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In silico design and enantioselective synthesis of functionalized monocyclic 3-amino-1-carboxymethyl-β-lactams as inhibitors of penicillin-binding proteins of resistant bacteria

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Abstract

As a complement to the renowned bicyclic β-lactam antibiotics, monocyclic analogues provide a breath of fresh air in the battle against resistant bacteria. In that framework, the present study discloses the *in silico* design and unprecedented ten-step synthesis of eleven nocardicin-like enantiomerically pure 2-{3-[2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-oxoazetidin-1-yl}acetic acids starting from serine as a readily accessible precursor. The capability of this novel class of monocyclic 3-amino-β-lactams to inhibit penicillin-binding proteins (PBPs) of various (resistant) bacteria was assessed, revealing the potential of α-benzylidenecarboxylates as interesting leads in the pursuit of novel PBP inhibitors. No deactivation by representative enzymes belonging to the four β-lactamase classes was observed, while weak inhibition of class C β-lactamase P99 was demonstrated.

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Introduction

β-Lactam antibiotics undoubtedly represent a cornerstone in global health care. Through their effectiveness in inhibiting penicillin-binding proteins (PBPs), the D,D-transpeptidase enzymes that catalyze the crosslinking of peptidoglycan (the major component of the bacterial cell wall), β-lactam antibiotics have gained worldwide attention.[1] More specifically, by functional mimicry of the natural substrate of these serine enzymes, *i.e.* the C-terminal D-alanyl-D-alanine moiety of the stem peptide, a stable acyl-enzyme complex is formed with β-lactam antibiotics. These act as suicide inhibitors and cause missteps in the cell wall biosynthesis, thus provoking growth inhibition or lysis.[2],[3] This powerful mechanism has made penicillins **1** and their analogues the most widely used antibiotics for any bacterial infectious disease over the past 70 years.[4] Disappointingly, however, β-lactam and other antibacterial agents have been challenged by the propagation of drug-resistant bacterial strains, developing alarmingly fast, while the pace of antibiotic discovery has dropped significantly.[5] In that framework, the development of innovative chemical entities is of paramount importance in order to effectively address and counteract microbial resistance. While some pathogens evade β-lactam action by the production of β-lactamases, others impede the uptake of antibiotics *via* decreased permeability of the outer membrane, or remove the antibiotics from the bacterium *via* efflux pumps. Finally, certain Gram-positive bacteria produce drug-insensitive PBPs.[6] *Via* so-called active-site distortion, the active-site size and hence the β-lactam acylation efficiency is dramatically reduced (*e.g*. PBP2x of *Streptococcus pneumoniae*, PBP2a of *Staphylococcus aureus* (MRSA), PBP5fm of *Enterococcus faecium)*.[4],[7] In order to overcome this intrinsic low acylation susceptibility, we envisioned the design of β-lactam compounds that are smaller than classic penicillins **1** and cephalosporins **2**, but still retain their acylation potential: monocyclic β-lactams. Indeed, as we demonstrated recently *via* a three-decade literature overview concerning the structure-activity relationships of diverse classes of monocyclic β-lactams (*e.g.* monobactams **3**), β-lactams do not require a conformationally constrained bicyclic scaffold to exert their antibacterial properties, suggesting that a suitably functionalized azetidin-2-one ring constitutes an adequate pharmacophore.[8],[9] Such a milestone achievement in the quest for monocyclic β-lactam inhibitors involved the development of aztreonam **4**, the first, and so far only, synthetic monobactam approved by the FDA in 1986.[10] Isolated from *Nocardia uniformis* in the late 70s, the first monocyclic β-lactams to be discovered, though, concerned the nocardicins **5**.[11] Originally, they possessed only limited antibacterial activity and required chemical modifications to increase their bioactive potential.[12] Still, however, this class of monocyclic β-lactams did not receive that much of attention.[8] In that respect, the promising potential of the nocardicins, combined with the clinical success of aztreonam **4**, prompted us to pursue the development of novel monocyclic 3-amino-1-carboxymethyl-β-lactam analogues with possibly enhanced biological activities. Within this

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larger framework, our first challenge comprised the successful synthesis of a library of new nocardicin-like analogues **5** (Figure 1) in which R^3 represents alkylic, olefinic, acylic or enolic side chains, in order to expand the library of aromatic features inherent to the nocardicin class.

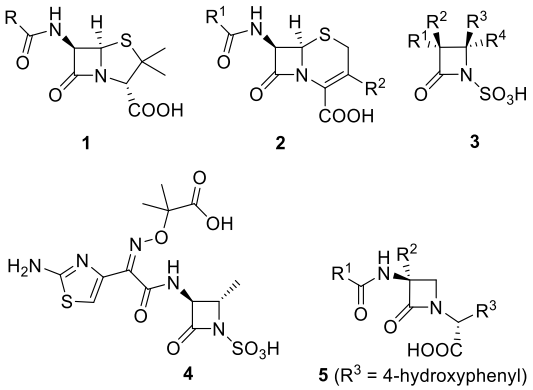

Figure 1. Penicillins **1**, cephalosporins **2**, monobactams **3**, aztreonam **4** and nocardicins **5**.

Results and discussion

1. *In silico* design

The vast structural modifications previously included in numerous penicillins **1** and cephalosporins **2** signify the possibility of incorporating these side chains in monocyclic azetidin-2-ones as well. Therefore, as a starting point for this study, *in silico* techniques were applied to construct a virtual combinatorial library of novel monocyclic 3-amino-1-carboxymethyl-β-lactams with potential activity against PBP5fm (*Enterococcus faecium*) (Figure 2, for more details concerning these computational techniques, see Supporting Information). The designed monocyclic β-lactam compounds all comprised scaffold **6**, as shown in Figure 2a. The pharmacophore consisted of i) a 4-unsubstituted 3-amino-1-carboxymethyl-β-lactam nucleus, securing the limited size of the core skeleton and mimicking the natural substrate of the PBPs, D-alanyl-D-alanine; the carboxylic acid moiety enables crucial molecular interactions with the enzyme's active site and the possibility to form a destabilizing and thus activating hydrogen bond with the β-lactam carbonyl group, which can, in a second stage, stabilize the ring-opened azetidin-2-one after acylation of the active site serine residue, as was postulated by Dobrowolski *et al*.[13]; ii) a renowned 2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(hydroxyimino)acetamido substituent linked to the β-lactam C3-position (this element will eventually be replaced by a similar 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido group for synthetic reasons)[8]; and finally, iii) a side chain 'R', a variable item in our combinatorial library which was carefully examined during the subsequent virtual screening process. Following further fine filtering and conformer generation, the

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resulting dataset was available for *ligand-based drug design*. A query pharmacophore was constructed from a substructure **8** of the fifth-generation cephalosporin ceftobiprole **7** and its monocyclic β-lactam analogue **9** (Figure 2b). The virtual structures were superimposed and a shared pharmacophore was calculated, after which each ligand was scored for its electronic and steric similarity with the query ('virtual screening', Figure 2c). This virtual screening campaign afforded 64 hits, which were visually inspected for their synthetic feasibility.

Additionally, the highest scoring compounds were selected for *in silico* covalent docking studies using PBP5fm, co-crystallized with a covalently bound ceftobiprole **7** molecule, in order to evaluate their binding mode (*structure-based* drug design, Figure 2d).[14] The resulting β-lactam hits, of which a small selection is presented in Figure 2e, show that a high variety of side chains is tolerated in the PBP's active site. In general, most side chains have in common that they reveal the preference for a side chain ('R') embedding a lipophilic linker and a terminal hydrogen bond-donating or -accepting functionality ('HBD/HBA'), enabling additional non-covalent interactions with the enzyme. In a next stage, keeping the latter in mind, the synthesis of a broad, model library of target compounds was accomplished at first instance, paving the way for potential fine-tuning of the selected side chains after preliminary biological evaluation.

