic cavity. This feature was analyzed by using different models of the R39 active site, including the three conserved motifs of this enzyme family. Of the synthesized bicycles, compound **4d** was a good inhibitor of the R39 enzyme (as theoretically predicted) and some compounds also exhibited significant activity against resistant PBP2a from MRSA. This activity could be related to the flexibility of the bicycles, which can be accommodated into the enzyme active site.

The activity of the 1,3-bridged macrocycles derived from bis-ω-alkenyl-3(*S*)-aminoazetidinone precursors suggested a way to design new lactam antibiotics with a planar amide bond and devoid of carboxylic group. Indeed, this acid functionality only plays a role in stabilizing the conformation of penicillin-like molecules by hydrogen bonds to residues of the third motif.

Experimental Section

General

Experiments were performed under an argon atmosphere in flame-dried glassware. All solvents, including anhydrous solvents, and reagents were purchased from Acros Organics, Alfa Aesar, Fluka, Sigma–Aldrich, or VWR, and used without any further purification. TLC analyses were performed on aluminum plates coated with silica gel $60F_{254}$ (Merck) and visualized with a solution of $KMnO_4$ and UV (254 nm) detection. Column chromatography was performed on silica gel (40–63 or 63–200 μm) purchased from Rocc. Melting points were determined on a Büchi B-540 apparatus calibrated with caffeine, vanillin, and phenacetin. $[\alpha]_D$ values were measured on Perkin–Elmer 241 MC polarimeter at 20 °C. Concentrations are given in g/100 mL. NMR (^1H and ^{13}C) spectra were recorded at 300 MHz for proton and 75 MHz for carbon (Bruker Avance 300 spectrometer) or 500 MHz for proton and 125 MHz for carbon (Bruker Avance 500 spectrometer). Chemical shifts are reported in parts per million relative to the residual solvent signal from the deuterated solvent or relative to peak of tetramethylsilane (TMS). NMR coupling constants (*J*) are reported in hertz. IR spectra were recorded by using an FTIR-8400S Shimadzu apparatus. Products were analyzed as thin films deposited on an Se–Zn crystal by evaporation from solutions in CH_2Cl_2. Low-resolution mass spectra were obtained by using ThermoFinnigan LCQ Quantum or FinniganMat TSQ7000 spectrometers. High-resolution mass spectrometry (HRMS) analyses were performed at University College London (UK).

*Bis-alkyl Precursor **2d***

NaH (60% dispersion in oil, 279 mg, 6.98 mmol) was added to a stirred solution of **1** (0.65 g, 3.49 mmol) in dry DMF (41 mL) at 0 °C. Alkyl bromide (1.17 mL, 7.68 mmol) was added after 30 min and the mixture was then slowly warmed to room temperature and stirred overnight. The mixture was quenched with water (30 mL) at 0 °C and then the medium was diluted with brine (50 mL). The resulting mixture was extracted with EtOAc (3×30 mL) and the organic layers were washed with brine (70 mL). After drying over $MgSO_4$ and removing the solvent under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc 4:1) to provide **2d** as a pale-yellow oil (515 mg, 39%). R_f=0.63 (hexane/EtOAc 3:2); $[\alpha]_D$=+3.0 (c=1.7 in CH_3OH); ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ=5.84–5.71 (m, 2H), 5.01–4.90 (m, 4H+0.4H; rotamer), 4.52 (brs, 0.6H; rotamer), 3.46–3.43 (m, 1H), 3.26–3.08 (m, 5H), 2.07–2.00 (m, 4H), 1.57–1.24 ppm (m, 21H); ^{13}C (125 MHz, $CDCl_3$, 20 °C): δ=167.1, 154.7, 138.9, 138.7, 114.7, 114.5, 80.9, 80.5, 63.2, 62.9, 47.9, 47.1, 46.7, 41.7, 33.8, 33.7, 29.6, 29.0, 28.7, 28.5, 27.5, 26.5, 26.4 ppm; IR: $\tilde{\nu}$=2974–2856, 1753, 1693, 1639, 1456 cm^{-1}; MS (ESI): *m*/*z* (%): 779 (12) $[2M+Na]^+$, 401 (100) $[M+Na]^+$; HRMS (ESI): *m*/*z* calcd for $C_{22}H_{38}N_2O_3Na$: 401.2780 $[M+Na]^+$; found: 401.2778.

