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Synthesis and Characterisation of Novel 3-Chloropiperidines: Secondary Derivatives and Ligands for Cisplatin Analogues

Kumulative Dissertation zur Erlangung des Grades „Doktor der Naturwissenschaften“ am Fachbereich Biologie und Chemie der Justus-Liebig-Universität Gießen

vorgelegt von

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“Science is the poetry of reality.”

Richard Dawkins

Kurzzusammenfassung

Seit ihrer Entdeckung Mitte des 20. Jahrhunderts spielen Alkylanzien eine entscheidende Rolle in der Krebstherapie. Stickstoff-Loste, die frühesten Vertreter dieser Klasse, zeichnen sich durch ihre einzigartige 2-Chlorethylamin-Gruppe aus. Diese funktionelle Gruppe ermöglicht die intramolekulare Bildung eines hochreaktiven Aziridinium-Intermediates, welches leicht von zellulären Nukleophilen wie beispielsweise DNA-Basen angegriffen wird. Die resultierenden kovalenten Addukte können Folgeschäden wie Depurinierungen und DNA-Strangbrüche verursachen, wodurch die DNA-Replikation gehemmt und letztendlich die Apoptose eingeleitet wird. Die Reaktivität dieser Substanzen ist allerdings nicht auf malignes Gewebe beschränkt, was zu schweren Nebenwirkungen führt und ihre therapeutische Anwendbarkeit einschränkt. Die weitere Entwicklung von neuen, verbesserten Derivaten ist daher unerlässlich, um Parameter wie Wirksamkeit und Verträglichkeit zu steigern.

Im Laufe der letzten Jahrzehnte kam es infolgedessen zur Entwicklung einer Vielzahl neuer Zytostatika. Es konnte gezeigt werden, dass eine Reduktion der Elektronendichte am zentralen Stickstoffatom die Bildung des gespannten Aziridinium-Ions verlangsamt und so die resultierende Reaktivität effektiv vermindert. Prominente Vertreter, welche diesen Ansatz nutzen, integrieren die 2-Chlorethylamin-Gruppe in ein Phosphonamid (Cyclophosphamid) oder setzen auf elektronenziehende aromatische Substituenten (Chlorambucil). In der Arbeitsgruppe *Göttlich* wurde die Integration der alkylierenden Einheit in eine zyklische Struktur untersucht, mit dem Ziel zusätzliche Ringspannung in dem nunmehr bicyclischen Intermediat herbeizuführen und analog die Rate der Aziridinium-Ionen-Bildung zu reduzieren. Die aus diesen Bemühungen hervorgegangenen 3-Chlorpiperidine haben in vorangegangen Studien eine vielversprechende Aktivität gegenüber mehreren Krebszelllinien gezeigt.

Im Rahmen dieser Arbeit wurde sich vormerklich auf die Synthese und biologische Untersuchung neuartiger 3-Chlorpiperidine konzentriert, welche im Gegensatz zu bereits publizierten Analoga ein sekundäres anstelle eines tertiären Amins im Heterozyklus enthalten. Sekundäre Stickstoff-Loste und ihre stabilen Aziridin-Intermediate finden sich in hochwirksamen Naturstoffen, sowie als aktiver Metabolit von Cyclophosphamid, dem klinisch erfolgreichsten Stickstoff-Lost, wieder. Die Synthese dieser sekundären Derivate stützt sich dabei auf eine neuentwickelte Methode zur selektiven Monochlorierung und ein *in situ* Abfangen des instabilen Endprodukts. Des Weiteren konnte gezeigt werden, dass sowohl die neuartigen sekundäre 3-Chlorpiperidine, als auch die entsprechenden Aziridine, ihre tertiären Analoga in DNA-Spaltungsassays übertreffen, was ihr Potential als vielversprechende Chemotherapeutika demonstriert.

Die zweite in dieser Arbeit enthaltene Publikation zielte darauf ab, unsere zuvor untersuchten zyklischen Stickstoff-Loste mit Cisplatin-basierten Wirkstoffen, einer weiteren unverzichtbaren Kategorie von Zytostatika, zu kombinieren. Die direkte Koordination der alkylierenden Einheit an das Metallzentrum verändert deren Reaktivität signifikant und verhindert eine vorzeitige Hydrolyse außerhalb des Zellkerns. Zusätzlich gelangen auf diese Weise gleichzeitig zwei zytostatisch wirksame Substanzen in die Zelle und induzieren gehäufte und potentiell irreparable DNA-Schäden. Darüber hinaus konnten die erhaltenen Komplexe die erworbene Cisplatin-Resistenz in Ovarialkarzinomzellen weitgehend überwinden und übertrafen in der resistenten Zelllinie sogar die Wirksamkeit von Cisplatin in absoluten Zahlen.

Abstract

Since their discovery in the mid-20th century, alkylating agents have played a crucial role in cancer treatment. Nitrogen mustards, the earliest representatives, are characterized by their unique 2-chloroethylamine moiety. This structural motif facilitates the intramolecular formation of a highly electrophilic aziridinium intermediate, which is readily attacked by cellular nucleophiles, such as DNA bases. The resulting covalent adducts can cause depurination and DNA strand cleavage, thereby inhibiting DNA replication and eventually leading to apoptosis. However, the reactivity of these agents is not confined to malignant tissues, resulting in severe side effects and limiting their therapeutic application. Therefore, the development of improved derivatives is essential to enhance efficacy and tolerability.

Consequently, a variety of drugs have been developed over the past decades. The reduction of the electron density on the central nitrogen atom has been shown to effectively lower the resulting reactivity, by decreasing the rate at of aziridinium ion formation. Prominent examples of this approach either incorporated the 2-chloroethylamine motif into a phosphonamide (Cyclophosphamide) or utilize electron withdrawing aromatic substituents (Chlorambucil). In the *Göttlich* group, the integrating the alkylating moiety into a cyclic structure has been investigated, aiming to introduce additional ring strain in the now bicyclic intermediate, similarly reducing the rate of aziridinium ion formation. The 3-chloropiperidines that emerged from this efforts have exhibited remarkable activity against multiple cancer cell lines in previous studies.

This work focused on the synthesis and biological evaluation of novel 3-chloropiperidines incorporating a secondary amine in the cyclic core, contrary to the tertiary analogues exclusively examined in prior reports. Secondary nitrogen mustards and their stable aziridine intermediates are found in potent natural compounds and as an active metabolite of cyclophosphamide, the clinically most successful nitrogen mustard. A new synthetic method was developed revolving around a novel selective mono-chlorination protocol and *in situ* trapping of the unstable final product. Concluding this publication, these novel secondary 3-chloropiperidines and the respective aziridines both outperformed their tertiary counterparts in DNA-cleavage assays, demonstrating their potential as promising drug candidates.

The second publication included in this work strived to combine our formerly reported cyclic nitrogen mustards with cisplatin based drugs, another invaluable class of anticancer agents. Direct coordination of the alkylating moiety to the metal centre significantly altered its reactivity, to prevent premature hydrolysation outside the nucleus. Additionally, this delivers two active chemotherapeutics simultaneously, possibly inducing clustered DNA-damage. Furthermore, the obtained complexes were able to largely overcome acquired cisplatin resistance in ovarian cancer cells, even surpassing the absolute potency of cisplatin in the resistant subline.

Preface

The present work is executed as a cumulative dissertation and based on the peer reviewed articles I was able to publish during my doctoral studies. The necessary research was conducted in the working group of *Prof. Dr. Richard Göttlich* at the Justus-Liebig-University Gießen. The first chapter aims to give a brief introduction of the general theoretical background of my research topic and summarizes previous ground breaking work. The second and third chapter deal with more recent and specific research, respectively culminating into my own contribution to the field. Finally this work is concluded by a short summary of the core findings, together with a perspective on potential future research. The structure of this thesis aims to present a conclusive overview, describe the relation between my individual publications and reflect the chronological and conceptual progression of the tackled scientific problems.

Part of the presented research has been done in cooperation with the working group of *Dr. Michael A. Jakupec* from the Institute of Inorganic Chemistry of the University of Vienna. Further cooperation partners relevant to this work include *Prof. Dr. Barbara Gatto* from the Department of Pharmaceutical and Pharmacological Sciences, University of Padova and *Prof. Dr. Peter Friedhoff* from the Institute of Biochemistry at the Justus-Liebig-University Gießen.

The original peer reviewed publications are attached in the final chapter, reprinted with permission of the owners of the respective copyrights. The respective supporting information are publicly accessible through the publisher's website.

Table of Contents

Eidesstattliche Erklärung I
Kurzzusammenfassung III
Abstract V
Preface VI
1. Introduction 1
  1.1 History of Alkylating Agents 1
  1.2 Molecular Mechanism of DNA Alkylation 3
  1.3 Secondary Nitrogen Mustards and Aziridines 5
2. 3-Chloropiperidines: Cyclic Nitrogen Mustard Analogues 7
  2.1 Synthesis of 3-Chloropiperidines 7
  2.2 Alkylation Mechanism of 3-Chloropiperidines 10
  2.3 Secondary 3-Chloropiperidines 11
3. Cisplatin and Platinum Analogues 13
  3.1 Cisplatin-Nitrogen Mustard Conjugates 15
  3.2 3-Chloropiperidine-Cisplatin Conjugates 16
4. Summary and Perspective 19
5. References 21
6. Publications 27
  6.1 Secondary 3-Chloropiperidines: Powerful Alkylating Agents 27
  6.2 Synthesis and antiproliferative activity of cisplatin-3-chloropiperdine conjugates 36
  6.3 Further Co-Authored Publications 44
7. Acknowledgement 45

1. Introduction

Historically, cancer therapy relied solely on surgical operations to locally remove tumorous tissue. Patients whose malignant cells had already metastasized were only eligible for palliative care.[1] The discovery of alkylating agents in the middle of the 20th century brought a fundamental change to this and the treatment of cancer in general.[2] Commonly known as chemotherapy, the application of various cytotoxic drugs is often regarded as one of the three pillars of modern oncology.[3] Contrary to surgery and radiation therapy as local methods, it represents a systematic approach aiming to inhibit cell growth, ultimately leading to apoptosis. While this enables the treatment of metastatic cancer it also poses serious drawbacks. The unselective nature of the deployed chemotherapeutic agents leads to a variety of severe side effects, especially harming highly proliferative cells and tissue.[4,5] Additionally, therapy administered at the maximum tolerated dose might lead to the rapid build-up of resistances. To mitigate these effects and achieve an optimal therapeutic profile, different anticancer drugs are often administered in combination.[6] Furthermore, chemotherapy is not only utilized as a standalone treatment but also as a follow-up or synergistic therapy with surgery or radiation therapy, aiming for full remission of malignant tumours.[7,8] Together with the wide divergence between different cancer types, this necessitates a broad portfolio of antineoplastic agents.[9] Consequently, further research is still required to develop novel anticancer agents and optimize existing drugs with regard to reducing side effects, circumventing resistances, and enhancing uptake profiles.

1.1 History of Alkylating Agents

The origin of modern chemotherapy can be traced back to chemical warfare agents developed during World War I. *Bis*(2-chloroethyl) sulphide (Figure 1), better known as mustard gas or sulphur mustard, was first used in Belgium in 1917.[10] In the following years, multiple systemic disorders, such as gastrointestinal ulcerations and leukopenia, a significant decrease in white blood cells, were diagnosed among affected victims.[11] Leveraging this phenomenon, sulphur mustards were clinically tested against leukemia. However, their high acute toxicity ultimately prevented their therapeutic use. In an effort to reduce this toxicity the less noxious nitrogen analogues were developed and clinical trials were started by Yale pharmacologists *Goodman* and *Gilman* in 1942.[12,13] While *tris*(2-chloroethyl) amine was the very first compound administered to a human, it was the bifunctional *bis*(2-chloroethyl) methylamine (Figure 1) tested by *L. O. Jacobson* from the University of Chicago that was finally granted approval and became the very first chemotherapeutic drug.[14] Today more commonly known under its trivial

and brand names, Mechlorethamine respectively Mustargen®, it is still in use for the treatment of Hodgkin´s lymphoma.

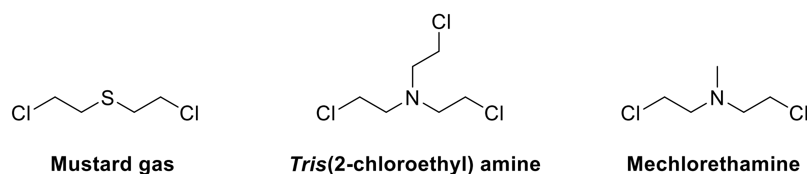


FIGURE 1: CHEMICAL STRUCTURE OF SULPHUR MUSTARD AND ITS NITROGEN ANALOGUES.

The correct mode of action, revolving around intramolecular formation of the highly reactive aziridinium ion and subsequent alkylation of a vital cellular constituent, was predicted by *Gilman* in 1946 after the wartime secrecy ended.[13] Owing to the discovery of DNA in the mid-20th century this postulate was subsequently refined and will be discussed in detail in a later section (compare Chapter 1.2).

However, aliphatic *bis*(2-chloroethyl) amines were quickly noticed to exhibit substantial side effects, which were attributed to their tendency to alkylate undesired biological targets such as proteins or enzymes. In 1949 *Everett* and *Ross* published a series of articles aiming to overcome these issues and to develop a more selective analogue. They reasoned that the introduction of an aromatic moiety would significantly reduce the electrophilicity of the compounds.[15–17] This assumption was proven by investigation of the hydrolysis rates of these novel mustards and correlated with a lowered toxicity in animal studies.[16,18] Furthermore the aromatic unit could be derivatized with various substituents, giving rise to a broad library of novel alkylating agents. In the end, utilizing a carboxyl group to increase the water solubility, combined with an aliphatic spacer to retain the desired reactivity led to the approval of Chlorambucil (Figure 2).[19].

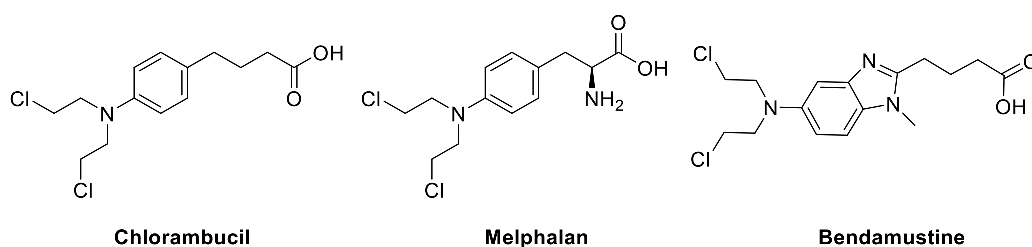
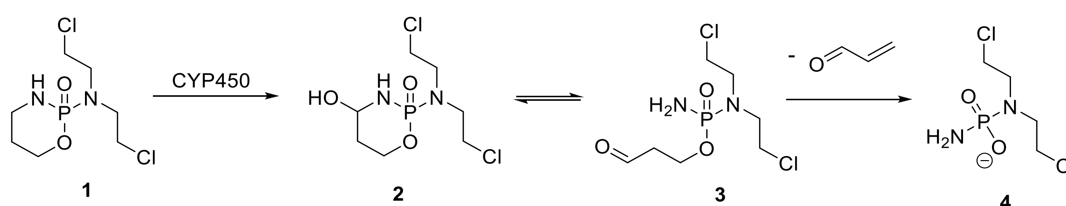


FIGURE 2: CHEMICAL STRUCTURES OF AROMATIC NITROGEN MUSTARDS; CHLORAMBUCIL, MELPHALAN AND BENDAMUSTINE

Inspired by this approach several successful aromatic nitrogen mustards have been synthesized. For example Melphalan[20] and Bendamustine[21] (Figure 2) are among the most essential chemotherapeutic agents to date. They aim to utilize amino acid transporters or introduce additional antimetabolite properties into the drug.[22] It is a noteworthy advantage of the aforementioned alkylating agents that they can be administered orally, in contrast to mechlorethamine, which is limited to intravenous injection.[23]

In the early 1960s, a slightly different approach to reduce reactivity incorporated the bis(2-chloroethyl) amino function into a phosphonamide structure. While the resulting compound showed promising *in vitro* results, being specifically active against cancer cells with high phosphamidase contents, it failed phase I clinical trials.[24,25] Further efforts finally gave rise to the cyclic derivate **1** functioning as a prodrug. Initially nontoxic upon uptake, cyclophosphamide **1** is oxidized in the liver by the enzyme cytochrome P450.[26] The resulting 4-hydroxy metabolite **2** can isomerize to aldophosphamide **3**, which in turn spontaneously eliminates acrolein to release the active substrate **4**. (Scheme 1) [25,27,28]



SCHEME 1: IN VIVO ACTIVATION OF THE PRODRUG CYCLOPHOSPHAMIDE ***1***

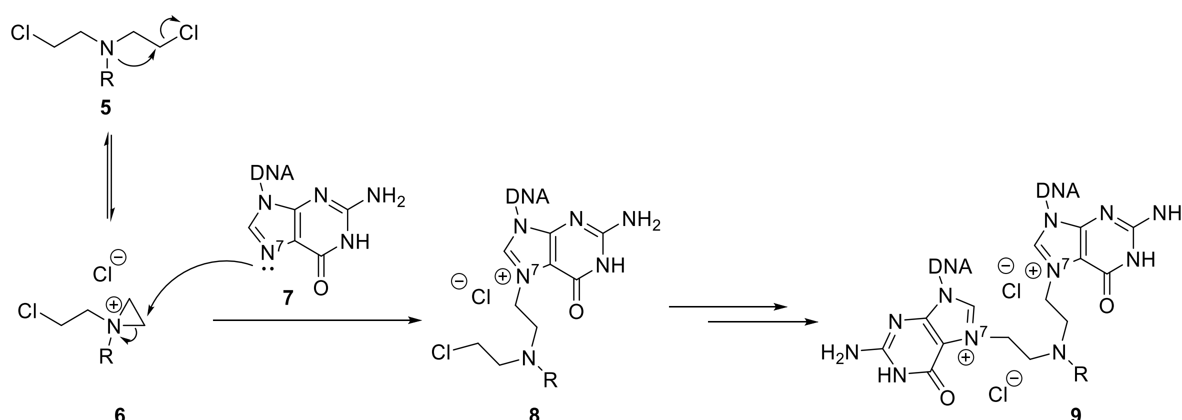
Over half a century after its initial synthesis, cyclophosphamide has been established as one of the most important chemotherapeutics, with clinical use against a multitude of neoplastic diseases. In fact, it stands out as the clinically most successful compound in the nitrogen mustard family. Despite the unique mode of activation the cytotoxic metabolite **4** ultimately relies on the same reactivity and mechanism of action as the previously depicted alkylating agents.[26,29]

1.2 Molecular Mechanism of DNA Alkylation

As mentioned in the previous chapter, *Gilman* correctly attributed the toxicity of nitrogen mustards to their ability to covalently transfer an alkyl group to cellular constituents.[13] Although this phenomenon, commonly referred to as alkylation, can occur with any given biological nucleophile, primarily the alkylation of DNA is responsible for the cytotoxic effects. Under physiological conditions alkyl chlorides are normally too unreactive to be sufficiently substituted by DNA bases for therapeutic use. The anchimeric assistance of the central nitrogen however leads to a significant increase in reactivity that allows for the alkylation of DNA under mild conditions.[9] The mechanism can be divided into two steps, beginning with the intramolecular substitution of one chloride by the nitrogen atom in an S_N2-like reaction, leading to the formation of a three membered cycle.[30,31] This highly strained, positively charged aziridinium ion **6** is a better electrophilic compared to the original chloroalkyl moiety **5**. It is therefore readily attacked, even by weak nucleophiles such as water or DNA bases. The N^7-postion of the guanine nucleobase **7** poses the most nucleophilic centre in the DNA and is consequently the primary alkylation target.[32] In case of a bifunctional alkylating agents this process can repeat

itself with the remaining 2-chloroethyl moiety, covalently linking two nucleobases **9**. The formed *mono* **8** or *bis* **9** DNA adducts either directly impact cellular replication processes or possibly induce further detrimental effects through follow-up reactions, for example depurination and strand cleavage (Scheme 2).[31,32] Accumulated and irreparable damage can either directly lead to necrosis or might be recognized by enzymes like p53 that ultimately initiate apoptosis.[33]

Depending on the location of the second nucleobase, either in the same or the complementary DNA strand, this leads to intrastrand or interstrand crosslinks respectively. Especially interstrand crosslinks have been proven to be particular detrimental due to the adversity of repair.[34] The aforementioned nitrogen mustards (compare Chapter 1.1) mainly form interstrand crosslinks, with each agent predominantly targeting a specific DNA sequence.[35]



SCHEME 2: ALKYLATION MECHANISM OF NITROGEN MUSTARDS

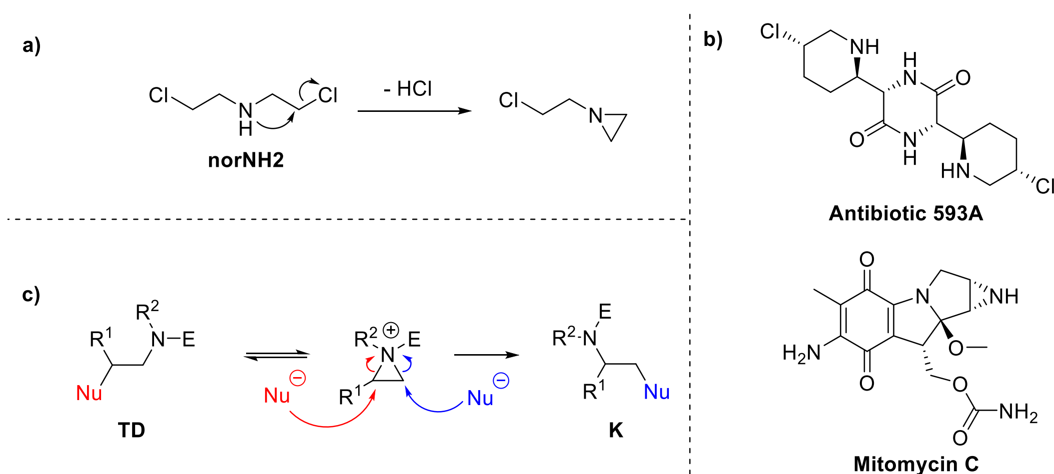
Since DNA serves as a blueprint during semiconservative replication, cells with alkylated DNA are primarily affected during their replication phase. Highly proliferative cells, such as malignant tissue, are therefore affected disproportionally strong. Nevertheless, this does not imply that chemotherapeutics, particularly alkylating agents, demonstrate selective toxicity exclusively towards malignant cells. Healthy tissues like bone marrow, hair follicles or mucous membranes are also strongly impaired, leading to a series of adverse side effects.[3,4,7,9]

Independent studies on the different nitrogen mustards have found the initial formation of the highly strained aziridinium ion in the reaction towards nucleophiles in non-acidic conditions as the overall rate determining step, regardless of the substituent. Chlorambucil, Melphalan and Bendamustine (Figure 2) undergo a much slower intramolecular substitution of the chlorine to form their respective intermediates compared to Mechlorethamine (Figure 1) or phosphonamide mustard **4**, the active form of cyclophosphamide **1** (Scheme 1).[36] This is accounted by the lower electron density on the nitrogen centre and explains the generally lower reactivity of the aromatic compounds, as predicted in the late 1950s (compare Chapter 1.1).[15,17,18] Utilizing the same correlation it is now possible to understand why the labile

Mechlorethamine can be stored in its protonated hydrochloride form without undergoing hydrolysis. Early theoretical research and more recent computational work have confirmed this experimental relationship. They have also revealed additional influencing factors, such as solvation and the electrophilicity of the aziridinium ion. Furthermore, these investigations have highlighted the difference in activation energy between the initial monoalkylation and the subsequent formation of the second aziridinium ion.[37] The latter provides a more detailed insight into the propensity of forming crosslinks, which is vital to comprehensively understand the resulting toxicity of nitrogen mustards.

