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Photochemical synthesis and functional transformations of bicyclic vinyl aziridines

Dissertação para obtenção do Grau de Mestre em Química Bioorgânica

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Indicação sobre os direitos de cópia

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Resumo

Aziridinas são moléculas extremamente utilizadas em química orgânica e medicinal, compostas por um heterocíclo de 3 membros com um átomo de azoto, sendo frequentemente usadas como versáteis percursores na síntese de compostos naturais. Além disso, muitas moléculas com actividade biológica possuem na sua estrutura o grupo funcional aziridina.

A reactividade da aziridina tem sido bastante investigada, como por exemplo, em reacções de abertura de anel com tióis. No entanto, existem poucos estudos em reacções de abertura de aziridinas em meio aquoso, apesar das numerosas vantagens provenientes da utilização de água como solvente em química orgânica.

Neste trabalho pretendeu-se estudar a reacção de abertura de aziridinas bicíclicas em meio aquoso na presença de nucleófilos de enxofre, azoto, carbono e oxigénio. As aziridinas foram preparadas por transformação fotoquímica de diversos sais de piridínio, de acordo com a metodologia de Kaplan. Seguidamente, as suas reacções de abertura de anel por nucleófilos foram investigadas. Os estudos mostram que tióis, anilinas e azida são bons nucleófilos na reacção com a aziridina, originando os produtos de abertura de anel com rendimentos moderados a altos. Os melhores resultados foram obtidos com tióis, mais concretamente com os bionucleófilos investigados, como a cisteína e a glutationa. Resultados preliminares demonstram que ocorre reacção de modificação da calcitocina, um péptido com 2 resíduos de cisteína, com a aziridina, revelando o seu potencial para bioconjugação como ligando de proteínas com resíduos de cisteína, ou mesmo como inibidor enzimático de, por exemplo, protéases de cisteína.

Foram ainda investigados métodos para a separação de ambos os enantiómeros da aziridina bicíclica de Kaplan, tirando partido de uma metodologia enzimática para a resolução de álcoois secundários racémicos. Estes enantiómeros são valiosos precursores para a síntese de moléculas enantiomericamente puras, não existindo nenhum método descrito para a sua separação.

Palavras-chave: Aziridinas, abertura de anel, condições fisiológicas, cisteína, resolução enantiomérica.

Abstract

Aziridines, a class of organic compounds containing a three membered heterocycle with a nitrogen atom, are extremely valuable molecules in organic and medicinal chemistry. They are frequently used as versatile precursors in the synthesis of natural products, and many biologically active molecules possess the aziridine moiety.

The reactivity of aziridines has been studied, for example, in ring-opening reactions with thiols. However, not much interest seems to be given to reactions of aziridines in aqueous media, despite the numberless advantages of using water as solvent in organic chemistry.

The nucleophilic ring-opening reaction of aziridines in aqueous media was here explored. Following the Kaplan aziridine synthetic methodology, in which pyridinium salts undergo a photochemical transformation to give bicyclic vinyl aziridines, new aziridines were synthetized. Their nucleophilic ring-opening reaction in water under physiological conditions was investigated and a range of sulphur, nitrogen, carbon and oxygen nucleophiles tested. Thiols, anilines and azide proved to be good nucleophiles to react with the aziridines, giving the ring-opening product in moderate to good yields. The best results were obtained with thiols, more specifically with cysteine-derived nucleophiles. Preliminary results show that these bicyclic vinyl aziridines can modify calcitonin, a peptide containing two cysteine amino acids residues, grating them the potential to be used in bioconjugation as ligands to cysteine-containing proteins, or even as enzyme inhibitors of, for example, cysteine proteases.

Additionally, exploratory investigations suggest that the separation of both enantiomers of the bicyclic vinyl aziridine can be performed by taking advantage of an enzymatic methodology for the resolution of racemic secondary alcohols. Both enantiomers would be highly valuable as precursors in the synthesis of enantiomerically pure molecules, as no other method is currently reported for their separation.

Keywords: Aziridine, ring-opening, physiological conditions, cysteine, enantiomeric resolution.

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Abbreviations

BOC- *tert*-Butyloxycarbonyl

CAL B- *Candida antarctica* lipase B

COSY- Correlation spectroscopy

DDBAB- Dodecyldimethylbezylammoium bromide

ee- Enantiomeric excess

EEACE- Electric eel acetylcholinesterase

GC- Gas chromatography

HPLC- High-performance liquid chromatography

HRMS- High resolution mass spectroscopy

HSQC- Heteronuclear single quantum coherence

MEM- 2-Methoxyethoxymethyl

MTBE- Methyl *tert*-butyl ether

NMR- Nuclear magnetic resonance

sCT- Calcitonin Acetyl salmon

TCEP- Tris(2-carboxyethyl)phosphine

TLC- Thin layer chromatography

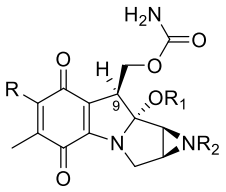
TMS- Trimethylsilyl

VOCs- Volatile organic compounds

1. Introduction

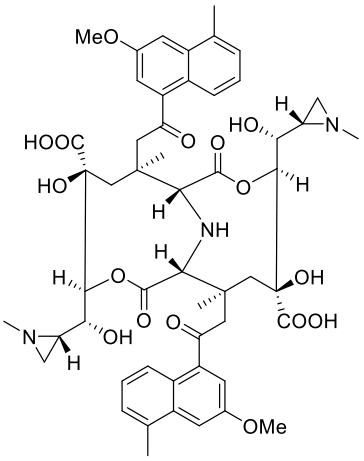
1.1. Aziridines

Aziridines are a class of organic compounds that contain a three membered heterocycle with a nitrogen atom. There are many molecules containing the aziridine moiety that have biological interest and can be used in a medicinal approach (Figure 1.1). Such compounds include mitomycins, that are potent antibiotics belonging to the family of antitumour quinones, first isolated from *Streptomyces caespitosus* in 1956[1] and clinically used for their activity against tumours; Carzinophilin, a carboxylic antibiotic active against Gram-positive bacteria and tumour cells, isolated from *Streptomyces sahachiroi* in 1954[2], and many others [3].



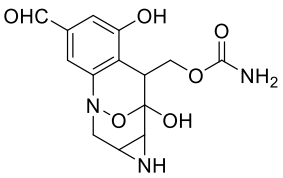
Mitomycin C: R = NH_2, R_1 = Me, R_2 = H
Porfiromycin: R = NH_2 R_1, R_2 = Me
Mitomycin A: R = OMe, R_1, R_2 = H
Mitomycin F: R = OMe, R_1, R_2 = Me
9a-Demethylmitomycin A: R = OMe, R_1, R_2 = H
9-epi mitomycin B: R = OMe, R_1 = H, R_2 = Me

Antibiotics from the family of anti-tumor quinones



Carzinophillin A

Carboxilic acid antibiotic, active against Gram+ bateria and tumor cells



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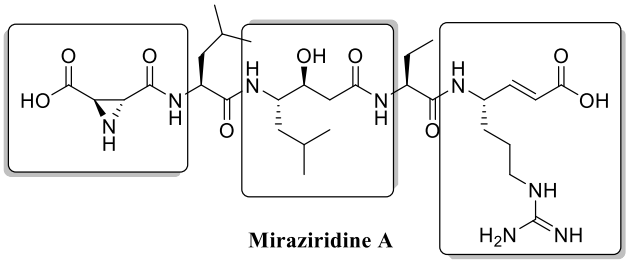
Mytomicin-like antitumor antibiotic

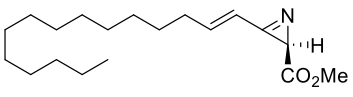


Miraziridine A

Inhibition of papain-like cystein proteases

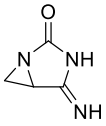
Inhibition of pepsin-like aspartyl proteases

Inhibition of trypsin-like serine proteases



R-Dyzidazirine

Cytotoxic azacyclopropene

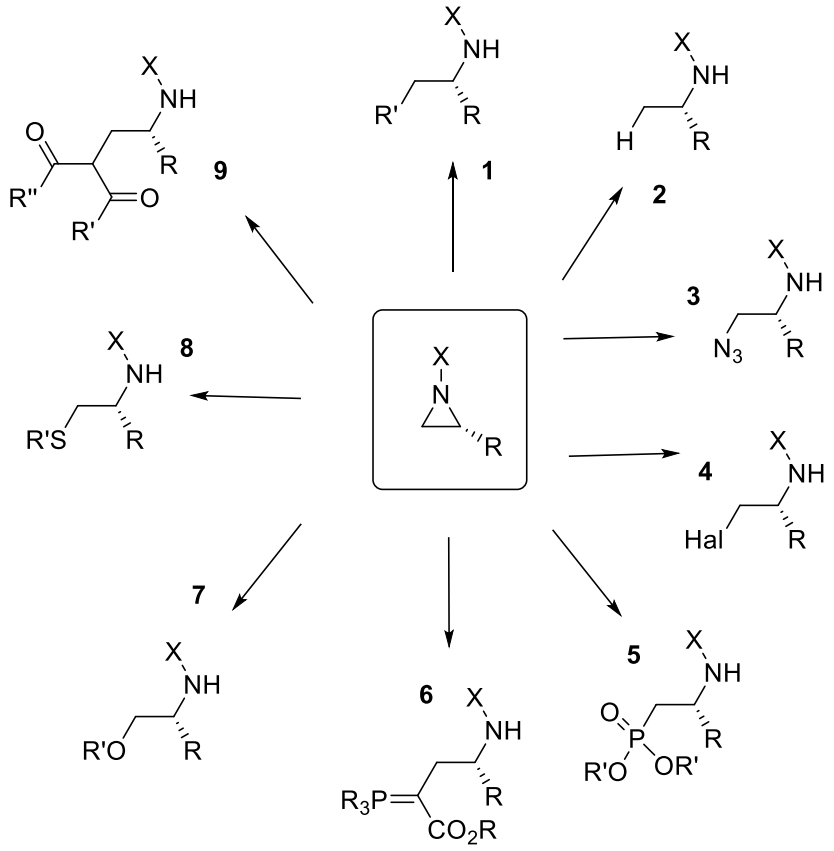


Imexon

B-lymphocyte activation surpressor

Figure 1.1: Natural and unnatural relevant aziridine–containing compounds[3a].

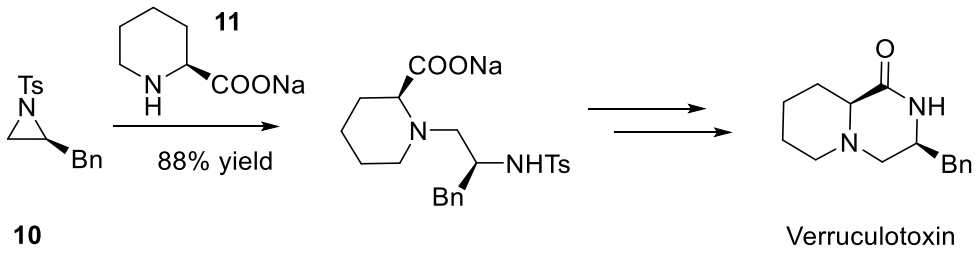
Aziridines are also extremely useful intermediates in organic and medicinal chemistry due to the high reactivity given by their high ring strain, result of the angle strain of the trigonal ring (≈60°), much smaller than the usual tetrahedral angle of 109.5º. This ring tension allows aziridine to undergo a large number of reactions, such as nucleophilic ring opening (Scheme 1.1)[4].



Scheme 1.1: Ring-opening reactions of aziridine with different nucleophiles[4].

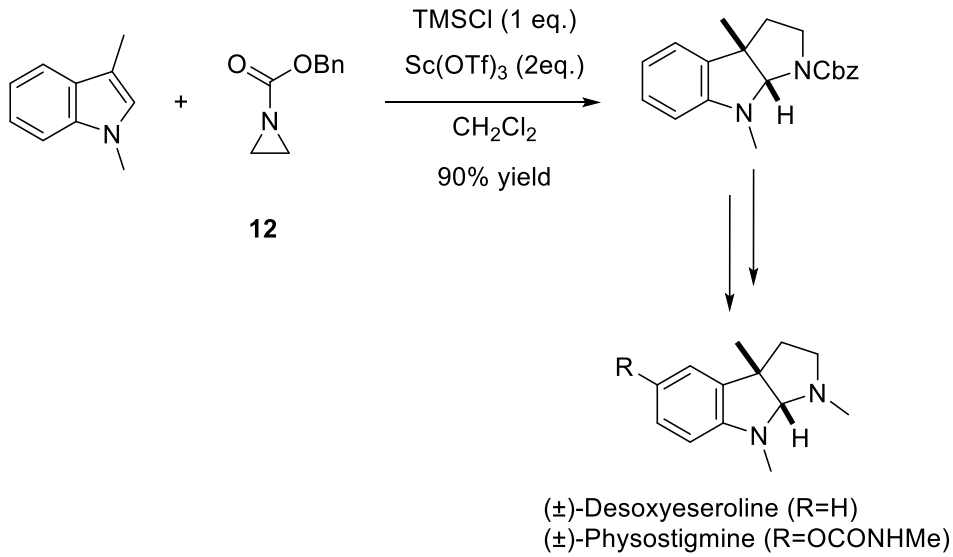
The nucleophilic ring-opening reaction of aziridines can support a large scope of nucleophiles, including organometallic compounds and hydrides (**1** and **2** from Scheme 1.1, respectively), strong nucleophiles, thiols (**8** from Scheme 1.1), and even weak alcohol nucleophiles (**7** from Scheme 1.1). Azide, halides and dicarbonyl compounds also open the aziridine group (**3**, **4** and **9** from Scheme 1.1, respectively), along with many other nucleophiles, making this reaction incredibly versatile.

In addition, aziridines can undergo cycloadditions, rearrangements and isomerizations[5], proving to be extremely important precursors for the synthesis of relevant *N*-containing molecules[3b]. For example, aziridines are used in the synthesis of Verruculotoxin, a highly toxin mycotoxin, in which one important step is the nucleophilic ring-opening of aziridine **10** by the cyclic amine **11**, in 80% yield (Scheme 1.2)[6].



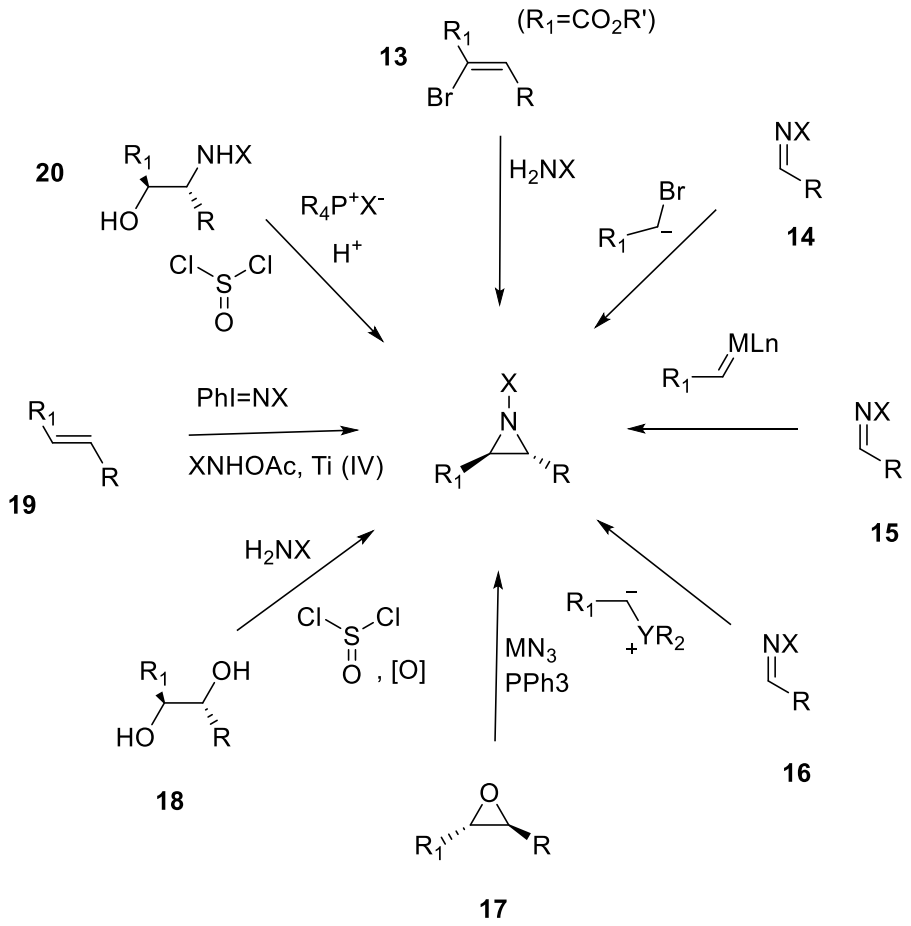
Scheme 1.2: Synthesis of natural product verruculotoxin, employing an aziridine precursor[6].

Also, desoxyeseroline, a precursor to the insecticidal alkaloid Physostigmine, can be synthesized by ring-opening of the aziridine **12** with 1,3-dimethylindole, followed by intramolecular nucleophilic attack by the nitrogen to the resulting indolenium, in 90% yield [7] (Scheme 1.3). Many more examples are present in the literature[3b].



Scheme 1.3: Synthesis of alkaloids Desoxyeseroline and Physostigmine, employing an aziridine precursor[7].

Because of their great versatility as precursors and biological utility as therapeutic agents, there has been a large interest in the scientific community to develop efficient synthetic methodologies to obtain aziridines. Many methods have been developed to synthetize aziridines[4], utilising simple alkenes (**19** from Scheme 1.4), imines (**14**, **15** and **16** from Scheme 1.4), epoxides (**17** from Scheme 1.4) and other molecules as starting materials. To perform the work of this master thesis, the aziridines were synthesised from the Kaplan methodology[8], that employs pyridinium salts as precursors to bicyclic vinyl aziridines. This methodology will be discussed in more detail in chapter 1.4.



Scheme 1.4: Different synthetic approaches to the formation of aziridines[4].

1.2. Aziridines and reactions in water

The previous chapter showed the value of aziridines in chemistry, either as versatile reactive compounds or bioactive molecules. For these reasons, aziridines are widely used in organic chemistry, and have been extensively studied over the years. However, reactions of aziridines in water have not been fully explored, despite the obvious advantages of using water as solvent in organic chemistry, as it is a cheaper, safer, cleaner and overall more environmental friendly molecule, compared to commonly used organic solvents. All of these solvents should be avoided when possible, as they are usually volatile organic compounds (VOCs), which contribute to the greenhouse effect and catalyse the destruction of the ozone layer, increasing global warming, in addition of being toxic to animals and plants[9]. Having a "green chemistry" philosophy in mind, we should always try to perform reactions as ecologically clean as possible, including replacing organic solvents with water when the reaction permits.

To the best of our knowledge there are only a few studies on aqueous nucleophilic ring-opening reactions of aziridines: in hot water[10] (Table 1.1); promoted by tributylphosphine[11]; in a silica-water system[12] and catalysed by cationic-micelle at room temperature[13] (Table 1.2).

Table 1.1: Ring-opening of aziridine with thiophenol in water at 60ºC[10].

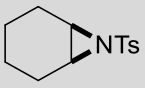
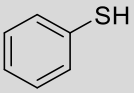
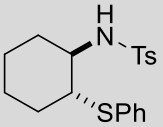
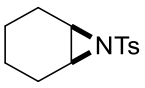
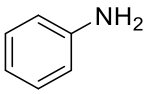
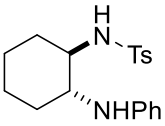
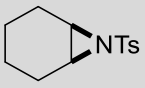
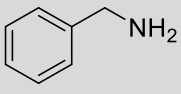
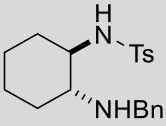
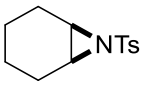
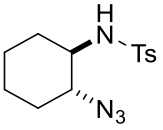
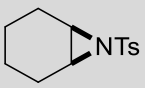
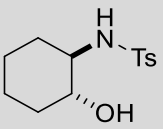
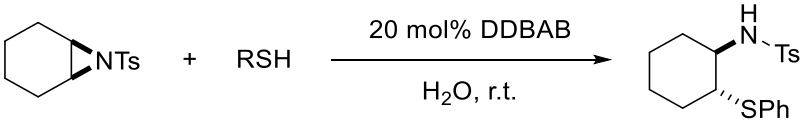
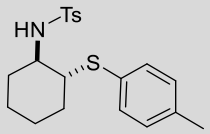
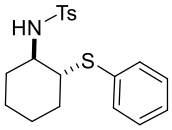
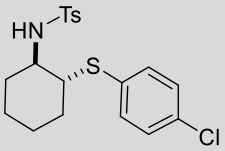
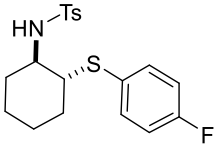
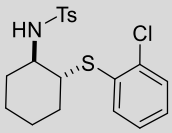
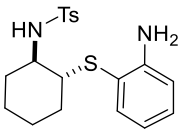
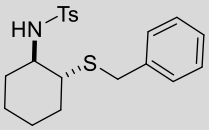
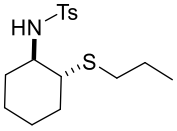
Substrate	Nucleophile	Product	Time (h)	Yield (%)
			72	73
			5	98
			5	99
	NaN_3		5	99
	H_2O		7	99

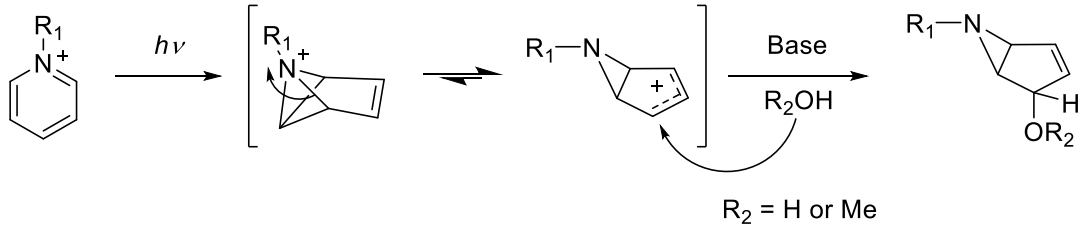
Table 1.2: Micelle catalysed ring-opening reactions of aziridine with various thiols in water with 20 mol% DDBAB (dodecyldimethylbezylammoium bromide).



Product	Time (h)	Yield (%)
	0.5	93
	0.5	97
	1	98
	1	86
	2.5	93
	3.5	80
	24	61
	24	0

1.3. Bicyclic vinyl aziridines from photohydration of pyridinium salts

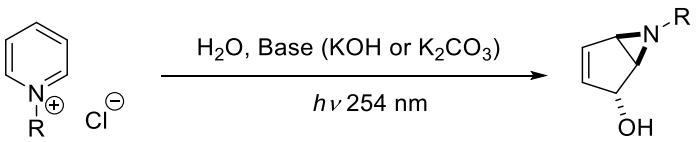
In 1972, Kaplan discovered a simple and useful procedure to synthesise valuable bicyclic vinyl aziridines via photochemical hydration of the pyridinium ion[8] (Scheme 1.5).



Scheme 1.5: Photochemical transformation of pyridinium salt[8].

From cheap and easily synthetized pyridinium salts, highly valuable aziridines can be obtained. The incidence of UV radiation onto the pyridinium salt leads to the tetra coordinated nitrogen cation intermediate, which rapidly rearranges to the aziridine group and the stabilized allylic carbocation. This carbocation is attacked by the nucleophilic solvent providing the bicyclic vinyl aziridine after deprotonation with a base (Scheme 1.5). A very large number of pyridinium salts can be used, as the scope of this reaction proved to be vast. It is though, limited to water and methanol as solvent nucleophiles, and to electro rich R_1 groups, eliminating the possibility of getting Boc, Acetyl, and Tosyl protected aziridines. Protecting groups with aromatic units like benzyl also can't be used as they absorb *hv* radiation that would otherwise be absorbed by the pyridinium moiety, resulting in low yields. Several different bicycle vinyl aziridines have been synthetized by this method (Table 1.3 and Table 1.4) and thoroughly investigated by Burger[14] and Mariano[15].

Table 1.3: Pyridinium salts photochemical reaction [8 14b].



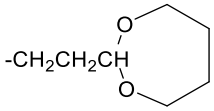
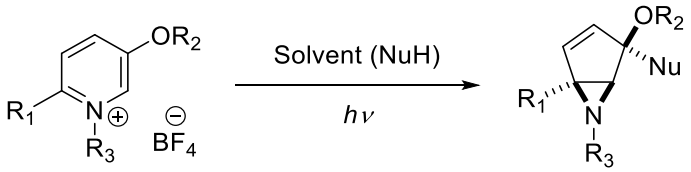
R	Yields (%)
$-CH_3$	-
$-(CH_2)_3OH$	82
$-CH_2CH_2OMe$	57
	55
$-CH_2OCH_2CH_2OCH_3$	60
$-(CH_2)_2COOK$	40

Table 1.4: Photochemical transformation of substituted pyridinium salts[16].



R_1	OR_2	R_3	NuH	Yield (%)
H	OMe	Me	MeOH	6
Me	OMe	Me	MeOH	41
Et	OMe	Me	MeOH	77
^{n}Pr	OMe	Me	MeOH	70
Me	OMe	Et	MeOH	65
Et	OMe	Et	MeOH	92
H	OMe	Et	^{n}PrOH	0
H	O ^{n}Pr	Et	MeOH	50
Me	OMe	Et	^{n}PrOH	42
Me	O ^{n}Pr	Et	MeOH	81

These molecules proved to be very versatile and useful in many synthetic approaches such as the synthesis of important carbocycles inclunding (+)-mannostatin, an α-mannosidade inhibitor, (-)-

swainsonine, a potent inhibitor of Golgi α-mannosidase II, (-)-cephalotaxine, a precursor to molecules with potential antileukemic activity, and others (Figure 1.2)[15].

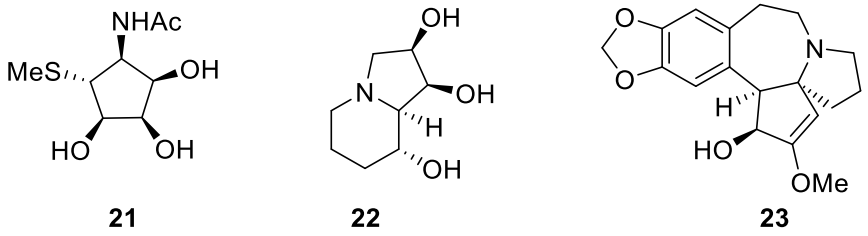
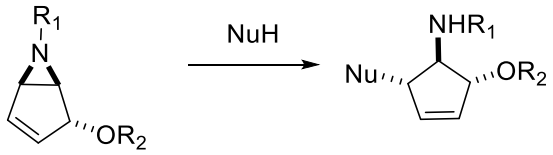
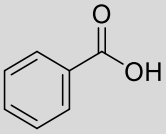
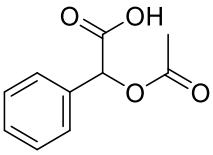
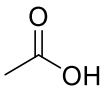


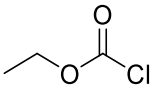
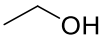
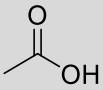
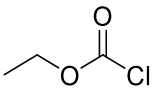
Figure 1.2: Carbocycles synthetized from Kaplan aziridine. Mariano[15]. 21: (+)-mannostatin; 22: (-)-swainsonine; 23: (-)-cephalotaxine.

Nucleophilic ring opening of these aziridines have also been investigated with several nucleophiles, mainly sulphur and oxygen nucleophiles, with satisfactory results (Table 1.5).

Table 1.5: Bicyclic vinyl aziridine ring-opening reactions with various nucleophiles to form trans-trans aminocyclopentenes.



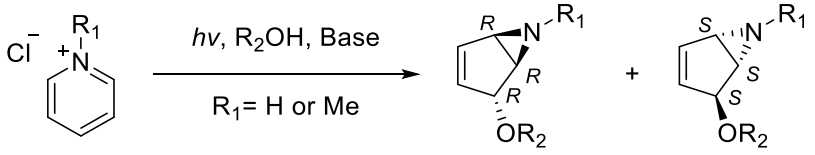
R_1	R_2	NuH	Conditions	Yield (%)
$(CH_2)_3OH$	H		$CHCl_3$, r.t.	92 [14b]
$(CH_2)_3OH$	H		$CHCl_3$, r.t.	87 [14b]
$(CH_2)_3OCbz$	PhCO	—SH	$BF_3{\cdot}OEt_2$, CH_2Cl_2, -48ºC	82 [17]
Pr	Me	—OH	HClO4, r.t.	42 [17]
Pr	Me	H_2O	HClO4, r.t	81 [17]
Pr	Me		HClO4, r.t	36 [17]

Pr	Me		$HClO_4$, r.t	74 [a] [17]
Pr	Me		$CHCl_3$, r.t.	90 [b] [17]
Pr	H		r.t.	67 [17]
Pr	H		r.t.	34 [17]
CH_2CONH_2	Me		$CHCl_3$, r.t.	93 [17]
CH_2CONH_2	Me		$HClO_4$, r.t	79 [17]
CH_2CONH_2	Me		MeCN, reflux	54 [17]
CH_2CONH_2	Me		$CHCl_3$, r.t.	18 [b, c] [17]
$(CH_2)_2OH$	Me		r.t.	99 [17]
$(CH_2)_2OH$	H		$HClO_4$, r.t	99 [17]

[a] Of the *N*-acylated derivative. [b] Of the *N*-ethoxycarbonyl derivative, Nu = Cl^-. [c] 40% of a six-membered azalactone also isolated.

