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SYNTHESIS OF POTENTIAL β-LACTAMASE INHIBITORS BASED ON A 2,6-DIAZABICYCLO[3.2.0]HEPTAN-7-ONE SCAFFOLD

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Preface

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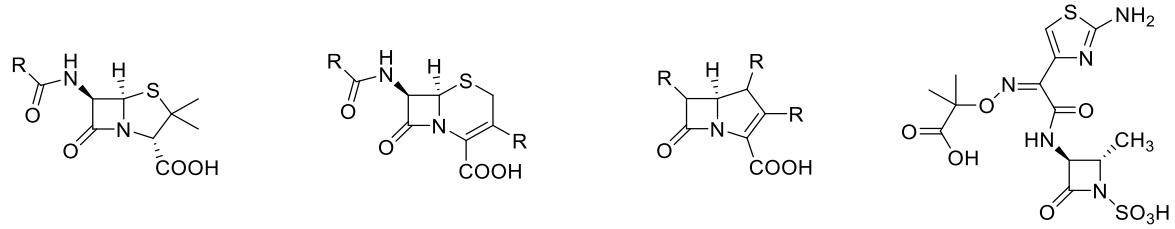
1 Scope and goal

1.1 Scope

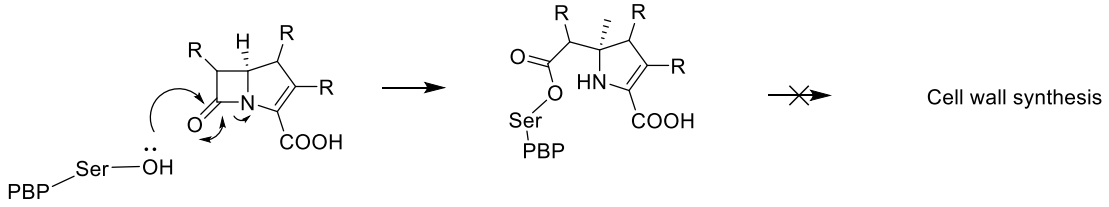
Antibiotics have proven to represent medical breakthrough in recent history since they revolutionized the practice of medicine in many different ways, including safer childbirth, surgical procedures and organ transplantation.[1] However, antimicrobial resistance (AMR) is an ever increasing problem that threatens to impede and even reverse some of this progress, and therefore must be continously monitored. The main causes for growing AMR in bacteria, viruses an fungi are the extensive and sometimes unnecessary use of antimicrobials, which drives the selective pressure towards resistance, and the globally connected human population that allows pathogens access to all of humanity.[2]

One of the most famous classes of antibiotics are the β-lactam antibiotics. After accidental discovery of penicillin **1** by Alexander Flemming in 1928, it took until the year 1946 for the drug to be available in the open market.[3] Since then many derivatives of this medicine were introduced and categorized into three other groups: cephalosporins **2**, carbapenems **3** and monobactams **4**.



1 **2** **3** **4**

The bactericidal properties of the β-lactam antibiotics are based on interference with the bacterial cell wall synthesis.[4] β-Lactam antibiotics will irreversibly bind with the penicillin-binding proteins which are needed in the final step of the cell wall synthesis in order to cross-link the peptidoglycan polymers. Inhibition of the PBPs, therefore, weakens the bacterial cell wall leading to growth inhibition or even cell lysis. The irreversible bond is established by the nucleophilic attack of the hydroxy moiety of the PBP's catalytic site serine on the azetidin-2-one ring, creating PBP-β-lactam complex **6**.



5 **6**

However, bacteria can counteract the working mechanism of β-lactam antibiotics via different adaptations,[5–8] which can be subdivided into four categories. A first possibility is the removal of the antibiotics that entered the cell via efflux pumps, before they can do any harm to the cell. Another way to achieve antimicrobial resistance is to prevent the antibiotics from entering the cell by modifying the outer cell membrane. A third option is a distortion of the PBPs active site, in a manner that the β-lactams cannot interact with it anymore and will lose their antimicrobial properties. A final possibility to become resistent against β-lactam antibiotics is the production of the enzym β-lactamase. These enzymes will hydrolyze the β-lactam structure, rendering it unable to irreversibly bind with the PBP. β-Lactamases can be divided into four classes, where the A, C and D enzymes contain a reactive serine hydroxy group at the active site, while β-lactamases from class B require a zinc ion for activity.[9]

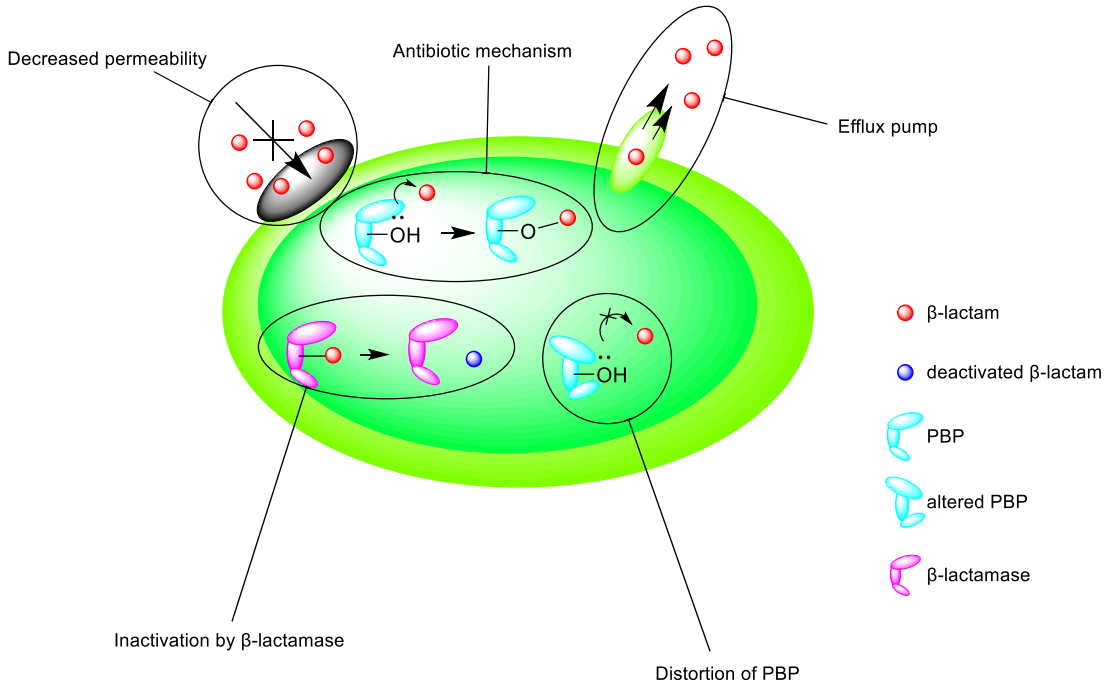
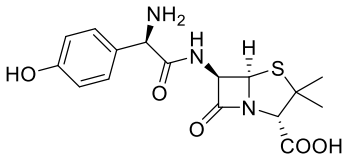


Figure 1 Visualisation of working mechanism of β-lactam antibiotics and different routes to β-lactam antimicrobial resistance

To counteract the negative effect of β-lactamases, two main strategies are employed. The first strategy consists of making slight modifications in the structure of existing antibiotics in a way that these new compounds are no longer recognized by the β-lactamases. The second strategy combines the existing antibiotic with a β-lactamase inhibitor that disarms the β-lactamase, thus restoring the β-lactam activity. A succesfull example of latter method is the combination of the antibiotic amoxicillin **7** with the β-lactamase inhibitor clavulanic acid **8**, which is sold under the name 'augmentin'.[10] This antibiotic cocktail's activity is however only limited to a subset of class A β-lactamases. Almost no class C β-lactamases are commercially available nowadays, with the exception of avibactam **9**, which is mixed with. the antibiotic ceftazidime **10** and is commercially available since 2015.[11]



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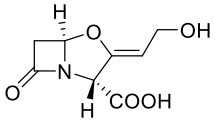
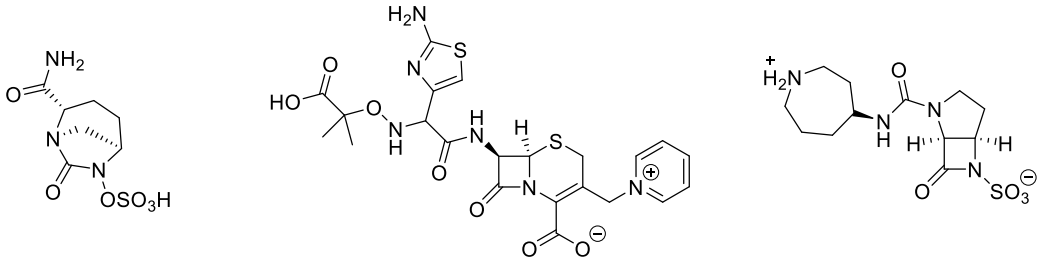


8

Looking at the current bicyclic β-lactamase inhibitors, it is observable that the additional ring structure is *N*-fused to the β-lactam, while much less attention is given to molecules where the ring is *C*-fused to the β-lactam core. A reason to more actively engage in the search for *C*-fused inhibitors is the discovery of several *C*-fused bicyclic β-lactams that showed promising results in inhibiting class C β-lactamases. One of these molecules is MK-8712 **11**, but the development was discontinued because of it's lack of class A β-lactamase inhibition while the attention of the research was focussed on finding molecules that can inhibit both classes.[12]

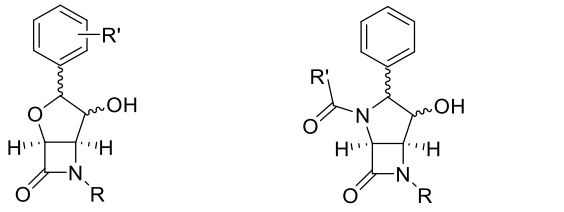


9 10 11

The low number of available class C β-lactamase inhibitors and the promising results of these 3,4-fused bicyclic β-lactams form a motive to explore this new approach to counteract antimicrobial resistance.

1.2 Goal

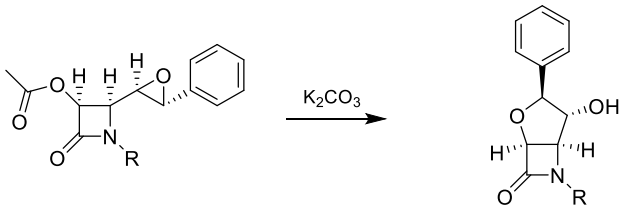
The goal of this Master thesis can be split up in two sections. The main domain of interest is the chemical synthesis of newly *C*-fused bicyclic β-lactams. In preliminary research at the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University), a synthetic route was constructed to obtain 3,4-oxolane-fused bicyclic lactams **12**.[13][14] In the present work, the goal is to synthesize the aza-analogues **13**, i.e. 2,6-diazabicyclo[3.2.0]heptan-7-ones. The second domain is screening the newly synthesized β-lactams for their potential β-lactamase inhibitory properties, since *C*-fused bicyclic β-lactams **12** were found to inhibit class C β-lactamases to a certain extent.[12]



12: R = *i*Pr, PMP, PMB, PMBz
R' = H, 3-CF_3

13: R = PMP, PMB, PMBz, *i*Pr
R' = Bn, Me

The choice to synthesize these aza-analogues was mainly based on the observation of preliminary research mentioned *in supra* that no addition of base to deprotonate the alcohol nor lewis acid to activate the oxirane ring in β-lactams **14** was necessary for the production of bicyclic β-lactam **15**.[13][14] Since nitrogen is trivalent, it also has the advantage that extra substituents on the molecule can be introduced that might have an interaction with the active site of the enzymes. Another consideration is the higher similarity the molecules have with the β-lactam antibiotics, which are the target molecules of the β-lactamases.

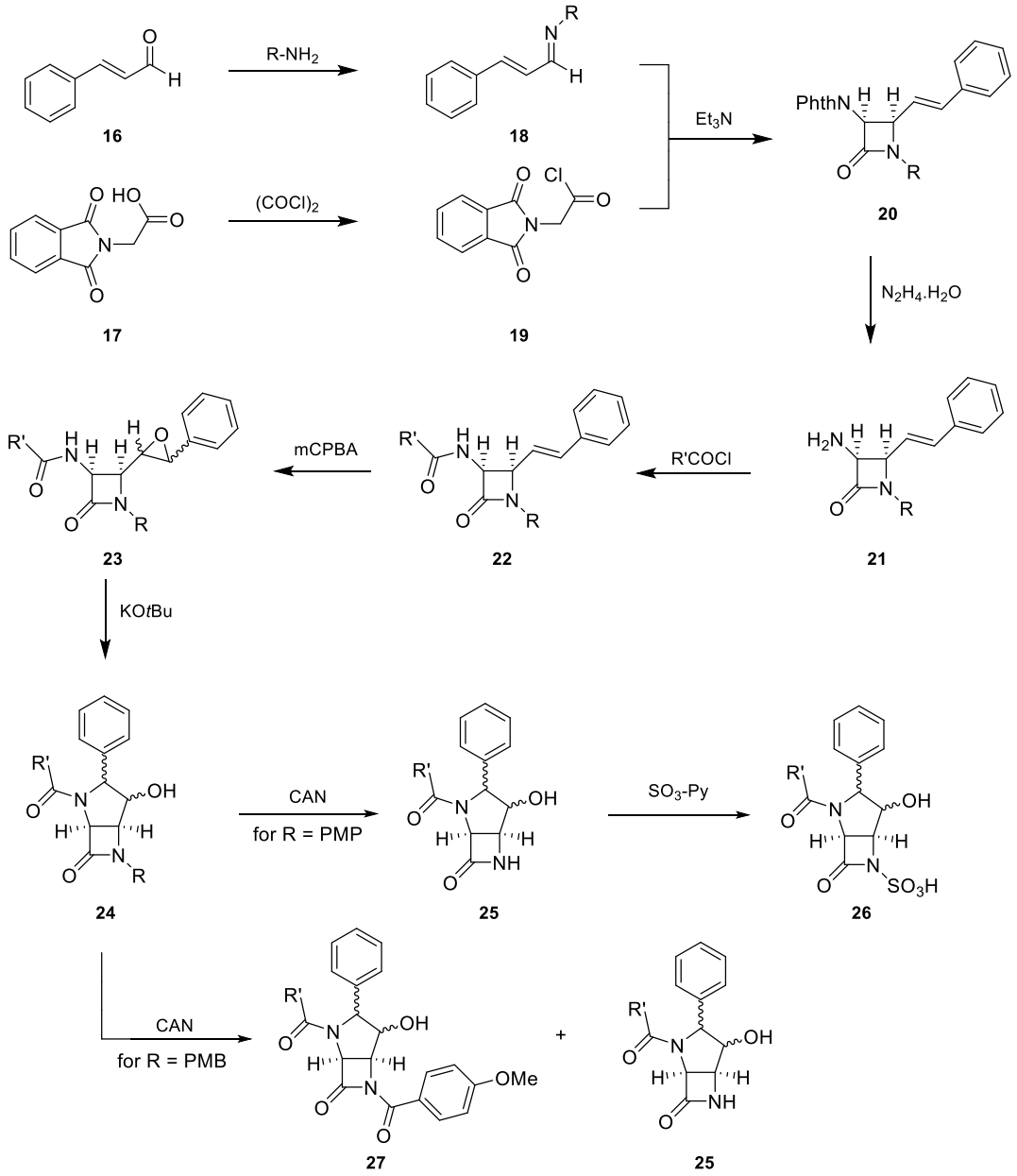


14 15

Preliminary research proposed a seven-step pathway towards the desired 3,4-pyrrolidine-fused bicyclic β-lactams **13**.[15] First, *cis*-3-phthalimido-4-((*E*)-styryl)azetidin-2-ones **20** will be synthesized via a triethylamine-mediated Staudinger synthesis, after the imination of cinnamaldehyde **16** towards *N*-substituted imines **18** and the reaction of *N*-phtaloylglycine **17** into *N*-phthaloylglycyl chloride **19** with

the help of oxalyl chloride. Afterwards, β-lactam **20** will be *N*-deprotected, after which *N*-acylation takes place to afford 3-acylamino-β-lactams **22**. The synthesis route enables us to introduce variations in the structure of the bicyclic β-lactams via this step, since different acyl chlorides can be used. Next, epoxidation takes place with the help of *m*-perchloroperoxybenzoic acid (mCPBA), after which bicyclic β-lactams **24** are formed through amido group-induced ring closure with potassium *tert*-butoxide. When the reaction pathways with both PMP and PMB seem to be successful, the route will be validated with an isopropyl moiety as *N*-protection. After yielding the bicyclic β-lactam **24**, another goal of this thesis is to synthesize *N*-sulfonic acid β-lactam **26** after deprotection with CAN and reaction with sulfur trioxide pyridine complex (SO_3-Py), since preliminary research has suggested that the sulfonic acid moiety contributes significantly to the β-lactamase inhibitory activity.[13] As seen in the synthesis route, different protecting groups can be introduced to protect the bicyclic β-lactam **24**. When choosing *p*-methoxy benzyl (PMB) as *N*-substitution, it has been observed that both *N*-deprotection and oxidation of the benzylic position adjacent to the β-lactam *N*-atom takes place to afford deprotected β-lactam **25** and *N*-(4-methoxybenzoyl)-substituted bicyclic β-lactam **27**, respectively, depending on the reaction conditions.[15]

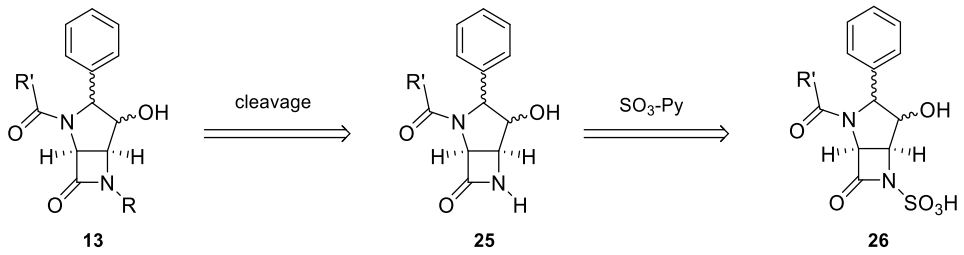
The newly synthesized bicyclic *N*-substituted molecules **24**, **25**, **26** and **27** will eventually be evaluated for their biological activities against class C β-lactamases in collaboration with prof. T. Desmet (Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University).


R-NH2
(COCl)2
Et3N
N2H4.H2O
mCPBA
R'COCl
KOtBu
CAN
for R = PMP
SO3-Py
CAN
for R = PMB
16
17
18
19
20
21
22
23
24
25
26
27
25

2 Literature overview

One of the final goals of this research is to obtain *N*-unsubstituted β-lactams **25**, and subsequent addition of the sulfonic acid group, yielding 4-hydroxy-7-oxo-3-phenyl-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **26**. Cleaving the R group of 3,4-pyrrolidine-fused bicyclic β-lactams **13** is a necessary step to achieve this goal. This literature overview will focus on different ways to deprotect the β-lactam *N*-atom, yielding the corresponding *N*-unsubstituted β-lactam. Many methods are available to cleave this bond, which will be divided in different categories based on the used principles. Within one category, a distinction can be made on the basis of the used reagent or the atom where the *N*-atom is bound to.

Since there is no restriction on possible other groups present on the β-lactam, some methods might not be effective on every substituted β-lactam in general.



13 **25** **26**

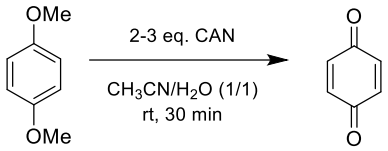
2.1 Oxidative cleavage

2.1.1 Ceric ammonium nitrate-mediated oxidative cleavage

A common method that is used to remove a substituent from the β-lactam *N*-atom is oxidative cleavage with CAN ($(NH_4)_2Ce(NO_3)_6$). The mild reaction conditions, the large reduction potential value, the experimental simplicity, and the low cost of the substrate makes the method attractive for this purpose.[17] The good oxidizing properties of ceric ammonium nitrate lies in the ability of the metal to form stable cerium(IV) and cerium(III) oxidation states. Most lanthanides have a +3 oxidation state, but since cerium(IV) has a vacant f-shell, it can also form a stable +4 oxidation state. A disadvantage of using ceric ammonium nitrate as oxidant is that it often requires more than two equivalents of CAN to complete the reaction. This makes the use of CAN not favourable for industrial processes.

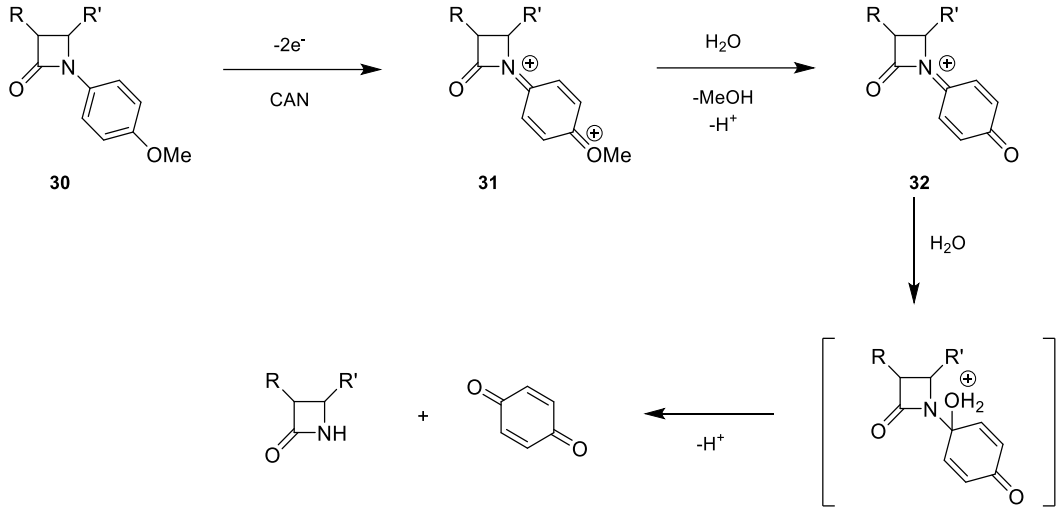
A remark that was pointed out in the research of Bull *et al.*[18] is that the substituent that will be removed with CAN requires electron-donating properties. The vast majority of the protecting groups that are cleaved by CAN are protecting groups with an aromatic moiety that is substituted with a methoxy group, which ensures the electron-donating properties.

The first β-lactam *N*-deprotections in the literature with CAN emerged in the early to mid 80's.[19–22] The method was used to remove aromatic groups with electron-donating properties (e.g. *p*-methoxyphenyl and *p*-methoxybenzyl) in good yields. Inspiration for the oxidative cleavage was found in the work of P. Jacob *et al*.[23] In their research, the group observed that ceric ammonium nitrate in aqueous acetonitrile is able to oxidize a variety of hydroquinone dimethyl ethers **28** to the coresponding quinones **29**. In 1982, some scientists reasoned that since CAN oxidatively demethylates hydroquinone dimethyl ether, it would be possible to remove the electron-rich PMP group and yield the *N*-deprotected β-lactam accompanied by the oxidated aromatic moiety 1,4-benzoquinone.[21]



28 **29**

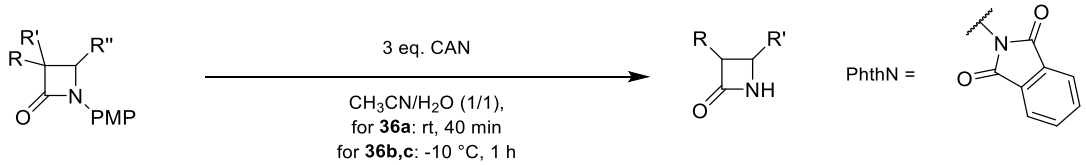
The generally accepted mechanism of the ceric ammonium nitrate-mediated oxidative cleavage is shown below.[24] The protecting group that is removed from β-lactam **30** is indeed oxidated to unsubstituted β-lactam **35** while methanol is also generated in the process.



