FACULTY OF BIOSCIENCE ENGINEERING

C3-C4 RING OPENING OF 3-OXO-4-TRIFLUOROMETHYL-BETA-LACTAMS

A COMPUTATIONAL STUDY

Number of words: 15.873

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

Academic year: 2016 - 2017

GHENT UNIVERSITY

If you're walking down the right path, and you're willing to keep walking, eventually you'll make progress

Barack Obama

Dankwoord

Na een intensieve periode van tien maanden is het dan zover. Met het schrijven van dit dankwoord leg ik de laatste hand aan mijn thesis. Uiteraard zou deze thesis niet tot stand gekomen zijn zonder de hulp van enkele andere personen, die ik dus ook graag wil vermelden.

Eerst en vooral wil ik mijn promotoren, professor Van Speybroeck en professor D'hooghe, bedanken. Bedankt voor de kans die jullie mij gegeven hebben om een gecombineerde thesis uit te voeren, waardoor ik het voorbije jaar zo enorm veel heb kunnen bijleren, en bedankt om mij dit jaar zo goed te begeleiden en bij te sturen wanneer nodig.

Naast mijn promotoren hebben uiteraard mijn begeleiders mij enorm goed bijgestaan dit jaar. Bedankt Hannelore en Dietmar, jullie waren gewoonweg veel meer dan begeleiders. We hebben elkaar gedurende het jaar ook op een meer persoonlijke manier leren kennen. Dietmar, jouw passie voor 'De Redenaar', en Hannelore, de liefdevolle manier waarop jij over je man en kindjes babbelt hebben mij geïnspireerd en mee mijn keuze voor volgend jaar bepaald.

I would also like to take a moment to thank my third tutor, Hang, for making this thesis subject possible and for the time you took to guide me in the lab.

Enkele andere personen die ik wil bedanken zijn mijn medethesissers, met wie ik slechts twee weken in het labo stond. Toch was de toon in die korte periode al gezet en maakten we met z'n allen heel wat hilarische momenten mee. In het bijzonder wil ik hierbij Robin bedanken. De afgelopen twee jaar hebben ben jij een enorme steun geweest voor mij. Bedankt om mij ontelbare keren van en naar de faculteit te brengen toen ik in een rolstoel zat en om keer op keer weer te luisteren naar mijn verhalen of frustraties.

Daarnaast wil ik graag 'de meisjes' van bij de bio-ingenieurs bedanken. Ook al koos elk van ons zeven doorheen de opleiding haar eigen weg, toch vinden we elkaar steeds terug, of het nu is met een lach of een traan. Onze etentjes, filmavondjes en feestjes zijn altijd de perfecte

ontspanning. Bedankt voor al die grappige gebeurtenissen, die onverwachte en veel te late avonden en voor al jullie steun gedurende de voorbije vijf jaar.

Er zijn dan ook nog enkele personen die wat minder te maken hebben met het schrijven van mijn thesis, maar die zeker zo belangrijk zijn om te vermelden. Mama en papa, jullie zijn volgens mij de beste ouders die iemand zich kan wensen. Steeds staan jullie klaar met raad en onvoorwaardelijke liefde. Jullie hebben mij gemaakt tot de persoon die ik nu ben en ook al is er mogelijks nog wat werk aan, ik hoop dat ik jullie toch al trots heb kunnen maken in de voorbije 23 jaar.

Julie en Maxime, 'zussielief' en 'broereman', jullie wil ik graag ook bedanken. Eigenlijk zijn jullie altijd al mijn twee grote voorbeelden geweest en ben ik blij dat we zo'n hechte band hebben. Bedankt om mij af en toe eens een wijze les te leren, ook al gebeurde dat vaak op een minder leuke manier ;-).

Karen, jou mag ik hier zeker niet vergeten. Jij bent al meer dan tien jaar mijn allerbeste vriendin en ik zou honderden pagina's kunnen schrijven over alles wat we samen beleefd hebben. Toch moet ik het hier kort houden en wil ik je daarom gewoon al eens bedanken voor de voorbije jaren. Ook al kozen we een andere studierichting en was ons thesisjaar enorm druk, je bent er steeds voor mij geweest. Bedankt voor alles!

Tenslotte wil ik een van de meest belangrijke personen in mijn leven bedanken. Jan-Klaas, ik zou niet weten wat ik hier kan schrijven om mijn dank juist uit te drukken. In de voorbije twee jaar heb je waarschijnlijk even veel afgezien van mijn operaties en thesis als ik. Zonder ook maar een moment te twijfelen heb je een taak opgenomen die niemand zou willen. Ik stond er versteld van hoe je elke dag 300 kilometer reed om mij te kunnen bezoeken in het ziekenhuis, hoe je mij hielp toen ik weer eens flauwviel tijdens de revalidatie, hoe je elke avond kookte toen ik weer thuis was en hoe je mij probeerde op te vrolijken als ik het weer even had gehad. Ik weet dat ik vooral een stresskip en niet steeds de meest aangename persoon ben geweest dit laatste jaar, maar toch stond jij altijd klaar met een luisterend oor (alé, of toch voor de helft luisterend ;-)). Merci! Om nog af te sluiten met jouw eigen woorden: Jij bent het beste resultaat dat ik de afgelopen jaren bereikt heb.

Valerie Otte

Gent, 3 juni 2017

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Chapter 1

Introduction and goals

1.1 Introduction

In 1928, Alexander Fleming discovered that the mould *Penicillium* possesses clear bacteriolytic properties.[1] Penicillin was the first naturally occurring antibiotic to be used in clinical medicine and is seen as the prototype for β-lactam antibiotics, recognised by their typical four-membered N-heterocyclic ring structure.[2] Later on, Guiseppe Brotzu discovered a second member of the β-lactam family, cephalosporin, extracted from *Cephalosporium acremonium.*[3] Up until 1970, these two antibiotics were the only examples of naturally occurring β-lactam compounds. Discovery of cephalosporin derivatives in 1971 further encouraged researchers to search for new antibiotics.[2]

Meanwhile, antimicrobial resistance became an important and worldwide threat. In the 1930s, organisms such as *Streptococcus pyogenes* became resistant to sulfonamide, especially in military hospitals, where the antibiotic was frequently used.[4–6] Further incidents with resistance of several bacteria followed in the next decades.[6–12] Bacterial resistance has led to the synthesis of novel β-lactam derivatives to tackle the defence mechanisms of resistant micro-organisms and to broaden their reactivity.

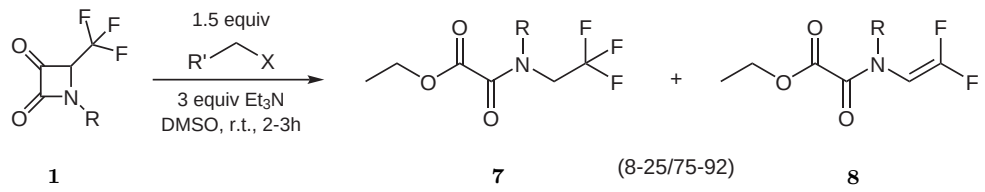
Next to their typical use as antibiotics, β-lactam compounds are exceptionally versatile and their derivatives display a lot of other biological activities. Hence, modification of the β-lactam ring can provide compounds that inhibit the absorption of cholesterol; inhibit human tryptase, thrombin and chymase; and act as anti-inflammatory, antiparkinsonian, antidiabetic

and anti-HIV components. Furthermore, they also posess serine protease, human leukocyte elastase and human cytomegalovirus protease enzyme inhibitor activity. Finally, they show moderate activity against some types of cancer.[13–17]

This Master's dissertation is a combined experimental and computational study to gain more insight into the reactivity of 3-oxo-4-trifluoromethyl-β-lactam derivatives. In the experimental part, the goal was to produce new types of 3-oxo-4-trifluoromethyl-β-lactams. The theoretical calculations on the other hand were performed to rationalise the experiments performed by H. Dao Thi. During these experiments, it was intended to deprotonate the C4 carbon atom of several 3-oxo-beta-lactam derivatives and to alkylate the formed anionic intermediates. The idea and motivation for performing these experiments originated from previous experimental work completed at the SynBioC research group (UGent), in which 1-tosyl-2-(trifluoromethyl)aziridine was deprotonated and subsequently alkylated.[18] However, deprotonation of 3-oxo-4-trifluoromethyl-β-lactams turned out to be very challenging and mixtures of several other compounds were repeatedly found. Based on the experimental results, possible reaction mechanisms were proposed, which will be evaluated in this work.

1.2 Goals

In Scheme 1.1, an overview of experimental results is shown. Three equivalents of triethylamine (Et_3N) were added in order to deprotonate the 3-oxo-4-trifluoromethyl-β-lactam **1**. Furthermore, several halide electropiles were added to achieve α-alkylation. The reactions resulted in the formation of the *N*-(2,2,2-trifluorovinyl) amido ester **7** as the minor product and of the *N*-(2,2-difluorovinyl) amido ester **8** as the major product.



1 **7** **8**

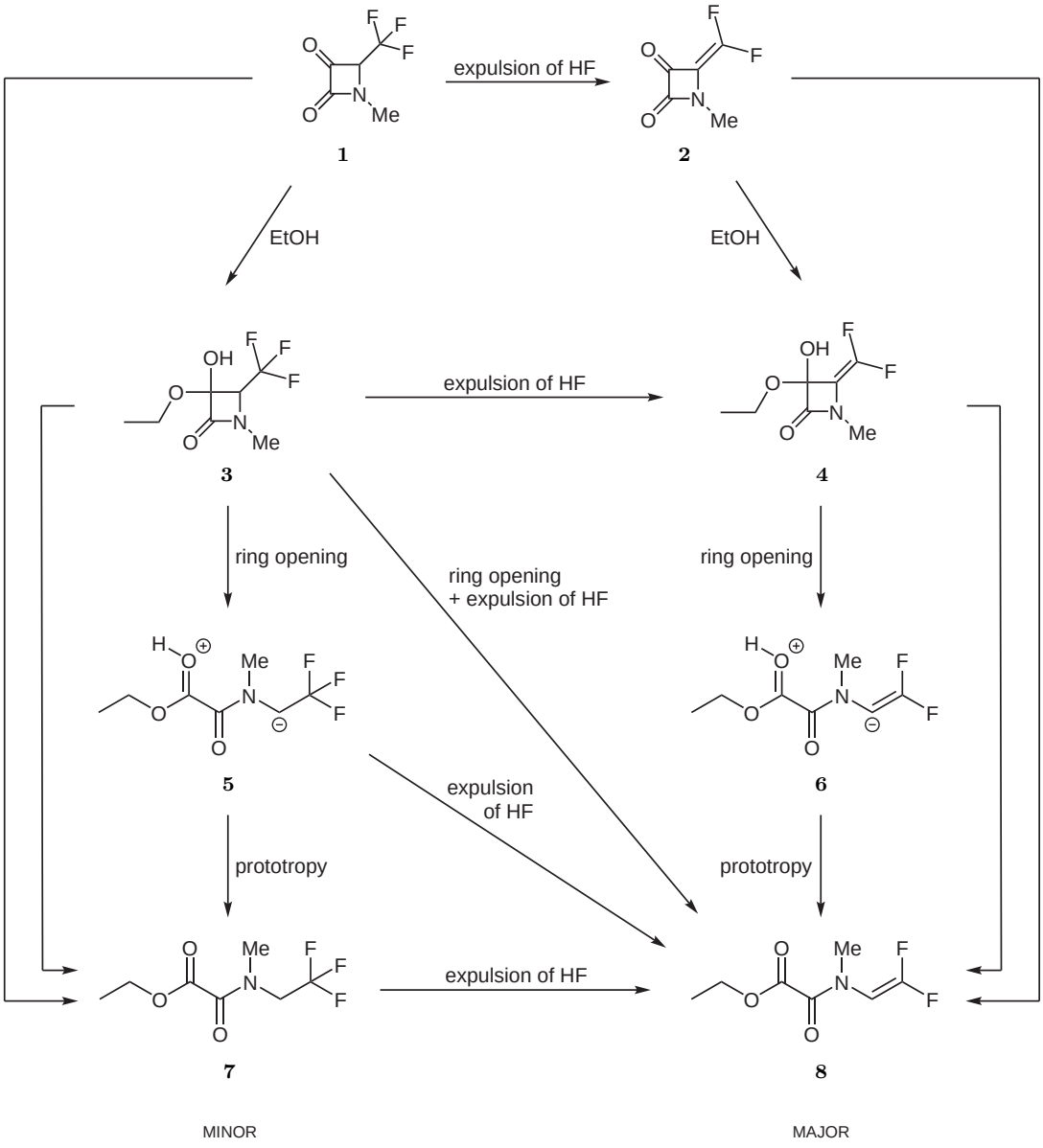
Scheme 1.1: Transformation of 3-oxo-4-trifluoromethyl-β-lactams **1** to products **7** and **8** (R = PMB, PMP; R' = Et, H, Ph, vinyl, 1-chloroethyl).

The experimentally observed reaction was unexpected, since deprotonation and subsequent alkylation of aziridines had been observed in previous research. In order to rationalise the reaction outcome, possible reaction mechanisms were proposed that could lead to the formation of products **7** and **8**. Scheme 1.2 shows all pathways under study (for 3-oxo-4-trifluoromethyl-β-lactam **1** with R = Me). First of all, formation of the β-lactam derivative **2** is investigated. This reaction could proceed through a concerted or a stepwise pathway. Secondly, reactions of ethanol with the starting compound **1** to form compound **3**, as well as with compound **2** to form compound **4** are investigated. Ethanol is formed in a reaction from the added electrophile, in this case an ethyl halide (R' = Me). Ring opening of these acetal compounds **3** and **4** towards their zwitterionic counterparts **5** and **6** is the subject of the next calculations. These zwitterions are assumed to readily lead to products **7** and **8** via proton transfer.

Besides the reactions described above, the assisting effects of several molecules are considered. Adding an extra ethanol molecule to the system under study is the first possibility to be examined. Assistance by a water molecule is a second possibility that is considered. Finally, triethylamine is an option for assistance as well, since this is the base added for the intended deprotonation.

Thus, the overall goal of this study is to unravel the reaction mechanism in order to explain the experimental results.

Chapter 2 gives a literature review, in which both experimental and computational techniques are applied to analyse the behaviour and ring opening of cyclopropanes and aziridines. Chapter 3 focusses on the experimental work. Experiments involved with the formation of β-lactam **1** are discussed and evaluated. Chapter 4 contains an in-depth computational analysis of the possible pathways, which β-lactams **1** can undergo when reacting with ethyl halides and triethylamine in dimethylsulfoxide (DMSO). The first part of this chapter analyses the reactions, without assistance of any molecules (Section 4.2.1). The second part discusses the reactions with assistance of water or one or two ethanol molecules (Section 4.2.2). During these calculations, it became clear that reactions without assistance or with ethanol or water assistance were insufficient. Therefore, assistance of triethylamine is described in detail in the last part, showing that these calculations give much better results (Section 4.2.3).



Scheme 1.2: Overview of possible pathways starting from compound **1**, towards end products **7** and **8** based on the experimental results provided by H. Dao Thi.

Chapter 2

Literature review

This literature review focusses on combined experimental and theoretical papers, in which molecular modeling was applied to unravel the behaviour of several nucleophilic ring-opening reactions of three-membered rings, since originally, the goal of this Master's thesis was to compare the reactivity of three and four-membered rings. More information on the computational techniques will be provided briefly in Section 4.1. The interested reader is guided to dedicated textbooks.[19–21]

In the first part, the nucleophilic S_N2-like ring-opening reaction of specific cyclopropanes by means of the azide ion is discussed. Therefore, both experimental and theoretical approaches are elaborated, giving a good insight in the observed reaction mechanism. Furthermore, the formation of other azaheterocyclic compounds is covered.

The second part includes the formation of aziridines with specific protecting groups and several other substituents as well as the ring-opening reaction of the newly synthesised aziridines using several nucleophiles. A comparison between experimental and theoretical results is also made.

2.1 Ring opening of cyclopropanes

Nucleophilic S_N2-like ring-opening reactions of three-membered cyclic compounds by means of the azide ion can be used in the synthesis of various nitrogen-containing compounds, both cyclic and acyclic. The use of the azide ion leads to a compound with an azido group, which can further be transformed by the loss of an N_2 molecule, e.g. by the Staudinger,

Curtius, Schmidt or Boyer reactions[22–26], or without the elimination of N_2, e.g. by the [3+2] cycloaddition to unsaturated reactants.[27–31] Besides these nucleophilic ring-opening reactions, electrophilic ring opening has been observed as well.[32–36]

2.1.1 Ring-opening reaction

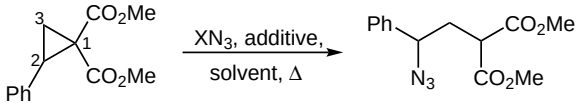
The ring-opening reaction of donor-acceptor (D-A) cyclopropanes **9** with the azide ion was investigated by Ivanov et al. (Scheme 2.1). The selected cyclopropanes **9** varied in both the electron-donating group (EDG) as well as the two vicinal electron-withdrawing groups (EWG). The EDG was typically an aryl group bearing a substituent, while the EWG was usually a carbonyl group or nitrogen-containing group.[37] The azide ion was added as the sodium or trimethylsilyl (TMS) azide and acts as a nucleophile, mostly attacking on the more substituted C2 atom in an S_N2-like pathway.[27]

9 **10**

Scheme 2.1: General S_N2-like ring-opening reaction of D-A cyclopropane **9**.[27]

Experimental results

In order to obtain a sufficient yield for the ring-opening reaction, appropriate conditions were determined by selecting a phenyl group as the EDG and two methyl ester groups as the vicinal EWG, as can be seen in Scheme 2.2.[27] A first requirement for the reaction is



9a **10a**

Scheme 2.2: Reaction performed for determination of optimal conditions for the ring-opening reaction of D-A cyclopropanes **9**.[27]

the activation of the donor-acceptor cyclopropane **9a**, using a Lewis acid.[38] In addition, conditions that are typical for S_N2 reactions are applied using TMS or sodium azide as source of the nucleophile and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a Lewis

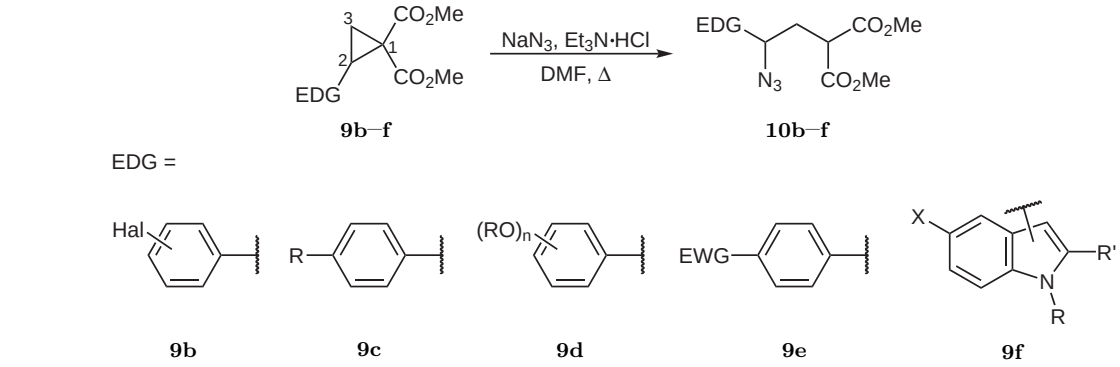
acid. In experiments where $TMSN_3$ was used as both the source of nucleophile as well as Lewis acid, no yield was obtained, since the acidic properties of $TMSN_3$ are relatively weak (Table 2.1). Using TMSOTf as a Lewis acid, the highest yield was 66 %, in contrast with the use of sodium azide without activation by a Lewis acid, leading to a yield of only 15 %. The reversibility of the reaction was demonstrated by performing the reverse reaction with NaH in DMF at 100 °C. Ultimately, the highest yield was obtained if the source of the azide ion was NaN_3 (2 equivalents), the Lewis acid was $Et_3N \cdot HCl$ (2 equivalents), the solvent was *N,N*-dimethylformamide (DMF) and the temperature was set at 100 °C for 4 hours. This reaction was essentially irreversible because of the strength of the Lewis acid, and the obtained yield was 94 %.

