SYNTHESIS OF PYRROLIDINE-FUSED β-LACTAMS AS POTENTIAL β-LACTAMASE INHIBITORS

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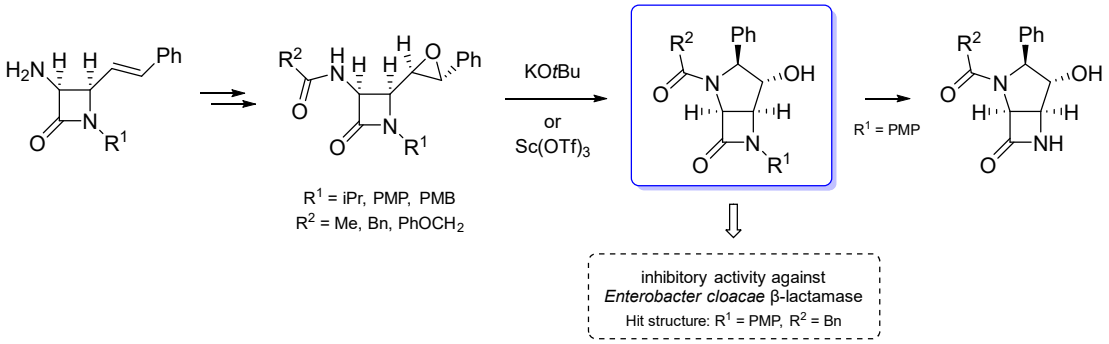
Graphical abstract



Abstract

A synthetic protocol for the preparation of novel 3,4-pyrrolidine-fused β-lactams was developed. The proposed 2,6-diazabicyclo[3.2.0]heptan-7-one scaffolds were constructed through an amido group-induced, potassium *tert*-butoxide-promoted intramolecular ring closure of 3-acylamino-4-oxiranyl-β-lactams as the key reaction step. Alternatively, the desired cyclization was also effected by means of a scandium triflate-mediated catalytic approach. In this way, a set of stereodefined 3,4-pyrrolidine-fused β-lactams was synthesized, which were preliminary evaluated as β-lactamase inhibitors. These first-line biological assessments led to the identification of a 2-benzoyl-6-(4-methoxyphenyl)-substituted diazabicyclo structure as an eligible starting point for further β-lactamase inhibitor optimization studies.

Introduction

Since their serendipitous discovery, antibiotics form a cornerstone of medical health care due to their clinical efficacy and safety. However, in spite of the optimism associated with the golden era of antibiotics in 1950's, the rise of antimicrobial resistance (AMR) has led to a global health crisis in recent decades. In 2019, an estimated 1.27 million deaths worldwide could be attributed to bacterial AMR, making it a prominent cause of death.[1] This number is only expected to increase in the future, hence the important challenge and pressing need to address AMR effectively.

Looking at the current utilization of antibiotics in European Economic Area countries, the class of β-lactam antibacterial agents leads the ranking, accounting for more than 50% of the total antibiotic consumption (community and hospital sector).[2] These antibiotics intervene in bacterial cell wall synthesis through chemical acylation, thereby inhibiting the penicillin-binding proteins (PBPs) which are involved in the biosynthesis of peptidoglycan, the main component of the cell wall. The success of β-lactam antibiotics lies in their resemblance to the natural substrate of the PBPs;[3] however, their effectiveness is severely hampered by the rapid propagation of drug-resistant bacterial strains. While some bacteria produce modified PBPs with lower β-lactam acylation efficiency, others impede the antibiotic uptake by reducing the outer cell membrane permeability or by actively removing the antibiotic from the cell by means of efflux pumps.[4] Finally, some bacteria produce β-lactamases, enzymes that are able to hydrolyze, and thus inactivate, β-lactam antibiotics. Two main strategies can be employed to address β-lactamase-mediated antibiotic resistance. On the one hand, new antibiotics can be developed that are not recognized by β-lactamases and therefore able to inhibit the PBPs. On the other hand, β-lactam antibiotics can be administered together with an additional β-lactamase inhibitor in a combination therapy approach, exemplified by the popular drug Augmentin. Indeed, several β-lactamase inhibitors are in clinical use; however, these agents are not able to inhibit all four classes of β-lactamases (based on the Ambler β-lactamase classification[5]).

In the literature, *C*-fused bicyclic β-lactams have been described as potential class C β-lactamase inhibitors. More specifically, MK-8712 **1** (Figure 1) has been subjected to preclinical studies in the past because of its efficacy in both *in vitro* and *in vivo* assays.[6] Safety studies, however, highlighted its insufficient therapeutic margin, and hence further development was aborted. Embarking on these insights, and bearing the lack of efficient class C β-lactamase inhibitors in mind, previous work in our group focused on the synthesis of 3,4-oxolane-fused bicyclic β-lactams **2**, which showed moderate inhibition of the *Enterobacter cloacae* β-lactamase enzyme (e.g. R^1 = 4-methoxybenzoyl, R^2 = H, CF_3).[7]

In an effort to further boost the β-lactamase inhibitory activity of this type of *C*-fused bicyclic β-lactams, we envisioned the construction of the aza-analogs **3** of the previously developed 3,4-oxolane-

fused bicyclic β-lactams **2**. The rationale for this strategy was based on the premise that the introduction of a nitrogen atom at C3 (instead of oxygen) would result in structures bearing a higher resemblance to the natural β-lactamase substrate. Furthermore, including a trivalent nitrogen atom allows for the introduction of an additional *N*-substituent. In this way, *N*-acylated structures (with an even higher degree of resemblance to the natural substrate) can be produced, which might enable additional beneficial interactions within the catalytic site of β-lactamase enzymes.

