Design, Synthesis, and Evaluation of Functionalized Chroman-4-one and Chromone Derivatives

Somatostatin receptor agonists and Sirt2 inhibitors

MARIA FRIDÉN-SAXIN

UNIVERSITY OF GOTHENBURG

Department of Chemistry and Molecular Biology
University of Gothenburg
2012

DOCTORAL THESIS

Submitted for partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Chemistry

Design, Synthesis, and Evaluation of Functionalized Chroman-4-one and Chromone Derivatives. Somatostatin receptor agonists and Sirt2 inhibitors

MARIA FRIDÉN-SAXIN

Cover picture: The crystal structure of the Sirt2 enzyme (PDB 1J8F).

ISBN: 978-91-628-8548-9
http://hdl.handle.net/2077/30223

Department of Chemistry and Molecular Biology
University of Gothenburg
SE-412 96 Göteborg
Sweden

Printed by Ineko AB
Kållered, 2012

Till Noel och Judith

Abstract

Peptides are involved in many physiological processes such as regulation of blood-pressure, food intake, pain transmission and blood-glucose levels. They consist of amino acids that are connected through amide bonds which make peptides hydrophilic and conformationally flexible. Peptides generally make poor oral drugs as amide bonds are easily cleaved by endogenous enzymes. One way to overcome the structural problems with peptides is to develop stabilized mimetics, so called peptidomimetics, via a scaffold approach. The amino acid side chains needed for activity are attached as substituents to the scaffold.

In this thesis, chroman-4-ones and chromones have been used as scaffolds for the development of peptidomimetics. These frameworks are naturally occurring derivatives containing an oxa-pyran ring. Depending of the substitution pattern they show different biological effects. Synthetic modifications in the 2-, 3-, 6-, and 8-positions of chromones and chroman-4-ones have been conducted. This work has included the development of an efficient synthetic route to obtain 2-alkyl chroman-4-one derivatives. Via bromination in the 3-position of chroman-4-one, various substituents (NH_2, Br, OAc, CN, CH_2NHCbz) have been introduced either through substitution reactions or via a Sm-mediated Reformatsky reaction. By incorporation of the appropriate substituents on the chromone-4-one and the chromone scaffolds, the biological applications have included the development of β-turn mimetics of the peptide hormone somatostatin. This has resulted in two compounds with agonistic properties for two subtypes of somatostatin receptors.

In addition, functionalized 2-alkyl substituted chroman-4-one and chromone derivatives were developed as selective inhibitors of the Silent information type 2 (Sirt2) enzyme. Sirt2 functions as a deacetylating enzyme using both histones and non-histone proteins (e.g. α-tubulin) as substrates. Sirt2 is located in the cytosol but enters the nucleus during mitosis. Evaluation of a number of chroman-4-one and chromone derivatives resulted in the identification of a series of novel Sirt2-selective inhibitors with IC_{50} values in the low μM range. Two chroman-4-one derivatives with 2-pyridylethyl substituents in the 2-position of the chroman-4-one showed significant reduction of the proliferation of breast and lung cancer cells using a fluorescent based assay. These results indicate that the synthesized chroman-4-one based Sirt2-selective inhibitors can be valuable in more detailed studies of the function of Sirt2 in cancer.

Keywords: Chroman-4-ones, Chromones, Inhibitors, Molecular Modeling, Peptidomimetic, Samarium, Sirtuin, Sirt2, Somatostatin, Structure-Activity Relationships, Tubulin, β-Turn.

List of Publications

The thesis is based on the following papers, which are referred to by Roman numerals I-VI. The publications **I**, **II**, **IV**, and **VI** are reprinted with kind permission from the publishers.

I **Synthesis of 2-Alkyl-Substituted Chromone Derivatives Using Microwave Irradiation**
Fridén-Saxin, M., Pemberton, N., Andersson, K. S., Dyrager, C., Friberg, A., Grøtli, M., Luthman, K.
Journal of Organic Chemistry **2009**, 7, 2755–2759.

II **KHMDS Enhanced SmI_2-mediated Reformatsky Type α-Cyanation**
Ankner, T., Fridén-Saxin, M., Pemberton, N., Seifert, T., Grøtli, M., Luthman, K., Hilmersson, G.
Organic Letters **2010**, 12, 2210-2213.

III **Substituted Chroman-4-one and Chromone Scaffolds: Design, Synthesis, and Evaluation of Somatostatin β-Turn Mimetics**
Fridén-Saxin, M., Seifert, T., Andersson, K. S., Pemberton, N., Dyrager, C., Friberg, A., Dahlén, K., Wallén, E. A. A., Grøtli, M., Luthman, K.
Submitted

IV **Synthesis and Evaluation of Substituted Chroman-4-one and Chromone Derivatives as Sirtuin 2 Selective Inhibitors**
Fridén-Saxin, M.,† Seifert, T.,† Rydén Landergren, M., Suuronen, T., Lahtela-Kakkonen, M. L., Jarho, E. M., Luthman, K.
Journal of Medicinal Chemistry **2012**, 55, 7104-7113.

V **Chroman-4-one Based Inhibitors of Sirtuin 2 with Antiproliferative Effects**
Seifert, T., Fridén-Saxin, M., Engen, K., Kokkola, T., Wallén, E. A. A., Suuronen, T., Lahtela-Kakkonen, M. L., Jarho, E. M., Luthman, K.
Manuscript

VI **Proline Mediated Formation of Novel Chroman-4-one Tetrahydropyrimidines**
Fridén-Saxin, M., Seifert, T., Hansen, L.K., Grøtli, M., Erdelyi, M., Luthman, K.
Tetrahedron **2012**, 68, 7035-7040.

† Equally contributing authors.

Publications related to, but not discussed in this thesis:

2,3,6-Trisubstituted 3-Hydroxychromone Derivatives as Fluorophores for Live-Cell Imaging
Dyrager, C., Friberg, A., Dahlén, K., Fridén-Saxin, M., Börjesson, K., Wilhelmsson, L.M., Smedh, M., Grøtli, M., Luthman, K.
Chemistry a European Journal **2009**, 15, 9417-9423.

Inhibitors and Promoters of Tubulin Polymerization: Synthesis and Biological Evaluation of Chalcones and Related Dienones as Potential Anticancer Agents
Dyrager, C., Wickström, M., Fridén-Saxin, M., Friberg, A., Dahlén, K., Wallén, E.A.A., Gullbo, J., Grøtli, M., Luthman, K.
Bioorganic and Medicinal Chemistry **2011**, 19, 2659-2665.

The Author's Contribution to Papers I-VI

I Formulated the research problem; performed or supervised most of the experimental work; interpreted the results, and wrote the manuscript.

II Contributed to the formulation of the research problem; performed or supervised a major part of the experimental work, the interpretation of the results, and to the writing of the manuscript.

III Formulated the research problem; performed or supervised most of the experimental work; interpreted the results, and wrote the manuscript.

IV Formulated the research problem; performed half of the experimental work, contributed considerably to the interpretation of the results, and to the writing of the manuscript.

V Contributed to the formulation of the research problem; contributed to the interpretation of the results, and to the writing of the manuscript.

VI Formulated the research problem; performed or supervised all experimental work; interpreted the results, and wrote the manuscript.

Table of Contents

1. Introduction 1

1.1 Bioactive peptides 1

1.1.1 Peptides as drugs and development of peptidomimetics 2

1.2 Targets for bioactive peptides relevant to this thesis 3

1.2.1 G-protein coupled receptors 3

1.2.2 Enzymes: Silent information regulator type (Sirt) enzymes 5

1.3 Chroman-4-ones and chromones as scaffolds for bioactive compounds 10

1.4 Computational calculations as tools in medicinal chemistry 13

2. Aims of the thesis 15

3. Synthesis of functionalized chroman-4-one/chromone scaffolds 16

3.1 Introduction of substituents in the 2-position: Base mediated aldol condensation (Paper I) 16

3.2 Introduction of substituents in the 3-position (Papers I and II) 20

3.2.1 Formation of 3-amino-, 3-bromo, and 3-acetoxychromones 20

3.2.2 Introduction of a 3-aminomethyl group in chroman-4-ones 24

3.4 Introduction of substituent in the 6-position of the chroman-4-one 29

3.4.1 Synthesis of chroman-4-one derivative useful as a building block in the synthesis of peptide analogs 29

3.5 Introduction of substituents in 8-position of chroman-4-ones and chromones 30

4. Substituted chroman-4-ones and chromones as β-turn peptidomimetics 32

4.1 Design of substituted chroman-4-one and chromone derivatives as peptidomimetics of somatostatin (Paper III) 32

4.2 Synthesis of substituted chroman-4-ones **52-55** 36

4.2.1 Synthesis of building block **57** 36

4.3 Biological evaluation of compounds **53** and **55** as mimetics of somatostatin 37

5. Substituted chroman-4-ones and chromones as Sirt2 inhibitors 38

5.1 Evaluation of compound **6** as a lead for novel Sirt2 inhibitors (Paper IV) 38

5.1.1 Synthesis of potential Sirt2 inhibitors based on **6** 39

5.2 Biological evaluation of chroman-4-one and chromone based Sirt2 inhibitors 41

5.3 Determination of the absolute configuration of the enantiomers of **6** 43
5.4. Synthesis of chroman-4-one based Sirt2 inhibitors with more hydrophilic substituents in the 2-position (Paper V) 45
5.5 Biological evaluation of the inhibitory activity towards Sirt2....47
5.6 Evaluation of the antiproliferative activity of pyridyl derivatives **77c** and **78c**....48
6. Proline mediated formation of novel chroman-4-one tetrahydropyrimidines....50
6.1 A proline catalyzed Mannich reaction for the incorporation of a 3-aminomethyl group (Paper VI) 50
6.2 Formation of tricyclic derivatives **81-83**....50
6.3 Conformational analysis of **81** and **81a** 53
7. Concluding remarks and future perspective 56
8. Acknowledgements 57
9. Populärvetenskaplig sammanfattning 58
10. References and Notes....59
Appendix 67

Abbreviations

Ac	Acetate
AcOH	Acetic acid
ADP	Adenosine diphosphate
aq.	Aqueous
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
CDI	*N,N'*-Carbonyldiimidazole
CNS	Central nervous system
COSY	Correlation spectroscopy
3D	Three-dimensional
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DCE	Dichloroethane
DFT	Density functional theory
DIPA	Diisopropylamine
DIPEA	Diisopropylethylamine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
equiv	Equivalents
Fmoc-ONSu	9-Fluorenylmethoxycarbonyloxy(succinimide)
GC	Gas chromatography
GDP	Guanosine diphosphate
GEP	Gastroenteropancreatic
GH	Growth hormone
GI	Gastro-intestinal
GPCR	G-protein coupled receptors
GTP	Guanosine triphosphate
h	Hours
HDAC	Histone deacetylase
HMBC	Hetero multiple bond correlation
HMDS	Hexamethyl disilazane
HPLC	High performance liquid chromatography
IC_{50}	The concentration of an inhibitor required to inhibit an enzyme by 50%
IUPAC	International Union of Pure and Applied Chemistry
IR	Infrared
Lys	Lysine
min	Minutes
MM	Molecular mechanics
MW	Microwave
NAD	Nicotinamide dinucleotide
NAM	Nicotinamide

NAMFIS	NMR analysis of molecular flexibility in solution
NBS	*N*-Bromosuccinimide
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser enhancement
n.d.	Not determined
o.n.	Overnight
p-TSA	*para*-Toluenesulfonic acid
PDB	Protein data bank
Phe	Phenylalanine
PMB	*para*-Methoxyphenyl
Pro	Proline
QM	Quantum mechanics
rt	Room temperature
SAR	Structure activity relationship
sat.	Saturated
SET	Single electron transfer
SD	Standard deviation
Sirt	Silent information regulator type
SRIF	Somatotropin release-inhibiting factor
7 TM	Seven transmembrane
TBMS	*tert*-Butylmethylsilyl
THF	Tetrahydrofuran
THP	Tetrahydropyran
Thr	Threonine
TMG	Trimethylguanidine
TMPA	Trimorpholinophosphortriamide
TPAP	Tetrapropylammonium perruthenate
TPPA	Tripyrrolidinophosphortriamide
Trp	Tryptophan
VCD	Vibrational circular dichroism
Xaa	Any arbitrary amino acid

1. Introduction

1.1 Bioactive peptides

Peptides are involved in a wide range of biological processes, e.g. regulation of blood pressure, food intake, pain transmission, and blood-glucose levels. Peptides consist of amino acids linked together via amide bonds (Figure 1).[1] There are 20 naturally occurring amino acids with side chains comprising both hydrophilic and hydrophobic groups. The combinations of amino acids with different side chains provide peptides with high structural variation and diverse biological functions. Several endogenous bioactive peptides have been identified such as vasopressin,[2] oxytocin,[3] enkephalin,[4] insulin,[5, 6] somatostatin,[7] and angiotensin II.[8] A peptide adopts its bioactive conformation upon binding to its target. It can then activate/deactivate the target, e.g. a G-protein coupled receptor (GPCR) or an enzyme.

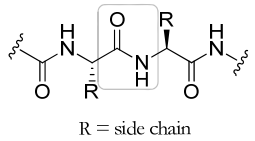


Figure 1. The primary structure of a peptide is defined by the order of the amino acids linked together via amide bonds.

Peptides interact with their targets via ionic and hydrogen bonds, π-π interactions, and van der Waal's interactions. The flexibility and the propensity to form intramolecular hydrogen bonds allow peptides to adopt secondary structures such as turns, sheets, and helices. Peptide turns, comprising α-, β-, and γ-turns, function as recognition sites when peptides bind to their target receptors.[9] The β-turn is the most prevalent secondary structure of peptides, classified according to ϕ and ψ torsion angles of amino acids i+1 and i+2. β-Turns are denoted type I, I´, II, II´ III, and VIII,[10-13] the type II β-turn (Figure 2) is defined by $\phi(i+1)= -60°$, $\psi(i+1)= -30°$, $\phi(i+2)= -120°$ and $\psi(i+2)= 120°$ and is of particular interest in this thesis.

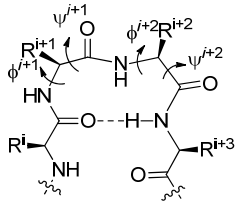


Figure 2. A β-turn is formed by a tetrapeptide fragment and functions as a recognition site between a peptide and its receptor. The turn is defined by ϕ and ψ angles.

1.1.1 Peptides as drugs and development of peptidomimetics

There are limitations for using peptides as oral drugs due to their physico-chemical properties such as high polarity and high conformational flexibility. In addition, peptides undergo rapid enzymatic degradation by cleavage of the amide bonds. These structural properties contribute to short half-life, low bioavailability, and lack of selectivity. However, peptides are still possible to use as drugs, this can be exemplified by the macromolecules insulin (blood glucose regulator), cyclosporin A (immunosuppressant), and oxytocin (smooth muscle contractile agent). With the exception of cyclosporin A, which is used as a peroral drug, insulin and oxytocin are intravenously administered due to the instability of the drugs in the gastro-intestinal (GI) tract.

The development of conformationally restricted analogs of peptides has been a successful approach in terms of improving selectivity and chemical stability of peptides towards enzymatic degradation. Such peptide mimicking agents are termed peptidomimetics.[14-18] The International Union of Pure and Applied Chemistry (IUPAC) has stated the following definition for peptidomimetics; *"A peptidomimetic is a compound containing non-peptidic structural elements that is capable of mimicking or antagonizing the biological action(s) of a natural peptide. A peptidomimetic does no longer have classical peptide characteristics such as enzymatically scissile peptidic bonds"*.[19] Approaches used for the development of peptidomimetics are depicted in Figure 3, where the starting points are either endogenous peptides or non-peptidic compounds e.g. natural products, or derivatives from synthetic collections.[14]

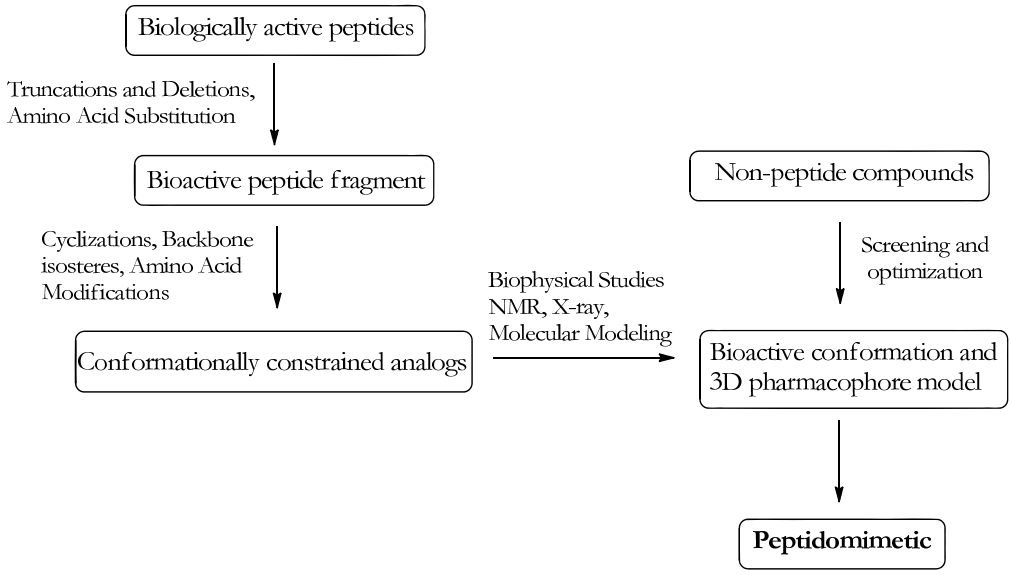


Figure 3. Design and development of peptidomimetics.[14]

There are various strategies to identify the amino acids that are essential for the activity of a peptide. The most common methods used include truncations, deletions, and substitutions

of amino acids. Then the conformational flexibility of the peptides can be reduced through the introduction of local or global constraints.[16] The local constraints involve the incorporation of modified amino acids (e.g. D-amino acids, *N*-methyl, cyclic or β-substituted amino acids) or replacement of amide bonds with bioisosteres (e.g. CH=CH, CH_2CH_2, $CH(OH)CH_2$, $COCH_2$ or CH_2NH).[20, 21] IUPAC defines a bioisostere as; *"A compound resulting from the exchange of an atom or group of atoms with another, broadly similar, atom or groups of atoms"*.[19] Global constraints comprise e.g. medium- or long range cyclizations including disulfide- or lactam bridges. Other modifications are the development of secondary structure mimetics such as β-turn mimetics.[17, 22, 23]

Altogether these types of modifications result in either i) a class I mimetic where the peptide backbone is modified using bioisosteres, ii) a class II mimetic where the entire framework is changed but the derivative has affinity to the same receptor as the parent peptide, or iii) a class III mimetic which encompasses a scaffold that places amino acid side chains crucial for activity in the same relative positions as in the parent peptide.[15] Figure 4 shows examples of successfully developed type III peptidomimetics.

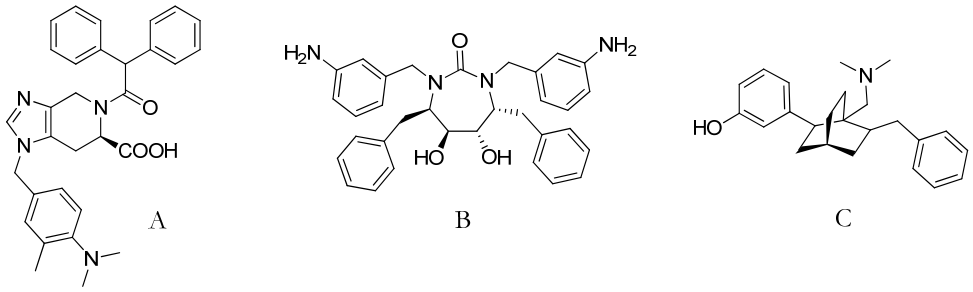


Figure 4. Examples of type III peptidomimetics. A) A selective antagonist at the AT-2 receptor,[24] B) the HIV protease inhibitor DuP450,[25] and C) the first published scaffold-based mimetic, it proved to act as an enkephalin mimetic.[18, 26]

1.2 Targets for bioactive peptides relevant to this thesis

1.2.1 G-protein coupled receptors

The G-protein coupled receptors (GPCRs) are one of the most common types of receptors and hence attractive drug targets. A broad range of ligands such as peptide hormones (e.g. glucagon, insulin, oxytocin, somatostatin), or peptide neurotransmitters (e.g. somatostatin, enkephalin, substance P) are recognized by GPCRs. GPCR´s are characterized by an extracellular *N*-terminus, seven transmembrane helices (7TM), an intracellular *C*-terminus in association with a heterotrimeric G protein complex (containing α-, β-, and γ-subunits) (Figure 5).[27, 28]

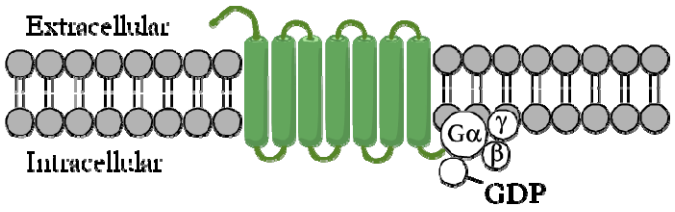


Figure 5. Schematic representation of a G-protein coupled receptor embedded in a phospholipid bilayer.