Table 2.1: Overview of performed reactions and corresponding yields for determination of optimal conditions for the ring-opening reaction in Scheme 2.2.[27]

XN_3 (equiv)	**Additive (equiv)**	**Solvent**	**T [°C]**	**t [h]**	**Yield [%]**
$TMSN_3$ (1)	-	PhCl	131	8	**-**
$TMSN_3$ (3)	TMSOTf (1.2)	PhCl	131	8	40
$TMSN_3$ (3)	TMSOTf (3)	PhCl	131	10	60
$TMSN_3$ (3)	TMSOTf (3.5)	PhCl	131	10	**66**
NaN_3 (2)	-	DMF	100	4	**15**
NaN_3 (2)	$Et_3N \cdot HCl$ (2)	DMF	50	4	-
NaN_3 (2)	$Et_3N \cdot HCl$ (2)	DMF	100	4	**94**
NaN_3 (1)	$Et_3N \cdot HCl$ (1)	DMF	100	9	90
NaN_3 (2)	$Et_3N \cdot HCl$ (2)	DMSO	100	4	92
NaN_3 (2)	NH_4Cl (2)	DMF	100	4	86

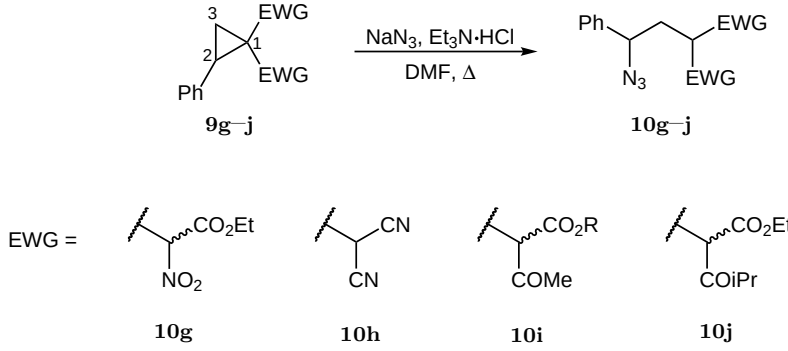
After optimisation of the conditions, the original phenyl electron-donating group was replaced with a wide variety of other substituents, including arylhalides **9b**, alkylaryls **9c**, alkoxyaryls **9d**, aryls carrying an EWG **9e** and two-ring structures **9f** (Scheme 2.3). This changed the electronic and steric properties of the cyclopropane and ultimately altered the reaction yield. Lowest yields were found if the aryl substituent carried an EWG such as a nitrile group (53 %), while the highest yield was encountered with the two-ring 1-benzyl-3-indolyl substituents (91 %). Important in all these reactions was the exceptional regioselectivity towards the more

substituted C2 atom instead of the C3 atom.



Scheme 2.3: Altered EDG for the S_N2-like ring-opening reaction of cyclopropane **9b**–**f** by means of sodium azide.[27]

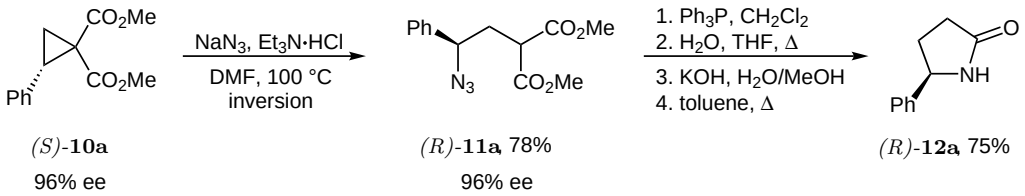
In the next part, the cyclopropanes were substituted with other EWG, such as esters, ketones, nitriles and nitro groups **9g**–**j** (Scheme 2.4). Here, it was shown that the yield was the lowest at 43 % when two cyano groups were chosen as EWG **9h**. The reason for this were side reactions towards a number of unidentified products. On the other hand, the highest yield of 90 % was found for isopropylketone and ethyl ester as EWG **9j**, although it must also be noted that the original phenyl EDG was in this case changed into a 4-fluorophenyl group.



Scheme 2.4: Altered EWG for the S_N2-like ring-opening reaction of cyclopropane **9g**–**j** by means of sodium azide.[27]

In all of the experiments described above, the ring-opening reaction involved the azide ion attacking on the more substituted C2 atom leading to full inversion of configuration at this chiral center. This was verified in an extra experiment, in which the starting product was

the cyclopropane *(S)*-**10a** in 96 % enantiomeric excess. After the ring opening, the same enantiomeric excess for the *(R)*-enantiomer **10a** was found and subsequent conversion resulted in the five-membered heterocyclic compound *(R)*-**12a**.

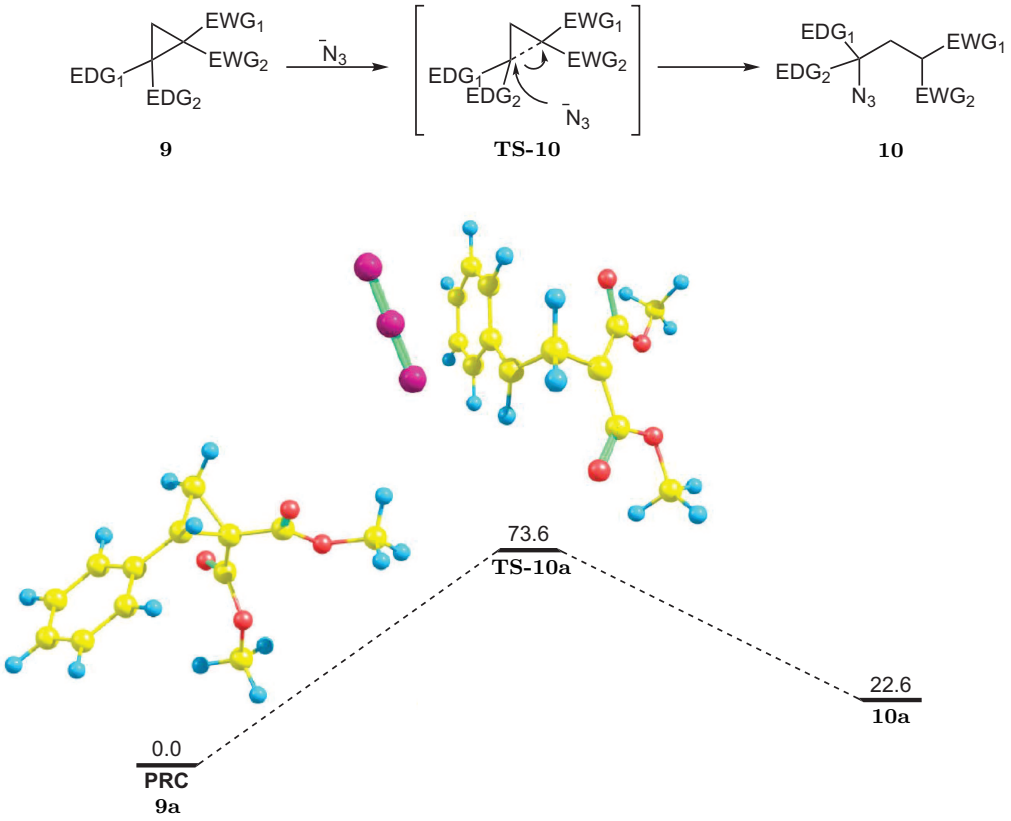


Scheme 2.5: Full inversion of configuration in the nucleophilic ring-opening reaction of D-A-cyclopropane *(S)*-**10a** to *(R)*-**11a** and *(R)*-**12a**.[27]

Theoretical calculations

In order to understand the outcome of the S_N2-like ring-opening reaction of cyclopropanes with use of the azide ion (Scheme 2.1), Ivanov et al. also performed density functional theory (DFT) calculations, using the ORCA 2.9 program package.[27] The B3LYP hybrid functional was selected with the Split Valence Polarization (SVP) basis set. To take into account the solvent DMF, the conductor-like screening model (COSMO) was used. This model is a continuum solvation model, introduced by Klamt and Schüürmann, in which the continuum is assumed to be a perfect conductor. This conductor screens the charge density of the solute and scales the charges down to a finite dielectric constant, which is then implemented in the system's Hamiltonian.[39,40]

Firstly, the transition states (TS) for the C1-C2 ring-opening reaction with the azide anion as nucleophile were optimised. The molecules used here had at least one electron-withdrawing group, mostly methyl esters, but also nitro groups, methyl ketones and nitriles. The electron-donating groups were phenyl groups, ether groups, indolyl groups or were left out (Scheme 2.6, Table 2.2). The angle of attack between the nucleophile, the carbon atom and the leaving group varied from 142 ° up to 148 °, whereas for S_N2 reaction mechanisms, this angle is typically 180 °.



Scheme 2.6: Representation of the ring-opening reaction of compound **9** towards compound **10** and visual representation of the pre-reactive complex and transition state of compound **9a** (Gibbs free energy profile in kJ/mol, B3LYP/SVP, COSMO(DMF, $\varepsilon = 38.0$))(Table 2.2)(3D structures reproduced from Ivanov et al.[27]; Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

The calculations showed that when a cyclopropane with only one electron-withdrawing substituent (methyl ester) was used for the ring-opening reaction, the activation enthalpy was the highest at 118.8 kJ/mol, which means that the reaction would not be able to proceed (Table 2.2). The presence of a second methyl ester group or second methyl ester and phenyl group significantly lowered the activation enthalpy to 73.6 kJ/mol and 81.6 kJ/mol, respectively. An even greater decrease of the activation barrier was encountered with a structure containing a methyl ketone EWG (60.2 kJ/mol) or a nitro EWG (47.7 kJ/mol). The lowest activation enthalpy (38.1 kJ/mol) was found when two cyano groups were used as EWG.

These computational findings are in good agreement with the experimental results, since the cyclopropane with only one EWG did not undergo ring opening, while the reaction with the

methyl ester and methyl ketone or nitro groups could be performed at a temperature lower than the standard 100 °C.

Furthermore, when the EDG was left out, the activation enthalpy was 79.1 kJ/mol, while an ether EDG gave an activation enthalpy of 63.6 kJ/mol. Finally, the indolyl EDG gave an activation enthalpy of 42.7 kJ/mol.

Table 2.2: Theoretical results with corresponding activation enthalpies for the ring-opening reaction of compound **9** towards compound **10** (Scheme 2.6).[27]

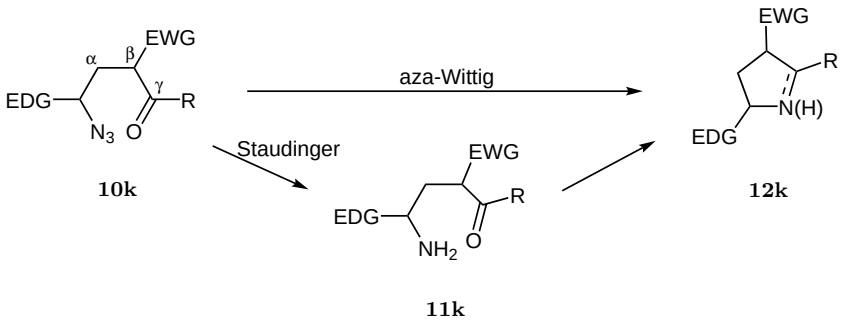
$\mathbf{EDG_1}$	$\mathbf{EDG_2}$	$\mathbf{EWG_1}$	$\mathbf{EDG_2}$	$\mathbf{\Delta H^{\ddagger}}$ **[kJ/mol]**
Ph	-	CO_2Me	-	118.8
Ph	-	CO_2Me	CO_2Me	73.6
Ph	Ph	CO_2Me	CO_2Me	81.6
Ph	-	CO_2Et	COMe	60.2
Ph	-	CO_2Et	NO_2	47.7
Ph	-	CN	CN	38.1
-	-	CO_2Me	CO_2Me	79.1
4-$MeOC_6H_4$	-	CO_2Me	CO_2Me	63.6
3-(*N*-Me)Ind	-	CO_2Me	CO_2Me	42.7

2.1.2 Formation of other azaheterocyclic compounds

After the ring opening of the cyclopropane **9** and the azido-functionalised compound **10** is formed, several consecutive reactions can prevail, of which some will be described in this part.

A first important follow-up reaction is the synthesis of five-membered *N*-heterocycles, especially derivatives of pyrrolidone and pyrroline. This intermolecular reaction occurs between the γ-placed carbonyl group and the azido group, which reacts as an amino function by elimination of an N_2 molecule through the Staudinger reaction (Scheme 2.7).

If the reaction described above was carried out for compound **10a**, a yield of 89 % was found. Next to high yields, the reaction also provides an easy pathway towards natural products, such as the total synthesis of *(S)*-nicotine starting from the corresponding *(R)*-reactant, as



Scheme 2.7: Formation of five-membered N-heterocycles **12k** from **10k** by Staudinger via **11k** or aza-Wittig reaction.[27]

well as other medicinal products such as the cholesterol lowering Lipitor, of which the calcium salt atorvastatin was produced (Figure 2.1).

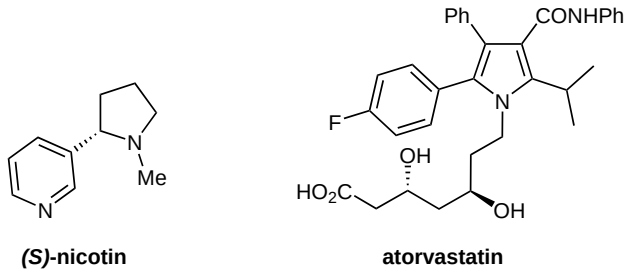
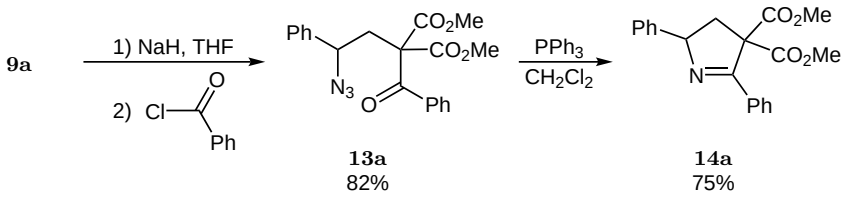


Figure 2.1: *(S)*-nicotine and atorvastatine as examples of the possibilities which the azide **10** provides.

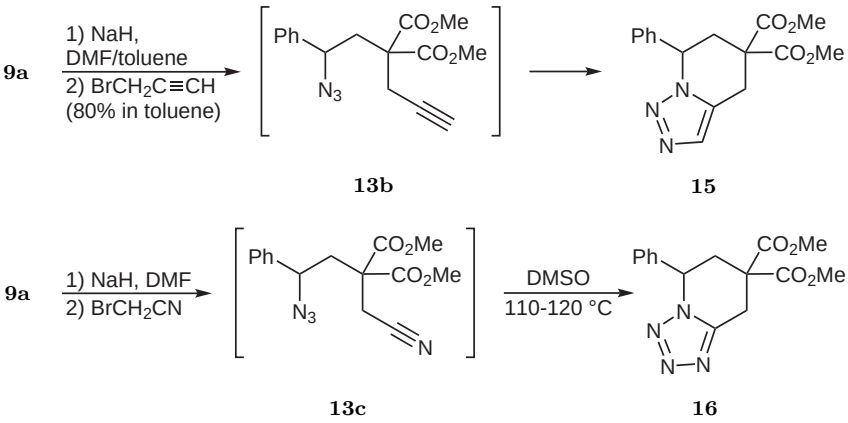
A second promising follow-up reaction is the functionalisation of the C3-carbon atom of the azide **10** via substitution of its two EWG. This methine group can undergo nucleophilic substitution or addition reactions, yielding a wide variety of highly functionalized acyclic compounds and *N*-heterocycles with different ring sizes. One example is the synthesis of 1-pyrroline **14** by means of nucleophilic substitution, as shown in Scheme 2.8. In this reaction, the azide **10a** was acylated with benzoyl chloride, after which PPh_3 was added in order to initiate the Staudinger or aza-Wittig reactions.

A third important reaction is the synthesis of triazolo- and tetrazolopyridines **15** and **16** by methine functionalisation and a subsequent [3+2] cycloaddition. The latter is the most important example of follow-up reactions proceeding without the loss of an N_2 molecule, for which reactions with alkyne and nitrile groups are of special importance. As can be seen



Scheme 2.8: Synthesis of 1-pyrroline **14** by nucleophilic substitution and following Staudinger or aza-Wittig reaction.[27]

in Scheme 2.9, using a propargyl bromide (2-propynyl bromide) reagent to functionalise the methine group even leads to a spontaneous azide-alkyne [3+2] cycloaddition, producing the triazolopyridines **15**.

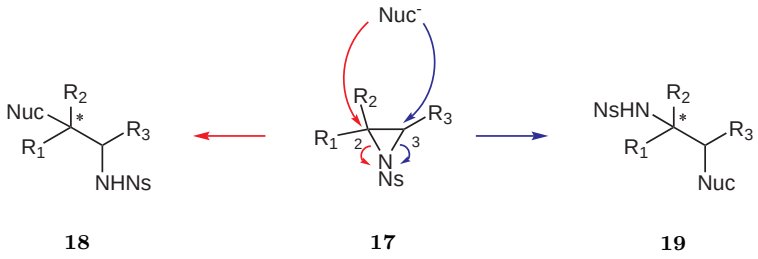


Scheme 2.9: Synthesis of triazolo- and tetrazolopyridines **15** and **16** by nucleophilic substitution and following [3+2] cycloaddition.[27]

2.2 Ring opening of aziridines

Nucleophilic ring opening of aziridines **17** provides β-substituted quaternary compounds **18** and **19** (Scheme 2.10), of which compound **18** is present in a number of different products, including β-substituted quaternary amino acids and important enzyme inhibitors.[41] These amino acids can be incorporated in peptides in order to alter secondary and tertiary conformations and eventually have improved catalytic and pharmacological properties.[42]

Another important application of the ring-opening reaction is the total synthesis of Ustiloxin D and several derivatives (Figure 2.2), which belong to the class of ustiloxins and phomopsins,



Scheme 2.10: General nucleophilic ring-opening reaction of trisubstituted aziridine **17** at the C2 (red) or C3 (blue) carbon atom, towards compounds **18** and **19**.[41]

naturally occurring heterodetic peptides (peptide containing only amino-acid residues but with other types of linkages besides peptide bonds) having antimitotic properties. In this way, eukaryotic cells can be inhibited by interference with the assembly and further functions of microtubule.[41,43–46]

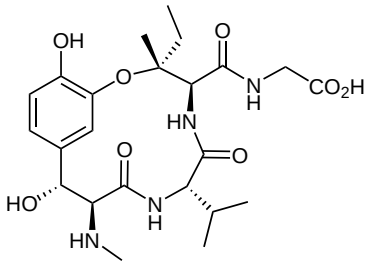


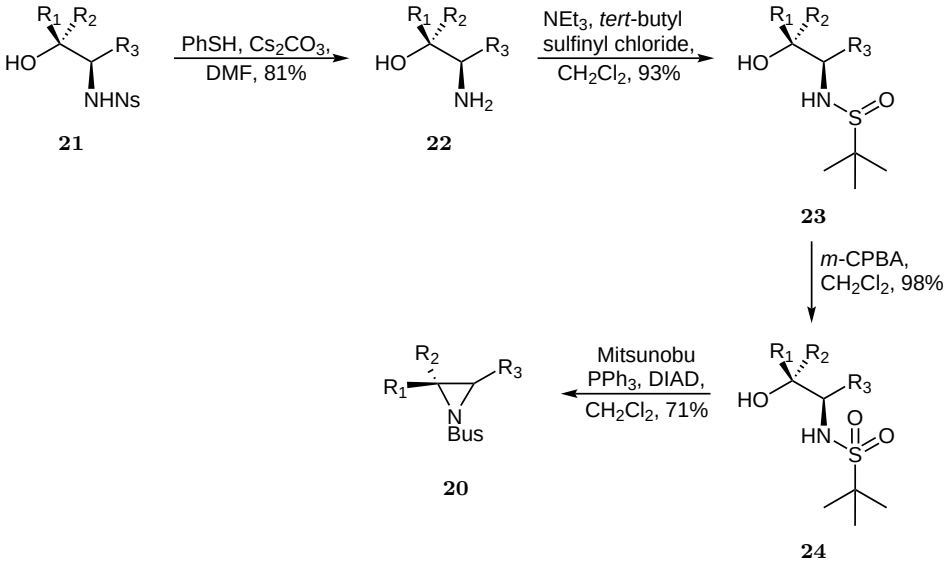
Figure 2.2: Ustiloxin D as example of the possibilities of the ring opening of aziridines **17**.

Due to the electronegative nitrogen atom and Baeyer distortion strain in the three-membered ring, aziridines tend to undergo nucleophilic ring opening even under mild conditions.[47] A collection of nucleophiles such as carbon nucleophiles, heteroatom nucleophiles, tertiary moieties and 1,2-diamines have been examined before. Most nucleophiles showed to be stereospecific and regioselective towards the less substituted carbon atom C3, and this reaction proceeded under non-Lewis acidic conditions.[48–50] In order to achieve ring opening at the more substituted carbon atom C2, Lewis acid catalysis or a π-bond-containing substituent (e.g. aryl group) should be employed. If carbon nucleophiles were used, ring opening was also observed at the more substituted carbon atom C2.[50] Furthermore, some trisubstituted aziridines itself feature a one-of-a-kind regioselectivity towards the more substituted carbon with inversion of

configuration. The reactivity of these last aziridines have been studied by Kelley et al. and will be discussed in the following sections.