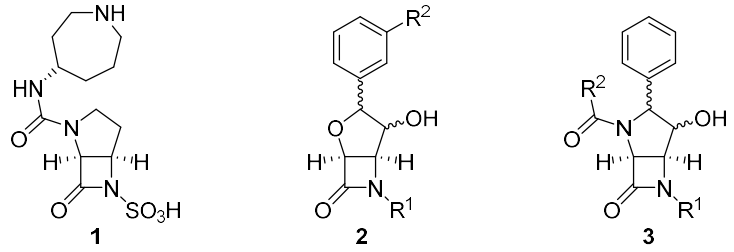


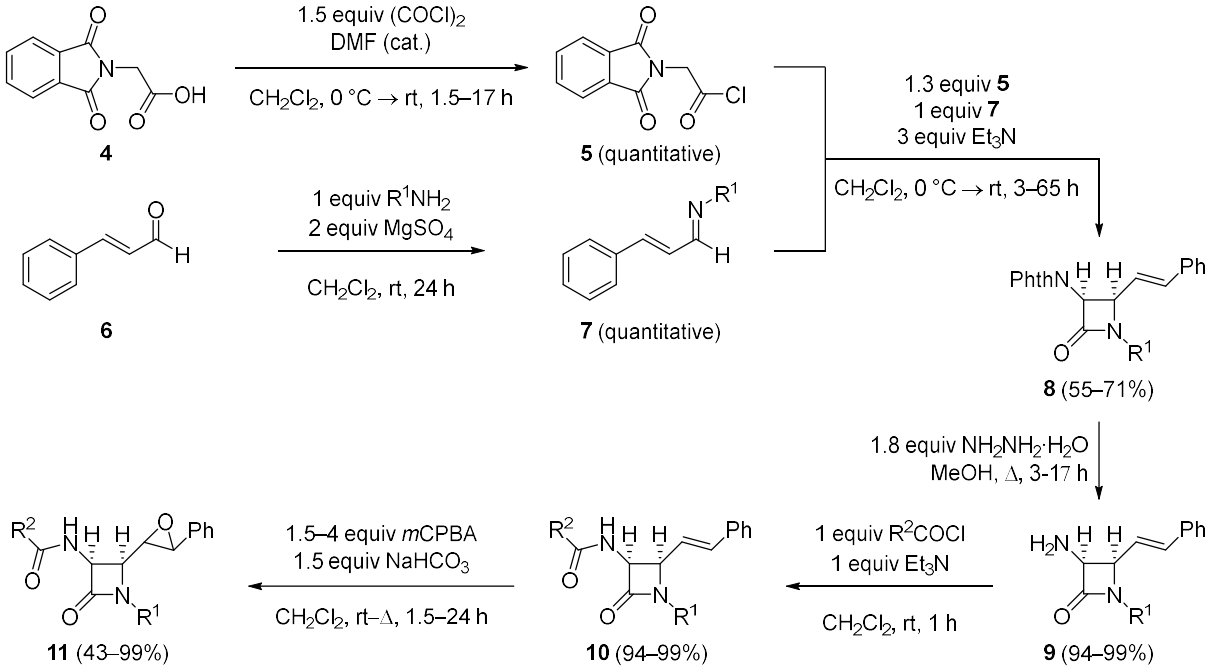
Figure 1. Literature compound **1**, oxolane-fused β-lactams **2** and proposed new pyrrolidine-fused β-lactams **3**.

Results and discussion

The envisioned pathway toward the proposed pyrrolidine-fused bicyclic β-lactams commenced with the synthesis of 4-(3-phenyloxiran-2-yl)azetidin-2-ones **11**. To obtain these key epoxide intermediates, our previously developed procedure for the synthesis of 3-acetoxy-4-oxiranyl-β-lactams was adapted (Scheme 1,

Table 1). In that respect, imination of cinnamaldehyde **6** and treatment of *N*-phthaloyl glycine **4** with oxalyl chloride delivered imines **7** and acid chloride **5**, respectively, which were deployed in the Staudinger synthesis to obtain *N*-phthaloyl-β-lactams **8** (predominantly *cis*). The observed diastereoselectivity was assigned *via* ^{1}H NMR analysis, as the recorded vicinal coupling constants of 5.1–5.6 Hz between the H3 and H4 protons were judged to be in accordance with literature values for *cis*-β-lactams. On a larger scale (50 mmol), the formation of *trans*-β-lactams was observed up to 15%, characterized by H3-H4 vicinal coupling constants of 0–2.4 Hz (based on ^{1}H NMR analyses of the crude reaction mixtures). Nonetheless, the major *cis*-β-lactam isomers **8** were isolated efficiently in pure form by means of column chromatography (SiO_2) in yields ranging from 55 to 71%. After deprotection with hydrazine monohydrate in methanol, the free 3-amino group was acylated using different acid chlorides in the presence of triethylamine, resulting in new 3-acylamino-β-lactams **10** in high yields (94–99%). Subsequently, the styryl double bond was epoxidated using *meta*-chloroperbenzoic acid (*m*CPBA) in a Prilezhaev approach to afford a diastereomeric mixture of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **11**. The relative stereochemistry of these structures could be