30 **31** **32** **33** **35** **34**

Research in the Department of Green Chemistry & Technology (Ghent university)[16] has shown that the cleavage of *p*-methoxyphenyl (PMP) from β-lactam **36a** provides *N*-deprotected β-lactam **37a** in moderate yields. To deprotect the PMP, very often a solvent mixture of acetonitrile/water is used. In this specific situation, the reaction was started in a solvent mixture of acetonitrile/water (1/1) at room temperature and proceeded for 40 minutes. This deprotection method can be optimised by varying both the ratio of acetonitrile/water and the amount of CAN added for different β-lactams.

The experiments of ceric ammonium nitrate-mediated oxidative cleavage with β-lactams found in the literature can differ a lot in terms of yield. The conducted experiments by Adam *et al*.[25], aimed at the synthesis of α-methylene-β-lactams **37b** and **37c**, attributes the moderate yield of the reaction products to the acid-sensitivity of the β-lactam. Both starting products reacted for one hour at -15 °C in the same solvent mixture as the compound **36a**. A possible explanation for the difference in yield is the different steric and electronic effects due to varying groups on the C3 and C4 positions.



36a: R = PhthN, R' = H, R'' = CF_3
36b: RR' = CH_2=, R'' = Me
36c: RR' = CH_2=, R'' = H

37a (28%)
37b (48%)
37c (57%)

More recent research has shown that the *N*-dearylation of β-lactams by silica-supported ceric ammonium nitrate (CAN-SiO_2) is possible and results in good yields.[26] Different reaction conditions were examined, and it was determined that CH_3CN/H_2O (3/1) at 25°C is the optimal condition. After these promising results, the set-up of the experiment was changed to a column filled with silica gel (**Figure 1**). β-lactams **38** were charged onto the column in a little dichloromethane and after a reaction time of 10 to 15 minutes, the column was eluted with a THF/H_2O (19/1) solution to afford first the *p*-benzoquinone, followed by *N*-unsubstituted β-lactam **39**. Since the reaction and the work-up are combined into one step, this is an attractive method to use.

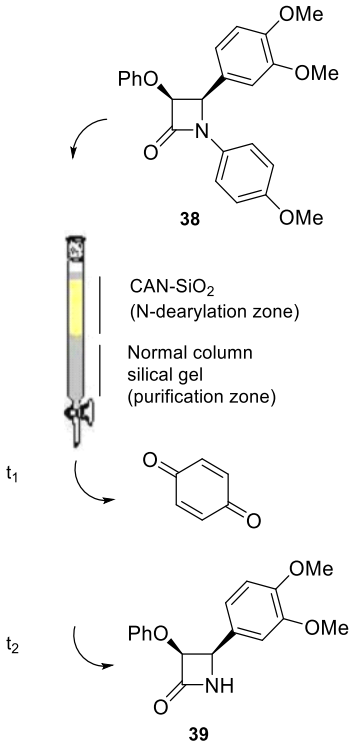
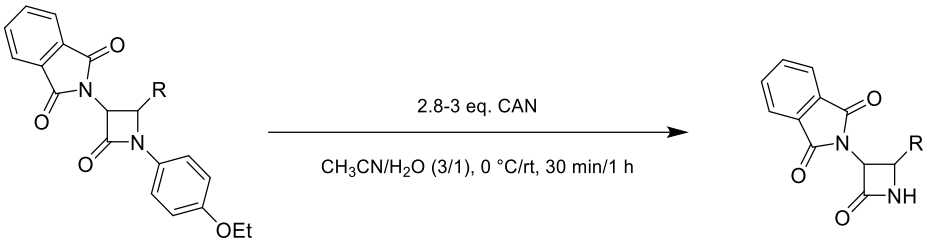


Figure 1 Reaction set-up for the on-column dearylation of the β-lactam

Another group that can be used as protecting group is the *p*-ethoxyphenyl group.[27] Since different substitutions on the β-lactam structure will have an influence on the yield obtained by deprotection under certain conditions, the reaction had to be optimized for every compound. The parameters that reesulted in the highest yield of *N*-unsubstituted β-lactam **41(a-e)** are shown. According to the mechanism, two equivalents of CAN are needed for the reaction, but in this experiment maximum conversion was reached when adding a higher amount of CAN.

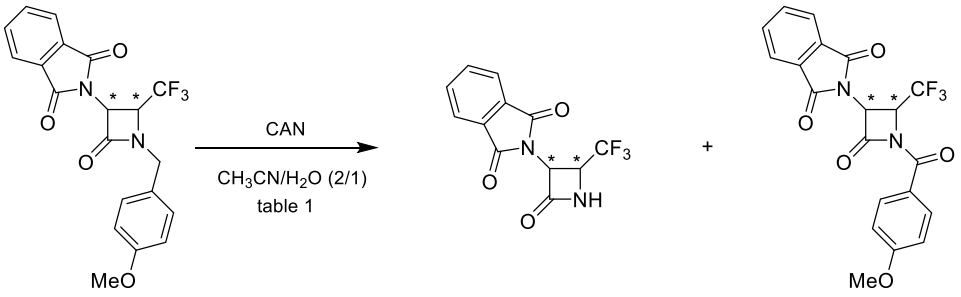
In conclusion, this study has shown that the *p*-ethoxyphenyl group can be considered a suitable *N*-protective group for the azetidin-2-ones and can be easily removed by CAN under mild conditions in good yield. Also note that according to the mechanism mentioned before, ethanol will be formed as side product instead of methanol, which is a less toxic side product that is friendlier for the environment.



40a: R = 4-$NO_2C_6H_4$
40b: R = 4-ClC_6H_4
40c: R =PMP
40d: R = 4-MeC_6H_4
40e: R = CH=CHC_6H_4

41a (78%)
41b (76%)
41c (82%)
41d (77%)
41e (83%)

The drawback of the oxidative cleavage is the possible oxidation of oxidative-sensitive positions on the β-lactam. An example of this is the oxidative-mediated cleavage of the *p*-methoxybenzyl (PMB) group. In the research within our department that was previously mentioned[16], the oxidative cleavage of the PMB-group was performed with various equivalents of CAN to investigate the effect on the ratio deprotected β-lactam **43**/side product **44**. It was observed that a higher amount of CAN added to the reaction (Table 1) results in a higer amount of overoxidated side product **44**. At nine equivalents of CAN, no PMB-cleaved product is formed anymore.

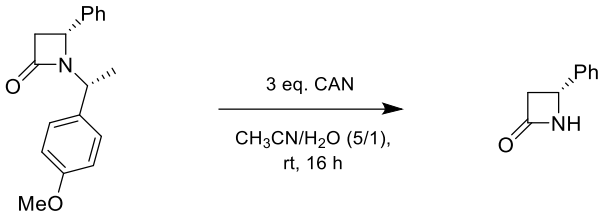


42 **43** **44**

*Table 1. Reaction conditions and results for the deprotection of 3-phthaloylazetidin-2-ones 1**8***

Reaction conditions deprotection:	**19/20**
18$_{trans}$: 1 eq. CAN, rt, 72 h; +2 eq. CAN, rt, 30 min	35/65
18$_{trans}$: 6.3 eq. CAN, rt, 10 min	15/85
18$_{cis}$: 9 eq. CAN, rt, 10 min	0/100
18$_{trans}$: 9 eq. CAN, rt, 10 min	0/100

To overcome the problem of the side product formation in the PMB cleavage, it is possible to use a protecting group that has no oxidative-sensitive sites. An example can be found in the work of Bull *et al.*,[28] where an α-methyl-*p*-methoxybenzyl is used as protecting group to obtain *N*-deprotected β-lactam **46** after cleavage in higher yields as compared to the deprotection of β-lactam **42**.

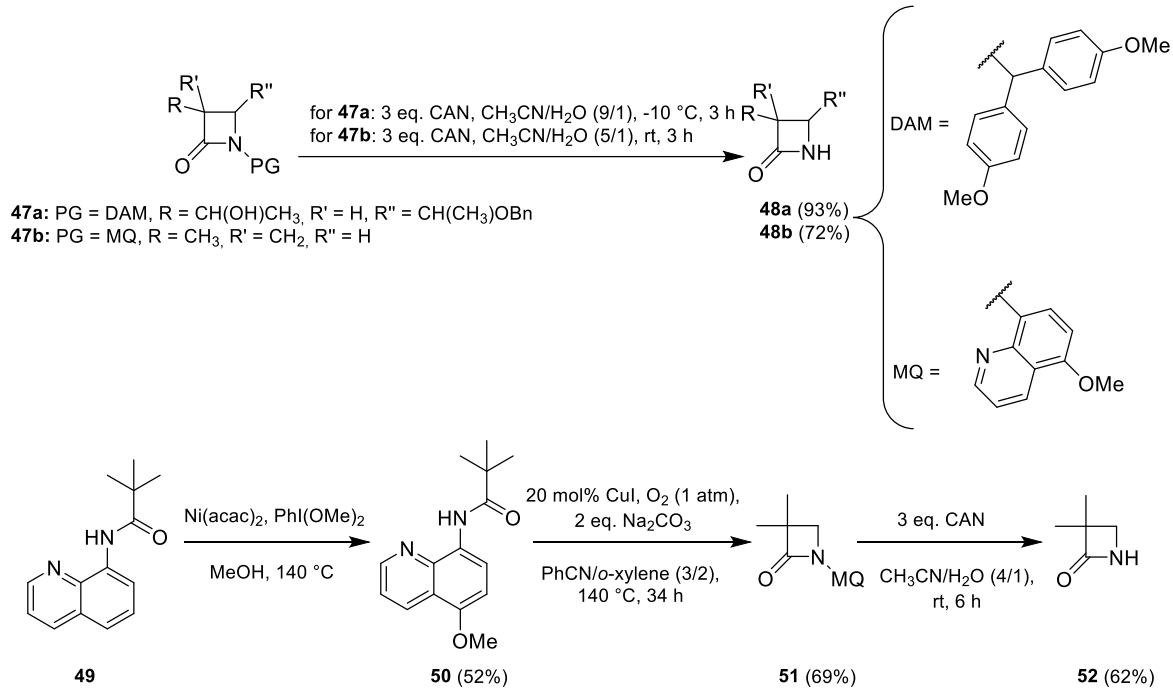


45 **46** (68%)

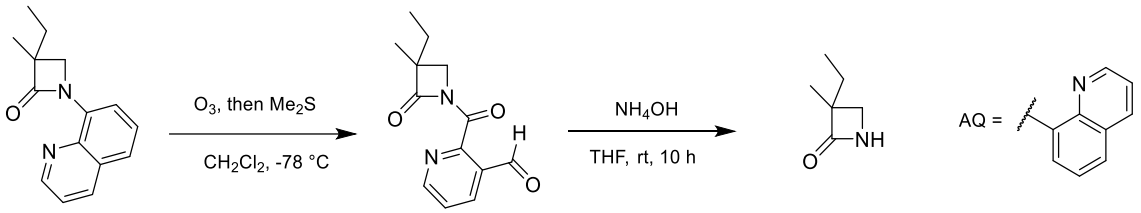
All examples given above mention a protecting group consisting of an aromatic moiety with only one ring structure, but this is not always the case. An example of a protecting group with two aromatic rings incorporated in the structure is the di-*p*-anisylmethyl (DAM) group. This method was demonstrated in the work of Ito *et al.*,[29] where the DAM group was removed in an oxidative way with CAN to obtain the DAM-removed β-lactam **48a** in high yield.

Another example of an electron-donating group containing an aromatic system with two ring structures is the 5-methoxyquinolin-8-yl (MQ) group. This group has been used as a directing group on an amide moiety in order to selectively perform an iodination on the desired carbon atom, whereafter a β-lactam is formed.[[30]] After β-lactam formation, the MQ group was cleaved by treatment with three equivalents of CAN and yielded the unsubstituted β-lactam **48b**.

It is also possible to first carry out the desired reactions with an unsubstituted quinolin-8-yl group on the β-lactam *N*-atom, after which it is substituted with a methoxy group on the C5-atom of the quinoline before deprotection with CAN. This method has been utilized in the research of Chunxia *et al.*[31] and was used because the production of free MQ is a multiple step synthetic route[32] that is more time consuming than latter procedure. In this research the desired amide **49** was formed where quinolin-8-yl served as protecting group. Thereafter the methoxy group was added to obtain the 5-methoxyquinolin-8-yl-protected amide **50**, after which ring closure found place to yield *N*-protected β-lactam **51** that is oxidatively cleaved with CAN to afford the deprotected reaction product **52**.

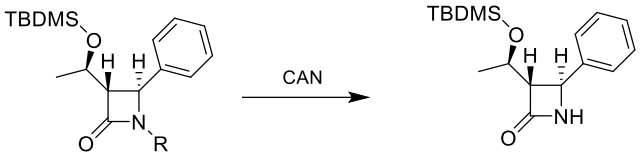


Above method can be an improvement for the deprotection of the quinolin-8-yl since the synthetic burden is decreased. However, it has been shown that the quinolin-8-yl can also be removed without attaching the methoxy group on the quinolin-8-yl moiety.[33] The principal of the cleavage is the transformation of robust amide **53** to labile imide **54** with the help of ozone at -78 °C, after which the ring-opened 8-aminoquinoline (AQ) can be cleaved through simple aminolysis with aqueous ammonia (NH_4OH). It was already shown before that the AQ group could indeed be cleaved without the help of the electron-donating methoxy on the fifth position, but these methods required strong acidic or basic conditions with high temperatures.[34] The ability to remove the AQ group under mild conditions is very useful since this protecting group is important in the view of C-H activation with transition metals.



53 **54** **55** (77%)

A very specific application of CAN deprotection is found in the work of Annunziata *et al.*[35] The goal of this research was the synthesis of β-lactams **56** on a solid support, where the monomethylether of polyethylene glycol (MeOPEG) is used to fulfill this function with the help of a linker. After β-lactam **56** was synthesized, the solid support and the linker had to be removed, which was achieved with the help of CAN. The deprotection step afforded free β-lactam **57** in low yield.

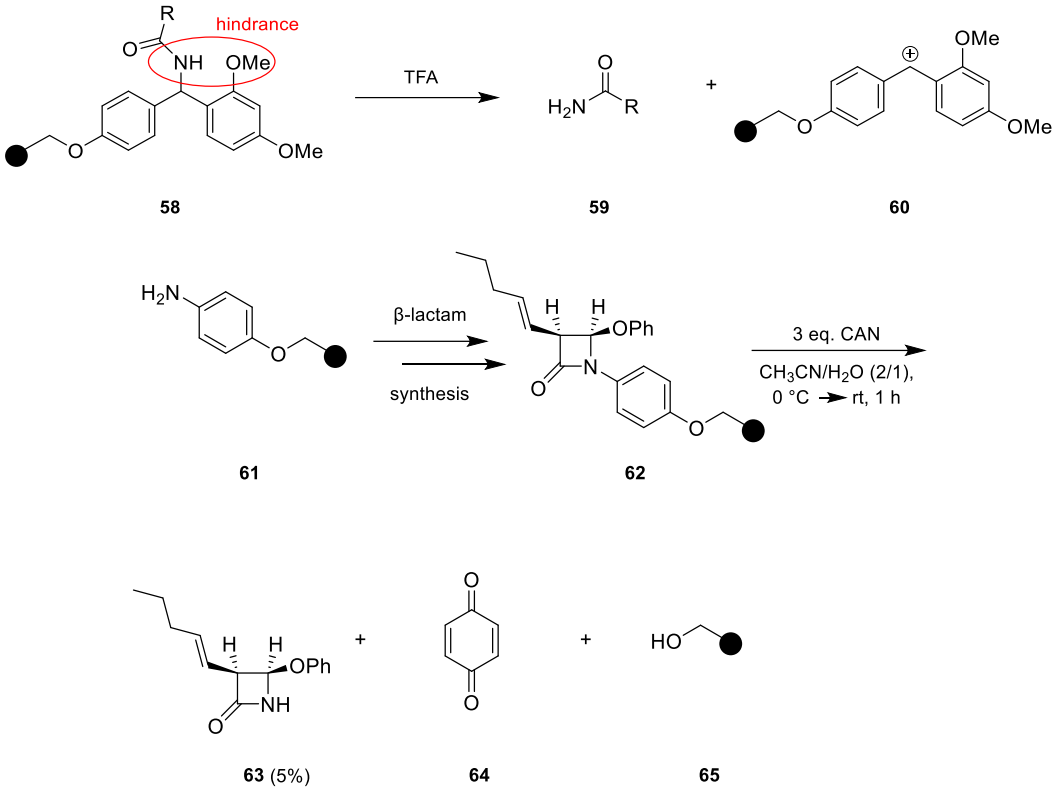


56 **57** (24%)

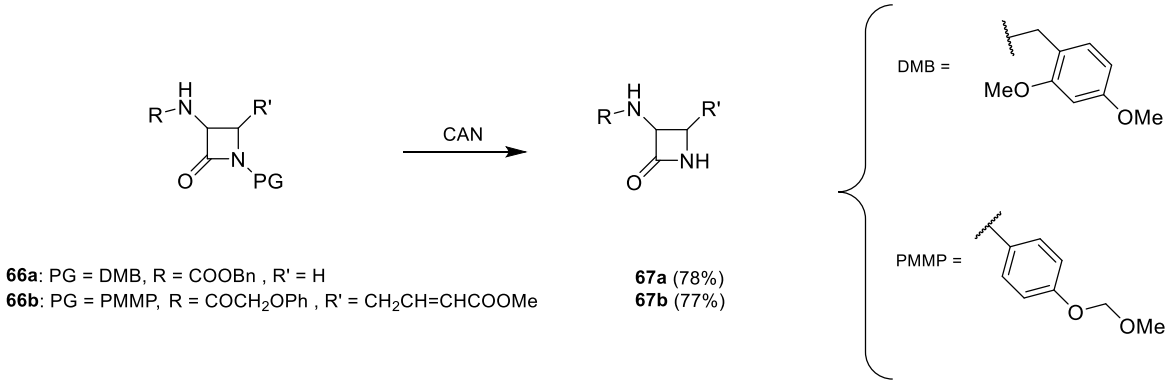
R = -C_6H_4-O-$(CH_2)_3$-C_6H_4-O-●

The group of Kirsteen *et al.*[36] investigated another polymeric approach for the synthesis of β-lactams. Here, a simple benzyloxyaniline linker **61** attached to a solid support was used as the protecting group for the β-lactam *N*-atom and was removed by oxidative cleavage with CAN. A number of amide releasing linkers that possess amino groups are already known. A few examples are Rink, Sieber amide, PAL, SASRIN, BAL and MAMP. However, these linkers are acid-labile linkers, which means that they need aromatic structures with electron-donating moieties (mostly methoxy groups) attached to it to stabilize cation **60** that is formed after cleavage. Disadvantages for this intended application are the sterically hindering properties of the methoxy groups on the aromatic moieties towards the amino anchor that is used to attach to the desired functionality. Another possible disadvantage is the acidic environment (TFA) that is necessary to cleave the linker, when other acid-sensitive groups are present.

Since the benzyloxyaniline linker is cleaved in an oxidative way instead of cleavage in an acidic environment, both disadvantages are countered: no methoxy groups are present near the amino functionality, and no acidic conditions are needed. The reaction on solid support proceeds in a same way as the solution-phase reaction. β-Lactam **62** attached to the linker will be deprotected forming *N*-unsubstituted β-lactam **63**, while formation of the benzoquinon **64** and alcohol **65** are also observed.



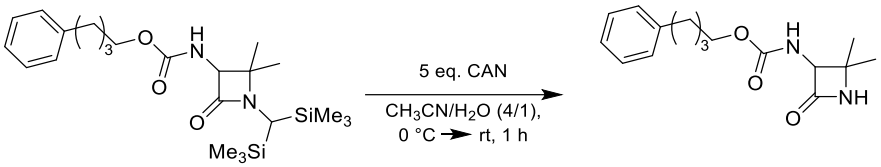
Since oxidative cleavage with CAN proceeds as long as there is an electron donating group present on the aromatics, it is also possible to remove derivatives from both PMB and PMP. One of those derivatives is the 2,4-dimethoxybenzyl (DMB) group. This protecting group has been utilized in the work of Overman *et al.*,[19] where the β-lactam **66a** was deprotected in high yields. An example of a PMP-derived protecting group that is cleaved with CAN is the *p*-(methoxymethoxy)phenyl group (PMMP),[37] which provided the deprotected β-lactam **67b** in good yield.



66a: PG = DMB, R = COOBn , R' = H
66b: PG = PMMP, R = $COCH_2OPh$, R' = $CH_2CH{=}CHCOOMe$

67a (78%)
67b (77%)

One last protecting group which will be mentioned, that is oxidatively cleaved with CAN, is the bis(trimethylsilyl)methyl group. The group of of Nuzzi *et al.*[38] treated *N*-substituted β-lactam **68** with five equivalents of CAN to obtain the final product in low yield.



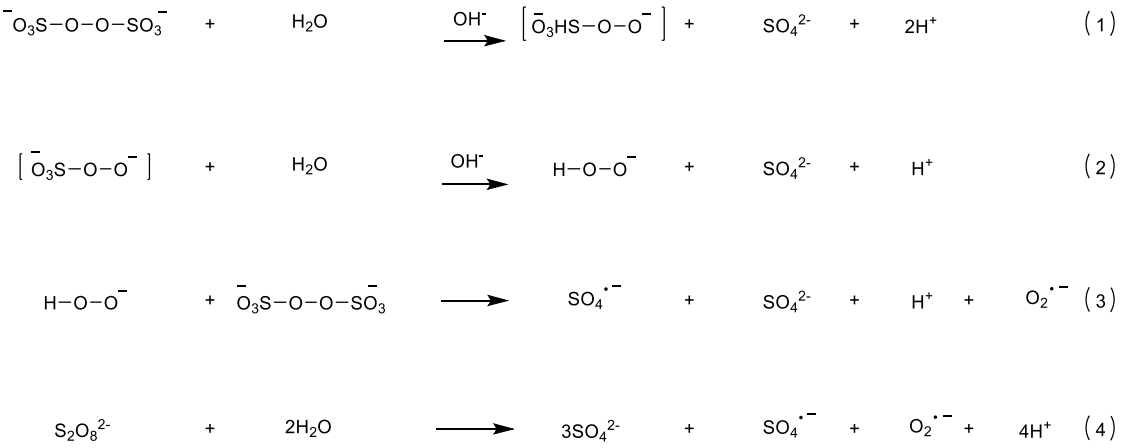
68 **69** (32%)

2.1.2 Persulfate-mediated oxidative cleavage

An alternative reagent to achieve the removal of a protecting group on the β-lactam *N*-atom is the persulfate ion ($S_2O_8^{2-}$), which is generally used as a strong inorganic oxidizing agent that can be utilized both with and without a metal catalyst. The advantages of this reagent are the cost-effectiveness and the wide variety of applications, which makes it suitable for industrial processes. After thermal, photolysis, radiolysis or redox decomposition of the peroxydisulfate ion $S_2O_8^{2-}$ under mild conditions, the sulfate radical ion $SO_4^{\bullet-}$ will be formed. This very strong one-electron oxidant will initiate the electron tranfer reactions. The persulfate ion often derives from $K_2S_2O_8$ instead of its other variants like $Na_2S_2O_8$ or $(NH_4)_2S_2O_8$ because the latter ones are less soluble, which results in a less efficient transformation to the ion.[39]

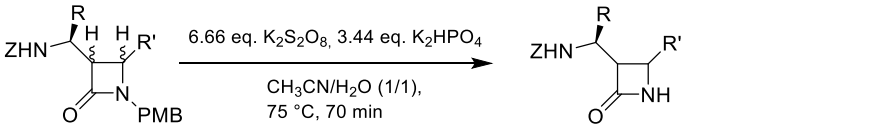
The sulfate radical is one of the strongest oxidizing agents. With a redox potential of 2.6 V, it is almost as strong as the hydroxyl radical, which has a redox potential of 2.7 V. The main advantages of the sulfate radical are the stability of the molecule, and the ability to oxidise the target molecule rather fast.[40] As mentioned above, there are several ways to activate the $S_2O_8^{2-}$ ion. Since metal and base activation are the most abundantly used activation methods, those will be looked at in depth. Also note that in similar fashion with the ceric ammonium nitrate-mediated oxidative cleavage, an electron-donating group on the β-lactam *N*-atom is necessary for a successful cleavage.