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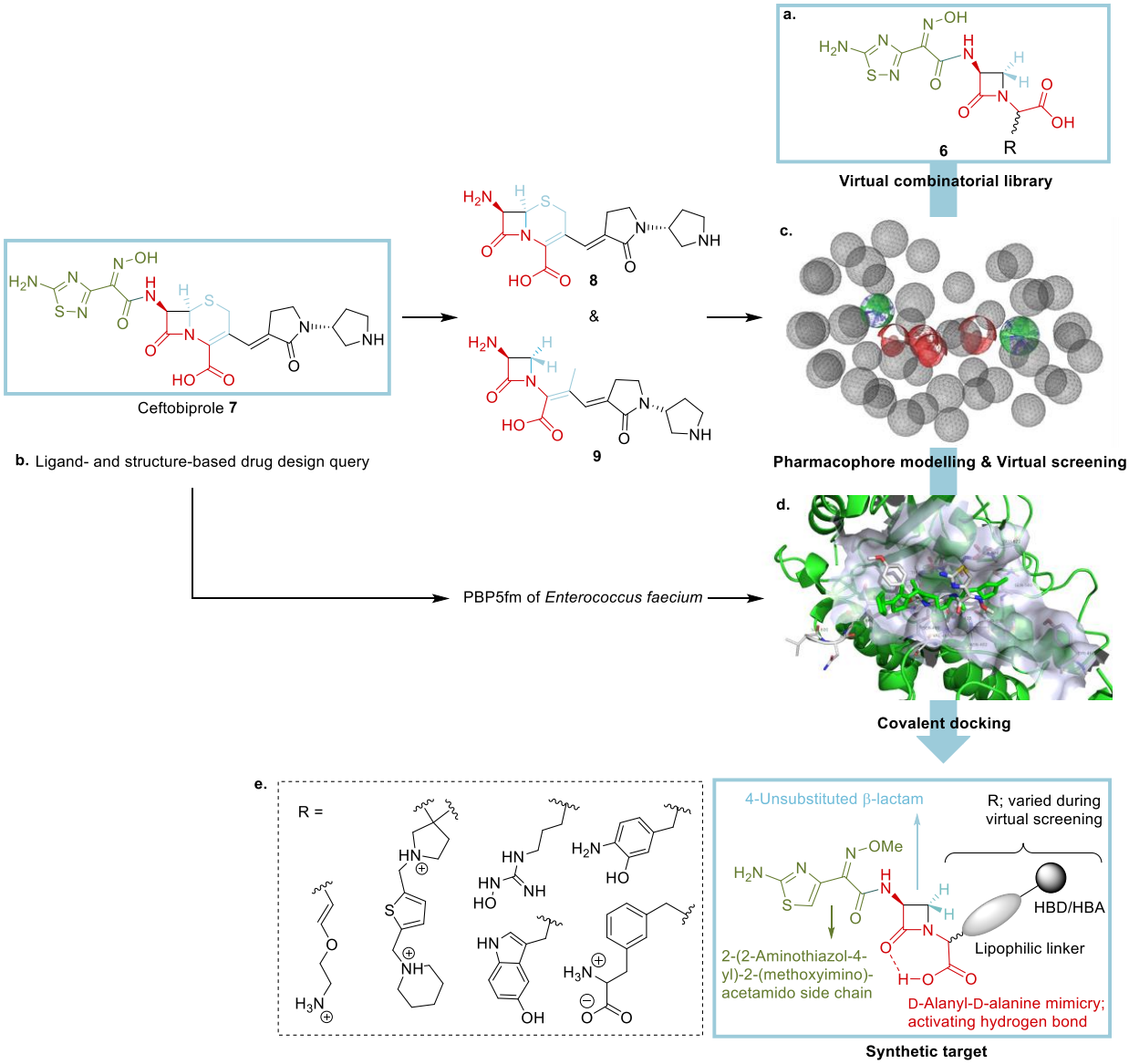
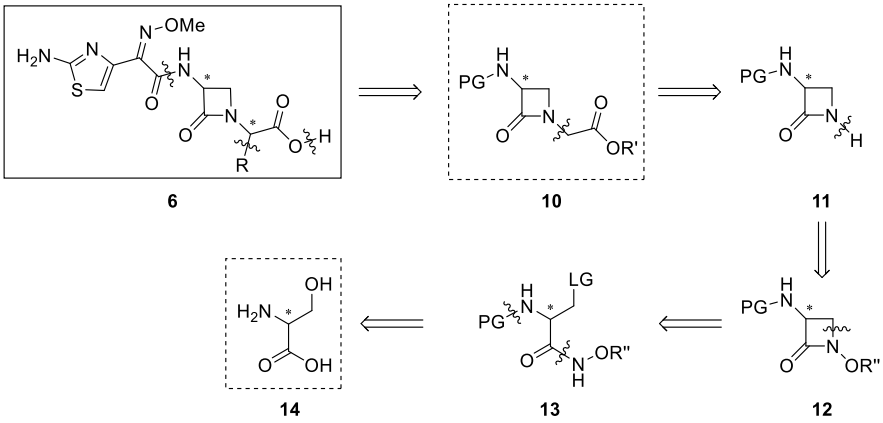


Figure 2. *Ligand- and structure-based drug design.* a: Virtual combinatorial library; b: Ceftobiprole **7** query, indicating the 3-amino side chain in green and the D-alanyl-D-alanine mimicry in red; c: Virtual screening, superimposing the virtual library compounds on the pharmacophore model, constructed using ceftobiprole substructures **8** and **9**; d: 3D-representation of covalent docking of a modeled inhibitor (grey) and ceftobiprole **7** (green) in PBP5fm; e: Design rationale and/or synthetic target of novel functionalized monocyclic 4-unsubstituted 3-amino-1-carboxymethyl-β-lactams and a selection of hits resulting from the virtual screening campaign.

2. Chemical synthesis

Inspired by the molecular structures discovered *via* virtual screening techniques, a new synthetic pathway was devised for the organic synthesis of these highly complex compounds, as presented in Scheme 1. The absence of a C4-β-lactam substituent complicates the synthesis of the contemplated 3-aminoazetidin-2-ones, as only few methodologies are described for their formation.[15] The proposed synthetic strategy to afford α-substituted 2-{3-[2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-

2-oxoazetidin-1-yl}acetic acids **6** adopts Miller's hydroxamate synthesis of 4-unsubstituted 3-amino-β-lactams **12**, starting from the amino acid serine **14** through Mitsunobu (N1-C4)-cyclization after the required modification and protection steps (Scheme 1).[15e] N1-Deprotection and -alkylation then enables derivatization of key building block **10** through α-functionalization, amino group deprotection and -acylation and final release of the essential carboxylic acid functionality.



Scheme 1. Retrosynthetic strategy for the synthesis of α-substituted 2-{3-[2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-oxoazetidin-1-yl}acetic acids **6**. PG = protecting group; LG = leaving group.

As such, upon thorough optimization and modification of literature procedures, key intermediates **10** were obtained after six reaction steps in up to 65% overall yield (Scheme 2). The biologically active form of final compounds **6** is supposed to have the 3'*S*-configuration. Nevertheless, both enantiomers were synthesized here for comparison of their bioactivity and to assess their optical purity. In that respect, after quantitative *tert*-butyloxycarbonyl (Boc) protection of easily available precursors L- and D-serine **14a-b** by means of Boc anhydride in a (1/1)-mixture of dioxane and aqueous sodium hydroxide (1 M),[16] hydroxamate synthesis was performed. To that end, after testing several alternatives (based on the use of DCC, EDC/HOBt and EDC/Oxyma Pure), a TBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate)-mediated coupling reaction was performed using the hydrochloric acid salt of *O*-benzylhydroxylamine and *N*-methylmorpholine (NMM) as a base, affording (*S*)- and (*R*)-*N*-benzyloxy-2-(*tert*-butyloxycarbonylamino)-3-hydroxypropanamides **16a-b** (Scheme 2).[17] Subsequently, cyclization-prone substrates **16a-b** were subjected to Mitsunobu conditions (DEAD, PPh_3), resulting in β-lactam ring formation.[18] As a second amino protecting group proved to be necessary during later reaction steps, a second Boc-group was introduced under DMAP catalysis (4-(dimethylamino)pyridine) to produce β-lactams **18**.[19] A final Raney Nickel-mediated hydrogenolysis[17a] and alkylation with methyl bromoacetate using caesium carbonate as a base, furnished 3-(di-Boc-amino)-1-(methoxycarbonylmethyl)azetidin-2-ones **20a-b** as central building blocks for further functionalization *en route* to the target β-lactams **6**.

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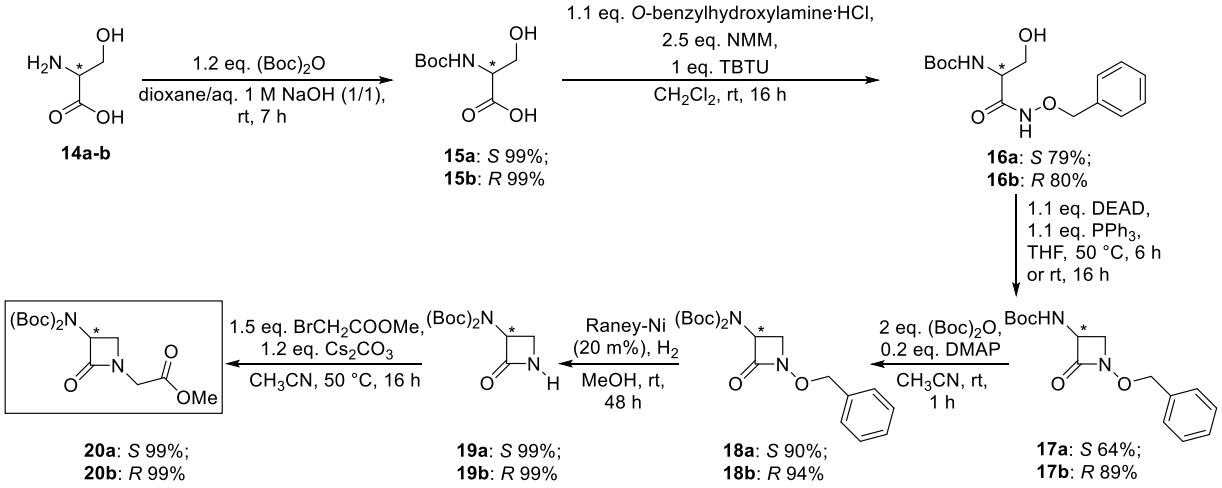

Scheme 2. Six-step synthesis of di-Boc-protected 3-amino-1-methoxycarbonylmethyl-β-lactams **20** in 44-65% overall yield.