2.2.1 Formation of aziridines

In order to perform ring-opening reactions of trisubstituted aziridines **17** with carbon nucleophiles, the 2-nitrobenzenesulfonyl (Ns) group of compound **17**, as seen in Scheme 2.10, was changed to a *tert*-butanesulfonyl (Bus) group, as this protecting group would be compatible with hard carbon nucleophiles, is stable in strong basic conditions and can easily be removed under mildly acidic solvolysis, in contrast to e.g. a 4-toluenesulfonyl group (Ts).[41,51,52]



Scheme 2.11: Synthesis of *N-tert*-butanesulfonylaziridine **20**, starting from Ns-protected intermediate **21** and using Mitsunobu conditions.[41]

The main problem with the Bus group is that the starting reactant *tert*-butanesulfonyl chloride is unstable and the protecting group thus had to be synthesized (Scheme 2.11).[51] Removal of the Ns group in the intermediate compound **21** afforded the free amine **22**, after which reaction with *tert*-butanesulfinyl chloride provided compound **23**. Finally, oxidation of the sulfinyl group by means of *meta*-chloroperoxybenzoic acid (*m*-CPBA) generated the protected amine **24**. Ring closure towards the protected aziridine **20** was then achieved under Mitsunobu conditions, using diisopropyl azodicarboxylate (DIAD) and PPh_3.[53]

Besides protecting the aziridine with the Bus group, several aziridines were produced in order to further investigate the ring-opening reaction (Figure 2.3). In every aziridine, the C2 atom had at least one methyl substituent and could carry an additional acetylenic (ethynyl) group or a 1-propynyl group. The C3 atom carried one substituent, namely a methyl, phenyl, *tert*-butyldimethylsiloxymethyl (OTBS-methyl) or OTBS-ethyl group.

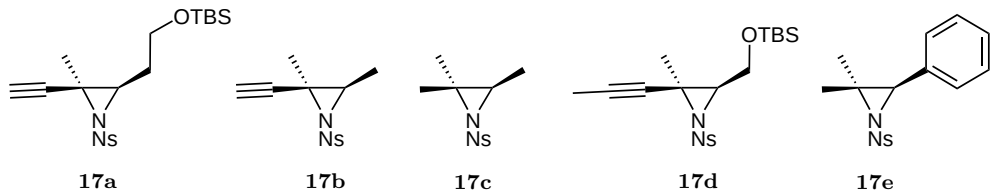


Figure 2.3: Newly synthesized aziridines **17a**–**e** used for further investigation of the nucleophilic ring opening.[41]

2.2.2 Ring-opening reaction

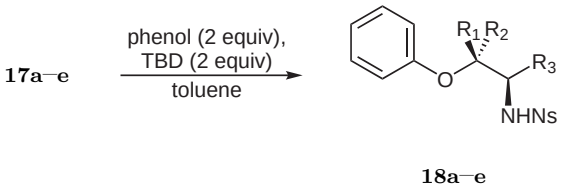
The ring-opening reaction of the aziridines mentioned above proceeds through attack of the nucleophile at the more substituted carbon atom. This atom however is fully substituted and the substituted groups would usually block S_N2 nucleophilic attack. Nevertheless, the angle methyl-C2-methyl in e.g. compound **17c** is 116 °, and is thus much bigger than that of a normal tetrahedral carbon and would therefore allow for the S_N2 attack to be carried out. Moreover, crystallographic experiments suggested that the C2-N bond is weaker than the C3-N bond, which again implies that the C2-N bond would be broken under nucleophilic conditions.[41]

In the following parts, the experiments performed by Kelley et al. will be discussed, after which a comparison will be made with their computational results.

Experimental results

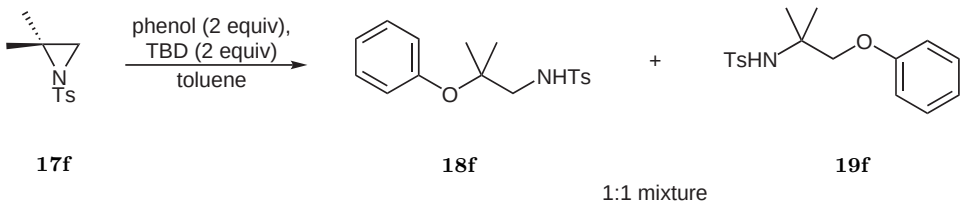
Upon treatment of the aziridines **17a**–**e** with a phenol nucleophile in presence of the base 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) and the solvent toluene, predictions on the regioselectivity were confirmed and only one regioisomer was formed (Scheme 2.12). Highest yields were found in the ring-opening reactions of compounds **17a** and **17c** (84 %), while the lowest yield was obtained using compound **17b** (68 %). Intermediate yields of 78 % and 77 % were encountered

with aziridines **17d** and **17e**, respectively.



Scheme 2.12: Performed nucleophilic ring-opening reactions of the trisubstituted aziridines **17a–e** to compounds **18a–e**.[41]

In order to test and influence the preference of the phenol nucleophile for the less substituted C3 atom, the known 2,2-dimethyl-N-tosyl aziridine compound **17f** was produced and the same conditions were applied for the ring opening.[54] In this case, two regioisomers **18f** and **19f** were isolated in a 1:1 ratio, meaning that the nucleophile had no specific preference for the C2 or C3 carbon atom (Scheme 2.13).

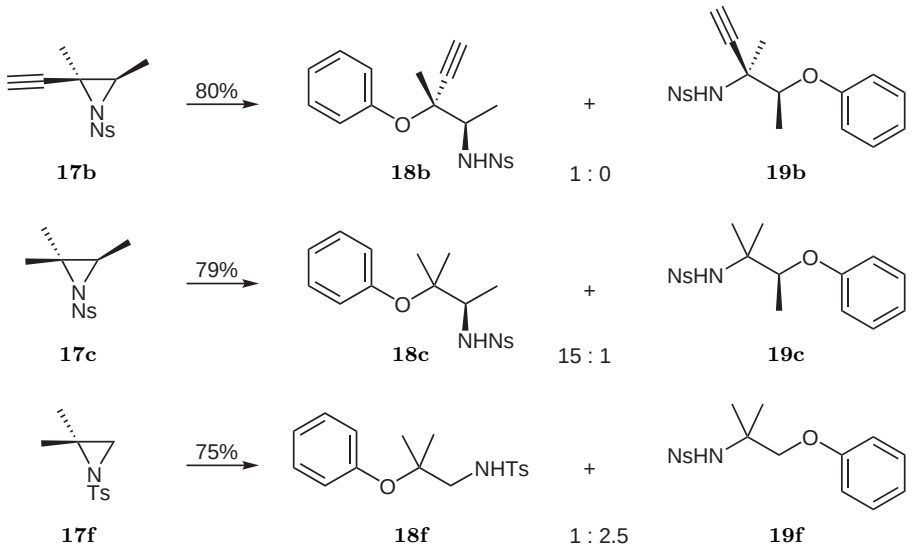


Scheme 2.13: Performed nucleophilic ring-opening reaction of the trisubstituted aziridines **17f** to compounds **18f** and **19f** in order to alter the preference of phenol to the C2 atom.[41]

As mentioned above, the ring-opening reaction of these unsymmetrical aziridines **17** proceeds with unusual regioselectivity. However, depending on both the aziridine and the nucleophile, unsymmetrical aziridines also tend to undergo ring opening via a Single Electron Transfer (SET) pathway.[54,55] In this reaction, the more stable radical is formed on the more substituted carbon. To rule out this radical reaction, a radical trap (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) was added in the reaction. The final product was again the known stereomeric compound **18**, which proved that no radical reaction prevailed.

In a final step, the TBD and phenol were changed to potassium phenoxide by adding potassium hydride (KH) and phenol in order to analyse the reaction even further (Scheme 2.14).

The absence of TBD in this reaction is an important factor, since it is a guanidine analogue which acts as a hydrogen bond donor and activator of the aziridines.[56]



Scheme 2.14: Ring-opening reaction of the trisubstituted aziridines **17b**, **17c** and **17f** to compounds **18b** and **19b**, **18c** and **19c** and **18f** and **19f**, respectively. Conditions: phenol (4 equiv), KH (4 equiv), 18-crown-6 (4 equiv, as ligand for potassium), THF.[41]

For aziridine **17b**, only one reaction product was observed, namely the compound resulting from reaction at the more substituted carbon atom. When aziridine **17c** was used, a 15 to 1 mixture of regioisomers was obtained, in which attack at the more substituted carbon atom gave the major isomer. In the reaction of compound **17f** however, a 1 to 2.5 mixture of isomers was found, meaning that the phenol nucleophile favors attack at the less substituted carbon atom.

Theoretical calculations

In order to understand the ring-opening reaction of trisubstituted aziridines **17** at the more substituted carbon atom, Kelley et al. performed several DFT calculations, using the Gaussian 09 program package.[41] The B3LYP/6-31+G* basis set was selected and the solvent THF was implemented using the self-consistent reaction field (SCRF). For the computational model, the same structures and potassium phenoxide conditions as in Scheme 2.14 were used.

For all three compounds **17b**, **17c** and **17f**, ground states, transition states and Gibbs free

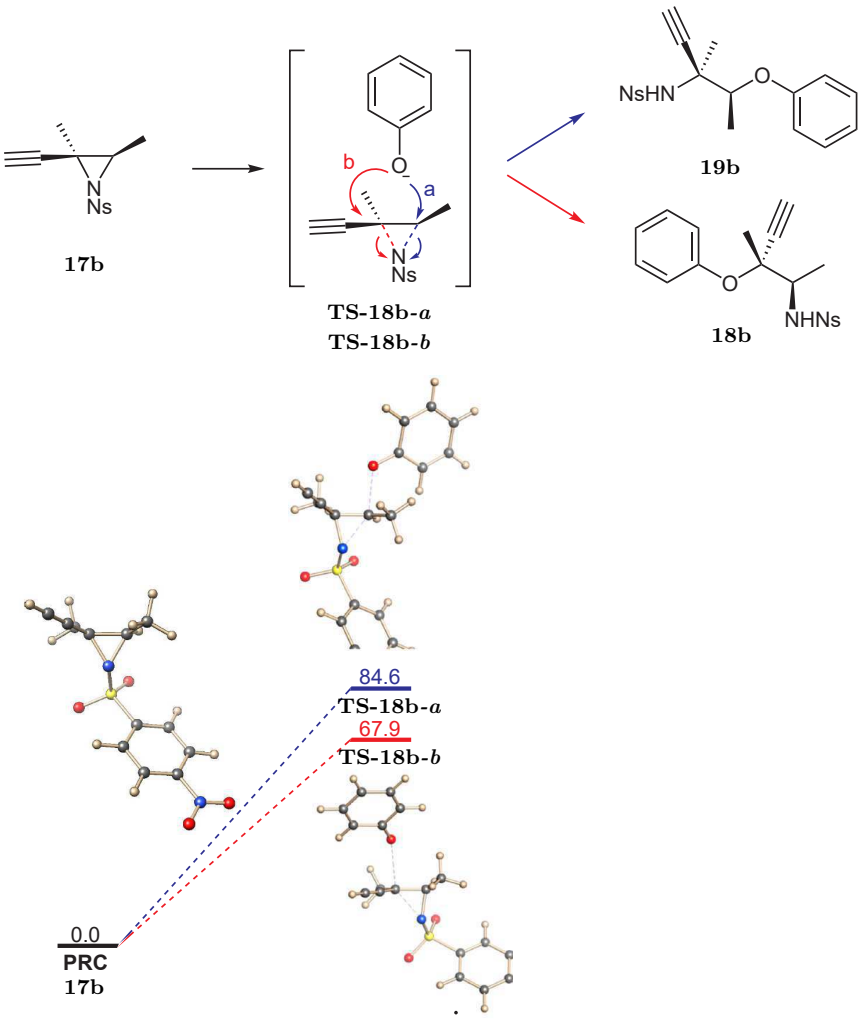
activation barriers were calculated, for reactions at both the more and less substituted carbon atom (Scheme 2.15 for compound **17b**). For compound **17b**, the ring-opening reaction at C2 had an activation barrier of 67.9 kJ/mol, while ring opening at C3 had a barrier of 84.6 kJ/mol. This clearly shows that the phenoxide nucleophile has a preference for the C2 atom, as was predicted by the experimental results. For the second aziridine **17c**, the Gibbs free energies were 76.3 kJ/mol and 84.7 kJ/mol for ring opening at the C2 and C3 atom, respectively, showing again the preference for the C2 atom. A possible cause for the higher transition state energies for attack at the less substituted carbon atom C3 is unfavorable electronics, but this falls beyond the scope of the research performed by Kelley et al. Finally, for compound **17f**, the Gibbs free activation barrier for the C2 atom was 90.0 kJ/mol. In this case however, the activation barrier for attack at C3 was 86.4 kJ/mol, which means that this reaction will be preferred. This is again in good agreement with the previously mentioned experimental results.

2.3 Conclusions

In the study of Ivanov et al., a novel method for the ring opening of donor-acceptor cyclopropanes is developed, providing easy access to a wide variety of azides. The reactions proceed with complete inversion of configuration at the C2 carbon atom. These compounds can give rise to the formation of several important azaheterocyclic compounds of biological importance e.g. *(S)*-nicotin, 1-pyrroline and triazolo- and tetrazolopyridines.

The research of Kelley et al. on the other hand involves stereoselective ring opening of 2,2,3-trisubstituted aziridines through an S_N2 nucleophilic attack of phenol. The reactions can be used in the production of a number of biologically active compounds e.g. Ustiloxin D.

For both studies, exceptional regioselectivity towards the more substituted carbon atom is observed. Furthermore, in both studies DFT calculations are performed, of which the results give a much better insight in the reaction mechanism. The calculations show for example the important effects of EDG and EWG on the reaction enthalpy and provide a clear comparison for ring opening at different carbon atoms. It is thus clear that the theoretical calculations provide a powerful tool to support the experimental outcome.

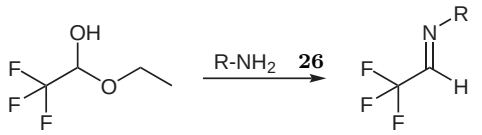


Scheme 2.15: Representation of the ring-opening reaction of the trisubstituted aziridine **17b** to compounds **18b** and **19b** with ground state and pre-reactive complex (Gibbs free energy profile in kJ/mol, B3LYP/6-31+G*, SCRF(THF, $\varepsilon = 7.4$))(Scheme 2.14)(3D structures reproduced from Kelley et al.[41]; Copyright 2014 American Chemical Society).

Chapter 3

Experimental results

The goal of the experiments was to produce new types of 3-oxo-4-trifluoromethyl-β-lactams **1**, starting from 1-ethoxy-2,2,2-trifluoroethanol **25** and a suitable amine **26** and explore the reactivity of the formed 3-oxo-4-CF_3-β-lactams **1**. However, because of time limits and difficulties in finding the appropriate reaction conditions, only the first step in the reaction pathway could be explored, with formation of *(E)-N*-(2,2,2-trifluoroethylidene)amines **27** (Scheme 3.1).



25 **27** (R = *i*Pr, Bn)

Scheme 3.1: Production of trifluoroaldimine **27**, starting from 1-ethoxy-2,2,2-trifluoroethanol **25** and an amine **26**.

3.1 Materials and methods

3.1.1 NMR-spectroscopy

NMR spectra (^{1}HNMR, ^{13}C-NMR and ^{19}F-NMR) were recorded on a Bruker Avance Nanobay III (400 MHz, 100 MHz and 376.5 MHz, respectively) high resolution NMR spectrometer. The compounds were solved in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as the internal standard. The used probe was a 5 mm BBFO Z-gradient high resolution probe

at room temperature.

3.1.2 Liquid chromatography - Mass spectroscopy

The samples were dissolved in acetonitrile and analysed using an Agilent 1200 series LS/MSD SL device. This device was equipped with a Supelco Ascentic Express C18 column (3 cm length, 4.6 mm internal diameter), a UV detector, an electrospray ionisation mass spectrometer (ESI, 70 eV) and a quadrupole detector.

3.1.3 Melting point determination

The melting point of the crystals was detected by means of a Kofler-melting point device of type WME Heizbank of Wagner and Munz. Calibration of the device proceeded with several standard substances. The crystals are applied from the cold side of the bench, which has a temperature gradient from 50 to 260 °C. The crystals are then slowly moved towards the warmer side. From the place where the crystals melt, the melting point temperature can be deduced.

3.1.4 Infrared spectroscopy

Infrared spectroscopy was performed using an IR Affinity-1 apparatus from Shimadzu, which is a compact Fourier Transform Infrared Spectrophotometer, offering a high S/N ratio of 30 000 : 1 and a maximum resolution of 0.5 cm^{-1}. After carrying out a background analysis, the analysis of the product was performed.

3.1.5 Dry solvents

Dry dichloromethane (CH_2Cl_2) was obtained using an MBraun SPS-800 solvent purification system equipped with different solvents. This system works with Pure-Pac solvent storage vessels of 17 liters. The solvents are pressurised by inert nitrogen gas and before use, the solvents flow through two filter/drying columns, which absorb moisture from the solvent. The columns have a volume of 4.8 liter and the filter particles depend on the type of solvent used. The dried solvents are dispensed in evacuated collection vessels. The vacuum in the collection vessels is created using an MPC 301 Zp type oil free diaphragm pump.

3.1.6 Safety

General safety features

All of the experiments in the laboratory were performed in agreement with the 'Safety Instructions' and 'Internal guidelines' of SynBioC (Synthesis, Bioresources and Bioorganic Chemistry Research Group, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University). Prior to the experiments, the Material Safety Data Sheet (MSDS) of used reagents and solvents were reviewed and appropriate safety measures were taken when demanded.

Specific safety risks

Some of the important hazardous reagents and solvents used during the experiments, requiring special precautions, are classified below and their chemical and physical effects are explained.

Isopropyl amine: Isopropyl amine is an extremely flammable liquid and has corrosive and toxic effects when swallowed or inhaled. It can cause severe skin burns and eye damage and therefore, protective gloves and goggles should be worn at all times.

Dichloromethane (CH_2Cl_2): Dichloromethane is suspected of causing cancer and may cause damage to organs. Moreover, it also causes skin, eye and respiratory irritation. Protective gloves and goggles are mandatory when handling dichloromethane.

3.2 Results and discussion

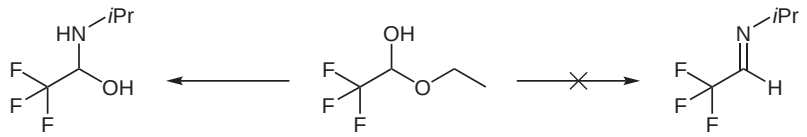
Synthesis of *(E)-N*-(2,2,2-trifluoroethylidene)amines 27

For the synthesis of (E)-*N*-(2,2,2-trifluoroethylidene)amines **27**, 1-ethoxy-2,2,2-trifluoroethanol **25** was dissolved in toluene or dry dichloromethane and 0.9 up to 1.0 equivalents of isopropylamine **26a** or benzylamine **26b** were added to the mixture. 1 mol% of *p*-toluenesulfonic acid monohydrate or 2 equivalents of $MgSO_4$ were also added. Several reaction conditions were examined for the production of the *(E)-N*-(2,2,2-trifluoroethylidene)amines **27** and are summarised in Table 3.1.

Table 3.1: Attempts towards the preparation of compounds **27**.

Entry	Amine	Reaction conditions
1	0.9 equiv **26a**	toluene, Δ, Dean-Stark, 22 h
2	1.0 equiv **26a**	2 equiv $MgSO_4$, dry CH_2Cl_2, Δ, 2 h
3	0.9 equiv **26b**	toluene, Δ, Dean-Stark, 5 h
4	1.0 equiv **26b**	1 mol% pTsOH $\cdot$ H_2O, toluene, Δ, Dean-Stark, 24 h

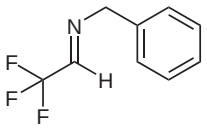
The production of *(E)-N*-(2,2,2-trifluoroethylidene)isopropylamine **27a** did not succeed. Instead, the 2,2,2-trifluoro-1-isopropylamino-ethanol **28a**, as shown in Scheme 3.2, was formed.[57]



28a 58 % **25** **27a**

Scheme 3.2: Production of compound **27a** from 1-ethoxy-2,2,2-trifluoroethanol **25** and an amine **26a** did not succeed, instead, compound **28a** was formed.

The production of the *(E)-N*-(2,2,2-trifluoroethylidene)benzylamine **27b** was however confirmed, as traces (3 %) of the substance could be indicated after detailed spectral analysis.[58]



27b

Figure 3.1: Compound **27b**, produced from 1-ethoxy-2,2,2-trifluoroethanol **25** and benzylamine **26b**.

Since this experimental part is very limited, follow-up reactions could include fine tuning of the reaction conditions in order to obtain a higher yield of the aldimine intermediate **27b** and the transformation of this compound into the desired 3-oxo-4-trifluoromethyl-β-lactam

1b. Production of other aldimines **27** is an interesting follow-up study as well, such as the attempted production of the *(E)-N*-(2,2,2-trifluoroethylidene)isopropylamine **27a**.

Chapter 4

Computational results

In this section, the theoretical calculations are discussed in order to unravel the pathways suggested in Chapter 1 and explain the experimental results. In the first part, the computational methods, which were used in this thesis to obtain insight into various reaction pathways, are discussed. Afterwards, these techniques are applied to investigate the reactivity of the 3-oxo-4-trifluoromethyl-β-lactams and the results are reported and discussed.