1.3 Secondary Nitrogen Mustards and Aziridines

Parallel to the development of aromatic and phosphorylated nitrogen mustards multiple analogues incorporating a secondary amine as the central nitrogen have been published. Even though early studies had shown a great cytotoxic potential, they were mostly used as building blocks for the synthesis of novel tertiary nitrogen mustards.[38] *Bis*(2-chloroethyl) amine, commonly named nornitrogen mustard or norNH2, was of particular interest as a precursor for the synthesis of cyclophosphamide and other phosphorylated or acylated compounds.[39] Originally proposed as the active form of cyclophosphamide, more detailed biological insight revealed phosphonamide mustard **4** as the main cytotoxic metabolite.[28,40] However, it has been shown that norNH2 is also an important metabolite of cyclophosphamide, formed by non-enzymatic cleavage of the phosphonamide bond. Even occurring after the mono alkylation of DNA to yield DNA bound nornitrogen mustard residues, their reactivity plays a crucial role in the subsequent formation of crosslinks. Therefore their reactivity towards various nucleophiles and ultimately the resulting cytotoxicity has been intensively investigated.[41–44] Compared to tertiary nitrogen mustards an inverse and much stronger dependency on the pH value has been observed. More precisely, the reaction towards DNA proceeds much faster at lower pH levels compared to physiological conditions. This is hardly surprising, as secondary nitrogen mustards tend to form stable aziridines rather than highly reactive aziridinium ions (Scheme 3a).[41] Multiple natural compounds incorporating secondary nitrogen mustards or directly aziridines in their structure are known, many of which have shown excellent antibacterial and cytostatic activity. In the context of this work, two of the most relevant examples are the bifunctional cyclic norNH2 derivative Antibiotic 593A and Mitomycin C. The latter, following reductive activation, alkylates DNA via a bicyclic aziridine.[45–48] (Scheme 3b)



SCHEME 3: A) FORMATION OF AZIRIDINE FROM NORNH2; B) NATURALLY OCCURRING ALKYLATING AGENTS ANTIBIOTIC 593A AND MITOMYCIN C; C) REGIOSELECTIVITY OF THE NUCLEOPHILIC RING OPENING OF AZIRIDINES.

These uncharged three membered heterocycles can be activated by the addition of an electrophile and can in turn be opened by a suitable nucleophile. This two-step mechanism explains the aforementioned pH-dependency, as the activating electrophile might just be a simple proton. In case of unsymmetrically substituted aziridines the regioselectivity depends on a variety of factors such as solvent, temperature, or the activating electrophile.[49,50] Generally, it has been found that hard nucleophiles prefer the attack on the less substituted carbon resulting in the kinetic product **K** (Scheme 3c). Soft nucleophiles on the other hand favour the thermodynamic product **TD**. This preference is especially pronounced if the nucleophile also represents a decent leaving group, resulting in the formation of an equilibrium between cyclized and open form almost exclusively leading to the thermodynamic product **TD**.[50,51]

These characteristics make aziridines not only relevant in a biological context but on a broader perspective they pose to be interesting substrates for organic synthesis. Allowing for the convenient introduction of further functional groups, ring expansions or cycloadditions, aziridines have been identified as highly valuable building blocks.[52] However, their synthesis from simple starting materials such as unactivated alkenes has remained challenging and in the past decade a multitude of different synthetic approaches has been published on this topic. Often making use of metal or organo catalysts, the aziridine is either directly formed via addition to an alkene or by intramolecular substitution of a *β*-chloro atom, similar to the mechanism depicted in Scheme 3a.[53] The continued efforts in this field clearly demonstrate the need for novel synthetic methods yielding structurally diverse aziridines, which can be used as constituents for more complex molecules or directly employed as potential new drugs.

2. 3-Chloropiperidines: Cyclic Nitrogen Mustard Analogues

Over the last decade various 3-chloropiperidines were developed in the *Göttlich* group as cyclic nitrogen mustard analogues. While conceptionally exhibiting the same reactivity as their acyclic counterparts, the formation of the electrophilic aziridinium ion leads to a bicyclic intermediate (Figure 3a). Due to the thereby introduced additional ring strain the intramolecular displacement of chlorine is much more reluctant, ultimately lowering the cytotoxicity. Inspired by the bifunctional natural compound Antibiotic 593A (Scheme 3b) *bis*-3-chloropiperidines (Figure 3b) were developed that enable crosslinking to induce further DNA damage and allow further derivatisation through variation of the linker structure.[54–58] Kinetic studies provided deeper insights into the mechanism of action and support the theoretical considerations mentioned above.[59] Furthermore, a direct comparison with the monofunctional nitrogen mustard 2-chloroethyl diethylamine confirmed the much lower reactivity of the cyclic analogues.[60] Nonetheless, both monofunctional and bifunctional analogues showed excellent DNA cleavage activity against double stranded plasmids.[55,61] Moreover, the cytotoxicity towards various cancer cell lines has been examined and especially compounds with aromatic functionalisation stood out as particular effective against pancreatic cancer cells. [54,62] A combination of kinetic, DNA cleavage, and MTT based studies gave rise to multiple structure activity relationships that can be leveraged to further optimize the 3-chloropiperidine structure.[59,61]

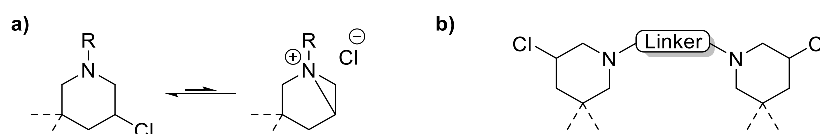


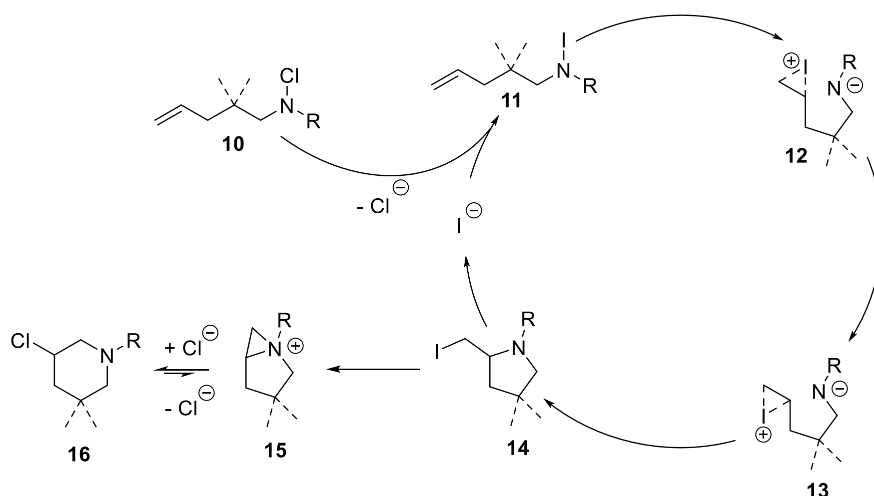
FIGURE 3: A) 3-CHLOROPIPERIDINE AND FORMATION OF THE BICYCLIC AZIRIDINE; B) BIFUNCTIONAL *BIS*-3-CHLOROPIPERIDINE.

Our most recent advances include the synthesis of 3-chloropiperidines incorporating a secondary amine, mimicking the lead structure Antibiotic 593A, and the isolation of the highly strained bicyclic aziridines.[63] Furthermore we succeeded in combining cisplatin, one of the world's leading chemotherapeutic agents, with our cyclic alkylating agents resulting in a novel complex effectively circumventing cisplatin resistance.[64] The following chapters convey a brief overview, arching from synthesis and early developments towards our latest findings.

2.1 Synthesis of 3-Chloropiperidines

The existing synthetic approaches towards 3-chloropiperidines can be divided into two different concepts. Starting from a linear precursor via cyclisation or through modification of an existing aza-heterocycle. The first approach starts from the isomeric unsaturated *N*-chloroamine as the

cyclisation precursor and has been used to yield a broad library of mono- and bifunctional 3-chloropiperidines. A variety of linker structures, for instance simple linear and cyclic aliphatic frameworks[55], lysine esters and amides,[57,58] as well as substituted and unsubstituted aromatic systems[54,55] have been successfully incorporated. The late stage cyclisation approach conveniently allows the introduction of various substituents, not only in the linker, but also on the heterocycle itself, through modification of the precursor. Especially the introduction of methyl groups (Scheme 4, indicated by dashed bonds) has been commonly employed, leveraging the *Thorpe-Ingold* effect to greatly enhance the rate of cyclisation.[65]

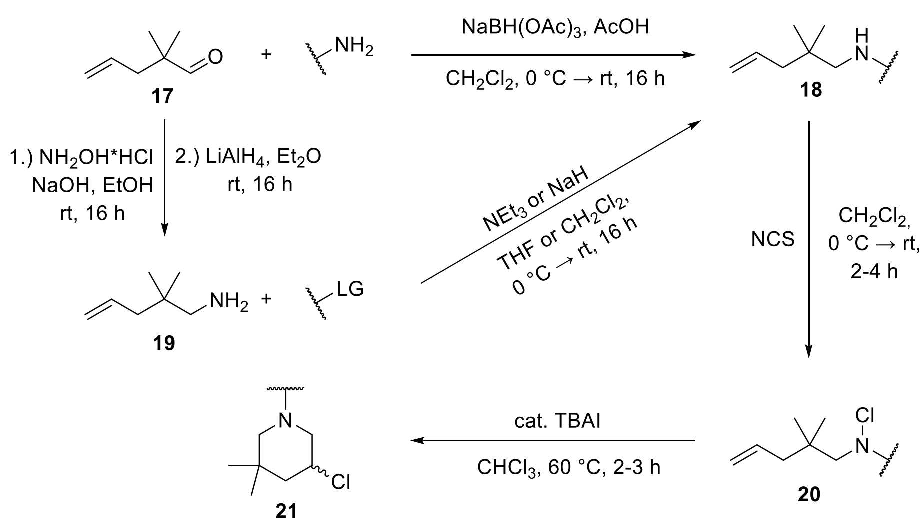


SCHEME 4: CATALYTIC CYCLE OF THE TETRABUTYLAMMONIUM IODIDE-MEDIATED CYCLISATION OF UNSATURATED *N*-CHLOROAMINES

Several catalytic systems, including Cu(I)[66], Pd(0)[67] and Sm(II)[68] that allow for the desired cyclisation have been reported. However the *Göttlich* group most frequently utilized tetrabutylammonium iodide as a metal free alternative to mediate the heterocycle formation.[69] The proposed mechanism (Scheme 4) involves a *Finkelstein*-type halogen exchange to form *N*-iodoamine **11.** This highly reactive intermediate adds to the alkene yielding the iodonium ion **12**. After rotation around the single bond, compound **13** can react intramolecular in an S_N2 ring opening reaction giving the kinetically favoured 2-iodomethyl pyrrolidine **14.** Formation of the bicyclic aziridinium ion **15** and nucleophilic attack of the previously liberated chlorine finally leads to the desired thermodynamic product **16** and closes the catalytic cycle. In addition, multiple one-pot chlorination/cyclisation reactions based on copper(II)chloride[70], palladium(II)acetate[71] and iodine[72] have been used to circumvent the need for strong oxidizing agents to form the necessary *N*-chloroamines. As the demand for greener and environmentally friendly chemistry rises, our group recently also reported an electrochemical chlorination/cyclisation method minimizing organic waste and avoiding precious metals.[73]

The necessary amine precursor **18** can be obtained through reductive amination from a suitable aldehyde such as 2,2-dimethylpent-4-enal **17**, which can be conveniently produced in a large scale one-pot synthesis.[74] Formation of the oxime, followed by reduction with lithium

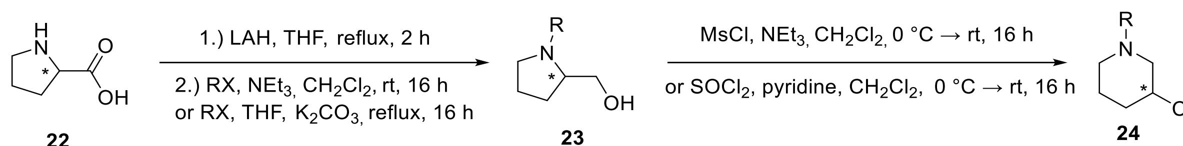
aluminium hydride yields the corresponding primary amine **19** that can be either used directly[63] or undergo *N*-functionalisation by nucleophilic substitution first. For the synthesis of *bis*-3-chloropiperidines **21** the same reactivity can be taken advantage of, by supplying the linker structure as the respective diamine or dihalogen. The broad spectrum of unsaturated amines **18** obtained this way can be chlorinated by *N*-chlorosuccinimide to give the desired precursors **20.** The efficient purification by column chromatography of these relatively unpolar *N*-chloroamines **20** allows for the final TBAI-initiated transition to the 3-chloropiperidine **21** to proceed smoothly, producing only sparse amounts of side products.



SCHEME 5: SYNTHESIS OF *(BIS)*-3-CHLORO-5,5-DIMETHYLPIPERIDINES **21** PROCEEDING FROM 2,2-DIMETHYL-4-ENAL

Albeit this method provided access to a wide range of different novel alkylating agents, the unselective nature of the intramolecular addition poses a serious drawback, merely leading to racemic products. In order to also investigate the influence of stereochemistry on the biological activity of 3-chloropiperidines, the second synthetic strategy mentioned above, modifying an existing nitrogen heterocycle, was employed.[61] Enantiopure aza-heterocycles such as 3-hydroxypiperidine could provide access to non-racemic 3-chloropiperidines, via enantioselective chlorination of the hydroxyl function. However, prolinol, derived from the naturally occurring amino acid proline **22**, is a feasible and less expensive alternative. Moreover, utilisation of the likewise abundant 4-hydroxyproline allows for the introduction of further functional groups, for example a second chlorine to form a bifunctional alkylating agent. The first step consists of the reduction of D- or L-proline **22** with lithium aluminium hydride. The amino group can subsequently be functionalized by nucleophilic substitution (Scheme 6). The amino alcohol **23** can then be chlorinated with metanesulfonyl chloride or thionylchloride in the presence of pyridine, yielding the 2-chloromethyl pyrrolidine.[75] In polar solvents this initial product quickly equilibrates to the thermodynamically more stable 3-chloropiperidine **24** (compare Scheme 4). Since the formation of the aziridinium ion as well as the following ring

opening proceeds stereo convergent the outcome is only dependent on the absolute configuration of the respective precursor.[50,76]



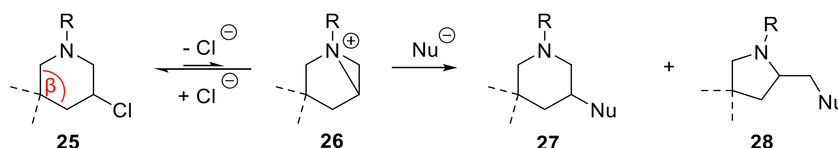
SCHEME 6: SYNTHESIS OF ENANTIOPURE 3-CHLOROPIPERIDINES **24** FROM *D*- OR *L*- PROLINE **22**

As mentioned above, this enantioselective synthesis enabled the investigation of the influence of stereochemistry on biological activity. A direct comparison between the two enantiomeric *N*-butyl functionalized derivatives revealed a striking difference in activity against isolated DNA and three different cancer cell lines.[61]

On a more general perspective 3-substituted piperidines are common moieties in many natural compounds that show a variety of different biological effects. Therefore, a multitude of synthetic pathways towards substituted piperidine scaffolds have been developed and continue to be of interest.[77] Albeit the presented synthetic methods here were predominantly used to inquire the biological activity and evaluate possible medical uses of 3-chloropiperidines[55,61], these compounds also represent possible building blocks for structurally more complex molecules.

2.2 Alkylation Mechanism of 3-Chloropiperidines

In general the reaction of 3-chloropiperidines **25** towards various nucleophiles, including cellular components, proceeds via a similar pathway as discussed for linear nitrogen mustards (compare Chapter 1.2, Scheme 2). In comparison, the intramolecular nucleophilic substitution of chloride leads to a highly strained bicyclic aziridinium ion **26**, which has been confirmed by NMR[75,78] and ESI-MS.[57] This reactive intermediate can in turn be opened by nucleophiles either in alpha or beta position to the nitrogen, leading to the isomeric piperidine **27** and methylpyrrolidine **28.** The exact ratio depends on the factors discussed in Chapter 1.3.



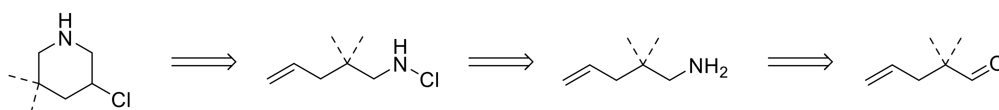
SCHEME 7: REACTION OF 3-CHLOROPIPERIDINES TOWARDS NUCLEOPHILES, VIA BICYCLIC AZIRIDINIUM ION INTERMEDIATE.

Isolation and crystallisation of the bicyclic aziridinium ion **26**, through halide abstraction utilizing silver salts with weakly coordinating counter ions enabled us to obtain the crystal structure of the two derivatives. Their boat-like structure is in accordance with results of previous computational studies[79] and the crystal structure of similar compounds.[80] Initial kinetic investigations using these isolated aziridinium ions showed an immediate reaction with

imidazole as a DNA model nucleophile at room temperature. This allowed us to employ the steady state approximation in the following examination of the multistep reaction of 3-chloropiperidines towards nucleophiles. The reaction with 2´-desoxyguanosine as the nucleophile was monitored by ^{1}H-NMR and resulted in simple first order kinetics. The measured rate constants k_1 matches the results obtained in previous DNA cleavage assays and therefore can be used for an easy initial determination of the reactivity of novel 3-chloro-piperidines.[60] Further leveraging this NMR-kinetic method, together with computational analysis, our group recently established a structure activity relationship for different substitution patterns on the piperidine cycle. The internal angle β (Scheme 7) was found to correlate linearly with the observed rate constant k_1. However, the cyclic structure of 3-chloropiperidines excludes any contribution of the reactive rotamer effect, therefore allowing for the first clear separation between this factor and the classic angle depended *Thorpe-Ingold* effect.[59]

2.3 Secondary 3-Chloropiperidines

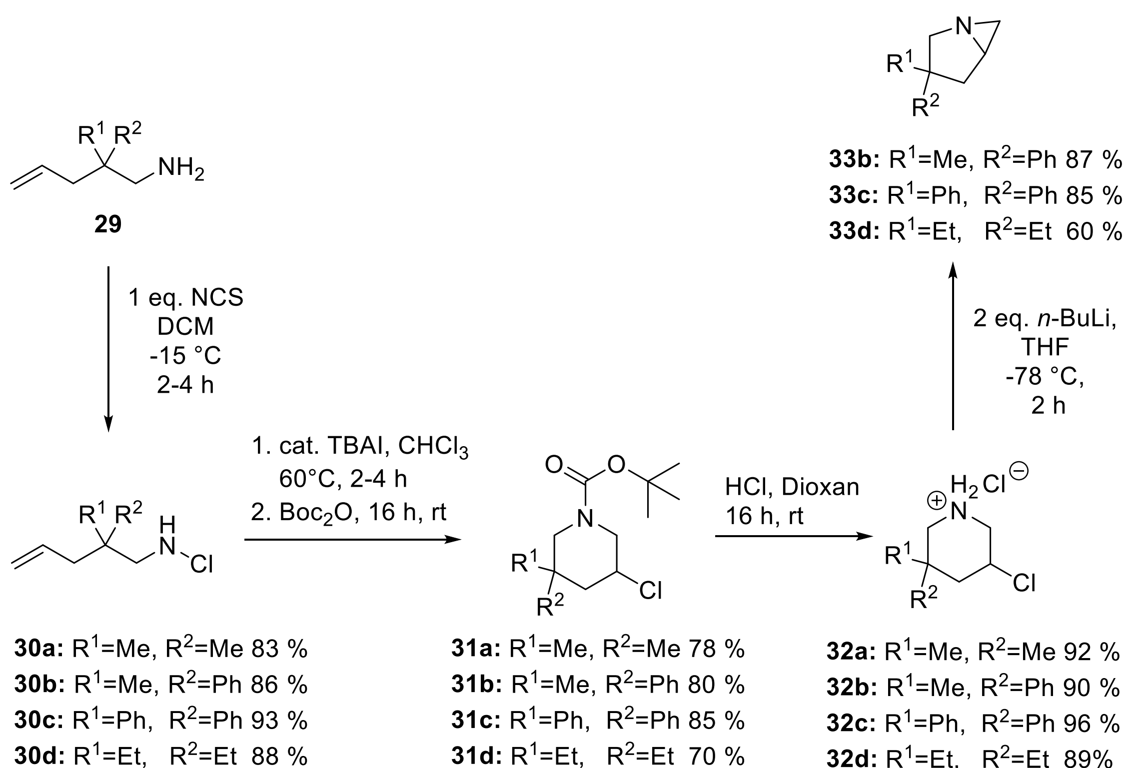
In early preclinical studies Antibiotic 593A (Chapter 1.3, Scheme 3b) has shown excellent activity against various cancer types both *in vitro* and *in vivo*.[46,81] A comparison of its structure with our previously published 3-chloropiperidines reveals a subtle but important distinction. The active moiety incorporates a secondary instead of a tertiary amine, similar to the cyclophosphamide metabolite norNH2. (Chapter 1.3, Scheme 3a) This drastically changes the reactivity towards nucleophiles, ultimately altering the cytotoxicity, and has been extensively studied in case of norNH2.[41–44] We therefore reasoned that secondary 3-chloropiperidines, which more closely resemble Antibiotic 593A as a lead structure, could be novel and powerful alkylating agents. In order to gain synthetic access to this class of compounds a simple retrosynthetic analysis (Scheme 8) revealed the need for a method to selectively monochlorinate unsaturated primary amines.