A crucial reaction step toward the desired 3-amino-1-carboxymethyl-β-lactams **6** concerned the introduction of a side chain in α-position with respect to the ester carbonyl group *via* enolate alkylation or acylation (Scheme 3). An overview of the final target structures with the various side chains introduced, obtained in this way, is presented in Figure 3. As the scientific literature only provides a few reports on the coupling of similar substrates with low-molecular-weight and highly reactive electrophiles such as short-chain aliphatic acid chlorides, alkyl halides and aldehydes,[20] significant optimization seemed obvious and necessary. Indeed, only after elaborate screening of i) numerous commercially available or newly synthesized electrophiles (alkyl halides, acid chlorides, aldehydes); ii) different bases (bis(trimethylsilyl)amide bases such as LiHMDS, KHMDS, NaHMDS); iii) temperatures (-96 °C up to reflux); iv) concentrations (0.01-0.1 M); v) additives (*e.g.* NaI to accomplish a Finkelstein reaction,[21] or lithium solvating agents such as HMPA (hexamethylphosphoramide), crown ethers or DMPU (1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone)) and vi) the order of addition and the number of equivalents of the reagents, optimal reaction conditions were established. Firstly, some model derivatives with less functionalized but chemically interesting side chains in α-position of the carboxylate were envisioned and considered as anchor points to validate the new synthetic methodology. Despite the absence of H-bond-donors or -acceptors, but with the potential to provoke π-stacking interactions in the enzyme's active site cavity, a benzyl derivative seemed convenient for that purpose. Unfortunately, it appeared not to be possible to selectively form the desired monoalkylated products **21A-a-b** (as a diastereomeric mixture), as a smaller amount of the corresponding dialkylated products **22A-a-b** was present in all cases (Table 1). In additional studies, it soon became clear that the choice of optimal reaction conditions for further application would be a trade-off between sufficient conversion of the starting material on the one hand, and a satisfying ratio of mono- *vs*. dialkylated product on the other hand. Nonetheless, the major monoalkylated substrates

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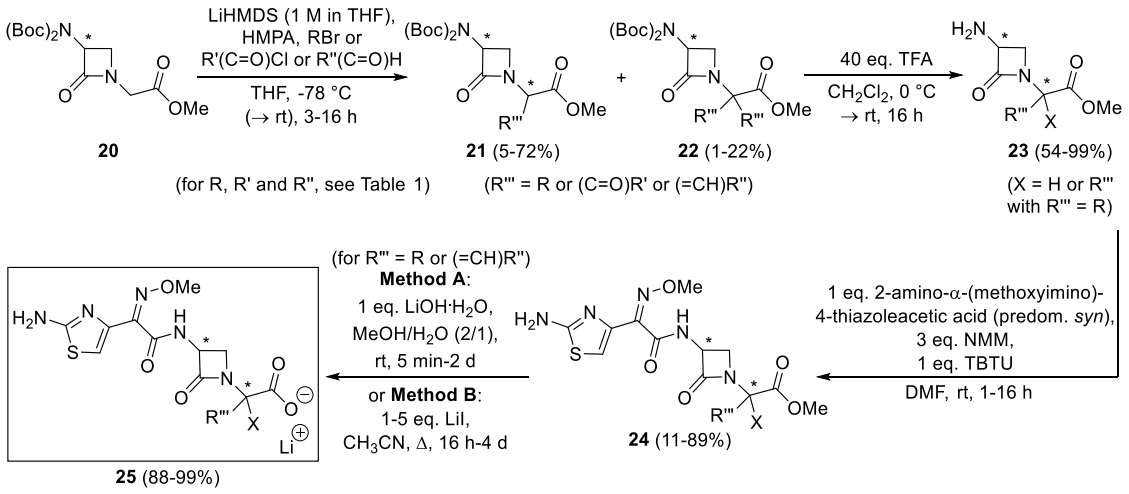
21A-a-b could be isolated in an analytically pure form in moderate yields *via* (reversed phase) column chromatography. In Table 1, the successful synthesis of the latter and eight other, gradually more complex derivatives containing the envisioned functionalities (*i.e.* lipophilic linker with HBD/HBA), is presented. Indicated by the isomeric ratios of the obtained monoalkylated adducts **21**, no effect of the stereochemistry at the C3-position of the β-lactam nucleus on the preferential formation of one of both isomers could be observed. The non-crystalline physical state of these products did not allow X-ray analysis to offer a decisive answer regarding the stereochemical identity with respect to the α-position of each of both isomers, nor could Nuclear Overhauser Effect (NOE) analysis, probably attributable to the free rotatability of the N1-substituent in these compounds. However, for α-unsaturated Boc-feruloyl (enol tautomer) and benzylidene analogues **21H-I** (Table 1), NOESY analysis provided clarity with respect to the configuration of the α-positioned double bond, as will be discussed in more detail in a next section.

Prior to decorating the 3-amino functional groups with the convenient side chain, deprotection of the monoalkylated **21A-I-a-b** and some dialkylated intermediates **22D-E-a-b** in trifluoroacetic acid (TFA)-environment for 16 hours at room temperature afforded 3-amino-β-lactams **23a-b**, the diastereomeric ratios and yields of which are displayed in Table 2. Subsequently, the free amino groups were acylated with commercially available 2-amino-α-(methoxyimino)-4-thiazoleacetic acid (predominantly *syn*) using TBTU as a coupling reagent and NMM as a base. Stirring in DMF at room temperature for 1 to 16 hours furnished 3-[2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-1-(methoxycarbonylmethyl)azetidin-2-ones **24a-b**, which were either further purified *via* conventional purification techniques resulting in isomeric mixtures, or additionally separated *via* preparative HPLC affording isolated isomers (Table 2).[22]



Scheme 3. Functionalization of 3-amino-1-methoxycarbonylmethyl-β-lactams **20** toward target products **25**.

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Following the docking experiments and crystallization studies of PBP5fm in the presence of ceftobiprole **7**, it was verified that the presence of a carboxylic acid group is beneficial, if not crucial, with respect to potential biological activity. Furthermore, an adequate distance between the latter functional group and the β-lactam core is indispensable in order to mimic the D-alanyl-D-alanine terminus of the natural substrate of the PBPs. In that respect, diastereomerically pure methyl ester **24A-a-2** was hydrolyzed as a final reaction step by means of lithium hydroxide monohydrate in a solution of methanol and water (2/1) at room temperature for five minutes to two days (follow-up *via* LC-MS analysis, Method A). The hydrolyzing reagent, however, caused epimerization at the α-position of the carboxylate, resulting again in a mixture of diastereomers **25A-a-1** and **25A-a-2** (*dr* = 43/57, as determined *via* LC-MS analysis). Multiple attempts toward separation of the isomers using preparative HPLC failed. Similar results were obtained upon treatment of methyl esters **24A-a-2** with one equivalent of sodium hydroxide (0.1 M) in THF/water (1/1) or 1.05 equivalents of tetrabutylammonium hydroxide in methanol/water (2/1), both at room tempature for 30 minutes. To circumvent the need for cumbersome purification steps at the level of these carboxylates, an alternative synthetic protocol was explored, relying on lithium iodide as a non-basic S_N2-type dealkylating agent (Method B). However, hydrolysis by means of lithium iodide seemed to demand harsh reflux conditions in acetonitrile to drive the reaction to completion, combined with a large excess of the reagent (up to 5 equivalents) and long reaction times. Nonetheless, thorough optimization efforts of both hydrolytic procedures gave rise to the final lithium carboxylates **25A-E,I**, either as single isomers or as mixtures. For further biological assessment, β-lactams **25A-E,I** were subjected to one equivalent of HCl (0.1 M) to afford final carboxylic acids **6** (Figure 3).