4.1 Computational methods

Molecular modeling techniques have been developed to unravel mechanistic aspects of chemical reactions, making it possible for researchers to rationalise experimental results and to thoroughly understand chemical reactivity. The techniques attempt to describe interactions of atoms and molecules at the (sub)nanoscale, i.e. at the level of covalent bonds and non-covalent interactions between molecules, and to elucidate chemical mechanisms.

The energy of a system of atoms or molecules can be described using the potential energy surface (PES), which represents the potential energy (PE) as a function of the nuclear geometries (Figure 4.1). Several techniques have been established to describe the PES and these can be divided into two main categories: *ab initio* and force field methods. *Ab initio* methods start from quantum mechanical principles, with no or a limited amount of empirical input, while force field methods are based on classical physics and are typically fitted to experimental (or *ab initio*) data. Intermediate semi-emperical techniques are based on quantum mechanical aspects in combination with empirical data.[19]

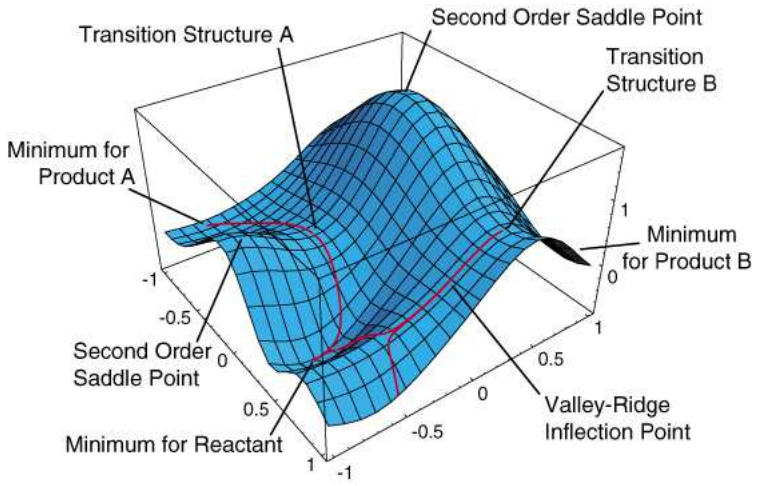


Figure 4.1: Representation of a potential energy surface, with two first order saddle points as transition states for the reaction of the reactants towards products A and B (Reproduced from Schlegel[59]; Copyright 2011 John Wiley & Sons, Ltd.).

In this Master's thesis, only *ab initio* methods were used. This method is based on quantum chemistry, in which the molecular system is determined by a many-electron wave function Ψ from which the probability of finding an electron at a certain coordinate can be deduced and which depends both on the position and the spin of the electrons. This wave function can be found by solving the time-independent Schrödinger equation (Equation 4.1). In this equation, $\hat{H}$ is the Hamiltonian and E the energy of the system.

$$\hat{H}\ \Psi = E\ \Psi \tag{4.1}$$

Using the Born-Oppenheimer (BO) approximation, the wave function is simplified, since it assumes that the motion of the electrons does not depend on the motion of the nuclei and vice versa.[60] In this way, the electronic Schrödinger equation is solved for a fixed configuration of the nuclei. Afterwards, a new configuration is selected and a new solution for the electronic Schrödinger equation is searched for. This complete cycle is repeated until convergence is reached. This way, the lowest energy of the system or minimum on the PES is found for stable species, while a first order saddle point is found for transition states.

Even with the BO approximation, the Schrödinger equation can only be solved exactly for one electron systems, e.g. H_2^+. For systems involving more electrons, several many-body

techniques have been developed to cope with this many-body problem: Hartree-Fock (HF), post-Hartree-Fock methods and density functional theory (DFT).[61]

4.1.1 Hartree-Fock and post-Hartree-Fock methods

The Hartree-Fock method is an approximation in which the wave function is approximated by a single Slater determinant of individual electron spin-orbitals or molecular orbitals.[62] These molecular orbitals are constructed as a linear combination of atomic orbitals (LCAO).[63]

The atomic orbitals can be represented by hydrogen-like Slater type orbitals (STO). Since these STOs are not computationally attractive, Gaussian type orbitals (GTO) are used, each consisting of one ore more Gaussian type functions. A distinction can also be made in the number of GTOs for the core and valence orbitals. Finally, several polarisation or diffuse functions can be applied.[19,20]

In order to determine the potential energy of a molecular system for a given set of nuclear coordinates, an initial guess of the Slater determinant is made, after which it is iteratively optimised through a self-consistent field method (SCF).[19] In Hartree-Fock however, correlation between electrons is left out, since only a single Slater determinant is used, meaning that the Hartree-Fock result will always be higher in energy than the exact solution.[20] Several extensions of the basic Hartree-Fock method have been developed in order to include some correlation effects.[64]

Post-Hartree-Fock methods like many-body perturbation theory (MBPT), coupled cluster (CC) or configuration interaction (CI) are extensions of Hartree-Fock in order to obtain better results. In these methods a linear combination of Slater determinants is made, each corresponding to an excitation of electrons. The number of these excitations determines the number of Slater determinants used and is denoted in the name e.g. MP2, MP3 (MP for Møller-Plesset), CCSD(T) (CC for single and double excitations, triple excitation based on perturbation).[19,65]

4.1.2 Density Functional Theory

As an alternative to Hartree-Fock and post-Hartree-Fock, density functional theory was developed. In this theory, Thomas and Fermi proposed to use the electron density to describe

molecular systems.[66,67] Hohenberg and Kohn provided the theoretical groundwork for DFT with two theorems. The first theorem states that the wave function is uniquely determined by the electron density of the ground state. The second Hohenberg-Kohn theorem gives the variational principle and states that the energy of a system is minimal for the ground state density of the system. In other words, every other electron density will result in a higher energy than in the ground state.[20,68] From these theorems it is clear that DFT is only applicable to ground states, since this is a requirement for the first Hohenberg-Kohn theorem. However, a lot of extensions have been made since the development of DFT.[69]

The Hohenberg-Kohn theorems form the basis of density functional theory, in which the electron density is thus used instead of the wave function to describe molecular systems. Kohn and Sham provided a work flow to obtain the electron density of the ground state, known as the Kohn-Sham scheme. In this Kohn-Sham scheme, the electronic energy is expressed as a functional of the electron density.[20] One of the terms in this expression is the exchange correlation functional describing electron-electron interactions. DFT would be exact if the expression of this functional was known. In practice, however, only guesses based on theoretical and empirical insights are available. For a chosen functional, ground state electron density and energy are determined variationally within in the Kohn-Sham scheme.[19]

4.1.3 Considering the solvent

Since most chemical reactions take place in a solvent environment and interaction with the solvent is of great importance, a method to take this solvent into account is necessary.[71–73] There are several methods to model the solvent. In an explicit solvent model, the solvent molecules interact with the reactant molecule(s) and are individually added to the chemically active species. The chemically active species and its surrounding solvent molecules form a so-called 'supermolecule'.[74] The main advantage of the explicit solvent model is that it is possible to describe specific interactions like hydrogen bonds. On the contrary, no long-range interactions are included in the model and this type of models is computationally expensive.

In an implicit solvent model (or continuum model) on the other hand, the solvent is modeled as a continuous medium. In this medium, the active species is placed in a cavity which is characterised by a dielectric constant (ε) and other variables. In contrast to the explicit

solvation, this model accurately describes long-range electrostatic interactions.[72,75] Explicit interactions such as hydrogen bonds however are not included.[74]

In implicit solvation, interaction between solvent and solute is also determined using the properties of the solute, e.g. polarisability and charge distribution. In the solute, polarisation will be induced, which creates a reaction field within the continuum. This changes the wave function and can be solved by a self-consistent reaction field (SCRF).[76] One type of continuum model is the Polarisable Continuum Model (PCM), a frequently used method which was developed in 1981 by Miertus et al.[77] The PCM method has been adjusted continuously since its original development in order to expand its use.[40,71,78,79]

Next to explicit and implicit solvation, other ways of including the solvent exist as well. A combination of implicit and explicit solvation is possible. The supermolecule is then placed in a dielectric continuum, combining the advantages of both methods. Another alternative, which is often used for complex systems for which the inclusion of a lot of solvent molecules is necessary, is to treat the most important part of the system quantum mechanically, while describing the larger solvent environment with force field methods.[80]

4.1.4 Methodology

In this Master's thesis, all DFT calculations were performed with the Gaussian 09 program package.[81] Both geometry optimisations and frequency calculations were performed. The latter was necessary in order to verify the nature of the stationary points, with zero or one imaginary frequencies for ground states and transition states, respectively. During the calculations, the M06-2X functional was used with a 6-31+G* basis set.[82,83] This functional includes dispersion effects, which are important for polarisable atoms. After determination of a transition state, the corresponding reactant and product complexes were found through Intrinsic Reaction Coordinate (IRC) analyses and subsequent full geometry optimisations.[87] In order to take into account the solvent, the PCM implicit solvation model was used. The Gibbs free energy was determined via frequency calculations or normal mode analyses (NMA), during which the thermal corrections to the electronic energy were calculated based on the vibrational frequencies or normal modes. This is done within the harmonic oscillator approximation. All Gibbs free energies are reported with respect to seperate reactants. The

pre-reactive complex, which is the configuration of the reactant complex before the start of the reaction, will be designated by its abbreviation PRC. The configuration of the product complex is the post-reactive complex and will be denoted as Post-RC. Visualisation of geometries was done using the CYLview program package.[88]

4.2 Results and discussion

Scheme 1.2 in Chapter 1 shows an overview of the possible pathways for the reactions of the starting compound 3-oxo-4-trifluoromethyl-β-lactam **1**. The purpose of the following theoretical study is to unravel the exact mechanism, in order to rationalise the experimental results. In the first part, the reactions are examined without assistance of an extra molecule, after which reactions with assistance of ethanol and water molecules will be discussed. The last part will examine the influence of triethyl amine on the reactions. Throughout the three parts, it will become clear that assistance of extra molecules is necessary to achieve a plausible reaction mechanism.

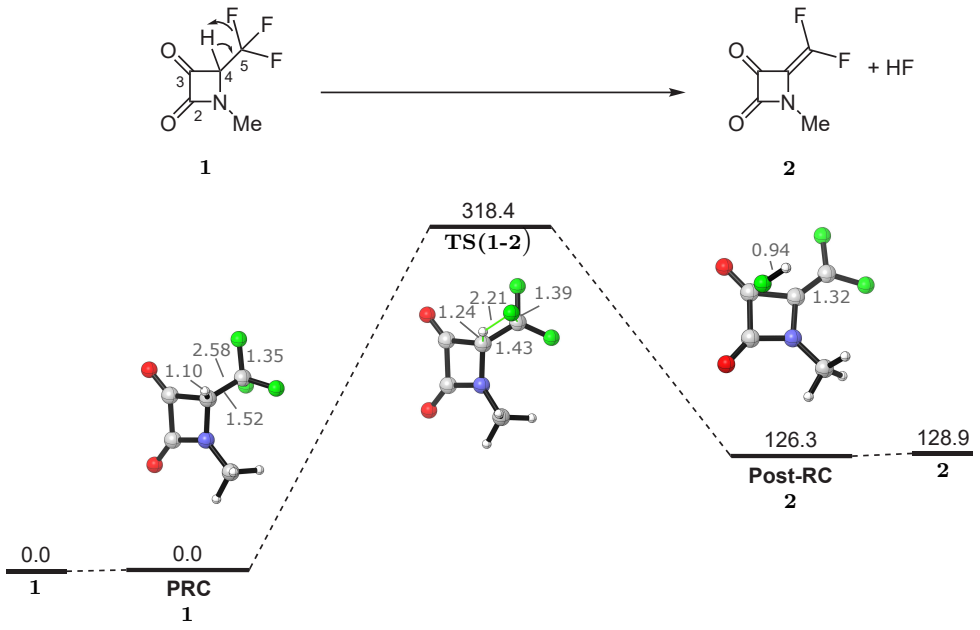
4.2.1 Calculations without assistance of an extra molecule

The calculations without assistance of extra molecules were a first attempt to unravel the pathways under study.

Elimination of hydrogen fluoride from β-lactam 1

The Gibbs free energy profile for the elimination of hydrogen fluoride (HF) by the starting compound β-lactam **1** is shown in Scheme 4.1. As can be seen, this reaction takes place in a concerted (single-step) E_2-fashion, meaning that simultaneous breaking of the C4-H (deprotonation) and C5-F bonds occurs, together with formation of an H-F bond and a double C4-C5 bond.

This double bond formation can also be seen in the profile, since the length of the C4-C5 bond has shortened from 1.52 Å in the PRC to 1.43 Å in the transition state and 1.32 Å in the post-reactive complex.

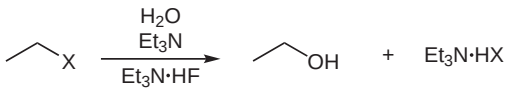


Scheme 4.1: Gibbs free energy profile for the reaction of compound **1** towards product **2** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

The activation barrier ($\Delta G^{\ddagger}$) for this reaction is 318.4 kJ/mol with respect to the seperate reactants. Since this barrier is very high, the elimination of HF is not plausible. This was expected, since compound **1** is a very stable compound as opposed to the product complex ($\Delta G_{rxn} = 126.3$ kJ/mol), making spontaneous deprotonation and release of the fluorine atom unlikely.

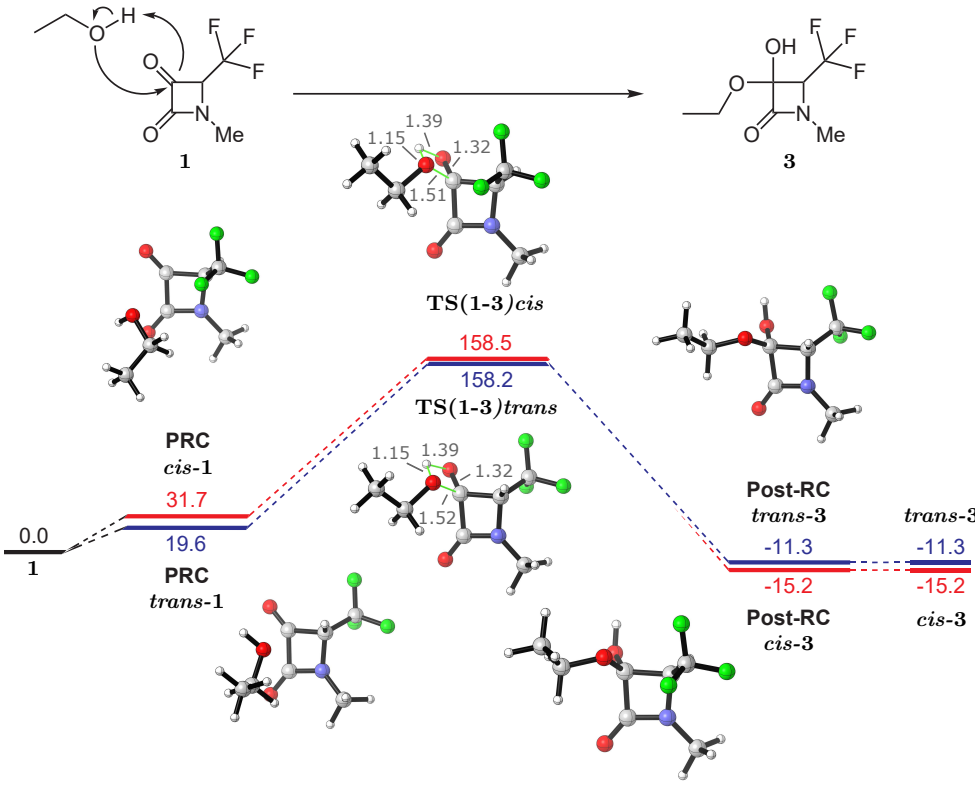
Attack of ethanol on β-lactam 1

Based on the experiments, it was proposed that ethanol is formed in a reaction from added ethyl halide, together with Et_3N, $Et_3N \cdot HF$ and water (Scheme 4.2).[89] The Et_3N was added in order to deprotonate the starting compound **1** as was the goal of the experiments. Water on the other hand was present since no dry solvents were used during the experiments. The formed ethanol can then react with the starting compound **1** to form compound **3**.



Scheme 4.2: Formation of ethanol from ethyl halide.[89]

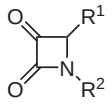
The Gibbs free energy profile for the reaction between ethanol and the starting β-lactam **1** is shown in Scheme 4.3. From the scheme it is clear that both a *cis* and *trans* attack of ethanol with respect to the trifluoromethyl group is possible. The *trans* reaction has an activation barrier of 158.2 kJ/mol and a reaction energy of -11.3 kJ/mol. The *cis* reaction has a similar activation barrier of 158.5 kJ/mol ($\Delta\Delta G^{\ddagger} =$ 0.3 kJ/mol) and has a slightly lower reaction energy of -15.2 kJ/mol ($\Delta\Delta G_{rxn} = 3.9$ kJ/mol). Since the difference in activation barriers and reaction energies is this small, it is not possible to conclude which pathway is preferred, but the activation barriers are both too high for the reactions to be plausible.



Scheme 4.3: Gibbs free energy profiles for the *cis* and *trans* reactions of compound **1** towards product **3** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

In order to analyse the effect of other substituents, the trifluoromethyl group on C4 and the methyl group on N were changed to several other substituents, which were used during the experiments as well. The Gibbs free activation barriers of these reactions can be seen in Table 4.1. As the table shows, the activation barriers of the reactions are higher than the original barrier for compound **1** ($\Delta G^{\ddagger}_{PRC} = 138.6\,\mathrm{kJ/mol}$), meaning that different and mostly larger substituents cause more steric hindrance and thus make the reaction even more difficult to proceed.

Table 4.1: Activation barriers with respect to the PRC for the *trans* attack of ethanol on compounds **1c**–**j** providing compounds **3c**–**j** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

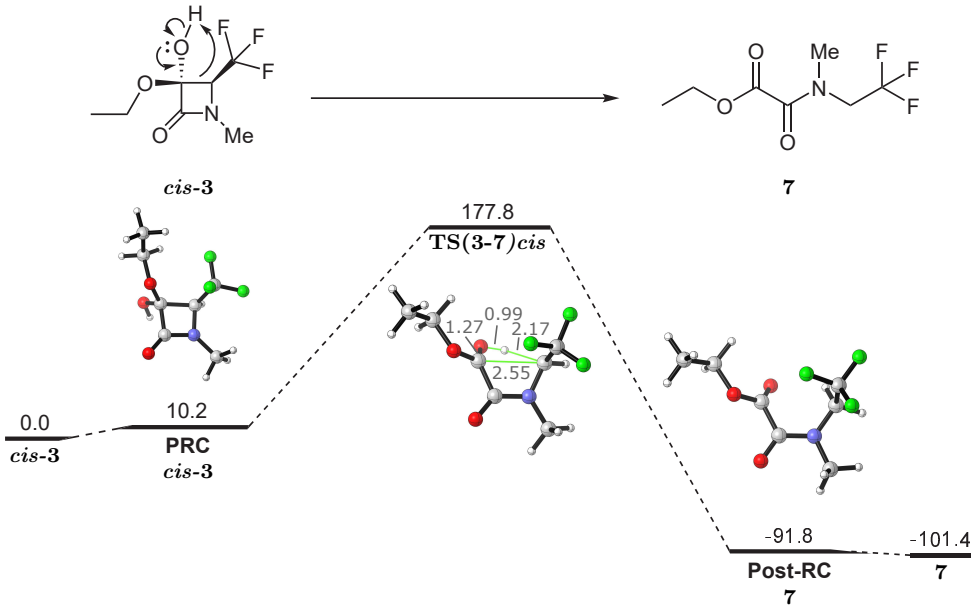


$\mathbf{R}^1$	$\mathbf{R}^2$	$\Delta\mathbf{G}^{\ddagger}_{PRC}$
-	PMP	168.2
-	PMB	177.0
*i*Pr	-	172.2
*i*Pr	PMP	171.1
*i*Pr	PMB	170.6
Ph	-	170.3
Ph	PMP	173.6
Ph	PMB	177.0

Ring opening of the ethoxy-β-lactam 3

After attack of ethanol on the starting compound **1**, leading to the ethoxy-β-lactam compound **3**, this intermediate can react further through ring opening. The proton of the hydroxyl group is simultaneously passed on to the C4 atom of the compound, resulting in the dicarbonyl product **7**. The Gibbs free energy profile is shown in Scheme 4.4 and the activation barrier of this reaction is 177.8 kJ/mol. Since preliminary calculations revealed that the difference in activation barriers between the formation of ***cis*-3** and ***trans*-3** was small, only one type of reaction was studied here.

Other reactions concerning the zwitterionic compounds **5** and **6** were neglected at this point, since these compounds are very unstable intermediates and no extra stabilisation was possible.



