FACULTY OF
BIOSCIENCE ENGINEERING

SYNTHESIS OF 3,4-PYRROLIDINE-FUSED BICYCLIC β-LACTAMS AS POTENTIAL β-LACTAMASE INHIBITORS

Bram Van Den Bossche
Student number: 01507249

Promoter: prof. dr. ir. Matthias D'hooghe
Tutor: ir. Sari Deketelaere

Master's Dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Science in Bioscience Engineering: Chemistry and Bioprocess Technology

Academic year: 2019-2020

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ACKNOWLEDGEMENTS

Tempus fugit. In the perpetuum mobile of everyday life, it is astonishing how time flies, and how quickly and unnoticeably days pass into weeks, which become months, and eventually turn into years. My journey of becoming a bioscience engineer, which set out five years ago, now comes to an end. Throughout this demi-decade of exploratory turmoil, I have grown substantially on both an intellectual and personal level. Student life at the "Boerekot" has given me all I could have asked for, and more. Intrigued by nature in all its aspects, the bachelor courses satisfied my curiosity by providing a broad basis in the various scientific fields, while the last two master years allowed me to dig deeper into the wondrous world of organic chemistry, for which I genuinely have grown a profound intrinsic passion. Furthermore, Fortuna bestowed on me the marvellous opportunity of gaining valuable experiences and forging some lifelong friendships. To conclude these joyful times in Ghent and beyond, I am delighted and extremely proud to present this Master's thesis as the pièce de résistance of my studies and as a token for what was, and what is to come. In that respect, I wish to acknowledge and express my gratitude to all people, without whom this work could not possibly have been accomplished.

In the first place, thanks are due to my promoter, prof. D'hooghe, for numerous reasons. To this very day, your reactivity in organic chemistry course remains my favourite, not in a small way because the vim and vigour, with which you taught, grasped my immediate attention and helped pass along your enthusiasm for and knowledge of the field. It is the cornerstone for my current scientific interests, and it made doing my Master's thesis at the SynBioC research group feel like the only right decision for me. I thank you for having granted me this incredible opportunity, and moreover for honouring my wish of getting a thesis subject, which fully focused on organic synthesis and the chemistry, per se. During the course of past year, you provided me with all kinds of helpful advice, and I really appreciate the positive ambience that was ever-present midst our monthly meetings.

Sari, you rightfully deserve a sincere thank you as I am very glad to be able to call you my tutor. I think that the combination of a cheerful vibe, a patient and supportive nature, a strong eye for detail, and keen intellect is all what one could ask for. I know it has been a busy year for you, having multiple thesis students, which is why your willingness to put aside your work when I was in need of help is appreciated even more, as well as your swift and thorough corrections of my thesis. In addition, I want to thank you for allowing me the freedom of exploring ideas of my own, and for the trust you have put in me by giving me this level of independence. However, when facing problems, whether it were difficult reactions, laborious purifications, or challenging structural elucidations, I could always count on your in-depth knowledge and chemical expertise, as I knew I could. For all this, I am truly grateful.

Next, I wish to properly thank everyone, who contributed to making the SynBioC laboratory not only a professional and energetic research environment, but also a place with a great atmosphere. To that end, I say thank you to prof. Stevens, prof. Mangelinckx, Pieter, Maarten, Els, Ans, and all PhD students. A special thanks goes to Carlos, who made my first few weeks of practical work even more pleasant, and whose chemical experience and preliminary research really kick-started my Master's thesis. Furthermore, I thank my fellow thesis students, with whom I have grown close in the past year. My fifth floor boys Jordy, Bjarne and Helder; my fifth floor girls Dalia and Cato; my fourth floor flow fellas Nick, Renaat, Stefan, Ewoud, Lies, Jef and Hanne: because of you all and the many laughs we shared, every moment in the lab was a joy, as were the unforgettable moments outside of it.

Lastly, I would like to mention my closest friends among the vast amount of happy souls and memorable people I was lucky to have encountered along the course of my student life. My Ghent buddies Annelien, Arthur, Cedric, Elias, Fien, Guillaume, Julie, Nick, Renaat, Stijn and Wolf; my Erasmus buddies Nicolas, Théo and Timon: I am extremely grateful for having met all of you, for the awesome times we have had, and for those which are still to come. To finish, I say the biggest thank you of all to my parents and sisters, whose aid, encouragement and support is unconditional and beyond question, as I know it always will be.

With this chapter of my life concluding, I look forward, excited. I seek to broaden my view, to explore the world, to further discover myself, to be amazed. Additionally, I wish to be engulfed by what organic chemistry undoubtedly still has to offer, and I hope to fulfil my ambition of pursuing a fruitful career of research in this fascinating field. How I wonder things will turn out.

Sic Parvis Magna.

Bram Van Den Bossche, June 5th, 2020

PREAMBLE

This preamble was drawn up in mutual agreement between the author and the promoter.

During the course of this Master's thesis, a new human coronavirus, *i.e.* SARS-CoV-2, emerged in Wuhan, China, in late 2019. Ever since, it spread rapidly on a global scale, making that by spring 2020 this virus, and the pneumonia disease it causes (COVID-19), had grown to pandemic proportions. In compliance with the stringent measures imposed by the Belgian government, safety measures were taken by Ghent University in an effort to slow down the spread of the virus as much as possible. All research activities within the context of a Master's thesis, which required physical presence on Ghent University premises, had therefore to be terminated immediately as of March 19$^{\text{th}}$, 2020.

Due to the early cease of lab work, *i.e.* one month prior to the expected date, some of the goals set for this Master's thesis could not be achieved. Concretely, it has resulted in: (i) incomplete characterisation data for some of the novel β-lactam compounds in this work; (ii) no further experiments in order to clarify the outcome of some reactions; (iii) no new experiments in order to synthesise some of the initial target structures. The relevance of constructing latter molecules and their proposed synthetic procedures will be elaborated among other perspectives near the close of this Master's thesis (section 3.4) as a guideline for future research that might wish to continue on the results achieved in this work.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Å	Ångström
Ac	acetyl
ACCN	1,1-azobis(cyclohexanecarbonitrile)
Ad	adamantane
AIBN	azobisisobutyronitrile
ALK	anaplastic lymphoma kinase
AMR	antimicrobial resistance
aq.	aqueous
Ar	aryl
ATR	attenuated total reflectance
ATRC	atom transfer radical cyclisation
BHT	2,6-dimethyl-4-*tert*-butylphenol
bmim	1-butyl-3-methylimidazolium
Bn	benzyl
br	broad
Bu	butyl
°C	degrees Celsius
CAN	cerium(IV) ammonium nitrate
cat.	catalytic amount
Cbz	benzyloxycarbonyl
CDI	1,1'-carbonyldiimidazole
*c*Hex	cyclohexyl
COSY	correlation spectroscopy
CSI	chlorosulphonyl isocyanate
CuAAC	copper(I)-catalysed azide-alkyne cycloaddition
δ	chemical shift
d	doublet *or* deuterated
Δ	reflux temperature *or* difference
2D	two-dimensional
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DG	directing group
DMAP	4-dimethylaminopyridine
DMB	3,4-dimethoxybenzyl
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
dr	diastereomeric ratio
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ee	enantiomeric excess
ELSD	evaporative light scattering detector
equiv.	equivalent(s)
ESI	electrospray ionisation
Et	ethyl
eV	electronvolt
EXSY	exchange spectroscopy
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectrophotometer
g	gram
h	hour(s)
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMPA	hexamethylphosphoramide
HSQC	heteronuclear single-quantum coherence spectroscopy
hv	light
Hz	hertz
IC_{50}	half-maximal inhibitory concentration
INAC	intramolecular nitrone-alkene cycloaddition
*i*Pr	isopropyl
IR	infrared spectroscopy
J	coupling constant
K	Kelvin
l	liquid
L	liter
λ	wavelength
LC-MS	liquid chromatography mass spectrometry
LDA	lithium diisopropylamide
LG	leaving group
LiHMDS	lithium hexamethyldisilazide
m	*meta*
m	multiplet *or* meter
M	mother ion *or* mol per liter
m/z	mass number (m) over charge number (z)
*m*CPBA	*meta*-chloroperbenzoic acid

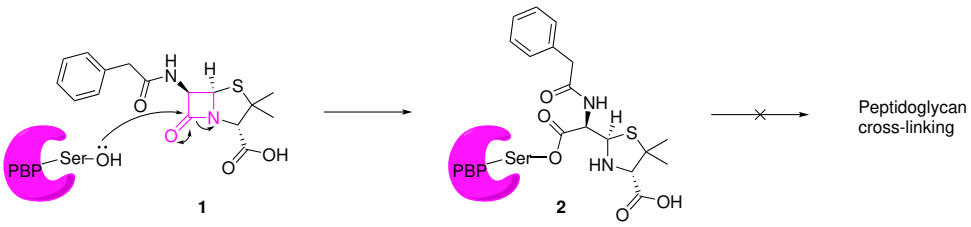
Me	methyl
Mes	mesitylene
MIC	minimal inhibitory concentration
min	minute(s)
Mp	melting point
MS	mass spectrometry
Ms	methanesulphonyl
MW	microwave conditions
NBS	*N*-bromosuccinimide
*n*Bu	*n*-butyl
NCS	*N*-chlorosuccinimide
NHC	*N*-heterocyclic carbene
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
NOE(SY)	nuclear Overhauser effect (spectroscopy)
*n*Pr	*n*-propyl
ν	wavenumber
ν_{max}	wavenumber of the band maximum
o	*ortho*
p	*para*
PBP	penicillin-binding protein
PCMO	polycentric molecular orbital
PE	petroleum ether
PG	protecting group
Ph	phenyl
Phth	phthaloyl
Piv	pivaloyl
PMB	*para*-methoxybenzyl
PMBz	*para*-methoxybenzoyl
PMDETA	*N*,*N*,*N*',*N*",*N*"-pentamethyl-diethylenetriamine
PMP	*para*-methoxyphenyl
ppm	parts per million
psi	pound-force per square inch
PTFE	polytetrafluoroethylene
Py	pyridine
ref.	reference
R_f	retention factor
rt	room temperature
s	singlet
sat.	saturated
SDS	safety data sheet
S_N2	nucleophilic substitution, second order reaction
t	triplet *or* time
t	*tert*
T	temperature
TBAF	tetra-*n*-butylammonium fluoride
TBDMS(Cl)	*tert*-butyldimethylsilyl (chloride)
*t*Bu	*tert*-butyl
TCAI	trichloroacetyl isocyanate
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
tet	tetrahydral
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane (*in NMR*) *or* trimethylsilyl (*in silylated compounds*)
TMSOTf	trimethylsilyl trifluoromethanesulphonate
Tr	triphenylmethyl
trig	trigonal
Ts	4-toluenesulphonyl
U-4C-3CR	Ugi four-center three-component reaction
U-4CC	Ugi four-component condensation
UV	ultraviolet
V	volt
vs.	versus
W	watt
Xantphos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

1 SCOPE AND GOAL

1.1 Scope

Antimicrobial resistance (AMR) is a severe issue of growing concern, threatening to jeopardise global public health and risking a full medicinal set-back to the pre-antimicrobial era.[1,2] AMR poses a serious threat to the effective treatment of infectious diseases caused by bacteria, fungi, parasites and viruses. Due to the ever-present process of evolution, these micro-organisms get resistant to drugs as they develop mechanisms, which allow them to endure a medicine's presence that would otherwise result in their death or at least hinder their growth. However, mankind accelerates this natural process with bad practices such as over-prescribing antimicrobials, over-using them in livestock and fish farming, being insufficiently hygienic, and not finishing drug treatments.[1–7] Nowadays about 700,000 human lives are forfeited each year because of AMR, but if the present situation of excessive antimicrobial use and inadequate development does not change, it is expected that by 2050 this number will rise to an appalling ten million deaths a year, equivalent to one person dying every three seconds, thereby surpassing cancer.[1,8,9] Hence, humanity has a profound need and urgent obligation to tackle the problem of AMR.

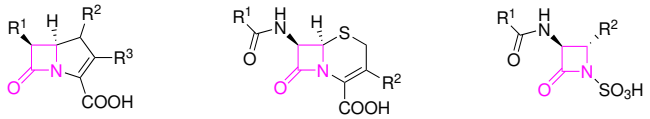
Among antibiotics, none are more well-known than the β-lactams or azetidin-2-ones. These strained four-membered amide ring systems are among the safest and most prescribed pharmaceuticals for the treatment of bacterial infections.[10] A medicinal milestone was reached when more than ninety years ago, in 1928, Alexander Fleming unwittingly discovered the mold-produced benzylpenicillin **1**, which is able to kill bacteria.[11] Building on the earlier work of Hermann Staudinger, who pioneered in 1907 with an even now still popular procedure for the synthesis of β-lactam rings,[12] soon a series of analogous bactericidal β-lactam drugs were produced. All of a sudden, common though fatal bacterial infections like pneumonia, tuberculosis and sepsis became readily treatable and, in addition, the risk of routine surgeries and childbirth was reduced substantially.[8] Penicillins were rightfully acclaimed a miracle drug during World War II, having considerably reduced the death toll among allied soldiers,[2,13] but also after the war they deserve this praise as the average life expectancy in the United States has increased from 47 to 75 years old over the past half century.[14] Going back to the pre-antibiotic era is therefore unacceptable and should be avoided at all costs.



The mode of action of β-lactam antibiotics lies in the effective and irreversible inhibition of the penicillin-binding proteins (PBPs). It is achieved by acylation of the PBPs' catalytic site serine residue due to the nucleophilic attack of its hydroxyl functionality on the antibiotic's azetidin-2-one ring, forming complex **2**. The PBPs are essential enzymes for bacterial cell wall synthesis as they are responsible for the cross-linking of peptidoglycan polymers during its final step. Consequently, inhibition of this reaction by β-lactam moieties significantly weakens the bacterial cell wall, causing growth inhibition or even cell lysis.[15–17]

It should be pointed out that, globally, the usefulness of β-lactams can be divided into two categories. On the one hand there is the direct biological use of these cyclic amides in a medicinal context, since, next to their predominant role in the realm of antibiotics as discussed *in supra*, these molecules can also possess other pharmocological properties, such as exerting antidiabetic, antiparkinson, anti-inflammatory and even anticarcinogenic activity.[18–22] Yet, besides their great biological relevance β-lactams play a significant role as building blocks in organic synthesis as well. Referred to as the "β-lactam synthon method", they form, due to the inherent reactivity of their strained four-membered amide ring, important precursors for a range of both acyclic and heterocyclic compounds.[23–29] This susceptibility of the azetidin-2-one core to nucleophiles is in the end also necessary for its bactericidal effect.

In 1942, a few years after the drug made its clinical debut, the first cases of bacterial resistance against penicillin G **1** were already reported.[6,30,31] Since then, it has been a never-ending race between antibiotics and bacteria, the former designed by mankind to circumvent the ever-adapting AMR mechanisms of the latter. Completely new classes of antibiotics were discovered (*e.g.* tetracyclines), but also within the β-lactams different scaffolds were found to be effective, such as the carbapenems **3**, cephalosporins **4** and monobactams **5**.[32–35]



3 **4** **5**

The observed bacterial AMR mechanisms can be subdivided into four classes according to whether they avoid the antibiotic, remove it, change its target, or destroy it (**Figure 2**).[31,36] The first option comprehends an impediment of the uptake of the antibiotic *via* decreased permeability of the bacterium's outer cell membrane. Another way of becoming resistant is removing the antibiotic, once taken up, with efflux pumps before it can cause any harm to the bacterial cell. This mechanism is often responsible for resistance against tetracycline antibiotics.[31] A third option involves the distortion of the PBPs' active site, causing the antibiotic not being able to acylate the serine residue there, or even impeding the initial binding of the β-lactam to the active site.[36–39] Lastly, bacteria can produce a new type of enzymes, β-lactamases, of which the structure is PBP-like and which are considered the most efficient mechanism of resistance. Instead of getting irreversibly acylated, they hydrolyse the azetidin-2-one ring, rendering the antibiotic inactivated as it is not able to interact with PBPs' catalytic site anymore. Afterwards, the β-lactamases release the inactivated antibiotic and are able to cleave the next β-lactam molecule.[31,36,39] Based on their structure and mode of action, four classes of β-lactamases can be identified: A, B, C and D. Class B enzymes are zinc-dependent hydrolases, while the other three are serine-β-lactamases.[39–41]

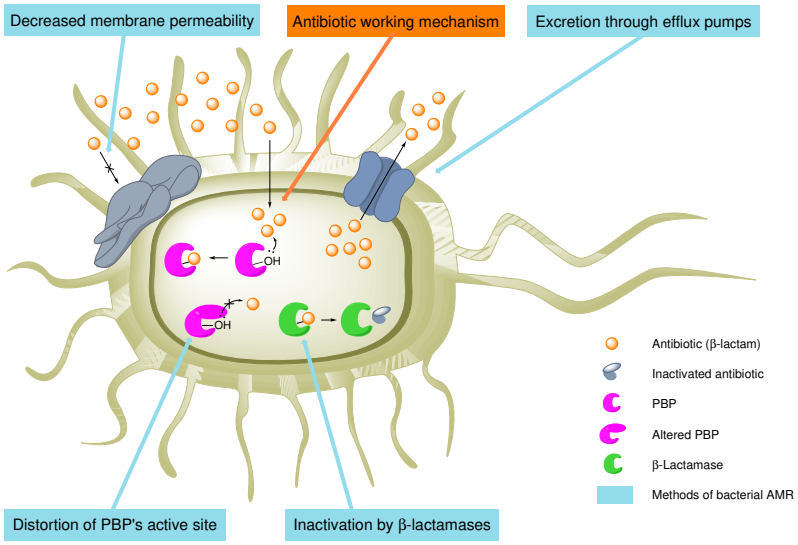
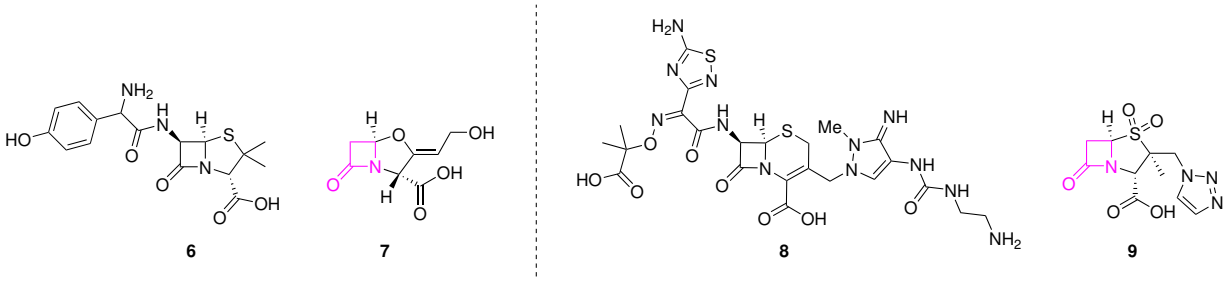


Figure 2: Visualisation of the uptake and working mechanism of β-lactam antibiotics in a bacterium, as well as the four major bacterial mechanisms of achieving antimicrobial resistance (AMR).

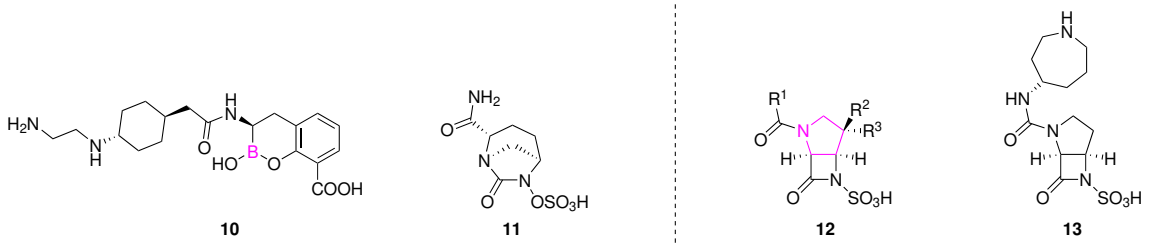
To overcome bacterial resistance against antibiotics, one can employ two major strategies: designing or combining. The first approach focuses on making slight alterations in the structure of the antibiotic itself, and this in such a way that it can still exert its activity, but it does not get hindered anymore in its efficacy by the mechanism of resistance.[42] Cephalosporins **4**, for example, have since their discovery in 1945 been altered over the decades, resulting in five generations of increasingly potent antibiotics.[31,43] Another example is the realisation, because of the discovery of monobactams **5**, that not a *N*-fused bicyclic β-lactam system, as present in penicillins and cephalosporins, but a single azetidin-2-one ring is sufficient for a β-lactam antibiotic to have bactericidal activity.[34,35,44] Therefore, these small compounds might still be able to acylate PBPs' catalytic serine residue in cases where resistance was achieved *via* distortion of the active site.

The second strategy in tackling bacterial AMR involves a combination therapy, in which the medicine contains both the antibiotic as well as a separate drug that acts as an inhibitor of resistance. The latter can be an outer membrane permeabiliser, an efflux pump inhibitor, or a β-lactamase inhibitor.[41,42] The advantage is that antibiotics, which have shown over the years to be both safe and effective for clinical use, can keep being employed without the need for new clinical trials, which would be required if the structure was modified even slightly. In the case of β-lactamase inhibitors, the catalytic site of β-lactamases gets irreversibly bound to the inhibitor, making it unable to further inactivate antibiotics. In recent years, this approach has become increasingly popular and proven successful with examples in the realm of β-lactams, such as the penicillin-type antibiotic amoxicillin **6** and clavulanic acid **7** as β-lactamase inhibitor, a combination which was already discovered in the late 1970s, or the fifth-generation cephalosporin ceftolozane **8** combined with the penicillin-based sulphone tazobactam **9** as a more recent example of a β-lactamase inhibitor.[41,43,45,46]



β-Lactamase inhibitors can differ widely in appearance, and it should be noted that containing an azetidin-2-one ring themselves is not a prerequisite at all. This structural variety is a logical consequence of there being four classes of β-lactamase enzymes, that have substantial structural and mechanistic differences. Finding an inhibitor that can cover all classes therefore seems unlikely, though there are compounds in the pipeline, *e.g.* the non-β-lactam boron-based taniborbactam **10**, which at the moment seems closer than ever to achieving this goal.[41,47–49]

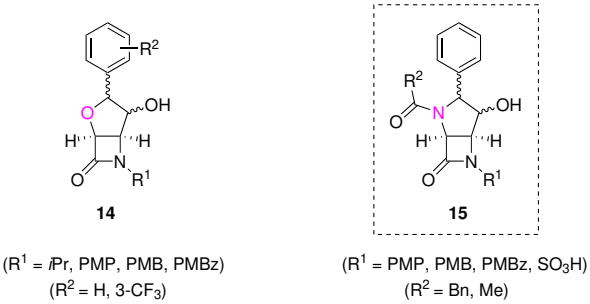
Looking at the already commercially available class A β-lactamase inhibitors clavulanic acid **7** and tazobactam **9**, which are β-lactams themselves, they highlight the original trend present in the search for new β-lactam compounds, *viz.* creating *N*-fused systems as inspired by penicillin G **1**.[44] Azetidin-2-one cores that are 3,4-fused to a ring system, *i.e.* *C*-fused β-lactams, have received much less attention in the literature.[28,45,50–52] Nevertheless they should, since 2,6-diazabicyclo[3.2.0]heptan-7-ones **12** were found to be potent and selective inhibitors to overcome class C β-lactamase-mediated resistance.[53–57] These class C enzymes target cephalosporin antibiotics and are unaffected by traditional class A β-lactamase inhibitors, such as clavulanic acid.[39,46] In addition, almost no class C β-lactamase inhibitors are commercially available, with the non-β-lactam compound avibactam **11**, clinically approved in 2015 by the FDA in combination with the third-generation cephalosporin ceftazidime, being one of the few exceptions.[41,46,58] A specific example of 3,4-pyrrolidine-fused bicyclic β-lactams **12** is MK-8712 **13**, which was selected for preclinical development from a range of analogous molecules because of its efficacy in both *in vitro* and *in vivo* assays, though during the safety studies it had an insufficient therapeutic margin and the development was thus aborted.[45,55,59]



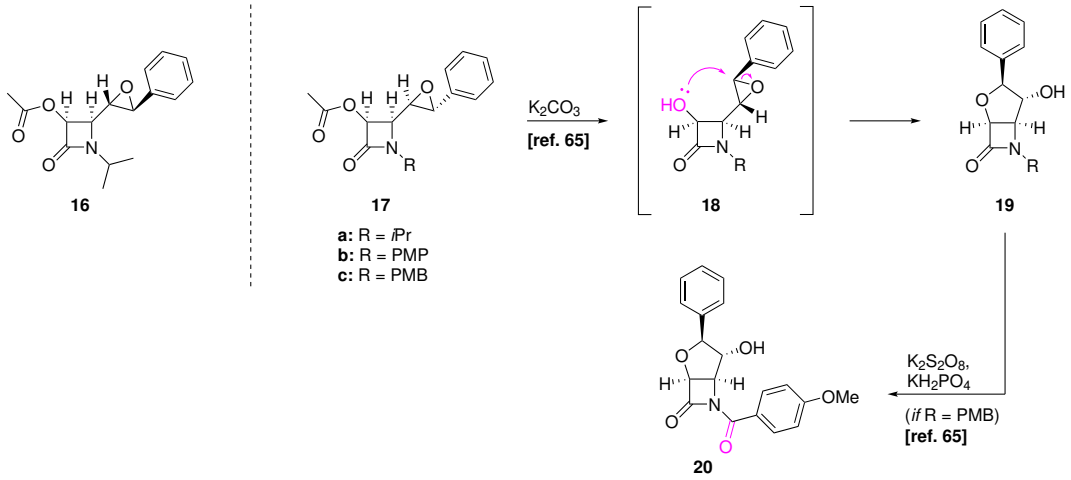
In the already worrisome exponential increase of the frequency of resistance against modern β-lactam antibiotics like third-generation cephalosporins,[31,60] the trend also exists that often a high level of expression of class C β-lactamases in the bacterium is to blame.[61–63] Considering this problematic development, additional research in the field of *C*-fused β-lactams, particularly derivatives of 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **12**, is of paramount importance and will be further elaborated in this Master's thesis.

1.2 Goal

The dire need for commercially available, potent and selective class C β-lactamase inhibitors, as well as the lack of diverse literature methods for synthesising derivatives of *C*-fused bicyclic β-lactams **12** form the inspiration for this Master's thesis. In preliminary research, conducted at the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University), a synthetic strategy for constructing 3,4-oxolane-fused bicyclic β-lactams **14** was developed.[64,65] The goal of this Master's thesis concerns the synthesis of their aza-analogues, *i.e.* 3,4-pyrrolidine-fused bicyclic β-lactams **15**.



The preliminary research, mentioned *in supra*, focused on the formation of *cis*-3-acetoxy-4-(3-aryloxiran-2-yl)azetidin-2-ones **16** and **17**, as intermediates for the synthesis of novel 3-aryl-4-hydroxy-2-oxa-6-azabicyclo-[3.2.0]heptan-7-one scaffolds **14**.[64,65] The strategy employed for accessing *C*-fused bicyclic β-lactams is the attack of a nucleophilic moiety in the β-lactam's C3-substituent on a functionality in the C4-substituent, resulting in the displacement of a leaving group in the C4-side chain and the subsequent construction of the 3,4-annulated ring. In this preliminary research, the leaving group displacement is realised by a hydroxyl group-induced intramolecular ring opening of an epoxide functionality at the benzylic position. *O*-Deacetylation of β-lactams **17**, thus affording the desired free hydroxyl group, proved sufficient in achieving full conversion towards 4-hydroxy-6-isopropyl-3-phenyl-2-oxa-6-azabicyclo[3.2.0]heptan-7-ones **19**. No addition of a base to deprotonate the alcohol, or a Lewis acid to activate the oxirane ring was necessary. Furthermore, it is noteworthy that epoxide diastereomer **16** needed a twentyfold increase in reaction time compared to the other diastereomer **17a** to be rearranged into a 3,4-oxolane-fused bicyclic β-lactam, which is probably due to steric hindrance exerted by the phenyl group during the S_N2-type oxirane ring opening.[64,65]



In an attempt to debenzylate 6-(4-methoxybenzyl)-substituted derivative **19c** (R = PMB), an unprecedented benzylic oxidation was observed, yielding 4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2-oxa-6-azabicyclo[3.2.0]-heptan-7-one **20**.[64,65] This transformation of the previously electron-donating 4-methoxybenzyl group into an electron-withdrawing functionality is interesting, since the former is preferable during Staudinger synthesis as it favours β-lactam formation,[66] while the latter is beneficial for its biological activity as it facilitates ring opening of the β-lactam core by *e.g.* β-lactamase enzymes.[67]

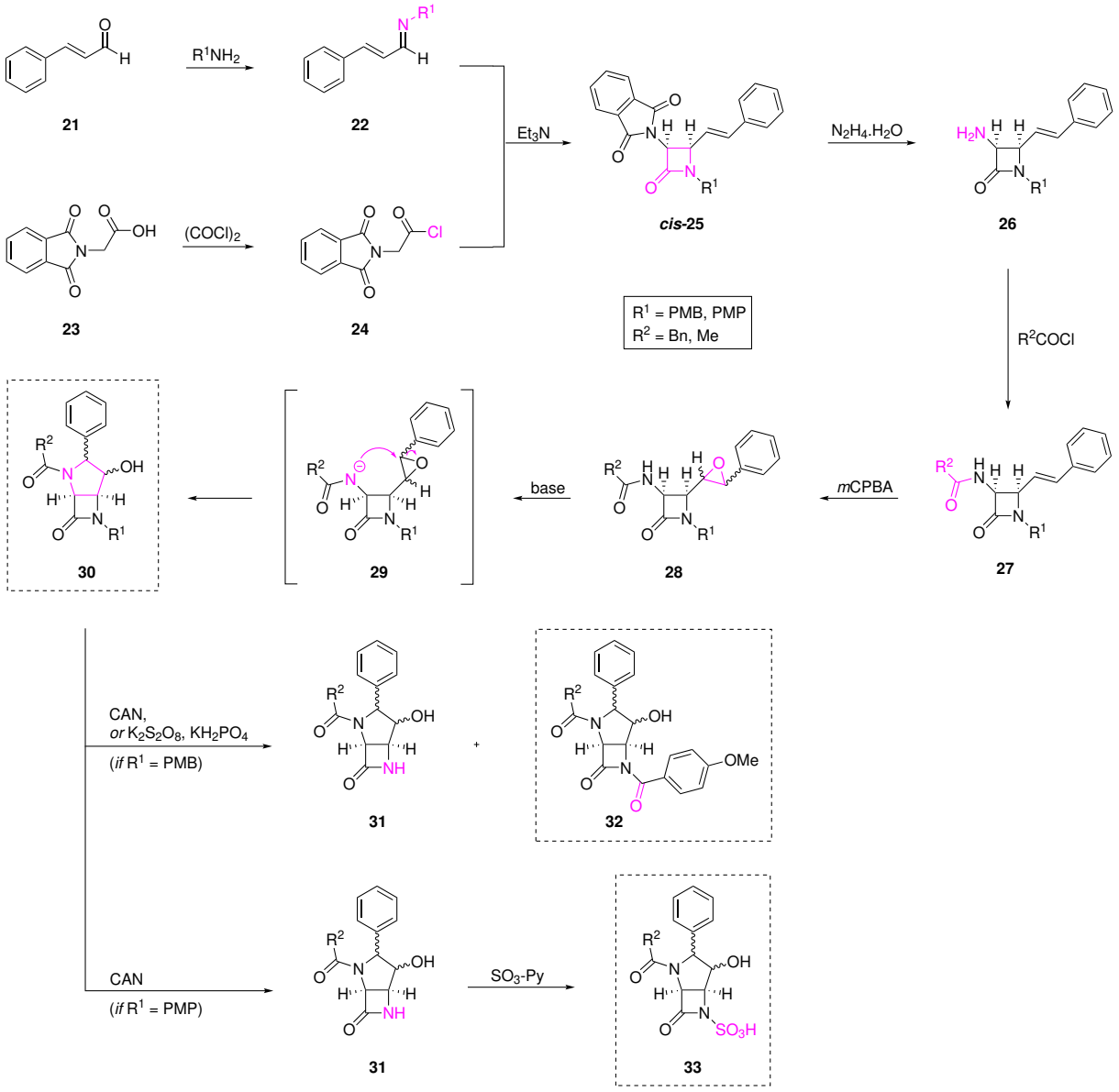
Some initial data concerning the β-lactamase inhibitory potential of the synthesised *C*-fused bicyclic lactams **19a-c** and **20** was obtained *via* incubation with β-lactamase from *Enterobacter cloacae*. No compounds performed better than the reference compound tazobactam **8** (16.2 ± 10.6 % residual β-lactamase activity), though the substantial better result of the *N*-benzoylated derivative **20** (58.7 ± 12.4 %), compared to compounds **19a** (99.3 ± 5.8 %), **19b** (108.8 ± 11.3 %) and **19c** (95.1 ± 12.9 %), supports the literature consensus that an electron-withdrawing group at the β-lactam nitrogen is a necessity for adequate β-lactamase inhibitory activity.[68,69] These results are a hopeful indication that, after further optimisation studies, selective and potent new 3,4-fused bicyclic azetidin-2-ones may be synthesised *via* this methodology, which will overcome class C β-lactamase-mediated resistance in bacteria.[64,65]

The preliminary research *in supra* was inspired by the promising *in vivo* class C β-lactamase inhibitory activity of MK-8712 **13**, but the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University) chose to first focus on constructing oxa-derivatives as these had not yet been synthesised in a context of β-lactamase inhibition. Since this research showed that the intramolecular nucleophilic attack towards 3,4-oxolane-fused bicyclic β-lactams **14** went smoothly, even without the addition of a base or Lewis acid to facilitate the cyclisation,[64,65] the construction of its aza-analogues, *i.e.* 3,4-pyrrolidine-fused bicyclic β-lactams **15**, was considered. The greater similarity with MK-8712 **13** put aside, our willingness to construct 2,6-diazabicyclo[3.2.0]heptan-7-ones β-lactams **15** has other motivations as well. A nitrogen atom connected to a β-lactam's C3 shows a higher resemblance to β-lactamases' natural substrate, *i.e.* β-lactam antibiotics such as cephalosporins **4**. Keeping in mind that class C enzymes are serine cephalosporinases, meaning that they selectively focus on cephalosporins as their substrate, this is an important factor.[41] Furthermore, incorporating a nitrogen instead of an oxygen in a molecule also results in a significant structural alteration as nitrogen is trivalent. Therefore, this modification will result in an additional substituent on the diazabicyclic scaffold, which might have benign interactions with the β-lactamases' catalytic site, hereby improving the efficacy of the inhibitor.

To that end, a seven-step synthesis is proposed for the construction of 2-acyl-4-hydroxy-2-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **30**. At first, *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-ones ***cis*-25** will be created *via* a triethylamine-mediated Staudinger [2+2] cyclocondensation after the imination of (*E*)-cinnamaldehyde **21** towards *N*-substituted (1*E*,2*E*)-3-phenylprop-2-en-1-imines **22** and the conversion of *N*-phthaloylglycine **23** into *N*-phthaloylglycyl chloride **24** upon reaction with oxalyl chloride. Acylation of the C3-amino substituent will be performed through deprotection of the *N*-phthaloyl group, affording 3-amino-β-lactams **26**, and the subsequent reaction of their free amino functionality with an acid chloride. Next, these *cis*-3-acylamino-4-((*E*)-styryl)azetidin-2-ones **27** will undergo epoxidation, for which 3-chloroperbenzoic acid (*m*CPBA) is selected as the oxidising agent, most probably yielding a diastereomeric mixture of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28**. Lastly, an amido group-induced intramolecular ring closure through ring opening of the epoxide functionality will result in the construction of the desired 3,4-pyrrolidine-fused bicyclic β-lactams **30**, which is the primary goal of this Master's thesis.

The key cyclisation step towards bicyclic structures **30** is expected to be complicated by the fact that an acyl group reduces the nucleophilicity of the C3-attached nitrogen. Nevertheless, this route is chosen as it is probably unfeasible in the presence of a free amino group to use a peracid for creation of the oxirane moiety without causing harm to the former. Therefore, a screening will be done to find the right base, for which the C3-amido functionality of epoxides **28** will get deprotonated and perform a nucleophilic attack at the benzylic position of the oxirane ring. Circumventing above complication by epoxidating before or just after Staudinger synthesis of the β-lactam moiety, is equally considered irreconcilable with the current synthetic approach. The rationale for this lies in the likely and probably unfavourable interaction of the hydrazine hydrate reagent with the reactive three-membered oxirane ring during the *N*-phthaloyl deprotection step. In that respect, epoxidation of the double bond has to occur after both C3-amino deprotection and *N*-acylation.

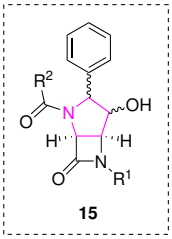
Since 3,4-oxolane-fused bicyclic azetidin-2-ones with an electron-withdrawing group at the β-lactam nitrogen have been shown in the preliminary research cited *in supra* to exhibit class C β-lactamase inhibitory activity,[64,65] incorporating such a functionality in 3,4-pyrrolidine-fused bicyclic β-lactams **15** forms a secondary goal of this Master's thesis. In particular, the introduction of a sulphonic acid group at the N6-position is especially interesting, as its electron-withdrawing capability not only facilitates nucleophilic attack at the β-lactam carbonyl,[67] but the anionic site, which it becomes *in vivo*, also appears to be essential for binding with the target enzymes,[70] and for the bioactivity. The reason lies respectively in its formation of a salt bridge with a lysine residue in the class C β-lactamases' active site, and in its displacement of the deacylating water molecule, as was proven by X-ray crystallography of enzyme-inhibitor complexes (section 2.4).[71–73]



Performing a selective benzylic oxidation of a 4-methoxybenzyl (PMB) substituent at nitrogen N6 towards 4-methoxybenzoyl (PMBz), affording β-lactams **32**, is desired as its inhibitory activity could then be compared with 3,4-oxolane-fused bicyclic β-lactams **20**. This reaction will be accomplished with a combination of potassium persulphate and potassium dihydrogen phosphate, as was employed in preliminary research,[64,65] but cerium(IV) ammonium nitrate (CAN) can be used as well. However, based on the literature, the latter might result in a mixture of PMBz-substituted and N6-deprotected compounds **32** and **31**.[74,75] When the N6-position contains a 4-methoxyphenyl (PMP) moiety, it is expected that upon reaction with CAN only a N6-deprotection will occur,[74,76–79] resulting in the exclusive formation of 4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **31**. The newly created free amino functionality will then be employed to get the desired sulphonic acid group attached to it *via* treatment with *e.g.* sulphur trioxide pyridine,[80–82] affording 4-hydroxy-7-oxo-3-phenyl-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33** after an overall synthesis of nine steps as the ultimate target structures of this Master's thesis. The newly synthesised 3,4-pyrrolidine-fused bicyclic β-lactams **30**, **32** and **33** will be evaluated for their biological activity against class C β-lactamases in collaboration with prof. T. Desmet (Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University).

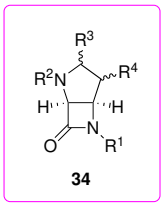
2 LITERATURE OVERVIEW

As the goal of this Master's thesis concerns the synthesis of 3,4-fused azetidin-2-ones, and more specifically 3,4-pyrrolidine-fused bicyclic β-lactams **15**, this literature overview will focus on the different existing synthesis routes leading towards various 2,6-diazabicyclo[3.2.0]heptan-7-ones **34**. These syntheses can be divided into three groups: routes that create a (hetero)cycle on the β-lactam core (section 2.1), routes that start with the ring structure and where only in a last step the β-lactam moiety is formed (section 2.2) or, thirdly, routes that transform acyclic precursors directly into a *C*-fused bicyclic β-lactam (section 2.3). Within each group the routes can be classified according to their reaction type (nucleophilic, pericyclic, radical or transition metal-catalysed). Albeit our main interest, not all literature discussed in this chapter particularly focuses on 3,4-pyrrolidine-fused systems **34**, though each time a five- or six-membered ring, whether or not containing a nitrogen, oxygen or sulphur heteroatom, is created that is *C*-fused to the azetidin-2-one core. Furthermore, since there exist a lot of divergent ways to form 3,4-annulated β-lactams, the relevance of some methods shall be pointed out, though they will not be discussed in full detail. In the last part of this chapter, the biological mechanism and the reason for the effectiveness of *C*-fused bicyclic β-lactams against class C β-lactamases will be elaborated (section 2.4).



15

(R^1 = PMP, PMB, PMBz, SO_3H)
(R^2 = Bn, Me)

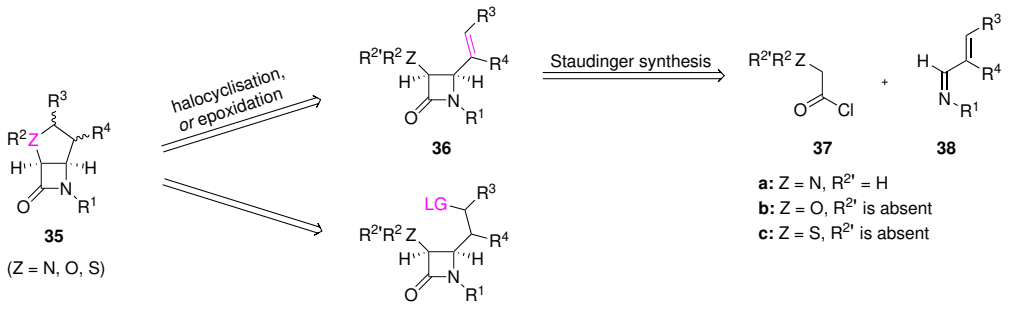


34

2.1 Synthesis of *C*-fused bicyclic β-lactams starting with the β-lactam

2.1.1 Intramolecular nucleophilic attacks

An often applied synthesis strategy towards *C*-fused bicyclic β-lactams **35** is a two-step cyclisation, in which after formation of the β-lactam ring either the C3-substituent attacks the C4-substituent, or the other way around, in a nucleophilic way.[65,71,83–94] In most of the cases *cis*-substituted β-lactams **36** are created *via* the Staudinger [2+2] cyclocondensation of ketenes, formed *in situ* by addition of a tertiary base to acid chlorides **37**, and imines **38**. Subsequently, when the imine is α,β-unsaturated, one can opt to halogenate or epoxidise the π-bond, after which a heteroatom originating from the acid chloride can perform a nucleophilic substitution, thus forming the bicyclic core. Evidently, any method that creates β-lactams **39**, of which the C3-substituent contains a (pro)nucleophilic heteroatom and the C4-substituent a good leaving group, or *vice versa*, can lead to the construction of *C*-fused heterocycles **35** *via* an intramolecular nucleophilic substitution.



35
(Z = N, O, S)

36

39
(LG = Cl, Br, I, OMs, OTs)

37 **38**

a: Z = N, $R^{2'}$ = H
b: Z = O, $R^{2'}$ is absent
c: Z = S, $R^{2'}$ is absent

2.1.1.1 Halocyclisations

The quite popular halocyclisation synthetic route towards 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **34** is in fact, as mentioned *in supra*, a double annulation strategy: α-aminoacetyl chlorides **37a** and α,β-unsaturated imines **38** undergo a classical Staudinger β-lactam synthesis, followed by the ring closure of the heterocycle onto *cis*-β-lactams **36a** through a halogen-promoted heterocyclisation process.[83] α-Alkoxy- **37b** or α-(alkylthio)acetyl chlorides **37c** can be used as well, though the latter do not possess the *cis*-selectivity that is usually present with its nitrogen and oxygen analogues and which is needed, according to the literature, to make a halogen-mediated cyclisation possible.[83–86] Benefits of the two-step halocyclisation method are its low number of steps required to access various [3.2.0]-bicyclic systems, as well as the freedom to easily alter the ring substituents (R^1, R^2, R^3 and R^4).[83]

In view of performing as β-lactam synthons for the synthesis of highly functionalised proline esters **44**, which can be employed as organocatalysts and are important building blocks in natural products and compounds of pharmaceutical interest, Kumar *et al.* developed a simple metal-free regio- and diastereoselective synthesis of 2,6-di(alkyl/aryl)-4-halo-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **43** *via* unfavourable intramolecular 5-*endo*-*trig* haloamination of *cis*-3-aminoazetidin-2-ones **40** in good yields.[87,88] They assessed the influence of different halogenating reagents, bases and solvents on the yield of halocyclised product **43a** (**Table 1**). Molecular halogens (Br_2, I_2) performed way better than their succinimide counterparts (NBS, NIS) and within the halogen group iodine performed clearly better than bromine. This is probably attributable to the mild nature of iodine, whereas the stronger acidity of bromine makes it participate in more side reactions. *N*-Chlorosuccinimide (NCS) did not give any reaction and substituting potassium carbonate by sodium carbonate lowered the yield. Using stronger bases, *viz.* sodium hydride and potassium *tert*-butoxide, resulted in deterioration of the product. Changing the solvent from dichloromethane to *N*,*N*-dimethylformamide (DMF) or tetrahydrofuran (THF) also lowered the yield. In conclusion, a iodocyclisation with potassium carbonate in dichloromethane performs the best. When considering different N1-substituents (R^1), generally not much difference in reactivity as well as in yield of the bicyclic product was observed (**Table 2**). Upon comparison of substituents of the 3-amino group (R^2 and R^3), the 5-*endo*-*trig* haloamination did not occur in the presence of an electron-withdrawing group (*e.g.* 4-toluenesulphonyl, compound **40d**), but it could with an electron-donating group (*e.g.* methyl, compound **40b**), though only a fair yield was obtained. The dimethylated compound **40c**, however, did not react due to steric hindrance during the halocyclisation.[87,88]

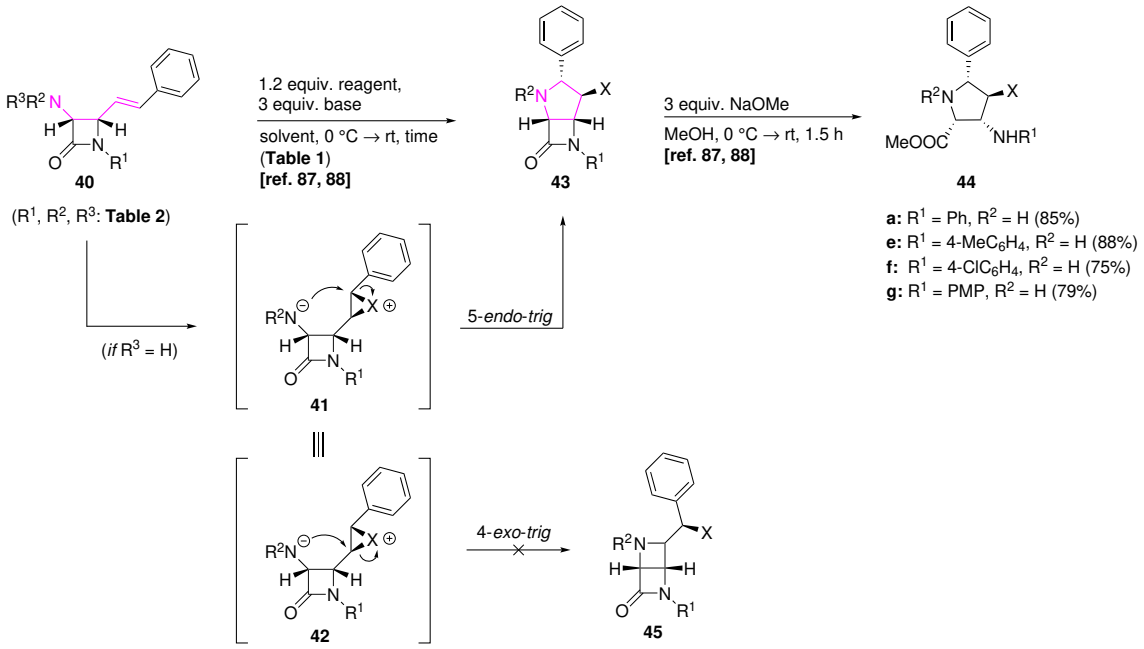


Table 1: Reaction conditions and yields for the intramolecular 5-*endo-trig* halocyclisation of *cis*-3-aminoazetidin-2-one **40a** towards 4-halo-3,6-diphenyl-2,6-diazabicyclo[3.2.0]heptan-7-one **43a** (R^1 = Ph, R^2 = H, R^3 = H).[87,88]

Reagent[a]	Base	Solvent[b]	Time [min]	Yield[c] [%]
I_2	K_2CO_3	CH_2Cl_2	90	90
NIS	K_2CO_3	CH_2Cl_2	30	40
Br_2	K_2CO_3	CH_2Cl_2	45	61
NBS	K_2CO_3	CH_2Cl_2	60	20
NCS	K_2CO_3	CH_2Cl_2	90	-[d]
I_2	Na_2CO_3	CH_2Cl_2	90	80
Br_2	Na_2CO_3	CH_2Cl_2	90	45
I_2	KO*t*Bu	CH_2Cl_2	90	-[e]
I_2	NaH	CH_2Cl_2	90	-[e]
I_2	K_2CO_3	DMF	80	55
I_2	K_2CO_3	THF	90	30

[a] NIS = *N*-iodosuccinimide, NBS = *N*-bromosuccinimide, NCS = *N*-chlorosuccinimide.