When the appropriate ligand binds to the receptor there is a conformational change in the receptor-ligand complex resulting in an activation cascade through the exchange of guanosine diphosphate (GDP) to guanosine triphosphate (GTP) on the α-unit in the G-protein. The α-subunit then splits off from the β- and γ-subunits and the free α-subunit and the β/γ complex mediate a second messenger response via different cellular effectors e.g. adenylate cyclase or protein phosphatases.[29-31]

1.2.1.1 Somatostatin (Somatotropin Release-Inhibiting Factor, SRIF)

Somatostatin is an inhibitory peptide hormone isolated in 1973 from ovine hypothalamus, it is expressed in the central nervous system (CNS), the GI tract, and in endocrine tissues.[7, 32, 33] Somatostatin comprises 14 or 24 amino acids and exerts its action through five structurally related GPCR subtypes (sst1-sst5).[34] The peptide functions as a neurotransmitter on e.g. the sst2 receptor, which is involved in the inhibition of the release of growth hormone (GH), glucagon and insulin.[33, 34] Figure 6 shows the structure of somatostatin-14 containing the tetrapeptide Phe7-Trp8-Lys9-Thr10 which adopts a type II´ β-turn as the bioactive conformation.[35, 36] Trp8 and Lys9 side chains are particularly important for the activity.[37]

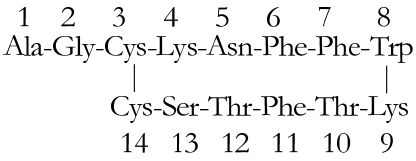


Figure 6. The primary sequence of somatostatin-14.[38]

The fact that somatostatin has a short half-life in plasma (<3 min) makes the peptide interesting for development of stabilized mimetics. The cyclic hexapeptide L-363,301 (Figure 7) was synthesized with a reduced ring size in comparison to somatostatin and was considered to be the lead compound in the development of more restricted analogs.[39, 40] It is a highly potent agonist and inhibits the release of GH, insulin and glucagon to a greater extent than somatostatin. Octreotide (or Sandostatin®) (Figure 7) is a peptide-based somatostatin agonist used in the treatment of hormone-secreting pituitary adenomas and gastroenteropancreatic (GEP) tumors.[41] Octreotide is stabilized by the introduction of D-Phe1, and D-Trp4, a disulfide bridge between Cys2 and Cys7, and an amino alcohol at the *C*-

terminus. The half-life of octreotide is 117 min.[42] An extensive number of peptidic and non-peptidic somatostatin agonists have received considerable attention over the years.[38] The non-peptidic derivatives of somatostatin are mainly scaffold-based β-turn mimetics to which appropriate side chains are attached. The first non-peptidic analog of somatostatin was based on a glucose scaffold.[43, 44] Other scaffolds such as benzodiazepines,[45] pyrrolidine[46] and catechol[47] having the crucial side chain moieties of Trp8 and Lys9 have also been evaluated for their agonistic activity (Figure 7).

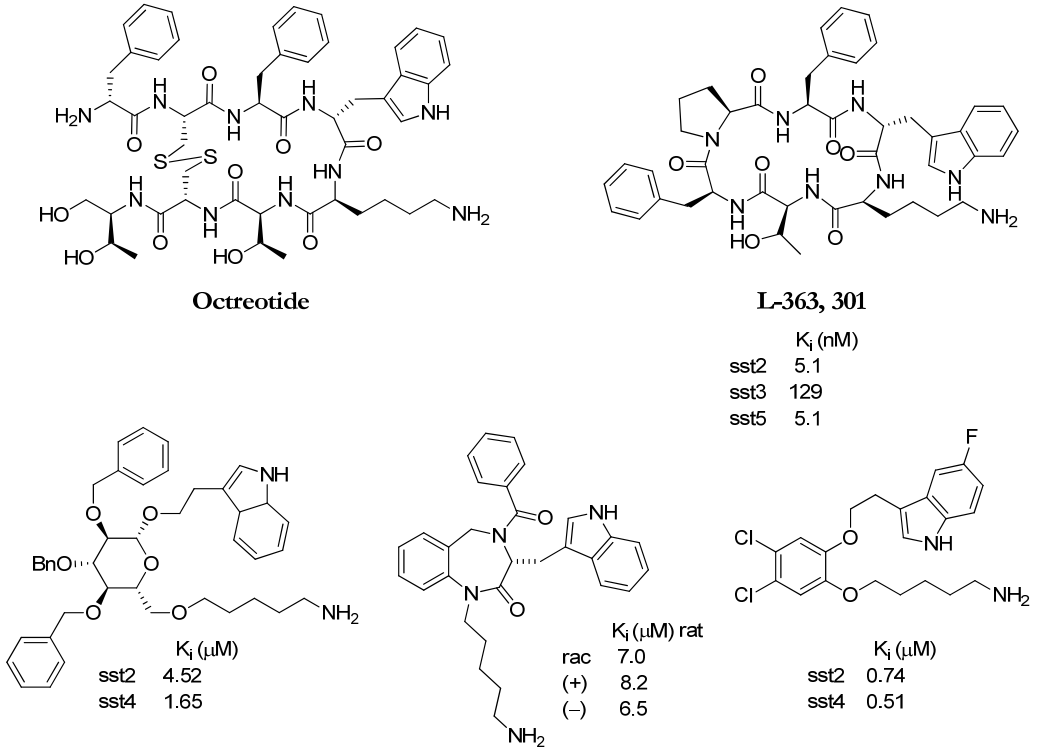


Figure 7. L-363,301 and octreotide[38] are peptidic analogs of somatostatin while the non-peptidic scaffold-based mimetics are represented by substituted glucose,[43, 44, 48] benzodiazepine,[45] and catechol[47] derivatives.

1.2.2 Enzymes: Silent information regulator type (Sirt) enzymes

The function of proteins is related to post-translational modifications including acetylation, methylation or phosphorylation. Protein complexes such as histones undergo ε-amino acetylation of lysine residues.[49] Histones bind DNA in the nucleosomes (Figure 8) and the lysine acetylation/deacetylation process affects gene regulation. The acetylation neutralizes the positive charge of lysine and affects the decondensation of the chromatin fibres. This leads then to alterations in DNA binding.[49, 50]

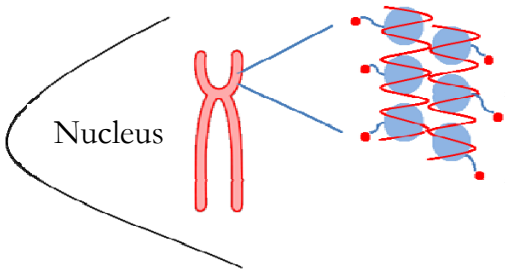


Figure 8. A chromosome is composed of DNA packed into chromatins. The chromatins are repeating units of nucleoseomes with DNA helices (red wires) wrapped around histones (blue filled circles) with acetyl groups on the surface (red filled circles).

The acetylation is a reversible process involving an acetyl transfer to an ε-amino group of lysine catalyzed by histone acetyltransferase (HAT).[51] The opposite deacetylation is catalyzed by histone deacetylases (HDACs). Sirtuins (Sirts or Silent Information Regulator Types) belong to the class III HDACs that require nicotinamide adenine dinucleotide (NAD^+) as a co-substrate. The name sirtuin refers to the originally found Sir2 homolog in yeast.[52] The sirtuins deacetylate not only histones but also non-histone substrates such as transcription factors (e.g. p53) or α-tubulin.[50, 53-56] There are seven mammalian Sirt isoforms (Sirt1-Sirt7)[57, 58] localized in the nucleus (Sirt1, -6, -7), cytoplasm (Sirt2), and the mitochondria (Sirt3, -4, -5).[52, 59] Sirt1-3, -5, and 6-7 catalyze deacetylations whereas Sirt4 and -6 catalyze an adenine diphosphate (ADP)-ribosyl transfer reaction (the latter mechanism is not discussed in this thesis).

In the deacetylation reaction the glycosidic bond in NAD^+ is believed to break through an S_N2-mechanism,[60, 61] and a deacetylated substrate, 2´-*O*-acetyl-ADP-ribose, and nicotinamide (NAM) are formed (Figure 9).[62] The acetyl group on 2´-*O*-acetyl-ADP-ribose equilibrates via an intramolecular transesterification with the 3´-*O*-acetylated regioisomer.[63] Nicotinamide functions as the physiological regulator of the deacetylation process.[64]

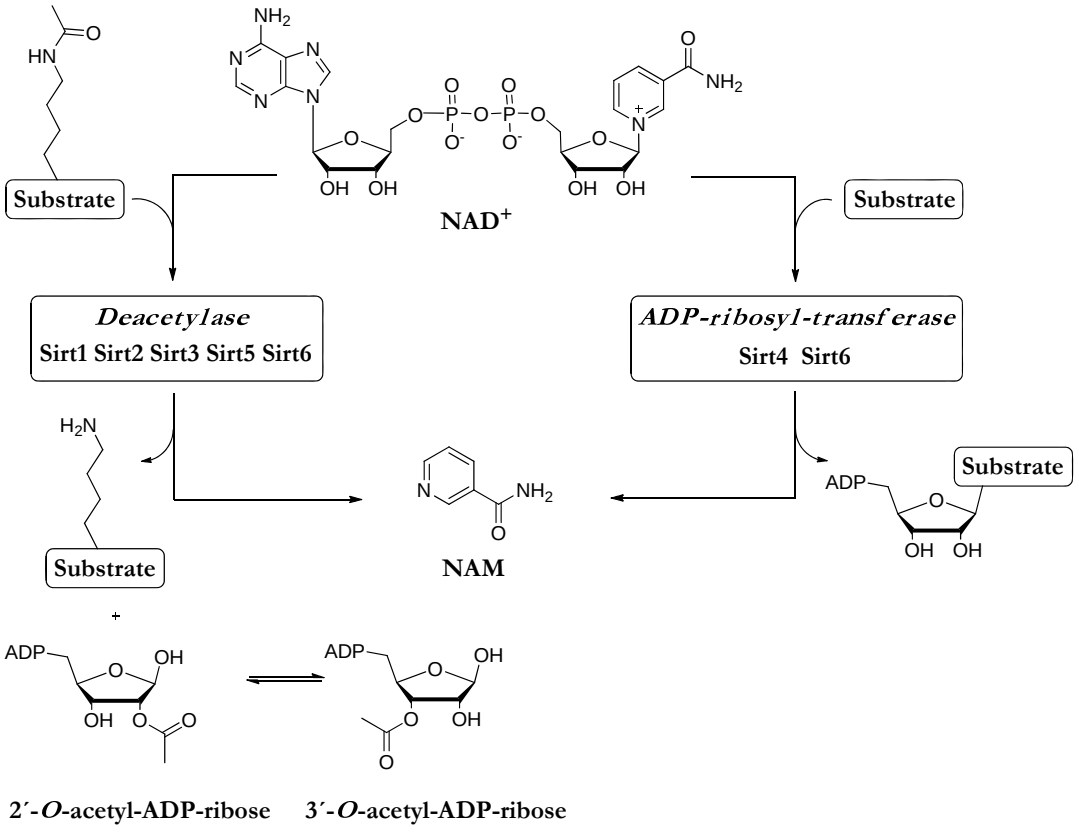


Figure 9. The function of mammalians sirtuins is either to catalyze the deacetylation of various protein substrates or an ADP-ribosyl transfer reaction.[65]

The sirtuins have recently become highly interesting targets for drug development as they are proposed to be involved in age-related diseases such as diabetes, cancer[58, 66, 67] and neurodegenerative disorders, e.g. Parkinson's and Alzheimer's disease.[58, 66, 68, 69] One of the aims of this thesis is to develop Sirt modulators.

1.2.2.1 Deacetylation by silent information regulator type 2 (Sirt2)

The microtubule network of a cell is composed of α-and β-tubulin proteins shaped as hollow cylinders in the cytosol (Figure 10).[70] The microtubule is involved in the movement of organelles in the cell, in cell division, and cell wall formation.[70, 71] Sirt2 colocalizes with the microtubule and hence with the α-tubulin both in vivo and in vitro.[55] Sirt2 is involved in cell cycle regulation[72] and inhibition of Sirt2 leads to hyperacetylation of α-tubulin and to reduced tumor growth in cancer tissues.[55, 58] In addition, reports have shown that Sirt2 inhibition leads to a decreased neuronal cell death which relates Sirt2 activity to Parkinson´s disease.[73]

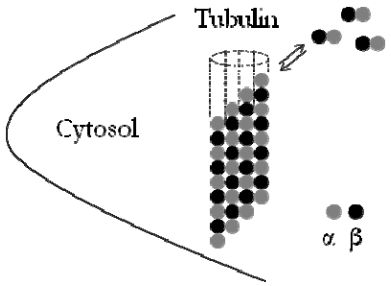


Figure 10. The microtubule is composed of α- and β-tubulin. The polymerization and depolymerization processes of the subunits are highly dynamic and crucial for e.g. mitosis.[70]

1.2.2.2 Structure of Sirt2

The crystal structure of human Sirt2 was solved in 2001 by Finnin et al.[74] The enzyme is composed of two domains connected by four polypeptide chains (Figure 11). The larger domain is a Rossmann fold domain present in many NAD(H)/(NADP(H) binding enzymes.[75] It includes six β-strands surrounded by six α-helices and constitutes the NAD^+ binding site. The Rossmann fold is characterized by a Gly-X-Gly sequence important for the NAD phosphate binding and a small pocket with charged residues to bind the ribose groups. Mutations in the large groove between the two domains disturb the deacetylation activity and this part is therefore considered as the catalytic site of the enzyme.[74] The smaller domain has a helical module and a structural zinc binding module. The structures of the two domains are conserved throughout the sirtuin family.

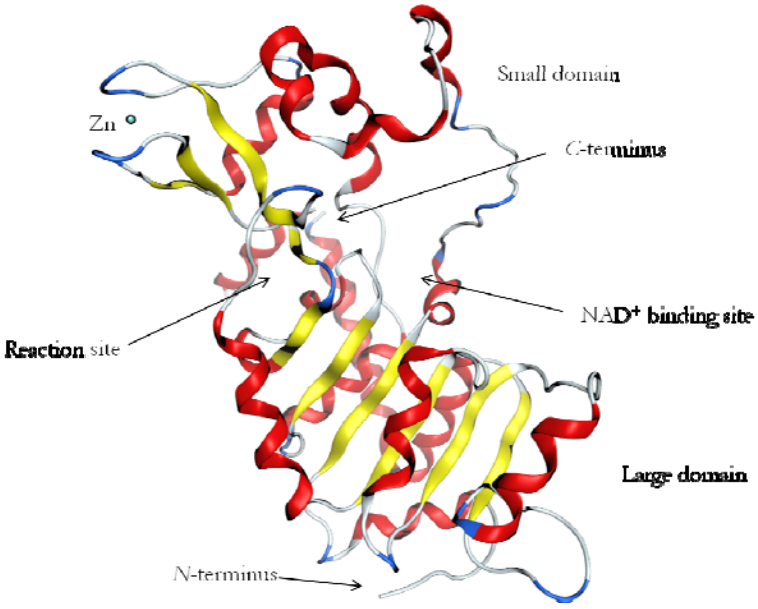


Figure 11. The apo structure of human Sirt2 (PDB 1J8F).[74] The secondary structure is represented by α-helices (red), β-strands (yellow), and loops (grey/blue).

1.2.2.3 Inhibitors of Sirt2

Nicotinamide is the physiological inhibitor of Sirt2 whereas sirtinol (Figure 12) was the first synthetic Sirt2 inhibitor explored in 2001 by Grotzinger et al.[76] A number of compounds have been synthesized and evaluated as Sirt2 selective inhibitors such as alkylated cambinol derivatives,[77] AGK2,[73, 78] tryptamide analogs,[79] and 2-anilinobenzamides.[80] The binding of the published inhibitors have been suggested to either occur in the catalytic site or in the NAD^+ binding site, however the binding modes of many of the developed inhibitors remain unknown. Substrate based inhibitors such as N^{ε}-thioacetyl-lysine containing peptides show high potency but so far no selectivity is observed for Sirt2 over Sirt1.[81, 82] However, a cyclic pentapeptide has recently been discovered as a selective Sirt2 inhibitor.[60]

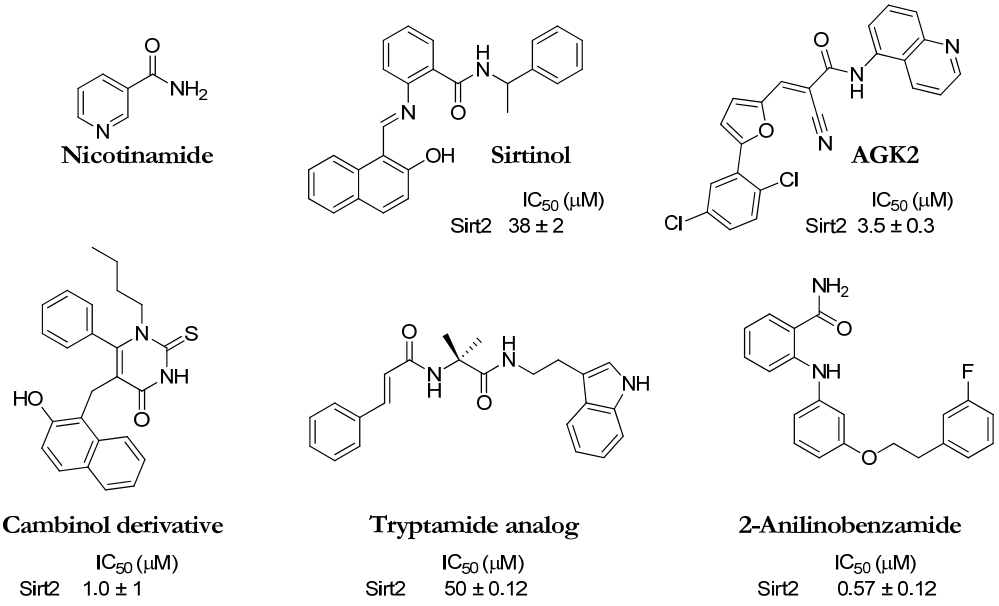


Figure 12. Structures of nicotinamide (natural regulator of Sirt2) and known Sirt2 inhibitors.

1.2.2.4 The proposed role of Sirt2 in cancer

Although Sirt2 is mainly located in the cytoplasm, the enzyme is shuttled into the nucleus during the mitosis.[72] The Sirt2 level increases in the G_2/M phase (Figure 13) and an overexpression of Sirt2 prolongs the mitotic phase in a normal cell cycle.[72] Sirt2 is believed to have an effect on the check-point in the G_2/M phase and ensures that the cell does not proceed through mitosis if exposed to any stress signal or if damaged DNA is present. [83-85]

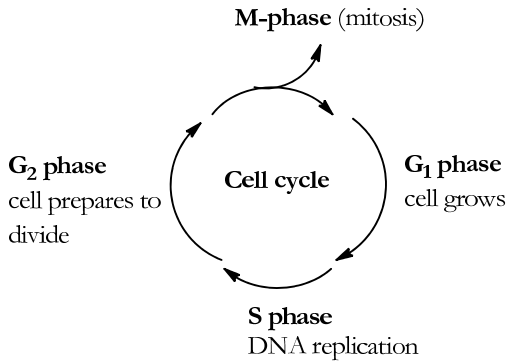


Figure 13. Schematic picture of the different stages in the cell cycle.[86]

Wang et al. have reported that Sirt2 activity facilitates apoptosis of damaged cells, and hence a decreased Sirt2 concentration is important for the mitotic exit in the cell cycle.[87] Certain cancer cell lines (e.g. HeLa cells) show a downregulation of Sirt2 which induces p53 accumulation and eventually apoptosis of the cell.[88] Sirt2 inhibitors have therefore become an interesting target in cancer research.[85, 89] Recently, the selective Sirt2 inhibitors sirtinol[90] and AGK2[91] (structures shown in Figure 12) induce apoptosis of e.g. MCF-7 breast cancer cells and C6 glioma cells.