For benzoyl-substituted compounds **24F-G**, multiple hydrolysis attempts resulted in chemoselectivity issues (Table 2). Therefore, analogues **24F-a-b** were converted to their reduced analogues *via* treatment with sodium borohydride resulting in compounds **24J-a-b**, introducing an additional hydrogen bond-donating group (Scheme 4). Similar reducing conditions resulted in a difficult purification in the case of compound **24G-a.** Therefore, reduction of precursor **21G-a** and subsequent Boc-deprotection were performed, which furnished compound **23K-a** that could subsequently be acylated (Table 2). Despite removal of the problematic β-ketone moiety, hydrolysis of β-hydroxyl-containing derivatives **24J-K-a-b** was still not straightforward, as opening of the β-lactam ring occurred under all hydrolyzing conditions tested, which might be attributed to the presence of an activating hydrogen bond between the β-lactam carbonyl and the β-hydroxyl group. Similarly, deprotection of feruloyl esters **24H-a-b** failed after multiple attempts. Therefore, we decided to submit methyl esters **24H,J,K** to preliminary biological evaluation instead, as the presence of the hydroxyl group might replace the carboxylic acid as activating moiety.[13]

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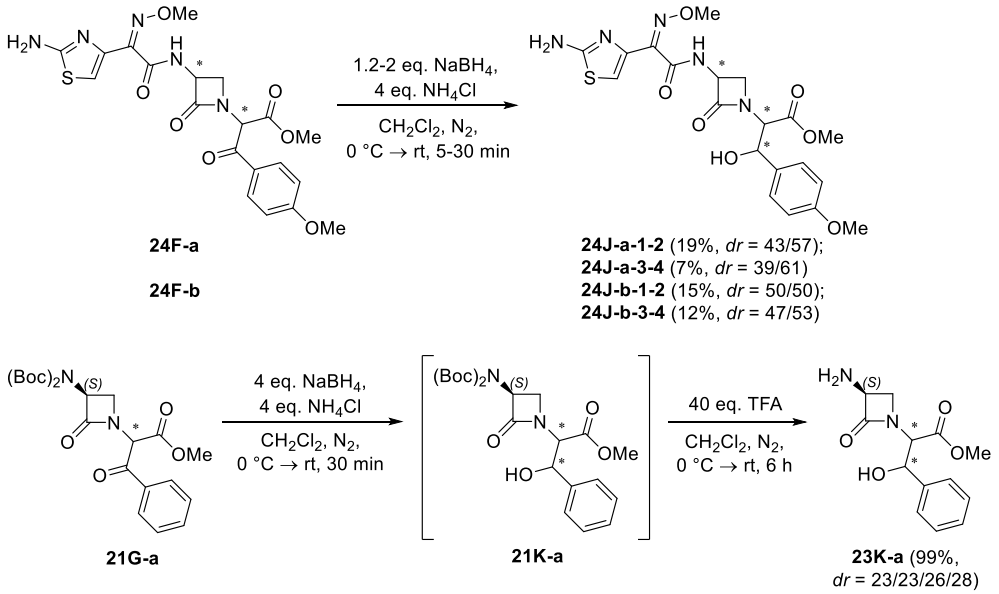

Scheme 4. Reduction of benzoyl-substituted analogues **24F** and **21G** to prepare hydroxy(phenyl)methyl analogues **24J** and **23K**.

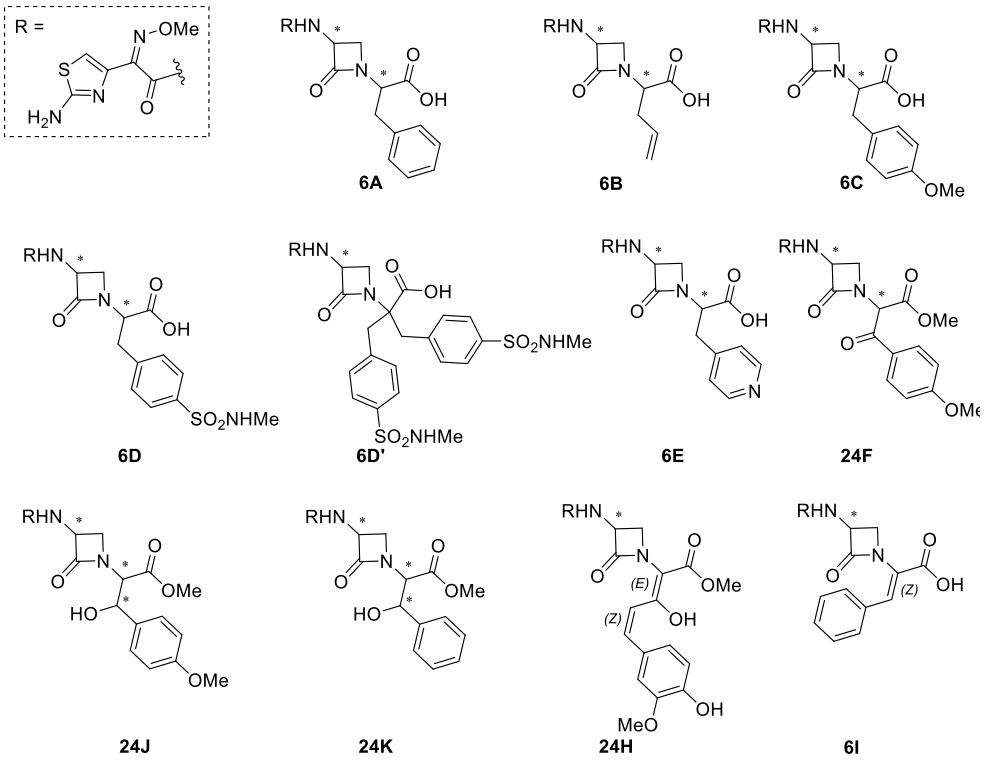

Figure 3. Overview of the newly synthesized 3-acetamidoazetidin-2-ones **6** and **24**.

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Table 1. Optimized synthesis of α-substituted 3-amino-1-(methoxycarbonylmethyl)azetidin-2-ones **21**.

Entry[a]	R'''	Reaction conditions[b]	Crude analysis 20/21/22[c]	21 (%)[d]	*dr* before purif. 21-1/21-2[e]	*dr* after purif. 21-1/21-2[e]	22 (%)
1a	Benzyl	1 eq. **20**, 1.5 eq. LiHMDS, 1.5 eq. HMPA, 1.5 eq. benzyl bromide, THF, -78 °C, 3 h	5/82/13	**21A-a** (30)	43/57	48/52	**22A-a** (7)
1b			3/78/19	**21A-b** (41)	48/52	47/53	**22A-b** (5)
2a	Allyl	1 eq. **20**, 1.5 eq. LiHMDS, 1.5 eq. HMPA, 1.5 eq. allyl bromide, THF, -78 °C, 8 h → rt, 16 h	3/56/41	**21B-a** (24)	49/51	43/57	**22B-a** (7)
2b			19/72/9	**21B-b** (13)	41/59	48/52	**22B-b** (3)
3a	4-MeO-benzyl	1 eq. **20**, 1.5 eq. LiHMDS, 1.5 eq. HMPA, 1.5 eq. 4-methoxybenzyl bromide, THF, -78 °C, 4 h → rt, 16 h	0/57/43	**21C-a** (11)	44/56	46/54	**22C-a** (19)
3b			4/87/9	**21C-b** (25)	47/53	47/53	**22C-b** (1)
4a	4-SO_2NHMe-benzyl	1 eq. **20**, 3.15 eq. LiHMDS, 2 eq. HMPA, 1.5 eq. 4-bromomethyl-*N*-methylbenzenesulfonamide **26**[f], THF, -78 °C → rt, 7 h	39/39/22	**21D-a** (5)	45/55	44/56	**22D-a** (15)
4b			28/55/17	**21D-b** (13)	49/51	46/54	**22D-b** (11)
5a	Pyridin-4-ylmethyl	1 eq. **20**, 1.5 eq. LiHMDS, 1.5 eq. HMPA, 1.5 eq. 4-(bromomethyl)pyridine hydrobromide[g], THF, -78 °C, 4 h → rt, 16 h	79/15/6	**21E-a** (10)	46/54	-[h]	**22E-a** (2)[i]
5b			81/8/11	**21E-b** (7)	47/53	49/51	**22E-b** (2)[i]
6a	4-MeO-benzoyl	1 eq. **20**, 3 eq. LiHMDS, 3 eq. HMPA, 1.5 eq. *p*-anisoyl chloride, THF, -78 °C → rt, 16 h	4/79/16	**21F-a** (40)	39/61	43/57	-[j]
6b			7/84/9	**21F-b** (72)	41/59	41/59	-[j]
7a	Benzoyl	1 eq. **20**, 3 eq. LiHMDS, 3 eq. HMPA, 3 eq. benzoyl chloride, THF, -78 °C, 3 h	1/69/30	**21G-a** (34)	-[k]	41/59 (keto)[k]	**22G-a** (22)
7b			8/68/24	**21G-b** (26)	-[k]	41/59 (keto)[k]	**22G-b** (17)
8a	Boc-Feruloyl	1 eq. **20**, 3 eq. LiHMDS, 3 eq. HMPA, 3 eq. Boc-feruloyl chloride **28**[l], THF, -84 °C, 3 h	0/100/0	**21H-a** (17)	0/100[m]	0/100[m]	-
8b			0/10l/0	**21H-b** (18)	0/100[m]	0/100[m]	-
9a	Benzylidene[n]	1 eq. **20**, 3 eq. LiHMDS, 3 eq. HMPA, 2 eq. benzaldehyde THF, -78 °C → rt, 24 h	0/100/0	**21I-a** (13)	0/100[o]	0/100[o]	-
9b			0/100/0	**21I-b** (14)	0/100[o]	0/100[o]	-