1.4. Enantiomeric resolution of secondary alcohols

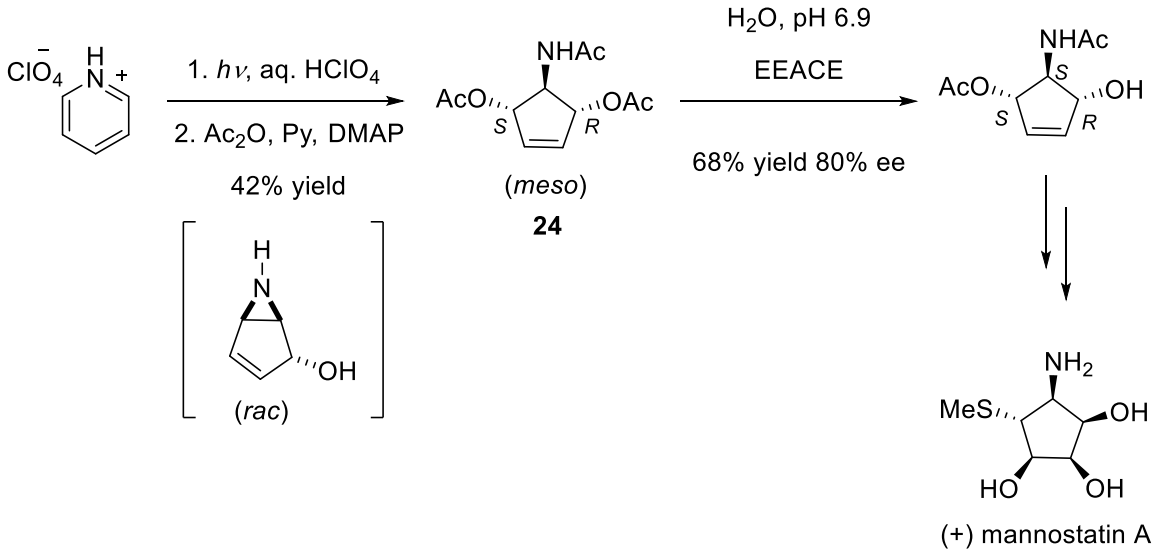
As shown in chapter 1.3, the aziridine obtained from the Kaplan methodology[8] can be very useful to synthetize natural products. However, this aziridine is formed as a racemic mixture of 2 enantiomers (Scheme 1.6).



Scheme 1.6: Synthesis of racemic vinyl bicyclic aziridines from photohydration of pyridinium salt.

In order to synthetize an enantiomeric pure molecule with this aziridine precursor, its two enantiomers would have to be separated via expensive separation methods like chiral HPLC. Alternatively, in a later stage in the synthetic methodology, the two resulting enantiomers or diastereoisomers (if a new chiral centre is added to the molecule) could be separated, which again may be expensive and complicated, and even if successful, 50% of the product (the undesired enantiomer or diastereoisomer) would go to waste, leading to a huge waste of material.

In the synthesis of (+)-mannostatin, Mariano[18] developed a very interesting solution to this problem. From the racemic bicyclic vinyl aziridine, they formed an achiral *meso* compound **24** (Scheme 1.7) by opening the aziridine in acidic aqueous conditions and acetylating both resulting alcohols and amine.

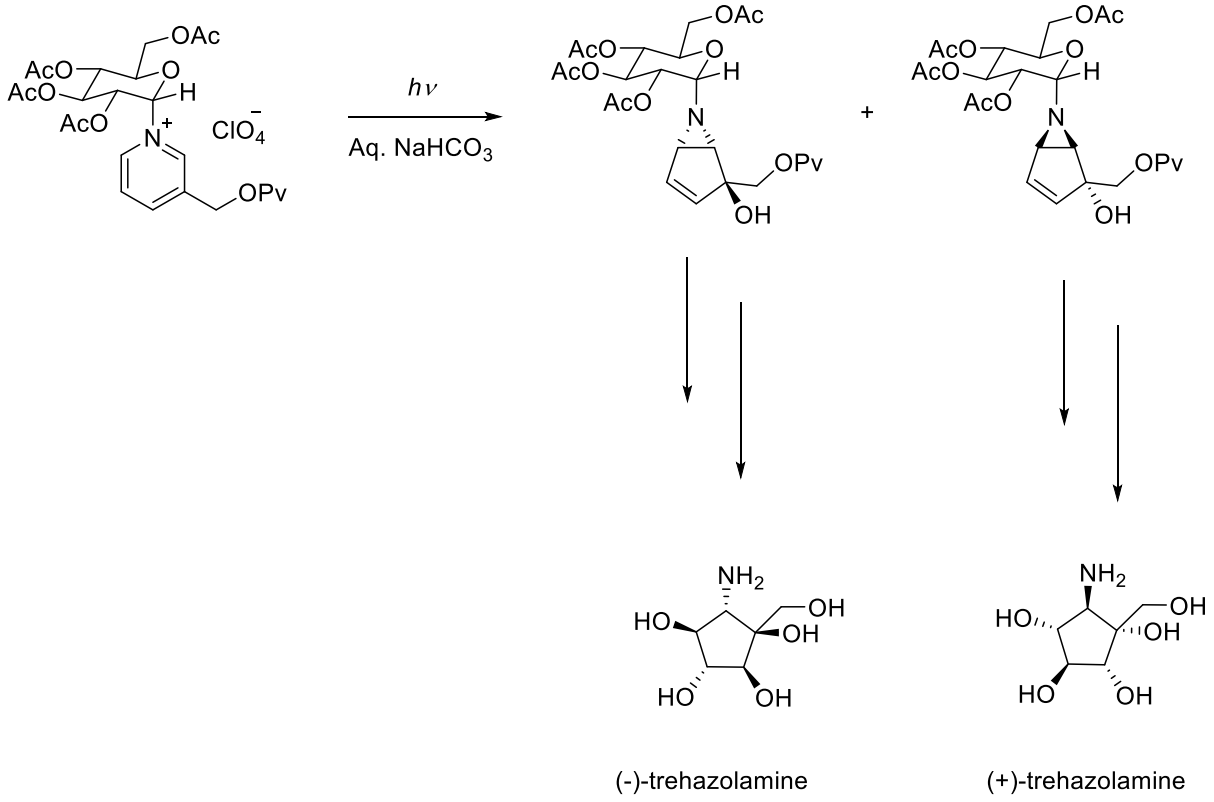


Scheme 1.7: Synthesis of enantiomerically pure (+) mannostatin A by Mariano, utilizing an acetylcholinesterase enzyme[18].

This *meso* molecule **24** was selectively hydrolysed through an electric eel acetylcholinesterase (EEACE) known to convert substrates similar to the amido-diacetate to the nonracemic acetoxy-alcohols. Indeed the enzyme selectively hydrolysed the *R* acetate centre, providing the chiral alcohol molecule in 80% enantiomeric excess, which was then converted to (+)-mannostatin A.

Despite being a very elegant strategy, it is limited to this particular case, using this precise substrate, as the enzyme is specific to these structures. Also, the stereochemical course of the enzymatic reaction is very unpredictable, and it is not always clear what stereogenic centre will react. For these reasons, this methodology can't be used in the synthesis of many other natural products.

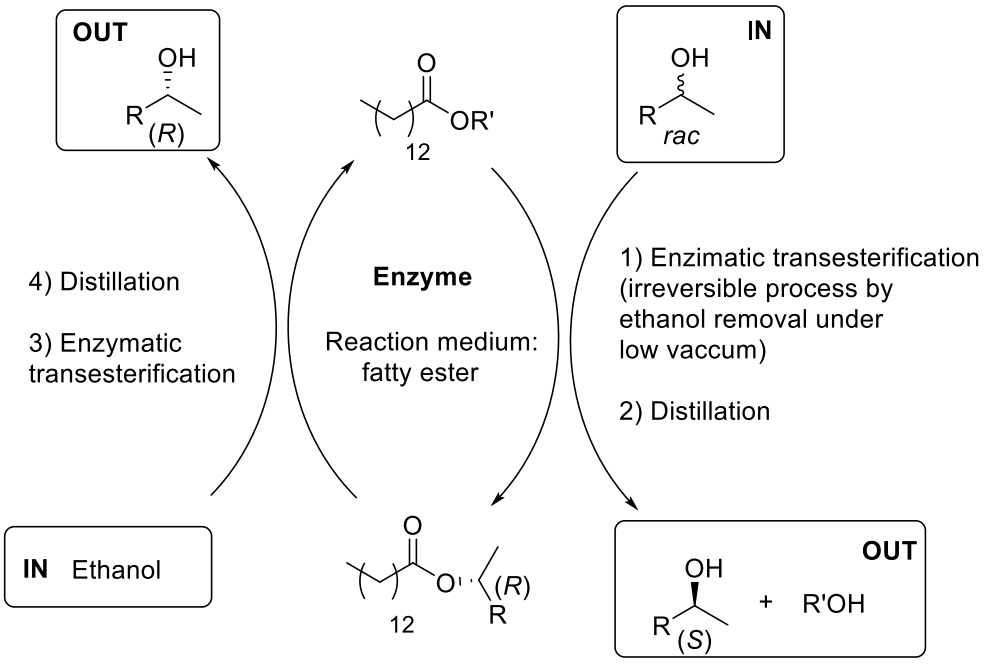
In fact, for the synthesis of (+)-trehazolamine, a potent trehelaze inhibitor, Mariano used a different approach (Scheme 1.8)[19]. The introduction of the *N*-glucosyl chiral auxiliary in the pyridinium ion provided the respective aziridine in a diasteoisomeric mixture (plus some undesired regioisomers). These diastereoisomers could easily be separated in a later stage by simple silica gel chromatography, followed by removal of the auxiliary chiral group. The group managed to obtain both enantiomers of trehazolamine, but since only the (+) enantiomer is desired and actually relevant and biological active, 50% of the reagents were wasted to synthetize the undesired (-) enantiomer.



Scheme 1.8: Synthesis of both enantiomers of trehazolamine by Mariano using a chiral auxiliar[19].

These problems could be avoided if there was an easy methodology to separate the enantiomers of the bicyclic vinyl aziridine. Both isolated isomers would have an incredible value for the synthesis of relevant enantiomerically pure natural products.

An in house methodology for the enzymatic resolution of racemic secondary alcohols[20] showed that using the lipase CAL B as a selective catalyst, it is possible to isolate both alcohol enantiomers with high *ees* (Scheme 1.9).

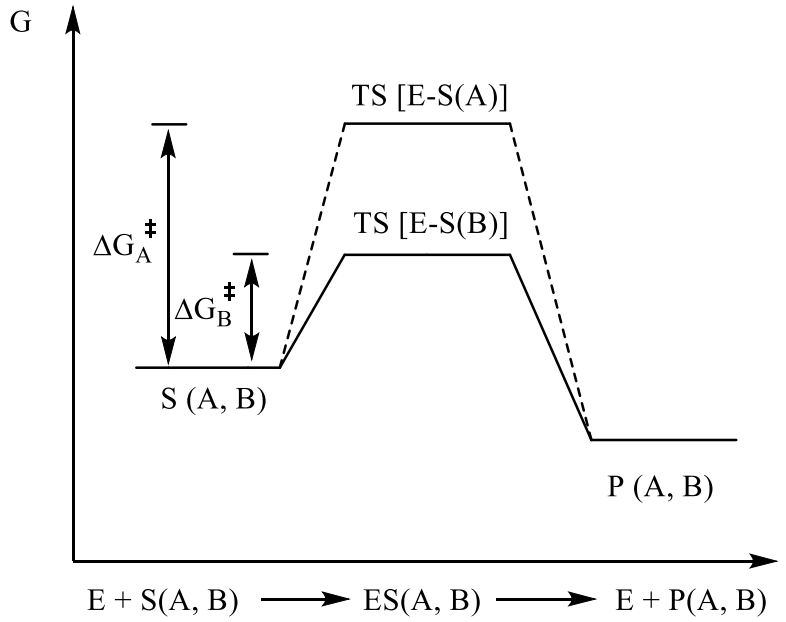


Scheme 1.9: One-pot resolution of sec-alcohols, based in the usage of fatty esters as solvent and acylating agent[20].

In *Monteiro et al.* experiment[20] (Scheme 1.9), a racemic mixture of a secondary alcohol is mixed with an acylation agent (a long chain fatty acid-derived ester) that also serves as solvent and CAL B, a lipase. This enzyme selectively catalyses the transesterification of the fatty acid ester with the *R* alcohol, while the *S* alcohol remains unchanged and is isolated by distillation. The resulting *R* ester is again transesterificated, regenerating the initial fatty acid ester and forming the *R* alcohol that is also isolated by distillation. In this simple cycle, where the acylation agent is reformed and the enzyme reused, is possible to isolate both alcohol enantiomers in good *ees* that can go as high as 98%.

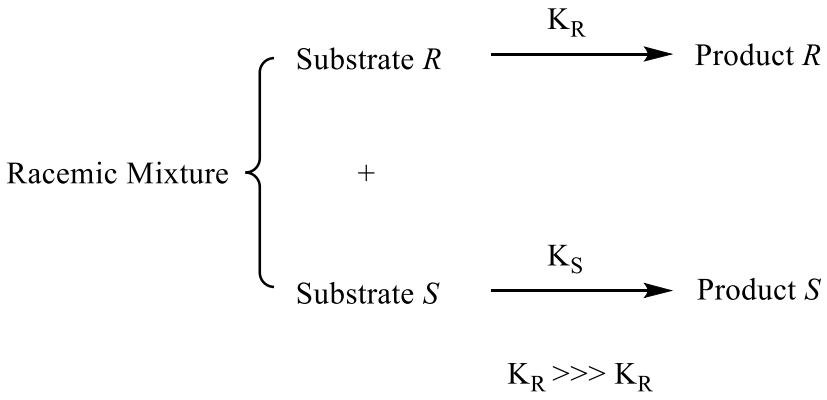
This kinetic resolution derives from the different reaction rates of each enantiomer, in a reaction catalysed by a chiral enzyme catalyst. This is due to their different activation energies. Being enantiomers, they both have the same free Gibbs energy, but the energy of their stabilized Enzyme-Substrate transition state is different. As the enzyme is enantiomerically pure, two diastereoisomers

are formed in the transition state and one of them is more stable than the other. This results in a lower activation energy and subsequent higher reaction rate of one enantiomer, allowing the resolution of the enantiomers (Scheme 1.10).



Scheme 1.10: Reaction energy profile for the enzymatic kinetic resolution of enantiomers A and B. E represents the enzyme, S(A, B) the substrates and P(A, B) the products. TS [E-S(B)] and TS [E-S(B)] represent the transition states of the enantiomers coordinated with the enzyme.

In these reactions, the enantiomeric excess increases as the reaction progresses and the most reactive enantiomer is converted, reaching a maximum. It then starts to decrease as the concentration of the most reactive enantiomer concentration decreases, and the lower rate of conversion of the less reactive enantiomer is no longer negligible. It is vital to isolate both reagent and product when the optimal enantiomeric excess and ratio of unreacted reagent and product is reached. Theoretically, full conversion of one enantiomer occurs while the other one stays unchanged, providing a maximum reaction yield of 50% and 100% *ee* of both product and reagent. However, the catalyst is not specific to one enantiomer but rather selective, consuming one enantiomer at a much faster rate than the other (Scheme 1.11) and eventually consuming both if the reaction time is not controlled.



Scheme 1.11: Reaction rate differences in kinetic resolution.

In order to evaluate the process two important parameters are need: the enantiomeric excess of both products and reagents (Equation 1.1), and the conversion (Equation 1.2).

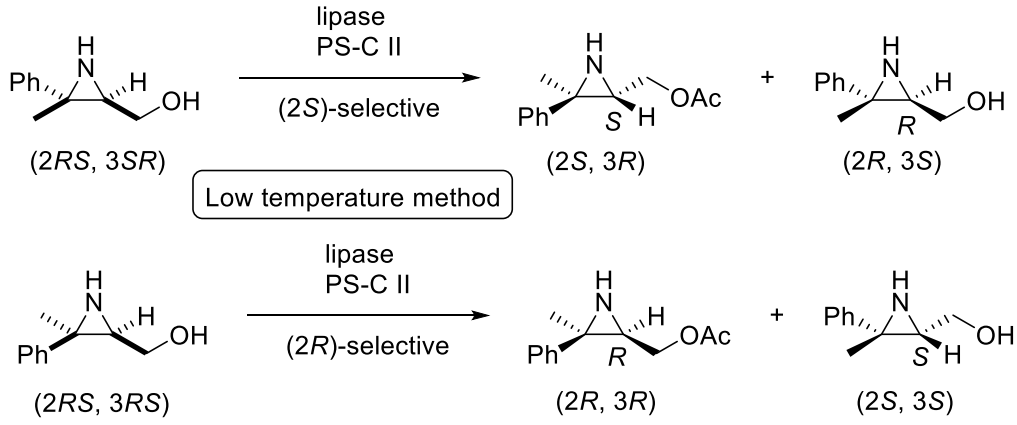
$$\%ee = \frac{[R] - [S]}{[R] + [S]} \times 100$$

Equation 1.1: Formula for enantiomeric excess (*ee*). [*R*] is the concentration of enantiomer *R* and [*S*] is the concentration of enantiomer *S*.

$$\%\mathrm{c} = \frac{ee_{\mathrm{R}}}{ee_{\mathrm{P}}} \times 100$$

Equation 1.2: Formula for the conversion (c). ee_{R} is the enantiomeric excess of the reagent and ee_{S} the enantiomeric excess of the product.

A similar methodology as the one in Scheme 1.9 was developed by Ema *et al.* for the separation of racemic aziridines that contain a primary alcohol group (Scheme 1.12)[21].



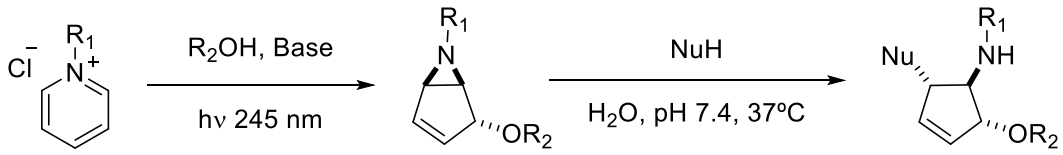
Scheme 1.12: Lipase-catalyzed resolution of (2*RS*,3*SR*)-3-methyl-3-phenyl-2-aziridinemethanol and (2*RS*,3*RS*)-3-methyl-3-phenyl-2-aziridinemethanol.

The authors also utilized an enzyme (lipase) to selectively acetylate the primary alcohol of one aziridine enantiomer, which can then be separated from the unchanged alcohol. Although this alcohol is not chiral because it is a primary alcohol, it is vicinal to a stereogenic centre, which is enough to grant one of the enantiomers high selectivity to the lipase enzyme, as the authors obtain very good *ees*, above 90%, after optimization of conditions.

In this work, the enzymatic resolution of the aziridines obtained from the Kaplan methodology[8] was explored, since this bicyclic vinyl aziridine has a chiral secondary alcohol that can be stereoselectively modified. This approach can lead to the synthesis of enantiomerically pure compounds.

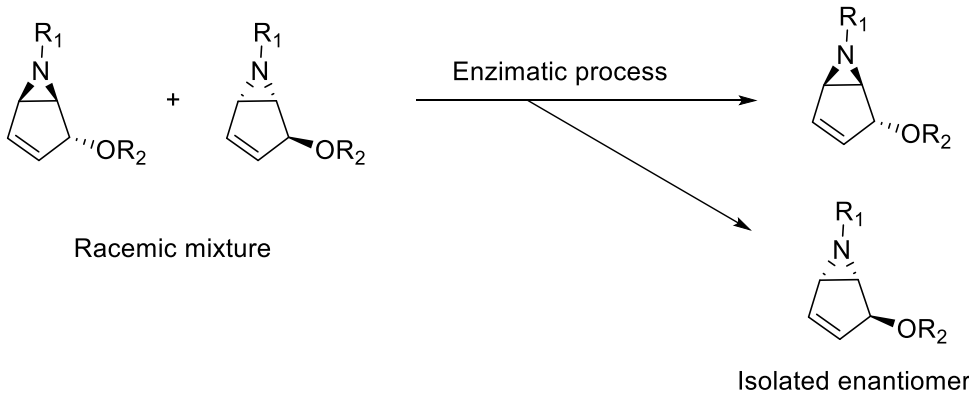
2. Objectives

In this work we synthesised new bicyclic vinyl aziridines from the Kaplan methodology[8] in order to study the scope of the nucleophilic ring-opening reaction of this aziridines with a range of nucleophiles (Scheme 2.1). These reactions were performed in aqueous phosphate buffer at pH 7.4 at 37ºC with the objective of understanding how these molecules would react in physiological media in a medicinal chemistry approach. These results eventually opened the way to study the bioconjugation of these molecules under physiological conditions.



Scheme 2.1: Synthesis of bicyclic aziridines from pyridinium salts.

Utilizing the methodology of enzymatic enantiomeric resolution of secondary alcohols discussed in chapter 1.4 we planned to separate both enantiomers of the bicyclic vinyl aziridine, which would be highly valuable as precursors to enantiomerically pure natural products.

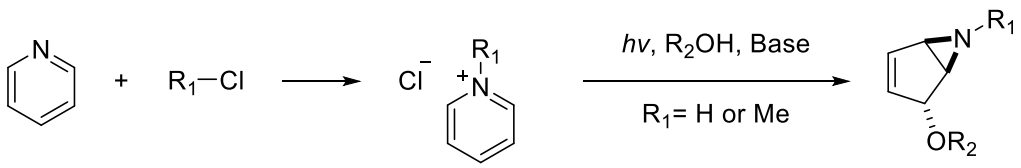


Scheme 2.2: Separation and isolation of both enantiomers of bicyclic vinyl aziridines.

3. Results and discussion

3.1. Aziridine synthesis

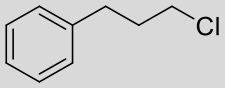
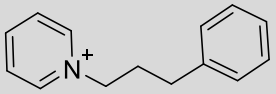
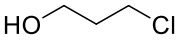
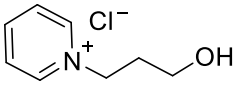
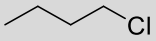
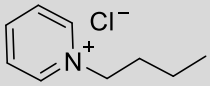
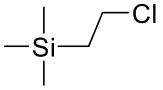
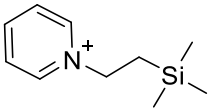
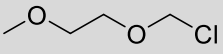
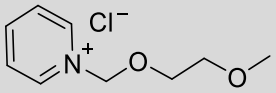
We synthetized bicyclic vinyl aziridines from the Kaplan methodology[8], following the general strategy presented in Scheme 3.1.



Scheme 3.1: Synthetic approach to the synthesis of vinyl bicyclic aziridines.

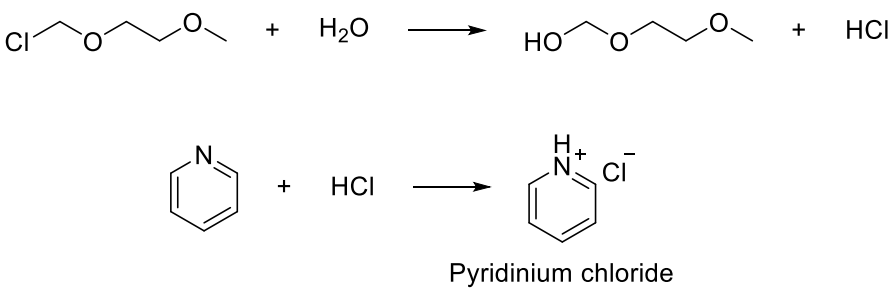
We prepared several pyridinium chloride salts by nucleophilic attack of pyridine to a range of alkyl chlorides (Table 3.1).

Table 3.1: Pyridinium salts synthetized or already available in the laboratory.

Entry	R_1-Cl	Yield (%)	Product
1		Quantitative	 **25**
2		Quantitative	 **26**
3		Quantitative	 **27**
4		NA	 **28**
5		33	 **29**

NA: compounds available in the laboratory.

These reactions were simply performed by addition of the pyridine (which also acted as solvent) to the alkyl chloride reagent, undergoing a S_N2 reaction. The products were easily obtained after 24 hours of reaction in quantitative yields (Entries 1 to 3 from Table 3.1), with no need of purification besides evaporation of excess pyridine in the rotary evaporator. The only exceptions were Entries 4 and 5 (Table 3.1) were there was a considerable amount of pyridinium chloride in the final product. A possible explanation for this is that some water could be present in the reaction media, maybe in the pyridine flask, as it was not freshly distilled, that attacked the alkyl chloride reagent forming the corresponding alcohol and HCl. Pyridine, as a base, would be easily protonated in the presence of such a strong acid (Scheme 3.2). This hypothesis is corroborated by the fact that when pyridine was added to MEM chloride (Entry 5 from Table 3.1), some gas was seen being formed, presumably HCl. This competitive reaction probably decreased the reaction yield, as the product **29** was isolated in 33% yield in Entry 5 (Table 3.1). Compound **28** was already available in laboratory, but contained the same pyridinium chloride impurity, that was removed. This pyridinium salt impurity was only found in reactions 4 and 5 (Table 3.1) probably because it utilised more reactive alkyl chlorides. Reagent (2-Chloroethyl)trimethylsilane (in Entry 4 from Table 3.1) is highly reactive because if the C-Cl bound is broken and chloride anion formed, a carbocation is left in the β position of the silicon atom, that is stabilized due to the β-silicon effect (a positive charge in a carbon in the β position to a silicon atom is stabilized by hyperconjugation). In reagent MEM chloride, the oxygen geminal to the chloride atom facilitates the breaking of the C-Cl bound by donating one of its two lone pair of electrons, forming the oxonium cation and the chloride anion. When in contact with water, despite it being a fairly weak nucleophile, the high reactivity of these two reagents allows the hydrolysis to occur, forming HCl).



Scheme 3.2: Consumption of MEM chloride by water in the reaction media, with subsequent formation of pyridinium salt.

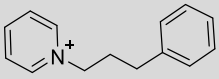
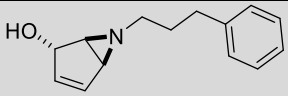
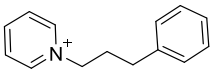
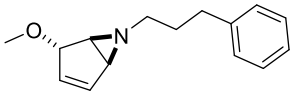
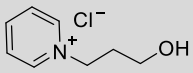
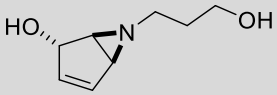
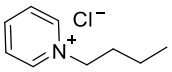
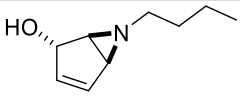
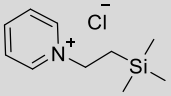
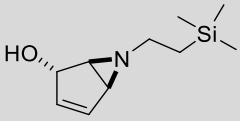
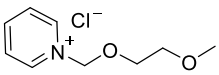
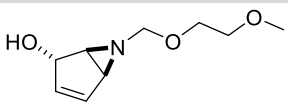
The pyridium chloride impurity could be easily separated from the products **28** and **29** by slowly adding an aqueous sodium bicarbonate solution until the pH of the products solution was about 8. The bicarbonate anion, as a weak base, deprotonated the pyridinium ion, forming pyridine and carbonic acid which decomposed in water and CO_2, as bubbles started to emerge from the solution. Pyridine was extracted with Et_2O and the aqueous phase evaporated. Acetonitrile was added to

dissolve the pyridinium salts **28** and **29** to give the final pure product, leaving the bicarbonate salts precipitated. Using carbonate as a base was also tried for both Entries but full degradation of the product occurred, as they were seemingly unstable in stronger basic conditions.

No reaction yields are presented for compound **28** as it was synthetized previously in the laboratory and information on the reaction was not found. This pyridinium salt was produced in large quantities and the reaction was not repeated.

Having synthetized the pyridinium salts, their photochemical transformation (Scheme 3.1) was studied and the results are presented in Table 3.2.

Table 3.2: Synthesis of bicyclic vinyl aziridines.

Entry	Pyridinium salt	Solvent	Product	Yield (%)	Reported yield (%)
1	 **25**	H_2O	 **30**	19	-
2	 **25**	MeOH	 **31**	10	-
3	 **26**	H_2O	 **32**	88	82[14b]
4	 **27**	H_2O	 **33**	65	-
5	 **28**	H_2O	 **34**	24	-
6	 **29**	H_2O	 **35**	23	60[14b]

The photochemical reactions were performed in a homemade reactor in the same scale as the Ryonet model and the bicyclic vinyl aziridines were obtained in low to excellent yields (Entries 3 and 4 in Table 3.2), one example even surpassing the yield obtained in the literature (Entry 3 from Table

3.2). Pure aziridines are simply obtained after evaporating the water and extraction with dichloromethane and diethyl ether, respectively. This reaction, though, does not seem to allow an aromatic ring on the pyridinium alkyl chain, as they provide low yields (Entries 1 and 2 in Table 3.2) and messy reactions. As the aromatic ring does absorb UV radiation around the same wavelength as the pyridinium moiety (around 255 nm) and the reactor lamps radiation wavelength (245 nm), it may compete with it for the radiation which would explain the low yields.

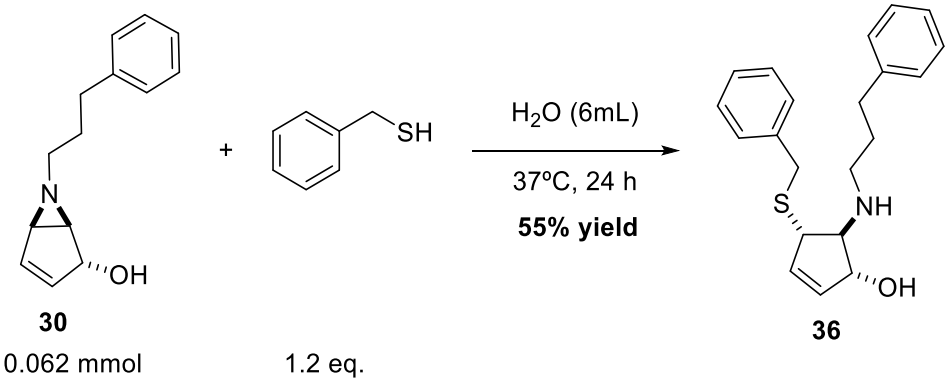
For Entries 5 and 6 (Table 3.2) bicarbonate was used as the base because it was observed that the pyridinium salts reagents were unstable in a potassium carbonate solution. Glarner also used this conditions for the same reaction as Entry 6[14b]. Unfortunately aziridines **34** and **35** were obtained in low yields, as they were the only *N*-protected aziridines that would be deprotected after ring-opening to give the primary amine, which would increase their value in a synthetic methodology.