1.3 Chroman-4-ones and chromones as scaffolds for bioactive compounds

In this thesis chroman-4-ones and chromones are used as scaffolds for the development of bioactive compounds. These frameworks are naturally occurring derivatives containing an oxa-pyran ring.[92, 93] Structures of chroman-4-one and chromone derivatives are illustrated in Figure 14. The most frequently found chromone-based natural products are the 2-aryl substituted chromones (flavonoids) carrying hydroxy and/or methoxy groups on the A and/or B rings.[94, 95] They are constituents of pigments in leaves and are present in a range of food sources such as olive oil, tea, fruits, and red wine.[96] Flavonoids are well represented in the literature whereas the 2-alkyl substituted chroman-4-ones and chromones are not as common. In addition, the literature regarding enantioselective synthesis of 2-alkyl chroman-4-ones is limited.[97, 98]

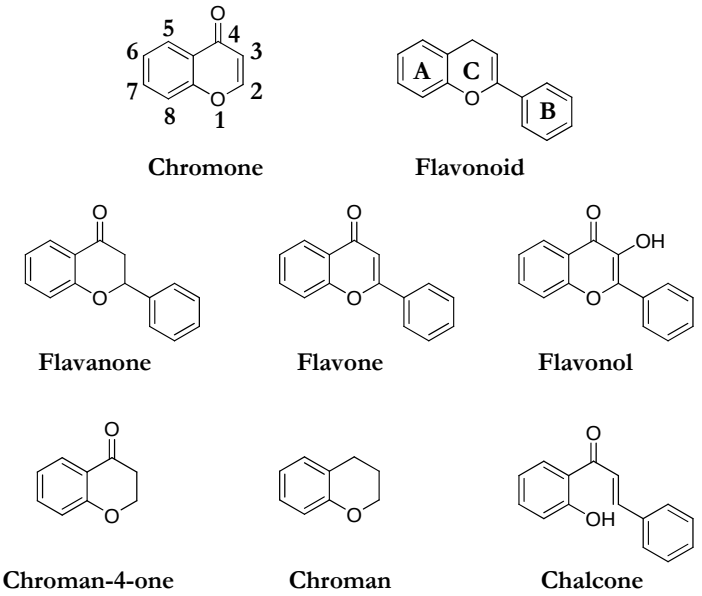


Figure 14. The chemical structures and numbering of chroman-4-one and chromone related derivatives.

The substitution pattern of the chroman-4-one and chromone scaffolds determines their different biological effects. Known effects of these types of compounds are antioxidant,[99, 100] antiviral,[101] antibacterial activities[102], or kinase inhibition.[103, 104] Hence, chroman-4-ones and chromones can be considered privileged structures, defined as "*a single molecular framework able to provide ligands for diverse receptors*".[105-107] This thesis is mainly based on 2,6- or 2,8-disubstituted chroman-4-ones and chromones and 2,3,6,8-tetrasubstituted chromones.

The first clinically used chromone was khellin (Figure 15) which was extracted from the seeds of *Ammi visnaga* and isolated in its pure form in the 1930´s.[94] It functioned as a relaxing agent in visceral smooth muscle and was later found to provide prolonged relief of bronchial asthma. There are currently a number of chroman/chromone based medical treatments in use, e.g. sodium cromoglycate (Lomudal®) which prevents the release of histamine from mast cells and is administrated as a disodium salt,[108] and nabilone (Cesamet®) which is a cannabinoid used as an antiemetic drug.[109] α-Tocopherol (vitamin E) occurs mainly in avocado, almond, and wheat germ and acts as an antioxidant and a radical scavenger.[109]

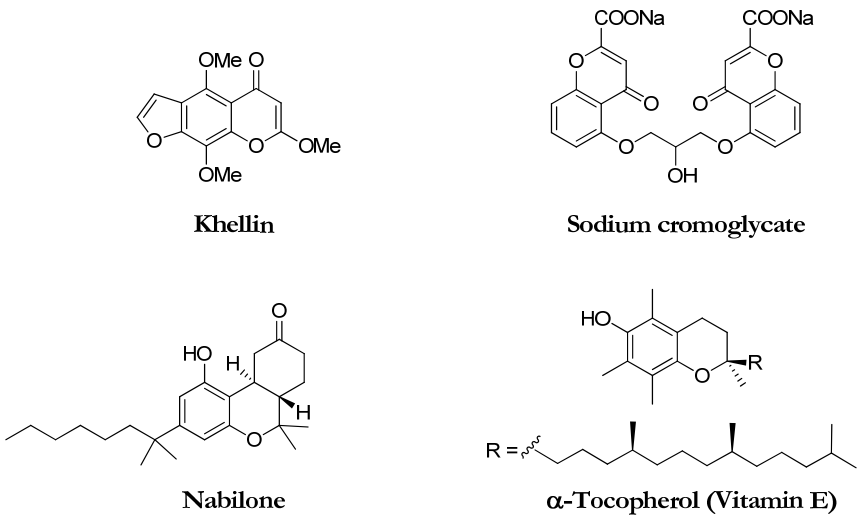


Figure 15. Khellin, sodium chromoglycate and nabilone are clinically used chromone and chroman derivatives. α-Tocopherol is a naturally occurring antioxidant in food.

Substituted chroman-4-one and chromone derivatives are formed either biosynthetically[110] or synthetically. The retrosynthetic analysis for the most common pathways to derive chroman-4-ones and chromones is shown in Figure 16. Routes 1 and 2 require acidic or basic conditions in order to form the desired chromone. Route 1 involves α,β-diketones formed through a Baker-Venkataraman rearrangement from *o*-acyloxyketones.[111, 112] Route 2 involves a chalcone intermediate synthesized in a Claisen-Schmidt condensation from an aldehyde and an acetophenone.[113, 114] As illustrated in route 3, prior an oxidation to the chromone the corresponding chroman-4-one derivative could be synthesized via a condensation reaction with an acetophenone and an aldehyde whereas route 4 involves a propargyl derivative formed from salicylic acid and an alkyne.[115] In order to synthesize the desired chroman-4-one and chromone derivatives route 3 was specifically applied in this thesis.

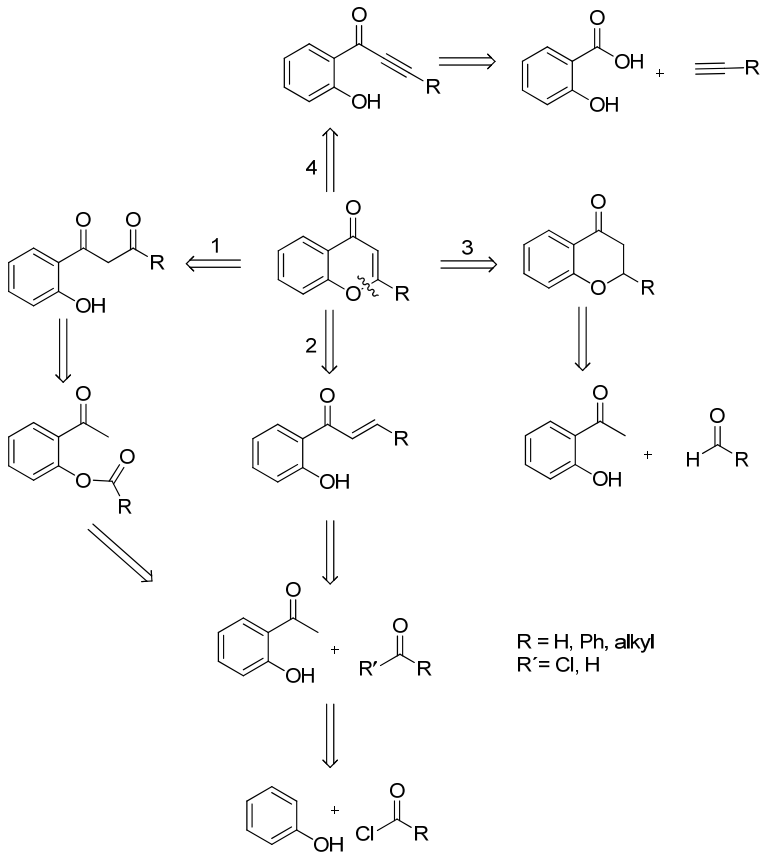


Figure 16. Retrosynthetic analysis of common synthetic pathways to obtain chroman-4-one and chromone derivatives. Route 3 is applied in this thesis.

1.4 Computational calculations as tools in medicinal chemistry

The bioactive conformation of a peptide is of great interest in order to understand how the peptide binds to the target and which of the individual amino acids that are involved in the binding. Peptides are highly flexible in solution and adopt a large number of conformations. A way to determine which conformations that are prevalent in solution is to use NMR spectroscopy. However, as individual conformations cannot be studied using this technique, computational calculations using simulated solvation has become increasingly important.

Computer based methods are of great value in medicinal chemistry in terms of calculations of energies and geometries of molecules.[116] Two common methods available for this purpose are molecular mechanics (MM) and quantum mechanics (QM) calculations. Molecular mechanics calculations are considered to be fast, have broad applicability for a range of compounds, and to be sufficiently accurate. Quantum mechanics calculations are suitable for modeling of transitions states, and for determination of reaction paths or geometries. The basic idea of quantum mechanics is to determine the exact electronic distribution of the molecules.

The concept of conformational search and energy minimization using MM is based on the potential energy of a molecule. The potential energy is determined by factors such as bond stretching, angle bending, torsional angles, non-bonding interactions including van der Waals interactions, electrostatics and coupled energy terms. These parameters are combined to provide the total energy (E_{tot}) as described in Eq. (1):

$$E_{\text{tot}} = E_{\text{str}} + E_{\text{bend}} + E_{\text{tors}} + E_{\text{vdw}} + E_{\text{elec}} + E_{\text{cross-term}} \quad (1)$$

Considering the first parameter in Eq. (1) the energy E_{str} is obtained for a bond stretch that deviates from an optimal geometry or unstrained value. Each deviation will increase the total energy. The E_{str} values for bonds between a large variety of atoms are empirically derived and are included in what is referred to as a *force field*.[116] The same is true for other terms in the equation. A conformational search in MM results in low energy conformations including global and local minima where a conformation is mainly related to changes in torsion angles around single bonds. Because the bioactive conformation is not necessarily at global energy minima of a ligand, other low energy conformations within 12 kJ/mol from the global minima in solution are of interest for the search of the bioactive conformation.[117]

2. Aims of the thesis

The general aim of this thesis was to synthesize compounds based on functionalized chroman-4-one and chromone scaffolds and evaluate their biological activities.

The specific objectives were:

- To develop synthetic methods to incorporate substituents in defined positions of the chroman-4-one and chromone frameworks (Papers I and II).

- To use chroman-4-one and chromone scaffolds for the development of β-turn mimetics using somatostatin as a model peptide (Paper III).

- To develop chroman-4-one and chromone based Sirt2 modulators (Papers IV and V).

- To perform a structural determination study of a proline mediated cyclization product based on chroman-4-one (Paper VI).

3. Synthesis of functionalized chroman-4-one/chromone scaffolds

As described in section 1.3, 2-phenyl substituted chroman-4-one/chromone derivatives are more prevalent in the literature than the corresponding 2-alkyl derivatives. The initial aim of this thesis was to develop an efficient method to synthesize 2-alkyl substituted chroman-4-ones e.g. 2,6,8-trisubstituted chroman-4-one **C** (Figure 17). These derivatives were considered to be key intermediates for the syntheses of functionalized chroman-4-ones and chromones such as **D** and **E**.

More specifically, the synthetic strategy was to react substituted acetophenones **A** and aliphatic aldehydes **B** to obtain the 2,6,8-trisubstituted chroman-4-ones **C**. A subsequent incorporation of a 3-substituent using appropriate methods would eventually give **D** or **E**. In the following section the development and optimization of various synthetic procedures to obtain the 2,6,8-trisubstituted chroman-4-ones **C** and 2,3,6,8-tetrasubstituted derivatives **D** and **E** will be discussed.

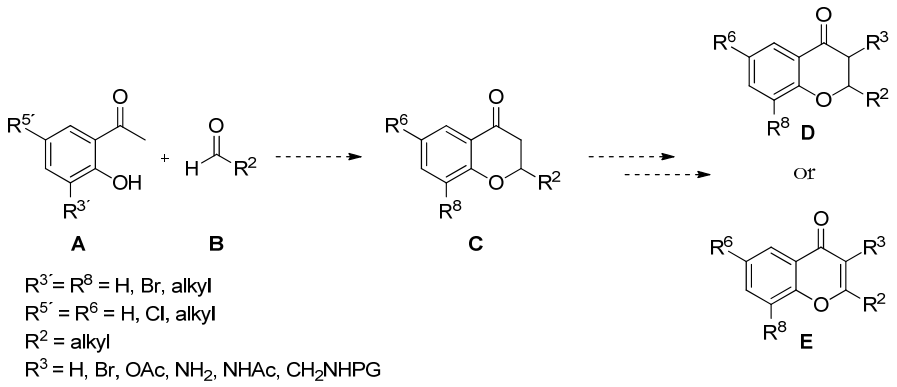


Figure 17. The synthetic strategy to obtain functionalized chroman-4-ones/chromones **C**, **D**, and **E**. The 2-alkyl chroman-4-one derivative **C** is considered to be a key compound for the subsequent introduction of substituents. PG = protecting group.

3.1 Introduction of substituents in the 2-position: Base mediated aldol condensation (Paper I)

One common method to obtain 2-alkyl chroman-4-ones is via an enamine catalyzed reaction to afford 2-mono- or 2,2-disubstituted chroman-4-ones using pyrrolidine in refluxing toluene as reported by Kabbe et al. in 1982.[118] The reactions involved *o*-hydroxyacetophenones and ketones or aldehydes (aromatic or aliphatic) (Figure 18). A majority of the alkyl derivatives were synthesized using acetophenones without other substituents, it was also reported that chroman-4-ones with phenethyl as the 2-substituent were formed in only low yields.[118] An alternative route to obtain 2-alkyl substituted

chroman-4-ones is to perform a Mukayiama aldol condensation which requires the use of $TiCl_4$.[119] Such harsh conditions are however not suitable if (acid) sensitive groups such as esters or nitriles are present in the acetophenone or the aldehyde.

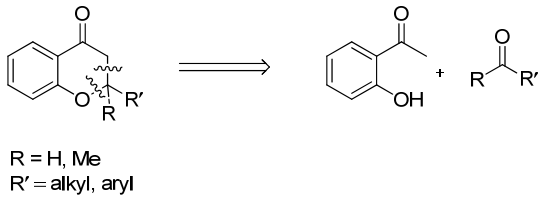
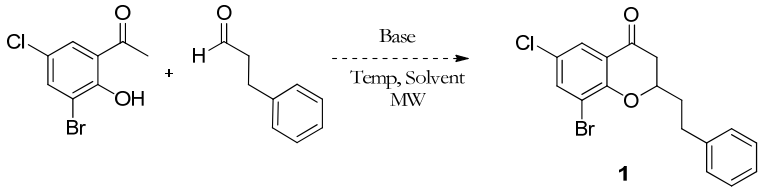


Figure 18. The retrosynthetic analysis of the formation of 2-alkyl substituted chroman-4-ones according to previously reported procedures. The reactions are mainly enamine catalyzed or involve the use of silyl enol ethers.[118, 119]

The main aim of Paper I was to develop an efficient synthetic procedure to obtain 2-alkyl substituted chroman-4-ones using microwave heating. Previously, L-proline was reported to catalyze the formation of flavanones in DMF at 80 °C.[120] The enantioselectivity obtained in the reaction was however low (<5%). As a starting point attempts to form the 2-alkyl substituted derivative **1** (Scheme 1) from 3'-bromo-5'-chloro-2'-hydroxyacetophenone and 3-phenylpropanal. The reaction was performed in DMF using various amounts of L-proline (0.3 or 1.1 equiv) under microwave conditions (120 or 170 °C) or classical heating (80 °C). Also different reaction times (1, 21 or 48 h) were examined. Independent on the choice of conditions the reaction resulted in low yields (8-38%) of product.



Scheme 1. The synthesis of derivative **1** was used as a model reaction for the optimization of the procedure to obtain 2-alkyl substituted chroman-4-ones.

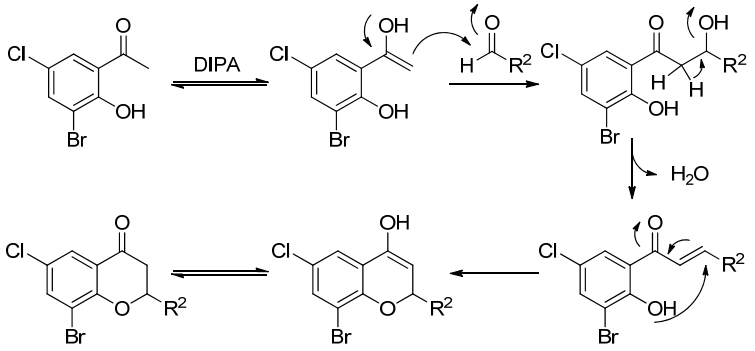
Instead other bases (pyrrolidine, diisopropylamine (DIPA), morpholine, piperazine, and piperidine), temperatures (100 °C or 170 °C), and solvents (EtOH, water, or toluene) were evaluated under microwave conditions for 1 hour (Table 1). The desired chroman-4-one **1** was obtained as a racemic mixture in low to moderate yields using pyrrolidine as the base (15-52%) (Table 1, entry 1). The yields of the reaction were improved (61-88%) using DIPA or morpholine in EtOH or toluene (Table 1, entries 2 and 3). Piperazine and piperidine (Table 1, entries 4-5) gave **1** in moderate to good yields in EtOH at 170 °C (61 and 63%, respectively).

Table 1. The screening of conditions for the formation of derivative **1**.[a]

		EtOH		Water		Toluene	
Entry	**Base**[b]	**100 °C**	**170 °C**	**100 °C**	**170 °C**	**100 °C**	**170 °C**
1	Pyrrolidine	52	16	36	15	30[f]	n.r.[g]
2	DIPA	71	88[c]	45	48	n.r.[g]	78
3	Morpholine	68	72	12	72	n.r.[g]	61
4	Piperazine		61[d]				
5	Piperidine		63[e]				
6	DIPEA		81				

[a]Isolated yields. [b]1.1 equiv of the base was used. [c]0.3 equiv of DIPA resulted in lower yield and formation of aldehyde condensation products. [d]Unreacted 3-bromo-5-chloro-2-hydroxyacetophenone was recovered. [e]0.3 equiv of piperidine resulted in lower yield and formation of aldehyde condensation products. [f]The yield was estimated from ^{1}H NMR spectra on the crude reaction mixture due to purification problems. [g]no reaction.

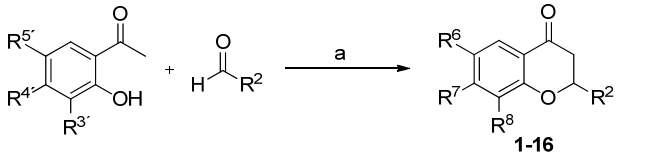
In summary, the reaction gave the highest yield, 88% of **1** (Table 1, entry 2) when using DIPA in EtOH with microwave heating at 170 ºC for 1 h. A control experiment with the tertiary amine diisopropylethylamine (DIPEA) also gave high yields (81%) of **1**, which implies that the reaction proceeds via an aldol condensation rather than an enamine mechanism. The proposed mechanism for the base mediated aldol condensation of the formation of the chroman-4-ones is shown in Scheme 2.



Scheme 2. The proposed mechanism for the base mediated formation of 2-alkyl substituted chroman-4-ones. The reaction involves an aldol condensation and a subsequent *oxa*-Michael addition.

The scope of the reaction was further investigated by screening different acetophenones and aldehydes as illustrated in Table 2.

Table 2. Screening of different acetophenones and aldehydes to obtain 2-alkyl substituted chroman-4-ones **1**-**16**.[a]



1-11 R^6 = Cl, R^7 = H, R^8 = Br
12 R^6 = H, R^7 = F, R^8 = H
13 R^6 = NO_2, R^7 = H, R^8 = H
14 R^6 = Me, R^7 = H, R^8 = H
15 R^6 = OMe, R^7 = H, R^8 = H
16 R^6 = R^7 = R^8 = H

Entry	R^2	Product	Yield (%)[b]
1	CH_2CH_2Ph	**1**	88
2	CH_2CH_2(1-naphthyl)	**2**	84
3	CH_2CH_2(3-indolyl)	**3**	86
4	CH_2CH_2(*N*-Bn)-3-indolyl	**4**	84
5	CH_2CH_2(*N*-Ts)-3-indolyl	**5**	74
6	$(CH_2)_4CH_3$	**6**	80
7	$CH(CH_3)_2$	**7**	43
8	cyclohexyl	**8**	46
9	Ph	**9**	24
10	4-OMePh	**10**	n.r.[c,e]
11	4-CF_3Ph	**11**	n.r.[e]
12	CH_2CH_2Ph	**12**	70[d]
13	CH_2CH_2Ph	**13**	38[d]
14	CH_2CH_2Ph	**14**	17[d]
15	$(CH_2)_4CH_3$	**15**	26
16	$(CH_2)_4CH_3$	**16**	37

[a]Reagents and conditions: a) DIPA, 170 °C, 1 h, EtOH, MW. [b]Isolated yields. [c]32% of the chalcone was isolated. [d]Estimated yield of product according to ^{1}H NMR spectra on the crude reaction mixture, the product could not be isolated due to purification problems. [e]no reaction

In general the reaction resulted in good to high yields using aliphatic aldehydes (entries 1-6). However branched aldehydes bearing isopropyl or cyclohexyl groups (entries 7-8) gave somewhat lower yields (43 and 46%, respectively). This is probably due to sterical hindrance in the aldol reaction. Also aryl aldehydes were evaluated (entries 9-11) but resulted in low yields when using benzaldehyde (entry 9) and gave no or only traces of product with 4´-substituted benzaldehydes (entries 10-11), instead chalcone intermediates were isolated.

