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Synthesis and Penicillin-binding Protein Inhibitory Assessment of Dipeptidic 4-Phenyl-β-lactams from α-Amino Acid-derived Imines

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Abstract: Monocyclic β-lactams revive the research field on antibiotics, which are threatened by the emergence of resistant bacteria. A six-step synthetic route was developed, providing easy access to new 3-amino-1-carboxymethyl-4-phenyl-β-lactams, of which the penicillin-binding protein (PBP) inhibitory potency was demonstrated biochemically.

In light of the rapidly escalating bacterial resistance problem, antibiotic discovery remains a highly important drug discovery endeavour.[1] To break away from traditional bicyclic β-lactam scaffolds, the research on monocyclic β-lactam antibiotics gains in importance, as we previously demonstrated through a three-decade literature overview.[2] Taking into account the assembled state-of-the-art, and combining the structural properties of aztreonam (the only FDA-approved monobactam), the nocardicins (the first described, natural monocyclic β-lactams) and late-generation cephalosporins, our group recently studied a new pharmacophore **1** to mimic D-alanyl-D-alanine, the natural substrate of the penicillin-binding proteins, playing a crucial role in the bacterial cell wall biosynthesis (Figure 1). Using a virtual screening approach as an onset, a ten-step synthetic pathway was developed towards a model library of 4-unsubstituted β-lactams **1**, characterised by diverse side chains 'R'. α-Benzylidene-substituted analogue **1A** was promoted to become our first hit, favoured with promising PBP inhibitory activity and high β-lactamase stability. In order to gain additional insights in the chemistry and structure–activity relationships of these complex β-lactams, an explorative study on synthetic routes towards C4-substituted analogues **2** was now initiated, holding on to the same design objectives (Figure 1). In light of synthetic feasibility, 4-phenyl-β-lactams **2** were targeted first to provide a proof of concept. Their synthesis was perceived to be achievable through functionalisation, in a similar way as previously described,[3] of 3-amino-1-carboxymethyl-β-lactams. As opposed to C4-unsubstituted β-lactams, C4-substituted variants can be easily prepared via Staudinger's ketene-imine [2+2]-cyclocondensation.[4] To date, this is one of the most renowned and versatile methods for the construction of the four-membered amide ring system and is often selected for its simplicity in reaction procedures and predictability of the stereochemical outcome.[5] For the synthesis of 3-amino-β-lactams specifically, phthalimido- (FtN) and azido-containing ketenes are typically employed.[6] As a means to shorten the synthetic pathway as much as possible, the variable side chain 'R' could be introduced directly in the amine part of the imine. Indeed, when protected α-amino acids would be used as cheap and easily available substrates and condensed with benzaldehyde, the α-functionalised carboxymethyl moiety could be installed quite easily onto the resulting β-lactam N1-position. Based on the in silico design criteria regarding the α-side chain 'R' (Figure 1c),[3] several divergent α-amino acids were selected to *i.* optimise the synthetic procedures (L-/DL-Phe); *ii.* introduce terminal hydrogen bond donors or acceptors (L-Glu, L-Trp and L-/D-Met, for oxidation to sulphones); and *iii.* change the linker type to aromatic nocardicin-like motifs (D-Phg).[2]

Taking all the above into account, L-phenylalanine **3A** (R = Bn) as a model substrate was converted to the corresponding methyl ester **4A** by means of 2,2-dimethoxypropane and hydrochloric acid (*Method A*, Scheme 1, Table 1). The resulting amine HCl salt was converted to the free base and subsequently condensed with benzaldehyde, affording aldimine **5A** in quantitative yield (*Method C*). Also the racemic starting material **3A'** (DL-Phe) was converted in this way in view of HPLC analysis using a chiral stationary phase ('chiral HPLC') at a later stage. Meanwhile, *N*-phthaloylglycine **6** was converted to the corresponding glycyl chloride **7**. After dehydrochlorination of acid chloride **7** using triethylamine, the in situ generated ketene was coupled with imines **5A-A'** to yield 4-phenyl-3-phthalimidoazetidin-2-ones **8A-A'**. Profound variation of the Staudinger reaction conditions was necessary to enhance the conversion rate and yield. The optimised set of reaction parameters and corresponding reaction outcomes are displayed in Table 1. As expected, comparison of the results of *Methods E* and *F*, showed that a higher amount of *trans*-isomer was ob-