Aziridines with the alcohol moiety (where water is used as nucleophile) were preferentially produced instead of aziridines with the methoxide group (where methanol is used as nucleophile), because the presence of a free hydroxyl group would allow extra functionalization of the molecule if needed.

Aziridines **32** and **33** will be used as model molecules for the ring-opening reaction of bicyclic vinyl aziridines as they were obtained in good yields.

3.2. Aziridine ring-opening with sulphur nucleophiles

To check the viability of aziridine ring-opening reactions with thiols in aqueous media, the reaction of aziridine **30** with benzyl mercaptan in water was performed (Scheme 3.3).



Scheme 3.3: Nucleophilic ring-opening of aziridine 30 with benzyl mercaptan, in water, at 37ºC.

The reaction worked surprisingly well since after SiO_2 column purification, the ring-opening product **36** was obtained in a moderate yield (55%). Comparing the results of Jin Qu *et al.* [10] (Entry 1 from Table 1.1), this result was very good considering that, although with a lower yield (reported yield was 73%), this reaction worked with a weaker nucleophile, a benzylic thiol, and the authors used a phenyl thiol (thiophenol) which is more acidic and nucleophilic. Also, it was used a much lower temperature (37ºC vs 60ºC) and a less reactive aziridine, as it is an electron-rich alkyl aziridine, that need to be either protonated or bound to a Lewis acid to open, in contrast to the reported activated sulfonyl aziridine, in which the forming negative charge on the nitrogen is stabilized by delocalization to the sulfonyl moiety.

Aziridine **32** was also left in water for 3 days to see if there was some degradation or other process that could explain the remaining 45% of aziridine molecules that did not provide the desired product. Figure 3.1 c) shows the formation of pyridinium salt after leaving the aziridine **32** in water, by the appearance of three signals at 8-9 ppm in c), corresponding to the aromatic hydrogens of pyridinium salt **26**. This reversion process may be catalysed by the slightly acidic distilled water (pH 5.6) and seems to be main cause of aziridine degradation (Scheme 3.4).

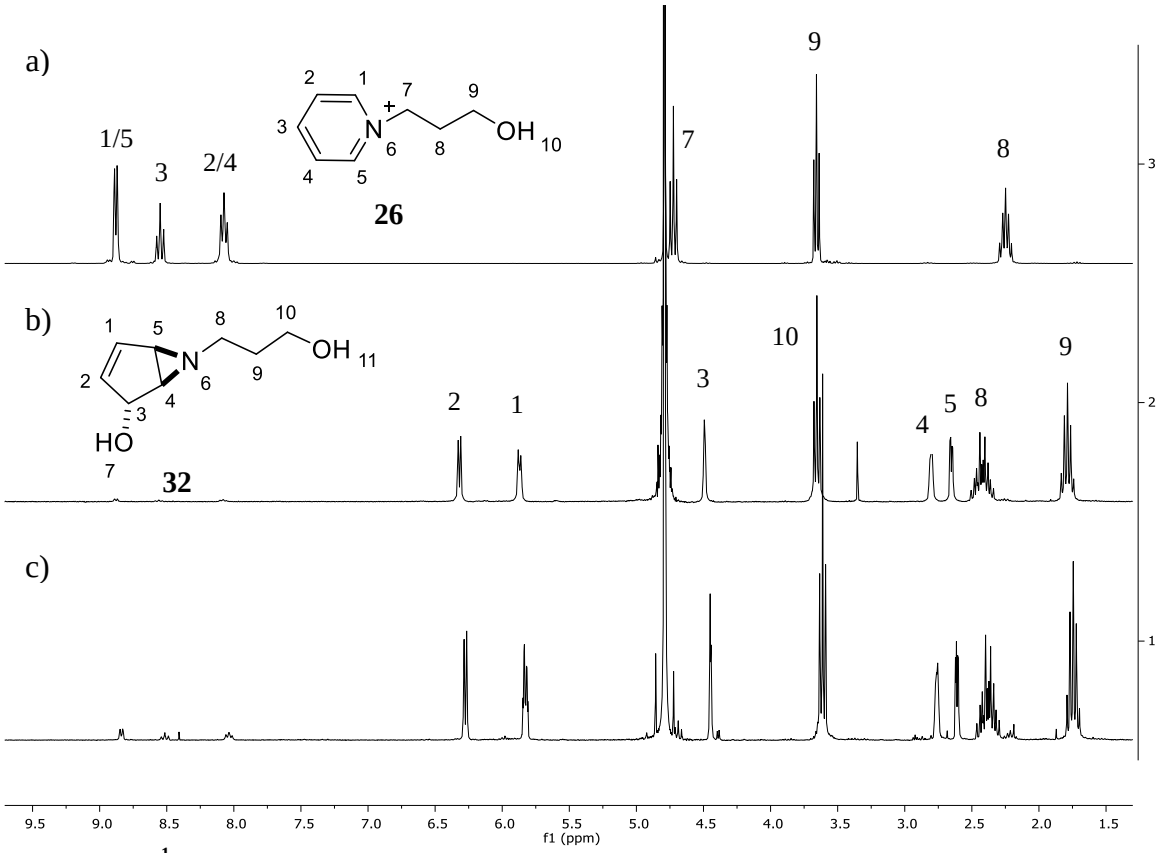
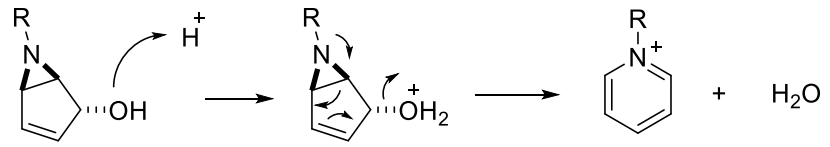
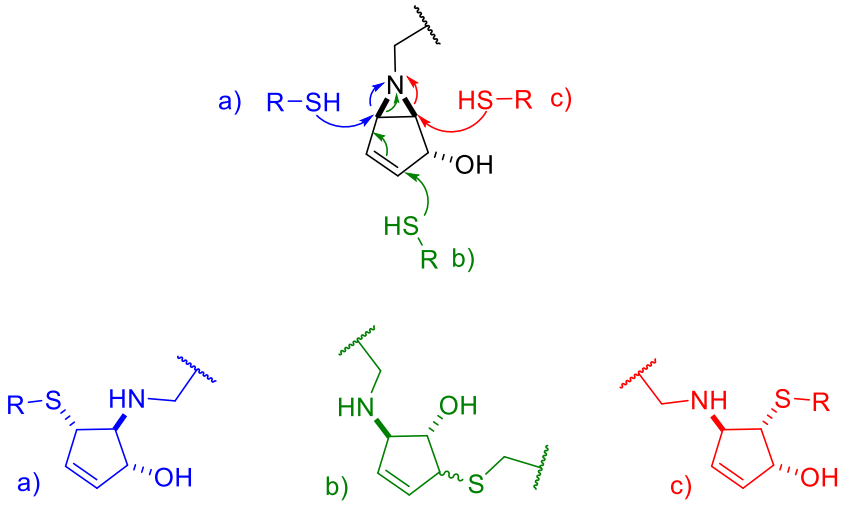


Figure 3.1: ^{1}H NMR spectra of a) pyridinium salt 26, b) aziridine 32 and c) aziridine 32 after 3 days in water (D_2O). Signal integration at 8-9 ppm indicates regression of 9% of the aziridine 32 to the pyridinium salt 26.



Scheme 3.4: Proposed mechanism for the bicyclic vinyl aziridine regression to pyridinium salt in acidic media.

This reaction could be much more complicated than presented in Scheme 3.3 because there are in fact three possible ways in which the nucleophile can attack this aziridine. The attack can happen on either of the two carbons of the aziridine moiety, and even on the double bound, with its migration to the aziridine carbon and opening of the 3-memebered ring (Scheme 3.5).



Scheme 3.5: Possible mechanisms for the nucleophilic attack on the bicyclic vinyl aziridine, with its respective products.

Previous studies from Burger and Mariano show that these reactions go via pathway a) (Table 1.5)[14b, 17], with attack of the nucleophile on the less sterically hindered carbon of the aziridine moiety. Nevertheless, to be sure our reaction followed the same pathway, we performed a 2D NMR experiment (COSY NMR) of product **36** (Figure 3.2).

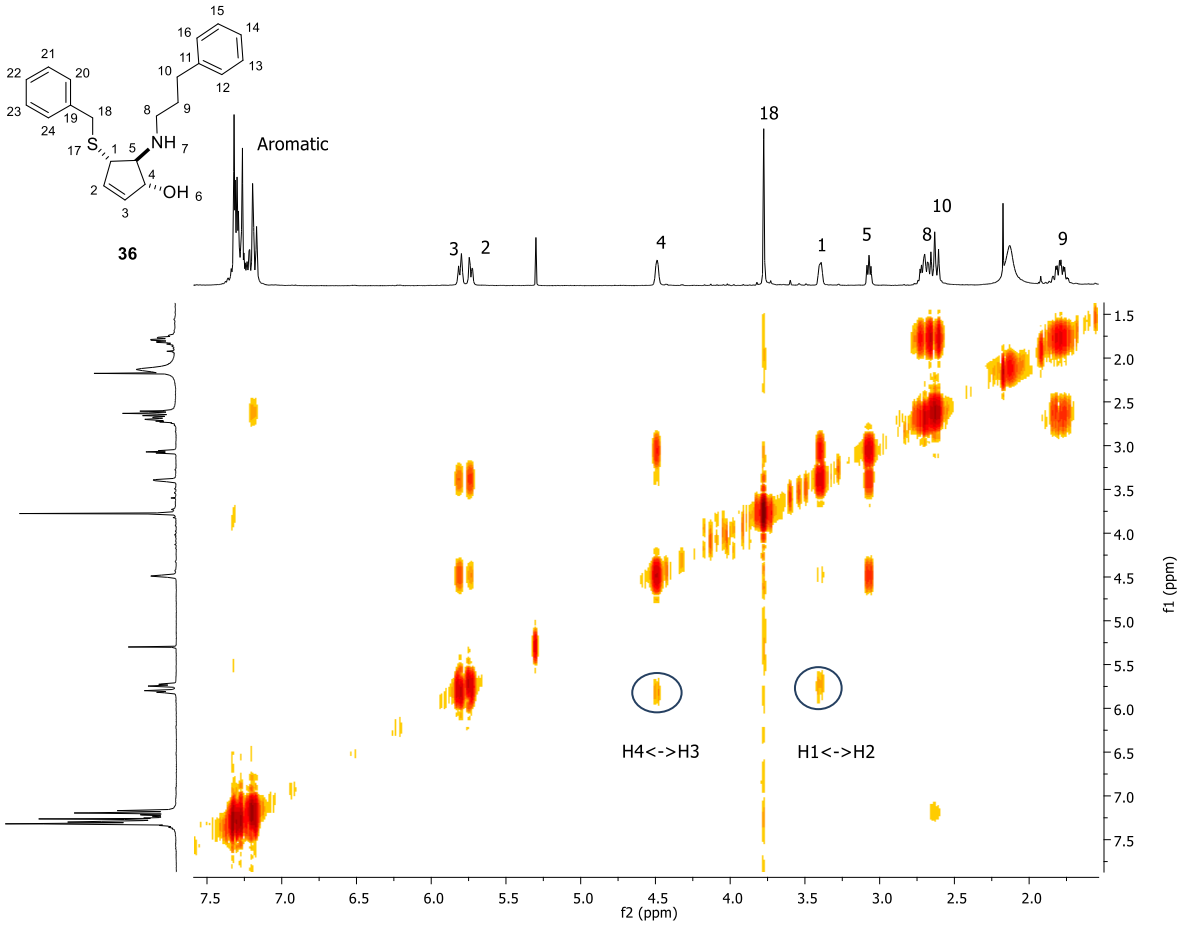


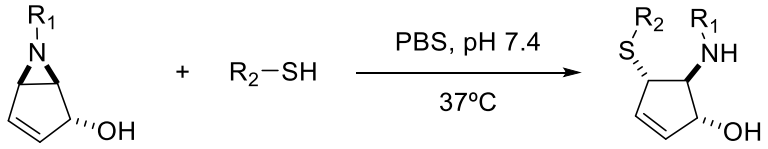
Figure 3.2: COSY NMR spectrum of product 36 ($CDCl_3$).

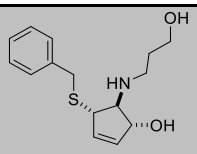
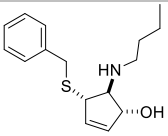
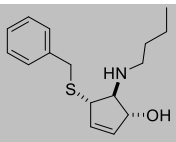
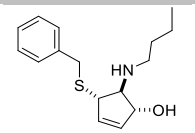
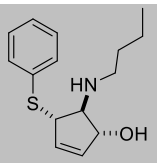
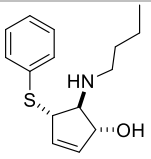
Analysing the spectra, we observe that for the hydrogens in the pentene ring, there is correlation between the hydrogen geminal to the hydroxyl (H4) and the vinyl hydrogen (H3) and correlation between the hydrogen geminal to the sulphur atom (H1) and the vinyl hydrogen (H2), both marked in blue circles in the spectra. The hydrogen geminal to the amine (H5) however shows no correlation to any of the vinyl hydrogens. Looking at Scheme 3.5, the structure where the nitrogen is connected to the most distant carbon from the double bound is a). This structure explains the lack of correlation between the double bound hydrogens and the hydrogen geminal to the amine, as they are separated by 4 covalent bounds, where the other two pentene hydrogens (geminal to sulphur and oxygen) each would have correlation to one hydrogen of the double bound, as they are separated by 3 covalent bounds.

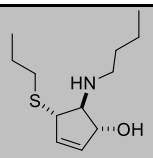
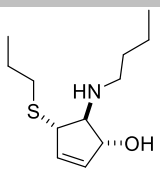
It was concluded that the mechanism from which the thiols open the aziridine is indeed a) (Scheme 3.5), which is in conformity with the literature[14b, 17].

This work also aimed to study the nucleophilic ring-opening reactions of aziridines in physiological conditions. For that a PBS buffer pH 7.4 was prepared and used as solvent for the

reaction. This buffer simulates the ionic strength and the pH of the cellular media. The scope of this reaction was extended to other thiols and the reaction conditions and isolation methodologies optimized in order to obtain better yields. The results are shown in Scheme 3.6.



Entry	Aziridine (0.065 mmol)	Thiol	Thiol eq.	Solvent	Reaction time (h)	Product	Yield (%)
1	**32**	Benzyl mercaptan	1.2	6 mL PBS	96	 **37**	61
2	**33**	Benzyl mercaptan	3	6 mL PBS	24	 **38**	41
3	**33**	Benzyl mercaptan	3	6 mL PBS	24	 **38**	61 [a]
4	**33**	Benzyl mercaptan	3	0.5 mL PBS	24	 **38**	75 [a]
5	**33**	Thiophenol	3	6 mL PBS	24	 **39**	65 [a]
6	**33**	Thiophenol	3	0.5 mL PBS	3	 **39**	86 [a]

7	**33**	Propanethiol	3	6 mL PBS	117	 **40**	61 [a]
8	**33**	Propanethiol	3	0.5 mL PBS	120	 **40**	8 [a]

[a] During silica column purification, triethylamine was used in the eluent (1% Et_3N in AcOEt).

Scheme 3.6: Aziridine ring-opening reactions with thiols in physiological conditions. Scope and optimization.

The first attempt at performing the aziridine ring-opening at physiological conditions (Entry 1 from Scheme 3.6) with 1.2 equivalents of thiol benzyl mercaptan gave the expected product in 61% yield, 6% higher than the previous result in water (Scheme 3.3) but with a much longer reaction time. This result is possible explained by the fact that distilled water, being somewhat acidic (pH 5.6), may catalyse the aziridine opening, by facilitating its protonation. In an attempt to decrease the reaction time, the equivalents of thiol were increased to three, which decreased the reaction time to 24 hours (Entry 2 from Scheme 3.6). Surprisingly, the reaction yield decreased to 41%, for which no reasonable explanation can be given. In an attempt to increase the yield, the purification step was optimized: because the final product is basic, since upon aziridine opening an amine group is formed, some product may be lost during silica column isolation, by bonding to the silica hydroxyl groups, which are acidic. To prevent this, triethylamine was added in 1% V/V to the eluent, to saturate the acidic silica sites. In fact, repeating the reaction while changing only the purification step increased the yield to 61% (Entry 3 from Scheme 3.6). After this result, in all silica column isolations of aziridines ring-opening products a base was used in the eluent, either ammonia or triethylamine. In order to increase the reaction yields, the reagents concentration was increased by 12 fold by reducing the solvent volume to 0.5 mL. The reaction yield in fact increased to 75%, 2% higher than reported in the literature for a similar reaction (Entry 1 from Table 1.1) [10].

Thiophenol was tested in the two best conditions of the reaction with benzyl mercaptan (Entries 3 and 4 from Scheme 3.6). Being a stronger nucleophile, it expectedly gave better yields in both conditions, and similar to the latter, when the solvent volume was decreased, the yield increased to a very satisfying 86% (Entry S6), higher than the reactions performed by Mariano with sulphur nucleophiles in organic solvents (Table 1.5)[17], proving the viability, and even benefit of using aqueous media in these reactions. Propanethiol was tested in the same way, and using 6 mL of PBS the

expected product was obtained in 61% yield (Entry 7 from Scheme 3.6), similar result to the benzyl mercaptan Entry 3 from Scheme 3.6. However, when the reagents concentration was increased by 12 fold the yield went down drastically to 8%. Presumably, because of the low solubility of propanethiol in water, the use of such a small volume of solvent didn't allow for proper mixing of the two immiscible liquids. Also, propanethiol is less reactive compared to the other two tested thiols, as its reaction time was significantly longer (Entry 7 from Scheme 3.6), which may have contributed to the low yield in Entry 8 from Scheme 3.6.

Encouraged by our thiols results, the ring-opening of these aziridines was tested with biological-relevant thiols, such as the amino acid cysteine, and the peptide glutathione (Figure 3.3).

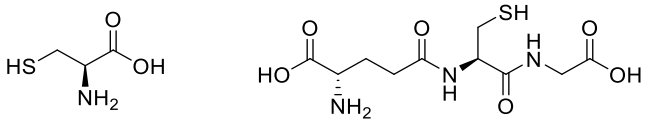
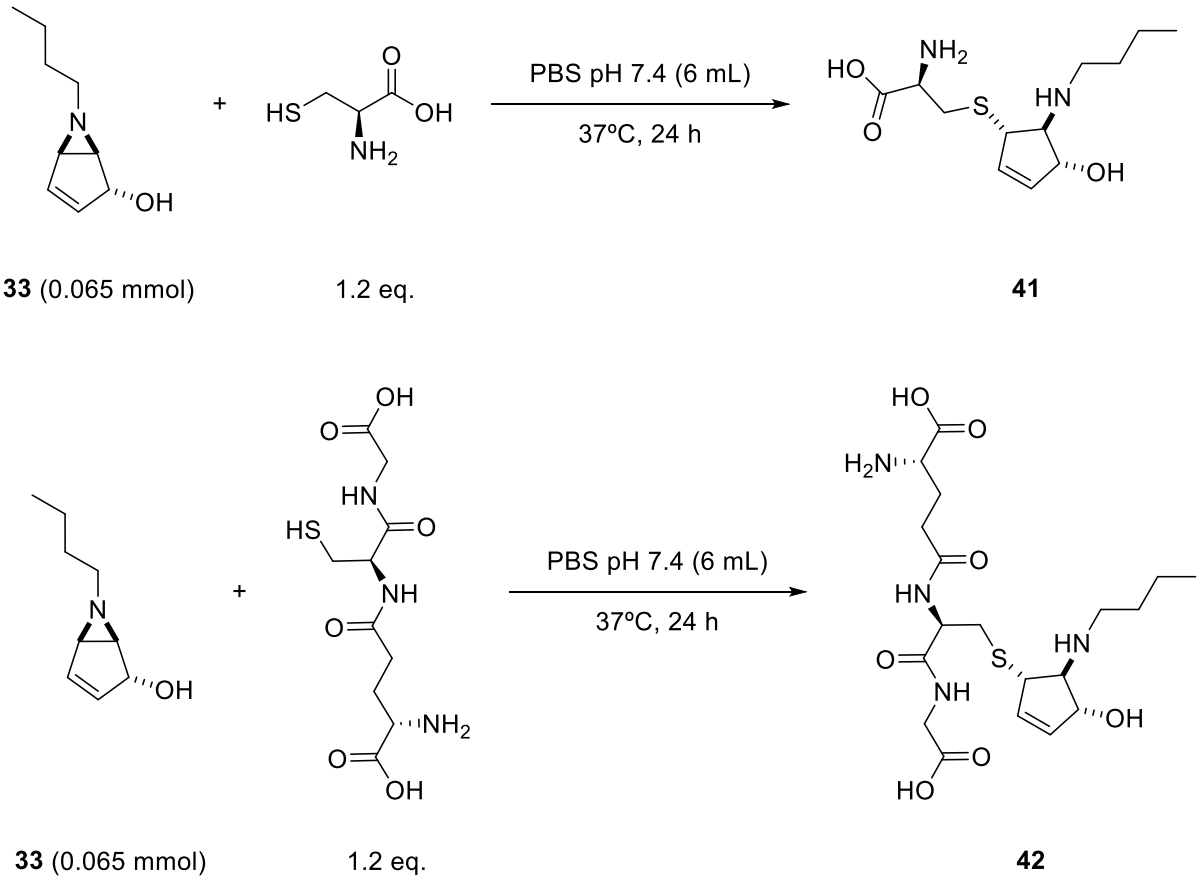


Figure 3.3: Biological thiols L-cysteine and glutathione.

As such, L-cysteine and glutathione were added to aziridine **33** in physiological conditions (Scheme 3.7):



Scheme 3.7: Ring-opening of aziridine 33 with cysteine and glutathione in physiological conditions.

The reactions were relatively fast (24 hours), and the ^{1}H NMR of both crudes clearly show the products, with just minor pyridium salt, as usually observed (Figure 3.4).

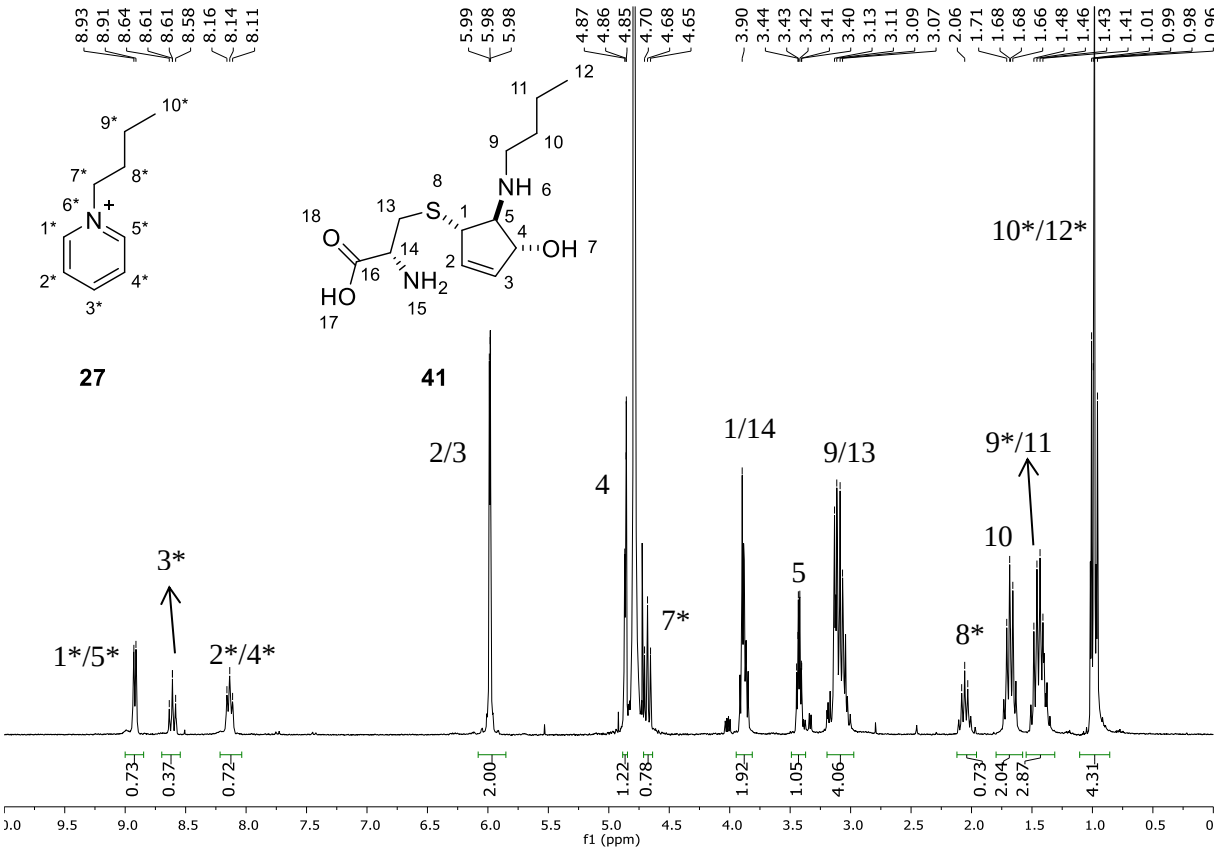
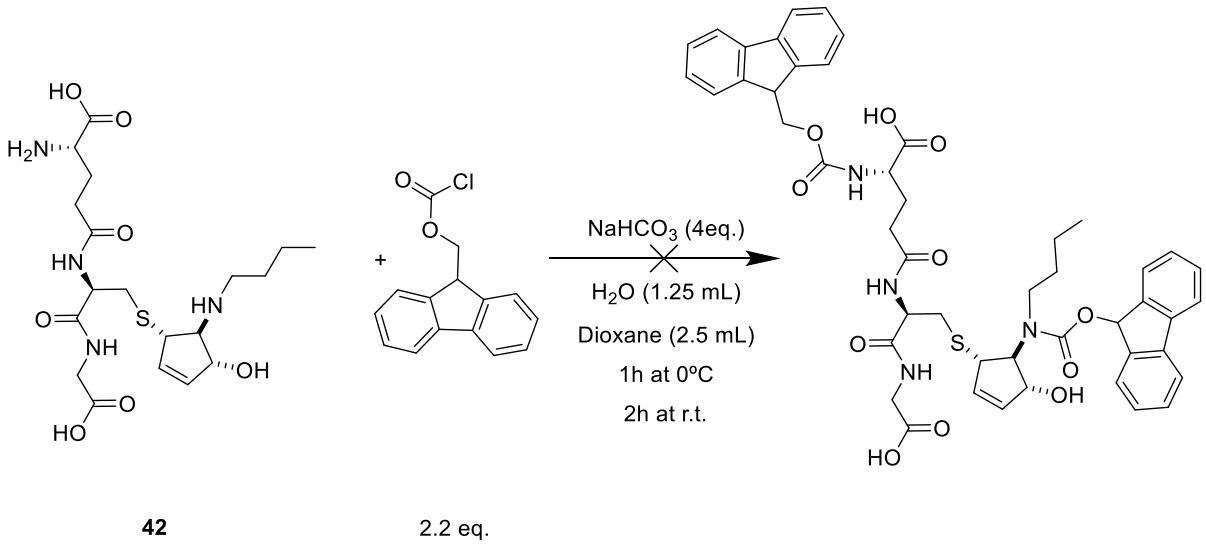


Figure 3.4: Crude ^{1}H NMR of the ring-opening reaction of aziridine 33 with cysteine in physiological conditions. Signals at 8-9 ppm show aziridine regression to pyridinium salt 27.

Both products **41** and **42** were very polar, possessing two amine groups, two alcohol groups and a carboxylic acid (two carboxylic acids in the case of **42** and two more amide groups), which made it impossible to separate them from other impurities, such as the pyridinium salt and buffer salts, using a normal silica column, as the compounds had a high affinity towards the polar stationary phase (In TLCs, they did not move from the application dot). As a result, other approaches had to be taken in order to isolate them. Reversed-phase chromatography seemed like a good choice as it is usually applied in isolation of hydrophilic compounds, and preparative column silica RP8 and RP18 were tested. However, the products eluted in the first water fraction together with the buffer salts. Dry methanol and ethanol extraction was tried, hoping that one of these two solvents would be hydrophilic enough to solubilize the desired product but not as much as to dissolve the buffer salts, leaving them precipitated. This method partially worked because clearly most of the salts were not being dissolved by the solvent (in both alcohols), but nevertheless, after evaporation of the solvent, some salts were still contaminating the product.