[b] DMF = *N,N*-dimethylformamide, THF = tetrahydrofuran.

[c] Isolated yield after purification. [d] No reaction occurred. [e] Deterioration of the products.

Regarding the mechanism an initial coordination of a halogen atom to the double bond of the β-lactam's C4-substituent is proposed, forming halonium ions **41**.[87–89] Subsequently, the nitrogen at carbon C3 performs a nucleophilic attack at the C6-position of the halonium ion. The [3.2.0]-bicyclic systems **43** are thus formed by a 5-*endo-trig* cyclisation and not a 4-*exo-trig* halocyclisation. Although the latter is favoured according to Baldwin's rules for ring closure reactions of aliphatic compounds based on orbital overlap requirements,[95] a 4-*exo-trig* cyclisation would result in severely strained [2.2.0]-bicyclic cores **45**, which are thermodynamically less stable than [3.2.0]-systems **43**.[89]

Table 2: Substitution pattern and yields for the intramolecular 5-*endo-trig* halocyclisation[a] of *cis*-3-aminoazetidin-2-ones **40** towards 2,6-di(alkyl/aryl)-4-iodo-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **43**.[87,88]

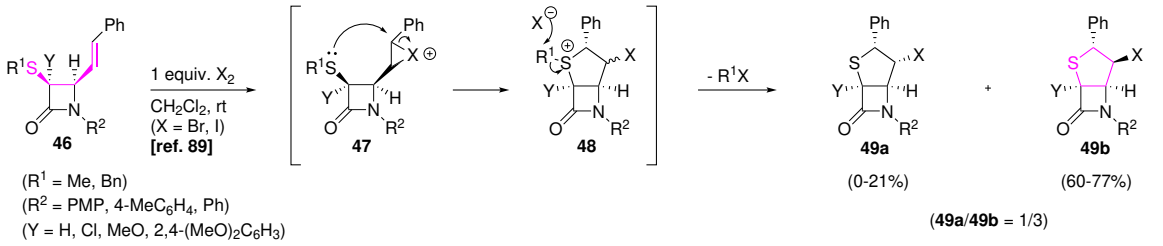
	R^1	R^2	R^3	Yield[b] [%]
40a	Ph	H	H	90
40b	Ph	Me	H	75
40c	Ph	Me	Me	-[c]
40d	Ph	Ts	H	-[c]
40e	4-MeC_6H_4	H	H	82
40f	4-ClC_6H_4	H	H	65
40g	PMP	H	H	66
40h	4-FC_6H_4	H	H	62
40i	*c*Hex	H	H	75
40j	Bn	H	H	60

[a] Reaction conditions: 1.2 equiv. I_2, 3 equiv. K_2CO_3, CH_2Cl_2, 0 °C → rt, 90 min.

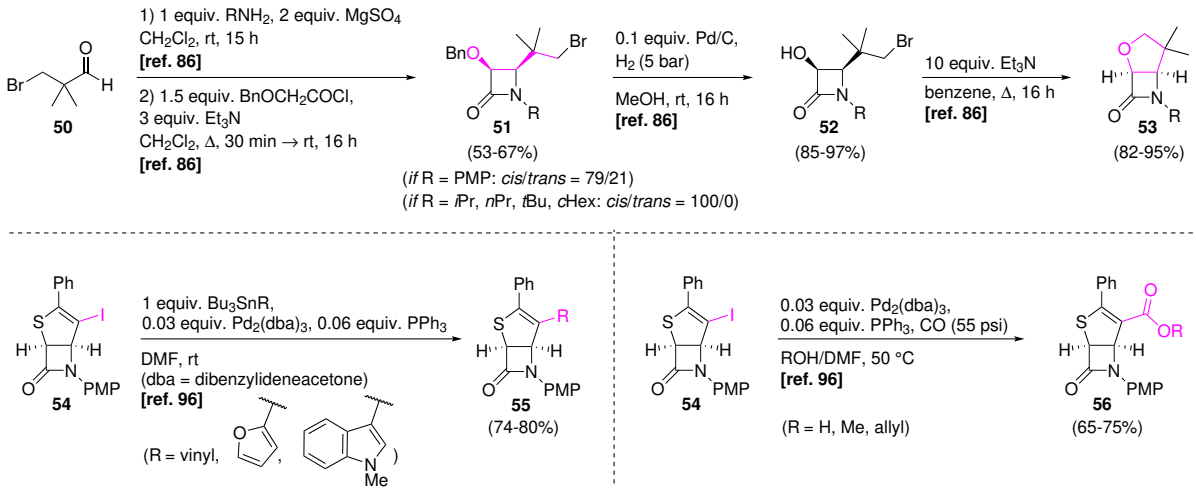
[b] Isolated yield after purification.

[c] No reaction occurred, even after stirring for several hours at different temperatures, and even when higher amounts of iodine were used, or other bases were employed.

Research by Bari and co-workers illustrates that halocyclisations are not intrinsically diastereoselective: *cis*-3-alkylthio-β-lactams **46** cyclised to a mixture of 4-halo-2-thia-6-azabicyclo[3.2.0]heptan-7-ones **49a**/**49b** in 1/3 ratios.[89] This favoured formation of isomers **49b** can be attributed to the interaction of the vinyl phenyl group with the C3-substituent, as well as to the positioning of sulphur's free electron pairs for attack at the terminus of halonium ions **47**.[83,84,89]



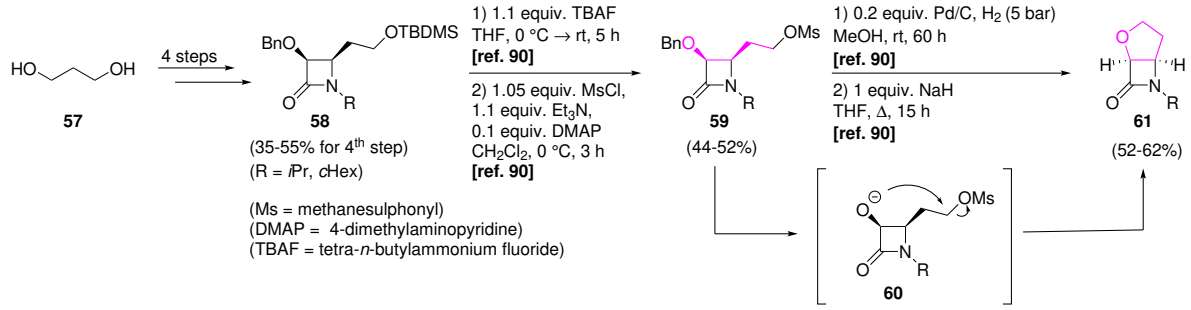
De Kimpe *et al.*'s research shows the possibility to circumvent a non-diastereoselective halocyclisation of the double bond by commencing the reaction with an already halogen-containing reagent, *e.g.* 3-bromo-2,2-dimethylpropanal **50**.[86] After palladium-catalysed hydrogenolysis of β-lactams' **51** benzyl ether substituent at the C3-position, a base-promoted nucleophilic attack by the hydroxyl group on the bromine resulted in formation of *cis*-2-oxa-6-azabicyclo[3.2.0]heptan-7-ones **53**. As expected, the attempted ring closure of a *trans*-β-lactam to the *trans*-bicyclic compound did not occur. It is noteworthy that this method avoids ending up with a halogen at the C4-position of the *C*-fused bicyclic β-lactam. Nonetheless, literature shows palladium(0)-promoted reactions can easily transform this halogen in another functional group.[96] A Stille cross-coupling of 4-iodo-*C*-fused penems **54** with various organotin compounds can introduce heteroaromatics, olefins and upon ozonolysis of the latter even aldehydes onto the bicyclic core. On the other hand, palladium(0)-catalysed CO insertions in alcohol/DMF and water/DMF solvent mixtures are able to prepare respectively esters and carboxylic acids **56** directly from the iodine substituent. These carboxylations need to be run for a few days at room temperature and with less than 1 % of water present to prevent substantial hydrolysis of the β-lactam ring.[96] The unsaturated bond present in the annulated ring system of 4-iodo-*C*-fused penems **54** can be achieved by performing a iodocyclisation on β-lactams with an alkyne-containing C4-side chain instead of an alkene functionality.[83]



2.1.1.2 Intramolecular nucleophilic substitutions of non-halogen leaving groups

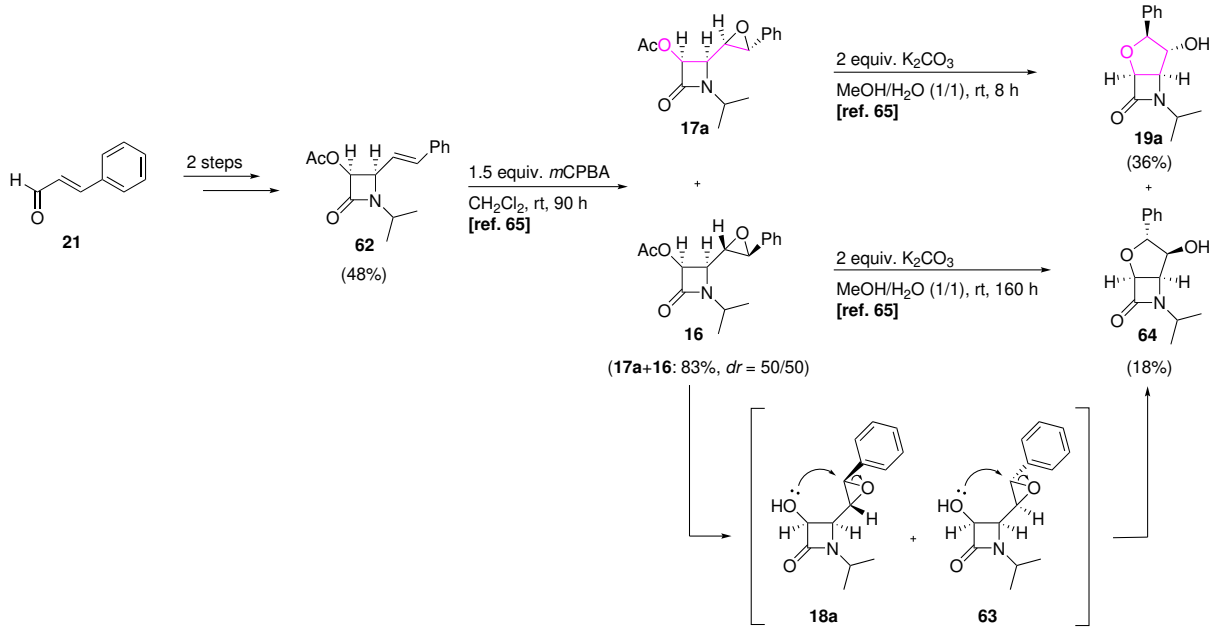
Nucleophilic substitution of mesylates and tosylates

The principle of the halocyclisation reaction can in a similar manner be applied to other leaving groups. Building on their research mentioned *in supra*, where starting material **50** of the halogen promoted heterocyclisation already contained bromine,[86] De Kimpe and co-workers used a similar strategy to obtain 3,4-oxolane-fused bicyclic β-lactams **61**, yet now the leaving group during the intramolecular nucleophilic substitution is a mesylate group.[90] Propane-1,3-diol **57** was monoprotected with *tert*-butyldimethylsilyl chloride (TBDMSCl) and afterwards the remaining hydroxyl moiety underwent a Swern oxidation and consecutive imination. Staudinger synthesis then afforded *cis*-4-[2-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-ones **58** in moderate yields. Liberation of the TBDMS-protected hydroxyl functionality and its subsequent mesylation towards β-lactams **59**, followed by palladium-catalysed ether hydrogenolysis and base-induced hydroxyl activation at the C3-position, resulted in formation of the desired *cis*-2-oxa-6-azabicyclo[3.2.0]heptan-7-ones **61** in high yields.[90] Other literature proves that above synthesis strategy works equally well with tosylate leaving groups.[91]



Nucleophilic substitution of epoxides

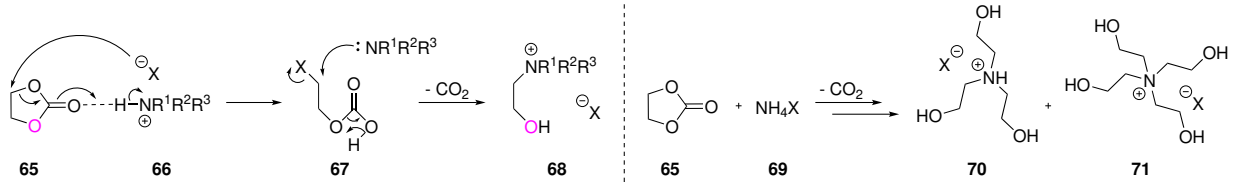
In preliminary research, conducted at the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University) and mentioned in the chapter "Scope and goal", the double bond of 4-((*E*)-styryl)azetidin-2-ones **62** was epoxidised with 3-chloroperbenzoic acid (*m*CPBA), generating a 1/1 diastereomeric mixture of *cis*-3-acetoxy-1-isopropyl-4-(3-phenyloxiran-2-yl)azetidin-2-ones **16**/**17a**.[64,65] If a diastereoselective synthesis is desired, the alkene functionality can be epoxidised prior to β-lactam formation, causing the aryloxirane moiety to impose steric hindrance during Staudinger synthesis. To construct 3,4-oxolane-fused bicyclic β-lactams **64**/**19a** out of β-lactams **16**/**17a**, a deprotection of the alcohol group *via* ester hydrolysis with potassium carbonate, followed by a hydroxyl group-induced intramolecular ring closure by ring opening of the epoxide moiety at the benzylic position, was proposed. The latter was envisioned to be facilitated by addition of a base to deprotonate the hydroxyl group, or a Lewis acid to activate the oxirane ring. Nevertheless, it was observed that *O*-deacetylation sufficed for the *C*-fused bicyclic β-lactam formation to occur spontaneously. When this procedure was applied to a mixture of 4-oxiranyl-β-lactams **16**/**17a** (1/1), it was apparent that for epoxide diastereomer **16** the rearrangement into 4-hydroxy-3-phenyl-2-oxa-6-azabicyclo[3.2.0]heptan-7-one **64** needed a twentyfold increase in reaction time compared to the other diastereomer **17a**, which is probably due to steric hindrance exerted by the phenyl group during the S_N2-type oxirane ring opening.[64,65]



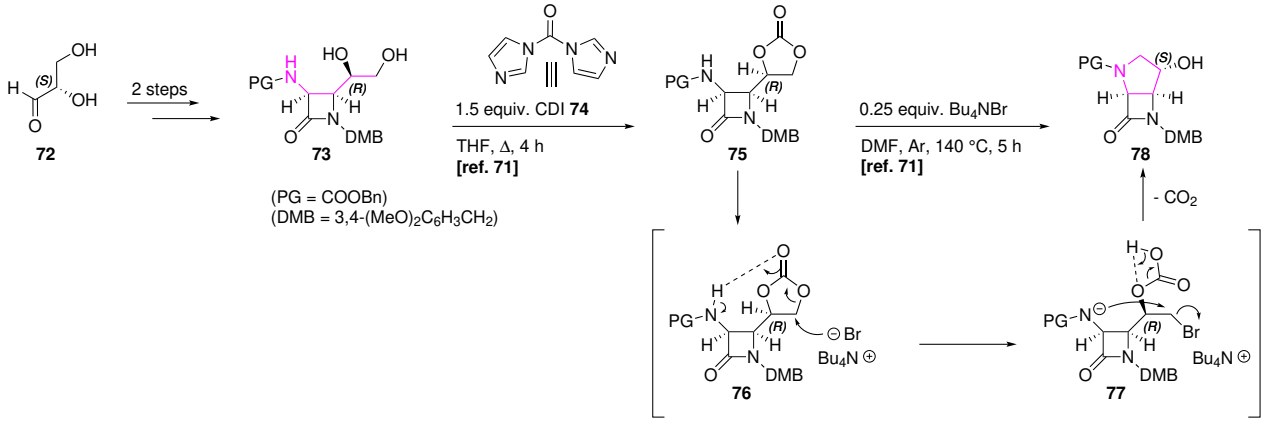
Nucleophilic substitution of ethylene carbonates with tetra-alkylammonium halides

A quite different way, yet relying on the same principle, to synthesise the 2,6-diazabicyclo[3.2.0]heptan-7-one core is based on the research by Yoshino *et al.* on the nucleophilic substitution reaction of amine hydrohalides **66** with ethylene carbonate **65**.[92,93] The proposed two-step mechanism consists of a nucleophilic attack by the halide ion on the carbonate moiety, which is activated by the amine hydrohalide's proton. Next, the formed amine uses the now available free electron pair of its nitrogen to substitute the halogen atom of intermediate **67**, while in the meantime a rearrangement with evolution of carbon dioxide gas creates the β-hydroxyl

functionality, affording β-hydroxyethylammonium halides **68**. If ammonium halides **69** are used instead of amine hydrohalides **66**, the molecules can take part in multiple reactions, resulting in the formation of tri- and tetra-β-hydroxyethylammonium halides **70** and **71**, considering for each hydrogen atom a deprotonation can occur and the subsequent attack of an ethylene halohydrine intermediate **67**.



Using this 2-hydroxyethylation reaction, 3,4-pyrrolidine-fused bicyclic β-lactams **78** can be created from the readily available (*S*)-glyceraldehyde **72**.[71] After its imination and subsequent use in a Staudinger [2+2] cyclocondensation, vicinal diol **73** was formed, which could be transformed into carbonate **75** by 1,1'-carbonyldiimidazole **74** (CDI). Key in the second cyclisation step is using a tetra-alkylammonium halide, *e.g.* tetrabutylammonium bromide, and not an amine hydrohalide as otherwise it will compete with the 3-amino substituent of the β-lactam to get β-hydroxyethylated. Now, only the C3-nitrogen can activate the carbonate by protonation and subsequently perform a S_N2-reaction, forming desired bicyclic product **78**.



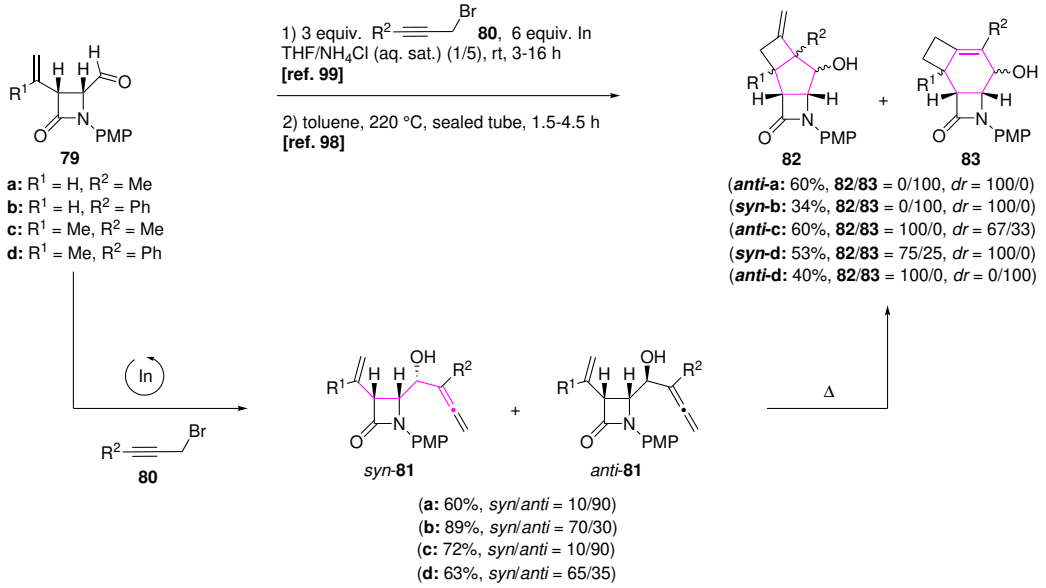
2.1.2 Intramolecular pericyclic reactions

A second group of reaction types, that can annulate a (hetero)cycle onto the azetidin-2-one core, are the pericyclic reactions. This subsection concisely gives an overview of the various mechanisms found in literature yielding *C*-fused β-lactams, which covers in fact three of the four major types of pericyclic reactions with only the electrocyclisations missing.

2.1.2.1 Alkene-allenol [2+2] cycloadditions

With their substantial resistance against β-lactamases and dehydropeptidases,[97] not only bicyclic azetidin-2-ones experience a renewed scientific interest, but in general novel polycyclic β-lactam systems do.[50] Alcaide and co-workers designed a synthesis pathway leading to strained tricyclic β-lactams **82** and **83** containing a cyclobutane ring.[98] An intramolecular thermal [2+2] cycloaddition of an alkene substituent at C3 and an allene moiety at C4 in azetidin-2-one-tethered enallenols **81** was envisioned. Starting from a racemic mixture of 4-oxoazetidine-2-carbaldehydes **79**, α-allenic alcohols **81** were created by an indium-mediated Barbier-type carbonyl allenylation of β-lactam aldehydes **79** with propargyl bromides **80** in aqueous media.[99] Both diastereomers were formed, though when a methyl is the allene substituent (R^2 = Me) more of diastereomers ***anti*-81** were formed, while with a phenyl ring (R^2 = Ph) the ratio shifted in favour of diastereomers ***syn*-81**. Next, a thermolysis of the major diastereomer in toluene was employed to let the allene group act as a 2π-electron donor in the [2+2] process, creating the desired tricyclic structures **82** and **83**. Surprisingly,

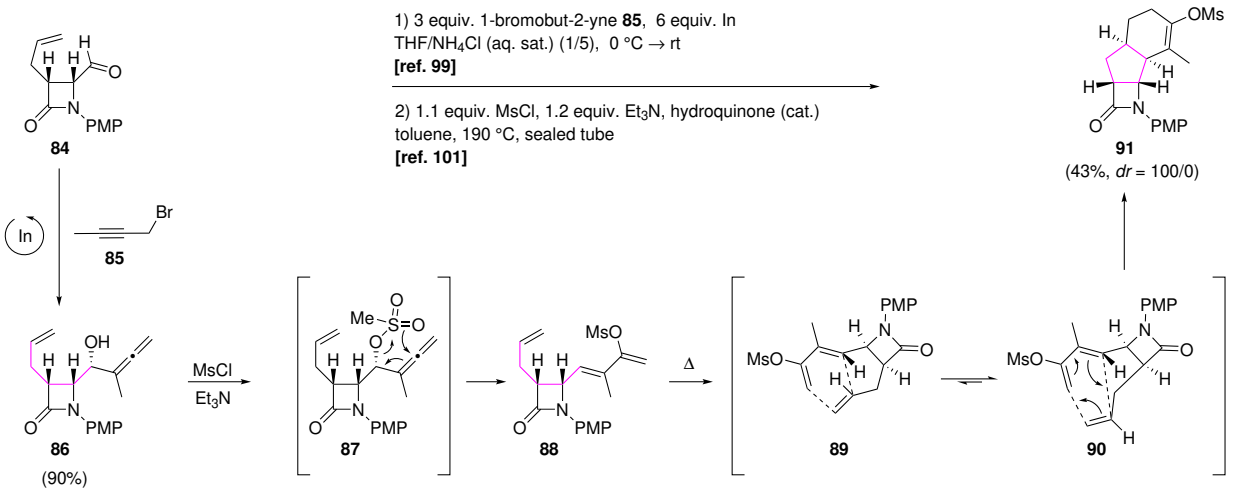
only a slight variation in the substituent of the alkene moiety (R^2) switched around the regioselectivity in the allene component, resulting in a preferred formation of the cyclobutane-annulated cyclopentane ring or -hexene ring *C*-fused to the β-lactam, depending whether the alkene respectively is an isopropenyl (R^1 = Me) or vinyl (R^1 = H) group. Furthermore, new diastereomers can be created during this second reaction, depending whether the *cis*-substituted R^1- and R^2-groups of the cyclopentane derivatives **82**, or just the R^1-substituent of the cyclohexene derivatives **83**, are oriented in-plane or out-of-plane. Some compounds (***anti*-81a**, ***syn*-81b** and ***anti*-81d**) yielded a single regio- and diastereomer, while others gave rise to a mixture of the regio- (***syn*-81d**) or diastereomers (***anti*-81c**). Substituents at the allene carbon (R^2) on the other hand did not influence the preferred regiochemistry of the cycloaddition, though they did effect the reaction rate.[98]



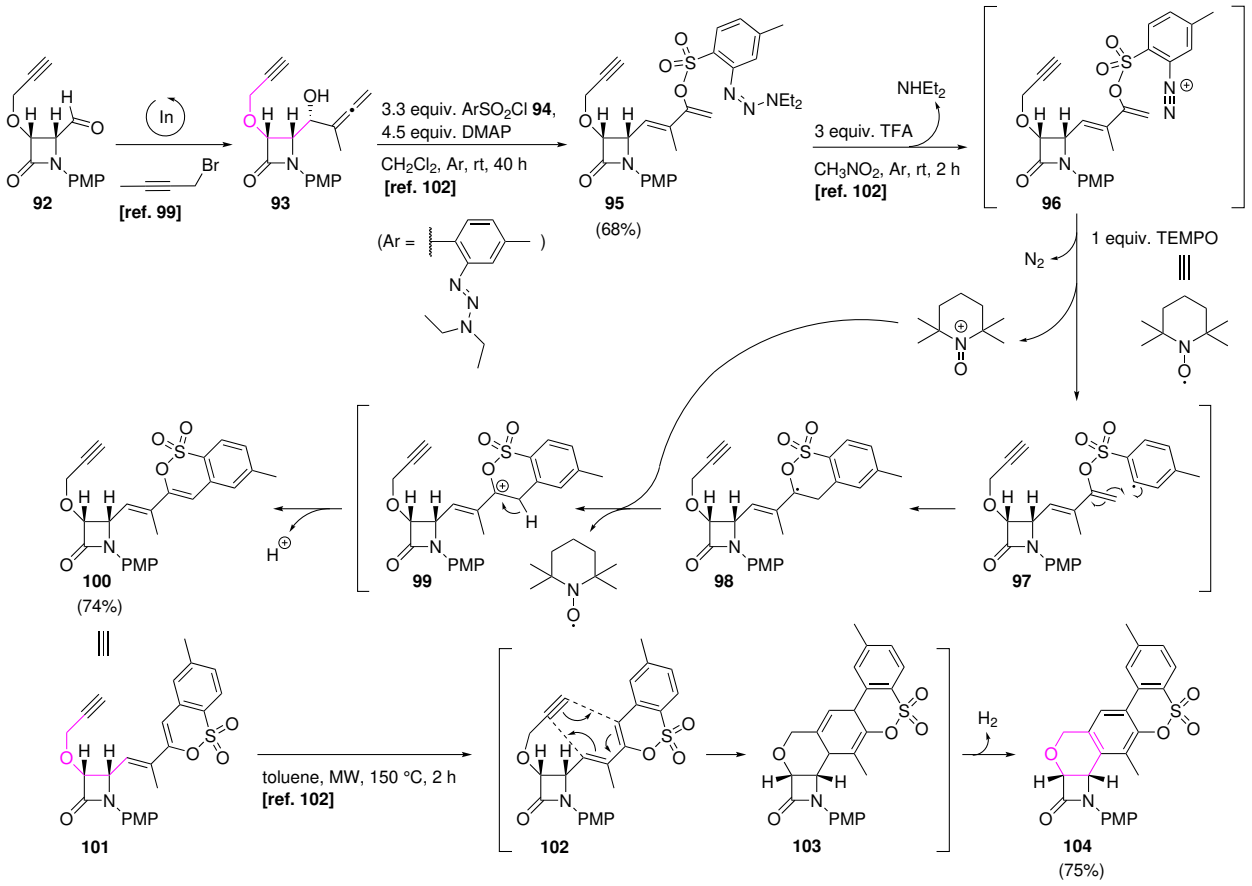
It is noteworthy that a thermal [2+2] cycloaddition is not that common, especially when compared with its photochemical counterpart. Obviously, the reason lies in the need for suprafacial-antarafacial orbital overlap of the orbitals of both engaging π-systems, as was originally stated by Woodward and Hofmann.[100] Normally, such a suprafacial-antarafacial approach is geometrically forbidden, but in some cases, as with ketenes in the Staudinger β-lactam synthesis or in this case with allenes, they are geometrically allowed. This is because some of the sterically hindering H-atoms around the π-bond of a normal alkene are removed in the case of a cumulated olefin bond. If such traditionally disallowed reactions are observed, they are usually occurring *via* a non-concerted mechanism, like a diradical or dipolar one. As for this reaction a drastic reduction in both reaction rate and yield was noticed upon addition of a catalytic amount of the radical quencher benzoquinone, the former seems to be the case.[98]

2.1.2.2 Domino [3+3] sigmatropic rearrangement/Diels-Alder reactions

In a similar way to the [2+2] cycloaddition procedure described above, fused tricycle **91** can be formed *via* a one-pot tandem allenol transposition/intramolecular Diels-Alder reaction of monocyclic α-allenol **86**, which is in fact a masked functionalised diene.[101,102] The β-lactam-tethered α-allenic alcohol **86** is prepared from racemic carbaldehyde **84** *via* the same procedure as depicted *in supra* with 1-bromobut-2-yne **85** and indium as a catalyst.[99] It was discovered that treatment of this α-allenic alcohol with methane sulphonylchloride and triethylamine at room temperature yielded 2,3-difunctionalised diene **88** in good yield (73 %) and with complete (*E*)-stereoselectivity. The extremely high selectivity of this allenol-diene transformation is a good indication of the pericyclic mechanism of the reaction. The allenol moiety forms α-allenic methanesulphonate intermediate **87**, which reacts *in situ* to mesyloxydiene **88** *via* a [3+3] sigmatropic rearrangement with a chair-like six-membered ring as transition state.



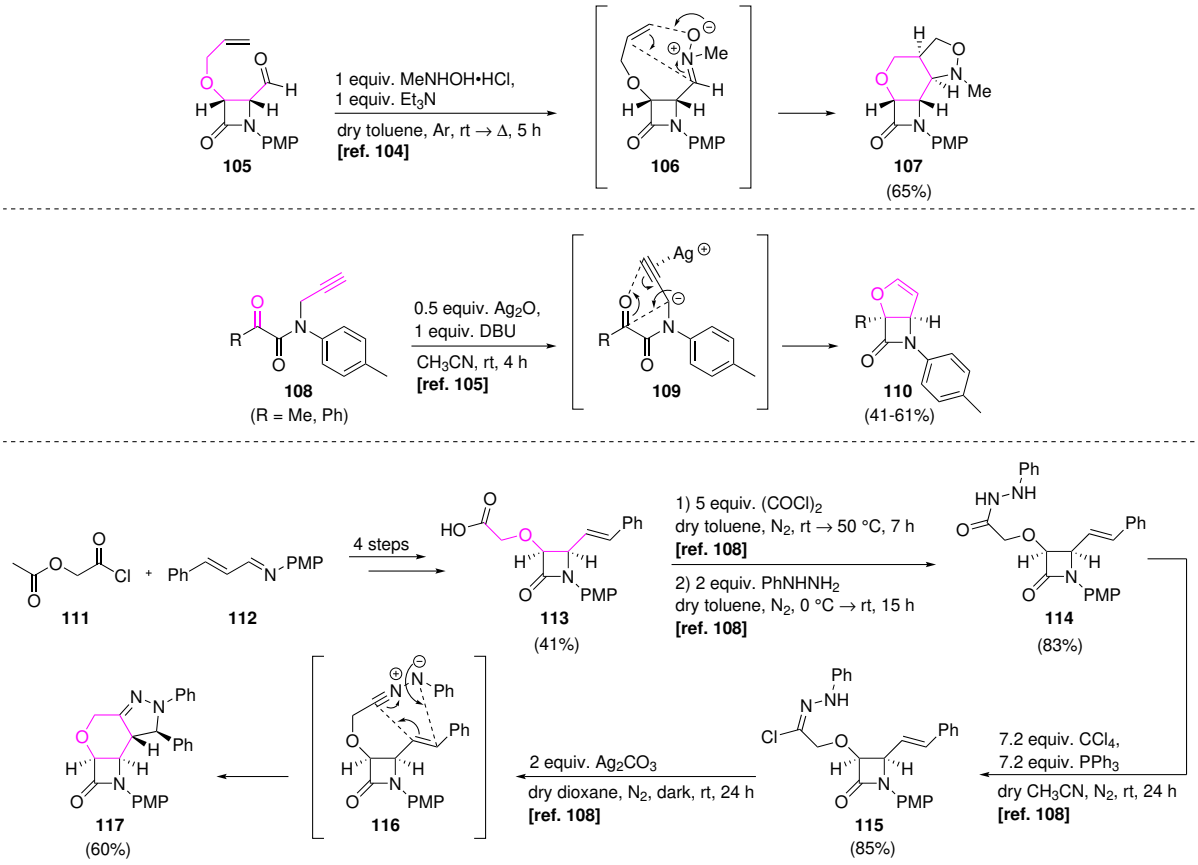
Since now a β-lactam with an alkene functionality at carbon C3 and a diene substituent at carbon C4 is created, an intramolecular thermal [4+2] Diels-Alder cycloaddition reaction can be used to generate *C*-fused tricyclic azetidin-2-one **91**. However, it appeared to be possible to combine both allenol transposition and Diels-Alder steps in a one-pot domino reaction. Tandem or domino reactions, in which the initial reagent is converted to a product that then becomes the substrate for the next reaction until a stable end product is formed, all of which happens in a one-pot process, are getting increasingly more popular due to their elegance, inherent efficiency in terms of reagent use and purification, and often excellent selectivity.[101] The reaction of α-allenyl alcohol **86** with methanesulphonyl chloride and triethylamine, though now performed at 190 °C in sealed tubes, afforded tricycle **91** in moderate yield, but with complete diastereoselectivity. This stereochemical outcome can be attributed to a preference for cyclic transition state **90** over transition state **89**. The mesylate functional group in the end product could, if desired, undergo further functionalisation *via* a transition metal-catalysed reaction.[101]



Other literature illustrates that this methodology can be used with some alterations and extra steps, as well as on different substrates, to generate complex structures, such as β-lactam-fused δ-sultone polycycle **104**.[102] Once more, the indium-mediated Barbier-type carbonyl-allenylation reaction on a carbaldehyde C4-substituent was employed for the synthesis of β-lactam-tethered hydroxyallenyne **93** as starting substrate. Sulphonylation with arenesulphonyl chloride **94** and 4-dimethylaminopyridine (DMAP) as base, immediately followed by a [3+3] sigmatropic rearrangement, afforded 1,3-diene **95**. The availability of the aryltriazene moiety however opens the possibility to make use of a cyclisation/dehydrogenation radical cascade by employing trifluoroacetic acid (TFA) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), resulting in enynyl benzo[*c*][1,2]oxathiine 1,1-dioxide **100**. Lastly, an intramolecular Diels-Alder cycloaddition under microwave conditions resulted, after *in situ* aromatisation of the intermediate 1,4-cyclohexadiene **103**, in pentacyclic δ-sultone **104**.[102]

2.1.2.3 1,3-Dipolar cycloadditions

The preparation of bridged polycyclic β-lactams can also occur *via* the [3+2] cycloaddition of a 1,3-dipolar compound with an alkene. Various alterations of the same principle can be found in the literature, differing from one another in the 1,3-dipole that is used. One option is the intramolecular nitrone-alkene cycloaddition (INAC) of azetidin-2-one-tethered alkenylaldehyde **105** towards 3,4-oxane-fused azetidin-2-one **107**.[103,104] Zhang *et al.* proved with the successful 1,3-dipolar cycloaddition of oxo-*N*-propargylamides **108**, using silver(I) oxide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), that all-carbon 1,3-dipoles can be generated as well and then employed for the construction of *e.g.* *C*-fused bicyclic β-lactam **110**.[105] It should be noted that this method forms the whole *C*-fused bicyclic β-lactam structure in one step, which means it actually belongs in section 2.3. However, for the purpose of illustrating the possible structural variations in 1,3-dipoles, it is shown here.

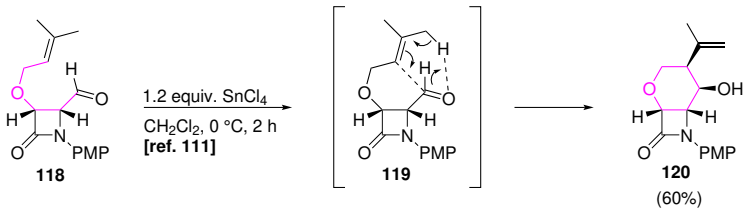


Other [3+2] cycloaddition pathways include intramolecular azide-olefin,[106] nitrile oxide-olefin,[107] and nitrilimine-olefin cycloadditions.[108] Latter method was illustrated by Del Buttero and co-workers in their seven-step stereoselective synthesis of tricyclic β-lactam **117**. Initial Staudinger [2+2] cyclocondensation between

acetoxyacetyl chloride **111** and 1-azadiene **112**, followed by hydrazinolysis, *O*-alkylation and ester hydrolysis yielded β-lactam **113**. After the subsequent transformation into acyl hydrazine **114**, chlorination with carbon tetrachloride gave hydrazonoyl chloride **115**. This compound generated *in situ* the key nitrilimine intermediate **116** upon addition of silver(I) carbonate, resulting in formation of the desired 3,4-oxane-fused β-lactam end product **117**.[108]

2.1.2.4 Lewis acid-promoted carbonyl-ene cyclisations

A third type of pericyclic reaction, that can be employed for the synthesis of functionalised bicyclic compounds, is the intramolecular carbonyl-ene reaction. Just like with intramolecular cycloadditions and sigmatropic rearrangements, its entropic benefit, operational simplicity and usually exquisite regio- and stereoselectivity are strong selling points. Alcaide *et al.* illustrate the application of a Lewis acid-promoted ene cyclisation in the construction of bicyclic β-lactam **120**.[109–111] The Lewis acid is essential in activating the enophile, *i.e.* the carbonyl, because of the inherent low nucleophilicity of an alkene. Treatment of azetidin-2-one-tethered alkenylaldehydes **118** with tin(IV) chloride resulted in the rapid and diastereospecific synthesis of 3,4-oxane-fused β-lactam **120** in good yield. However, switching the Lewis acid to boron trifluoride diethyl etherate yielded a complex mixture of compounds with total disappearance of the 4-oxoazetidine-2-carbaldehyde substrate **118**. Once more, this concerted reaction proceeds through a specific chair-like six-membered transition state, which explains the single stereochemical outcome.[109,111]

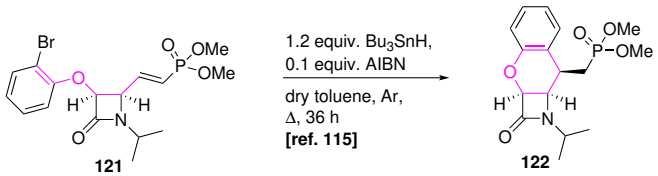


2.1.3 Other reaction types

This subsection will briefly, and without going in full detail, point out various other pathways that 3,4-annulate a ring structure onto a β-lactam core. The mechanisms featured here do not employ the above-mentioned intramolecular nucleophilic substitutions or pericyclic reactions, but feature different reaction options in organic chemistry, such as radicals, transition metals, rearrangements and oxidations.

R_3SnH/AIBN-mediated radical cyclisations

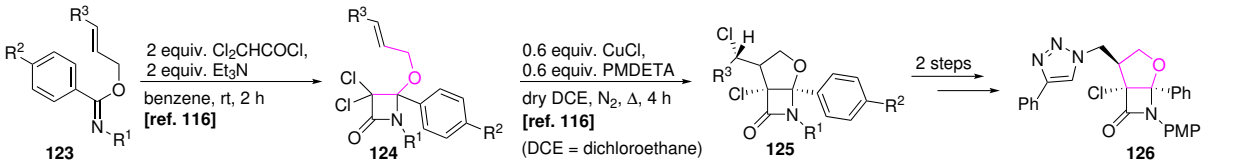
Different studies in the literature employ a trialkyltin hydride reagent in combination with azobisisobutyronitrile (AIBN) in an intramolecular radical cyclisation process, that creates a bicyclic 3,4-annulated azetidin-2-one compound.[112–115] The occurrence of an alkene and a halovinyl or haloaryl moiety at the C3 and C4 positions seems to be a prerequisite. An example is the synthesis of phosphono-substituted benzocarbacephem **122** from haloarene **121**.[115]



Atom transfer radical cyclisations

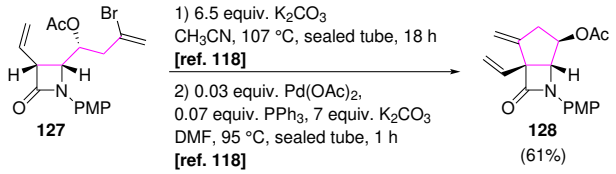
A more exotic entry in the β-lactam literature is Ram and co-workers' higly diastereoselective preparation of chlorinated tetrahydrofuro[3,2-*c*]azetidin-2-ones **126** by a 5-*exo*-*trig* chlorine atom transfer radical cyclisation (ATRC).[116,117] Appealing to the ATRC process is that it does not suffer from the traditional drawbacks of the *in supra* mentioned organotin hydride-mediated radical cyclisations, *viz.* a dreary purification procedure to

get rid of the toxic organotin halide side products, or loss of the halogen functionality. The reaction is catalysed by copper(I) chloride and works best with *N*,*N*,*N*',*N*",*N*"-pentamethyldiethylenetriamine (PMDETA) as a ligand. The ATRC-substrate, α,α-dichloro-β-lactams **124**, are created through Staudinger synthesis of readily and inexpensively preparable allylic imidates **123** with dichloroacetyl chloride. A chlorine substituent at the β-lactam's α-position is expected to be beneficial for its chemical reactivity and biological activity. Besides, a side chain halogen can be used in the derivatisation of ATRC-formed bicyclic compounds **125** as it can be substituted by another functional group. When for example an azide moiety is introduced, a click reaction, *i.e.* the copper(I)-catalysed Huisgen azide-alkyne 1,3-dipolar cycloaddition (CuAAC), can be used to create 1,2,3-triazolyl β-lactam **126**. Since triazole-containing molecules are known for often exhibiting biological activity, *e.g.* the class A β-lactamase inhibitor tazobactam **9**, this is an interesting functionalisation.[116]

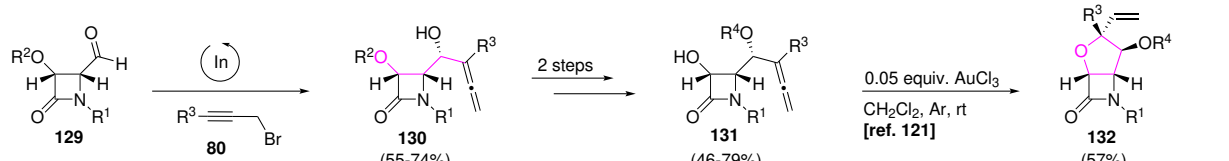


Noble metal-catalysed reactions

Alcaide *et al.* found that β-lactam **127** with both an alkene and a vinyl bromide tether at respectively the C3- and C4-position can undergo a palladium(II)-catalysed Heck cyclisation, affording 3,4-cyclopentane-fused bicyclic azetidin-2-one **128**. The protected bromohomoallyl alcohol **127** can be prepared *via* a metal-promoted reaction (with indium, tin, bismuth or zinc) of a 4-oxo-azetidine-2-carbaldehyde.[118]

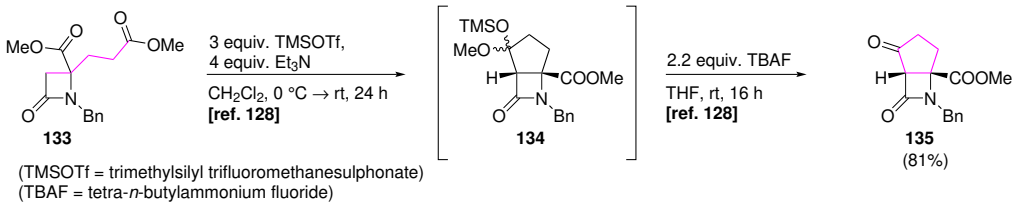


Furthermore, carbaldehydes **129** can also, as already discussed *in supra*, undergo an indium-mediated Barbier-type carbonyl allenylation with propargyl bromides **80** to form protected α-allenols **130**. Manipulation of the protecting groups yields γ-allenols **131**, of which the hydroxyl substituent at carbon C3 is now free. This hydroxyl group can be employed in a gold(III) chloride-catalysed hydroalkoxylation reaction, resulting in the heterocyclisation of the γ-allenol towards tetrahydrofuran-annulated β-lactams, such as compound **132**. Apparently, the choice of noble metal catalyst, the α-hydroxy protecting group (R^4), as well as the allene substituents (R^3) all influence the regioselectivity of the cyclisation, making the synthesis of fused six- and seven-membered rings equally possible.[119–122]



Cationic Dieckmann rearrangements

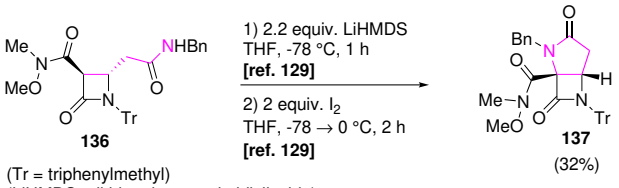
Continuing with the realm of uncommon reaction mechanisms, we mention for the sake of completeness some specific but rare pathways found in the literature, which yield 6-azabicyclo[3.2.0]heptan-7-one derivatives. These include the intramolecular cationic Dieckmann-type condensation, in some literature referred to as an Mukaiyama aldol-like reaction,[123] between an ester and a silyl imidate functionality, which afforded *C*-fused bicyclic azetidin-2-one **135** starting from diester-tethered β-lactam **133**.[123–128]



(TMSOTf = trimethylsilyl trifluoromethanesulphonate)
(TBAF = tetra-*n*-butylammonium fluoride)

Oxidative coupling of dianions

Another example is the construction of β-lactam **136** with a Weinreb amido (*i.e.* *N*-methoxy-*N*-methylamido) functionality at carbon C3 by Konopelski and co-workers.[129] The desired bicyclic product **137** is obtained through oxidative coupling of the C3-carbanion, *i.e.* the Weinreb amide α-anion, and the C4-appended benzylamide's deprotonated nitrogen. When molecular iodine was used as a reagent, this oxidative C-N bond formation yielded bridged compound **137** with modest yield.