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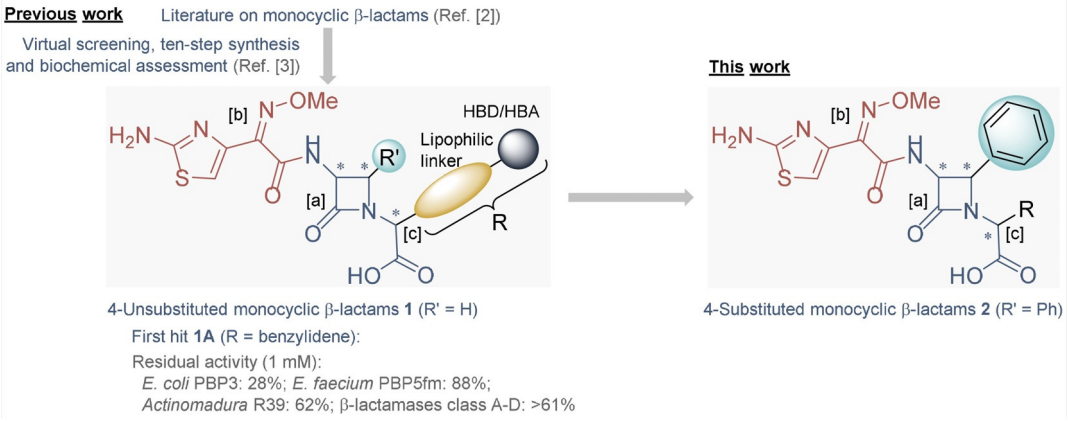
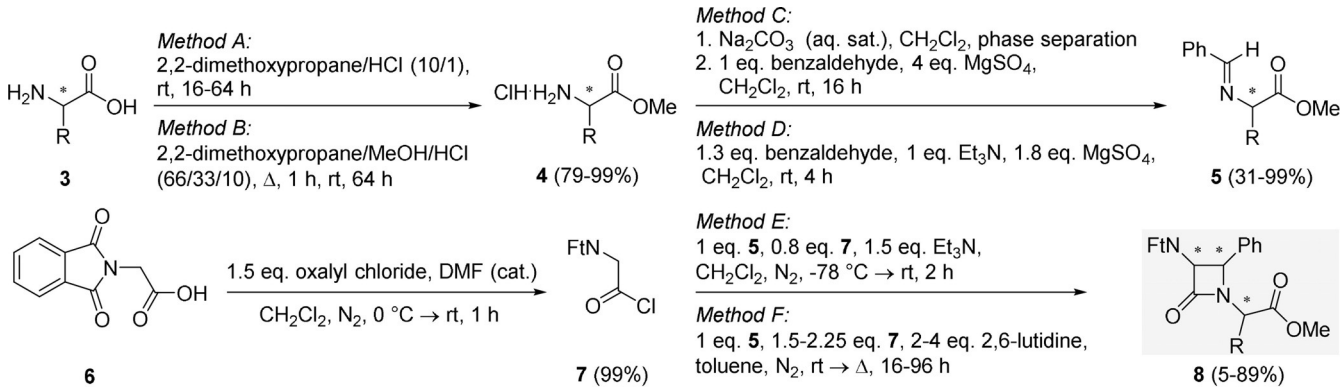


Figure 1. Design strategy. [a] 3-Amino-1-carboxymethyl-β-lactam, mimicking the natural PBP substrate, D-alanyl-D-alanine, and enabling crucial molecular interactions with the enzyme's active site; [b] Optimised 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido substituent;[2] [c] Variable side chain embedding a lipophilic linker and terminal hydrogen bond-donating or -accepting functionalities, enabling additional non-covalent interactions with the key active site residues.



Scheme 1. Staudinger synthesis of Ft-protected 3-amino-1-methoxycarbonylmethyl-4-phenyl-β-lactams **8**.

Table 1. Optimised synthesis of α-substituted 3-amino-1-methoxycarbonylmethyl-4-phenylazetidin-2-ones **8**.