SCHEME 8: RETROSYNTHETIC ANALYSIS OF SECONDARY 3-CHLOROPIPERIDINES

Although general methods for such monochlorinations have already been established, unsaturated amines however where reported as too unstable.[82] Treating a primary amine with *N*-chlorosuccinimide under the conditions described above (Scheme 5) led to undesired *N,N*-dichlorination. Optimizing the conditions in view of temperature, reaction time and equivalents of oxidizing agent finally led to almost exclusive formation of the desired product. Purification of the crude product by column chromatography was unsuccessful, presumably due to disproportionation. However simple precipitation and filtration of the succinimide side

product with pentane proved to be sufficient, resulting in the isolation of the pure product **30** in high yields. While the following cyclisation under established conditions[66,69] resulted in conversion to the desired secondary 3-chloropiperidine, isolation was challenging. Due to volatility of the corresponding aziridines only derivatives with higher molecular weight could be obtained directly, albeit only in mediocre yields. To circumvent the premature formation of aziridines and subsequently supress side reactions like self-alkylation, the reactive secondary 3-chloropiperidines were trapped *in situ* with a carbamate protecting group **31**. The removal of the employed *tert*-butyloxycarbonyl group gave the desired products **32** as bench stable HCl salts in excellent yields. Exploiting this strategy, multiple aryl- and alkyl- substituted analogues have been prepared. Furthermore, the controlled addition of *n*-butyllithium as a strong base led to the formation of the corresponding aziridines **33**. These bicyclic aziridines bear a structural similarity to the highly active and clinically successful chemotherapeutic Mitomycin C.[47,48] (Scheme 3b)



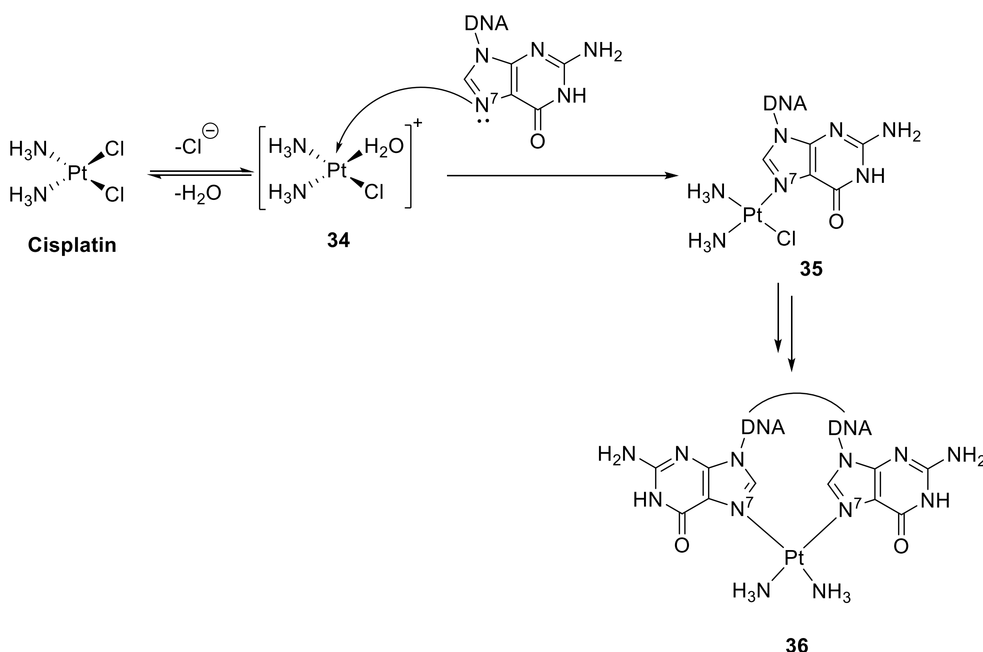
SCHEME 9: OPTIMIZED SYNTHESIS OF SECONDARY 3-CHLOROPIPERDINES AND CORRESPONDING AZIRIDINES

With not only the sought after secondary 3-chloropiperidines, but also their matching aziridines at hand we strived to examine their activity against isolated DNA. Both novel compounds **32** and **33** have been demonstrated to be highly active, cleaving DNA even more effectively than their previously published tertiary analogues (see Chapter 6.1).[60,61]

The results briefly summarized in this chapter have been published in *ChemistryOpen* and serve as an integral part of this work.[63] They can be viewed in detail in the attached publication. (Chapter 6.1)

3. Cisplatin and Platinum Analogues

Cisplatin was first synthesized in 1844 by *Michele Peyrone* and initially became known as Peyrones chloride.[83] Over one hundred years later in the 1960s its cytotoxic potential was accidentally discovered by *Barnett Rosenberg* during electrochemical experiments on bacterial cells utilizing platinum electrodes.[84] First examined against various bacteria, the attention quickly shifted towards cancer treatment, which culminated in 1978 with the approval for clinical use showing exceptional activity against testicular and ovarian cancer cells.[85] Nowadays it serves as a model for treatable neoplastic diseases with excellent cure rates up to 90 %.[86,87] The mechanism of action involves hydrolysation of the chlorine ligands to form strongly electrophilic platinum-aqua intermediate **34** (Scheme 10). The aqua ligands are in turn replaced by suitable nucleophiles, including cellular constituents and DNA bases. Similar to nitrogen mustards the N^7-position of guanine as the most nucleophilic centre in the DNA represents the preferred target, leading to the mono platinum adduct **35**. [88,89]



SCHEME 10: CISPLATIN MECHANISM OF ACTION, ACTIVATION THROUGH HYDROLYSATION FOLLOWED BY NUCLEOPHILIC SUBSTITUTION OF THE AQUA LIGAND.

In order to prevent premature hydrolysation in the stomach, cisplatin is solely administered through intravenous infusion of a chloride containing solution. High chloride concentrations in these solutions and extracellular fluids suppress hydrolytic activation, whereas the lower chloride concentration inside cells promotes it.[89] Hydrolysis and substitution of the second chlorine ligand predominantly leads to 1,2-intrastrand crosslinks **36** between adjacent guanosine bases that account for about 90 % of DNA lesions caused by cisplatin.[90]

These lesions distort the DNA, leading to interruptions in replication and transcription that ultimately result in apoptosis.[91] The less frequently formed interstrand crosslinks are believed to also contribute to the resulting toxicity. However, as they do not distort the DNA helix as significantly, their impact is generally regarded smaller. The clinically less efficient isomer transplatin majorly forms interstrand crosslinks, explaining its lower toxicity.[92] As the covalent adducts inhibit vital cellular reproduction mechanisms, highly proliferative tissue is affected disproportionally. Comparable to nitrogen mustards, this leads to a wide variety of adverse effects, with high nephrotoxicity often posing a dose-limiting factor. While pre-hydration of the patient or the administration of sulphur containing chemoprotective agents have been shown to reduce these side effects, research mainly focused on the development of less toxic cisplatin derivatives.[89] The lability of the leaving ligands plays a crucial role and is proportionally related to the overall toxicity.[93] Introduction of more stable leaving ligands to reduce side effects and enhance tolerability lead to the worldwide approval of carboplatin (Figure 4). Often regarded to as the second generation platinum drug it utilizes a bidentate carboxylic acid ligand and shows a greatly improved therapeutic profile with a significantly lower nephrotoxicity. However, the overall potency is also negatively affected, necessitating an escalation of the administered doses.[89] Additionally, carboplatin suffers from extensive cross-resistance with cisplatin hampering its clinical application against patients that had previously been treated with another platinum based chemotherapeutic.[94,95] Consequently, the third generation of platinum drugs has been developed with focus on overcoming these limitations. Four main factors underlying resistance have been identified. Firstly the reduction of drug uptake, and secondly the overexpression of glutathione or metallothionein production diminish the concentration of active drug in the cell. Similar to the previously mentioned chemoprotective agents these sulphur containing structures substitute the leaving group of platinum drugs and render them inactive. The other two main mechanisms revolve around increases in DNA repair mechanisms and enhanced tolerance towards DNA-platinum adducts.[89,95] Substituting the ammonia ligands with *trans*-1,2-diaminocyclohexane (DACH) increases the lipophilicity and therefore amplifies passive transport through the cell membrane. Simultaneously, it counteracts important DNA repair mechanisms, most noteworthy the mismatch repair commonly abbreviated as MMR. Besides hindering recognition by MMR, the DACH ligand also effects or completely abolishes enlarged DNA-platinum adduct tolerance and effectively counteracts acquired cisplatin resistance.[95] Oxaliplatin (Figure 4), the most recent platinum based drug gaining worldwide approval, utilizes enantiopure (-)-DACH in combination with oxalate as the leaving ligand.

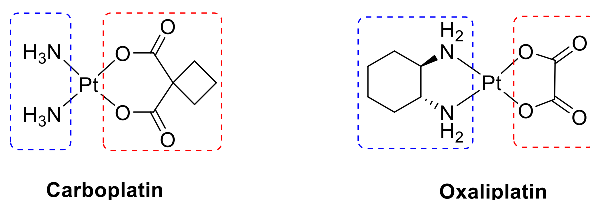


FIGURE 4: CHEMICAL STRUCTURES OF CARBOPLATIN AND OXALIPLATIN; STABLE CARRIER LIGAND MARKED IN BLUE, LABILE LEAVING LIGAND MARKED IN RED.

To achieve an optimal therapeutic profile and further circumvent resistances, the presented cisplatin drugs are often administered together with other chemotherapeutic agents (compare Chapter 1). For example the combination of Oxaliplatin with the antimetabolite 5-Fluoruracil is particularly effective in treating advanced and metastatic colorectal cancer. [96] Carboplatin is approved for use in multiple treatment protocols, but it is most frequently paired with paclitaxel, a microtubule inhibitor from the taxane family. The therapeutic regimen has demonstrated significant efficiency against various cancer types and is employed as a first-line treatment for both ovarian and lung cancer.[97] In addition to the three compounds discussed above, a plethora of other platinum-based drug candidates has been synthesized and evaluated over the past decades.[98] Some of these drug candidates have even obtained national approvals [86]. Strategies have included the introduction of bulkier and more lipophilic ligands[99], coupling with essential nutrients to enhance cellular uptake[100], and the utilization of platinum(IV) complexes[101] serving as prodrugs which are activated via reduction.[102] These diverse approaches have broadened the scope of platinum-based chemotherapy research, highlighting the continuous effort in this field and underscoring the need for ongoing research to develop more effective treatments in the future.

3.1 Cisplatin-Nitrogen Mustard Conjugates

A particularly promising and pertinent approach, especially relevant to this work, is advancing the principle of combination therapy by directly conjugating cisplatin with other cytotoxic or synergetic molecules.[103] Aiming to overcome cisplatin resistance these complexes can induce further clustered DNA damage that is proposed to be repaired less efficiently.[104] The combination of cisplatin with nitrogen mustards is a promising approach that has only been scarcely investigated so far. Early attempts employed simple monofunctional 2-chloroethylamines **37** and **38** [105,106] as well as their ethylene-bridged chelating counterparts as ligands **39** (Figure 5).[107] However, the resulting compounds did not demonstrate improved activity compared to other platinum-based drugs. Notably, complexes with monofunctional ligands were shown to exhibit significant cross-resistance with cisplatin.[105] A possible explanation for this relative inactivity is the withdrawal of electron density from the nitrogen upon coordination, drastically altering the reactivity of the nitrogen mustard moiety.

As discussed in Chapter 1.2, Scheme 2, the nitrogen lone pair is essential for the formation of the electrophilic aziridinium intermediate. Consequently, subsequent research introduced a flexible ethylene glycol linker between the metal centre and the active alkylating unit (Scheme 5, structure **39**). As a result both the platinum and the *N*-mustard moiety were capable of reacting with guanine residues and the complex effectively inhibited cell division in *E. coli* cells.[104] The most recent studies conjugated a bifunctional mustard via introduction of pyridine as a second coordinating site. The resulting complex **40** (Figure 4) has shown an enhanced stability towards glutathione, which works as a cisplatin scavenger and is a major factor in the build-up of resistances. Detailed investigations revealed a slow dissociation that allowed for delayed formation of the reactive aziridinium ion, possibly even occurring after the platinum has been consumed by cellular nucleophiles.[108]

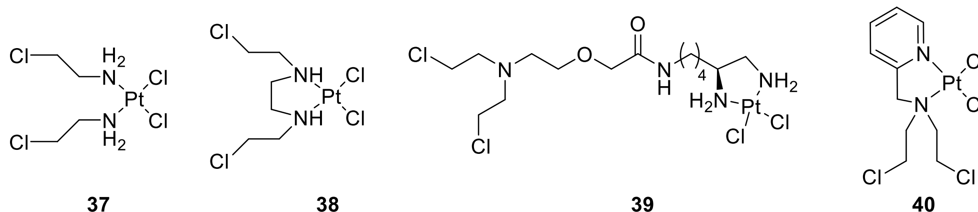


FIGURE 5: CHEMICAL STRUCTURES OF PREVIOUSLY INVESTIGATED *N*-MUSTARD CISPLATIN CONJUGATES.

3.2 3-Chloropiperidine-Cisplatin Conjugates

In previous works from the *Göttlich* group an inverse correlation between the reactivity in DNA cleavage assays and the cytoxicity against cancer cells was found for some compounds. This indicates that besides the pure reactivity other factors contribute to cellular activity, for example premature hydrolysis.[54,109] Inspired by preceding studies (Chapter 3.1) we assumed that direct conjugation to platinum could not only deliver two active drugs to the nucleus, but also help keeping the alkylating moiety intact. Preliminary experiments with various metals (Cu, Co, Ni, Fe, and Zn) revealed that 3-chloropiperidines exhibit a strong reluctance to form the respective complexes. We hypothesized that a weakly coordinating ligand is more readily replaced, leading to a release of the active alkylating moiety similar to what has been described for compound **40**. Suitable ligands for platinum have predominantly utilized primary or secondary amines, while tertiary amines are only found sparsely in literature.[86,98] A possible explanation for this preference could be the increased steric demand of tertiary amines that seems to play a crucial role in the square planar geometry of platinum(II) complexes.[110] Consequently building on our previous work,[63] we first attempted to utilize novel secondary 3-chloropiperidines such compound **41** as ligands to form the desired complexes. Potassium tetrachloroplatinate(II) was chosen as a metal source to ensure the *cis* configuration of the

resulting conjugate. Following the first exchange of one halogen ligand, the stronger *trans*-effect of the chloride group compared to amines would direct the second substitution in *cis* position relative to the initially introduced amine.[111]

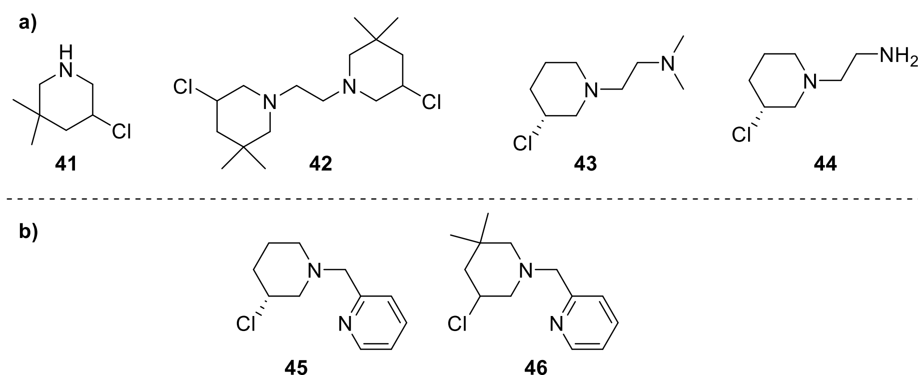


FIGURE 6: A) SYNTHESISED UNSUCCESSFUL LIGANDS **41**-**44**; B) SUCCESSFUL LIGANDS **45** AND **46**

However, employment of ligand **41** did not lead to any observable complex formation. We attributed this to the lack of any chelating effect and switched our attention to bifunctional ligands. As efforts to produce a secondary *bis*-3-chloropiperidine remained unsuccessful, we opted for compound **42** as a substitute. The ethylene linker was chosen due to its ability to form a favourable five-membered chelate ring in the resulting complex.[112] Nonetheless, no complex formation was observed yet again. In the following attempt we gradually reduced the steric demand on the second coordination site (Figure 6; **43** and **44**). The addition of ligand **44** to the red tetrachloroplatinate solution resulted in a short term colour change to yellow, indicating the temporary formation of the desired complex which seemed to be unstable. Introduction of pyridine as a secondary coordination site (Figure 6b, **45** and **46**), bearing similarity to compound **40**[108], finally yielded two novel 3-chloropipidine-cisplatine conjugates, characterized by ESI-HRMS, elemental analysis and single crystal X-ray diffraction.

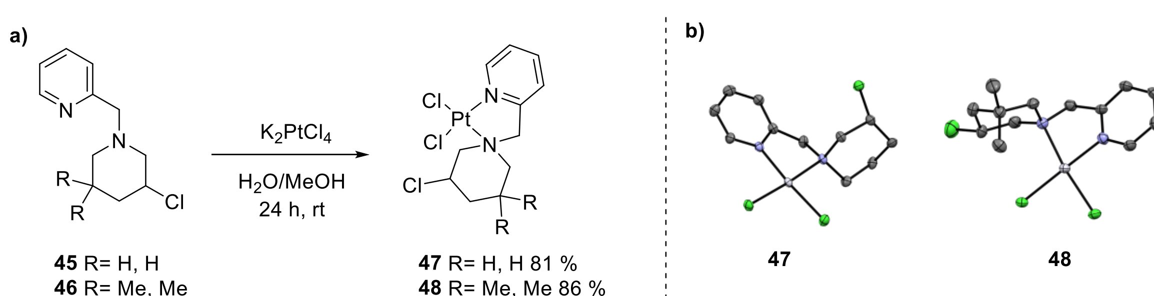


FIGURE 7: A) SYNTHESIS OF CISPLATIN CONJUGATES **47** AND **48**; B) ORTEP DIAGRAMS OF **47** AND **48**, ELLIPSOIDS DRAWN AT 50% PROBABILLTY, HYDROGENS OMMITED FOR CLEARITY

To evaluate the biological activity of the obtained complexes **47** and **48,** they were tested against multiple human cancer cell lines, alongside their respective ligands **45** and **46**. Although the ligands themselves exhibited minimal activity, their conjugates achieved IC_{50}

values comparable to those of cisplatin. Their ability to overcome acquired cisplatin resistance was assessed in an isogenic pair of ovarian cancer cell lines. While cisplatin showed better activity against the parental A2780 cell line, both complexes exhibited 8 to 12 fold lower resistance factors, with compound **48** even surpassing cisplatin in absolute potency against the resistant cell line A2780cisR (Figure 8). Additionally, both conjugates where shown to reliably induce apoptic cell death in a dose depended manner in the isogenic cell pair. Possible explanations for this effective circumvention of resistances include the steric bulk exhibited by the 3-chloropiperidin, similar to the widely utilized DACH-ligand and the additional DNA-damage introduced by the alkylating moiety. Another rationalisation is based on previous findings, showing reduced susceptibility towards deactivation by glutathione in the comparable cisplatin conjugate **40**.[108] The exact mechanism however remains to be elucidated and is therefore a potential subject of further investigations.

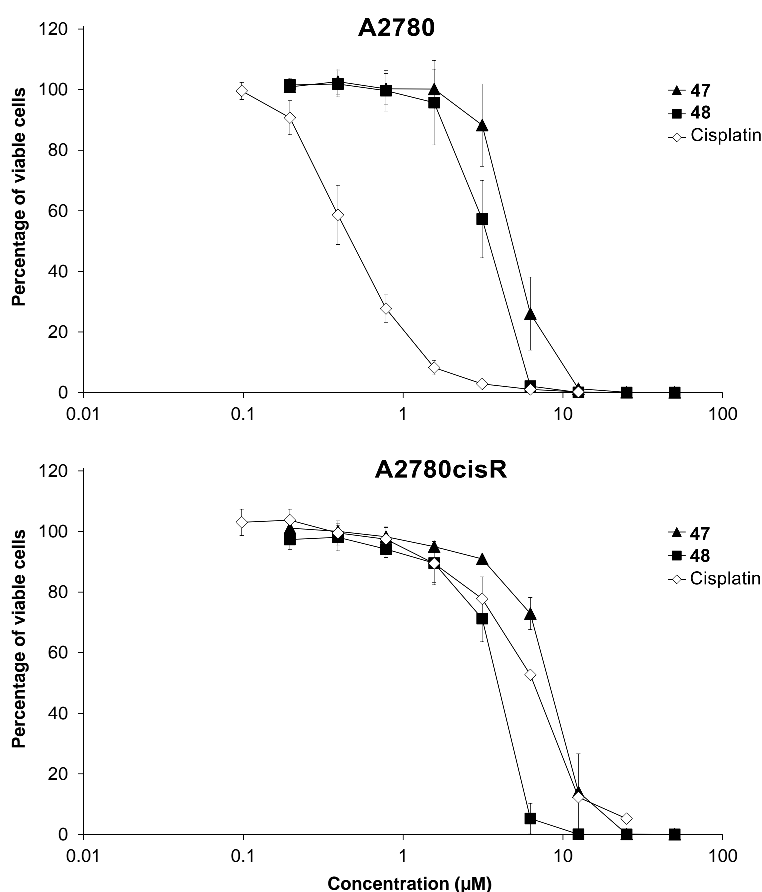


FIGURE 8: CONCENTRATION-EFFECT CURVE IN A2780 (TOP) AND A2780CISR (BOTTOM) CELL LINES RELATIVE TO UNTREATED CONTROLS.

The results briefly summarized in this chapter have been published in *ChemBioChem* and serve as the second integral part of this work.[64] They can be viewed in detail in the attached publication (Chapter 6.2).

4. Summary and Perspective

Non-cyclic secondary nitrogen mustards, such as the potent Cyclophosphamide metabolite norNH2, have been shown to form uncharged aziridines, contrary to the aziridinium ions exhibited by their tertiary counterparts (Chapter 1.3, Scheme 3a). This subtle change in the mechanism of action strongly impacted the resulting reactivity and ultimately the toxicity. Reasoning that this could be transferred to the cyclic *N*-mustards developed in our group we strived to investigate 3-chloropiperidines incorporating a secondary amine in their cyclic core. Compared to previously inquired tertiary 3-chloropiperidines these novel alkylating agents also resemble the strongly cytotoxic lead structure Antibiotic 593A (Chapter 1.3, Scheme 3b) even closer.

In this work, a synthetic approach towards secondary 3-chloropiperidines has therefore been devised. As a first step a new method to selectively monochlorinate unsaturated primary amines has been developed. Thorough control of the reaction temperature and utilized equivalents of oxidizing agent were key to access these synthetically valuable compounds. The following cyclisation was carried out under iodine catalysis and the highly reactive products trapped *in situ* with boc-anhydride to prevent degradation and premature formation of the aziridine. Removal of the protection group transferred the secondary 3-chloropiperidines directly into the respective store able HCl salts. Isolation of the corresponding bicyclic aziridines was achieved by addition of a suitable base at low temperatures (compare Chapter 2.3, Scheme 9).

As a proof of principle, the reactivity of the novel compounds was compared to a tertiary analogue in a DNA cleavage assays. Both, the secondary 3-chloropiperidine as well as the respective aziridine exhibited excellent activity, surpassing their tertiary counterpart's potency. (see Chapter 6.1) We believe that these novel secondary 3-chloropiperidines are therefore promising candidates for the development of new and powerful chemotherapeutic agents. Additionally, we presume that the isolated bicyclic aziridines, as well as the monochlorinated amines are of high synthetic value. These compounds could potentially be leveraged as substrates for novel transformations producing structurally complex aza-heterocycles and other nitrogen containing motifs (compare Chapter 1.3).

The second part of this work focused on the synthesis and the antiproliferative activity of 3-chloropiperidine cisplatin conjugates. In the past, coordination of nitrogen mustards to cisplatin has been explored in multiple publications, aiming to deploy multiple chemotherapeutic agents simultaneously (compare Chapter 3.1). The clustered DNA damage potentially induced by this is believed to be less efficiently repaired and therefore possibly circumvents cisplatin

resistances. We strived to adapt this principle to our previously reported 3-chloropiperidines and opted for a direct coordination of the piperidine heterocycle to the metal centre. Lowering the electron density on the nitrogen, efficiently reduced the reactivity of the alkylating moiety (compare Chapter 1.1). We reasoned that this approach therefore prevents premature hydrolysis outside the nucleus, keeping the 3-chloropiperidine motif intact (compare Chapter 3.2). Given that platinum-based drugs predominantly utilize primary and secondary amines as ligands, we initially employed our novel secondary 3-chloropiperidines, logically extending our previous research (see Chapter 6.1). However, no complex formation was observed, likely due to the absence of any chelating effect. In the end, the introduction of pyridine as an additional coordination site led to the isolation of the desired conjugate, characterized by ESI-MS, elemental analysis and single crystal XRD (Chapter 3.2; Figure 7). The obtained 3-chloropiperidine-cisplatin complexes were tested against various different cell lines in MTT assays and showed slightly lower, yet comparable activity to cisplatin (see Chapter 6.2). Finally, their ability to circumvent acquired resistance was examined in an isogenic pair of ovarian cancer cell lines. Both complexes were able to largely overcome cisplatin resistance, as reflected by resistance factors about ten-fold lower compared to cisplatin. In the resistant subline A2780cisR, one compound came close to the absolute potency of cisplatin, with the other even surpassing it by a factor of 1.7 in terms of IC_{50} values (compare Chapter 3.2; Figure 8). Additionally, both novel conjugates were shown to reliably induce apoptosis in a dose depended manner in both examined cell lines.