To investigate whether the method is general regarding the substitution in the acetophenone also 4´-fluoro-, 5´-nitro-, 5´-methyl-, and 5´-methoxyacetophenone were used as starting materials (entries 12-15). The desired products were formed in low to good yields (17-70%) as estimated from ^{1}H NMR spectra of the crude reaction mixtures. In addition, the 2-hydroxyacetophenone without any other substituents gave chroman-4-one **16** in 37% yield (entry 16). Thus, the developed method seems to be general for aliphatic aldehydes but results in lower yields when bulky or aromatic aldehydes are used. Higher yields of the chroman-4-ones are obtained when electron withdrawing groups on the acetophenone are present.

3.2 Introduction of substituents in the 3-position (Papers I and II)

The synthetic strategy for further functionalization of the chroman-4-one scaffold was planned to go via 3-bromo substituted 2-alkyl chroman-4-ones (Figure 19). The bromine could then serve as a handle in e.g. substitution and elimination reactions. In addition, halogens such as Cl and Br in the 6- and 8-positions, respectively, were considered as handles for further Pd mediated reactions.

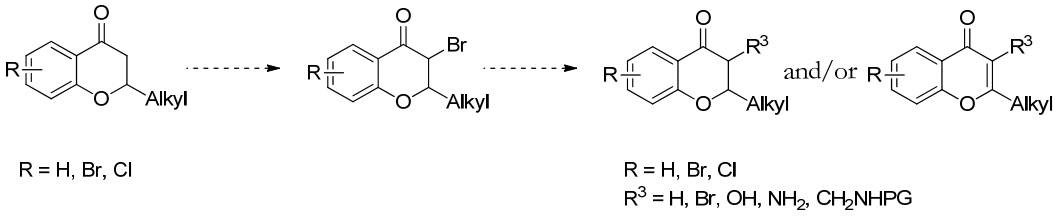
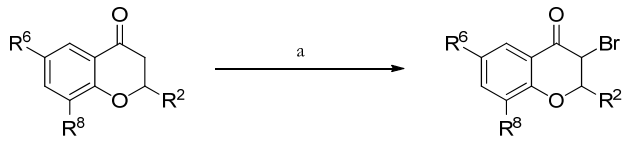


Figure 19. The strategy to synthesize 2-alkyl-3-bromochroman-4-ones in order to functionalize the 3-position and form substituted chroman-4-one and/or chromone derivatives.

3.2.1 Formation of 3-amino-, 3-bromo, and 3-acetoxychromones

The synthesis towards further functionalized chroman-4-ones was performed via the formation of 3-bromo derivatives **1a**-**2a**, **6a**, **8a**-**9a**, and **15a**-**19a** (Scheme 3) starting from chroman-4-ones **1**-**2**, **6**, **8**-**9**, and **15**-**19**. The chroman-4-ones **17**-**19** were commercially available.



1 $R^2 = CH_2CH_2Ph$, $R^6 = Cl$, $R^8 = Br$
2 $R^2 = CH_2CH_2$(1-naphthyl), $R^6 = Cl$, $R^8 = Br$
6 $R^2 = (CH_2)_4CH_3$, $R^6 = Cl$, $R^8 = Br$
8 $R^2 = C_6H_{11}$, $R^6 = Cl$, $R^8 = Br$
9 $R^2 = Ph$, $R^6 = Cl$, $R^8 = Br$
15 $R^2 = (CH_2)_4CH_3$, $R^6 = OMe$, $R^8 = H$
16 $R^2 = (CH_2)_4CH_3$, $R^6 = H$, $R^8 = H$
17 $R^2 = H$, $R^6 = H$, $R^8 = H$
18 $R^2 = H$, $R^6 = Cl$, $R^8 = H$
19 $R^2 = H$, $R^6 = Me$, $R^8 = H$

1a $R^2 = CH_2CH_2Ph$, $R^6 = Cl$, $R^8 = Br$ (92%)
2a $R^2 = CH_2CH_2$(1-naphthyl), $R^6 = Cl$, $R^8 = Br$ (90%)
6a $R^2 = (CH_2)_4CH_3$, $R^6 = Cl$, $R^8 = Br$ (81%)
8a $R^2 = C_6H_{11}$, $R^6 = Cl$, $R^8 = Br$ (58%)
9a $R^2 = Ph$, $R^6 = Cl$, $R^8 = Br$ (82%)
15a $R^2 = (CH_2)_4CH_3$, $R^6 = OMe$, $R^8 = H$ (68%)
16a $R^2 = (CH_2)_4CH_3$, $R^6 = H$, $R^8 = H$ (86%)
17a $R^2 = H$, $R^6 = H$, $R^8 = H$ (70%)
18a $R^2 = H$, $R^6 = Cl$, $R^8 = H$ (63%)
19a $R^2 = H$, $R^6 = Me$, $R^8 = H$ (60%)

Scheme 3. The formation of 3-bromo chroman-4-one derivatives. Reagents and conditions: (a) $CuBr_2$, $CHCl_3$/EtOAc, reflux, 2-6 h (compounds **1a**-**2a**, **6a**, **8a**-**9a**, **15a**-**16a**) or $Py{\cdot}Br_3$, CH_2Cl_2, rt, 30 min (compounds **17a**-**19a**).

Interestingly, the formation of 3-brominated derivatives gave *cis*-isomers as the major products as shown in Table 3. For instance, derivative **1a** (entry 1) resulted in a diastereomeric ratio of 80:20 according to ^{1}H NMR spectroscopy.

Table 3. The *cis:trans* ratio obtained in the 3-bromination reaction to obtain the chroman-4-ones **1a**-**2a**, **6a**, **8a**-**9a**, and **15**-**16a**.[a]

Entry	*cis:trans ratio*	Product
1	80:20	**1a**
2	70:30	**2a**
3	75:25	**6a**
4	99:1	**8a**
5	75:25	**9a**
6	75:25	**15a**
7	78:22	**16a**

[a]The *cis:trans* ratio observed according to ^{1}H NMR spectroscopy after purification.

The results obtained for derivative **1a** were confirmed using computational calculations. Figure 20 shows a simplified structure of the 3-brominated chroman-4-one. After a molecular mechanics based conformational search (MacroModel v. 8.0, MM3* force field),[121] one low energy conformation of each isomer was reevaluated using density functional theory (DFT) (B3LYP/LACVP*).[122] The results were in full agreement with the ^{1}H NMR spectral

interpretation and showed that the *cis*-isomer was thermodynamically more stable than the *trans*-isomer.

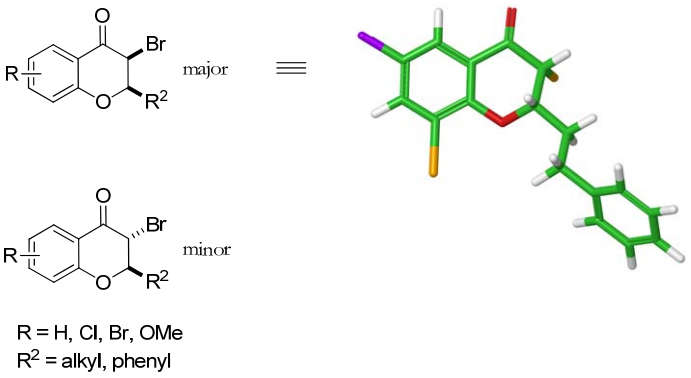
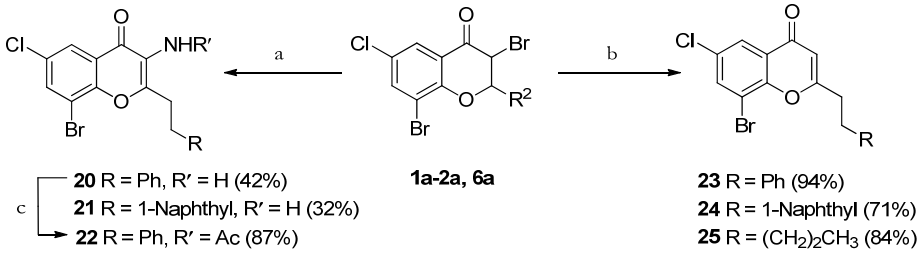


Figure 20. The *cis*-isomer is the dominating product in the bromination reaction using $CuBr_2$. The conformation having bromine in an axial position and the phenethyl substituent in the equatorial position was favored.

The 3-brominated products **1a**-**2a**, and **6a** were used in further functionalizations of the scaffold. Attempts to introduce an amino group in the 3-position to form **20** via the diastereomeric mixture of **1a** were first performed (Scheme 4). Using NaN_3 in DMF the desired amine **20** was obtained in 39% yield accompanied with the chromone derivative **23** (49% yield).

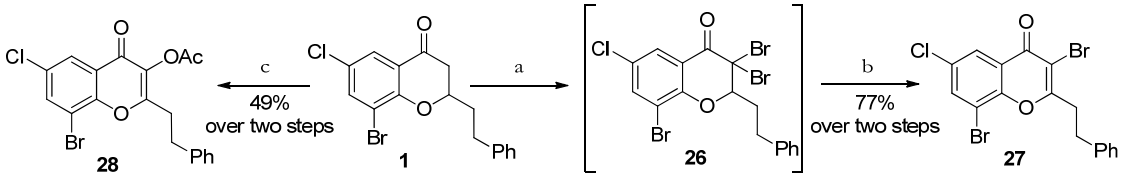


Scheme 4. Formation of chromones. Reagents and conditions: (a) NaN_3, DMSO, rt, 3 h; (b) $CaCO_3$, DMF, 100 °C, 10 min; (c) Acetic anhydride, pyridine, rt, o.n.

Attempts were performed to improve the yields of **20** by e.g. increasing the amount of *trans*-isomer of **1a** using other bromination methods (Br_2 in AcOH or pyridinium tribromide (Py·Br_3) in AcOH or THF). By changing solvent, Py·Br_3 in dichloromethane at room temperature gave **1a** in 92% yield with a *cis*:*trans* ratio of 40:60 according to ^{1}H NMR spectra. The result may be explained by pyridine preventing enolization of the *trans*-isomer and thereby avoiding the epimerization to the *cis*-isomer. However, an epimerization occurred instead during the purification by column chromatography on silica resulting in a *cis*:*trans* ratio of 60:40. Interestingly, when repeating the NaN_3 experiment in DMF using the *cis*:*trans* (40:60) mixture it was shown that the diastereomeric ratio of **1a** did not affect the ratio of

compounds **20** and **23**, chromone **23** was still the major product. The azide reaction was therefore evaluated further in attempts to favor the formation of **20** over **23**. In this effort, different azide sources (NaN_3, $TMSN_3$ or $TMGN_3$), solvents (DMF, DMSO, THF, acetone or MeCN) and temperatures were tested. Unfortunately, this did not substantially improve the yield of **20**, the best result was obtained by using 10 equiv of NaN_3 in DMSO which provided **20** in 42% and **23** in 43% yields. A subsequent acetylation of **20** gave the **22** in 87% yield. The azide method was also applied to the naphthyl derivative **2a** and resulted in 32% of the amine **21** and 51% yield of the chromone **24**. The outcome of the amination reaction was probably due to an epimerization of the *trans*- to the *cis*-isomer when using NaN_3, which then promotes an E2-reaction. Alternatively, an azide ion attacks the *trans*-isomer forming the *cis*-2-alkyl-3-azido derivative which then eliminates HN_3 to form the corresponding chromone.

Also the 3-substituted chromone **27** was synthesized (Scheme 5). A dibromination of **1** with $Py{\cdot}Br_3$ at 80 °C using microwave heating gave a smooth conversion to the dibrominated intermediate **26**. An HBr-elimination of crude **26** yielded the brominated chromone **27** in 77% (over two steps) using $CaCO_3$ in DMF.



Scheme 5. Formation of chromones **27** and **28**. Reagents and conditions: (a) i) $Py{\cdot}Br_3$, CH_2Cl_2, 80 °C, 70 min, MW; b) $CaCO_3$, DMF, 100 °C, 10 min, MW; (c) i) Isoamyl nitrite, HCl, THF, 60 °C, 7 h, MW, ii) AcCl, TEA, CH_2Cl_2, 2 h, rt.

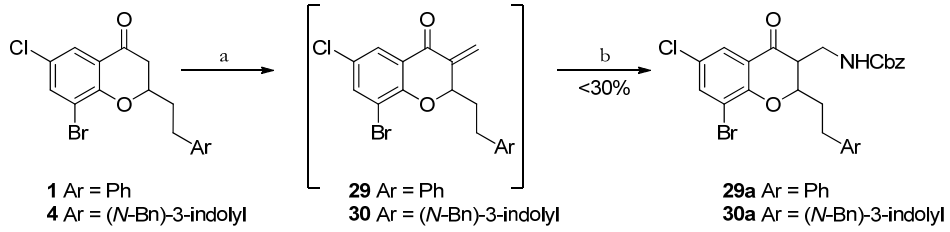
Other bases such as DBU or TEA in dichloromethane or Cs_2CO_3 in DMF also gave **27** but were always accompanied with other impurities. $CaCO_3$ in DMF was then used to prepare chromone **23**-**25** in 71-94% yields from the mono-brominated compounds **1a**-**2a**, and **6a** (Scheme 4). Finally, the 3-hydroxychromone analog was synthesized from **1** using isoamyl nitrite and HCl in EtOH.[123] The reaction was performed at different temperatures (60, 70, 100, and 120 °C) using microwave as well as conventional heating sources and by using different solvents (EtOH, iPrOH or THF). For most of the experiments, the desired alcohol was obtained in significant amounts however always accompanied with several by-products, which complicated the purification. The best result was obtained at 60 °C in EtOH using conventional heating. To facilitate the purification, the alcohol was acetylated using acetyl chloride and triethylamine, which gave the 3-acetoxy chromone **28** in 49% yield over two steps.

3.2.2 Introduction of a 3-aminomethyl group in chroman-4-ones

The introduction of an aminomethyl group in the 3-position of chroman-4-ones can be achieved using various methods, e.g. via a combined Mannich/Michael reaction or a metal mediated Reformatsky type reaction. Both approaches have been tested and the results will be discussed below. Further studies on the use of the Mannich reaction will be discussed in Chapter 7.

3.2.2.1 The Mannich/Michael addition approach

Wallén et al. earlier reported the introduction of a Cbz-protected 3-aminomethyl group via a 3-methylenechroman-4-one intermediate using an efficient microwave assisted Mannich reaction followed by an *aza*-Michael addition.[113] We applied this method on derivatives **1** and **4** (Scheme 6). The initial Mannich reaction was run at 165 °C for 10 min in a microwave cavity and resulted in the formation of the 3-methylene substituted products **29** and **30**, respectively, together with approximately 40% of the starting materials according to ^{1}H NMR spectroscopy of the crude reaction mixtures.



Scheme 6. Reagents and conditions: (a) $Me_2NH{\times}HCl$, $(CH_2O)_n$, dioxane, 165 °C, 10 min, MW; (b) $CbzNH_2$, Tf_2NH, MeCN, rt, o.n.

Similar results were obtained after variation of the amounts of amine and aldehyde (0.3, 2, and 4 equiv), amine sources ($Me_2NH{\times}HCl$, morpholine, piperidine) and solvents (MeCN, CH_2Cl_2, dioxane, THF, EtOH). Instead of attempting to isolate the methylene derivatives **29** and **30**, the crude product mixtures were used directly to obtain the protected primary amine in the 3-position. The *aza*-Michael addition using Cbz-NH_2 in MeCN at room temperature overnight gave however only low yields (<30%) of the desired products. Neither variation of the amounts of $CbzNH_2$, addition of other amine nucleophiles (Me_3SiN_3 or NaN_3), choice of solvents (MeCN, $AcOH/H_2O$, CH_2Cl_2), addition of Lewis acids (Tf_2NH, $ZrOCl_2{\times}H_2O$ or NAFION SAC-13) nor different temperatures (60 °C, rt, -20 °C) did improve the yield of the amine-containing derivatives **29a** or **30a**.

Instead the focus was directed to the 3-bromo derivatives for the introduction of the aminomethyl group in the 3-position. The idea was to use the 3-brominated chroman-4-ones in a Reformatsky type reaction with SmI_2 and a suitable cyano-containing electrophile

(Figure 21).[124] Eventually the nitrile was to be reduced to the corresponding aminomethyl group.

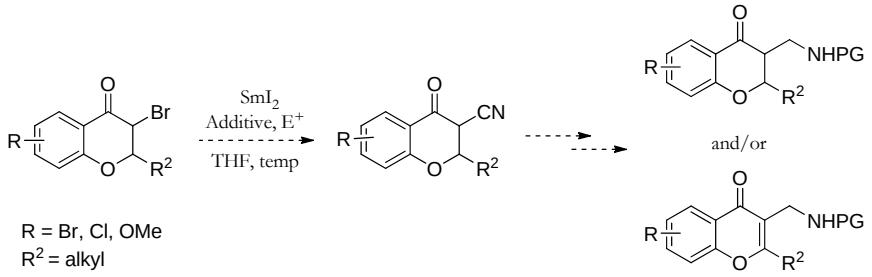
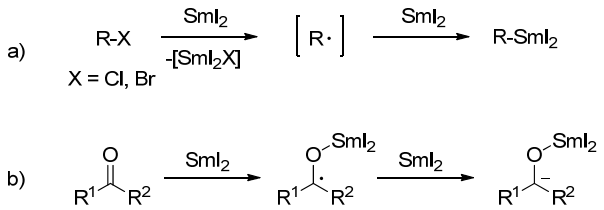


Figure 21. The synthetic strategy to introduce an aminomethyl group as the substituent in the 3-position of chroman-4-one using a SmI_2 mediated Reformatsky reaction. PG=protecting group

3.3.2.2 Sm(II) medited α-cyanation of 3-bromo-chroman-4-ones (Paper II)

Samarium (Sm) belongs to the lanthanides. The metal possesses a high oxidation potential (-1.41 V and -1.55 V in THF and water, respectively) and salt equivalents of Sm are common reagents in single electron transfer (SET) reactions.[125-128] Samarium diiodide (SmI_2) has become a useful samarium reagent in coupling reactions of alkyl halides and ketones (Barbier reactions)[129] and aromatic carbonyls (pinacol reactions),[130] reductions of ketones,[131] and nitro groups,[132] deoxygenations,[133] and in Reformatsky type reactions.[128, 134] The mechanism of SmI_2 promoted reactions with alkyl halides or carbonyl compounds is proposed to proceed in a two-step process (Scheme 7).[128]



Scheme 7. a) A reaction between SmI_2 and an alkyl halide occurs via stepwise one-electron transfer reactions and b) SmI_2 mediated activation of carbonyl compounds.

The reactivity of SmI_2 varies depending on choice of solvents (THF, tetrahydropyran (THP), acetonitrile, water, benzene), co-solvents (hexamethylphosphoramide (HMPA), *N*,*N*'-dimethylpropyleneurea (DMPU), *N*-methylpyrrolidone (NMP)), proton-donors (amines, alcohols, water, glycol), or addition of metal salts (e.g. LiCl, NiI_2).[127, 135] For the initial screening of the desired conditions, 3-bromo-chroman-4-one **17a** was used as the model compound. Tosyl cyanide (TsCN)[136] was selected as the electrophile and THF as the solvent. The experiments were run at room temperature or -78 °C and SmI_2 was used with or without the additives tetramethylguanidine (TMG), trimorpholinophosphortriamide (TMPA), or tripyrrolidinophosphortriamide (TPPA) (Figure 22). According to GC/MS analysis the reactions resulted in a mixture of the desired product **17b** accompanied by the

dehalogenated product **17**. Using bromide ($SmBr_2$) or hexamethyl disilazane ($Sm(HMDS)_2$) as the counter ions resulted mainly in the dehalogenated product **17**.

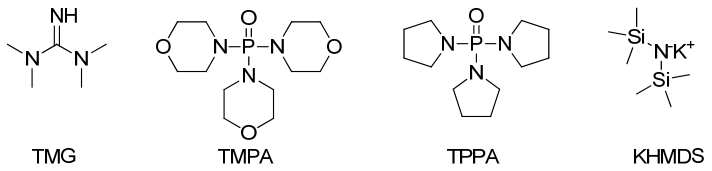
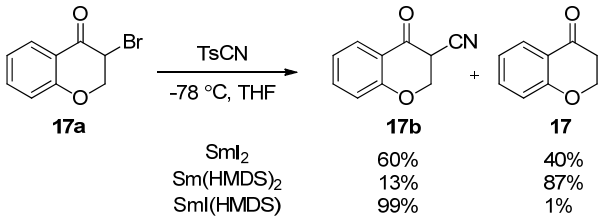


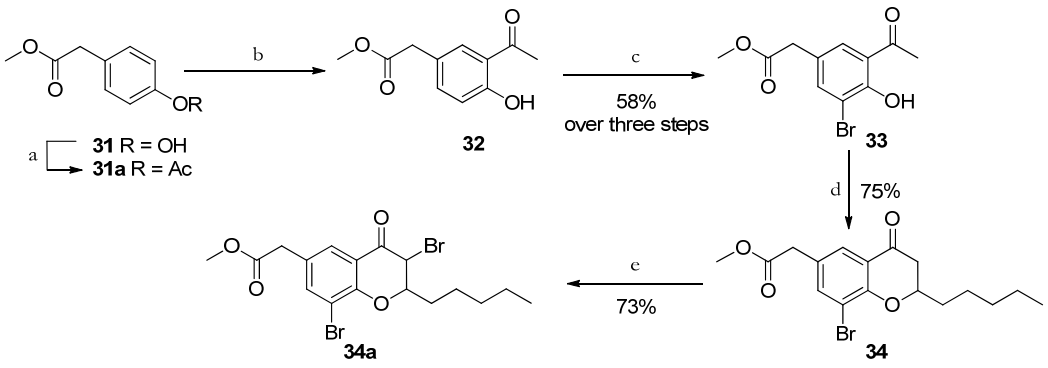
Figure 22. Structure of the additives TMG, TMPA, TPPA, and KHMDS used in the optimization of the SmI_2 mediated Reformatsky reaction.