Compound (Amino acid)	Esterification		Imination		Staudinger synthesis		
	Method	**4** [%] (*ee*)[a]	Method	**5** [%] (*ee*)[a]	Method (Eq. base; Eq. **7**)	*dr* before purif.	**8** [%] (*dr*)
3A (L-Phe)	A	99 (99)	C D	46-99 (6) 99 (86)	E (1.5; 0.8)	56/44/0/0	**8A**$_{cis}$: 5 (58/42)[b]
3A′ (DL-Phe)	A	99 (0)	C	64 (2)	F (4; 1.5)	3/2/53/42	**8A′**$_{cis}$: 6 (59/41); **8A′**$_{trans}$: 64 (55/45)[b]
3B (L-Glu)	B	79	D	31	F (2; 1.5)	2/1/51/46	**8B**$_{trans}$: 89 (52/48)[c]
3C (L-Trp)	–[d]	–[d]	D	77	F (2; 1.5)	0/0/63/37	**8C**$_{trans}$: 85 (59/41)[c]
3D (L-Met)	A	96	D	99	F (2; 1.5)	4/4/53/39	**8D**$_{trans}$: 64 (56/44)[c]
3D′ (D-Met)	–[d]	–[d]	D	87	F (2; 1.5)	4/4/53/39	**8D′**$_{trans}$: 60 (57/43)[c]
3E (D-Phg)	A	99	D	69	F (3; 2.25)	–[b,e]	–[b,e]

[a] Chromatographic conditions: see Supporting information. [b] Purified via automated column chromatography (C18). [c] Purified via automated column chromatography (SiO_2). [d] Commercially available. [e] Complex mixture, containing 29% of coupling product of **4E** and **7**, 4% **8E**$_{cis}$ (*dr* = 62/38) and 11% **8E**$_{trans}$ (*dr* = 51/49).

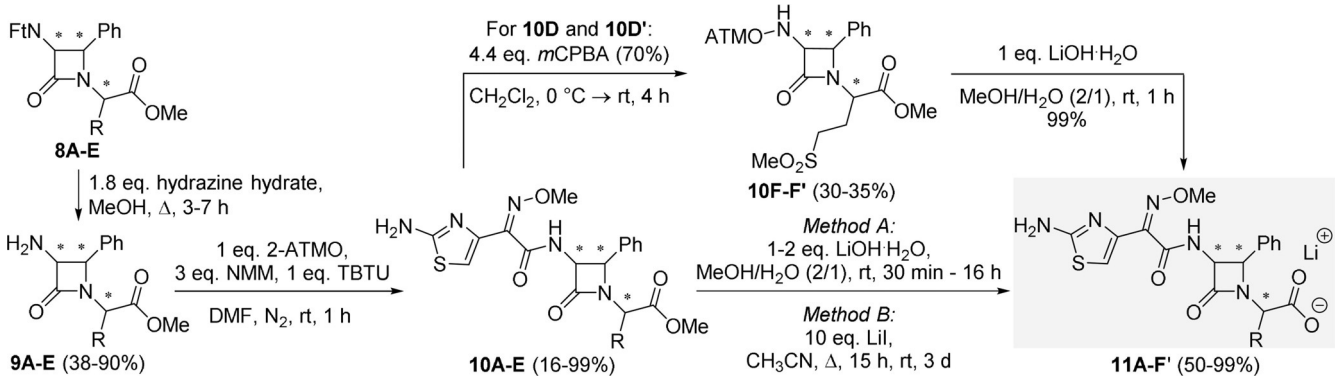
tained when 2,6-lutidine was used as a base in boiling toluene.[5] The accessibility of both *cis*- and *trans*-isomers was considered an asset with respect to future evaluation of the biological potency of the corresponding target products **2**.

Next, phthaloyl deprotection of compounds **8A-A′** using hydrazine hydrate furnished 3-aminoazetidin-2-ones **9A-A′** (Table 2, Scheme 2).[7] In the next step, the obtained free amines were coupled with 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid (2-ATMO, predominantly *syn*) in alkaline environment using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as a coupling reagent. The resulting cyclic amides **10A-A′** were obtained in variable yields of

Table 2. Yields and diastereomeric ratios of intermediate and target products **9**–**11**.

Compound	AA	Deprotection **9** [%] (*dr*)	Acylation **10A-E** [%] (*dr*)	Oxidation **10F-F′** [%] (*dr*)	Hydrolysis Method	**11** [%] (*dr*)
8A$_{trans}$[a]	L-Phe	61 (53/47)	35 (53/47)[b,c]	–	A (on **10A**$_1$); B (on **10A**$_1$)	99 (83/17); 50 (96/4)
8A′$_{cis}$	DL-Phe	90 (59/41)	36 (60/40)[b]	–	A	99 (67/33)
8A′$_{trans}$		90 (58/42)	69 (54/46)[b,c]	–	A (on **10A′**$_{1\text{-}2}$); A (on **10A′**$_2$)	99 (69/31); 99 (58/42)
8B$_{trans}$	L-Glu	70 (53/47)	16 (51/49)[b]	–	A[d]	99 (62/38)
8C$_{trans}$	L-Trp	89 (51/49)	22 (56/44)[e]	–	A	99 (54/46)
8D$_{trans}$	L-Met	67 (59/41)	35 (55/45)[f]	–	A (on **10D**)	99 (66/34)
				10F: 30 (62/38)[g,h]	A (on **10F**)	99 (61/39)
8D′$_{trans}$	D-Met	84 (55/45)	69 (54/46)[f,h]	–	A (on **10D′**)	99 (58/42)
				10F′: 35 (53/47)[f]	A (on **10F′**)	99 (57/43)
8E$_{cis}$	D-Phg	38 (66/34)	99 (60/40)[g,h]	–	A	99 (74/26)
8E$_{trans}$		79 (62/38)	38 (52/48)[g,h]	–	A	99 (70/30)