Seeing different isolation techniques fail mainly because of the high polarity of the products, many attempts were made in order to transform the final products into other molecules, more hydrophobic, that could be easily isolated by a regular silica column. The amino acid moiety of the

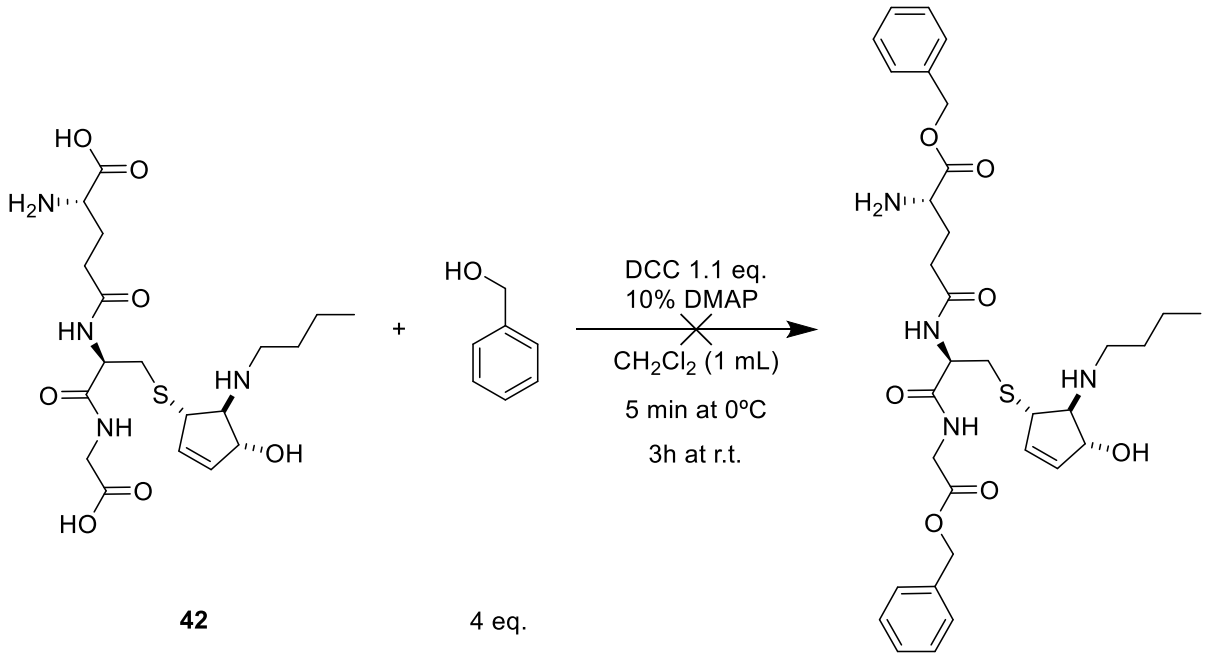
molecule seemed like the ideal target for chemical protection with protecting groups that would make them more apolar. A search in the literature[22] revealed many approaches towards amino acid protection. Protection of the amine group with Fmoc group appeared to be an easy procedure[23]. Seeing as both products have amine groups, we hoped that they could be protected by Fmoc, decreasing the polarity of the compounds. This protection reaction was tested with the crude **42** (Scheme 3.8):



Scheme 3.8: Attempt at Fmoc protection of crude 42.

Unfortunately, no reaction occurred on the glutathione derivative molecule. Maybe the bulkiness of this molecule prevented binding to the also relatively bulky Fmoc chloride molecule, or for some reason the amine groups were not reactive enough to attack the carbonyl group of Fmoc chloride.

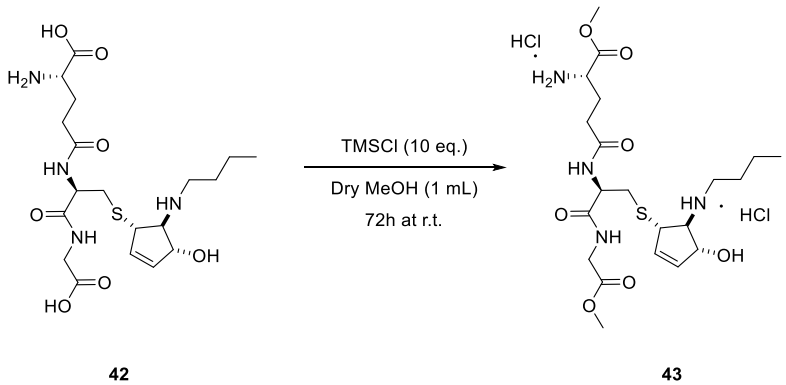
With amine protection seemingly failing, protection of the carboxylic acid moiety was attempted, using the Steglish esterification protocol (Scheme 3.9) [24].



Scheme 3.9: Attempt at Fmoc protection of crude 42.

This protection was also unsuccessful, since this type of reaction is incompatible with substrates with unprotected amines as they are more nucleophilic than alcohol and therefore lead to an intramolecular lactam formation, or even intermolecular amide formation, leading to polymerization. In fact the ^{1}H NMR spectrum of the reaction crude was very confusing with many different products so this method was abandoned.

Inspired on a simple protocol developed by Sha and Li[25], one last attempt at protecting the product **42** was made to prepare the corresponding methyl esters the from carboxylic acids (Scheme 3.10).

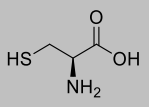
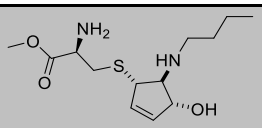
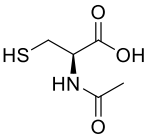
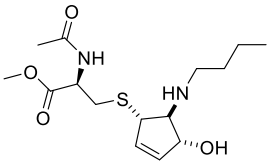
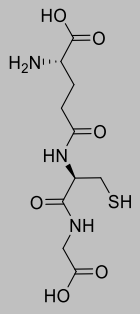
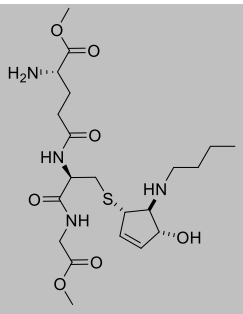
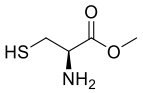
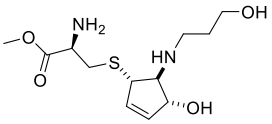


Scheme 3.10: Esterification of crude 42 with TMSCl and dry methanol.

Gratifyingly, this reaction did work nicely and the corresponding product **43** was possible to isolate via silica column chromatography albeit with a very polar eluent (AcOEt: MeOH: NH_3 25%aq.

= 8:2:0.5). Other cysteine nucleophiles were used in the nucleophilic ring-opening of bicyclic vinyl aziridines, and their products isolated with this methodology (Table 3.3).

Table 3.3: Ring-opening of aziridines with cysteine derived nucleophiles in physiological conditions.

Entry	Aziridine	Nucleophile (1.2 eq.)	Solvent	Reaction time (h)	Isolated product	Yield (%)
1	**33** (0.065 mmol)		6 mL Phosphate buffer 1M pH 7.4	24	 **44**	81 [a]
2	**33** (0.065 mmol)		6 mL Phosphate buffer 1M pH 7.4	24	 **45**	86 [a]
3	**33** (0.065 mmol)		6 mL Phosphate buffer 1M pH 7.4	24	 **46**	50 [a]
4	**32** (0.2 mmol)		6 mL PBS pH 7.4	4	 **47**	47 [b]

[a] Yield from 2 steps, after esterification of carboxylic acids to allow isolation. [b] Isolated by reversed phase chromatography, silica RP8.

All cysteine nucleophiles successfully opened the aziridine ring. The methyl ester protocol proved reproducible for all examples (Entries 1 to 3 from Table 3.3) and the products were isolated. Surprisingly, even in suboptimal conditions tested for other thiols (Scheme 3.6), where only 1.2 equivalents of thiol were used and the reagents concentrations were relatively low as the solvent volume was 6 mL (Entries 1-3 from Table 3.3), the reactions were fast (24 hours for Entries 1 and 3 and just 3 hours for Entry 2 from Table 3.3). Even after transformation of the carboxylic acid to methyl ester with a procedure that is reported to have yields of 76-97%[25], our combined reactions yields were very good (Entries 1 and 2 from Table 3.3, 81% and 86% respectively), although only

50% was obtained using glutathione as nucleophile (Entry 3 from Table 3.3). Maybe because glutathione is a relatively big molecule and the thiol group could be somewhat hindered and not as available to attack the aziridine as in the amino acid cysteine. The high yield obtained in these reactions compared to other thiols (benzyl mercaptan, thiophenol and propanethiol) may be explained by the differences in solubility in water of the thiols. The cysteine derived molecules are extremely soluble in water while the former are only partially soluble, which certainly decreases the reaction rates and consequently lowers the yields.

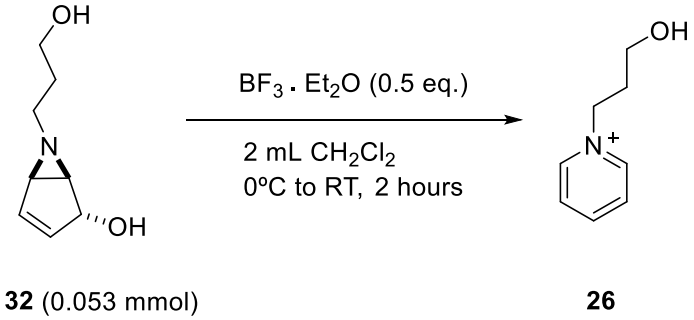
All of these methyl esters products were found to be instable in water as the ester moiety is hydrolysed when the compounds were left in an NMR tube in D_2O. In fact, when the same reaction was attempted with cysteine methyl ester, only the carboxylic acid product was obtained after 24 hours. This time is enough for the full hydrolysis of the methyl ester, so we increased the aziridine concentration in order to decrease the reaction time so that the hydrolysis would be minimal (Entry 4 from Table 3.3). The reaction was fast, only 4 hours, and the reaction product, possessing already the methyl ester moiety, could be possible to isolate like the previous examples via column chromatography, but instead a reversed phase chromatography with silica RP8 was used. The product eluted in the second fraction (10% MeOH in water), as it is less polar than the corresponding carboxylic acid, free from buffer salts. The yield however, was very low comparing to the Entry 1 from Table 3.3 where cysteine was the nucleophile. It's likely that in the 4 hours of reaction there might have been some hydrolysis of the ester and some compound lost due to that.

Regarding the solvent used in these reactions, a phosphate buffer 1M pH 7.4 was used instead of the usual PBS buffer. This is because in a previous reaction of aziridine **33** with glutathione and cysteine (Scheme 3.7), when PBS buffer was used, a large amount of pyridinium salt was observed in the reaction crude (Figure 3.4). In the case of glutathione, as it is an acidic molecule (has two carboxylic acids groups and only one amine group), PBS may have not efficiently neutralised the reaction medium, because it is a relatively weak buffer (only has 11.8 mM of phosphates, and the concentration of 1.2 eq. glutathione is 13 mM after addition). In fact, with the use of phosphate buffer pH 7.4 1M as solvent, only traces of pyridinium salt were observed in the reaction crude, by ^{1}H NMR. As such, for these reactions, the stronger phosphate buffer pH 7.4 1M was used.

Thiols proved to be very good nucleophiles in the ring-opening reaction of the bicyclic vinyl aziridines, giving the expected products in moderate to good yields. Cysteine nucleophiles were especially reactive because of their high water solubility. The next step of this work would be testing the ring-opening of aziridines with nitrogen nucleophiles.

3.3. Aziridine regression to the pyridinium salt.

The unexpected observation that the bicyclic vinyl aziridine regresses to the corresponding pyridinium salt, possibly catalysed by slightly acidic medium of water (Figure 3.1), we decided to further explore this reactivity before moving on to the ring-opening of the aziridines in physiological conditions with other nucleophiles. Aziridine **32** was dissolved in CH_2Cl_2 and the Lewis acid BF_3 was added (Scheme 3.11). After only 2 hours of reaction, full regression of the aziridine to the pyridinium salt was observed (Figure 3.5).



Scheme 3.11: Reaction of aziridine 32 with BF_3, with full regression to the corresponding pyridinium salt 26.

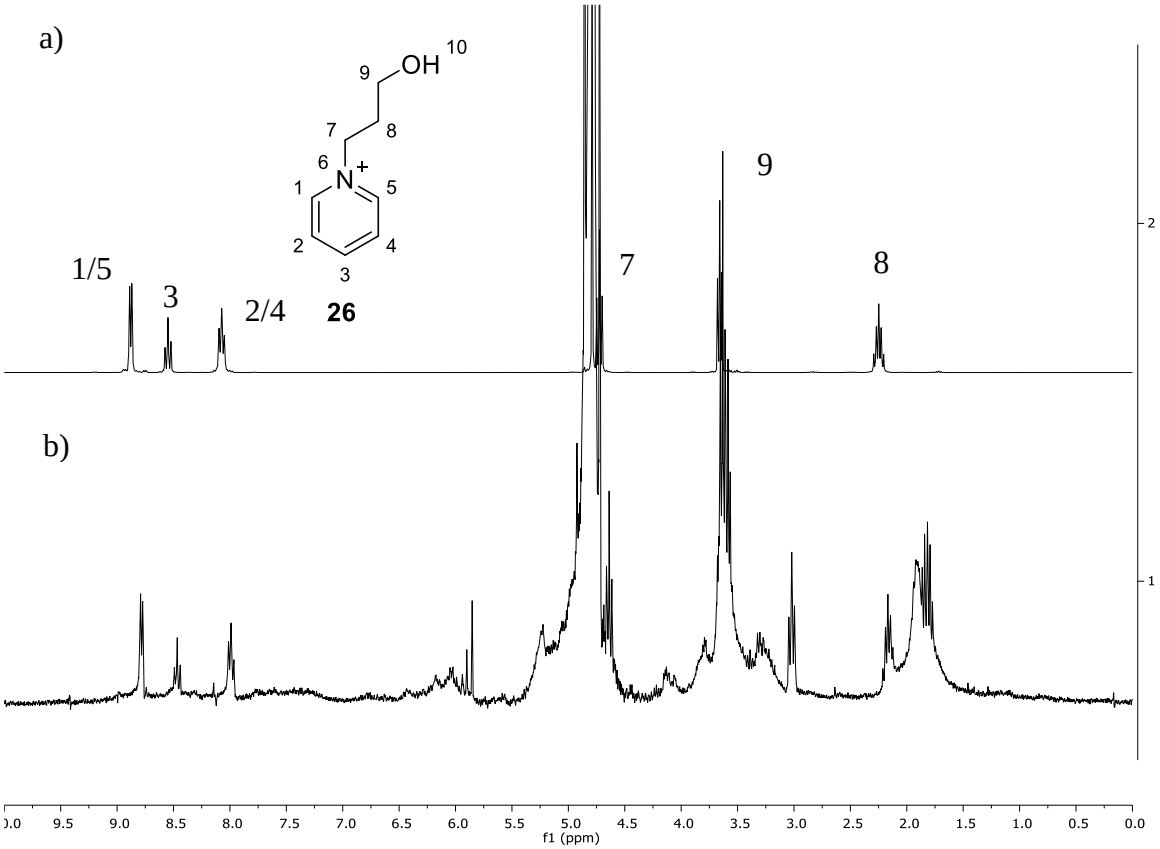
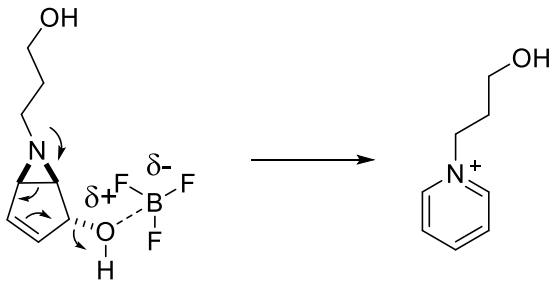


Figure 3.5: ^{1}H NMR spectra (D_2O) of a) pyridinium salt 26 and b) crude of reaction between aziridine 32 with BF_3 (Scheme 3.11).

Being BF_3 a strong Lewis acid, with high affinity towards oxygen, it probably catalyses the regression of the bicyclic vinyl aziridine to the corresponding pyridinium salt in the same mechanism as an acidic medium (represented in Scheme 3.4). BF_3 most likely coordinates with the alcohol oxygen in the pentane ring, activating it and making it a good leaving group, with consequent rearrangement of the aziridine to the pyridinium salt (Scheme 3.12)



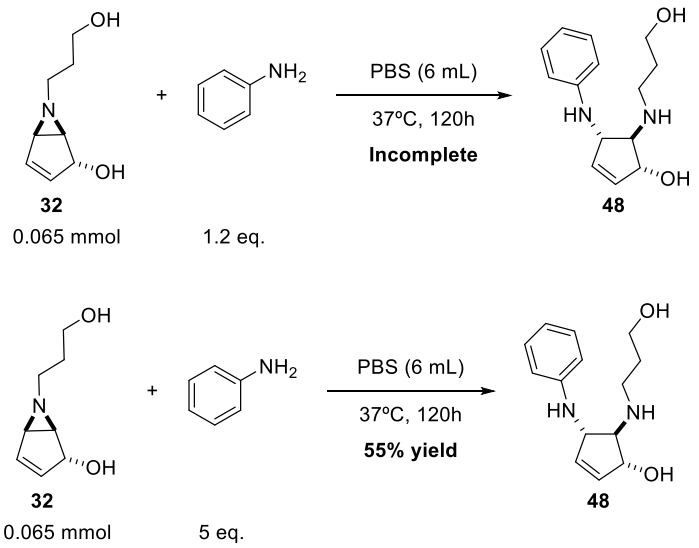
Scheme 3.12: Proposed mechanism for the coordination of aziridine 32 with BF_3 followed by regression to the pyridinium salt 26.

We proved that the regression of the bicyclic vinyl aziridine to the corresponding pyridinium salt can be promoted by Lewis acids in a very fast reaction. We also observed this reaction occurring in aqueous media, at a slower rate. To the best of our knowledge, it is the first time that that this reaction is described, and it will be further explored in the future.

3.4. Aziridine ring-opening with nitrogen nucleophiles

3.4.1. Ring-opening with amines

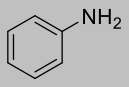
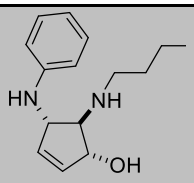
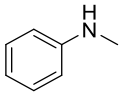
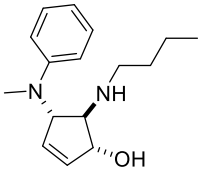
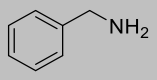
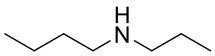
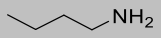
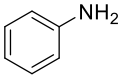
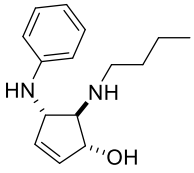
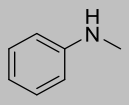
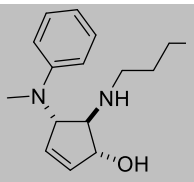
The scope of the nucleophilic ring-opening reaction of aziridines in close to physiological conditions was expanded by testing several nitrogen nucleophiles. The first nitrogen nucleophile tested was aniline (Scheme 3.13).



Scheme 3.13: Nucleophilic ring-opening of aziridine 32 with aniline, in PBS buffer, at 37ºC.

In the first reaction, the reaction was very slow and even after 120 hours there was still starting material in the reaction medium (seen by TLC). The equivalents of aniline were increased to 5 in order to drive the reaction to completion, which worked well, giving a modest 55% yield. After addition of aniline the solution pH increased to 11 (seen by paper pH indicator). The stronger buffer (used in Table 3.3) was used in order to maintain the reaction medium around pH 7.4. Other amines were tested at this stage and the results are presented in Table 3.4.

Table 3.4: Ring-opening of aziridine with amines at physiological conditions.

Entry	Aziridine (0.065 mmol)	Nucleophile (5 eq.)	Solvent	Reaction time (h)	Product	Yield (%)
1	**33**		6 mL Phosphate buffer 1M pH 7.4	48	 **49**	43
2	**33**		6 mL Phosphate buffer 1M pH 7.4	48	 **50**	34
3	**33**		6 mL Phosphate buffer 1M pH 7.4	48	Degradation	-
4	**33**		6 mL Phosphate buffer 1M pH 7.4	120	Degradation	-
5	**33**		6 mL Phosphate buffer 1M pH 7.4	120	Degradation	-
6	**33**		1 mL Phosphate buffer 1M pH 7.4	120	 **49**	Incomplete
7	**33**		1 mL Phosphate buffer 1M pH 7.4	120	 **50**	Incomplete

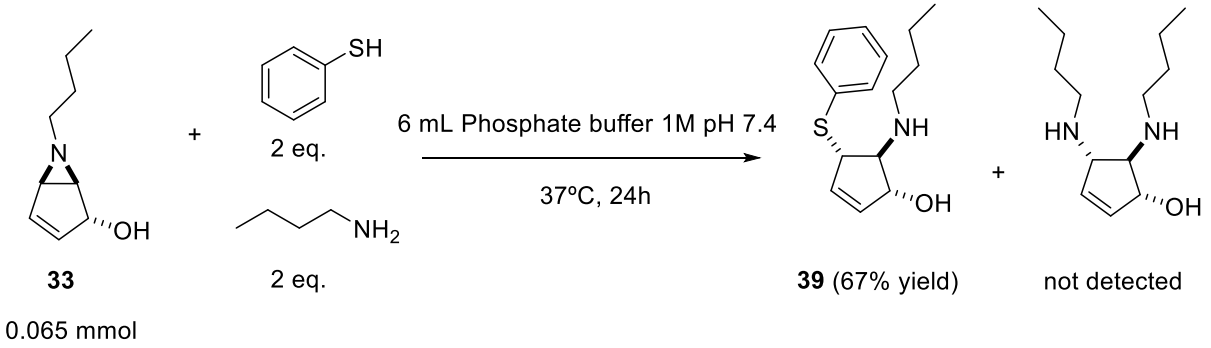
These results seem to limit the reaction scope of amines to aromatic amines such as aniline and *N*-methylaniline (Entries 1 and 2 from Table 3.4), and even those presented low to moderate

yields (43% and 34%, respectively). Primary and secondary amines (Entries 3-5 from Table 3.4) did not react to give expected products and only degradation of the aziridine was observed. Too confirm if the aziridine degradation is due to the amines in solution or the high molarity of the phosphate buffer, a 10.8 mM solution of aziridine **33** in phosphate buffer 1M 7.4 pH was left stirring at 37ºC for 72 hours. There was complete degradation of the aziridine, suggesting that the aziridine degradation was a result of the strong buffer. Also, the same reaction as the Entry 3 from Table 3.4 was performed using PBS pH 7.4 as the solvent, and no reaction occurred, confirming that the amines do not promote aziridine degradation.

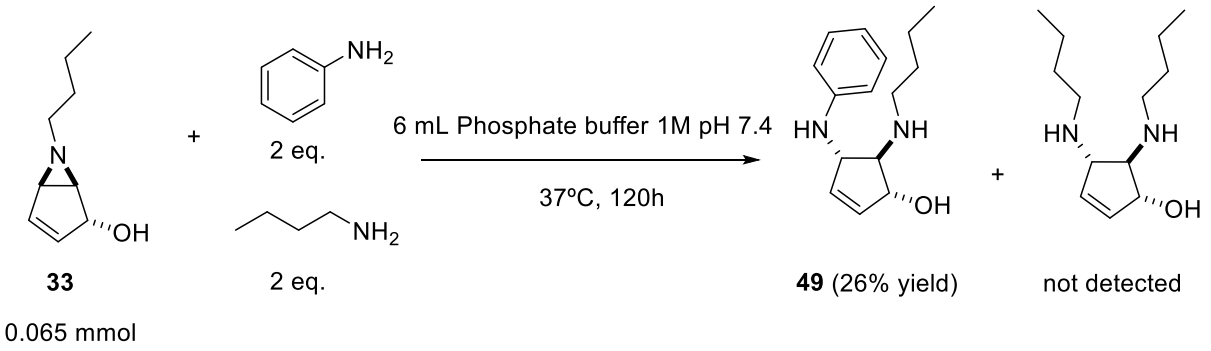
As with thiols, the reaction volume of Entries 1 and 2 from Table 3.4 was decreased (increasing reagents concentration) in an attempt to increase reaction yields. However, due to the low solubility and reactivity of the two anilines, the reaction was incomplete and only traces of the ring-opening product were seen by TLC.

3.4.2 Competitive reactions

Competitive reactions were performed to confirm if primary amines such as butylamine are in fact non-reactive towards the bicyclic vinyl aziridine in physiological conditions. Thiophenol or aniline (nucleophiles that were proved to open the aziridine) and butylamine were simultaneously added to the aziridine **33** (Scheme 3.14 and Scheme 3.15, respectively).



Scheme 3.14: Competitive study between thiophenol and butylamine nucleophiles.



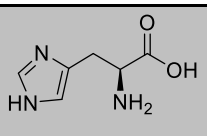
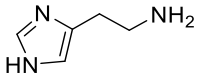
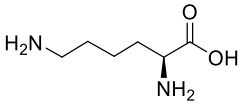
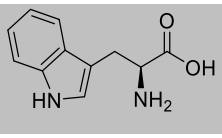
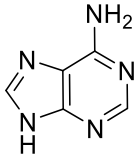
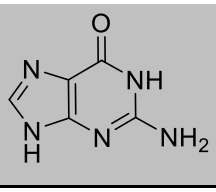
Scheme 3.15: Competitive study between aniline and butylamine nucleophiles.

In the competitive study between thiophenol and butylamine nucleophile (Scheme 3.14), only the aziridine ring-opening product with thiophenol (**39)** was obtained, in 67% yield. The theoretical ring-opening product with butylamine was not detected, neither by TLC or ^{1}H NMR. Using aniline as the nucleophile, in competition with butylamine (Scheme 3.15), provided the aziridine ring-opening product with aniline (**49**) in 26% yield. Again, the ring-opening product with butylamine was not detected, proving that alkyl amines are not nucleophilic towards the bicyclic vinyl aziridine in physiological conditions, and do not compete with nucleophilic ring-opening reactions when present in the reaction medium.

3.4.3 Ring-opening with other nitrogen nucleophiles

Other different nitrogen nucleophiles such as imidazole derivatives, purines and indole derivatives were tested but apparently with no success (Table 3.5), as no ring-opening products with the tested nucleophiles were obtained.

Table 3.5: Ring-opening of aziridine with several nitrogen nucleophiles at physiological conditions.

Entry	Aziridine	Nucleophile (1.2 eq.)	Solvent	Reaction time (h)	Product
1	**32**		PBS	27	Degradation
2	**32**		PBS	52.8	Degradation
3	**32**		PBS	65	Starting material with minor degradation
4	**32**		PBS	54	Starting material
5	**32**		PBS	81	Starting material
6	**32**		PBS	57	Starting material
7	**32**		PBS + NaOH 1M aqueous	67	Starting material

Surprisingly, when using histidine and histamine as nucleophiles (Entries 1 and 2 from Table 3.5, respectively) there was complete consumption of the aziridine, but from ^{1}H NMR and mass spectrometry analysis, the expected product was not being formed. Instead, by ^{1}H NMR, for both cases, formation of pyridinium salt can be observed, which is already expected, and signals from other

unidentifiable product were observed. By mass spectroscopy a peak corresponding to the aziridine opening with water is detected at 173.9 m/z (Figure 3.6).

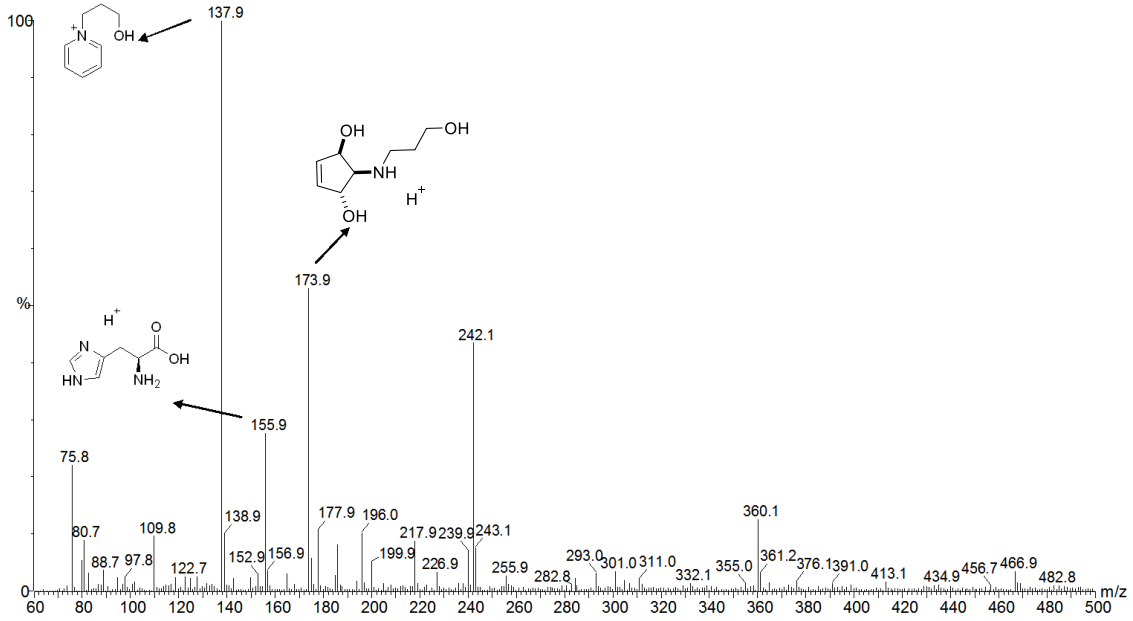
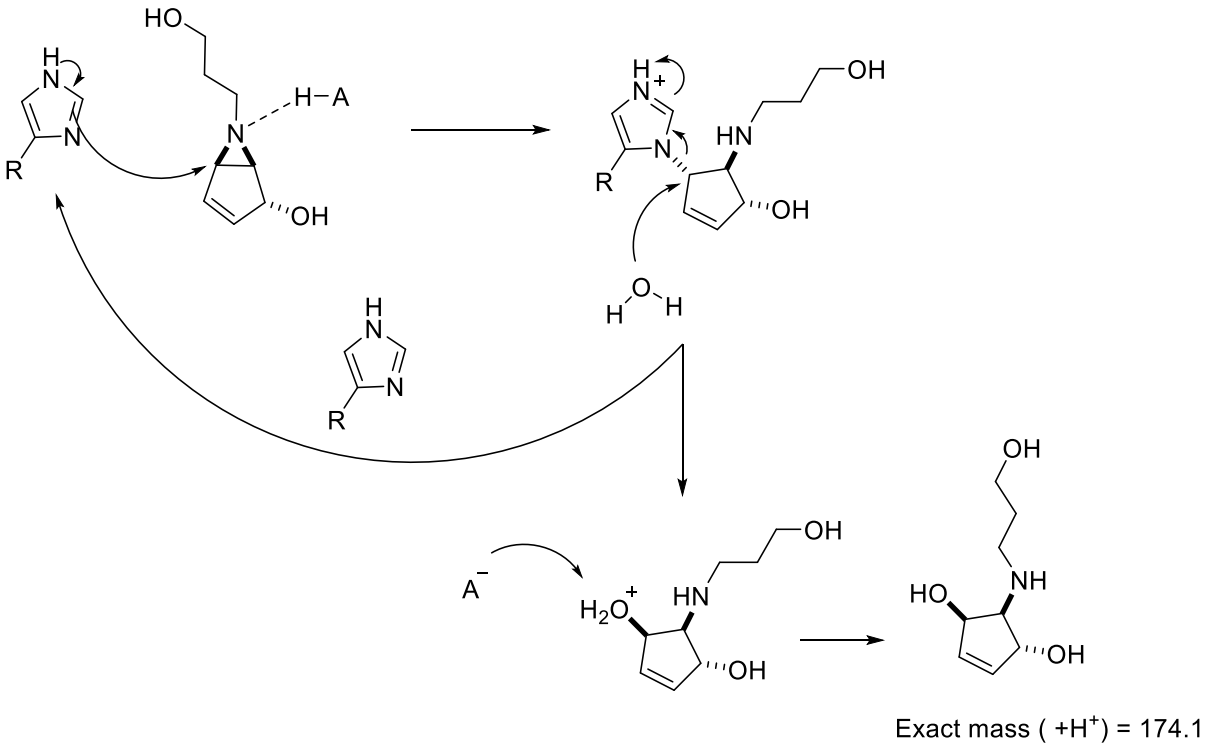


Figure 3.6: ESI+ spectrum of reaction crude of the reaction of aziridine 32 with histidine (Entry 1 from Table 3.5).