In light of these promising results, we believe that further optimization of the ligand to induce DNA damage more effectively is essential. Additionally, a thorough investigation of the underlying mechanism that enables the circumvention of resistances, followed by subsequent structural refinements, could lead to the development of a powerful new anticancer drug.

5. References

[1] a) D. J. T. Wagener, *The history of oncology*, Springer, Houten, **2009**; b) B. A. Chabner, T. G. Roberts, *Nat. Rev. Cancer.* **2005**, *5*, 65.

[2] a) B. A. Chabner, T. G. Roberts Jr., *Nat. Rev. Cancer.* **2005**, *5*, 65; b) R. K. Singh, S. Kumar, D. N. Prasad, T. R. Bhardwaj, *Eur. J. Med. Chem.* **2018**, *151*, 401.

[3] M. J. Lind, *Medicine* **2016**, *44*, 20.

[4] Alison M. R. P. D., *The cancer handbook*, **2002**.

[5] a) Edward Chu,Vincent T. DeVita Jr., *Physicians' Cancer Chemotherapy Drug Manual 2015*, Jones & Bartlett Learning, Burlington, **2015**; b) Dragony Fu, Jennifer A. Calvo and Leona D. Samson, *Nat. Rev. Cancer.* **2012**, *12*, 104.

[6] a) K. O. Alfarouk, C.-M. Stock, S. Taylor, M. Walsh, A. K. Muddathir, D. Verduzco, A. H. H. Bashir, O. Y. Mohammed, G. O. Elhassan, S. Harguindey et al., *Cancer cell international* **2015**, *15*, 71; b) X. Wang, H. Zhang, X. Chen, *Cancer Drug Resist.* **2019**, *2*, 141.

[7] R. Airley, *Cancer chemotherapy*, Wiley-Blackwell, Chichester, **2009**.

[8] R. Bayat Mokhtari, T. S. Homayouni, N. Baluch, E. Morgatskaya, S. Kumar, B. Das, H. Yeger, *Oncotarget* **2017**, *8*, 38022.

[9] C. Avendaño, J. C. Menéndez, *Medicinal chemistry of anticancer drugs*, Elsevier, Amsterdam, Netherlands, Oxford, England, Waltham, Massachusetts, **2015**.

[10] Bignold L. P., *Anticancer Res.* **2006**, *26*, 1327.

[11] a) E. B. Krumbhaar, H. D. Krumbhaar, *J. Med. Res.* **1919**, *40*, 497-508.3; b) K. W. Kohn, *Cancer Res.* **1996**, *56*, 5533.

[12] a) L. S. Goodman, M. M. Wintrobe, *J. Am. Med. Assoc.* **1946**, *132*, 126; b) A. Gilman, *Am. J. Surg.* **1963**, *105*, 574.

[13] A. Gilman, F. S. Philips, *Science (New York, N.Y.)* **1946**, *103*, 409.

[14] L. O. Jacobson, C. L. Spurr, *J. Am. Med. Assoc.* **1946**, *132*, 263.

[15] J. L. Everett, W. C. J. Ross, *J. Chem. Soc.* **1949**, 1972.

[16] G. A. R. Kon, W. C. J. Ross, A. Haddow, *Nature* **1948**, *162*, 824.

[17] W. C. J. Ross, *J. Chem. Soc.* **1949**, 183.

[18] W. C. J. Ross, *J. Chem. Soc.* **1949**, *0*, 2589.

[19] J. L. Everett, J. J. Roberts, W. C. J. Ross, *J. Chem. Soc.* **1953**, 2386.

[20] a) F. Bergel, V. C. E. Burnop, J. A. Stock, *J. Chem. Soc.* **1955**, *0*, 1223; b) F. Bergel, J. A. Stock, *J. Chem. Soc.* **1954**, 2409.

[21] a) W. Ozegowski, D. Krebs, *J. Prakt. Chem.* **1963**, *20*, 178; b) W. Ozegowski, D. Krebs, *J. Prakt. Chem.* **1965**, *29*, 18; c) W. Ozegowski, D. Krebs, M. Wunderwald, *J. Prakt.*

Chem. **1963**, *20*, 166; d) W. Ozegowski, M. Wunderwald, D. Krebs, *J. Prakt. Chem.* **1959**, *9*, 54.

[22] G. L. Plosker, N. J. Carter, *Drugs* **2008**, *68*, 2645.

[23] T. Shimizu, K. Nakagawa, H. Hayashi, T. Iwasa, H. Kawakami, S. Watanabe, N. Yamamoto, K. Yonemori, T. Koyama, J. Sato et al., *Invest. New Drugs* **2023**, *41*, 1.

[24] S. L. Maddock, A. H. Handler, O. M. Friedman, *Cancer chemotherapy reports* **1966**, *50*, 629.

[25] O. M. Colvin, *Curr. Pharm. Des.* **1999**, *5*, 555.

[26] T. K. H. Chang, G. F. Weber, C. L. Crespi, D. J. Waxman, *Cancer Res.* **1993**, *53*, 5629.

[27] a) M. Colvin, R. B. Brundrett, M.-N. N. Kan, I. Jardine, C. Fenselau, *Cancer Res.* **1976**, *36*, 1121; b) C. Fenselau, M.-N. N. Kan, S. S. Rao, A. Myles, O. M. Friedman, M. Colvin, *Cancer Res.* **1977**, *37*, 2538; c) O. M. Friedman, I. Wodinsky, A. Myles, *Cancer Treat. Rep.* **1976**, *60*, 337.

[28] R. F. Struck, M. C. Kirk, M. H. Witt, W. R. Laster, *Biomedical mass spectrometry* **1975**, *2*, 46.

[29] A. Emadi, R. J. Jones, R. A. Brodsky, *Nat. Rev. Clin. Oncol.* **2009**, *6*, 638.

[30] a) C. C. Price, *Ann. N. Y. Acad. Sci.* **1958**, *68*, 663; b) C. C. Price, H. Akimoto, R. Ho, *J. Org. Chem.* **1973**, *38*, 1538; c) M. H. Benn, P. Kazamier, C. Watanatada, L. N. Owen, *J. Chem. Soc. D* **1970**, 1685.

[31] P. Brookes, P. D. Lawley, *Biochem J.* **1961**, *80*, 496.

[32] M. R. Osborne, D. E. Wilman, P. D. Lawley, *Chem. Res. Toxicol.* **1995**, *8*, 316.

[33] a) B. J. Aubrey, G. L. Kelly, A. Janic, M. J. Herold, A. Strasser, *Cell Death Differ.* **2018**, *25*, 104; b) W.-X. Zong, D. Ditsworth, D. E. Bauer, Z.-Q. Wang, C. B. Thompson, *Genes & development* **2004**, *18*, 1272.

[34] a) N. Kondo, A. Takahashi, K. Ono, T. Ohnishi, *J. Nucleic Acids* **2010**, *2010*, 543531; b) L. H. Hurley, *Nat. Rev. Cancer.* **2002**, *2*, 188.

[35] a) Q. Dong, D. Barsky, M. E. Colvin, C. F. Melius, S. M. Ludeman, J. F. Moravek, O. M. Colvin, D. D. Bigner, P. Modrich, H. S. Friedman, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 12170; b) S. M. Rink, M. S. Solomon, M. J. Taylor, S. B. Rajur, L. W. McLaughlin, P. B. Hopkins, *J. Am. Chem. Soc.* **1993**, *115*, 2551; c) J. T. Millard, S. Raucher, P. B. Hopkins, *J. Am. Chem. Soc.* **1990**, *112*, 2459; d) G. B. Bauer, L. F. Povirk, *Nucleic Acids Res.* **1997**, *25*, 1211.

[36] a) C. Golumbic, J. S. Fruton, M. A. Bergmann, *J. Org. Chem.* **1946**, *11*, 518; b) C. E. Williamson, B. Witten, *Cancer Res.* **1967**, *27*, 33; c) E. Watson, P. Dea, K. K. Chan, *J. Pharm. Sci.* **1985**, *74*, 1283.

[37] a) B. Neog, S. Sinha, P. K. Bhattacharyya, *Comput. Theor. Chem.* **2013**, *1018*, 19; b) D. J. Mann, *J. Phys. Chem. A* **2010**, *114*, 4486; c) O. Larrañaga, A. de Cózar, F. P. Cossío,

ChemPhysChem **2017**, *18*, 3390; d) I.-S. Han, C. K. Kim, C. K. Kim, B.-S. Lee, I. Lee, *J. Comput. Chem.* **1997**, *18*, 1773.

[38] a) O. M. Friedman, E. Boger, *J. Am. Chem. Soc.* **1956**, *78*, 4659; b) A. M. Rutenburg, L. Persky, O. M. Friedman, A. M. Seligman, *J. Pharmacol. Exp. Ther.* **1954**, *111*, 483.

[39] a) O. M. Friedman, H. Sommer, E. Boger, *J. Am. Chem. Soc.* **1960**, *82*, 5202; b) O. M. Friedman, A. M. Seligman, *J. Am. Chem. Soc.* **1954**, *76*, 655.

[40] T. A. Connors, P. J. Cox, P. B. Farmer, A. B. Foster, M. Jarman, *Biochem. Pharmacol.* **1974**, *23*, 115.

[41] K. Hemminki, *Chem. Biol. Interact.* **1987**, *61*, 75.

[42] O. M. Friedman, E. Boger, *Anal. Chem.* **1961**, *33*, 906.

[43] K. Hemminki, *Cancer Res.* **1985**, *45*, 4237.

[44] B. Hartley-Asp, F. Hyldig-Nielsen, *Carcinogenesis* **1984**, *5*, 1637.

[45] B. H. Arison, J. L. Beck, *Tetrahedron* **1973**, *29*, 2743.

[46] C. O. Gitterman, E. L. Rickes, D. E. Wolf, J. Madas, S. B. Zimmerman, T. H. Stoudt, T. C. Demny, *J. Antibiot.* **1970**, *23*, 305.

[47] S. T. Crooke, W. T. Bradner, *Cancer Treat. Rev.* **1976**, *3*, 121.

[48] M. Tomasz, R. Lipman, D. Chowdary, J. Pawlak, G. L. Verdine, K. Nakanishi, *Science* **1987**, *235*, 1204.

[49] H. Goossens, D. Hertsen, K. Mollet, S. Catak, M. D’hooghe, F. de Proft, P. Geerlings, N. de Kimpe, M. Waroquier, V. van Speybroeck in *Topics in Heterocyclic Chemistry* (Eds.: F. de Proft, P. Geerlings), Springer Berlin Heidelberg, Berlin, Heidelberg, **2014**, pp. 1–34.

[50] E. B. Boydas, G. Tanriver, M. D'hooghe, H.-J. Ha, V. van Speybroeck, S. Catak, *Org. Biomol. Chem.* **2018**, *16*, 796.

[51] a) M. D'hooghe, S. Catak, S. Stanković, M. Waroquier, Y. Kim, H.-J. Ha, V. van Speybroeck, N. de Kimpe, *Eur. J. Org. Chem.* **2010**, *2010*, 4920; b) M. D'hooghe, V. van Speybroeck, M. Waroquier, N. de Kimpe, *Chem. Commun.* **2006**, 1554; c) S. Stanković, M. D'hooghe, S. Catak, H. Eum, M. Waroquier, V. van Speybroeck, N. de Kimpe, H.-J. Ha, *Chem. Soc. Rev.* **2012**, *41*, 643.

[52] a) C. Dank, L. Ielo, *Org. Biomol. Chem.* **2023**, *21*, 4553; b) J. Dolfen, N. N. Yadav, N. De Kimpe, M. D'hooghe, H.-J. Ha, *Adv. Synth. Catal.* **2016**, *358*, 3485; c) R. Kapoor, R. Chawla, A. Verma, T. Keshari, I. R. Siddiqui, *Synlett* **2023**, *34*, 1621.

[53] a) L. Legnani, G. Prina-Cerai, T. Delcaillau, S. Willems, B. Morandi, *Science* **2018**, *362*, 434; b) A. A. Pinarci, N. Daniecki, T. M. TenHoeve, B. Dellosso, R. Madiu, L. Mejia, S. E. Bektas, G. Moura-Letts, *Chem. Commun.* **2022**, *58*, 4909; c) Q.-Q. Cheng, Z. Zhou, H. Jiang, J. H. Siitonen, D. H. Ess, X. Zhang, L. Kürti, *Nat. Catal.* **2020**, *3*, 386; d) Z. Ma, Z. Zhou, L. Kürti, *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 9886; e) J. L. Jat, M. P. Paudyal,

H. Gao, Q.-L. Xu, M. Yousufuddin, D. Devarajan, D. H. Ess, L. Kürti, J. R. Falck, *Science* **2014**, *343*, 61.
[54] T. Helbing, C. Carraro, A. Francke, A. Sosic, M. de Franco, V. Gandin, R. Göttlich, B. Gatto, *ChemMedChem* **2020**, *15*, 2040.
[55] I. Zuravka, R. Roesmann, A. Sosic, W. Wende, A. Pingoud, B. Gatto, R. Göttlich, *ChemMedChem* **2014**, *9*, 2178.
[56] A. Sosic, I. Zuravka, N.-K. Schmitt, A. Miola, R. Göttlich, D. Fabris, B. Gatto, *ChemMedChem* **2017**, *12*, 1471.
[57] I. Zuravka, R. Roesmann, A. Sosic, R. Göttlich, B. Gatto, *Bioorg. Med. Chem.* **2015**, *23*, 1241.
[58] I. Zuravka, A. Sosic, B. Gatto, R. Göttlich, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4606.
[59] T. Helbing, M. Kirchner, J. Becker, R. Göttlich, *Eur. J. Org. Chem.* **2022**, *2022*.
[60] T. Helbing, M. Georg, F. Stöhr, C. Carraro, J. Becker, B. Gatto, R. Göttlich, *Eur. J. Org. Chem.* **2021**, *2021*, 5905.
[61] C. Carraro, A. Francke, A. Sosic, F. Kohl, T. Helbing, M. de Franco, D. Fabris, R. Göttlich, B. Gatto, *ACS Medicinal Chemistry Letters* **2019**, *10*, 552.
[62] C. Carraro, L. Bonaguro, J. Schulte-Schrepping, A. Horne, M. Oestreich, S. Warnat-Herresthal, T. Helbing, M. de Franco, K. Haendler, S. Mukherjee et al., *eLife* **2022**, *11*.
[63] M. Georg, L. A. Laping, V. Billo, B. Gatto, P. Friedhoff, R. Göttlich, *ChemistryOpen* **2023**, e202300181.
[64] M. Georg, A. A. Legin, M. Hejl, M. A. Jakupec, J. Becker, R. Göttlich, *ChemBioChem* **2024**, e202400519.
[65] A. J. Kirby in *Advances in Physical Organic Chemistry*, Elsevier, **1980**, pp. 183–278.
[66] R. Göttlich, *Synthesis* **2000**, *2000*, 1561.
[67] J. Helaja, R. Göttlich, *Chem. Commun.* **2002**, 720.
[68] R. Göttlich, M. Noack, *Tetrahedron Lett.* **2001**, *42*, 7771.
[69] M. Noack, R. Göttlich, *Eur. J. Org. Chem.* **2002**, *2002*, 3171.
[70] a) R.-L. Li, G.-Q. Liu, W. Li, Y.-M. Wang, L. Li, L. Duan, Y.-M. Li, *Tetrahedron* **2013**, *69*, 5867; b) G.-Q. Liu, W. Li, Y.-M. Li, *Adv. Synth. Catal.* **2013**, *355*, 395.
[71] G. Yin, T. Wu, G. Liu, *Chemistry* **2012**, *18*, 451.
[72] W. Li, G.-Q. Liu, B. Cui, L. Zhang, T.-T. Li, L. Li, L. Duan, Y.-M. Li, *RSC Adv.* **2014**, *4*, 13509.
[73] M. Kirchner, Y. Dubinina, R. Göttlich, *Org. Chem. Front.* **2023**, *10*, 4631.
[74] K. C. Brannock, *J. Am. Chem. Soc.* **1959**, *81*, 3379.
[75] C. F. Hammer, S. R. Heller, J. H. Craig, *Tetrahedron* **1972**, *28*, 239.

[76] a) A. Cochi, D. G. Pardo, J. Cossy, *Organic letters* **2011**, *13*, 4442; b) D. Gomez Pardo, J. Cossy, *Chemistry* **2014**, *20*, 4516; c) S. Rioton, A. Orliac, Z. Antoun, R. Bidault, D. Gomez Pardo, J. Cossy, *Organic letters* **2015**, *17*, 2916.

[77] a) N. A. Frolov, A. N. Vereshchagin, *Int. J. Mol. Sci.* **2023**, *24*; b) S. B. D. Jarvis, A. B. Charette, *Organic letters* **2011**, *13*, 3830; c) Y. Du, A. Yu, J. Jia, Y. Zhang, X. Meng, *Chem. Commun.* **2017**, *53*, 1684; d) A. I. Subota, S. V. Ryabukhin, A. O. Gorlova, O. O. Grygorenko, D. M. Volochnyuk, *J. Fluor. Chem.* **2019**, *224*, 61; e) A. Bari, A. Iqbal, Z. A. Khan, S. A. Shahzad, M. Yar, *Synth. Comm.* **2020**, *50*, 2572.

[78] C. F. Hammer, S. R. Heller, *Chem. Commun.* **1966**, 919.

[79] Y. Lam, K. N. Houk, J. Cossy, D. Gomez Pardo, A. Cochi, *Helv. Chim. Acta* **2012**, *95*, 2265.

[80] M. A. Graham, M. Thornton-Pett, C. M. Rayner, A. H. Wadsworth, *Chem. Commun.* **2001**, 966.

[81] a) G. S. Tarnowski, F. A. Schmid, D. J. Hutchison, C. C. Stock, *Cancer chemotherapy reports* **1973**, *57*, 21; b) T. Fukuyama, R. K. Frank, C. F. Jewell, *J. Am. Chem. Soc.* **1980**, *102*, 2122.

[82] J.-C. Guillemin, J.-M. Denis, *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 690.

[83] G. B. Kauffman, R. Pentimalli, S. Doldi, M. D. Hall, *Johnson Matthey Technol. Rev.* **2010**, *54*, 250.

[84] B. Rosenberg, L. Vancamp, T. Krigas, *Nature* **1965**, *205*, 698.

[85] L. H. Einhorn, *J. Clin. Oncol.* **1990**, *8*, 1777.

[86] N. J. Wheate, S. Walker, G. E. Craig, R. Oun, *Dalton Trans.* **2010**, *39*, 8113.

[87] M. S. Galanski, M. A. Jakupec, B. K. Keppler, *Curr. Med. Chem.* **2005**, *12*, 2075.

[88] N. Farrell, L. R. Kelland (Eds.) *Cancer Drug Discovery and Development*, Humana Press, Totowa, New Jersey, **2000**.

[89] M. A. Jakupec, M. S. Galanski, B. K. Keppler, *Rev. Physiol. Biochem. Pharmacol.* **2003**, *146*, 1.

[90] A. Moretton, J. Slyskova, M. E. Simaan, E. A. Arasa-Verge, M. Meyenberg, D. A. Cerrón-Infantes, M. M. Unterlass, J. I. Loizou, *Front. Oncol.* **2022**, *12*, 874201.

[91] S. Acklin, F. Xia, *Int. J. Mol. Sci.* **2021**, *22*.

[92] a) T. Kishimoto, Y. Yoshikawa, K. Yoshikawa, S. Komeda, *Int. J. Mol. Sci.* **2019**, *21*; b) M. Frybortova, O. Novakova, V. Brabec, *J. Biol. Inorg. Chem.* **2014**, *19*, 1203; c) J. Kasparkova, S. Pospisilova, V. Brabec, *J. Biol. Chem.* **2001**, *276*, 16064.

[93] a) M. J. Cleare, J. D. Hoeschele, *Johnson Matthey Technol. Rev.* **1973**, *17*, 2; b) M. J. Cleare, J. D. Hoeschele, *Bioinorg. Chem.* **1973**, *2*, 187.

[94] a) M. E. Gore, I. Fryatt, E. Wiltshaw, T. Dawson, B. A. Robinson, A. H. Calvert, *Br. J. Cancer.* **1989**, *60*, 767; b) H. Calvert, *Inorg. Chim. Acta* **2019**, *498*, 118987.

[95] R. Fu, B. Zhao, M. Chen, X. Fu, Q. Zhang, Y. Cui, X. Hu, W. Zhou, *Med. Oncol.* **2023**, *41*, 9.

[96] a) E. Healey, G. E. Stillfried, S. Eckermann, J. P. Dawber, P. R. Clingan, M. Ranson, *Anticancer Res.* **2013**, *33*, 1053; b) H. K. Sanoff, D. J. Sargent, M. E. Campbell, R. F. Morton, C. S. Fuchs, R. K. Ramanathan, S. K. Williamson, B. P. Findlay, H. C. Pitot, R. M. Goldberg, *J. Clin. Oncol.* **2008**, *26*, 5721.

[97] a) A. Du Bois, H.-J. Lück, W. Meier, H.-P. Adams, V. Möbus, S. Costa, T. Bauknecht, B. Richter, M. Warm, W. Schröder et al., *J. Natl. Cancer. Inst.* **2003**, *95*, 1320; b) G. Fountzilas, A. Athanassiades, V. Papadimitriou, M. A. Dimopoulos, D. Bafaloukos, G. Aravantinos, C. Nicolaides, H. Kalofonos, P. Papakostas, N. Xiros et al., *Oncology (Williston Park)* **1998**, *12*, 45.

[98] M. G. Apps, E. H. Y. Choi, N. J. Wheate, *Endocr. Relat. Cancer.* **2015**, *22*, R219-33.

[99] V. V. Bakhonsky, A. A. Pashenko, J. Becker, H. Hausmann, H. J. M. de Groot, H. S. Overkleeft, A. A. Fokin, P. R. Schreiner, *Dalton Trans.* **2020**, *49*, 14009.

[100] O. Aronov, A. T. Horowitz, A. Gabizon, D. Gibson, *Bioconjug. Chem.* **2003**, *14*, 563.

[101] E. Fokkema, H. J. M. Groen, M. N. Helder, E. G. E. de Vries, C. Meijer, *Biochem. Pharmacol.* **2002**, *63*, 1989.

[102] E. Wexselblatt, D. Gibson, *J. Inorg. Biochem.* **2012**, *117*, 220.

[103] a) B. S. Iyengar, T. Takahashi, W. A. Remers, W. T. Bradner, *J. Med. Chem.* **1986**, *29*, 144; b) W. Neumann, B. C. Crews, M. B. Sárosi, C. M. Daniel, K. Ghebreselasie, M. S. Scholz, L. J. Marnett, E. Hey-Hawkins, *ChemMedChem* **2015**, *10*, 183; c) C. Camacho, D. Maciel, H. Tomás, J. Rodrigues, *Pharmaceutics* **2023**, *15*.