Interestingly, when SmI(HMDS) was used instead, 3-cyanochroman-4-one **17b** was obtained in 99% yield (Scheme 8). The results indicate that the ligands on the samarium had a great impact on the competing dehalogenation reaction.



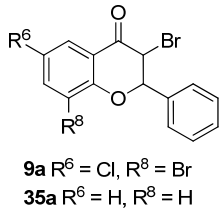
Scheme 8. The optimization procedure for the formation of the 3-cyanochroman-4-one **17b**. The counter ions had a crucial impact on the outcome on the 3-cyanation reaction. The highest selectivity was obtained using 1:1 equiv of SmI_2 and KHMDS, respectively. The yields were determined by GC/MS analysis after work up using dodecane as internal standard.

To expand the scope of the reaction a number of electrophiles such as benzoyl chloride, acetyl chloride, benzyl bromide, cyanic bromide, 1-chlorobutane, ethynyl *p*-tolyl sulfone, benzyl isocyanate, and benzaldehyde were evaluated. Unfortunately only the corresponding dehalogenated product, traces of products and/or recovered starting material could be detected. Also a selection of structurally diverse 3-bromochroman-4-ones was synthesized as substrates for the reaction. For example, it was desired to incorporate a hydrolysis labile group such as an ester to evaluate the scope of the method. It was therefore decided to synthesize the 3-bromo-chroman-4-one derivative **34a**. The product was synthesized in a five-step procedure including an acetylation, a Fries-rearrangement, and a bromination in the aromatic ring to form **33** (Scheme 9). After the cyclization to the chroman-4-one **34** the 3-bromination resulted in a *cis:trans* ratio of 77:23 of **34a** which is in agreement with previous bromination experiments (section 3.2.1).



Scheme 9. Formation of 2,3,6,8-tetrasubstituted derivative **34a** used as starting material in the SmI_2 mediated Reformatsky reaction. Reagents and conditions: (a) AcCl, TEA, CH_2Cl_2, rt, o.n.; (b) $AlCl_3$, DCE, 30 min, 170 °C, MW; (c) NBS, DMF, 0 °C→rt, 12 h; (d) Hexanal, DIPA, EtOH, 1 h, 170 ºC, MW; (e) $CuBr_2$, EtOAc/CH_2Cl_2, reflux, 2 h.

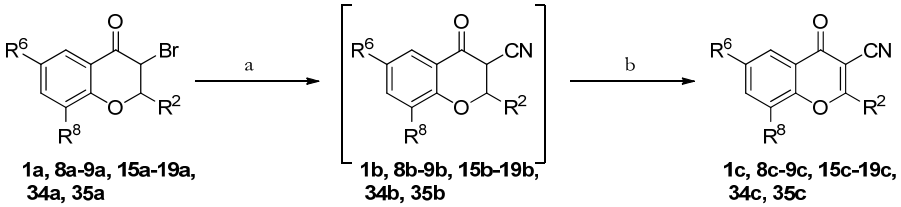
Moreover 2-aryl substituted derivatives were of great interest in the evaluation of the method. The flavanone **9a** and the 3-bromo substituted flavanone **35a**[113, 137] were included in the study.



Scheme 10. The flavones **9a** and **35a** used in the SmI_2 mediated Reformatsky reaction.

Using various 3-brominated derivatives the corresponding 3-cyanochroman-4-ones could be synthesized followed by an oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to the 3-cyanochromones (Table 4). Interestingly, in contrast to the corresponding brominated products where the *cis*-isomer was favored, the *trans*-isomers of 3-cyanochroman-4-ones **1b**, **8b**-**9b**, **15b**-**19b**, and **34b**-**35b** were the major isomers. The result is most likely due to sterical hindrance from the nitrile functionality that favor the *trans*-isomer over the *cis*-isomer. The mechanism for the initial 3-cyanation reaction is believed to proceed via a carbonyl activation as described in Scheme 7.

Table 4. The formation of 3-cyano substituted chromone derivatives.[a]

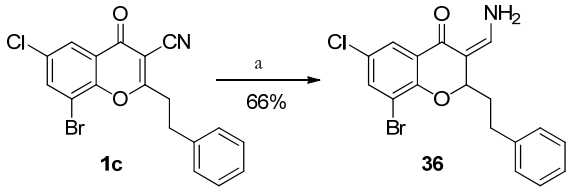


Substrate	R^2	R^6	R^8	cis:trans 3-bromo	cis:trans 3-cyano[b]	Product	Yield (%)[c]
17a	H	H	H	n/a	n/a	**17c**	49
18a	H	Cl	H	n/a	n/a	**18c**	77
19a	H	Me	H	n/a	n/a	**19c**	62
15a	$(CH_2)_4CH_3$	OMe	H	75:25	45:55	**15c**	42
16a	$(CH_2)_4CH_3$	H	H	78:22	18:82	**16c**	75
8a	C_6H_{11}	Cl	Br	99:1	25:75	**8c**	76
1a	CH_2CH_2Ph	Cl	Br	80:20	45:55	**1c**	59
34a	$(CH_2)_4CH_3$	CH_2COOMe	Br	77:23	32:68	**34c**	61
35a	Ph	H	H	65:35	25:75	**35c**	65
9a	Ph	Cl	Br	75:25	40:60	**9c**	61

[a]Reagents and conditions: (a) i) SmI_2, KHMDS, TsCN, THF, -78 °C, 2 h; (b) DDQ, dioxane, rt, 2 h. [b]The *cis:trans* ratio was obtained from ^{1}H NMR spectra. [c]Isolated yields over two steps.

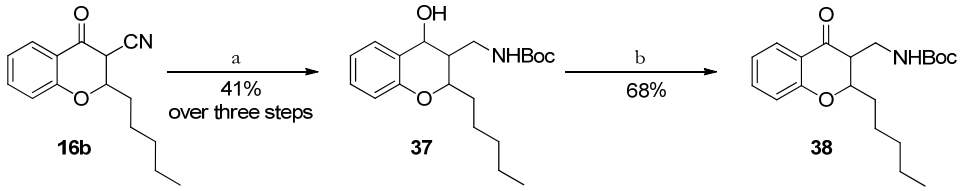
3.3.2.3 Reduction of 3-cyanochromone to afford 3-aminomethylchroman-4-one

The 3-cyanochromone **1c** was used as a model compound in the investigations of the reduction of the nitrile to the corresponding primary amine. Attempts using $NaBH_4/CoCl_2\times6H_2O$[138] or $BH_3\cdot SMe_2$[139] resulted in traces of enaminone **36** (Scheme 11) together with a mixture of unidentified products, whereas DIBAL-H[140] in THF at -78 °C gave **36** in 66% yield. Attempts to reduce the nitrile moiety in **1c** with $LiAlH_4$ at -78 °C only gave a selective reduction of the double bond to the saturated 3-cyanochroman-4-one **1b**. An attempt to hydrolyze the nitrile function in **1c** using conc. H_2SO_4 at 90 °C gave the corresponding amide together with a sulfonation in the *para*-position on the phenyl ring in the 2-position.



Scheme 11. Formation of enaminone **36**. Reagents and conditions: (a) DIBAL-H, CH_2Cl_2, -78 °C, 3 h.

3-Cyanochromone **17c** without any substituents in the 2-, 6- or 8-positions gave a mixture of unidentified products using DIBAL-H or $LiAlH_4$. Instead the 2-alkyl-3-cyanochroman-4-one **16b** was used as a model compound (Scheme 12). Catalytic hydrogenation of **16b** using an H-cube® apparatus charged with column-based Pd/C (10%) with EtOH as solvent, resulted in a selective reduction of the carbonyl group to the alcohol. A subsequent reduction of the nitrile group of the crude product with Ra/Ni in MeOH/THF followed by a Boc-protection of the primary amine afforded a diastereomeric mixture of **37** in 41% yield over three steps. Eventually an oxidation of the alcohol to the ketone using TPAP/NMO was made. This gave the desired 3-aminomethylated derivative **38** in 68% yield and a *cis:trans* ratio of 75:25 according to ^{1}H NMR spectroscopy.



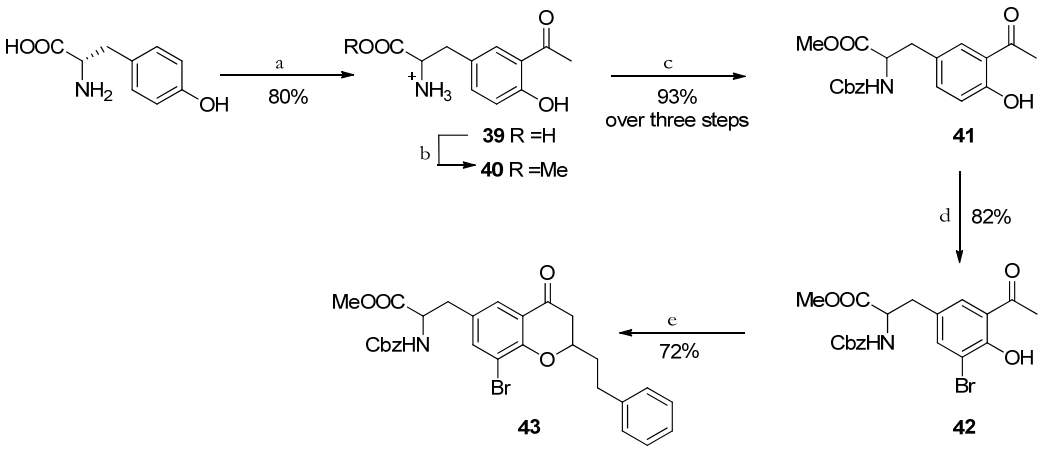
Scheme 12. Synthesis of compound **38**. Reagents and conditions: (a) i) H_2, 10% Pd/C, EtOH, rt, ii) H_2, Ra/Ni, MeOH/THF, rt, iii) Boc_2O, TEA, THF, rt, o.n.; (b) TPAP, NMO, CH_2Cl_2, MeCN, rt, 6 h.

3.4 Introduction of substituent in the 6-position of the chroman-4-one

So far, the chroman-4-one and/or chromone scaffolds have been functionalized with alkyl groups in the 2-position, an amine, a bromine, an acetoxy, and an aminomethyl group in the 3-position. We were also interested in the functionalization of the 6-position of the chroman-4-one.

3.4.1 Synthesis of chroman-4-one derivative useful as a building block in the synthesis of peptide analogs

In addition to derivative **34a** which possesses a methyl acetate moiety in the 6-position a tyrosine based model analog (**43**) was also synthesized (Scheme 13). The 2-phenethyl substituted chroman-4-one **43** was synthesized from L-tyrosine[141, 142] in an efficient five-step sequence involving acetylation/Fries rearrangement, esterification, amine protection, bromination and eventually an aldol condensation/cyclization. The chroman-4-one **43** was obtained as diastereomeric mixture in an overall yield of 44%. This amino acid derivative can be used for incorporation into a peptide sequence as such or can after appropriate substitutions e.g. act as a fluorescent label in peptides.[143]



Scheme 13. Synthesis of a tyrosine based chroman-4-one. Reagents and conditions: (a) AcCl, $AlCl_3$, 4-nitrobenzene, 100 °C, 7 h; b) $SOCl_2$, MeOH, -8 °C→rt; c) Benzyl chloroformate, Na_2CO_3, EtO_2/H_2O, rt, o.n.; d) NBS, MeCN, 0 °C→rt, o.n.; e) 3-Phenylpropanal, MeOH, 1 h, 170 °C, MW.

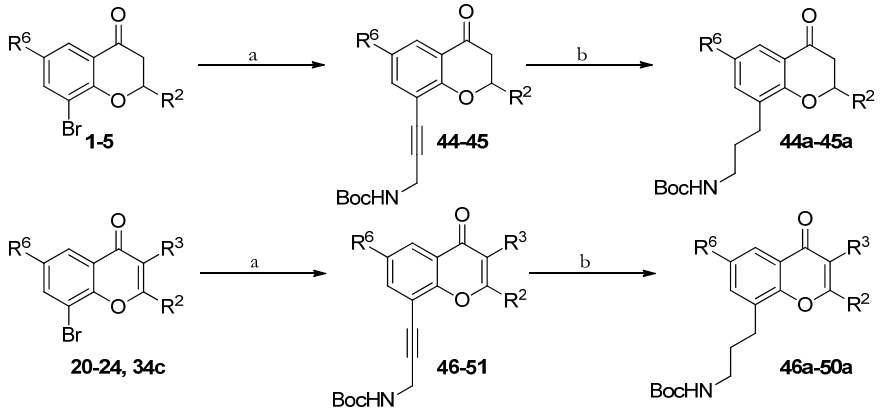
3.5 Introduction of substituents in 8-position of chroman-4-ones and chromones

In one subproject the incorporation of an alkyl group in the 8-position of the chroman-4-one and chromone scaffolds was considered to be of great interest. One strategy was to incorporate substituents in the 8-position using the Br-substituent in a Sonogashira reaction.[144, 145] A Sonogashira reaction is a coupling between aryl or alkenyl halides or triflates and terminal alkynes as illustrated in Figure 23.

Figure 23. Schematic overview of a Sonogashira reaction. The substrates comprise an aryl or vinyl halide or triflate and a terminal alkyne.

To introduce a Boc-protected propargylamine moiety in the 8-position the reaction was performed using *N*-Boc-progargylamine in the presence of CuI, $PdCl_2(PPh_3)_2$, TEA, and the chroman-4-ones **1**-**5** or chromones **20**-**24**, and **34c** in THF. The mixture was heated in a microwave cavity for 30 min at 120 ºC to obtain the desired products **44**-**51** in moderate to good yields (41-69%) (Table 5). Disappointingly, using chroman-4-ones **3**-**5** did not result in any product formation. A subsequent reduction using catalytic hydrogenation of the alkyne moiety to the alkane in the 8-position resulted in products **44a**-**50a**, respectively, in moderate to high yields (41-80%). The tetrasubstituted derivative **51** resulted in a reduction of the alkyne to the alkane and a reduction of the carbonyl to the corresponding alcohol in one step (for structures see Scheme 15).

Table 5. Formation of derivatives **44a**-**50a** after a Sonogashira reaction and a subsequent catalytic hydrogenation.[a]



Substrate	R^2	R^3	R^6	Product alkyne	Yield (%)[b]	Product alkane	Yield (%)[b]
1	CH_2CH_2Ph	H,H	Cl	**44**	50	**44a**	80
2	CH_2CH_2(1-naphthyl)	H,H	Cl	**45**	62	**45a**	56
3	CH_2CH_2(3-indolyl)	H,H	Cl		n.r.[c]		
4	CH_2CH_2(*N*-Bn)-3-indolyl	H,H	Cl		n.r.[c]		
5	CH_2CH_2(*N*-Ts)-3-indolyl	H,H	Cl		n.r.[c]		
20	CH_2CH_2Ph	NH_2	Cl	**46**	59	**46a**	54
22	CH_2CH_2Ph	NHAc	Cl	**47**	63	**47a**	60[d]
23	CH_2CH_2Ph	H	Cl	**48**	69	**48a**	80
21	CH_2CH_2(1-naphthyl)	NH_2	Cl	**49**	41	**49a**	41
24	CH_2CH_2(1-naphthyl)	H	Cl	**50**	61	**50a**	71
34c	$(CH_2)_5CH_3$	CN	CH_2COOMe	**51**	48	**51a**	75[e]

[a]Reagents and conditions: (a) *N*-Boc-progargylamine (4.0 equiv), CuI (0.1 equiv), $PdCl_2(PPh_3)_2$ (0.1 equiv), TEA (10 equiv), THF, 30 min, 120 ºC, MW; (b) H_2, 10% Pd/C, MeOH, rt, 2-4 h. [b]Isolated yields. [c]No reaction. [d]Yields obtained from ^{1}H NMR spectra on the crude reaction mixture. [e]The carbonyl was also reduced under the catalytic hydrogenation. Yield was obtained from ^{1}H NMR spectra on the crude reaction mixture (the structure of **51a** is shown in Scheme 15).

4. Substituted chroman-4-ones and chromones as β-turn peptidomimetics

The 2,3,6,8-tetrasubstituted chromone system can adopt a conformation that is similar to that of a β-turn of a peptide (Figure 24). By using somatostatin as a model peptide the objective of this study was to develop β-turn mimetics using chroman-4-ones and chromones substituted with amino acid side chain equivalents.

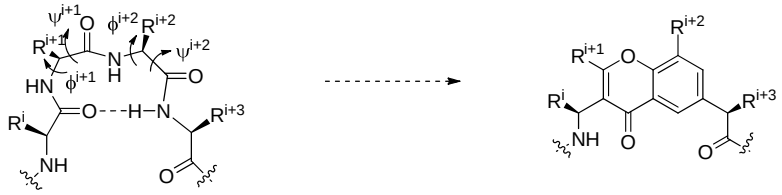


Figure 24. A 2,3,6,8-tetrasubstituted chromone scaffold as a potential β-turn mimetic.

4.1 Design of substituted chroman-4-one and chromone derivatives as peptidomimetics of somatostatin (Paper III)

The β-turn of somatostatin is composed of the amino acids Phe7, Trp8, Lys9, and Thr10 (Figure 25). By using somatostatin as the model peptide the idea was to introduce substituents in the 2- and 8-positions on the chroman-4-one/chromone resembling the crucial tryptophan and lysine side chains. Glycine moieties representing *N*- and *C*-terminals (Figure 25) were positioned as 3- and 6-substituents. They could for example be used as handles for incorporation of the substituted scaffold into a peptide chain.

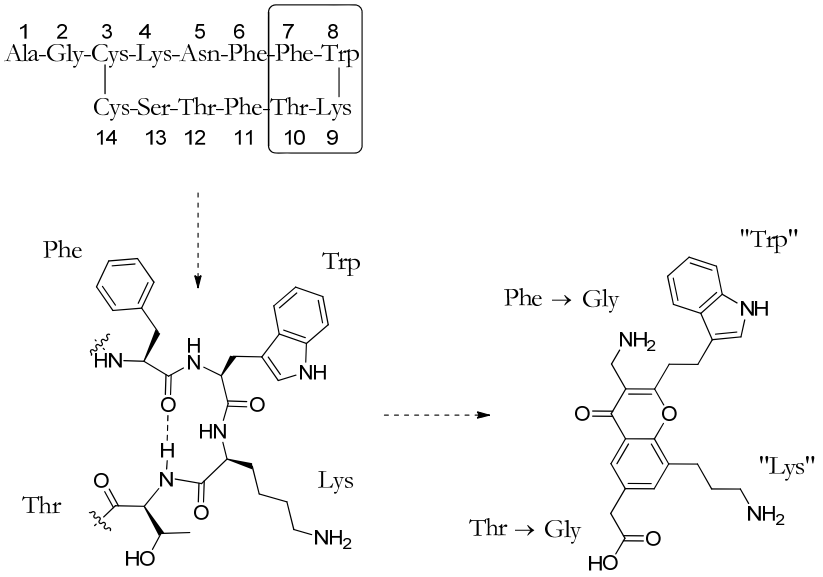


Figure 25. The β-turn in somatostatin is composed of Phe7, Trp8, Lys9, and Thr10. Phe7 and Thr10 are replaced by glycine residues representing *N*- and *C*-terminals in the 3- and 6-positions of the chromone scaffold. The side chains of Trp8 and Lys9 residues are somewhat modified when introduced in the 2- and 8-positions, respectively, of the chromone scaffold.

In order to confirm the assumptions that substituted chroman-4-ones and chromones could mimic a β-turn of somatostatin computational studies were performed. Five different β-turn structures (I, I´, II, II´ and VIII)[10] comprising the Phe7-Trp8-Lys9-Thr10 sequence of somatostatin were selected for modeling studies in order to investigate if any of these were similar to the chroman-4-one and chromone scaffolds.

For structure simplification the Trp8 and Lys9 side chains in the *i*+1 and *i*+2 positions in all turns were replaced by methyl groups (Figure 26a) for structural simplification. Further, Phe7 and Thr10 in the *i*+2 and *i*+3 positions were replaced by glycine residues to give the sequence Ac-Gly-Ala-Ala-Gly-NHMe. The chroman-4-one and chromone scaffolds were simplified in the same way having methyl groups in the 2- and 8-positions, an acetylated *N*-terminus in the 3-position and a methylamidated *C*-terminus in the 6-position (Figure 26b).