established based on vicinal coupling constant analysis in the ^{1}H NMR spectra ($CDCl_3$) (Figure 2), confirming that the *cis* stereochemistry of the β-lactam moiety was retained throughout the synthesis pathway (because of H3-H4 vicinal coupling constants ranging from 4.9 to 5.7 Hz). The *m*CPBA epoxidation strategy is known to result in *trans*-epoxides, corroborated by vicinal coupling constants of 1.3–2.0 Hz recorded for our compounds. Discrimination between diastereomers **11A** and **11B** was possible based on analysis of the coupling constants between the C4 β-lactam and the C1' epoxide proton. The major isomer proved to have a H4-H1' *cis* coupling with values of 6.2–8.1 Hz, while for the minor *trans* diastereomer a vicinal coupling constant of 4.6–5.7 Hz was noted, which are both in accordance with values reported in the literature.[7] The observed diastereoselectivity can be attributed to steric interactions exerted by the β-lactam substituents (e.g. R^1) during the epoxidation, giving rise to the preferential formation of structures **11A** as the major isomers.



Scheme 1. Synthesis of 4-oxiranyl-β-lactams **11** starting from *N*-phthaloyl glycine **4** and cinnamaldehyde **6** (

Table **1**).

Table 1. Overview of the synthesis of 4-oxiranyl-β-lactams **11** and intermediates (Scheme 1).

Entry	R^1	**7** (%)	**8** (%)	**9** (%)	R^2	**10** (%)	**11** (%)	*dr* **11A/11B**[a]
1	*i*Pr	**7a** (99)	**8a** (55)	**9a** (98)	Bn	**10a** (94)	**11a** (59)	64/36
2	4-$MeOC_6H_4CH_2$	**7b** (99)	**8b** (71)	**9b** (99)	Bn	**10b** (99)	**11b** (63–93)	59-85/15-41
3					Me	**10c** (99)	**11c** (83–99)	57-59/41-43
4	4-$MeOC_6H_4$	**7c** (99)	**8c** (89)	**9c** (99)	Bn	**10d** (94)	**11d** (30–48)	81-100/0-19
5					$PhOCH_2$	**10e** (94)	**11e** (79–84)	66-100/0-34
6					Me	**10f** (86)	**11f** (68)	81/19

[a] Based on ^{1}H NMR analysis ($CDCl_3$); for compounds **11b-e**, a range is provided.

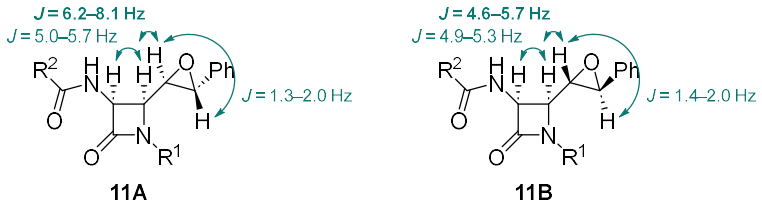
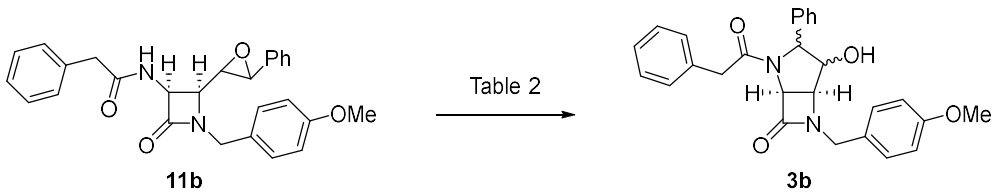


Figure 2. Vicinal coupling constants between the protons at C3 and C4, C4 and C1', and C1' and C2' of *cis*-3-acetamido-4-(3-phenyloxiran-2-yl)azetidin-2-ones **11A** and **11B** in ^{1}H NMR ($CDCl_3$).

The key step in the formation of the target compounds concerns the intramolecular ring closure of intermediate 3-acylamino-4-oxiranyl-β-lactams to construct the desired bicyclic scaffold. While cyclization for the oxa derivatives in previous research occurred spontaneously upon *O*-deacetylation, this step proved to be much more challenging in case of the aza analogs. One of the main reasons for that relates to the low nucleophilicity of an amido nitrogen, in addition to steric hindrance. However, acylation of the 3-amino group was shown to be crucial for the molecule to withstand the reaction conditions required to install the epoxide portion, rendering the direct preparation of *N*-unprotected 3-amino-4-oxiranyl-β-lactams impossible.