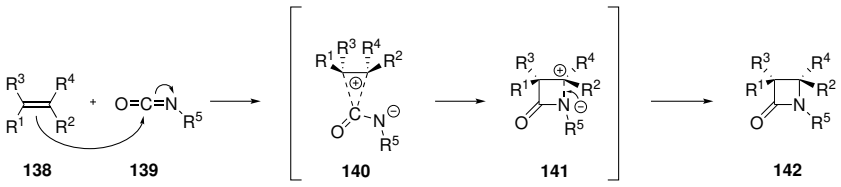


(Tr = triphenylmethyl)
(LiHMDS = lithium hexamethyldisilazide)

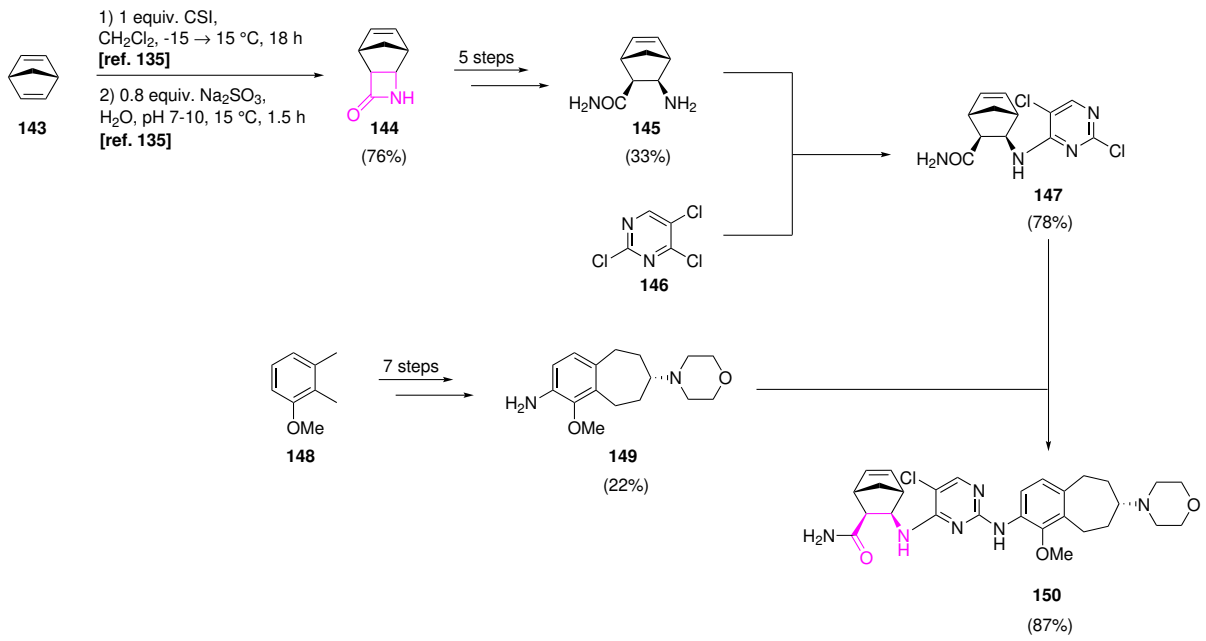
2.2 Synthesis of *C*-fused bicyclic β-lactams starting with the ring structure

2.2.1 Alkene-isocyanate [2+2] cyclocondensations

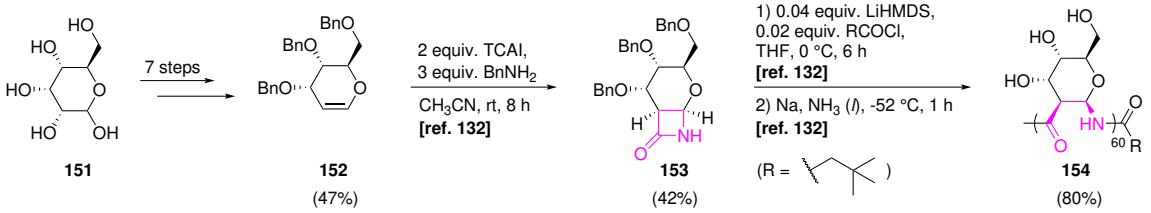
A frequently used, though very straightforward method for synthesising 3,4-annulated azetidin-2-ones is the simple [2+2] cyclocondensation of an alkene and an isocyanate. Chlorosulphonyl isocyanate (CSI), which can be readily prepared through reaction of sulphur trioxide with cyanic chloride,[130] is a reagent well-known to easily lead to β-lactam formation upon addition to an olefin. However, if it fails, other isocyanate reagents such as trichloroacetyl isocyanate (TCAI) can be used as well.[131,132] Since no UV lamp is needed to drive the reaction, this cycloaddition is another example of an exception to the Woodward-Hoffman selection rules[100] and thus must have a non-concerted mechanism. Similar to the two-step mechanism of the Staudinger β-lactam synthesis, a stepwise 1,2-dipolar mechanism is proposed here as well, hence the term cyclocondensation. Attack of the π-electrons of dipolarophilic olefin **138** on isocyanate **139** results in π-complex **140**. This intermediate rearranges itself to 1,4-dipole **141**, of which the charged atoms lie adequately aligned for performing a ring closure, yielding β-lactam moiety **142**.[130,133]



In the literature this type of reaction is often chosen as one of the first steps during the total synthesis of a pharmaceutical compound, that contains a β-lactam ring,[134] or where the reactivity of this functionality is used during the subsequent reaction step.[132,135,136] Usually the latter is the case, of which one example is that of ALK inhibitor CEP-28122 **150**. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase and, without going in anymore detail, can be shown to be involved with the proliferation and survival of various human cancers.[137] Two halves of the pharmaceutical were synthesised, one starting from 2,5-norbornadiene **143** and one from 2,3-dimethylanisole **148**, and then put together with a linking 2,4,5-trichloropyrimidine ring **146** in between.[135] Using CSI, 2,5-norbornadiene **143** was transformed into a chlorosulphonamide, which got reduced with sodium sulphite to β-lactam **144**. Next, the azetidin-2-one ring was hydrolysed in order to ultimately form after four more steps the desired amino- and amido-functionalised norbornene **145**, which makes up one third of ALK inhibitor CEP-28122 **150**.



Another reoccurring application in the literature is to use this [2+2] cycloaddition on an unsaturated carbohydrate, *e.g.* D-allopyranose derivative **152**.[132,136] The resulting *C*-fused bicyclic β-lactam **153** can be employed as monomer during an anionic ring-opening polymerisation in order to create polyamidosaccharides, such as compound **154**.



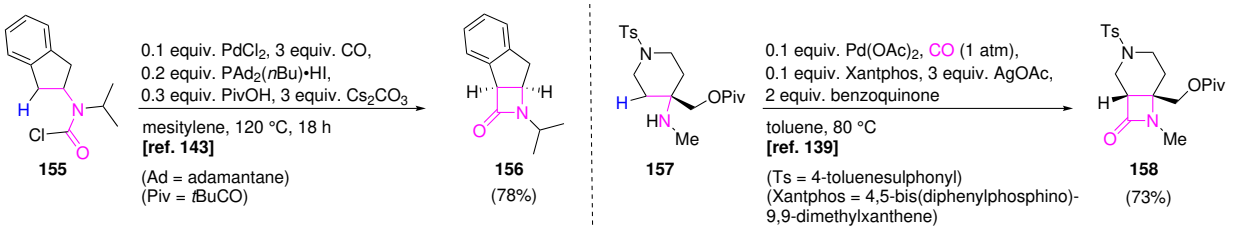
2.2.2 Transition metal-catalysed cyclisations

With their ability to directly and catalytically functionalise normally unreactive aliphatic C-H bonds, transition metals can form a very elegant solution for a manifold of limitations and challenges classical organic synthesis routes have to cope with, such as toxic reagents or expensive starting materials, long steps and the removal of typical by-products like phosphine oxide and hydrazinodicarboxylate. In particular, the fact that the C-H bond does not need any prefunctionalisation and that no stoichiometric amounts of organometallic reagents are necessary, results in a significantly improved step economy of the synthesis route, which in turn has great economical and ecological advantages.[138–141] It is thus no wonder that this field of organic chemistry has received quite a lot of interest over the past two decades.[142] Nonetheless, one major issue is the discrimination the metal has to make between the copious C-H bonds, that are present in an organic molecule and which only very slightly differ from one another (*e.g.* steric and inductive environment, hybridisation, number of geminal hydrogens, and strain of the bond).[139] This subsection will briefly cover some successful methods, that can be used to synthesise *C*-fused bicyclic β-lactams.

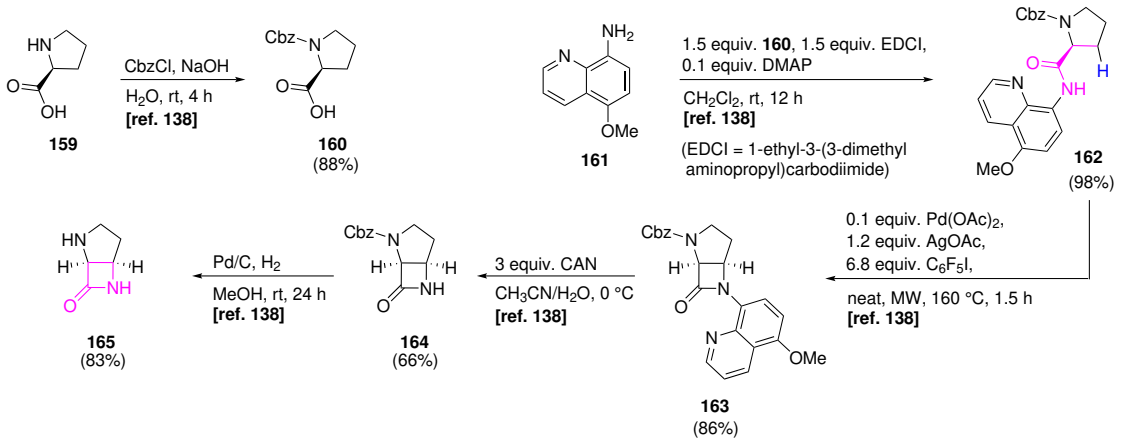
2.2.2.1 Palladium- and copper-catalysed reactions *via* C(sp^3)-H bond activation

Although the greater part of the successfully developed catalytic cycles focus on activation of C(sp^2)-H bonds,[142] C(sp^3)-H functionalisation would be especially interesting in view of coupling a heterocycle's carbon atom with a vicinal amido functionality, in this way producing an immediately *C*-fused β-lactam

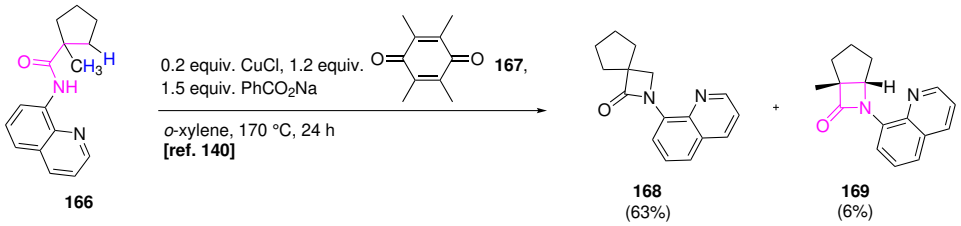
ring. It is evident from literature that the palladium-catalysed β-lactam formations can be categorised according to which two atoms of the azetidin-2-one are linked by palladium, which is determined by the substrates, reaction conditions, and the palladium catalyst used. [143] For example, the palladium(0)-catalysed carbamoylation of carbamoyl chlorides **155** links C2 and C3 together, [143] while the palladium(II)-catalysed carbonylation of secondary amines **157** connects N1 and C3 by inserting carbon monoxide as the C2 carbonyl in between. [139,142]



The most-known method and, in the context of synthesising diazabicyclic β-lactams, the one with the most promising results is the palladium(II)-catalysed intramolecular amidation of a methylene group at the β-position of a carboxamide, *i.e.* forming the azetidin-2-one's N1-C4 bond. [138,144] The commercially available substrate L-proline **159** was protected with benzyl chloroformate and coupled with 5-methoxyquinolin-8-amine **161**, which acts as a directing group during the catalytic cycle. The intramolecular amidation of this L-proline derivative **162** using palladium(II) acetate afforded compound **163** in high yield. The methoxyquinoline and benzyloxycarbonyl (Cbz) groups could readily be cleaved off respectively upon treatment with cerium(IV) ammonium nitrate (CAN) and by hydrogenation over palladium on carbon. It should be noted that now the key 3,4-pyrrolidine-fused bicyclic intermediate **165** for the formation of promising pseudomonal class C β-lactamase AmpC inhibitor MK-8712 **13** has been synthesised in only five steps with an overall yield of 41 %. [138]

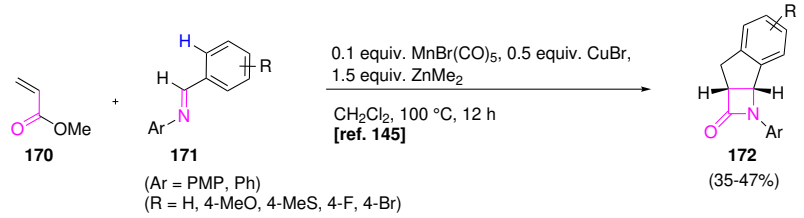


A similar reaction can be performed with copper as well, though it was observed that this intramolecular dehydrogenative amidation favours a β-methyl's C-H bonds over the C-H bonds of a methylene group. [140] It accounts for the results of the copper(I) chloride catalysed reaction of 1-methyl-*N*-(quinolin-8-yl)cyclopentane-1-carboxamide **166** with duroquinone **167** as oxidant and sodium benzoate as base: the major product was spiro compound **168**, while only little of the 3,4-cyclopentane-fused bicyclic β-lactam **169** was formed.



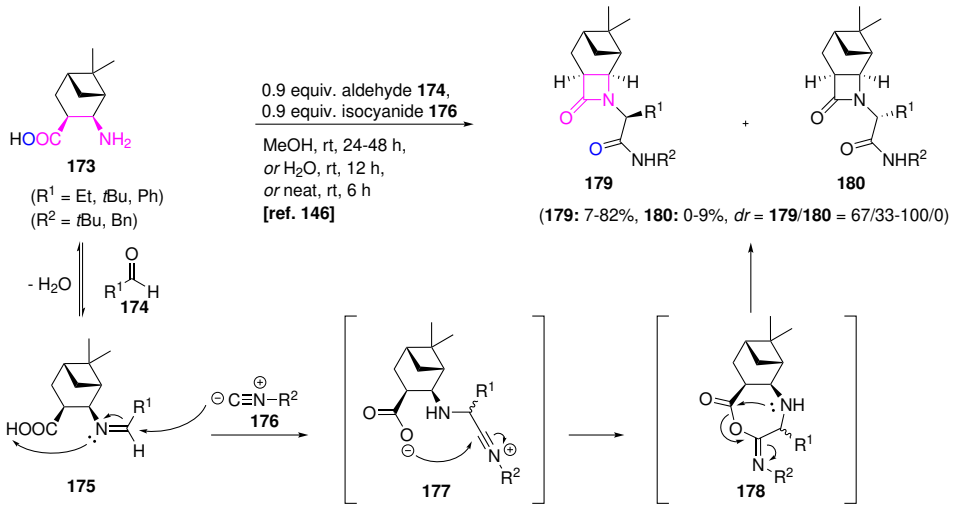
2.2.2.2 Manganese-catalysed reactions *via* C(sp^2)-H bond activation

In the contemporary context of sustainability, a shift in the catalyst used during direct C-H bond functionalisations from the mostly studied 4d and 5d transition metals (Pd, Rh, Ru, Ir) to third row elements (Ni, Co, Fe, Cu, Mn) would be a good thing. Being the third most abundant transition metal after iron and titanium, as well as being easy accessible, in combination with having varied oxidation states and a low toxicity, makes manganese in particular a promising candidate.[141,145] An interesting entry in the literature is the research by Ackermann *et al.*[141] and Wang *et al.*[145] on the bicyclic annulation of α,β-unsaturated esters **170** and aromatic aldimines **171** *via* activation of a C(sp^2)-H bond *ortho* to the imine. The one-step process exhibits high efficiency, is robust to different functional groups and both aldimines and ketimines can act as substrates. The addition of dimethyl zinc as base appeared to be indispensable, since it not only assists the manganese catalyst in forming the essential azamanganacycle intermediate, but it also plays a role at a later point in the catalytic cycle during the second intramolecular nucleophilic cyclisation reaction that leads to the 3,4-cyclopentane-fused β-lactam ring, affording tricycles **172**.



2.2.3 Ugi four-center three-component reactions

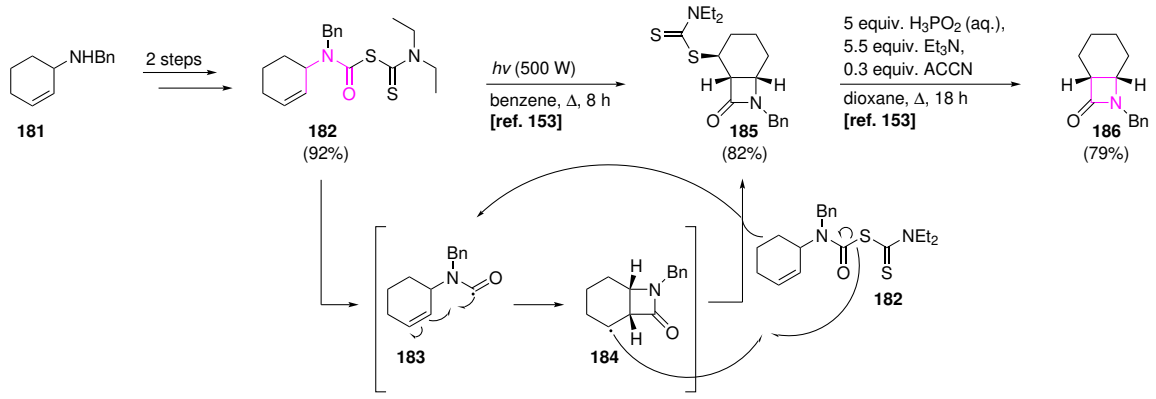
In light of the pharmaceutical interest in generating large libraries of diverse and potential biological active compounds, one-pot multicomponent reactions, like the Ugi and Passerini condensations, are highly appealing.[146] The traditional Ugi four-component condensation (U-4CC), where an isocyanide, carboxylic acid, amino and carbonyl functionality react to α-aminoacyl amide derivatives, can be modified in a way that it affords heterocycles, although yields seem best with only acyclic compounds. Rings are formed simply by incorporating two of the four functional groups in one reagent, *i.e.* an intramolecular Ugi or so-called Ugi four-center three-component reaction (U-4C-3CR), but when this reagent is a cycle itself the synthesis of bridged heterocycles can be achieved.[146–148] Most of the literature, involving the application of this strategy in creating bicyclic *cis*-azetidin-2-one derivatives, makes use of cyclic *cis*-β-amino acids.[146,148–150] For example, the U-4C-3CR of apopinane **173** proceeded well, resulting in the construction of *C*-fused bicyclic β-lactam diastereomers **179** and **180** with good yields and high diastereoselectivities.[146]



Instead of using the classical methanol during U-4CCs, an environmentally more benign solvent like water, or no solvent at all, can be employed. Often yields and diastereoselectivities are comparable, though water works rate accelerating and offers a facile isolation of precipitated products. The shorter reaction times can be attributed to factors as hydrogen bonding in the transition state, the high cohesive energy density of water, and the hydrophobic effect. The later explains why addition of a solute like glucose often improves the reaction rate even more *via* salting out, *i.e.* the hydrophobic effect is intensified.[146–148] This also enables the efficient use of cyclic *cis*-β-keto acids in a U-4C-3CR, when working in a 1 M glucose containing aqueous solution, which was previously impossible in an organic solvent.[147]

2.2.4 Group transfer radical cyclisations with dithiocarbamates

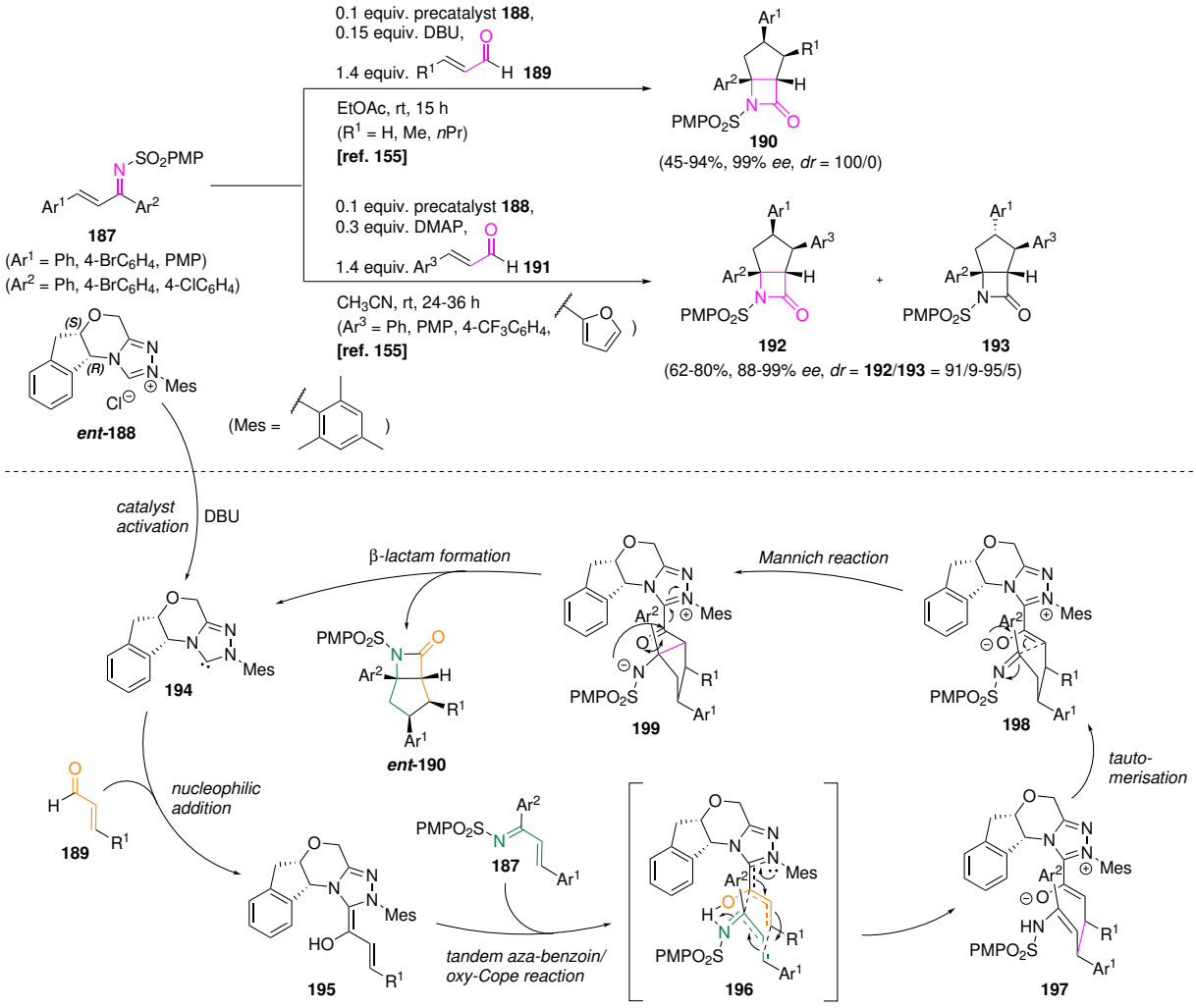
A bit more exotic considering its substrate and mechanism, the photomediated tandem 4-*exo*-*trig* carbamoyl radical cyclisation-dithiocarbamate group transfer reaction can generate β-lactams annulated with five-, six- and seven-membered rings in good yields.[151–154] This light-driven reaction proceeds *via* a two-step radical mechanism, in which first a cyclohexyl radical **184** is formed through irradiation of the readily preparable carbamoyl dithiocarbamate **182** with visible light, using a 500 W halogen lamp. Next, this intermediate induces a diastereoselective dithiocarbamate group transfer from a new substrate molecule **182** to the cyclohexyl radical on the sterically less hindered face of the bicyclic compound. Reductive desulphurisation of dithiocarbamate **185** with hypophosphorous acid, triethylamine and 1,1'-azobis(cyclohexanecarbonitrile) (ACCN) yielded the unsubstituted 3,4-cyclohexane-fused azetidin-2-one **186** without deterioration of the strained four-membered β-lactam core. Dithiocarbamate **185** can also be used in other reactions, allowing introduction of for example hydroxyl functionalities onto the cyclohexane ring of 7-azabicyclo[4.2.0]octan-8-one **186**.[153,154]



2.3 Synthesis of *C*-fused bicyclic β-lactams starting with acyclic precursors

2.3.1 Organocatalysis with *N*-heterocyclic carbenes

Keeping their possible pharmaceutical applications in mind, it is no wonder that catalytic enantioselective synthesis strategies are among the most prised targets for reaction development. It was found that *N*-heterocyclic carbene (NHC) catalysts can afford enantio- and diastereoenriched 3,4-cyclopentane-fused β-lactams **190** and **192** starting from 3-substituted enals **189** and **191**, and α,β-unsaturated *N*-4-methoxyphenyl sulphonyl imines **187**.[155] Using organocatalysis for the formation of enantiomerically pure bicyclic β-lactams was originally dismissed as not viable due to a high risk of competing side reactions, such as the aza-Diels-Alder reaction or dimerisation of the enal. Studies showed, however, that the reaction outcome can be modulated through proper choice of precatalyst (*e.g.* triazolium or imidazolium based), amine base (*e.g.* triethylamine or DBU), which *in situ* generates the carbene, and substrates (electron-withdrawing or -donating substituents).

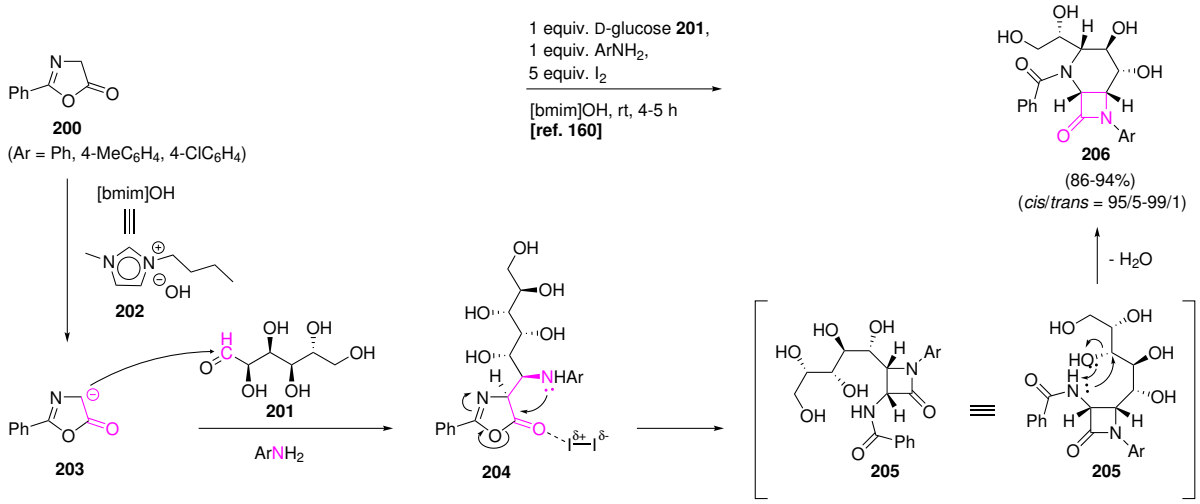


Notably, the base influenced the diastereoselectivity, with 3-alkyl enals **189** in ethyl acetate and in the presence of DBU generally yielding only one single diastereomer **190**, in contrast to 3-aryl enals **191** which gave a mixture of diastereomers **192**/**193** for the Ar^1-substituent. This ratio could be boosted though to at least 91/9, when DMAP in acetonitrile was chosen, although this lowered the reaction rate. The preferred stereochemical configuration of the *C*-fused β-lactam product can be entirely linked to how the two reagents are oriented towards each other and the *N*-mesityl-substituted triazolium catalysts **188** or ***ent*-188** during the catalytic cycle. Examples are the preferred boat oxy-Cope transition state **196** due to maximal secondary orbital overlap during the key tandem aza-benzoin/oxy-Cope bonding step, or how only one stereochemical outcome **199** of the subsequent Mannich reaction, which closes the cyclopentane ring, allows the third and last bond forming step of the bicyclic compound leading to β-lactam ***ent*-190**.[155] It should be noted that in the literature there exist many more examples of NHC-catalysed reactions, yielding 3,4-cyclopentane- or 3,4-cyclohexane-fused azetidin-2-ones. Although the NHC-catalyst can vary widely from simple proline derivatives[156] to the more often used imidazolium and triazolium derivatives,[147,157–159] an observable trend is the presence of enals or α,β-unsaturated imines among the used reagents.

2.3.2 One-pot [1C+2C+1N] three-component synthesis in ionic liquids

A quite different manner of constructing the β-lactam ring was illustrated by Rai and co-workers and consists of a three-component one-pot approach, which is catalysed by iodine and an ionic liquid.[160] In view of their medicinal importance,[161,162] the goal was to synthesise iminosugars **206** (*i.e.* sugars with an endocyclic nitrogen atom instead of oxygen) *via* the classical Staudinger approach of a [2+2] cyclocondensation with an imine, formed out of an amine (1N-source) and a carbohydrate like D-glucose **201**, as aldehyde equivalent (1C-source), and a ketene, generated from an α-amino acid (2C-source). Since this did not work, the

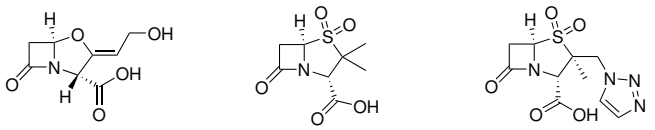
[1C+2C+1N] one-pot three-component strategy was successfully tested, though only successful when the free amino functionality was masked by conversion into 2-phenyl-1,3-oxazolan-5-one **200**, which also activated the methylene group at carbon C4. This transformation did not only result in the creation of a β-lactam core, but also in an annulation of the sugar chain, yielding target *C*-fused β-lactams **206**. The best catalyst system appeared to be molecular iodine in combination with the highly stable ionic liquid [bmim]OH, *i.e.* 1-butyl-3-methylimidazolium hydroxide **202**. When compared to some regular solvents, such as *N*,*N*-dimethylformamide and acetonitrile, it was clear that the ionic liquid acted superior both in reaction rate as in yield.



The proposed mechanism for the formation of polyhydroxyiminosugar-annulated β-lactams **206** starts with creation of an increased nucleophilicity at carbon C4 of the masked amino acids **200** by simple interaction with the ionic liquid medium. The formed carbanions **203** go through Knoevenagel-hydroamination cascades, affording adducts **204**, which then undergo the key intramolecular *N*-nucleophilic attack at the C5 carbonyl functionality. The iodine presumably plays its catalytic role here by polarising the carbonyl group, thus making it more electrophilic. Immediately following this azetidin-2-one ring construction is the fourth and last step: an aminoacetylative ring transformation of intermediates **205** with expulsion of water, resulting in desired products **206**. The operational simplicity, excellent yields, high *cis*-diastereoselectivity and the fact that the [bmim]OH ionic liquid can be recycled and reused without any loss of efficiency are salient features of this synthesis method.[160]

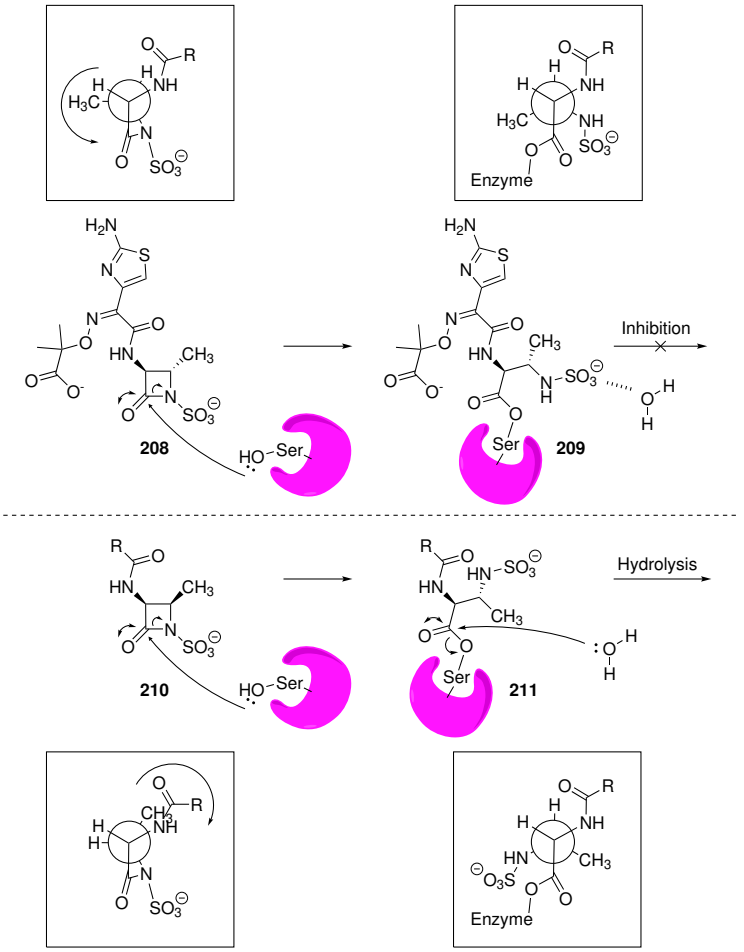
2.4 Biological activity of *C*-fused bicyclic β-lactams

As previously mentioned in the chapter "Scope and Goal", a problematic trend in the already worrisome exponential increase of the frequency of resistance against modern β-lactam antibiotics like third-generation cephalosporins,[31,60] is that often the high level of expression of class C β-lactamases is to blame.[61–63] Known β-lactamase inhibitors that are available on the market, such as clavulanic acid **7** and penam sulphones like sulbactam **207** and tazobactam **9**, are unsuited to tackle this issue.[39,46] Their mode of action lies in undergoing a chemical rearrangement after attack of the β-lactamase's catalytic serine residue on the azetidin-2-one core, ultimately forming acrylic esters which are inherently more stable to hydrolysis.[163–167] No hydrolysis means, as with the interaction between a β-lactam antibiotic and a penicillin-binding protein, that the catalytic site remains acylated by the inhibitor, thus impeding the β-lactamase to inactivate any more β-lactam antibiotics. The catch is that the rearrangement of class A inhibitors to acrylic esters has to occur more rapidly than the deacylation by a water molecule that is bound in the catalytic site. Because of a different mechanism of hydrolysis compared to their class A brothers, this prerequisite is not fulfilled with class C β-lactamase enzymes, thus making class A inhibitors like tazobactam **9** ineffective against them. Hence, the efficacy of successful class C β-lactamase inhibitors, for example aztreonam **208**, must be the result of a different working mechanism.



7 **207** **9**

Molecular modelling and X-ray crystallography of acyl-enzyme complexes formed with aztreonam **208** and class C β-lactamase from *Citrobacter freundii* let Heinze-Krauss *et al.* deduce the working mechanism of class C inhibitors, which is much simpler since it does not involve secondary rearrangements as is the case for class A β-lactamase inhibitors.[71,72] Essential is the rotation about the C3-C4 bond of the azetidin-2-one ring. As can be visualised by its Newman projection, *trans*-substituted monobactam aztreonam **208** in its intact form has an energetically unfavourable eclipsed conformation. When acylation of the serine residue had taken place, a counterclockwise rotation about the C3-C4 bond by ±70° to the more relaxed gauche form **209** was observed. In this rotamer, both nitrogen N1 and its attached sulphonate group, which forms a salt bridge with lysine 315 (**Figure 3**), block one face of the ester bond between serine residue 64 and the inhibitor. Furthermore, they also displace water molecule 192 near tyrosine 150, which in the enzyme's native structure is postulated to be critical for its catalytic mechanism, away from the position where it can be activated.[71–73] The alternative gauche form, achieved by clockwise rotation, was not formed because of steric conflict between the methyl C4-substituent and the side chains of tyrosine 150 and leucine 119. However, for penicillin-like, cephalosporin-like and *cis*-substituted monobactam inhibitors **210**, this clockwise rotation can occur without conflict with amino acid side chains. The resulting gauche form **211** does now not displace a water molecule (WAT192 in **Figure 3**), nor does it sterically block the enzyme-inhibitor ester bond from getting attacked, therefore allowing hydrolysis to readily take place.[71,72]



The bottom line is that hindering, but preferably just totally preventing rotation about the C3-C4 bond from occurring, should block water molecule 192's access to the enzyme-inhibitor ester bond, thus vastly increasing the stability and half-life of the complex. It was envisioned that linking the 3-amino group to C4 by a two-carbon bridge, *i.e.* forming *C*-fused bicyclic β-lactams, yields structures that are potent, mechanism-based inhibitors of class C β-lactamases.[71,72] An additional advantage is the increased interaction of the N2-acyl side chain of, for example, sodium 2-[(4-hydroxyphenyl)carbamoyl]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonate **212c** with the enzyme's parallel lying β-strand, originating in a rotation about the amide side chain bond which is caused by the pyrrolidine ring. As long as the side chain is not too bulky, optimisation of three interactions can mount up to a ten thousandfold increase in affinity, as well as a substantial increase in enzyme-inhibitor complex stability. These interactions are, as can be seen in **Figure 3**, a hydrogen bond between the N2-side chain's carbonyl and asparagine residue 152, one between the α-amino group of the R^1-substituent and the backbone carbonyl of serine 318 in the β-strand, and thirdly hydrophobic π-stacking interactions between an aromatic group in the N2-side chain and tyrosine 221.[71,72]

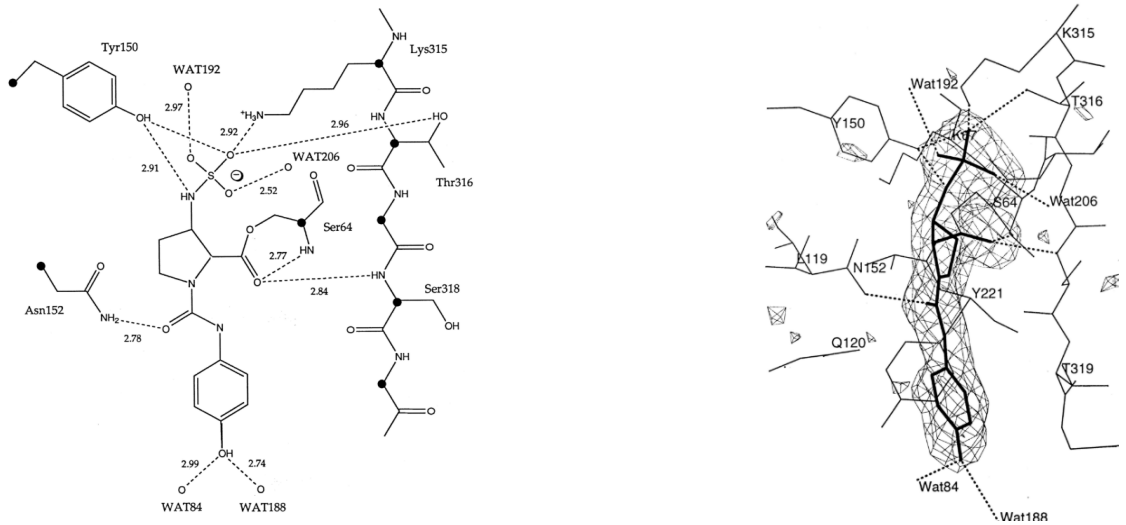
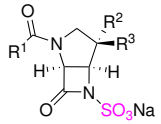
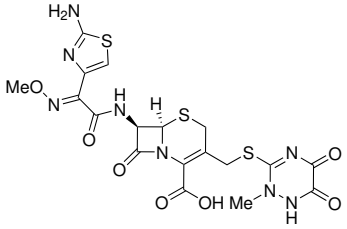


Figure 3: X-ray crystallographic structure determination of the acyl-enzyme complex, formed upon binding of 3,4-pyrrolidine-fused bicyclic β-lactam **212c** with catalytic serine residue 64 in the active site of *Citrobacter freundii* class C β-lactamase. (*Left*) Schematic representation. Visualisation of hydrogen bonding interactions of covalently bound inhibitor **212c** with the enzyme and with ordered solvent molecules, *i.e.* water, (dashed lines, distance in Å) and of C_α positions (filled circles). (*Right*) Stereoview of inhibitor **212c** together with an omit electron density map (contour level 0.12 electrons/$Å^3$). Visualisation of hydrogen bonding interactions (dashed lines).[71,72]

When the *in vitro* efficacy of various sodium 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonates **212** was tested,[71] no intrinsic antibacterial activity was detected. Next, the inhibition of isolated enzymes from the β-lactamase producing strains *Citrobacter freundii* 1982 (class C), *Pseudomonos aeruginosa* 18 SH (class C) and *Escherichia coli* TEM-3 (class A), and the synergy with the third-generation cephalosporin antibiotic ceftriaxone **213** (ratio 4/1) against the bacteria themselves were tested (**Table 3**). As expected, 3,4-pyrrolidine-fused bicyclic β-lactams **212** are effective and selective class C β-lactamase inhibitors, of which the half-inhibition constants (IC_{50}) can be as low as 3 nM (compound **212g**). However, class A enzymes exhibit low affinity for *C*-fused bicyclic β-lactams with an IC_{50} usually larger than 100 µM. Examination of the class A β-lactamase crystal structure explains why: the hydrolysing attack occurs here from the opposite side of the ester, with the water molecule being activated by a hydrogen bond network that is absent in class C enzymes. Hence, C3-C4 bond rotation is in class A β-lactamases not essential for their deacylation mechanism, and restricting that rotation by *C*-fusing a cycle onto the azetidin-2-one core has therefore very few impact on prolonging the stability of the acyl-enzyme complex.[71,72]



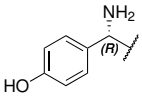
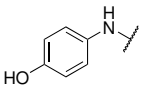
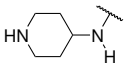
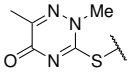
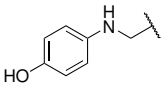
212
(R^1, R^2, R^3: **Table 3**)



213

Interestingly, the magnitude of synergy observed against *P. aeruginosa* for monosubstituted derivatives **212b-g** was not as strong as expected. Although multiple mechanisms may lay at the origin of this observation, it seems that several compounds struggle to enter the periplasmic space of *P. aeruginosa*. Despite exercising potent enzyme inhibition, this phenomenon was especially apparent with disubstituted derivative **212a**, which appears to suffer from limited penetration through the outer membrane of all three bacteria. The strong synergy of the monosubstituted derivatives with ceftriaxone **213** against *C. freundii* suggests that penetration of the outer membrane is not such a barrier in class C β-lactamase producing species of the *Enterobacteriaceae*. With the exception of β-lactams **212e** and **212g**, the observed MIC values here correlated quite well with the IC_{50} values.[71,72]

Table 3: Substitution pattern,[a] β-lactamase enzyme inhibition and *in vitro* synergy with β-lactam antibiotic ceftriaxone **213** of sodium 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonates **212**.[71,72]

		IC_{50}[b] [nM]			MIC[c] [µg/mL]		
	R^1	*C. freundii* 1982 (class C)	*P. aeruginosa* 18 SH (class C)	*E. coli* TEM-3 (class A)	*C. freundii* 1982 (class C)	*P. aeruginosa* 18 SH (class C)	*E. coli* TEM-3 (class A)
ref.[d]	-	-	-	-	128	128	16
9[e]	-	900	800	15	8	>32	0.25
212a	BnO	13	380	511	>16	>16	16
212b		500	90	>100000	2	8	8
212c		109	155	>100000	1	8	8
212d		2200	3900	>100000	2	8	16
212e	*t*BuO	225	1500	>100000	8	8	16
212f		6	55	>100000	0.25	16	16
212g		3	25	79200	8	128	8

[a] For compound **212a**: R^2 = F, R^3 = H. For compounds **212b-g**: R^2 = H, R^3 = H.

[b] Inhibition of isolated β-lactamase enzymes.

[c] Inhibition of strains that produce β-lactamases. The inhibitor to ceftriaxone ratio is 4/1.

[d] MICs of ceftriaxone in the absence of any inhibitor.

[e] IC_{50}s and MICs of the class A β-lactamase inhibitor tazobactam.