[104] S. Schiesser, B. Hackner, M. Vrabel, W. Beck, T. Carell, *Eur. J. Org. Chem.* **2015**, *2015*, 2654.

[105] A. R. Khokhar, Q. Xu, R. A. Newman, Y. Kido, Z. H. Siddik, *J. Inorg. Biochem.* **1992**, *45*, 211.

[106] T. A. Connors, M. Jones, W. C. Ross, P. D. Braddock, A. R. Khokhar, M. L. Tobe, *Chem. Biol. Interact.* **1972**, *5*, 415.

[107] a) K. Lee, T. Tashiro, M. Noji, *Chem. Pharm. Bull.* **1994**, *42*, 702; b) Heinz P. Fritz, Friedemann Tiedt, *Z. Naturforsch. B* **1973**, 176.

[108] S. Karmakar, K. Purkait, S. Chatterjee, A. Mukherjee, *Dalton Trans.* **2016**, *45*, 3599.

[109] C. Carraro, T. Helbing, A. Francke, I. Zuravka, A. Sosic, M. de Franco, V. Gandin, B. Gatto, D. R. Göttlich, *ChemMedChem* **2021**, *16*, 860.

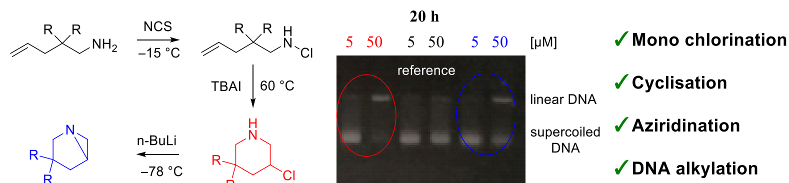
[110] R. F. Fenske, D. S. Martin, K. Ruedenberg, *Inorg. Chem.* **1962**, *1*, 441.

[111] A. Pidcock, R. E. Richards, L. M. Venanzi, *J. Chem. Soc. A* **1966**, *0*, 1707.

[112] M. Bazargan, M. Mirzaei, A. Franconetti, A. Frontera, *Dalton Trans.* **2019**, *48*, 5476.

6. Publications

6.1 Secondary 3-Chloropiperidines: Powerful Alkylating Agents



The synthesis of secondary 3-chloropiperidines and highly strained bicyclic aziridines is reported, including a new method for the selective mono-chlorination of unsaturated primary amines. The novel compounds, which closely resemble natural alkylating agents, proved to be more active than previously reported 3-chloropiperidines in a DNA cleavage assay, highlighting their potential as powerful alkylating agents.

Reference

Mats Georg, Lina Alexandra Laping, Veronica Billo, Barbara Gatto, Peter Friedhoff, Richard Göttlich, *ChemistryOpen* **2023**, 13, e202300181. (doi.org/10.1002/open.202300181)

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Secondary 3-Chloropiperidines: Powerful Alkylating Agents

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In previous works, we demonstrated that tertiary 3-chloropiperidines are potent chemotherapeutics, alkylating the DNA through the formation of bicyclic aziridinium ions. Herein, we report the synthesis of novel secondary 3-chloropiperidine analogues. The synthesis incorporates a new procedure to monochlorinate unsaturated primary amines utilizing *N*-chlorosuccinimide, while carefully monitoring the temperature to prevent dichlorination. Furthermore, we successfully isolated highly strained bicyclic aziridines by treating the secondary 3-chloropiperidines with a sufficient amount of base. We conclude this work with a DNA cleavage assay as a proof of principle, comparing our previously known substrates to the novel compounds. In this, the secondary 3-chloropiperidine as well as the isolated bicyclic aziridine, proved to be more effective than their tertiary counterpart.

Introduction

Being the first class of chemotherapeutics, alkylating agents have played a major role in the treatment of cancer.[1] One of the simplest representatives is mechlorethamine, which has been developed in the mid-20th century and is still in use today.[2,3] Its mode of action involves the intramolecular formation of a highly reactive aziridinium ion, by substitution of the chloride. This electrophilic intermediate is readily attacked by nucleophiles like the guanine bases of the DNA.[4] This results in the formation of a covalent adduct which potentially initiates further steps like depurination, strand cleavage and eventually apoptosis of the affected cell.[5] Despite serious side effects, a wide variety of alkylating agents have been developed in the past and to this day they are indispensable therapeutics. Besides synthetic compounds like mechlorethamine or chlorambucil[2] (Figure 2a), numerous natural analogues with similar modes of action have been discovered and isolated. In the context of this paper the most relevant compounds would be azinomycins, mitomycins as well as the antibiotic 593A (Figure 1).[6–8]

Especially the latter one shows a remarkable similarity to the synthetic nitrogen mustards with its active moiety being a 3-chloropiperidine. Its total synthesis, however, has demonstrated to be very challenging.[9] Therefore, in the past years, our research group has developed a multitude of simplified analogues. Previous publications have proven that these novel 3-chloropiperidines are capable of alkylating the DNA in vitro and confirmed that they are preferentially attacked by the N^7-Position of the guanine nucleobase.[10,11] Our most recent advancements included the isolation of the bicyclic aziridinium ion as the key intermediate to gain further inside in the mechanism of action by NMR kinetics.[12]

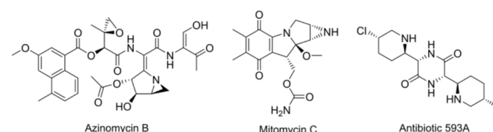

Figure 1. Natural compounds with known alkylating properties as lead structures.

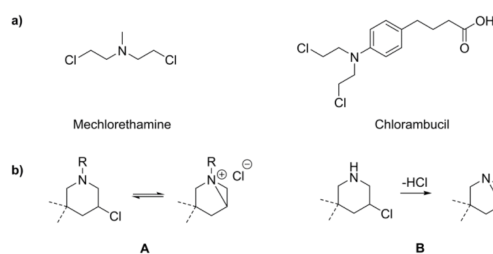

Figure 2. a) Structures of mechlorethamine and chlorambucil. b) General structure of tertiary 3-chloropiperidines (A) and of secondary 3-chloropiperidines (B).

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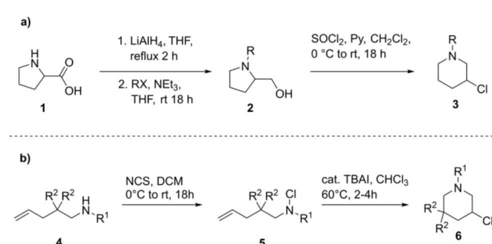
Looking back at the antibiotic 593A (Figure 1) as the lead structure, one might quickly notice that it incorporates a 3-chloropiperidine containing a secondary amine. In our previous studies we reported the synthesis and evaluation of tertiary 3-chloropiperidines (Figure 2b, A), using the nitrogen as a simple and convenient position to modify the structure. To resemble the lead structure more closely, in the present work we focus on the synthesis and characterisation of secondary 3-chloropiperidines (Figure 2b, B). In contrast to previous compounds, we expect that these novel alkylating agents could form the stable aziridines instead of the highly reactive aziridinium ions.[12] Their stability should make them less prone to side reactions such as hydrolysis and ultimately lead to a higher concentration of the active intermediate. Aziridines are also common in a variety of natural compounds and serve as the active moiety in clinical used compounds such as mitomycin C and azinomycin B (Figure 1).[6,7] Taking this into account,t we hypothesized that, despite being chemically less reactive, secondary 3-chloropiperidines possibly represent more potent alkylating agents then their tertiary counterparts.

Results and Discussion

In our previous publications, we have established two main synthetic pathways towards 3-chloropiperidines.[11] The first approach (Scheme 1) is utilizing the already cyclic amino acid proline **1**, which in the first step is reduced to proline with lithium aluminium hydride and functionalized at the nitrogen through a nucleophilic substitution. In the final step, the pyrrolidine precursor 2 is chlorinated with thionyl chloride to the thermodynamically more stable 3-chloropiperidine **3**, which is formed via a ring expansion reaction utilizing the aforementioned aziridinium ion. Since the ring expansion proceeds in a stereoselective way this approach can lead to enantiopure compounds, whose stereochemistry depends on the choice of the starting material, i.e. either D- or L-proline.[13,14] While this pathway bears obvious advantages it lacks the opportunity of any further functionalization of the 3-chloropiperidine.

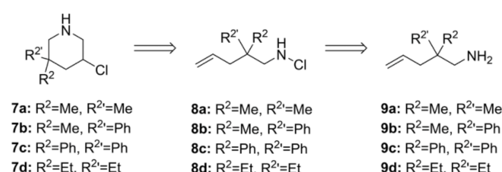
The second synthetic approach starts from a linear amine precursor **4**, which is chlorinated utilizing NCS and then transformed into the desired product **6** via an Iodine catalysed cyclisation (Scheme 1b). While leading to stereoisomers, this route allows the introduction of various different groups in the R^2-position, such as methyl, phenyl or even cyclic groups like cyclopropanyl and cyclobutanyl.[15]



Scheme 1. a) Pathway 1, starting from D- or L-proline resulting in the respective enantiopure 3-chloropiperidine; b) pathway 2, starting from a linear amine precursor, which is chlorinated to the corresponding *N*-chloroamine followed by TBAI-mediated cyclisation.

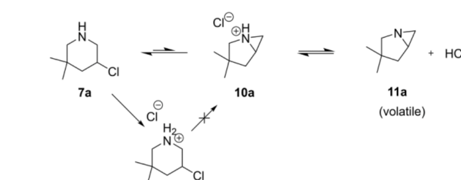
The primary amine **9a** used as a starting material in this approach (Scheme 2) is readily available through a literature known procedure from 2,2-dimethylpent-4-enal.[16] Transformation to the oxime and subsequent reduction with LAH gives the desired amine.[17] The mono chlorination to the cyclisation precursor **8a** posed a bigger challenge. Although GUILLEMIN described a gas/solid phase based procedure to obtain monochlorinated primary amines, he also reported that unsaturated amines are too unstable to be chlorinated under his conditions.[18] Utilizing the same conditions as described above in Scheme 1b inadvertently led to the unwanted *N,N*-dichloroamine. While the desired product could be observed in a TLC reaction control, it was not possible to isolate it through column chromatography. Finally, a reduction of the reaction temperature to −15 °C, plus adjustment of the used equivalents, combined with a careful monitoring via TLC to prevent disproportionation, led to a full conversion into the sought-after product **8a**. The succinic imide, formed as a side product, could be filtered off after precipitation with pentane, resulting in very high yields without the need for any further purification. With the precursor **8a** at hand we turned to the final cyclisation. While multiple catalysts (TBAI, $CuCl_2$ and $BF_3 \cdot Et_2O$) led to a conversion, which could be observed with GC/MS, it was not possible to isolate the desired product **7a**. We reasoned this might be due to the formation of the possibly volatile aziridine **11a** (Scheme 3).

To circumvent this problem, we increased the molecular weight of the product by introducing phenyl groups in the R^2-Position. The necessary starting material **9b** can be obtained as described above.[16,17] The diphenyl substituted amine **9c** can be



7a: R^2=Me, $R^{2'}$=Me	**8a**: R^2=Me, $R^{2'}$=Me	**9a**: R^2=Me, $R^{2'}$=Me
7b: R^2=Me, $R^{2'}$=Ph	**8b**: R^2=Me, $R^{2'}$=Ph	**9b**: R^2=Me, $R^{2'}$=Ph
7c: R^2=Ph, $R^{2'}$=Ph	**8c**: R^2=Ph, $R^{2'}$=Ph	**9c**: R^2=Ph, $R^{2'}$=Ph
7d: R^2=Et, $R^{2'}$=Et	**8d**: R^2=Et, $R^{2'}$=Et	**9d**: R^2=Et, $R^{2'}$=Et

Scheme 2. Planned synthetic pathway, adapted from pathway 2.



Scheme 3. Formation of the azirinium ion, followed by the release of HCl to the aziridine.

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produced starting from commercially available diphenylacetonitrile by allylation and reduction.[19] This attempt proved to be successful and compounds **7b** and **7c** could be isolated over two steps in 44% and 51% yield respectively.

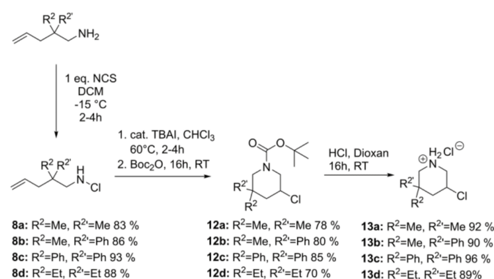
Transforming 3-chloropiperidines into their HCl salts effectively brings their reactivity to a halt, by lowering the electron density at the nitrogen centre and therefore stopping the formation of the aziridinium ion. This led to an optimized approach, trapping the cyclized product in situ with a suitable reagent, which lowers the electron density of the nitrogen. First experiments were done with acetic anhydride, giving the acetylated piperidine in 92% yield. In order to release the trapping agent more easily, we switched to Boc-anhydride, which after purification via column chromatography can easily be removed with HCl·dioxane leading directly to the stable HCl salt in nearly quantitative yields.

In summary this optimized synthetic pathway gives easy access to a variety of novel 3-chloropiperidines incorporating a secondary amine in good yields up to >90%. Due to the unspecific cyclisation all products are obtained racemic, with the exception of **13b** giving two diastereomers that can be separated through column chromatography prior to deprotection. The synthesis of diphenyl functionalized compound **12c** also led to the isolation of the corresponding 2-(chloromethyl)pyrrolidine in 19% yield, bringing the cyclisation to a combined yield of 85% (Scheme 4).

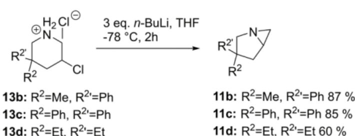
The aforementioned azinomycin B (Figure 1) also has been shown to alkylate DNA with its two active centres, one of them being a bicyclic aziridine,[20] similar to compound **11a** shown in Scheme 3. This observation, together with the high synthetic value of aziridines,[21] sparked our interest in accessing bicyclic aziridines from the secondary 3-chloropiperidines discussed above. Multiple sources have shown that treating β-chlorinated amines with sufficient base leads to the formation of aziridines.[22] With compounds **13a–d** at hand we turned our attention to finding suitable reaction conditions. Treating compounds **13b–d** with a slight excess of *n*-BuLi at −78 °C in THF, followed by an aqueous workup resulted in good yields up to 87% without the need for any further purification (Scheme 5). Compound **11a** could not be isolated, which is attributed to its low molecular weight and therefore low boiling point.

With the availability of the novel compounds better resembling the lead natural compound, we examined their reactivity in comparison to our previously published tertiary amine compound **6**. A classical DNA cleavage assay was chosen, adopting the workflow employed in our previous publications. Briefly, single- or double-strand breakage of DNA, as a follow up reaction to previous alkylation, leads to a change in the topology of a supercoiled (SC) DNA plasmid. The resulting open chain (OC) or linearized (L) forms show a different electrophoretic mobility in agarose gel relative to the control unreacted DNA. This evidence can be used to evaluate the ability to alkylate DNA of our novel compounds. DNA plasmid pGEM1 was incubated with two distinct concentrations (5 and 50 μM) of the 3-chloropiperidine or the respective aziridine in a BPE (biphosphate-EDTA) buffer solution at 37 °C. While compounds containing two methyl groups in the R^2 position have previously been in the focus of our research, compounds **7c**, **11c** and **6c** have been chosen for this proof of principle, allowing us to also evaluate the reactivity of the bicyclic aziridine.

While in the lower concentration of 5 μM none of the compounds show differences in cleavage activity to allow for a clear distinction of potency, at the higher concentration of 50 μM and prolonged incubation time of 20 h substances **7c** and **11c** proved to be much more active than their tertiary



Scheme 4. Optimized synthetic pathway leading directly to the storable HCl salts.



Scheme 5. Synthesis of bicyclic aziridines from secondary 3-chloropiperidine.

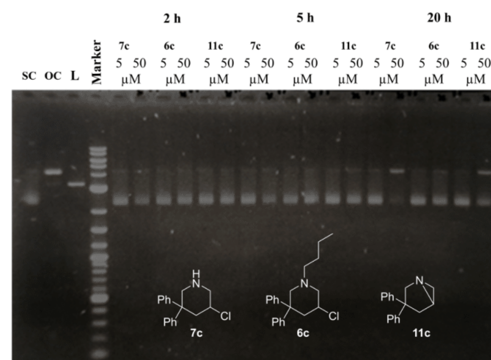


Figure 3. DNA cleavage activity comparison between **7c**, **14c** and **11c** in two different concentrations (5 μM and 50 μM). The supercoiled pGEM1 was incubated for 2 h, 5 h and 20 h at 37 °C in BPE buffer. Cleavage of supercoiled DNA into its open circular (OC) and linearized (L) forms was monitored utilizing gel electrophoresis (1.4% agarose in TAE buffer). Visualization was achieved upon staining with Intas HDGreen™ under UV-light.

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counterpart **6c**. **7c** seems to be slightly more active than **11c**, which is somewhat surprising considering that **11c** is believed to be an intermediate in the reaction of **7c** with nucleophiles. A possible explanation for this might be a premature hydrolysation of the highly reactive aziridine. These results demonstrate that the novel compounds are capable of alkylating and cleaving DNA, and also proves our initial hypothesis regarding the reactivity of secondary 3-chloropiperidines in comparison to our previously published compounds.[11]

Conclusions

In conclusion we report the synthesis of novel 3-chloropiperidines incorporating a secondary amine. In the first step, we developed a new and easy method for the mono-chlorination of unsaturated primary amines with NCS at lowered temperatures. The cyclisation to the desired 3-chloropiperidines proceeded through the use of catalytic amounts of TBAI. Our method utilizing Boc-anhydride to trap the product in situ allows for an easy workup and prevents the formation of highly reactive and volatile side products. The protection group can afterwards be cleaved easily with HCl·dioxane, to give the unreactive and therefore storable HCl salts in almost quantitative yields, without the need for any further purification. Treatment with 2 equivalents of *n*-BuLi gave the synthetically interesting bicyclic aziridines in good yields over 80%. Finally, we concluded this work proving by DNA alkylating assay that the secondary 3-chloropiperidine as well as the corresponding aziridine showed a higher activity compared to previously reported compounds. In the future we plan on further investigating the alkylating properties of the novel secondary 3-chloropiperidines and the isolated bicyclic aziridines, as well as exploring the synthetic applications of the mono-chlorinated primary amines.

Experimental Section

2,2-Dimethylpen-4-enal

Freshly distilled isobutyraldehyde (109.508 g, 1.50 mol, 1.5 equiv.), allyl alcohol (59.121 g, 1.0 mol, 1 equiv.) and *p*-toluenesulfonic acid (0.263 g, 1.50 mmol) are added in *p*-cymene (200 g). The mixture is heated at reflux for 24 h under a Dean–Stark apparatus, until no more water is separated and the temperature at the reaction sump reached 140 °C. The product is separated as a colourless liquid in 73% yield (82.339 g, 0.73 mol) by vacuum distillation through a 50 cm Vigreux column (62–69 °C at 150 mbar). ^{1}H NMR (400.1 MHz, $CDCl_3$): 9.46 (s, 1H), 5.76–5.62 (m, 1H), 5.10–4.98 (m, 2H), 2.20 (dt, J = 7.5, 1.2 Hz, 2H), 1.04 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 205.88, 133.13, 118.45, 45.72, 41.44, 21.16. HRMS (ESI): m/z = 135.0783 [M + Na]$^+$ (calculated for $C_7H_{12}O + Na^+$: 135.0780).

2,2-Dimethylpent-4-enal oxime

A solution of hydroxylammonium chloride (15.04 g, 216.0 mmol, 1.2 equiv.) in 20 mL of distilled water and a solution of sodium hydroxide (7.23 g, 181.0 mmol, 1 equiv.) in 80 mL of distilled water/ethanol 1:3 are added to a solution of 2,2-dimethylpent-4-enal (20.26 g, 181 mmol, 1 equiv.) in 300 mL of ethanol. The mixture is left standing for 16 h at room temperature. The ethanol is removed under reduced pressure and 300 mL of TMBE are added to the mixture. The phases are separated, and the aqueous phase is extracted by the addition of TMBE (3×100 mL) and the organic phase is then dried with $MgSO_4$. The solvent is removed under reduce pressure and the crude product is purified by vacuum distillation (94 °C at 40 mbar). The product is obtained in 81% yield (18.696 g, 0.147 mol). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 9.00 (br s, 1H), 7.34 (s, 1H), 5.81–5.70 (m, 1H), 5.10–5.02 (m, 2H), 2.16 (dt, J = 7.4, 1.2 Hz, 2H), 1.09 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 158.68, 133.99, 118.27, 45.36, 36.76, 25.13 (ESI): m/z = 150.0890 [M + Na]$^+$ (calculated for $C_7H_{13}NO + Na^+$: 150.0889).

2,2-Dimethylpent-4-en-1-amine (9a)

Under nitrogen atmosphere, to a suspension of $LiAlH_4$ (8.38 g, 221.0 mmol, 1.5 equiv.) in 200 mL of anhydrous diethyl ether, a solution of 2,2-dimethylpent-4-enal oxime (18.696 g, 147.0 mmol, 1.0 equiv.) in 80 mL of anhydrous diethyl ether is added dropwise at 0 °C. The mixture is stirred at room temperature for 18 h. The latter is then cooled down to 0 °C and carefully quenched with water (8.40 mL), 15% NaOH solution (8.40 mL), and then water again (25 mL). The mixture is stirred for 30 min at 0 °C and filtered. The precipitate is suspended again in diethyl ether, heated to reflux for 30 min, and filtered while it is still warm. The combined organic filtrate is dried over Na_2SO_4 and the solvent is removed under reduced pressure. The product is purified by vacuum distillation (50–60 °C at 80 mbar), obtaining a colourless oily liquid in 56% yield (9.347 g, 82.6 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 5.75 (ddt, J = 15.9, 11.3, 7.5 Hz, 1H), 4.99–4.92 (m, 2H), 2.38 (s, 1H), 1.90 (dt, J = 7.5, 1.2 Hz), 0.98 (s), 0.79 (s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 135.33, 116.90, 52.65, 44.01, 34.89, 24.59. HRMS (ESI): m/z = 114.1277 [M + H]$^+$ (calculated for $C_7H_{15}N + H^+$: 114.1277).

N-Chloro-2,2-dimethylpent-4-en-1-amine (8a)

2,2-dimethylpent-4-en-1-amine (1.136 g, 10.00 mmol, 1.0 equiv.) is added to a solution of dry DCM (70 mL) under nitrogen atmosphere. The solution is cooled down to −15 °C (ethylene glycol/liquid nitrogen bath) and then 1 equiv. of NCS is added (1.333 g, 10.00 mmol, 1 equiv.). The mixture is stirred for 2–4 h at −15 °C. When a TLC control (pentane/TBME 10:1) confirms the end of the reaction, the solvent is evaporated. 50 mL of pentane are added to make the succinic imide precipitate. The solution is filtered and the solvent is removed under reduced pressure resulting in a pale yellow oil in 83% yield (1.227 g, 8.31 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 5.81–5.69 (m, 1H), 5.03–4.94 (m, 2H), 4.27–3.93 (m, 1H), 2.83 (t, J = 2.8 Hz, 2H), 1.98–1.94 (m, 2H), 0.88 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 134.57, 117.72, 67.15, 44.66, 35.55, 25.35. HRMS (ESI): m/z = 148.0890 [M + H]$^+$ (calculated for $C_7H_{14}ClN + H^+$: 148.0888).