Different possibilities exist to form the sulfate radical. The addition of a base is one of the possibilities to transform the persulfate ion $S_2O_8^{2-}$ to the sulfate radical $SO_4^{\bullet-}$. The proposed mechanism[41] for the formation of $SO_4^{\bullet-}$ consists of multiple steps, which starts with the base-catalyzed hydrolysis of the persulfate ion to peroxomonosulfate (SO_5^{2-}) and sulfate (1). It is likely that the persulfate ion forms a complex with the hydroxide that weakens the S-O bond, which ultimately is removed from the persulfate ion. In similar fashion, the remaining S-O bond is cleaved and results in the formation of a hydroperoxide (2). In the proposed mechanism the formed hydroperoxide reduces another persulfate ion, generating a sulfate ion, sulfate radical and a superoxide (3). The net reaction (4) shows that two molecules of persulfate result in one activated sulfate radical that is available for the oxidative cleavage. A compound that is often used as the base is K_2HPO_4 because of its buffering capacities.



Another way to activate the persulfate ion is with a metal (5). Although this method is rarely used, in some situations this is the prefered way of activation. One example where metals are used as the activator is in the treatment of groundwater, where organic contaminants will be oxidated. Since groundwater naturally consists of Fe(III) and Mn(IV) that can serve as activator, the persulfate will be in the contaminated water as such.

After activation of the persulfate, the oxidative cleavage of the protecting groups can start. A protecting group that is often removed by persulfate is the *p*-methoxybenzyl (PMB) group.[42] Just like the ceric ammonium nitrate-mediated oxidative cleavage, this method is in need of an electron-donating moiety on the protecting group. The deprotection of *N*-PMB-β-lactam **70** is carried out with potassium peroxodisulfate in a buffered (K_2HPO_4) mixture of acetonitrile and water, resulting in *N*-unsubstituted azetidin-2-one **71**. In contrast with the CAN-mediated cleavage, the production of overoxidated product is significantly reduced and the β-lactam **71** is formed in medium yields.



70a: R = Me, R' = Ph
70b: R = Me, R' = Ph
70c: R = Me, R' = 4-ClC_6H_4
70d: R = Me, R' = 4-ClC_6H_4
70e: R = Me, R' = 1,3-dioxolan-2-yl
70f: R = *i*Pr, R' = Ph
70g: R = *i*Pr, R' = 4-ClC_6H_4
70h: R = Bn, R' = Ph
70i: R = Bn, R' = Ph

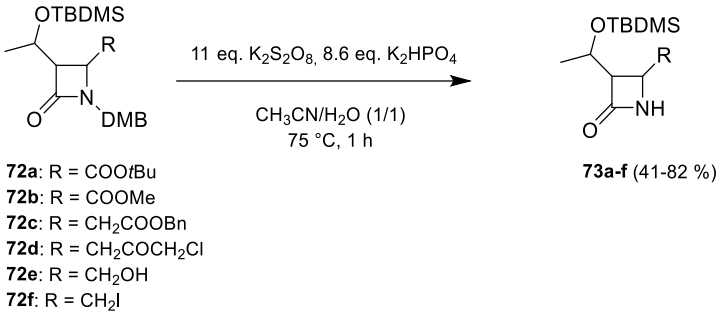
71a-i (32-56%)
R = Me, *i*Pr, Bn
R' = Ph, 4-ClC_6H_4, 1,3-dioxolan-2-yl

Z group is a non-specified group in order to make the compound UV-detectable
*3R, 4S (**70a,c,e-h**)*

Another advantage of this method is the protection of acid-sensitive moieties by the basic buffer that is used to catalyze the reaction, such as the 1,3-dioxolan-2-yl group that is present in β-lactam **70e**. A drawback on the other hand is the impossibility to use electron-rich aromatic side chains, since persulfate is a very strong oxidant.

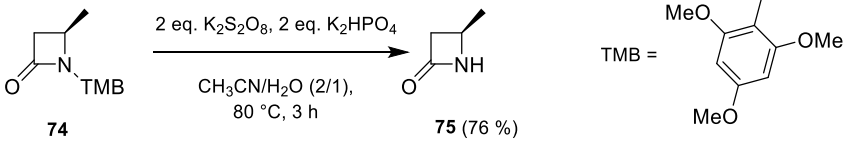
Another group that is often cleaved with persulfate is the 1,3-dimethoxybenzyl group (DMB).[43] It has been shown that the deprotection of the DMB group from β-lactams **72a-f** with different C4-substituents results in reaction products **73a-f** in medium to good yields. The yields of the deprotection can vary a lot depending on the substituents that are present on

β-lactam **72**. In addition, the deprotection of 4-methyl-β-lactam **74** bearing a *N*-substituent with three methoxy groups, has also been performed with high yields.[44]



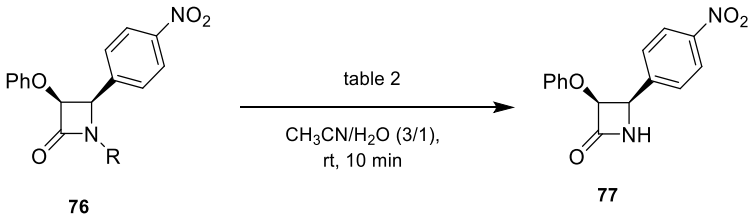
72a: R = COO*t*Bu
72b: R = COOMe
72c: R = CH_2COOBn
72d: R = CH_2COCH_2Cl
72e: R = CH_2OH
72f: R = CH_2I

73a-f (41-82 %)



74 **75** (76 %)

Although the *p*-methoxyphenyl protecting group cannot be cleaved with the persulfate anion, it is possible to perform the cleavage in combination with Ag(II) as a complex. This method has been used in the research of M. Zarei.[45] Since persulfate has a reduction potential of 2.01 V, and Ag(II) has a reduction potential of only 1.98 V, it is possible to convert two moles of Ag(I) to Ag(II) with one mole of persulfate. Ag(II) itself is not stable enough to exist on itself for a very long time, so it is brought into a complex with organic ligands, e.g. $[Ag(Py)_4]S_2O_8$, which can be stored in a bottle for several months. The *p*-methoxyphenyl and *p*-methoxybenzyl group cleavage has been performed with three different complexes, $K_2S_2O_8$ and AgO. As mentioned above, the PMP group cannot be cleaved with $K_2S_2O_8$, which is once again confirmed by this research. Although the yield of the reaction performed with the complexed reagents is comparable with the yield of the AgO-mediated deprotection, this is preferable since this method is not in need of a mineral acid, and is also a lot less expensive than the latter one. It is also noteworthy that the reaction with the complexes delivers a higher yield than with $K_2S_2O_8$.



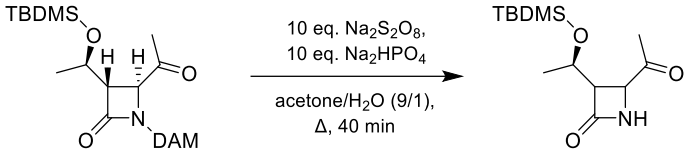
76 **77**

Table 2. Reaction conditions and results for the deprotection of 3-phenoxy-4-nitrophenylazetidin-2-ones ***52***

Substrate	R	Reactants	Yield (%)
52a	PMP	3 eq. $K_2S_2O_8$	0
52b	PMP	3 eq. AgO	83
52c	PMP	3 eq. $[Ag(Py)_4]S_2O_8$	81
52d	PMP	3 eq. $[Ag(Bipy)_2]S_2O_8$	80
52e	PMP	3 eq. $[Ag(Phen)_2]S_2O_8$	77
52f	PMB	3 eq. $K_2S_2O_8$	51[a]
52g	PMB	3 eq. $[Ag(Py)_4]S_2O_8$	70
52h	PMB	3 eq. $[Ag(Bipy)_2]S_2O_8$	71
52i	PMB	3 eq. $[Ag(Phen)_2]S_2O_8$	73

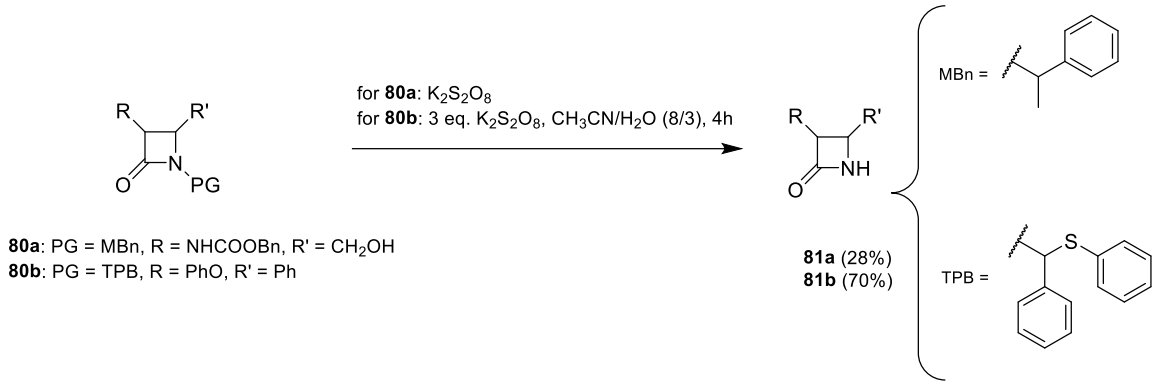
[a]*The reaction was performed at 70 °C for 1h*

The di-*p*-anisylmethyl (DAM) group, which can be cleaved off by ceric ammonium nitrate, can also be removed through the help of persulfate. In the work of Ito *et al.*[29] this deprotection was performed with $Na_2S_2O_8$ in a buffer of Na_2HPO_4 in aqueous acetone. The deprotection proceeded smoothly resulting in β-lactam **79**, which was comparable with the yield of 93 % obtained with the CAN-mediated method. Since no harsh reaction conditions are needed, the *t*-butyldimethylsilyl (TBDMS) group is not removed.



78 **79** (89%)

the work of Richard C. Thomas[46] showed that the α-methylbenzyl (MBn) group can be removed with persulfate-mediated oxidative cleavage. This protecting group has no strong electron-donating moieties, which might explain the poor yield of β-lactam **81a**. Better yields where obtained in the research of Karupaiyan *et al.*,[47] where the thiophenylbenzyl group was reported as a novel *N*-protecting group in the synthesis of β-lactams. Treatment of β-lactam **80b** with three equivalents of $K_2S_2O_8$ resulted in *N*-unsubstituted β-lactam **81b** in a yield of 70 %.

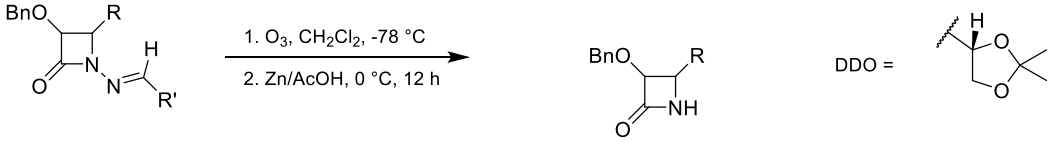


80a: PG = MBn, R = NHCOOBn, R' = CH_2OH
80b: PG = TPB, R = PhO, R' = Ph

81a (28%)
81b (70%)

2.1.3 Other oxidative cleavages

It has also been shown that an imine moiety attached to the β-lactam *N*-atom can be oxidatively removed by ozone.[48] β-Lactams **82** with different substituents were treated with ozone, after which the mixture was quenched with zinc in acetic acid. Zinc in acetic acid was preferred because other quenching agents like dimethyl sulfoxide or dimethyl sulfide produced complex reaction mixtures.



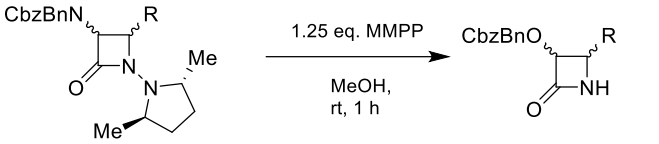
82a: R = PMP, R' = PMP
82b: R = *p*-MeC_6H_4, R' = *p*-MeC_6H_4
82c: R = DDO, R' = DDO
82d: R = DDO, R' = *t*Bu

83a-d (30-92%)

In some cases, the protecting group has more purpose than just protecting the susceptible *N*-atom. In the case of the dialkylhydrazone group, it also ensures the stereoselective formation of the β-lactam

84 with the 3*R*-configuration in the Staudinger synthesis.[49] However, the main reason why hydrazones are used in the Staudinger synthesis instead of aldimines is the poor stability of the latter ones.

The reaction was carried out with different C4-substituents and it was found out that the (3*R*,4*R*) configuration is formed. After the β-lactams **84a-f** were formed, the *N*-unsubstituted product **85** is obtained by oxidatively cleaving the N-N bond with magnesium monoperoxyphthalate (MMPP).



85a-f (59-74%)

84a: R = Me
84b: R = *i*Pr
84c: R = *i*Bu
84d: R = CH_2Bn
84e: R = *n*-C_5H_{11}
84f: R = CH_2OBn

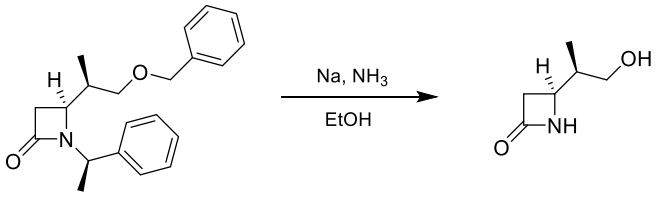
*compounds **60a-e** have the (3R,4R) configuration, compound **60f** has the both the (3R,4R) and (3R, 4S) configuration*

2.2 Reductive cleavage

Since the methods described above need strong electron-donating properties on the protecting groups, a simple benzyl group cannot be removed from the β-lactam *N*-atom in that way. A possibility to perform the *N*-debenzylation is via a metal reduction.

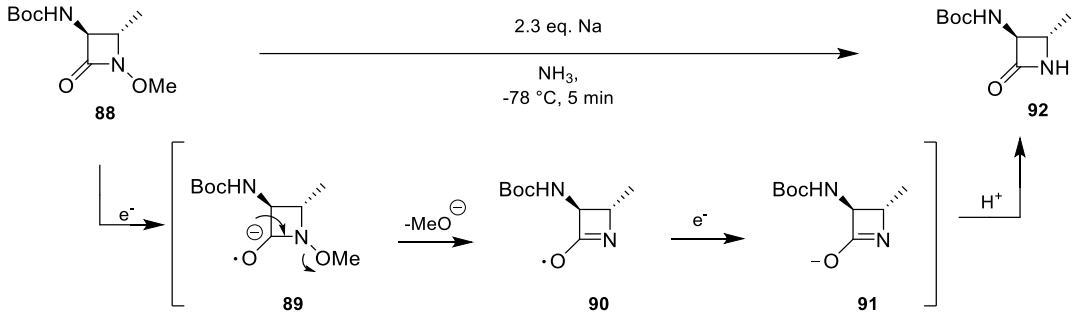
A first method to perform a reductive cleavage is with an alkali metal dissolved in aqueous ammonia, which is called the Birch reduction. Although sodium is used the most in this application, it is also possible to perform the reduction with lithium.[50] The mixture of the alkali metal and ammonia will perform the reduction by providing the substrate with free electrons present in the solution. In reality, the free electrons come in the form of three species: actual free but solvated electrons, electrons in the form of metal anions ($Na^{\bullet-}$) and an electride which consists of a complexed alkali metal cation with an electron trapped inside of it (e.g. $[Na(NH_3)_6]^+e$).[51] This reduction reaction is not the method of choice, since alkali metals are dangerous because of the ability to form hydrogen gas in contact with water. The harsh temperature range of -78 °C to -33 °C to maintain the ammonia in liquid state also posesses a challenge for the scale-up. [52]

Davies *et al.*[53] applied a Birch reduction with sodium on β-lactam **86** to remove an α-methylbenzyl group on the *N*-atom, resulting in azetidin-2-one **87** with 92 % yield. Since the benzyl groups attached to the *O*-atom will also be removed, this has to be kept into account when considering this method because this is not always desirable.



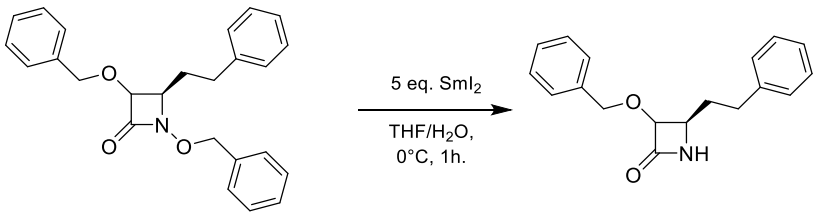
86 **87** (92%)

Beside a debenzylation, this method is also able to remove a methoxy group from the β-lactam *N*-atom, which has been performed by Floyd *et al.*[54] and resulted in a yield of 94 % of *N*-deprotected β-lactam **92**. The proposed mechanism shows how the carbonyl in the four-membered ring might facilitate the deprotection. Although only two equivalents of sodium are required according to the mechanism, in practice 10-15 % excessive sodium has to be used.

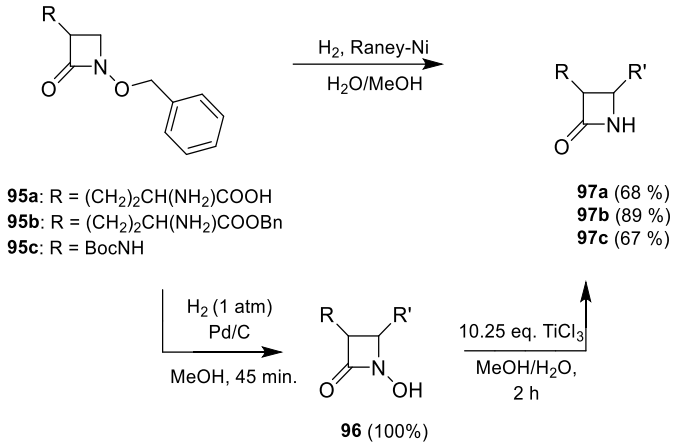


Another method to cleave an N-O bond, in particular a benzyloxy protecting group, is a reductive cleavage with samarium(II) iodide (SmI_2). This has been described in the research of Yang *et al.*[55] where a mixture of THF and water was used as a solvent to yield 4-phenylethyl-*N*-unsubstitued β-lactam **94**. Note that SmI_2 cleaves the N-O bond selectively, while not affecting the benzyloxy group on the β-lactam *C*-atom. This is noteworthy since the cleavage of the bond between the oxygen and the benzylic carbon was observed previously for α-oxygenated esters in the work of Gary Molander.[56]

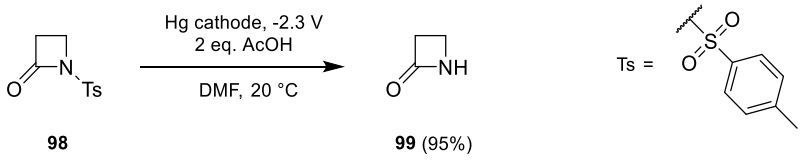
Other possible methods to remove the benzyloxy group make use of metal catalysts.[57][58] The first method consists of two different reduction steps to finally obtain the deprotected product **97c**. First the *N*-benzyloxy-azetidin-2-one is hydrogenated in methanol with a 10 % palladium on carbon catalyst to obtain *N*-hydroxy-β-lactam **96** with full conversion. After hydrogenation, titanium(III) chloride is added to the mixture that will remove the hydroxy group in good yield. The acid-sensitive *t*-butoxycarbonyl group and the base-sensitive C3 position are not affected by this method. Another method is the reduction with Raney nickel to yield the desired reaction products **97a** and **97b**. This method is much more efficient since it is a one-step method that doesn't require an intermediate work-up.



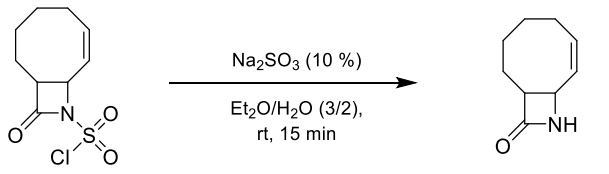
93 **94** (91%)



A totally different approach to achieve reductive removal of the protecting group is with the help of electrochemical methods. The advantages of electrochemistry are the mild conditions and the chemo- and regioselectivity, the latter being achieved by changing the applied voltage. In the work of Casadei *et al.*,[59] attempts to reductively cleave the tosyl group were undertaken. It shows that the electrochemical reduction to obtain the *N*-unsubstituted β-lactam should be considered as a valid option for deprotection of this protecting group since the obtained yields are very high. The optimal conditions consisted of a Hg cathode with two equivalents of acetic acid as proton donor in DMF.



Another N-S deprotection method can be found in research conducted by the group of Dust *et al.*[60] in the early 70's, where the chlorosulfonyl group was removed. Sodium sulfite was chosen as the reducing agent since it was already known that sodium is capable of transforming sulfonyl chlorides to the corresponding sulfinic acids. In the case of chlorosulfonyl, the reduction led to the corresponding *N*-sulfunic acid, which could readily lose the sulfur dioxide to yield the *N*-unsubstituted β-lactam **101**.

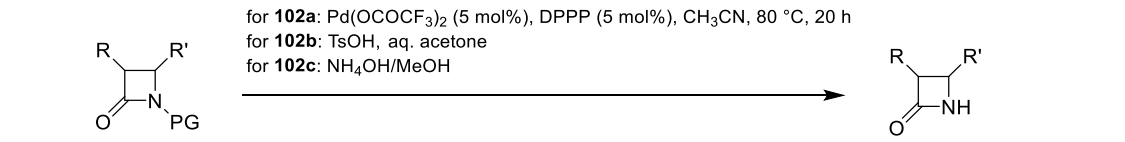


100 **101** (97%)

2.3 Other cleavages

Both oxidative and reductive cleavage have proven to be effective methods to remove the *N*-substituent, and many possibilities are to be found in the literature. However, also other methods exist to effectively remove a protecting group to obtain the *N*-unsubstituted β-lactam. One of the possibilities is hydrolytic cleavage. An example of a group that can be cleaved with hydrolysis is the allyl group, which is shown in the work of Ohmura *et al.*[61] The allyl group is often used to protect alcohols, phenols, carboxylic acids, amines and amides and has the advantage that it is stable in both acidic and basic conditions. The traditional approach for deprotection was the isomerization to the 1-propenyl group and subsequent hydrolysis or oxidation, but the research mentioned above has found a one-step deallylation method. The deprotection, carried out with the help of catalysts $Pd(OCOCF_3)_2$

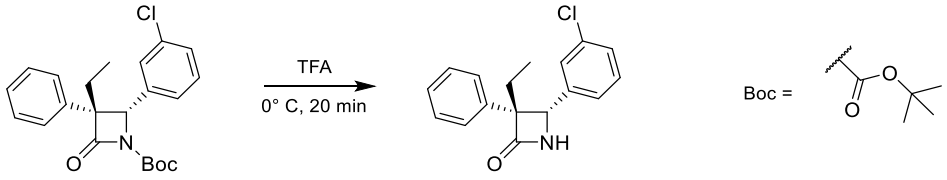
and 1,3-bis(diphenylphosphanyl)propane (DPPP), takes place in acetonitrile in the presence of 20 equivalents of water and delivered 4-methyl-β-lactam **103a** in moderate yields. A same moderate yield was obtained when cleaving the α-methoxybenzyl group with tosylic acid.[62] This hydrolysis is facilitated through the stabilisation of the intermediate carbenium ion that is formed after cleavage due to the aromatic ring and the methoxy group present. Another possible protecting group that can be cleaved by hydrolysis is the hydroxymethyl group with the help of aqueous ammonia (NH_4OH) in methanol.[63] Under the applied conditions the trimethylsilyl (TMS) group, which is present in the C4 substituent, is also removed.