Apparently, histidine, histamine, and to a lesser degree imidazole promoted ring opening of the aziridine, with addition of water. It is known that molecules with the imidazole moiety can be used as catalytic nucleophiles[26] and nature even uses them in enzymes as histidine amino acids to catalyse numerous chemical processes[27]. A hypothesis is that the imidazole group attacks and opens the aziridine like a normal nucleophile, and afterwards the intermediate molecule suffers a nucleophilic attack of the solvent in a S_N2 type reaction, regenerating the imidazole molecule, as in a catalytic cycle (Scheme 3.16).



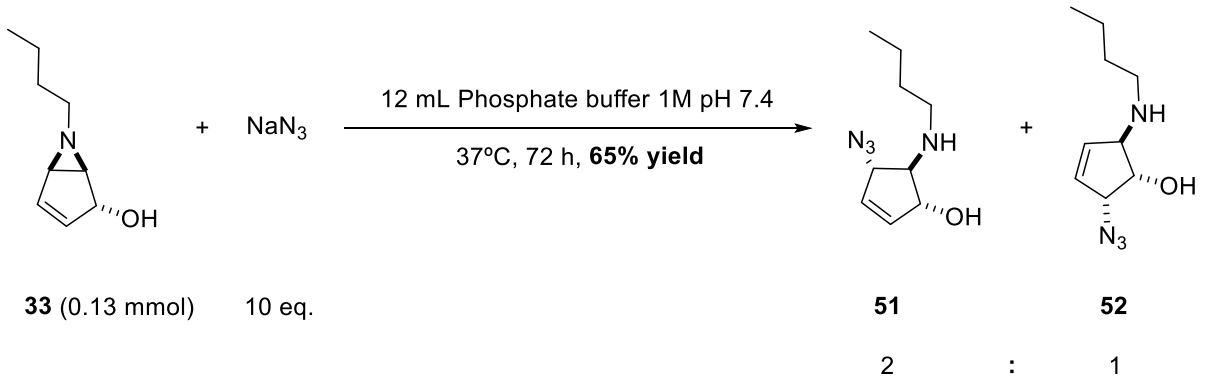
Scheme 3.16: Possible mechanism for the water addition to the aziridine, catalysed by molecules with imidazole moiety.

This mechanism would explain the ESI+ signal at 173.9 m/z (close to 174.1) and why the predicted product (opening of the aziridine with histidine, histamine and imidazole) was never detected by NMR or ESI+. The conversion of aziridine with imidazole was not complete unlike the reaction with histidine or histamine, which may suggest that a substituted imidazole is much more reactive, which makes sense considering the charge donating effect of an alkylic group in an aromatic moiety, granting it extra nucleophilicity. The catalytic property of substituted imidazoles in this specific reaction can be very useful as a tool to add a weak nucleophile to the bicyclic vinyl aziridine, in an otherwise difficult reaction. This topic was not expanded during this year but will be in due time.

Other nitrogen nucleophiles were tested, namely lysine and tryptophan amino-acids and the purines adenine and guanine. Unfortunately they showed no reactivity, expected in the case of lysine, being an amino acid with an amine side chain, which were already shown non-reactive. Guanine had to be dissolved in a basic NaOH solution because it proved to be insoluble in the PBS buffer pH 7.4. It did increase the pH of the reaction medium but since no reaction was observed there was no reason to do the reaction in a more controlled way, regarding the pH.

We also investigated the azide as nucleophile for the ring-opening reaction of aziridines. Azide was not very reactive, so 10 equivalents were used for full starting material conversion. The reaction was followed by TLC, and 2 products were observed, with close retention factors, and a 2 to 1 ratio (seen by ^{1}H NMR of the reaction crude). Upon isolation by silica column, the products **51** and **52**

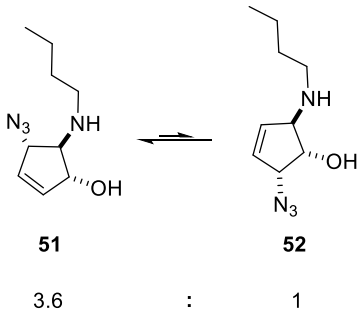
(Scheme 3.17) were quantified and identified by 1D and 2D NMR techniques (^{1}H, ^{13}C, COSY and HSQC NMR)



Scheme 3.17: Reaction of aziridine 33 with azide. Two different products were formed.

The major product formed, **51,** is the one usually observed in this type of reaction with these bicyclic vinyl aziridines: nucleophile attack on the less hindered α carbon of the aziridine. The minor product, **52**, probably derived from the attack of the nucleophile on the double bound, followed by its migration and opening of the aziridine, which was never observed before with other nucleophiles.

However, when both isolated isomers **51** and **52** where left separately in their NMR tubes, dissolved in $CDCl_3$, for one week, the products interconverted in each other, and in both tubes an equilibrium of 3.6:1 was reached (Scheme 3.18 and Figure 3.7).



Scheme 3.18: Interconversion of the products from the azide addition to aziridine 33, in $CDCl_3$.

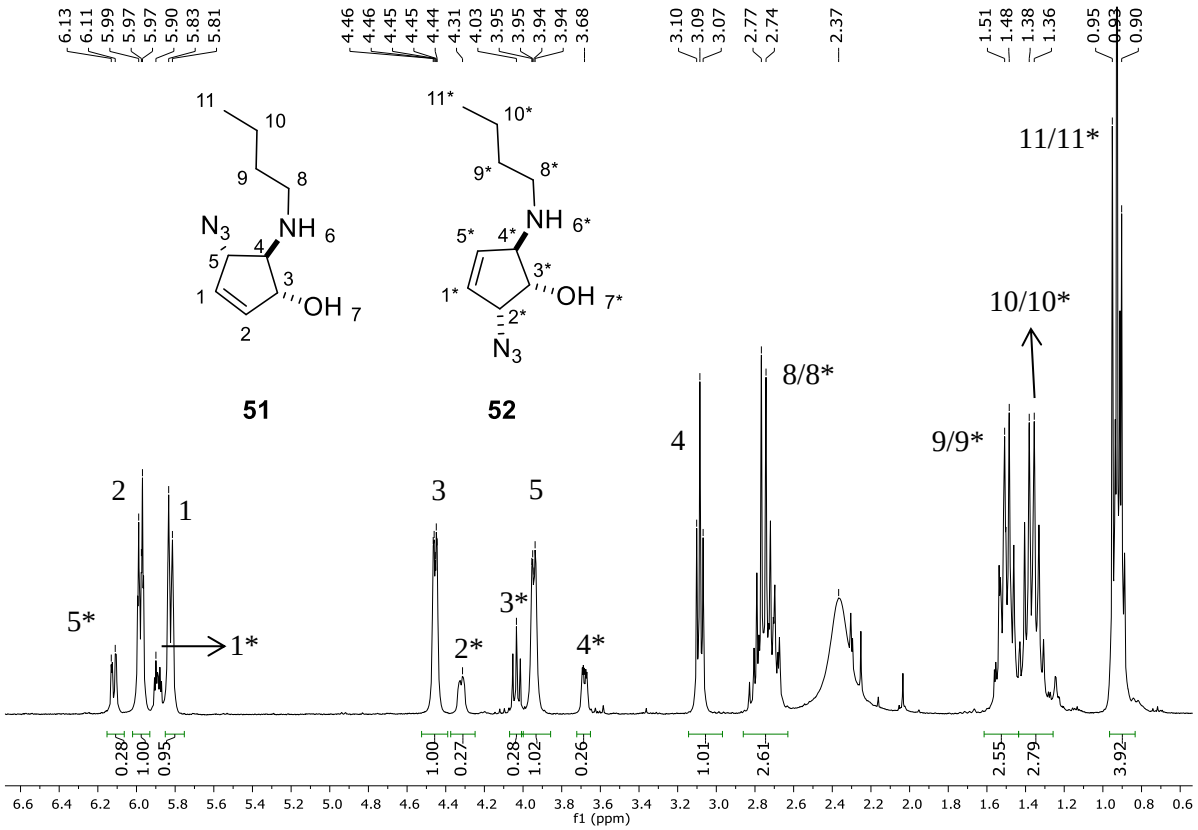
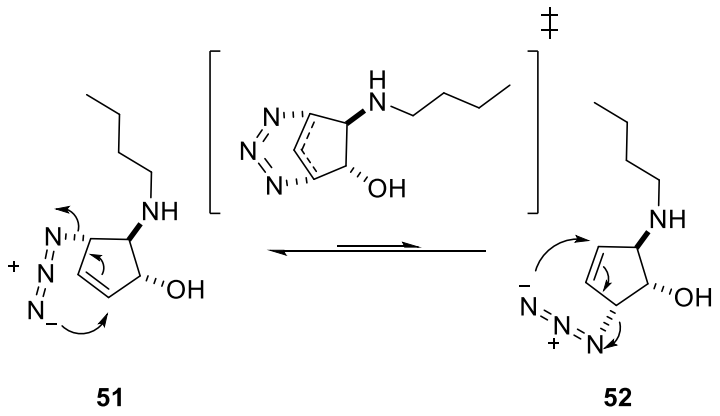


Figure 3.7: ^{1}H NMR spectrum of compounds 51 and 52 after reaching equilibrium in the NMR tube ($CDCl_3$).

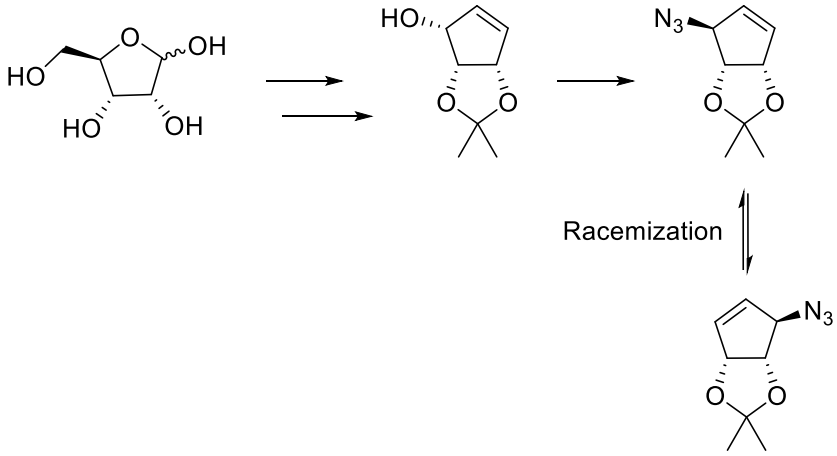
This equilibrium did not change when the molecules were dissolved in D_2O, maintaining the same 3.6:1 ratio. However, when dissolved again in phosphate buffer 1M pH 7.4 the equilibrium shifted to a 1:1 proportion, which indicates that the presence of concentrated phosphate salts interferes somehow with the interconversion rates of **51** and **52**.

A search in the literature provided insight on why this interconversion was happening. In fact, allylic azides are known to undergo a [3.3]-sigmatropic rearrangement since 1960[28] and several studies have been made to understand this reaction[29]. The equilibrium constant seems to be independent of the solvent, which was also observed. It can be assumed that the azide only attacks the aziridine in its less hindered α carbon, and that this product, **51**, rearranges to **52** and vice versa reaching equilibrium, as illustrated in Scheme 3.19.



Scheme 3.19: Azide allyl rearrangement seen in the product from the azide addition to aziridine 33.

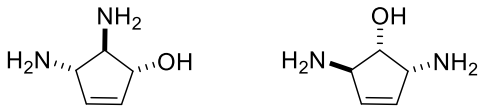
This equilibrium between the isomers of the allylic azide occasionally poses problems to some authors. Carell *et al.*[30], in the synthesis of enantiomerically pure queuosine derivatives, observed racemization of their allylic azide intermediate (Scheme 3.20), destroying any enantiomeric excess previously obtained.



Scheme 3.20: Racemization of an enantiomerically pure allylic azide intermediate, observed by Carrel *et al.*[30].

The authors lowered the temperatures of the reactions involving the allylic azide to 0ºC as they observed effective suppression of the sigmatropic rearrangement at this temperature, avoiding the undesired racemization.

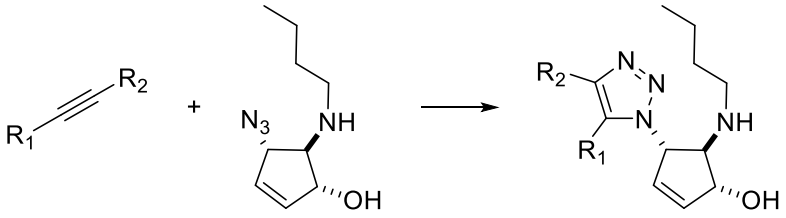
We see, however, the value of compounds **51** and **52** as new valuable precursors for the synthesis of 4-5-diaminocyclopent-2-enols and 2-5-diaminocyclopent-3-enols, which will explored in the near future (Figure 3.8).



53 **54**

Figure 3.8: 4,5-Diaminocyclopent-1-enol (53) and 2,5-diaminocyclopent-3-enol (54).

In addition, taking advantage of the azide group, molecules **51** and **52** can be attached to other molecules via "click chemistry"[31], as the azide can undergo a cycloaddition to an alkyne forming a triazole ring connecting both molecules (Scheme 3.21). An application for the azide products **51** and **52** in this topic will be eventually pursued.

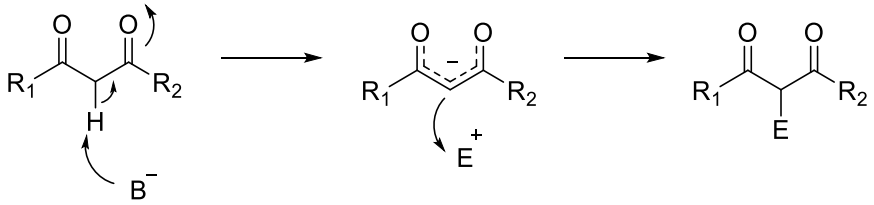


51

Scheme 3.21: Possible "Click reaction" between compound 51 and an alkyne.

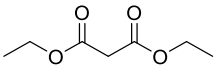
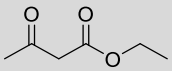
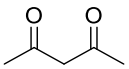
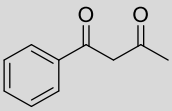
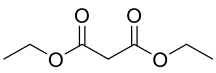
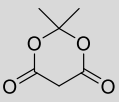
3.5. Aziridine ring-opening with carbon nucleophiles

The aziridine ring-opening reaction was also investigated in the presence of a range of known carbon nucleophiles, mainly dicarbonyl compounds, because the α position of both carbonyl groups is fairly acidic and nucleophilic (Scheme 3.22). Hopefully, the nucleophilic attack of this position on the aziridine carbon would occur. Unfortunately, no reaction products were obtained (Table 3.6).



Scheme 3.22: Nucleophilic attack of a dicarbonyl nucleophile to an electrophile.

Table 3.6: Ring-opening of aziridine with dicarbonyl nucleophiles and cyanide at physiological conditions.

Entry	Aziridine	Nucleophile	Nuc eq.	Solvent	Product
1	**32**	$\bar{C}N$	1.2	PBS	Starting material
2	**32**		1.2	PBS	Degradation
3	**32**		1.2	PBS	Starting material and degradation
4	**32**		1.2	PBS	Starting material and degradation
5	**32**		1.2	PBS	Starting material and degradation
6	**33**		2	Phosphate buffer 1M pH 7.4	Degradation
7	**33**		2	Phosphate buffer 1M pH 7.4	Degradation

Several dicarbonyl compounds were tested for the aziridine ring opening reaction in a PBS buffer, however, no product was observed being formed and some aziridine degradation occurred. It

was assumed that the buffer used was not strong enough to neutralise the acidic properties of the dicarbonyl molecules which led to aziridine opening with the solvent and also reformation of the respective pyridinium salt, catalysed by acidic medium. In Entries 6 and (Table 3.6) the stronger phosphate buffer 1M pH 7.4 was used to see if a better control of the pH would yield the desired product. Unfortunately, only degradation due to the strong buffer was detected, as observed before. In fact, an HRMS analysis of the reaction crudes only showed aziridine opening with water and phosphates, not with the dicarbonyl compounds.

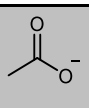
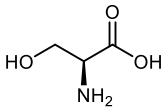
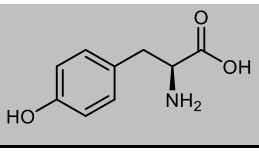
Cyanide was a too weak of a nucleophile to open the aziridine as well.

In this scenario, all carbon nucleophiles proved to be ineffective in opening the aziridine ring in physiological conditions.

3.6. Aziridine ring-opening with oxygen nucleophiles

The aziridine ring-opening reaction was performed with oxygen nucleophiles under physiological conditions. Once more, no reaction products were detected and only starting material was observed (Table 3.7).

Table 3.7: Ring-opening of aziridine with oxygen nucleophiles at physiological conditions.

Entry	Aziridine	Nucleophile (1.2 eq.)	Solvent	Product
1	**32**		PBS	Starting material
2	**32**		PBS	Starting material
3	**32**		PBS	Starting material

The results from ring-opening reactions with oxygen, carbon, nitrogen and sulphur nucleophiles in physiological conditions showed that these bicyclic vinyl aziridines react very specifically with thiols, anilines and azide. Also, cysteine amino acids proved to be the most reactive nucleophiles, undergoing the nucleophilic ring-opening reaction with good yields and relatively fast reaction times. Naturally, the step forward would be to study the reaction of these aziridines with cysteine containing proteins, to assess their potential to be used in biological targeting of such proteins.

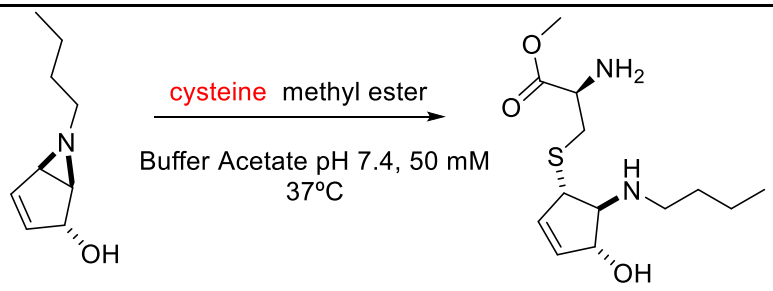
3.7. Aziridine ring-opening with cysteine containing peptides

Inspired by our good results obtained with biological thiols like cysteine in the ring-opening reaction of the

bicyclic vinyl aziridine, we tested this reaction with a cysteine-containing peptide.

To better simulate physiological conditions, where proteins and substrates are present in very low concentrations, the aziridine and nucleophile concentrations were decreased to confirm if the reaction occurs in diluted conditions. These optimizations were performed using cysteine methyl ester as model nucleophile.

Table 3.8: Optimization of conditions of ring-opening reaction of aziridine 33 with methyl ester cysteine in physiological conditions. Conversion was obtained from ^{1}H NMR analysis of reaction crudes.



33 **53**

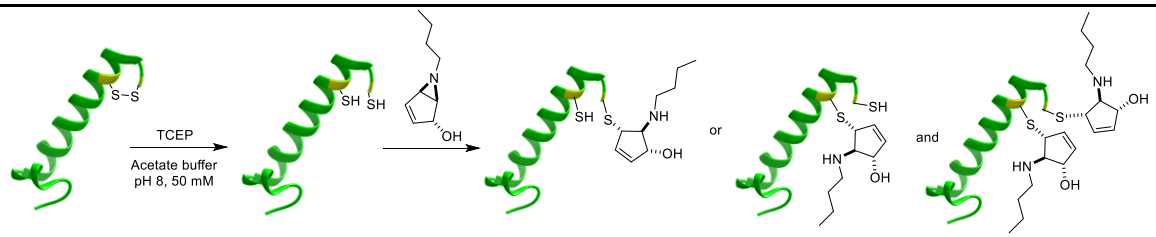
Entry	Aziridine 33 concentration (mM)	Cys.OMe (mol eq.)	Reaction time (h)	RMN crude analysis
1	33	1.2	21	100% conversion of aziridine
2	10	1.2	24	100% conversion of aziridine
3	5	1.2	24	100% conversion of aziridine
4	3	1.2	48	100% conversion of aziridine
5	1	1.2	72	50% conversion of aziridine
6	8.5 (10 eq.)	1	72	100% conversion of cysteine methyl ester
7	5 (10 eq.)	1	48	100% conversion of cysteine methyl ester

Results from Table 3.8 show that the nucleophilic ring-opening reaction of the bicyclic vinyl aziridine with methyl ester cysteine supports dilution of the reagents. Full conversion of aziridine was observed at concentrations as low as 3 mM (Entry 4 from Table 3.8). Even at aziridine concentration of 1mM, 50 % conversion of the aziridine was observed by ^{1}H NMR (Entry 5 from Table 3.8) after 72 hours of reaction. The aziridine concentration was not further decreased due to the low sensibility of the NMR equipment.

In order to mimic the reactions conditions what will be tested with the cysteine-containing peptide, new conditions were tested using methyl ester cysteine in 1 equivalent, and excess aziridine, in order to obtain full modification of the thiol compound. Satisfactory conditions were reached with 500 μM of methyl ester cysteine and 5 mM of aziridine **33**, observing full modification of the thiol (Entry 7 from Table 3.8).

After confirming that the nucleophilic ring-opening of the aziridine with biological thiols supports low concentrations of reagents, we then focused on the modification of the peptide calcitonin acetyl salmon (sCT). This peptide hormone is secreted by neuroendocrine cells of thyroid in mammals and is used for the treatment of postmenopausal osteoporosis, Paget's disease and hypercalcaemia. sCT has a molecular weight of 3473.89 Da, contains 32-amino acids residues and a disulphide bridge (Cys1-Cys7) that can be reduced in the presence of tris(2-carboxyethyl)phosphine (TCEP) [32]. The bioconjugation reaction of aziridine **33** with the sCT after disulphide reduction with TCEP was investigated (Table 3.9). The best result of sequential reduction-conjugation approach was obtained using 10 μM of sCT with 5 mol eq. of TCEP for 3h, followed by the addition of aziridine **33** (500 μM) (Entry 2 of Table 3.9). Peptide modifications were observed following the change of the peptide mass in MS as is modified by the aziridine. Comparing the mass spectrum of the unmodified sCT with the bioconjugation product, a mass increase of approximately 153 Da was observed, in the peak 3630.2 Da, attributed to the modification of one Cys residue (Figure 3.9). The peak 3781.1 Da was assigned to the increment of 2 aziridines molecules in the sCT. In order to promote full modification of both Cys residues of the peptide, the aziridine concentration was gradually increased (Entries 4 to 8 in Table 3.9). Surprisingly, no cysteine modification occurred in these experiments. Only in Entry 6 (Table 3.9) a vestigial peak corresponding to the modification of the 2 cysteine residues of sCT was observed. These preliminary results will be further explored in the near future and full modification of sCT will be attempted.

Table 3.9: Reduction of sCT disulphide bound and S-alkylation of its cysteine amino acids residues with aziridine 33. Protein image: PBD ID: 2JXZ[33].



Entry	Aziridine Conc.	TCEP Conc. (μM)	sCT Conc. (μM)	Reaction time (h)	MS analysis
1	100 μM	12	10	72 [a]	-
2	500 μM	50 [b]	10	24	Modification of 1 and 2 residues
3	500 μM	50	10	24	Modification of 1 and 2 residues
4	1 mM	12	10	48	-
5	1 mM	12 [b]	10	48	-
6	1 mM	30 [b]	10	24	Modification of 2 residues
7	5 mM	50 [b]	10	24	-
8	10 mM	100 [b]	10	24	-

[a] This modification was also performed in water. [b] The aziridine was added after 3 hours of reduction with TCEP.

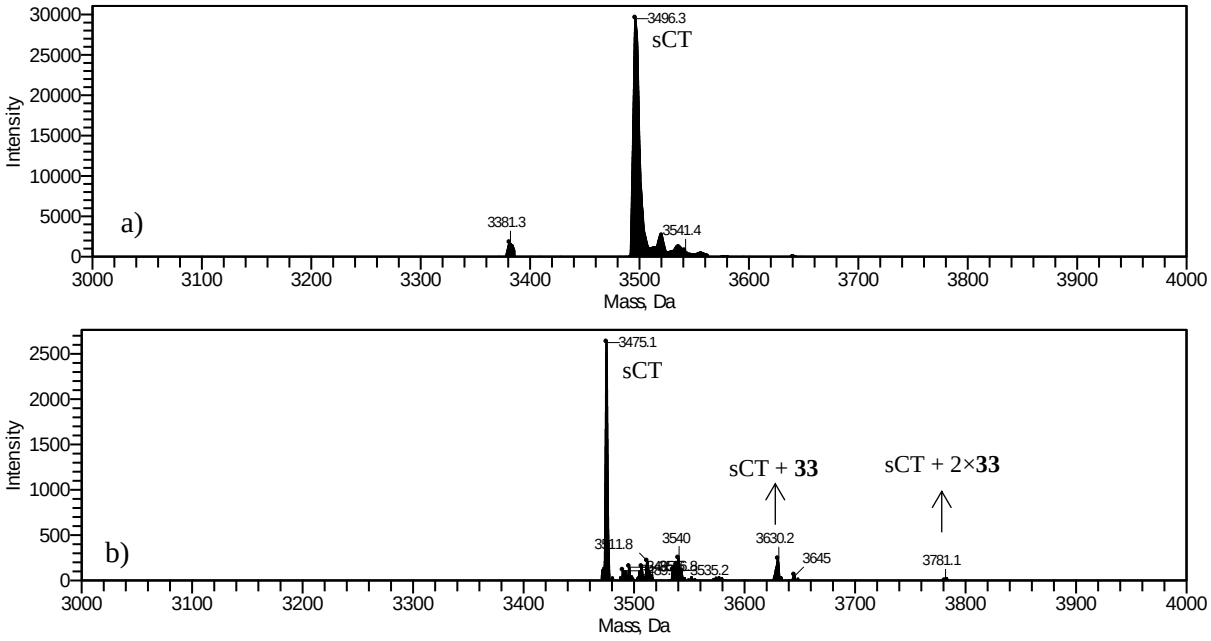
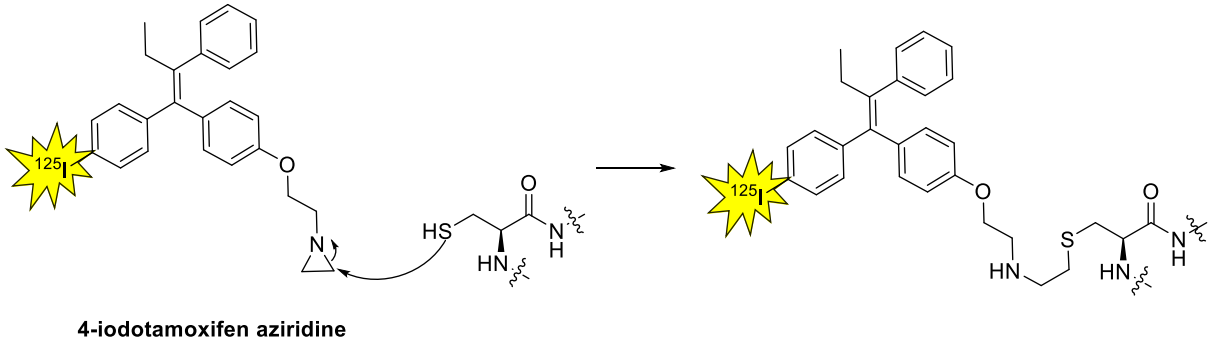


Figure 3.9: a) Unmodified sCT and b) sCT (10 μM) after modification with 500 μM aziridines, and reduction with TCEP 50 μM (Erro! A origem da referência não foi encontrada.**).**

We proved that the bicyclic vinyl aziridine can modify cysteine-containing peptides like calcitocin. These results show the potential of this aziridine to be used in a medicinal chemistry approach. It could be used, after coordination with a probe, for detection of cysteine-containing proteins. A similar mechanism is shown by 4-iodotamoxifen aziridine, a radioiodinated probe that can be used for the detection of the estrogen receptor (ER) that is present in cytosols from breast carcinomas[34]. The detection of this receptor is an important predictor for the hormone therapy response. The aziridine moiety of 4-iodotamoxifen aziridine can alkylate the cysteine residues of the ER, which is then detected by radiography (Scheme 3.23).



Scheme 3.23: 4-iodotamoxifen aziridine binding to cysteine residue from estrogen receptor.