N-Boc-3-chloro-5,5-dimethylpiperidine (12a)

Under nitrogen atmosphere, 230 mg (1.56 mmol, 1 equiv.) of *N*-chloro-2,2-dimethylpent-4-en-amine is dissolved in 15 mL of anhydrous $CHCl_3$. 0.408 g of TBAI (0.31 mmol, 0.2 equiv.) is added to the solution at room temperature. The mixture is heated at 60 °C for 3 h. Boc-anhydride (1.87 g, 1.87 mmol, 1.2 equiv.) is added to the solution at room temperature. The mixture is stirred for 16 h at room temperature. After purification by column chromatography (pentane/TBME, 10:1) the product is isolated as a mixture of rotamers as colourless crystals in 78% yield (300 mg, 1.21 mmol)

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^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 4.59–4.24 (m, 1H), 3.96 (tt, *J* = 11.5, 4.6 Hz, 1H), 3.86–3.54 (m, 1H), 2.75–2.55 (m, 1H), 2.54–2.34 (m, 1H), 2.02–1.95 (m, 1H), 1.49 (d, *J* = 12.4 Hz, 1H), 1.45 (s, 9H), 0.94 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 154.55, 80.01, 54.96, 51.97, 50.66, 48.69, 34.13, 28.37, 28.25, 23.53. HRMS (ESI): *m/z* = 270.1229 $[M+Na]^+$ (calculated for $C_{12}H_{22}ClNO+Na^+$: 270.1231).

3-Chloro-5,5-dimethylpiperidine hydrochloride (13 a)

150 mg (0.60 mmol, 1 equiv.) *N*-Boc-3-chloro-5,5-dimethylpiperidine are dissolved in 5 mL of dioxane and 3 mL 4 N HCl*dioxane are added. The solution is stirred for 16 h at room temperature. 5 mL of diethyl ether are added and the suspension is centrifuged. The resulting white solid is washed two times with diethyl ether and dried in vacuo. The product is obtained in 92% yield (102 mg, 0.55 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 10.33 (s, 1H), 9.48 (s, 1H), 4.37–4.24 (m, 1H), 3.77–3.68 (m, 1H), 3.07 (d, *J* = 12.9 Hz, 1H), 2.82–2.69 (m, 1H), 2.71–2.60 (m, 1H), 2.15–2.06 (m, 1H), 1.57 (t, *J* = 12.7 Hz, 1H), 1.26 (s, 3H), 1.07 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = ^{13}C NMR (101 MHz, $CDCl_3$) δ 49.25, 48.06, 46.33, 32.05, 29.01, 24.38.

2-Methyl-2-phenylpent-4-enal

12.63 g (217.38 mmol, 1.07 equiv.) allylic alcohol and 27.76 g (203.16 mmol, 1 equiv.) 2-phenyl-propionaldehyde are dissolved in 15 mL of toluene in a 100 mL two necked flask with an attached 30 cm Vigreux column and a Dean–Stark trap. 0.103 g (0.6 mmol, cat.) *p*-toluenesulfonic are added and the mixture is refluxed, until 3.7 mL of water have been released. The crude product is distilled directly from the mixture at 3 mbar and 77 °C. The product is obtained in 83% yield (29.522 g, 169.4 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 9.62 (s), 7.41–7.35 (m), 7.31–7.23 (m), 5.60–5.45 (m), 5.09–5.00 (m), 2.73–2.59 (m), 1.44 (s) ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = δ 202.09, 139.62, 133.31, 128.99, 127.47, 127.31, 118.73, 53.75, 40.74, 18.98 HRMS (ESI): *m/z* = 193.0935 $[M+Na]^+$ (calculated for $C_{12}H_{14}O+Na^+$: 197.0937).

2-Methyl-2-phenylpent-4-enal oxime

A solution of 6.25 g (90 mmol, 1.2 equiv.) of hydroxylamine hydrochloride in 6.6 mL of water is added to a solution of sodium hydroxide (3.0 g, 75 mmol, 1 equiv.) in water/ethanol (7 mL/25 ml). 2-methyl-2-phenylpent-4-enal (13.07 g, 75 mmol, 1 equiv.) is dissolved in 170 mL of ethanol and added to the mixture. The latter is left stirring overnight. 350 mL of TBME are added and the phases are separable. The organic phase is washed with brine and then dried over $MgSO_4$. After evaporation of the solvent, the product is obtained in 91% yield (12.97 g, 68.55 mmol). ^{1}H NMR (400.1 MHz, CDCl3): δ [ppm] = 8.85 (br s), 7.48 (s), 7.32–7.22 (m), 7.21–7.15 (m), 5.54 (ddt, J = 17.3, 10.2, 7.2 Hz), 5.02–4.95 (m), 2.59 (d, J = 1.1 Hz), 1.41 (s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 157.24, 143.88, 133.74, 128.53, 126.75, 126.62, 118.41, 43.98, 40.62, 23.45. HRMS (ESI): *m/z* = 190.1042 $[M+H]^+$ (calculated for $C_{12}H_{15}NO+H^+$: 190.1227).

2-Methyl-2-phenylpent-4-en-1-amine (9 b)

Under nitrogen atmosphere, 3.90 g (103 mmol, 1.5 equiv.) of lithium aluminium hydride are suspended in 125 mL of dry diethyl ether at 0 °C. 12.94 g (68.37 mmol, 1 equiv.) of 2-methyl-2-phenylpent-4-enal oxime are dissolved in 50 mL of anhydrous diethyl ether and added carefully to the LAH suspension at 0 °C. The mixture is stirred at room temperature for 16 h. The mixture is quenched with 3.76 mL of water, 3.76 mL of 20% $NaOH_{aq}$ and 11.28 mL of water at 0 °C. After filtration the solid is resuspended and heated to reflux in 100 mL TBME. After filtration the organic phases are combined and evaporated. The crude product is purified by vacuum distillation (100–105 °C at 3 mbar) obtaining a colourless liquid in 75% yield (8.99 g, 51.3 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.38–7.29 (m), 7.24–7.17 (m), 5.63–5.50 (m), 5.06–4.95 (m), 2.96 (d, *J* = 13.1 Hz), 2.73 (d, *J* = 13.2 Hz), 2.52 (ddt, *J* = 13.8, 6.4, 1.5 Hz), 2.28 (ddt, *J* = 13.8, 8.1, 1.1 Hz), 1.30 (s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 134.75, 128.36, 126.74, 125.96, 117.31, 53.37, 44.60, 22.21. HRMS (ESI): *m/z* = 176.1438 $[M+H]^+$ (calculated for $C_{12}H_{17}N+H^+$: 176.1434).

N-Chloro-2-methyl-2-phenylpent-4-en-1-amine (8 b)

2-Methyl-2-phenylpent-4-en-1-amine (1.753 g, 10 mmol, 1.0 equiv.) is added to a solution of dry DCM (70 mL) under nitrogen atmosphere. The solution is cooled down to −15 °C and 1 equiv. of NCS is added (1.335 g, 10 mmol, 1 equiv.). The mixture is stirred for 2 h at −15 °C. When a TLC control (pentane/TBME 10:1, Rf = 0.54) confirms the end of the reaction, the solvent is evaporated. 50 mL of pentane are added to make succinimide precipitate. The solution is filtered and evaporated to obtain the product as a pale yellow oil in 86% yield. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.34–7.26 (m, 4H), 7.21–7.16 (m, 1H), 5.50 (dddd, *J* = 16.8, 10.1, 7.9, 6.6 Hz, 1H), 5.03–4.92 (m, 2H), 3.77–3.66 (m, 1H), 3.36–3.28 (m, 1H), 3.16–3.07 (m, 1H), 2.51–2.44 (m, 1H), 2.36–2.29 (m,1H), 1.34 (s, 3H). ^{13}C NMR (100.6 MHz, CDCl3): δ [ppm] = 144.03, 133.81, 128.73 (d, *J* = 2.5 Hz, C-9 or C-10), 126.59, 126.30, 118.20, 67.23, 45.18, 42.57, 23.06. HRMS (ESI): *m/z* = 210.1048 $[M+H]^+$ (calculated for $C_{12}H_{16}ClN+H^+$: 210.1044).

3-Chloro-5-methyl-5-phenylpiperidine (7 b)

Under nitrogen atmosphere, 1.750 g (8.34 mmol, 1 equiv.) of *N*-chloro-2-methyl-2-phenylpent-4-en-amine is dissolved in 30 mL of anhydrous chloroform. 0.308 g of TBAI (0.83 mmol, 0.1 equiv.) are added to the solution at room temperature. The mixture is heated to 60 °C for 3 h. The product is purified by flash chromatography (DCM/acetone 20:1, Rf = 0.13) and obtained as a mixture of diastereomers (762 mg. 44%). *cis*-Diastereomer: ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.35–7.23 (m), 7.20–7.12 (m), 3.75–3.64 (m), 3.49 (ddd, *J* = 13.9, 2.8, 1.0 Hz), 3.22–3.14 (m), 2.87–2.79(m), 2.67–2.60 (m), 2.58–2.53 (m), 1.75 (dd, *J* = 13.4, 12.1 Hz), 1.06 (s).^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 144.78, 129.11, 126.28, 126.13, 55.21, 54.51, 54.17, 46.96, 42.15, 31.33. *trans*-Diastereomer: ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.35–7.23 (m), 7.20–7.12 (m), 4.15–4.05 (m), 3.39–3.33 (m), 2.97–2.92 (m), 2.79–2.74 (m) 2.59–2.55 (m), 2.40–2.33 (m), 1.97–1.91 (m), 1.33 (s). ^{13}C NMR (100.6 MHz, CDCl3): δ [ppm] = 147.82, 128.52, 126.44, 124.96, 55.65, 54.51, 54.24, 46.57, 40.25, 24.08. HRMS (ESI): *m/z* = 210.1043 $[M+H]^+$ (calculated for $C_{12}H_{16}ClN+H^+$: 210.1044).

5-Methyl-5-phenyl-1-azabicycle[3.1.0]hexane (11 b)

Under nitrogen atmosphere, 0.212 g (0.86 mmol, 1 equiv.) of 3-chloro-5-methyl-5-phenylpiperidine hydrochloride are dissolved in 10 mL of dry THF. The solution is cooled down at −78 °C with an acetone/liquid nitrogen bath. 2 equiv. of *n*-BuLi (0.70 mL, 1.72 mmol) are added carefully. The reaction mixture is stirred at −78 °C for 2 h and then quenched with 5–10 mL of NH_4Cl. Diethyl ether is added and the phases are separated. The latter is extracted with diethyl ether (3×25 mL) and combined organic phases are dried over $MgSO_4$ and evaporated. The product is obtained in 87% yield (0.103 g, 0.75 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] =

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7.40–7.29 (m), 7.22–7.16 (m), 3.68 (d, J = 12.2 Hz), 2.71 (d, J = 12.2 Hz), 2.54–2.47 (m), 2.51–2.47 (m), 2.10–2.08 (m), 2.07–2.05 (m), 1.60–1.57 (m), 1.41 (s). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 150.65, 128.37, 125.76, 125.63, 69.07, 52.93, 42.96, 42.23, 40.53, 30.82. HRMS (ESI): m/z = 174.1274 [M + H]$^+$ (calculated for $C_{12}H_{15}N + H^+$: 174.1277).

2,2-Diphenylpent-4-en-nitrile

A solution of diphenylacetonitrile (10.013 g, 51.83 mmol, 1.0 equiv.) in 30 mL of anhydrous THF is added dropwise at 0 °C to a suspension of sodium hydride (2.714 g, 67.37 mmol, 1.3 equiv.) in anhydrous THF (20 mL) under nitrogen atmosphere. The mixture is stirred for 1 h at room temperature. 4.9 mL of allyl bromide (57.01 mmol, 1.1 equiv.) are added dropwise at 0 °C and the mixture is stirred for 2 h at room temperature. After the careful addition of NH_4Cl (40 mL), the organic phase is separated by extraction of the aqueous phase with diethyl ether (3×25 mL). The organic phase is dried over $MgSO_4$ and distilled in the Kugelrohr. The product is obtained in 99% yield (11.951 g, 51.22 mmol) as a colourless oil. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.44–7.26 (m, 10H), 5.80–5.65 (m, 1H), 5.33–5.14 (m, 2H), 3.19–3.11 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 139.77, 131.82, 128.89, 127.98, 127.09, 121.99, 120.44, 51.75, 43.97. HRMS (ESI): m/z = 256.1098 [M + Na]$^+$ (calculated for $C_{17}H_{15}N + Na^+$: 256.1096).

2,2-Diphenylpent-4-en-1-amine (9 c)

Under nitrogen atmosphere, 11.868 g (50.87 mmol, 1.0 equiv.) of 2,2-diphenylpent-4-en-nitrile **2** is dissolved in 60 mL of anhydrous diethyl ether and added dropwise to a suspension of LAH (3.513 g, 92.57 mmol, 1.8 equiv.) in 100 mL of anhydrous diethyl ether at 0 °C. The mixture is stirred for 18 h at room temperature. The blend is cooled down to 0 °C and water (3.5 mL), 20% NaOH aqueous solution (3.5 mL) and water again (10.5 mL) are added carefully. Na_2SO_4 is added to the mixture and after filtration the solvent is removed und reduced pressure. The product is obtained as a colourless oil in 96% yield (11.643 g, 49.05 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.32–7.23 (m, 4H), 7.21–7.13 (m, 6H), 5.45–5.31 (m, 1H), 5.10–4.92 (m, 2H), 3.32 (s, 2H), 2.92 (dt, J = 7.0, 1.3 Hz, 2H), 1.22 (s, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 146.27, 134.67, 128.31, 128.22, 126.23, 117.90, 51.39, 48.59, 41.23. HRMS (ESI): m/z = 238.1591 [M + H]$^+$ (calculated for $C_{17}H_{19}N + H^+$: 238.1590).

N-Chloro-2,2-diphenylpent-4-en-1-amine (8 c)

2,2-Diphenylpent-4-en-1-amine **3** (2.718 g, 10 mmol, 1.0 equiv.) is added to a solution of dry DCM (70 mL) under nitrogen atmosphere. The solution is cooled down to −15 °C and 1 equiv. of NCS is added (1.336 g, 10 mmol, 1 equiv.). The mixture is stirred for 4 h at −15 °C. When a TLC control (pentane/TBME 10:1, Rf = 0.61) confirms the end of the reaction, the solvent is evaporated. 50 mL of pentane are added to make succinic imide precipitate. The solution is filtered and evaporated to obtain the product in 93% yield (2.530 g, 9.31 mmol). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.34–7.21 (m, 7H), 7.18–7.15 (m, 4H), 5.41–5.30 (m, 1H), 5.14–5.01 (m, 2H), 3.73–3.67 (m, 2H), 2.99 (d, J = 7.1 Hz, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 145.20, 134.06, 128.48, 127.95, 126.73, 118.78, 62.90, 50.87, 41.94.

3-Chloro-5,5-diphenylpiperidine (7 c)

Under nitrogen atmosphere, 9.150 g (30.3 mmol, 1 equiv.) of *N*-chloro-2,2-diphenylpent-4-en-amine **4** is dissolved in 40 mL of anhydrous $CHCl_3$. 1.120 g of TBAI (3.03 mmol, 0.1 equiv.) are added to the solution at room temperature. The mixture is heated at 60 °C for 3 h. The product is purified by flash chromatography (DCM/acetone 50:1, Rf = 0.13). 50% Yield (4.220 g, 15.50 mmol) ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.34–7.01 (m, 10H), 3.85–3.81 (m, 1H), 3.81–3.76 (m, 2H), 3.29–3.21 (m, 1H), 3.16–3.09 (m, 1H), 2.95 (d, J = 14.1 Hz, 1H), 2.72 (dd, J = 12.9, 11.1 Hz, 1H), 2.34 (dd, J = 13.2, 12.0 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 146.80, 143.00, 129.22, 128.50, 127.71, 126.59, 126.44, 126.11, 77.24, 54.37, 54.21, 53.99, 49.35, 45.71, 30.94. HRMS (ESI): m/z = 272.1197 [M + H]$^+$ (calculated for $C_{17}H_{18}ClN + H^+$: 272.1201).

N-Boc-3-chloro-5,5-diphenylpiperidine (12 c)

Under nitrogen atmosphere, 1.00 g (3.68 mmol, 1 equiv.) of *N*-chloro-2,2-diphenylpent-4-en-amine is dissolved in 20 mL of anhydrous chloroform. 1.120 g of TBAI (0.37 mmol, 0.1 equiv.) are added to the solution at room temperature. The mixture is heated at 60 °C for 3 h. 2 equiv. of di-*tert*-butyldicarbonate (1.61 g, 7.36 mmol) are added at room temperature and the mixture is stirred for 16 h. 20 mL of water are added and the aqueous phase is extracted with DCM (3×25 mL) The organic phase is dried with $MgSO_4$ and then filtered. The product is purified by column chromatography (pentane/TBME 30:1) and obtained as a mixture of rotamers in 66% yield (998 mg, 2.68 mmol) together with the pyrrolidine isomer in 19% yield (279 mg, 0.75 mmol). Piperidine-Isomer: ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.42–7.35 (m, 2H,), 7.34–7.23 (m, 4H), 7.23–7.10 (m, 4H), 5.22–4.82 (m, 1H), 4.62–4.27 (m, 1H), 3.97–3.60 (m, 1H), 3.19–3.12 (m, 1H),3.11–3.00 (m, 1H), 2.92–2.70 (m, 2H), 2.49–2.28 (m, 1H),1.49 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 153.98, 146.01, 128.61, 127.61, 126.29, 80.64, 52.06, 51.41, 50.94, 48.79, 45.93, 28.38. HRMS (ESI): m/z = 394.1548 [M + Na]$^+$ (calculated for $C_{22}H_{26}ClNO_2 + Na^+$: 394.1544). Pyrrolidine-Isomer: ^{1}H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.26–7.06 (m, 20H), 4.65 (dd, J = 11.6, 2.2 Hz, 1H), 4.45 (dd, J = 11.5, 2.1 Hz, 1H), 3.93–3.81 (m, 1H), 3.79–3.64 (m, 4H), 3.59–3.44 (m, 3H), 2.82–2.70 (m, 2H), 2.67–2.57 (m, 2H), 1.42 (s, 9H), 1.38 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = δ 154.65, 145.26, 128.71, 126.76, 126.59, 80.50, 57.11, 56.07, 52.99, 46.85, 42.81, 28.54.

3-Chloro-5,5-diphenylpiperidine hydrochloride (13 c)

372 mg (1 mmol, 1 equiv.) *N*-Boc-3-chloro-5,5-diphenylpiperidine are dissolved in 5 mL dioxane. 2.5 mL 4 N HCl·dioxane are added and the solution is stirred for 16 h at room temperature. 5 mL of diethyl ether are added and the suspension is centrifuged. The resulting white solid is washed two times with diethyl ether and dried in vacuo. The product is obtained in 96% yield (296 mg, 0.96 mmol). ^{1}H NMR (400.1 MHz, DMSO-d_6): δ [ppm] = 7.62–7.56 (m, 2H), 7.46–7.39 (m, 2H), 7.33–7.26 (m, 3H), 7.24–7.16 (m, 3H), 4.45 (d, J = 13.6 Hz, 1H), 4.08–3.98 (m, 1H), 3.49 (d, J = 13.4 Hz, 1H), 3.46–3.39 (m, 1H), 3.39–3.36 (m, 1H), 3.23 (t, J = 11.6 Hz, 1H), 2.53 (d, J = 12.8 Hz, 1H).^{13}C NMR (100.6 MHz, DMSO-d_6): δ [ppm] = 145.21, 140.14, 129.35, 128.54, 127.15, 126.82, 125.78, 49.84, 48.28, 46.57, 41.62.HRMS (ESI): m/z = 272.1199 [M–Cl$^-$]$^+$ (calculated for $C_{17}H_{18}ClN + H^+$: 272.1201).

5,5-Diphenyl-1-azabicycle[3.1.0]hexane (11 c)

Under nitrogen atmosphere, 2.00 g (6.49 mmol, 1 equiv.) of 3-chloro-5,5-diphenylpiperidine hydrochloride are dissolved in 20 mL

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of THF. The solution is cooled down to −78 °C. 3 equiv. of *n*-BuLi (7.80 mL of a 2.5 M solution, 19.46 mmol) are added carefully. The reaction mixture is stirred at −78 °C for 2 h and then quenched with 15 mL of NH_4Cl. Diethyl ether is added and the phases are separated. The organic phase is separated, dried over $MgSO_4$ and evaporated. The product is obtained in 85% yield (1.302 g, 5.53 mmol). ^1H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.32–7.22 (m, 9H), 7.21–7.10 (m, 3H), 3.96 (d, *J* = 12.2 Hz, 1H), 3.48 (d, *J* = 12.3 Hz, 1H), 2.94 (dd, *J* = 13.4, 2.5 Hz, 1H), 2.77–2.67 (m, 1H), 2.59–2.50 (m, 1H), 1.88 (d, *J* = 1.2 Hz, 2H), 1.45 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = δ 149.35, 147.50, 128.66, 128.36, 126.83, 126.76, 126.15, 125.81, 66.09, 59.99, 40.30, 39.99, 25.64. HRMS (ESI): *m/z* = 236.1432 $[M+H]^+$ (calculated for $C_{17}H_{17}N+H^+$: 236.1434).

2,2-Diethylpen-4-enal

To a solution of 5.00 g (50 mmol, 1 equiv.) ethylbutyraldehyde in 220 mL DCM 7.31 g (65 mmol, 1.3 equiv.), potassium *tert*-butoxide are added followed by 8.7 mL (100 mmol, 2 equiv.) of allyl bromide. The mixture is stirred for 2 h at room temperature. 100 mL water is added, the phases are separated and the organic phase is washed with 100 mL of water, dried over $MgSO_4$ and the solvent is removed under reduced pressure. After distillation (102–104 °C, 100 mbar), the product is obtained as a colourless oil in 60% yield (4.20 g, 29.95 mmol). ^1H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 5.73–5.47 (m, 1H), 5.14–4.98 (m, 2H), 2.19 (dt, *J* = 7.4, 1.3 Hz, 2H), 1.48 (q, *J* = 7.6 Hz, 4H), 0.74 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 206.88, 133.12, 118.05, 52.49, 35.06, 24.44, 7.87.

2,2-Diethylpent-4-enal oxime

2.495 g (35.91 mmol, 1.2 equiv.) hydroxylammonium chloride in 2.5 mL H_2O are added to a solution of 1.177 g (29.92 mmol, 1 equiv.) NaOH in 2.5 mL H_2O. After 5 min, 4.196 g 2,2-diethylpen-4-enal in 70 mL ethanol are added. The solution is left standing for 16 h and the ethanol is removed under reduced pressure. 50 mL of H_2O and 150 mL of TBME are added and the phases are separated. The aqueous phase is extracted with 3×50 mL of TBME. The combined organic phases are dried over Na_2SO_4 and the solvent is removed under reduced pressure. The product is obtained as a colourless oil in 94% yield (4.381 g, 28.22 mmol). ^1H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 7.23 (s, 1H), 5.72 (ddt, *J* = 16.5, 11.0, 7.4 Hz, 1H), 5.12–5.01 (m, 2H), 2.20 (dt, *J* = 7.3, 1.3 Hz, 2H), 1.46 (qd, *J* = 7.4, 1.7 Hz, 4H), 0.82 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 157.81, 133.87, 117.87, 43.01, 38.44, 27.55, 7.94. HRMS (ESI): *m/z* = 178.1203 $[M+Na]^+$ (calculated for $C_9H_{17}NO+Na^+$: 178.1202).

