Development of 2-Pyridone–Based Central Fragments

Affecting the Aggregation of Amyloid Proteins

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Doctoral Thesis

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Title
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Abstract
There are many applications of small organic compounds, e.g. as drugs or as tools to study biological systems. Once a compound with interesting biological activity has been found, medicinal chemists typically synthesize small libraries of compounds with systematic differences to the initial “hit” compound. By screening the new ensemble of compounds for their ability to perturb the biological system, insights about the system can be gained. In the work presented here, various ways to synthesize small libraries of ring-fused 2-pyridones have been developed. Members of this class of peptidomimetic compounds have previously been found to have a variety of biological activities, e.g. as antibacterial agents targeting virulence, and as inhibitors of the aggregation of Alzheimer β-peptides. The focus in this work has been to alter the core skeleton, the central fragment, of the previously discovered biologically active 2-pyridones and evaluate the biological effects of these changes. Several new classes of compounds have been constructed and their preparations have included the development of multi-component reactions and a method inspired by diversity-oriented synthesis.

Some of the new compounds have been evaluated for their effect on the fibrillation of different amyloid proteins. Both the Parkinson-associated amyloid protein α-synuclein and the bacterial protein CsgA that is involved in bacterial biofilm formation are affected by subtle changes of the compounds’ central fragments. This is an example of the usefulness of central-fragment alterations as a strategy to probe structure-activity relationships, and the derived compounds may be used as tools in further study of the aggregation of amyloid proteins.

Keywords
2-Pyridone, central fragment alteration, multi-component reactions, directed diversity-oriented synthesis, peptidomimetics, amyloid, protein aggregation, pilicide, curlicide
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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV  Sellstedt, M., Prasad, G. K., Krishnan, K. S., and Almqvist, F. Directed Diversity-Oriented Synthesis. Ring-Fused 5 to 10-Membered Rings from a Common 2-Pyridone Precursor. *2012, Submitted*


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Other papers by the author not appended to the thesis


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Å</td>
<td>Ångström</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>All</td>
<td>allyl</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>Cys</td>
<td>cystein</td>
</tr>
<tr>
<td>DCE</td>
<td>1,2-dichloroethane</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DOS</td>
<td>diversity-oriented synthesis</td>
</tr>
<tr>
<td>dppf</td>
<td>1,1'-bis(diphenylphosphino)ferrocene</td>
</tr>
<tr>
<td>dr</td>
<td>diastereomeric ratio</td>
</tr>
<tr>
<td>e.g.</td>
<td>exempli gratia</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>hex</td>
<td>hexanes</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>iPr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography – mass spectrometry</td>
</tr>
<tr>
<td>LHMDS</td>
<td>lithium hexamethyldisilazane</td>
</tr>
<tr>
<td>mCPBA</td>
<td>meta-chloroperbenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MS</td>
<td>molecular sieves</td>
</tr>
<tr>
<td>MWI</td>
<td>microwave irradiation</td>
</tr>
<tr>
<td>n.d.</td>
<td>not determined</td>
</tr>
<tr>
<td>NMP</td>
<td>N-methyl-2-pyrrolidinone</td>
</tr>
<tr>
<td>NMR</td>
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</tr>
<tr>
<td>NOE</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethyleneglycol</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>Pin</td>
<td>pinacolato</td>
</tr>
<tr>
<td>PMB</td>
<td>para-methoxybenzyl</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PyBOP</td>
<td>(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>rac</td>
<td>racemic</td>
</tr>
<tr>
<td>RaNi</td>
<td>Raney nickel</td>
</tr>
<tr>
<td>RFU</td>
<td>relative fluorescence unit</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>S$_{N}$Ar</td>
<td>nucleofilic aromatic substitution</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>ThT</td>
<td>thioflavine T</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
</tr>
</tbody>
</table>
1. Introduction

The use of synthetic organic molecules revolutionized medicine during the 20th century. Chloral hydrate 1 (figure 1), first synthesized in the early 1830s from ethanol and chlorine gas,1 was recognized as a useful anesthetic in 18692 and became thereby one of the first synthetic drugs. Until then, organic drugs had been obtained by isolation from various natural sources. In 1899, acetylsalicylic acid, or aspirin 2, was marketed as an anti-inflammatory medicine.3, 4 Aspirin is perhaps the most successful drug in history and its sales were still strong in 2010 at over 770 million euro.5 Modern drug discovery emerged at the beginning of the 1900s. One of the most prominent pioneers in the field was Paul Ehrlich, a pharmacologist who, during his PhD studies, had used organic dyes to stain cell tissues.6 He realized that the chemical structure of a compound determined its specificity to different cell structures7 and thereby that compounds could be created with selective toxicity toward microorganisms.8 In a systematic search for such drugs, hundreds of organoarsenic compounds were synthesized and tested for their ability to treat syphilis-infected animals.9, 10 The resulting drug, salvarsan 3, was used clinically in 1910.11 A few decades later the sulfa antibiotics, such as 4, were discovered under the lead of Gerhard Domagk in a similar systematic search for substances active against Streptococci infections.12, 13

![Chemical structures](image)

**Figure 1.** Chloral hydrate 1 was a common anesthetic in the 1870s. Aspirin 2 is still a common painkiller. Salvarsan 3 is an oligomeric mixture where n is predominantly 3 or 5.14 Sulfamidchrysoidine 4 was the first sulfa antibiotic. The naturally occurring penicillins have the general structure 5. Eribulin 6 was approved in 2010 as an anticancer drug.
The discovery of the naturally occurring penicillins, when Alexander Fleming noticed how bacterial growth was inhibited by the presence of contaminating mould on a culture plate, is on the other hand an example of how serendipity can have a productive influence on research.

The early synthetic drugs, such as chloral hydrate and acetyl salicylic acid, were prepared in one or a few synthetic steps. Today, complex molecules synthesized in multiple steps are frequently used as drugs. The most advanced example is undoubtedly the anticancer agent eribulin, which is inspired by the natural product halichondrin B, but manufactured synthetically in 62 steps.

The methods for finding compounds that affect biological processes are many and can today range from rational computer-aided design, targeted on a specific protein, to high-throughput screening of hundreds of thousands of substances. In addition to synthetic compounds, natural products from e.g. plants, fungi, and bacteria can be valuable in drug discovery. When a compound with the desired effect, termed a “hit”, is found, analogs of that compound are synthesized and evaluated in order to establish structure-activity relationships and improve the activity and/or other properties, such as membrane permeability, solubility, and metabolism. Analog can be synthesized by utilizing the intrinsic reactivity of the hit compound to alter or extend its functionalities. If the hit compound is complex, it can be difficult to make analogs. Hence, it is important to design a flexible synthetic route that allows systematic variation of different parts of the molecules when creating compounds intended for biological studies. A common approach to accomplish this is to synthesize compounds by combining two independently variable building blocks. A powerful extension of this method is the use of multi-component reactions where three or more building blocks are combined in a single step. Another strategy is diversity-oriented synthesis (DOS), where the aim is to create compounds with many different molecular skeletons, rather than only varying substituents on a shared scaffold. One way to achieve this is the reagent-based diversity-generating strategy, where a single substrate is converted into several different scaffolds in a few synthetic steps by reaction with different reagents (figure 2).

![Figure 2](image-url)

**Figure 2.** A schematic representation of the reagent-based approach to diversity-oriented synthesis (DOS).
Although the vast majority of compounds prepared in laboratories around the world will never reach the market as drugs – because of their toxicity, poor efficacy, or other reasons – some are still useful in basic medicinal research. With the development of molecular and chemical biology, small organic molecules have become important tools in the study of fundamental biochemical phenomena such as kinase activity, and fluxes of calcium and other metal ions. To the same end, fluorescent probes have been designed to selectively label recombinant proteins in the study of protein function in live cells.

In the effort to create new biologically interesting compounds, different synthetic strategies have in this thesis been applied to create a variety of substances based on 2-pyridones.

2-Pyridones

2-Pyridones are the major tautomers of 2-hydroxypyridines and have versatile reactivity. As indicated in figure 3A, positions 3 and 5 of the 2-pyridone are nucleophilic and readily halogenated, nitrated or formylated. Positions 4 and 6 on the other hand are electrophilic and accept nucleophiles, e.g. as in Gallagher’s synthesis of the natural product cytisine (figure 3B). Nucleophilic aromatic substitutions are also possible if these positions are halogenated.

A

B

Figure 3. A) Tautomers and resonance structures of a 2-pyridone. B) An intramolecular nucleophilic addition to a 2-pyridone was used in a synthesis of the natural product cytisine.

The pyridone carbonyl also activates methyl groups in positions 4 and 6; alkoxide bases can be sufficient to allow acylation of these positions, especially if the pyridone has electron-withdrawing groups. Stronger bases are usually required for alkylations (figure 4).
Figure 4. Examples of the reactivities of 4- and 6-methyl substituted 2-pyridones.