Additionally, the bicyclic vinyl aziridine can potentially be used as an inhibitor for enzymes that possess a cysteine residue in its active site. Cysteine proteases, for example, exploit the nucleophilicity of the thiol side chain of a cysteine residue to catalyse the hydrolysis of peptide bounds. In fact, Schirmeister *et al.*[35] reported the targeting of the major cysteine protease expressed by *Trypanosoma brucei* with aziridine-2,3-dicarboxylate inhibitors (Figure 3.10). This protozoan parasite causes African trypanosomiasis, that can be fatal. The studied aziridines alkylate the cysteine residues of the parasite cysteine proteases, inhibiting them irreversibly, leading to the parasite death.

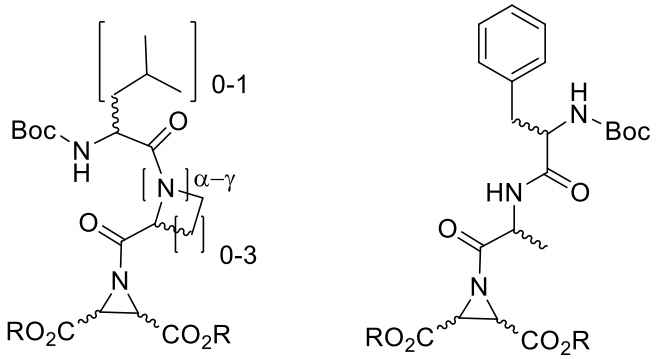
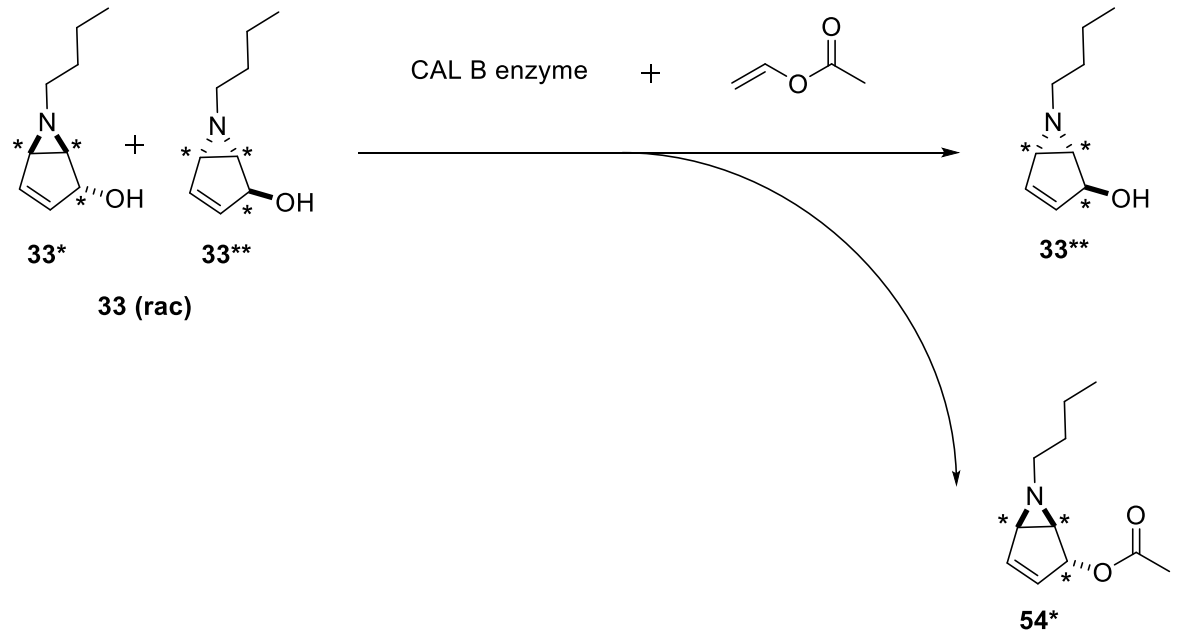


Figure 3.10: Aziridines tested by Schirmeister et al.[35] for the inhibition of *Trypanosoma brucei* cysteine proteases.

The bicyclic vinyl aziridines show great promise to be used in diverse biological strategies, which is currently being explored.

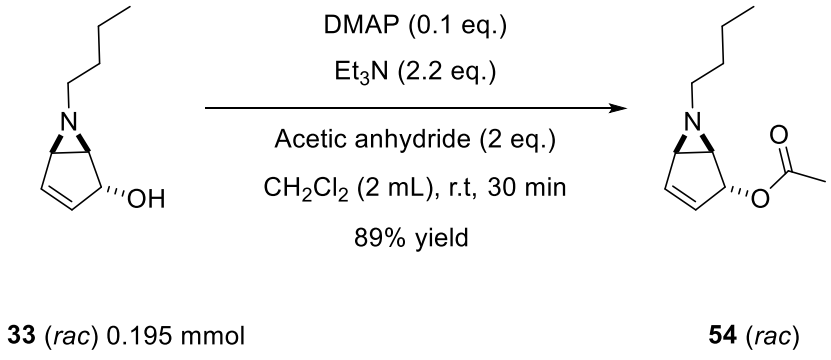
3.8. Enantiomeric resolution of the bicyclic vinyl aziridine

Inspired on the work of Monteiro *et al.* from this laboratory[20], we attempted to separate both enantiomers of the bicyclic vinyl aziridine. The objective was to use an enzymatic catalytic process that would selectively esterify the secondary alcohol of one of the aziridine enantiomers, leaving the other enantiomer unaltered, which could then be isolated. For preliminary testing, a simple scheme was designed (Scheme 3.24).



Scheme 3.24: Preliminary strategy for the isolation both aziridine 35 enantiomers.

The racemic acetyl aziridine was synthetized as a model compound through acetylation of the hydroxyl moiety of aziridine **33** (Scheme 3.25).



Scheme 3.25: Synthesis of racemic acetyl aziridine 54.

The reaction proved to be very robust, as the acetyl aziridine was obtained in 89% yield after isolation with silica column chromatography, in a very fast reaction at room temperature. After having this compound **54** and the corresponding aziridine **33** with the free alcohol in hand, both were injected in a GC equipped with a chiral column to hopefully be able to separate the enantiomers from both molecules in order to analyse the enantiomeric excesses of future enzymatic reactions.

Several chiral columns were tested and only one, after optimization, separated the aziridine enantiomers with acceptable resolution, HYDRODEX beta-6TBDM (Figure 3.11).

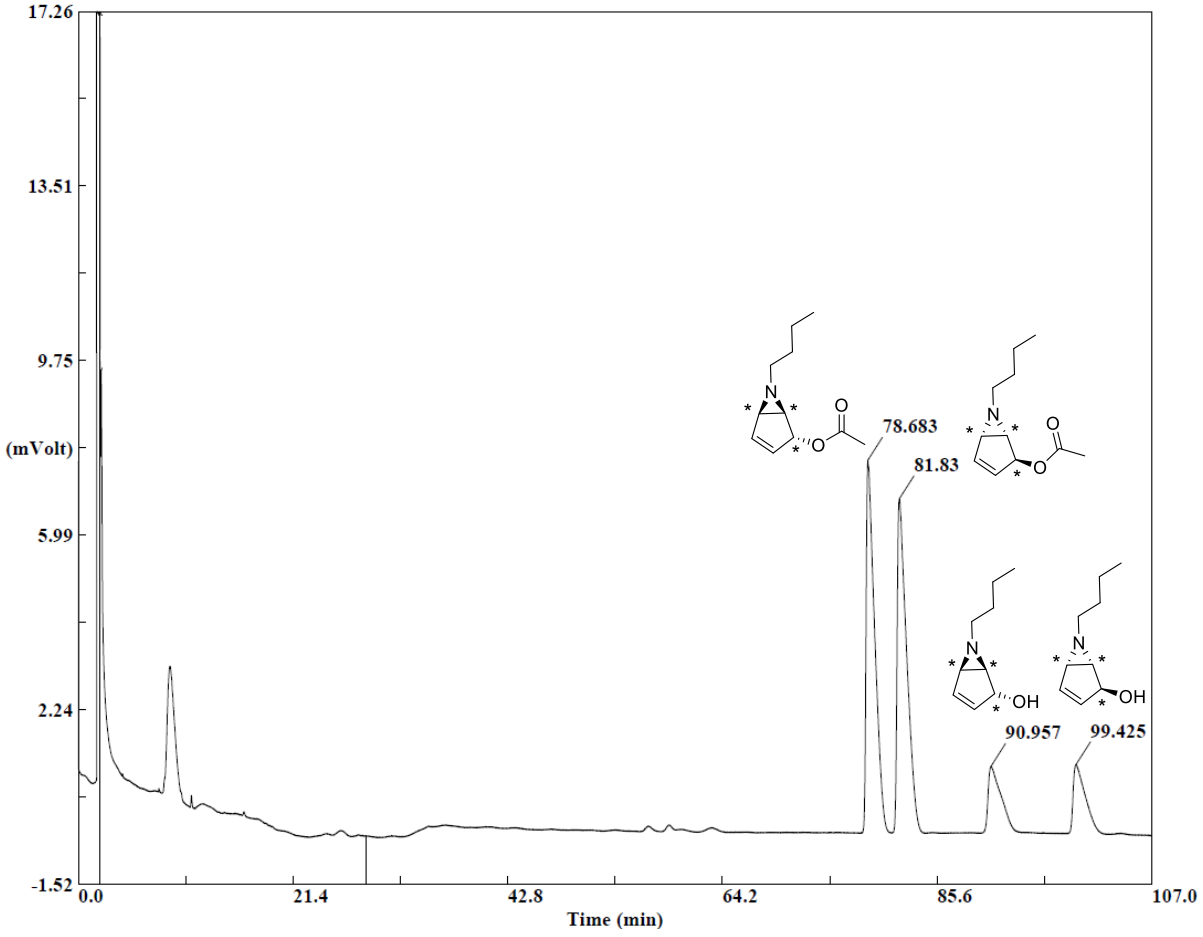
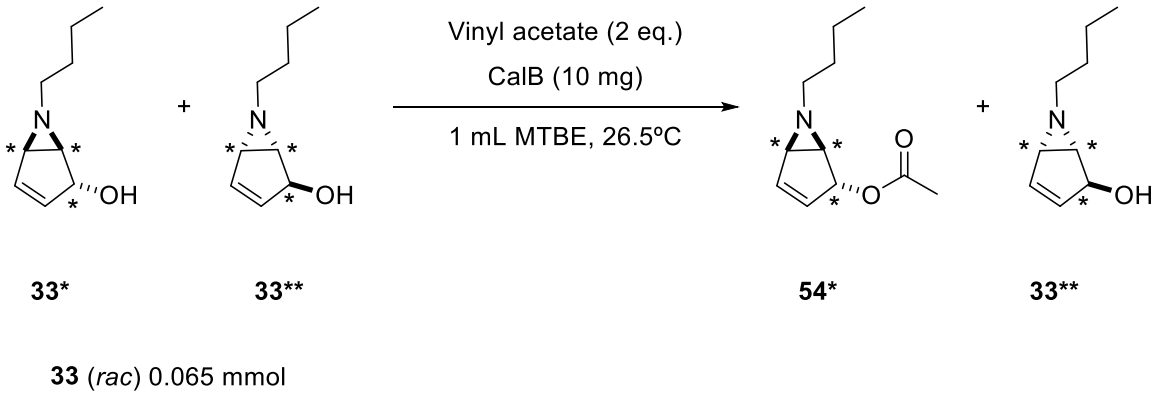


Figure 3.11: Separation of racemic aziridine 33 and acetyl aziridine 54 enantiomers by chiral GC.

After successfully separating both aziridine **33** and acetyl aziridine **54** enantiomers via chiral GC, an enzymatic reaction was tested to see if there was indeed some selectivity towards one of the aziridines enantiomers to the lipase enzyme. Aziridine **33** was dissolved in methyl *tert*-buthyl ether, and the enzyme CALB was added to the solution, followed by addition of the alkylating agent, vinyl acetate (Scheme 3.26). Hopefully CALB could selectively acetylate one of the enantiomers of the aziridine, and the enantiomeric excesses of both product and aziridine reagent were analysed with the chiral GC method optimized previously.



Scheme 3.26: Preliminary reaction of an enzymatic resolution of bicyclic vinyl aziridine 33, with enzyme CAL B and acetylating agent vinyl acetate.

The reaction was followed for 24 hours, injecting 1 µL of the reaction medium in the chiral GC to analyse if there was any enantiomeric selectivity as intended (Table 3.10).

Table 3.10: Enantiomeric excesses obtained in the enantioselective enzymatic reaction of Aziridine 33 with enzyme CAL B and acetylating agent vinyl acetate. *ee* (enantiomeric excess) and conversion were calculated from Equation 1.1 and Equation 1.2, respectively.

Entry	Reaction time (h)	Ester 54* *ee* (%)	Alcohol 33** *ee* (%)	Conversion (%)
1	3	99.5	27	21.4
2	6	94.0	53.4	26.2
3	24	84.1	91.4	52.1

Gratifyingly, after 3 hours of reaction (Entry 1 of Table 3.10) we detected that one of the enantiomers of the acetylated aziridine product was being formed in an enantiomeric excess of 99.5%, proving an enantioselectivity of the CAL B enzyme to the corresponding enantiomer of the aziridine **33**. We arbitrarily defined this more reactive aziridine enantiomer as compound **33*** and the less reactive one **33**,** as we do not know yet which exact enantiomer is being acetylated selectively. A Mosher ester ^{1}H NMR analysis will eventually be performed in order to know the exact configuration of the reactive and the less reactive enantiomer.

At a reaction time of 3 hours, the conversion is still low (21.4%), so naturally there is still large quantity of the reactive alcohol enantiomer (**33***) that is not fully acetylated and therefore the enantiomeric excess of the aziridine **33**** is only 27%, which is very low. After 6 hours of reaction, the conversion increased as expected, and the enantiomeric excess of the less reactive enantiomer of the aziridine (**33****) increased to 53.4%, while its enantiomer **33*** was being selectively acetylated. However, as **33*** is consumed and its concentration decreases, the rate at which the enzyme catalyses its acetylation decreases, while it continues to acetylate the **33**** enantiomer, although in a much

slower rate because it's less selective towards it. This leads to a slight decrease of the enantiomeric excess of the acetylated product **54*** to 94%. When the reaction was left for 24 hours, this excess decreased even more to 84.1% but the enantiomeric excess of the free alcohol **33**** increased to 91.4%, while the conversion was 52.1%. Eventually, if the reaction continues for many days the conversion would reach 100% and the enantiomeric excess of the acetylated aziridine would be 0%, as all the initial racemic mixture of alcohols would be acetylated. So, one has to make a compromise in order to have both alcohol and acetyl aziridine in a good enantiomeric excess. Stopping the reaction too soon and the alcohol reagent is still with a very low enantiomeric excess and stopping the reaction too late the acetyl product no longer has a high enantiomeric excess due to full conversion of the racemic substrate.

This promising result will be further investigated in order to optimize the reaction conditions such as the chosen enzyme, acylation reagent, temperature, solvent, etc. to obtain higher *ees*. This methodology will have great utility due to the high value of the enantiomerically pure bicyclic vinyl aziridine in the synthesis of natural products.

4. Conclusions

New bicyclic vinyl aziridines were synthetized based on the Kaplan aziridine synthesis[8] in low to good yields, expanding the scope and application of this methodology.

The scope of nucleophilic ring-opening of these aziridines in physiological conditions was extensively studied. Moderate to good results were obtained with thiols, anilines and azide nucleophiles, and their ring-opening products were characterised by ^{1}H, ^{13}C NMR and MS.

It was proved that water can be an effective solvent for this kind of reactions, sometimes providing better yields than similar reactions in organic solvents, in addition of being a cheaper and more environmental friendly solvent.

The best results were obtained with biological thiols like cysteine, which prompted the study of cysteine-containing peptides modification with the bicyclic vinyl aziridines. Preliminary results show that the bicyclic vinyl aziridine can potentially be used for bioconjugation by specifically binding to the thiol moiety of cysteine-containing peptides. More is being done in this topic in order to find a useful biological strategy taking advantage of this property.

Exploratory results show that employing an enzymatic methodology for the enantiomeric resolution of secondary alcohols, it is possible to separate both enantiomers of the bicyclic vinyl aziridine. This topic will be further explored in order to optimize the reaction conditions to obtain the best enantiomeric excesses possible. The main objective will be to separate the enantiomers of a *N*-protected bicyclic vinyl aziridine, to obtain two extremely valuable precursors for enantiomerically pure natural products or other molecules.

5. Materials and methods

General remarks.

All chemicals, reagents and solvents for the synthesis of the compounds were of analytical grade, purchased from commercial sources, namely Sigma-Aldrich®, Merck and Alfa Aesar and these were used without further purification.

Calcitonin Acetyl salmon [MM=3473.8], was purchased from Sigma Aldrich and was stored at --20ºC. **Amino Acid Sequence:**

Ac-**Cys**-Ser-Asn-Leu-Ser-Thr-**Cys**-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH2 [Disulfide bridge: 1-7]

^{1}H and ^{13}C NMR spectra were measured on an Ultrashield Bruker Avance II 300 spectrometer. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak.

Low resolution mass spectroscopy was performed in a triple quadrupole mass spectrometer Micromass Quattro Micro API, Waters. High resolution mass spectroscopy performed a LTQ Orbitrap XL mass spectrometer, Thermo Fischer Scientific, Bremen, Germany.

Low resolution ESI mass spectra of the calcitocin experiments were carried on a ion trap mass analyser (Thermo Scientific LCQ Fleet Ion Trap LC/MS) equipped with an electrospray interface. Pro Mass for Xcalibur (Version 2.8) was used as the deconvolution software.

UV radiation was performed on a home-made equivalent Rayonet reactor (Model RPR-200), containing 16 UV lamps (8W, 2W at 254 nm).

GLC analysis of aziridine **33** was performed using Trace Focus Unicam, FID detection, using capillary column HYDRODEX beta-6TBDM (25m x 0.4 mm x 0.25 mm). Injector: 200ºC; Detector 200ºC; Split ratio: 20; Split flow: 10 mL/min, 80kPa; Oven: 100ºC.

Buffer preparation.

PBS Buffer pH 7.4.

2g of NaCl, 0.05g of KCl, 0.36g of Na_2HPO_4 and 0.06g of KH_2PO_4 were dissolved in 200 mL of H_2O and the pH adjusted to 7.4 with a diluted solution of HCl. Water was added until the volume of the solution reached 250 mL.

Phosphate buffer 1M pH 7.4.

33.1g of K_2HPO_4 and 8.17g of KH_2PO_4 were dissolved in 200 mL of H_2O and the pH adjusted to 7.4 with a diluted solution of HCl. Water was added until the volume of the solution reached 250 mL.

Acetate buffer 50 mM pH 7.4.

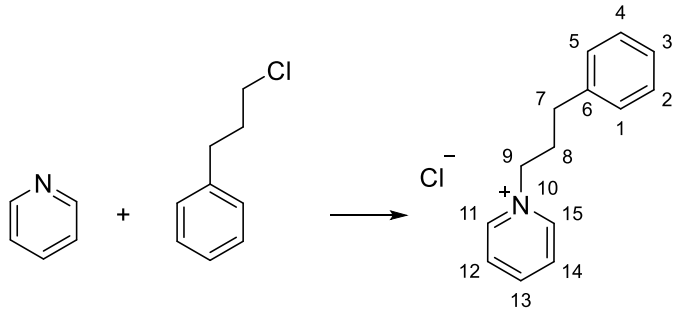
1.025 g of Sodium acetate was dissolved in 250 mL of Milli-Q water and the pH adjusted to 7.4 with a diluted ammonia solution.

Acetate buffer 50 mM pH 8.

1.025 g of Sodium acetate was dissolved in 250 mL of Milli-Q water and the pH adjusted to 8 with a diluted ammonia solution.

Pyridinium salt synthesis 25-29.

1-(3-Phenylpropyl)pyridinium chloride (25).



25

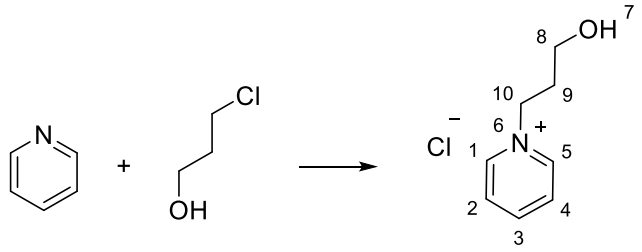
Pyridine (3 mL, 37.2 mmol, 7.6 eq.) was slowly added to 3-chloropropylbenzene (0.716 mL, 4.9 mmol). The solution was kept in a closed vessel at 124ºC for 24 hours. After cooling, the excess pyridine was evaporated under vacuum and the pyridinium salt **25** was obtained as a yellow oil in quantitative yield.

^{1}H NMR (300 MHz, D_2O) δ 8.75 (d, *J* = 5.5 Hz, 2H, H11 and H15), 8.48 (tt, *J* = 7.9, 1.3 Hz, 1H, H13), 7.98 (t, *J* = 7.1 Hz, 2H, H12 and H14), 7.35 – 7.22 (m, 5H, H1-5), 4.61 (t, *J* = 7.2 Hz, 2H, H9), 2.75 (t, *J* = 7.4 Hz, 2H, H7), 2.44 – 2.31 (m, 2H, H8).

^{13}C NMR (100 MHz, D_2O) δ 145.5 (CAr), 144.1 (CAr), 140.2 (CAr), 128.8 (CAr), 128.4 (CAr), 128.1 (CAr), 126.5 (CAr), 61.3 (C9), 31.5 (C7), 31.3 (C8).

MS(ESI) m/z: 197.8 **[M]$^+$**

1-(3-Hydroxypropyl)pyridinium chloride (26).



26

Pyridine (0.52 mL, 6.45 mmol, 1.1 eq.) was slowly added to 3-chloropropanol (0.54 mL, 5.86 mmol). The solution was kept in a closed vessel at 124ºC for 24 hours. After cooling, the excess pyridine was evaporated under vacuum and the pyridinium salt **26** was obtained as a yellow oil in quantitative yield.

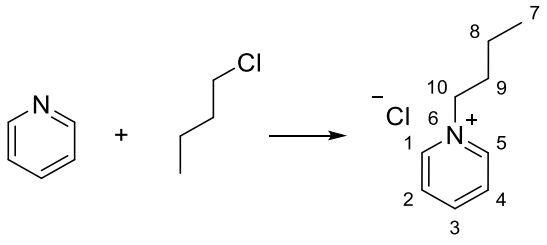
^{1}H NMR (300 MHz, D2O) δ 8.88 (d, J= 5.6Hz, 2H, H1 and H5), 8.55 (tt, J= 7.9, 1.2 Hz, 1H, H3), 8.07 (t, J= 7.1 Hz, 2H, H2 and H4), 4.72 (t, J=7.2 Hz, 2H, H10), 3.66 (t, J= 6.0 Hz, 2H, H8), 22.30 – 2.20 (m, 2H, H9).

13C NMR (100 MHz, D2O) δ 145.7 C3 or C1/C5), 144.4 (C3 or C1/C5), 128.2 (C2 and C4), 59.0 (C8 or C10), 57.7 (C8 or C10), 32.6 (C9).

MS(ESI) m/z: 137.7 **[M]$^+$**

Spectral data were in accordance with the literature[14a].

1-Butylpyridinium chloride (27).



27

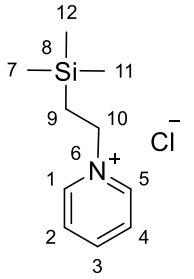
Pyridine (0.52 mL, 6.45 mmol, 1.1 eq.) was slowly added to chlorobutane (0.67 mL, 5.86 mmol). The solution was kept in a closed vessel at 124ºC for 24 hours. After cooling, the excess pyridine was evaporated under vacuum and the pyridinium salt **27** was obtained as a colorless oil in quantitative yield.

^{1}H NMR (300 MHz, D_2O) δ 8.87 (d, *J* = 5.4 Hz, 2H, H1 and H5), 8.55 (tt, *J* = 7.9, 1.3 Hz, 1H, H3), 8.08 (t, *J* = 6.8 Hz, 2H, H2 and H4), 4.63 (t, *J* = 7.4 Hz, 2H, H10), 2.06 – 1.96 (m, 2H, H9), 1.43 – 1.31 (m, 2H, H8), 0.94 (t, *J* = 7.4 Hz, 3H, H7).

^{13}C NMR (100 MHz, D_2O) δ 145.4 (C3 or C1/C5), 144.2 (C3 or C1/C5), 128.2 (C2 and C4), 61.7 (C10), 32.5 (C9), 18.7 (C8), 12.6 (C7).

MS(ESI) m/z: 135.8 **$[M+H]^+$**

1-(2-(Trimethylsilyl)ethyl)pyridinium chloride (28).



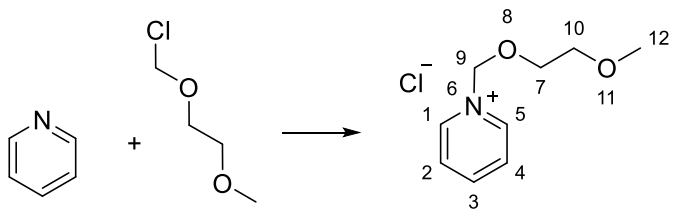
28

Pyridinium salt **28** was available in the laboratory contaminated with pyridinium chloride. The compound was dissolved in water and a saturated solution of sodium bicarbonate was added until the solution pH reached 8. Following ether extraction, the aqueous phase was evaporated giving the product **28** contaminated with bicarbonate salts. Acetonitrile was added and filtered. Evaporation of the acetonitrile gave pure pyridinium salt **28** as a yellow oil.

^{1}H NMR (300 MHz, D_2O) δ 8.88 (d, J = 5.4 Hz, 2H, H1 and H5), 8.52 (tt, J = 7.9, 1.4 Hz, 1H, H3), 8.05 (t, J = 6.9 Hz, 2H, H2 and H4), 4.72 – 4.64 (m, 2H, H10), 1.47 – 1.38 (m, 2H, H9), 0.13 (s, 9H, H7, H11 and H12).

^{13}C NMR (100 MHz, D_2O) δ 145.2 (C3 or C1/C5), 143.5 (C3 or C1/C5), 128.1 (C2 and C4), 59.8 (C10), 19.5 (C9), -2.9 (C7, C11 and C12).

1-((2-Methoxyethoxy)methyl)pyridin-1-ium chloride (29).



29

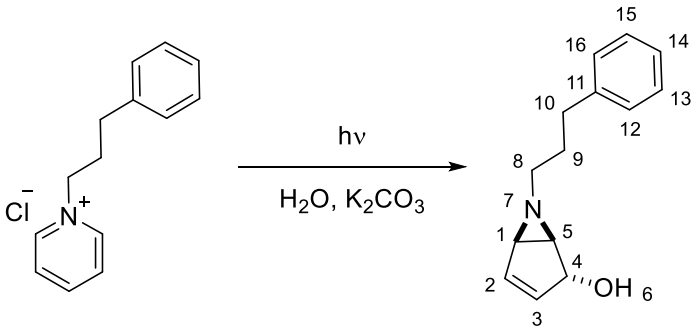
Pyridine (0.142 mL, 1.76 mmol) was slowly added to 2-methoxyethoxymethyl chloride (1 mL, 8.8 mmol, 5 eq.) in an ice bath under N_2. After 5 minutes the solution was kept at room temperature for 24 hours. After cooling, the excess pyridine was evaporated under vacuum and the resulting oil dissolved in 2 mL of H_2O. A saturated solution of sodium bicarbonate was added until the

solution pH reached 8. Following ether extraction (3x5mL), the aqueous phase was evaporated giving the product **29** contaminated with bicarbonate salts. Acetonitrile (2 mL) was added and filtered. Evaporation of the acetonitrile gave pure pyridinium salt **29** as an orange oil in 33% yield (118 mg).

^{1}H NMR (300 MHz, D_2O) δ 8.99 (d, *J* = 6.3 Hz, 2H, H1 and H5), 8.68 (t, *J* = 7.3 Hz, 1H, H3), 8.18 (t, *J* = 6.7 Hz, 2H, H2 and H4), 5.98 (s, 2H, H9), 3.88 (dd, *J* = 4.2, 2.6 Hz, 2H, H7, H7 or H10), 3.65 (dd, *J* = 4.2, 2.5 Hz, 2H, H7 or H10), 3.32 (d, *J* = 1.3 Hz, 3H, H12).
^{13}C NMR (100 MHz, D_2O) δ 147.5 (C3 or C1/C5), 142.6 (C3 or C1/C5), 128.2 (C2 and C4), 89.0 (C9), 70.5 (C7 or C10), 70.0 (C7 or C10), 58.0 (C12).
Spectral data were in accordance with the literature[14b].

Synthesis of bicyclic vinyl aziridines (30-35).

(1*SR*,2*SR*,5*SR*)-6-(3-Phenylpropyl)-6-azabicyclo[3.1.0]hex-3-en-2-ol (30).

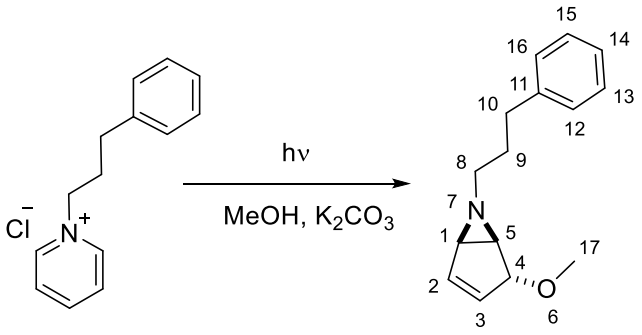


25 **30**

Pyridinium salt **25** (128 mg, 0.55 mmol) and potassium carbonate (93mg, 0.67 mmol, 1.14 eq.) were dissolved in 10 mL of water, inside a quartz tube (Ø = 12mm) The solution was deoxygenised under N_2 for 30 minutes, placed inside the Rayonet equivalent reactor, and irradiated for 100 hours at room temperature. The solvent was evaporated under vacuum and the solid dissolved in 20 mL of dichloromethane. The solution was stirred for 15 minutes and filtered. The crude solid was washed again with 2x20 mL of dichloromethane, stirred and filtered. The solvent was evaporated under vacuum and the resulting oil purified by silica gel column chromatography (EtOAc) to give the bicyclic vinyl aziridine **30** as a brown oil in 19% yield (22.5 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 – 7.16 (m, 5H, H12-16), 6.29 (d, *J* = 5.5 Hz, 2H, H3), 5.88 (d, *J* = 5.0 Hz, 2H, H2), 4.49 (s, 1H, H4), 2.69 – 2.64 (m, 2H, H10), 2.48 (s, 1H, H5), 2.44 (s, 1H, H1), 2.41 – 2.23 (m, 2H, H8), 1.96 – 1.86 (m, 2H, H9).
^{13}C NMR (100 MHz, $CDCl_3$) δ 142.0 (C11), 137.2 (C2), 136.1 (C3), 128.5 (C13 and C15), 128.4 (C12 and C16), 126.0 (C14), 75.3 (C4), 57.8 (C8), 50.6 (C1), 46.8 (C5), 33.7 (C10), 31.2 (C9).