2.5 Conclusion

In this literature overview different synthetic routes leading towards *C*-fused bicyclic azetidin-2-ones were elaborated. One possibility is to start with a β-lactam precursor and get a cyclic structure to form on it, but another option is to first prepare the (hetero)cycle and construct the β-lactam functionality at the end. The former method always distils down to creating two different functionalities at the C3- and C4-position of the β-lactam, which then will react with each other in a nucleophilic, pericyclic, radical or transition metal-catalysed way. The latter method also can make use of these four reaction types. A third alternative is not having formed the β-lactam ring, nor the to-be-fused (hetero)cycle in advance, but starting with acyclic precursors and making use of organocatalysis or ionic liquids. The goal of this Master's thesis concerns the synthesis of 3,4-pyrrolidine-fused bicyclic β-lactams **34**. A theoretical retrosynthetic overview on their preparation *via* several of the in this chapter discussed reaction pathways is visualised in **Figure 4**, but only very few of them have already been employed to actually construct this diazabicyclic scaffold (methods A1, A3 and B2).

Transition metals like palladium can swiftly create the azetidin-2-one core onto a cyclic structure *via* C-H bond activation, though they are expensive as are the often numerous extra reagents, *e.g.* special bulky ligands, that are needed. While organocatalysis or the Ugi reaction seem very elegant, most of the literature focuses on intramolecular substitutions and the cycloaddition of an isocyanate to a double bond-containing ring structure to form *C*-fused bicyclic β-lactams. Especially the latter seems to be a frequently used method, when the *C*-fused β-lactam formation is only the first step in a longer synthetic route leading to certain pharmaceuticals. They both have the advantages of a simple methodology and easily variable substituents. Additionally, allowed to be run at room temperature, they have an energetic advantage over for example thermal pericyclic reactions. Drawbacks, however, are the lack of enantioselectivity due to the racemic *cis*-mixtures that the Staudinger or isocyanate [2+2] cyclocondensations usually yield, and the often poor diastereoselectivity. These pharmaceutically critical factors, combined with their one-pot and cascade nature, which minimise the amount of purification steps and reagents needed, make multicomponent reactions and organocatalysis so attractive on both an economical and ecological level. The other discussed synthesis routes that lead towards 3,4-annulated β-lactams all have their individual flaws, ranging from the complications of working with ionic liquids, over the need of costly noble metals, to the often narrow or insufficiently investigated substrate scope of the reaction.

The 3,4-pyrrolidine-fused bicyclic β-lactams **34** aimed for in this Master's thesis will be synthesised in the context of trying to overcome class C β-lactamase-mediated resistance in bacteria, which is a severe global issue of growing concern. They ought to serve as potential class C β-lactamase inhibitors. The class C β-lactamase crystallographic structure-based design of inhibitors concludes that *C*-fused azetidin-2-ones with a sulphonate functionality at the β-lactam nitrogen are excellent class C enzyme inhibitor candidates. Their impediment of C3-C4 bond rotation after acylation of the enzyme both prevents the activation of a deacylating water molecule in the catalytic site, as it sterically blocks the pathway the incoming water would have to take for its nucleophilic attack on the enzyme-inhibitor ester bond. Furthermore, choosing a pyrrolidine cycle as the *C*-fused ring structure is especially interesting, because it results in an additional *N*-substituent on the diazabicyclic scaffold. This side chain might have benign hydrogen-bonding or π-stacking interactions with the β-lactamase's catalytic site, hereby increasing the affinity between enzyme and inhibitor as well as the stability of the enzyme-inhibitor complex, thus vastly improving the efficacy of the inhibitor. However, limited penetration through the target bacterium's outer membrane or periplasmic space can form an important obstacle for a β-lactamase inhibitor in obtaining good synergy with a β-lactam antibiotic.

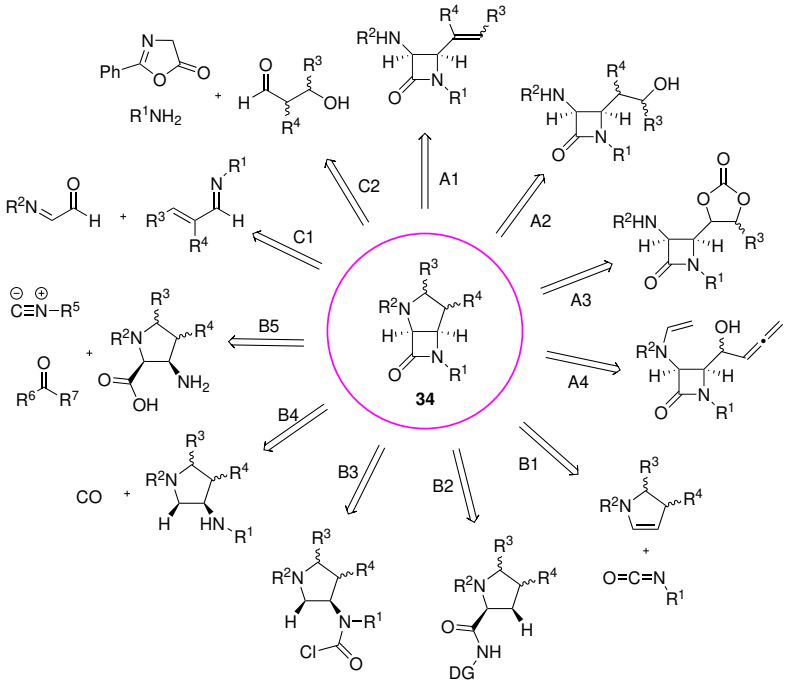
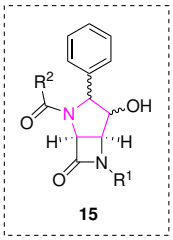


Figure 4: Possible synthetic approaches towards 2,6-diazabicyclo[3.2.0]heptan-7-ones **34**. The A-methods construct the pyrrolidine heterocycle on a through Staudinger synthesis obtained β-lactam; the B-methods do the opposite and create the β-lactam core on a pyrrolidine compound; the C-methods start from acyclic precursors and directly synthesise the whole *C*-fused bicyclic β-lactam in a one-pot process. **A1**: halocyclisation *or* intramolecular nucleophilic substitution of an epoxide moiety, **A2**: intramolecular nucleophilic substitution of a mesylate or tosylate moiety, **A3**: intramolecular nucleophilic substitution of an ethylene carbonate moiety, **A4**: domino [3+3] sigmatropic rearrangement/Diels-Alder reaction, **B1**: alkene-isocyanate [2+2] cyclocondensation, **B2**: palladium(II)-catalysed N1-C4 bond formation (DG = directing group), **B3**: palladium(0)-catalysed C2-C3 bond formation, **B4**: palladium(II)-catalysed N1-C3 linkage *via* CO insertion, **B5**: Ugi four-center three-component condensation, **C1**: NHC-organocatalysis, **C2**: [C+2C+N] three-component synthesis in an ionic liquid.

3 RESULTS AND DISCUSSION

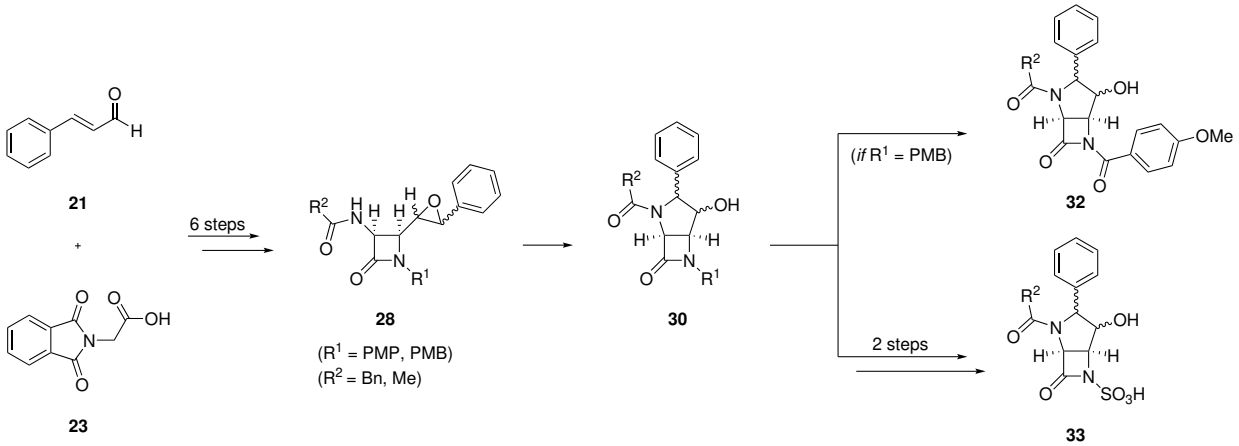
In this Master's thesis novel 3,4-pyrrolidine-fused bicyclic β-lactams **15** with potential class C β-lactamase inhibitory activity will be synthesised. These compounds will contribute filling a gap both pharmaceutical-wise as literature-wise, since there not only exists a dire need for commercially available, potent and selective class C β-lactamase inhibitors, but there also is a lack of diverse literature methods for synthesising 2,6-diazabicyclo[3.2.0]heptan-7-ones **34** in general.



15

(R^1 = PMP, PMB, PMBz, SO_3H)
(R^2 = Bn, Me)

The proposed synthesis pathway, affording the construction of all desired bicyclic β-lactams **15** starting from the readily available (*E*)-cinnamaldehyde **21** and *N*-phthaloylglycine **23**, consists of nine steps. However, the discussion of this synthesis route will comprise three parts, representing the three key synthetic milestones: (i) preparation of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)-β-lactams **28** in six steps (section 3.1); (ii) cyclisation towards 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **30** *via* amido group-induced intramolecular ring closure through epoxide ring opening (section 3.2); (iii) functionalisation towards 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **32** in one step *via* benzylic oxidation, or towards 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33** in two steps *via* subsequent deprotection and sulphonation of nitrogen N6 (section 3.3).



As stated in the chapter "Scope and Goal", the primary goal of this Master's thesis concerns the synthesis of the diazabicyclic scaffold of 3,4-pyrrolidine-fused bicyclic β-lactams **30** *via* the intramolecular nucleophilic attack at an epoxide functionality. Although it becomes clear from the chapter "Literature overview" that intramolecular nucleophilic attacks are a frequently applied synthetic strategy towards *C*-fused bicyclic azetidin-2-ones, there exists only one instance in the literature, in which oxirane moieties are employed for the construction of the 3,4-annulated ring, *i.e.* aforementioned preliminary research.[64,65] However, there the intramolecular ring closure was hydroxyl group-induced and led towards the formation of 3,4-oxolane-fused bicyclic β-lactams **14**. This Master's thesis will explore the aza-variant of that reaction, hereby also providing a new route towards 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **34**, for which only very few literature protocols exist until now, *viz.* a halocyclisation (section 2.1.1.1),[87,88] a nucleophilic substitution of a carbonate with a tetra-alkylammonium halide (section 2.1.1.2),[71] an oxidative coupling of a dianion (section 2.1.3),[129] and a palladium(II)-catalysed N6-C5 bond formation (section 2.2.2.1).[138] The further derivatisation of 2,6-diazabicyclo[3.2.0]heptan-7-ones **30** towards compounds **32** and **33** forms an important secondary objective as their functional groups (R^1 = PMBz, SO_3H) exert most probably a significant influence on the biological activity against class C β-lactamase enzymes.

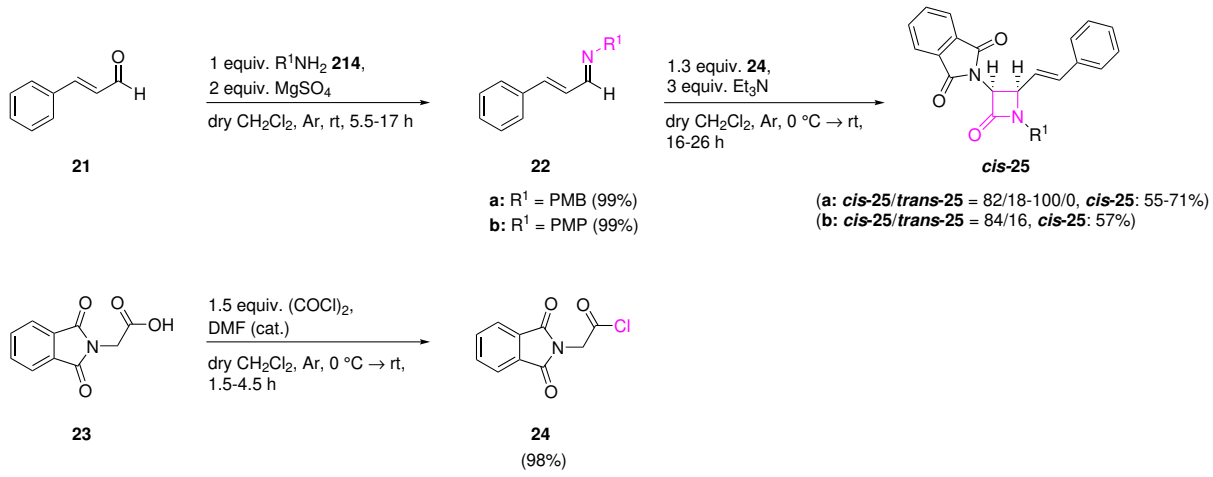
3.1 Synthesis of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones

The first part of the synthesis route towards 3,4-pyrrolidine-fused bicyclic β-lactams **15** comprises the six-step synthesis of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28**. Containing both a C3-attached amido functionality and a C4-tethered epoxide moiety, these novel compounds form the precursors for the intramolecular construction of a pyrrolidine ring.

3.1.1 Staudinger β-lactam synthesis of *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-one building blocks

In the first three steps of the envisioned synthesis route, the construction of the β-lactam core is realised. Different methodologies are available in the literature, but the original Staudinger β-lactam synthesis,[12] of which the mechanism has been studied extensively over the past century in both experimental and computational ways, remains very popular as a result of its broad substrate scope and operational simplicity.[66,168–170] At first, it appears as a rare example of a thermal [2+2] cycloaddition, for which the usually geometrically forbidden Woodward-Hoffman demand of suprafacial-antarafacial orbital overlap is met, due to the absence of two sterically hindering hydrogen atoms around the non-carbonyl π-bond of a ketene.[100] However, the Staudinger β-lactam synthesis is nowadays generally considered to be a two-step [2+2] cyclocondensation between an imine and a ketene, with the latter generated *in situ* from an acid chloride.[66,168–170]

In that respect, imination of (*E*)-cinnamaldehyde **21** was performed in anhydrous dichloromethane at room temperature and under inert argon atmosphere, using one equivalent of primary amines **214** in the presence of two equivalents magnesium sulphate as the drying agent. Upon full conversion of the starting material, as determined by ^{1}H NMR analysis (in $CDCl_3$) through disappearance of the aldehyde proton signal at 9.71 ppm, crude imines **22** were obtained quantitatively. After filtration of the drying agent they were, due to their hydrolytic instability and since further purification was also deemed unnecessary (purity > 98 %, as determined by ^{1}H NMR analysis in $CDCl_3$), used as such in the subsequent Staudinger cyclocondensation. Formation of the acid chloride proceeded by treatment of *N*-phthaloylglycine **23** with 1.5 equivalents oxalyl chloride and a catalytic amount of *N*,*N*-dimethylformamide (DMF) in ice-cooled (0 °C) anhydrous dichloromethane under inert argon atmosphere, achieving 98 % conversion towards *N*-phthaloylglycyl chloride **24** after stirring at room temperature for several hours. Follow-up of the conversion and determination of the yield was done by ^{1}H NMR analysis (in $CDCl_3$) through the ratio in chemical shift of the methylene group's protons, which was 4.50 ppm and 4.83 ppm for carboxylic acid **23** and acid chloride **24**, respectively. Also being sensitive to hydrolysis and not in need of purification (purity > 98 %, as determined by ^{1}H NMR analysis in $CDCl_3$), work-up of *N*-phthaloylglycyl chloride **24** was limited to partial evaporation of the solvent, and the resulting solution was immediately used in the Staudinger β-lactam synthesis as well.



21

22

a: R^1 = PMB (99%)
b: R^1 = PMP (99%)

***cis*-25**

(**a:** ***cis*-25**/***trans*-25** = 82/18-100/0, ***cis*-25**: 55-71%)
(**b:** ***cis*-25**/***trans*-25** = 84/16, ***cis*-25**: 57%)

23

24
(98%)

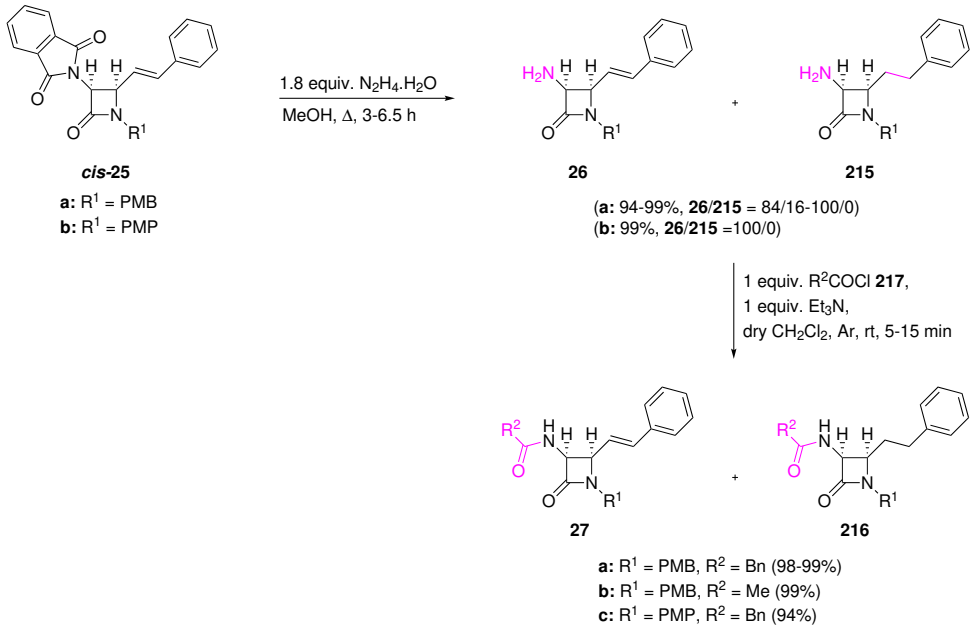
To that end, an ice-cooled (0 °C) solution in anhydrous dichloromethane of *N*-substituted (1*E*,2*E*)-3-phenylprop-2-en-1-imines **22** was treated with 1.3 equivalents phthaloyl-protected acid chloride **24** in anhydrous dichloromethane and in the presence of three equivalents triethylamine as base, to give racemic 3-phthalimido-4-((*E*)-styryl)azetidin-2-ones **25** after stirring overnight at room temperature. Over the course of this work, two different substituents at the β-lactam nitrogen (R^1) were investigated, *i.e.* 4-methoxybenzyl (PMB) and 4-methoxyphenyl (PMP). Analysis of the crude reaction products revealed that the Staudinger β-lactam syntheses in this Master's thesis were clean reactions with little non-β-lactam side products present after work-up, affording azetidin-2-ones of high purity (purity > 95 %, as determined by ^{1}H NMR analysis in $CDCl_3$). Assignment of the relative stereochemistry of β-lactams **25** was established *via* the ^{1}H NMR spectrum, in which the major reaction product's observed vicinal coupling constants of 5.1 Hz (R^1 = PMB) and 5.6 Hz (R^1 = PMP) between the proton signals at carbons C3 and C4 were in accordance with the literature data for *cis*-β-lactams, *i.e.* generally J_{cis} = 5-8 Hz and J_{trans} = 0-2 Hz.[65,86,171,172] During some experiments on a larger scale, *i.e.* 50 mmol starting materials **22** instead of 10 mmol, formation of *trans*-β-lactams was detected as well, but only to a rather small extend (0-18 %). *Cis*-β-lactams ***cis*-25** were successfully purified by means of manual or automated column chromatography on silica gel in 55-71 % yields. It is stated in the literature that so-named Sheehan ketenes, *e.g.* a *N*-phthaloyl-substituted ketene derived from acid chloride **24**, do not possess a strong *cis*- or *trans*-diastereoselectivity.[168] Hence, the strongly favoured formation of *cis*-azetidin-2-ones ***cis*-25** during above reaction is most likely the result of a rather complex interaction between the many factors that are known to influence the stereochemical outcome of the Staudinger β-lactam synthesis, *viz.* substituents, base, solvent, temperature and order of reagent addition.[66,168,170,173] In particular, PMP-substituted imines are known to boost the selectivity towards *trans*-azetidin-2-ones,[168] while (*E*)-cinnamaldehyde-derived imines on the other hand tend to increase the amount of *cis*-product.[173,174] The observed *cis*-diastereoselectivity in this Master's thesis is both fortunate and important, since an intramolecular ring closure towards 3,4-annulated bicyclic β-lactams appears to be impossible with the *trans*-diastereomers.[83–86]

It should be noted that during Staudinger β-lactam synthesis, besides the possibility of *trans*-β-lactam formation, another undesired side reaction might take place as well. Imines **22** are α,β-unsaturated and, therefore, their use in the Staudinger cyclocondensation could yield both β- and δ-lactams. The absence of this [2+2] *vs.* [4+2] periselectivity problem during the synthesis of azetidin-2-ones **25** can be attributed to the steric interaction between the bulky phthalimido and phenyl groups at respectively carbons C3 and C4 during the second step of the mechanism, which is an electrocyclic ring closure. Since the disrotatory electrocyclisation, yielding δ-lactams, experiences higher steric hindrance than the β-lactam-affording conrotatory one, exclusive formation of the latter can be observed.[169,170] It is evident from the literature though, that the outcome of the δ-lactam side reaction is not only influenced by steric and electronic effects, but by the reaction conditions and presence of additional reagents as well.[66,168–170] In that respect, it is fortunate that these undesired δ-lactams were not formed during any experiments. Note that the β-lactams synthesised in this step are racemic, with all depicted stereochemistry thus being relative and not absolute. Therefore, all azetidin-2-ones, which will be synthesised in the coming steps, are racemic as well, since no enantioselective reactions or purifications will be employed.

3.1.2 *N*-phthaloyl deprotection and subsequent *N*-acylation of *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-ones

With *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-one building blocks ***cis*-25** in hand, their conversion towards *cis*-3-acylamino-4-oxiranylazetidin-2-ones **28** was investigated. Being a well-known and successful method, the epoxidation of the double bond was decided to be achieved by addition of a peracid,[65,175–177] *e.g.* 3-chloroperbenzoic acid (*m*CPBA), which unlike most peracids is stable at moderate temperatures for prolonged periods.[177] As pointed out in the chapter "Scope and Goal", incorporation of the desired 3-acylamino functionality, involving *N*-phthaloyl deprotection and subsequent *N*-acylation, needs to occur prior to epoxidation, due to the incompatibility of the functional groups with the employed reagents. In particular, this concerns the interaction of hydrazine hydrate with the reactive three-membered oxirane ring, if epoxidation would occur before *N*-phthaloyl deprotection, or the interaction of peracid *m*CPBA with the 3-amino substituent, should epoxidation take place prior to *N*-acylation. Some undisclosed initial experiments, in which this preferential order of steps was altered, gave non-encouraging results, as expected. Therefore, confidence in the reaction order of the envisioned synthesis pathway was strengthened, and the *N*-phthaloyl deprotection was righteously determined to be the fourth step.

To that end, newly synthesised *cis*-3-phthalimido-β-lactams ***cis*-25** were used as a substrate for *N*-deprotection *via* hydrazinolysis.[74] Hydrazine was already employed in the 1970s for dephthaloylation of phthalimido-substituted cephalosporins and penicillins.[178] Formation of the premised *cis*-3-amino-β-lactams **26** required reaction between *cis*-3-phthalimidoazetidin-2-ones ***cis*-25** and 1.8 equivalents hydrazine monohydrate in methanol for several hours under reflux conditions. After work-up, crude products **26** were obtained in excellent yields (94-99 %) and no purification was deemed necessary upon investigation of the ^{1}H NMR spectrum (purity > 95 %, in $CDCl_3$). The benefit of using hydrazine for cleaving off the protecting group lies in the formed phthalhydrazide, which precipitates and can thus easily be removed from the crude reaction mixture by filtration afterwards. It was discovered that this deprotection step sometimes suffers from a side reaction, *i.e.* a hydrogenation of the 4-((*E*)-styryl) substituent's double bond, but confirmation of hydrogenated side products **215** being formed, was only attained after isolation and characterisation of their *N*-acylated derivatives **216** during the epoxidation step (section 3.1.3). Fortunately, it can be said that, in general, this side reaction during dephthaloylation never presented a huge issue, since most of the time it only occurred to a small extend (0-3 %). The highest fraction of starting materials ***cis*-25**, that ended up as 4-(2-phenylethyl)-β-lactams **215**, was 16 % after an unnecessarily long reaction time of twenty hours.



An explanation for the observed side reaction might lie in the process of transfer hydrogenation, which is the addition of hydrogen to an unsaturated system from another source than hydrogen gas. It is apparent from the literature that hydrazine makes a popular hydrogen donor to be used in this process and, in addition, methanol is a frequently used solvent for these kind of reactions.[179–182] In such transfer hydrogenation systems, hydrazine is known to decompose to either molecular hydrogen (H_2) or to the short-lived diimide (HN=NH), with the former usually occurring over metals like palladium, while the latter is formed upon interaction with oxidising agents, such as hydrogen peroxide or air.[182] In fact, already more than a century ago, diimide was employed for the reduction of unsaturated fatty acids in open vessels containing aqueous or alcoholic solutions of hydrazine.[183] Also more recent research by Pieber and co-workers, in which a catalyst-free continuous-flow process for selectively reducing alkenes has been developed with hydrazine monohydrate and molecular oxygen being the only reagents, clearly illustrates the hydrogenating capability of a hydrazine-oxygen system.[181] Therefore, it is very likely that the employed reaction conditions during the *N*-phthaloyl deprotection in this Master's thesis, *i.e.* hydrazine monohydrate in methanol under air, resulted in the undesired transfer hydrogenation of the 4-alkenyl functionality to some extend. Another indication, that the *in situ* formation of diimide is responsible for the observed side reaction, is the characteristic property of this molecule to only add hydrogen to non-polar π-systems, *e.g.* alkenes, and not to polar double bonds like a carbonyl group.[182,183] In order to avoid transfer hydrogenation in future experiments, it is advised to use degassed methanol *via* treatment with nitrogen gas and subsequent sonification, and to perform the *N*-deprotection under an inert atmosphere, *i.e.* nitrogen gas or argon.

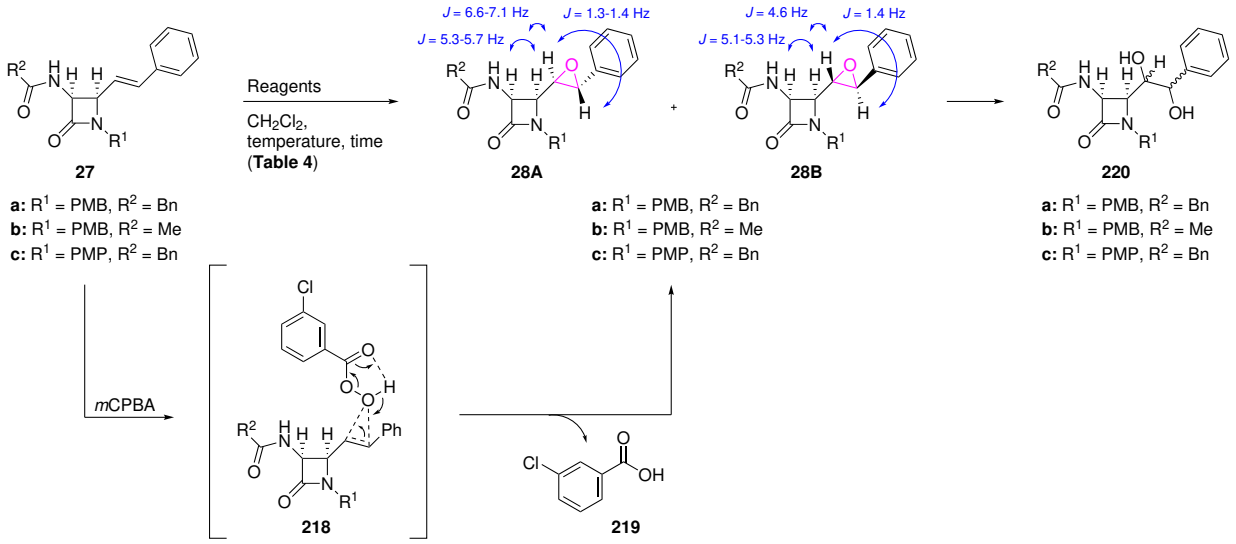
The fifth reaction step in the envisioned synthesis pathway concerns the *in supra* mentioned *N*-acylation of the newly created free amino group at carbon C3 of the azetidin-2-one ring. In that respect, addition of one equivalent acid chlorides **217** to a solution of *cis*-3-aminoazetidin-2-ones **26** in anhydrous dichloromethane and in the presence of one equivalent triethylamine, afforded *cis*-3-acylamino-β-lactams **27** after stirring for several minutes at room temperature and under inert argon atmosphere. After work-up, crude products **27** were obtained in excellent yields (94-99 %) and, once again, no purification was deemed necessary upon investigation of the ^{1}H NMR spectrum (purity $>$ 90 %, in $CDCl_3$) When an experiment was performed without the addition of triethylamine, complete conversion of starting material **26b** was still not achieved after ninety minutes, which otherwise already was the case after less than ten minutes. When one equivalent of triethylamine base was added at last, conversion proceeded rapidly to completion. This confirms the necessity of adding base, since it quenches the hydrogen chloride acid, which is formed upon nucleophilic attack of the 3-amino functionality onto acid chlorides **217**. If not quenched, the hydrogen chloride would otherwise protonate the 3-amino groups of the remaining starting materials **26**, therefore removing their nucleophilic character and thus impeding the reaction towards products **27** from achieving completion. It should be noted that any hydrogenated side products **215**, that were present in the starting material along compounds **26**, got acylated as well towards β-lactams **216**. However, their separation from the desired *N*-acylated β-lactam products **27** was considered cumbersome because of the very similar chromatographic retention times.

3.1.3 Epoxidation of *cis*-3-acylamino-4-((*E*)-styryl)azetidin-2-ones

After deprotection and acylation of the 3-amino moiety on the β-lactam core, the next step in the envisioned synthesis pathway concerns the epoxidation of the double bond in *cis*-3-acylamino-4-((*E*)-styryl)azetidin-2-ones **27** with a peracid, most probably yielding a mixture of major **28A** and minor *cis*-4-oxiranylazetidin-2-one diastereomers **28B**. As stated previously, 3-chloroperbenzoic acid (*m*CPBA) was chosen as the oxidising agent, since it is quite stable for being a peracid and, in addition, it is well-known to usually give successful epoxidations.[65,175–177] The goal of this section is finding robust epoxidation conditions, which give a high yield and consistent diastereoselectivity for varying substituents (R^1 and R^2), and under which, in addition, complete conversion of starting material **27** is achieved readily.

In that respect, various experiments were performed, in which a solution of *cis*-4-((*E*)-styryl)-β-lactams **27** in dichloromethane was treated with variable equivalents *m*CPBA, while stirring at room or reflux temperature. **Table 4** gives an overview of the yields and diastereomeric ratios *inter alia* of these epoxidation experiments. Initial reactions on compounds **27a** (R^1 = PMB, R^2 = Bn), based on a protocol in preliminary research,[64,65] indicated that a single addition of 1.5 equivalents *m*CPBA at room temperature was insufficient, resulting in incomplete conversion of the starting material (entries 1-3). However, each time a reproducible diastereomeric ratio of 2/1 was attained, no side reactions were observed, and *cis*-4-oxiranyl-β-lactams **28a** were obtained in moderate yields (37-63 %) after purification by means of column chromatography on silica gel. It was discovered that under these conditions, multiple additions of 1.5 or two equivalents *m*CPBA over a time span of a couple days were necessary in order to drive the conversion to completion (entries 3, 4 and 10). Sodium bicarbonate was added in an initial attempt to speed up the reaction, because it was thought deprotonation of the peracid would enhance its reactivity (entry 3). That this was not the case is evident from the reaction mechanism (Prilezhaev reaction), in which the peracid's proton is essential for creating the intramolecular hydrogen bond in intermediates **218**.[184,185]

Our interest was sparked by the occurrence of experiments, in which major **28Aa** and minor epoxide diastereomers **28Ba** were initially formed in a 1/1 ratio, but after reacting for a couple days at room temperature they were recovered in 2/1 to 6/1 ratios (**Table 4**: entries 3-5). The hypothesis of interconversion between the epoxide diastereomers, with the favoured formation of a probably thermodynamically more stable major diastereomer, was further investigated and is discussed *in infra*. However, one of the tests, *i.e.* checking whether a change in the thermodynamic equilibrium (*dr*) and/or interconversion rate occurred by elevating the temperature, revealed that full epoxidation of 4-((*E*)-styryl)-β-lactams **27a** could be achieved after only three hours by a single addition of four equivalents *m*CPBA at reflux temperature (entry 6). This finding was very interesting, because until now epoxidation reactions with complete conversion of the starting material **27a** needed a rather long reaction time of four to eight days. When six additional similar experiments, employing three equivalents *m*CPBA, were performed for compounds **27a** (entries 7-9) and **27b** (entries 11-13), it was discovered that the applied conditions gave excellent and reproducible results: a complete



epoxidation after three hours under reflux conditions, a consistent diastereomeric ratio of 3/2, and obtaining crude reaction products after work-up in high yields (83-99 %) and of high purity (purity > 95 %, as determined by ^{1}H NMR analysis in $CDCl_3$). It should be noted that, when no immediate work-up of the reaction mixture had taken place upon full conversion, and when it was kept under reflux conditions for more than seven hours, deterioration of the epoxide products **28** was observed (entries 8 and 12). During the epoxidation of PMP-substituted *cis*-4-((*E*)-styryl)-β-lactam **27c**, a different behaviour was detected (entry 14). Even though four equivalents *m*CPBA were employed at reflux temperature, no complete conversion was observed after more than four hours. In addition, the diastereoselectivity (dr = 5/1) was different compared to the synthesis of compounds **28a** and **28b** (dr = 3/2). Nonetheless, letting it react four more days at room temperature with two extra equivalents *m*CPBA led to full conversion and exclusively afforded crude major epoxide diastereomer **28Ac** in 88 % yield after work-up (purity > 70 %, as determined by ^{1}H NMR analysis in $CDCl_3$) and 43 % yield after purification by means of reversed phase automated column chromatography (C18). It should be mentioned that in some experiments, the loss of the formed epoxides' **28a** (R^1 = PMB, R^2 = Bn) LC-MS signals coincided with the appearance of a strong LC-MS signal, which had an increase in mass of eighteen dalton (entries 5, 6 and 8). Purification by means of reversed phase automated column chromatography (C18) allowed identification of this signal by NMR spectroscopy (in $CDCl_3$) as a 5/2 mixture of two *anti*-vicinal diols **220a** (R^1 = PMB, R^2 = Bn), which were then characterised (section 6.3). The acid- or base-catalysed ring opening of oxiranes towards *anti*-vicinal diols is a well-known phenomenon in aqueous solvents.[186] Hence, it is a possibility that the absence of anhydrous conditions during the epoxidation step might have caused this reaction. In order to avoid diol formation, it is advised to use anhydrous dichloromethane, and to perform the reaction under inert atmosphere, *i.e.* nitrogen gas or argon.

Assignment of the relative stereochemistry of major **28A** and minor *cis*-4-oxiranylazetidin-2-one diastereomers **28B** was established *via* ^{1}H NMR spectroscopy (in $CDCl_3$), since it can be easily deduced from the vicinal coupling constants between the proton signals at carbons C3 and C4 of the β-lactam core, C1' and C2' of the oxirane moiety, and C4 and C1'. Their measured values were in accordance with those reported in the literature for similar azetidin-2-ones, and allowed full stereochemical characterisation of the major **28A** ($J_{\text{C3-C4, }cis}$ = 5.3-5.7 Hz, $J_{\text{C1'-C2', }trans}$ = 1.3-1.4 Hz, $J_{\text{C4-C1', }cis}$ = 6.6-7.1 Hz) and minor epoxide diastereomers **28B** ($J_{\text{C3-C4, }cis}$ = 5.1-5.3 Hz, $J_{\text{C1'-C2', }trans}$ = 1.4 Hz, $J_{\text{C4-C1', }trans}$ = 4.6 Hz).[65,176,187,188] During work-up of the reaction mixture, in which a saturated sodium sulphite solution was added to quench the remaining *m*CPBA and then multiple washes with sodium bicarbonate were performed, the formation of a strong emulsion was noted in the initial experiments (**Table 4**: entries 1-5 and 10), hereby considerably prolonging the procedure as even addition of brine was of little influence. Surprisingly, these emulsion problems were almost absent during work-up of reaction mixtures, obtained by stirring for three hours at reflux temperature (entries 7-9 and 11-13). Furthermore, it should be noted that complete separation of the two diastereomers by means of manual (SiO_2) or reversed phase automated (C18) column chromatography proved to be impossible, since their chromatographic retention times were very similar. In fact, only isolation of the first eluting major epoxides **28A** was achieved, but due to substantial tailing, minor epoxides **28B** could never be separated from their major counterparts.

Table 4: Overview of the reaction conditions, *viz.* scale, choice and amount of reagents, temperature, reaction time, diastereomeric ratio of the crude reaction product and yield, of the epoxidation of *cis*-3-acylamino-4-((*E*)-styryl)azetidin-2-ones **27** towards *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28**.

	Scale [mmol]	Reagents	Temperature, time	dr[a] = **28A**/**28B**	**28** [%]
R^1 = PMB, R^2 = Bn			(Starting material: 4-((*E*)-styryl)-β-lactams **27a**)		
1	0.4	1.5 equiv. *m*CPBA	0 °C → rt, 3 days	67/33	41[c,d]
2	2.6	1) 1.5 equiv. *m*CPBA	0 °C → rt, 18 h	-	-[d]
		2) 0.5 equiv. *m*CPBA	rt, 2 days	67/33	37[c,d]
3	0.5	1) 1.5 equiv. *m*CPBA, 1.5 equiv. $NaHCO_3$	rt, 6 days	50/50[e]	-[d]
		2) 1.5 equiv. *m*CPBA	rt, 1 day	52/48[e]	-[d]
		3) 1.5 equiv. *m*CPBA	**rt, 1 day**	**67/33**	**63[c]**
4	3.6	1) 2 equiv. *m*CPBA	0 °C → rt, 1 day	50/50[e]	-[d]
		2) 2 equiv. *m*CPBA	**rt, 3.5 days**	**85/15**	**84[b,c]**
5	2.6	3 equiv. *m*CPBA	1) 0 °C → rt, 7 h	50/50[e]	-[d]
			2) rt, 5 days	75/25	33[b,f,g]
6	5.3	4 equiv. *m*CPBA	1) rt → Δ, 1 h	56/44[e]	-[d]
			2) Δ, 1 day	80/20[e]	-[h]
			3) Δ, 4 days	-	-[b,g,i]
7	**1.8**	**3 equiv. *m*CPBA**	**Δ, 7 h**	**59/41**	**93[j]**
8	1.8	3 equiv. *m*CPBA	1) Δ, 7 h	59/41[e]	-
			2) Δ, 1 day	77/23[e]	-[h]
			3) Δ, 1 day	92/8	15[g,h,k]
9	**6.6**	**3 equiv. *m*CPBA**	**Δ, 3 h**	**54/46**	**89[j]**
R^1 = PMB, R^2 = Me			(Starting material: 4-((*E*)-styryl)-β-lactams **27b**)		
10	2.7	1) 2 equiv. *m*CPBA	0 °C → rt, 1 day	50/50[e]	-[d]
		2) 2 equiv. *m*CPBA	rt, 1 day	50/50[e]	-
		3) 2 equiv. *m*CPBA	rt, 2.5 days	100/0	40[b,c]
11	**1.8**	**3 equiv. *m*CPBA**	**Δ, 7 h**	**57/43**	**99[j]**
12	1.8	3 equiv. *m*CPBA	1) Δ, 7 h	59/41[e]	-
			2) Δ, 1 day	79/21[e]	-[h]
			3) Δ, 1 day	98/2	83[h]
13	**6.6**	**3 equiv. *m*CPBA**	**Δ, 3 h**	**59/41**	**83[j]**
R^1 = PMP, R^2 = Bn			(Starting material: 4-((*E*)-styryl)-β-lactams **27c**)		
14	3.9	1) 4 equiv. *m*CPBA	rt → Δ, 4.5 h	84/16[e]	-[d]
		2) -	rt, 1 day	90/10[e]	-[d]
		3) 2 equiv. *m*CPBA	rt, 3 days	100/0	43[k]

[a] The diastereomeric ratio of crude reaction products **28** after work-up, as determined by ^{1}H NMR analysis (in $CDCl_3$) or by LC-MS analysis in the absence of the former.

[b] Contamination of starting materials **27** with hydrogenated side products **216**.

[c] Purification *via* column chromatography on silica gel (petroleum ether/ethyl acetate 1/2 (entries 1, 2 ,3) or 3/2 (entry 10) or gradient petroleum ether/ethyl acetate 67/33-33/67 (entry 4)).

[d] Incomplete conversion of the starting material, as determined by LC-MS analysis and/or ^{1}H NMR analysis (in $CDCl_3$).

[e] Determined during follow-up of the reaction by ^{1}H NMR (in $CDCl_3$), or by LC-MS analysis in the absence of the former.

[f] Purification *via* normal phase automated column chromatography (SiO_2, gradient petroleum ether/ethyl acetate 100/0-0/100).

[g] Appearance of a new LC-MS signal with a m/z value, that has a mass increase of 18 dalton compared to the m/z value of the 4-oxiranyl-β-lactams **28**. The epoxides have reacted towards *anti*-vicinal diols **220**.

[h] Appearance of a lot of impurities, as determined by ^{1}H NMR analysis (in $CDCl_3$) and/or LC-MS analysis.
[i] Complete decomposition of the formed 4-oxiranyl-β-lactams **28**, as determined by ^{1}H NMR analysis (in $CDCl_3$) and/or LC-MS analysis.
[j] Clean reaction, as determined by ^{1}H NMR analysis (in $CDCl_3$) after work-up. No purification was performed.
[k] Purification *via* reversed phase automated column chromatography (C18, gradient H_2O/CH_3CN 100/0-0/100 (entry 8) or 90/10-0/100 (entry 14)).

As mentioned *in supra*, the epoxidation step also allowed isolation of hydrogenated impurities **216** as without the 4-((*E*)-styryl) functionality they could not take part in this reaction. Consequently, the difference in retention time between side products **216** and 4-oxiranylazetidin-2-ones **28** was found to be larger than it was between compounds **26** and **215**, or between β-lactams **27** and **216**, which was attributed to the fact that the similarity in chromatographic behaviour between the two molecules had decreased substantially with the introduction of an extra heteroatom in compounds **28**. By means of reversed phase automated column chromatography (C18) and subsequent recrystallisation from ethanol, the purity of β-lactam **216a** (R^1 = PMB, R^2 = Bn) was enhanced substantially (purity of 88 %, as determined by ^{1}H NMR analysis in $CDCl_3$), after which spectral data confirmed the suspicion of a hydrogenation side reaction as *cis*-4-(2-phenylethyl)azetidin-2-one **216a** was identified and characterised (section 6.3). Isolation of *N*-acetylated side product **216b** (R^1 = PMB, R^2 = Me), after synthesis of 4-oxiranyl-β-lactams **28b**, by means of preparative TLC with a mixture of petroleum ether and ethyl acetate (1/2) failed. No attempts at the isolation of non-acylated side products, *i.e.* *cis*-3-amino-4-(2-phenylethyl)-β-lactams **215**, were made.

Lastly, a further investigation of the suspected, but unconfirmed phenomenon of interconversion between the *cis*-4-oxiranylazetidin-2-one diastereomers **28A**/**28B** was done in order to explore its nature. As stated *in supra*, an attempt was made to check whether a change in the thermodynamic equilibrium (*dr*) and/or interconversion rate would occur at elevated temperatures (**Table 4**: entries 6, 8 and 12). It was found that reflux conditions drove the epoxidation to completion in three hours and afforded a lower diastereoselectivity (*dr* = 3/2) compared to the "multiple addition method" (*dr* = 2/1, entries 1-3), but that four more hours at reflux temperature did not change this diastereomeric ratio whatsoever. When keeping the reaction mixture at reflux for even longer, *i.e.* multiple days, a gradual increase of the diastereoselectivity towards major epoxides **28A** was observed. These results should however be interpreted with caution as gradual decomposition of the epoxide products, and particularly the minors **28B**, was noted as well. Therefore, an alternate hypothesis to the interconversion theory concerns the deterioration of mainly the minor epoxides **28B**, hereby increasing the diastereomeric ratio. A third, and maybe the most plausible option, is that both phenomena, *viz.* interconversion and decomposition, occur at the same time upon prolonged exposure of the reaction mixture to reflux conditions.

To that end, the follow-up of the epoxidation of *cis*-4-((*E*)-styryl)-β-lactam **27c** (R^1 = PMP, R^2 = Bn) was not done by LC-MS analysis as was usually the case, but by a quantitative method, *i.e.* NMR spectroscopy (in $CDCl_3$), of which the results are given in **Table 5**. Most interesting are the following transitions: (i) between entries 2 and 3, where the conversion increased with 7 %, but the fraction of major epoxide increased with 18 % while the minor decreased with 11 %, resulting in a change in diastereomeric ratio from 3/2 to 5/1; (ii) between entries 6 and 7, where conversion went from 75 % to 100 %, but in the end only major epoxide was recovered. Furthermore, this major epoxide **28Ac** was recovered in a yield of 88 % after work-up (purity > 70 %, as determined by ^{1}H NMR analysis in $CDCl_3$), and in 43 % yield after purification (**Table 4**: entry 14). It should be noted that during this experiment the chromatographic apparatus' solvent was later proven to be contaminated with ethyl acetate, which might explain the significant loss in yield. Given that during this follow-up experiment NMR spectra with rather few impurities were obtained, thus showing no significant deterioration of any epoxide products, above findings support the idea of interconversion between the two *cis*-4-oxiranylazetidin-2-ones **28Ac** and **28Bc**. In the end, it should however be stressed that no irrefutable proof of interconversion by means of a mass balance, *i.e.* a yield of major 4-oxiranyl-β-lactams **28A** that exceeds 50 % after purification, was attained. As the primary goal of this Master's thesis concerned the synthesis of bicyclic target compounds **30**, no further investigation of the suspected interconversion phenomenon was performed. However, the section "Future work and Perspectives" provides some suggestions how this could be approached (section 3.4).