In the work presented here, the reactivity of the 2-pyridones has been used to construct small libraries of compounds, based on the bicyclic 2-pyridones 7, 8, and 9 (figure 5A), but with new central fragments. These bicyclic 2-pyridones were originally designed as structural peptidomimetics to mimic the C-terminal of a bacterial protein involved in the biosynthesis of pili (figure 5B). Pili are extracellular fibers that some bacteria use e.g. to adhere to and invade host cells, and are hence important virulence factors. By targeting the bacterium’s ability to cause disease without affecting growth, drug resistance may be less likely to emerge than it is with classical antibiotics.

Figure 5. A) Three biologically active bicyclic 2-pyridones that constitute the starting point for the work presented in this thesis. B) The bicyclic pyridones mimic the C-terminal of PapG (10), a bacterial protein.

In addition to functioning as antibacterial agents through inhibition of the assembly of pili, compounds 8 and 9 also target curli, another bacterial
surface organelle. Curli are functional amyloids, β-sheet–rich protein fibers that can mediate the formation of bacterial communities, biofilms. Compounds 8 and 9 also inhibit the aggregation of other amyloid-forming biomolecules such as the Alzheimer β-peptides. The effect on the amyloid protein α-synuclein, which is associated with Parkinson’s disease, will be discussed in chapter eight.

**Previous synthetic efforts**

The bicyclic 2-pyridones in figure 5 are synthesized via a cyclocondensation reaction between thiazolines 13 and acyl ketenes; the latter formed in situ by heating an acyl ketene precursor (12) under acidic conditions (figure 6).

![Figure 6](image)

**Figure 6.** The bicyclic pyridones 11 are synthesized from acyl Meldrum’s acids 12 (synthesized from Meldrum’s acid and carboxylic acids) and thiazolines 13 (prepared from cysteine and nitriles).

Previous work has focused on variation of the two R-groups of 11 to improve potency as antibacterial agents and on the introduction of fluorescent tags for studies of virulence mechanisms. The use of other acyl ketene sources and imines has also been investigated. This has resulted in the synthesis of other ring-fused 2-pyridone structures, e.g. compounds 14-17 (figure 7A). Further, more substituents have been introduced on the bicyclic central fragment; e.g. by formylation of the 2-pyridone ring followed by reductive amination (18) and by oxidation and lithiation of the thiazolone ring (19) (figure 7B).

![Figure 7](image)

**Figure 7.** A) Examples of cyclocondensation products from different building blocks. B) Two compounds with an additional substituent on the bicyclic scaffold.
2. Objectives

The aim of this work was to use previously developed chemistry to construct thiazolo ring-fused 2-pyridones and then alter or extend the ring-fused system while keeping the dipeptidomimetic part of the molecules (figure 8A). Ring-fused 2-pyridones are commonly found in nature and often display biological activity; e.g. the cytotoxic camptothecin\textsuperscript{76} 20 and the acetylcholinesterase inhibitor huperzine A\textsuperscript{77} 21 (figure 8B).

A

B

\textbf{Figure 8.} A) Peptidomimetic thiazolo ring-fused 2-pyridones have been altered in different ways to produce compounds with new central fragments and a maintained or extended peptidomimetic backbone (highlighted). B) Two examples of biologically active, naturally occurring ring-fused 2-pyridones.

New heterocyclic ring structures are valuable in drug discovery as they can lead to biologically potent and patentable compounds\textsuperscript{78} and scaffold modifications are desirable in the lead generation process as they expand the candidate pool.\textsuperscript{79} By altering the central fragments of the biologically active pyridones in figure 5A, the positions of their substituents can become shifted or constrained to certain conformations. For a less flexible analog, the affinity for a target protein can increase if the preferred conformation of the new compound is similar to the binding mode, since there will be a decrease in loss of entropy upon protein binding.\textsuperscript{80} In addition, the new central fragments themselves may offer more interaction points, such as hydrogen-bond donors and acceptors. With an extended central fragment, introduction of additional substituents is also possible. These strategies increase the diversity of available compounds and allow for better tuning of their properties; with the primary goal being to provide tools to study different biological systems, in particular the aggregation of amyloid proteins.
3. Pyrazoles (paper I)

The bicyclic 2-pyridones 7–9 have a peptidomimetic backbone (figure 5). To further extend the peptidomimetic structure, an amine functionality was introduced into the pyridones by a nitration-reduction sequence\(^3\) (table 1).

**Table 1.** Extension of the peptidomimetic backbone by introduction of an amine functionality.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>Ph</td>
<td>22</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>isobutyl</td>
<td>Ph</td>
<td>23</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>1-naphthyl</td>
<td>cyclopropyl</td>
<td>24</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>1-naphthyl</td>
<td>3-CF(_3)Ph</td>
<td>25</td>
<td>55</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) (i) 1.05 equiv. NaNO\(_2\), DCM:TFA 25:1, O\(_2\)(g), rt, overnight. (ii) 5 equiv. Zn(dust), AcOH, rt, 4 h.

We had previously functionalized this type of amines by acylation and sulfonylation\(^81\) and were now interested in making a nitrogen-to-oxygen exchange by diazotation and a Sandmeyer reaction.\(^82, 83\) However, applying standard diazotation conditions (NaNO\(_2\) and H\(_2\)SO\(_4\)(aq.)) resulted in ring closure, forming pyrazoles. This type of reaction had previously been reported for electron-deficient 2-methylanilines in acetic acid.\(^84\) The produced pyrazolopyridones had a dipeptidomimetic backbone and were more conformationally constrained than the parent amines or hydroxyls. As a result of this, the attention shifted to synthesis of a small series of ring-fused pyrazoles, and the use of acetic acid and 5% sulfuric acid were compared for a few amines (table 2).
Table 2. Ring closure to pyrazoles under diazotation conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>Product</th>
<th>Yield (%) Method A</th>
<th>Yield (%) Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>Ph</td>
<td>26</td>
<td>31</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>isobutyl</td>
<td>Ph</td>
<td>27</td>
<td>54</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>1-naphthyl</td>
<td>cyclopropyl</td>
<td>28</td>
<td>61</td>
<td>46/82*</td>
</tr>
<tr>
<td>4</td>
<td>1-naphthyl</td>
<td>3-CF$_3$-Ph</td>
<td>29</td>
<td>n.d.</td>
<td>75*</td>
</tr>
</tbody>
</table>

*Reagents and conditions: (a) Method A: 1.05 equiv. 0.2 M NaNO$_2$(aq.), AcOH, rt, 1 h. Method B: 1.05 equiv. 0.2 M NaNO$_2$, 5% H$_2$SO$_4$(aq.), rt, 30 min. (*) THF was used as a co-solvent.*

In general, sulfuric acid was superior to acetic acid, at least if THF was used as a co-solvent to improve the solubility of the substrate and the product. This was especially evident for the methyl-substituted pyridone 22. The yield of the corresponding pyrazole 26 was 85% in sulfuric acid, but only 31% in acetic acid. In acetic acid, concomitant formation of nitrosated pyrazole and a dimerization product were detected with LC-MS. Dimerization of ring-fused pyrazoles by reaction with intermediate diazonium salts has previously been reported for other systems.\cite{85}

Compound 26 is unsubstituted in the pyrazole ring, which opens up for introduction of a substituent on the central fragment late in the synthetic sequence. This can be useful if a more thorough investigation of the biological importance of this substituent is undertaken. Compound 26 was first brominated to allow for further functionalization by palladium-catalyzed coupling reactions (scheme 1).

Scheme 1. Bromination of pyrazole 26. *Reagents and conditions: (a) 1.1 equiv. KOAc, 1.05 equiv. Br$_2$, THF, rt, 1 h, 83%.*

Compound 30 was arylated using the Suzuki-Miyaura reaction.\cite{86,87} Ring-fused pyrazoles with NH-bonds had previously been coupled using Pd(dppf)Cl$_2$·CH$_2$Cl$_2$ in dioxane.\cite{88} These conditions were however not conducive and even the use of 20 mol% palladium did not result in more than approximately 60% coupling with phenyl boronic acid according to $^1$H-NMR. Different palladium sources were screened and Pd(OAc)$_2$ with BINAP gave similar results as Pd(dppf)Cl$_2$·CH$_2$Cl$_2$, while Herrmann’s
catalyst\(^{89}\) and \(\text{Pd(OAc)}_2\) with S-Phos\(^{90, \ 91}\) did not result in any notable conversion of starting material. The solvent and base were also found to be important. Both DMF and acetonitrile as solvent resulted in partial methylation of the pyrazole nitrogen when used with carbonate or phosphate bases. Finally, methanol and potassium fluoride\(^92\) were chosen as solvent and base, and a series of boronic acids were coupled (table 3).