102a: R = H, R' = Me, PG = allyl
102b: RR' = $(CH_2)_5$, PG = α-(OMeBn)
102c: R = H, R' = C≡C-TMS, PG = CH_2OH

103a (40%)
103b (50%)
103c (61%): R' = C≡CH

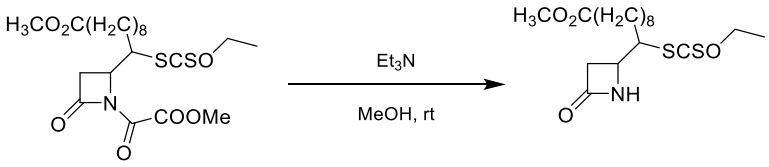
When no acid-sensitive moieties are present on the azetidin-2-one, one might think about protecting the β-lactam nitrogen with a *tert*-butyloxycarbonyl (Boc) group. This protecting group is abundantly used in applications where the final step is the removal of the protecting group with a strong acid. An acid that is often used to perform this deprotection is trifluoroacetic acid, which is shown in the work of Zhang *et al.*[64] The hydrolytic cleavage of the Boc group will yield *tert*-butanol, CO_2 and *N*-deprotected β-lactam **105**.



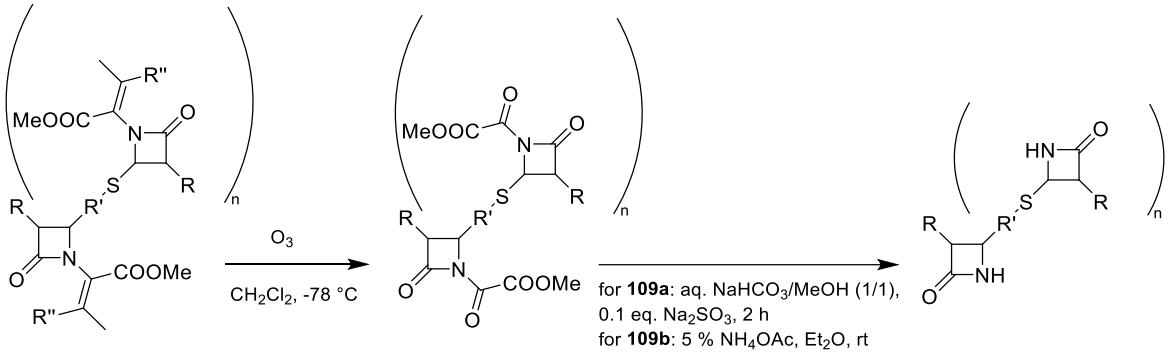
104 **105** (94 %)

When no harsh conditions are tolerated, a possible protecting group to consider is a methyl glyoxylate group. The latter one can be removed in many different ways. A first approach is to add the β-lactam **106** to a solution of methanol and triethylamine.[65]

Another approach is shown in the work of Vittorio *et al.*[66] Here the *N*-atom is first protected with a methyl but-2-enoate, which is transformed into methyl glyoxylate through ozonolysis. The methyl glyoxylate group will be removed in aqueous sodium bicarbonate and methanol to obtain 3-acetoxy-4-phenylazetidin-2-one **110a** in high yield. This method is also seen in the work of Hou *et al*.[67] but here the final step includes a cleavage with ammonium acetate in diethyl ether to yield deprotected β-lactam dimer **110b**.



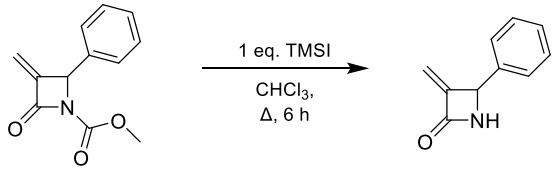
106 **107**



108a: R = OAc, R' = Ph, R'' = H, n = 0 **109a**
108b: R = Et-1-OTBDMS, R' = S, R'' = Me, n = 1 **109b**

110a (80 %)
110b (79 %)

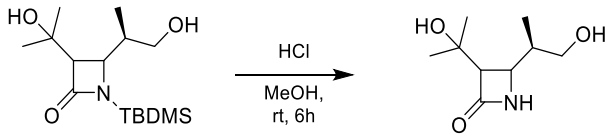
A completely different way of cleaving an *N*-carbonyl bond is with the help of trimethylsilyl iodide (TMSI).[68] The driving force is the evaporation of the iodomethane that will drive the reaction to completion. After heating under reflux for six hours 3-methylidene-4-phenylazetidin-2-one **112** was obtained in quantitative yield.



111 **112** (quantitative)

Previously, various ways have been developed how to cleave an N-C bond, N-S bond as well as an N-O bond. Another protecting group that is abundantly used is the *tert*-butyldimethylsilyl (TBDMS) group. This group is mainly used for the protection of hydroxy groups, but it can also serve as a protecting group for the β-lactam *N*-atom.

The advantage of this protecting group is the simplicity of the cleavage, where the TBDMS group is cleaved with hydrochloric acid in methanol as solvent. This method has been used in the research of Honda *et al.*,[69] where the TBDMS-protected β-lactam **113** was converted to unsubstituted β-lactam **114** in a yield of 44 % after stirring for six hours at room temperature.



113 **114** (44%)

The disadvantage of this method is the use of an acidic medium to deprotect the TBDMS group. Therefore this method can only be used if there are no acid-sensitive centers in the molecule that might undergo undesired reactions.

Conclusion

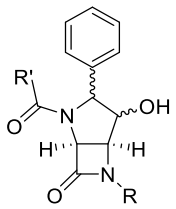
Many different protecting groups and deprotecting methods are available to obtain the *N*-unsubstituted β-lactam. When searching the literature, it is clear that the PMP and PMB are the most abundantly used protecting groups, while oxidative cleavage is the most employed method to obtain the *N*-unsubstitued β-lactam. The choice of protecting group, however, will not solely depend on the simplicity of the deprotection. For example, the protecting group can also serve as a directing group in C-H activation.

Choosing the deprotection method will mostly depend on the properties of the environment wherein the synthesis takes place. When the synthesis of an acid-sensitive molecule is desired, one will not choose a deprotection with a strong acid and vice versa. Some methods that were used in earlier years are not desirable anymore because of the toxic properties and/or intrinsic danger that goes with the used compounds.

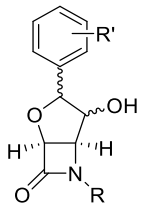
Choosing the *N*-substituent and the deprotection method will also depend on the scale of the reaction. Deprotections that are in need of multiple equivalents of a compound will not be favourable for industrial processes but can be used for small scale applications if needed.

3 Results and discussion

In this Master thesis, new 2,6-diazabicyclo[3.2.0]heptan-7-ones **13** will be synthesized. The creation of these molecules has both a chemical and a biological purpose. First of all, there is a lack of literature methods towards 2,6-diazabicyclo[3.2.0]heptan-7-ones in general. Another reason of interest in these bicyclic β-lactams is the possible β-lactamase inhibitory activity they might possess, with selectivity towards class C β-lactamases.[12]

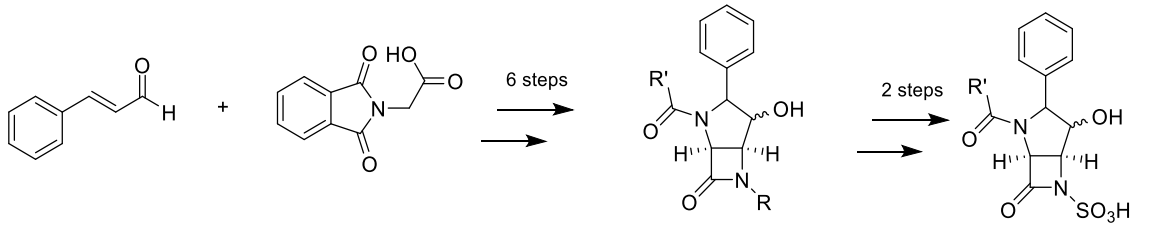


13: R = PMP, PMB, *i*Pr, PMBz, SO_3H
R' = Me, Bn



12: R = *i*Pr, PMP, PMB, PMBz
R' = H, 3-CF_3

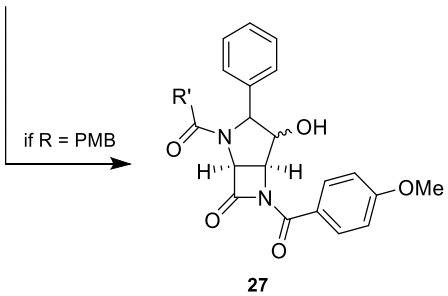
The pathway towards the PMP-, PMB- and *i*Pr-substituted bicyclic β-lactams **13**, which was established in preliminary research at the Department of Green Chemistry & Technology (Ghent University) [15], consist of seven steps, starting from (*E*)-cinnamaldehyde **16** and *N*-phthaloylglycine **17**. When sulfonic-acid substituted derivative **26** is the desired product, the proposed continuation of the pathway is the cleavage of the PMP or PMB group, whereafter the sulfonic acid moiety is attached to the β-lactam *N*-atom to yield the SO_3H-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **26**. The PMBz-substituted derivative **27** is formed due to overoxidation of the PMB group in the deprotection step. The latter SO_3H- and PMBz-substituted derivatives are of high interest, since there is a literature consensus that electron-withdrawing groups are needed on the β-lactam *N*-atom to enhance the β-lactamase inhibitory activity.[70] [71] This explains the choice to initially synthesize the PMP and PMB β-lactam derivatives. This Master's thesis will continue on this preliminary research,[15] which focussed on the synthesis of PMP and PMB derivatives. The first objective, however, in this Master's thesis will be the synthesis of the *i*Pr-derivative of compound **13**, which has the purpose of validating this synthetic pathway. Since the *i*Pr group is not electron withdrawing, it is expected to have a low β-lactamase inhibitory activity, but its biological activity towards β-lactamases will still be tested anyway.



16 **17**

13: R = PMP, PMB, *i*Pr
R' = Me, Bn

26



27

3.1 Synthesis of isopropyl-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one

Preliminary research in this department mainly focussed on the PMP-and PMB-substituted derivatives of β-lactam **13**, since these protecting groups are the keys towards the desired PMBz-and SO_3H-substituted β-lactams. In this research, the first objective was to synthesize *i*Pr-substituted compound **13** in order to validate the synthetic pathway towards these bicyclic β-lactams. Although the main goal of synthesizing the *i*Pr-substituted derivative concerns the validation of the synthetic pathway towards β-lactam **13,** this molecule will still be evaluated for its β-lactamase inhibitory properties.

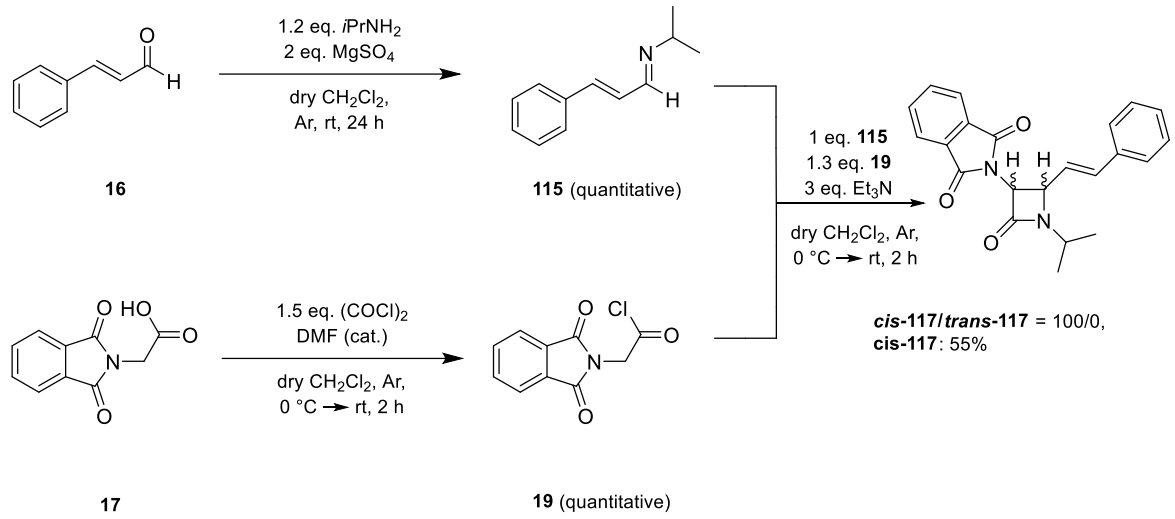
3.1.1 Staudinger synthesis of 1-isopropyl-3-phthalimido-4-((*E*))-styryl)azetidin-2-one

The first step towards synthesizing the desired molecule is the formation of the β-lactam core through the Staudinger synthesis. This reaction concerns a [2 + 2] cyclocondensation between an imine and an acid chloride, yielding the desired β-lactam. First, imine **115** was formed via the reaction of (*E*)-cinnamaldehyde **16** with 1 equivalent of isopropylamine in dry dichloromethane at room temperature under inert argon atmosphere with two equivalents of $MgSO_4$ as drying agent. The follow-up of the reaction was done by ^{1}H NMR since the signal of the aldehyde proton at 9.72 ppm (in $CDCl_3$) is very easily distinguishable of the imine proton signal at 8.05 ppm. After the reaction proceeded for 24 hours, it was observed that some 15 % of the aldehyde was still left. A possible explanation for this is the evaporation of the isopropylamine, which is very volatile with a boiling point of 32 °C. After adding extra 0.2 equivalents of isopropylamine the reaction proceeded for one additional hour, transforming all aldehyde to imine while no impurities were visible on ^{1}H NMR, so a quantitative yield was assumed. With this in mind, the reaction was repeated where 1.2 equivalents of isopropylamine were added from the start. After stirring for two hours, the reaction was completed.

Conversion of *N*-phthaloylglycine **17** to the corresponding acid chloride **19** was performed with 1.5 equivalents of oxalylchloride and dimethylformamide as catalyst in ice-cooled dry dichloromethane, which then evolved to room temperature since the ice-bath was removed after the addition of the reagents. As the reaction proceeds, the signal of the methylene protons near the carbonyl will shift from 4.50 ppm to 4.83 ppm. After stirring for two hours under inert argon atmosphere, it was observed that all the *N*-phthaloyl glycine was converted to the acid chloride and the solvent was evaporated to remove the excess of oxalyl chloride. Since no impurities are observable on ^{1}H NMR, a quantitative yield was assumed. Simultaneously with the acid chloride formation, the oxalyl chloride will be converted to carbon monoxide, carbon dioxide and hydrogen chloride.[59] In contact with air, the hydrogen chloride will be converted to hydrochloric acid that forms a white fume which can be seen at the start of the reaction.

In order to prevent the degradation of the rather unstable imine **115** and acid chloride **19**, both imination and acid chloride formation were executed in parallel to make sure that possible degradation of these compounds was minimised. Since both reactions reached almost full conversion with a high purity, no further purification was needed. To perform the subsequent Staudinger synthesis, the route to the β-lactam scaffold, the drying agent of the imination reaction mixture was filtered off and half of the solvent was evaporated. When the reaction mixture was cooled down to 0 °C, the acid chloride, which was dissolved in dry dichloromethane, was added dropwise to the reaction mixture under inert argon atmosphere as well as three equivalents of triethylamine (TEA). After the addition of the acid chloride, the ice bath was taken away and the reaction proceeded for three hours. Looking at the ^{1}H NMR of the crude reaction mixture, purification was necessary as some small impurities emerged, and this was performed by means of automated column chromatography on silica gel (SiO_2, gradient PE/EtOAc 67/33-0/100) with a yield of 55 %.

The most widely accepted mechanism for the Staudinger synthesis is that the ketene-imine cyclocondensation reaction is a stepwise reaction and not a concerted one. The reaction is initiated by a nucleophilic attack of the imine towards the ketene to form a zwitterionic intermediate, and a conrotatory electrocyclic ring closure is then responsible for the formation of the final β-lactam.[73] This means that the synthesis can be *cis* or *trans* stereoselective, but it can also be a mixture of *cis* and *trans*. Different factors like the reaction temperature, electrophilicity of the substituents and many other parameters can influence the stereoselectivity. The diastereoselectivity (***cis*-117/*trans*-117**) can be determined by ^{1}H NMR analysis. In this case, only one type of diastereomer was observed, where the coupling between the protons on the C3 and C4 position is 5.1 Hz ($CDCl_3$), which corresponds to the *cis* isomer according to literature data.[74,75]



3.1.2 Synthesis of 4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one

When the Staudinger synthesis was performed, the β-lactam scaffold was formed and four additional reaction steps are needed to yield the desired 2,6-diaza-bicyclo[3.2.0]heptan-7-one **13**. The next steps consist of a deprotection of the *N*-phthalimido group, a subsequent acylation of the free amine that has arised, an epoxidation of the double bond on the styryl moiety, followed by an intramolecular ring closure to finally obtain PMP-bicyclic β-lactam **13**.

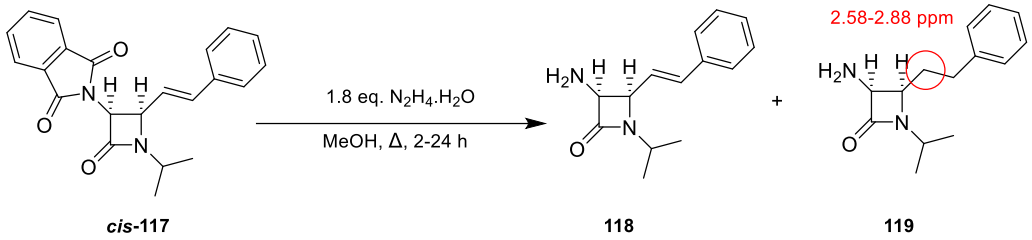
3.1.2.1 *N-phthaloyl deprotection*

After the formation of ***cis*-117**, the *N*-phthaloyl group had to be removed in order to perform an acylation in the subsequent step. The deprotection was performed via hydrazinolysis in methanol at reflux temperature. The proposed mechanism[76] shows that the hydrazine molecule performs a nucleophilic attack on the carbonyl of the phthalimido moiety, after which the free NH_2 of the hydrazine can perform a second nucleophilic attack, this time on the other carbonyl group. The insoluble phthalhydrazide that is formed will precipitate and can be removed by a simple filtration step after the reaction. Following the reaction by LC-MS, it was observed that the starting product was first converted to an intermediate, whereafter the desired phthaloyl-deprotected β-lactam **118** was formed in almost quantitative yields.

In preliminary research at this department[15], it was seen that during the deptrotection step a side reaction occurs which involves the reduction of the double bond of the 4-((*E*)-styryl) substituent,

resulting in compound **119**. This side product was also formed in this reaction, and it was observed that longer reaction times result in more side product formation. When the reaction lasted for only two hours, no side product **119** was formed. After an unnecessarily long reaction time of 24 hours, however, 12 % of side product was formed.

Although isolation and characterization of the compound was not achieved, the ratio of hydrogenated product can be determined by integrating the ^{1}H NMR signal of the newly formed methylene at the C1′ position, which appears as a two multiplets that ranges from 2.58-2.88 ppm ($CDCl_3$). This signal is almost identical to the methylene signal of the hydrogenated *N*-PMB substituted product on ^{1}H NMR in preliminary research, which ranged from 2.50-2.71 ppm.

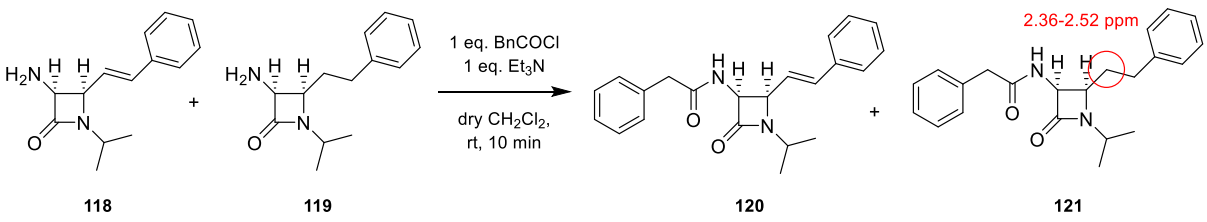


(87-98%, **118/119** = 88/12-100/0)

3.1.2.2 Acylation of 4-((*E*)-styryl)azetidin-2-ones

The following step consists of an acylation of the free amine site created in the last step. *cis*-3-Amino-1-(4-methoxyphenyl)-4-((*E*)-styryl)azetidin-2-one **118** was treated with one equivalent of phenylacetyl chloride in the presence of one equivalent of triethylamine. The base is necessary since the reaction releases hydrogen chloride, which would protonate the free amine and make it unable to perform its nucleophilic attack on the phenylacetyl chloride. The reaction was carried out in dry dichloromethane under inert argon atmosphere and proceeded very smoothly with full conversion in only ten minutes. Close to quantitative yields were obtained, while almost no impurities were formed. This made purification unnecessary and *N*-acylated **120** could advance to the next reaction step as such.

Hydrogenated compound **119**, which was still present in the reaction mixture because no purification step was performed after the *N*-phthaloyl deprotection, was acylated as well. The ratio **120/121** was again determined by integrating the ^{1}H NMR signals of the newly formed methylene at the C1' position, which appears as a multiplet that ranges from 2.36-2.52 ppm ($CDCl_3$). This signal was almost identical to the methylene signal of the hydrogenated *N*-PMB substituted product that was characterized in preliminary research, which ranged from 2.31-2.44 ppm.[56] The ratio of hydrogenation in this step was identical to the previous step, which confirms that all hydrogenated free amine **119** was acylated.



118/119 = 88/12-100/0

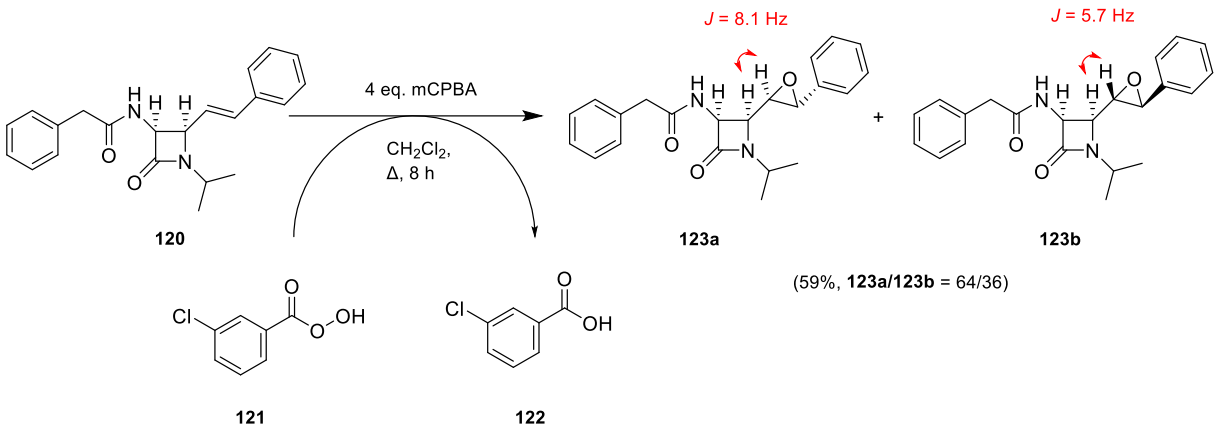
(94%, **120/121** = 88/12-100/0)

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

The *N*-acylated β-lactam **120** obtained in the final step has to be epoxidized in order to be able to perform the intramolecular ring closure to yield the desired bicyclic β-lactam. Since preliminary research with PMP-and PMB-substituted *cis*-3-acylamino-4-((E)-styryl)azetidin-2-ones has shown that *meta*-chloroperoxybenzoic acid (*m*CPBA) affored the epoxide in high yields, the epoxidation step was performed with the latter compound as oxidizing agent. This reaction, known as the Prilezhaev reaction, was performed in dichloromethane at reflux temperature. The reaction was being monitored via LC-MS analysis, and it was observed that four equivalents of *m*CPBA were needed to complete the reaction. The *meta*-chloroperoxybenzoic acid **121**, which will donate an oxygen atom to effect the epoxidation, will be transformed towards *meta*-chlorobenzoic acid **122** during the reaction. During work-up, a saturated solution of sodium sulfite was added to the reaction to quench the remaining *m*CPBA. The formed *meta*-chlorobenzoic acid was removed by addition of aqueous sodium bicarbonate, that will convert the chlorobenzoic acid to the corresponding sodium salt, which will remain in the aqueous phase when extracting the reaction mixture with dichloromethane to obtain the racemic mixture of epoxides in good yield.