Different bases were screened to enhance the nucleophilicity of the amide nitrogen and to enable cyclization, which is summarized in Table 2. Using a catalytic amount of pyridine in ethanol under reflux resulted in full recovery of the starting epoxide **11b**. Similarly, potassium carbonate or cesium carbonate, two popular bases for ring opening of epoxides by amides, were not able to induce pyrrolidine ring formation as well. Heating up the latter reaction to reflux temperature allowed the detection of traces (<5%) of the bicyclic product **3b** *via* LC-MS analysis; however, microwave irradiation for an additional 20 minutes did not induce an increased conversion. Heating an ethanol solution of epoxide **11b** and a large excess of potassium hydroxide at reflux temperature resulted in decomposition of the starting material. Reducing the amount of KOH and lowering the temperature could not prevent degradation. By switching from ethanol to acetonitrile as a solvent, degradation was avoided, and this approach even resulted in traces (<5%) of the desired bicyclic product **3b**. However, further trials with potassium hydroxide were unsuccessful, and thus our focus shifted to other bases. In the literature, only a few reports on amide-induced intramolecular ring openings of epoxides are available. For example, the groups of Spurlock and Dolby both performed an amide-epoxide cyclization reaction using potassium *tert*-butoxide.[8] Although these literature procedures started from conformationally constrained bicyclic precursors, KO*t*Bu also proved to be successful for the synthesis of δ-lactams.[9] Therefore, it was hypothesized that this base might induce the desired ring opening of β-lactam epoxides **11** as well. To that end, oxirane **11b** was treated with two

equivalents of KO*t*Bu in *tert*-butanol under reflux conditions. After two hours, however, only traces (<5%) of bicyclic β-lactam **3b** were observed. In a next attempt, the same amount of KO*t*Bu was used and the reaction was allowed to stir for 19 hours at room temperature, resulting in the successful formation of the desired cyclized β-lactam **3b**, which was isolated in pure form and 28% yield by means of column chromatography. However, the rather long reaction time and challenging purification approach made us look into other bases anyway. A further literature search revealed the total synthesis of the marine alkaloid lepadiformine, in which a similar transformation was accomplished to afford a tricyclic scaffold by using sodium hydride in THF,[10] and a related methodology had been elaborated for the synthesis of *A*-nor-*B*-homo-5-azasteroid analogs.[11] In addition, in the work of Powell, sodium hydride was used to induce intramolecular epoxide ring opening by an amido moiety for the synthesis of 6-hydroxymethyl-2-ketopiperazines.[12] Keeping these findings in mind, sodium hydride was evaluated as a base to obtain the desired 2,6-diazabicyclo[3.2.0]heptan-7-one framework. Unfortunately, applying sodium hydride at room temperature, as well as at reflux temperature, resulted in full recovery of starting epoxide **11b**. After addition of one equivalent of lithium diisopropylamide at room temperature, no reaction was observed as well. Performing the reaction at reflux temperature, however, did result in the observation of traces (<5%) of the desired bicyclic β-lactam **3b**. An additional hour with an extra 1.5 equivalents of base delivered a good conversion toward the cyclized product **3b**, unfortunately as part of a complex reaction mixture. Treatment of epoxide **11b** with two equivalents of *tert*-butyllithium at room temperature left the starting epoxide unaffected. When applying three equivalents and higher temperatures, traces (<5%) of bicyclic β-lactam **3b** were observed after two hours. Unfortunately, after an additional hour at reflux temperature, degradation of both starting and end product was observed. In order to facilitate the cyclization process, some additives were evaluated as well. First, the addition of hexamethylphosphoramide (HMPA) to enhance nucleophilicity through lithium ion complexation was tested, but no effect was observed and only traces (<5%) of the bicyclic end product were detected by LC-MS analysis. In a final attempt, the combination of HMPA with boron trifluoride etherate, deployed to activate the epoxide toward ring opening, was assessed, but also this combination did not result in any improvement.



Scheme 2. Base-induced intramolecular ring closure of 1-(4-methoxybenzyl)-3-phenylacetamido-4-(3-phenyloxiran-2-yl)-β-lactam **11b** (Table 2).

Table 2. Overview of the screened conditions for the intramolecular ring closure of 1-(4-methoxybenzyl)-3-phenylacetamido-4-(3-phenyloxiran-2-yl)-β-lactam **11b** (Scheme 2).