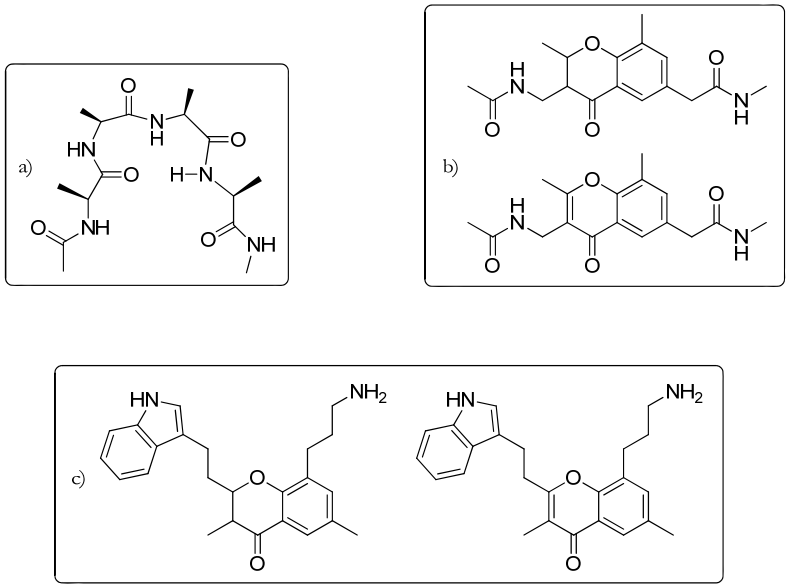
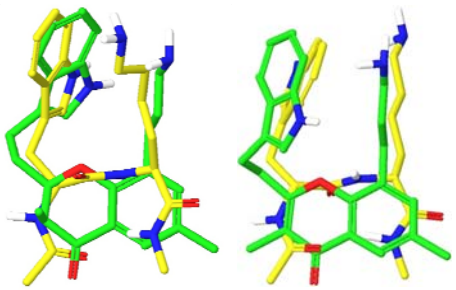


Figure 26. a) The simplified β-turn used in the computational calculations; b) Substituted and simplified chroman-4-one and chromone derivatives used in the initial molecular mechanics calculations; b) Chroman-4-one and chromone derivatives substituted with Lys and Trp side chains in the 2- and 8-positions, respectively, used in the final molecular mechanics calculations.

Molecular mechanics calculations were used for energy minimization of the selected β-turn structures using the OPLS2005 force field as implemented in the MacroModel program v.9.7.[121] Conformational constraints were introduced to keep the desired peptide turn structure during the energy minimization procedure. The energy minimized conformations were manually superimposed with different low energy conformations identified in conformational analyses of the 2,3,6,8-tetrasubstituted chromone and the four different stereoisomers of the chroman-4-one scaffolds, respectively. Of the energy minimized tetrapeptide structures the type II and II´ β-turns gave good alignments with the global minimum conformations of the chroman-4-one and the chromone scaffolds (results not shown).

In order to investigate the preferred conformations of the crucial Trp8 and Lys9 amino acid side chains in the *i*+1 and *i*+2 positions of the tetrapeptide, conformational analyses were performed on the type II and II´ β-turns. Further, conformational analyses were also performed on the different stereoisomers of the 2,3,6,8-tetrasubstituted chroman-4-one scaffold with methyl groups in the 3- and 6-positions, a 2-(3-indolyl)ethyl group in the 2-position and a 3-aminopropyl moiety in the 8-position (Figure 26c).



(a) (b)

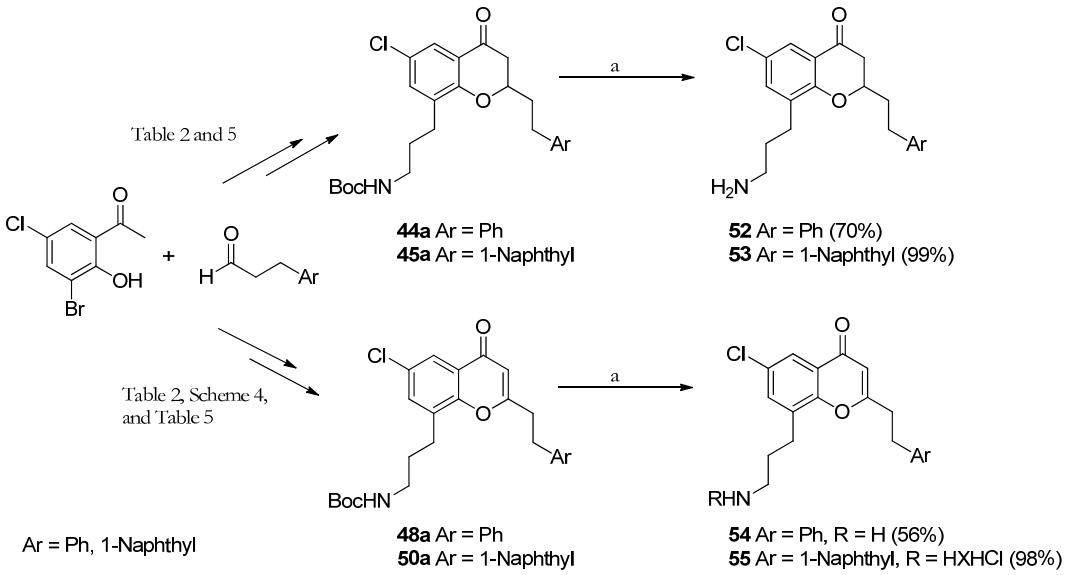
Figure 27. (a) Alignment of one conformation (ΔE= 12.2 kJ/mol) of the 2*R*,3*S* stereoisomer of the 2,3,6,8-tetrasubstituted chroman-4-one (green) with a low energy conformation (ΔE= 5.1 kJ/mol) of the type II β-turn (yellow); (b) Alignment of a low energy conformation (ΔE = 6.2 kJ/mol) of a 2,3,6,8-tetrasubstituted chromone derivative (green) and the global minimum conformation of the type II´ β-turn (yellow).

Conformations with relative energies above 21 kJ/mol were discarded. Interestingly, all conformations of the 2*S*,3*S*- and 2*R*,3*R* disubstituted chroman-4-ones that had ΔE < 7.8 kJ/mol preferentially adopted a diaxial relationship between the 2- and 3-substituents. A conformational analysis with dihedral constraints was performed on the type II and type II´ β-turns of Ac-Gly-Trp-Lys-Gly-NHMet. Different low energy conformations of these β-turns and the four different stereoisomers of the chroman-4-one scaffold were manually superimposed and gave good alignments with no significant difference between the stereoisomers. Figure 27a shows a selected alignment of a low energy conformation of the 2*R*,3*S*-isomer of the chroman-4-one and a low energy conformation of the type II β-turn. The alignment of the global minimum conformation of the II´ β-turn and a low energy conformation of the more rigid 2,8-disubstituted chromone ring (ΔE = 6.2 kJ/mol) is shown in Figure 27b.

Thus, molecular mechanic calculations on the chroman-4-one and chromone scaffolds show that they mimic type II and type II´ β-turn structures, respectively. The same types of β-turns have been identified in previous studies of other bicyclic systems used as potential β-turn mimetics of somatostatin.[23] These results prompted us to synthesize chroman-4-one and chromone derivatives and test them for affinity at the somatostatin receptors sst2 and sst4. Studies of the binding mode and the π-π-interactions between somatostatin and its receptor using molecular modeling have shown that it is feasible to replace the indole moiety in Trp8 with either a phenyl or a naphthyl group without any decrease in activity.[146] To simplify the synthesis the 2-(3-indolyl)ethyl moiety was therefore replaced by phenethyl or 2-(1-naphthyl)ethyl groups in the 2-position of the chroman-4-one and the chromone scaffolds.

4.2 Synthesis of substituted chroman-4-ones 52-55

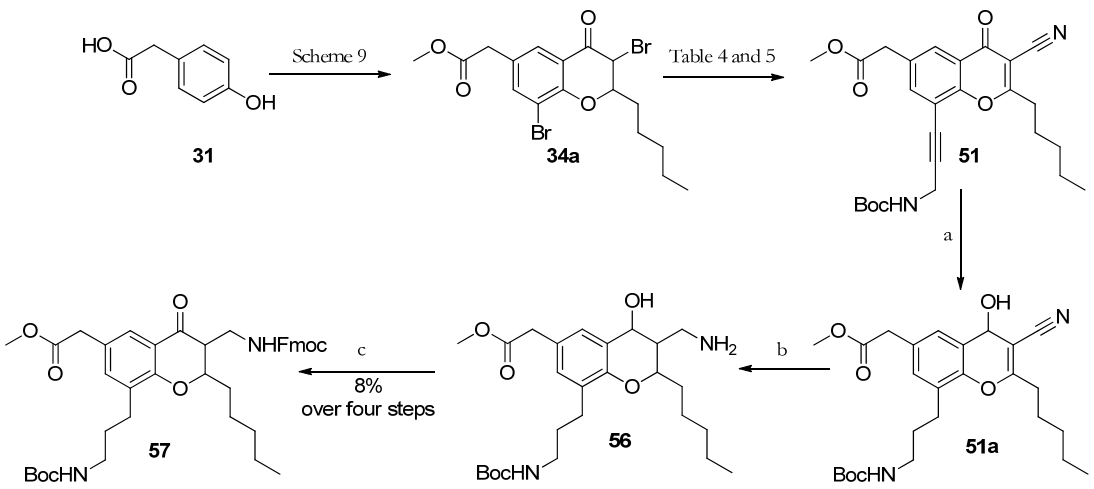
The chroman-4-ones **44a**-**45a** and chromones **48a** and **50a** previously synthesized (Table 5) were selected for testing as potential β-turn mimetics of somatostatin. A Boc-deprotection using HCl in MeOH afforded the unprotected alkylamine derivatives **52**-**55** (Scheme 14). The biological evaluation is described in section 4.3.



Scheme 14. Synthesis of the potential somatostatin β-turn mimetics **52**-**55**. Reagents and conditions: (a) 3M HCl in MeOH, rt, o.n.

4.2.1 Synthesis of building block 57

In order for the developed β-turn mimetic scaffold to be useful as a building block in peptide synthesis, the 2,3,6,8-tetrasubstituted chroman-4-one **57** was synthesized as a model compound (Scheme 15). A catalytic hydrogenation (Pd/C) of **51** led to a reduction of both the alkyne moiety and the carbonyl group (**51a**). The crude mixture was directly used in the next step when the nitrile functionality in the 3-position was reduced with Ra/Ni under a H_2 atmosphere to give **56**. Due to solubility problems the primary amine was Fmoc-protected prior the oxidation of the secondary alcohol to afford **57** as a mixture of diastereomers. The derivative contains a Fmoc-protected aminomethyl group in the 3-position and a methyl acetyl moiety in the 6-position that could be used as handles in the synthesis of modified peptides. The derivative corresponding to a building block useful for the synthesis of somatostatin based pseudopeptides has not yet been synthesized.



Scheme 15. Formation of tetrasubstituted chroman-4-one **57**. Reagents and conditions: (a) H_2, 10% Pd/C, EtOH, rt; (b) H_2, Ra/Ni, MeOH/THF, rt; (c) i) Fmoc-ONSu, $NaHCO_3$, dioxane/water, rt, o.n., ii) TPAP, NMO, CH_2Cl_2, MeCN, rt, 6 h.

4.3 Biological evaluation of compounds 53 and 55 as mimetics of somatostatin

Derivatives **53** and **55** were selected and sent to Euroscreen[147] for testing of their affinities for human sst2 and sst4 receptors using a radioligand binding assay with sst28 (a natural agonist) as a reference.[148] Interestingly, **53** and **55** showed similar affinities for the two receptors (Table 6). The activity of the derivatives was also comparable to that of other non-peptidic somatostatin β-turn mimetics (Figure 7).

Table 6. Affinities of **53** and **55** at the human sst2 and sst4 receptors.[a]

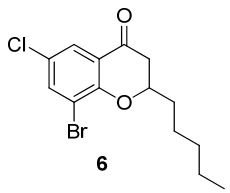
ligand	K_i, sst2	K_i, sst4
sst28	0.030 nM	1.34 nM
53	6.85 µM	7.09 µM
55	2.66 µM	1.17 µM

[a] ^{125}I-Tyr11-SRIF was used as the radiolabeled ligand.

5. Substituted chroman-4-ones and chromones as Sirt2 inhibitors

5.1 Evaluation of compound 6 as a lead for novel Sirt2 inhibitors (Paper IV)

In an initial study, a set of compounds based on the chroman-4-one and chromone scaffolds were tested against human Sirt2 to see if these privileged structures could serve as scaffolds for sirtuin modulators (data not shown). Interestingly, 8-bromo-6-chloro-2-pentylchroman-4-one **6** (Figure 28) showed excellent inhibition (88%) of Sirt2 at 200 μM concentration in a fluorescence-based assay.[149] A more detailed determination of the potency gave an IC_{50} value of 4.5 μM. Compound **6** was also tested against Sirt1 and Sirt3 at 200 μM concentration resulting in less than 10% inhibition of these sirtuin subtypes. Initial experiments to investigate whether **6** was substrate competitive showed that the chroman-4-one derivative acts via non-competitive binding with the substrate (the corresponding NAD^+ competitive experiments are ongoing).



6

Figure 28. The 2-alkyl substituted chroman-4-one **6** acts as a selective Sirt2 inhibitor with 88% inhibition at 200 μM and an IC_{50} value of 4.5 μM.

In collaboration with a research group at the University of Eastern Finland in Kuopio, the Sirt2 inhibition was verified with two different methods. First, a western blot analysis of the Sirt2-mediated deacetylation of acetylated α-tubulin was carried out and inhibition of the Sirt2 catalyzed reaction by **6** was observed (Figure 29A). Secondly, a Sirt2 activity assay based on the release of radioactive ^{14}C-nicotinamide was performed in the presence of an acetylated peptidic substrate (RSTGGK(Ac)APRKQ) without a fluorophore (Figure 29B). In this assay **6** gave 66% inhibition. Taken together, **6** was able to inhibit the deacetylation of three different substrates; an artificial substrate with a fluorophore, and a peptide and a protein substrate without a fluorophore. Based on these results a series of analogs of **6** was synthesized and evaluated as Sirt2 inhibitors.

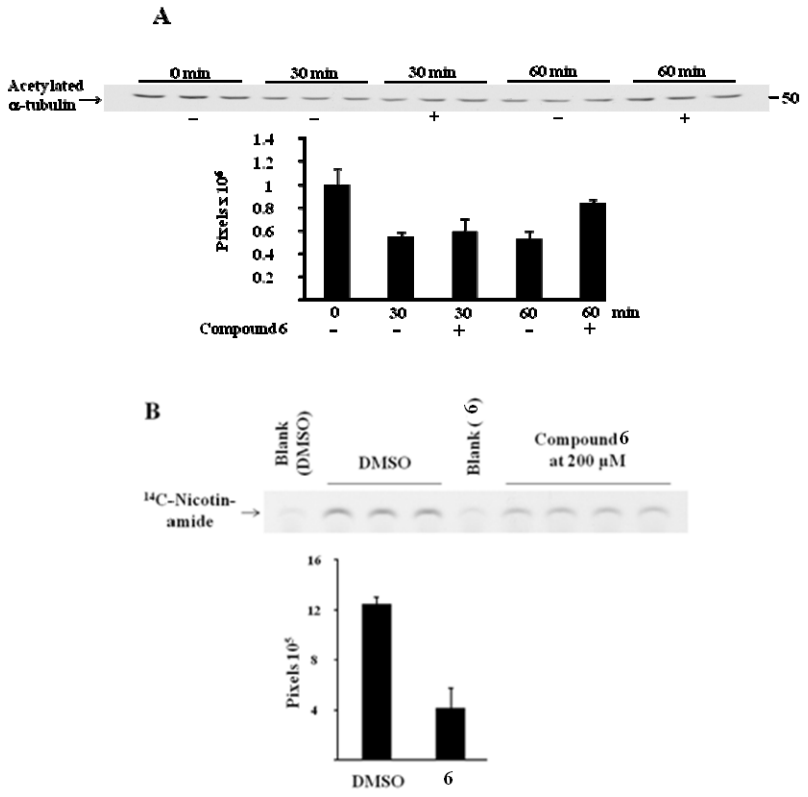
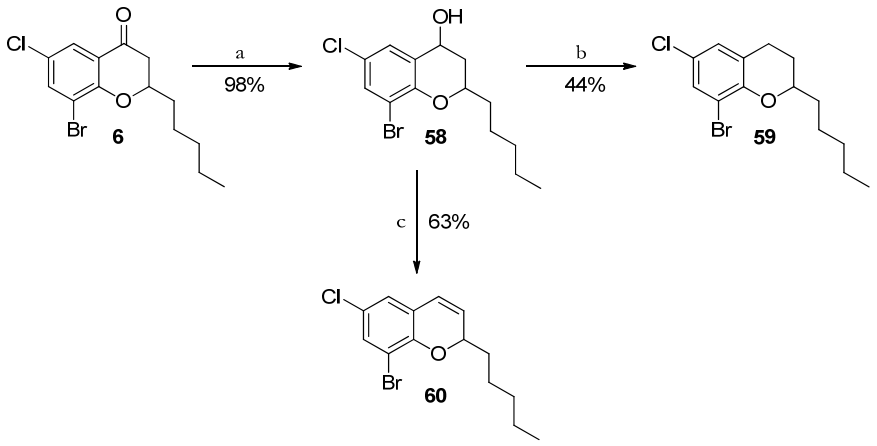


Figure 29. Inhibition of Sirt2 mediated deacetylation reactions by compound **6**. (A) Western blot analysis of the inhibition of Sirt2 mediated α-tubulin deacetylation by **6**. The concentration of **6** was 200 μM, measurements were done at 30 min and 1 hour. (B) Inhibition by **6** of Sirt2 mediated deacetylation of the acetylated peptide RSTGGK(Ac)APRKQ. The reaction was detected by formation of the reaction product ^{14}C-nicotinamide.

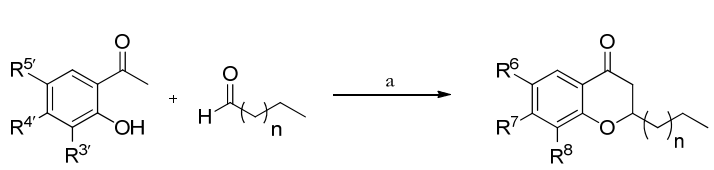
5.1.1 Synthesis of potential Sirt2 inhibitors based on 6

An initial structure activity relationship (SAR) study based on **6** was performed. The alcohol **58**, the chroman derivative **59** and the chromen **60** were synthesized (Scheme 16) to investigate if the carbonyl group in the chroman-4-one was necessary for Sirt2 inhibition.



Scheme 16. Formation of alcohol derivative **58**, chroman **59**, and chromen **60**, respectively. Reagents and conditions: (a) $NaBH_4$, MeOH/THF, 0 °C→rt, 15 min; (b) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, -78 °C→rt, 19 h; (c) *p*-TSA (cat.), $MgSO_4$, toluene, 90 °C, 1.5 h.

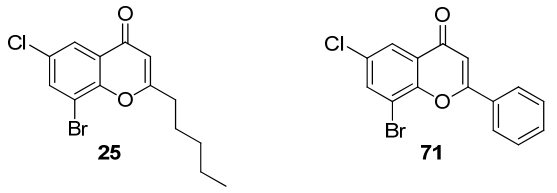
Thereafter, to reveal whether the inhibitory effect of **6** is caused by steric or electrostatic properties of the substituents, various substitution patterns in the aromatic ring system of 2-pentylchroman-4-ones (**61**-**68**) were investigated (Scheme 17). In addition, the alkyl length of the 2-substituents was modified to three (**69**) and seven carbons (**70**). As previously described (section 3.1) the synthesis was performed by reacting the appropriate acetophenone with an aldehyde in a base promoted aldol condensation using DIPA as a base in EtOH under microwave conditions. This resulted in low to good yields (17-71%) of products.



61 n = 3, $R^6 = R^8$ = Br, R^7 = H (56%)
62 n = 3, $R^6 = R^8 = CH_3$, R^7 = H (17%)
63 n = 3, $R^6 = R^8$ = F, R^7 = H (23%)
64 n = 3, R^6 = Cl, $R^7 = R^8$ = H (51%)
65 n = 3, $R^6 = NO_2$, $R^7 = R^8$ = H (58%)
66 n = 3, $R^6 = OCH_3$, $R^7 = R^8$ = H (17%)
67 n = 3, $R^6 = R^7$ = H, R^8 = Br (42%)
68 n = 3, $R^6 = R^8$ = H, R^7 = F (20%)
69 n = 1, R^6 = Cl, R^7 = H, R^8 = Br (71%)
70 n = 5, R^6 = Cl, R^7 = H, R^8 = Br (56%)

Scheme 17. Formation of chroman-4-ones **61**-**70**. Reagents and conditions: (a) Appropriate aldehyde, DIPA, EtOH, 160-170 °C, 1 h, MW.

Also the 2-alkyl substituted chromone **25** and flavone **71**[150] (Scheme 18) were selected for testing for their inhibitory activity of Sirt2.



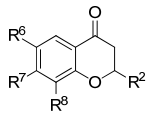
Scheme 18. The chromones **25** and **71** selected for testing of the inhibitory activity towards Sirt2.