*Unsaturated Bicycle **3d***

Grubbs II catalyst (56 mg, 66.09 μmol) was added to a stirred solution of **2d** (0.50 g, 1.32 mmol) in dry CH_2Cl_2 (264 mL) and the solution was stirred at reflux under argon for 4 h. Then a second addition of Grubbs II catalyst (56 mg, 66.09 μmol) was made and the mixture was additionally stirred at reflux for 20 h. The solvent was removed under reduced pressure and the crude product was purified three times by column chromatography (hexane/EtOAc 4:1) to provide **3d** as a pale-brown solid (416 mg, 90%). R_f=0.43 (hexane/EtOAc 3:2); m.p. 90.1–90.9 °C; $[\alpha]_D$=−24.4 (c=1.2 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, 20 °C): δ=5.51 (brs, 0.6H; rotamer), 5.34–5.18 (m, 2H+0.4H; rotamer), 3.73–3.67 (m, 1H), 3.42–3.35 (m, 1H), 3.25–2.93 (m, 3H), 2.81–2.76 (m, 1H), 2.13–1.94 (m, 4H), 1.69–1.17 ppm (m, 21H); ^{13}C (125 MHz, $CDCl_3$, 20 °C): δ=167.6, 167.4, 155.1, 154.3, 131.9, 131.7, 130.4, 130.2, 80.6, 80.3, 62.5, 61.9,

46.9, 45.4, 45.1, 41.2, 41.0, 40.8, 32.2, 30.5, 28.8, 28.4, 28.1, 27.9, 27.2, 27.1, 26.9, 26.7, 26.2, 26.1, 25.1, 25.0, 24.8, 24.5 ppm; IR: $\tilde{\nu}$ = 2924–2852, 1755, 1697, 1454 cm^{-1}; MS (ESI): *m/z* (%): 373 (100) $[M+Na]^+$, 317 (23) $[M+Na\text{-}C_4H_8]^+$, 295 (25) $[M+H\text{-}C_4H_8]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{34}N_2O_3Na$: 373.2467 $[M+Na]^+$; found: 373.2455.

*Saturated Bicycle **4d***

10% Pd/C (10 mg) was added to a stirred solution of **3d** (110 mg, 0.31 mmol) in methanol (10 mL). After being stirred under a hydrogen atmosphere (P = 1 atm) for 3 h at room temperature, the mixture was filtered through a short pad of Celite and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 4:1) to provide **4d** as a white solid (108 mg, 98%). R_f = 0.48 (hexane/EtOAc 3:2); m.p. 55.4–56.2 °C; $[\alpha]_D$ = −24.9 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 20 °C): δ = 5.44 (brs, 0.6H; rotamer), 5.18 (brs, 0.4H; rotamer), 3.68 (brs, 1H), 3.43 (brs, 1H), 3.26–2.99 (m, 3H), 2.84–2.79 (m, 1H), 1.75–1.69 (m, 1H), 1.59–1.20 ppm (m, 28H); ^{13}C (125 MHz, $CDCl_3$, 20 °C): δ = 167.4, 167.1, 155.2, 155.1, 80.5, 62.6, 62.1, 48.0, 47.7, 45.1, 41.9, 29.1, 28.5, 28.2, 26.9, 26.8, 26.6, 26.5, 26.0, 25.4 ppm; IR: $\tilde{\nu}$ = 2924–2854, 1757, 1697, 1458 cm^{-1}; MS (ESI): *m/z* (%): 375 (100) $[M+Na]^+$, 319 (10) $[M+Na\text{-}C_4H_8]^+$, 297 (26) $[M+H\text{-}C_4H_8]^+$; HRMS (ESI): *m/z* calcd for $C_{20}H_{36}N_2O_3Na$: 375.2624 $[M+Na]^+$; found: 375.2621.