2,2-Diethylpent-4-en-1-amine (9 d)

1.246 g (21.84 g, 1.5 equiv.) LAH are suspended in 50 mL dry diethyl ether. At 0 °C, 3.399 g (21.84 mmol, 1 equiv.) 2,2-diethylpent-4-enal oxime in 20 mL dry diethyl ether are added dropwise. The suspension is stirred for 16 h at room temperature. The reaction is quenched by the subsequent addition of 1.25 mL H_2O, 1.25 mL 20% $NaOH_{aq}$ and 3.75 mL H_2O. The mixture is dried over Na_2SO_4 and filtered through a Büchner funnel. After distillation (92–96 °C, 50 mbar), the product is obtained as a colourless oil in 63% yield (1.945 g, 13.77 mmol). ^1H NMR (600.1 MHz, $CDCl_3$): δ [ppm] = 5.89–5.61 (m, 1H), 5.08–4.93 (m, 2H), 2.44 (s, 2H), 1.94 (d, *J* = 7.4 Hz, 2H), 1.21 (q, *J* = 7.5 Hz, 5H), 0.77 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ [ppm] = 135.13, 116.88, 46.46, 39.69, 38.50, 26.02, 7.47. HRMS (ESI): *m/z* = 142.1594 $[M+H]^+$ (calculated for $C_9H_{19}N+H^+$: 142.1590).

N-Chloro-2,2-diethylpent-4-en-1-amine (8 d)

In 20 mL dry DCM, 500 mg (3.54 mmol, 1 equiv.) 2,2-diethylpent-4-en-1-amine are dissolved. At −15 °C 473 mg NCS (3.54 mmol, 1 equiv.) are added and the solution is stirred at −15 °C for 4 h. After TLC showed completion of the reaction, the solvent is removed under reduced pressure. 20 mL pentane are added and the precipitating succinic imide is filtered of. The solvent is removed under reduced pressure and the product is obtained as a pale blue oil in 88% yield (549 mg, 3.12 mmol). ^1H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 5.89–5.67 (m, 1H), 5.19–4.95 (m, 2H), 2.90 (s, 2H), 2.01 (d, *J* = 7.5 Hz, 2H), 1.30 (q, *J* = 7.5 Hz, 4H), 0.82 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ [ppm] = 134.60, 117.76, 62.28, 40.91, 39.24, 26.79, 7.54. HRMS (ESI): *m/z* = 176.1220 $[M+H]^+$ (calculated for $C_9H_{18}ClN+H^+$: 176.1201).

N-Boc-3-chloro-5,5-diethylpiperidine (12 d)

In 50 mL dry $CHCl_3$, 1.116 g (6.35 mmol, 1 equiv.) *N*-chloro-2,2-diethylpent-4-en-1-amine are dissolved and 235 mg (0.64 mmol, 0.1 equiv.) TBAI are added at room temperature. The solution is heated to 60 °C for 3 h. 1.2 equiv. of di-*tert*-butyldicarbonate (1.66 g, 7.62 mmol) are added at room temperature and the mixture is stirred for 16 h. 20 mL of water are added and the aqueous phase is extracted with DCM (3×20 mL) The organic phase is dried with $MgSO_4$ and then filtered. The product is purified by column chromatography (pentane/TBME 50 : 1) and obtained as a mixture of rotamers in 70% yield (1.23 g, 4.46 mmol). ^1H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 4.59–4.26 (m, 1H), 4.06–3.74 (m, 2H), 2.78–2.27 (m, 2H), 2.22–2.07 (m, 1H), 1.45 (s, 9H), 1.40–1.17 (m, 5H), 0.85–0.77 (m, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 154.65, 52.18, 51.45, 51.07, 45.20, 29.29, 28.49, 23.65, 7.73, 7.15. HRMS (ESI): *m/z* = 298.1548 $[M+Na]^+$ (calculated for $C_{14}H_{26}ClNO_2+Na^+$: 298.1544).

3-Chloro-5,5-diethylpiperidine hydrochloride (13 d)

500 mg (1 mmol, 1 equiv.) *N*-Boc-3-chloro-5,5-diethylpiperidine are dissolved in 4.5 mL 4 N HCl·dioxane and the solution is stirred for 16 h at room temperature. 5 mL of diethyl ether are added and the suspension is centrifuged. The resulting white solid is washed two times with diethyl ether and dried in vacuo. The product is obtained in 89% yield (335 mg, 1.59 mmol). ^1H NMR (400.1 MHz, $CDCl_3$): δ [ppm] = 10.28 (s, 1H), 9.43 (s, 1H), 4.32–4.20 (m, 1H), 3.77–3.65 (m, 1H), 3.23–3.08 (m, 1H), 2.84–2.69 (m, 1H), 2.65–2.52 (m, 1H), 2.26–2.16 (m, 1H), 1.71–1.61 (m, 2H), 1.45 (t, *J* = 12.8 Hz, 1H), 1.39–1.30 (m, 2H), 0.89–0.81 (m, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] = 50.11, 49.60, 48.11, 42.27, 37.23, 29.85, 24.14, 7.34, 6.97. HRMS (ESI): *m/z* = 176.1201 $[M-Cl^-]^+$ (calculated for $C_9H_{19}ClN$-Cl^-: 176.1201).

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Conflict of Interests

The authors declare no conflict of interest.

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Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: alkylation · antitumor agents · aziridine · primary chloroamine · secondary 3-chloropiperidine

[1] M. R. P. D. Alison, (Ed.) *The cancer handbook*, John Wiley & Sons, Ltd, London, **2002**.
[2] B. A. Chabner, T. G. Roberts, *Nat. Rev. Cancer* **2005**, *5*, 65.
[3] K. W. Kohn, *Cancer Res.* **1996**, *56*, 5533.
[4] a) G. P. Warwick, *Cancer Res.* **1963**, *23*, 1315; b) L. H. Hurley, *Nat. Rev. Cancer* **2002**, *2*, 188.
[5] a) L. P. Bignold, *Anticancer Res.* **2006**, *26*, 1327; b) K. S. Gates, T. Nooner, S. Dutta, *Chem. Res. Toxicol.* **2004**, *17*, 839.
[6] S. Ishizeki, M. Ohtsuka, K. Irinoda, K.-I. Kukita, Nagaoka Katsuhiko, T. Nakashima, *J. Antibiot.* **1987**, *40*, 60.
[7] S. T. Crooke, W. T. Bradner, *Cancer Treat. Rev.* **1976**, *3*, 121.
[8] R. M. Folk, A. C. Peters, K. L. Pavkov, J. A. Swenberg, *Cancer Chemotherapy Reports. Part 3*, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, **1974**.
[9] T. Fukuyama, R. K. Frank, C. F. Jewell Jr., *J. Am. Chem. Soc.* **1980**, *102*, 2122.
[10] a) I. Zuravka, R. Roesmann, A. Sosic, W. Wende, A. Pingoud, B. Gatto, R. Göttlich, *ChemMedChem* **2014**, *9*, 2178; b) A. Sosic, I. Zuravka, N.-K. Schmitt, A. Miola, R. Göttlich, D. Fabris, B. Gatto, *ChemMedChem* **2017**, *12*, 1471; c) T. Helbing, C. Carraro, A. Francke, A. Sosic, M. de Franco, V. Gandin, R. Göttlich, B. Gatto, *ChemMedChem* **2020**, *15*, 2040.
[11] C. Carraro, A. Francke, A. Sosic, F. Kohl, T. Helbing, M. de Franco, D. Fabris, R. Göttlich, B. Gatto, *ACS Med. Chem. Lett.* **2019**, *10*, 552.
[12] T. Helbing, M. Georg, F. Stöhr, C. Carraro, J. Becker, B. Gatto, R. Göttlich, *Eur. J. Org. Chem.* **2021**, *2021*, 5905.
[13] M. D'hooghe, S. Catak, S. Stanković, M. Waroquier, Y. Kim, H.-J. Ha, V. van Speybroeck, N. de Kimpe, *Eur. J. Org. Chem.* **2010**, *2010*, 4920.
[14] J. Dolfen, N. N. Yadav, N. De Kimpe, M. D'hooghe, H.-J. Ha, *Adv. Synth. Catal.* **2016**, *358*, 3485.
[15] T. Helbing, M. Kirchner, J. Becker, R. Göttlich, *Eur. J. Org. Chem.* **2022**, *2022*, e202200597.
[16] K. C. Brannock, *J. Am. Chem. Soc.* **1959**, *81*, 3379.
[17] M. Noack, R. Göttlich, *Eur. J. Org. Chem.* **2002**, *2002*, 3171.
[18] J.-C. Guillemin, J.-M. Denis, *Angew. Chem. Int. Ed.* **1982**, *21*, 690.
[19] a) H. M. Lovick, F. E. Michael, *J. Am. Chem. Soc.* **2010**, *132*, 1249; b) F. Liu, K. M. Worthy, L. Bindu, A. Giubellino, D. P. Bottaro, R. J. Fisher, T. R. Burke Jr., *Org. Biomol. Chem.* **2007**, *5*, 367.
[20] R. S. Coleman, R. J. Perez, C. H. Burk, A. Navarro, *J. Am. Chem. Soc.* **2002**, *124*, 13008.
[21] S. Stanković, M. D'hooghe, S. Catak, H. Eum, M. Waroquier, V. van Speybroeck, N. de Kimpe, H.-J. Ha, *Chem. Soc. Rev.* **2012**, *41*, 643.
[22] a) L. Legnani, G. Prina-Cerai, T. Delcaillau, S. Willems, B. Morandi, *Science* **2018**, *362*, 434; b) G. Berger, M. Gelbcke, E. Cauët, M. Luhmer, J. Nève, F. Dufrasne, *Tetrahedron Lett.* **2013**, *54*, 545; c) A. Cruz, I. I. Padilla-Martínez, E. V. García-Báez, *Tetrahedron: Asymmetry* **2010**, *21*, 909.

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6.2 Synthesis and antiproliferative activity of cisplatin-3-chloropiperdine conjugate

Synthesis and characterization of two novel cisplatin- alkylating agents conjugates. Combining cisplatin with a sterically demanding cyclic nitrogen mustard. Cytotoxicity of the ligands and the complexes examined with MTT assays. The novel conjugates displayed a high antiproliferative potency against 5 human cancer cell lines. Both complexes were able to largely circumvent the acquired cisplatin resistance of A2780cisR ovarian cancer cells.

Reference

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Synthesis and antiproliferative activity of cisplatin-3-chloropiperidine conjugates

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Abstract: We report the synthesis and characterization of two novel cisplatin- alkylating agents conjugates. Combining a platinum based cytostatic agent with a sterically demanding alkylating agent could potentially induce further DNA damage, block cell repair mechanisms and keep the substrate active against resistant tumor cell lines. The 3-chloropiperidines utilized as ligands in this work are cyclic representatives of the *N*-mustard family and were not able to coordinate platinum on their own. The introduction of a second coordination site, in form of a pyridine moiety, led to the isolation of the desired conjugates. They were characterized with HRMS, CHN-analyses and XRD. We concluded this work by examining the cytotoxicity of the ligands and the obtained complexes with MTT assays in human cancer cell lines. While the ligands showed hardly any activity, the novel conjugates both displayed a high antiproliferative and cytotoxic potency in a panel of three cell lines. Moreover, both complexes were able to largely circumvent the acquired cisplatin resistance of A2780cisR ovarian cancer cells, both in the MTT assay and a flow-cytometric apoptosis assay.

Introduction

Cisplatin (Fig 1a) was first synthesized by *Michele Peyrone* in 1844 and became known as Peyrones chloride.[1] Over hundred years later in the 1960s *Barnett Rosenberg* accidently discovered its cytotoxic potential during electrochemical experiments on bacterial cells by utilizing platinum electrodes. Cisplatin dramatically changed cancer treatment after its worldwide approval in 1978.[2,3] Together with its analogs carboplatin (Fig 1b) and oxaliplatin (Fig 1c) it forms an indispensable class of drugs administered in at least half of all cancer chemotherapies, with excellent cure rates of up to 90% for testicular cancer.[4,5,6]

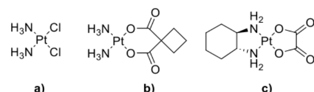

Figure 1. Cisplatin (a), Carboplatin (b) and Oxaliplatin (c)

The mechanism of action is believed to involve a hydrolysis leading to a cationic complex, which quickly forms crosslinks between two adjacent nucleobases. The N^7-position of the guanine nucleobase appears to be the main target, forming predominantly guanosine guanosine crosslinks.[7,8] These platinum nucleobase adducts interfere with different enzymes such as DNA and RNA polymerases and can ultimately lead to apoptosis.[9]
In spite of the therapeutic success it became apparent that the application of cisplatin leads to a variety of adverse effects, such as nephrotoxicity, peripheral neurotoxicity and hematological toxicity.[10,11] In an effort to reduce these side effects the aforementioned carboplatin, often referred to as second generation platinum drug, was developed. While showing greatly reduced toxicity, it also shows a lower anti-cancer potency, generally requiring much higher dosage which is limited by the still occurring myelosuppression.[5,11,12] Another drawback of carboplatin is the cross resistance with cisplatin that many cancer types exhibit. Consequently, oxaliplatin, the third generation platinum drug, has been developed and was first approved in

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1996. The bulky (1*R*, 2*R*)-1,2-diaminocyclohexane (DACH) ligand introduced in this compound hinders DNA repair mechanisms and the complex remains effective against many cisplatin resistant tumor cells.[13,14,15] Besides DNA repair mechanisms other factors, such as lesion tolerance or platinum deactivation by glutathione have been shown to contribute towards cisplatin resistance.[3,16] Consequently a plethora of other platinum-based drug candidates has been synthesized and evaluated over the past decades,[17,17,18] with some compounds even obtaining national approvals.[6] Today cisplatin and its analogs are frequently administered in combination with other chemotherapeutic agents such as 5-fluorouracil or gemcitabine, in order to enhance efficacy and minimize side effects.[3,8,13,14]

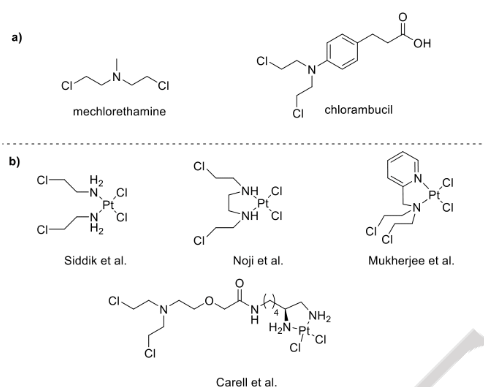

Figure 2. a) Approved *N*-mustards mechlorethamine and chlorambucil; b) cisplatin *N*-mustard conjugates by Z. H. Siddik[19], M. Noji[20], T. Carell[21] and Muhkerjee[22]

Taking this combination approach one step further, various groups have explored the idea of linking an *N*-mustard moiety to platinum in order to induce further DNA damage (Fig 2).[19–21] *N*-Mustard, often cited as the first cancer chemotherapeutic drugs[23], also target the N^7-position of guanine to covalently transfer an alkyl unit and form DNA crosslinks.[23,24] Contrary to platinum induced lesions these alkylation products can lead to depurination, which in turn cause DNA strand breaks, ultimately leading to apoptosis.[25] While the conjugates reported by *Siddik et al.*[19] and *Noji et al.*[20] coordinate the metal center directly with the nitrogen of the mustard moiety (Fig 2b), *Carell* reasoned that this would drastically diminish the reactivity of the *N*-mustard. Due to the coordination towards the metal center the lone pair of the nitrogen is no longer available and therefore the critical aziridinium intermediate cannot be formed. Consequently *Carell et al.* opted for an indirect coordination (Fig 2b) through the use of an ethylene glycol spacer. With this approach both moieties, the platinum as well as the alkylating agent, remain active as demonstrated via LC-ESI-HRMS.[21] Recently, *Mukherjee et al.* introduced a cisplatin conjugate featuring *bis*-(2-chloroethyl) pyridylmethylamine as a ligand[22], along with derivatives incorporating carboxylic acids as leaving groups.[26] (Fig 2b) These conjugates exhibited markedly reduced susceptibility to deactivation by thiol-containing molecules, such as glutathione, a tripeptide linked to the development of cisplatin resistance. Furthermore, they demonstrated a delayed release of the alkylating moiety even after Pt(II) was sequestered by other cellular nucleophiles, leading to promising cytotoxicity against various cancer cell lines.[22,26]

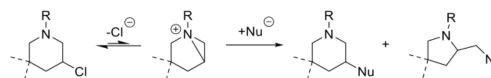

Figure 3. 3-Chloropiridenes: cyclic *N*-mustard analogues utilizing a bicyclic aziridinium intermediate

In our previous publications we have reported cyclic *N*-mustard analogs.[27–29] These 3-chloropiperidines react with nucleophiles in a similar mode of action[30] (Fig 3) and have shown good activity in DNA cleavage assays as well as high potency against pancreatic tumor cell lines.[31] Their cyclic nature allows for an easy adjustment of their reactivity through the exploitation of ring strain and the *Thorpe-Ingold* effect.[32] The aforementioned works by *Siddik*, *Noji* and *Carell*[19–21] inspired us to investigate possible 3-chloropiridine platinum conjugates.

Results and Discussion

As a consequence of the high reactivity of the 3-chloropiperidine compared to other *N*-mustards, like chlorambucil (Fig 2a), and the resulting susceptibility towards hydrolysation[31] we reasoned that a direct coordination, without the use of a linker unit, might be beneficial in order to keep the alkylating moiety intact. The sterically demanding 3-chloropiperidine could also function similar to the DACH ligand in oxaliplatin, blocking potential DNA repair mechanisms.[13,14] From preliminary experiments with various metals, only being able to obtain complexes from the likes of copper and cobalt (see supporting information), we concluded that the 3-chloropiperidine unit only poorly coordinates to metal centers. We hypothesized that a weakly coordinating ligand is more readily replaced, releasing the active alkylating moiety in the process. With these theoretical considerations we set our focus on finding suitable 3-chloropiperidine ligands. Noticing that most published novel platinum analogs used primary or secondary amines to coordinate [19,20,33], we started from our recently published secondary 3-chloropiperidines. [28] Similar to *Siddik et al.* we tried to conjugate potassium tetrachloroplatinate with two separate monofunctional 3-chloropiridines **A** (Fig 4a) but were unable to observe any complex formation. Attributing this to lack of any chelating effect we switched our efforts to bifunctional ligand **B** (Fig 4a). The synthesis along the already established route of chlorinating and TBAI mediated cyclization of a suitable diamine[29] proved to be unexpectedly challenging, resulting in low yield of only 25 % over 2 steps. The low yield might be explained by one piperidine ring forming the bicyclic aziridinium ion and subsequently getting attacked by the second piperidine moiety, leading to an inherent instability. With chelating ligand **B** at hand we tried to form the corresponding platinum complex, but yet

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again could not observe any formation, which might be due to steric hindrance. We reasoned that substituting one of the 3-chloropiperidine units with a less sterically demanding and therefore better coordinating group might be beneficial.

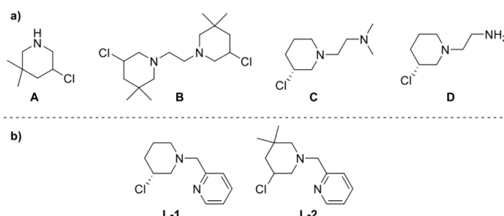

Figure 4. a) Synthesized unsuccessful ligands A-D; b) successful ligands L-1 and L-2

The synthesis of ligand **C** (Fig 4a) starting from *S*-prolinol via functionalization of the nitrogen, followed by chlorination with thionylchlorid was plagued with problems similar to ligand **B**, resulting in 22 % yield. To prevent this instability and further side reactions with thionylchlorid the primary amine in ligand **D** (Fig 4a) was protected with tert-butyloxycarbonyl during the synthesis and stored as an HCl salt after deprotection, resulting in a total yield of 54 % over 5 steps. Attempting to convert ligands **C** and **D** to their respective platinum complexes led to no isolable product, but the reaction of the HCl salt from 3-chloropiperidine **D** with potassium tetrachloroplatinate and 2 equivalents of NaOH (or NaOD in D_2O as reported by *Carell et al.*)[21] resulted only in a short term color change to yellow, indicating the temporary formation of the desired product. Reinforced by this observation we introduced pyridine as a coordinating unit, similar to the ligands utilized by *Muhkerjee et al.*[22,26]. Ligands **L-1** and **L-2** (Fig 4b) could be synthesized in good yields, starting from *S*-prolinol and 2,2-dimethyl-4-pentenal, respectively. Finally reacting **L-1** and **L-2** with equimolar amounts of potassium tetrachloroplatinate in H_2O/MeOH gave the desired complex as a yellow precipitate (Fig 5).

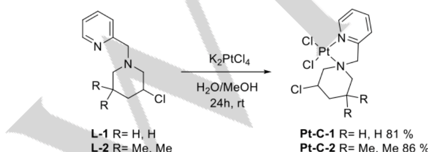

Figure 5. Synthesis of novel platinum 3-chloropiperidine conjugates Pt-C-1 and Pt-C-2

Platinum conjugates **Pt-C-1** and **Pt-C-2** (Fig 5) were purified by repeated precipitation from DMF/H_2O and characterized by ESI-MS, elemental analysis and single crystal X-ray diffraction.

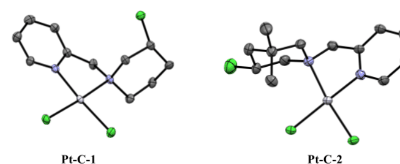

Figure 6. Molecular structures of the novel cisplatin analogues Pt-C-1 and Pt-C-2

To evaluate the biological activity of the novel complexes we first conducted some preliminary experiments to assess their DNA-damaging properties. Compound **Pt-C-1** was dissolved in DMF and incubated at 37 °C with 10 eq of 2'-deoxyguanosine. After 72 h the solution was analyzed with HRMS and mono- and di-2'-deoxyguanosine adducts where confirmed by matching mass values and isotope patterns. Having established the reactivity towards DNA-bases we turned our attention to study their antiproliferative activity, which was assessed over 96 h by the MTT assay in monolayer cultures of three human malignant tumor cell lines. The ligands **L-1** and **L-2** alone hardly showed any inhibitory effects on the growth of cancer cell lines below a concentration of 100 µM (Fig. 7) and yielded IC_{50} values below 200 µM only in the broadly chemosensitive CH1/PA-1 cells (Table 1). Previous studies have shown that 3-chloropiperidines, particularly aromatic-functionalized *bis*-3-piperidine derivatives, exhibit high efficacy against BxPC-3 pancreatic cancer cell lines, surpassing the potency of the reference compound chlorambucil.[31] However, their potency was markedly reduced against other cancer cell lines and for mono-3-chloropiperidines. Additionally, chirality has been demonstrated to significantly influence cytotoxicity.[27] Together with premature hydrolysation of the alkylating unit these factors could account for the lower-than-expected activity observed for **L-1** and **L-2**. Their respective platinum(II) complexes **Pt-C-1** and **Pt-C-2** on the other hand yielded IC_{50} values in the low micromolar range in CH1/PA-1 ovarian teratocarcinoma as well as SW480 colon carcinoma cells and in the low two-digit micromolar range in the intrinsically multidrug-resistant lung adenocarcinoma cell line A549 (Table 1). **Pt-C-2** seems to be very slightly (1.4–1.8 times at the IC_{50}) but consistently more potent than **Pt-C-1**, contrary to the tendency shown by the corresponding ligands at concentrations higher than 100 µM. Concentration-effect curves of the complexes consistently approached the zero line at concentrations just about three times the respective IC_{50} in all three cell lines, indicating a high cell-killing potency (Fig. 7).