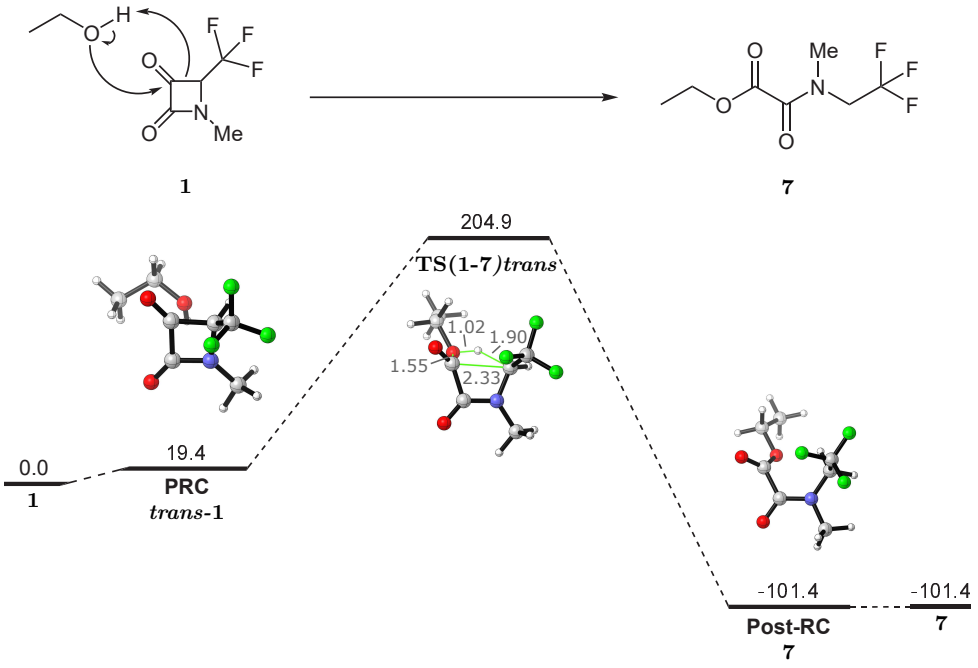
Scheme 4.4: Gibbs free energy profiles for the ring opening reaction of compound ***cis*-3** towards product **7** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

Reactions of compounds **3** and **7** to products **4** and **8**, respectively, are reactions with the elimination of HF. These reactions are expected to have high activation barriers as well, since preliminary calculations showed that the spontaneous elimination of HF had a very high activation barrier.

Simultaneous attack of ethanol and ring opening of the β-lactam compound 1

Besides attack of ethanol on compound **1**, resulting in compound **3**, and subsequent ring opening of this compound towards compound **7**, a concerted alternative for the two previously described reactions could take place. This reaction is shown in Scheme 4.5 and consists of simultaneous formation of the O_{EtOH} - C3 and H_{EtOH} - C4 bonds and breaking of the C3 - C4 and O_{EtOH} - H_{EtOH} bonds, providing compound **7**.

From the scheme it can be seen that the activation barrier for this reaction is much higher ($\Delta G^{\ddagger} = 204.9$ kJ/mol) than the previous activation barriers for the first step ($\Delta G^{\ddagger}_{trans}$ =-158.2 kJ/mol and $\Delta G^{\ddagger}_{cis} = 158.5$ kJ/mol), meaning that this reaction is even less probable than these previous reactions.



Scheme 4.5: Gibbs free energy profiles for the reaction of compound **1** towards product **7** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

The reason for this could be that in the transition state, a bicyclic ring is formed causing a lot of strain. In the first reaction of the two-step pathway on the other hand, the four-membered rings are connected with only one mutual atom, making this a more likely transition state. The same phenomenon is observed looking at the second step in this pathway, having again a much higher activation barrier ($\Delta G^{\ddagger} = 177.8\,\text{kJ/mol}$). This could be the confirmation for this theory, since the bicyclic ring is formed here as well.

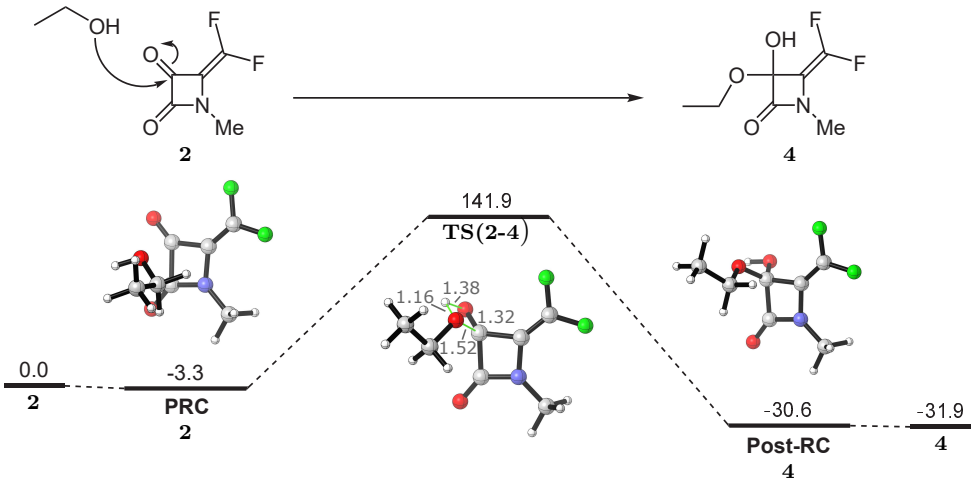
Subsequent reactions from the difluoromethenyl-β-lactam 2

The reactions as presented in the three previous sections, all have similar counterparts starting from compound **2**. The Gibbs free energy profiles of these reactions are shown in Scheme 4.6, Scheme 4.7 and Scheme 4.8.

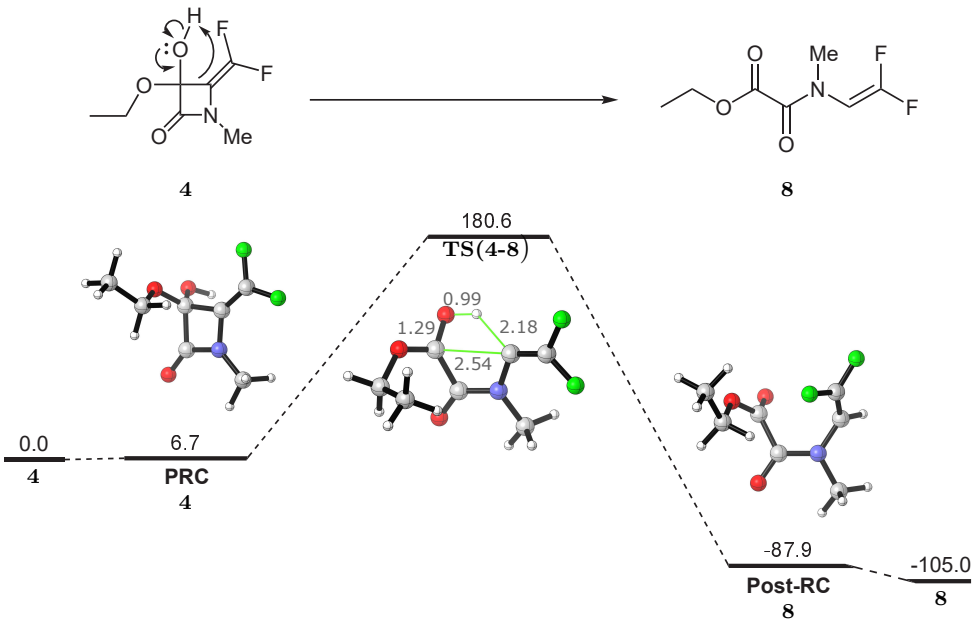
When comparing the reaction from **1** to **3** with the similar reaction from **2** to **4**, it can be seen that the latter reaction has a slightly lower activation barrier of 141.9 kJ/mol than the former ($\Delta G^{\ddagger}_{cis} = 158.5\,\text{kJ/mol}$).

For the ring-opening reactions, **3** to **7** and **4** to **8**, the contrast is smaller, with a difference in activation barriers $\Delta\Delta G^{\ddagger}$ of 2.8 kJ/mol (177.8 kJ/mol for **3** to **7**, 180.6 kJ/mol for **4** to **8**).

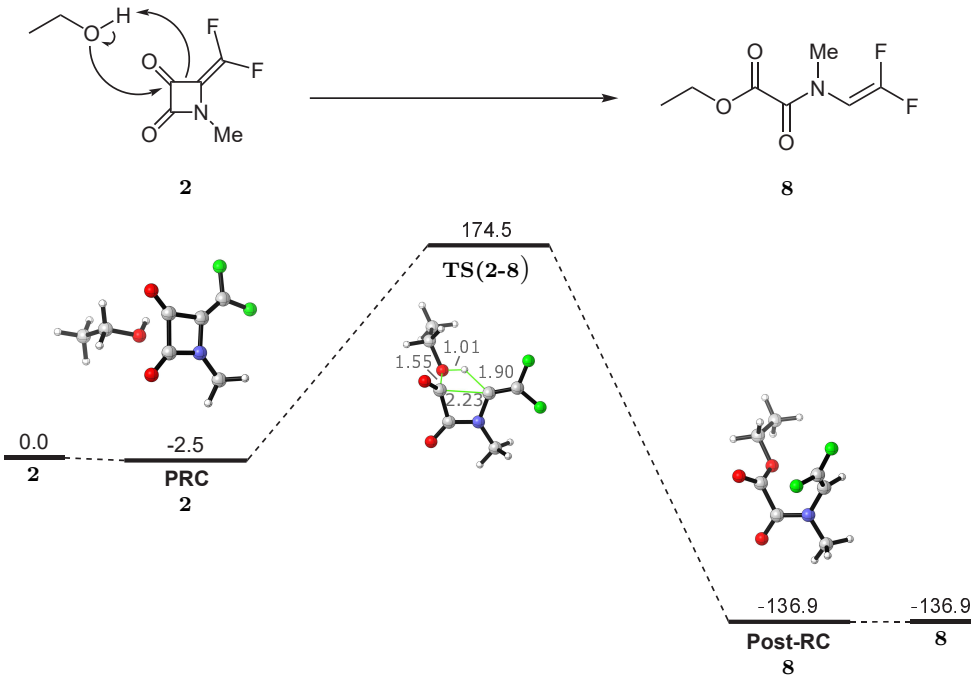
For the final concerted reaction starting from compound **2** the difference is much more significant, with a $\Delta\Delta G^{\ddagger}$ of 30.4 kJ/mol (204.9 kJ/mol for **1** to **7**, 174.5 kJ/mol for **2** to **8**). A possible explanation for this is the reduced steric hindrance because of two fluorine atoms instead of three. These differences thus show that reactions starting from the original compound **1** are mostly similar in activation barriers to reactions starting from the difluoromethyl compound **2**, except for the concerted mechanism.



Scheme 4.6: Gibbs free energy profile for the reaction of compound **2** towards product **4** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

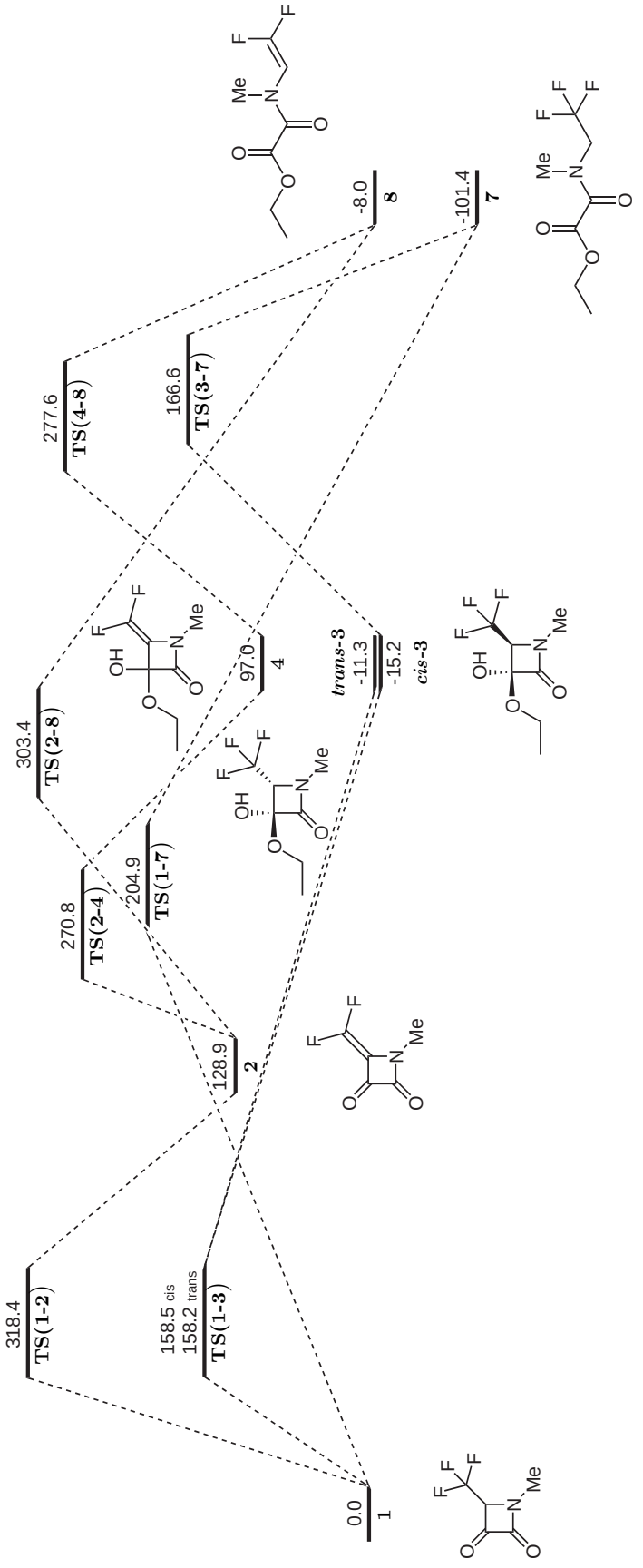


Scheme 4.7: Gibbs free energy profile for the reaction of compound **4** towards product **8** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).



Scheme 4.8: Gibbs free energy profile for the reaction of compound **2** towards product **8** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

To provide a summary of the analysed mechanisms in this section, an overview can be seen in Scheme 4.9. From this scheme, the conclusion is that none of the reactions will proceed without assistance, since all the activation barriers are too high.



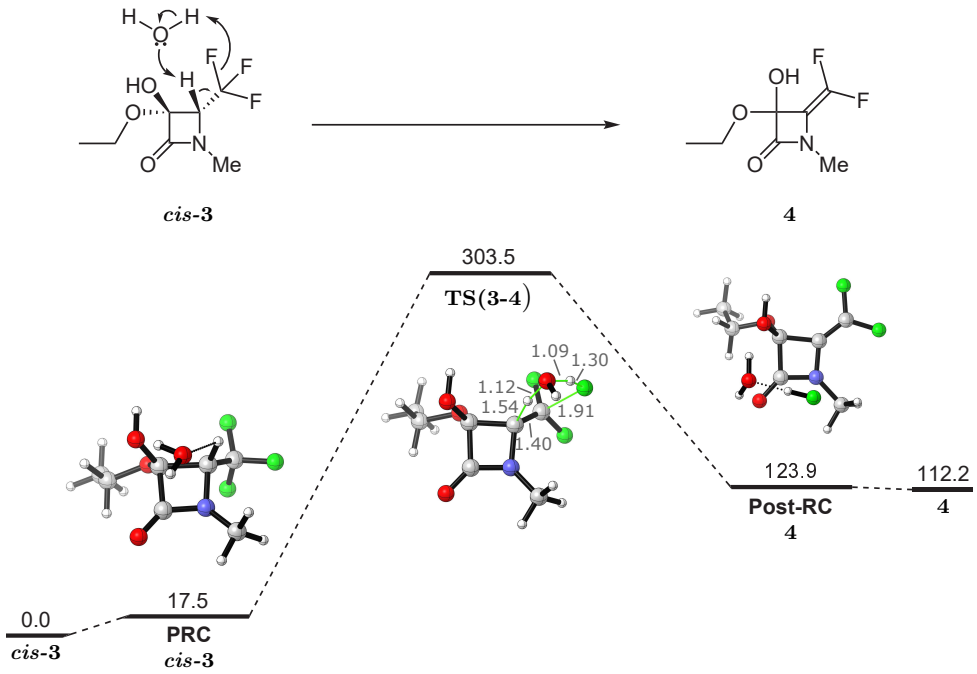
Scheme 4.9: Overall Gibbs free energy profile for the reactions without assistance, starting from compound **1** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

4.2.2 Calculations with assistance of extra ethanol or water molecules

In the previous section, it became clear that the unassisted activation barriers were too high for the reactions to proceed as expected. Therefore, assistance of ethanol and water molecules is investigated. As mentioned before, ethanol can be formed during the reaction described in Scheme 4.2, while water is present in the solvent.

Elimination of hydrogen fluoride

In the previous section, it was shown that the elimination of HF from the starting compound **1** was not a plausible reaction, since the activation barrier was 318.4.5 kJ/mol. In this section, the influence of assistance of ethanol and water is investigated. For the elimination of HF however, the assistance of both types of molecules is very limited, since both are weak bases ($pKb_{EtOH} = 16.4$, $pKb_{H_2O} = 15.7$ in water), not able to deprotonate the starting compound **1**.[90,91] The assisted elimination of HF will thus be very similar to the spontaneous deprotonation mentioned above and is expected to have a high activation barrier.

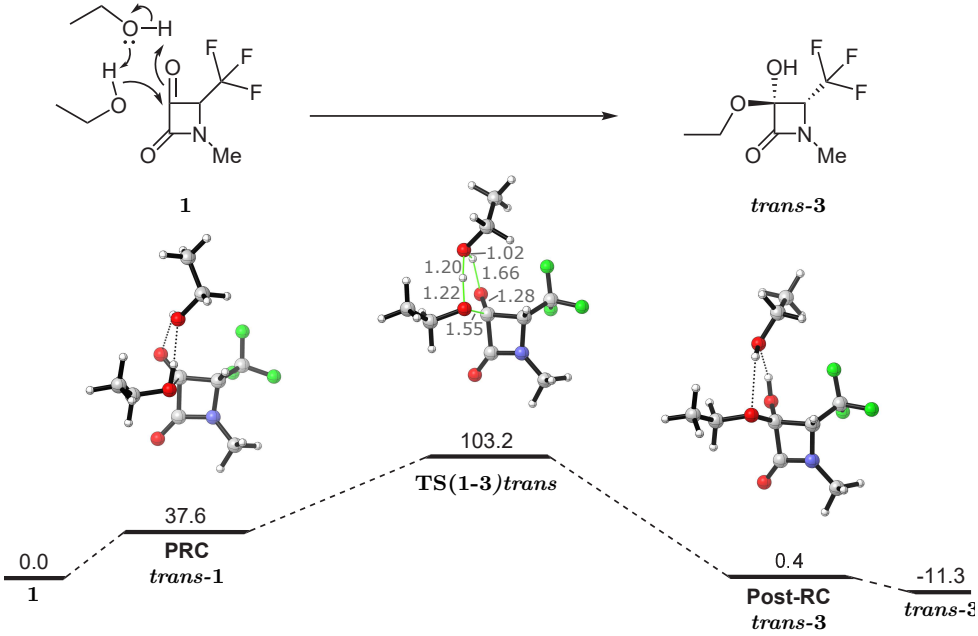


Scheme 4.10: Gibbs free energy profile for the reaction of compound ***cis*-3** towards product **4**, with the assistance of a water molecule (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

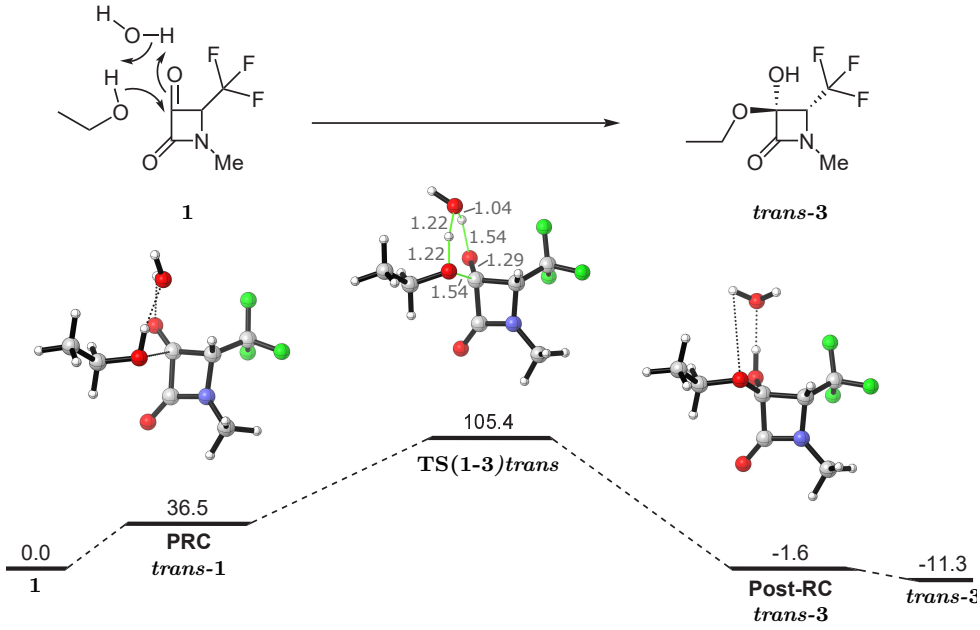
The Gibbs free energy profile for the reaction of ***cis*-3** to **4** with the assistance of a single water molecule is shown in Scheme 4.10. As was expected, the activation barrier has a high value of 303.5 kJ/mol and the reaction is not plausible.