Entry	**11A/11B**	Conditions	Results[a]
1		pyridine (cat.), EtOH, Δ, 3 h	No reaction
2		3 equiv K_2CO_3, CH_3CN, rt, 5 days	No reaction
3		3 equiv Cs_2CO_3, CH_3CN, rt, 4 days	No reaction
		→ Δ, 16 h	Traces of **3b**
		→ MW, 85 °C, 20'	Traces of **3b**
4		4 equiv Cs_2CO_3, CH_3CN, MW, 100 °C, 10'	No reaction
5		25 equiv KOH, EtOH, Δ, 1 h	Decomposition
6		3.5 equiv KOH, EtOH, rt, 3 h	Decomposition
7		1.2 equiv KOH, CH_3CN, rt, 5 days	Traces of **3b**
8		2 equiv KOH, CH_3CN, rt, 5 days	No reaction
9		3 equiv KOH, CH_3CN, Δ, 7 h	No reaction
10		2 equiv KO*t*Bu, *t*BuOH, Δ, 2 h	Traces of **3b**
11		2 equiv KO*t*Bu, *t*BuOH, rt, 19 h	Formation of **3b**[b]
12		2 equiv NaH, THF, rt, 4 h	No reaction
		→ Δ, 19 h	No reaction
13		1 equiv LDA, THF, rt, 6 days	No reaction
14		1 equiv LDA, Δ, 1.5 h	Traces of **3b**
		+ 1.5 equiv LDA, Δ, 1 h	Formation of **3b**[c]
15		2 equiv *t*BuLi, THF, rt, 4 days	No reaction
16		3 equiv *t*BuLi, THF, Δ, 2 h	Traces of **3b**
		→ Δ, 1 h	Decomposition
17		3 equiv *t*BuLi, 1 equiv HMPA, THF, rt, 3 h	Traces of **3b**
		→ Δ, 3 h	Traces of **3b**
18		3 equiv *t*BuLi, 1 equiv HMPA, THF, Δ, 1 day	Traces of **3b**
19		3 equiv *t*BuLi, 1 equiv HMPA, 1 equiv $BF_3{\cdot}Et_2O$, THF, Δ, 1 day	Traces of **3b**

[a] Based on LC-MS analysis.

[b] Purified by means of column chromatography (C18), 28% isolated yield of compound **3Ab**.

[c] **3b** formed as part of a complex reaction mixture.

Bearing the results of this extensive base screening in mind, potassium *tert*-butoxide was selected as the base of choice, as that strategy did afford the desired bicyclic target structure in an acceptable yield and high purity (Table 2, Entry 11). After further optimization in terms of reaction temperature,

(co-)solvents and stoichiometry, ring closure of epoxide **11b** was accomplished utilizing three equivalents of KO*t*Bu in *tert*-butanol at 35 °C to deliver 3,4-pyrrolidine-fused bicyclic β-lactam **3Ab** in 34% yield after column chromatography (C18). Only one diastereomer was formed, which was believed to be attributable to the S_N2-type epoxide ring opening of major diastereomer **11Ab**. Irrefutable proof for the molecular framework and the relative stereochemistry was provided by means of single crystal X-ray analysis of (1*S**,3*S**,4*S**,5*S**)-4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **3Ab**. Next, the same conditions were applied to all other epoxides **11** with the intention to prepare the corresponding 3,4-pyrrolidine-fused bicyclic β-lactams **3**. Fortunately, only one diastereomer was observed for all derivatives due to the preferred reactivity of major diastereomers **11A** over their isomeric counterparts **11B**, and purification by means of column chromatography (C18) delivered pure samples of unprecedented bicyclic β-lactams **3A**.

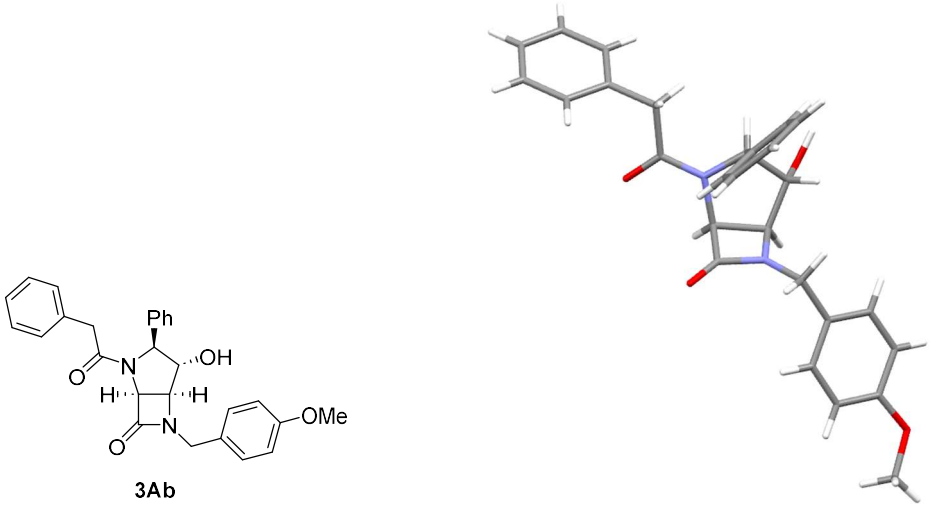
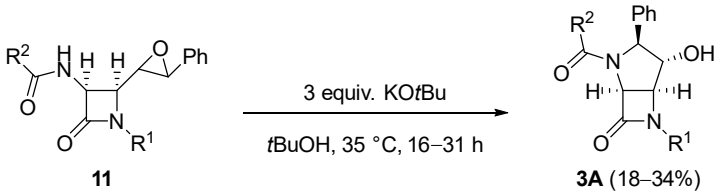


Figure 3. X-ray analysis molecular structure in stick representation of (1*S**,3*S**,4*S**,5*S**)-4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **3Ab**.



Scheme 3. Intramolecular ring closure of epoxide **11** toward 3,4-pyrrolidine fused β-lactam **3A**.

Table 3. Synthesis of bicyclic β-lactams **3A**, starting from epoxides **11**.