*(2S)-3-Hydroxy-2-(2-phenoxyacetamido)propanoic Acid (**6**)*

Phenoxyacetyl chloride (6.57 mL, 47.58 mmol) was added to a solution of **5** (5.00 g, 47.58 mmol) in a saturated aqueous solution of $NaHCO_3$ (200 mL) and MeCN (40 mL). The reaction mixture was stirred vigorously overnight at room temperature and the aqueous phase was extracted with diethyl ether (2 × 200 mL). The aqueous solution was acidified to pH 2–3 with HCl 36% and extracted with EtOAc (4 × 200 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was dissolved in a minimum of EtOAc and hexane was added until the appearance of a precipitate. After 5–6 h the suspension was filtered, washed with hexane, and dried under reduced pressure to provide **6** as a white solid (8.08 g, 71%); m.p. 30.1–130.9 °C; $[\alpha]_D$ = +30.4 (*c* = 1.1 in $(CH_3)_2CO$); ^{1}H NMR (300 MHz, $(CD_3)_2CO$, 20 °C): δ = 7.62–7.60 (brd, *J* = 6.5 Hz, 1H), 7.35–7.30 (m, 2H), 7.04–6.98 (m, 3H), 4.65–4.60 (m, 1H), 4.55 (s, 2H), 4.06–4.00 (dd, *J* = 4.1, 11.1 Hz, 1H), 3.95–3.90 ppm (dd, *J* = 3.6, 11.1 Hz, 1H); ^{13}C (75 MHz, $(CD_3)_2CO$, 20 °C): δ = 171.7, 168.6, 158.7, 130.5 (2C), 122.5, 115.7 (2C), 68.0, 62.7, 55.0 ppm; IR: $\tilde{\nu}$ = 3393–3060, 2754–2469, 1731, 1643, 1539, 1495 cm^{-1}; MS (ESI): *m/z* (%): 262 (100) $[M+Na]^+$, 240 (38) $[M+H]^+$, 222 (52) $[M+H-H_2O]^+$; HRMS (CI): *m/z* calcd for $C_{11}H_{14}NO_5$: 240.08720 $[M+H]^+$; found: 240.08649.

*(2S)-N-(Benzyloxy)-3-hydroxy-2-(2-phenoxyacetamido)propanamide (**7**)*

A solution of DCC (2.72 g, 13.17 mmol) in THF (10 mL) was added dropwise, at 0 °C, to a well-stirred solution of **6** (3.00 g, 12.54 mmol) and *O*-(phenylmethyl)hydroxylamine (1.46 mL, 12.54 mmol) in THF (120 mL). The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The obtained white precipitate was separated by filtration and the resulting clear reaction mixture was concentrated under reduced pressure. After addition of Et_2O (100 mL), the precipitate was filtered, washed with Et_2O, and dried under reduced pressure to provide **7** as a white solid (3.76 g, 87%). R_f = 0.30 (CH_2Cl_2/MeOH 95:5); m.p. 145.0–145.7 °C; $[\alpha]_D$ = −1.5 (*c* = 1.0 in $(CH_3)_2SO$); ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 20 °C): δ = 11.33 (brs, 1H), 8.01 (brd, *J* = 7.9 Hz, 1H), 7.39–7.28 (m, 7H), 6.99–6.95 (m, 3H), 5.08 (brs, 1H), 4.78 (s, 2H), 4.55 (s, 2H), 4.23–4.29 (m, 1H), 3.59 ppm (brd, *J* = 5.5 Hz, 2H); ^{13}C (75 MHz, $(CD_3)_2SO$, 20 °C): δ = 167.6, 166.7, 152.7, 135.8, 129.5 (2C), 128.9 (2C), 128.3 (3C), 121.2, 114.7 (2C), 76.9, 66.6, 61.4, 52.8 ppm; IR: $\tilde{\nu}$ = 3313–2999, 1650, 1556, 1499 cm^{-1}; MS (ESI): *m/z* (%): 367 (100) $[M+Na]^+$, 345 (30) $[M+H]^+$, 222 (12) $[M+H-NH_2OBn]^+$; HRMS (CI): *m/z* calcd for $C_{18}H_{21}N_2O_5$: 345.14505 $[M+H]^+$; found: 345.14649.