Attack of ethanol on the β-lactam 1

For the attack of ethanol on the starting compound **1**, two scenarios were analysed. In Scheme 4.11, the Gibbs free energy profile is shown for the assistance of an ethanol molecule, having an activation barrier of 103.2 kJ/mol. In Scheme 4.12 on the other hand, the same reaction with water as the assisting molecule, is shown. In this case, the activation barrier is 105.4 kJ/mol. From both of the schemes it is clear that the activation barriers are very close to the limit of feasibility and that the assistant molecules lower the activation barriers significantly ($\Delta\Delta G^{\ddagger}_{EtOH} = 55.0$ kJ/mol, $\Delta\Delta G^{\ddagger}_{H_2O} = 52.8$ kJ/mol). Hence, it seems that assistant molecules are necessary to model the formation of the final products **7** and **8** from reactant **1**.



Scheme 4.11: Gibbs free energy profile for the reaction of compound **1** towards product ***trans*-3**, with the assistance of an ethanol molecule (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).



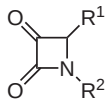
Scheme 4.12: Gibbs free energy profile for the reaction of compound **1** towards product ***trans*-3**, with the assistance of a water molecule (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

It is not clear whether EtOH or H_2O assistance will be preferred for this reaction, however. First of all, the difference in activation barriers is only 2.2 kJ/mol. Secondly, the difference in reaction energy is insignificant as well ($\Delta\Delta G_{rxn} = 2.0$ kJ/mol).

To investigate the effect of substitutions, the trifluoromethyl group on C4 and the methyl group on *N* were changed for this reaction as well. The activation barriers and reaction energies for the formation of **3c**, **3e** and **3h** can be seen in Table 4.2.

Assisting EtOH molecules lower the activation barriers with respect to the original reaction **1** to **3** ($\Delta G^{\ddagger}_{PRC} = 68.9$ kJ/mol). This is in contrast with the results without assistance, where other substituents caused even higher activation barriers. For $R^2 =$ PMP, the activation barrier was the lowest at 58.7 kJ/mol. For $R^1 =$ Ph, on the other hand, the most stable product complex was obtained ($\Delta G_{rxn} =$ -45.2 kJ/mol).

Table 4.2: Activation barriers with respect to the PRC for the *trans* attack of ethanol on compounds **1c**,**e**,**h** with the assistance of an ethanol molecule, forming compounds **3c**,**e**,**h** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).



$\mathbf{R}^1$	$\mathbf{R}^2$	$\Delta\mathbf{G}^{\ddagger}_{PRC}$	$\Delta\mathbf{G}_{rxn}$
-	PMP	58.7	-38.2
*i*Pr	-	68.5	-30.0
Ph	-	65.5	-45.2

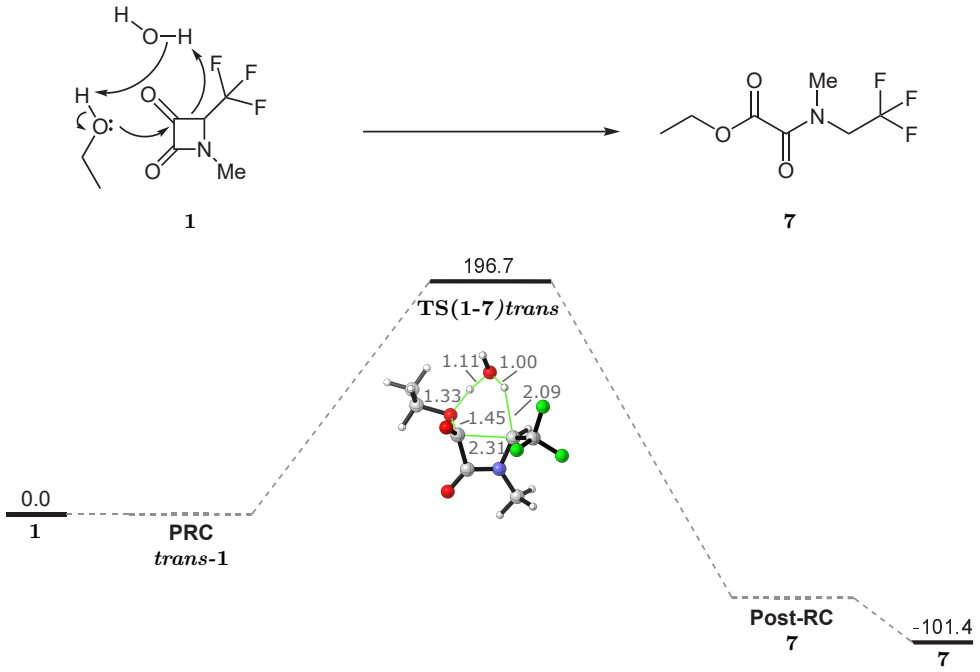
Ring opening of the ethoxy-β-lactam compound 3

The ring opening following the attack of ethanol on compound **1** was the next reaction under study. Even after close investigation however, no correct transition states were found for this reaction.

Simultaneous attack of ethanol and ring opening of the β-lactam 1

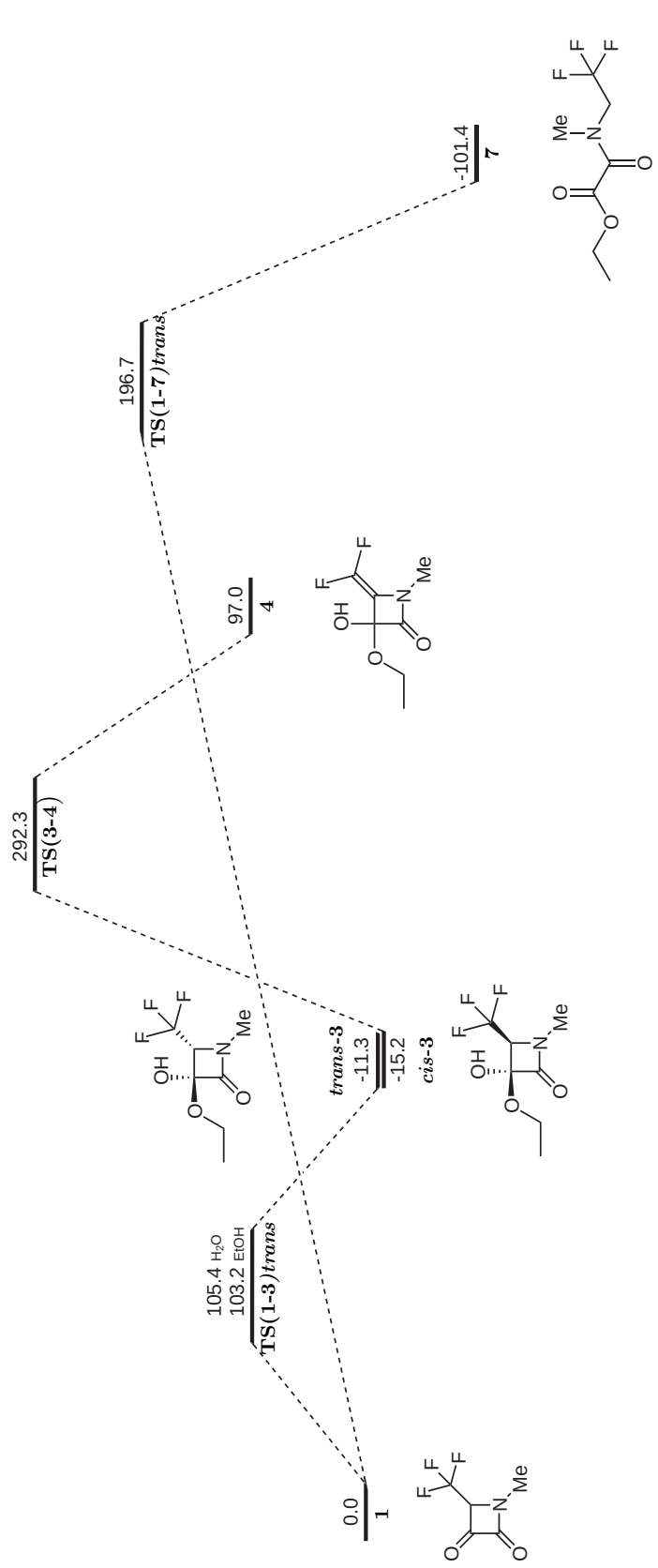
Comparable to the ring opening above, the calculations for the concerted reaction of compound **1** to **7** were not straightforward. Only a single transition state was found and the partial Gibbs free energy profile of this reaction is shown in Scheme 4.13. As can be seen, the pre- and post-reactive complexes are missing in this scheme, since the IRC analyses have not converged. The representation of the separate reactants and products can replace these to some extent.

With an activation barrier of 196.7 kJ/mol, this reaction is not plausible, even after the addition of assistant molecules. The barrier has been lowered by only 8.2 kJ/mol ($\Delta G^{\ddagger} = 204.9$ kJ/mol for the reaction without assistance). It is expected that the ring opening from **3** to **7**, discussed in the previous section behaves similarly, i.e. assistance will have little influence on the barriers and the reaction is not likely to proceed. This should however be confirmed by extra calculations.



Scheme 4.13: Partial Gibbs free energy profile for the reaction of compound **1** towards product **7**, with the assistance of a water molecule, missing data in grey (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

Scheme 4.14 provides an overview of the ethanol and water assisted reactions reviewed in this section. From this scheme, it is clear that only the addition of EtOH or H_2O to **1** has come close to the limit of plausibility, with activation barriers of 103.2 and 105.4 kJ/mol for assistance by an ethanol and water molecule, respectively. However, because of the time limitation in this study, some data is still missing. Further calculations should be performed in order to complete this Gibbs free energy profile.



Scheme 4.14: Overall Gibbs free energy profile for the reactions with assistance of water and ethanol, starting from compound **1** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

4.2.3 Calculations with assistance of a triethylamine molecule

In the previous part, the assistance of ethanol and water was investigated. The influence of these molecules was rather limited, since their basic properties are relatively weak. In this part, the influence of triethylamine (Et_3N), added to deprotonate the starting compound **1**, is examined. This substance is a much stronger base (pKb = 3.25 in water).[91,92]

Et_3N has several possible conformations, two of which are shown in Figure 4.2. In the conformation on the left side the ethyl groups are more planar, while they are more perpendicular to the plane on the right side. Combinations of these orientations are possible as well, e.g. with one planar and two perpendicular ethyl groups. Calculations showed however that the conformation with only planar ethyl groups was the most stable one ($\Delta G = 13.7\,kJ/mol$ for planar versus perpendicular). This conformation is used for further calculations.

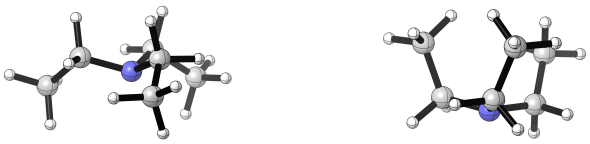
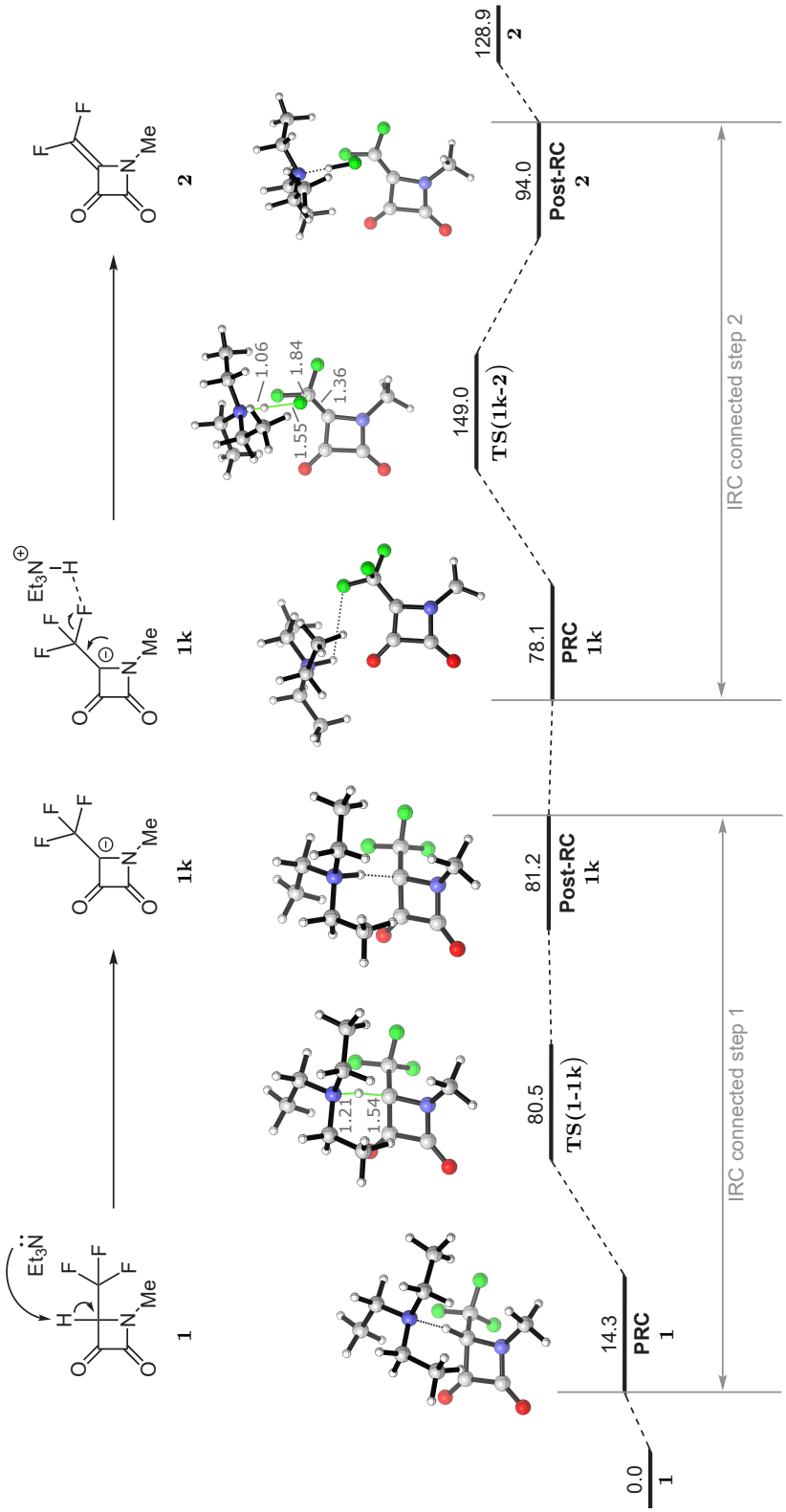


Figure 4.2: Two conformations of Et_3N, with the conformation on the left side being the most stable (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

Elimination of hydrogen fluoride

The influence of Et_3N assistance on the elimination of HF is investigated. In this case, the elimination of HF was found to be a two-step reaction E_1, which can be explained because Et_3N is a strong base and will first deprotonate the β-lactam compound **1**. Afterwards, the protonated Et_3NH^+ complex helps to extract a fluorine atom from the β-lactam anion **1k**. The Gibbs free energy profile for the two-step reaction from compound **1** to **2** is shown in Scheme 4.15.



Scheme 4.15: Gibbs free energy profile for the two-step reaction of compound **1** towards product **2**, with the assistance of Et_3N (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

From the scheme, it can be seen that the activation barrier of the first step is 80.5 kJ/mol, which makes it a plausible reaction pathway. The formed anionic intermediate **1k** is metastable and will immediately react further. The second reaction is the rate-determining step and has an activation barrier of 149.0 kJ/mol. This barrier is however too high for the second step to be plausible. It is most likely that the intermediate **1k** will react back to **1** or will be degraded towards other compounds. However, these reactions are beyond the scope of this Master's thesis.

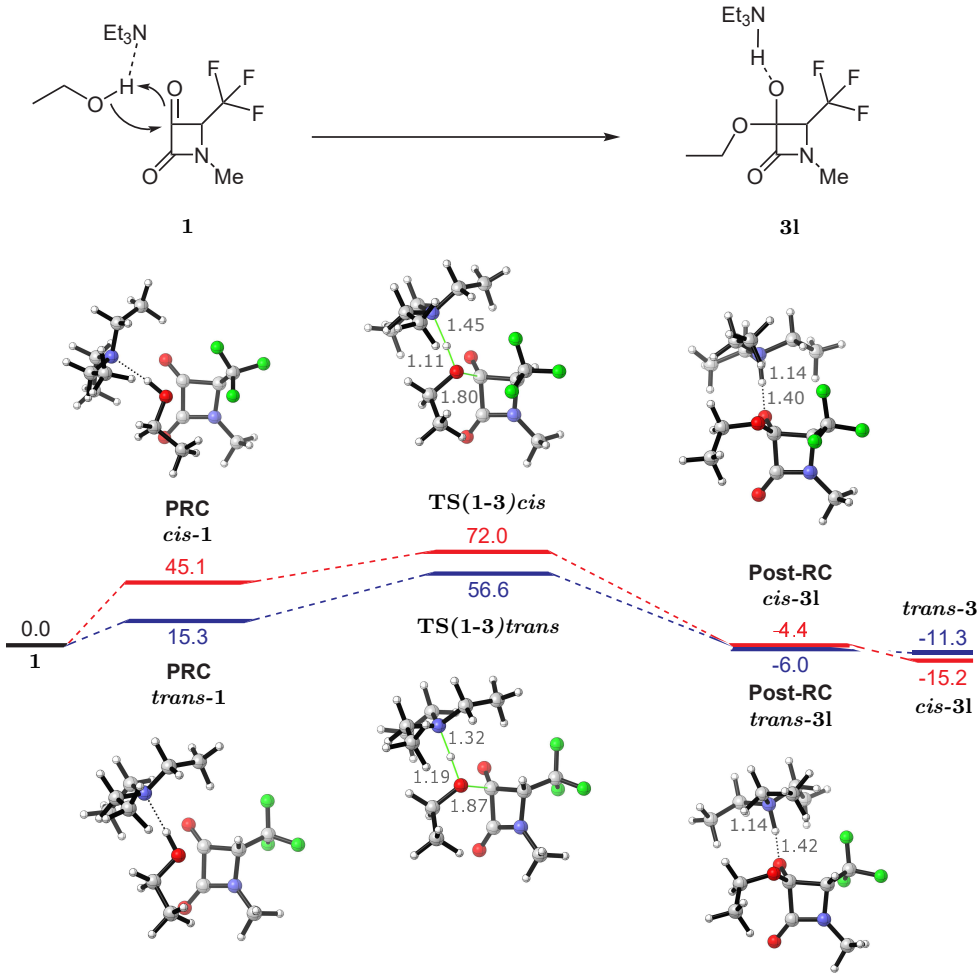
It is expected that all reactions involving the elimination of HF will have a high barrier. This hypothesis was examined for the reaction from **3** to **4**. For the second step in this reaction, an activation barrier of 126.8 kJ/mol with respect to the PRC was found, even higher than the second step shown in Scheme 4.15 ($\Delta G^{\ddagger}_{PRC} = 70.9$). This suggests that the elimination reactions **1** to **2**, **3** to **4** and **7** to **8** will not proceed.

Attack of ethanol on the β-lactam 1

In the previous part, it was shown that the assistance of ethanol or water significantly lowered the activation barriers for the attack of ethanol on compound **1** ($\Delta G^{\ddagger}_{EtOH} = 103.2$ kJ/mol, $\Delta G^{\ddagger}_{H_2O} = 105.4$ kJ/mol). In this part, the assistance of Et_3N in the attack is investigated. In Scheme 4.16, the Gibbs free energy profile for this reaction is shown, both for the *cis* and *trans* pathways.

From the scheme, it is clear that the activation barriers for both reactions are low enough for the reactions to proceed ($\Delta G^{\ddagger}_{cis} = 72.0$ kJ/mol and $\Delta G^{\ddagger}_{trans} = 56.6$ kJ/mol). These barriers are even lower than the barriers with assistance of ethanol or water ($\Delta\Delta G^{\ddagger} = 46.6$ kJ/mol versus assistance of ethanol, $\Delta\Delta G^{\ddagger} = 48.8$ kJ/mol versus assistance of water), i.e. Et_3N assistance is preferred.

The difference between the *cis* and *trans* activation barriers is 15.5 kJ/mol, making ***trans*-3l** the kinetically preferred product. However, since for both pathways, the activation barrier of the backward reaction is low enough, it cannot be concluded which pathway, *cis* or *trans*, will be preferred.