Table 3. Suzuki-Miyaura couplings and hydrolysis of methyl esters.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>31</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>4-MeOPh</td>
<td>32</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>3-NO(_2)Ph</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>3-furyl</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>2-tolyl</td>
<td>35</td>
<td>32(^*)</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) (i) 2 equiv. \(\text{ArB(OH)}_2\), 10 mol\% \(\text{Pd(OAc)}_2\), 10 mol\% \(\text{rac-BINAP}\), 1.9 equiv. \(\text{KF}\), \(\text{MeOH}\), MWI 50 °C, 3 min, then 140 °C, 20 min. (ii) 1.5 equiv. 0.1 M \(\text{LiOH(aq.)}\), \(\text{MeOH:pyridine 3:1}\), rt, overnight. (*MWI 60 min at 140 °C in step i.

Both electron-rich and electron-poor aryls could be coupled. A 2-substituted boronic acid (entry 5, table 3) could also be coupled, although this required prolonged heating. For solubility reasons, the produced compounds were only partially purified as methyl esters and instead hydrolyzed to acids using lithium hydroxide and then purified with reversed-phase HPLC.

The carboxylic acids are more interesting than the methyl esters from a biological perspective and they can be viewed as C-terminal \(\beta\)-strand mimetics.\(^93\) \(\beta\)-Strands are extended peptide chains that most often participate in hydrogen-bonded networks, forming protein secondary structures called \(\beta\)-sheets (figure 9).

![Figure 9. A schematic representation of two peptide chains forming: A) a parallel \(\beta\)-sheet–motif and B) an anti-parallel \(\beta\)-sheet–motif. Hydrogen-bonds are indicated with dashed lines.](image)

19
4. Fluorescent compounds (paper II)

The ease with which ring-fused pyrazoles were formed highlights the reactivity of the methyl group in position 7 of the bicyclic pyridones. This reactivity could potentially be used to construct other heterocycles. It was hypothesized that a formylated derivative could be used as a masked diene in a Diels-Alder reaction\(^{94}\) (figure 10). Similar diene-systems, generated by cheletropic eliminations of sulfur dioxide from sulfolene ring-fused 2-pyridones have previously been used in Diels-Alder reactions.\(^{95}\)

![Figure 10. A considered route to new ring-fused compounds.](image)

For benzaldehydes and benzophenones, the type of enolizations described in figure 10 typically require photoexcitation.\(^{96,98}\) In our case, no Diels-Alder reaction was observed under UV-irradiation. However, the acidity of the methyl protons in compound 36 made enolization possible in the absence of UV-irradiation. This was evidenced by heating 36 in deuterated methanol with morpholinium acetate as catalyst, which induced the expected hydrogen-deuterium exchange (figure 11). Unfortunately, when compound 36 was heated with either maleimide or dimethyl acetylenedicarboxylate the dienophile, no Diels-Alder reaction was detected with LC-MS.

![Figure 11. Hydrogen-deuterium exchange suggested that derivatization of the methyl group in 36 was possible.](image)

Next, TiCl\(_4\) in dichloromethane was tested as a catalyst, but again no conversion was seen for 36. When the naphthyl-substituted compound 38\(^{81}\) was used instead of 36 no reaction was observed with morpholinium acetate as catalyst, but with TiCl\(_4\) compound 38 underwent a Bradsher reaction\(^{99,100}\).
to give compound 39 (scheme 2). In the absence of a dienophile, 39 was isolated in 79% yield.

Scheme 2. A TiCl₄ induced Bradsher reaction. Reagents and conditions: (a) 2.0 equiv. TiCl₄, DCM, MWI 100 ℃, 10 min., 79%.

Although compound 39 is less polar than ideal for use in biological systems, with a calculated octanol-water partition coefficient over five, it was clearly fluorescent, making it easy to trace the compound. This could provide insight into its mode of action if it elicited a response in any biological assay, and it was decided to examine this substance class further. Smaller ring-fused 2-pyridones such as carbostyrils (40) and even the isoquinolinone 41 have been reported to be somewhat fluorescent (figure 12). Naphthalimides (42) are also well known fluorescent probes.

Figure 12. Examples of fluorescent 2-pyridone–like ring systems.

We aimed to synthesize naphthalene ring-fused 2-pyridones to examine their potential fluorescence. A series of differently substituted bicyclic 2-pyridones were prepared in analogy with previously prepared compounds (table 4).

Table 4. Synthesis of a series of formylated bicyclic pyridones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>R²</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Product</th>
<th>Yield (%)</th>
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<td>88</td>
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<td>2</td>
<td>Ph</td>
<td>Me</td>
<td>46</td>
<td>63</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>2-MePh</td>
<td>H</td>
<td>47</td>
<td>92</td>
<td>52</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>2-MeOPh</td>
<td>H</td>
<td>48</td>
<td>96</td>
<td>53</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>4-FPh</td>
<td>H</td>
<td>49</td>
<td>93</td>
<td>54</td>
<td>69</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) 2.5 equiv. 43, 2.5 equiv. TFA, DCE, MWI 125 ℃, 3 min. (b) 5 equiv. DMF, 5 equiv. oxalyl chloride, MeCN, reflux, 4 h.
By heating compounds 50–53 at 100 °C in dichloromethane in the presence of TiCl₄, a series of naphthalene ring-fused pyridones were obtained (table 5).

**Table 5.** Bradsher reactions of formylated pyridones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R¹</th>
<th>Reaction time (min)</th>
<th>Equiv. TiCl₄</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>50</td>
<td>5</td>
<td>55</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>Me</td>
<td>20</td>
<td>2</td>
<td>56</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>10-Me</td>
<td>H</td>
<td>20</td>
<td>2</td>
<td>57</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>10-MeO</td>
<td>H</td>
<td>70</td>
<td>8</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>8-F</td>
<td>H</td>
<td>120*</td>
<td>10</td>
<td>59</td>
<td>51</td>
</tr>
</tbody>
</table>

*Reagents and conditions: (a) TiCl₄, DCM, MWI 100 °C. (**) MWI 160 °C, then 10 equiv. TMSCl, MeOH, rt, overnight.*

In the case of the electron-deficient fluoro-substituted compound 54, prolonged heating at 160 °C was required to convert all of the starting material. These conditions also hydrolyzed the methyl ester, and the initially obtained crude acid was re-methylated using TMSCl in methanol (table 5, entry 5).

**Evaluation of fluorescent properties and toxicity**

The fluorescent properties of these polyaromatic compounds were examined in more detail. All compounds 55–59 showed similar absorption and fluorescence spectra, the latter with broad fluorescence bands centered at approximately 500 nm. The absorption spectra were also quite broad, but the overlap was small, enabling the use of high dye concentrations without significant reabsorption of the emitted light. The quantum yield of fluorescence, Φᵢ, of a compound is the ratio of the number of photons emitted to the number of photons absorbed. The quantum yield and the fluorescence lifetime – the average time the compounds are in an excited state before emitting light – were determined for the compounds in two solvents (table 6).
Table 6. Quantum yields ($\Phi_f$) and fluorescence lifetimes ($\tau_f$) in glycerol at 277 K and in ethanol at 293 K. The excitation wavelengths were 410 nm for quantum yield measurements and 404 nm for lifetime measurements.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>$\Phi_f$ glycerol</th>
<th>$\tau_f$ (ns) glycerol</th>
<th>$\Phi_f$ ethanol</th>
<th>$\tau_f$ (ns) ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>0.06*</td>
<td>1.6</td>
<td>0.11</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>0.72</td>
<td>17.0</td>
<td>0.44</td>
<td>14.0</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>0.46</td>
<td>11.3</td>
<td>0.29</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>0.66</td>
<td>17.1</td>
<td>0.38</td>
<td>14.4</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>0.56</td>
<td>14.0</td>
<td>0.34</td>
<td>8.1</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>0.57</td>
<td>16.2</td>
<td>0.27</td>
<td>14.3</td>
</tr>
</tbody>
</table>

*1,2-propanediol was used as solvent instead of glycerol due to poor solubility in glycerol.

In glycerol the fluorescence lifetimes of compound 55–59 were slightly longer than for most small organic fluorophores\(^{106}\) while compound 39 had a shorter lifetime. The quantum yields were relatively high for all compounds except 39, enabling their use as fluorescent markers in biological applications. In ethanol the lifetimes and quantum yields were somewhat lower. Similar values were recorded in dichloromethane.\(^{107}\)

We wished to demonstrate the use of one of the most fluorescent compounds as a fluorescent dye and to visualize its distribution in a mammalian cell-line. For this purpose, HeLa cells were incubated in the presence of compound 55 and fluorescence microscopy was used to record the location of fluorescence the cells (figure 13).