Since the double bond of the 4-((*E*)-styryl) moiety was epoxidized and the carbon atoms attached to the oxygen get an additional substituent, two new chiral centers are formed. The (*E*)-styryl moiety will give rise to two different diastereomers in unequal amounts, resulting in major epoxide **123a** and minor epoxide **123b** in a ratio of 64/36. The ^{1}H NMR signals indicated that some impurities were present, so it was decided to purify the product by means of normal phase column chromatography over silica gel (gradient petroleum ether/ethyl acetate 67/33-0/100), which delivered a mixture of the two diastereomers with a yield of 59 %.

Since the two diastereomers showed a similar retention time on LC-MS, these diastereomers could not be fully seperated via column chromatography. The minor epoxide eluted first, which made it possible to separate it in pure form and characterize it. Soon after, the major diastereomer eluted, but due to tailing of the minor diastereomer, a mixture of **123a/123b** (68/32) was obtained. To try and isolate the major diastereomer, it was decided to perform recrystallization in order to hopefully isolate it in the form of pure crystals. To perform the recrystallization, a solvent mixture of ethylacetate/dichloromethane (9/1) was chosen since the solubility in pure ethylacetate was too low, which would result in a lot of solvent waste. After adding the compound to the solvent mixture, the solvent was partly evaporated after which the mixture was cooled to obtain crystals. The diastereomer that will crystallize first is hard to predict, since diastereomers have different physical properties.[77] In this case, the minor epoxide **123b** crystallized first, which resulted in an enrichment of the major epoxide in solution as a racemic mixture of **123a/123b** (9/1), which is used for the characterization of major epoxide **123a**. The main characteristic to distinguish the two diastereomers on ^{1}H NMR is the different coupling constant between the hydrogen atoms on the C4 position of the β-lactam and the C1' postion of the oxirane moiety. Since one diastereomer will experience a C4-C1 *cis* coupling, and the other a C4-C1 *trans* coupling, a significant difference was observed. The coupling constant in compound **123a** is 8.1 Hz, while the coupling constant of compound **123b** is observed to be 5.7 Hz. These vicinal coupling constants match with data for a similar 1-isopropyl-4-((*E*)-styryl)azetidin-2-one diastereomer found in the literature, which were observed to be 8.1 and 6.2 Hz, respectively.[65]

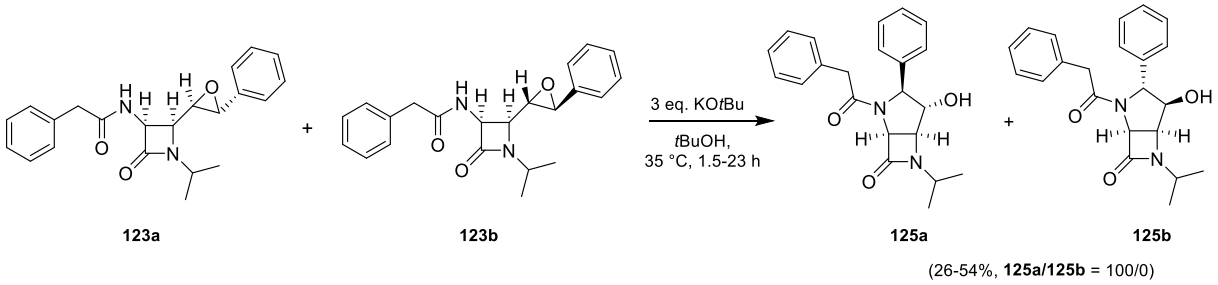


3.1.2.4 Intramolecular ring closure towards 4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one

With the epoxides **123a/123b** (68/32) available, the intramolecular ring closure towards the corresponding bicyclic β-lactams **125a** and **125b** was investigated. It is worth noting that the acylation of the free amine moiety with phenyl acetyl chloride has reduced the electron density around the nitrogen atom, which makes it less nucleophilic. Therefore, a base was used to deprotonate the amido moiety to make it a strong nucleophile that will attack the oxirane moiety to achieve the ring closure towards **125a** and **125b**. Another possible way to initiate the intramolecular ring closure would be the activation of the epoxide by a Lewis acid, but this possible route has not been investigated in this Master's thesis.

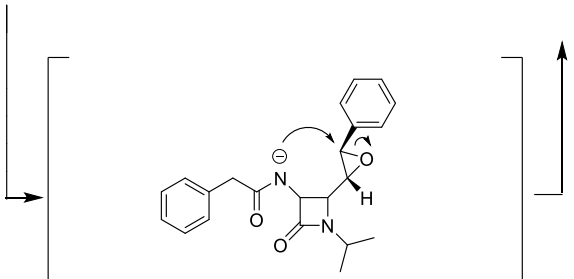
In theory, the nucleophilic attack can result in two different compounds. The *exo* attack results in the formation of a four-membered ring, while the *endo* attack results in a five-membered ring, which is desired in this case. However, only the formation of the five-membered ring was established, which can be explained by the increased tension of the formed [2.2.0]-bicyclic core that makes the occurrence of the *exo* attack thermodynamically unfavourable. In preliminary research, different bases were screened and it was shown that potassium *tert*-butoxide (KO*t*Bu) proved to be an effective base for this purpose, so it was decided to use this base for the intramolecular ring closure.[15] The reaction was performed with three equivalents of KO*t*Bu that were added in small portions to diastereomeric mixture of epoxides **123a** and **123b** dissolved in *tert*-butanol. After the reaction was completed, hydrochloric acid (1 M) was added to the reaction mixture and dichloromethane was used for the work-up. Follow-up of the reaction by LC-MS analysis showed that as time progressed, an accumulation of side product was formed that had a mass which corresponds to the mass of the epoxide with an additional 18 dalton. This observation, and also the low retention time which indicates a stronger polarity of this side product, made us believe that there is a possibility of hydrolytic β-lactam ring opening that occurs during the reaction towards compound **127a**, while it is also possible that the epoxide hydrolyzes towards the corresponding diol **126a**. The alleged hydrolysis of the epoxides and the ring opening is only possible if water is present, which is possible since traces of water in the solvent can never be excluded. Unfortunately, the side products could not be isolated and characterized. In a first entry, the reaction proceeded for 23 hours, which was too long since a lot of side product formation was visible. After work-up the signals of the impurities were reduced, probably due to the more polar character of the impurities that will result in a higher fraction that is left behind in the washing water. The ^{1}H NMR signals still showed a lot of impurity, so the experiment was repeated with shorter reaction times. When the reaction proceeded for three hours, the amount of side product

formation was reduced significantly. After work-up and further purification, a yield of 26 % was obtained. Since longer reaction times result in more side product, we had to find a reaction time where almost all starting product is reacted while side product formation is minimised. The best yield was obtained after a reaction time of 1.5 hours. After work-up without further purification, a yield of 54 % with only minor impurities visible on ^{1}H NMR (purity > 90 %) was obtained. On the LC-MS there was only one clear signal that can be observed, indicating that only one diastereomer of the bicyclic β-lactam was formed. The ^{1}H NMR however shows 'double' signals in an unequal amount (67/33), which are attributed to two different rotamers of the major 2,6-diazabicyclo[3.2.0]heptan-7-one, probably caused by the reduced mobility of the bond between nitrogen N2 and the carbonyl due to the partial double bond character of the amide. A factor beside the preliminary research that indicates that only the major epoxide was converted, is the yield of the reaction, which exceeds the share of minor epoxide present in the initial racemic product.

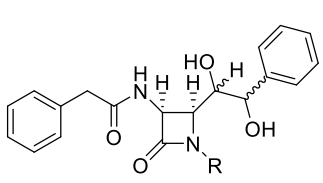


123a **123b** **125a** **125b**

(26-54%, **125a/125b** = 100/0)

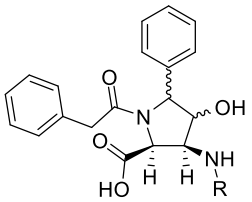


124



126

(**a**: *i*Pr)
(**b**: PMP)
(**c**: PMB)



127

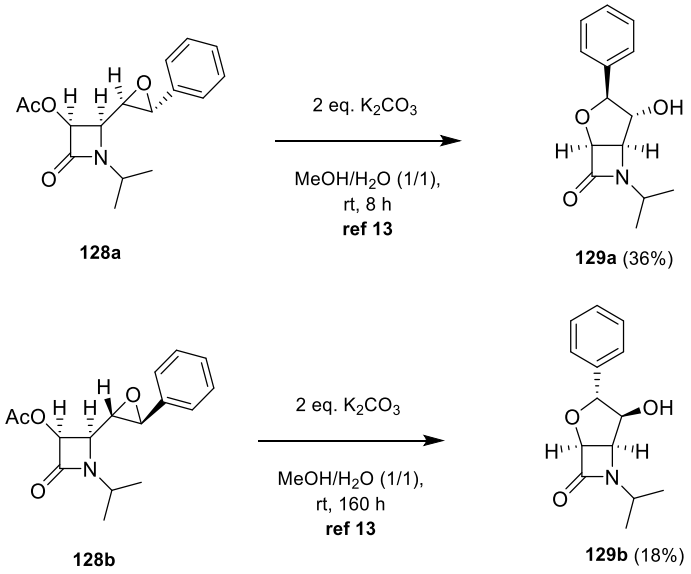
(**a**: *i*Pr)
(**b**: PMP)
(**c**: PMB)

*Table 3. Reaction conditions and results for the intramolecular ring closure of 1-isopropyl-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-ones **123a/123b** towards 4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one **125***

	Scale (mmol)	dr = **123a/123b**	Base	conditions	results
1	1.12	68/32	3 eq. KO*t*Bu	*t*BuOH, 35 °C, 23 h	Traces of **125a**[a]
2	0.91	68/32	3 eq. KO*t*Bu	*t*BuOH, 35 °C, 3 h	**125a** in 26 % yield[b]
3	0.89	68/32	3 eq. KO*t*Bu	*t*BuOH, 35 °C, 1.5 h	**125a** in 54 % yield[c]

[a]ya was observed on both ^{1}H NMR and LC-MS analysis, but a lot of impurities were formed
[b]After work-up, the reaction product was washed with CH_3CN to remove the impurities that were visible on ^{1}H NMR
[c]Very little impurities were seen via ^{1}H NMR analysis after work-up, no further purification (purity: > 90 %)

It is also worth noting that LC-MS and ^{1}H NMR analysis showed that beside the major epoxide, the minor epoxide was also fully converted. Since the corresponding bicyclic product **125b** was not observed, it is believed that the minor epoxide was degraded towards the side products, after which it was transferred to the water phase after the washing step. The difference in reactivity between the diastereomeric epoxides has also been observed in preliminary research, conducted at the Department of Green Chemistry and Technology (Faculty of Bioscience Engineering, Ghent University). Here an intramolecular ring closure towards the 3,4-oxolane-fused bicyclic β-lactam **129b** was only achieved after stirring the reaction for 160 hours, while the major epoxide **128a** was converted after only eight hours.[13] It is possible that the reaction conditions for the *N*-analogues were too harsh, causing the minor epoxide to degrade before it was able to form the bicyclic β-lactam **129b**.



The goal of the first part of this Master's thesis was to prove that the proposed pathway, starting with (*E*)-cinnamaldehyde and *N*-phthaloylglycine on route to the 2,6-diazabicyclo[3.2.0]heptan-7-ones after seven reaction steps, can be generalised for different *N*-substituted imines. The steps until the Staudinger synthesis to obtain the β-lactam core do not pose major problems, as only one purification was needed where a yield of 55 % was obtained. The two subsequent steps, where the phthaloyl moiety was removed and the free amine was treated with an acyl chloride to obtain acylated β-lactam **120** did not need purification and almost reached quantitative yields. Apart from the need for purification, the epoxidation step was also proven to be robust enough to allow other substituents at the β-lactam nitrogen. After optimization of the intermolecular ring closure towards the desired 4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **125a**, it was shown that with a sufficient short reaction time, the bicyclic β-lactam was obtained in a yield of 54 % (purity > 90 %). The observation that ring closure at the minor epoxide **123b** towards the corresponding bicyclic β-lactam was not successful was to be expected, since preliminary research with similar compounds had come to the same conclusion. In this case, it has been proven that the pathway can also be used in case of isopropyl as *N*-substitution.

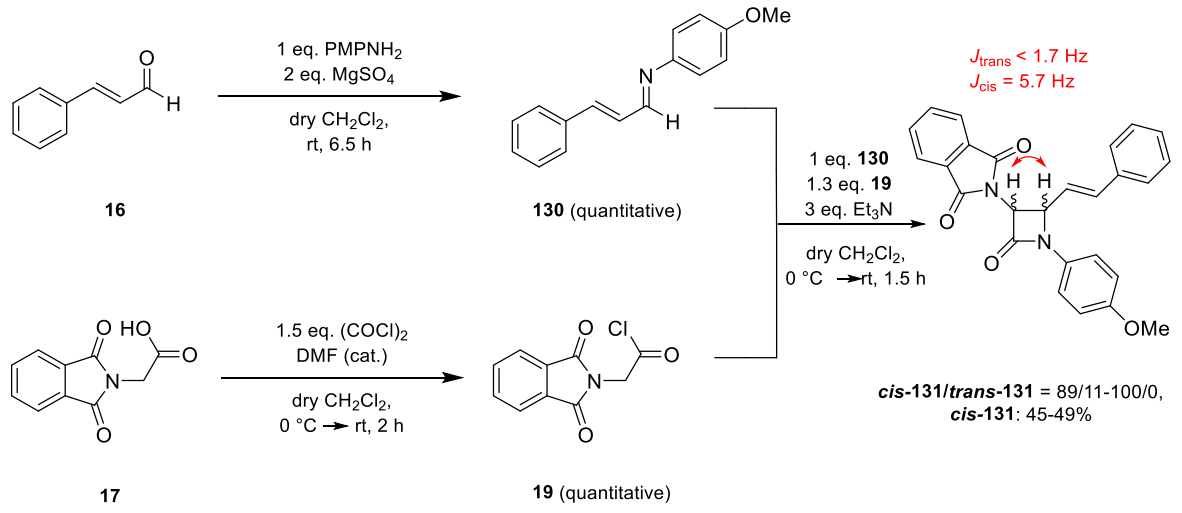
3.2 Synthesis and further derivatization of *para*-methoxyphenyl (PMP) and *para*-methoxybenzyl- (PMB) substituted 2,6-diazabicyclo[3.2.0]heptan-7-one and subsequent *N*-deprotection

Another goal of this Master's thesis was the synthesis of sulfonic acid-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **26**. As mentioned before, interest in this molecule originates from preliminary research that suggests that an electron-withdrawing group such as sulfonic acid will facilitate the nucleophilic attack on the β-lactam carbonyl *in vivo*, which is necessary for its β-lactamase inhibitory activity. Beside this, it is also believed that the anionic sulfonate site which is formed *in vivo* also seems to be essential to bind to the target enzyme.[78,79]

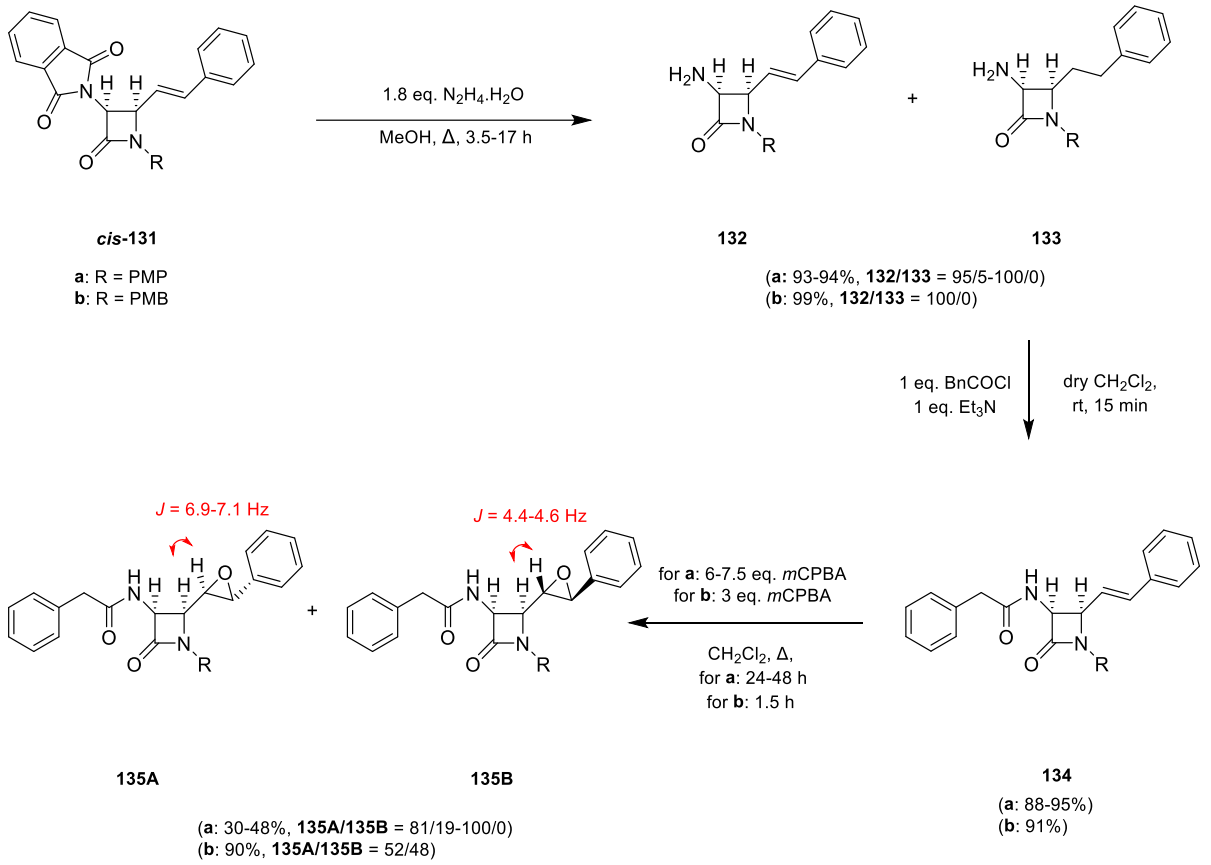
The proposed pathway towards this compound involves the synthesis of PMP-and PMB-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **13**, whereafter the *N*-substituent moiety is removed with the help of ceric ammonium nitrate (CAN) and the sulfonic acid group is introduced through a reaction with sulfur trioxide pyridine complex (SO_3-Py). The synthesis of PMP and PMB substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **13** is also a goal on it's own, since they will also participate in the biological tests to measure the β-lactamase inhibitory activity. Preliminary research has already investigated the first part of the synthetic pathway towards the racemic mixture of epoxides **135Aa/135Ba** for the PMP derivative, while this Master's thesis will mainly focus on the additional steps. Nonetheless, the previous steps that were performed to obtain the racemic mixture of epoxides **135Aa/135Ba** will also be discussed briefly. The route towards PMB-substituted 2,6-diazabicyclo[3.2.0]heptan-7-one **136A** has also been inverstigated in previous research, and will be further looked at. The first three steps towards the β-lactam scaffold were already performed for the PMB-substituted derivative in previous research, thus will only be discussed for the PMP derivative.

3.2.1 Synthesis of *cis*-4-hydroxy-6-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one

To obtain the β-lactam scaffold, a Staudinger synthesis was performed with imine **130** and acid chloride **19**. To obtain the imine **130**, an imination reaction proceeded, this time with one equivalent of *p*-methoxy-phenylamine in dry dichloromethane at room temperature. To form the acid chloride, the same procedure as mentioned in the pathway towards 4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one **125** was used. The obtained acid chloride **19** was then dissolved in dry dichloromethane and was added dropwise to the imine mixture together with three equivalents of triethylamine. After stirring at room temperature for one and a half hours, the imine was fully converted. Looking at the ^{1}H NMR signals, it was observed that some signals are doubled. The most remarkable difference is the presence of a doublet at 5.69 ppm ($CDCl_3$) with a coupling constant of 5.7 Hz, and also a broad singlet at 5.33 ppm. The signals are allocated to the proton at the C3 position of β-lactam **131**. The explanation for this is that the Staudinger synthesis is not 100 % stereoselective, and some *trans* product has been formed as well. This has also been observed in preliminary research and is supported by literature data that states that the *trans* coupling constant is below 1.7 Hz, which explains why the signal was seen as a broad singlet.[80] Due to the similarity in retention time of the two diastereomers, it was decided to try to separate them via recrystallization in a mixture of ethyl acetate/dichloromethane (9/1), which appeared to be successful since only the *cis*-isomer ***cis*-131** was seen upon ^{1}H NMR analysis of the newly formed crystals. When repeating the experiments on a larger scale, it was observed that no ***trans*-131** was formed.



Having *cis*-1-*p*-methoxybenzyl-3-phthalimido-4-((*E*))-styryl)azetidin-2-one ***cis*-131b** from previous research[15] and newly synthesized *cis*-1-*p*-methoxyphenyl-3-phthalimido-4-((*E*))-styryl)azetidin-2-one ***cis*-131a** in hand, the next steps are the deprotection of the 3-phthaloyl group and subsequent acylation of this site. These reactions proceed without any major problems in almost quantitative yield. During hydrazonolysis of β-lactam ***cis*-131a** and ***cis*-131b**, less to none side reaction towards the hydrogenated product **133** was observed compared to its isopropyl counterpart. The ratio of hydrogenation was again determined by the ^{1}H NMR signals of the newly formed methylene at the C1' position.