(1*SR*,4*SR*,5*SR*)-4-Methoxy-6-(3-phenylpropyl)-6-azabicyclo[3.1.0]hex-2-ene (31).

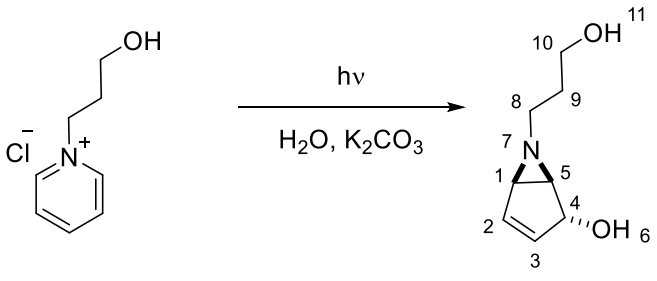


25 **31**

Pyridinium salt **25** (350 mg, 1.5 mmol) and potassium carbonate (248.8 mg, 1.8 mmol, 1.2 eq.) were dissolved in 30 mL of methanol, inside a quartz tube (Ø = 12mm) The solution was deoxygenised under N_2 for 30 minutes, placed inside the Rayonet equivalent reactor, and irradiated for 48 hours at room temperature. The solvent was evaporated under vacuum and the solid dissolved in 20 mL of dichloromethane. The solution was stirred for 15 minutes and filtered. The crude solid was washed again with 2x20 mL of dichloromethane, stirred and filtered. The solvent was evaporated under vacuum and the resulting oil purified by silica gel column chromatography (EtOAc: Hex = 6:4) to give the bicyclic vinyl aziridine **31** as a brown oil in 10% yield (34.4 mg).

^{1}H NMR (300 MHz, D_2O) δ 7.33 – 7.13 (m, 5H, H11-12), 6.32 (d, *J* = 5.7 Hz, 1H, H3), 5.90 (dd, *J* = 5.7, 1.2 Hz, 1H, H2), 4.17 (d, *J* = 1.7 Hz, 1H, H4), 3.41 (s, 3H, H17), 2.75 – 2.58 (m, 2H, H10), 2.48 (s, 2H, H1 and H5), 2.46 – 2.23 (m, 2H, H8), 2.00 – 1.83 (m, 2H, H9).
MS(ESI) m/z: 229.8 **[MH]$^+$**

(1*SR*,2*SR*,5*SR*)-6-(3-Hydroxypropyl)-6-azabicyclo[3.1.0]hex-3-en-2-ol (32).



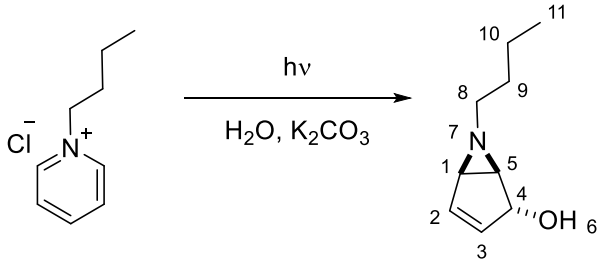
26 **32**

Pyridinium salt **26** (167 mg, 0.96 mmol) and potassium carbonate (159.6 mg, 1.115 mmol, 1.2 eq.) were dissolved in 10 mL of water, inside a quartz tube (Ø = 12mm). The solution was deoxygenised under N_2 for 30 minutes, placed inside the Rayonet equivalent reactor, and irradiated for

20 hours at room temperature. The solvent was evaporated under vacuum and the solid dissolved in 20 mL of dichloromethane. The solution was stirred for 15 minutes and filtered to a balloon. The crude solid was washed again with 2x20 mL of dichloromethane, stirred and filtered. The solvent was evaporated under vacuum to give the bicyclic vinyl aziridine **32** as a brown oil in 88% yield (131.1 mg), higher than the 82% yield reported in the literature[14a].

^{1}H NMR (300 MHz, D_2O) δ 6.32 (dt, *J* = 5.7, 1.2 Hz, 1H, H3), 5.88 – 5,86 (m, 1H, H2), 4.49 (d, *J* = 1.8Hz, 1H, H4), 3.65 (t, *J* = 6.6 Hz, 2H, H10), 2.80 (s, 1H, H5), 2.65 (dd, *J* = 4.4, 1.8 Hz, 1H, H1), 2.48 – 2.34 (m, 2H, H8), 1.83 – 1.74 (m, 2H, H9).
^{13}C NMR (100 MHz, D_2O) δ 136.2 (C2), 135.5 (C3), 73.9 (C4), 59.6 (C10), 53.2 (C8), 49.6 (C1), 46.9 (C5), 31.0 (C9).
MS(ESI) m/z: 155.8 **[MH]$^+$**
Spectral data were in accordance with the literature[14a].

(1*SR*,2*SR*,5*SR*)-6-Butyl-6-azabicyclo[3.1.0]hex-3-en-2-ol (33).

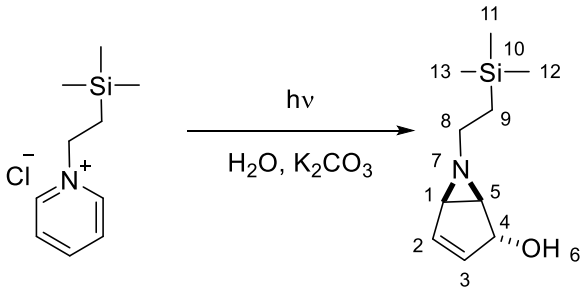


27 **33**

Pyridinium salt **27** (165 mg, 0.96 mmol) and potassium carbonate (159.6 mg, 1.115 mmol, 1.2 eq.) were dissolved in 10 mL of water, inside a quartz tube (Ø = 12mm). The solution was deoxygenised under N_2 for 30 minutes, placed inside the Rayonet reactor, and irradiated for 20 hours at room temperature. The solvent was evaporated under vacuum and the solid dissolved in 20 mL diethyl ether. The solution was stirred for 15 minutes and filtered to a balloon. The crude solid was washed again with 2x20 mL of diethyl ether, stirred and filtered. The solvent was evaporated under vacuum to give the bicyclic vinyl aziridine **33** as a brown oil in 65% yield (95.6 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.29 (dd, *J* = 5.6, 1.2 Hz, 1H, H3), 5.95 – 5.78 (m, 1H, H2), 4.48 (d, *J* = 7.1 Hz, 1H, H4), 2.48 (s, 1H, H5), 2.43 (dd, *J* = 4.3, 1.8 Hz, 1H, H1), 2.40 – 2.11 (m, 2H, H8), 1.64 – 1.46 (m, 2H, H9), 1.34 (dq, *J* = 14.3, 7.2 Hz, 2H, H10), 0.90 (t, *J* = 7.3 Hz, 3H, H11).
^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2 (C2), 136.1 (C3), 75.2 (C4), 58.2 (C8), 50.6 (C1), 46.8 (C5), 31.8 (C9), 20.7 (C10), 14.2 (C11).
HRMS-ESI m/z calcd. for $C_9H_{16}NO$ **[MH]$^+$**: 154.12319; **obtained** 154.12239.

(1*SR*,2*SR*,5*SR*)-6-(2-(Trimethylsilyl)ethyl)-6-azabicyclo[3.1.0]hex-3-en-2-ol (34).

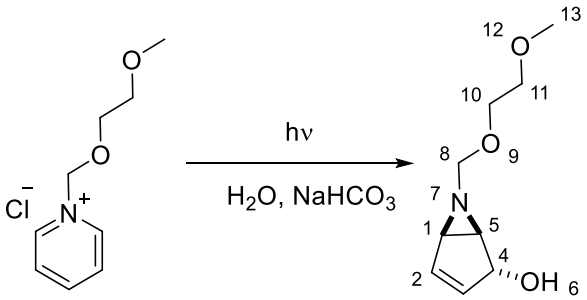


28 **34**

Pyridinium salt **28** (126.4 mg, 0.59 mmol) was dissolved in 3.33 mL of water and 6.66 mL of an aqueous saturated solution of $NaHCO_3$ was added. The solution transferred to a quartz tube (Ø = 12mm). The solution was deoxygenised under N_2 for 30 minutes, placed inside the Rayonet reactor, and irradiated for 20 hours at room temperature. The solvent was evaporated under vacuum and the solid dissolved in 20 mL diethyl ether. The solution was stirred for 15 minutes and filtered to a balloon. The crude solid was washed again with 2x20 mL of diethyl ether, stirred and filtered. The solvent was evaporated under vacuum to give the bicyclic vinyl aziridine **34** as a brown oil in 24% yield (27.4 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (d, *J* = 5.6 Hz, 1H, H3), 5.90 – 5.77 (m, 1H, H2), 4.44 (d, *J* = 1.3 Hz, 1H, H4), 2.46 (s, 1H, H5), 2.40 (dd, *J* = 4.4, 1.8 Hz, 1H, H1), 2.38 – 2.12 (m, 2H, H8), 0.94 – 0.72 (m, 2H, H9), -0.04 (s, 9H, H11-13).
^{13}C NMR (100 MHz, $CDCl_3$) δ 137.3 (C2), 135.7 (C3), 75.0 (C4), 54.0 (C8), 50.6 (C1), 46.8 (C5), 17.3 (C9), -1.6 (C11-13).

(1*SR*,2*SR*,5*SR*)-6-((2-Methoxyethoxy)methyl)-6-azabicyclo[3.1.0]hex-3-en-2-ol (35).



29 **35**

Pyridinium salt **29** (121.5 mg, 0.60 mmol) was dissolved in 3.33 mL of water and 6.66 mL of an aqueous saturated solution of $NaHCO_3$ was added. The solution transferred to a quartz tube (Ø = 12mm). The solution was deoxygenised under N_2 for 30 minutes, placed inside the Rayonet reactor,

and irradiated for 20 hours at room temperature. The solvent was evaporated under vacuum and the solid dissolved in 20 mL dichloromethane. The solution was stirred for 15 minutes and filtered to a balloon. The crude solid was washed again with 2x20 mL of dichloromethane, stirred and filtered. The solvent was evaporated under vacuum to give the bicyclic vinyl aziridine **35** as a brown oil in 23% yield (25.6 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.25 (d, *J* = 5.5 Hz, 1H, H3), 5.89 (d, *J* = 4.7 Hz, 1H, H2), 4.48 (s, 1H, H4), 3.93 (dd, *J* = 37.8, 8.3 Hz, 2H, H8), 3.76 – 3.70 (m, 2H, H10 or H11), 3.54 – 3.50 (m, 2H, H10 or H11), 3.35 (s, 3H, H13), 2.75 (s, 1H, H5), 2.68 (dd, *J* = 4.3, 1.6 Hz, 1H, H1).
Spectral data was in accordance with the literature[14b].

Stability experiments of aziridines.

Aziridine 32 in water.

Aziridine **32** (10 mg, 0.065 mmol) was dissolved in 6 mL of water. The solution was left stirring at 37ºC for 67 hours. The solvent was evaporated and the compound analysed by ^{1}H NMR (solvent D_2O), showing a regression of 9% of the aziridine **32** to the pyridinium salt **26.**

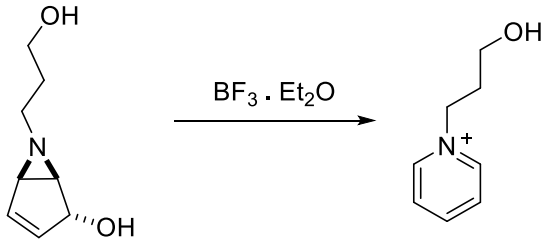
Aziridine 32 in PBS.

Aziridine **32** (10 mg, 0.065 mmol) was dissolved in 6 mL of PBS. The solution was left stirring at 37ºC for 67 hours. The solvent was evaporated and the compound analysed by ^{1}H NMR (solvent D_2O), showing a regression of 12% of the aziridine **32** to the pyridinium salt **26.**

Aziridine 33 in phosphate buffer 1M pH 7.4.

Aziridine **32** (10 mg, 0.065 mmol) was dissolved in 6 mL of phosphate buffer 1M pH 7.4. The solution was left stirring at 37ºC for 67 hours. The solvent was evaporated and the compound analysed by ^{1}H NMR (solvent D_2O), showing complete degradation of the aziridine **33**.

Aziridine 32 in a reaction medium with Lewis acid BF_3

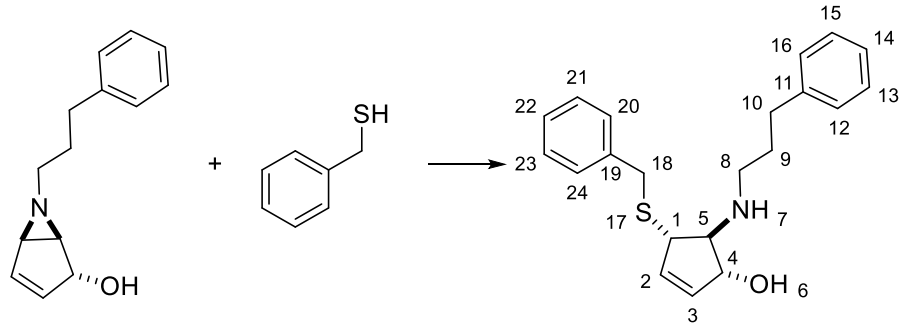


32 **26**

Aziridine **32** (8.3 mg, 0.053 mmol) was dissolved in 2 mL of CH_2Cl_2. The solution was kept stirring in an ice bath and BF3 · Et_2O (3.3 µL, 0.5 eq.) was added and left at room temperature for 2 hours. The solvent was evaporated and the crude analysed by ^1H NMR (solvent D_2O), showing full conversion of the aziridine to the pyridinium salt **26**.

Aziridine ring-opening with thiols (36-40).

(1*SR*,4*RS*,5*RS*)-4-(Benzylthio)-5-((3-phenylpropyl)amino)cyclopent-2-en-1-ol (36).

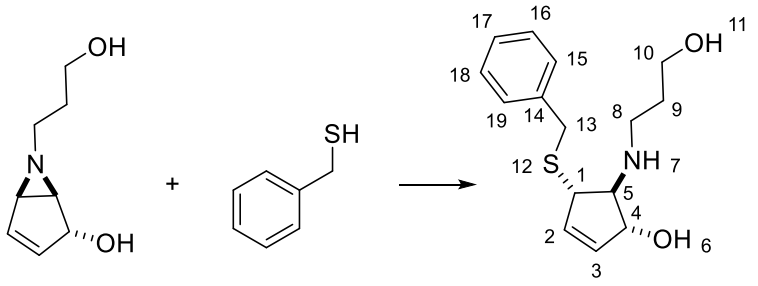


30 **36**

Aziridine **30** (13.4 mg, 0.062 mmol) was dissolved in 6 mL of water. Benzyl mercaptan (8.7 µL, 0.074 mmol, 1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC for 24 hours. The aqueous phase was extracted with 3x10 mL dichloromethane and the organic phases combined and evaporated under vacuum, following purification by silica gel column chromatography (EtOAc) to give the ring-opening product **36** as a yellow amorphous solid in 55% yield (11.7 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 – 7.17 (m, 10H, Ar), 5.81 (d, *J* = 5.7 Hz, 1H, H3), 5.74 (d, *J* = 5.8 Hz, 1H, H2), 4.49 (s, 1H, H4), 3.78 (s, 2H, H18), 3.39 (d, *J* = 1.7 Hz, 1H, H1), 3.07 (t, *J* = 4.0 Hz, 1H, H5), 2.74 – 2.67 (m, 2H, H8), 2.63 (t, *J* = 7.7 Hz, 2H, H10), 1.84 – 1.74 (m, 2H, H9).
^{13}C NMR (100 MHz, $CDCl_3$) δ 142.0 (C19 or C11), 138.4 (C19 or C11), 134.0 (C2), 133.9 (C3), 129.1 (Ar), 128.7 (Ar), 128.5 (Ar), 127.3 (C22 or C14), 126.0 (C22 or C14), 81.9 (C4), 74.2 (C5), 53.6 (C1), 47.7 (C8), 35.9 (C18), 33.6 (C10), 31.7 (C9).
HRMS-ESI m/z calcd. for $C_{15}H_{22}NO_2S$ $[MH]^+$: 340.17351; found 340.17111.

(1*SR*,4*RS*,5*RS*)-4-(Benzylthio)-5-((3-hydroxypropyl)amino)cyclopent-2-en-1-ol (37).



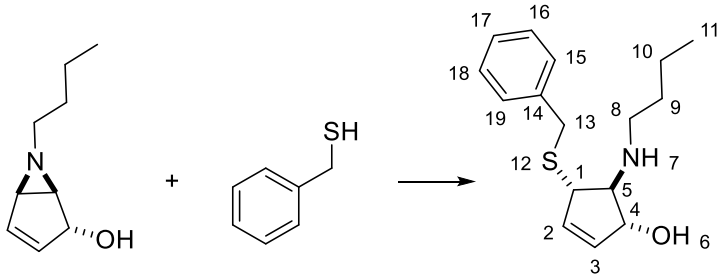
32 **37**

Aziridine **32** (10 mg, 0.065 mmol) was dissolved in 6 mL of PBS buffer. Benzyl mercaptan (9 µL, 0.077 mmol, 1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC for 96

hours. The aqueous phase was extracted with 3x10 mL dichloromethane and the organic phases combined and evaporated under vacuum, following purification by silica gel column chromatography (CH_2Cl_2: MeOH = 9:1) to give the ring-opening product **37** as a yellow amorphous solid in 61% yield (10.9 mg, 0.039 mmol).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.22 (m, 5H, H14-H17), 5.80 (dt, *J* = 5.7, 1.8 Hz, 1H, H3), 5.72 – 5.69 (m, 1H, H2), 4.59 (d, *J* = 1.8 Hz, 1H, H4), 3.78 (d, *J* = 0.8 Hz, 2H, H13), 3.74 (t, *J* = 5.6 Hz, 2H, H10), 3.47 – 3.45 (m, 1H, H1), 3.11 – 3.08 (m, 1H, H5), 2.94 (t, *J* = 6.0 Hz, 2H, H8), 1.70 (dd, J = 9.5, 5.4 Hz, 2H, H9).
^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3 (C14), 133.8 (C2 and C3), 129.1 (C16 and C18), 128.8 (C15 and C19), 127.4 (C17), 81.4 (C4), 74.0 (C5), 63.4 (C10), 53.1 (C1), 47.8 (C8), 35.8 (C13), 31.0 (C9).
HRMS-ESI m/z calcd. for $C_{15}H_{22}NO_2S$ $[MH]^+$: 280.13712; found 280.13491.

(1*SR*,4*RS*,5*RS*)-4-(Benzylthio)-5-(butylamino)cyclopent-2-en-1-ol (38).

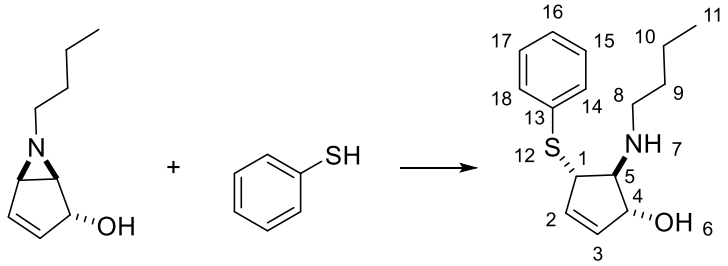


33 **38**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 0.5 mL of PBS buffer. Benzyl mercaptan (22.9 µL, 0.195 mmol, 3 eq.) was added and the solution was left stirring in a water bath at 37ºC for 24 hours. The aqueous phase was extracted with 3x10 mL dichloromethane and the organic phases combined and dried over magnesium sulphate, filtered and evaporated under vacuum, following purification by silica gel column chromatography (EtOAc: Et_3N = 10:0.1) to give the ring-opening product **38** as a yellow amorphous solid in 75% yield (13.5 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.19 (m, 5H, H14-H17), 5.83 (dt, *J* = 5.7, 1.8 Hz, 1H, H3), 5.76 (d, *J* = 5.7 Hz, 1H, H2), 4.48 (d, *J* = 1.2 Hz, 1H, H4), 3.80 (d, *J* = 1.4 Hz, 2H, H13), 3.38 (s, 1H, H1), 3.09 – 3.05 (m, 1H, H5), 2.73 – 2.55 (m, 2H, H8), 1.50 – 1.20 (m, 4H, H9 and H10), 0.91 (t, *J* = 7.2 Hz, 3H, H11).
^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4 (C14), 134.1 (C2), 133.9 (C3), 129.1 (C16 and C18), 128.7 (C15 and C19), 127.3 (C17), 82.1 (C4), 74.4 (C5), 53.6 (C1), 48.1 (C8), 35.9 (C13), 32.4 (C9), 20.6 (C10), 14.1 (C11).
HRMS-ESI m/z calcd. for $C_{16}H_{24}NOS$ $[MH]^+$: 278.15786; found 278.15553.

(1*SR*,4*RS*,5*RS*)-5-(Butylamino)-4-(phenylthio)cyclopent-2-en-1-ol (39).

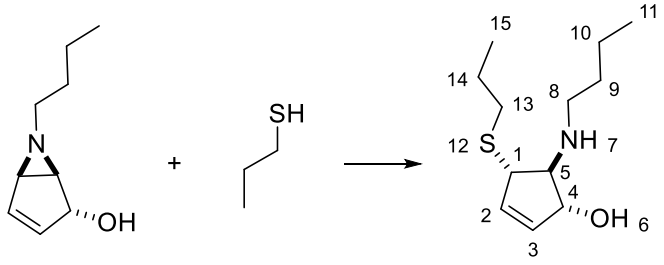


33 **39**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 0.5 mL of PBS buffer. Thiophenol (20 µL, 0.195 mmol, 3 eq.) was added and the solution was left stirring in a water bath at 37ºC for 3 hours. The aqueous phase was extracted with 3x10 mL dichloromethane and the organic phases combined and dried over magnesium sulphate, filtered and evaporated under vacuum, following purification by silica gel column chromatography (EtOAc: Et_3N = 10:0.1) to give the ring-opening product **39** as a yellow amorphous solid in 86% yield (14.7 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 – 7.43 (m, 2H, H14 and H18), 7.36 – 7.24 (m, 3H, H15-H17), 5.90 (ddd, *J* = 5.7, 1.8, 1.0 Hz, 1H, H3), 5.83 (dt, *J* = 5.7, 1.7 Hz, 1H, H2), 4.47 – 4.43 (m, 1H, H4), 3.83 (d, *J* = 1.0 Hz, 1H, H1), 3.13 (t, *J* = 3.6 Hz, 1H, H5), 2.67 (t, *J* = 7.2 Hz, 2H, H8), 1.45 (dt, *J* = 14.1, 4.2 Hz, 2H, H9), 1.30 (td, *J* = 14.1, 7.1 Hz, 2H, H10) 0.89 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 134.3 (C2), 134.2 (C3), 134.0 (C13), 133.3 (C14 and C18), 129.2 (C15 and C17), 128.0 (C16), 82.3 (C4), 73.8 (C5), 57.1 (C1), 48.0 (C8), 32.4 (C9), 20.5 (C10), 14.1 (C11)
HRMS-ESI m/z calcd. for $C_{15}H_{22}NOS$ $[MH]^+$: 264.14221; found 264.14010.

(1*SR*,4*RS*,5*RS*)-5-(butylamino)-4-(propylthio)cyclopent-2-en-1-ol (40).



33 **40**

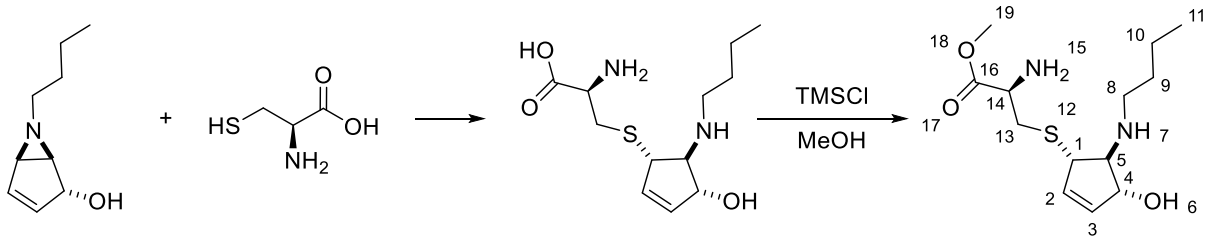
Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL of PBS buffer. Propanethiol (17.7 µL, 0.195 mmol, 3 eq.) was added and the solution was left stirring in a water bath at 37ºC for 117

hours. The aqueous phase was extracted with 3x10 mL dichloromethane and the organic phases combined and dried over magnesium sulphate, filtered and evaporated under vacuum, following purification by silica gel column chromatography (EtOAc: Et_3N = 10:0.1) to give the ring-opening product **40** as a yellow amorphous solid in 61% yield (9.1 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.88 – 5.79 (m, 2H, H2 and H3), 4.55 (d, *J* = 3.3 Hz, 1H, H4), 3.47 (d, *J* = 4.3 Hz, 1H, H1), 3.15 – 3.09 (m, 1H, H5), 2.78 (t, *J* = 7.2 Hz, 2H, H8), 2.59 – 2.52 (m, 2H, H13), 1.71 – 1.57 (m, 2H, H14), 1.57 – 1.47 (m, 2H, H9), 1.37 (dq, *J* = 14.2, 7.2 Hz, 2H, H10), 1.00 (t, *J* = 7.3 Hz, 3H, H15), 0.93 (t, *J* = 7.3 Hz, 3H, H11).
^{13}C NMR (100 MHz, $CDCl_3$) δ 134.6 (C2), 133.6 (C3), 82.1 (C4), 74.3 (C5), 53.5 (C1), 48.2 (C8), 32.9 (C13), 32.3 (C9), 23.4 (C14), 20.6 (C10), 14.1 (C11), 13.7 (C15).
HRMS-ESI m/z calcd. for $C_{12}H_{24}NOS$ $[MH]^+$: 230.15786, found 230.15592.

Aziridine ring-opening with cysteines (44-47).

Methyl *S*-((1*SR*,4*RS*,5*SR*)-5-(butylamino)-4-hydroxycyclopent-2-en-1-yl)-*L*-cysteinate (44).



33 **44**

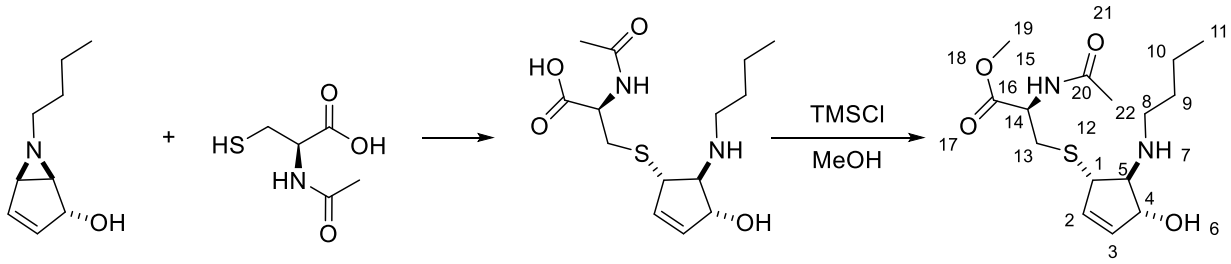
Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL of phosphate buffer 1 M pH 7.4. Cysteine (9.5 mg, 0.078 mmol, 1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC for 24 hours. Water was evaporated and the product extracted with methanol and filtered. Methanol was evaporated and 1 mL of dry methanol was added. TMSCl (49.5 µL, 0.39 mmol, 6 eq.) was added slowly at 0ºC and the solution was left stirring at room temperature for 48h. The solvent was evaporated and the red oil purified by silica gel column chromatography (EtOAc: MeOH: NH_3aq.25%= 10:0.3:0.2) to give the two diastereoisomers of the cysteine methylester derivative **44** as a yellow oil in 81% combined yield (15.2 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 – 5.83 (m, 1H, H3), 5.81 – 5.69 (m, 1H, H2), 4.54 – 4.53 and 4.49 – 4.47 (m, 1H, H4), 3.74 (s, 3H, H19), 3.72 – 3.64 (m, 1H, H14), 3.57 – 3.45 (m, 1H, H1), 3.19 – 3.06 (m, 1H, H5), 3.05 – 2.78 (m, 2H, H13), 2.84 – 2.69 (m, 2H, H8), 1.54 – 1.45 (m, 2H, H9), 1.44 – 1.26 (m, 2H, H10), 1.11 – 0.44 (m, 3H, H11).

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5 and 174.3 (C16), 134.8 and 134.5 (C3), 133.8 and 133.5 (C2), 82.0 and 81.7 (C4), 75.0 (C5), 54.9 and 54.5 (C14), 54.21 and 54.18 (C1), 52.5 (C19), 48.1 and 48. 0 (C8), 35.4 and 34.9 (C13), 32.4 and 32.2 (C9), 20.6 (C10), 14.1 (C11).

MS(ESI) m/z: 288.8 **[MH]$^+$**

Methyl *N*-acetyl-*S*-((1*SR*,4*RS*,5*SR*)-5-(butylamino)-4-hydroxycyclopent-2-en-1-yl)-*L*-cysteinate (45).