Table 5: Investigation of the suspected interconversion of major and minor 4-oxiranylazetidin-2-one diastereomers by follow-up[a] of an experiment (**Table 4**: entry 14), concerning the epoxidation of *cis*-1-(4-methoxyphenyl)-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one **27c** (R^1 = PMP, R^2 = Bn) towards *cis*-1-(4-methoxyphenyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-one diastereomers **28Ac**/**28Bc**.[b]

	Sample time[c]	Δt[d]	*m*CPBA[e] [equiv.]	Temperature[e]	**27c**[f] [%]	**28Ac** [%]	**28Bc** [%]
1	40 min	40 min	4	Δ	72	13	15
2	2 h	80 min	4	Δ	45	33	22
3	4.5 h	2.5 h	4	Δ	38	51	11
4	22 h	17.5 h	4	rt[g]	37	57	6
5	28 h	6 h	4	rt	35	59	6
6	28.5 h	30 min	6[g]	rt	25	67	8
7	4 days	3 days	6	rt	0	100	0

[a] As determined by ^{1}H NMR analysis (in $CDCl_3$) of an equivalent proton signal at carbon C1' (*i.e.* (C=O)(CH)$_2$C<u>H</u>).

[b] During the whole course of the experiment, no significant decomposition of the formed epoxides **28c** is observed.

[c] Time passed since the start of the experiment.

[d] Time passed since taking the previous sample.

[e] Conditions, *i.e.* amount of oxidising reagent or temperature, present in the reaction mixture at the moment of sampling.

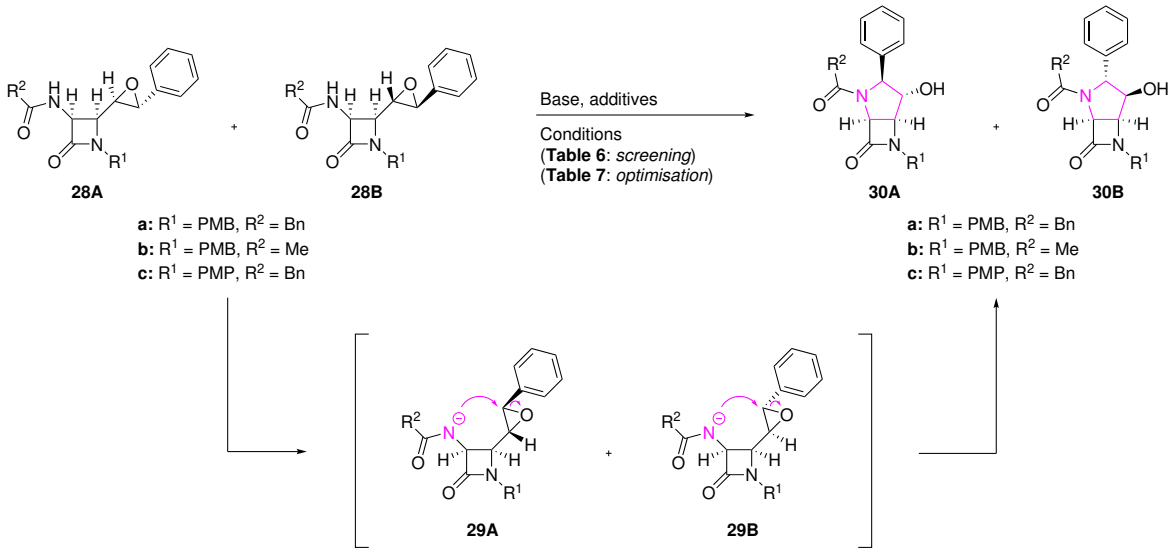
[f] The alkene proton signal of starting material **27c** is an accurate measure of the conversion of the reaction.

[g] Conditions, *i.e.* amount of oxidising reagent or temperature, were changed immediately after taking the previous sample.

In retrospect, the goal of this section, *i.e.* finding robust epoxidation conditions, which give a high yield and consistent diastereoselectivity for varying substituents (R^1 and R^2), is achieved. For the synthesis of PMB-substituted epoxides **28a** and **28b**, two different conditions were deemed successful and reproducible: (i) working at room temperature and using a smaller amount of *m*CPBA (1.5 to two equivalents), which has to be added two or three times, over a course of several days, in order to drive the reaction to completion (**Table 4**: entries 1-4 and 10); (ii) working at reflux temperature and using a larger amount of *m*CPBA (three equivalents), which needs to be added only one time, and where full conversion is already achieved after several hours (entries 7, 9, 11 and 13). The diastereomeric ratio of the major and minor epoxides tends to be respectively 2/1 and 3/2. However, whenever a reaction takes place over several days, which is always the case with former "multiple addition method", the suspected phenomenon of epoxide diastereomer interconversion has to be taken into account, thus resulting in different diastereomeric ratios (entries 3-5, 10 and 14). The latter "reflux method" achieves full epoxidation of *cis*-4-((*E*)-styryl)azetidin-2-ones **27** much more readily, but if the reaction is not stopped in time, deterioration of the formed epoxides commences (entries 6, 8 and 12). In the end, this Master's thesis concludes that the "reflux method" is preferential, due to its short reaction time (three hours), good yields after work-up (83 % and higher), consistent diastereoselectivity (dr = 3/2), and above all its tendency to provide clean crude reaction products after a more facile work-up (purity > 95 %), hereby allowing their immediate use, without the need for chromatographic purification, in the subsequent intramolecular cyclisation reaction towards bicyclic β-lactams **30**. The synthesis of PMP-substituted epoxides **28c** behaved differently under reflux conditions, *i.e.* slower and giving a different diastereomeric ratio, but delivered a satisfying result nonetheless (88 % yield after work-up, dr = 1/0) after the addition of extra *m*CPBA (entry 14).

3.2 Intramolecular ring closure of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)-azetidin-2-ones towards 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]-heptan-7-ones

The second synthetic milestone of this Master's thesis concerns assessing the reactivity of the newly synthesised *cis*-3-acylamino-4-oxiranylazetidin-2-ones **28** towards the preparation of synthetically and biologically interesting 3,4-pyrrolidine-fused bicyclic β-lactams **30**. For this transformation, the proposed synthetic strategy is envisioned to proceed by an amido group-induced intramolecular ring closure through oxirane ring opening at the benzylic position. Such a 5-*endo-tet* cyclisation is actually disfavoured, according to Baldwin's rules for ring closure reactions of aliphatic compounds based on orbital overlap requirements,[95] but the favoured 4-*exo-tet* cyclisation would result in a severely strained and thus thermodynamically less stable [2.2.0]-bicyclic core. An important note is that *N*-acylation has reduced the nucleophilicity of the C3-attached nitrogen atom, which obviously might complicate the ring closure. However, achieving the simultaneous presence of a C3-attached free amino group and an epoxide moiety on the β-lactam was deemed difficult in the current synthetic approach. The rationale for this lies, as mentioned *in supra*, in the likely and probably unfavourable interaction of the hydrazine hydrate reagent with the reactive three-membered oxirane ring, if epoxidation would occur before *N*-phthaloyl deprotection, or interaction of the peracid with the 3-amino substituent, should epoxidation take place prior to *N*-acylation. Therefore, this reaction step will be base-mediated, whether or not with the help of additional reagents, *e.g.* a Lewis acid to activate the oxirane functionality.



In that respect, a large screening was done to find the right base, by which the C3-amido functionality of epoxides **28** gets deprotonated and performs *via* intermediates **29** a nucleophilic attack at the benzylic position of the oxirane ring, affording construction of the diazabicyclic scaffold. Different solvents and temperatures were assessed as well. **Table 6** gives an overview of these screening experiments, which were all performed on epoxides **28a** (R^1 = PMB, R^2 = Bn), and of which some were already performed during preliminary research, but are also discussed here for the sake of completeness. The first attempts at obtaining bicyclic β-lactams **30a** consisted of employing a catalytic amount of pyridine base in ethanol under reflux conditions (entry 1). Since it led to full recovery of starting material **28Aa**, a large amount of potassium hydroxide (twenty five equivalents) was added, but this resulted in deterioration of the epoxide. A less extreme variation of above test reaction, *i.e.* using 3.5 equivalents potassium hydroxide in ethanol at room temperature, only resulted in decomposition after three hours with no indication of *C*-fused β-lactams **30a** (entry 5). Switching solvents to acetonitrile and even less base (1.2 equivalents) was not of significant improvement, though it did result in the formation of a small LC-MS signal with the expected m/z value of compounds **30a** (entry 6). At a later point this signal was indeed proven to originate from the desired bicyclic β-lactam **30Aa**. However, conversion was very low and a lot of impurities were found present upon investigation by LC-MS and ^{1}H NMR analysis (in $CDCl_3$). In an attempt to increase the conversion, some alterations of above experiment were performed, testing a larger amount of potassium hydroxide and working in refluxing acetonitrile, but without result (entries 7 and 8). In addition, also the use of carbonates was

evaluated, *viz.* potassium and caesium carbonate, in acetonitrile (entries 2-4). In both cases, stirring at room temperature for multiple days did not convert any starting materials **28a**, but going to reflux conditions did once again make the expected m/z value of compounds **30a** during LC-MS analysis appear for the caesium carbonate experiment (entry 3). The hope of increasing this signal by going to a different manner of applying heat to the reaction mixture, *i.e.* using a microwave instead of an oil bath, did not pan out as the composition of the reaction mixture had not changed afterwards.

The outcome of all these initial reactions confirmed the expected trickiness of an amido group-induced ring closure, as mainly decomposition or full recovery of the starting material were observed. Therefore, it was decided to try more extreme reaction conditions, which could be achieved on four increasingly harsh levels: choosing a stronger base (*e.g.* organolithium compounds), performing the reaction at reflux temperature, employing an organolithium base in combination with a solvating agent in order to increase its reactivity even more (*e.g.* hexamethylphosphoramide (HMPA)), and finally also adding a Lewis acid to activate the epoxide moiety (*e.g.* boron trifluoride diethyl etherate). To that end, a 2/1 diastereomeric mixture of 4-oxiranylazetidin-2-ones **28Aa**/**28Ba** was treated with one equivalent lithium diisopropylamide (LDA) in anhydrous tetrahydrofuran (THF) at room temperature under argon atmosphere (**Table 6**: entry 12). However, no reaction was observed and the starting material was once more fully recovered. Repeating this experiment under reflux conditions did however result after ninety minutes in a small amount of bicyclic products **30a** being detected by LC-MS analysis (entry 13). When another 1.5 equivalents LDA were added to the reaction mixture, the sole signal detected by LC-MS analysis of the crude reaction product after work-up had indeed the desired m/z value, making this one of the most promising results of the screening. Using two equivalents *tert*-butyllithium at room temperature resulted, as in the case of LDA, in full recovery of the epoxides (entry 14). Yet, combining three equivalents of this extremely strong base with reflux conditions (entry 15) or the solvating agent HMPA (entry 16) did again afford the desired LC-MS signal in small intensity. The same could be said for the most severe experimental conditions, employing *tert*-butyllithium at reflux temperature with HMPA (entries 17-18) and even the Lewis acid boron trifluoride diethyl etherate (entry 19). Nonetheless, even though indications of the formation of 3,4-pyrrolidine-fused bicyclic β-lactams **30a** were present, it is of little surprise that the harsh environment, that was applied in all above screening reactions, resulted in significant deterioration and thus many impurities present during both LC-MS and ^{1}H NMR analysis (in $CDCl_3$).

Upon consultation of the literature, it was clear that intermolecular nucleophilic ring openings of epoxides by amido moieties are well established, but very few examples exist, which concern an intramolecular cyclisation by the nucleophilic attack of an amido functionality at an unactivated oxirane ring.[189] An interesting entry was found, concerning the intramolecular 5-*exo*-epoxy ring opening towards a tricyclic δ-lactam *via* deprotonation of the δ-lactam's nitrogen by sodium hydride in refluxing tetrahydrofuran.[190] Another example is the research by Powell and co-workers, which also employed sodium hydride as a base, and achieved a successful 6-*exo*-*tet* cyclisation towards 2-ketopiperazines in *N*,*N*-dimethylformamide at 50 °C.[189] Inspired by these methodologies, two equivalents of sodium hydride were added to a solution of major *cis*-3-acylamino-4-oxiranyl-β-lactam **28Aa** in anhydrous tetrahydrofuran (**Table 6**: entry 11). As four hours of stirring at room temperature had no effect, the reaction mixture was kept under reflux conditions for nineteen hours. Even then, the starting material was recovered completely with no new LC-MS signals formed whatsoever. It was with the discovery of a literature entry, in which Schultz *et al.* performed a 5-*exo*-*tet* amide-epoxide cyclisation towards heteronorbornanes, that results changed for the better.[191] It should be noted that the authors used conformationally constrained bicyclic precursors, hereby facilitating the cyclisation due to the close proximity of the amide nitrogen to the epoxide-bearing ring carbon. Nonetheless, the conditions employed there were not yet tested in this work's screening experiments, *i.e.* working with potassium *tert*-butoxide in refluxing *tert*-butanol. Applying them to *cis*-4-oxiranyl-β-lactams **28a** did once again afford the desired LC-MS signal, but, as with multiple screening conditions until now, only in low intensity (entry 9). At that point still unsure whether this signal originated from the construction of desired compounds **30a** in small quantity, it was nonetheless intriguing that exactly the same LC-MS signal with the correct m/z value reappeared during different experiments. In a serendipitous next attempt, a new reaction with a slight change of the conditions of last experiment was performed, treating major epoxide **28Aa** with two equivalents potassium *tert*-butoxide in *tert*-butanol, but now at room temperature (entry 10). After nineteen hours of stirring, the results were extremely promising, because for the first time a complete conversion of the starting material to one single product was observed without any significant impurities present, as determined by LC-MS analysis. Purification by means of column chromatography on silica gel afforded this product, later confirmed as the desired 3,4-pyrrolidine-fused bicyclic β-lactam **30Aa**, in 28 % yield.

Table 6: Overview of the reaction conditions, *viz.* scale, diastereomeric ratio, choice and amount of base and additives, solvent, temperature, reaction time and yield, of the cyclisation of *cis*-1-(4-methoxybenzyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28Aa**/**28Ba** (R^1 = PMB, R^2 = Bn) towards 4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **30a**. The entries are ordered by ascending base strength.

	Scale [mmol]	*dr*[a] = **28Aa**/**28Ba**	Base, additives	Conditions	Results[b]
1[c]	0.07	100/0	1) pyridine (cat.)	EtOH, Δ, 3 h	No reaction
			2) 25 equiv. KOH	EtOH, Δ, 1 h	Decomposition
2[c]	0.04	67/33	3 equiv. K_2CO_3	CH_3CN, rt, 5 days	No reaction
3	0.05	61/39	3 equiv. Cs_2CO_3	dry CH_3CN, Ar, rt, 4 days	No reaction
				→ Δ, 16 h	Traces of **30Aa**[d]
				→ MW, 85 °C, 10 min	Traces of **30Aa**[e]
				→ MW, 85 °C, 20 min	Traces of **30Aa**[e]
4	0.04	100/0	4 equiv. Cs_2CO_3	dry CH_3CN, Ar, rt, 1 h	No reaction
				→ MW, 100 °C, 10 min	No reaction
5[c]	0.09	63/37	3.5 equiv. KOH	EtOH, rt, 3 h	Decomposition
6[c]	0.04	67/33	1.2 equiv. KOH	CH_3CN, rt, 5 days	Traces of **30Aa**[d]
7[c]	0.04	67/33	2 equiv. KOH	CH_3CN, rt, 5 days	No reaction
8[c]	0.04	67/33	3 equiv. KOH	CH_3CN, Δ, 7 h	No reaction
9	0.05	50/50	2 equiv. KO*t*Bu	*t*BuOH, Δ, 2 h	Traces of **30Aa**[d]
10	**0.23**	**100/0**	**2 equiv. KO*t*Bu**	***t*BuOH, rt, 19 h**	**30Aa in 28 % yield[f]**
11	0.06	100/0	2 equiv. NaH	dry THF, Ar, rt, 4 h	No reaction
				→ Δ, 19 h	No reaction
12[c]	0.07	67/33	1 equiv. LDA	dry THF, Ar, rt, 6 days	No reaction
13[c]	0.12	100/0	1) 1 equiv. LDA	dry THF, Ar, Δ, 1.5 h	Traces of **30Aa**[d]
			2) 1.5 equiv. LDA	dry THF, Ar, Δ, 1 h	Formation of **30Aa**[g]
14[c]	0.05	67/33	2 equiv. *t*BuLi	dry THF, Ar, rt, 4 days	No reaction
15[c]	0.08	67/33	3 equiv. *t*BuLi	dry THF, Ar, Δ, 2 h	Traces of **30Aa**[d]
				→ Δ, 1 h	Decomposition
16	0.05	67/33	3 equiv. *t*BuLi, 1 equiv. HMPA	dry THF, Ar, rt, 3 h	Traces of **30Aa**[d]
				→ Δ, 1 day	Traces of **30Aa**[h]
17	0.01	100/0	3 equiv. *t*BuLi, 1 equiv. HMPA	dry THF, Ar, Δ, 1 day	Traces of **30Aa**[d]
18	0.05	67/33	3 equiv. *t*BuLi, 1 equiv. HMPA	dry THF, Ar, Δ, 1 day	Traces of **30Aa**[d]
19[c]	0.03	67/33	3 equiv. *t*BuLi, 1 equiv. HMPA, 1 equiv. $BF_3 \cdot Et_2O$	dry THF, Ar, Δ, 3 h	Traces of **30Aa**[d]

[a] The diastereomeric ratio of the starting material, as determined by ^{1}H NMR analysis (in $CDCl_3$).

[b] As determined by ^{1}H NMR analysis (in $CDCl_3$) and/or LC-MS analysis.

[c] Performed during preliminary research.

[d] Appearance of a new LC-MS signal with the expected m/z value, later confirmed to be 3,4-pyrrolidine-fused β-lactam **30Aa**. Low conversion and presence of many impurities during LC-MS analysis and/or ^{1}H NMR analysis (in $CDCl_3$).

[e] No quantitative, nor qualitative change in LC-MS signals occurred, compared to before applying these conditions.

[f] Clean conversion towards the desired LC-MS signal, later confirmed to be 3,4-pyrrolidine-fused β-lactam **30Aa**. Purification *via* column chromatography on silica gel (petroleum ether/ethyl acetate 1/1).

[g] Appearance of a new LC-MS signal with the expected m/z value, later confirmed to be 3,4-pyrrolidine-fused β-lactam **30Aa**. LC-MS analysis of the crude product after work-up gave a chromatographic spectrum, which solely contained that single signal.

[h] The new LC-MS signal with the expected m/z value of compound **30Aa**, that appeared after applying prior conditions, did increase in intensity. Only the major epoxide diastereomer **28Aa** converted; minor epoxide **28Ba** did not react.

However, initial structural elucidation of the compound proved difficult, since the ^{1}H NMR spectrum (in $CDCl_3$) gave a larger amount of signals than expected. The fact that LC-MS analysis showed that the epoxide had been transformed into a compound with the same m/z value, for which next to the desired ring closure no immediate other reactions could be responsible, additionally strengthened the belief in having found successful cyclisation conditions. Furthermore, the sudden appearance of signals in the 60-70 ppm region during ^{13}C NMR analysis (in $CDCl_3$), in combination with a broad infrared absorption band at 3374 cm^{-1}, were obvious indications of a hydroxyl functionality being present in the molecule. An interesting observation was that the signals in the ^{1}H NMR spectrum (in $CDCl_3$) all came in "doubles" with an integration ratio of 62/48, making it look like two highly similar compounds were present, even though LC-MS analysis clearly suggested that one compound was isolated by chromatography. Since only the major epoxide **28Aa** was present in the starting material (dr = 1/0), only the major bicyclic product **30Aa** could have formed, unless interconversion of the epoxide diastereomers had taken place. Yet, interconversion is unlikely to be the cause for the observations as the data in previous epoxidation step suggested that major diastereomers **28A** are favoured by thermodynamics. With the exception of deuterated dimethyl sulphoxide, the compound was found to readily crystallise in deuterated chloroform, deuterated acetonitrile and deuterated methanol. Recovery of the white, needle-shaped crystals from latter solvent allowed structure determination *via* X-ray diffraction. The single crystal X-ray analysis was performed by prof. K. Van Hecke (XStruct, Department of Inorganic and Physical Chemistry, Faculty of Sciences, Ghent University) and delivered irrefutable proof that indeed (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 **30Aa** had been synthesised successfully by above procedure (**Figure 5**). Furthermore, the compound was confirmed to be racemic and it was found that a hydrogen bond network is formed between the C4-attached hydroxyl group and the N2-connected acyl group's carbonyl, yielding chains of molecules in the b direction of the monoclinic crystal.

Figure 5: Single crystal X-ray diffraction 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 **30Aa**.

The integration problem of the "double" signals in all recorded NMR spectra (in $CDCl_3$, CD_3OD, CD_3CN and d_6-DMSO) was finally attributed to the presence of two rotamers within compound **30Aa**. Most probably, the bond between nitrogen N2 and the acyl chain is responsible as it has partial double bond character, due to the PCMO between nitrogen and the carbonyl. The literature entry by Hu *et al.* stresses the problematic NMR analysis of an analyte solution, which contains rotamers, but also might contain diastereomers, which is the case in this Master's thesis.[192] Traditional techniques to differentiate equilibrating species, *e.g.* rotamers, from normally non-equilibrating diastereomers are variable temperature NMR experiments, switching to another NMR solvent, or emloying a complexing agent. However, a way easier and often overlooked solution to simply determine whether rotamers are present in the NMR spectrum, regardless of the presence of diastereomers, is chemical exchange NMR spectroscopy (EXSY). In essence, performing a nuclear Overhauser effect (NOE) experiment should confirm rotamers being present or not, since normal NOE enhancements will appear in the opposite phase from the irradiated peaks, while signals due to *e.g.* rotameric chemical exchange should appear in the same phase.[192,193] Indeed, EXSY analysis *via* a 2D NOESY experiment (in CD_3OD) of compound **30Aa** resulted in same phase cross peaks between the "double" signals, meaning that prior integration problem actually represented the ratio between both rotamers. Interestingly, in d_6-DMSO the chemical exchange between the two rotamers was less visible, but the exchange between the hydroxyl group's proton and the signal of residual water present in the hygroscopic deuterated solvent was. Furthermore, some variable temperature NMR experiments were performed in an attempt to find the point of coalescence of the rotamer signals, *i.e.* achieving fast exchange on the saturation time scale. It was found that 80 °C, *i.e.* the maximum temperature which the spinners allowed, was not a high enough temperature for coalescence to occur as only broadening of the signals was observed. The ratio between both rotamers did change however, *e.g.* 67/33 at 25 °C and 64/36 at 50 °C (in d_6-DMSO), which is in agreement with the expectation that a higher amount of available thermal energy would result in a larger fraction of the molecules in the energetically less favourable rotamer state.

In order to find robust cyclisation conditions, slight alterations of the most promising hit during screening of bases, *i.e.* the use of potassium *tert*-butoxide in *tert*-butanol (**Table 6**: entry 10, or **Table 7**: entry 1), were tested on all formed epoxides **28**. The goal was finding that combination of conditions, *viz.* solvent, equivalents of base added, and temperature, for which consistently: (i) a clean and complete conversion towards bicyclic products **30** is readily achieved; (ii) no practical problems during the course of the reaction and work-up are encountered. **Table 7** gives an overview of these optimisation experiments. One of the practical drawbacks of employing *tert*-butanol is its water miscibility, hereby resulting in a tedious work-up procedure, which involves addition of dichloromethane and multiple washes with water to remove all the *tert*-butanol that equilibrates between the organic and aqueous phase. Initial optimisation trials therefore assessed the influence of different solvents, which would allow facile aqueous work-ups, on the reaction rate and yield of the potassium *tert*-butoxide-mediated cyclisation. Working in anhydrous tetrahydrofuran at room temperature resulted in formation of *C*-fused β-lactam **30Aa**, but at a significantly slower pace, with conversion remaining unchanged after one day (entry 2). Since addition of one extra equivalent base and one more day of stirring was of little influence, a drastic four equivalents potassium *tert*-butoxide were added, but this resulted over weekend in complete decomposition of both starting material **28Aa** and product **30Aa**. When above experiment was tried with major *N*-acetylated *cis*-4-oxiranylazetidin-2-one **28Ab**, no reaction was observed at all, until so much base had been added (eight equivalents) that partial decomposition occurred (entry 10). With tetrahydrofuran out of the question, next, using dichloromethane was attempted. However, treatment of epoxides **28a** with three equivalents of potassium *tert*-butoxide in dichloromethane at room temperature led to full recovery of the starting material, even after six days of stirring (entry 3). It was proven that these epoxides **28a** were not inactivated in any way, since performing the reaction on the recovered starting material in *tert*-butanol resulted in formation of the desired LC-MS signal of major bicyclic product **30Aa**. In the end, it was concluded that alleviating the work-up problem of *tert*-butanol's water miscibility should not be achieved by altering the solvent to tetrahydrofuran or dichloromethane, as this switch had a profound negative effect on the cyclisation. Additionally, it was discovered that the work-up of *C*-fused β-lactams **30** suffered from emulsion formation. Brine did not break these emulsions, but starting the work-up with an initial wash using a hydrogen chloride solution (1 M), and afterwards employing tepid water (40 °C) for the other washes, proved to significantly diminish the emulsion problem.

A second practical problem, that was encountered with *tert*-butanol as a solvent, concerns the poor solubility of epoxides **28** in it. Changing the solvent to acetonitrile allowed rapid dissolution of the starting materials **28a**, but it resulted in a complex LC-MS spectrum during follow-up of the reaction, in which throughout the six to eight days of reacting the diastereomeric ratio altered in favour of the minor epoxide (**Table 7**: entries 4 and 11). ^{1}H NMR analysis (in $CDCl_3$) after work-up revealed that deterioration of the starting material is most probably to blame. Above experiments led to the conclusion that the combination of the correct base and solvent, *i.e.* potassium *tert*-butoxide in *tert*-butanol, is key during the desired intramolecular pyrrolidine ring closure. In that respect, trials were performed, in which a solvent mixture of *tert*-butanol and acetonitrile in 4/1 ratio was employed, hereby attempting to achieve both a clean and complete cyclisation as well as facile dissolution of the epoxides. It proved indeed successful in tackling the solubility problem and did result in the formation of major bicyclic β-lactams **30A**, but the mixture gave less clean and less reproducible reactions (entries 5, 12 and 15). For example, in the case of 4-methoxyphenyl-substituted β-lactam **28Ac** (R^1 = PMP, R^2 = Bn), usage of the 4/1 *tert*-butanol/acetonitrile mixture resulted in rapid formation of the expected signal of bicyclic product **30Ac** after only thirty minutes, as determined by LC-MS analysis, but after two hours and more, a lot of impurities started to appear (entry 15). The fact that a side product with a mass increase of eighteen dalton was found, raised the question whether degradation of the epoxide **28Ac** towards *anti*-vicinal diols **220c** is a side reaction in this synthesis step as well. Because of the less desirable results, it was decided to abandon the solvent mixture approach. In the end, it appeared that making sure the starting materials **28** were completely covered by the solvent at the beginning of the reaction was all one had to do, as the formed 2,6-diazabicyclo[3.2.0]heptan-7-ones **30** dissolved just fine in *tert*-butanol.

Table 7: Overview of the reaction conditions, *viz.* scale, diastereomeric ratio, amount of potassium *tert*-butoxide base, solvent, temperature, reaction time and yield, for various optimisation experiments, concerning the cyclisation of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28** towards 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **30**.

	Scale [mmol]	dr[a] = **28A**/**28B**	KO*t*Bu	Conditions	Results[b]
R^1 = PMB, R^2 = Bn					(Starting material: 4-oxiranyl-β-lactams **28a**)
1	**0.23**	**100/0**	**2 equiv.**	***t*BuOH, rt, 19 h**	**30Aa in 28 % yield[c,d]**
2	0.89	100/0	1) 2 equiv.	dry THF, Ar, rt, 32 h	Incomplete conversion
			2) 1 equiv.	dry THF, Ar, rt, 2 days	Incomplete conversion
			3) 4 equiv.	dry THF, Ar, rt, 3 days	Decomposition
3	0.15	20/80	1) 3 equiv.	CH_2Cl_2, rt, 6 days	No reaction
			2) -	*t*BuOH, rt, 1.5 h	No reaction
			3) 3 equiv.	*t*BuOH, rt, 4.5 h	Formation of **30Aa**[e]
4	0.04	77/23	3 equiv.	CH_3CN, rt, 8 days	Decomposition
5	0.18	59/41	3 equiv.	1) *t*BuOH/CH_3CN (4/1), 35 °C, 4 days	Formation of **30Aa**[f]
				2) *t*BuOH/CH_3CN (4/1), Δ, 6 h	Decomposition[g]
6	0.13	100/0	1) 2 equiv.	*t*BuOH, 35 °C, 2 days	Incomplete conversion
			2) 1 equiv.	*t*BuOH, 35 °C, 1 day	Incomplete conversion
			3) 1 equiv.	***t*BuOH, 35 °C, 6 days**	**30Aa in 14 % yield[c,h]**
7	**0.40**	**86/14**	**3 equiv.**	***t*BuOH, rt, 31 h**	**30Aa in 13 % yield[c,i]**
8	**0.42**	**95/5**	**3 equiv.**	***t*BuOH, rt, 17 h**	**30Aa in 34 % yield[h]**
9	1.52	59/41	3 equiv.	*t*BuOH, 35 °C, 20 h	Formation of **30Aa**[j]
R^1 = PMB, R^2 = Me					(Starting material: 4-oxiranyl-β-lactams **28b**)
10	0.08	100/0	1) 2 equiv.	dry THF, Ar, rt, 1 day	No reaction
			2) 2 equiv.	dry THF, Ar, rt, 2 days	No reaction
			3) 4 equiv.	dry THF, Ar, rt, 3 days	Partial decomposition
11	0.04	100/0	2 equiv.	CH_3CN, rt, 6 days	Decomposition
12	**0.47**	**100/0**	**3 equiv.**	***t*BuOH/CH_3CN (4/1), 35 °C, 6 h**	**30Ab in 6 % yield[c,h]**
13	0.05	100/0	2 equiv.	*t*BuOH, rt, 6 days	Incomplete conversion
14	**1.84**	**57/43**	**3 equiv.**	***t*BuOH, 35 °C, 20 h**	**30Ab in 18 % yield[h,k]**
R^1 = PMP, R^2 = Bn					(Starting material: 4-oxiranyl-β-lactams **28c**)
15	0.77	100/0	3 equiv.	*t*BuOH/CH_3CN (4/1), 35 °C, 19 h	Decomposition[l]
16	**0.05**	**100/0**	**3 equiv.**	***t*BuOH, 35 °C, 2 h**	**Formation of 30Ac[m]**
17	0.77	100/0	1) 3 equiv.	*t*BuOH, 35 °C, 2 days	Incomplete conversion
			2) 2 equiv.	*t*BuOH, 35 °C, 3 days	Decomposition[n]

[a] The diastereomeric ratio of the starting material, as determined by ^{1}H NMR analysis (in $CDCl_3$), or by LC-MS analysis in the absence of the former.

[b] As determined by ^{1}H NMR analysis (in $CDCl_3$) and/or LC-MS analysis.

[c] Contamination of starting materials **28** with hydrogenated side products **216**, but this was taken into account during calculation of the yield.

[d] Purification *via* column chromatography on silica gel (petroleum ether/ethyl acetate 1/1).

[e] Full disappearance of starting material's LC-MS signals, both major **28Aa** and minor epoxide **28Ba**, though only formation of major *C*-fused β-lactam **30Aa** was observed. No work-up, nor purification was performed to isolate this bicyclic compound.

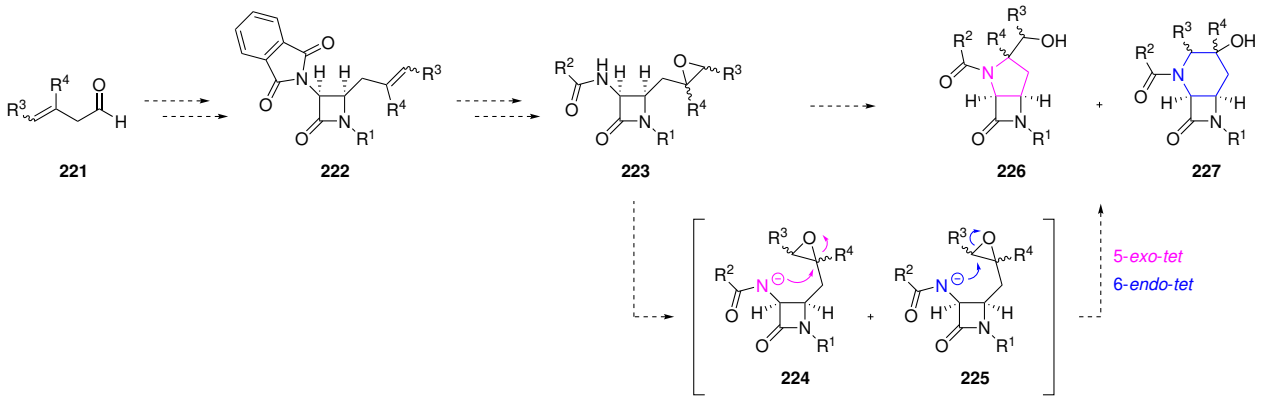
[f] Full conversion of major epoxide **28Aa** towards *C*-fused β-lactam **30Aa**, but also recovery and no obvious deterioration of minor epoxide **28Ba**, as determined by LC-MS analysis.
[g] Most probably complete decomposition of minor epoxide **28Ba**, and substantial decomposition major bicyclic β-lactam **30Aa**.
[h] Purification *via* reversed phase automated column chromatography (C18, gradient H_2O/CH_3CN 100/0-0/100).
[i] Purification *via* normal phase automated column chromatography (SiO_2, gradient petroleum ether/ethyl acetate 50/50-0/100).
[j] Formation of only major 3,4-pyrrolidine-fused bicyclic β-lactam **30Aa**, as determined by LC-MS analysis. Decomposition of this bicyclic product probably occurred during work-up.
[k] Full disappearance of the starting material's LC-MS and ^{1}H NMR (in $CDCl_3$) signals, for both major **28Ab** (fast) and minor epoxide **28Bb** (slow), though in the end only formation of major 3,4-pyrrolidine-fused bicyclic β-lactam **30Ab** was observed and isolated. Clean reaction; almost no side products formed.
[l] Initial clean formation of major 3,4-pyrrolidine-fused bicyclic β-lactam **30Ac**, as determined by LC-MS analysis. However, after two hours of reaction, decomposition of product **30Ac** seemed to commence, yielding a complex crude product of which the ^{1}H NMR spectrum (in $CDCl_3$) contained many impurities, among which most probably *anti*-vicinal diols **220c**.
[m] Formation of major 3,4-pyrrolidine-fused bicyclic β-lactam **30Ac**, as determined by LC-MS analysis. Clean reaction; almost no other LC-MS signals appear. However, work-up of the small amount of product failed.
[n] Loss of LC-MS signal of major 3,4-pyrrolidine-fused bicyclic β-lactam **30Ac**, and most probably formation of *anti*-vicinal diols **220c**. In addition, samples were too diluted, since too much solvent was used during this experiment.

Now aware that the base, nor the solvent of the successful screening conditions (**Table 7**: entry 1) should be changed, their reproducibility was tested in combination with an assessment of the number of equivalents potassium *tert*-butoxide, which needs to be added in order to achieve full conversion. Minimising this number is attractive on both an economical and ecological level. It was shown that two equivalents potassium *tert*-butoxide were not enough in order to drive the reaction to completion (entries 6 and 13). To that end, two additional experiments were performed in *tert*-butanol at room temperature, though in the presence of once again three equivalents potassium *tert*-butoxide instead of two (entries 7 and 8). The results were auspicious, giving a clean reaction with no notable formation of side products, and affording major 2,6-diazabicyclo[3.2.0]heptan-7-one **30Aa** in 13 % (entry 7) and 34 % yield (entry 8) after purification *via* respectively normal phase (SiO_2) and reversed phase (C18) automated column chromatography. Lastly, also the assessment of a third parameter, *i.e.* the reaction temperature, was performed. The reason lies in the freezing point of *tert*-butanol, which is around room temperature (23-26 °C), and which complicated some of above reactions as the solvent had partially frozen overnight. In that respect, the cyclisation was attempted at an elevated temperature (35 °C), and fortunately, this higher reaction temperature was of no noticeable negative effect to the results of the intramolecular ring closure reaction (entries 5, 6, 9, 12, 14, 16 and 17).

Above optimisation experiments (**Table 7**) lead us to the conclusion that robust cyclisation conditions have been found. Preparation of 3,4-pyrrolidine-fused bicyclic β-lactams **30A** from major *cis*-4-oxiranylazetidin-2-ones **28A** requires reaction with three equivalents of potassium *tert*-butoxide in *tert*-butanol at 35 °C for several hours. No addition of any other reagents, such as a Lewis acid to activate the oxirane ring, is needed. Nevertheless, it is quite peculiar that this specific base-solvent combination exclusively delivered a clean reaction towards the desired bicyclic products. Employing other bases only resulted in a lot of impurities and insignificant amounts of the desired bicyclic products **30** being formed, while working in different solvents made potassium *tert*-butoxide give less reproducible reactions, resulted in decomposition of the starting material, or in no reaction occurring at all. It should be noted that only major PMB-substituted 2,6-diazabicyclo[3.2.0]heptan-7-ones **30Aa** and **30Ab** could be isolated and characterised within the time frame of this Master's thesis. The small scale test reaction on PMP-substituted compound **28Ac** in just *tert*-butanol was promising, since it showed clear and clean conversion towards the expected bicyclic product's LC-MS signal, but no product **30Ac** could be recovered after work-up (entry 16). A reaction on larger scale failed, because too much solvent was added to allow a clear follow-up of the reaction, and because the second portion of base added (two equivalents) probably resulted in decomposition of all material present in the reaction mixture (entry 17). Once again, side products with a mass increase of eighteen dalton were found, suggesting the formation of *anti*-vicinal diols **220c**. It should also be noted that, from the moment that *C*-fused β-lactams **30Aa** and **30Ab** were characterised, re-examination of the starting epoxides' LC-MS spectra showed that often trace amounts of the major 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **30A** had spontaneously formed during the epoxidation step.

Lastly, very interesting observations were made in the experiments, in which a mixture of major and minor 4-oxiranylazetidin-2-one diastereomers **28A** and **28B** were submitted to the cyclisation conditions. In some cases, the major epoxides **28A** had completely cyclised after reacting for a few hours, but all minor starting materials **28B** were still present, as determined during follow-up of the reaction (**Table 7**: entry 5) or after work-up (entry 8). It is however known from the preliminary research, conducted at the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University), that cyclisation of minor 3,4-oxolane-fused bicyclic β-lactam **64** needed a twentyfold increase in reaction time, due to steric hindrance exerted by the phenyl group at carbon C3 in intermediate **63**.[64,65] In one of this work's test reactions, three additional days of stirring the reaction mixture at room temperature still gave no result, yielding a mixture of the major cyclised compound **30Aa** and the minor epoxide **28Ba** (entry 5). It was decided to check whether elevating the temperature might induce cyclisation, but deterioration of both structures was the only observation after six hours under reflux conditions. Even more interesting are the other cases, in which full conversion of a starting mixture of epoxide diastereomers **28A**/**28B** was achieved, but only the major bicyclic β-lactams **30A** were recovered and no minor epoxides **28B**, nor minor bicyclic azetidin-2-ones **30B** were found (entries 7, 9 and 14). The follow-up during these experiments revealed a fast conversion of the major 4-oxiranylazetidin-2-ones **28A** and a slow disappearance of the minors' **28B** signal, as determined by LC-MS analysis. Nevertheless, as stated *in supra*, LC-MS and ^{1}H NMR analysis (in $CDCl_3$) of the crude reaction product after work-up did not detect any signals belonging to the minor bicyclic product **30B** and, in addition, no minor starting material **28B** was recovered at all. Furthermore, no obvious degradation of these minor epoxides **28B** had taken place: major 2,6-diazabicyclo[3.2.0]heptan-7-ones **30A** were the sole products recovered (entry 14: purity $>$ 90 %, as determined by ^{1}H NMR analysis in $CDCl_3$). Above observations could support the suggested phenomenon of epoxide diastereomer interconversion, which was discussed in the previous section. If true, it would mean that the major diastereomers **28A** have both the property of being the thermodynamically most stable epoxide diastereomers, as well as converting much more readily to ring closed products **30A**, consequently resulting in a starting mixture of both epoxides **28A**/**28B** solely yielding major cyclised structures **30A**. However, there is not enough empirical data to make clear statements about the formation and reactivity of minor 2,6-diazabicyclo[3.2.0]heptan-7-ones **30B**. To clarify, in none of the reactions, that were performed in this Master's thesis, construction of these minor 3,4-pyrrolidine-fused bicyclic β-lactams **30B** was unambiguously observed.

Even though the attempted 5-*endo*-epoxy ring opening is thermodynamically preferred to its 4-*exo* alternative, it still has the problem of inefficient orbital overlap, as stated by Baldwin.[95] In research by Schultz and co-workers, directly after its epoxidation with *m*CPBA, a bicyclic epoxyamide ring structure closed with unexpected ease towards an aza-adamantol without the need of adding any base.[191] Apparently, the close proximity of the amide nitrogen to the epoxide-bearing ring carbon resulted in the facile cyclisation. This literature entry proves that, even with its reduced nucleophilicity, an acylamino functionality can still perform the desired S_N2-type oxirane ring opening. Furthermore, the fact that in this Master's thesis the amido group-induced cyclisation did not proceed more readily for *N*-acetylated epoxides **28b** (R^1 = PMB, R^2 = Me) demonstrates that steric hindrance exerted by the 3-acylamino substituent (R^2) also is not the main problem. Therefore, it is possible that Baldwin's rules, or rather them not being followed in this work, are responsible for the difficulty with which the *C*-fused pyrrolidine cycle is created. In order to facilitate the preparation of 3,4-pyrrolidine-fused bicyclic azetidin-2-ones *via* oxirane ring opening in future experiments, it is advised to attempt a 5-*exo-tet* cyclisation by starting the synthesis route from β,γ-unsaturated aldehydes **221** instead, *e.g.* the commercially available pent-3-enal (R^3 = Me, R^4 = H). After incorporated in a β-lactam core and being epoxidised towards compounds **223**, the oxirane moiety will open to the other side during cyclisation, which is favoured orbital overlap-wise and which will afford 2,6-diazabicyclo[3.2.0]heptan-7-ones **226** with the hydroxyl group now not tethered to the pyrrolidine cycle. It should however be noted that competition with a 6-*endo-tet* reaction *via* intermediates **225** is possible, yielding 3,4-piperidine-fused bicyclic β-lactams **227**. The formation of latter molecules is not preferred, according to Baldwin's rules, but they do construct thermodynamically favoured six-membered rings, and it is known from the literature that both these reactions can simultaneously take place.[190]



In retrospect, the goal of this section, *i.e.* finding robust cyclisation conditions, which allow the construction of 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **30** *via* an unprecedented intramolecular 5-*endo-tet* amide-epoxide ring closure of *cis*-3-acylamino-4-oxiranyl-β-lactams **28** for varying substituents (R^1 and R^2), is achieved. It appears that using potassium *tert*-butoxide in *tert*-butanol is the key. No cyclisation of any minor epoxides **28B** was unambiguously observed in this Master's thesis. The fact that minor epoxides **28B** do not cyclise readily, is probably due to steric hindrance exerted by the phenyl group at carbon C3 in intermediates **29B**, and was expected based on the results of preliminary research.[64,65] However, the observation of a mixture of both epoxide diastereomers **28A**/**28B** achieving full conversion, but with only the major 3,4-annulated bicyclic β-lactam **30A** recovered, was surprising and should be further investigated. It forms however an additional argument for the suspected interconversion between 4-oxiranyl-β-lactam majors **28A** and minors **28B**. The practical problems of employing *tert*-butanol as a solvent, *e.g.* freezing at room temperature and doing a poor job at dissolving epoxides **28**, could be overcome by respectively working at 35 °C and by making sure all starting material is covered by the solvent as the formed 2,6-diazabicyclo[3.2.0]heptan-7-ones **30** dissolve just fine. However, nothing could be done about its water miscibility and the cumbersome work-up procedure this induces. The literature, respectively the results of this work suggest that not the reduced nucleophilicity of an acylated amine, nor the sterics of the N2-acylamino substituent (R^2) should necessarily be the main cause for this reaction step being difficult, but an inefficient orbital overlap could be responsible as well. It is believed that altering the reaction to a Baldwin-favoured 5-*exo*-epoxy ring opening, instead of the current 5-*endo* variant, might facilitate the cyclisation towards 3,4-pyrrolidine-fused bicyclic β-lactams **30**. Therefore, future research concerning the preparation of 2,6-diazabicyclo[3.2.0]heptan-7-ones **15** is advised to start the synthesis route from β,γ-unsaturated aldehydes **221**, which should be compatible with the optimised procedures of all discussed reaction steps thus far.