[a] Entries **a** and **b** refer to the 3'*S*- and 3'*R*-configuration, respectively. [b] LiHMDS: 1 M solution in THF; addition of electrophile after 30 min. [c] Determined *via* LC-MS analysis of the crude reaction mixture (MS signal ratio). [d] After automatic column chromatography (C18). [e] Determined *via* ^{1}H NMR analysis ($CDCl_3$). [f] Prepared *via* radical bromination of *N*,4-dimethylbenzenesulfonamide using 4.1 eq. NBS and 0.046 eq. AIBN (CH_2Cl_2, Δ, 4 d; 41% yield).[23] [g] Deprotected prior to addition using 1.6 eq. DIPEA (CH_2Cl_2, 0 °C, 30 min). [h] Mixture of 27% di-Boc- and 73% mono-Boc-protected product **21E-a**, both as mixtures of diastereomers. The *dr*'s are 37/63 and 40/60, respectively. [i] **22E-a**: Mixture of 10% di-Boc- and 90% mono-Boc-protected product; **22E-b**: Mixture of 66% di-Boc- and 34% mono-Boc-protected product. [j] Product not isolated. [k] *dr* before purification could not be determined due to complexity regarding keto-enol tautomerism: keto/enol **21G-a**: 86/14; **21G-b**: 87/13. [l] Prepared *via* Boc protection of *trans*-ferulic acid (**27**, 37% yield), followed by activation of **27** with oxalyl chloride (**28**, 99% yield). [m] Isomeric ratio (100% enol tautomer with (2*E*,4*E*)-stereochemistry); determination of stereochemistry *via* NOESY analysis, see below. [n] Formed *via* hydroxy(phenyl)methyl intermediate, followed by dehydration. [o] Isomeric ratio (100% (2*Z*)-stereochemistry); determination of stereochemistry *via* NOESY analysis, see below.

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Table 2. Yields and diastereomeric ratios of intermediate and target products **25**.

Cpd[a]	R'''	X	23 (%)	*dr* 23-1/23-2 (/23-3/23-4)[b]	24 (%)	*dr* before purif. 24-1/24-2[b]	*dr* after purif. 24-1/24-2[b]	Separation of diastereo-mers (%)[e]	Reaction conditions of hydrolysis Method A or B	25 (%)	*dr* 25-1/25-2[b]
21A-a	Benzyl	H	**23A-a** (82)	46/54	**24A-a** (86)[c]	43/57	-	**24A-a-1** (26) **24A-a-2** (32)	B: 3 eq. LiI, CH_3CN, Δ, 16 h	**25A-a-1** (95) **25A-a-2** (92)	- -
21A-b			**23A-b** (75)	47/53	**24A-b** (77)[c]	48/52	-	**24A-b-1** (25) **24A-b-2** (38)		**25A-b-1** (94) **25A-b-2** (93)	- -
21B-a	Allyl	H	**23B-a** (54)	49/51	**24B-a** (82)[c]	49/51	-	**24B-a-1** (4) **24B-a-2** (4)	A[f]	**25B** (99)[f]	28/72
21B-b			**23B-b** (64)	47/53	**24B-b** (89)[c]	46/54	-	**24B-b-1** (4) **24B-b-2** (5)			
21C-a	4-MeO-	H	**23C-a** (98)	48/52	**24C-a** (22)[d]	43/57	46/54	-	A	**25C-a** (97)	39/61
21C-b	benzyl		**23C-b** (88)	42/58	**24C-b** (20)[d]	43/57	46/54	-		**25C-b** (99)	36/64
21D-a	4-SO_2-	H	**23D-a** (99)	49/51	**24D-a** (16)[d]	43/57	45/55	-	A	**25D-a** (88)	39/61
21D-b	NHMe-benzyl		**23D-b** (99)	46/54	**24D-b** (25)[d]	41/59	43/57	-		**25D-b** (98)	35/65
22D-a	4-SO_2-	4-SO_2-	**23D'-a** (99)	-	**24D'-a** (47)[d]	-	-	-	A *or* B: 4.6 eq. LiI, CH_3CN, Δ, 4 d	**25D'-a** (99)	-
22D-b	NHMe-benzyl	NHMe-Benzyl	**23D'-b** (99)	-	**24D'-b** (11)[d]	-	-	-	A	**25D'-b** (99)	-
21E-a	Pyridin-4-	H	**23E-a** (99)	38/62	**24E-a** (20)[d]	40/60	37/63	-	A	**25E-a** (99)	34/66
21E-b	ylmethyl		**23E-b** (99)	32/68	**24E-b** (21)[d]	26/74	21/79	-		**25E-b** (99)	30/70
22E-a	Pyridin-4-ylmethyl	Pyridin-4-ylmethyl	**23E'-a** (99)	-	-	-	-	-	-	-	-
21F-a	4-MeO-	H	**23F-a** (99)	47/53	**24F-a** (32)[d]	49/51	46/54	-	A: 3 eq. $LiOH{\cdot}H_2O$, CH_3OH/H_2O (2/1), rt, 24 h *or* B: 5 eq. LiI, CH_3CN, Δ, 20 h	-[g] -[h]	- -
21F-b	benzoyl		**23F-b** (99)	50/50	**24F-b** (56)[d]	42/58	43/57	-	A: 1-10 eq. $LiOH{\cdot}H_2O$, CH_3OH/H_2O (2/1), rt, 24 h *or* B: 1-3 eq. LiI, CH_3CN, Δ, 20 h	-[g] -[h]	- -
21H-a	Feruloyl	-	**23H-a** (99)	0/100[i] (2*E*,4*E*)	**24H-a** (23)[d]	0/100[i] (2*E*,4*Z*)[j]	0/100[i] (2*E*,4*Z*)	-	*Multiple reagents and conditions:*	-[k]	-
21H-b			**23H-b** (99)	0/100[i] (2*E*,4*E*)	**24H-b** (14)[d]	0/100[i] (2*E*,4*Z*)[j]	0/100[i] (2*E*,4*Z*)	-	*e.g.* LiOH, LiI, NaOH, HCl, CH_3SO_3H, $(CH_3)_3SnOH$, $(nBu_3Sn)_2O$	-[k]	-
21I-a	Benzyli-	-	**23I-a** (99)	0/100[i] (2*Z*)	**24I-a** (25)[m]	0/100[i] (2*Z*)	0/100[i] (2*Z*)	-	A	**25I-a** (99)	0/100[i] (2*Z*)
21I-b	dene		**23I-b** (54)	0/100[i] (2*Z*)	**24I-b** (18)[m]	0/100[i] (2*Z*)	0/100[i] (2*Z*)			**25I-b** (99)	0/100[i] (2*Z*)
-	Hydroxy-(4-MeO-	H	-	-	**24J-a** (26)[d]	-[n]	43/57 39/61	**24J-a-1-2** (19) **24J-a-3-4** (7)	A	-[o]	-[o]
-	phenyl)-methyl		-	-	**24J-b** (27)[d]		50/50 47/53	**24J-b-1-2** (15) **24J-b-3-4** (12)		-[o]	-[o]
21K-a	Hydroxy-(phenyl)-methyl	H	**23K-a** (99)	23/23/26/28	**24K-a** (32)[d]	-[n]	34/66 49/51	**24K-a-1-2** (6) **24K-a-3-4** (8)	*Multiple reagents and conditions:* 1 eq. $LiOH{\cdot}H_2O$ *or* NaOH, CH_3OH/H_2O (2/1), rt, 10 min *or* 3-12 eq. LiI, CH_3CN, Δ, 24 h *or* 1.06 eq. K_2CO_3, THF/H_2O (6/7), rt, 10 min	-[o]	-[o]

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[a] Compounds **a** and **b** have the 3'*S*- and 3'*R*-configuration, respectively. [b] Determined *via* ^{1}H NMR analysis ($CDCl_3$, CD_3OD or D_2O). [c] Crude yield. [d] After automatic column chromatography (C18). [e] Separation of the two diastereomers *via* preparative HPLC (Zorbax Eclipse XDB-C18 and Supelco Ascentis C18 column coupled in series) (**24A-B**) or reversed phase column chromatography (**24J-K**). [f] Hydrolysis performed on a mixture of the four isomers **24B-a-1**, **24B-a-2**, **24B-b-1** and **24B-b-2** considering the small amount of the corresponding methyl esters available. [g] Hydrolysis of the ketone. [h] Decarboxylation of the β-ketocarboxylic acid. [i] Isomeric ratio (100% enol tautomer). [j] Isomerization of double bond upon base-catalyzed acylation reaction: (4*E*)- to (4*Z*)-stereochemistry (based on decrease of vicinal coupling constants in ^{1}H NMR spectra (MeOD-d_4): J = 15.6-15.8 → 8.1 Hz). [k] Complex reaction mixtures, product not isolated. [l] Isomeric ratio. [m] After preparative TLC (EtOAc/MeOH/H_2O 30/5/4). [n] *dr* before purification could not be determined due to overlap of the spectral signals of the four diastereoisomers in ^{1}H NMR and LC-MS spectra. [o] Hydrolysis of the β-lactam ring.