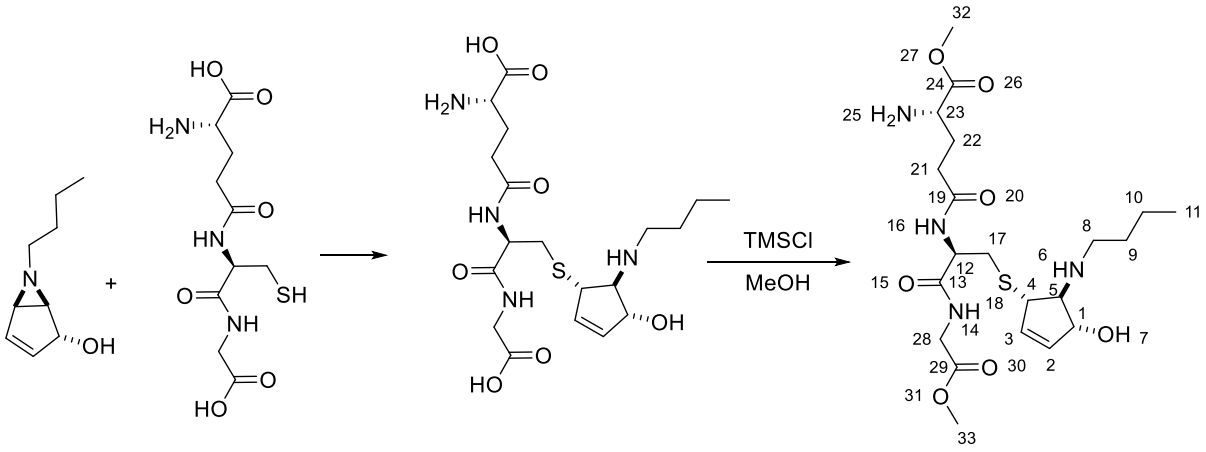


33 **45**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL of phosphate buffer 1 M pH 7.4. *N*-acetylcysteine (12.7 mg, 0.078 mmol, 1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC for 24 hours. Water was evaporated and the product extracted with methanol and filtered. Methanol was evaporated and 1 mL of dry methanol was added. TMSCl (49.5 µL, 0.39 mmol, 6 eq.) was added slowly at 0ºC and the solution was left stirring at room temperature for 48h. The solvent was evaporated and the yellow oil purified by silica gel column chromatography (EtOAc: MeOH: NH_3aq.25%= 8:1.5:0.1) to give the two diastereoisomers of the *N*-acetylcysteine methylester derivative **45** as a yellow oil in 86% combined yield (18.5 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.94 – 5.81 (m, 1H, H3), 5.83 – 5.74 (m, 1H, H2), 4.58 – 4.37 (m, 1H, H2), 3.75 (s, 3H, H19), 3.74 – 3.61 (m, 1H, H14), 3.53 – 3.50 (m, 1H, H1), 3.23 – 3.08 (m, 1H, H5), 3.07 – 2.81 (m, 2H, H13), 2.81 – 2.68 (m, 2H, H8), 2.04 (s, 3H, H22), 1.55 – 1.47 (m, 2H, H9), 1.40 – 1.33 (m, 2H, H10), 0.93 (t, *J* = 7.3 Hz, 3H, H11).

Methyl N^5-(3-(((1*SR*,4*RS*,5*SR*)-5-(butylamino)-4-hydroxycyclopent-2-en-1-yl)thio)-1-((2-methoxy-2-oxoethyl)amino)-1-oxopropan-2-yl)-*L*-glutaminate (46).

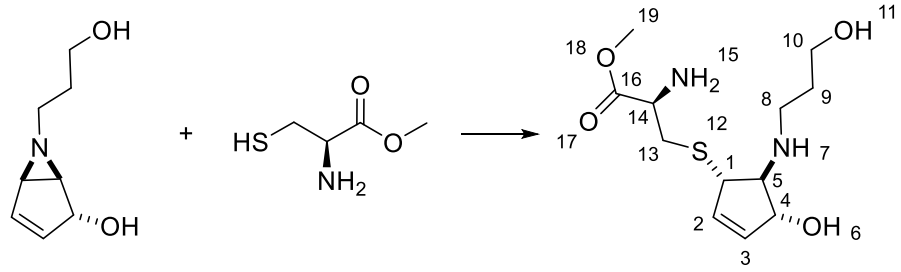


33 **46**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL of phosphate buffer 1 M pH 7.4. Glutathione (24 mg, 0.078 mmol, 1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC for 24 hours. Water was evaporated and the product extracted with methanol and filtered. Methanol was evaporated and 1 mL of dry methanol was added. TMSCl (49.5 µL, 0.39 mmol, 5 eq.) was added slowly at 0ºC and the solution was left stirring at room temperature for 48h. The solvent was evaporated and the yellow oil purified by silica gel column chromatography (EtOAc: MeOH: NH_3aq.25%= 8:2:0.5) to give the two diastereoisomers of the glutathione methylester derivative **46** as a yellow oil in 50% combined yield (15.9 mg).

^{1}H NMR (300 MHz, D_2O) δ 6.10 – 5.59 (m, 2H, H2 and H3), 4.61 – 4.56 (m, 2H, H1 and H12), 4.00 (s, 2H, H28), 3.72 (s, 6H, H32 and H33), 3.64 – 3.42 (m, 2H, H4 and H23), 3.12 – 3.03 (m, 1H, H5), 2.91 – 2.84 (m, 2H, H17), 2.76 – 2.68 (m, 2H, H8), 2.47 – 2.29 (m, 2H, H21), 2.24 – 1.69 (m, 2H, H22), 1.53 – 1.43 (m, 2H, H9), 1.38 – 1.26 (m, 2H, H10), 0.88 (t, *J* = 7.3 Hz, 3H, H11).
MS(ESI) m/z: 244.9 **$[MH_2]^{2+}$**, 488.8 **$[MH]^+$**

Methyl *S*-((1*SR*,4*RS*,5*SR*)-4-hydroxy-5-((3-hydroxypropyl)amino)cyclopent-2-en-1-yl)-*L*-cysteinate (47).



33 **47**

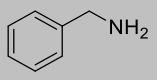
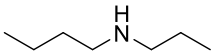
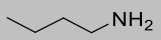
Aziridine **32** (30.5 mg, 0.2 mmol) was dissolved in 6 mL of PBS pH 7.4, followed by the addition of L-cysteine methyl ester hydrochloride (41 mg, 0.24 mmol, 1.2 eq.). The reaction mixture was stirred in a water bath at 37ºC for 4 hours, and the solvent was carefully evaporated under reduced pressure. The resulting oil was dissolved in water and purified by reverse-phase (RP-8) modified silica gel column chromatography, using water (20 mL) and 10% MeOH (10 mL) as eluent. The fraction containing the product eluted with 10% MeOH. The solvent was evaporated to give two diastereoisomers of the the cysteine methylester derivative **47** as a yellow oil in 47% yield (26.9 mg).

^{1}H NMR (300 MHz, D_2O) δ 6.02 – 5.84 (m, 2H, H2 and H3), 3.79 – 3.73 (m, 5H, H1, H14 and H19), 3.68 (t, *J* = 6.2 Hz, 2H, H10), 3.33 – 3.30 (m, 1H, H5), 3.17 – 3.01 (m, 2H, H8), 3.01 – 2.85 (m, 2H, H13), 1.98 – 1.75 (m, 2H, H9).

MS(ESI) m/z: 290.9 **[MH]$^+$. HRMS-ESI m/z calcd. for** $C_{12}H_{22}N_2O_4S$**[MH]$^+$:** 291.1300**; obtained** 291.1396**.**

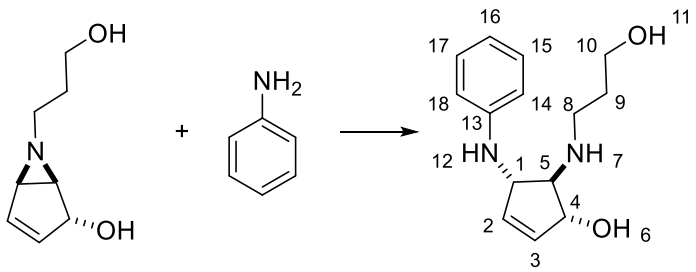
General procedure for the aziridine ring-opening with amines.

Bicyclic vinyl Aziridine (0.065 mmol) was dissolved in 6 mL of phosphate buffer 1M pH 7.4. Amine (5 eq.) was added and the solution was left stirring in a water bath at 37ºC. The reaction was followed by TLC (CH_2Cl_2:MeOH = 9: 1). After maximum 120 hours of reaction, the solvent was evaporated. The reaction crude was extracted with methanol, evaporated, and analysed by ^{1}H NMR. The performed reactions are presented in the following table.

Aziridine	Nucleophile (5 eq.)	Solvent	Reaction time (h)	Product	Yield (%)
33		6 mL Phosphate buffer 1M pH 7.4	48	Degradation	-
33		6 mL Phosphate buffer 1M pH 7.4	120	Degradation	-
33		6 mL Phosphate buffer 1M pH 7.4	120	Degradation	-

Aziridine ring-opening with anilines (48-50).

(1*SR*,4*RS*,5*SR*)-5-((3-Hydroxypropyl)amino)-4-(phenylamino)cyclopent-2-en-1-ol (48).



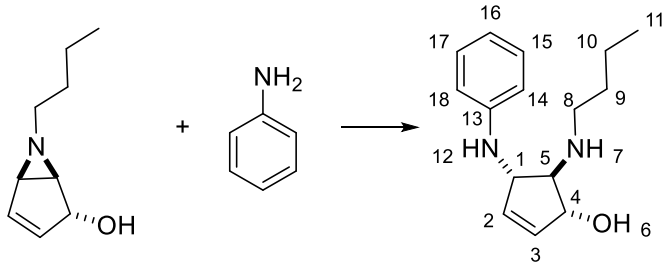
32 **48**

Aziridine **32** (10 mg, 0.065 mmol) was dissolved in 6 mL of PBS buffer pH 7.4. Aniline (29.6 µL 0.325 mmol, 5 eq.) was added and the solution was left stirring in a water bath at 37ºC for 120

hours. The solvent was evaporated under vacuum and the yellow oil was purified by silica gel column chromatography (CH_2Cl_2: MeOH = 8: 2) to give the ring-opening product **48** as a yellow oil in 55% yield (8.9 mg).

^{1}H NMR (300 MHz, D_2O) δ 7.27 (dd, *J* = 8.9, 7.1 Hz, 2H, H15 and H170), 6.95 – 6.78 (m, 3H, H14, H16, H18), 5.99 (dt, *J* = 6.0, 1.6 Hz, 1H, H2 or H3), 5.92 (dt, *J* = 6.0, 1.8 Hz, 1H, H2 or H3), 4.93 – 4.82 (m, 1H, H4), 4.60 (dd, *J* = 5.7, 1.4 Hz, 1H, H1), 3.62 (t, *J* = 6.1 Hz, 2H, H10), 3.56 – 3.45 (m, 1H, H5), 3.26 (td, *J* = 7.2, 3.9 Hz, 2H, H8), 2.01 – 1.73 (m, 2H, H9).
^{13}C NMR (100 MHz, $CDCl_3$) δ 146.2 (C13), 133.0 (C2 or C3), 132.9 (C2 or C3), 129.7 (C15 and C17), 119.5 (C16), 115.0 (C14 and C18), 76.2 (C4), 71.0 (C5), 60. 9 (C1), 58.8 (C10), 44.8 (C8), 28.0 (C9).
HRMS-ESI m/z calcd. for $C_{14}H_{21}N_2O_2$ $[MH]^+$: 249.15975, found 249.15841.

(1*SR*,4*RS*,5*SR*)-5-(Butylamino)-4-(phenylamino)cyclopent-2-en-1-ol (49).

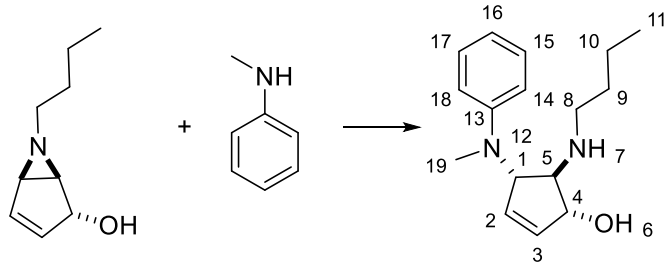


33 **49**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL of phosphate buffer 1M pH 7.4. Aniline (29.6 µL 0.325 mmol, 5 eq.) was added and the solution was left stirring in a water bath at 37ºC for 48 hours. The solvent was evaporated under vacuum and the yellow oil was purified by silica gel column chromatography (CH_2Cl_2: MeOH: Et_3N = 9.8:0.2:0.1) to give the ring-opening product **49** as a yellow oil in 43% yield (6.9 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 – 7.11 (m, 2H, H15 and H17), 6.75 (t, *J* = 7.3 Hz, 1H, H16), 6.72 – 6.66 (m, 2H, H14 and H18) 5.90 (dd, *J* = 12.5, 5.9 Hz, 2H, H2 and H3), 4.79 (d, *J* = 3.8 Hz, 1H, H4), 4.48 (d, *J* = 5.4 Hz, 1H, H1), 3.16 – 3.10 (m, 1H, H5), 2.97 – 2.81 (m, 2H, H8), 1.69 – 1.51 (m, 2H, H9), 1.4 – 1.28 (m, 2H, H10), 0.89 (t, *J* = 7.3 Hz, 3H, H11).
^{13}C NMR (100 MHz, $CDCl_3$) δ 146.6 (C13), 133.8 (C2 and C3), 133.4 (C2 and C3), 129.7 (C15 and C17), 118.7 (C16), 114.0 (C14 and C18), 78.5 (C4), 75.2 (C5), 62.1 (C1), 48.0 (C8), 30.6 (C9), 20.3 (C10), 13.9 (C11).
MS(ESI) m/z: 246.8 **$[MH]^+$**

(1*SR*,4*RS*,5*SR*)-5-(Butylamino)-4-(methyl(phenyl)amino)cyclopent-2-en-1-ol (50).



33 **50**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL of phosphate buffer 1M pH 7.4. *N*-methylaniline (35.2 µL, 0.325 mmol, 5 eq.) was added and the solution was left stirring in a water bath at 37ºC for 48 hours. The solvent was evaporated under vacuum and the yellow oil was purified by silica gel column chromatography (CH_2Cl_2: MeOH: Et_3N = 9.8:0.2:0.1) to give the ring-opening product **50** as a yellow oil in 34% yield (5.8 mg).

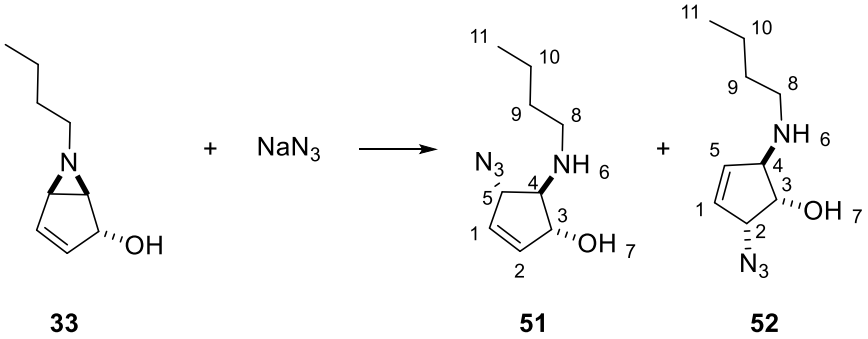
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 – 7.21 (m, 2H, H15 and H17), 6.93 (d, *J* = 8.1 Hz, 2H, H14 and H18), 6.79 (t, *J* = 7.3 Hz, 1H, H16), 5.96 (dt, *J* = 5.9, 1.8 Hz, 1H, H2 or H3), 5.79 (d, *J* = 6.0 Hz, 1H, H2 or H3), 5.38 (d, *J* = 5.4 Hz, 1H, H4), 5.14 (d, *J* = 4.0 Hz, 1H, H1), 3.51 – 3.43 (m, 1H, H5), 2.95 – 2.67 (m, 2H, H8), 2.80 (s, 3H, H19), 1.74 – 1.58 (m, 2H, H9), 1.30 – 1.14 (m, 2H, H10), 0.79 (t, *J* = 7.3 Hz, 3H, H11).

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.0 (C13), 133.9 (C2 or C3), 133.2 (C2 or C3), 129.8 (C15 and C17), 118.4 (C16), 133.9 (C14 and C18), 77.0 (C4), 71.2 (C5), 66.7 (C1), 47.7 (C8), 32.4 (C19), 29.2 (C9), 20.0 (C10), 13.5 (C11).

MS(ESI) m/z: 260.9 **[MH]$^+$**

Aziridine ring-opening with azide (51-52).

(1*RS*,4*S*R,5*RS*)-4-Azido-5-(butylamino)cyclopent-2-en-1-ol 51 and (1*SR*,2*RS*,5*RS*)-2-azido-5-(butylamino)cyclopent-3-en-1-ol 52



33 **51** **52**

Aziridine **33** (20 mg, 0.13 mmol) was dissolved in 12 mL of phosphate buffer 1M pH 7.4. Sodium azide (84.5 mg, 1.3 mmol, 10 eq.) was added and the solution was left stirring in a water bath at 37ºC for 72 hours. The solvent was evaporated under vacuum and products were purified by silica gel column chromatography (EtOAc: MeOH = 9.5: 0.5) to give the ring-opening products **51** and **52** as yellow amorphous solids in 65% yield (16.6 mg) in a 2:1 ratio (**51**: **52)**.

51:

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98 (dt, *J* = 5.8, 1.7 Hz, 1H, H2), 5.82 (d, *J* = 5.8 Hz, 1H, H1), 4.48 – 4.38 (m, 1H, H3), 3.95 (dd, *J* = 3.5, 1.6 Hz, 1H, H5), 3.09 (t, *J* = 5.0 Hz, 1H, H4), 2.84 – 2.65 (m, 2H, H8), 1.59 – 1.42 (m, 2H, H9), 1.43 – 1.30 (m, 2H, H10), 0.95 – 0.82 (m, 3H, H11)

^{13}C NMR (100 MHz, $CDCl_3$) δ 136.9 (C2), 130.1 (C1), 80.7 (C3), 74.7 (C4), 70.3 (C5), 48.2 (C8), 32.5 (C9), 20.5 (C10), 14.1 (C11).

MS(ESI) m/z: 196.9 **$[MH]^+$**

52:

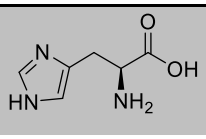
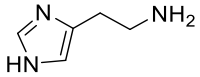
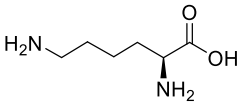
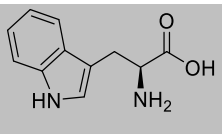
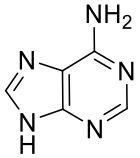
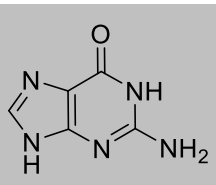
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 – 6.08 (m, 1H, H5), 5.94 – 5.89 (m, 1H, H1), 4.37 (d, *J* = 3.7 Hz, 1H, H2), 4.05 (dd, *J* = 6.1, 5.4 Hz, 1H, H3), 3.73 – 3.65 (m, 1H, H4), 2.74 – 2.69 (m, 2H, H8), 1.56 – 1.42 (m, 2H, H9), 1.41 – 1.32 (m, 2H, H10), 0.92 (t, *J* = 7.2 Hz, 3H, H11)

^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4 (C5), 128.0 (C1), 80.7 (C3), 69.0 (C4), 66.9 (C2), 47.9 (C8), 32.5 (C9), 20.5 (C10), 14.1 (C11).

MS(ESI) m/z: 196.9 **$[MH]^+$**

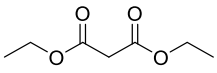
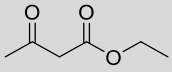
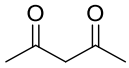
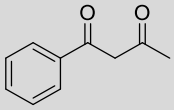
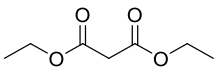
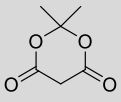
General procedure for the aziridine ring-opening with other nitrogen nucleophiles.

Bicyclic vinyl Aziridine (0.065 mmol) was dissolved in 6 mL of PBS pH 7.4. Nitrogen nucleophile (1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC. The reaction was followed by TLC (CH_2Cl_2:MeOH = 9: 1). After full aziridine consumption or after at least 50 hours of reaction the solvent was evaporated. The reaction crude was analysed by ^{1}H NMR. The performed reactions are presented in the following table.

Aziridine	Nucleophile (1.2 eq.)	Solvent	Reaction time (h)	Product
32		6 mL PBS	27	Degradation
32		6 mL PBS	52.8	Degradation
32		6 mL PBS	65	Starting material with minor degradation
32		6 mL PBS	54	Starting material
32		6 mL PBS	81	Starting material
32		6 mL PBS	57	Starting material
32		6 mL PBS + 1 mL NaOH 1M aqueous	67	Starting material

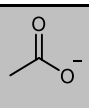
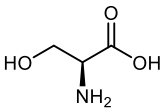
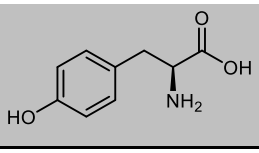
General procedure for the aziridine ring-opening with carbon nucleophiles.

Bicyclic vinyl Aziridine (0.065 mmol) was dissolved in 6 mL of PBS pH 7.4. Carbon nucleophile (1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC. The reaction was followed by TLC (CH_2Cl_2:MeOH = 9: 1). After full aziridine consumption or after at least 50 hours of reaction the solvent was evaporated. The reaction crude was analysed by ^1H NMR. The performed reactions are presented in the following table. (Note: For nucleophiles diethyl malonate and Meldrum's acid 2 equivalents of nucleophiles were used and the solvent was a phosphate buffer 1M pH 7.4).

Nucleophile	Nuc eq.	Solvent	Product
	1.2	6 mL PBS	Starting material
	1.2	6 mL PBS	Degradation
	1.2	6 mL PBS	Starting material and degradation
	1.2	6 mL PBS	Starting material and degradation
	1.2	6 mL PBS	Starting material and degradation
	2	6 mL Phosphate buffer 1M pH 7.4	Degradation
	2	6 mL Phosphate buffer 1M pH 7.4	Degradation

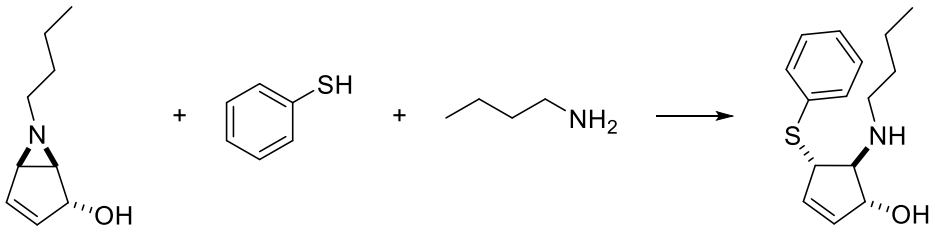
General procedure for the aziridine ring-opening with oxygen nucleophiles.

Bicyclic vinyl Aziridine (0.065 mmol) was dissolved in 6 mL of PBS pH 7.4. Oxygen nucleophile (1.2 eq.) was added and the solution was left stirring in a water bath at 37ºC. The reaction was followed by TLC (CH_2Cl_2:MeOH = 9: 1). After full aziridine consumption or after at least 50 hours of reaction the solvent was evaporated. The reaction crude was analysed by ^{1}H NMR. The performed reactions are presented in the following table.

Aziridine	Nucleophile (1.2 eq.)	Product
32		Starting material
32		Starting material
32		Starting material

Competitive experiments.

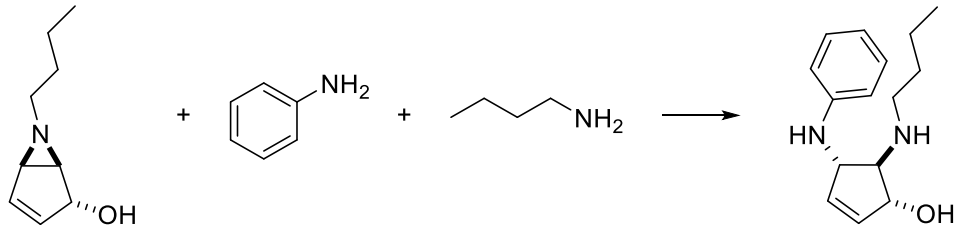
Thiophenol and butylamine.



33 **39**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL phosphate buffer 1M pH 7.4. Thiophenol (13.3 µL, 0.13 mmol, 2 eq.) and butylamine (12.8 µL, 0.13 mmol, 2 eq.) were added and the solution was left stirring for 24 hours. The solvent was evaporated and purification by silica gel column chromatography (EtOAc: MeOH: Et_3N = 10: 0.1: 0.1) provided the aziridine ring-opening product with thiophenol **39** in 67% yield (11.5 mg), confirmed by comparison of the ^{1}H NMR (solvent $CDCl_3$) with authentic sample.

Aniline and butylamine.



33 **49**

Aziridine **33** (10 mg, 0.065 mmol) was dissolved in 6 mL phosphate buffer 1M pH 7.4. Aniline (11.9 µL, 0.13 mmol, 2 eq.) and butylamine (12.8 µL, 0.13 mmol, 2 eq.) were added and the solution was left stirring for 120 hours. The solvent was evaporated and purification by silica gel column chromatography (EtOAc: MeOH: Et_3N = 10: 0.1: 0.1) provided the aziridine ring-opening product with aniline **49** in 26% yield (4.2 mg), confirmed by comparison of the ^1H NMR (solvent $CDCl_3$) with authentic sample.

Optimization of ring-opening of aziridine 33 with methyl ester cysteine for the peptide modification experiment.

Example for Entry 1 from Table 3.8: A 33 mM of aziridine **33** and 39.6 mM of methyl ester cysteine (1.2 eq.) solution was prepared in 2 mL of acetate buffer 50 mM pH 7.4. The solution was left stirring at 37ºC for 21 hours. A solution aliquot was extracted and analysed by water suppression ^{1}HNMR.

For the other conditions tested, resort to Table 3.8.

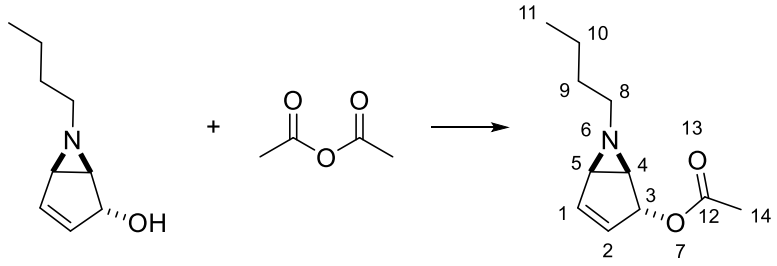
General Procedure to sCT modification.

Example for Entry 3 from Table 3.9. A solution of sCT 10 µM, TCEP 50 µM and aziridine **33** 500 µM was prepared in ammonium acetate buffer 50.0 mM pH8.0 to a final volume of 500 µL. The mixture was left at room temperature, overnight and evaluated on a Thermo LCQ ESI ion-trap mass spectrometer.

For the other conditions tested, resort to Table 3.9.

Aziridine acetylation (54).

(1*SR*,2*SR*,5*SR*)-6-Butyl-6-azabicyclo[3.1.0]hex-3-en-2-yl acetate (54).

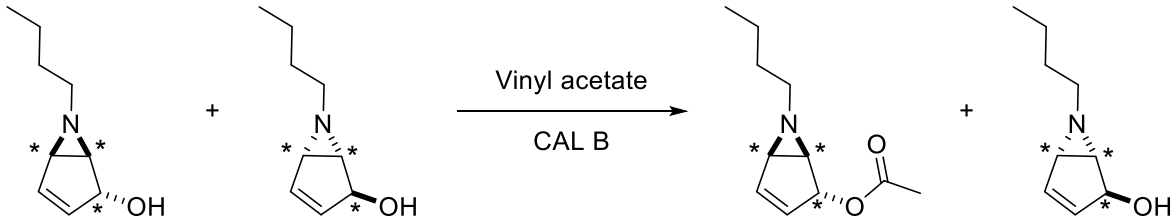


33 (*rac*) **54** (*rac*)

Aziridine **33** (30 mg , 0.195 mmol) was dissolved in 1 mL of dry dichloromethane. Et_3N (59.8 µL, 0.429 mmol, 2.2 eq.) and DMAP (2.4 mg, 0.020 mmol, 0.1 eq.) were added to the solution. Then, acetic anhydride (36.8 µL 0.39 mmol, 2 eq.) was added and the solution left stirring at room temperature for 30 minutes. 2 mL of water as added following extraction with 3x2 mL dichloromethane. The combined organic phases combined and dried over magnesium sulphate, filtered and evaporated under vacuum, following purification by silica gel column chromatography (CH_2Cl_2: Et_3N = 49:1). The acetyl aziridine **54** was obtained in 89% yield (33.9 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.38 (d, *J* = 5.7 Hz, 1H, H2), 5.82 (dd, *J* = 5.6, 1.1 Hz, 1H, H1), 5.51 – 5.36 (m, 1H, H3), 2.62 – 2.50 (m, 1H, H4), 2.48 (dd, *J* = 4.3, 1.9 Hz, 1H, H5), 2.42 – 2.18 (m, 2H, H8), 2.08 (s, 3H, H14) 1.63 – 1.47 (m, 2H, H9), 1.43 – 1.15 (m, 2H, H10), 0.90 (t, *J* = 7.3 Hz, 3H, H11).

Enzymatic resolution of aziridine 33:



33* **33**** **54*** **33****

Racemic aziridine **33** (10 mg, 0.065 mmol) was dissolved in 1 mL of TBME and 10 mg of enzyme CAL B was added to the solution. Vinyl acetate (12.1 µL, 0.13 mmol, 2 eq.) was then added to the solution, which was left with minimal stirring in a water bath at 26.5ºC. 1 µL aliquots were taken at 3, 6 and 24 reaction hours and injected in the GC equipped with a chiral column.

6. References

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