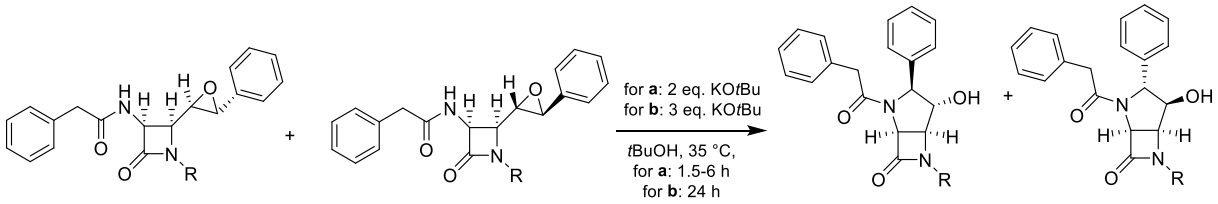


After obtaining 3-acylamino-4-((*E*)-styryl)azetidin-2-one **134**, the next step in the reaction pathway was the epoxidation of this compound towards its corresponding epoxides **135A** and **135B**. The reaction is similar as seen before and it was observed that acylated β-lactam **134a** required more *m*CPBA and a longer reaction time compared to it's PMB counterpart **134b**. It is worth noting that in this reaction, the major epoxide **135Aa** was formed abundantly, which is fortunate given that the major epoxide is the one that undergoes the next step of intermolecular ring closure without problem. PMB derivative **135b**, however, does not include a strong stereoselectivity. To identify the compound as the major epoxide **135A**, the coupling constant of the protons at the β-lactam C4' and oxiranyl C1' position were determined, where the coupling constant is 6.9-7.1 Hz for major epoxide **135A** and 4.4-4.6 Hz for minor epoxide **135B**, which corresponds with literature data from similar compounds.[14][80] Since some impurities were observed in ^{1}H NMR after conversion of **134a**, the crude compounds were subjected to automated column chromatography (SiO_2), obtaining yields between 30 and 48 %. Epoxide mixture **135Ab/135Bb** did not need purification.

Having successfully produced the epoxides **135A** and **135B**, the intramolecular ring closure towards the corresponding bicyclic β-lactam with potassium *tert*-butoxide was attempted. The reaction was carried out in *tert*-butanol at slightly elevated temperatures of 35 °C, in order to keep the solvent in liquid state. The reaction was carried out with both the pure epoxide **135A** and a mixture of diastereomers **135A/135B**. After finishing the reaction, LC-MS signals show that only one bicyclic β-lactam was formed and a signal that corresponds to the mother ion with an additional 18 dalton appear. This confirmed that minor epoxide **135B** does not participate in the reaction and was likely degraded towards a side product **126** or **127**, but unfortunately the side product could not be isolated

to confirm this statement. Different experiments showed that too less equivalents of KO*t*Bu resulted in incomplete conversion, while too much of the base resulted in the degradation of the formed bicyclic β-lactams. The optimal amount of base was determined to be two and three equivalents of KO*t*Bu for the PMP and PMB derivative, respectively, where full conversion was almost achieved while an acceptable amount of impurities were observed on LC-MS.

After purification of both reaction mixtures, pure 4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one **136A** was obtained in 19 to 26 % yield. Consulting the ^{1}H NMR signals, it was again observed that 'doublets' of signals appear, which are contributed to the rotamers probably caused by the reduced mobility of the bond between nitrogen N2 and the carbonyl due to the partial double bond character of the amide.



135A **135B** **136A** **136B**

(**a:** R = PMP, **135A/135B** = 81/19-100/0)
(**b**: R = PMB, **135A/135B** = 52/48)

(**a**: 20-26%, **136A/136B** = 100/0)
(**b**: 19%, **136A/136B** = 100/0)

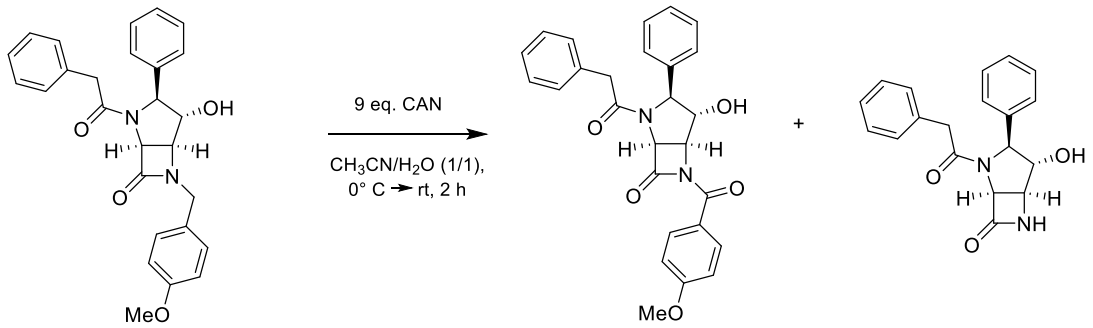
The goal of these experiments was to obtain 4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-ones **136Aa** and **136Ab**, which will be subjected to biological tests for their potential inhibitory activity against β-lactamase inhibitors. It is concluded that the proposed pathway is valid to meet this goal. However, it is observed that the pathway only works well for epoxide diastereomer **135A**, while the other diastereomer **135B** is supposedly degraded.

3.2.2 Synthesis of 4-hydroxy-6-(4-methoxybenzoyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one

In the previous part, it was explained that N-PMP-and N-PMB-protected bicyclic β-lactam **136A** is believed to present a possible path towards N-sulfonic acid β-lactam **26**. In this part, the attempted deprotection of the PMB moiety with CAN will be discussed. Although preliminary research from this department that was mentioned above[16] showed that in experiments with similar β-lactams most of the compound would be transformed into the corresponding *p*-methoxybenzoyl (PMBz) counterpart **138** instead of the deprotected form **139**, it was decided to further investigate this. The formation of the oxidized benzoyl compound, however, is interesting as well, since its N-PMBz 3,4-oxolane-fused bicyclic β-lactam **12** counterpart has shown β-lactamase inhibitory activity, which makes it interesting to compare it with the N-analogue **138**.

3.2.2.1 Benzylic oxidation and deprotection of 4-hydroxy-6-(4-methoxybenzyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones

Starting from compound **137**, ceric ammonium nitrate was used, which resulted in a mixture of the deprotected compound **139** and the oxidated compound **138**. After dissolving bicyclic β-lactam **137** in an ice-cooled solvent mixture, nine equivalents of CAN were added in small portions. After stirring for two hours at room temperature, the reaction was stopped. The ratio **138/139** can be determined by integrating the new signal that appeared arisen at 9.89 ppm ($CDCl_3$). This signal is believed to be an aldehyde signal, corresponding to *p*-methoxybenzaldehyde derived from the PMB moiety that is removed.[17] Investigating the ^1H NMR signals, it was also seen that 'doublets' of signals appear, again indicating that two different rotamers of compound **138** are present.



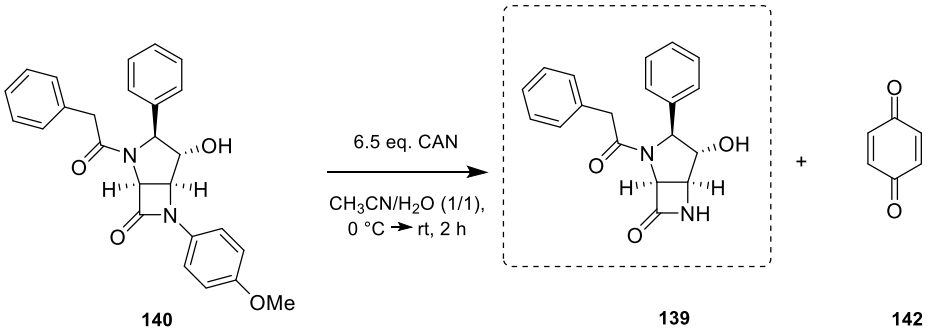
137 **138** **139**

(41-70%, **138/139** = 95/5-73/27)

From these experiments, it is concluded that the PMB moiety is not the optimal protecting group to yield deprotected β-lactam **139**, but the synthesis route does however proved to be useful to yield the corresponding *p*-methoxybenzoyl (PMBz) counterpart **138**, which will be tested for its β-lactamase inhibitory activity.

3.2.3 *N*-deprotection of 4-hydroxy-6-(4-methoxyphenyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one

In order to obtain the desired 7-aza-2,6-diaza-bicyclo[3.2.0]heptane-6-sulfonic acid **26**, The *N*-substituent present on the bicyclic β-lactam has to be removed. In this case, it concerns the *p*-methoxyphenyl (PMP) or *p*-methoxybenzyl (PMB) moiety. In this Master's thesis, it was seen that PMB deprotection does not seem to be a desired approach to yield 4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one **139**, since this reaction mainly yield the PMBz derivative **138**. Now the focus was placed on the PMP deprotection. Looking into the literature, a lot of information can be found on the deprotection of a *N*-PMP group-substituted β-lactam. It was observed that the reaction is carried out in a solvent mixture of acetonitrile and water, but the ratio of the solvent mixture differs depending on the specific β-lactam that is deprotected. In this case, a solvent mixture of acetonitrile/water (1/1) was used. After the β-lactam was dissolved in the solvents, it was cooled down with the help of an ice bath, after which the ceric ammonium nitrate was added and the ice bath was removed. It was observed that full conversion was reached after adding a total of 6.5 equivalents of CAN to the reaction mixture, after which the reaction was stopped and extracted with ethyl acetate. The organic phase was washed again with water to mainly remove the solid parts of the 1,4-benzoquinone **142** that was formed after the PMP group is removed. It was also noted that the starting compound does not dissolve well in the solvent mixture, but this does not seem to give any problems.



140 139 142

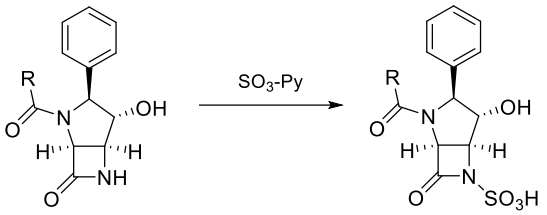
^{1}H NMR analysis showed the presence of some impurities, so it was decided to purify the compound by washing it in ethyl acetate, yielding pure deprotected bicyclic β-lactam **139** in 29 % yield. On the ^{1}H NMR signals, the ratio of rotamers has slightly shifted from 65/35 in the previous step towards 70/30.

The goal of these experiments was to obtain deprotected β-lactam **139**, which will be the building block for 7-aza-2,6-diaza-bicyclo[3.2.0]heptane-6-sulfonic acid **26**. It is concluded that the proposed pathway is valid to meet this goal.

3.3 Future work and perspectives

3.3.1 Sulphonation towards 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids

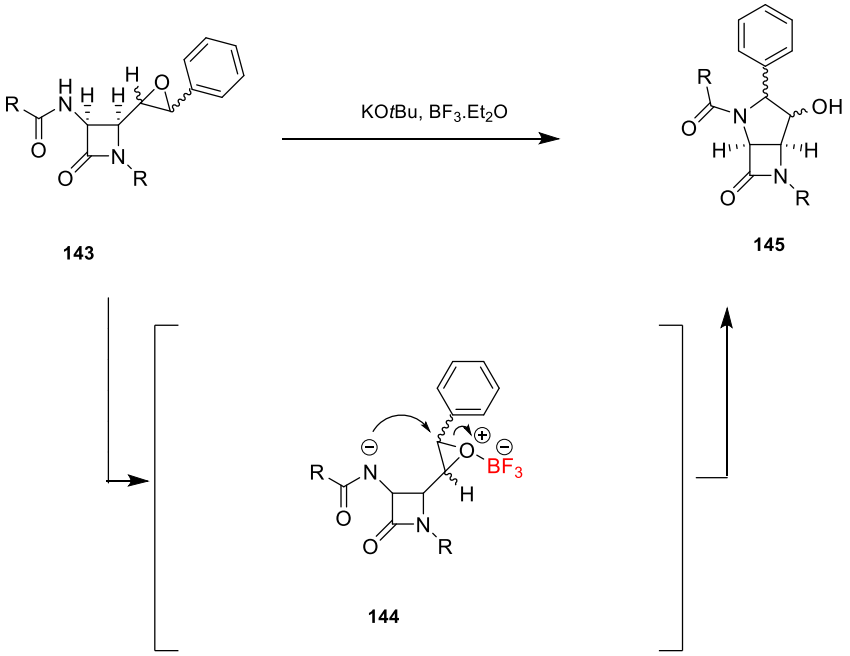
It has been proven that the PMP group attached to the bicyclic β-lactam **13** is susceptible to a deprotection with CAN. A next step is to attach a sulfonic acid group to the free β-lactam N-atom. As mentioned before, the 7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulphonic acids **26** are of special interest, since the sulfonic acid moiety is believed to play an active role in the β-lactamase inhibitory activity.[66] The proposed reagent to realise this reaction step is sulfur trioxide pyridine complex (SO_3-Py). When consulting the literature, numerous simple one-step procedures can be found to achieve the desired sulfonylation. It is observed that the reaction has been achieved with different possible solvents like pyridine, DMF, dichloromethane and dioxane.[81][82][83]



13 (R = Me, Bn) **26**

3.3.2 Lewis acid-mediated intramolecular ring closure

The base-mediated intramolecular ring closure towards bicyclic β-lactam **145** with KO*t*Bu has proven to be successful, as the reaction proceeded smoothly and the work-up was straightforward. A remark that has to be noted however, is the inevitable formation of side product. The side product is believed to be mainly the ring-opened β-lactam, which is catalysed by the base that is added. A promising possibility in order to reduce the side product formation would be the addition of a non-protonic acid, also known as a Lewis acid. It is reasoned that this would facilitate the intramolecular ring closure, since not only the amide will be activated, but also the epoxide. An example of such a Lewis acid is boron trifluoride diethyl etherate.

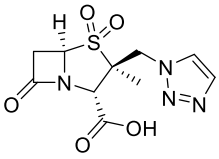


143 **145**

144

3.3.3 Biological testing of formed 2-acyl-4-hydroxy-3-phenyl-2,6-diazabicyclo[3.2.0]heptan-7-ones

After the succesfull formation and purification of newly formed bicyclic β-lactams **147** that is described in this Master's thesis, the compounds will be tested regarding their β-lactamase inhibitory activity, together with bicyclic β-lactams **148** formed in previous research.[15] A very interesting comparison is the inhibitory activity of the *N*-PMBz-substituted bicyclic β-lactam **148** and its oxo derivative **12**, which has already been tested in previous research.[66] Since results will be compared with earlier tests, it is important that the same procedure is used for each compound. In the earlier tests, the potential β-lactamase inhibitors were incubated with β-lactamase from *Enterobacter cloacae*, whereafter the residual enzymatic activity was measured by quantifying the rate of hydrolysis of nitrofecin. The reference compound used for these tests was tazobactam **146**.



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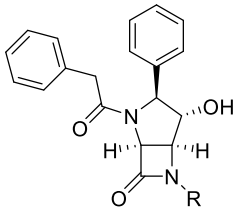
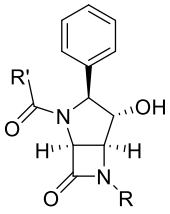
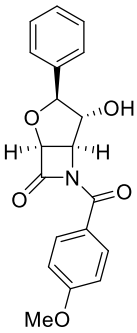


147 (R = H, PMP, *i*Pr)



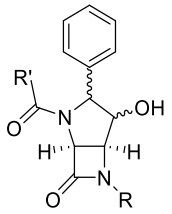
148 (R = PMB, PMBz
R' = Bn, Me)



12

4 Summary and conclusion

Antibiotics have proven to be a medical breakthrough in the recent history of mankind, enabling the safer practice of different medical procedures and playing a major role in combatting bacterial infections. One of the most widely known group of antibiotics are the β-lactam antibiotics, discovered in 1928 and available for the open market since 1946. However, the progress that is made through antibiotics is being threatened by antimicrobial resistance (AMR), which is mainly caused by the extensive and sometimes unnecessary use of antimicrobials, driving the selective pressure towards resistance. In recent years, a new method to combat AMR has emerged, where an additional drug is added in combination with the antibiotic to counteract resistance. One of the resistance mechanisms that can be opposed in this way, is the production of β-lactamase by the bacterial cell. In this thesis, the synthesis of 3,4-pyrrolidine-fused bicyclic azetidin-2-ones **i** that possibly possess β-lactamase inhibitory activity, is envisioned.



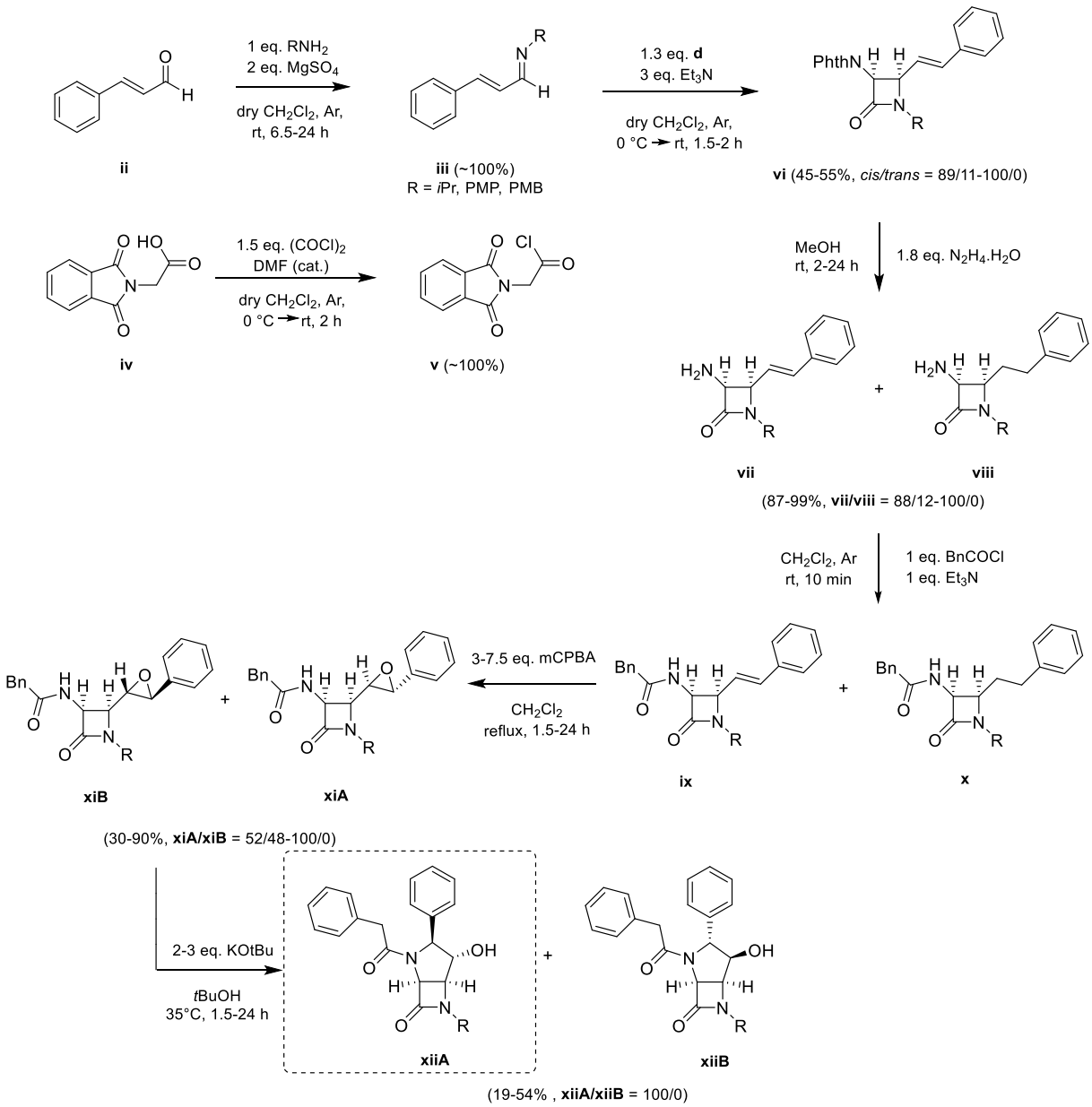
i: R = PMP, PMB, *i*Pr, PMBz, SO_3H
R' = Bn

4.1 Summary

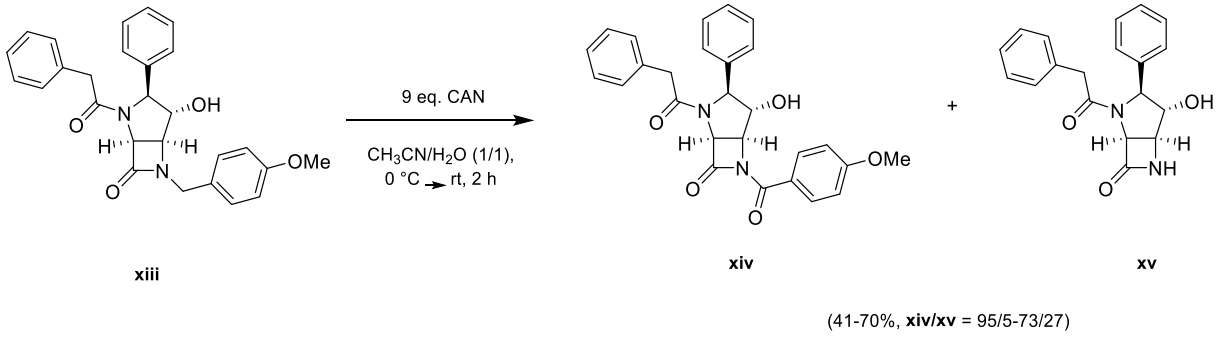
To synthesize the desired compound **i**, a seven-to nine-step pathway, depending on the selected *N*-substitution, was developed starting from (*E*)-cinnamaldehyde **ii** and *N*-phthaloylglycine **iv**. Preliminary research already succeeded in synthesizing the PMB and PMBz derivative of **i**, while also investigating a part of the pathway towards the PMP derivative. The first goal of this Master's thesis was validating this pathway by synthesizing the isopropyl derivative of compound **i**, which will also be tested for its β-lactamase inhibitory activity. The first step towards the latter compound was the formation of β-lactam scaffold **vi**. This was achieved by converting (*E*)-cinnamaldehyde **ii** towards imine **iii**, parallel with the formation of acid chloride **v**. With these two building blocks in hand, the Staudinger synthesis was performed to yield β-lactam **vi**. The subsequent steps first removed the phthaloyl moiety via hydrazinolysis, whereafter an *N*-acylation took place to yield *cis*-3-acylaminoazetidin-2-ones **ix**. It was observed that during the hydrazinolysis, a side reaction occurs that hydrogenated the (*E*)-styryl double bond, forming hydrogenated compound **viii**. The latter compound could not be seperated from its non-hydrogenated counterpart and was therefore acylated as well, yielding *N*-acylated compound **x**. The next step in the synthesis route was the formation of a mixture of epoxides by *m*CPBA, where it was observed that this step was stereoselective, yielding a major and a minor epoxide **xiA** and **xiB**. The final step towards desired compound **i** was the intramolecular ring closure. This ring closure has been achieved with the help of KO*t*Bu, which served as a base to deprotonate the amide function that will facilitate the 5-*endo-tet* cyclisation to obtain bicyclic β-lactam **xiiA**. It was seen that only the major epoxide **xiA** is able to perform the ring closure, while the minor epoxide **xiB** is believed to degrade during the process. This synthetic route has also been used to yield the *N*-PMP-and *N*-PMB-substituted bicyclic β-lactam **i**, where the reaction parameters had to be optimized for each derivative. The PMP

and *i*Pr bicyclic β-lactams **i** are molecules that were never synthesized before, thus they were isolated and characterized in this work.

The desired 4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one and 4-hydroxy-6-(4-methoxyphenyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **xiiA** will also be tested for their β-lactamase inhibitory activity in the near future.