Entry	R^1	R^2	*dr* **11A**/**11B**	**3A** (%)[a]
1	*i*Pr	Bn	68/32	**3Aa** (26)
2	4-$MeOC_6H_4CH_2$	Bn	95/5	**3Ab** (34)
3		Me	57/43	**3Ac** (18)
4	4-$MeOC_6H_4$	Bn	81/19–100/0[b]	**3Ad** (20–26)[b]
5		$PhOCH_2$	68/32	**3Ae** (31)[c]
6		Me	83/17	**3Af**[d]

[a] After purification by column chromatography (C18), unless mentioned otherwise.

[b] Repetition of the reaction led to small variations in terms of yield and selectivity.

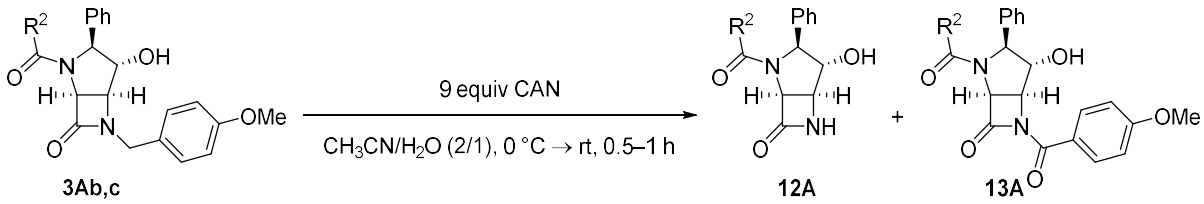
[c] **3Ae** was obtained in 45% yield by the $Sc(OTf)_3$-promoted cyclization method: 0.2 equiv $Sc(OTf)_3$, toluene, 60 °C, 6 h.

[d] Due to the small scale of the reaction, bicyclic β-lactam **3Af** could not be isolated.

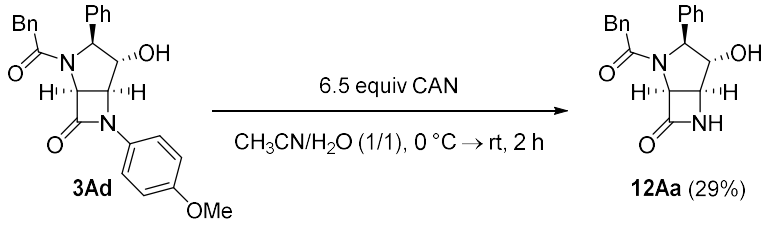
Nonetheless, in an attempt to further enhance the efficiency of this synthetic protocol, additional efforts were made to look for an improved procedure for the cyclization of 3-acylamino-4-oxiranyl-β-lactams **11**. As mentioned above, most literature precedents apply bases for amide-induced ring openings of epoxides, in addition to a few spontaneous cyclizations of e.g. transient 1-(oxiran-2-ylmethyl)guanidines.[13] In that respect, instead of activating the carbamoyl group, an alternative strategy could involve the activation of the epoxide moiety by a Lewis acid to render it more electrophilic. In one of the above-mentioned attempted cyclization procedures, boron trifluoride etherate was added to facilitate epoxide ring opening (Table 2, entry 19), albeit without effect. With the intention to further investigate the potential Lewis acid-catalyzed intramolecular ring opening of our epoxides **11**, a literature screening was performed. This effort revealed an interesting approach developed by Johnson and co-workers concerning the total synthesis of pactamycin, in which a $Sc(OTf)_3$-promoted epoxide ring opening by 3-acetylaniline was realized. Inspired by these findings, a similar strategy was applied to compound **11e** as a model substrate for all epoxides **11**. In a first attempt, using one equivalent of $Sc(OTf)_3$ in toluene at room temperature, a small amount (~15%) of the desired bicyclic product **3** was formed, but further elaboration eventually led to the efficient construction of phenoxyacetyl-substituted analog **3Ae** upon treatment of epoxide **11e** with 0.2 equivalents of $Sc(OTf)_3$ in toluene at 60 °C. This method thus offers an attractive alternative for the KO*t*Bu-promoted approach, in which work-up is complicated by the presence of *tert*-butanol.

As established in the literature and confirmed by our previous studies, the presence of an electron-withdrawing group at the N1 β-lactam position enhances the chemical reactivity of the β-lactam ring system, and thereby also its biological activity. In order to install different electron-withdrawing