Figure 13. A and B) Confocal fluorescence microscopy of HeLa cells incubated in the presence of 100 µM 55. C) HeLa cells stained with 100 µM 55 after methanol fixation. D) The same cells as in picture C, but stained with antibodies against α-tubulin. E) Pictures C and D overlayed. Scale bars are 10 µm.
The compound was found in most parts of the cell and its distribution was compared to that of microtubules, which are also found throughout the cell. The cells were cross-stained with antibodies against α-tubulin, one of the major components of microtubules, to reveal a partial overlap with a larger proportion of the compound than α-tubulin being found near the cell nucleus.

Although it might be more interesting if the fluorescent compounds elicited a biological response in themselves, they could in principle also be used as fluorescent tags by linking them via the ester to a biologically active molecule. In that case it is important to ensure that the fluorescent probe has limited toxicity. In collaboration with associate professor Joachim Gullbo’s group at Uppsala University, three different human cell-lines were cultured in the presence of compound 55, and the cell viability after 72 hours was measured at different concentrations of 55 (figure 14).

![Cell toxicity test](image)

**Figure 14.** Viability (percentage of cells surviving) of three different cell-lines after 72 hours exposure to six different concentrations of the fluorescent pyridone 55.

CCRF-CEM is a T-cell–leukemia cell-line, U937 is a lymphoma cell-line, and PBMC are normal peripheral-blood mononuclear cells. All these cell-lines are commonly used in cytotoxicity tests. The graph in figure 14 shows the percentage of cells surviving after 72 hours exposure to increasing concentrations of compound 55. A similar trend was observed after 24 hours exposure. The concentration of 55 required to cause 50% cell mortality (LD₅₀) was estimated to be 23 µM for PMBCs, 37 µM for U937, and more than 50 µM for the CCRF-CEM cells. These cell lines are rather sensitive and the toxicity of compound 55 is unlikely to be a problem if used as a fluorescent tag. The LD₅₀ values can be compared with that of the commercial fluorescent probe calcein-AM, which has an LD₅₀ of 0.25 µM for U937.
5. Naphthyridones (paper III)

As mentioned above, morpholinium acetate was found to promote the scrambling of hydrogens in compound 36 (figure 11), but no further reaction was observed. However, when a primary ammonium acetate salt was reacted with the pyridone 36, LC-MS analysis implied that two pyridones reacted with one amine to form a dimer. It was hypothesized that these dimeric compounds had the general structure 60 (figure 15). The reactivity of 36 contrasts with that of the naphthyl substituted analog 38, which mainly gives the anticipated imine when treated with primary amines.

The formation of 60 was found to be reversible, and heating 60 with benzaldehyde resulted in the formation of the dihydro naphthyridone 61. The reversibility of the reaction made these species difficult to isolate. However, some of the dihydro naphthyridones turned out to be sensitive to air, and stirring 36 with benzaldehyde and ethanolamine in air for 24 hours gave 42% of the naphthyridonium salt 62 after treatment with hydrochloric acid (scheme 3).

The resulting naphthyridonium salt was structurally similar to the natural products lophocladine A (63) and B (64) (figure 16). Lophocladine A and B are biologically interesting compounds with, respectively, \( \delta \)-opioid

\[
\begin{align*}
\text{36} & \quad \text{R} \quad \text{N} \quad \text{H} \\
\text{Ph} & \quad \text{S} \\
\text{CO}_2\text{Me} & \quad \text{Ph} \\
\text{R} \quad \text{N} \quad \text{H} & \quad \text{Ph} \\
\text{Ph} & \quad \text{S} \\
\text{CO}_2\text{Me} & \quad \text{Ph} \\
\text{R} \quad \text{N} \quad \text{H} & \quad \text{Ph} \\
\text{Ph} & \quad \text{S} \\
\text{CO}_2\text{Me} & \quad \text{Ph} \\
\end{align*}
\]

**Figure 15.** Suggested equilibria between the pyridone 36, primary amines, and benzaldehyde.

\[
\begin{align*}
\text{36} & \quad \text{R} \quad \text{N} \quad \text{H} \\
\text{Ph} & \quad \text{S} \\
\text{CO}_2\text{Me} & \quad \text{Ph} \\
\text{R} \quad \text{N} \quad \text{H} & \quad \text{Ph} \\
\text{Ph} & \quad \text{S} \\
\text{CO}_2\text{Me} & \quad \text{Ph} \\
\text{R} \quad \text{N} \quad \text{H} & \quad \text{Ph} \\
\text{Ph} & \quad \text{S} \\
\text{CO}_2\text{Me} & \quad \text{Ph} \\
\end{align*}
\]

**Scheme 3.** Formation of a naphthyridonium salt. *Reagents and conditions:* (a) 1.5 equiv. ethanolamine, 2.5 equiv. PhCHO, air, AcOH:MeOH:MeCN 1:10:10, rt, 24 h. 42%.
antagonist activity, and cytotoxicity against breast cancer cells. Related naphthyridones are also present in other natural products e.g. nauclefine \(65\) and angustine \(66\) (figure 16).

![Figure 16. Lophocladine A (63) and B (64) and two other natural products that contain the 2,7-naphthyridin-1-ones, nauclefine (65) and angustine (66).](image)

2,7-Naphthyridin-1-ones are most frequently prepared from nicotinamides or nitriles, and an efficient but harsh synthesis of the lophoclades had previously been reported (scheme 4). Also 4-substituted analogs had been prepared by Suzuki-Miyaura, Stille, and Sonogashira couplings of the corresponding bromides.

![Scheme 4. A previously reported synthesis of lophocladine A (63) and B (64). Reagents and conditions: (a) DMF, 160 °C, 3 h. (b) AcOH:H\(_2\)SO\(_4\) 1:1, reflux, 4 h, 72% over 2 steps. (c) NH\(_4\)OAc(s), 114 °C, 2 h, 87% over 2 steps.]()
Table 7. Evaluation of different ammonia sources.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Conditions step b</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NH₄OAc*</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>PMBNH₂</td>
<td>TFA:DCM:H₂O 10:20:1, MWI 100 °C, 40 min.</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>β-alanine</td>
<td>MeCN:AcOH 2:1, MWI 140 °C, 10 min.</td>
<td>78</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) (i) 1.5 equiv. amine, 2.5 equiv. PhCHO, AcOH:MeOH:MeCN 1:25:25, MWI 80 °C, 10 min. (ii) 1.5 equiv. chloranil, rt, 1 h. (*) 3.5 equiv. NH₄OAc.

Primary amines gave cleaner reactions than ammonium acetate, and a strategy of using a protected form of ammonia was evaluated. The use of paramethoxybenzyl amine followed by acidic deprotection increased the yield to 68% and by using β-alanine as the ammonia source, followed by thermal deprotection, the yield was increased further to 78% (table 7). The thermal deprotection is believed to proceed by elimination of acrylic acid. These conditions were then applied in an investigation into the range of aldehydes that can be used in this one-pot, three-step reaction (table 8).

Table 8. Variation of aldehydes in the synthesis of naphthyridones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>RCHO</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>68</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>69</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>70</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>71</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>72</td>
<td>65</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>73</td>
<td>56</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>74</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>75</td>
<td>71</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) 1.5 equiv. β-alanine, 2.5 equiv. RCHO, AcOH:MeOH:MeCN 1:10:10, MWI 80 °C, 10 min. (b) 1.5 equiv. chloranil, rt, 1 h. (c) MeCN:AcOH 2:1, MWI 140 °C, 10 min.
A wide range of different aldehydes could be used, including alkyl and heteroaromatic aldehydes. The resulting naphthyridones are all thiazolo ring-fused analogs of lophocladine A. To make closer analogs, and to determine if also other pyridones could be used in this reaction, pyridone 80 (scheme 5) was prepared.

Scheme 5. Preparation of pyridone 80. Reagents and conditions: (a) 2.8 equiv. Br₂, 3.0 equiv. NaOAc, AcOH, 80 °C, overnight, 97%. (b) (i) 1.0 equiv. 1.6 M BuLi (hex), Et₂O, –70 °C, 15 min. (ii) 2.0 equiv. HCO₂Me, –70 °C, 30 min, 79%. (c) 1.5 equiv. PhB(OH)₂, 1.5 equiv. KF, 1 mol% Pd(OAc)₂, PEG-400:MeOH 2:1, rt, 90 min, 94%. (d) 2.5 equiv. 47% HBr (aq.), AcOH, 40 °C, overnight, 89%.

First, the picoline 76 was dibrominated and then formylated with methyl formate by selective lithiation. Similar chemistry had been reported for 2-methoxypyridine. Compound 78 was then functionalized using a slight modification of the conditions reported for ligand-free Suzuki reactions in polyethylene glycol. By adding methanol as co-solvent in the coupling, the reaction rate increased and the amount of palladium could be halved, while keeping the reaction time to 90 minutes. If methanol was used as the sole solvent the reaction proceeded to approximately 70% in a few minutes, but then stopped. Finally, the methyl ether was deprotected with hydrogen bromide in acetic acid to obtain pyridone 80.