5.2 Biological evaluation of chroman-4-one and chromone based Sirt2 inhibitors

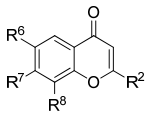
Table 7 shows a summary of the results of the synthesized chroman-4-one derivatives when tested for their inhibitory activity at Sirt2 in the Fluor-de-Lys assay.[79, 149] The assay is based on a combination of a fluorescently labeled p53 derived substrate containing an acetylated lysine residue (Gln-Pro-Lys- Lys(Ac)) and a developer (trypsin) and involves two main steps: first the Sirt2 substrate is incubated with Sirt2 together with NAD^+ and inhibitor. Deacetylation of the substrate sensitizes the substrate so that in the second step, treatment with trypsin produces a fluorophore. The readout is a fluorescence signal (460 nm) which is proportional to the amount of deacetylated substrate produced by Sirt2 action.

The IC_{50} values were measured on derivatives with >70% inhibitory effect at 200 μM. The unsaturated analog of **6**, chromone **25**, was insignificantly less active than **6** with an IC_{50} value of 5.5 μM. Interestingly, the difluorinated **63** with smaller but more electronegative substituents was considerably less active than the other dihalogenated derivatives (**6** and **61**) but more potent than the unsubstituted **16**. This suggests that electron-withdrawing groups in general enhance activity but that electrostatic properties are not exclusively responsible for strong inhibition. Replacement of the halogens with methyl groups (**62**) caused a slight decrease in activity compared to the chloro- and bromo-substituted **6** but a clear increase in activity compared to the difluorinated **63**. This supports the previous finding that larger substituents in the 6- and 8-positions are necessary to achieve significant inhibition. Derivatives **58**-**60** with no carbonyl present show low inhibition (31-38% at 200 μM), which indicates a need of a hydrogen acceptor group in that position.

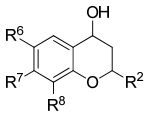
Table 7. The in vitro activity of chroman-4-one/chromone based derivatives against Sirt2.



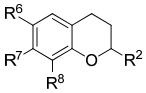
1, 3, 5-7, 16 , 61-70



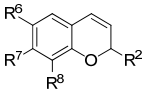
25, 71



58



59



60

Cmpd	R^2	R^6	R^7	R^8	Inhib. 200 μM (%)[a]	IC_{50} (μM)[b,c]
6	$(CH_2)_4CH_3$	Cl	H	Br	88 ± 0.9	4.3 (3.5-5.4)
16	$(CH_2)_4CH_3$	H	H	H	4.9 ± 4.8	n.d.
61	$(CH_2)_4CH_3$	Br	H	Br	92 ± 1.2	1.5 (1.3-1.7)
62	$(CH_2)_4CH_3$	CH_3	H	CH_3	83 ± 0.7	6.2 (4.7-8.1)
63	$(CH_2)_4CH_3$	F	H	F	30 ± 1.3	n.d.
64	$(CH_2)_4CH_3$	Cl	H	H	55 ± 2.4	n.d.
65	$(CH_2)_4CH_3$	NO_2	H	H	58 ± 0.7	n.d.
66	$(CH_2)_4CH_3$	OCH_3	H	H	20 ± 4.1	n.d.
67	$(CH_2)_4CH_3$	H	H	Br	28 ± 1.1	n.d.
68	$(CH_2)_4CH_3$	H	F	H	18 ± 1.0	n.d.
69	$(CH_2)_2CH_3$	Cl	H	Br	76 ± 1.8	10.6 (9.0-12.5)
70	$(CH_2)_6CH_3$	Cl	H	Br	57 ± 2.5	n.d.
1	CH_2CH_2Ph	Cl	H	Br	81 ± 0.7	6.8 (5.8-8.0)
7	$CH(CH_3)_2$	Cl	H	Br	52 ± 1.0	n.d.
3	CH_2CH_2(3-indolyl)	Cl	H	Br	53 ± 1.7	n.d.
5	CH_2CH_2(*N*-Ts)(3-indolyl)	Cl	H	Br	27 ± 1.6	n.d.
25	$(CH_2)_4CH_3$	Cl	H	Br	82 ± 0.4	5.5 (4.8-6.2)
71	Ph	Cl	H	Br	20 ± 1.4	n.d.
58	$(CH_2)_4CH_3$	Cl	H	Br	31 ± 3.0	n.d.
59	$(CH_2)_4CH_3$	Cl	H	Br	38 ± 1.3	n.d.
60	$(CH_2)_4CH_3$	Cl	H	Br	38 ± 1.2	n.d.

[a]SD, standard deviation (n = 3). [b]IC_{50} (95% confidence interval). IC_{50} values were determined for compounds showing >70% inhibition of Sirt2 at 200 μM concentration. [c]n.d. = not determined.

To probe the importance of the 6- and 8-substituents for inhibitory potency, derivatives lacking one of these groups were synthesized (**64**-**67**). Compound **64** that only contains the 6-chloro substituent showed a decrease in activity (55% at 200 μM). No change in activity was observed when the 6-chloro substituent was replaced with an electron-withdrawing nitro group **65** (58% at 200 μM). Interestingly, with an electron-donating methoxy group in the same position (**66**) the inhibitory activity decreased to 20% at 200 μM. This particular example shows that the activity can be altered by the electronic nature of the substituent. Compound **67** lacking a substituent in the 6-position was significantly less potent than the lead compound **6**. Thus, the substituent in the 6-position is more important for activity than that in the 8-position.

It was also clarified that electron-rich chroman-4-ones generally are less potent inhibitors than electron-poor compounds. The unsubstituted 2-pentylchroman-4-one **16** lost all inhibitory activity, indicating that substituents in the aromatic system are necessary to achieve any inhibition**.** One example of substitution in the 7-position was explored with the fluorinated **68** which showed only weak inhibitory activity (18% at 200 μM). In summary, among the investigated modifications it was found that an alkyl chain with three to five carbons in the 2-position, larger, electron-withdrawing groups in the 6- and 8-positions and the carbonyl group intact were crucial for high potency. The summary of the SAR is illustrated in Figure 30.

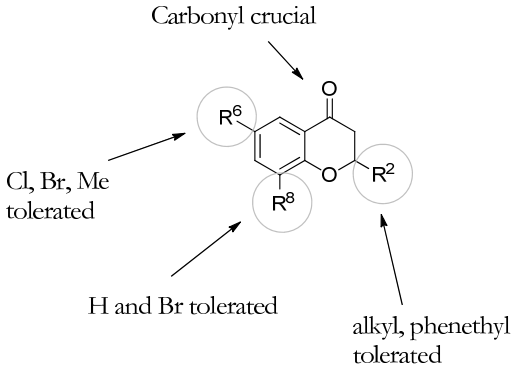


Figure 30. Results of the SAR study of chroman-4-one based Sirt2 inhibitors.

5.3 Determination of the absolute configuration of the enantiomers of 6

The enantiomers of **6** were separated by preparative chiral HPLC. The absolute configuration was intended to be determined by means of X-ray crystallography. Unfortunately, all attempts to obtain useful crystals of the enantiomers failed. A valid alternative for the determination of the absolute configuration of small molecules is to compare experimental and calculated vibrational circular dichroism (VCD) spectra.[151] To predict the VCD spectra of low energy conformers of a molecule, density functional theory

(DFT) calculations can be used. Highly flexible groups in a molecule lead to many conformers which have to be considered in the calculation of the VCD data. Therefore, to facilitate the configurational determination, the calculations were done on a slightly truncated structure (2-ethylchroman-4-one instead of 2-pentyl, **Figure 31**) as the change in the alkyl group is not expected to alter the VCD spectra to any greater extent.[152] The experimental spectra of the two enantiomers of **6** were compared with the calculated spectra of the *R*- and the *S*-enantiomers of the modified structure.

Figure 31. Structures used for the DFT calculations of VCD spectra to determine the absolute configuration of the enantiomers of 6. To simplify the *ab initio* calculations the 2-pentyl group in 6 was truncated to an ethyl group.

As can be seen in the VCD spectra (Figure 32) several bands in the frequency region from 1500 to 1100 cm^{-1} show good alignment between the experimental spectrum of (-)**-6** and the calculated spectrum of the *S*-enantiomer of the 2-ethyl analog. Equally good alignment in the same region is obtained when comparing the experimental spectrum of (+)**-6** and the calculated spectrum of the *R*-enantiomer. Thus, from this study it can be concluded that (-)**-6** most likely has the *S*-configuration and (+)**-6** the *R*-configuration.

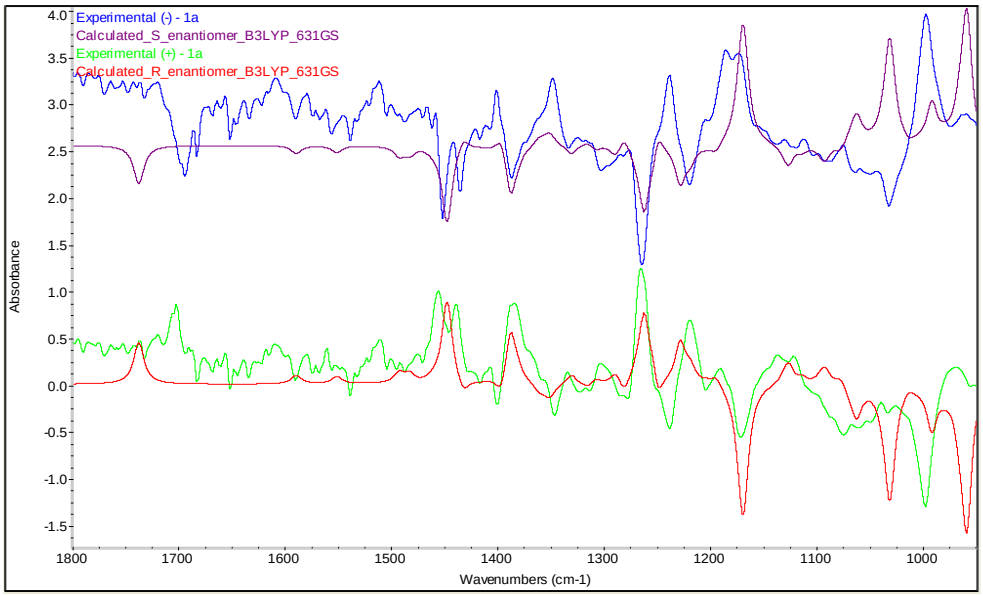


Figure 32. Comparison of the experimental VCD spectra of (-)-6 (blue) and (+)-6 (green) with the calculated spectra of the *S*-enantiomer (purple) and the *R*-enantiomer (red) of 8-bromo-6-chloro-2-ethylchroman-4-one, respectively.

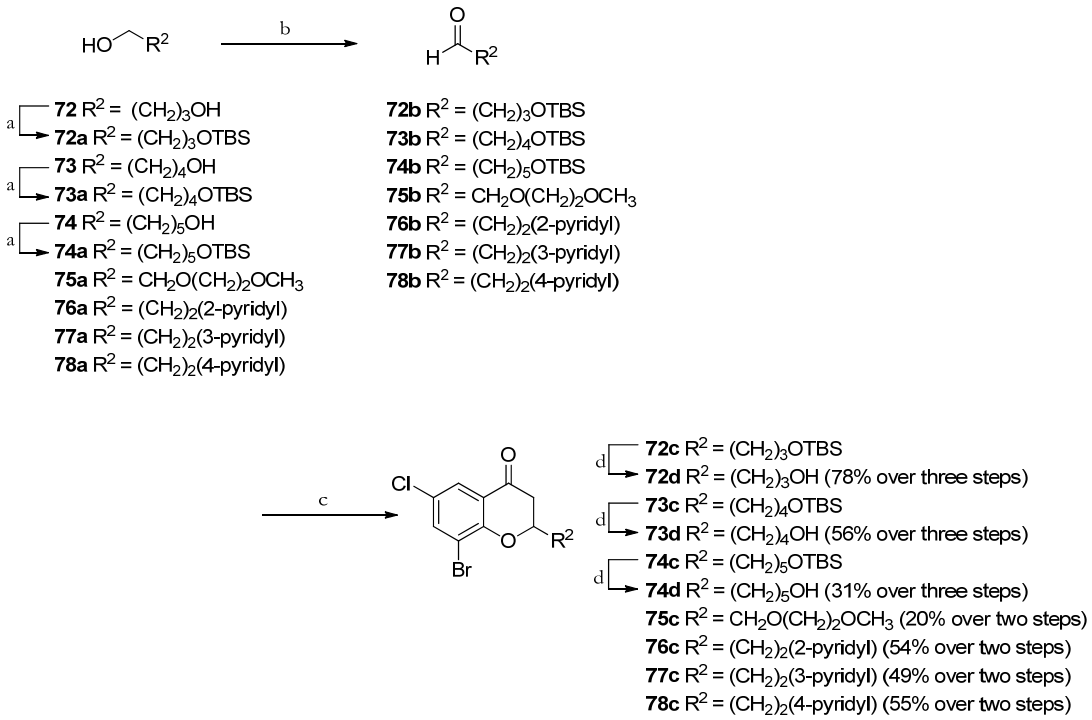
The individual enantiomers of the lead compound **6** were tested. It turned out that the enantiomers had only slightly different inhibitory activities. The Sirt2 inhibition for the (-)-**6**, and (+)-**6** enantiomers showed 91±0.8%, and 70±0.8% inhibition of Sirt2, respectively. The (-)-**6** enantiomer was a slightly more potent inhibitor with an IC_{50} value of 1.5 μM (1.3±1.7) compared to (+)-**6** with an IC_{50} of 4.5 μM (3.5±5.9).

5.4. Synthesis of chroman-4-one based Sirt2 inhibitors with more hydrophilic substituents in the 2-position (Paper V)

The previous findings that 2-alkyl substituted chroman-4-ones could act as selective Sirt2 inhibitors were very interesting, there was however a need to increase the solubility of the derivatives prior testing on cells. The intention was to incorporate heterofunctional groups such as amines, amides, alcohols, acids or esters in the structures. The substituent in the 2-position was selected for the desired modifications.

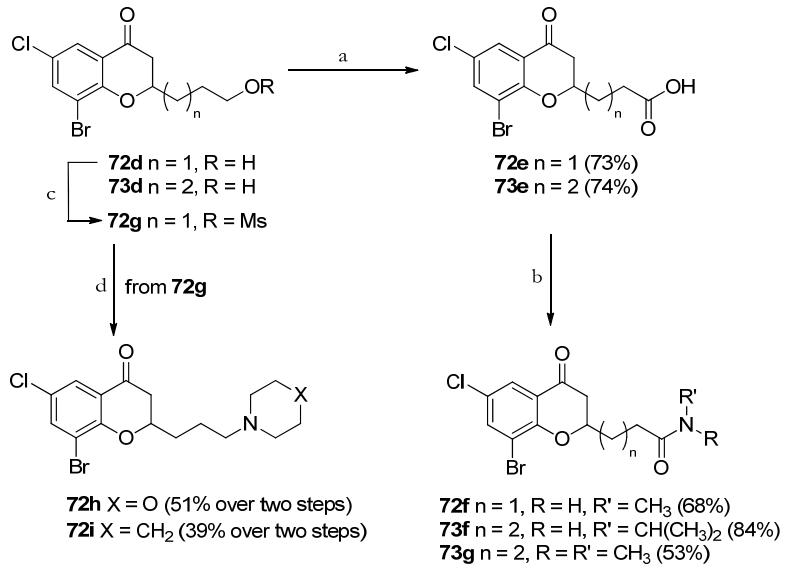
The initial aim was to synthesize 2-hydroxyalkyl substituted chroman-4-ones with different chain lengths (Scheme 19). The synthetic strategy was to construct the chroman-4-one framework using the base-mediated method described in section 3.1. The synthetic pathway to the appropriate aldehydes started with a mono-protection of the diols **72**-**74** using NaH and tri-butylsilyl chloride (TBSCl) (Scheme 19) according to a procedure reported by McDougal et al. which gave **72a**-**74a**.[153] A subsequent Swern oxidation gave the corresponding aldehydes **72b**-**74b**. Swern oxidations were also performed on a hydroxy-containing polyethyleneglycol (PEG) derivative **75** to give **75b** and three commercially available pyridylalcohols **76**-**78** to afford **76b**-**78b**.

The chroman-4-one derivatives **72c**-**78c** were prepared from the synthesized aldehydes via the base-promoted aldol-condensation followed by *oxa*-Michael ring closure reaction using DIPA as the base. The reaction was run in EtOH under microwave heating at 150-170 °C for 1 h. Eventually the TBS group in **72c**-**74c** was removed by treatment with Selectflour® in a microwave-assisted reaction furnishing the deprotected chroman-4-ones **72d**-**74d** in 31-78% yield over three steps.



Scheme 19. Synthesis of **72d**-**74d** and **75c**-**78c**. Reagents and conditions: (a) TBDMSCl, NaH, THF, 1 h, rt; (b) $(COCl)_2$, DMSO, TEA, THF, -78 °C→rt or Dess-Martin periodinane, CH_2Cl_2, 0 °C, 1.5 h; (c) 3'-Bromo-5'-chloro-2'-hydroxyacetophenone, DIPA, EtOH, MW, 170 °C, 1 h; (d) Selectfluor®, MeOH, 30 min, 150 °C, MW.

By using compounds **72d** and **73d** in a Swern oxidation and a subsequent Pinnick oxidation the corresponding carboxylic acids **72e**-**73e** were obtained (Scheme 20). The amide analogs **72f** and **73f**-**g** were then synthesized via activation of the acids with *N,N'*-carbonyldiimidazole (CDI) and a subsequent substitution with the appropriate amines. The alcohol **72d** was mesylated and reacted in a substitution reaction with either morpholine or piperidine to give the cyclic amines **72h**-**i**.



Scheme 20. Synthesis of acid, amide, and amine functionalized 2-substituents in the chroman-4-ones. Reagents and conditions: (a) i) Dess-Martin periodinane, CH_2Cl_2, rt, 45 min, ii) $NaClO_2$, $NaH_2PO_4 \cdot 2H_2O$, amylene, H_2O, THF, 0 °C→rt; (b) appropriate amine, CDI, CH_2Cl_2, DMF, 0 °C→rt; c) MsCl, TEA, CH_2Cl_2, 0 °C, 2 h; d) Morpholine or piperidine, THF, 120-150 °C, 1 h MW.

In addition, the methyl ester **79** (Figure 33) was also included in the study.[154]

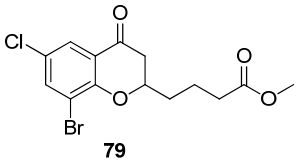
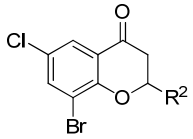


Figure 33. The structure of **79** included in the biological evaluation.

5.5 Biological evaluation of the inhibitory activity towards Sirt2

The evaluation of the Sirt2 inhibitory potency of the synthesized derivatives was performed in the same fluorescent based Fluor-de-Lys assay as described previously (section 5.2). The introduction of alcohols in the 2-position (**72d**-**74d**), the PEG moiety (**75c**), carboxylic acids (**72e**-**73e**), or the amides (**72f**, **73f**, and **73g**) did not improve the inhibition of Sirt2 at 200 μM in comparison to the lead compound **6** (Table 8). However, for the 3-pyridyl analog **77c** (86% at 200 μM), the inhibition was comparable with **6** and the phenethyl inhibitor **1**. For the 2-, and 4-pyridyl moieties (**76c** and **78c**), the inhibition of Sirt2 was somewhat lower (73-74% at 200 μM). The morpholine and piperidine derivatives **72h**-**72i** were found to be activators in the Fluor-de-Lys assay but weak inhibitors in a SIRTainty™ assay.[155] From the tested compounds the ester analog **79** was the most potent Sirt2 inhibitor at 200 μM with an inhibition of 90±0.6% and an IC_{50} value of 1.9 μM.

Table 8. The in vitro activity of chroman-4-ones based derivative against Sirt2.



Comp.	R^2	% Inhibition at 200 μM[a]	IC_{50} (μM)[b,c]
1	CH_2CH_2Ph	81 ± 0.7	6.8 (5.8-8.0)
6	$(CH_2)_4CH_3$	88 ± 0.9	4.3 (3.5-5.4)
72d	$(CH_2)_3OH$	18 ± 1.1	n.d.
73d	$(CH_2)_4OH$	52 ± 0.9	n.d.
74d	$(CH_2)_5OH$	69 ± 0.5	n.d.
75c	$CH_2O(CH_2)_2OCH_3$	33 ± 2.0	n.d.
76c	CH_2CH_2(2-pyridyl)	74 ± 0.5	n.d.
77c	CH_2CH_2(3-pyridyl)	86 ± 1.9	3.7 (3.1-4.5)
78c	CH_2CH_2(4-pyridyl)	73 ± 1.8	10.02 (8.3-12.2)
72e	$(CH_2)_2COOH$	6.8 ± 0.5	n.d.
73e	$(CH_2)_3COOH$	7.6 ± 1.5	n.d.
72f	$(CH_2)_2CONHCH_3$	4.8 ± 0.2	n.d.
73f	$(CH_2)_3CONHCH(CH_3)_2$	23 ± 1.8	n.d.
73g	$(CH_2)_3CON(CH_3)_2$	53 ± 1.4	n.d.
72h	$(CH_2)_3$(1-morpholinyl)	20 ± 10.7[d]	n.d.
72i	$(CH_2)_3$(1-piperidyl)	40 ± 2.8[d]	n.d.
79	$(CH_2)_3COOCH_3$	90 ± 0.6	1.9 (1.6-2.5)

[a]SD, Standard Deviation (n = 3). [b]IC_{50} (95% confidence interval). IC_{50} values were determined for compounds showing >70% inhibition of Sirt2 at 200 μM concentration. [c]n.d. = not determined. [d]The inhibitory activity was determined in a SIRTainty™ assay.[155]

The in vitro activities indicate that hydrogen bond acceptor properties are desirable in the 2-position. Further testing of analogs including ester isosteres and mono-methyl amide with the same length as the corresponding ester **79** are of great interest. Also substituted pyridyl analogs and alcohols with longer chain lengths could be valuable.