3.3 Functionalisation of 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]-heptan-7-ones

The third and last synthetic milestone of this Master's thesis concerns the further functionalisation of novel 2,6-diazabicyclo[3.2.0]heptan-7-ones **30** towards biologically more promising derivatives. It comprises the synthesis of 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **32** *via* a benzylic oxidation, or the preparation of 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33** *via* *N*-deprotection and subsequent sulphonation at the β-lactam nitrogen. These transformations of the previously electron-donating N6-substituents (R^1 = PMB, PMP) into electron-withdrawing functionalities (R^1 = PMBz, SO_3H) are important, since the former are preferable during Staudinger synthesis as they favour β-lactam formation,[66] while the latter are beneficial for the biological activity as they facilitate ring opening of the β-lactam core by *e.g.* β-lactamase enzymes.[67] In that respect, the current 4-methoxybenzyl (PMB) and 4-methoxyphenyl (PMP) protecting groups of newly synthesised major 3,4-pyrrolidine-fused bicyclic β-lactams **30** will be modified and/or cleaved off, for which some literature protocols already exist.[64,65,71,74,76,77,80–82,138,194,195]

Benzylic oxidation

In preliminary research, conducted at the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University), 3,4-oxolane-fused bicyclic azetidin-2-one **20** with a 4-methoxybenzoyl (PMBz) substituent at nitrogen N6 was synthesised.[64,65] As this imide compound has been shown in the preliminary research cited *in supra* to exhibit class C β-lactamase inhibitory activity, the construction of its aza-analogues, *i.e.* PMBz-substituted 3,4-pyrrolidine-fused bicyclic β-lactams **32**, is desired as their bioactivity could then be compared with compound **20**. It is clear from the literature that 4-methoxybenzyl and 4-methoxyphenyl are among the most popular moieties employed for protection of the β-lactam nitrogen.[77,194,196] Cleaving them off again to give *N*-unsubstituted azetidin-2-ones can be achieved in many different ways, such as Lewis or Brönsted acid-mediated hydrolysis,[197,198] catalytic hydrogenation,[199] and persulphate-mediated oxidative cleavage.[65,74,200,201] Yet, the most widely used method involves the use of cerium(IV) ammonium nitrate to oxidatively remove this *N*-PMB or *N*-PMP group. Advantages include the relatively low cost of CAN, the simplicity of the experimental procedure, and its tolerance towards the presence of divergent functional groups. In general, CAN is reported to cleanly cleave off both PMP and PMB protecting groups, solely affording *N*-unsubstituted β-lactams.[74,77–79,194–196] However, in some cases attempted CAN-mediated PMB-deprotection has suffered from side reactions, for example, one yielding *N*-hydroxymethyl substituents,[194] or a benzylic oxidation towards imide functionalities occurred.[65,74,75] Latter over-oxidation is desired in this section in order to obtain 6-(4-methoxybenzoyl)-substituted *C*-fused β-lactams **32**. Preliminary research has shown that enlarging the number of equivalents of CAN added, increased the ratio of imide to *N*-unsubstituted azetidin-2-one.[74]

To that end, and inspired by the protocol of the preliminary research *in supra*,[74] an ice-cooled (0 °C) solution in acetonitrile of major 3,4-pyrrolidine-fused bicyclic β-lactam **30Aa** (R^1 = PMB, R^2 = Bn) was treated with nine equivalents CAN in water, while ensuring the acetonitrile/water volume to volume ratio was 2/1. Stirring one hour at room temperature resulted in complete conversion of the starting material, as determined by LC-MS analysis, primarily affording 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **32Aa**, which was purified in 52 % yield by means of reversed phase automated column chromatography (C18). The same procedure was successfully applied to major 2,6-diazabicyclo[3.2.0]heptan-7-one **30Ab** (R^1 = PMB, R^2 = Me), which after purification provided imide **32Ab** in 16 % yield. **Table 8** gives an overview of the yields *inter alia* of above benzylic oxidations. The work-up methodology comprised evaporation of the acetonitrile and an extraction of the remaining aqueous phase with ethyl acetate. However, in the case of N2-acetylated compound **32Ab**, these extractions suffered from strong emulsion formation, hereby significantly lowering the yield of the crude product after work-up, as was noticed during a test reaction. Therefore, switching the extracting solvent to dichloromethane was attempted, combined with increasing the number of extractions from two to five, but after work-up crude product **30Ab** was still only recovered in a moderate yield of 51 % (entry 2).

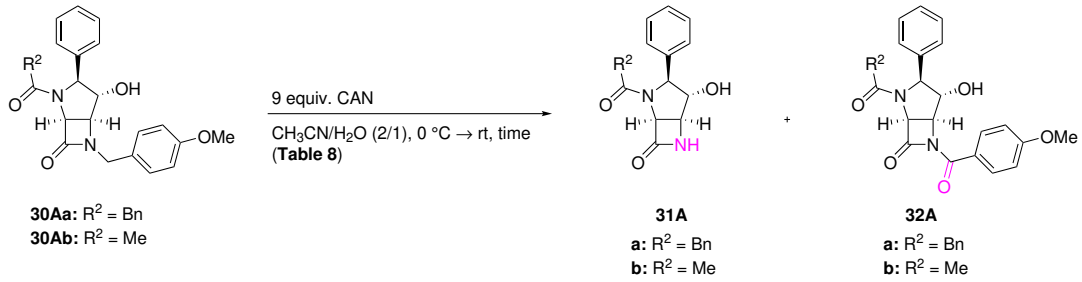


Table 8: Substitution pattern, scale, reaction time and yield for the PMB-deprotection and/or benzylic oxidation at N6 of 6-(4-methoxybenzyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **30Aa** and **30Ab** towards N6-unsubstituted 2,6-diazabicyclo[3.2.0]heptan-7-ones **31Aa** and **31Ab**, and/or 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **32Aa** and **32Ab**.

	R^1	R^2	Scale [mmol]	Time	**31A**+**32A**[a] [%]	**31A**[b] [%]	**32A**[b] [%]
1	PMB	Bn	**30Aa**: 0.07	1 h	92[c]	7[d]	52
2	PMB	Me	**30Ab**: 0.20	30 min	51[c,e]	-	16

[a] Based on the crude reaction product after work-up. For the calculation, only the molecular weight of imide compounds **32A** is used, since they form by far the major products.

[b] After purification *via* reversed phase automated column chromatography (C18, gradient H_2O/CH_3CN 90/10-0/100 (entry 1) or 100/0-0/100 (entry 2)).

[c] Both N6-unsubstituted **31A** and imide compounds **32A** were formed during the reaction, as determined by LC-MS analysis, with imides **32A** as the major products.

[d] Due to the low amount of purified product (1.5 mg), no full characterisation could be performed. However, the available data strongly hints that this is N6-unsubstituted compound **31Aa**.

[e] N6-unsubstituted compound **31Ab** was not present anymore in the crude reaction product after work-up, as determined by LC-MS analysis.

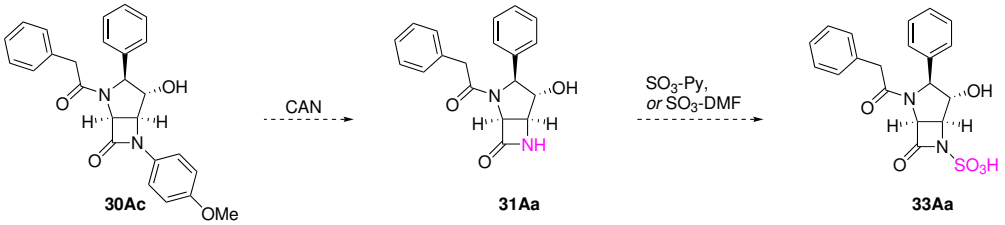
Structural elucidation of 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **32Aa** and **32Ab** proved no problem with the spectral insights, that were acquired during characterisation of 3,4-pyrrolidine-fused bicyclic β-lactams **30**. Investigation of the ^{1}H NMR spectrum (in $CDCl_3$ and/or d_6-DMSO), which showed great similarity with that of unoxidised compounds **30**, revealed that a solution of 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **32** also has two rotamers present. The chemical exchange between the rotamers' equivalent proton signals was confirmed *via* EXSY, for which the necessary 2D NOESY experiment could be recorded in deuterated chloroform. Variable temperature NMR experiments were performed for imides **32** as well, but as with structures **30**, a temperature of 80 °C proved insufficiently high to achieve fast exchange on the saturation time scale, *i.e.* witness coalescence of both rotamers' signals.

Even though a large excess of CAN reagent was employed during above reactions, hereby primarily yielding over-oxidised products **32** as expected, follow-up of the conversion by LC-MS analysis suggested that still a small amount of N6-unsubstituted bicyclic β-lactams **31** was formed. These suspicions were strengthened by the sudden presence of an aldehyde signal at 9.88 ppm in the ^{1}H NMR spectrum (in $CDCl_3$) of the crude reaction product after work-up, which would originate from the cleaved off PMB-substituent as upon interaction with CAN and water the methylene group gets oxidised to an aldehyde functionality.[194,196] The suspected N6-unsubstituted product **31Ab** (R^2 = Me) was lost during the *in supra* mentioned tedious work-up procedure (**Table 8**: entry 2), as determined by LC-MS analysis. However, compound **31Aa** (R^2 = Bn) was still present after work-up (entry 1), and could be purified in 7 % yield *via* reversed phase automated column chromatography (C18). Due to the low amount of product (1.5 mg), no full characterisation could be achieved. Nonetheless, a ^{1}H NMR spectrum (in $CDCl_3$) was recorded, albeit with a low signal-to-noise ratio, in which the high level of similarity with the spectra of confirmed 3,4-pyrrolidine-fused bicyclic β-lactams **30Aa** and **32Aa** strongly hinted towards the formation of compound **31Aa**. The NMR data, combined with the presence of an aldehyde functionality and the correct m/z value during LC-MS analysis, leave little doubt that indeed PMB-deprotected compound **31Aa** was formed and isolated.

The formation of PMP-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **30Ac** *via* intramolecular ring closure was proven by LC-MS analysis during previous reaction step, but within the time frame of this Master's thesis it could not be isolated and characterised. Therefore, no CAN-mediated deprotection of β-lactam **30Ac** was tested, which according to literature data should result in a full conversion towards the N6-unsubstituted bicyclic β-lactam **31Aa** (R^2 = Bn) with the PMP group cleaved off as 1,4-benzoquinone.[74,77–79,195,196] In light of their importance as intermediates towards 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33**, which are the ultimate target compounds of this Master's thesis, future experiments should focus on obtaining N6-unsubstituted 2,6-diazabicyclo[3.2.0]heptan-7-ones **31** in good yields.

Sulphonation

Above benzylic oxidation is interesting, considering its link with preliminary research and the opportunity it gives to compare the bioactivity of the 6-(4-methoxybenzoyl)-substituted bicyclic β-lactams from both works, *i.e.* 3,4-oxolane-fused **20** and 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **32**. Nonetheless, the most promising target molecules to possibly exhibit potent and selective class C β-lactamase inhibitory activity remain 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33**. The chapter "Literature overview" illustrated the biological importance of a sulphonic acid moiety, which becomes a sulphonate group *in vivo*, through its formation of a salt bridge with the enzyme and its crucial ability to displace the deacylating water molecule to a position in the catalytic site, where it cannot be activated (section 2.4).[71–73] Introduction of this sulphonic acid functionality at nitrogen N6 of 3,4-pyrrolidine-fused bicyclic β-lactams **30** was in the envisioned synthesis pathway proposed to be achieved through N6-deprotection, affording compounds **31**, which are then to be sulphonated *via* a literature-based protocol employing, for example, sulphur trioxide pyridine,[80–82] or sulphur trioxide *N,N*-dimethyl formamide.[71,81,202] Since complete deprotection of PMP-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **30Ac** is expected upon reaction with CAN, as was stated *in supra*, this compound is preferred as a precursor over PMB-protected bicyclic β-lactams **30Aa** and **30Ab**, because latter structures easily over-oxidise towards imides **32**. However, as explained in the "Preamble", this Master's thesis' lab work was ceased early due to the COVID-19 pandemic. The resulting reduced time frame did not permit isolation of N6-deprotected β-lactam **31Aa** in a significant amount and, therefore, no sulphonation towards compound **33Aa** could be attempted.



3.4 Future work and Perspectives

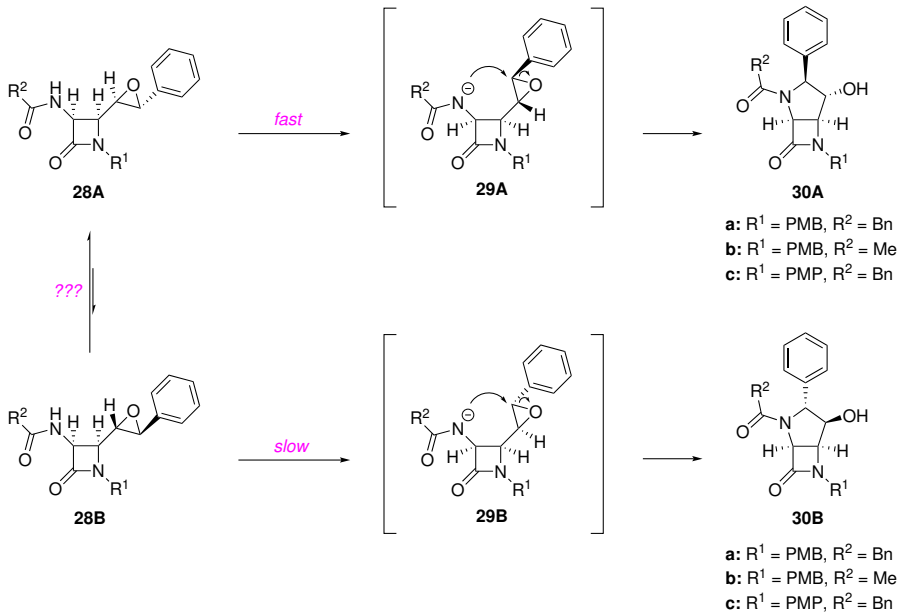
In this Master's thesis, ultimately nineteen β-lactam compounds were synthesised and isolated along the course of an eight-step reaction pathway. With the exception of compounds ***cis*-25b** and **26b**, these structures were not yet described in the literature. As clarified in the "Preamble", due to the COVID-19 pandemic, lab work was ceased one month earlier than expected, and therefore some of the goals set for this Master's thesis could not be achieved. One consequence is that not all seventeen novel β-lactam compounds, that were synthesised and isolated, could be fully characterised with all required physical and spectral data. What data is missing for which compounds will be mentioned at a later point (section 6.3). This section will elaborate which experiments seem worthwhile to further investigate in order to clarify their outcome, but also explain the relevance of certain derivatisations of 3,4-pyrrolidine-fused bicyclic β-lactams **30** as well as what their proposed synthetic procedures for future research are.

3.4.1 Interconversion and cyclisation of *cis*-4-oxiranylazetidin-2-one diastereomers

Additional experiments should be performed in order to further explore the outcome of following reactions and the phenomena that are suspected to be responsible: (i) the interconversion of *cis*-4-oxiranylazetidin-2-one diastereomers **28A**/**28B**; (ii) the intramolecular ring closure of the minor epoxides **28B** towards 2,6-diazabicyclo[3.2.0]heptan-7-ones **30B**.

Some results, acquired during this Master's thesis' epoxidation reactions, suggest an interconversion between major **28A** and minor epoxide diastereomers **28B** might take place, meaning a favoured formation of the probably thermodynamically more stable major diastereomers **28A**. For example, reactions were observed with an initial diastereomer formation in 1/1 ratio, but they were recovered in higher ratios (dr = 3/2-6/1) after a couple days of reacting at room temperature. Reaction under reflux conditions also resulted after one day in a change of the diastereomeric ratio, but the fact that deterioration of especially the minor epoxides **28B** was observed as well, complicated the interpretation. It should be noted that both phenomena, *i.e.* interconversion and decomposition, could well take place at the same time. A follow-up experiment by ^{1}H NMR spectroscopy (in $CDCl_3$) gave indications of the suspected interconversion (**Table 5**). Only major crude epoxide **28Ac** was recovered after work-up in a high yield of 88 %, which, given that little formation of impurities was detected during follow-up, excludes significant deterioration of any epoxide product and thus supports the idea of interconversion between the two initially formed *cis*-4-oxiranylazetidin-2-ones **28Ac**/**28Bc**. However, no absolute confirmation by means of a mass balance, *i.e.* a yield of major 4-oxiranyl-β-lactams **28A** that exceeds 50 % after purification, was obtained as it was purified in only 43 % yield after reversed phase automated column chromatography. Nonetheless, the fact, that the chromatographic apparatus' solvent was later proven to be contaminated with ethyl acetate at the time, might explain this low yield.

The experiments, that are proposed for future research regarding the suspected interconversion, are combined with another phenomenon that is associated with *cis*-3-acylamino-4-oxiranylazetidin-2-ones **28**, *i.e.* the cyclisation of minor epoxides **28B** towards minor 3,4-pyrrolidine-fused bicyclic β-lactams **30B**. In none of the reactions of this Master's thesis, the construction of these minor 2,6-diazabicyclo[3.2.0]heptan-7-ones **30B** was unambiguously observed. It is expected from preliminary research that the minors' cyclisation could need a twentyfold increase in reaction time.[64,65] However, experiments were performed, in which full conversion of a starting mixture of epoxide diastereomers **28A**/**28B** was achieved, but only the major bicyclic β-lactams **30A** were recovered and no minor epoxides **28B**, nor minor bicyclic azetidin-2-ones **30B** were found. As during follow-up of the reaction no obvious deterioration of the epoxides was detected as well, these observations support the suggested phenomenon of epoxide diastereomer interconversion. It would mean that the major diastereomers **28A** have both the property of being the thermodynamically most stable epoxide diastereomer, as well as converting much more readily to ring closed products **30A**, consequently resulting in a starting mixture of both epoxides **28A**/**28B** solely yielding major cyclised structures **30A**.



It is recommended for future research to primarily focus on isolating minor epoxides **28B**, which cannot be achieved from a mixture of both epoxides **28A**/**28A** due to substantial tailing of the first eluting major diastereomer **28A**. However, since the cyclisation reaction proceeds faster for the major diastereomer and since it substantially changes the chromatographic retention time of the compound, it is proposed to separate the major 2,6-diazabicyclo[3.2.0]heptan-7-ones **30A** from minor *cis*-4-oxiranylazetidin-2-ones **28B** after having stopped the cyclisation reaction early. With pure minor epoxides **28B** in hand, following tests should be performed: (i) dissolving them in dichloromethane and stirring them at room temperature, while regularly taking samples and determine by ^{1}H NMR analysis (in $CDCl_3$) whether any signals belonging to the major diastereomers **28A** appear; if no reaction is observed, the temperature could be elevated and/or *m*CPBA could be added to simulate the epoxidation conditions; (ii) attempting cyclisation towards minor bicyclic β-lactams **30B** and track how long this takes, as well as whether interconversion towards the major diastereomers **28A** and subsequent reaction towards their cyclised forms **30A** occurs.

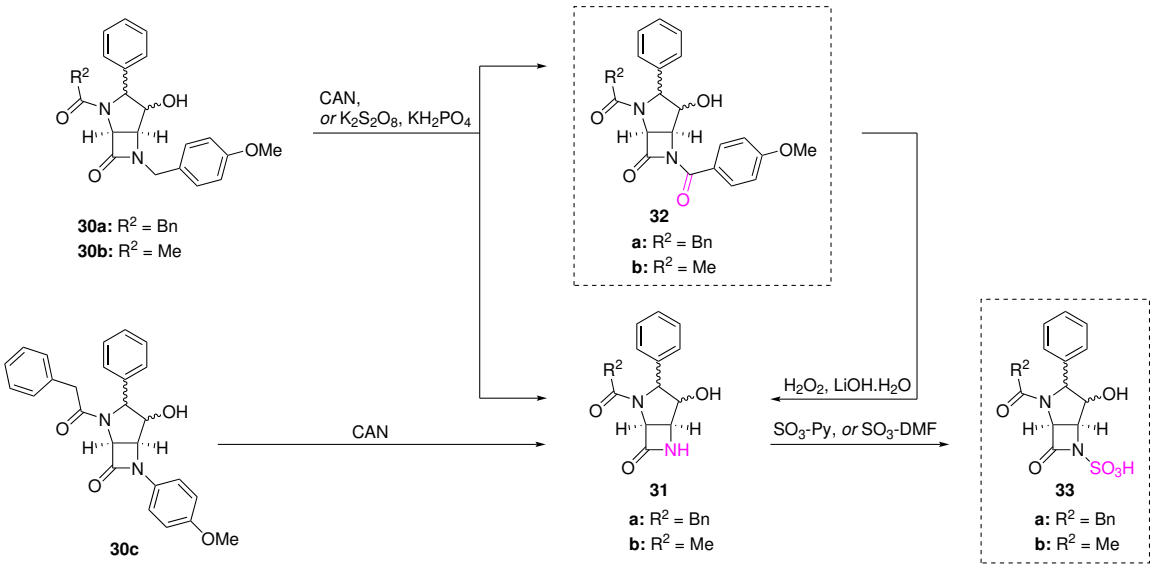
3.4.2 Functionalisation of 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones

Benzylic oxidation of 6-(4-methoxybenzyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones

In view of the pronounced effect of the azetidin-2-one *N*-substituent on the β-lactamase inhibitory activity, novel 3,4-pyrrolidine-fused bicyclic β-lactams are to be derivatised towards compounds, of which the N6-attached functional group should result in an improved biological activity. In this Master's thesis, a successful benzylic oxidation of 6-(4-methoxybenzyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **30Aa-Ab** towards imides **32Aa-Ab** was achieved by treatment with a large excess (nine equivalents) of CAN. Nonetheless, still a small amount of *N*-unsubstituted bicyclic β-lactam **31** was formed. In the future, a procedure employing potassium persulphate in the presence of potassium dihydrogen phosphate might be attempted. In preliminary research, it was shown to result in a full benzylic oxidation of PMB-substituted β-lactam **19c**,[64,65] but other literature shows it giving complete oxidative cleavage of the PMB group, affording *N*-unsubstituted azetidin-2-ones without any *N*-benzoylated product present.[200,201] It is thus unknown whether this methodology would solely yield PMBz-substituted **32** or N6-deprotected bicyclic azetidin-2-ones **31**, or a mixture of both. Regarding the CAN-procedure, additional experiments should be attempted, in which the number of equivalents CAN added is varied, since preliminary research has proven its influence on the imide to *N*-deprotected β-lactam ratio.[74] The rationale for this lies in the desired isolation of N6-unsubstituted bicyclic β-lactams **31** as they play an intermediate role in the synthesis of target sulphonic acids **33**, which is discussed *in infra*.

Deprotection and sulphonation towards 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids

Although it results in the installation of a favourable electron-withdrawing substituent at the β-lactam nitrogen, the motivation for above benzylic oxidation is mainly its link with preliminary research and the opportunity to compare the bioactivity of 3,4-oxolane-fused **20** and 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **32**. Purely based on the mechanism-based design of class C β-lactamase inhibitors by Heinze-Krauss *et al.*,[71,72] 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33** remain by far the most promising candidates from this work's target molecules **15** to possibly exhibit potent and selective class C enzyme inhibitory activity. It became clear in the chapter "Literature overview" that the reason for the interest in replacing the protecting groups at nitrogen N6 (R^1 = PMB, PMP) of 2,6-diazabicyclo[3.2.0]heptan-7-ones **30** by a sulphonic acid functionality goes beyond its electron-withdrawing capability, which facilitates nucleophilic attack at the β-lactam carbonyl by *e.g.* a β-lactamase enzyme.[67] It appears that the anionic site, which a sulphonic acid would become *in vivo*, is essential for binding with the target enzymes as a salt bridge between the sulphonate group and a lysine residue in the class C β-lactamases' active site is formed.[70–73] Furthermore, it is key in preventing deacylation of the enzyme, which would restore its activity against β-lactam antibiotics, since a sulphonate moiety displaces the active site-bound deacylating water molecule to a position where it cannot be activated. The findings *in supra* were proven by X-ray crystallography of enzyme-inhibitor complexes and they led, together with the need of preventing C3-C4 bond rotation in an azetidin-2-one ring and the possible benign interactions of an acyl side chain, to compounds **212** being excellent mechanism-based inhibitor candidates of class C β-lactamases (section 2.4).[71–73] Possessing the same three key structural features, but with structural variety due to a different acyl chain as well as the presence of 3-phenyl and 4-hydroxyl groups, the synthesis of compounds **33** was declared the ultimate goal of this Master's thesis.



The synthesis of target molecules **33** requires sulphonation of N6-unsubstituted 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **31**. Latter compounds can, as already mentioned, be prepared from PMB-substituted β-lactams **30a-b** by altering the number of equivalents CAN added to a level where benzylic oxidation is minimal and the ratio of N6-deprotected **31** to PMBz-substituted compounds **32** is thus maximised. Another option is cleaving of the 6-(4-methoxybenzoyl) substituent by treatment with hydrogen peroxide in combination with lithium hydroxide monohydrate, but it would increase the synthesis route with an additional step. Furthermore, this *N*-deprotection was concluded irreproducable, when attempted in preliminary research.[74] A third way of obtaining precursors **31** is starting from PMP-substituted β-lactams **30c** and this procedure is deemed the most efficient, since literature data suggests their CAN-mediated deprotection will result in a full conversion towards N6-unsubstituted bicyclic azetidin-2-ones **31** without competing side reactions.[74,77–79,195,196] The newly created free amino functionality can then be employed to get the desired sulphonic acid group attached to it, for which literature protocols exist using the sulphur trioxide pyridine complex,[80–82] but successful β-lactam sulphonation has also been achieved employing sulphur trioxide *N*,*N*-dimethylformamide.[71,81,202]

3.4.3 Biological evaluation of 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones

The newly synthesised 3,4-pyrrolidine-fused bicyclic β-lactams **30**, **32** and **33** are planned to be evaluated for their biological activity against class C β-lactamases in collaboration with prof. T. Desmet (Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University). Although not foreseen within the frame of this Master's thesis, eventually biological experiments will be commenced, including evaluation of the four in this work isolated 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones, *i.e.* major PMB-substituted compounds **30Aa-Ab** and major PMBz-substituted compounds **32Aa-Ab**.

As one of the goals was to compare the bioactivity of 3,4-oxolane-fused bicyclic azetidin-2-ones **14** with their aza-analogues **15**, some of the performed experiments should utilise the same protocol as was used in the preliminary research.[64,65] Concretely, inhibitor candidates **14** were incubated with β-lactamase from *Enterobacter cloacae*, after which the residual enzymatic activity was determined by following the rate of hydrolysis of the chromogenic cephalosporin nitrocefin. For this procedure, tazobactam **8** was employed as the reference compound, of which the residual activity was 16.2 ± 10.6 %. None of the tested bicyclic structures performed better than tazobactam, but one of the most promising results was the substantially decreased residual enzymatic activity, when 4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2-oxa-6-azabicyclo[3.2.0]heptan-7-one **19c** (95.1 ± 12.9 %) underwent a benzylic oxidation towards PMBz-substituted β-lactam **20** (58.7 ± 12.4 %). Another biological experiment, which could be interesting, would be to attempt determining the half-inhibition constants (IC_{50}) and minimal inhibitory concentrations (MIC), as was done in the research of Heinze-Krauss and co-workers (section 2.4).[71,72] In that respect, the inhibition of isolated enzymes from the β-lactamase producing strains *Citrobacter freundii* 1982 (class C), *Pseudomonos aeruginosa* 18 SH (class C) and *Escherichia coli* TEM-3 (class A), respectively the synergy with the third-generation cephalosporin ceftriaxone **213** in a 4/1 inhibitor to antibiotic ratio against the bacteria themselves should be tested. It would allow comparing the bioactivity of bicyclic β-lactams **15**, that were synthesised in this Master's thesis, with that of highly similar sodium 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonates **212** (**Table 3**).

Concerning the results of the biological tests, the interpretation should particularly focus on following expected structure-activity relationships: (i) the influence of the 3,4-annulated ring, *i.e.* oxolane *vs.* pyrrolidine, the latter resulting in an additional *N*-substituent on the bicyclic scaffold; (ii) the influence of this N2-attached acyl substituent, *i.e.* phenylacetyl (R^2 = Bn) *vs.* acetyl (R^2 = Me), both possibly having benign hydrogen-bonding interactions with the β-lactamase's active site due to the carbonyl moiety, but phenylacetyl's benzene ring might additionally result in favourable π-stacking interactions; (iii) the influence of the N6-attached substituent, *i.e.* the electron-donating PMP and PMB *vs.* the electron-withdrawing PMBz and sulphonic acid, the latter two facilitating the enzyme's nucleophilic attack at the β-lactam carbonyl, but the sulphonate should additionally form a salt bridge with the enzyme and have an essential role in preventing deacylation of the inhibitor; (iv) the influence of the stereochemistry of the 3-phenyl and 4-hydroxyl substituents, *i.e.* the major *vs.* minor diastereomers. Above expected structure-activity relationships are summarised in **Figure 6**. However, as with compound **212a** in the chapter "Literature overview", an effective inhibition of isolated β-lactamase enzymes (*i.e.* low IC_{50}) does not necessarily mean a good synergy with an antibiotic against a bacterium in its whole (*i.e.* low MIC).[71,72] A trade-off possibly exists between decoration of the 3,4-annulated pyrrolidine ring with substituents at positions N2, C3 and C4, which may have benign interactions with the enzyme's catalytic site, and effective penetration of the inhibitor through the outer membrane and periplasmic space of bacteria. In such cases, it is advised to see whether adding a membrane permealiser has any effect. Lastly, since there is a difference between a drug's potency and its human oral bioavailability, estimation of the theoretical "druglikeness" of compounds **15** is also important.[203] The latter can be estimated by using various criteria, *e.g.* Lipinski's "rule of five",[204] and calculated parameters such as the fractional sp^3 character, molecular flexibility, and polar surface area.[205–207]

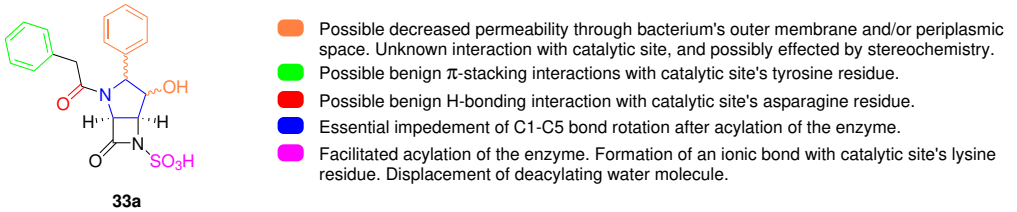
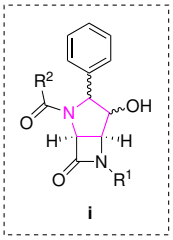


Figure 6: Overview of the expected structure-activity relationships of 4-hydroxy-7-oxo-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **33a**, based on literature results concerning X-ray crystallographic structure determination of enzyme-inhibitor complexes.[71,72]

4 SUMMARY AND CONCLUSION

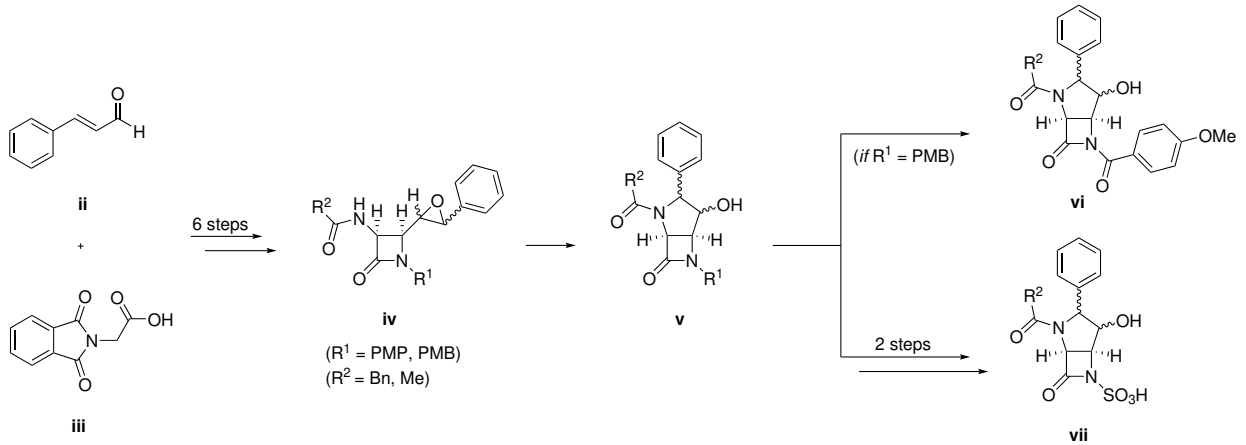
Since their serendipitous discovery in 1928, β-lactams have grown into a heterocyclic class of great importance. Besides their significant role within organic chemistry as flexible synthons towards a wide range of compounds, they possess numerous interesting pharmacological properties. Being among the most well-known, most prescribed and safest pharmaceuticals for the treatment of bacterial infections, they helped achieve the high life expectancy humanity is used to nowadays. However, the natural phenomenon of antimicrobial resistance (AMR), intensified due to mankind's excessive usage of antibiotics and their inadequate development after the 1990s, risks a full medicinal set-back to the pre-antibiotic era, which would jeopardise global public health. As such a situation is to be avoided at all costs, there exists a profound need to come up with innovative strategies in anti-infective drug discovery. In recent years, an excellent approach identified by the pharmaceutical industry concerns a combination therapy, in which the medicine contains both an antibiotic as well as a separate drug, that acts as an inhibitor of resistance. This Master's thesis focuses on tackling the most efficient bacterial AMR mechanism, *i.e.* the production of β-lactamase enzymes, with the construction of 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **i** as potential class C β-lactamase inhibitors. Since often a high level of expression of class C enzymes is to blame for resistance against modern β-lactam antibiotics, and since almost no potent and selective class C inhibitors are commercially available, the preparation of such compounds is of paramount biological importance. In addition, the chemical synthesis of *C*-fused bicyclic β-lactams has received much less attention compared to their renowned *N*-fused analogues, hereby making the in this work employed strategy a valuable entry in the literature.



i

(R^1 = PMP, PMB, PMBz, SO_3H)
(R^2 = Bn, Me)

In that respect, all desired 2,6-diazabicyclo[3.2.0]heptan-7-ones **i** were envisioned in this Master's thesis to be synthesised along the course of a nine-step reaction pathway, starting from (*E*)-cinnamaldehyde **ii** and *N*-phthaloylglycine **iii**. *Cis*-3-acylamino-4-oxiranylazetidin-2-ones **iv** were designated the precursors for the intramolecular construction of a pyrrolidine ring, affording *C*-fused β-lactams **v**, after which functionalisation would lead to final *N*-benzoylated **vi** and sulphonated **vii** target molecules.



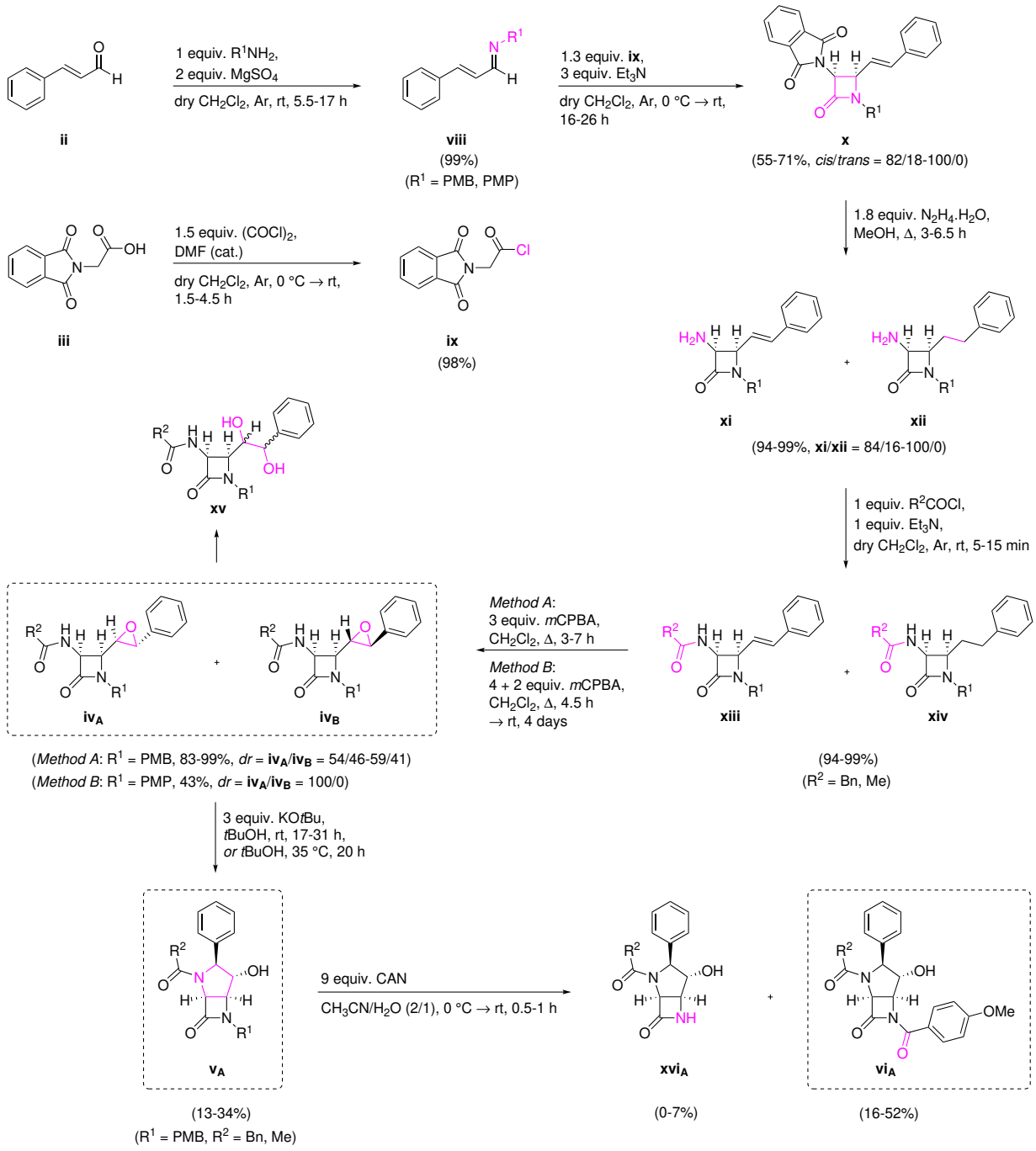
Of the available literature methods towards various 3,4-annulated β-lactams, a great many could theoretically be used for the specific synthesis of 2,6-diazabicyclo[3.2.0]heptan-7-ones **i**, but only very few of them have already been employed to actually construct this diazabicyclic scaffold, *viz.* a halocyclisation, a nucleophilic substitution of a carbonate with a tetra-alkylammonium halide, an oxidative coupling of a dianion, and a palladium(II)-catalysed N6-C5 bond formation. In that light, the in this work used synthetic strategy of an amido-induced 5-*endo*-epoxy ring opening adds a fifth methodology to the literature for the preparation of 3,4-pyrrolidine-fused bicyclic azetidin-2-ones. It should be noted that, in general, above intramolecular amide-epoxide cyclisation strategy has only sporadically been employed in the literature, and solely the 5-*exo*, 6-*exo* and 6-*endo* variants. For the synthesis of *C*-fused β-lactams in particular, even the use of an oxirane ring opening to induce the intramolecular cyclisation is rare. In fact, there exists only one entrance in the literature, in which oxirane moieties are used for the construction of the 3,4-annulated ring, *i.e.* the preparation of 3,4-oxolane-fused bicyclic azetidin-2-ones *via* a 5-*endo*-*tet* hydroxyl-epoxide cyclisation. The goal of this Master's thesis concerns employing above strategy for the synthesis of their aza-analogues **i**.

The aimed for novel 3,4-pyrrolidine-fused bicyclic β-lactams **i** are considered potent, mechanism-based inhibitor candidates for overcoming class C β-lactamase-mediated resistance in bacteria. Three structural features of these compounds should be responsible for a vast increase in the half-life of the enzyme-inhibitor complex, and therefore lie at the basis of their inhibitory potential: (i) the bicyclic core, which is essential to impede bond rotation between bridgehead carbons C1 and C5 after acylation of the enzyme's catalytic serine residue, thus preventing deacylation as the pathway, that an incoming water molecule in the active site would have to take for attacking the enzyme-inhibitor ester bond, is sterically blocked; (ii) the sulphonic acid functionality ($R^1 = SO_3H$) at nitrogen N6, which with its electron-withdrawing capability not only facilitates the initial nucleophilic attack by serine at the β-lactam carbonyl, but after *in vivo* deprotonation also forms an ionic bond with a lysine residue, and thirdly prevents the activation of the deacylating water molecule by displacing it; (iii) the acyl side chain at nitrogen N2, which can have benign hydrogen-bonding and π-stacking interactions with amino acids in the catalytic site, such as asparagine and tyrosine.

4.1 Summary

The chemical synthesis of synthetically and biologically interesting 3,4-pyrrolidine-fused bicyclic β-lactams **i** comprised three parts, each representing the construction of one of the structures **iv**, **v** or **vi**/**vii**, that are considered the key synthetic milestones of this work.

In the first part, preparation of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **iv** was achieved *via* a six-step synthesis. In that respect, imination of (*E*)-cinnamaldehyde **ii** and DMF-catalysed treatment of *N*-phthaloylglycine **iii** with oxalyl chloride were performed in parallel. The resulting phthaloyl-protected acid cloride **ix** was condensed with imines **viii** during a triethylamine-mediated Staudinger [2+2] cyclocondensation towards racemic β-lactam building blocks **x** with high *cis*-diastereoselectivity. The subsequent *N*-phthaloyl deprotection of *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-ones **x** *via* hydrazinolysis towards β-lactams **xi** was shown to occasionally suffer from a transfer hydrogenation side reaction, yielding 4-(2-phenylethyl)-substituted compounds **xii**. In order to avoid this undesired loss of the alkene functionality, future experiments are advised to remove the presence of all molecular oxygen from the system. Next, *N*-acylation of *cis*-3-aminoazetidin-2-ones **xi** by reaction with an acid chloride in the presence of triethylamine afforded *cis*-3-acylaminoazetidin-2-ones **xiii**. The sixth synthesis step concerned epoxidation of the 4-((*E*)-styryl) functionality of β-lactams **xiii** with 3-chloroperbenzoic acid (*m*CPBA). A side reaction was sometimes noticed during this step as well, affording *anti*-vicinal diols **xv**. Optimisation experiments led to finding two robust epoxidation strategies, *i.e.* a "multiple addition method" and a "reflux method", with the latter being the preferred one because of its shorter reaction time, consistent diastereoselectivity, and good yields of clean crude reaction product. Some results hinted towards an interconversion between the formed major $\mathbf{iv_A}$ and minor epoxide diastereomers $\mathbf{iv_B}$, but this could not be irrefutably proven by *e.g.* mass balance and should therefore be further investigated. In the end, five different *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones **iv** were isolated and characterised: the majors $\mathbf{iv_A}$ and minors $\mathbf{iv_B}$ of the PMB-substituted derivatives (R^2 = Bn, Me), and the major $\mathbf{iv_A}$ of the PMP-substituted derivative (R^2 = Bn).

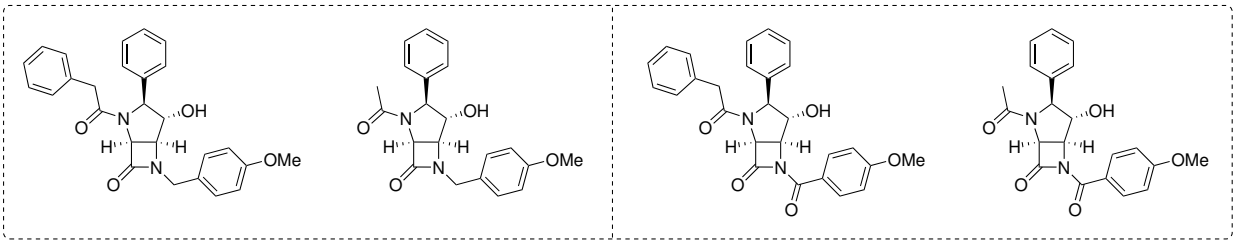


The second part of this Master's thesis comprised the key cyclisation step in forming the desired 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **v**. It involved an unprecedented amido group-induced intramolecular ring closure through oxirane ring opening at the benzylic position, *i.e.* a 5-*endo-tet* cyclisation. After numerous screening and optimisation experiments, robust cyclisation conditions employing potassium *tert*-butoxide in *tert*-butanol were found. No ring closure of minor epoxides $\mathbf{iv_B}$ was achieved in this work. They are expected to cyclise more slowly compared to major diastereomers $\mathbf{iv_A}$, but the observation where a mixture of both epoxide diastereomers $\mathbf{iv_A}/\mathbf{iv_B}$ achieved full conversion, though only formation of the major 3,4-annulated bicyclic β-lactam $\mathbf{v_A}$ could be detected, was surprising and should be further investigated. In the end, two 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones **v** were isolated and characterised: the majors $\mathbf{v_A}$ of the PMB-substituted derivatives (R^2 = Bn, Me), of which the structure of one (R^2 = Bn) was irrefutably proven by single crystal X-ray diffraction analysis.

In the third and last part, novel 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **v** were further functionalised towards biologically more promising derivatives. A benzylic oxidation of PMB-substituted bicyclic β-lactam majors $\mathbf{v_A}$ employing cerium(IV) ammonium nitrate (CAN) primarily afforded 6-(4-methoxybenzoyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones $\mathbf{vi_A}$, but also formation of N6-unsubstituted *C*-fused β-lactams $\mathbf{xvi_A}$ was detected to a small extend. No isolation of these N6-unsubstituted 2,6-diazabicyclo[3.2.0]heptan-7-ones **xvi** in a significant amount was achieved in this work and, therefore, no sulphonation towards 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **vii** could be attempted. Latter compounds were set as the ultimate target structures of this Master's thesis' envisioned nine-step synthesis, because the sulphonic acid moiety is expected to exert a significant influence on the biological activity against class C β-lactamase enzymes. Therefore, the introduction of this promising functional group at nitrogen N6 should be investigated in future research. In the end, two derivatives of 3,4-pyrrolidine-fused bicyclic β-lactams **v** were isolated and characterised: the PMBz-substituted majors $\mathbf{vi_A}$ (R^2 = Bn, Me).

4.2 Conclusion

In light of the never-ending race between antibiotics and bacteria, the former designed by mankind to circumvent the ever-adapting AMR mechanisms of the latter, the most recent successful approach in anti-infective drug discovery concerns a combination therapy, in which the antibiotic is administered together with an inhibitor of the mechanism of resistance. In particular, employing this strategy to overcome class C β-lactamase-mediated resistance is of paramount importance, since often a high level of expression of these class C enzymes is to blame for the nowadays exponentially increasing frequency of antibiotic resistance. As very few commercially available class C β-lactamase inhibitors exist, the synthesis of 2,6-diazabicyclo[3.2.0]heptan-7-ones, which are viable mechanism-based class C inhibitor candidates, was pursued in this Master's thesis. Ultimately, following four final bicyclic structures were synthesised and isolated as racemic mixtures.