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3. Determination of stereochemistry

In order to evaluate the optical purity of β-lactams **25** with respect to their crucial β-lactam-C3-position, the four stereoisomers of 3-(acylamino)azetidin-2-ones **24** were synthesized and isolated, whenever possible or necessary. The enantiomeric excess (*ee*) values of a selection of compounds **24**, belonging to the various types synthesized, clearly demonstrate that the C3-protons remain untouched during the entire nine-step synthesis (Table 3).

Table 3. Enantiomeric excesses (*ee*) of 3-(acylamino)azetidin-2-ones **24A-E,H-I**.

Cpd	a-1 (2*R*,3'*S*) or (2*S*,3'*S*)	a-2 (2*S*,3'*S*) or (2*R*,3'*S*)	b-1 (2*S*,3'*R*) or (2*R*,3'*R*)	b-2 (2*R*,3'*R*) or (2*S*,3'*R*)	**Chromatographic conditions** ChiralPak® IA column
24A	>92	>92	>99	>98	1 mL/min; 35 °C; hexane/EtOH (70/30);
24B	>94	>97	>98	>97	210.2 nm (**24A**) or 220.8 nm (**24B-D**)
24C[a]	>99	>99	>99	>99	
24D[b]	–[c]			>99	
24E[b]	>95			>98	0.5 mL/min; 30 °C; hexane/EtOH (75/25); 210.8 nm
Cpd	**a (3'*S*)**		**b (3'*R*)**		**Chromatographic conditions** ChiralPak® IA column
24D'	>97		>97		1 mL/min; 35 °C; hexane/EtOH (70/30); 220.8 nm
24H	>99		>99		1 mL/min; 35 °C; hexane/EtOH (70/30); 368 nm
24I	>99		>96		0.3 mL/min; 35 °C; hexane/EtOH (85/15); 220.8 nm

[a] The isolated diastereomers of 4-methoxybenzyl derivatives **24C-a-b** have only been obtained on an analytical scale after prep. HPLC using a Phenomenex® Luna C18(2) column. [b] Diastereomers have not been isolated. [c] Not to be determined due to overlap of diastereomers and enantiomers using different chromatographic conditions.

The optical purity of the target β-lactams **25**, however, could not be determined *via* chiral HPLC, even after testing multiple chiral columns and chromatographic conditions. Nevertheless, based on the reaction mechanism behind the conversion of methyl esters **24** to lithium carboxylates **25** by means of lithium iodide as a dealkylating reagent, assuming the S_N2-reaction type and considering the non-basic reaction conditions, the enantiopurity follows as a direct result and no change in *ee* values could be expected. *Per contra*, the conservation of the C3 stereochemical integrity could not be guaranteed during lithium hydroxide-mediated hydrolysis.[24] Therefore, another strategy was pursued, using a chiral resolving agent. (-)-Menthyloxyacetic acid, for example, has a fixed stereochemistry at three aliphatic positions, and is hence insensitive to the alkaline conditions prevailing during the hydrolysis reaction.[25] Bearing this in mind, C3-menthyloxyacetamido-linked analogues **30** were synthesized lacking a substituent in α-position of the methyl ester. Upon LiOH-mediated hydrolysis, a change in the stereochemistry at the C3-position would now easily be observed through the formation of two diastereomers **31**, as this is the only acidic proton left in the scaffold. A comprehensive report on the synthesis of the latter derivatives **30** and demonstration of their optical purity before and after LiOH-mediated hydrolysis is provided in the Supporting Information. This study therefore provides an indirect proof for the stereochemical purity of the final carboxylates **25** as well.

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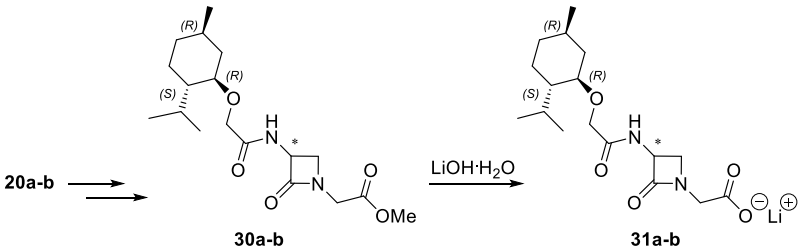

Scheme 5. Synthesis of (-)-menthyloxyacetamido coupled analogues **31**. For synthetic details, see supporting information).

As mentioned earlier, methyl esters **24A-E** were synthesized as pairs of diastereomers, which could be separated by means of preparative HPLC. For example, the four different isomers of benzylic derivatives **24A** were obtained as such in 25-38% isolated yield. In order to have a clue of the spatial configuration and, more specifically, of the stereochemistry of the unknown chiral center in α-position of the ester moiety, numerous attempts were made to create crystalline material for X-ray diffraction analysis. None of these, regrettably, proved to be successful and the same holds for all other derivatives **24-25**. As an alternative strategy, 2D NOESY analysis was performed on diastereomers **24A-b-1** and **24A-b-2** as model compounds in combination with inspection of the ^{1}H NMR chemical shifts and coupling constants, and energy-minimized molecular models, in analogy with literature reports.[26]

Based on the coupling constants of the multiplet signals in the ^{1}H NMR spectra ($CDCl_3$) of compounds **24A-b-1** and **24A-b-2**, corresponding to literature data,[18b] the relative stereochemistry of the β-lactam protons H_{a-c} could be derived and a clear distinction could be made regarding their chemical shift (Figure 4, Figure 5). Moreover, protons H_{d-f} form an AMX system of which H_e and H_f are diastereotopic. There is a large difference in their vicinal coupling constant (J_{de} = ±10 Hz and J_{df} = ±5 Hz), indicating that, presumably, conformational rotation around the (CH_d)-(CH_eH_fPh)-bond is restricted by high energy barriers (Figure 4).[26d],[27]

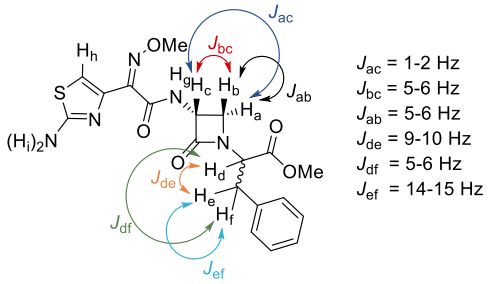

Figure 4. NMR coupling constants in benzylic derivatives **24A-b-1** and **24A-b-2**.

Upon inspection of the NOESY spectroscopic data of diastereomer **24A-b-2** (for NOESY spectrum, see supporting information), a clear interaction could be observed between proton H_b and the phenyl ring as well as between H_b and H_e. After OPLS3 force field minimization and *ab initio* QM optimization of a conformer,[28] arbitrarily assigned the (2*R*,3'*R*)-configuration and with its aromatic ring pointing to the

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$H_{(c\text{-}b)}$-side of the β-lactam ring system, H_b was indeed located within NOE-distance of H_e (2.97 Å) and the aromatic ring (2.77 Å). This was not the case for a 3D model of the other diastereomer. Furthermore, a moderate shielding effect on protons H_b and H_c, most plausibly caused by their proximity to the aromatic ring system (anisotropic effect), could be noted in the ^{1}H NMR spectra of this diastereomer, in comparison with the corresponding proton signals in diastereomer **24A-b-1** (Figure 5).

Likewise, a correlation exists between both H_a and $N(H_i)_2$ with respect to the aromatic carbocycle as observed in the 2D NOESY NMR spectra of the other diastereomer **24A-b-1** (for NOESY spectrum, see supporting information). Considering the additional NOESY interactions, two conformers ((2*S*,3'*R*) and (2*R*,3'*R*), respectively) were designed and energy-minimized. Only in the 3D-model with the former configuration, H_a and the aromatic ring were indeed positioned closely at only 2.79 Å apart from each other. The same holds for protons H_h and H_c (3.94 Å) which, considering the energetically possible rotation of the substituted thiazole moiety (superimposed in Figure 5), confirms the imposed stereochemistry based on the NOESY signals. Furthermore, H_a and the 3-amino (H_g) ^{1}H NMR signal are slightly shifted upfield in comparison with the second diastereomer **24A-b-2**, again due to the proximity of the aromatic system, although we realize that the effect on the latter is not fully reliable.