Compound 80 was subjected to the same conditions as described in table 8. With benzaldehyde as the second aldehyde the anticipated naphthyridine 81 was formed (table 9). However, with formaldehyde compound 82 was formed rather than the expected lophocladine A (63, figure 16). Chloranil could be excluded in this case.

Table 9. Synthesis of substituted lophocladines.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>R²</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>H</td>
<td>81</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>Me</td>
<td>82</td>
<td>54*</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) (i) 1.5 equiv. β-alanine, 2.5 equiv. R¹CHO, AcOH:MeOH:MeCN 1:25:25, MW1 80 °C, 10 min. (ii) 1.5 equiv. chloranil, rt, 1 h. (iii) MeCN:AcOH 2:1, MW1 140 °C, 10 min. (*) Step ii was excluded.
Compound 82 is believed to form by reaction of the intermediate dihydro naphthyridone with a second equivalent of formaldehyde followed by dehydration and isomerization. Unfortunately, with only one equivalent of formaldehyde, 82 and unreacted 80 were obtained. To make lophocladine A, ethyl glyoxylate was used as a protected formaldehyde source. The intermediate ethyl ester 83 was hydrolyzed and then decarboxylated simultaneously with the pyridine nitrogen being deprotected (scheme 6).

Scheme 6. A route to lophocladine A. Reagents and conditions: (i) 2.0 equiv. EtO₂CCHO, 3.0 equiv. β-alanine, AcOH:MeOH:MeCN 1:25:25, MWI 80 °C, 10 min. (ii) 3.0 equiv. chloranil, rt, overnight. (iii) 12 equiv. 2.0 M NaOH (aq.), THF, rt, 3 h. (iv) MeCN:AcOH 2:1, MWI 140 °C, 10 min, 16%.

The overall yield of this transformation was only 16%, mainly due to a more sluggish oxidation step. This is of course not the preferred method of producing lophocladine A given the previously reported much more efficient synthesis (scheme 4), but it suggests that ethyl glyoxylate could be used to make other lophocladine analogs that are unsubstituted in the pyridine ring.

Tetrahydro naphthyridones

The reaction between aldehyde either 36 or 80, an amine, and a second aldehyde constitutes a three-component reaction forming dihydro naphthyridones. Instead of oxidizing the dihydro naphthyridones as described above, there is also the possibility of reducing these reactive intermediates to tetrahydro naphthyridones. This could be accomplished with sodium borohydride, but in most cases formic acid was found to be a more convenient reducing agent and allowed a single-step conversion of pyridones into tetrahydro naphthyridones (table 10). Nevertheless, with alkyl aldehydes the sodium borohydride method had to be used to minimize self-condensation of the aldehyde. Neither was formic acid an efficient reducing agent when ammonia was used as the amine; but it did, unlike sodium borohydride, provide selective reduction of the dihydro naphthyridonium salts in the presence of ketones. This was exemplified by the synthesis of the ketone-containing compound 93. For the three-component reaction to work with aryl amines it was necessary to increase the amount of amine to match the total amount of aldehydes. Hence, three equivalents of the amine and two equivalents of the second aldehyde were used. With aminopyridine as the amine component the equilibration between dimeric and non-dimeric compounds was slow, and the yield of the pyridine-substituted tetrahydro
naphthyridone 94 could be improved from 30 to 54% if the reaction was first heated with acetic acid as catalyst and then reduced with formic acid (table 10, entries 11 and 12).

**Table 10.** Synthesis of tetrahydro naphthyridones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>( R^1 )</th>
<th>( R^2 \text{NH}_2 )</th>
<th>Method</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>iPr</td>
<td>MeNH2</td>
<td>A</td>
<td>84</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Ph</td>
<td>NH3</td>
<td>A</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Ph</td>
<td>MeNH2</td>
<td>B</td>
<td>86</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>Ph</td>
<td>MeNH2</td>
<td>B</td>
<td>87</td>
<td>69</td>
</tr>
<tr>
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<td>Ph</td>
<td>NH3</td>
<td>B</td>
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<td>43</td>
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<tr>
<td>6</td>
<td>Ph</td>
<td>NH3</td>
<td>B</td>
<td>89</td>
<td>23 + 26*</td>
</tr>
<tr>
<td>7</td>
<td>Ph</td>
<td>NH3</td>
<td>B</td>
<td>90</td>
<td>81</td>
</tr>
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<td>NH3</td>
<td>B</td>
<td>91</td>
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<td>9</td>
<td>Ph</td>
<td>NH3</td>
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<td>B</td>
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</tr>
<tr>
<td>11</td>
<td>Ph</td>
<td>NH3</td>
<td>B</td>
<td>94</td>
<td>30</td>
</tr>
<tr>
<td>12</td>
<td>Ph</td>
<td>NH3</td>
<td>C</td>
<td>94</td>
<td>54</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) Method A: (i) 2.0 equiv. \( R^1 \text{CHO} \), 3.0 equiv. \( R^2 \text{NH}_2 \), AcOH:MeOH:MeCN 1:25:25, MWI 80 °C, 10 min. (ii) 7.0 equiv. NaBH4, 0 °C → rt, 1 h. Method B: 2.0 equiv. \( R^1 \text{CHO} \), 3.0 equiv. \( R^2 \text{NH}_2 \), HCO2H:MeOH:MeCN 1:10:10, MWI 100 °C, 15 min. Method C: (i) 2.0 equiv. \( R^1 \text{CHO} \), 3.0 equiv. \( R^2 \text{NH}_2 \), AcOH:MeOH:MeCN 1:25:25, MWI 100 °C, 2.5 h. (ii) 27 equiv. HCO2H, MWI 100 °C, 30 min. (*) isolated as separate diastereomers.
Mechanistic considerations

Efforts were also made to provide a reasonable mechanism for this three-component reaction. Two different mechanistic pathways were considered (figure 17).

![Mechanistic Considerations Diagram](image)

**Figure 17.** Outline of two considered mechanisms.

Both of the hypothesized paths involve an initial imine formation followed by a tautomerization to enamine 96. In path A, the second aldehyde then reacts to form the iminium ion 97 followed by an electrocyclization to 99. In path B on the other hand, the enamine 96 reacts with another imine in a Mannich-type reaction, producing 98. Of course, a second aldehyde could also react like the suggested imine in an aldol reaction, but no such adducts were detected with LC-MS. Neither have intermediates like 98 been detected, but these intermediates are more likely to rapidly form the dihydro naphthyridone 99. To get more information about the mechanism, a competition experiment was designed where a preformed imine (benzylideneaniline 100) and a half equivalent of aniline were reacted with 80 at room temperature (figure 18).

![Kinetic Product Analysis](image)

**Figure 18.** Kinetic product analysis in an imine/aldehyde competitive experiment. 
*Reagents and conditions*: 1.0 equiv. 100, 0.5 equiv. PhNH₂, AcOH:MeOH:MeCN 1:25:25, rt, 30 min.

LC-MS showed that the main product after 30 minutes was a dimer that upon further reaction equilibrated to the non-dimeric product. The kinetic product in this particular case was found to be the hemiaminal ether 101. Its structure was determined by LC-MS and extensive NMR experiments of the crude product that formed when 80 was reacted with a half equivalent of
aniline in the absence of any other aldehyde or imine. The fact that the kinetic product was a dimer rules out path B in figure 17 as the kinetically favored mechanism because the proposed intermediate 96 reacts more rapidly with excess 80 than benzylideneaniline 100. Although this does not fully prove the hypothesis of path A, we suggest this to be the most plausible mechanistic pathway.

A Diels-Alder adduct (appendix I)
As discussed above for the formylated pyridones (figure 10), there should similarly exist an equilibrium between the dihydro naphthyridonium salts 99 (figure 17) and an uncharged exocyclic diene that potentially could undergo a Diels-Alder reaction. Indeed, recently the three-component reaction to dihydro naphthyridones was successfully extended to involve a dienophile as a fourth component. Pyridone 80 was reacted with benzaldehyde, methylamine, and maleimide (102) to generate compound 103 (scheme 7).

```
Scheme 7. A four-component reaction to produce ring-fused pyridones. Reagents and conditions: (a) 1.2 equiv. 102, 2.0 equiv. PhCHO, 3.0 equiv. 1.6 M MeNH₂ (MeOH), AcOH:MeOH:MeCN 1:10:10, MWI 100 °C 15 min, 24%.
```

Compound 103 was obtained as a single observable diastereomer by NMR. The anticipated endo Diels-Alder cyclization from the least sterically hindered face of the in situ generated diene 104 was confirmed by NOE cross-coupling of proton H₄ and H₅ in the product 103 (figure 19). Although only a single example of this four-component reaction has been demonstrated and the yield is low, given the complexity generated in the reaction and the excellent diastereoselectivity, it deserves further investigation in the future.

```
Figure 19. The structure of the obtained diastereomer was confirmed by NOE observation.
```
6. Medium-sized rings (paper IV)

The formation of dihydro naphthyridones in a three-component reaction was described in the previous chapter. It was possible to reduce the dihydro naphthyridones or to react them with dienophiles with relative ease, but oxidation to naphthyridones was less straightforward. On small scales, some of the dihydro naphthyridones could be oxidized with air, but typically a second step with chloranil as oxidant was required. In addition, to efficiently make naphthyridones a protected ammonia source had to be used, followed by deprotection. It was reasoned that use of a bromomethyl- or chloromethyl-substituted pyridone would provide a more straightforward route to the naphthyridones as this would abolish the need of oxidation.