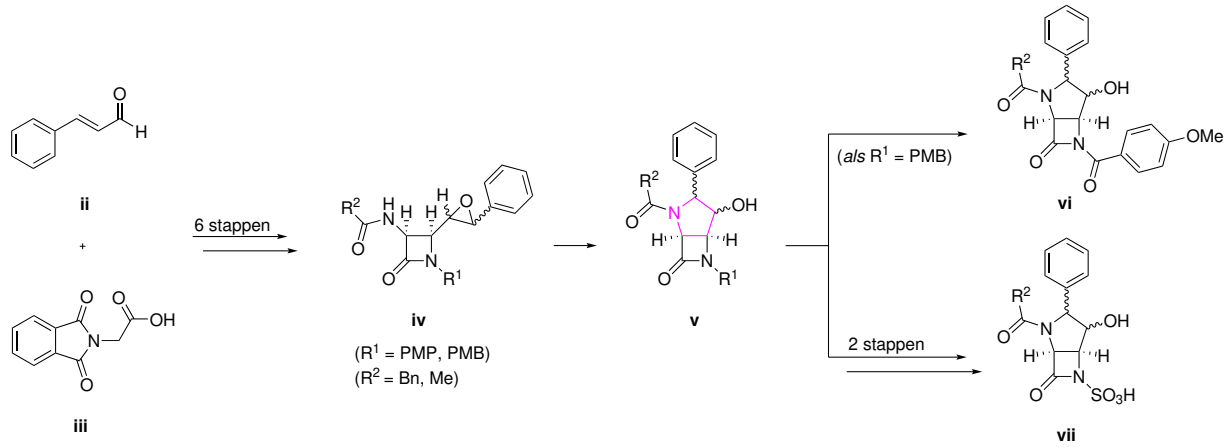


In retrospect, this work provides an optimised six-step reaction pathway towards *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)-β-lactams, which form key precursors in the intramolecular construction of a pyrrolidine ring, affording 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones *via* an unprecedented base-mediated 5-*endo-tet* amide-epoxide cyclisation. Robust epoxidation and cyclisation conditions for varying substituents were found, and it was shown that the reactivity between epoxide diastereomers differs. Therefore, this Master's thesis achieves its primary goal of constructing 3,4-pyrrolidine-fused bicyclic azetidin-2-ones in an attempt to contribute filling the gap, that is apparent both pharmaceutical-wise as literature-wise. The secondary objective concerned the functionalisation of these *C*-fused β-lactams towards biologically more promising derivatives. It has been partially achieved, since 6-(4-methoxybenzoyl)-substituted bicyclic compounds were obtained *via* a benzylic oxidation, though within this Master's thesis' reduced time frame no attempts at sulphonation towards 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids could be made.

In the end, nineteen racemic β-lactam compounds were synthesised and isolated in this work, of which seventeen were not yet described in the literature, and with four of them incorporating the desired diazabicyclic scaffold. These four novel 3,4-pyrrolidine-fused bicyclic β-lactams will be evaluated in the future for their biological activity against class C β-lactamases in collaboration with prof. T. Desmet (Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University).

5 SAMENVATTING

Sinds ze in 1928 bij toeval ontdekt werden, zijn β-lactamen of azetidin-2-onen uitgegroeid tot een vooraanstaande klasse van heterocyclische verbindingen. Niet enkel spelen ze een significante rol binnenin de organische chemie als veelzijdige bouwstenen voor de synthese van allerlei acyclische en heterocyclische verbindingen, maar daarnaast bezitten ze eveneens tal van interessante farmacologische eigenschappen. De voornaamste toepassing omvat hun gebruik in de behandeling van bacteriële infecties, waarvoor de β-lactamantibiotica tot de veiligste en meest voorgeschreven geneesmiddelen behoren. Ze hebben bijgedragen aan de hoge levensverwachting, die de mensheid tegenwoordig gewend is, maar die lang niet altijd vanzelfsprekend was. Echter, hun bruikbaarheid wordt vandaag de dag bedreigd door de heersende resistentieproblematiek, dewelke één van de grootste uitdagingen vormt voor de wereldwijde gezondheid, zoals gesteld door de Wereldgezondheidsorganistie. Hoewel bacteriële resistentie tegen antibiotica een natuurlijk en onvermijdbaar evolutionair fenomeen is, wordt dit proces versneld door onder andere het verkeerdelijk en overmatig gebruik ervan, alsook een ontoereikende ontwikkeling van nieuwe antibiotica of klassen met nieuwe werkingsmechanismen sinds de jaren '90. Gezien de nooit eindigende wedloop tussen bacteriën en antibiotica, en gezien een terugkeer naar het pre-antibioticum tijdperk onaanvaardbaar is, heerst er een dringende nood aan innovatieve strategieën voor de ontwikkeling van nieuwe anti-infectieve geneesmiddelen. De meest recente, succesvolle aanpak binnenin de farmaceutische industrie omvat een combinatietherapie, waarbij het antibioticum tezamen met een stof, die het mechanisme van resistentie bekampt, wordt toegediend. De focus van deze Masterproef ligt op de bestrijding van het meest efficiënte bacteriële resistentiemechanisme, nl. de productie van β-lactamasen, en de constructie van 3,4-pyrrolidine-gefuseerde bicyclische azetidin-2-onen als op mechanisme gebaseerde, potentiële klasse C β-lactamase inhibitoren. Aangezien de resistentie tegen moderne β-lactamantibiotica vaak te wijten is aan een hoge expressie van klasse C β-lactamasen, gecombineerd met het feit dat er nauwelijks krachtige en selectieve klasse C inhibitoren op de markt zijn, is de synthese van zulke verbindingen van enorm biologisch belang. Daarnaast is vanuit synthetisch oogpunt de chemische bereiding van *C*-gefuseerde bicyclische β-lactamen ook bijzonder interessant, vermits de synthese hiervan in de literatuur beduidend minder aandacht geniet, vergeleken met hun vermaarde *N*-gefuseerde analogen.



In dat opzicht werd in deze Masterproef een syntheseroute ontwikkeld, dewelke uit drie delen bestond. In een eerste luik werden *cis*-3-acylamino-4-(3-fenyloxiran-2-yl)azetidin-2-onen **iv** bereid via een zesstapsmethode, vertrekkende vanuit (*E*)-kaneelaldehyde **ii** en ftaalimidoazijnzuur **iii**. Deze gefunctionaliseerde β-lactamen dienden als precursoren voor de intramoleculaire constructie van een pyrrolidinering, resulterend in de gewenste 2-acyl-3-fenyl-4-hydroxy-2,6-diazabicyclo[3.2.0]heptaan-7-onen **v**, hetgeen werd onderzocht in een tweede luik. Bovenstaande door base geïnitieerde 5-*endo-tet* amide-epoxide cyclisatie is ongekend binnen de literatuur, en voorziet aldus een vijfde strategie voor de synthese van 2,6-diazabicyclo[3.2.0]heptaan-7-onen. Het derde en laatste luik van dit werk omvatte de verdere functionalisatie van 3,4-pyrrolidine-gefuseerde bicyclische β-lactamen **v** naar biologisch meer beloftevolle verbindingen, zijnde de finale *N*-gebenzoyleerde **vi** en gesulfoneerde **vii** doelmoleculen. Echter, binnen het tijdsbestek van deze Masterproef konden geen sulfonatiereacties uitgevoerd worden, en bijkomend onderzoek naar deze laatste stap is zodus wenselijk. Uiteindelijk werden vier nieuwe 3,4-pyrrolidine-gefuseerde bicyclische azetidin-2-onen gesynthetiseerd en geïsoleerd als racemische mengsels, waarvan de biologische activiteit tegen klasse C β-lactamasen in de toekomst getest zal worden in samenwerking met prof. T. Desmet (Vakgroep Biotechnologie, Faculteit Bio-ingenieurswetenschappen, Universiteit Gent).

6 EXPERIMENTAL CHAPTER

6.1 General analytical methods and laboratory equipment

All employed solvents and reagents were purchased from common chemical suppliers, and used as received without further purification.

6.1.1 Thin Layer Chromatography (TLC)

Thin layer chromatography was employed for the analysis of crude reaction mixtures or isolated compounds in order to determine their composition and/or R_f values (*i.e.* retention factors), hereby allowing identification of the most adequate solvent mixture for column chromatography. Furthermore, thin layer chromatography allowed follow-up of reactions, and of separations and/or purifications by means of column chromatography as well. To that end, glass-backed silica plates (Merck silicagel 60 F_{254}, precoated, thickness 0.25 mm) were used in combination with an experimentally determined eluent. Afterwards, visualisation of the compounds was achieved by means of UV irradiation (254 nm) and/or a cerium molybdate stain with subsequent heat treatment.

6.1.2 Preparative Thin Layer Chromatography (Prep. TLC)

Preparative thin layer chromatography was employed for the separation and/or purification of maximum 100 mg of crude reaction mixtures or compounds. To that end, Analtech silica plates GF (precoated, with an UV indicator (254 nm), dimensions 20 cm x 20 cm x 2 mm) were used in an elution chamber in combination with an appropriate experimentally determined eluent. Afterwards, visualisation of the compounds was achieved by means of UV irradiation (254 nm) and/or a cerium molybdate stain with subsequent heat treatment.

6.1.3 Column Chromatography

Column chromatography was employed for the separation and/or purification of crude reaction mixtures or compounds. To that end, depending on the amount of product being purified, glass columns with an appropriate diameter were used and filled with silica gel (particle diameter 35–70 µm, pore diameter 6 nm) as a stationary phase. An appropriate solvent mixture, which had been experimentally determined by means of thin layer chromatography, was chosen as eluent, and a constant elution speed of approximately 5 cm min^{-1} was maintained.

6.1.4 Automated Column Chromatography

Automated column chromatography was employed for the separation and/or purification of crude reaction mixtures or compounds. To that end, a Büchi Reveleris® X2 Flash Chromatography system was used for normal phase (SiO_2) purifications, while reversed phase (C18) purifications were executed with a Grace™ Reveleris™ Flash Chromatography system. Depending on the amount of product being purified, reusable prepacked Reveleris® silica (particle diameter 40-63 µm) or Reveleris® C18 (particle diameter 20-40 µm) cartridges of an appropriate size (4-120 g) were employed, and the elution rate was modified accordingly (18-80 mL min^{-1}). Real-time detection of the compounds occurred *via* two UV detectors and an Evaporative Light Scattering Detector (ELSD) for the Grace™ Reveleris™ Flash Chromatography system, or *via* three UV detectors and an ELSD for the Büchi Reveleris® X2 Flash Chromatography system. The appropriate UV wavelengths had been determined beforehand by means of LC-MS analysis.

6.1.5 Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid chromatography mass spectrometry was employed for the follow-up of reactions and for the analysis of crude reaction mixtures. To that end, an Agilent 1200 Series LC/MSD SL device, equipped with a Supelco Ascentis Express C18 column (internal diameter 4.6 mm, length 3 cm, fused core particles diameter 2.7 µm, fused core particles pore size 90 Å), was used. Additionally, the device was equipped with a Phenomenex Guard column (SecurityGuard Standard), and a UV-DAD detector. Furthermore, the chromatographic apparatus was coupled to an Agilent 1100 Series MSD SL mass spectrometer with electrospray ionisation (ESI, 4000 V, 70 eV) and with a single quadrupole detector. A solvent mixture of acetonitrile and water (5 mM NH_4OAc) in different ratios, depending on the selected method, was chosen as eluent.

6.1.6 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR and ^{13}C NMR spectroscopy were employed for the follow-up of reactions, for the analysis of crude reaction mixtures, and for the characterisation of isolated compounds. To that end, a Bruker Avance Nanobay III NMR spectrometer, equipped with a ^{1}H/BB z-gradient high resolution probe (BBO, 5 mm), was used. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100.6 MHz, respectively. All spectra were acquired through the standard sequences available in the Bruker pulse program library, and they were processed using TOPSPIN 3.5 software. The compounds were dissolved in deuterated solvents with tetramethylsilane (TMS) as an internal standard. Generally, deuterated chloroform was chosen as the NMR solvent, but in the case of low solubility deuterated dimethyl sulphoxide was used. ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) downfield of TMS, and are referenced to the residual solvent signal ($CDCl_3$ $\delta_H = 7.26$, $\delta_C = 77.16$; d_6-DMSO $\delta_H = 2.50$, $\delta_C = 39.52$). Coupling constants (J) are reported in hertz (Hz). Aided by COSY, HSQC, HMBC and 2D NOESY spectra, the assignment of the signals could take place, and rotameric chemical exchange processes were identified *via* EXSY by employing 2D NOESY experiments.

6.1.7 Infrared Spectroscopy (IR)

Infrared spectroscopy was employed for the characterisation of isolated compounds. To that end, a Shimadzu IRAffinity-1S Fourier Transform Infrared Spectrophotometer (FTIR) was used, in combination with a Quest Attenuated Total Reflectance (ATR) accessory with diamond crystal puck. All spectra were acquired with a signal-to-noise ratio of 30,000/1, and they were processed using LabSolutions IR software. Only selected absorbances (ν_{max}) are reported in cm^{-1}.

6.1.8 Mass Spectrometry (MS)

Mass spectrometry (low resolution) was employed for the characterisation of isolated compounds. To that end, an Agilent 1100 Series MSD SL mass spectrometer with electrospray ionisation (ESI, 4000 V, 70 eV) and with a single quadrupole detector was used.

6.1.9 Single crystal X-ray diffraction analysis

Single crystal X-ray diffraction analysis was employed for the characterisation of isolated, crystalline compounds. It was performed by prof. K. Van Hecke (XStruct, Department of Inorganic and Physical Chemistry, Faculty of Sciences, Ghent University), and used for structural elucidation of 3,4-pyrrolidine-fused bicyclic β-lactam (1*S**,3*S**,4*S**,5*S**)-**30Aa**. To that end, a suitable crystal was selected, mounted on a LithoLoop, and X-ray intensity data were collected at 100(1) K on a four-circle Agilent Supernova Dual Source (Cu at home/near) diffractometer, equipped with an Atlas CCD detector using ω scans and CuKα ($\lambda = 1.54184$ Å) radiation, and a Cryojet5 liquid nitrogen cooling device. The images were interpreted and integrated with the program CrysAlisPRO.[208] Using Olex2,[209] the structure was solved by direct methods using the ShelXT structure solution program,[210] and refined by full-matrix least-squares on F^2 using the ShelXL program package.[211] Non-hydrogen atoms were anisotropically refined and the hydrogen atoms in the riding mode and isotropic temperature factors fixed at 1.2 times U(eq) of the parent atoms (1.5 times for methyl and hydroxyl groups).

6.1.10 Melting point determination (Mp)

Melting points were determined for the characterisation of isolated, solid compounds. To that end, a Kofler heating bench system of Wagner and Munz (type WME, accuracy ± 1 °C, gradient 50-260 °C) was used. A small amount of pure, solid sample was applied at the cold side of the heating bench and moved slowly to the hot side until the melting point was reached.

6.1.11 Microwave reactor (MW)

Microwave reactions were performed in a CEM FocusedTM Microwave Synthesis System, Model Discover, with adaptable power (0-300 W), and they were monitored using Synergy 1.32 software. Reagents were held in a 10 mL microwave Pyrex vial, under continuous stirring, and sealed with a snap-on PTFE septum.

6.1.12 Anhydrous solvents

In order to avoid undesired moisture-induced side reactions, chemical reactions were executed under dry conditions, *i.e.* in an anhydrous solvent and under inert nitrogen or argon atmosphere. Five different anhydrous solvents were provided *via* the MBraun SPS-800 solvent purification system: acetonitrile, diethyl ether, tetrahydrofuran, dichloromethane, and toluene. The solvents were stocked in a safety closet in Pure-Pac storage tanks (17 L), in which they were put under pressure by means of inert gas (N_2) and afterwards sent through two filtering/drying columns. These stainless steel columns (1.4301/US 304, internal volume 4.8 L) were filled with filtering material (*i.e.* molecular sieves), of which the specific properties varied for each solvent. In the case of tetrahydrofuran, the solvent was first sent through an extra filtering element prior to coming in contact with the drying columns. Before the anhydrous solvents could be collected under inert atmosphere from separate flasks, a vacuum had to be created in these flasks by means of a membrane pump (type MPC 301 Zp).

6.2 Safety aspects

6.2.1 General safety aspects

In the framework of health and safety, the lab work of this Master's thesis was performed in compliance with the "Internal guidelines" of the SynBioC Research Group (Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University) and with the aid of the internal safety document "Safety Instructions: How to work with chemicals". Furthermore, two additional documents needed to be read and signed antecedent to the start of the practical work, *i.e.* "Safety and hygiene in chemical laboratories" and "Welzijns- en Milieugids UGent". Moreover, a presentation was given with regard to highlighting the main safety aspects, and a tour around the laboratory pointed out the position of emergency equipment, *viz.* fire extinguishers of various types, fire blankets, eye washing products, and emergency showers. Lastly, a "safety introduction test" had to be taken in order to verify whether this knowledge had been acquired. By means of above measures, Master students were made aware *inter alia* of all safety and health regulations, the general safety guidelines, and the specific safety risks of reagents, solvents and other chemicals. During the actual course of the experimental work, a specific dress code was compulsory, *i.e.* lab goggles, a lab coat, long trousers, and closed shoes had to be worn at all times in the laboratory. Additionally, in order to guarantee personal and others' protection, operating sensitive machinery and employing hazardous chemicals was always done with proper caution, *e.g.* wearing gloves and working in a fume hood. A reaction could only be performed with the appropriate preparation, *i.e.* when all risks were known and all necessary safety measurements were taken. In that respect, it was obligatory to consult the Safety Data Sheet (SDS) of all involved reagents and solvents prior to an experiment.

6.2.2 Specific safety risks

In view of the health and safety scheme *in supra*, it was attempted during the course of this Master's thesis to avoid and/or substitute any hazardous or toxic materials by safer and greener alternatives. An overview of the most hazardous chemicals, which were employed in this work, as well as the hazards they pose and the precautions that should be taken with respect to safety, human health, and the environment, is given below in alphabetical order. It is mainly limited to providing the most harmful hazards and their corresponding precautions for each chemical, *i.e.* the "**category 1**" hazards, as defined in European Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. This information was obtained from the respective SDS files, which can be retrieved from the website of the supplier.

Acid chlorides (acetyl chloride, oxalyl chloride, phenylacetyl chloride). Cause severe skin burns and eye damage. **Acetyl chloride** is a highly flammable liquid. **Oxalyl chloride** releases in contact with water flammable gases, which may ignite spontaneously.

***p*-Anisidine**. Fatal in contact with skin or if inhaled. May cause cancer. Very toxic to aquatic life. Avoid contact with eyes and skin. Wear protective gloves and clothing.

Boron trifluoride diethyl etherate ($BF_3 \cdot Et_2O$). Causes severe skin burns and eye damage. Fatal if inhaled. Flammable liquid. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources.

***tert*-Butyllithium (*t*BuLi)**. Catches fire spontaneously, if exposed to air. In contact with water releases flammable gases, which may ignite spontaneously. May be fatal if swallowed and enters airways. Causes severe skin burns and eye damage. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Handle and store contents under inert gas. Protect from moisture. Wear protective gloves and clothing, wear eye and face protection.

Caesium carbonate (Cs_2CO_3). Causes serious eye damage. Wear eye or face protection.

Cerium(IV) ammonium nitrate (CAN). May be corrosive to metals. May intensify fire. Causes severe skin burns and eye damage. May cause an allergic skin reaction. Very toxic to aquatic life with long lasting effects. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Avoid breathing dust. Avoid release to the environment.

3-Chloroperbenzoic acid (*m*CPBA). May cause an allergic skin reaction. Wear protective gloves.

***N*,*N*-Dimethylformamide (DMF)**. May damage the unborn child.

Halogenated solvents (deuterated chloroform ($CDCl_3$), dichloromethane (CH_2Cl_2)). Cause skin and serious eye irritation. Suspected of causing cancer. Avoid inhalation and release to the environment. **Deuterated chloroform** causes damage to organs (liver and kidney) through prolonged or repeated exposure.

Hexamethylphosphoramide (HMPA). May cause cancer. May cause genetic defects.

Hydrazine monohydrate ($N_2H_4 \cdot H_2O$). May cause cancer. Causes severe skin burns and eye damage. May cause an allergic skin reaction. Very toxic to aquatic life with long lasting effects. Avoid release to the environment. Wear protective gloves and clothing, wear eye and face protection.

Hydrochloric acid (HCl). May be corrosive to metals. Causes severe skin burns and eye damage.

4-Methoxybenzylamine. Causes severe skin burns and eye damage.

Non-halogenated solvents (acetone, acetonitrile (CH_3CN), *tert*-butanol (*t*BuOH), ethanol (EtOH), ethyl acetate (EtOAc), methanol (CH_3OH), petroleum ether (PE), tetrahydrofuran (THF)). (Highly) flammable liquids and vapours. Harmful if swallowed, in contact with skin, or if inhaled. Cause specific target organ toxicity following single or repeated exposure. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Avoid inhalation. **Petroleum ether** may be fatal if swallowed and enters airways.

Potassium *tert*-butoxide (KO*t*Bu). Flammable solid. In contact with water releases flammable gases, which may ignite spontaneously. Causes severe skin burns and eye damage. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Protect from moisture.

Sodium hydride (NaH). In contact with water releases flammable gases, which may ignite spontaneously. May be corrosive to metals. Causes severe skin burns and eye damage. Protect from moisture.

Triethylamine (Et_3N). Highly flammable liquid. Causes severe skin burns and eye damage.

6.3 Synthetic procedures and spectral data

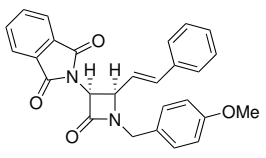
The synthetic procedures and characterisation data of isolated obtained compounds, which are not yet described in the literature, are presented below. As explained in the "Preamble", due to the COVID-19 pandemic, the reduced time frame of this Master's thesis' lab work did not permit that all seventeen novel β-lactams, that were synthesised and isolated, could be fully characterised with all required physical and spectral data. In particular, following data is not provided: the melting point of compounds ***cis*-25a**, **27c**, **28Ab**, **28Ac**, **220a**, **30Ab**, and **32Ab**; the retention factor of compounds **26a**, **27a**, **28Bb**, **30Ab**, and **32Ab**; the infrared absorptions of compounds **28Ab**, **28Bb**, **220a**, and **32Ab**.

6.3.1 Staudinger β-lactam synthesis of *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-ones *cis*-25

The synthesis of *cis*-1-(4-methoxybenzyl)-3-phthalimido-4-((*E*)-styryl)azetidin-2-one ***cis*-25a** and *cis*-1-(4-methoxyphenyl)-3-phthalimido-4-((*E*)-styryl)azetidin-2-one ***cis*-25b** was identical for both compounds and based on reported procedures for similar structures.[64] As a representative example for the Staudinger [2+2] cyclocondensation, the synthesis of *cis*-1-(4-methoxybenzyl)-3-phthalimido-4-((*E*)-styryl)azetidin-2-one ***cis*-25a** is described. Imine **22** and phthalimidoacetyl chloride **24** reagents were prepared quantitatively *via* reported procedures for similar,[64] respectively identical[74] structures and used as such in the Staudinger β-lactam synthesis. Their work-up is limited to filtration of the drying agent for imines **22**, and partial evaporation of the solvent for phthalimidoacetyl chloride **24**. Syntheses of PMP-substituted β-lactam ***cis*-25b** are already available in the literature, and spectral data are in accordance with those reported there.[173,212–214]

General procedure: to an ice-cooled solution (0 °C) of 12.57 g imine **22a** (50 mmol, 1 equiv.) and 20.80 mL triethylamine (150 mmol, 3 equiv.) in anhydrous CH_2Cl_2 (150 mL), a solution of 14.53 g phthalimidoacetyl chloride **24** (65 mmol, 1.3 equiv.) in anhydrous CH_2Cl_2 (100 mL) was added dropwise. After stirring for eighteen hours at room temperature, CH_2Cl_2 (150 mL) was added and the resulting mixture was washed with a saturated aqueous $NaHCO_3$ solution (400 mL) and brine (400 mL). Drying of the organic phase with $MgSO_4$, filtration of the drying agent and removal of the solvent *in vacuo* afforded crude *cis*-β-lactam ***cis*-25a** (racemic), which was purified in 55 % yield by means of column chromatography over silica gel (gradient petroleum ether/ethyl acetate 67/33-0/100).

***cis*-1-(4-Methoxybenzyl)-3-phthalimido-4-((*E*)-styryl)azetidin-2-one *cis*-25a**



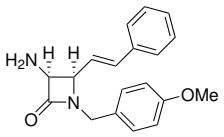
Light-brown amorphous solid. R_f = 0.06 (PE/EtOAc 2/1). Yield after column chromatography (SiO_2, gradient PE/EtOAc 67/33-0/100): 55 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.77 (3H, s, CH_3O); 4.31 (1H, d, J_{AB} = 14.9 Hz, (HCH)N); 4.46 (1H, d x d, J = 9.0, 5.1 Hz, CHCH=CH); 4.65 (1H, d, J_{AB} = 14.9 Hz, (HCH)N); 5.52 (1H, d, J = 5.1 Hz, (C=O)CH); 6.08 (1H, d x d, J = 15.9, 9.0 Hz, CH=CHPh); 6.51 (1H, d, J = 15.9 Hz, CHPh); 6.88 (2H, d, J = 8.4 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.15-7.24 (5H, m, 5 x (CH)CH_{arom}); 7.26 (2H, d, J = 8.4 Hz, 2 x O(CH_{arom})$_{meta}$); 7.68-7.70 (2H, m, 2 x (C=O)(CH_{arom})$_{meta}$); 7.81-7.83 (2H, m, 2 x (C=O)(CH_{arom})$_{ortho}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 44.6 (NCH_2); 55.3 (CH_3O); 58.2 ((C=O)CH); 60.9 (CHCH=CH); 114.2 (2 x O(HC_{arom})$_{ortho}$); 122.8 (CH=CHPh); 123.7 (2 x (C=O)(HC_{arom})$_{ortho}$); 126.7 (2 x CH(HC_{arom})$_{ortho}$); 127.3 ((NCH_2)$C_{arom,quat}$); 128.4 (CH(HC_{arom})$_{para}$); 128.6 (2 x CH(HC_{arom})$_{meta}$); 129.9 (2 x O(HC_{arom})$_{meta}$); 131.6 (2 x (C=O)$C_{arom,quat}$); 134.4 (2 x (C=O)(HC_{arom})$_{meta}$); 135.6 (CH$C_{arom,quat}$); 137.4 (CHPh); 159.3 ($OC_{arom,quat}$); 163.6 ((C=O)CH); 167.4 (2 x (C=O)$C_{arom,quat}$). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1759, 1717; ν_{max} = 1612, 1514, 1387, 1248, 910, 733. **MS** (70 eV): m/z (%) 439 ($[M + H]^+$, 100).

6.3.2 Synthesis of *cis*-3-amino-4-((*E*)-styryl)azetidin-2-ones 26

The synthesis of *cis*-3-amino-1-(4-methoxybenzyl)-4-((*E*)-styryl)azetidin-2-one **26a** and *cis*-3-amino-1-(4-methoxyphenyl)-4-((*E*)-styryl)azetidin-2-one **26b** was identical for both compounds and based on reported procedures for similar structures.[74] As a representative example, the synthesis of *cis*-3-amino-1-(4-methoxybenzyl)-4-((*E*)-styryl)azetidin-2-one **26a** is described. No characterisation data of obtained hydrogenated side products **215** is provided as none were isolated. Syntheses of PMP-substituted β-lactam **26b** are already available in the literature,[76,212,215] but no spectral data was found to verify the data obtained in this Master's thesis, which is therefore provided here as well.

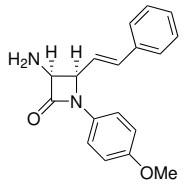
General procedure: 3.07 g *cis*-3-phthalimido-β-lactam ***cis*-25a** (7 mmol, 1 equiv.) was dissolved in methanol (80 mL) and treated with 0.61 mL hydrazine monohydrate (100 %, 12.6 mmol, 1.8 equiv.). After stirring for five hours at reflux temperature, a filtration was performed to remove the precipitated phthalhydrazide side product. The filtrate was evaporated, after which the residue was redissolved in a mixture of tepid water (100 mL, 40 °C) and ethyl acetate (20 mL), and extracted with ethyl acetate (3 x 50 mL). Drying of the combined organic phases with $MgSO_4$, filtration of the drying agent and removal of the solvent *in vacuo* afforded crude *cis*-3-amino-β-lactam **26a** (racemic) quantitatively, which was used without further purification in the subsequent reaction step (C3-amino acylation). During the deprotection, 3 % of starting material ***cis*-25a** was converted to the hydrogenated side product **215a**.

cis-3-Amino-1-(4-methoxybenzyl)-4-((*E*)-styryl)azetidin-2-one 26a



Light-brown oil. Yield: 99 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.79 (2H, br s, NH_2); 3.78 (3H, s, CH_3O); 4.04 (1H, d, J_{AB} = 14.8 Hz, (HCH)N); 4.21 (1H, d x d, J = 7.8, 5.1 Hz, CHCH=CH); 4.34 (1H, d, J = 5.1 Hz, (C=O)CH); 4.61 (1H, d, J_{AB} = 14.8 Hz, (HCH)N); 6.05 (1H, d x d, J = 16.0, 7.8 Hz, CH=CHPh); 6.56 (1H, d, J = 16.0 Hz, CHPh); 6.85 (2H, d, J = 8.1 Hz, 2 x $O(CH_{arom})_{ortho}$); 7.16 (2H, d, J = 8.1 Hz, 2 x $O(CH_{arom})_{meta}$); 7.26-7.34 (5H, m, 5 x (CH)CH$_{arom}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.9 (NCH_2); 55.3 (CH_3O); 60.0 (CHCH=CH); 63.8 ((C=O)CH); 114.2 (2 x $O(HC_{arom})_{ortho}$); 123.7 (CH=CHPh); 126.6 (2 x $CH(HC_{arom})_{ortho}$); 127.7 ($(NCH_2)C_{arom,quat}$); 128.3 ($CH(HC_{arom})_{para}$); 128.7 (2 x $CH(HC_{arom})_{meta}$); 129.9 (2 x $O(HC_{arom})_{meta}$); 135.8 (CHPh); 135.9 ($CHC_{arom,quat}$); 159.2 ($OC_{arom,quat}$); 170.0 (C=O). **IR** (ATR, cm^{-1}): ν_{NH2} = 3277, 3269; $\nu_{C=O}$ = 1738; ν_{max} = 1657, 1599, 1512, 1458, 1246, 1011, 750, 731, 692. **MS** (70 eV): m/z (%) 309 ($[M + H]^+$, 60); 617 ($[2M + H]^+$, 100).

cis-3-Amino-1-(4-methoxyphenyl)-4-((*E*)-styryl)azetidin-2-one 26b



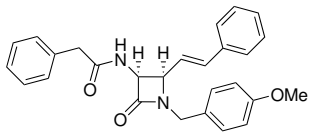
Light-brown oil. R_f = 0.12 (PE/EtOAc 1/2). Yield: 99 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.79 (2H, br s, NH_2); 3.77 (3H, s, CH_3O); 4.52 (1H, d, J = 5.4 Hz, (C=O)CH); 4.82 (1H, ∼t, J = 6.2 Hz, CHCH=CH); 6.27 (1H, d x d, J = 16.1, 7.0 Hz, CH=CHPh); 6.73 (1H, d, J = 16.1 Hz, CHPh); 6.84 (2H, d, J = 8.6 Hz, 2 x $O(CH_{arom})_{ortho}$); 7.26-7.34 (3H, m, $CH(CH_{arom})_{para}$ and 2 x $CH(CH_{arom})_{meta}$); 7.39-7.42 (4H, m, 2 x $CH(CH_{arom})_{ortho}$ and 2 x $O(CH_{arom})_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 55.5 (CH_3O); 60.3 (CHCH=CH); 63.5 ((C=O)CH); 114.4 (2 x $O(HC_{arom})_{ortho}$); 118.5 (2 x $O(HC_{arom})_{meta}$); 123.4 (CH=CHPh); 126.7 (2 x $CH(HC_{arom})_{ortho}$); 128.4 ($CH(HC_{arom})_{para}$); 128.7 (2 x $CH(HC_{arom})_{meta}$); 131.3 ($NC_{arom,quat}$); 135.7 (CHPh and $CHC_{arom,quat}$); 156.2 ($OC_{arom,quat}$); 167.3 (C=O). **IR** (ATR, cm^{-1}): ν_{NH2} = 3385, 3316; $\nu_{C=O}$ = 1732; ν_{max} = 1510, 1244, 829, 694. **MS** (70 eV): m/z (%) 295 ($[M + H]^+$, 90); 589 ($[2M + H]^+$, 100).

6.3.3 Synthesis of *cis*-3-acylamino-4-((*E*)-styryl)azetidin-2-ones 27

The synthesis of *cis*-1-(4-methoxybenzyl)-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one **27a**, *cis*-3-acetamido-1-(4-methoxybenzyl)-4-((*E*)-styryl)azetidin-2-one **27b** and *cis*-1-(4-methoxyphenyl)-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one **27c** was identical for all three compounds. As a representative example, the synthesis of *cis*-1-(4-methoxybenzyl)-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one **27a** is described. Characterisation data of isolated obtained hydrogenated side product *cis*-1-(4-methoxybenzyl)-3-(2-phenylacetamido)-4-(2-phenylethyl)azetidin-2-one **216a** is provided as well.

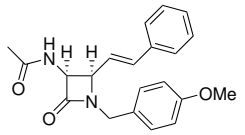
General procedure: 1.23 g *cis*-3-amino-β-lactam **26a** (4 mmol, 1 equiv.) was dissolved in anhydrous CH_2Cl_2 (40 mL) under inert atmosphere (Ar), after which 0.55 mL triethylamine (4 mmol, 1 equiv.) was added. Next, 0.53 mL phenylacetyl chloride (4 mmol, 1 equiv.) was added to the mixture. After stirring for ten minutes at room temperature, the mixture was washed with a saturated aqueous $NaHCO_3$ solution (3 x 50 mL). Drying of the organic phase with $MgSO_4$, filtration of the drying agent and removal of the solvent *in vacuo* afforded crude *cis*-3-acylamino-β-lactam **27a** (racemic) quantitatively, which was used without further purification in the subsequent reaction step (epoxidation). Originating from the hydrogenation side reaction in the previous reaction step (C3-amino deprotection), 3 % of *N*-acylated hydrogenated side product **216a** was formed.

cis-1-(4-Methoxybenzyl)-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one 27a



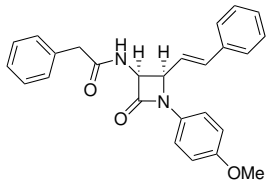
Pale yellow solid. Mp = 104 °C. Yield: 99 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.51 (1H, d, J_{AB} = 16.3 Hz, (C=O)(HCH)); 3.52 (1H, d, J_{AB} = 16.3 Hz, (C=O)(HCH)); 3.77 (3H, s, CH_3O); 3.98 (1H, d, J_{AB} = 14.8 Hz, N(HCH)); 4.29 (1H, d x d, J = 7.3, 5.1 Hz, CHCH=CH); 4.60 (1H, d, J_{AB} = 14.8 Hz, N(HCH)); 5.31 (1H, d x d, J = 8.3, 5.1 Hz, (C=O)CH); 5.76 (1H, d x d, J = 16.0, 7.3 Hz, CH=CHPh); 6.07 (1H, d, J = 8.3 Hz, NH); 6.43 (1H, d, J = 16.0 Hz, CHPh); 6.84 (2H, d, J = 8.2 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.03-7.08 (4H, m, 4 x (C=O)CH_2CH$_{arom}$); 7.11-7.15 (1H, m, (C=O)CH_2(CH$_{arom}$)$_{para}$); 7.12 (2H, d, J = 8.2 Hz, 2 x O(CH_{arom})$_{meta}$); 7.21-7.23 (2H, m, 2 x CH(CH$_{arom}$)$_{ortho}$); 7.30-7.37 (3H, m, CH(CH$_{arom}$)$_{para}$ and 2 x CH(CH$_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.4 ((C=O)CH_2); 44.3 (NCH_2); 55.3 (CH_3O); 59.2 (CHCH=CH); 60.0 ((C=O)CH); 114.3 (2 x O(HC_{arom})$_{ortho}$); 122.3 (CH=CHPh); 126.7 (2 x CH(HC$_{arom}$)$_{ortho}$); 126.9 ((NCH_2)C$_{arom,quat}$); 127.4 ((C=O)CH_2(HC$_{arom}$)$_{para}$); 128.4 (CH(HC$_{arom}$)$_{para}$); 128.7 (2 x CH(HC$_{arom}$)$_{meta}$); 129.0 (2 x (C=O)CH_2(HC$_{arom}$)$_{meta}$); 129.3 (2 x (C=O)CH_2(HC$_{arom}$)$_{ortho}$); 129.8 (2 x O(HC_{arom})$_{meta}$); 133.9 ((C=O)CH_2C$_{arom,quat}$); 135.7 (CHC$_{arom,quat}$); 135.8 (CHPh); 159.3 (OC$_{arom,quat}$); 166.1 ((C=O)CH); 171.0 ((C=O)CH_2). **IR** (ATR, cm^{-1}): ν_{NH} = 3277; $\nu_{C=O}$ = 1763, 1651; ν_{max} = 1512, 1495, 1271, 1252, 1175, 1026, 756, 725, 694, 567, 529. **MS** (70 eV): m/z (%) 252 ($[C_{17}H_{17}NO + H]^+$, 100); 427 ($[M + H]^+$, 6).

cis-3-Acetamido-1-(4-methoxybenzyl)-4-((*E*)-styryl)azetidin-2-one 27b



Pale brown solid. Mp = 139 °C. R_f = 0.27 (PE/EtOAc 1/1). Yield: 99 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.95 (3H, s, (C=O)CH_3); 3.78 (3H, s, CH_3O); 4.10 (1H, d, J_{AB} = 14.8 Hz, N(HCH)); 4.34 (1H, d x d, J = 7.6, 5.1 Hz, CHCH=CH); 4.60 (1H, d, J_{AB} = 14.8 Hz, N(HCH)); 5.32 (1H, d x d, J = 8.1, 5.1 Hz, (C=O)CH); 5.94 (1H, d x d, J = 15.9, 7.6 Hz, CH=CHPh); 6.29 (1H, d, J = 8.1 Hz, NH); 6.53 (1H, d, J = 15.9 Hz, CHPh); 6.85 (2H, d, J = 8.0 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.17 (2H, d, J = 8.0 Hz, 2 x O(CH_{arom})$_{meta}$); 7.26-7.35 (5H, m, 5 x (CH)CH$_{arom}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 22.9 ((C=O)CH_3); 44.4 (NCH_2); 55.3 (CH_3O); 59.7 (CHCH=CH); 60.1 ((C=O)CH); 114.3 (2 x O(HC_{arom})$_{ortho}$); 122.6 (CH=CHPh); 126.6 (2 x CH(HC$_{arom}$)$_{ortho}$); 127.1 ((NCH_2)C$_{arom,quat}$); 128.4 (CH(HC$_{arom}$)$_{para}$); 128.7 (2 x CH(HC$_{arom}$)$_{meta}$); 129.9 (2 x O(HC_{arom})$_{meta}$); 135.8 (CHC$_{arom,quat}$); 136.1 (CHPh); 159.4 (OC$_{arom,quat}$); 166.4 ((C=O)CH); 170.1 ((C=O)CH_3). **IR** (ATR, cm^{-1}): ν_{NH} = 3289; $\nu_{C=O}$ = 1748, 1649; ν_{max} = 1510, 1246, 1177, 1024, 957, 747, 691, 596, 565, 515. **MS** (70 eV): m/z (%) 252 ($[C_{17}H_{17}NO + H]^+$, 100); 351 ($[M + H]^+$, 20).

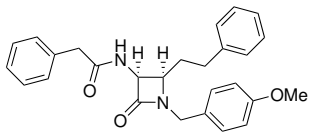
cis-1-(4-Methoxyphenyl)-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one 27c



Pale brown solid. R_f = 0.25 (PE/EtOAc 1/1). Yield: 94 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.55 (1H, d, J_{AB} = 16.5 Hz, (C=O)(HCH)); 3.56 (1H, d, J_{AB} = 16.5 Hz, (C=O)(HCH)); 3.75 (3H, s, CH_3O); 4.88 (1H, ∼t, J = 6.0 Hz, CHCH=CH); 5.50 (1H, d x d, J = 8.4, 5.3 Hz, (C=O)CH); 5.99 (1H, d x d, J = 16.0, 6.8 Hz, CH=CHPh); 6.05-6.07 (1H, m, NH); 6.59 (1H, d, J = 16.0 Hz, CHPh); 6.80 (2H, d, J = 8.3 Hz, 2 x $O(CH_{arom})_{ortho}$); 7.04-7.07 (4H, m, 4 x $(CH_2)CH_{arom}$); 7.09-7.14 (1H, m, $CH_2(CH_{arom})_{para}$); 7.26-7.37 (7H, m, 5 x (CH)CH_{arom} and 2 x $O(CH_{arom})_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.5 (CH_2); 55.5 (CH_3O); 59.4 ((C=O)CH); 59.6 (CHCH=CH); 114.4 (2 x $O(HC_{arom})_{ortho}$); 118.5 (2 x $O(HC_{arom})_{meta}$); 122.0 (CH=CHPh); 126.7 (2 x $CH(HC_{arom})_{ortho}$); 127.5 ($CH_2(HC_{arom})_{para}$); 128.5 ($CH(HC_{arom})_{para}$); 128.7 (2 x $CH(HC_{arom})_{meta}$); 129.0 (2 x $CH_2(HC_{arom})_{meta}$); 129.3 (2 x $CH_2(HC_{arom})_{ortho}$); 130.8 ($NC_{arom,quat}$); 133.8 ($CH_2C_{arom,quat}$); 135.5 ($CHC_{arom,quat}$); 135.8 (CHPh); 156.5 ($OC_{arom,quat}$); 163.0 ((C=O)CH); 171.2 ((C=O)CH_2). **IR** (ATR, cm^{-1}): ν_{NH} = 3264; $\nu_{C=O}$ = 1751, 1653; ν_{max} = 1510, 1497, 1389, 1242, 974, 826, 756, 694, 529. **MS** (70 eV): m/z (%) 238 ($[C_{16}H_{15}NO + H]^+$, 100); 413 ($[M + H]^+$, 20).

cis-1-(4-Methoxybenzyl)-3-(2-phenylacetamido)-4-(2-phenylethyl)azetidin-2-one 216a

Spectral data derived from the mixture of hydrogenated side product **216a** and epoxide major **28Aa** (88/12).



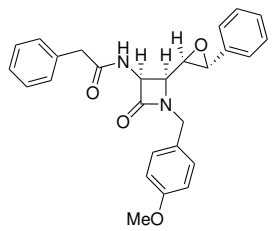
Light-yellow solid. Mp = 128 °C. R_f = 0.19 (PE/EtOAc 1/1). Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 100/0-0/100) and recrystallisation from EtOH: 58 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.46-1.55 (1H, m, (HCH)CH_2Ph); 1.65-1.74 (1H, m, (HCH)CH_2Ph); 2.31-2.44 (2H, m, CH_2CH_2Ph); 3.58 (1H, d, J_{AB} = 16.1 Hz, (C=O)(HCH)); 3.60 (1H, d, J_{AB} = 16.1 Hz, (C=O)(HCH)); 3.61-3.64 (1H, m, CH$(CH_2)_2$); 3.79 (3H, s, CH_3O); 4.04 (1H, d, J_{AB} = 15.1 Hz, N(HCH)); 4.51 (1H, d, J_{AB} = 15.1 Hz, N(HCH)); 5.24 (1H, d x d, J = 8.1, 4.9 Hz, (C=O)CH); 6.47 (1H, d, J = 8.1 Hz, NH); 6.84 (2H, d, J = 8.3 Hz, 2 x $O(CH_{arom})_{ortho}$); 6.93 (2H, d, J = 7.5 Hz, 2 x $(CH_2)_2(CH_{arom})_{ortho}$); 7.09 (2H, d, J = 8.3 Hz, 2 x $O(CH_{arom})_{meta}$); 7.18-7.26 (8H, m, $(CH_2)_2(CH_{arom})_{para}$, 2 x $(CH_2)_2(CH_{arom})_{meta}$ and 5 x (C=O)CH_2CH_{arom}). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 30.2 (CH_2CH_2Ph); 31.8 (CH_2CH_2Ph); 43.5 ((C=O)CH_2); 44.4 (NCH_2); 55.3 (CH_3O); 57.4 ($CHCH_2CH_2$); 58.2 ((C=O)CH); 114.3 (2 x $O(HC_{arom})_{ortho}$); 126.1 ($(CH_2)_2(HC_{arom})_{para}$); 127.0 ($(NCH_2)C_{arom,quat}$); 127.5 ($(C=O)CH_2(HC_{arom})_{para}$); 128.1 (2 x $(CH_2)_2(HC_{arom})_{ortho}$); 128.5 and 129.1 (2 x $(C=O)CH_2(HC_{arom})_{meta}$ and 2 x $(CH_2)_2(HC_{arom})_{meta}$); 129.3 (2 x $(C=O)CH_2(HC_{arom})_{ortho}$); 129.4 (2 x $O(HC_{arom})_{meta}$); 134.2 ($(C=O)CH_2C_{arom,quat}$); 140.7 ($(CH_2)_2C_{arom,quat}$); 159.3 ($OC_{arom,quat}$); 166.6 ((C=O)CH); 171.4 ((C=O)CH_2). **IR** (ATR, cm^{-1}): ν_{NH} = 3273; $\nu_{C=O}$ = 1748, 1655; ν_{max} = 1541, 1512, 1248, 1030, 725, 694, 644, 575, 552, 513. **MS** (70 eV): m/z (%) 429 ($[M + H]^+$, 100).

6.3.4 Synthesis of *cis*-3-acylamino-4-(3-phenyloxiran-2-yl)azetidin-2-ones 28

The synthesis of *cis*-1-(4-methoxybenzyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28Aa** and **28Ba**, *cis*-3-acetamido-1-(4-methoxybenzyl)-4-(3-phenyloxiran-2-yl)azetidin-2-ones **28Ab** and **28Bb**, and *cis*-1-(4-methoxyphenyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-one **28Ca** was identical for all five compounds. As a representative example, the synthesis of *cis*-1-(4-methoxybenzyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-one diastereomers **28Aa** and **28Ba** is described. Wherever possible, characterisation data of a single isolated obtained epoxide diastereomer was collected, though when separation proved to be unfeasible, characterisation was based on data of the diastereomeric mixture. Characterisation data of two isolated obtained *anti*-vicinal diol side products, *i.e.* *cis*-4-(1,2-dihydroxy-2-phenylethyl)-1-(4-methoxybenzyl)-3-(2-phenylacetamido)azetidin-2-ones **220a**, is provided as well.

General procedure: to a refluxing solution of 0.85 g *cis*-3-acylamino-4-((*E*)-styryl)-β-lactam **27a** (2 mmol, 1 equiv.) in CH_2Cl_2 (20 mL), 1.04 g 3-chloroperbenzoic acid (6 mmol, 3 equiv.) was added in small portions. After stirring for three hours at reflux temperature, CH_2Cl_2 (20 mL) and a saturated aqueous Na_2SO_3 solution (40 mL) were added. After stirring for another ten minutes at room temperature, the resulting mixture was washed with a saturated aqueous $NaHCO_3$ solution (2 x 40 mL) and brine (2 x 40 mL), after which the combined aqueous phases were extracted again with CH_2Cl_2 (60 mL). Drying of the combined organic phases with $MgSO_4$, filtration of the drying agent and removal of the solvent *in vacuo* afforded a crude diastereomeric mixture of *cis*-3-acylamino-4-oxiranyl-β-lactams **28Aa** and **28Ba** (racemic, *dr* = 59/41) in 93 % yield, which was used without further purification in the subsequent reaction step (amido group-induced intramolecular ring closure through epoxide ring opening). Complete separation of the two diastereomers **28Aa** and **28Ba** by means of column chromatography over silica gel (gradient petroleum ether/ethyl acetate 67/33-33/67) or reversed phase automated column chromatography (C18, gradient water/acetonitrile 100/0-0/100) both proved to be impossible. However, isolation of the major epoxide diastereomer **28Aa** could be achieved in 38 % yield *via* column chromatography over silica gel (gradient petroleum ether/ethyl acetate 67/33-33/67), or in 24 % yield *via* reversed phase automated column chromatography (C18, gradient water/acetonitrile 100/0-0/100).