*N-[(3S)-1-(Benzyloxy)-2-oxoazetidin-3-yl]-2-phenoxyacetamide (**8**)*

A solution of PPh_3 (2.09 g, 7.98 mmol) in dry MeCN (35 mL) was added dropwise, at 0 °C and under an Ar atmosphere, to a stirred solution of **7** (2.50 g, 7.26 mmol) in dry CH_3CN (20 mL), containing CCl_4 (0.77 mL, 7.98 mmol) and anhydrous triethylamine (1.52 mL, 10.89 mmol). The resultant reaction mixture was stirred for 2 h at 0 °C and then overnight at room temperature. After completion of the reaction, the obtained white precipitate ($OPPh_3$) was separated by filtration and the resulting clear reaction mixture was concentrated under reduced pressure. The residue was dissolved with EtOAc (50 mL), washed with a saturated aqueous solution of NH_4Cl (2 × 40 mL) and brine (40 mL). After drying over $MgSO_4$ and removing the solvent under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc 1:1) to provide **8** as a white solid (1.51 g, 64%). R_f = 0.41 (hexane/EtOAc 4:6); m.p. 138.8–139.4 °C; $[\alpha]_D$ = −6.2 (*c* = 1.0 in $(CH_3)_2CO$); ^{1}H NMR (300 MHz, $(CD_3)_2CO$, 20 °C): δ = 8.19 (brd, *J* = 7.0 Hz, 1H), 7.49–7.28 (m, 7H), 7.01–6.96 (m, 3H), 4.98 (s, 2H), 4.91–4.85 (m, 1H), 4.53 (brd, *J* = 1.4 Hz, 2H), 3.72–3.69 (m, 1H), 3.53 ppm (dd, *J* = 2.4, 4.3 Hz, 1H); ^{13}C (75 MHz, $(CD_3)_2CO$, 20 °C): δ = 169.1, 163.4, 158.7, 136.5, 130.4 (2C), 130.0 (2C), 129.5, 129.3 (2C), 122.4, 115.6 (2C), 77.9, 67.9, 52.6, 52.4 ppm; IR: $\tilde{\nu}$ = 3363–3193, 1776, 1677, 1598, 1531, 1495 cm^{-1}; MS (ESI): *m/z* (%): 365 (8) $[M+K]^+$, 349 (100) $[M+Na]^+$, 327 (10) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{18}H_{18}N_2O_4Na$: 349.1164 $[M+Na]^+$; found: 349.1151.

*N-[(3S)-2-Oxoazetidin-3-yl]-2-phenoxyacetamide (**9**)*

Compound **8** (1.00 g, 3.06 mmol) was dissolved in methanol (18 mL) and EtOAc (18 mL) and placed under H_2 (1 atm) at room temperature in the presence of Raney-Ni (50% in water) catalyst for 12 h. Then the mixture was filtered through a pad of Celite and concentrated under reduced pressure to provide **9** as a white solid (0.67 g, 100%), which was used without further purification. R_f = 0.42 (EtOAc/MeOH 95:5); m.p. 155.7–156.6 °C; $[\alpha]_D$ = −19.1 (*c* = 1.0 in CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, 20 °C): δ = 7.32–7.28 (m, 2H), 7.01–6.97 (m, 3H), 5.04 (dd, *J* = 2.7, 5.4 Hz, 1H), 4.54 (brd, *J* = 1.2 Hz, 2H), 3.58–3.56 (m, 1H), 3.39 ppm (dd, *J* = 2.6, 5.5 Hz, 1H); ^{13}C (125 MHz, CD_3OD, 20 °C): δ = 171.5, 170.8, 159.2, 130.6 (2C), 122.9, 115.9 (2C), 68.1, 57.8, 44.1 ppm; IR: $\tilde{\nu}$ = 3281–3020, 1776, 1703, 1664, 1628, 1556, 1498 cm^{-1}; MS (ESI): *m/z* (%): 243 (100) $[M+Na]^+$; HRMS (CI): *m/z* calcd for $C_{11}H_{13}N_2O_3$: 221.09262 $[M+H]^+$, found: 221.09253.