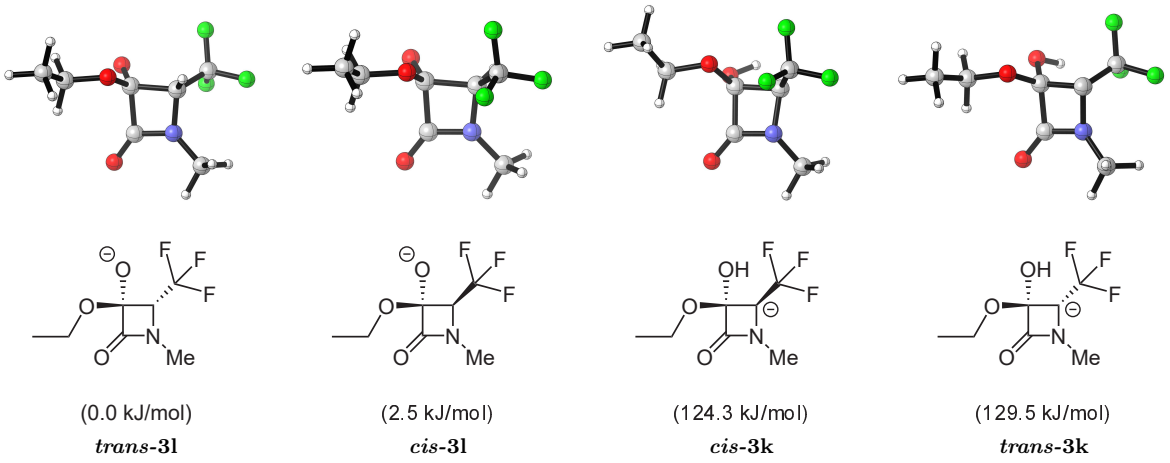


Scheme 4.16: Gibbs free energy profile for the *cis* and *trans* reaction of compound **1** towards product **3l**, with the assistance of Et_3N (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

Note that in the product complex, the proton originally bound to oxygen is actually bound to the nitrogen atom of Et_3N, with a distance of 1.14 Å in both the *cis* and *trans* product complexes. This means that the computationally calculated product **3l** is actually anionic.

Ring opening of the ethoxy-β-lactam compound 3

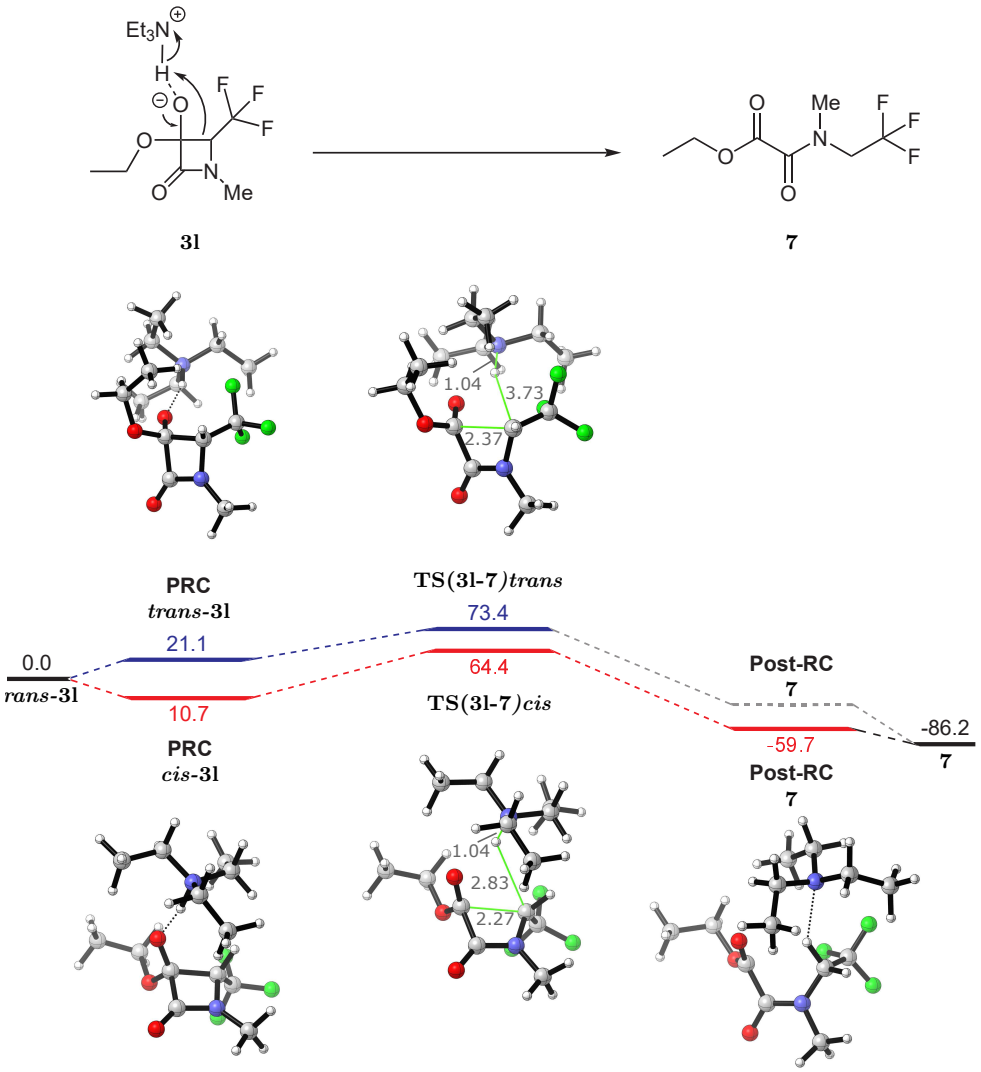
The ring opening of **3** starts from the anionic compounds ***cis*-3l** and ***trans*-3l**. These compounds are deprotonated at the hydroxy group and must be compared to their C4 deprotonated forms ***cis*-3k** and ***trans*-3k** (see Scheme 4.17), since their stability differs. Without stabilisation by assisting molecules, it is clear that **3k** is at least 121.8 kJ/mol less stable than **3l**. The latter will be used below.



Scheme 4.17: The most stable anion ***trans*-3l** (left), the least stable anion ***trans*-3k** (right) and intermediate anions ***cis*-3l** and ***cis*-3k** (two central structures) (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), 298 K, 1 atm).

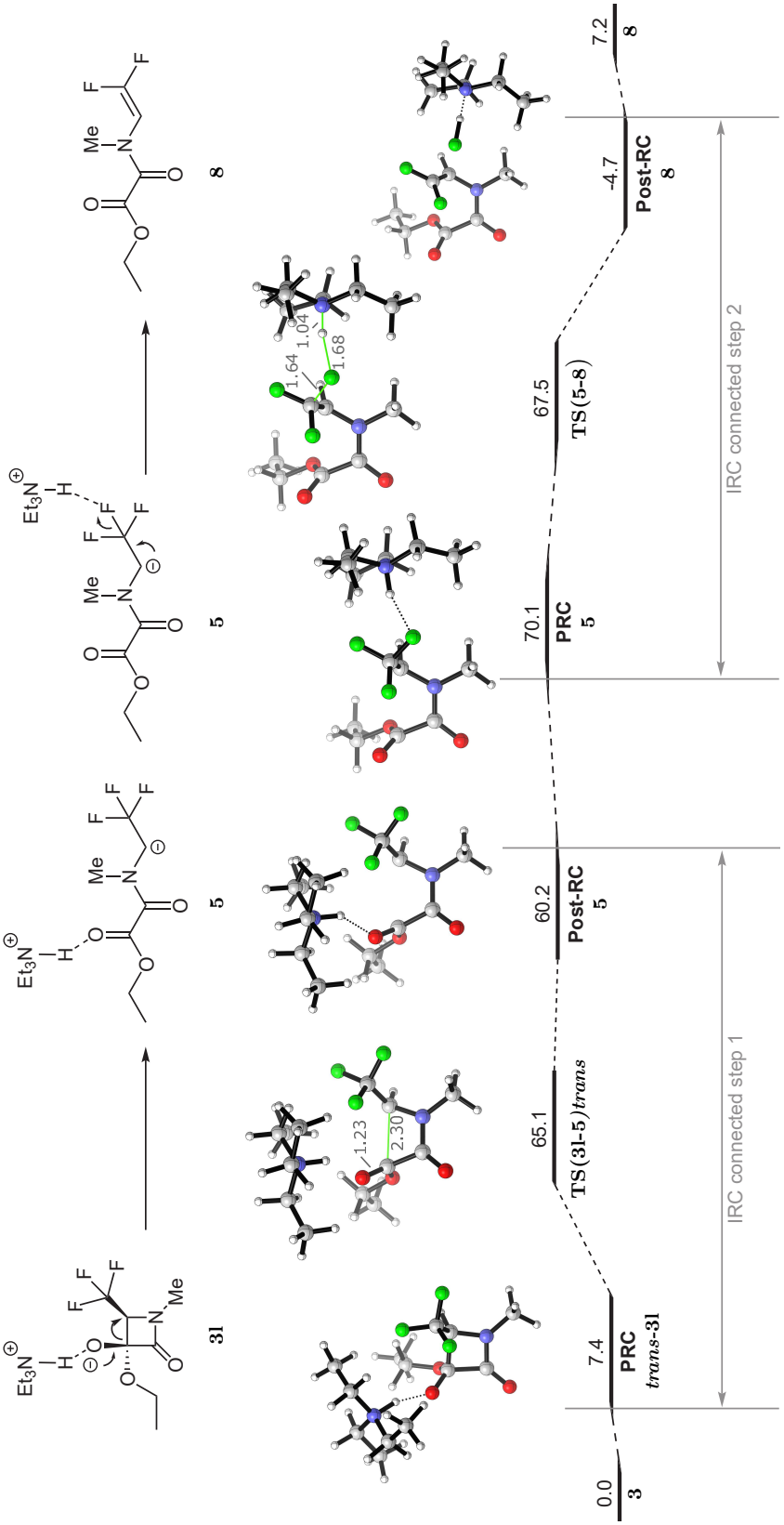
The *cis* and *trans* ring-opening reactions of compound **3** are shown in Scheme 4.18. These *cis* and *trans* reactions have activation barriers of 64.4 kJ/mol 73.4 kJ/mol, respectively. Even though both reactions are plausible, the *cis* reaction is preferred.

Besides the ring opening shown in the previous scheme, **3l** can also react towards **8** in a two-step reaction as shown in Scheme 4.19.



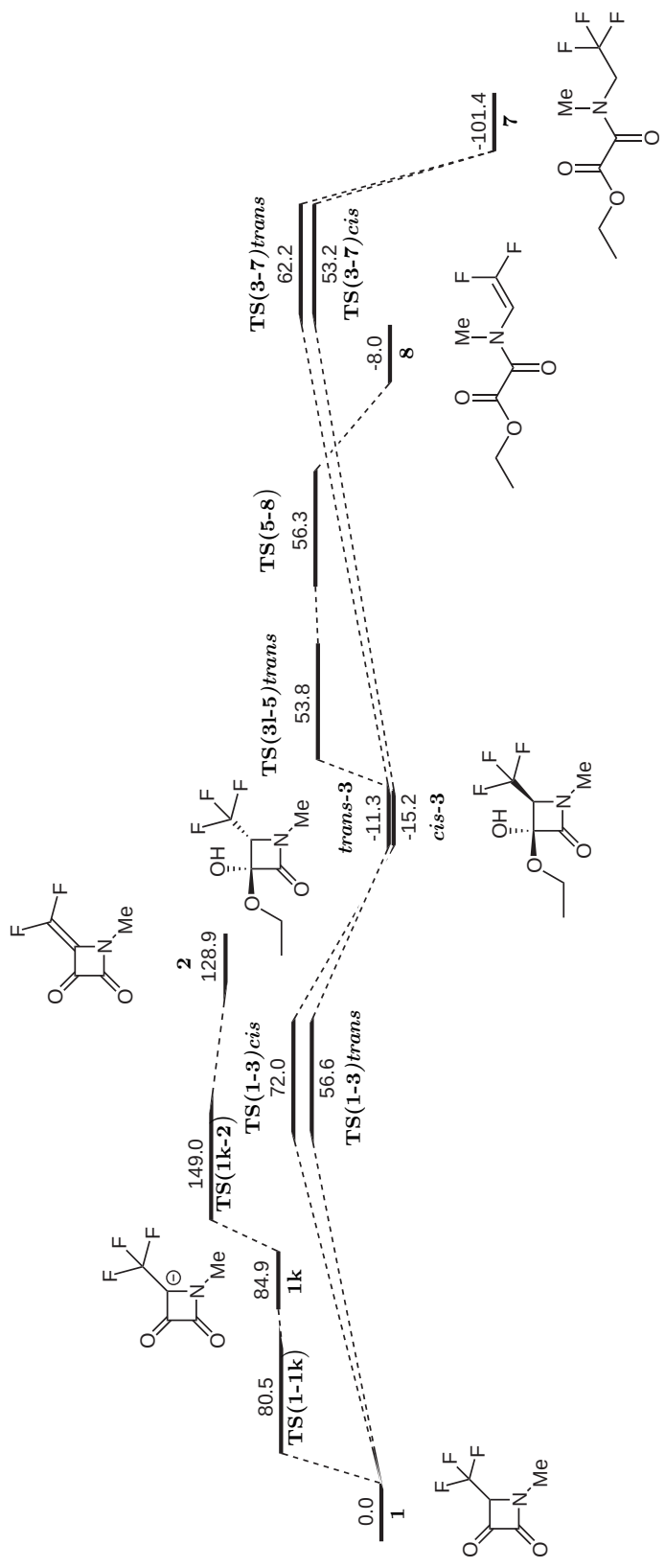
Scheme 4.18: Gibbs free energy profile for the reaction of compound **3l** towards product **7**, with the assistance of Et_3N, missing data in grey (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

As can be seen from Scheme 4.19, the activation barrier is 65.1 kJ/mol for the first step and 67.5 kJ/mol for the second step. No other two-step reaction mentioned above was feasible, except this one. Intermediate **5** is very unstable and will immediately react further. The proton bound to Et_3N in both **3l** and **5** is oriented towards the carbonyl group in **5**. It should also be noted that the PRC of the second step has a higher energy than the transition state itself, which is caused by an anomaly in the calculations. The concerted pathway of this reaction was investigated as well. However, no transition states were found.



Scheme 4.19: Gibbs free energy profile for the reaction of compound **31** towards product **8**, with the assistance of Et_3N (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

Scheme 4.20 provides an overview of the reactions reviewed in this section. It is clear that the two-step elimination of HF from compound **1** towards **2** will not proceed, since the activation barrier of the second step is too high. The attack of EtOH on **1** leading to **3** on the other hand will be able to proceed, just like the further reactions towards compounds **7** and **8**. The difference in activation barriers is relatively low for these reactions, making it hard to predict whether **7** or **8** will be formed. However, since product **8** is much less stable than **7**, this compound will be more likely to react back with eventual formation of **7**. Nonetheless, it should be noted that this is not completely in line with the experimental results. Further calculations should be performed here in order to provide this question with a final answer and to complete this Gibbs free energy profile.



Scheme 4.20: Overall Gibbs free energy profile for the reactions with assistance of Et_3N, starting from compound **1** (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, distances in Å, 298 K, 1 atm).

Chapter 5

Summary, conclusion and outlook

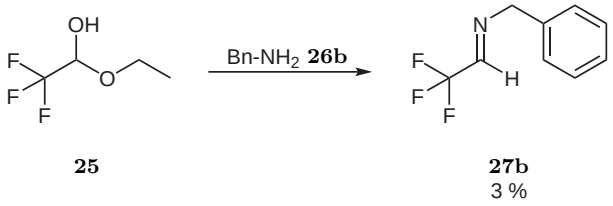
Since the discovery of penicillin in 1928, the spectrum of β-lactam antibiotics has been broadened tremendously. Not only do these structures function as antibiotics, they display several other biological activities, such as an anti-inflammatory action, cholesterol absorption inhibition and anti-cancer activity.

However, because of the increased prevalence of antimicrobial resistance, new β-lactam derivatives are needed urgently. This Master's dissertation tries to contribute to that. To that end, the reactivity of 3-oxo-4-trifluoromethyl-β-lactams towards ethanol and triethylamine was investigated, in order to rationalise the experiments performed by H. Dao Thi. The goal of these experiments was to produce, deprotonate and perform an α-alkylation on the C4 carbon atom of several 3-oxo-4-trifluoromethyl-β-lactams. Subsequently, computations were performed to obtain more insight into the various pathways. First of all, reactions without assistance of any other molecules were investigated. Secondly, the influence of assisting molecules such as ethanol, water and Et_3N on the reactions and activation barriers was examined.

5.1 Summary

During the experiments in this Master's thesis, the first step in the production of new derivatives of 3-oxo-4-trifluoromethyl-β-lactams was explored. In this reaction, 1-ethoxy-2,2,2-trifluoroethanol **25** was transformed using either isopropylamine **26a** or benzylamine **26b**. The production of *(E)-N*-(2,2,2-trifluoroethylidene)isopropylamine **27a** did not succeed

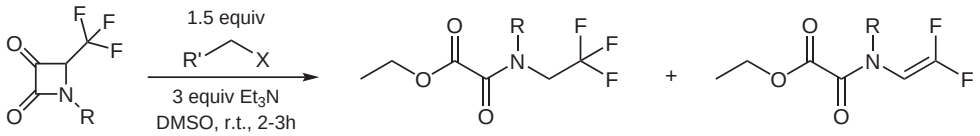
and the 2,2,2-trifluoro-1-isopropylamino-ethanol **28a** was formed instead. The production of *(E)-N*-(2,2,2-trifluoroethylidene)benzylamine **27b** however did succeed and is shown in Scheme 5.1.



25 **27b** 3 %

Scheme 5.1: Reaction of 1-ethoxy-2,2,2-trifluoroethanol **25** towards *(E)-N*-(2,2,2-trifluoroethylidene)benzylamine **27b**, using benzylamine **26b**.

In the computational part, the reaction of 3-oxo-4-trifluoromethyl-β-lactam **1** towards dicarbonyl products **7** and **8** was investigated in order to unravel the reaction mechanism and rationalise the experimental outcome as shown in Scheme 5.2.



1 **7** **8**

Scheme 5.2: Transformation of 3-oxo-4-trifluoromethyl-β-lactams **1** to products **7** and **8** (R = PMB, PMP; R' = Et, H, Ph, vinyl, 1-chloroethyl).

In order to limit the computational cost of the calculations, a methyl group was chosen as the substituent on the nitrogen atom (R = Me). An ethyl halide was chosen as the electrophile. Throughout the calculations, other substituents were used in order to examine their influence on the activation barrier.

Firstly, the calculations were performed without the assistance of other molecules. These reactions and their corresponding activation barriers and reaction energies are summarised in Table 5.1.

Table 5.1: Activation barriers and reaction energies with respect to their seperate reactants, for the reactions without assistance (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

Reaction	**Reactant**	**Product**	$\Delta G^{\ddagger}$	ΔG_{rxn}
HF expulsion	**1**	**2**	318.4	126.3
Ethanol attack	**1**	***trans*-3**	158.2	-11.3
	1	***cis*-3**	158.5	-15.2
	2	**4**	141.9	-30.6
Ring opening	***cis*-3**	**7**	177.8	-91.8
	4	**8**	180.6	-87.9
Ethanol attack and ring opening	**1**	**7**	204.9	-101.4
	2	**8**	174.5	-136.9

The HF expulsion follows a concerted mechanism, involving simultaneous deprotonation and extraction of the fluorine atom. Next, the attack of ethanol is a reaction which can take place both in a *cis* and *trans* manner. In this reaction, the proton from ethanol is immediately passed to the keto group at C3, transforming it into a hydroxy group. The C3-C4 bond of **3** or **4** opens subsequently. The concerted alternative for these reactions involves the simultaneous attack of ethanol and ring opening.

None of the unassisted reactions have an activation barrier low enough to be feasible. Assistance is clearly necessary in order to obtain realistic barriers for these reactions. This is in good agreement with the experimental results.

In the second part, ethanol and water, both present during the experiments, were used as assistant molecules. An overview of these results is shown in Table 5.2. The ethanol and water assisted attacks of ethanol on compound **1** have activation barriers close to the limit of plausibility. The activation barrier for the expulsion of HF on the other hand is too high for the reactions to proceed, because of the weak basic properties of both ethanol and water ($pKb_{EtOH} = 16.4$, $pKb_{H_2O} = 15.7$). The activation barrier for the concerted ethanol attack and ring opening is too high as well, although water assistance has slightly lowered the barrier.

Table 5.2: Activation barriers and reaction energies with respect to their seperate reactants, for the reactions with assistance of ethanol or water (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

Reaction	**Reactant**	**Product**	**Assisting compound**	$\Delta G^{\ddagger}$	ΔG_{rxn}
HF expulsion	**3**	**4**	H_2O	303.5	123.9
Ethanol attack	**1**	***trans*-3**	EtOH	103.2	0.4
	1	***trans*-3**	H_2O	105.4	-1.6
Ethanol attack and ring opening	**1**	**7**	H_2O	196.7	-

The last part of the computational study examined the assistance of triethylamine in the reactions described above. During the experiments, this compound was added to achieve deprotonation at the C4 atom of the β-lactam **1**. The results of the calculations are summarised in Table 5.3.