An additional observation concerns the (CH_d)-(CH_eH_fPh)-bond in the energy-minimized and *ab initio* optimized models of both diastereomers. In each of them, H_d adopts an anti-relationship with respect to H_e with dihedral angles of 56.4° and 71.5° between H_d and H_f (gauche), which is reflected by very similar vicinal coupling constants in the ^{1}H NMR spectra of both diastereomers (J_{de} = ±10 Hz and J_{df} = ±5 Hz). To summarize, by means of evaluation of the energy-minimized molecular models of the two possible diastereomers and their compatibility with experimental NMR coupling constants and NOESY analysis, a credible suggestion could be made with respect to the stereochemical identity of both isomers **24A-b-1** (2*S*,3'*R*) and **24A-b-2** (2*R*,3'*R*).

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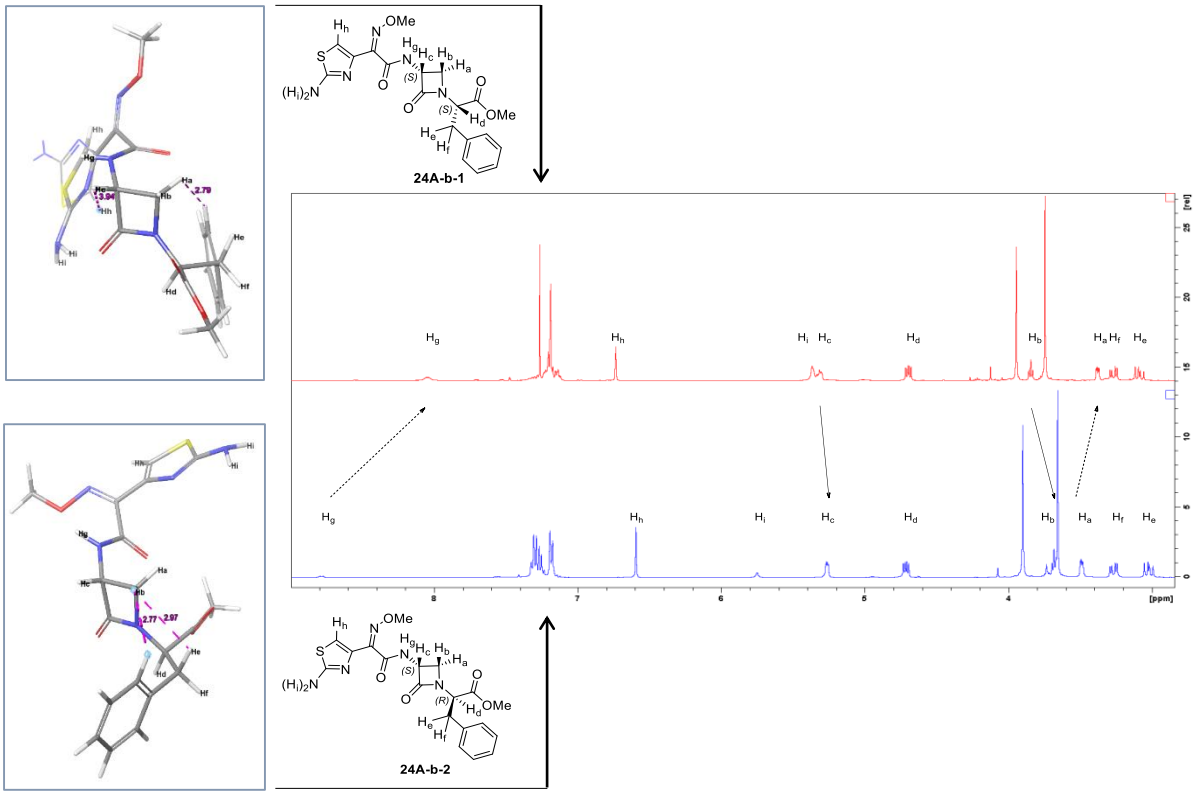

Figure 5. *Ab initio* optimized 3D-models of diastereomers **24A-b-1-2**, calculated using Jaguar software (version 9.5, Schrödinger, Inc., New York, NY, 2017),[29] corresponding molecular structures with suggested stereochemistry and corresponding ^{1}H NMR spectra.

Additionally, a similar strategy was used to determine the configuration of the α-positioned double bond in feruloyl- and benzylidene-substituted derivatives **21H-24H** and **21I-24I**. In case of compound **21I**, a significant NOESY interaction ($CDCl_3$) was observed between β-lactam proton H_a (δ = 3.88 ppm, $CDCl_3$) and the aromatic protons, as well as between double bond proton H_d (δ = 7.49 ppm, $CDCl_3$) and the methoxy protons (for NOESY spectrum, see supporting information). Translation of these interactions to OPLS3 energy-minimized and *ab initio* optimized 3D models of both the (3'*S*)- and (3'*R*)-enantiomer **21I-a-b** resulted in a preference for the (2*Z*)-stereochemistry based on smaller spatial distances between the relevant protons in the models (Figure 6). To confirm this unexpected outcome, the procedure was repeated for the more complex aminothiazoleoxime-substituted analogues **24I-a-b**, again showing that only the (2*Z*)-stereochemistry could reflect the experimental NMR interactions. Similar observations were made for feruloyl derivatives **21H**, suggesting a (2*E*,4*E*)-configuration. In a later stage, however, isomerization of the non-enolic double bond occurred during the base-catalyzed acylation reaction to form compounds **24H**, as proven by a significant decrease in the value of the vicinal coupling constants in the ^{1}H NMR spectra (*J* = 15.6-15.8 Hz for compounds **21H-23H**; *J* = 8.1 Hz for compound **24H**, MeOD-d_4).

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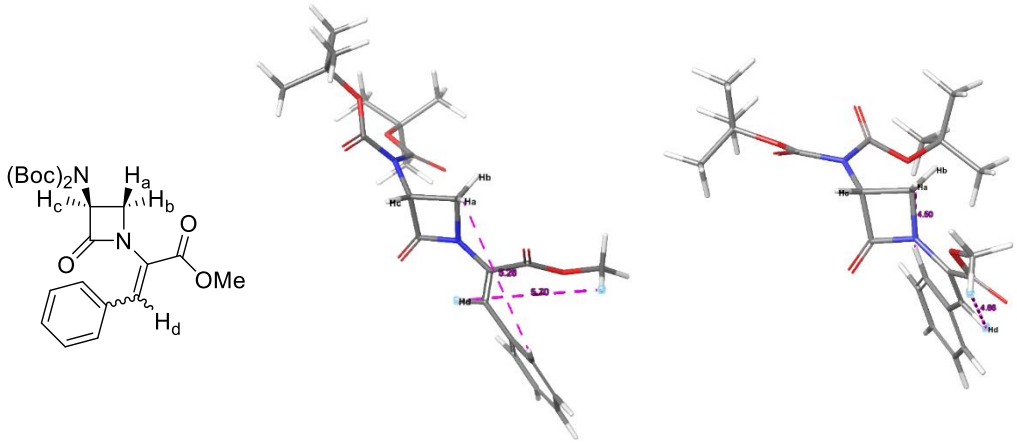

Figure 6. *Ab initio* optimized 3D-models of (*E*)- and (*Z*)-isomer of compound **21I-a**, calculated using Jaguar software (version 9.5, Schrödinger, Inc., New York, NY, 2017),[29] indicating preference for the (*Z*)-isomer.

4. Biological evaluation

In order to provide some preliminary data on their biological potential, the synthesized set of compounds was subjected to a PBP binding and competition assay. The purified PBP3 of *E. coli* K12, PBP5fm of *Enterococcus faecium* D63r and R39 DD-carboxypeptidase of *Actinomadura* spp.[30] were therefore incubated with compounds **6** and some of the corresponding methyl esters **24**, after which their residual activity (RA) was determined by labelling the free enzyme with Bocillin FL, a fluorescent reporter molecule and penicillin V analogue (for more information, see supporting information).[31] Although no exceptional residual activity values were found, careful inspection of the results presented in Table 4 revealed the inhibitory potential of derivatives **24A**, **6E** (albeit a chemical instability issue was detected in this case) and **6I** (Figure 3). Overall, benzylidene derivative **6I-a** showed a promising inhibitory potential on PBP3, a lethal target of *E. coli* (28% residual activity). Slight inhibition of the other two PBPs (88% RA of PBP5fm and 62% RA of R39) and a moderate PBP3 IC_{50} value in the micromolar range prompted us to promote this derivative to become the first hit of this study. Note that the IC_{50} value of 130 µM (54 µg/mL) fits in a recently reported series of IC_{50} values belonging to various commercial antibiotics ranging from 0.01 to >1000 µg/mL on the same PBP of *E. coli* DC2, a hypersusceptible mutant.[32] On the other hand, commercial monobactam aztreonam **4** has a very high affinity for PBP3 of *E. coli* strains DC2 and MC4100 (IC_{50} of 0.02 and 0.03 µg/mL, respectively).[33]

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Table 4. Residual enzymatic activities after incubation of PBP3, PBP5fm and R39 (2.5 µM) with selected compounds **24**/**6** (1 mM, pH 7, 30 °C, 3 h) and IC_{50} values on PBP3.