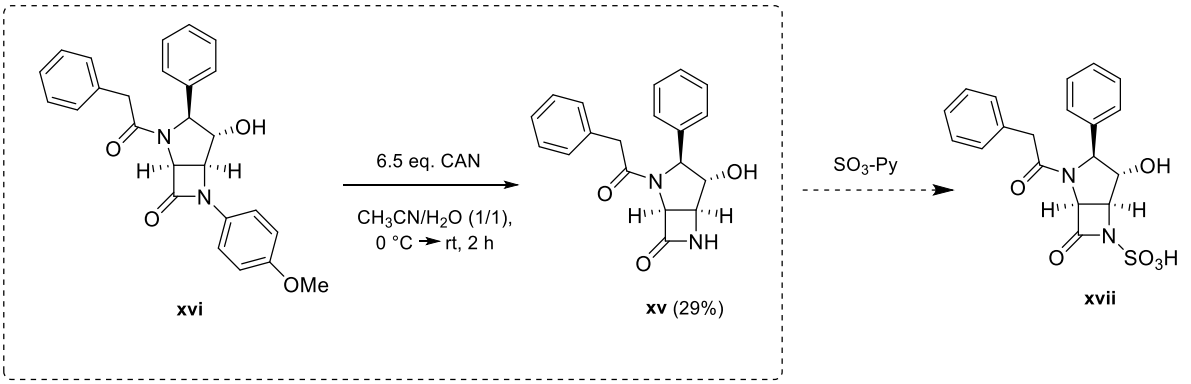
Another goal was to further investigate the derivatisation of *N*-PMB bicyclic β-lactam **xiii**. Initially the PMB group was considered as a possible way to achieve *N*-sulfonic acid bicyclic β-lactam **xvii** by deprotecting the PMB moiety with CAN. It was seen from previous research that a small amount of deprotected β-lactam was formed by treatment of compound **xiii** with CAN. After further investigation, however, it was observed that the conversion towards deprotected β-lactam **xv** could not be increased, and the starting product is mostly oxidized to the corresponding *N*-PMBz bicyclic β-lactam **xiv**. This overoxidated product, however, is also interesting to test for its β-lactamase inhibitory activity.



xiii **xiv** **xv**

(41-70%, **xiv/xv** = 95/5-73/27)

Beside the testing for its β-lactamase inhibitory activity, the other reason why *N*-PMP bicyclic β-lactam **xvi** is formed is to remove the PMP group and replace it with a sulfonic acid group, because the latter moiety is believed to enhance the β-lactamase inhibitory activity. The envisioned pathway to achieve this is removing the PMP group with the help of ceric ammonium nitrate (CAN) and letting deprotected β-lactam **xv** react with sulfur trioxide pyridine complex (SO_3-Py). The contemplated deprotection was achieved in this Master's thesis by adding 6.5 equivalents of CAN to a solution of *N*-PMP bicyclic β-lactam **xvi** in acetonitrile/water (1/1). The sulfonation of the β-lactam nitrogen with SO_3-Py, however, will be investigated in further research.



xvi **xv** (29%) **xvii**

4.2 Conclusion

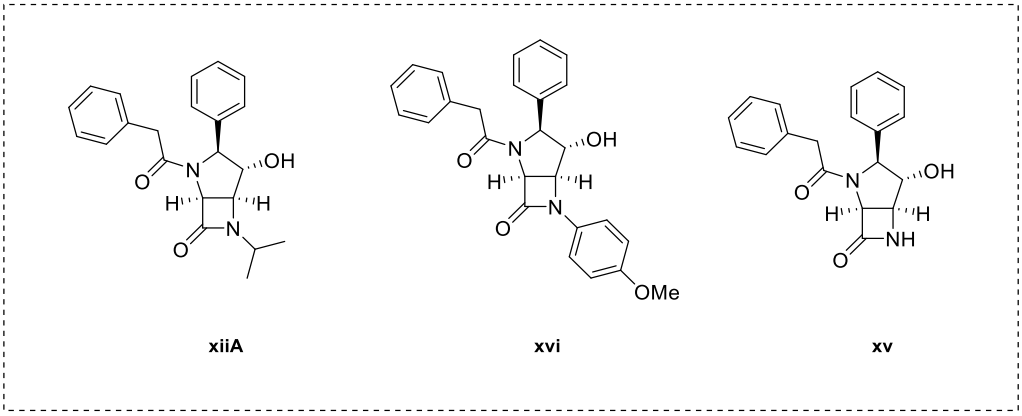
The aim of this Master's thesis was to synthesize different *cis*-4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-ones **i** as possible new class C β-lactamase inhibitors. The synthetic route towards these molecules had only been fully proven with PMB as *N*-substitution, which could

also yield the PMBz derivative after reaction with CAN. In this work, we were able to continue the previous research to finally obtain *N*-PMP substituted bicyclic β-lactam **xvi**, which was isolated and characterized. Another achievement was the validation of the synthetic pathway by synthesizing *N*-isopropyl substituted derivative **xiiA**. The Latter molecule and all intermediate compounds of the synthetic pathway that led to this bicyclic β-lactam were isolated and characterized.

The derivatisation of *N*-PMB-protected β-lactam was also further investigated, and it was seen that when the starting product is treated with CAN, a mixture of deprotected β-lactam **xv** and *N*-PMBz derivative **xiv** is formed. A more selective conversion towards the latter molecule is desired but was not achieved.

In addition, the deprotection of *cis*-4-hydroxy-6-(4-methoxyphenyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **xvi** towards 4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one **xv** was achieved, which has also been isolated and characterized. It was proved that PMP is a suitable protecting group for the synthesis of 3,4-pyrrolidine-fused bicyclic β-lactams. *N*-unsubstituted bicyclic β-lactam **xv** will be used as a building block in future research in an attempt to achieve the synthesis of *cis*-7-aza-4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic acid **xvii**.

In this Master's thesis, eight new β-lactams were synthesized and isolated, which have not been described before in the literature. Three of these new compounds possess the bicyclic β-lactam scaffold, which will be tested in the future for their β-lactamase inhibitory activity against class C β-lactamases with prof. T. Desmet (Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University).



xiiA **xvi** **xv**

5 Experimental part

5.1 General analytic methods and laboratory instruments

5.1.1 Thin layer chromatography (TLC)

To measure the R_f-values and to analyse column chromatography eluent fractions, thin layer chromatography was used. The R_f-values of the compounds are either used for characterization of new compounds, or to determine a suitable solvent mixture to use for the column chromatography. Silica plates, which consist of a glass back (Merck Silica gel 60 F_{254}, precoated, thickness 0.25 mm) were used in combination with the desired solvent mixture. To visualise the compound spots, both UV irradiation ($\lambda = 254$ nm) and staining the plate with potassium permanganate was used.

5.1.2 Automated column chromatography

Purification of the compounds after reaction was obtained by automated column chromatography. A Büchi Reveleris® X2 Flash Chromatography system was used for normal phase column chromatography (SiO_2), while the Grace™ Reveleris™ Flash Chromatography system was used when purification was obtained with a reversed phase silica column (C18). The systems made use of different reusable columns (SiO_2, particle diameter 0.040-0.063 mm and C18, particle diameter 0.020-0.040 mm). Detection of the compound was obtained by UV-detection at three selected wavelengths.

5.1.3 Column chromatography

To manually purify a reaction mixture, a glass chromatographic column was used, stuffed with a plug of cotton wool at the bottom and covered with a layer of sand. Hereafter, the mobile phase which consisted of a specific solvent mixture, and silica powder were added. After this, a thin layer of sand was added on top. Hereafter, the crude product was coated on silica and placed on top. To make the column ready for use, a thin layer of sand of one centimeter was applied again. The solvent mixture was then added to elute the different fractions.

5.1.4 Liquid Chromatography Mass Spectrometry (LC-MS)

To follow up reactions and analyse crude reaction mixtures, liquid chromatography mass spectrometry was used. The 1200 Series LC/MSD SL is equipped with a Supelco ascentis express C18 column with an internal diameter of 4.6 mm. Additionally, the instrument possesses a UV-DAD detector and there was an Agilent 1100 Series MSD SL mass spectrometer with electrospray ionisation (ESI, 4000 V, 70 eV) and with a single quadrupole detector coupled to the machine. To elute the components, a solvent mixture of acetonitrile and water in different ratios is used.

5.1.5 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance was both used for the follow-up of reactions and the characterization of the newly synthesized compounds. The spectra were taken by a Bruker Avance Nanobay III NMR spectrometer with a ^{1}H/BB z-gradient high resolution probe. The ^{1}H NMR was taken at 400 MHz, while the ^{13}C NMR spectra were taken at 100.6 MHz. The software used to process and display the spectra was TOPSPIN version 3.5. To prepare the samples for usage, the compounds were dissolved in deuterated solvents. In this Master's thesis, the solvent of choice was $CDCl_3$, but when it was seen that compounds were poorly soluble in the latter solvent, d_6-DMSO was chosen. Characterization of newly

synthesized compounds was possible with the help of COSY, HSQC and HMBC spectra, and coupling constants (*J*) are reported in hertz (Hz).

5.1.6 Mass Spectrometry (MS)

Mass spectrometry (low resolution) was used for the characterization of newly synthesized compounds. The spectra were recorded with an Agilent 1100 Series MSD SL mass spectrometer with electrospray ionisation (ESI, 4000 V, 70 eV), equipped with a single quadrupole detector.

5.1.7 Infrared spectroscopy (IR)

Infrared spectra of newly synthesized solid compounds were obtained with a Shimadzu IRAffinity-1S device with an Attenuated Total Reflectance (ATR) crystal. The software used to process the measurments was LabSolutions IR.

5.1.8 Melting point determination (Mp)

To characterize newly synthesized compounds, the melting point was measured. A Kofler heating bench system of Wagner and Munz was used. To measure the melting point, the sample is applied to the cold site of the instrument, while slowly moving it to the hot side until it melts. Calibration of the instrument was achieved by using one of the many calibration compounds.

5.1.9 Dry solvents

When reactions were performed that were sensitive to any water, dry solvents were used. The Mbraun SPS-800 solvent purfication system provided us with five different dry solvents: dichloromethane, toluene, tetrahydrofuran, diethyl ether and acetonitrile. To obtain the dry solvents, a vacuum is created inside a flask after which the solvent is pumped in it after previously passing through drying columns.

5.2 Safety aspects

5.2.1 General safety aspects

To ensure safe laboratory work and to minimize the risk of injuries, the SynBioC Research Group (Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University) has set up it's own internal guidelines. The internal guidelines and internal safety instructions ('Safety instructions: how to work with chemicals') had to be followed. In addition to these internal instructions, two other documents: "Safety and hygiene in chemical laboratories" and "Welzijns- en Milieugids UGent" had to be read and singed before the practical work began. To further highlight the safety aspects, a presentation was given before the laboratory experiments started. To put the safety aspects in practice, a tour around the laboratory was given, highlighting all the emergency equipment present. To be able to start the practical experiments, a test had to be taken in order to prove the safety aspects are known. In the lab, it was mandatory to wear a lab coat, long trousers, closed shoes and lab goggles (or normal glasses). It was also mandatory to consult the Safety Data Sheet (SDS) of all products that were needed before starting the experiments.

5.2.2 Specific safety risks

The use of hazardous chemicals was prevented at all cost and has been substituted by greener alternatives where possible. However, in some instances no alternative was available. An overview of the most important toxic chemicals and precautions that had to be taken, is given below.

Acid chlorides (**oxalyl chloride and phenylacetyl chloride):** Cause severe skin burns and eye damage.

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

Ceric ammonium nitrate (CAN): Causes eye damage and skin burns. May cause allergic skin reaction. Toxic to aquatic life. Avoid release to the environment. Keep away from ignition sources.

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

Dichloromethane (CH_2Cl_2): Causes necrosis of surrounding muscle and skin tissue when injected. Causes eye irritation. May cause cancer.

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

Hydrochloric acid (HCl): Causes severe skin burns and eye damage.

Isopropylamine: Extremely flammable liquid and vapor. May cause respiratory irritation. Causes severe skin burns and eye damage. Toxic if swallowed, in contact with skin or if inhaled.

Liquid nitrogen: Used for the cooling trap at the high vacuum installation. Can cause severe cryogenic burns to the skin and eyes. May cause suffocation by displacing oxygen in the air.

Potassium *tert*-butoxide (KO*t*Bu): Flammable solid. Releases flammable gases in contact with water. Causes eye damage and skin burns. Keep away from heat and moisture.

Silica gel (SiO_2): is used as carrier material in chromatography applications. Because of it's small particle size, a dust mask has to be worn in order to avoid inhalation.

Triethylamine (Et_3N): Flammable liquid. Causes eye damage and skin burns.

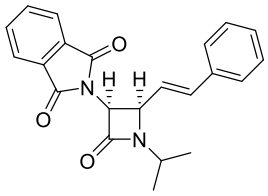
5.3 Synthetic procedures and spectral data

Standard procedures for the synthesis of compounds and their characterization is given below. It should be noted that the scale of the reaction did not influence the ratio of reagents that are used.

5.3.1 Staudinger β-lactam synthesis of *cis*-1-isopropyl-3-phthalimido-4-((*E*))-styryl)azetidin-2-one

3.47 g imine **115** (20 mmol, 1 eq.) was dissolved in ice-cooled (0 °C) anhydrous CH_2Cl_2 (60 ml) together with triethylamine (60 mmol, 3 eq.). After this, 5.81 g phthalimidoacetyl chloride **19** (26 mmol, 1.3 eq.) was dissolved in anhydrous CH_2Cl_2 (40 mL), after which it was added dropwise to the imine **115** solution. After the reaction proceeded for three hours at room temperature, CH_2Cl_2 (60 mL) was added, after which the resulting mixture was washed with a saturated aqueous $NaHCO_3$ solution (160 mL) and brine (160 mL). After this, the organic phase was dried with $MgSO_4$, the drying agent was filtered off and the solvent was evaporated. *cis*-β-Lactam **117** (racemic) was obtained in 55 % yield after purification by means of automated column chromatography over silica gel (gradient petroleum ether/ethyl acetate 67/33-0/100) and recrystallization from EtOAC.

***cis*-1-Isopropyl-3-phthalimido-4-((*E*)-styryl)azetidin-2-one 117**

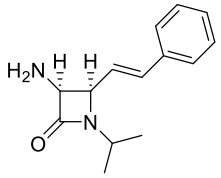


Pale white solid. R_f = 0.20 (PE/EtOAc 2/1). Mp= 191 °C. Yield after automated column chromatography (SiO_2, gradient PE/EtOAc 67/33-0/100), and recrystallization from EtOAC: 55 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.34 (6H, d, *J* = 6.7 Hz, 2 x CH_3); 4.04 (1H, sept, *J* = 6.7 Hz, C<u>H</u>$(CH_3)_2$); 4.66 (1H, d x d, *J* = 9.1, 5.2 Hz, C<u>H</u>CH=CH); 5.47 (1H, d, *J* = 5.2 Hz, (C=O)CH); 6.29 (1H, d x d, *J* = 16.0, 9.1 Hz, C<u>H</u>=CHPh); 6.65 (1H, d, *J* = 16.0 Hz, C<u>H</u>Ph); 7.19-7.29 (5H, m, 5 x (CH)C<u>H</u>$_{arom}$); 7.70-7.74 (2H, m, 2 x (C=O)$(CH_{arom})_{meta}$); 7.80-7.86 (2H, m, 2 x (C=O)$(CH_{arom})_{ortho}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 20.0 (CH_3); 21.7 (CH_3); 44.8 (<u>C</u>H$(CH_3)_2$); 57.4 ((C=O)<u>C</u>H); 60.1 (<u>C</u>HCH=CH); 123.7 (2 x (C=O)(H<u>C</u>$_{arom})_{ortho}$); 124.4 (<u>C</u>H=CHPh); 126.7 (2 x CH(H<u>C</u>$_{arom})_{ortho}$); 128.4 (CH(H<u>C</u>$_{arom})_{para}$); 128.7 (2 x CH(H<u>C</u>$_{arom})_{meta}$); 131.6 (2 x (C=O)<u>C</u>$_{arom,quat}$); 134.4 (2 x (C=O)(H<u>C</u>$_{arom})_{meta}$); 135.7 (CH<u>C</u>$_{arom,quat}$); 136.6 (<u>C</u>HPh); 163.0 ((<u>C</u>=O)CH); 167.5 (2 x (<u>C</u>=O)$C_{arom,quat}$). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1749, 1714; ν_{max} = 1387, 1202, 977, 721, 711, 696, 528. **MS**: m/z (%) 361 ($[M+H]^+$, 100).

5.3.2 Synthesis of *cis*-3-amino-1-isopropyl-4-((*E*))-styryl)azetidin-2-one

1.08 g *cis*-3-phthalimido-β-lactam **117** (3 mmol, 1 eq.) was dissolved in methanol (20 mL) together with 0.26 mL hydrazine monohydrate (100 %, 5.40 mmol, 1.8 eq.). After the reaction proceeded for two hours at reflux temperature, the precipitated phthalhydrazide was filtered off and the filtrate was evaporated. The residue was redissolved in tepid water (25 mL, 40 °C) and ethyl acetate (5 mL), after which it was extracted with ethyl acetate (3 x 15 mL). After this, the organic phase was dried with $MgSO_4$, the drying agent was filtered off and the solvent was evaporated. Crude *cis*-β-lactam **118** was obtained quantitatively, which was used without purification in the next reaction step. During the deprotection, 12 % of starting material **117** was converted to the hydrogenated side product **119**.

***cis*-3-Amino-1-isopropyl-4-((*E*))-styryl)azetidin-2-one 118**

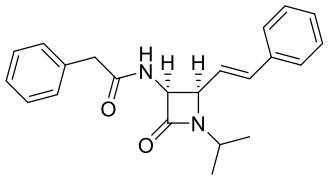


Pale white solid. R_f = 0.04 (PE/EtOAc 1/2). Mp = 85 °C. Yield 87 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.22 (3H, d, *J* = 6.7 Hz, CH($C\underline{H}_3$)(CH_3)); 1.28 (3H, d, *J* = 6.7 Hz, CH(CH_3)($C\underline{H}_3$)); 1.47 (2H, br d, *J* = 8.4 Hz, NH_2); 3.87 (1H, sept, *J* = 6.7 Hz, $C\underline{H}(CH_3)_2$); 4.30 (1H, t x d, *J* = 8.4, 5.1 Hz, (C=O)CH); 4.38 (1H, d x d, *J* = 8.5, 5.1 Hz, $C\underline{H}$CH=CH); 6.18 (1H, d x d, *J* = 15.9, 8.5 Hz, $C\underline{H}$=CHPh); 6.70 (1H, d, 15.9 Hz, $C\underline{H}$Ph); 7.26-7.30 (1H, m, $CH_{arom,para}$); 7.33-7.37 (2H, m, $CH_{arom,meta}$); 7.40-7.43 (2H, m, $CH_{arom,ortho}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 20.3 (CH(CH_3)($\underline{C}H_3$)); 21.8 (CH($\underline{C}H_3$)(CH_3)); 44.3 ($\underline{C}H(CH_3)_2$); 59.6 ($\underline{C}$HCH=CH); 62.8 ((C=O)$\underline{C}$H); 125.7 ($\underline{C}$H=CHPh); 126.6 (2 x $HC_{arom,ortho}$); 128.3 ($HC_{arom,para}$); 128.7 (2 x $HC_{arom,meta}$); 135.3 ($\underline{C}$HPh); 136.0 ($C_{arom,quat}$); 169.6 (C=O). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1734; ν_{max} = 968, 748, 690, 484. **MS**: m/z (%) 461 ($[2M+H]^+$, 100), 231 ($[M+H]^+$, 46).

5.3.3 Synthesis of *cis*-1-isopropyl-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one

1.84 g *cis*-3-amino-β-lactam **118** (8 mmol, 1 eq.) was dissolved in anhydrous CH_2Cl_2 (60 mL) under inert argon atmosphere together with 1.12 mL triethylamine (8 mmol, 1 eq.). Next, 1.06 mL phenylacetyl chloride (8 mmol, 1 eq.) was added to the mixture. After the reaction proceeded for ten minutes at room temperature, the reaction mixture was washed with a saturated aqueous $NaHCO_3$ solution (3 x 80 mL). After this, the organic phase was dried with $MgSO_4$, the drying agent was filtered off and the solvent was evaporated. Crude *cis*-β-lactam **120** was obtained quantitatively, which was used without purification in the next reaction step.

***cis*-1-isopropyl-3-(2-phenylacetamido)-4-((*E*)-styryl)azetidin-2-one 120**



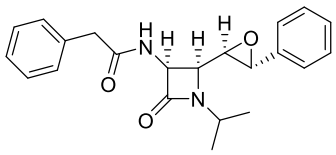
Pale white solid. R_f = 0.12 (PE/EtOAc 1/2). Mp = 122 °C. Yield 94 %. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.18 (3H, d, *J* = 6.7 Hz, CH(C<u>H</u>$_3$)(CH$_3$)); 1.26 (3H, d, *J* = 6.7 Hz, CH(CH$_3$)(C<u>H</u>$_3$)); 3.52 (1H, d, J_{AB} = 16.2 Hz, (<u>H</u>CH)); 3.54 (1H, d, J_{AB} = 16.2 Hz, (HC<u>H</u>)); 3.83 (1H, sept, *J* = 6.7 Hz, C<u>H</u>(CH$_3$)$_2$); 4.48 (1H, d x d, *J* = 8.2, 5.0 Hz, C<u>H</u>CH=CH); 5.26 (1H, d x d, *J* = 8.4, 5.0 Hz, (C=O)CH); 5.82 (1H, d x d, *J* = 16.0, 8.2 Hz, C<u>H</u>=CHPh); 5.87 (1H, br d, *J* = 8.4 Hz, NH); 6.59 (1H, d, *J* = 16.0 Hz, C<u>H</u>Ph); 7.03-7.06 (2H, m, 2 x CH$_2$(C<u>H</u>$_{arom}$)$_{ortho}$); 7.08-7.12 (2H, m, 2 x CH$_2$(C<u>H</u>$_{arom}$)$_{meta}$); 7.15-7.19 (1H, m, CH$_2$(C<u>H</u>$_{arom}$)$_{para}$); 7.25-7.30 (2H, m, 2 x CH(C<u>H</u>$_{arom}$)$_{ortho}$); 7.31-7.39 (3H, m, CH(C<u>H</u>$_{arom}$)$_{para}$ and 2 x CH(H<u>C</u>$_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 20.2 (CH(CH$_3$)(<u>C</u>H$_3$)); 21.5 (CH(<u>C</u>H$_3$)(CH$_3$)) 43.5 (CH$_2$); 44.9 (<u>C</u>H(CH$_3$)$_2$); 58.8 (<u>C</u>HCH=CH); 59.1 ((C=O)<u>C</u>H); 124.1 (<u>C</u>H=CHPh); 126.6 (2 x CH(H<u>C</u>$_{arom}$)$_{ortho}$); 127.5 (CH$_2$(H<u>C</u>$_{arom}$)$_{para}$); 128.4 (CH(H<u>C</u>$_{arom}$)$_{para}$); 128.7 (2 x CH(H<u>C</u>$_{arom}$)$_{meta}$); 129.0 (2 x CH$_2$(H<u>C</u>$_{arom}$)$_{meta}$); 129.4 (2 x CH$_2$(H<u>C</u>$_{arom}$)$_{ortho}$); 133.9 (CH$_2$<u>C</u>$_{arom,quat}$); 135.4 (<u>C</u>HPh); 135.7 (CH<u>C</u>$_{arom,quat}$); 165.6 (<u>C</u>=O(CH)); 171.0 (<u>C</u>=O(CH$_2$)). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1765, 1649; ν_{max} = 1530, 1368, 1315, 752, 696. **MS**: m/z (%) 174 ([$C_{12}H_{15}N$ + H]$^+$, 100), 349 ([M+H]$^+$, 5).

5.3.4 Synthesis of *cis*-3-acylamino-1-isopropyl-4-(3-phenyloxiran-2-yl)azetidin-2-one

A small fraction of the minor diastereomer **123b** could be isolated, which made it possible to characterize it. The major diastereomer **123a,** however, could not be isolated, which led us to characterize it using spectra from a mixture of major and minor diastereomer.