groups, N1-unsubstituted derivatives (i.e. NH-β-lactams) are desired. To that end, the removal of 4-methoxybenzyl and 4-methoxyphenyl protecting groups is mostly performed oxidatively by means of cerium ammonium nitrate (CAN). However, it is known that treatment of 1-(4-methoxybenzyl)-β-lactams with CAN often results in (partial) benzylic oxidation, affording the corresponding 1-(4-methoxybenzoyl)-β-lactams. Anyhow, because of the electron-withdrawing character of this benzoyl-type N1 substituent, and given our previous work on 3,4-oxolane-fused bicyclic *N*-benzoyl-β-lactams, we were interested in the biological activity of 1-(4-methoxybenzoyl)-substituted derivatives as well. In our experience, benzoyl formation is promoted when using a high amount of CAN (nine equivalents). Thus, with the intention to produce 1-(4-methoxybenzoyl)-β-lactams, 1-(4-methoxybenzyl)-β-lactams **3Ab,c** were treated with nine equivalents of cerium ammonium nitrate (CAN) (Scheme 4). After one hour, a mixture of *N*-unsubstituted **12A** and *N*-benzoyl-β-lactam **13A** was obtained in ratio's ranging from 13/87 to 18/82. The major product, i.e. the desired 1-(4-methoxybenzoyl)-β-lactam **13A**, was purified and isolated by means of column chromatography (C18). To obtain the free NH analogs, the *N*-(4-methoxyphenyl) derivative **3Ad** was treated with 6.5 equivalents of CAN over the course of the reaction, resulting in the formation of bicyclic NH-β-lactam **12Aa** in 29% yield (Scheme 5). The low yield of this step can be attributed to the small scale and the difficult work-up afterward. Nevertheless, the successful formation and isolation of this NH-β-lactam **12Aa** creates opportunities for the installation of different electron-withdrawing groups at this position in follow-up studies in order to enhance and/or finetune the reactivity of the resulting β-lactams and the associated biological activity.



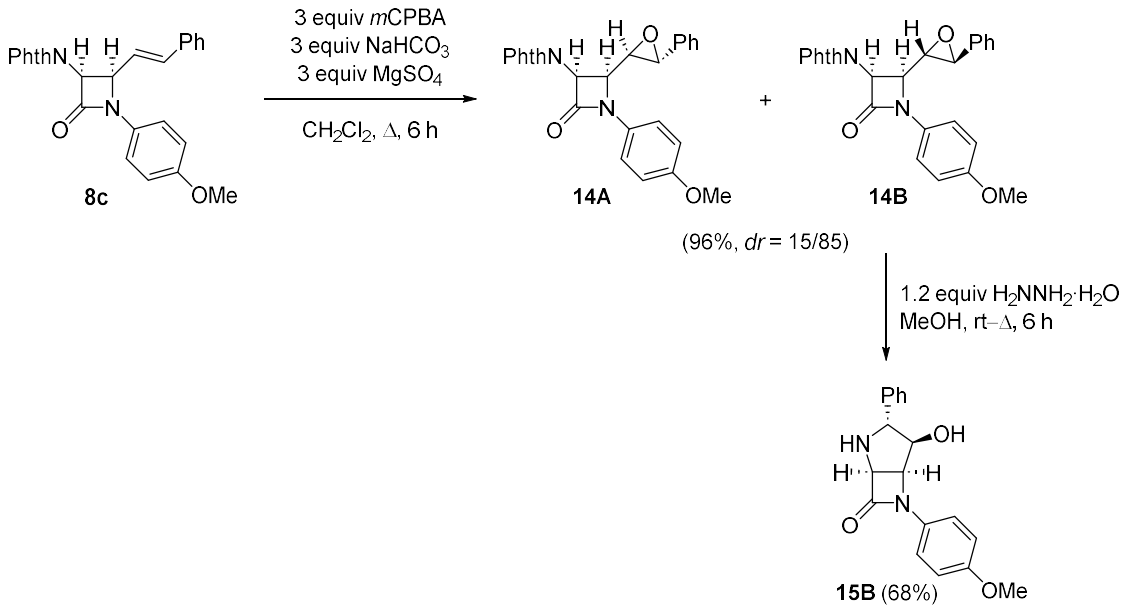
Scheme 4. Treatment of 4-methoxybenzyl substituted β-lactams **3Ab,c** with CAN toward 4-methoxybenzoyl substituted analogs **13A**.



Scheme 5. Removal of the 4-methoxyphenyl group toward N-unsubstituted analog **12Aa**.

Due to the high reactivity of epoxides toward ring opening, hydrazinolysis of the phthaloyl group was performed prior to epoxidation of the styryl double bond (Scheme 1). However, the ability of the epoxide to withstand ring opening under hydrazinolysis conditions was investigated as well. In that respect, 3-phthaloyl-4-styryl-β-lactam **8c** was epoxidized using *meta*-chloroperbenzoic acid in the presence of sodium carbonate and magnesium sulfate, resulting in 4-oxiranyl-β-lactam **14** as a diastereomeric mixture (*dr* = 15/85) (Scheme 6). Interestingly, in contrast to the 3-acetamido analogs **11**, the major epoxide concerned the (1*S**,3*R**,4*R**,5*S**)-isomer **14B** and not the (1*S**,3*S**,4*S**,5*S**)-isomer **14A**, as determined by ^{1}H NMR analysis ($CDCl_3$). With these epoxides in hand, the deprotection of the 3-amino group was investigated next using hydrazine monohydrate, as described for 3-phthaloyl-β-lactams **8**. In a first trial, ring opening of the epoxide by hydrazine was observed (based on LC-MS analysis), which was expected due to the reactive nature of the epoxide. However, addition of only 1.2 equivalents of hydrazine monohydrate and reaction at room temperature resulted in a mixture of the hydrazine-3-phthaloyl-β-lactam adduct and fully deprotected β-lactam. The mixture was then heated to reflux temperature to effect full deprotection of the amino functionality. ^{1}H NMR analysis ($CDCl_3$) showed the formation of bicyclic β-lactam **15B**, which is the result of direct intramolecular ring closure through epoxide ring opening by the amino group.