Figure 20. The bromomethyl-substituted pyridone 105 is prepared from acyl Meldrum’s acid 106 and thiazoline 107.

The bromomethyl substituted compound 105 had previously been synthesized (figure 20). However, the preparation of the bromomethyl acyl ketene source 106 was cumbersome, owing to its reactivity, and we made instead the readily prepared chloro analog 109. The chloromethyl formyl pyridone 111 was then synthesized in a similar way to previously prepared compounds (scheme 8).

Scheme 8. Preparation of a formyl and chloromethyl substituted pyridone. Reagents and conditions: (a) 1.0 equiv. Meldrum’s acid, 1.1 equiv. DCC, 1.2 equiv. DMAP, DCM, 0 °C → rt, overnight, 85%. (b) 0.4 equiv. 107, 0.8 equiv. TFA, DCE, MWI 120 °C, 3 min, 78% (based on 107). (c) 11 equiv. DMF, 10 equiv. oxalyl chloride, MeCN, 80 °C, 4 h, 54%.
Gratifyingly, compound 111 reacted as predicted and gave the naphthyridonium salt 112 and the naphthyridone 113 when reacted with benzaldehyde and different amines (table 11).

Table 11. One-step syntheses of naphthyridones and naphthyridonium salts.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeNH₂</td>
<td>112</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>NH₃</td>
<td>113</td>
<td>61</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) 1.5 equiv. PhCHO, 2.5 equiv. amine, AcOH:MeOH:MeCN 1:25:25, MWI 80 °C, 10 min.

Compound 111 has two electrophilic sites with different reactivities and was recognized as a useful precursor for the preparation of other heterocyclic systems as well. A 2-fluorobenzenesulfonyl chloride has been used as a double electrophile in the construction of a DOS-library. Here, an approach of a directed diversity-oriented synthesis was taken, keeping the peptidomimetic pyridone part of the compound constant while allowing large variation of the other part. As the thiazolo ring-fused 2-pyridones have several different biological activities depending on their substituents, hence occupying a biologically interesting place in the chemical space, we hope that generating related compounds with higher diversity than what is possible using substituent variation will result in many new compounds with various biological effects.

While reaction of compound 111 with primary amines under slightly acidic conditions gave naphthyridones, basic conditions led to ring-fused pyrroles (table 12). Analogous chemistry had been reported for related systems. The corresponding thiophene 116 was made by reaction with potassium thioacetate followed by deacylation and dehydration.

Table 12. Synthesis of ring-fused 5-membered heterocycles.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Conditions</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeN</td>
<td>a</td>
<td>114</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>PhN</td>
<td>b</td>
<td>115</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>S</td>
<td>c</td>
<td>116</td>
<td>70</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) 1.5 equiv. MeNH₂, 1.5 equiv. K₂CO₃, MeCN, rt, overnight. (b) 2.5 equiv. PhNH₂, 1.5 equiv. K₂CO₃, MeCN, 70 °C, overnight. (c) (i) 1.0 equiv. KSAc, MeCN, rt, 2 h. (ii) MeOH, rt, 2 h. (iii) AcOH, rt, 1 h.
So far, the majority of the ring-forming reactions presented in this thesis have produced extended aromatic central fragments. There is, however, evidence that molecules with a larger proportion of saturated carbons and higher stereochemical complexity, on average, interact more specifically with proteins and are more aqueous soluble than their unsaturated counterparts. With this in mind, compound 111 was used to make a series of different central fragments with more saturated carbons. The aim was to prepare medium-sized rings in order to allow the introduction of $sp^3$-hybridized atoms, while constraining the flexibility of the compounds in comparison to macrocyclic and acyclic analogs.

Seven-membered rings
To make seven-membered rings, the double electrophile 111 was converted to amino alcohols 118 and 119 by first reducing the aldehyde to an alcohol and then displacing the chloride with amines. The amino alcohols were then reacted with triphosgene to give the seven-membered cyclic carbamates 120 and 121 (scheme 9).

Scheme 9. Reagents and conditions: (a) 1.2 equiv. NaBH₄, MeOH:MeCN 1:1, rt 15 min, 79%. (b) 3.0 equiv. 1.6 M MeNH₂(MeOH), 1.5 equiv. K₂CO₃, MeCN, rt overnight, 59%. (c) 3.0 equiv. BnNH₂, 1.5 equiv. K₂CO₃, MeCN, rt, overnight, 64%. (d) 0.5 equiv. triphosgene, 6.0 equiv. TEA, DCM, rt, 1 h. 57% 120, 61% 121.

Eight-membered rings
Eight-membered rings were synthesized from compound 111 by $S_N2$ reactions of the chloride and reductive amination of the aldehyde. Compound 122 was prepared by allowing $N$-Boc-cystein methyl ester to react with 111, and after Boc-deprotection the ring was formed by an intramolecular reductive amination (scheme 10). If non-protected cystein was used, other products, e.g. pyroles were formed. The triazole-containing compound 123 was prepared by an $S_N2$ reaction with sodium azide, reductive amination with $N$-methylpropargylamine, and then ring closure to an eight-membered ring by a thermally induced intramolecular Huisgen cyclization.
Scheme 10. Reagents and conditions: (a) (i) 1.2 equiv. N-Boc-Cys-OMe, 1.0 equiv. K₂CO₃, MeCN:MeOH 2:1, rt 2 h. (ii) TFA:DCM 1:1, rt 10 min. (iii) 15 equiv. NaBH₄, MeOH, rt overnight. (b) (i) 1.1 equiv. NaN₃, MeCN:MeOH 3:1, MWI 80 °C, 10 min. (ii) 1.2 equiv. N-Me-propargylamine, 2.0 equiv. NaBH(OAc)₃, rt overnight. (iii) dioxane, 100 °C, 24 h, 37%. (c) 1.1 equiv. NaN₃, MeCN:MeOH 3:1, MWI 100 °C, 30 min, 62%.

In the first step in the synthesis of 123, small amounts of by-product 124 were observed; and when compound 111 was reacted with sodium azide at a somewhat elevated temperature, compound 124 could be isolated in 62% yield (scheme 10). Acid-catalyzed intramolecular Schmidt reactions had previously been shown to give similar ring closures, but in our case heat was much more efficient than acid in promoting the formation of 124.

A nine-membered ring
A nine-membered ring was prepared using ring-closing metathesis. First, the aldehyde was allylated under Hosomi-Sakurai conditions (table 13). Although the chiral center in compound 111 is four bonds away from the reacting aldehyde, some diastereoselectivity was induced. Several allylating agents, solvents, and Lewis acids were examined and the best results were obtained with BF₃·Et₂O in dioxane, which yielded after purification 73% of a 1:3.7 diastereomeric mixture. The choice of solvent was important, but the most striking difference was observed when stannous chloride was used as the Lewis acid, since this reversed the selectivity compared to boron trifluoride diethyl etherate or titanium tetrachloride. Other tested Lewis acids such as zirconium chloride and bismuth triflate were less effective at promoting the allylation.
Next, compound 126 was reacted with allylamine to introduce a second olefin for the ring-closing metathesis. The resulting compound 127 was acylated and the crude reaction mixture was then heated with Grubb’s 2nd generation catalyst to provide the nine-membered ring-fused compound 128 (scheme 11).

Scheme 11. Reagents and conditions: (a) 3.0 equiv. allylamine, 2.0 equiv. $\text{K}_2\text{CO}_3$, MeCN, MWI 100 °C, 1 h, 83% 1:3:1 dr. (b) 1.2 equiv. Ac$_2$O, DCM, rt, 1.5 h. (ii) 5 mol% Grubb’s 2nd generation catalyst, PhMe (20 mM), 100 °C, overnight, 72%.
A ten-membered lactone

A strategy to make a ten-membered lactone was also planned. Medium-sized lactones are usually difficult to form owing to a combination of transannular ring-strain and loss of entropy upon formation. This outweighs the increase in speed often seen for intramolecular reactions and high dilution and slow addition of the substrate to the reaction mixture is usually required to suppress formation of dimeric and polymeric species. There is however a strong dependence on the structure of the substrate, and groups that favor conformations similar to the ring-closing transition state can significantly improve lactonization efficiency. Here we aimed for the synthesis of compound 129 from the hydroxy acid 130 (figure 21).