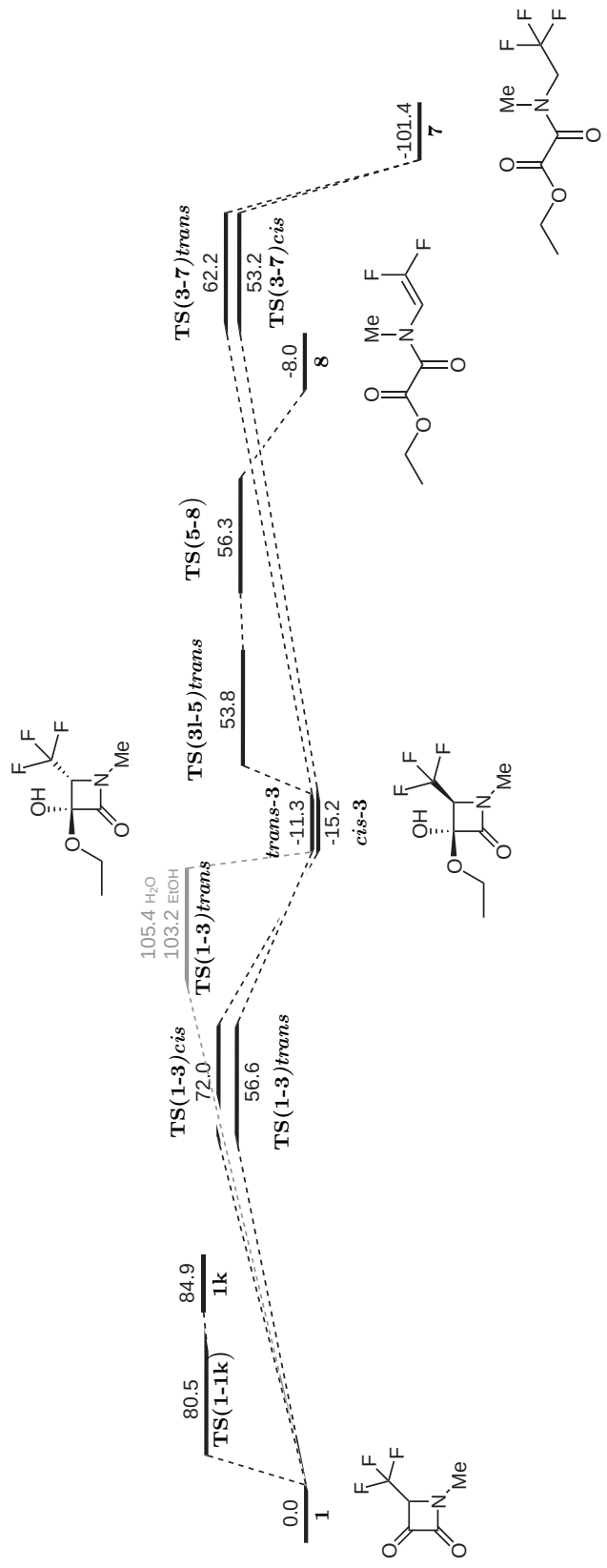
Table 5.3: Activation barriers and reaction energies with respect to their seperate reactants, for the reactions with assistance of Et_3N (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

Reaction		**Reactant**	**Product**	$\Delta G^{\ddagger}$	ΔG_{rxn}
HF expulsion	Step 1	**1**	**1k**	80.5	81.2
	Step 2	**1k**	**2**	149.0	94.0
Ethanol attack		**1**	***trans*-3**	56.6	-6.0
		1	***cis*-3**	72.0	-4.4
Ring opening		***trans*-3l**	**7**	73.4	-
		***cis*-3l**	**7**	64.4	-59.7
Ring opening and expulsion of HF	Step 1	***trans*-3l**	**5**	65.1	60.2
	Step 2	**5**	**8**	67.5	-4.7

It is clear that HF expulsion will not proceed, since the activation barrier for the second step is still too high ($\Delta G^{\ddagger} = 149.0$ kJ/mol). Ethanol attack on compound **1** however is plausible.

For the ring opening, both *trans* and *cis* reactions are feasible, although the *cis* reaction is kinetically preferred. For the final reactions, involving ring opening and subsequent expulsion of HF, the reaction pathway is plausible. It should be noted however that the intermediate **5** is relatively unstable and will quickly react towards other compounds.

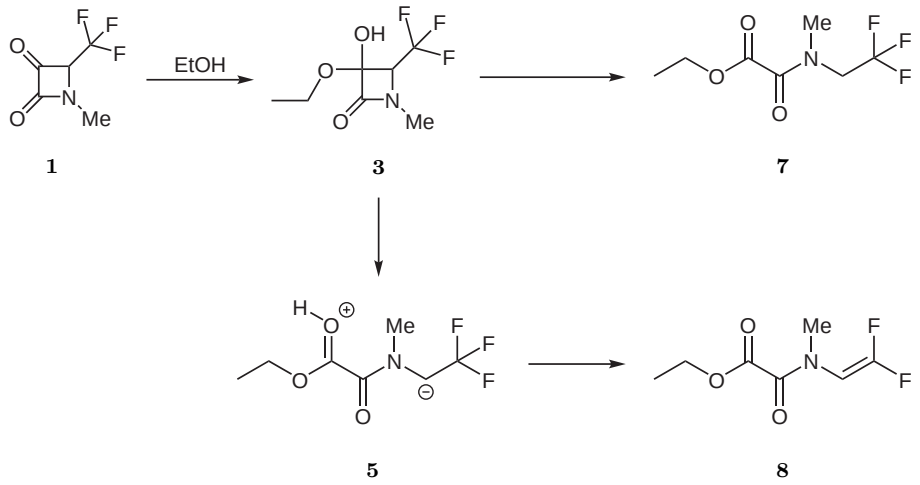
In order to provide an overview, the Gibbs free energy profiles for all probable reactions are shown again in Scheme 5.3. The only plausible reactions are the reactions with assistance of Et_3N. The two reactions of **1** to **3** with assistance of ethanol and water are shown as well, since the activation barriers were close to the limit of plausibility.



Scheme 5.3: Gibbs free energy profile of the plausible reactions, starting from compound **1**, all with assistance of Et_3N, except for the reactions with assistance of ethanol and water, having activation barriers close to the limit of plausibility, which are shown in grey (M06-2X/6-31+G*, IEF-PCM ($\varepsilon = 46.7$, DMSO), energies in kJ/mol, 298 K, 1 atm).

5.2 Conclusion and outlook

From the calculations performed on the reactivity of 3-oxo-4-trifluoromethyl-β-lactams **1**, plausible pathways towards the dicarbonyl products **7** and **8** were found and are shown in Scheme 5.4. The reaction starts at the β-lactam compound **1** and proceeds through an ethoxy-β-lactam intermediate **3**. From this intermediate, the dicarbonyl product bearing a trifluorovinyl substituent **7** is formed immediately from the ethoxy compound **3** through a ring-opening reaction. The product with the difluorovinyl substituent **8** on the other hand, is formed through ring opening towards the unstable the zwitterionic intermediate **5** and subsequent expulsion of a fluorine atom. Throughout this entire pathway, triethylamine assistance is necessary to lower the activation barriers, which is in good agreement with experimental results.



Scheme 5.4: Plausible pathway for the reaction from compound **1** to **7** and **8**, all with assistance of Et_3N.

These calculations do not provide an explanation for the formation of the difluoro compound **8** as the major product. Furthermore, the influence of the use of other electrophiles and bases as well as the effect of temperature and solvent were not investigated. Further calculations are necessary to unravel these mysteries and to fill in the blanks in this study.

Bibliography

[1] Fleming, A. *Brit. J. Exp. Pathol.* **1929**. *10*, 226.

[2] Page, M. I. *The chemistry of beta-lactams.* **2012**.

[3] Brotzu, G. Research on a new antibiotic. Tech. rep., **1948**.

[4] Levy, S. B. *Lancet* **1982**. *320*, 83–88.

[5] Levy, S. B. *Sci. Am.* **1998**. *278*, 46–53.

[6] Levy, S. B. & Marshall, B. *Nat. Med.* **2004**. *10*, S122–S129.

[7] Barber, M. & Rozwadowska-dowzenko, M. *Lancet* **1948**. *252*, 641–644.

[8] Crofton, J. & Mitchison, D. A. *Brit. Med. J.* **1948**. *2*, 1009–1015.

[9] Watanabe, T. *Bacteriol. Rev.* **1963**. *27*, 87–115.

[10] Levy, S. B. *Clin. Infect. Dis.* **2001**. *33*, S124–S129.

[11] Elwell, L. P., Roberts, M., Mayer, L. W. & Falkow, S. *Antimicrob. Agents Chemother.* **1977**. *11*, 528–533.

[12] Van Klingeren, B., Van Embden, J. D. A. & Dessens-Kroon, M. *Antimicrob. Agents Chemother.* **1977**. *11*, 383–387.

[13] Mehta, P. D., Sengar, N. P. S. & Pathak, A. K. *Eur. J. Med. Chem.* **2010**. *45*, 5541–5560.

[14] Firestone, R. A., Barker, P. L., Judith, M. P., Ashe, B. M. & Dahlgren, M. E. *Tetrahedron* **1990**. *46*, 2255–2262.

[15] Han, W. T., Trehan, A. K., Wright, J. J. K., Federici, M. E., Seller, M. & Meanwelp, N. A. *Bioorg. Med. Chem.* **1995**. *3*, 1123–1143.

[16] Slusarchyk, W. A., Bolton, S. A., Hartl, K. S., Huang, M.-H., Jacobs, G., Meng, W., Ogletree, M. L., Pi, Z., Schumacher, W. A., Seiler, S. M., Sutton, J. C., Treuner, U., Zahler, R., Zhao, G. & Bisacchi, G. S. *Bioorg. Med. Chem. Lett.* **2002**. *12*, 3235–3238.

[17] Veinberg, G., Vorona, M., Shestakova, I., Kanepe, I., Zharkova, O., Mezapuke, R., Turovskis, I., Kalvinsh, I. & Lukevics, E. *Bioorg. Med. Chem.* **2000**. *8*, 1033–1040.

[18] Dolfen, J., Kenis, S., Van Hecke, K., De Kimpe, N. & D'hooghe, M. *Chem. Eur. J.* **2014**. *20*, 10650–10653.

[19] Jensen, F. *Introduction to computational chemistry.* Wiley, **2007**.

[20] Tsuneda, T. *Density Functional Theory in Quantum Chemistry.* Springer, **2014**.

[21] Wolfram, K. & Holthausen, M. C. *A chemist's guide to density functional theory.* **2015**.

[22] Zhang, F.-L., Wang, Y.-f., Lonca, G. H., Zhu, X. & Chiba, S. *Angew. Chem. Int. Ed.* **2014**. *53*, 4390–4394.

[23] Wang, Y.-f. & Chiba, S. *Org. Lett.* **2014**. *16*, 4272–4275.

[24] Abaev, V. T., Plieva, A. T., Chalikidi, P. N., Uchuskin, M. G., Trushkov, I. V. & Butin, A. V. *Org Lett* **2014**. *16*, 4150–4153.

[25] Zhou, F. & Driver, T. G. *Org. Lett.* **2014**. *16*, 2916–2919.

[26] Xuan, J., Xia, X.-D., Zeng, T.-T., Feng, Z.-J., Chen, J.-R., Lu, L.-Q. & Xiao, W.-J. *Angew. Chem. Int. Ed.* **2014**. *126*, 5759–5762.

[27] Ivanov, K. L., Villemson, E. V., Budynina, E. M., Ivanova, O. A., Trushkov, I. V. & Melnikov, M. Y. *Chem. Eur. J.* **2015**. *21*, 4975–4987.

[28] Shie, J.-J., Liu, Y.-C., Lee, Y.-M., Lim, C., Fang, J.-M. & Wong, C.-H. *J. Am. Chem. Soc.* **2014**. *136*, 9953–9961.

[29] Li, X., Fang, T. & Boons, G.-j. *Angew. Chem. Int. Ed.* **2014**. *53*, 7179–7182.

[30] Gold, B., Batsomboon, P., Dudley, G. B. & Alabugin, I. V. *J. Org. Chem.* **2014**. *79*, 6221–6232.

[31] Wang, D., Etienne, L., Echeverria, M., Moya, S. & Astruc, D. *Chem. Eur. J.* **2014**. *20*, 4047–4054.

[32] Ok, T., Jeon, A., Lee, J., Lim, J. H., Hong, C. S., Lee, H.-S. & May, R. V. *J. Org. Chem.* **2007**. *72*, 7390–7393.

[33] Budynina, E. M., Ivanova, O. A., Averina, E. B., Kuznetsova, T. S. & Zefirov, N. S. *Tetrahedron Lett.* **2006**. *47*, 647–649.

[34] Izquerdo, M. L., Arenal, I., Barnabé, M. & Fernández Alvarez, E. *Tetrahedron* **1985**. *41*, 215–220.

[35] Yu, M. & Pagenkopf, B. L. *Tetrahedron* **2005**. *61*, 321–347.

[36] Cavitt, M. A., Phun, L. H. & France, S. *Chem. Soc. Rev.* **2014**. *43*, 804–818.

[37] De Nanteuil, F., De Simone, F., Frei, R., Benfatti, F., Serrano, E. & Waser, J. *Chem. Commun.* **2014**. *50*, 10912–10928.

[38] Reissig, H.-U. & Zimmer, R. *Chem. Rev.* **2003**. *103*, 1151–1196.

[39] Sinnecker, S., Rajendran, A., Klamt, A., Diedenhofen, M. & Neese, F. *J. Phys. Chem. A* **2006**. *110*, 2235–2245.

[40] Klamt, A. & Schüürmann, G. J. *J. Chem. Soc. Perkin Trans.* **1993**. *2*, 799–805.

[41] Kelley, B. T., Carroll, P. & Joullié, M. M. *J. Org. Chem.* **2014**. *79*, 5121–5133.

[42] Formaggio, F., Baldini, C., Moretto, V., Crisma, M., Kaptein, B., Broxterman, Q. B. & Toniolo, C. *Chem. Eur. J.* **2005**. *11*, 2395–2404.

[43] Li, P., Evans, C. D. & Joullie, M. M. *Org. Lett.* **2005**. *7*, 5325–5327.

[44] Li, P., Evans, C. D., Forbeck, E. M., Park, H., Bai, R., Hamel, E. & Joullie, M. M. *Bioorg. Med. Chem. Lett.* **2006**. *16*, 4804–4807.

[45] Li, P., Evans, C. D., Wu, Y., Cao, B., Hamel, E. & Joullie, M. M. *J. Am. Chem. Soc.* **2008**. *130*, 2351–2364.

[46] Joullié, M. M., Berritt, S. & Hamel, E. *Tetrahedron Lett.* **2011**. *52*, 2136–2139.

[47] Cox, J. D. *Tetrahedron* **1963**. *19*, 1175–1184.

[48] Forbeck, E. M., Evans, C. D., Gilleran, J. A., Li, P. & Joullie, M. M. *J. Am. Chem. Soc.* **2007**. *129*, 14463–14469.

[49] Kelley, B. T. & Joullie, M. M. *Org. Lett.* **2010**. *12*, 4244–4247.

[50] Kelley, B. T. & Joullié, M. M. *Tetrahedron: Asymmetry* **2013**. *24*, 1233–1239.

[51] Sun, P. & Weinreb, S. M. *J. Org. Chem.* **1997**. *62*, 8604–8608.

[52] Sakakibara, K. & Nozaki, K. *Org. Biomol. Chem.* **2009**. *7*, 502–507.

[53] Mitsunobu, O. *Synthesis* **1981**. *1981*, 1–28.

[54] Stamm, H., Asslthlanakls, P., Buchholz, B. & Weiss, R. *Tetrahedron Lett.* **1982**. *23*, 5021–5024.

[55] Lin, P.-Y., Bellos, K., Stamm, H. & Onistschenko, A. *Tetrahedron* **1992**. *48*, 2359–2372.

[56] Corey, E. J. & Grogan, M. J. *Org. Lett.* **1999**. *1*, 157–160.

[57] Gong, Y. & Kato, K. *J. Fluor. Chem.* **2001**. *108*, 83–86.

[58] Carroccia, L., Fioravanti, S., Pellacani, L. & Tardella, P. A. *Synthesis* **2010**. *23*, 4096–4100.

[59] Schlegel, H. B. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* **2011**. *1*, 790–809.

[60] Born, M. & Oppenheimer, R. *Ann. Phys.* **1927**. *389*, 457–484.

[61] Fock, V. *Z. Phys. A* **1930**. *61*, 126–148.

[62] Slater, J. C. *Phys. Rev.* **1929**. *34*, 1293–1322.

[63] Lennard-Jones, J. E. *J. Chem. Soc. Faraday Trans.* **1929**. *25*, 668–686.

[64] Heitler, W. & London, F. *Z. Phys. A* **1927**. *44*, 455–472.

[65] Moller, C. & Plesset, M. S. *Phys. Rev.* **1934**. *46*, 618–622.

[66] Thomas, L. H. *Math. Proc. Cambridge Philos. Soc.* **1927**. *23*, 542–548.

[67] Fermi, E. *Z. Phys.* **1928**. *48*, 73–78.

[68] Hohenberg, P. & Kohn, W. *Phys. Rev.* **1964**. *136*, B864–B871.

[69] Parr, R. G. & Yang, W. *Annu. Rev. Phys. Chem.* **1995**. *46*, 701–728.

[70] Kohn, L. & Sham, W. *Phys. Rev.* **1965**. *140*, A1133–A1138.

[71] Mennucci, B. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* **2012**. *2*, 386–404.

[72] Cossi, M., Rega, N., Scalmani, G., Barone, V., Chimica, D., Ii, F. & Angelo, C. M. S. *J. Comput. Chem.* **2003**. *24*, 669–681.

[73] Bochove, M. A. V. & Bickelhaupt, F. M. *J. Org. Chem.* **2008**. *2008*, 649–654.

[74] Goossens, H., Vervisch, K., Catak, S., Stankovic, S., D'hooghe, M., De Proft, F., Geerlings, P., De Kimpe, N., Waroquier, M. & Van Speybroeck, V. *J. Org. Chem.* **2011**. *76*, 8698–8709.

[75] Cramer, C. J. & Truhlar, D. G. *Chem. Rev.* **1999**. *99*, 2161–2200.

[76] Marten, B., Kim, K., Cortis, C., Friesner, R. A., Ringnalda, M. N., Murphy, R. B., Sitkoff, D. & Honig, B. *J. Phys. Chem.* **1996**. *100*, 11775–11788.

[77] Miertus, S., Scrocco, E. & Tomasi, J. *J. Chem. Phys.Chem. Phys.* **1981**. *55*, 117–129.

[78] Barone, V. & Cossi, M. *J. Phys. Chem. A* **1998**. *102*, 1995–2001.

[79] Mennucci, B., Cance, E. & Tomasi, J. *J. Phys. Chem. B* **1997**. *101*, 10506–10517.

[80] Warshel, A. & Levitt, M. *J. Mol. Biol.* **1976**. *103*, 227–249.

[81] Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J., Scalmani, G., Barone, V., Mennucci, B., Petersson, G. A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H. P., Izmaylov, A. F., Bloino, J., Zheng, G., Sonnenberg, J. L.,

Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J. A., Peralta, J. E., Ogliaro, F., Bearpark, M., Heyd, J. J., Brothers, E., Kudin, K. N., Staroverov, V. N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J. C., Iyengar, S. S., Tomasi, J., Cossi, M., Reaga, N., Millam, J. M., Klene, M., Knox, J. E., Cross, J. B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R. E., Yazyev, O., Austin, A. J., Cammi, R., Pomelli, C., Ochterski, J. W., Martin, R. L., Morokuma, K., Zakrzewski, V. G., Voth, G. A., Salvador, P., Dannenberg, J. J., Dapprich, S., Daniels, A. D., Farkas, O., Foresman, J. B., Ortiz, J. V., Cioslowski, J. & Fox, D. J. Gaussian 09, revision D.01, **2009**.

[82] Miehlich, B., Savin, A., Stoll, H. & Preuss, H. *Chem. Phys. Lett.* **1989**. *157*, 200–206.

[83] Zhao, Y. & Truhlar, D. G. *Theor. Chem. Acc.* **2008**. *120*, 215–241.

[84] Becke, A. D. *Phys. Rev. A* **1988**. *38*, 3098–3100.

[85] Becke, A. D. *J. Chem. Phys.* **1993**. *98*, 1372–1377.

[86] Lee, C., Yang, W. & Parr, R. G. *Phys. Rev. B* **1988**. *37*, 785–789.

[87] Fukui, K. *Acc. Chem. Res.* **1981**. *14*, 363–368.

[88] Legault, C. Y. CYLview, 1.0b, **2009**.

[89] Kim, D. W., Jeong, H.-j., Lim, S. T. & Sohn, M.-h. *Tetrahedron Letters* **2010**. *51*, 432–434.

[90] Chauhan, B. S. *Principles of Biochemistry and Biophysics.* **2008**.

[91] Ripin, D. H. & Evans, D. A. pKa's of Inorganic and Oxo-acids. Tech. rep., **2005**.

[92] Riddick, J. A. & William, B. *Organic solvents: physical properties and methods of purification.* **1986**.