By this route, new 4-hydroxy-6-(4-methoxyphenyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-one **15B** was synthesized and isolated, which can be deployed as a universal building block toward 2-acyl-substituted analogs in future work. Furthermore, the opposite diastereomer is obtained as compared to the base-mediated approach, allowing the comparison of the β-lactamase inhibitory activity of the two diastereomers.



Scheme 6. Synthesis of N2-unsubstituted diazabicyclo[3.2.0]heptan-7-one **15B**.

In order to gain a preliminary insight into the potential of 2,6-diazabicyclo[3.2.0]heptan-7-ones as β-lactamase inhibitors, an initial screening of their inhibitory activity against *Enterobacter cloacae* β-lactamase was performed. In that respect, compounds **3Aa–e**, **12Aa**, **13Aa–b** and **15B** (500 μM) were incubated with β-lactamase (final concentration of 2.5 μg/mL), after which the residual activity (RA) was measured by following the rate of nitrocefin hydrolysis. Although none of these molecules exhibited higher activity than the reference compound tazobactam, some trends can be noted based on the obtained data. Phenylacetyl-substituted analogs **3Ab** and **13Aa**, with residual activities of 62 ± 9% and 49 ± 6%, respectively, performed better than their acetyl-substituted counterparts **3Ac** and **13Ab** which showed no inhibition at the tested concentration, suggesting that the acetyl group does not result in additional interactions or is too small to prevent β-lactam hydrolysis. The introduction of an additional oxygen atom in the *N*-acyl group (2-phenoxyacetyl substituent) did not elicit enhanced inhibitory activity either, as no inhibition was observed under the applied conditions for compound **3Ae**. Based on the lack of β-lactamase inhibition by compounds **12Aa** and **15B**, substitution of both nitrogens seems to be desirable for inhibitory activity.

A more pronounced enzyme inhibition effect was observed for 4-methoxybenzoyl-substituted derivative **13Aa** as compared to its non-oxidized analog **3Ab**, which was expected as an electron-withdrawing group at the β-lactam nitrogen is known to enhance the reactivity of this four-membered ring system. Surprisingly, the highest inhibitory activity was measured for 4-methoxyphenyl-β-lactam **3Ad** (RA = 23 ± 2%), which was better than all previously evaluated oxa analogs. Due to the mild

electron-donating character of the 4-methoxyphenyl group, ring opening of the β-lactam core, needed for β-lactamase inhibition by formation of a covalent adduct, was expected to proceed less efficiently than with *N*-benzoyl-substituted analogs. In a final experiment, the 50% inhibitory concentration (IC_{50}) for this best-performing 2,6-diazabicyclo[3.2.0]heptan-7-one compound **3Ad** and tazobactam were determined, and IC_{50} values of 120 µM and 4.5 nM were obtained, respectively. Although follow-up research is certainly necessary to further boost the β-lactamase inhibitory activity of these bicyclic scaffolds, the identification of structure **3Ad**, showing micromolar activity and outperforming the best-in-class 3,4-oxolane-fused bicyclic β-lactam from our previous study, provides an eligible starting point for further elaborations *en route* to more potent inhibitors.

Table 4. Residual enzymatic activity after incubation of 4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **3Aa–e**, **12Aa**, **13Aa–b**, and **19B** (500 µM) with β-lactamase from *Enterobacter cloacae*.

Compound	R^1	R^2	Residual activity (%)[a]
3Aa	*i*Pr	Bn	103 ± 4
3Ab	4-$MeOC_6H_4CH_2$	Bn	62 ± 9
3Ac		Me	99 ± 8
3Ad	4-$MeOC_6H_4$	Bn	23 ± 2
3Ae		$PhOCH_2$	97 ± 6
12Aa	H	Bn	102 ± 10
13Aa	4-$MeOC_6H_4CO$	Bn	49 ± 6
13Ab		Me	103 ± 4
15B	4-$MeOC_6H_4$	/	104 ± 3

[a] Tazobactam as reference compound (complete inhibition of activity was observed). All reactions were performed in tripiclate.

Conclusion

In summary, a synthetic approach toward novel stereodefined 3,4-pyrrolidine-fused bicyclic β-lactams was developed, based on a potassium *tert*-butoxide-mediated tandem cyclization-ring opening of intermediate 3-acylamino-4-oxiranyl-β-lactams as the key reaction step. Further investigations resulted in a scandium triflate-mediated catalytic approach as an attractive alternative route toward these unprecedented bicyclic structures. Benzylic oxidation of *N*-(4-methoxybenzyl)-β-lactams delivered 2-acyl-4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones, changing the electronic properties at the N6 position with the intention to facilitate β-lactam ring opening. Preliminary screening of β-lactamase inhibitory activity revealed the potential of a 6-(4-methoxyphenyl)-2-(2-phenylacetyl)-substituted analog, demonstrating micromolar activity, as an eligible starting point for future work. Furthermore, the successful formation of a 6-unsubstituted 2,6-

diazabicyclo[3.2.0]heptan-7-one system provides opportunities for further chemical modifications to enhance β-lactamase inhibitory activity in follow-up studies.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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