![Figure 21](image)

**Figure 21.** The ten-membered lactone 129 was prepared from the hydroxy acid 130.

It was hypothesized that lactonization of 130 would suffer less from the above-mentioned problems, in part because the pyridone locks out many of the possible conformations of a fully saturated unconstrained hydroxy acid and thereby decreases the entropy cost in the transition state. Another contribution would be a decrease in transannular strain owing to the presence of more sp²-carbons. Compound 131 was first prepared from 117 by reaction with N-methyl-GABA allyl ester (scheme 12). The attempted reaction with the corresponding free acid in non-protic solvents gave O-alkylation rather than N-alkylation, and multiple products were observed in protic solvents. The allyl ester 131 was deprotected under palladium catalysis to give compound 130 and then lactonized with PyBOP as coupling reagent. The lactone was obtained in good yield, and despite modest dilute conditions (50 mM) without slow addition of substrate, no dimeric compound could be observed with LC-MS.

![Scheme 12](image)

**Scheme 12. Reagents and conditions:** (a) 3.0 equiv. N-Me-GABA-allyl ester HCl salt, 4.0 equiv. K₂CO₃, DMF, 70 °C, 24 h, 72%. (b) (i) 5 mol% Pd(PPh₃)₄, 15 equiv. Et₂NH, MeOH, rt, 1.5 h. (ii) 1.5 equiv. PyBOP, 1.5 equiv. DMAP, DCM (50 mM), rt, overnight, 66%.
7. Sulfonamides (paper V)

All new central fragments described up to this point in the thesis have been extensions of the thiazolo ring-fused bicyclic 2-pyridones that constitute the foundation of this work. This chapter describes instead the alteration of the existing thiazolo part of compound 132 to form the bicyclic sulfonamides 133 (figure 22A). The reaction that initiated the chemistry that lead to the sulfonamides presented here was an attempted nucleophilic aromatic substitution on a sulfone,\(^{149}\) to get ring-opened 6-aminopyridones such as 135 (figure 22B).

![Figure 22](image.png)

**Figure 22.** A) Synthesis of the sulfonamides 133 from the thiazolo ring-fused pyridone 132. B) An attempted nucleophilic aromatic substitution on compound 134 resulted instead in the formation of 136.

The outcome when 134 was heated for 15 minutes at 120 °C in neat morpholine was compound 136 rather than the expected S\(_\text{N}\)Ar product 135. It was noted that compound 136 was optically inactive. The same observation was made when the methyl ester 137 was hydrolyzed with lithium hydroxide, which also generated a racemic compound (scheme 13).
It was hypothesized that 137 undergoes a reversible elimination of the sulfone to give the ring-opened sulfinate and α,β-unsaturated ester 139 (scheme 14). In the presence of an amine, a conjugate addition can occur, and sulfur dioxide is released if this product is heated. Indeed, compound 140 could be isolated in 53% yield when 137 was treated with sodium methoxide and benzylamine at room temperature (scheme 14).

Scheme 14. Reaction of 137 with benzylamine in presence of sodium methoxide. Reagents and conditions: (a) (i) 1.2 equiv. BnNH₂, 1.2 equiv. NaOMe, MeOH, 0 °C → rt, overnight. (ii) TFA, 53%.

Compound 140 was rather unstable and lost sulfur dioxide and to some extent also benzylamine upon heating. It was however realized that the crude product could be directly oxidized to a more stable sulfonamide via the corresponding sulfonbromide by reaction with bromine. This type of oxidative coupling had previously been accomplished with potassium ferricyanide, but compound 140 proved unreactive in these conditions. For the oxidative ring closure with bromine to work properly, the reaction had to be performed in a non-alcoholic solvent – otherwise the sulfinate was oxidized to the corresponding sulfate. The first step, in contrast, benefitted from being conducted in methanol. The scope of this one-pot two-step conversion of a cyclic sulfone to the corresponding ring-expanded sulfonamide was examined by reaction with different amines (table 14).
Table 14. One-pot synthesis of differently substituted sulfonamides.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bn</td>
<td>141</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>Me</td>
<td>142</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>iPr</td>
<td>143</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>CH₂CH₂OH</td>
<td>144</td>
<td>61</td>
</tr>
<tr>
<td>5</td>
<td>PMB</td>
<td>145</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>146</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Ph</td>
<td>147</td>
<td>-</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) (i) 1.2 equiv. RNH₂, 1.2 equiv. 0.2 M NaOMe (MeOH), MeOH, 0 °C → rt, overnight. (ii) 1.0 equiv. pyridine, 1.2 equiv. Br₂, MeCN, 0 °C → rt, 70 min.

Primary amines worked best, while ammonia and aniline failed to provide the expected product. The unsubstituted product could though be obtained by acidic deprotection of the PMB-substituted sulfonamide 145 (scheme 15).

Scheme 15. Acidic removal of the PMB-group in compound 145. Reagents and conditions: (a) TFA:H₂O 95:5, MWI 80 °C, 10 min, 95%.

Aryl-substituted sulfonamides were then aimed for by using copper-catalyzed arylation of 146. The initial attempts using copper(I)-catalyzed conditions (CuI, sarcosine, K₂PO₄, DMF, PhI, 100 °C) were unsuccessful and no coupling was observed. Instead the focus turned to Chan-Lam couplings, which gave coupling-products with an electron-rich and with an electron-neutral aryl, but yielded only trace amounts with the electron-poor 3-nitrophenyl boronic acid (table 15).
Table 15. Chan-Lam couplings to give $N$-aryl sulfonamides.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>147</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>4-MeOPh</td>
<td>148</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>3-NO$_2$Ph</td>
<td>149</td>
<td>trace</td>
</tr>
</tbody>
</table>

Reagents and conditions: (a) 2.0 equiv. ArB(OH)$_2$, 1.1 equiv. Cu(OAc)$_2$, 3.0 equiv. TEA, 3Å MS, DCM, air, rt 20 h.

The presence of carboxylic acids or carboxylic acid isosteres on the 2-pyridone–based scaffolds have been found to be imperative for biological activity, both as inhibitors of pili-formation$^{155, 156}$ and of Aβ-peptide aggregation.$^{53}$ Many of the methyl esters presented in this thesis have been hydrolyzed with aqueous lithium hydroxide and protonated with ion-exchange resin to give the corresponding acids in good yields. The sulfonamides prepared in this chapter were also hydrolyzed and the obtained racemates have in most cases been tested as such. It was however found that enantiorerichment by co-crystallization with a chiral amine was possible for compound 150 (figure 23). A few 5-mg set-ups were tested with different amines like brucine, cinchonidine, and (S)-1-phenylethylamine. With ethyl acetate/heptane as solvent and (S)-1-phenylethylamine as amine, an enantiomeric excess of approximately 60% of each enantiomer in the mother liquor and crystals were obtained (figure 23). The corresponding acid of compound 142 has also been resolved by preparative chiral chromatography in collaboration with Carl Johan Arewång at AstraZeneca, Södertälje (appendix II).

Figure 23. Chiral chromatography of co-crystallized 150. Crystals (left), 58% ee, and mother liquor (right), 67% ee. Column: (S, S)-Whelk-O1. Eluent: Hex:EtOH:AcOH:TEA 600:400:1:2. (S)-1-phenylethylamine was not observed under the chosen conditions.
Amyloids are ordered protein fibers with β-sheet–rich structures that are denaturation-resistant, and can be found as deposits e.g. in the brain tissue of patients suffering from neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease. Although the exact mechanism of their formation is unknown, these disease-associated amyloids are thought to be a consequence of protein misfolding leading to soluble aggregation-prone oligomers that nucleate the formation of the amyloids. The oligomeric structures are considered to be the most neurotoxic forms of the amyloid proteins. In some cases however, amyloids are the desired protein form and have an important function, e.g. as in the case of bacterial curli. Curli are extracellular structures that many bacteria use to colonize hosts by adhering to cell tissues and to each other, as well as to inert surfaces, forming bacterial communities known as biofilms. The main protein of curli is CsgA, which is secreted in a soluble form by the bacteria and then polymerized in association with the nucleator protein CsgB, a bacterial cell-surface associated protein. CsgA also self-aggregates into amyloid fibers in vitro. In Alzheimer’s disease the main component of the amyloid deposits is the Alzheimer β-peptide (Aβ-peptide), and in Parkinson’s disease it is the protein α-synuclein. α-Synuclein is mainly unordered in aqueous solutions, but able to adopt both α-helical and β-sheet–rich ordered structures. Helical structures dominate in presence of lipids. The fibrillation of α-synuclein is believed to proceed via β-sheet–rich oligomers that nucleate the aggregation into amyloid fibers. Recently, compound 9 (figure 24) was shown to promote the formation of α-synuclein amyloids by initially increasing the population of β-sheet–rich soluble oligomers. The opposite effect was observed on Aβ-peptide aggregation and on curli-mediated biofilm formation, which were inhibited by compound 9. In the work described below, analogs of 9 were examined for their ability to affect the fibrillation of α-synuclein and of CsgA in vitro in order to establish structure-activity relationships.