*Bis-alkyl Precursor **10d***

Compound **10d** was prepared by the same procedure as that described for **2d**. Yield: 30%; R_f = 0.47 (hexane/EtOAc 1:1); $[\alpha]_D$ = +3.1 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 20 °C): δ = 7.33 (t, *J* = 7.7 Hz, 2H), 7.03 (t, *J* = 7.3 Hz, 1H), 6.93 (brd, *J* = 8.0 Hz, 2H), 5.84–5.75 (m, 2H), 5.12–4.95 (m, 4H + 0.3H; rotamer), 4.76–4.62 (m, 2H + 0.7H; rotamer), 3.53–3.10 (m, 6H), 2.07–2.03 (m, 4H), 1.71–1.52 (m, 4H), 1.44–1.25 ppm (m, 8H); ^{13}C (125 MHz, $C_2D_2Cl_4$, 90 °C): δ = 167.9, 165.1, 157.9, 138.3, 129.4, 121.7, 114.8, 114.4, 67.6, 62.9, 46.8, 41.9, 33.2, 28.3, 28.2, 27.1, 26.3, 26.1 ppm; IR: $\tilde{\nu}$ = 3003–2854, 1748, 1668, 1599, 1587, 1497 cm^{-1}; MS (ESI): *m/z* (%): 435 (100) $[M+Na]^+$, 413 (6) $[M+H]^+$, 288 (10); HRMS (ESI): *m/z* calcd for $C_{25}H_{36}N_2O_3Na$: 435.2624 $[M+Na]^+$; found: 435.2625.

*Unsaturated Bicycle **11d***

Compound **11d** was prepared by the same procedure as that described for **3d**. Yield: 67%; R_f = 0.42 (hexane/EtOAc 3:7); m.p. 86.7–87.3 °C; $[\alpha]_D$ = −2.8 (*c* = 1.9 in $CHCl_3$); ^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 20 °C): δ = 7.34–7.30 (m, 2H), 7.02 (t, *J* = 7.3 Hz, 1H), 6.93–6.91 (m, 2H), 5.84–5.74 (m, 0.4H; rotamer), 5.37–5.16 (m, 2H + 0.6H; rotamer), 4.79–4.62 (m, 2H), 3.72–3.66 (m, 1H), 3.55–3.08 (m, 4H), 2.88–2.66 (m, 1H), 2.16–1.85 (m, 4H), 1.73–1.19 ppm (m, 12H); ^{13}C (125 MHz, $C_2D_2Cl_4$, 120 °C): δ = 167.8, 165.5, 158.0, 131.8, 131.1, 130.3, 130.1, 129.4, 121.7, 115.0, 67.9, 62.3, 49.1, 47.1, 46.7, 45.2, 44.2, 41.8, 33.7, 31.7, 31.5, 31.3, 30.5, 28.4, 28.2, 28.0, 27.8, 27.4, 27.2, 27.1, 26.5, 26.1, 25.5, 25.2, 24.6 ppm; IR: $\tilde{\nu}$ = 3003–2853, 1740, 1664, 1497 cm^{-1}; MS (ESI): *m/z* (%): 407 (100) $[M+Na]^+$, 385 (89) $[M+H]^+$, 357 (21) $[M+H-CO]^+$; HRMS (ESI): *m/z* calcd for $C_{23}H_{32}N_2O_3Na$: 407.2311 $[M+Na]^+$; found: 407.2322.

*Saturated Bicycle **12d***

Compound **12d** was prepared by the same procedure as that described for **4d**. Yield: 93%; R_f = 0.33 (hexane/EtOAc 1:1); m.p. 85.2–85.7 °C;

$[\alpha]_D = -1.0$ ($c = 1.7$ in $CHCl_3$); ^1H NMR (500 MHz, $C_2D_2Cl_4$, 120 °C): $\delta =$ 7.40–7.36 (m, 2H), 7.10–7.03 (m, 3H), 5.46 (br s, 1H), 4.83–4.74 (m, 2H), 4.05 (br s, 0.4H; rotamer), 3.81–3.75 (m, 1H), 3.60–3.50 (m, 3H + 0.6H; rotamer), 3.38–3.36 (m, 1H), 2.93–2.89 (m, 1H), 2.03–1.47 ppm (m, 19H); ^{13}C (125 MHz, $C_2D_2Cl_4$, 120 °C): $\delta = 167.8$, 165.3, 158.0, 129.3, 121.7, 115.0, 67.9, 62.2, 49.1, 47.7, 45.0, 42.1, 33.7, 28.8, 27.7, 27.0, 26.9, 26.8, 26.3, 26.2, 26.1, 25.6, 25.5, 24.6 ppm; IR: $\tilde{\nu} = 3028$–2856, 1740, 1662, 1437 cm^{-1}; MS (ESI): *m/z* (%): 409 (100) $[M+Na]^+$, 387 (25) $[M+H]^+$, 359 (7) $[M+H-CO]^+$; HRMS (ESI): *m/z* calcd for $C_{23}H_{34}N_2O_3Na$: 409.2467 $[M+Na]^+$; found: 409.2439.