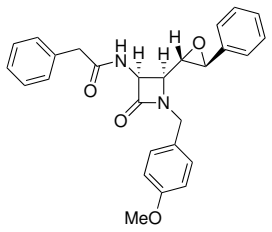
(3*S,4*S**,2'*R**,3'*R**)-1-(4-Methoxybenzyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)-azetidin-2-one 28Aa**



Pale white solid. Mp = 141 °C. R_f = 0.07 (PE/EtOAc 2/1). Yield after column chromatography (SiO_2, gradient PE/EtOAc 67/33-33/67): 38 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.72 (1H, d x d, J = 7.1, 1.3 Hz, C$\underline{H}$OCHPh); 3.30 (1H, d, J_{AB} = 16.3 Hz, (C=O)(C$\underline{H}$CH)); 3.37 (1H, d, J_{AB} = 16.3 Hz, (C=O)(HC$\underline{H}$)); 3.46 (1H, d x d, J = 7.1, 5.3 Hz, C$\underline{H}$CHOCH); 3.60 (1H, d, J = 1.3 Hz, C$\underline{H}$Ph); 3.80 (3H, s, CH_3O); 4.18 (1H, d, J_{AB} = 14.7 Hz, N(C$\underline{H}$CH)); 4.61 (1H, d, J_{AB} = 14.7 Hz, N(HC$\underline{H}$)); 5.13 (1H, d x d, J = 6.9, 5.3 Hz, (C=O)CH); 6.17 (1H, d, J = 6.9 Hz, NH); 6.70 (2H, d, J = 7.4 Hz, 2 x (C=O)CH_2(C$\underline{H}_{arom}$)$_{ortho}$); 6.89 (2H, d, J = 8.4 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.11-7.17 (2H, m, 2 x (C=O)CH_2(C$\underline{H}_{arom}$)$_{meta}$); 7.17-7.22 (3H, m, (C=O)CH_2(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.24 (2H, d, J = 8.4 Hz, 2 x O(CH_{arom})$_{meta}$); 7.34-7.40 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.2 ((C=O)$\underline{C}H_2$); 45.3 (NCH_2); 55.3 (CH_3O); 55.5 ($\underline{C}$HPh); 58.4 ((C=O)$\underline{C}$H); 59.3 ($\underline{C}$HCHOCH); 61.0 ($\underline{C}$HOCHPh); 114.3 (2 x O(HC_{arom})$_{ortho}$); 125.6 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 126.8 ((NCH_2)$\underline{C}_{arom,quat}$); 127.5 ((C=O)$CH_2$(H$\underline{C}_{arom}$)$_{para}$); 128.65 and 128.66 (CH(H$\underline{C}_{arom}$)$_{para}$ and 2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 129.1 (2 x (C=O)CH_2(H$\underline{C}_{arom}$)$_{meta}$); 129.3 (2 x (C=O)CH_2(H$\underline{C}_{arom}$)$_{ortho}$); 130.0 (2 x O(HC_{arom})$_{meta}$); 133.5 ((C=O)$CH_2\underline{C}_{arom,quat}$); 136.0 (CH$\underline{C}_{arom,quat}$); 159.5 ($OC_{arom,quat}$); 165.4 (($\underline{C}$=O)CH); 171.4 (($\underline{C}$=O)$CH_2$). **IR** (ATR, cm^{-1}): ν_{NH} = 3291; $\nu_{C=O}$ = 1759, 1655; ν_{max} = 1512, 1267, 1250, 1175, 1028, 831, 745, 725, 692, 571, 542, 513. **MS** (70 eV): m/z (%) 443 ($[M + H]^+$, 100).

(3*S,4*S**,2'*S**,3'*S**)-1-(4-Methoxybenzyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)-azetidin-2-one 28Ba**

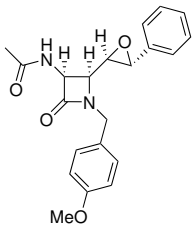
Spectral data derived from the mixture of two diastereomers **28Aa**/**28Ba** (*dr* = 59/41).



Golden yellow oil. R_f = 0.16 (PE/EtOAc 1/1). Yield: 93 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.96 (1H, d x d, J = 4.6, 1.4 Hz, C$\underline{H}$OCHPh); 3.38 (1H, d, J_{AB} = 15.3 Hz, (C=O)(H$\underline{C}$H)); 3.40 (1H, d, J_{AB} = 15.3 Hz, (C=O)(HC$\underline{H}$)); 3.66 (1H, d, J = 1.4 Hz, C$\underline{H}$Ph); 3.72 (3H, s, CH_3O); 3.74-3.76 (1H, m, C$\underline{H}$CHOCH); 4.09 (1H, d, J_{AB} = 14.6 Hz, N($\underline{H}$CH)); 4.48 (1H, d, J_{AB} = 14.6 Hz, N(HC$\underline{H}$)); 5.07 (1H, d x d, J = 6.9, 5.1 Hz, (C=O)CH); 6.79 (2H, d, J = 8.4 Hz, 2 x O(CH_{arom})$_{ortho}$); 6.98-7.00 (2H, m, 2 x (C=O)CH_2(C$\underline{H}_{arom}$)$_{ortho}$); 7.07-7.11 (2H, m, 2 x (C=O)CH_2(C$\underline{H}_{arom}$)$_{meta}$); 7.13-7.15 (2H, m, 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.13-7.16 (1H, m, (C=O)CH_2(C$\underline{H}_{arom}$)$_{para}$); 7.14-7.16 (2H, m, 2 x O(CH_{arom})$_{meta}$); 7.29-7.31 (1H, m, NH); 7.31-7.36 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.1 ((C=O)$\underline{C}H_2$); 45.4 (NCH_2); 55.2 ($\underline{C}$HPh and CH_3O); 56.7 ($\underline{C}$HCHOCH); 58.2 ((C=O)$\underline{C}$H); 60.3 ($\underline{C}$HOCHPh); 114.3 (2 x O(HC_{arom})$_{ortho}$); 125.8 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 126.6 ((NCH_2)$\underline{C}_{arom,quat}$); 127.2 ((C=O)$CH_2$(H$\underline{C}_{arom}$)$_{para}$); 128.5 (2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 128.6 (CH(H$\underline{C}_{arom}$)$_{para}$); 128.8 (2 x (C=O)$CH_2$(H$\underline{C}_{arom}$)$_{meta}$); 129.3 (2 x (C=O)$CH_2$(H$\underline{C}_{arom}$)$_{ortho}$); 129.8 (2 x O($HC_{arom}$)$_{meta}$); 134.2 ((C=O)$CH_2\underline{C}_{arom,quat}$); 136.0 (CH$\underline{C}_{arom,quat}$); 159.4 ($OC_{arom,quat}$); 166.5 (($\underline{C}$=O)CH); 171.8 (($\underline{C}$=O)$CH_2$). **IR** (ATR, cm^{-1}): ν_{NH} = 3281; $\nu_{C=O}$ = 1744, 1659; ν_{max} = 1512, 1497, 1244, 1177, 1030, 750, 725, 696, 519. **MS** (70 eV): m/z (%) 443 ($[M + H]^+$, 100).

(3*S,4*S**,2'*R**,3'*R**)-3-Acetamido-1-(4-methoxybenzyl)-4-(3-phenyloxiran-2-yl)-azetidin-2-one 28Ab**

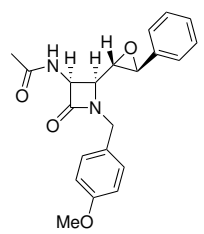
The product is contaminated with 43 % 2,6-dimethyl-4-*tert*-butylphenol (BHT) due to solvent contamination during column chromatography with tetrahydrofuran, which contained BHT as an anti-oxidant.



Pale white solid. R_f = 0.18 (PE/EtOAc 1/2). Yield after column chromatography (SiO_2, gradient PE/EtOAc 60/40-33/67): 40 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.79 (3H, s, (C=O)CH_3); 2.83 (1H, d x d, J = 6.6, 1.3 Hz, C$\underline{H}$OCHPh); 3.57 (1H, d x d, J = 6.6, 5.4 Hz, C$\underline{H}$CHOCH); 3.63 (1H, d, J = 1.3 Hz, C$\underline{H}$Ph); 3.82 (3H, s, CH_3O); 4.30 (1H, d, J_{AB} = 14.7 Hz, N($\underline{H}$CH)); 4.61 (1H, d, J_{AB} = 14.7 Hz, N(HC$\underline{H}$)); 5.21 (1H, d x d, J = 6.7, 5.4 Hz, (C=O)CH); 6.20 (1H, d, J = 6.7 Hz, NH); 6.91 (2H, d, J = 8.4 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.18-7.19 (2H, m, 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.27-7.30 (2H, m, 2 x O(CH_{arom})$_{meta}$); 7.30-7.34 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 22.6 ((C=O)$\underline{C}H_3$); 45.4 (NCH_2); 55.3 (CH_3O); 55.5 ($\underline{C}$HPh); 58.5 ((C=O)$\underline{C}$H); 59.5 ($\underline{C}$HCHOCH); 60.6 ($\underline{C}$HOCHPh); 114.4 (2 x O(HC_{arom})$_{ortho}$); 125.4 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 126.9 ((NCH_2)$\underline{C}_{arom,quat}$); 128.4 (CH(H$\underline{C}_{arom}$)$_{para}$); 128.5 (2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 130.0 (2 x O($HC_{arom}$)$_{meta}$); 135.9 (CH$\underline{C}_{arom,quat}$); 159.5 ($OC_{arom,quat}$); 165.8 (($\underline{C}$=O)CH); 170.5 (($\underline{C}$=O)$CH_3$). **MS** (70 eV): m/z (%) 367 ($[M + H]^+$, 100).

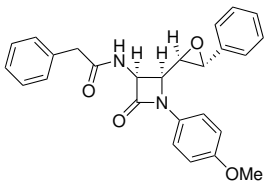
(3*S,4*S**,2'*S**,3'*S**)-3-Acetamido-1-(4-methoxybenzyl)-4-(3-phenyloxiran-2-yl)-azetidin-2-one 28Bb**

Spectral data derived from the mixture of two diastereomers **28Ab**/**28Bb** (dr = 57/43), which had a purity of 90 % as determined by ^{1}H NMR spectroscopy (in $CDCl_3$).



Golden yellow oil. Yield: 99 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.84 (3H, s, (C=O)CH_3); 3.05 (1H, d x d, J = 4.6, 1.4 Hz, C$\underline{H}$OCHPh); 3.73 (1H, br s, C$\underline{H}$Ph); 3.74 (3H, s, CH_3O); 3.81-3.83 (1H, m, C$\underline{H}$CHOCH); 4.17 (1H, d, J_{AB} = 14.6 Hz, N(C$\underline{H}$H)); 4.52 (1H, d, J_{AB} = 14.6 Hz, N(HC$\underline{H}$)); 5.14 (1H, d x d, J = 6.8, 5.3 Hz, (C=O)CH); 6.80 (2H, d, J = 8.3 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.18-7.21 (4H, m, 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$ and 2 x O(CH_{arom})$_{meta}$); 7.28-7.32 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$); 7.40-7.42 (1H, m, NH). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 22.5 ((C=O)$\underline{C}H_3$); 45.4 (NCH_2); 55.1 ($\underline{C}$HPh); 55.2 (CH_3O); 56.8 ($\underline{C}$HCHOCH); 58.3 ((C=O)$\underline{C}$H); 60.2 ($\underline{C}$HOCHPh); 114.3 (2 x O(HC_{arom})$_{ortho}$); 125.6 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 126.6 ((NCH_2)$\underline{C}_{arom,quat}$); 128.49 and 128.52 (CH(H$\underline{C}_{arom}$)$_{para}$ and 2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 129.8 (2 x O(HC_{arom})$_{meta}$); 135.9 (CH$\underline{C}_{arom,quat}$); 159.4 ($OC_{arom,quat}$); 166.7 ((C=O)CH); 170.9 ((C=O)CH_3). **MS** (70 eV): m/z (%) 367 ($[M + H]^+$, 100).

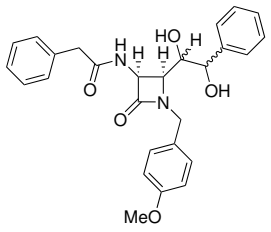
(3*S,4*S**,2'*R**,3'*R**)-1-(4-Methoxyphenyl)-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)-azetidin-2-one 28Ac**



Pale brown solid. R_f = 0.09 (PE/EtOAc 2/1). Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 90/10-0/100): 43 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.94 (1H, d x d, J = 6.9, 1.4 Hz, C$\underline{H}$OCHPh); 3.31 (1H, d, J_{AB} = 16.4 Hz, (C=O)(C$\underline{H}$H)); 3.41 (1H, d, J_{AB} = 16.4 Hz, (C=O)(HC$\underline{H}$)); 3.78 (3H, s, CH_3O); 3.79 (1H, ∼s, C$\underline{H}$Ph); 4.01 (1H, ∼t, J = 6.1 Hz, C$\underline{H}$CHOCH); 5.36 (1H, d x d, J = 7.1, 5.7 Hz, (C=O)CH); 6.28 (1H, d, J = 7.1 Hz, NH); 6.69 (2H, d, J = 7.2 Hz, 2 x CH_2(C$\underline{H}_{arom}$)$_{ortho}$); 6.85 (2H, d, J = 8.7 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.14-7.20 (3H, m, 2 x CH_2(C$\underline{H}_{arom}$)$_{meta}$ and CH_2(C$\underline{H}_{arom}$)$_{para}$); 7.25-7.27 (2H, m, 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.37-7.41 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$); 7.44 (2H, d, J = 8.7 Hz, 2 x O(CH_{arom})$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.3 (CH_2); 55.5 (CH_3O); 56.2 ($\underline{C}$HPh); 57.9 ((C=O)$\underline{C}$H); 60.0 ($\underline{C}$HCHOCH); 60.7 ($\underline{C}$HOCHPh); 114.5 (2 x O(HC_{arom})$_{ortho}$); 118.5 (2 x O(HC_{arom})$_{meta}$); 125.7 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 127.6 (CH_2(H$\underline{C}_{arom}$)$_{para}$); 128.7 (2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 128.8 (CH(H$\underline{C}_{arom}$)$_{para}$); 129.1 (2 x CH_2(H$\underline{C}_{arom}$)$_{meta}$); 129.4 (2 x CH_2(H$\underline{C}_{arom}$)$_{ortho}$); 130.6 ($NC_{arom,quat}$); 133.4 ($CH_2\underline{C}_{arom,quat}$); 135.8 (CH$\underline{C}_{arom,quat}$); 156.8 ($OC_{arom,quat}$); 162.3 ((C=O)CH); 171.6 ((C=O)CH_2). **IR** (ATR, cm^{-1}): ν_{NH} = 3273; $\nu_{C=O}$ = 1748, 1655; ν_{max} = 1510, 1248, 1113, 1030, 831, 739, 700, 525. **MS** (70 eV): m/z (%) 429 ($[M + H]^+$, 100).

(3*S,4*S**,1'*R**,2'*S**)- and (3*S**,4*S**,1'*S**,2'*R**)-4-(1,2-Dihydroxy-2-phenylethyl)-1-(4-methoxybenzyl)-3-(2-phenylacetamido)azetidin-2-ones 220a**

Spectral data derived from the mixture of two *anti*-diastereomers (*dr* = 79/21), which had a purity of 90 % as determined by ^{1}H NMR spectroscopy (in $CDCl_3$).



Golden yellow solid. R_f = 0.13 (PE/EtOAc 2/1). Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 90/10-0/100): 16 %. *Major diastereomer.* **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.27 and 2.69 (2H, br s, 2 x OH); 3.33 (1H, d, J = 6.0 Hz, C$\underline{H}$CHPh); 3.51 (1H, d, J_{AB} = 15.4 Hz, (C=O)(N$\underline{H}$CH)); 3.54 (1H, d, J_{AB} = 15.4 Hz, (C=O)(HC$\underline{H}$)); 3.79 (3H, s, CH_3O); 3.88 (1H, d, J = 5.3 Hz, C$\underline{H}$(CHOH)$_2$); 4.27 (1H, d, J_{AB} = 15.2 Hz, N($\underline{H}$CH)); 4.47 (1H, d, J = 6.0 Hz, C$\underline{H}$Ph); 4.62 (1H, d, J_{AB} = 15.2 Hz, N(HC$\underline{H}$)); 5.32 (1H, d x d, J = 9.8, 5.3 Hz, (C=O)CH); 6.69 (1H, d, J = 9.8 Hz, NH); 6.85 (2H, d, J = 8.2 Hz, 2 x O(C$\underline{H}_{arom}$)$_{ortho}$); 7.02-7.05 (2H, m, 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.09-7.15 (1H, m, (C=O)CH$_2$(C$\underline{H}_{arom}$)$_{para}$); 7.11-7.21 (6H, m, 2 x O(C$\underline{H}_{arom}$)$_{meta}$ and 2 x (C=O)CH$_2$(C$\underline{H}_{arom}$)$_{ortho}$ and 2 x (C=O)CH$_2$(C$\underline{H}_{arom}$)$_{meta}$); 7.24-7.37 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.6 ((C=O)$\underline{C}$H$_2$); 45.5 (NCH$_2$); 55.3 (CH_3O); 56.5 ($\underline{C}$H(CHOH)$_2$); 56.8 ((C=O)$\underline{C}$H); 72.7 ($\underline{C}$HCHPh); 75.0 ($\underline{C}$HPh); 114.2 (2 x O(H$\underline{C}_{arom}$)$_{ortho}$); 126.3 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 127.2 ((C=O)CH$_2$(H$\underline{C}_{arom}$)$_{para}$); 128.1 ((NCH$_2$)$\underline{C}_{arom,quat}$); 128.2 (CH(H$\underline{C}_{arom}$)$_{para}$); 128.6 (2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 128.9 (2 x (C=O)CH$_2$(H$\underline{C}_{arom}$)$_{meta}$); 129.3 (2 x (C=O)CH$_2$(H$\underline{C}_{arom}$)$_{ortho}$); 129.4 (2 x O(H$\underline{C}_{arom}$)$_{meta}$); 134.6 ((C=O)CH$_2\underline{C}_{arom,quat}$); 139.9 (CH$\underline{C}_{arom,quat}$); 159.3 (O$\underline{C}_{arom,quat}$); 168.7 (($\underline{C}$=O)CH); 171.6 (($\underline{C}$=O)CH$_2$). *Minor diastereomer.* **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.82 (2H, br s, 2 x OH); 3.58 (2H, s, (C=O)CH$_2$); 3.77 (3H, s, CH_3O); 3.77-3.79 (1H, m, C$\underline{H}$CHPh); 4.10-4.14 (2H, m, N($\underline{H}$CH) and C$\underline{H}$(CHOH)$_2$); 4.27-4.29 (1H, m, C$\underline{H}$Ph); 4.40 (1H, d, J = 15.1 Hz, N(HC$\underline{H}$)); 5.50 (1H, d x d, J = 9.8, 5.3 Hz, (C=O)CH); 6.82 (2H, d, J = 8.5 Hz, 2 x O(C$\underline{H}_{arom}$)$_{ortho}$); 7.02-7.05 (2H, m, 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.09-7.11 (1H, m, NH); 7.11-7.21 (6H, m, 2 x O(C$\underline{H}_{arom}$)$_{meta}$ and 2 x (C=O)CH$_2$(C$\underline{H}_{arom}$)$_{ortho}$ and 2 x (C=O)CH$_2$(C$\underline{H}_{arom}$)$_{meta}$); 7.24-7.26 (1H, m, (C=O)CH$_2$(C$\underline{H}_{arom}$)$_{para}$); 7.24-7.37 (3H, m, CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 43.6 ((C=O)$\underline{C}$H$_2$); 44.1 (NCH$_2$); 55.3 (CH_3O); 57.1 ((C=O)$\underline{C}$H); 57.8 ($\underline{C}$H(CHOH)$_2$); 72.2 ($\underline{C}$HCHPh); 73.6 ($\underline{C}$HPh); 114.3 (2 x O(H$\underline{C}_{arom}$)$_{ortho}$); 126.7 (2 x CH(H$\underline{C}_{arom}$)$_{ortho}$); 127.2 ((NCH$_2$)$\underline{C}_{arom,quat}$); 127.4 ((C=O)CH$_2$(H$\underline{C}_{arom}$)$_{para}$); 128.4 (CH(H$\underline{C}_{arom}$)$_{para}$); 128.6 (2 x CH(H$\underline{C}_{arom}$)$_{meta}$); 129.0 (2 x (C=O)CH$_2$(H$\underline{C}_{arom}$)$_{meta}$); 129.5 and 129.6 (2 x (C=O)CH$_2$(H$\underline{C}_{arom}$)$_{ortho}$ and 2 x O(H$\underline{C}_{arom}$)$_{meta}$); 134.1 ((C=O)CH$_2\underline{C}_{arom,quat}$); 141.0 (CH$\underline{C}_{arom,quat}$); 159.2 (O$\underline{C}_{arom,quat}$); 167.9 (($\underline{C}$=O)CH); 171.4 (($\underline{C}$=O)CH$_2$). **MS** (70 eV): m/z (%) 461 ([M + H]$^+$, 100).

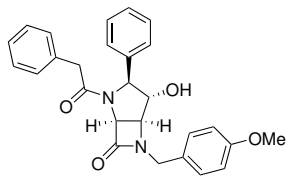
6.3.5 Synthesis of 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones 30

The synthesis 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 **30Aa** and (1*S**,3*S**,4*S**,5*S**)-2-acetyl-4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-one **30Ab** was identical for both compounds. As a representative example, the synthesis of (1*S**,3*S**,4*S**,5*S**)-2-acetyl-4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2,6-diazabicyclo[3.2.0]-heptan-7-one **30Ab** is described. Even though the starting material contained the two epoxide diastereomers for both compounds (**28Aa** /**28Ba** = 95/5 and **28Ab**/**28Bb** = 57/43), only conversion of the major diastereomers **28Aa** and **28Ab** towards the 3,4-pyrrolidine-fused bicyclic β-lactams **30Aa**, respectively **30Ab** was observed. In addition, a loss of signals of the minor epoxides **28Ba** and **28Bb** occurred. The structure of compound **30Aa** was confirmed by X-ray crystallography.

General procedure: 0.73 g *cis*-3-acylamino-4-oxiranyl-β-lactam mixture **28Ab**/**28Bb** in 57/43 ratio (2 mmol, 1 equiv.) was dissolved in *tert*-butanol (80 mL), after which 1.04 g potassium *tert*-butoxide (6 mmol, 3 equiv.) was added in small portions. After stirring for twenty hours at 35 °C, HCl (1 M, 40 mL) was added. After stirring for another ten minutes at room temperature, CH_2Cl_2 (80 mL) was added. The resulting mixture was washed with tepid water (5 x 80 mL, 40 °C) and brine (80 mL), after which the combined aqueous phases were extracted again with CH_2Cl_2 (80 mL). Drying of the combined organic phases with $MgSO_4$, filtration of the drying agent and removal of the solvent *in vacuo* afforded crude 3,4-pyrrolidine-fused bicyclic β-lactam **30Ab** (racemic), which was purified in 18 % yield by means of reversed phase automated column chromatography (C18, gradient water/acetonitrile 100/0-0/100).

(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 30Aa**

Spectral data derived from the mixture of two rotamers (62/38 in $CDCl_3$, 67/33 in d_6-DMSO).



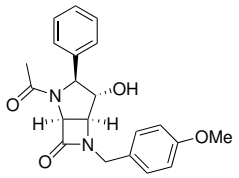
White crystalline solid. Mp = 200 °C. R_f = 0.13 (PE/EtOAc 1/2). Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 100/0-0/100): 34 %. *Major rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.72 (3H, s, CH_3O); 3.79 (1H, d, J_{AB} = 14.1 Hz, N(HCH)); 3.90 (1H, d, J_{AB} = 15.2 Hz, (C=O)(HCH)); 3.93 (1H, d, J_{AB} = 14.1 Hz, N(HCH)); 3.95 (1H, d, J_{AB} = 15.2 Hz, (C=O)(HCH)); 4.03 (1H, d, J = 4.0 Hz, (C=O)CHCH); 4.06 (1H, d, J = 3.3 Hz, CHOH); 5.40 (1H, s, NCHPh); 5.67 (1H, d, J = 4.0 Hz, (C=O)CH); 5.70 (1H, d, J = 3.3 Hz, OH); 6.69-6.77 (4H, m, 2 x $O(CH_{arom})_{ortho}$ and 2 x $O(CH_{arom})_{meta}$); 7.07-7.09 (2H, m, 2 x $NCH(CH_{arom})_{ortho}$); 7.20-7.23 (1H, m, $(C=O)CH_2(CH_{arom})_{para}$); 7.25-7.29 (2H, m, 2 x $NCH(CH_{arom})_{meta}$); 7.25-7.29 (1H, m, $NCH(CH_{arom})_{para}$); 7.31-7.33 (2H, m, 2 x $(C=O)CH_2(CH_{arom})_{meta}$); 7.32-7.33 (2H, m, 2 x $(C=O)CH_2(CH_{arom})_{ortho}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.8 ($(C=O)CH_2$); 44.1 (NCH_2); 55.5 (CH_3O); 64.9 ($(C=O)CHCH$); 69.5 ($(C=O)CH$); 72.0 (NCHPh); 75.1 (CHOH); 114.4 (2 x $O(HC_{arom})_{ortho}$); 125.3 (2 x $NCH(HC_{arom})_{ortho}$); 126.99 and 127.01 ($(C=O)CH_2(HC_{arom})_{para}$ and $NCH(HC_{arom})_{para}$); 127.3 ($(NCH_2)C_{arom,quat}$); 128.6 (2 x $NCH(HC_{arom})_{meta}$); 128.7 (2 x $(C=O)CH_2(HC_{arom})_{meta}$); 129.8 (2 x $O(HC_{arom})_{meta}$); 130.0 (2 x $(C=O)CH_2(HC_{arom})_{ortho}$); 135.7 ($(C=O)CH_2C_{arom,quat}$); 139.2 ($(NCH)C_{arom,quat}$); 158.9 ($OC_{arom,quat}$); 165.3 ($(C=O)CH$); 169.4 ($(C=O)CH_2$). *Minor rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.33-3.39 (1H, m, (C=O)(HCH)); 3.57 (1H, d, J = 15.6 Hz, (C=O)(HCH)); 3.72 (3H, s, CH_3O); 3.73-3.77 (1H, m, N(HCH)); 3.88 (1H, d, J = 16.2 Hz, N(HCH)); 3.90-3.92 (1H, m, (C=O)CHCH); 4.11 (1H, d, J = 3.8 Hz, CHOH); 5.40 (1H, s, NCHPh); 5.61 (1H, d, J = 3.9 Hz, (C=O)CH); 5.75 (1H, d, J = 3.8 Hz, OH); 6.69-6.75 (4H, m, 2 x $O(CH_{arom})_{ortho}$ and 2 x $O(CH_{arom})_{meta}$); 7.07-7.09 (2H, m, 2 x $(C=O)CH_2(CH_{arom})_{ortho}$); 7.20-7.23 (2H, m, 2 x $NCH(CH_{arom})_{ortho}$); 7.25-7.29 (2H, m, 2 x $NCH(CH_{arom})_{meta}$); 7.31-7.33 (2H, m, 2 x $(C=O)CH_2(CH_{arom})_{meta}$); 7.32-7.33 (1H, m, $(C=O)CH_2(CH_{arom})_{para}$); 7.38-7.41 (1H, m, $NCH(CH_{arom})_{para}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.3 ($(C=O)CH_2$); 44.0 (NCH_2); 55.5 (CH_3O); 63.2 ($(C=O)CHCH$); 68.9 ($(C=O)CH$); 73.2 (NCHPh); 76.7 (CHOH); 114.4 (2 x $O(HC_{arom})_{ortho}$); 125.3 (2 x $NCH(HC_{arom})_{ortho}$); 126.9 ($(C=O)CH_2(HC_{arom})_{para}$); 127.4 ($(NCH_2)C_{arom,quat}$); 127.6 ($NCH(HC_{arom})_{para}$); 128.7 (2 x $(C=O)CH_2(HC_{arom})_{meta}$); 129.1 (2 x $NCH(HC_{arom})_{meta}$); 129.76 and 129.79 (2 x $(C=O)CH_2(HC_{arom})_{ortho}$ and 2 x $O(HC_{arom})_{meta}$); 135.4 ($(C=O)CH_2C_{arom,quat}$); 139.3 ($(NCH)C_{arom,quat}$); 158.9 ($OC_{arom,quat}$); 165.2 ($(C=O)CH$); 170.0 ($(C=O)CH_2$). **IR** (ATR, cm^{-1}): ν_{OH} = 3374; $\nu_{C=O}$ = 1740, 1628; ν_{max} = 1512, 1429, 1408, 1246, 1177, 735, 708, 698, 584, 569. **MS** (70 eV): m/z (%) 443 ($[M + H]^+$, 100).

Single crystal X-ray data of compound (1*S,3*S**,4*S**,5*S**)-30Aa**

$C_{27}H_{26}N_2O_4$, M = 442.50 g mol^{-1}, T = 100(1) K, monoclinic, space group P2$_1$/n, a = 17.1940(10) Å, b = 6.3085(2) Å, c = 22.0858(11) Å, α= 90 °, β= 111.815(6) °, γ = 90 °, V = 2224.1(2) Å^3, Z = 4, ρ_{calc} = 1.322 g cm^{-3}, μ = 0.721 mm^{-1}, $F(000)$ = 936.0, CuKα radiation (λ = 1.54184 Å), 16737 reflections collected (5.612 ° ≤ 2Θ ≤ 133.194 °), 3928 independent reflections (R_{int} = 0.1376, R_{sigma} = 0.1147), which were used in all calculations. The goodness-of-fit on F^2 was 0.983, the final *R1* was 0.0677 ($I \geq 2\sigma$ (I)) and 0.1302 (all data), and the final *wR2* was 0.1579 ($I \geq 2\sigma$ (I)) and 0.2063 (all data). The absolute configuration of the structure was established as C1(R), C3(R), C4(R) and C5(R), but because of the centrosymmetric space group P2$_1$/n, 50 % of the product has an absolute configuration of C1(S), C3(S), C4(S) and C5(S), thus confirming that the obtained compound is indeed racemic. It was found that a hydrogen-bond network is formed between the hydroxyl group, attached to carbon C4, and the carbonyl of the acyl group, attached to nitrogen N2, yielding chains of molecules in the b direction of the crystal.

(1*S,3*S**,4*S**,5*S**)-2-Acetyl-4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-one 30Ab**

Spectral data derived from the mixture of two rotamers (60/40 in $CDCl_3$).



Pale white solid. Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 100/0-0/100): 18 %. *Major rotamer.* **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.31 (3H, s, (C=O)CH_3); 3.60 (1H, d, J_{AB} = 14.8 Hz, N(C$\underline{H}$CH)); 3.76 (3H, s, CH_3O); 3.95 (1H, d, J_{AB} = 14.8 Hz, N(HC$\underline{H}$)); 4.03 (1H, d, J = 3.7 Hz, (C=O)CHC$\underline{H}$); 4.17 (1H, s, C$\underline{H}$OH); 4.87 (1H, br s, OH); 5.15 (1H, d, J = 3.7 Hz, (C=O)CH); 5.63 (1H, s, NC$\underline{H}$Ph); 6.66-6.74 (4H, m, 2 x O(CH_{arom})$_{ortho}$ and 2 x O(CH_{arom})$_{meta}$); 7.08 (2H, d, J = 7.6 Hz, 2 x NCH(C$\underline{H}_{arom}$)$_{ortho}$); 7.21-7.23 (1H, m, NCH(C$\underline{H}_{arom}$)$_{para}$); 7.26-7.30 (2H, m, 2 x NCH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 22.6 ((C=O)C$\underline{H}_3$); 44.5 (NCH_2); 55.3 (CH_3O); 64.4 ((C=O)CH$\underline{C}$H); 69.7 ((C=O)$\underline{C}$H); 71.4 (N$\underline{C}$HPh); 75.3 (CHOH); 114.3 (2 x O(HC_{arom})$_{ortho}$); 124.9 (2 x NCH(H$\underline{C}_{arom}$)$_{ortho}$); 125.9 ((NCH_2)$\underline{C}_{arom,quat}$); 127.1 (NCH(H$\underline{C}_{arom}$)$_{para}$); 128.7 (2 x NCH(H$\underline{C}_{arom}$)$_{meta}$); 129.7 (2 x O(H$C_{arom}$)$_{meta}$); 137.2 ((NCH)$\underline{C}_{arom,quat}$); 159.2 (O$C_{arom,quat}$); 164.3 ((C=O)CH); 170.4 ((C=O)CH_3). *Minor rotamer.* **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.91 (3H, s, (C=O)CH_3); 3.71 (1H, d, J_{AB} = 14.8 Hz, N(C$\underline{H}$CH)); 3.76 (3H, s, CH_3O); 3.84 (1H, d, J = 3.7 Hz, (C=O)CHC$\underline{H}$); 3.88 (1H, d, J_{AB} = 14.8 Hz, N(HC$\underline{H}$)); 4.09 (1H, s, C$\underline{H}$OH); 4.87 (1H, br s, OH); 5.23 (1H, s, NC$\underline{H}$Ph); 5.61 (1H, d, J = 3.7 Hz, (C=O)CH); 6.66-6.74 (4H, m, 2 x O(CH_{arom})$_{ortho}$ and 2 x O(CH_{arom})$_{meta}$); 7.18 (2H, d, J = 7.8 Hz, 2 x NCH(C$\underline{H}_{arom}$)$_{ortho}$); 7.28-7.30 (1H, m, NCH(C$\underline{H}_{arom}$)$_{para}$); 7.34-7.38 (2H, m, 2 x NCH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 21.8 ((C=O)C$\underline{H}_3$); 44.5 (NCH_2); 55.3 (CH_3O); 63.3 ((C=O)CH$\underline{C}$H); 68.4 ((C=O)$\underline{C}$H); 73.7 (N$\underline{C}$HPh); 77.1 (CHOH); 114.2 (2 x O(HC_{arom})$_{ortho}$); 124.8 (2 x NCH(H$\underline{C}_{arom}$)$_{ortho}$); 126.1 ((NCH_2)$\underline{C}_{arom,quat}$); 127.6 (NCH(H$\underline{C}_{arom}$)$_{para}$); 129.0 (2 x NCH(H$\underline{C}_{arom}$)$_{meta}$); 129.7 (2 x O(H$C_{arom}$)$_{meta}$); 137.7 ((NCH)$\underline{C}_{arom,quat}$); 159.1 (O$C_{arom,quat}$); 164.8 ((C=O)CH); 171.1 ((C=O)CH_3). **IR** (ATR, cm^{-1}): ν_{OH} = 3372; $\nu_{C=O}$ = 1748, 1622; ν_{max} = 1516, 1441, 1395, 1246, 1175, 1069, 1024, 733, 698, 642, 559, 488. **MS** (70 eV): m/z (%) 367 ([M + H]$^+$, 100).

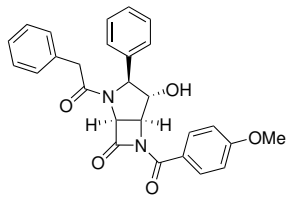
6.3.6 Synthesis of 2-acyl-4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones 32

The synthesis of (1*S**,3*S**,4*S**,5*S**)-4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **32Aa** and (1*S**,3*S**,4*S**,5*S**)-2-acetyl-4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-one **32Ab** was identical for both compounds and based on reported procedures.[74] As a representative example, the synthesis of (1*S**,3*S**,4*S**,5*S**)-4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **32Aa** is described. The work-up procedure of compound **32Aa** differs from compound **32Ab** in the organic phase used to extract the reaction mixture as respectively ethyl acetate and dichloromethane were employed.

General procedure: to an ice-cooled solution (0 °C) of 0.04 g 3,4-pyrrolidine-fused bicyclic β-lactam **30Aa** (0.1 mmol, 1 equiv.) in acetonitrile (8 mL), a solution of 0.48 g cerium(IV) ammonium nitrate (0.9 mmol, 9 equiv.) in water (4 mL) was added dropwise. After stirring for one hour at room temperature, the acetonitrile was evaporated and the remaining solution was extracted with ethyl acetate (2 x 10 mL). The combined organic phases were washed with a saturated aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL). Drying with $MgSO_4$, filtration of the drying agent and removal of the solvent *in vacuo* afforded crude 6-(4-methoxybenzoyl)-substituted bicyclic β-lactam **32Aa** (racemic), which was purified in 52 % yield by means of reversed phase automated column chromatography (C18, gradient water/acetonitrile 90/10-0/100).

(1*S,3*S**,4*S**,5*S**)-4-Hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one 32Aa**

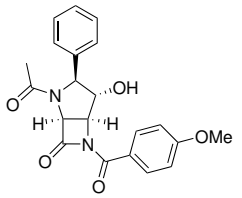
Spectral data derived from the mixture of two rotamers (55/45 in $CDCl_3$, 58/42 in d_6-DMSO).



Colourless solid. Mp = 97 °C. R_f = 0.10 (PE/EtOAc 1/1). Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 90/10-0/100): 52 %. *Major rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.85 (3H, s, CH_3O); 4.03 (1H, d, J_{AB} = 15.7 Hz, (C=O)(HCH)); 4.10 (1H, d, J_{AB} = 15.7 Hz, (C=O)(HCH)); 4.70 (1H, d, J = 3.6 Hz, CHOH); 4.76-4.77 (1H, m, (C=O)CHCH); 5.63 (1H, s, NCHPh); 5.82 (1H, d, J = 4.8 Hz, (C=O)CH); 6.12 (1H, d, J = 3.6 Hz, OH); 6.91 (2H, d, J = 8.2 Hz, 2 x $O(CH_{arom})_{ortho}$); 7.19-7.22 (2H, m, 2 x $O(CH_{arom})_{meta}$); 7.22-7.26 (2H, m, 2 x $NCH(CH_{arom})_{ortho}$); 7.26-7.28 (1H, m, $NCH(CH_{arom})_{para}$); 7.32-7.35 (1H, m, $(C=O)CH_2(CH_{arom})_{para}$); 7.37-7.41 (2H, m, 2 x $NCH(CH_{arom})_{meta}$); 7.39-7.42 (4H, m, 2 x $(C=O)CH_2(CH_{arom})_{meta}$ and 2 x $(C=O)CH_2(CH_{arom})_{ortho}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.8 ($(C=O)CH_2$); 56.0 (CH_3O); 62.8 ($(C=O)CHCH$); 67.1 ($(C=O)CH$); 70.4 ($NCHPh$); 75.5 (CHOH); 113.8 (2 x $O(HC_{arom})_{ortho}$); 123.8 ($N(C=O)C_{arom,quat}$); 125.2 (2 x $NCH(HC_{arom})_{ortho}$); 127.1 ($(C=O)CH_2(HC_{arom})_{para}$); 127.4 ($NCH(HC_{arom})_{para}$); 128.8 (2 x $(C=O)CH_2(HC_{arom})_{meta}$); 129.0 (2 x $NCH(HC_{arom})_{meta}$); 130.0 (2 x $(C=O)CH_2(HC_{arom})_{ortho}$); 131.8 (2 x $O(HC_{arom})_{meta}$); 135.4 ($(C=O)CH_2C_{arom,quat}$); 138.4 ($(NCH)C_{arom,quat}$); 162.4 ($(C=O)CH$); 163.6 ($OC_{arom,quat}$); 164.7 ($(C=O)C_{arom,quat}$); 170.0 ($(C=O)CH_2$). *Minor rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.59 (1H, d, J_{AB} = 15.6 Hz, (C=O)(HCH)); 3.79 (1H, d, J_{AB} = 15.6 Hz, (C=O)(HCH)); 3.85 (3H, s, CH_3O); 4.64 (1H, d, J = 4.8 Hz, (C=O)CHCH); 4.76-4.77 (1H, m, CHOH); 5.67 (1H, s, NCHPh); 5.76 (1H, d, J = 4.8 Hz, (C=O)CH); 6.19 (1H, d, J = 4.0 Hz, OH); 6.90 (2H, d, J = 8.4 Hz, 2 x $O(CH_{arom})_{ortho}$); 7.17 (2H, d, J = 8.4 Hz, 2 x $O(CH_{arom})_{meta}$); 7.24-7.26 (2H, m, 2 x $(C=O)CH_2(CH_{arom})_{ortho}$); 7.28-7.30 (1H, m, $(C=O)CH_2(CH_{arom})_{para}$); 7.30-7.37 (2H, m, 2 x $(C=O)CH_2(CH_{arom})_{meta}$); 7.32-7.35 (1H, m, $NCH(CH_{arom})_{para}$); 7.34-7.39 (2H, m, 2 x $NCH(CH_{arom})_{ortho}$); 7.45-7.49 (2H, m, 2 x $NCH(CH_{arom})_{meta}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.2 ($(C=O)CH_2$); 56.0 (CH_3O); 61.0 ($(C=O)CHCH$); 66.1 ($(C=O)CH$); 71.8 ($NCHPh$); 77.3 (CHOH); 113.7 (2 x $O(HC_{arom})_{ortho}$); 123.9 ($N(C=O)C_{arom,quat}$); 125.3 (2 x $NCH(HC_{arom})_{ortho}$); 127.0 ($(C=O)CH_2(HC_{arom})_{para}$); 127.9 ($NCH(HC_{arom})_{para}$); 128.6 (2 x $(C=O)CH_2(HC_{arom})_{meta}$); 129.3 (2 x $NCH(HC_{arom})_{meta}$); 129.9 (2 x $(C=O)CH_2(HC_{arom})_{ortho}$); 131.8 (2 x $O(HC_{arom})_{meta}$); 135.3 ($(C=O)CH_2C_{arom,quat}$); 138.6 ($(NCH)C_{arom,quat}$); 162.3 ($(C=O)CH$); 163.6 ($OC_{arom,quat}$); 164.3 ($(C=O)C_{arom,quat}$); 170.7 ($(C=O)CH_2$). **IR** (ATR, cm^{-1}): ν_{OH} = 3379; $\nu_{C=O}$ = 1794, 1649, 1603; ν_{max} = 1512, 1420, 1306, 1258, 1169, 1028, 727, 696. **MS** (70 eV): m/z (%) 457 ($[M + H]^+$, 100).

(1*S,3*S**,4*S**,5*S**)-2-Acetyl-4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-one 32Ab**

Spectral data derived from the mixture of two rotamers (56/44 in $CDCl_3$). Due to deuterium exchange with the solvent, no hydroxyl proton signals were observed.



Colourless solid. Yield after automated column chromatography (C18, gradient H_2O/CH_3CN 100/0-0/100): 16 %. *Major rotamer.* **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.12 (3H, s, (C=O)CH_3); 3.80 (3H, s, CH_3O); 4.70 (1H, d, J = 4.9 Hz, (C=O)CHC$\underline{H}$); 4.91 (1H, s, C$\underline{H}$OH); 5.41 (1H, s, NC$\underline{H}$Ph); 5.85 (1H, d, J = 4.9 Hz, (C=O)CH); 6.73 (2H, d, J = 8.6 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.18-7.26 (3H, m, 2 x O(CH_{arom})$_{meta}$ and NCH(C$\underline{H}_{arom}$)$_{para}$); 7.29-7.31 (2H, m, 2 x NCH(C$\underline{H}_{arom}$)$_{ortho}$); 7.34-7.38 (2H, m, 2 x NCH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 21.9 ((C=O)$\underline{C}H_3$); 55.4 (CH_3O); 61.0 ((C=O)CH$\underline{C}$H); 65.5 ((C=O)$\underline{C}$H); 72.1 (N$\underline{C}$HPh); 77.9 (CHOH); 113.3 (2 x O(HC_{arom})$_{ortho}$); 123.0 (N(C=O)$\underline{C}_{arom,quat}$); 124.9 (2 x NCH(H$\underline{C}_{arom}$)$_{ortho}$); 127.9 (NCH(H$\underline{C}_{arom}$)$_{para}$); 129.2 (2 x NCH(H$\underline{C}_{arom}$)$_{meta}$); 131.9 (2 x O($HC_{arom}$)$_{meta}$); 137.1 ((NCH)$\underline{C}_{arom,quat}$); 161.2 (($\underline{C}$=O)CH); 163.9 ($OC_{arom,quat}$); 165.4 (($\underline{C}$=O)$C_{arom,quat}$); 170.9 (($\underline{C}$=O)$CH_3$). *Minor rotamer.* **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.43 (3H, s, (C=O)CH_3); 3.81 (3H, s, CH_3O); 4.86 (1H, d, J = 4.8 Hz, (C=O)CHC$\underline{H}$); 4.93 (1H, s, C$\underline{H}$OH); 5.28 (1H, d, J = 4.8 Hz, (C=O)CH); 5.87 (1H, s, NC$\underline{H}$Ph); 6.73 (2H, d, J = 8.6 Hz, 2 x O(CH_{arom})$_{ortho}$); 7.16-7.21 (3H, m, NCH(C$\underline{H}_{arom}$)$_{para}$ and 2 x NCH(C$\underline{H}_{arom}$)$_{ortho}$); 7.18-7.25 (2H, m, 2 x O(CH_{arom})$_{meta}$); 7.26-7.29 (2H, m, 2 x NCH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 22.6 ((C=O)$\underline{C}H_3$); 55.4 (CH_3O); 62.3 ((C=O)CH$\underline{C}$H); 67.0 ((C=O)$\underline{C}$H); 69.8 (N$\underline{C}$HPh); 75.7 (CHOH); 113.3 (2 x O(HC_{arom})$_{ortho}$); 123.0 (N(C=O)$\underline{C}_{arom,quat}$); 125.0 (2 x NCH(H$\underline{C}_{arom}$)$_{ortho}$); 127.4 (NCH(H$\underline{C}_{arom}$)$_{para}$); 128.9 (2 x NCH(H$\underline{C}_{arom}$)$_{meta}$); 131.9 (2 x O($HC_{arom}$)$_{meta}$); 136.6 ((NCH)$\underline{C}_{arom,quat}$); 161.0 (($\underline{C}$=O)CH); 163.9 ($OC_{arom,quat}$); 165.2 (($\underline{C}$=O)$C_{arom,quat}$); 170.2 (($\underline{C}$=O)$CH_3$). **MS** (70 eV): m/z (%) 381 ($[M + H]^+$, 100).

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