Four compounds (8, 138, 151, and 152, figure 24) with variations in four different parts of the molecule compared to 9 were initially evaluated. The compounds were tested in fibrillation assays, where each compound was dissolved in DMSO, diluted with phosphate buffer (to pH 7.4), and mixed...
with thioflavin T (ThT) and either α-synuclein or CsgA. The fluorescence of the system was monitored over time; ThT becomes fluorescent when it interacts with amyloid fibers and the fluorescence signal is hence a measurement of the amount of amyloid fibers that is formed.

Figure 24. The effect on fibrillation of CsgA and of α-synuclein was initially evaluated for compound 9 and four close analogs (8, 138, 151 and 152).

Time-dependent fibrillation data of these compounds showed that the inhibitory effect on CsgA aggregation was significantly diminished for compounds 8, 138, and 151 compared to compound 9. Compound 152 with an extended peptidomimetic backbone appeared on the contrary to be somewhat more potent than 9 (figure 25A). Even larger effects were observed on α-synuclein aggregation, where compounds 8, 138, and 151 completely lost the promoting effect observed for compound 9. Compound 152 on the other hand showed a reversed effect compared to 9, and clearly inhibited amyloid formation (figure 25B).

Figure 25. A) Fibrillation of CsgA (5 µM) in the presence of 50 µM of the different compounds in 0.5% DMSO, and 20 µM ThT at 20 °C. B) Fibrillation of α-synuclein (70 µM) in the presence of 100 µM of the different compounds in 0.05% DMSO, and 20 µM ThT at 37 °C. The data represent average curves for at least three runs. The fluorescence signal was corrected for background fluorescence of ThT in the presence of the compounds and normalized against the DMSO-control of each run.
As alterations of the two aromatic substituents of compound 9 significantly decreased its biological activity, it was decided to focus on analogs with these groups unchanged. The sulfide in compound 9 is likely to undergo metabolic oxidation in higher organisms and we wished to explore more analogs with central fragment changes around this position. Both the corresponding sulfoxide 154 (obtained as a diastereomeric racemic mixture) and the desulfurized compound 156 were prepared (scheme 16). The sulfonamides 150, 161 and 162 (figure 26) were also tested. The introduction of an amine – as in compound 152 – strongly influenced the activity; and the amino sulfone 160 was prepared as described in scheme 16 to examine if the amine functionality could recover the loss of activity observed for the sulfone 138. The ring-fused pyrazole 163, which can be regarded as a sterically constrained analog of amine 152, was also evaluated.

Scheme 16. Synthesis of analogs with changes around the sulfur and position 6 of compound 9. Reagents and conditions: (a) 1.1 equiv. mCPBA, DCM, 0 °C → rt 1 h, 92%. (b) (i) 1.0 equiv. 0.1 M LiOH (aq.), THF:MeOH 1:1, 0 °C → rt overnight. (ii) Amberlite® IR-120 (H⁺), MeOH, rt 10 min, 98% of 154 and 89% of 156. (c) RaNi, MeOH, reflux overnight, 56%. (d) 1.0 equiv. NaNO₂, O₂(g), TFA:DCM 1:19, rt overnight, 54%. (e) 4 equiv. mCPBA, DCM, reflux 7 h, 75%. (f) 8 equiv. Zn(dust), AcOH, rt overnight, 50%. (g) 1.15 equiv. 0.1 M LiOH (aq.), THF:MeOH 1:1, 0 °C → rt overnight, 61%.
The sulfoxide 154 had intermediate CsgA-fibrillation–inhibiting properties compared to the sulfone 138 and the sulfide 9, while the ring-opened desulfurized compound 156 was worse at inhibiting CsgA fibrillation (figure 27A). The amino sulfone 160 performed similarly as 154, showing that some of the loss of activity observed for the sulfone 138 could be regained by extension of the peptidomimetic backbone. The pyrazole ring-fused compound 163 performed well and was similar in activity to the more flexible amino analog 152. Also the sulfonamides 161 and 162 were very good, while the larger benzyl substituted sulfonamide 150 was much less active. The separated enantiomers of compound 161 were also tested and they behaved similarly as the racemic mixture.

**Figure 27.** A) Fibrillation of CsgA (5 µM) in the presence of 50 µM compound and 20 µM ThT. B) Fibrillation of α-synuclein (70 µM) in the presence of 100 µM compound and 20 µM ThT. C) AFM images of α-synuclein incubated ~40 h in the presence of DMSO, compound 9, and compound 163, respectively from left to right.
For α-synuclein, the pyrazole 163 inhibited fibrillation with similar effectiveness as the amine 152. The sulfonamides gave conflicting results, with some replicates showing a clear inhibitory effect while other replicates revealed no difference to the DMSO-control. The reason for this behavior is currently under investigation. None of the other compounds affected α-synuclein fibrillation to any great extent (figure 27B). The ThT-monitored fibrillation assay was complemented with AFM pictures of the α-synuclein aggregates that were formed after incubation with the compounds. The AFM pictures showed that amyloid fibers were formed in the presence of 0.05% DMSO and in the presence of compound 9 (in addition to 0.05% DMSO), but smaller more globular species were formed in the presence of inhibiting compounds such as 163 (figure 27C). Although α-synuclein fibrillation was more sensitive to structural changes of the compounds, the data presented above suggests, except for compound 9, a related structure-activity relationship between these compounds for both CsgA and α-synuclein. For α-synuclein, subtle differences such as oxidation of the sulfur in compound 9 rendered the compounds completely inactive, while the introduction of an amine completely reversed the activity from a templating to an inhibiting compound. This indicates a delicate balance between different states of α-synuclein, where some state leads to fibrillation and others are aggregation-resistant. The more robust and rapid aggregation of CsgA might be explained by the fact that CsgA has evolved to function as an amyloid, while α-synuclein amyloids likely are products of misfolded proteins. Preliminary data indicate that the amine 152 and the pyrazole 163, as for α-synuclein and CsgA, also inhibit Alzheimer-β peptide aggregation. Several other small molecules have previously shown similar dual effects on both α-synuclein and Aβ-peptide aggregation, and antibodies prepared against soluble Aβ-peptide oligomers showed cross-reactivity with several other amyloidal oligomers, suggesting that these have similar structures. Compounds 152 and 163 are β-strand mimetics, and a possible explanation for their activity as inhibitors of aggregation of several different amyloid proteins could be an interaction with the β-sheet–rich oligomers. Considering the distinct effect of these small organic compounds on amyloid protein aggregation, one may speculate about the existence of small natural metabolites in vivo, that are relevant to amyloid-associated diseases.

By making alterations of the bicyclic central fragment of compound 9 several compounds with improved or reversed activity as modulators of protein aggregation were found. This demonstrates how central fragment alterations of hit compounds can be a valuable complement to the variation of substituents as means to study structure-activity relationships. The produced compounds can be used to study the aggregation process of different amyloid proteins and a more thorough examination of their effects is ongoing.
9. Conclusions

In this thesis synthetic methodologies have been developed to construct new 2-pyridone–based central fragments. The produced compounds are structurally diverse and inspired by biologically active 2-pyridones (figure 28). The synthetic strategies involved both directed diversity-oriented synthesis and the development of new multi-component reactions. These syntheses are flexible and allow variation of both the compounds substituents and their core skeletons, which is valuable in the drug discovery lead generation process. One compound class displayed prominent fluorescence, and its use as a fluorescent stain was demonstrated in HeLa-cells. Some of the synthesized compounds were evaluated for their ability to affect aggregation of amyloid proteins, and new inhibitors of both the biofilm associated CsgA and the Parkinson’s associated α-synuclein proteins were found. The identification of these inhibitors demonstrates how central fragment alterations successfully can complement substituent variation to establish structure-activity relationships and generate biologically active compounds. Subtle changes of the structure of these inhibitors render inactive or promoting compounds, which might provide insight into the aggregation process of amyloid proteins upon further study.

Figure 28. A variety of compounds with 2-pyridone motifs have been synthesized.
10. Acknowledgements

Det är självlklart en mängd personer som har bidragit till att jag har kunnat slutföra denna avhandling:

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Tack!
11. References