[a] *dr* = 68/32. [b] Purified via automated column chromatography (C18). [c] Additional purification via prep. HPLC permitted to separate the two diastereomers **10A**$_{trans\text{-}1}$ and **10A**$_{trans\text{-}2}$ (6% *ee* each) and **10A′**$_{trans\text{-}1}$ and **10A′**$_{trans\text{-}2}$ (0% *ee* each). [d] 2 equiv. LiOH·H_2O were used. [e] Purified via prep. TLC. [f] Purified via automated column chromatography (SiO_2). [g] Purified via prep. TLC. [h] Chiral HPLC analysis was performed on compounds **10D′**$_{trans}$ (*ee* > 76%), **10E**$_{cis,trans}$ (*ee* = 0%) and **10F**$_{trans}$ (*ee* = 85%).



Scheme 2. Functionalisation of key intermediates **8** towards target products **11**.

35–69% after conventional purification techniques. Additional separation of the obtained diastereomeric mixtures via preparative HPLC permitted to isolate the individual diastereomers **10A**$_1$, **10A**$_2$ and **10A′**$_1$, **10A′**$_2$ (all *trans*). The isolated isomers were used to assess the optical purity of the compounds with respect to the α-position after the different reaction steps via chiral HPLC. This technique, however, demonstrated a low enantiomeric excess (6%) for compounds **10A**$_1$ and **10A**$_2$, which implied epimerisation of the α-position of the methyl ester derived from optically pure L-phenylalanine. Retrospective analysis of all intermediates revealed that epimerisation took place already during imination (Scheme 1, Table 1, *Method C*), which is not in accordance with preceding literature reports, stating conservation of the chirality of similar amino acid-derived imines using comparable or even harsher reaction conditions (alkaline environment, elevated temperatures).[8] For this reason, a new imination procedure was tested (*Method D*), which did give rise to imine **5A** with a considerably higher *ee* of 86%.[9] Consequently, this method was applied for the synthesis of the other derivatives **5**,**8**-**10B-E** as well. As such, our target library was enlarged by converting the above-mentioned α-amino acids **3B–E** to β-lactams **10B-E** via similar chemical transformations (Scheme 1, Scheme 2, Table 1, Table 2). Despite the (presumed) use of chiral imines, a more or less equimolar amount of the two *trans*-isomers **8B-E** was obtained in each case, which is in good agreement with the statement that a chiral imine derived from a chiral amine and an achiral aldehyde is not an appropriate chiral inductor in the Staudinger synthesis.[10] Both L- and D-methionine **3D-D′** were employed individually to be able to use the enriched mixtures of compounds **10D-D′** for chiral HPLC method optimisation and determine if the configuration of the chiral centre in α-position of the carboxylic acid was now conserved throughout the pathway (Table 2). When compared with L-Phe-derived β-lactams **10A** (synthesised using imination *Method C*, Table 1, *ee* = 6%), the epimerisation issue of most other derivatives was far more limited (e.g., *ee* > 76% for **10D′**, *Method D*). As expected, only D-Phg-derived compounds **5**,**8**-**10E** were completely racemised as a result of the formation of an extensive conjugated system after deprotonation at the benzylic position.[11] In previous work,[3] it was shown that epimerisation at the α-position can also take place during subsequent hydrolysis, depending on the reagent used. The deprotection reaction of diastereomerically pure *trans* ester **10A**$_1$ using LiOH·H_2O indeed caused slight epimerisation (*Method A*, Table 2). S_N2-type dealkylation with LiI resulted in high stereoselectivity but

lower conversion (*Method B*). Similar results were obtained for the racemic analogues **10A′**. In either way, the loss of enantiopurity at the α-position was not a major issue as long as the biological potency of these compounds had not been proven. Analogues **10B-E** were therefore hydrolysed using the LiOH-mediated procedure.