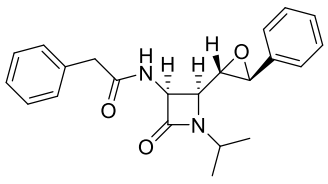
2.79 g *cis*-3-acylamino-4-((*E*)-styryl)-β-lactam **120** (8 mmol, 1 eq.) was dissolved in CH_2Cl_2 (60 mL) at refluxing temperature, after which 5.52 g 3-chloroperbenzoic acid (32 mmol, 4 eq.) was added in small portions. After the reaction proceeded for eight hours at reflux temperature, a mixture of CH_2Cl_2 (60 mL) and saturated aqueous Na_2SO_3 solution (120 mL) was added. After this, the quenched reaction mixture was stirred for another ten minutes at room temperature, after which the mixture was washed with a saturated aqueous $NaHCO_3$ solution (2 x 120 mL) and brine (2 x 120 mL). Subsequently, the combined aqueous phases were extracted again with CH_2Cl_2 (180 mL). After this, the organic phase was dried with $MgSO_4$, the drying agent was filtered off and the solvent was evaporated. A diastereomeric mixture of *cis*-3-acylamino-1-isopropyl-4-(3-phenyloxiran-2-yl)azetidin-2-one **123a/123b** (racemic, **123a/123b** = 64/36) was obtained in 59 % combined yield after purification by automated column chromatography over silica gel (gradient petroleum ether/ethyl acetate 67/33-0/100).

(3*S,4*S**,2'*R**,3'*R**)-1-Isopropyl-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-one 123a**



Spectral data derived from the mixture of two diastereomers **123a/123b** (*dr* = 90/10), which had a purity of 90 % as determined by ^{1}H NMR spectroscopy ($CDCl_3$). Pale white solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.27 (3H, d, *J* = 6.7 Hz, CH(C<u>H</u>$_3$)(CH$_3$)); 1.32 (3H, d, *J* = 6.7 Hz, CH(CH$_3$)(C<u>H</u>$_3$)); 2.70 (1H, d x d, *J* = 8.1, 2.0 Hz, C<u>H</u>OCHPh); 3.24 (1H, d, J_{AB} = 16.5 Hz, (HC<u>H</u>)); 3.34 (1H, d, J_{AB} = 16.5 Hz, (<u>H</u>CH)); 3.52 (1H, d x d, *J* = 8.1, 5.0 Hz, C<u>H</u>CHOCH); 3.76 (1H, d, *J* = 2.0 Hz, C<u>H</u>Ph); 3.95 (1H, sept, *J* = 6.7 Hz, C<u>H</u>(CH$_3$)$_2$); 5.07 (1H, d x d, *J* = 6.8, 5.0 Hz, (C=O)CH); 6.06 (1H, d, *J* = 6.8 Hz, NH); 6.58-6.60 (2H, m, 2 x CH$_2$(C<u>H</u>$_{arom}$)$_{ortho}$); 7.10-7.14 (2H, m, 2 x CH$_2$(C<u>H</u>$_{arom}$)$_{meta}$); 7.17-7.20 (1H, m, CH$_2$(C<u>H</u>$_{arom}$)$_{para}$); 7.26-7.31 (2H, m, 2 x CH(C<u>H</u>$_{arom}$)$_{ortho}$); 7.35-7.44 (3H, m, CH(C<u>H</u>$_{arom}$)$_{para}$ and 2 x CH(C<u>H</u>$_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 20.0 (CH(<u>C</u>H$_3$)(CH$_3$)); 21.4 (CH(CH$_3$)(<u>C</u>H$_3$)); 43.1 (CH$_2$); 44.4 (<u>C</u>H(CH$_3$)$_2$); 56.7 (<u>C</u>HPh); 57.4 ((C=O)<u>C</u>H); 59.1 (<u>C</u>HCHOCH); 62.1 (<u>C</u>HOCHPh); 125.6 (2 x CH(H<u>C</u>$_{arom}$)$_{ortho}$); 127.5 (CH$_2$(H<u>C</u>$_{arom}$)$_{para}$); 128.66 (CH(H<u>C</u>$_{arom}$)$_{para}$); 128.69 (2 x CH(H<u>C</u>$_{arom}$)$_{meta}$); 129.1 (2 x CH$_2$(H<u>C</u>$_{arom}$)$_{meta}$); 129.4 (2 x CH$_2$(H<u>C</u>$_{arom}$)$_{ortho}$); 133.4 (CH$_2$<u>C</u>$_{arom,quat}$); 136.2 (CH<u>C</u>$_{arom,quat}$); 164.7 ((<u>C</u>=O)CH); 171.5 ((<u>C</u>=O)CH$_2$). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1749, 1647; ν_{max} = 1516, 1497, 1369, 1204, 1024, 740, 696, 546. **MS**: m/z (%) 365 ([M+H]$^+$, 100).

(3*S,4*S**,2'*S** ,3'*S**)-1-Isopropyl-3-(2-phenylacetamido)-4-(3-phenyloxiran-2-yl)azetidin-2-one 123b**



Pale white solid. R_f = 0.13 (PE/EtOAc 1/1). Mp= 146 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.17 (3H, d, *J* = 6.7 Hz, CH(C<u>H</u>$_3$)(CH$_3$)); 1.26 (3H, d, *J* = 6.7 Hz, CH(CH$_3$)(C<u>H</u>$_3$)); 2.94 (1H, d x d, *J* = 5.7, 2.0 Hz, C<u>H</u>OCHPh); 3.51 (1H, d, J_{AB} = 16.1 Hz, (HC<u>H</u>)); 3.54 (1H, d, J_{AB} = 16.1 Hz, (<u>H</u>CH)); 3.72 (1H, d, *J* = 2.0 Hz, C<u>H</u>Ph); 3.85 (1H, sept, *J* = 6.7 Hz, C<u>H</u>(CH$_3$)$_2$); 3.89 (1H, ~t, *J* = 5.3 Hz, C<u>H</u>CHOCH); 5.14 (1H, d x d, *J* = 7.5, 4.9 Hz, (C=O)CH); 6.14 (1H, d, *J* = 7.5 Hz, NH); 7.10-7.14 (2H, m, 2 x

$CH_2(C\underline{H}_{arom})_{ortho}$); 7.19-7.21 (2H, m, 2 x $CH(C\underline{H}_{arom})_{ortho}$); 7.25-7.28 (3H, m, 2 x $CH_2(C\underline{H}_{arom})_{meta}$ and $CH_2(C\underline{H}_{arom})_{para}$); 7.36-7.40 (3H, m, 2 x $CH(C\underline{H}_{arom})_{meta}$ and $CH(C\underline{H}_{arom})_{para}$). **^{13}C NMR** (100 MHz, ref = $CDCl_3$): δ 20.0 ($CH(CH_3)(\underline{C}H_3)$); 21.5 ($CH(\underline{C}H_3)(CH_3)$); 43.4 ($CH_2$); 45.0 ($\underline{C}H(CH_3)_2$); 56.1 ($\underline{C}HPh$) 56.7 ($\underline{C}HCHOCH$); 57.6 ($(C=O)\underline{C}H$); 60.4 ($\underline{C}HOCHPh$); 125.6 (2 x $CH(H\underline{C}_{arom})_{ortho}$); 127.5 ($CH_2(H\underline{C}_{arom})_{para}$); 128.71 and 128.73 (2 x $CH(H\underline{C}_{arom})_{meta}$ and $CH(H\underline{C}_{arom})_{para}$); 129.1 (2 x $CH_2(\underline{H}C_{arom})_{meta}$); 129.5 (2 x $CH_2(H\underline{C}_{arom})_{ortho}$); 133.9 ($CH_2\underline{C}_{arom,quat}$); 135.7 ($CH\underline{C}_{arom,quat}$); 165.5 ($(\underline{C}=O)CH$); 171.4 ($(\underline{C}=O)CH_2$). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1755, 1649; ν_{max} = 1391, 1020, 762, 698. **MS**: m/z (%) 365 ($[M+H]^+$, 100).

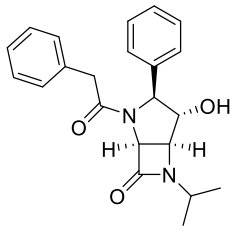
5.3.5 Synthesis of 4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-ones

The synthetic procedure for both (1*S**,3*S**,4*S**,5*S**)-4-hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one **125a** and (1*S**,3*S**,4*S**,5*S**)-4-hydroxy-6-(4-methoxyphenyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one **140** were similar, so only the procedure for compound **125a** is described as a representative example.

0.36 g *cis*-3-acylamino-1-isopropyl-4-(3-phenyloxiran-2-yl)azetidin-2-one as a mixture **123a**/**123b** in a ratio of 68/32 (1 mmol, 1 eq.) was dissolved in *tert*-butanol (30 mL), after which 0.30 g potassium *tert*-butoxide (10 mmol, 3 eq.) was added in small portions. After the reaction proceeded for one and a half hour at 35 °C, hydrochloric acid (1 M, 10 mL) was added and the reaction was stirred for another ten minutes at room temperature. After this, CH_2Cl_2 (30 mL) was added and the mixture was washed with tepid water (5 x 30 mL, 40 °C) and brine (30 mL). After this, the organic phase was dried with $MgSO_4$, the drying agent was filtered off and the solvent was evaporated. Without further purification, 3,4-pyrrolidine-fused bicyclic β-lactam **x** (racemic) was obtained in 54 % yield.

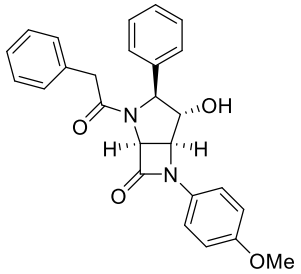
(1*S,3*S**,4*S**,5*S**)-4-Hydroxy-6-isopropyl-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one 125a**

Spectral data derived from the mixture of two rotamers (69/31 in d_6-DMSO).



Pale white solid. R_f = 0.56 (MeOH/EtOAc 1/19). Mp = 216 °C. Yield: 54 %. *Major rotamer:* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 0.51 (3H, d, *J* = 6.7 Hz, CH(C$\underline{H}_3$)(CH$_3$)); 0.86 (3H, d, *J* = 6.7 Hz, CH(CH$_3$)(C$\underline{H}_3$)); 3.26-3.37 (1H, m, C$\underline{H}$(CH$_3$)$_2$); 3.90 (1H, d, J_{AB} = 15.5 Hz, HC$\underline{H}$); 3.96 (1H, d, J_{AB} = 15.5 Hz, $\underline{H}$CH); 4.16 (1H, br d, *J* = 4.1 Hz, (C=O)CHC$\underline{H}$); 4.41 (1H, d, *J* = 3.5 Hz C$\underline{H}$OH); 5.48 (1H, br s, C$\underline{H}$Ph); 5.52 (1H, d, *J* = 4.1 Hz, (C=O)CH), 5.78 (1H, d, *J* = 3.5 Hz, OH); 7.11-7.30 (6H, m, 5 x CH(C$\underline{H}_{arom}$) and CH$_2$(C$\underline{H}_{arom}$)$_{para}$); 7.33-7.34 (4H, m, 2 x CH$_2$(C$\underline{H}_{arom}$)$_{ortho}$ and 2 x CH$_2$(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 19.8 (CH(CH$_3$)(C$\underline{H}_3$)); 20.8 (CH(C$\underline{H}_3$)(CH$_3$)); 40.8 (CH$_2$); 44.1 (C$\underline{H}$(CH$_3$)$_2$); 63.3 ((C=O)CHCH); 68.2 ((C=O)C$\underline{H}$); 71.5 (C$\underline{H}$Ph); 76.5 (CHOH); 125.3 (2 x CH(HC$\underline{C}_{arom}$)$_{ortho}$); 127.0 (CH(HC$\underline{C}_{arom}$)$_{para}$ and CH$_2$(HC$\underline{C}_{arom}$)$_{para}$); 128.6 (2 x CH(HC$\underline{C}_{arom}$)$_{meta}$); 128.7 (2 x CH$_2$(HC$\underline{C}_{arom}$)$_{meta}$); 130.0 (2 x CH$_2$(HC$\underline{C}_{arom}$)$_{ortho}$); 135.7 (CH$_2$C$\underline{C}_{arom,quat}$); 139.4 (CHC$\underline{C}_{arom,quat}$); 164.5 ((C=O)CH); 169.5 (C=O(CH$_2$)). *Minor rotamer:* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 0.47 (3H, d, *J* = 6.6 Hz, CH(C$\underline{H}_3$)(CH$_3$)); 0.85 (3H, d, *J* = 6.6 Hz, CH(CH$_3$)(C$\underline{H}_3$)); 3.26-3.37 (1H, m, C$\underline{H}$(CH$_3$)$_2$); 3.39 (1H, d, J_{AB} = 15.6 Hz, HC$\underline{H}$); 3.61 (1H, d, J_{AB} = 15.6 Hz, $\underline{H}$CH); 4.03 (1H, br d, *J* = 4.0 Hz, (C=O)CHC$\underline{H}$); 4.48 (1H, d, *J* = 3.9 Hz, C$\underline{H}$OH); 5.47-5.48 (1H, m, (C=O)CH); 5.49 (1H, br s, C$\underline{H}$Ph); 5.83 (1H, d, *J* = 3.9 Hz, OH); 7.11-7.32 (8H, m, 5 x CH$_2$(CH$_{arom}$), CH(C$\underline{H}_{arom}$)$_{para}$ and 2 x CH(C$\underline{H}_{arom}$)$_{ortho}$); 7.34-7.38 (2H, m, 2 x CH(C$\underline{H}_{arom}$)$_{meta}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 19.8 (CH(CH$_3$)(C$\underline{H}_3$)); 20.8 (CH(C$\underline{H}_3$)(CH$_3$)); 40.3 (CH$_2$); 43.9 (C$\underline{H}$(CH$_3$)$_2$); 61.6 ((C=O)CHC$\underline{H}$); 67.5 ((C=O)C$\underline{H}$); 72.7 (C$\underline{H}$Ph); 78.1 (CHOH); 125.4 (2 x CH(HC$\underline{C}_{arom}$)$_{ortho}$); 126.9 (CH(HC$\underline{C}_{arom}$)$_{para}$); 127.5 (CH(HC$\underline{C}_{arom}$)$_{para}$); 128.6 (2 x CH$_2$(HC$\underline{C}_{arom}$)$_{meta}$); 128.9 (2 x CH(HC$\underline{C}_{arom}$)$_{meta}$); 129.8 (2 x CH$_2$(HC$\underline{C}_{arom}$)$_{ortho}$); 135.4 (CH$_2$C$\underline{C}_{arom,quat}$); 139.5 (CHC$\underline{C}_{arom,quat}$); 164.4 ((C$\underline{=}$O)CH); 170.1 ((C$\underline{=}$O)CH$_2$). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1743, 1626; ν_{max} = 1535, 1391, 1234, 1078, 716, 579. **MS**: m/z (%) 365 ([M + H]$^+$, 100).

(1S*,3S*,4S*,5S*)-4-hydroxy-6-(4-methoxyphenyl)-3-phenyl-2-(2-phenylacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one 140

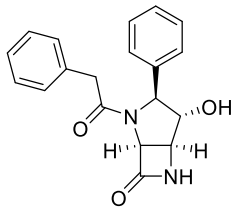


Spectral data derived from the mixture of two rotamers (65/35 in d_6-DMSO). Pale white solid. R_f = 0.78 (EtOAc/MeOH 19/1). Mp = 258 °C. Yield after automated column chromatography (SiO_2, gradient PE/EtOAc 67/33-0/100): 26 %. *Major rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.67 (3H, s, CH_3O); 3.96 (1H, d, J_{AB} = 15.6 Hz, HCH); 4.03 (1H, d, J_{AB} = 15.6 Hz, HCH); 4.46 (1H, d, *J* = 3.6 Hz, CHOH); 4.68 (1H, d x d, *J* = 4.3, 0.8 Hz, (C=O)CHCH); 5.50 (1H, br s, CHPh); 5.81 (1H, d, *J* = 4.3 Hz, (C=O)CH); 5.94 (1H, d, *J* = 3.6 Hz, OH); 6.81-6.84 (2H, m, 2 x $O(CH_{arom})_{ortho}$); 7.01-7.03 (2H, m, 2 x $O(CH_{arom})_{meta}$); 7.08-7.12 (3H, m, 2 x $CH(CH_{arom})_{ortho}$ and $CH(CH_{arom})_{para}$); 7.18-7.30 (3H, m, 2 x $CH(CH_{arom})_{meta}$ and $CH_2(CH_{arom})_{para}$); 7.35-7.36 (4H, m, 2 x $CH_2(CH_{arom})_{ortho}$ and 2 x $CH_2(CH_{arom})_{meta}$). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.8 (CH_2); 55.7 (CH_3O); 64.6 ((C=O)CHCH); 68.8 ((C=O)CH); 71.5 (CHPh); 74.8 (CHOH); 114.9 (2 x $O(HC_{arom})_{ortho}$); 118.5 (2 x $O(HC_{arom})_{meta}$); 125.2 ($CH(HC_{arom})_{ortho}$) ; 127.0 ($CH(HC_{arom})_{para}$ and $CH_2(HC_{arom})_{para}$); 128.6 (2 x $CH(HC_{arom})_{meta}$); 128.7 (2 x $CH_2(CH_{arom})_{meta}$); 129.99 ($NC_{arom,quat}$); 130.01 (2 x $CH_2(HC_{arom})_{ortho}$); 135.6 ($CH_2C_{arom,quat}$); 139.0 ($CHC_{arom,quat}$); 156.4 ($OC_{arom,quat}$); 162.3 ((C=O)CH); 169.6 ((C=O)CH_2). *Minor rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.41 (1H, d, *J* = 15.5 Hz, HCH); 3.62-3.65 (1H, m, HCH); 3.67 (3H, s, CH_3O); 4.52 (1H, d, *J* = 3.8 Hz, CHOH); 4.54 (1H, d, *J* = 4.0 Hz, (C=O)CHCH); 5.53 (1H, s, CHPh); 5.75 (1H, d, *J* = 4.0 Hz, (C=O)CH); 6.00 (1H, d, *J* = 3.8 Hz, OH); 6.79-6.82 (2H, m, 2 x $O(CH_{arom})_{ortho}$); 6.96-6.99 (2H, m, 2 x $O(CH_{arom})_{meta}$); 7.10-7.30 (10H, m, 10 x CH_{arom}). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.3 (CH_2); 55.7 (CH_3O); 62.9 ((C=O)CHCH); 68.1 ((C=O)CH); 72.6 (CHPh); 76.5 (CHOH); 114.8 (2 x $O(HC_{arom})_{ortho}$); 118.3 (2 x $O(HC_{arom})_{meta}$); 125.2 (2 x $CH(HC_{arom})_{ortho}$); 127.0 ($CH_2(HC_{arom})_{para}$); 127.6 ($CH(HC_{arom})_{para}$); 128.7 (2 x $CH_2(HC_{arom})_{meta}$); 129.0 (2 x $CH(HC_{arom})_{meta}$); 129.8 (2 x $CH_2(HC_{arom})_{ortho}$); 130.1 ($NC_{arom,quat}$); 135.3 ($CH_2C_{arom,quat}$); 139.0 ($CHC_{arom,quat}$); 156.3 ($OC_{arom,quat}$); 162.2 ((C=O)CH); 170.3 ((C=O)CH_2). **IR** (ATR, cm^{-1}): $\nu_{C=O}$ = 1741, 1625; ν_{max} = 1512, 1421, 1247, 825, 715, 700. **MS**: m/z (%) 429 ($[M+H]^+$, 100).

5.3.6 Synthesis of 4-hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one

0.04 g 3,4-pyrrolidine-fused bicyclic β-lactam **140** (0.1 mmol, 1 eq.) was dissolved in ice-cooled (0° C) acetonitrile (15 mL). After this, 0.36 g cerium(IV) ammonium nitrate (0.65 mmol, 6.5 eq.) was dissolved in water (15 mL), after which it was added dropwise to the bicyclic β-lactam **140** solution. After the reaction proceeded for two hours at room temperature, the acetonitrile was evaporated and the remaining reaction mixture was extracted with ethyl acetate (5 x 20 mL). The organic phases were washed with a saturated aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL). After this, the organic phase was dried with $MgSO_4$, the drying agent was filtered off and the solvent was evaporated. Deprotected β-lactam **139** (racemic) was obtained in 29 % yield after washing the crude compound with ethyl acetate (5 mL).

(1*S,3*S**,4*R**,5*S**)-4-Hydroxy-3-phenyl-2-(2-phenylacetyl)-2,6-diaza-bicyclo[3.2.0]heptan-7-one 139**



Spectral data derived from the mixture of two rotamers (70/30 in d_6-DMSO). Pale white solid. Mp = 226 °C. Yield: 29 %. *Major rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.89 (1H, d, J_{AB} = 15.6 Hz, HCH); 3.95 (1H, d, J_{AB} = 15.6 Hz, HCH); 4.06 (1H, d, J = 4.0 Hz, (C=O)CHCH); 4.26 (1H, d, J = 3.4 Hz, CHOH); 5.47 (1H, br s, CHPh); 5.61 (1H, ~t, J = 3.6 Hz, (C=O)CH); 5.77 (1H, d, J = 3.4 Hz, OH); 7.17-7.22 (3H, m, 2 x CH(CH_{arom})$_{ortho}$ and CH(CH_{arom})$_{para}$); 7.22-7.36 (7H, m, 2 x CH(CH_{arom})$_{meta}$ and 5 x CH_2(CH_{arom})); 8.24 (1H, d, J = 2.9 Hz, NH). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.8 (CH_2); 60.7 ((C=O)CHCH); 70.8 ((C=O)CH); 72.3 (CHPh); 77.9 (CHOH); 125.3 (2 x CH(HC_{arom})$_{ortho}$); 126.9 and 127.0 (CH(HC_{arom})$_{para}$ and CH_2(HC_{arom})$_{para}$); 128.6 (2 x CH(HC_{arom})$_{meta}$); 128.7 (2 x CH_2(HC_{arom})$_{meta}$); 130.0 (2 x CH_2(HC_{arom})$_{ortho}$); 135.7 ($CH_2C_{arom,quat}$); 139.9 ($CHC_{arom,quat}$); 166.2 ((C=O)CH); 169.3 ((C=O)CH_2). *Minor rotamer.* **^{1}H NMR** (400 MHz, d_6-DMSO): δ 3.33-3.37 (1H, m, HCH); 3.58 (1H, d, J_{AB} = 15.7 Hz, HCH); 3.93-3.94 (1H, m, (C=O)CHCH); 4.33 (1H, d, J = 4.0 Hz, CHOH); 5.48 (1H, s, CHPh); 5.58 (1H, ~t, J = 3.6 Hz, (C=O)CH); 5.84 (1H, d, J = 4.0 Hz, OH); 7.09-7.10 (2H, m, 2 x CH_2(CH_{arom})$_{ortho}$); 7.17-7.39 (8H, m, 5 x CH(CH_{arom}), CH_2(CH_{arom})$_{para}$ and 2 x CH_2(CH_{arom})$_{meta}$); 8.13 (1H, d, J = 2.7 Hz, NH). **^{13}C NMR** (100 MHz, ref = d_6-DMSO): δ 40.3 (CH_2); 59.1 ((C=O)CHCH); 70.1 ((C=O)CH); 73.5 (CHPh); 79.7 (CHOH); 125.3 (2 x CH(HC_{arom})$_{ortho}$); 126.9 (CH_2(HC_{arom})$_{para}$); 127.5 (CH(HC_{arom})$_{para}$); 128.6 (2 x CH_2(HC_{arom})$_{meta}$); 129.0 (2 x CH(HC_{arom})$_{meta}$); 129.8 (2 x CH_2(HC_{arom})$_{ortho}$); 135.4 ($CH_2C_{arom,quat}$); 140.0 ($CHC_{arom,quat}$); 166.2 ((C=O)CH); 170.0 ((C=O)CH_2). **IR** (ART, cm^{-1}): $\nu_{C=O}$ = 1768, 1627; ν_{max} = 1423, 723, 696, 527, 433. **MS**: m/z (%) 323 ($[M+H]^+$, 100).

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