Computational Chemistry

All of the calculations were performed with the Gaussian 03 suite of programs.[17] The geometry was optimized by analytical gradient energy minimization. The nature of the located extrema was defined by the inertia of the second-derivative energy (Hessian matrix). For the minima, all of the eigenvalues were positive; in the case of a first-order saddle point as the TS structures, the first eigenvalue was negative and was associated to the imaginary frequency. In the models studied, the related eigenvector components were the geometric variables involved in hydrogen transfer in the pseudo-eight-membered ring.

All absolute energies of the precursors, unsaturated bicycles, saturated bicycles, and all the absolute energies of the TS structures in the simple model (Scheme 6) computed for the unsaturated bicycles and saturated bicycles in the selected conformations can be found in the Supporting Information.

Assay with Resistant PBPs

Purified PBP5 from *E. faecium* D63r and PBP2a from MRSA ATCC 43300 were used as target proteins to test the inhibitory activity of synthesized β-lactams. Each of the purified PBPs (2.5 μM) were first incubated with 1 mM potential inhibitor in 100 mM phosphate buffer, 0.01% Triton X-100,[18] pH 7, for 4 h at 30 °C. Then, 25 μM fluorescein-labeled ampicillin[19] was added to detect the residual penicillin binding activity (RA). The samples were further incubated for 30 min at 37 °C in a total volume of 20 μL. Denaturation buffer was added (0.1 M Tris/HCl, pH 6.8, containing 25% glycerol, 2% SDS, 20% β-mercaptoethanol, and 0.02% bromophenol blue) and the samples were heated to 100 °C for 1 min. The samples were then loaded onto a 10% SDS acrylamide gel (10 × 7 cm) and electrophoresis was performed for 45 min at 180 V (12 mA). Detection and quantification of the RAs were done with Molecular Image FX equipment and Quantity One software (BioRad, Hercules, CA, USA). Three independent experiments were carried out for each inhibitor.

Assay with R39

All assays with R39 were performed in 96-well microtiter plates (Brand, Wertheim, Germany). 20 mM of the tested compounds were dissolved in DMF. Finally, 7.5 μL of the solution were used in the assay. The final concentration of the compounds in the assays was 100 μM. The final concentration of DMF in the assays was 0.25%. R39 (3.5 nM) was incubated in the presence of the potential inhibitors in 10 mM sodium phosphate buffer (pH 7.2) with 100 mM NaCl, 100 mM D-alanine, 0.01 mg mL^{-1} BSA, and 0.01% Triton for 60 min at 25 °C. This preincubation was realized so that slow-binding inhibitors were also detected. After preincubation, the RA value of R39 was determined by observing the hydrolysis of the thioester S2d substrate, in the presence of DTNB, catalyzed by the uninhibited enzyme. The initial rate of hydrolysis of 1 mM S2d in the presence of 1 mM DTNB was determined by monitoring the increase in absorbance at 412 nm (DTNB: $\varepsilon[\Delta\varepsilon] = 13600\ M^{-1}s^{-1}$) by using a microplate absorbance reader (Power Wave X, Biotek Instruments, Winooski, USA). The rate of spontaneous hydrolysis of S2d in the presence of the inhibitors was also determined in absence of R39. All assays were performed three times. The determination of RA of R39 in absence of inhibitors was performed six times on each plate. To detect false positives, which could be slow-binding, noncompetitive promiscuous inhibitors, the assays were performed in the presence of 0.01% Triton-X-100.[20]

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