As mentioned before, in case of methionine-derived analogues **10D-D′**, sulphide oxidation was performed prior to hydrolysis using *meta*-chloroperbenzoic acid to bring along the insertion of two additional hydrogen bond acceptors in a terminal position, which is one of the premised design requirements (Figure 1).[3,12] Sulphones **10F-F′** were obtained in this way without significant loss of enantiopurity at the α-position (*ee*(**10F**$_{trans}$) = 85%, Table 2), and could subsequently be hydrolysed in quantitative yields relying on LiOH·H_2O. As such, this pathway enabled the preparation of a set of novel *cis*- and/or *trans*-3-acetamido-1-carboxymethyl-4-phenylazetidin-2-ones **11** in a limited amount of six (or seven for **11F-F′**) steps in up to 30% overall yield, remarkably more efficient compared with the synthesis of their C4-unsubstituted analogues (ten steps, up to 2% overall yield).[3]

The reactant scope of the synthetic pathway can be expanded towards other aromatic or aliphatic aldehydes, combined with readily available (un)natural α-amino acids, if desired.

In order to provide some preliminary data on the biological potential of this series of functionalised 3-amino-1-carboxymethyl-β-lactams **2** (after neutralisation of **11** with HCl to provide the corresponding carboxylic acids), the compounds were subjected to a PBP binding and competition assay. The purified PBP3 of *E. coli* K12, PBP5fm of *E. faecium* D63r and R39 DD-carboxypeptidase of *Actinomadura spp.*[13] were incubated with acids **2**, after which their residual activity (%RA) was determined by labelling the free enzymes with Bocillin FL, a fluorescent reporter molecule and penicillin V analogue (Table 3).[14]

The results revealed the remarkably low residual activity of PBP3 (10% RA), a lethal target of *E. coli*, provoked by compound **2E**$_{trans}$. The inhibitory potency is higher than that of our initial hit **1A** (28% RA),[3] confirming the potential of *C4*-substituted, α-aromatic–and therefore nocardicin-like–3-amino-1-carboxymethyl β-lactam scaffolds for further PBP inhibitor design. The unprecedented and highly straightforward six-step synthetic route described in this work, converting simple substrates into highly functionalised C4-(phenyl-)substituted nocardicin analogues, might therefore be used to further guide expansion studies in the future starting from 1-carboxymethyl-β-lactam **2E**$_{trans}$ as a promising new hit structure (Figure 2).

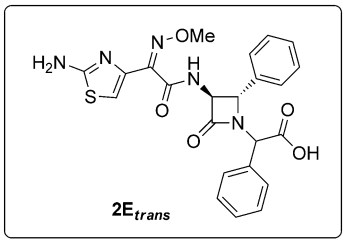


Figure 2. Structure of **2E**$_{trans}$.

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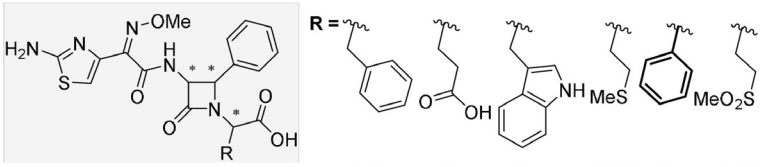
Conflict of interest

The authors declare no conflict of interest.

Table 3. Residual enzymatic activities after incubation of PBP3, PBP5fm and R39 (2.5 μM) with compounds **2** (1 mM, pH 7, 30 °C, 3 h) and IC_{50} values.



2 A-A' B C D-D' E F-F'

Compound	%RA PBP3 (IC_{50} [μM])	%RA PBP5 fm	%RA R39 (IC_{50} [μM])
Aztreonam	0 (0.004–0.022)[a]	–[b]	0 (4)[c]
Blank	100	100	100
2A′$_{cis}$; **2A′**$_{trans}$	87; 77	–	–
2B$_{trans}$	88	–	–
2C$_{trans}$	79	–	–
2D′$_{trans}$	89	–	–
2E$_{cis}$; **2E**$_{trans}$	73; **10** (720)[d]	–; 100	–; 100
2F$_{trans}$	83	78	99

[a] Computed from literature data.[15] [b] Aztreonam is not active against Gram-positive bacteria. [c] Computed from literature data.[16] [d] Indicatory value due to low solubility at >0.8 mM.

Keywords: amino acids · antibiotics · biological activity · Staudinger synthesis · β-lactams

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