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Table 1. Antiproliferative activity in three human cancer cell lines. Values are means ± standard deviations from at least three independent MTT assays (exposure time: 96 h).

Compound	A549	IC_{50} value (µM) CH1/PA-1	SW480
L-1	> 200	140 ± 15	> 200
L-2	> 200	≈ 200	> 200
Pt-C-1	20.6 ± 0.4	2.5 ± 0.3	2.9 ± 0.6
Pt-C-2	11.4 ± 0.7	1.8 ± 0.1	2.0 ± 0.3
Cisplatin [a]	3.8 ± 1.0	0.073 ± 0.001	2.3 ± 0.2

[a] taken from ref.[34]

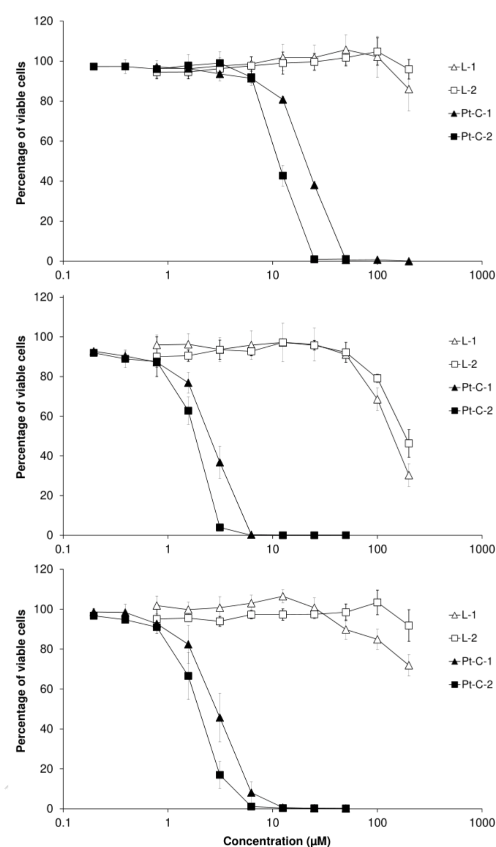

Figure 7. Concentration-effect curves in A549 (top), CH1/PA-1 (center) and SW480 cells (bottom) relative to untreated controls (100%). Values are means ± standard deviations from at least three independent MTT assays (exposure time: 96 h).

In an isogenic pair of ovarian cancer cell lines, consisting of cisplatin-sensitive parental A2780 cells and a cell subline with acquired cisplatin resistance, A2780cisR, **Pt-C-1** and **Pt-C-2** were able to largely overcome cisplatin resistance, as reflected by resistance factors as low as 1.7 and 1.1, respectively (compared to 13.8 for cisplatin). In the resistant subline, **Pt-C-1** came close to the absolute potency of cisplatin and **Pt-C-2** even surpassed it by 1.7 times in terms of IC_{50} values (Table 2, Figure 8).

Table 2. Antiproliferative activity in A2780 and A2780cisR cancer cell lines. Values are means ± standard deviations from three independent MTT assays (exposure time: 96 h).

Compound	IC_{50} value (µM) A2780	A2780cisR	Resistance factor
L-1	173 ± 9	> 200	(> 1.2)
L-2	*n.d.*	*n.d.*	*n.d.*
Pt-C-1	4.8 ± 0.6	8.3 ± 0.9	1.7
Pt-C-2	3.4 ± 0.5	3.9 ± 0.2	1.1
Cisplatin	0.47 ± 0.08	6.5 ± 0.1	13.8

n.d. not determined

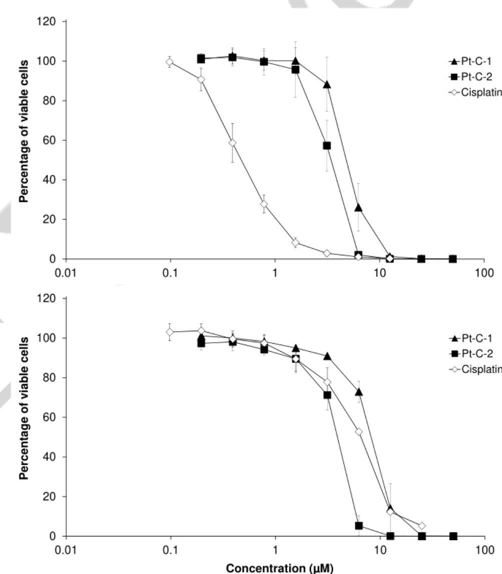

Figure 8. Concentration-effect curves in A2780 (top) and A2780cisR cells (bottom) relative to untreated controls (100%). Values are means ± standard deviations from three independent MTT assays (exposure time: 96 h).

Finally, complexes **Pt-C-1** and **Pt-C-2** were tested for their potency to induce apoptotic cell death in the isogenic cell pair following 24 h exposure. In good agreement with cytotoxicity results, novel conjugates and cisplatin showed a tendency to induce programmed cell death in a dose-dependent manner (Figure 9, A-C, Table S1). The cisplatin-resistant subline demonstrated a lower sensitivity towards all three compounds, however, the resistance towards cisplatin remained the largest (with almost 80% viable cells after exposure to 25 µM, Figure 9, C). The concentrations higher than IC_{50} values had to be applied to compensate for relatively short exposure time (24 h vs. 96 h in MTT assay).

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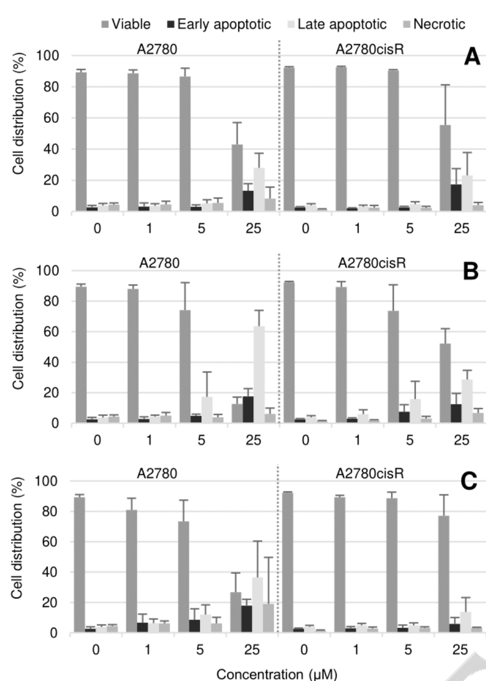

Figure 9. Induction of apoptosis/necrosis in A2780 and A2780cisR cells. **Pt-C-1** (**A**), **Pt-C-2** (**B**) and cisplatin (**C**) induced cell death detected by means of flow cytometry upon double annexin-FITC/propidium iodide staining. Values are means ± standard deviations from three independent experiments (exposure time: 24 h).

Conclusion

3-Chloropiperidines have already been shown to effectively alkylate DNA and inhibit cell growth of pancreatic cancer cells. In this work we strived to combine cisplatin, a well-known cytotoxic drug, with these cyclic alkylating agents. Their sterically demanding structure seems to be only poorly coordinating to metal centers, which could result in a cleavage of the coordination and a release of the highly reactive alkylating moiety in the cell, but also made the synthesis very challenging. Secondary 3-chloropiperidines as well as a suitable *bis*-3-chloropiperidine have not been forming any conjugation with platinum whatsoever. Introducing a second coordination site in the form of a pyridine unit finally led us to the path of success. Resulting in the formation of two novel platinum alkylating agent conjugates, which have been characterized by HRMS and CHN-analysis and their structure confirmed by single crystal XRD. We concluded this work by assessing the DNA damaging potential of said compounds, by MTT assays of three different tumor cell lines (A549, CH1/PA-1 and SW480). Both complexes showed a high antiproliferative and cell killing potential in all three tested cell lines, while the 3-chloropiperdine ligands alone hardly showed any activity. Furthermore, both complexes were able to overcome the acquired cisplatin resistance of A2780cisR ovarian cancer cells, yielding IC_{50} values in a range of low micromolar concentrations similar to cisplatin in this cell subline. Finally, the complexes were demonstrated to induce programmed cell death in both the parental A2780 cell line and the resistant subline, clearly surpassing the potency of cisplatin in the latter. Several factors must be considered and thoroughly investigated in future studies to understand the mechanisms by which these structures circumvent cisplatin resistance. Potential explanations include the steric bulk of the piperidine moiety and the delayed release of the alkylating unit, which may lead to additional DNA damage. Additionally, given the structural similarity to the conjugates reported by *Mukherjee et al.* (Fig 2b), the enhanced stability against hydrolysis and deactivation by glutathione should also be taken into account. We believe that further optimization of the ligand, such as utilizing bifunctional bis-3-chloropiperidines or exploiting chirality to more effectively induce DNA damage, along with a careful investigation into the activity of the coordinated ligand, could lead to the development of a potent new anticancer drug.

Materials and methods

Pt-C-1:
207 mg (0.50 mmol, 1 eq) potassium tetrachloroplatinate were dissolved in 2 ml of water and 105 mg (0.5 mmol, 1 eq) of **L-1** in 1 ml of methanol were added. The solution was stirred at room temperature for 24 h. The yellow precipitate was filtered of and washed with acetone. The crude product was finely dispersed in an ultrasonic bath and washed with water, before redissolving in DMF and precipitating it with water. The latter was repeated two times. The product was obtained as a yellow powder in 81 % yield (193 mg, 0.41 mmol). HRMS (ESI): *m*/*z* calcd for $C_{11}H_{15}Cl_3N_2PtNa^+$: 497.9841; found 497.9849 [M+Na+]; *m*/*z* calcd for $C_{11}H_{15}Cl_3N_2PtK^+$: 513.9580; found 513.9560 [M+K+], elemental analysis calcd (%) for $C_{11}H_{15}Cl_3N_2Pt$: C 27.72, H 3.17, N 5.88; found: C 27.78, H 3.14, N 5.78.

Pt-C-2:
299 mg (0.72 mmol, 1 eq) potassium tetrachloroplatinate were dissolved in 3 ml of water. 172 mg (0.72 mmol, 1 eq) of **L-2** dissolved in 1.5 ml methanol were added and the solution was stirred at room temperature for 24 h. The yellow precipitate was filtered of, finely dispersed in an ultrasonic bath and washed with water. The crude product was redissolved in DMF and precipitated with water. The latter was repeated two times. The product was obtained as a yellow powder in 86 % yield (312 mg, 0.62 mmol) HRMS (ESI): *m*/*z* calcd for $C_{13}H_{19}Cl_3N_2PtNa^+$: 526.0154; found 526.0156 [M+Na+]; *m*/*z* calcd for $C_{13}H_{19}Cl_3N_2PtK^+$: 541.9893; found 541.9898 [M+K+], elemental analysis calcd (%) for $C_{13}H_{15}Cl_3N_2Pt$: C 30.94, H 3.79, N 5.55; found: C 31.31, H 3.84, N 5.36.

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Ligand **A** was prepared according to literature.[28] The synthesis of ligands **B**-**D** as well as Ligands **L-1** and **L-2** can be found in the supporting information.

Cell culture

CH1/PA-1 cells were provided by L. R. Kelland, CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK, and confirmed by STR profiling as PA-1 ovarian teratocarcinoma cells at Multiplexion, Heidelberg, Germany. SW480 colon carcinoma and A549 non-small cell lung cancer cells as well as A2780 and A2780cisR ovarian cancer cells were obtained from the Institute of Cancer Research, Department of Medicine I, Medical University of Vienna. All media and supplements were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. All cell lines were grown as adherent cultures in 75 cm² culture flasks (Starlab, Hamburg, Germany) under a humidified atmosphere with 5% CO_2 in air at 37 °C. CH1/PA-1, SW480 and A549 cells were grown in minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS; BioWest, Nuaillé, France), 1 mM sodium pyruvate, 4 mM L-glutamine and 1% (v/v) nonessential amino acids (from a 100× stock solution), A2780 and A2780cisR cells in RPMI 1640 medium supplemented with 4 mM L-glutamine and 10% heat-inactivated FBS.

MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to assess the antiproliferative activity of the compounds. For this purpose, cells were harvested from culture flasks by using trypsin/EDTA (Sigma-Aldrich), and the following cell numbers were seeded into 96-well tissue culture plates (Starlab, Hamburg, Germany): 1 × 10^3 (CH1/PA-1), 1.5 × 10^3 (A2780), 2 × 10^3 (SW480) 2.5 × 10^3 (A2780cisR) and 3 × 10^3 (A549) cells per well, each in 100 µL of the appropriate supplemented medium (as specified in the section above). Cells were incubated for 24 h to re-adhere and resume exponential growth and then treated with concentration series of the test compounds. Compounds were dissolved in DMF (Fisher Scientific, Hampton, NH, USA), diluted in the appropriate medium, and 100 µL of each dilution were added to the respective wells in triplicates. After 96 h, the medium was replaced with 100 µL per well of an MTT/medium mixture, i.e., 5 mg/mL MTT (Acros Organics, Geel, Belgium) in phosphate-buffered saline (PBS), diluted 1:7 in RPMI 1640 medium (supplemented with 4 mM L-glutamine and 10% heat-inactivated FBS). Upon conversion of MTT into the formazan product by viable cells for 4 h, 150 µL DMSO were added per well, and absorbance at 550 nm (and at 690 nm as a reference) was measured with a microplate reader (ELx808) and Gen5 software, version 3.08 (both from BioTek, Winoosky, VT, USA). Blank-corrected optical densities were graphically evaluated, and IC_{50} values were interpolated from concentration-effect curves and each result averaged from at least three independent experiments.

Apoptosis assay

The potency to induce apoptotic cell death was quantitatively analyzed via flow cytometry using double staining with FITC-conjugated annexin V (eBioscience, San Diego, CA, USA) and propidium iodide (Sigma-Aldrich). A2780 and A2780cisR cells were seeded into 12-well plates (8 × 10^4 cells/well) in 1 mL of supplemented RPMI 1640 medium per well and allowed to settle for 24 h. Consecutively, cells were exposed to a range of concentration (1–5–25 µM) of the compounds prepared from DMF stocks not to exceed 0.25% DMF on cells. Following treatment, the supernatant media were separately collected, and cells were washed once with 37 °C warm PBS and harvested via trypsinization. The corresponding cell suspensions were added to the pre-collected media and probes were centrifuged (300 g, 3 min). The supernatant was discarded, and the cell pellets were resuspended in 150 µL of binding buffer (10 mM HEPES/NaOH pH 7.4, 140 mM NaCl and 2.5 mM CaCl2) supplemented with 1.5 µL FITC-conjugated annexin V per probe. Samples were stained for 15 min in the dark at 37 °C. An amount of 1 µg of propidium iodide dissolved in 150 µL of binding buffer (for each probe) was added shortly before the measurement. The flow-cytometric analysis was conducted using a Guava easyCyte 8HT instrument (Merck Millipore, Burlington, MA, USA) with InCyte software. Results were quantified by means of FlowJo software (TreeStar). At least three independent experiments were analyzed.

Keywords: Medicinal chemistry • antitumor agents • platinum • cytoxicity • 3-chloropiperidines

[1] G. B. Kauffman, R. Pentimalli, S. Doldi, M. D. Hall, *Platin Met. Rev.* **2010**, *54*, 250.
[2] a) B. Rosenberg, *Cancer* **1985**, *55*, 2303; b) S. Ghosh, *Bioorg. Chem.* **2019**, *88*, 102925.
[3] S. Shruthi, K. Bhasker Shenoy, *ChemistrySelect* **2024**, *9*.
[4] a) V. Gandin, J. D. Hoeschele, N. Margiotta, *Int. J. Mol. Sci.* **2023**, *24*; b) M. S. Galanski, M. A. Jakupec, B. K. Keppler, *Curr. Med. Chem.* **2005**, *12*, 2075; c) I. Ali, W. A. Wani, K. Saleem, A. Haque, *Anti-Cancer Agents Med. Chem.* **2013**, *13*, 296.
[5] L. Kelland, *Nat. Rev. Cancer.* **2007**, *7*, 573.
[6] N. J. Wheate, S. Walker, G. E. Craig, R. Oun, *Dalton Trans.* **2010**, *39*, 8113.
[7] A. Eastman, *Pharmacol. Ther.* **1987**, *34*, 155.
[8] S. Dasari, P. B. Tchounwou, *Eur. J. Pharmacol.* **2014**, *740*, 364.
[9] G. E. Damsma, A. Alt, F. Brueckner, T. Carell, P. Cramer, *Nat. Struct. Mol. Biol.* **2007**, *14*, 1127.
[10] a) I. Arany, R. L. Safirstein, *Semin. Nephrol.* **2003**, *23*, 460; b) S. Quasthoff, H. P. Hartung, *J. Neurol.* **2002**, *249*, 9; c) A. A. Argyriou, J. Bruna, P. Marmiroli, G. Cavaletti, *Crit. Rev. Oncol. Hematol.* **2012**, *82*, 51; d) X. L. Cheng, H. Q. Liu, Q. Wang, J. G. Huo, X. N. Wang, P. Cao, *Front. pharmacol.* **2015**, *6*, 234.
[11] M. H. Hanigan, P. Devarajan, *Cancer Ther.* **2003**, *1*, 47.
[12] S. Dilruba, G. V. Kalayda, *Cancer Chemother. Pharmacol.* **2016**, *77*, 1103.

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[13] O. Rixe, W. Ortuzar, M. Alvarez, R. Parker, E. Reed, K. Paull, T. Fojo, *Biochem. Pharmacol.* **1996**, *52*, 1855.
[14] E. Raymond, S. G. Chaney, A. Taamma, E. Cvitkovic, *Ann. Oncol.* **1998**, *9*, 1053.
[15] a) E. Raymond, S. Faivre, S. Chaney, J. Woynarowski, E. Cvitkovic, *Mol. Cancer. Ther.* **2002**, *1*, 227; b) H. Ouzon-Shubeita, M. Baker, M.-C. Koag, S. Lee, *Biochem. J.* **2019**, *476*, 747.
[16] a) Edward L. Mamenta, Eric E. Poma, William K. Kaufmann, Dean A. Delmastro, Heather L. Grady, Stephen G. Chaney, *Cancer Res.* **1994**, *54*, 3500; b) M. R. Albertella, A. Lau, M. J. O'Connor, *DNA repair* **2005**, *4*, 583; c) D.-W. Shen, L. M. Pouliot, M. D. Hall, M. M. Gottesman, *Pharmacol. Rev.* **2012**, *64*, 706; d) L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo, G. Kroemer, *Oncogene* **2012**, *31*, 1869.
[17] M. G. Apps, E. H. Y. Choi, N. J. Wheate, *Endocr. Relat. Cancer.* **2015**, *22*, R219-33.
[18] a) E. Wexselblatt, D. Gibson, *J. Inorg. Biochem.* **2012**, *117*, 220; b) J. Kasparkova, H. Kostrhunova, V. Novohradsky, J. Pracharova, A. Curci, N. Margiotta, G. Natile, V. Brabec, *Dalton Trans.* **2017**, *46*, 14139; c) O. Aronov, A. T. Horowitz, A. Gabizon, D. Gibson, *Bioconjug. Chem.* **2003**, *14*, 563.
[19] A. R. Khokhar, Q. Xu, R. A. Newman, Y. Kido, Z. H. Siddik, *J. Inorg. Biochem.* **1992**, *45*, 211.
[20] K. Lee, T. Tashiro, M. Noji, *Chem. Pharm. Bull.* **1994**, *42*, 702.
[21] S. Schiesser, B. Hackner, M. Vrabel, W. Beck, T. Carell, *Eur. J. Org. Chem.* **2015**, *2015*, 2654.
[22] S. Karmakar, K. Purkait, S. Chatterjee, A. Mukherjee, *Dalton Trans.* **2016**, *45*, 3599.
[23] L. H. Hurley, *Nat. Rev. Cancer.* **2002**, *2*, 188.
[24] a) J. T. Millard, S. Raucher, P. B. Hopkins, *J. Phys. Chem. Ref. Data* **1990**, *112*, 2459; b) R. J. Goldacre, A. Loveless, W. C. J. Ross, *Nature* **1949**, *163*, 667.
[25] a) K. S. Gates, *Chem. Res. Toxicol.* **2009**, *22*, 1747; b) J. R. Mehta, M. Przybylski, D. B. Ludlum, *Cancer Res.* **1980**, *40*, 4183.
[26] M. Maji, S. Karmakar, Ruturaj, A. Gupta, A. Mukherjee, *Dalton Trans.* **2020**, *49*, 2547.
[27] C. Carraro, A. Francke, A. Sosic, F. Kohl, T. Helbing, M. de Franco, D. Fabris, R. Göttlich, B. Gatto, *ACS Med. Chem. Lett.* **2019**, *10*, 552.
[28] M. Georg, L. A. Laping, V. Billo, B. Gatto, P. Friedhoff, R. Göttlich, *ChemistryOpen* **2023**, e202300181.
[29] T. Helbing, C. Carraro, A. Francke, A. Sosic, M. de Franco, V. Gandin, R. Göttlich, B. Gatto, *ChemMedChem* **2020**, *15*, 2040.
[30] T. Helbing, M. Georg, F. Stöhr, C. Carraro, J. Becker, B. Gatto, R. Göttlich, *Eur. J. Org. Chem.* **2021**, *2021*, 5905.
[31] C. Carraro, T. Helbing, A. Francke, I. Zuravka, A. Sosic, M. de Franco, V. Gandin, B. Gatto, D. R. Göttlich, *ChemMedChem* **2021**, *16*, 860.
[32] T. Helbing, M. Kirchner, J. Becker, R. Göttlich, *Eur. J. Org. Chem.* **2022**, *2022*.
[33] a) V. V. Bakhonsky, A. A. Pashenko, J. Becker, H. Hausmann, H. J. M. de Groot, H. S. Overkleeft, A. A. Fokin, P. R. Schreiner, *Dalton Trans.* **2020**, *49*, 14009; b) A. E.-G. E. Amr, K. A. Ali, M. M. Abdalla, *Eur. J. Med. Chem.* **2009**, *44*, 901; c) J. de Mier-Vinué, M. Gay, A. M. Montaña, R.-I. Sáez, V. Moreno, J. Kasparkova, O. Vrana, P. Heringova, V. Brabec, A. Boccarelli et al., *J. Med. Chem.* **2008**, *51*, 424; d) N. Margiotta, C. Marzano, V. Gandin, D. Osella, M. Ravera, E. Gabano, J. A. Platts, E. Petruzzella, J. D. Hoeschele, G. Natile, *J. Med. Chem.* **2012**, *55*, 7182; e) J. D. Hoeschele, H. D. Showalter, A. J. Kraker, W. L. Elliott, B. J. Roberts, J. W. Kampf, *J. Med. Chem.* **1994**, *37*, 2630.
[34] K. Cseh, H. Geisler, K. Stanojkovska, J. Westermayr, P. Brunmayr, D. Wenisch, N. Gajic, M. Hejl, M. Schaier, G. Koellensperger et al., *Pharmaceutics* **2022**, *14*.

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6.3 Further Co-Authored Publications

Understanding the Alkylation Mechanism of 3-Chloropiperidines – NMR Kinetic Studies and Isolation of Bicyclic Aziridium Ions

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