Compound	%RA PBP3 (IC_{50} (µM))	%RA PBP5fm	%RA R39
24A-a-1-2	50 (~1000)	87	100
6A-a-1	92	94	>100
6A-a-2	94	>100	>100
6A-b-1	96	>100	99
6A-b-2	93	>100	100
6B-a-b-1-2	80	-	-
24C-a-1	71	-	-
24C-a-2	82	-	-
6C-a-1-2	90	>100	100
6C-b-1-2	92	>100	79
24D-a-b-1-2	87	-	-
6D-a-1-2	91	>100	74
6D-b-1-2	95	>100	79
24D'-a	83	-	-
6D'-a	87	>100	73
6D'-b	96	>100	77
24E-a-1-2	73	-	-
24E-b-1-2	65	-	-
6E-a-1-2	33-96[a] (983)	93	79
6E-b-1-2	80	-	-
24F-a-1-2	73	-	-
24H-a	80	-	-
24H-b	71	-	-
24I-a	71	-	-
24I-b	81	-	-
6I-a	**28 (130)**	**88**	**62**
6I-b	47 (~1000)	100	100
24J-a-1-2	74	-	-
24K-a-1-2	78	-	-
24K-a-3-4	82	-	-
Blanc	100	100	100

[a] Variability due to stability issue upon conservation of the compound.

Albeit not the only requisite, our presumption regarding an increased electron flow through the additional double bond and the aromatic ring upon acylation by the active site serine residue indeed seems to elevate the inhibitory activity of compound **6I-a** compared to all other and even very close derivatives, such as for example benzyl-substituted compound **6A-a**. Similar observations of elevated biological activity are made upon comparison of Δ^3-cephalosporins **2a** and their unnatural Δ^2-isomers **2b**, which lack a conjugated, α-positioned double bond and are almost completely devoid of activity (Figure 7).[34] Besides, though still a controversial topic, if the cephalosporin C3'-substituent 'X' represents an adequate leaving group (*e.g.* pyridinium, acetate, heterocyclic thiols), it can act as an

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electron sink. Its elimination, whether or not concerted with β-lactam acylation and ring opening, can then further enhance the antibacterial activity or β-lactamase inhibitory potential of certain cephalosporins, but is certainly not essential.[35]

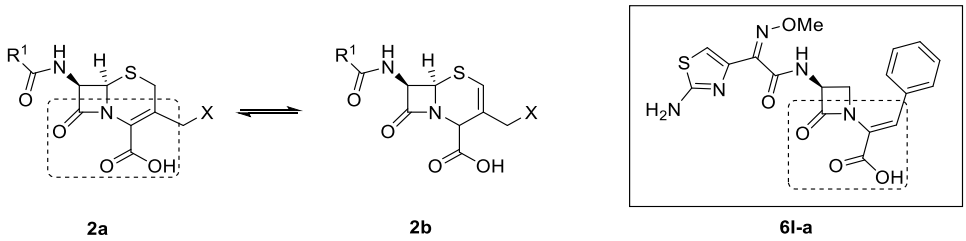

Figure 7. Comparison of double bond position in cephalosporins **2a-b** and monocyclic β-lactam **6l-a**.

Additionally, the inhibitory activity of benzylidene-β-lactam **6l-a** on β-lactamases was determined using a colorimetric assay. The residual activity of one or two representative enzymes of each of the four classes of β-lactamases was measured, after incubation in the presence of 1 mM of compound **6l-a**, by following the rate of hydrolysis of nitrocefin as a chromogenic cephalosporin reporter substrate.[36] Compound **6l-a** was found not to be a substrate for most β-lactamases tested. In addition, class C β-lactamase P99 was inhibited under the conditions tested, with about 40% loss of catalytic activity (Table 5). The specific inhibition mechanism, either suicidal or competitive, of P99, a chromosomally encoded, clavulanate-resistant cephalosporinase from *Enterobacter cloacae*, by **6l-a** is subject of ongoing research.

Table 5. Residual enzymatic activities after incubation of different β-lactamases with compound **6l-a** (1 mM, 37 °C, 1.5 min).

Class	Enzyme	%RA
A	CTX-M15	103
B	Imp1	109
B	Imp4	97
C	P99	61
C	AmpC-HD	109
D	Oxa48	98

Based on the encouraging results regarding PBP3 inhibition, stability against class A, B and D β-lactamases and weak inhibition of class C β-lactamase P99, additional structure-activity relationship studies around benzylidene-like compounds, aiming at an increased electron flow and additional non-covalent interactions with the PBP's active site cavity, will be performed in due time, based on the synthetic methodology that has been developed in the current work. In the best case, these efforts will culminate in lead compounds with low micromolar to nanomolar inhibitory activities against the target PBPs of resistant bacteria.

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Conclusion

The never faded, but – more than ever – upcoming attention for the need to tackle bacterial resistance, has stimulated both governments and the scientific community to intervene and blow the whistle. To address a critical resistance mechanism in Gram-positive bacteria, *e.g*. active-site distortion in mutated PBPs, a possible strategy comprises the synthesis of monocyclic β-lactam analogues, which are more restricted in volume, and thus more prone to enter a PBP active site, in contrast to their well-known bicyclic counterparts. In that framework, the current study focused in the first place on the synthetic development of a library of novel nocardicin-like analogues in the quest for new PBP inhibitors and extension of the knowledge on their structure-activity relationships. In summary, employing phamacophore modeling and covalent docking approaches as an onset, 4-unsubstituted 3-amino-1-carboxymethyl-β-lactams were designed, functionalized in α-position with a side chain comprised of a lipophilic linker moiety and, in most cases, a terminal hydrogen bond-donating or -accepting functional group. An unprecedented ten-step synthetic procedure was successfully elaborated toward this novel class of highly functionalized, enantiomerically pure monocyclic β-lactams, starting from L- and D-serine as easily available substrates. This protocol thus offers a valuable way to accomplish the enantioselective synthesis of unprecedented 4-unsubstituted 3-amino-1-carboxymethyl-β-lactams, which remain highly underexplored when compared to established monocyclic and of course bicyclic β-lactam antibacterials. Both evident and more complex side chains can be introduced. The former, however, offer multiple opportunities for chemical derivatization by means of *e.g.* oxidation, electrophilic addition or aromatic cross-coupling reactions. Preliminary screening of the PBP inhibitory potential of the synthesized β-lactams did not result in noteworthy residual activity values for all compounds, though benzylidene-substituted azetidin-2-one **6I-a** showed good inhibition of PBP3 of *E. coli*, thereby emerging as a lead structure for further inhibitor design. A colorimetric assay showed stability against representatives of the four classes of β-lactamases, while a weak inhibition of class C β-lactamase P99 was observed. In summary, an elaborate synthetic protocol was established for the construction of an introducing library of unprecedented 2-{3-[2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-oxoazetidin-1-yl}acetic acids, inspired by pharmacophore modelling and covalent docking, and biological assessment revealed α-benzylidenecarboxylates to be eligible lead structures *en route* to a novel class of antibacterials with PBP inhibitory activity. Encouraged by the fact that an appropriate lipophilic linker has now been identified, a follow-up library will be synthesized in due time using the established synthetic protocol and relying on the criteria set by the *in silico* design study (additional introduction of HBD/HBA).

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Conflict of interest

The authors declare no conflict of interest.

Keywords

Antibiotics • Drug design • Virtual screening • Lactams • Chiral pool • Configuration determination • Biological activity

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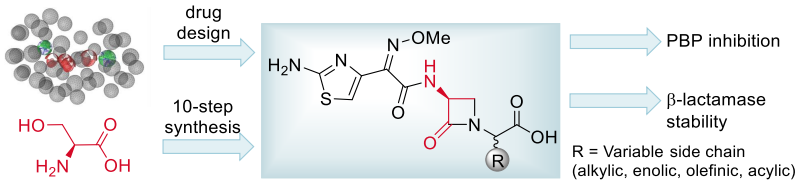
FULL PAPER



No action today, no cure tomorrow. Inspired by a virtual screening drug design approach toward new inhibitors of penicillin-binding proteins of resistant bacteria, a ten-step chiral pool strategy was elaborated for the synthesis of a library of enantiomerically pure, highly functionalized monocyclic 3-amino-1-carboxymethyl-β-lactams.

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***In silico* design and enantioselective synthesis of functionalized monocyclic 3-amino-1-carboxymethyl-β-lactams as inhibitors of penicillin-binding proteins of resistant bacteria**

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