5.6 Evaluation of the antiproliferative activity of pyridyl derivatives 77c and 78c

The 3-pyridyl derivative **77c** and the 4-pyridyl analog **78c** (Figure 34) were tested for their effects in a proliferation/cytotoxicity assay on MCF-7 breast cancer cells and A549 lung cancer cells.

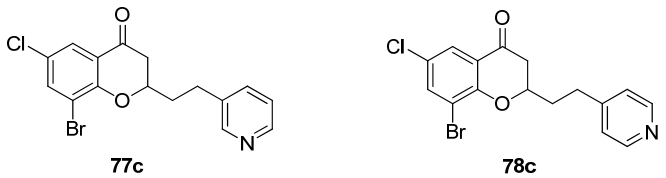


Figure 34. The 3- and 4-pyridyl derivatives were selected for antiproliferative/cytotoxicity effects on MCF-7 breast cancer cells and A549 lung cancer cells.

The protein mass of the living cells was measured using a sulforhodamine B based fluorescence assay.[90, 156] Interestingly, **77c** and **78c** showed a significant reduction of the proliferation of the two cancer cell lines at 10 and 50 μM (Figure 35).

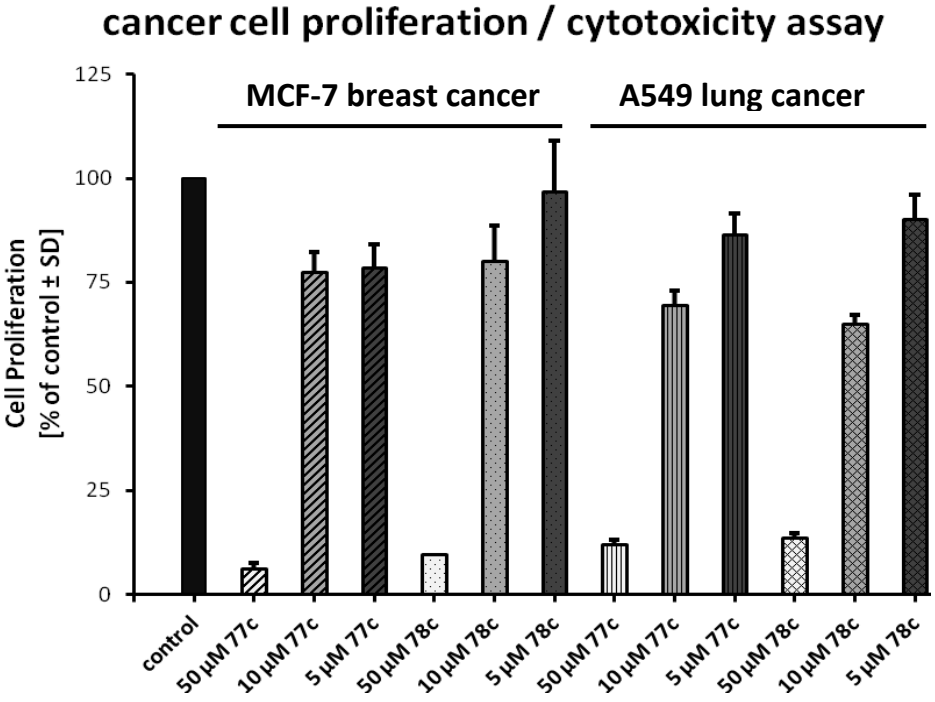


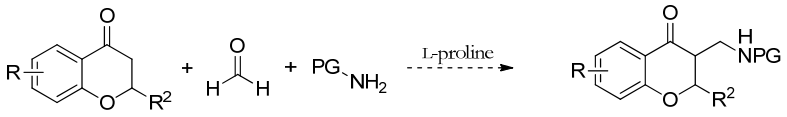
Figure 35. Results from the MCG-7 breast cancer cells and A549 lung cancer cells cytotoxicity assays where **77c** and **78c** show a significant decrease in the cell proliferations at 10 and 50 μM.

Further clarification of the mechanism behind the inhibition of the chroman-4-one based Sirt2 inhibitors **77c** and **78c** of the MCG-7 breast cancer cells and the A549 lung cancer cells is needed. Preliminary tests of the pyridyl derivatives on HEK cells also showed decreased cell proliferation (data not shown) but this finding also needs to be investigated further.

6. Proline mediated formation of novel chroman-4-one tetrahydropyrimidines

6.1 A proline catalyzed Mannich reaction for the incorporation of a 3-aminomethyl group (Paper VI)

As previously described (section 3.2.2) the introduction of an aminomethyl group in the 3-position of chroman-4-one was of great interest in our project (Figure 36). Although the Mannich reaction using $(CH_2O)_n$ and $CbzNH_2$ did not result in the desired product this reaction was further investigated. Of special interest was the fact that L-proline is known to efficiently catalyze asymmetric Mannich reactions, so for the additional studies of this reaction L-proline was chosen as the catalyst.[157-159]

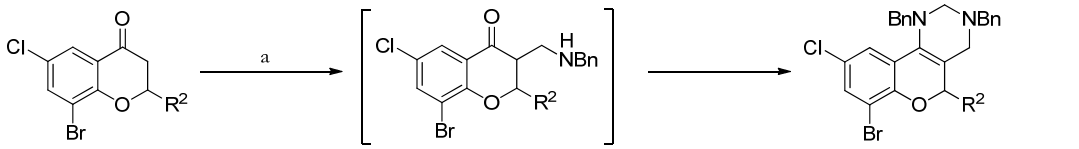


R = Cl, Br, H
R^2 = alkyl
PG = Bn, PMB

Figure 36. The planned synthetic route to introduce an aminomethyl group in the 3-position of 2-alkyl substituted chroman-4-one using an L-proline catalyzed Mannich reaction.

6.2 Formation of tricyclic derivatives 81-83

Interestingly, when the racemate of **5** was reacted with a catalytic amount of L-proline (0.3 equiv) and an excess of *N*-methylenebenzylamine[160] (5 equiv) in DMSO at 50 ºC for 48 h the novel tricylic derivative **81** was formed in 52% yield (Scheme 21). The chroman-4-one **1** substituted with a phenethyl group in the 2-position and the chroman-4-one **80**[161] with a considerably smaller 2-methyl substituent were also used as starting materials (Scheme 21). Applying the identical reaction conditions, the products **82** and **83** were formed but in lower yields (26% and 15%, respectively) as compared to **81**. An attempt to synthesize a derivative with a 2-phenyl substituent was unsuccessful.



5 $R^2 = CH_2CH_2$(*N*-Ts)-3-indolyl
1 $R^2 = CH_2CH_2Ph$
80 R^2 = Me

81 $R^2 = CH_2CH_2$(*N*-Ts)-3-indolyl (52%)
82 $R^2 = CH_2CH_2Ph$ (26%)
83 R^2 = Me (15%)

Scheme 21. Formation of novel tricyclic derivatives. Reagents and conditions: (a) *N*-methylenebenzylamine, L-proline, DMSO, 50 °C, 48 h.

The structure of the tricyclic derivative **81** was confirmed with a crystal structure, where the large 2-substituent was shown to be axially positioned (Figure 37).

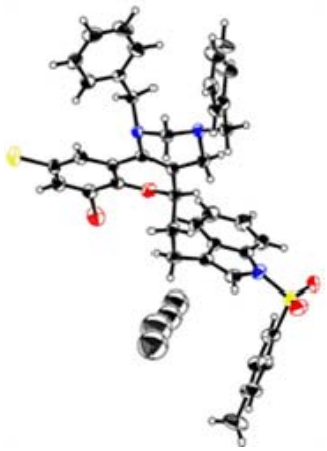
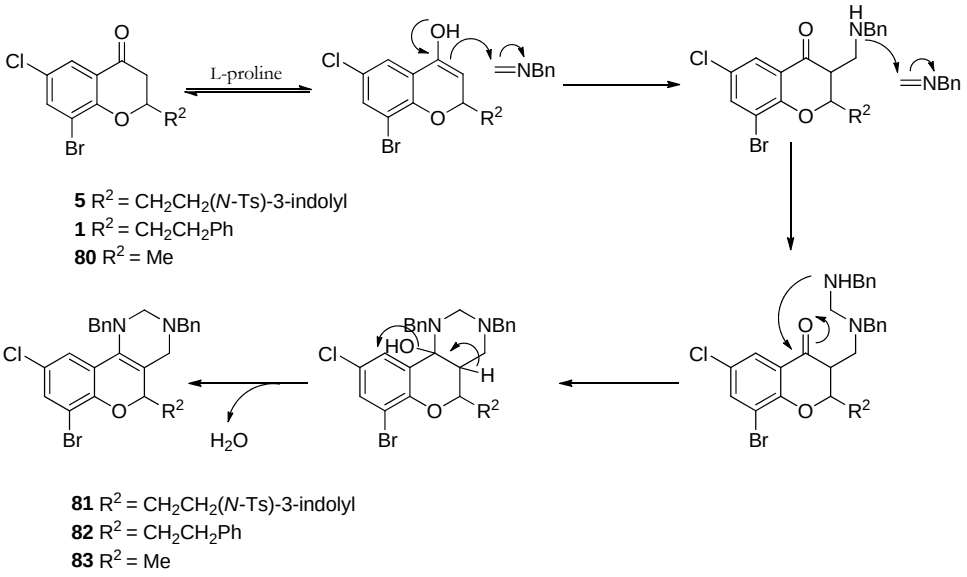


Figure 37. Crystal structure of tricyclic derivative **81**.

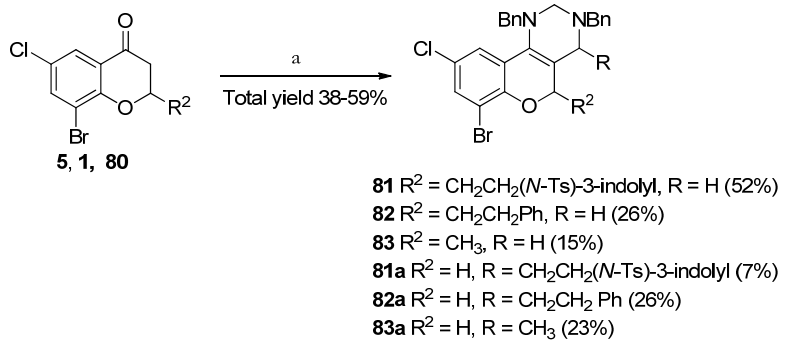
In this reaction, proline is suggested to catalyze the enolization of the chroman-4-one (Scheme 22) instead of mediating an enamine formation, which was previously proposed for L-proline.[159] This conclusion is based on experiments using other secondary amine sources such as DIPA and pyrrolidine which were shown to mainly react as nucleophiles leading to ring opening of the chroman-4-one ring (according to ^{1}H NMR spectroscopic analysis of the crude reaction mixture). In addition, upon heating a mixture of L-proline and chroman-4-one **5** at 50 °C no enamine formation was observed by ^{1}H NMR spectroscopy. Hence, in the proposed mechanism the enols of **1**, **5**, and **80** attack the preformed *N*-methylenebenzylamine providing the Mannich product as an intermediate. The subsequent nucleophilic attack of the newly formed amino function on a second *N*-methylenebenzylamine gives the aminal of which one amino group attacks the carbonyl functionality in the chroman-4-one. Subsequent dehydration provides the tetrahydropyrimidine ring and thus the final product.



Scheme 22. The proposed mechanism for the formation of the tricyclic derivatives.

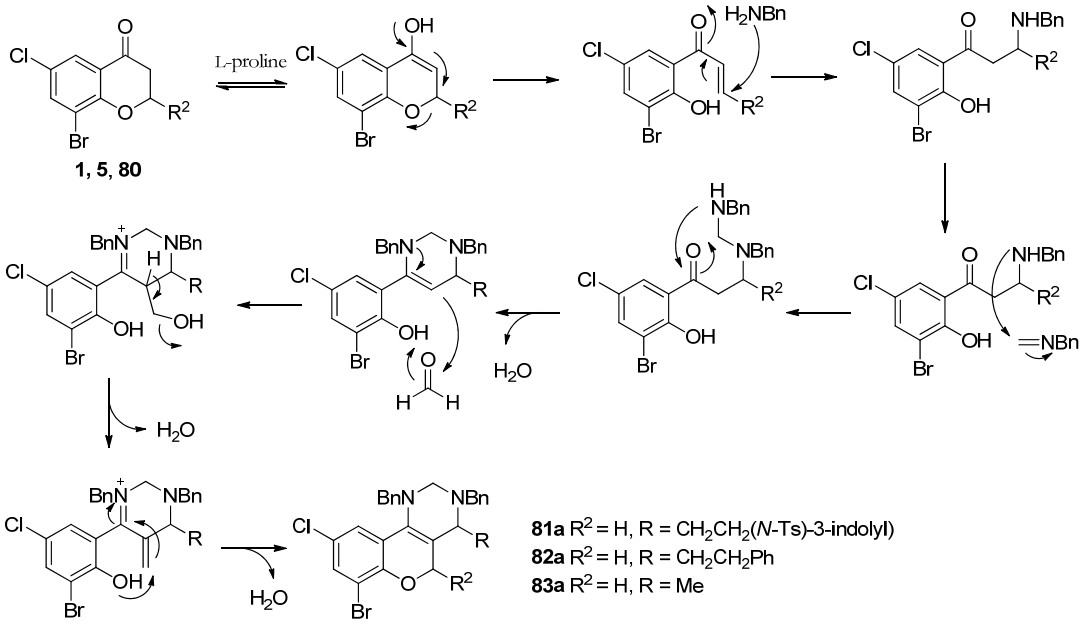
In an attempt to optimize the yield of the tricyclic derivatives **81**-**83** a series of reaction conditions were examined. The use of smaller amounts of *N*-methylenebenzylamine, shorter reactions times or higher temperatures (20 min or 2 h at 80, 120 or 150 ºC under microwave irradiation) resulted only in lower conversions. Similar observations were made upon variation of the chiral catalysts (sarcosine, L-pipecolic acid), the use of achiral catalysts (glycine, DIPA, DIPEA or pyrrolidine), racemic catalyst (D/L-proline) or alteration of solvents (THF or DMF). Neither the change of substrate structure by removal of aromatic substituents or by introduction of electron donating (OMe) or electron withdrawing (NO_2 or Cl) groups in the 6-position of the chroman-4-one resulted in improved yields. Further attempts on reacting **5** with other electrophiles such as *N*-methylene *p*-anisidine imine provided only traces of the Mannich product along with numerous impurities. Using dibenzyl imine as the electrophile resulted only in recovered starting material.

The isolated yields of derivatives **81**-**83** were moderate due to the competing formation of additional heterocyclic products. For example, the synthesis of **81** also yielded **81a** in 7% isolated yield (Scheme 23). As expected the formation of the corresponding products was detected in the synthesis of **82**-**83** which gave **82a**-**83a** in 26% and 23% yield, respectively. Compound **81** was found to have identical molecular weight to **81a**, but showed a different ^{1}H NMR spectrum and chromatographic behavior. Therefore additional HMBC and NOESY-based NMR spectroscopic investigations were performed, as described in more detail below.



Scheme 23. Summary of the formation of the tricyclic derivatives of **81**-**83** and **81a**-**83a**. Reagents and conditions: (a) *N*-methylenebenzylamine, L-proline, DMSO, 50 °C, 48 h.

The mechanism for the formation of compounds **81a**-**83a** is suggested to occur via a nucleophilic attack by benzylamine on the chroman-4-one ring system as shown in Scheme 24. Benzylamine is most likely formed by partial hydrolysis of *N*-methylenebenzylamine. However, using dry DMSO as the solvent and molecular sieves (4Å) or $MgSO_4$ as drying agents did not prevent the decomposition of *N*-methylenebenzylamine and hence the formation of the heterocyclic products **81a**-**83a**.



Scheme 24. A proposed mechanism for the formation of **81a**-**83a**.

6.3 Conformational analysis of 81 and 81a

In order to determine the most likely conformations of **81** and **81a** in solution a combined computational and NMR spectroscopic approach was utilized. A Monte Carlo conformational search followed by molecular mechanics calculations was performed using the OPLS2005 force field[162] as implemented in the MacroModel program.[121] Conformations

of **81** and **81a** within 21 kJ/mol from the global minimum were kept and resulted in two distinct conformational families. In addition to the predicted conformations a subsequent NMR analysis of molecular flexibility in solution (NAMFIS)[163] analysis was performed. Distances were determined from nuclear Overhauser enhancement (NOE) spectra. Despite the few available protons on **81** and **81a** a sufficient number of NOEs were observed for description of the orientation of their flexible fragments (Figure 38).

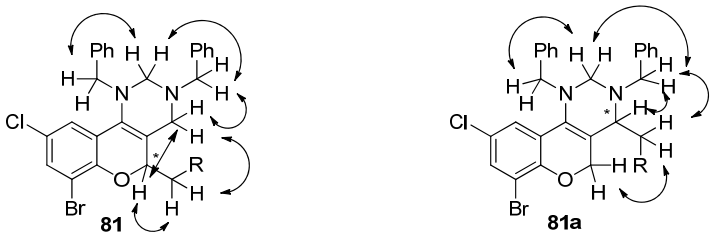


Figure 38. NOE correlations observed in NMR-spectra of **81** and **81a** in chloroform.

Taken together, using the theoretically predicted conformations including dihedral angles in combination with NMR derived distances (NOEs) the NAMFIS protocol was used. The analysis indicated one preferred conformation of **81** in solution. An overlap between that conformation and the solid state X-ray is illustrated in Figure 39a. For **81a** the 4-substituent is equatorially positioned in solution (Figure 39b).

a) b)

Figure 39. a) The solution structure of the core of **81** (yellow) overlapped with its X-ray derived conformation (green). b) The solution conformation of the tricyclic core of **81a**, as identified by NAMFIS analysis.

As described earlier the use of choman-4-one/chromone scaffolds as novel mimetics of bioactive peptides was of great interest (section 4.1). For evaluation of the potential applicability of these novel ring systems as peptidomimetics their most stable conformations were compared to that of various β-turn conformations of peptides. An initial analysis indicated that the tricyclic ring system of **81** could mimic a type VIII β-turn. Hence, computer based studies were made on a truncated tricyclic derivative and interestingly the tricyclic core of **81** efficiently mimics a native type VIII β-turn (Figure 40).

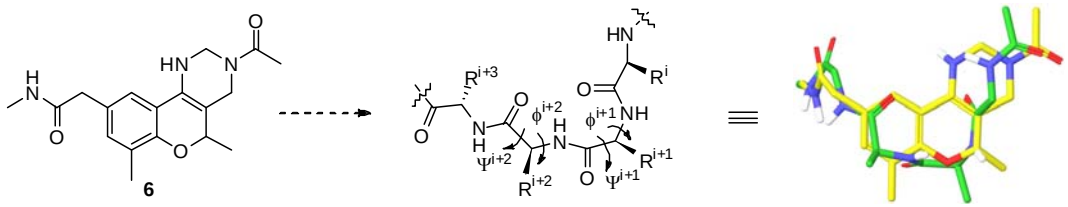


Figure 40. Alignment of a modified structure of the tricyclic derivative **81** and a type VIII β-turn ($\phi(i+1)$=-60°, $\psi(i+1)$=-30°, $\phi(i+2)$=-120° and $\psi(i+2)$=120°).

7. Concluding remarks and future perspective

This thesis describes the use of a scaffold approach for the development of biologically active substituted chroman-4-one and chromone derivatives. The thesis also describes extensive synthetic work to obtain such compounds, for example the development and use of an efficient microwave assisted reaction to facilitate the formation of 2-alkyl substituted chroman-4-ones. In addition, developed methods for the incorporation of substituents in the 3-position of chroman-4-ones (amine, aminomethyl, bromine, hydroxyl, and cyano) provide possibilities for further modifications e.g. via Pd-mediated couplings, alkylations or reductive aminations.

Two biological applications of functionalized chroman-4-one and chromones are described in the thesis. In one project, a combination of a computational analysis and affinity studies resulted in two naphthyl-containing chroman-4-one and chromone scaffolds, acting as β-turn mimetics at the somatostatin sst2 and sst4 receptors. The other application covers the ability of substituted chroman-4-ones and chromones to be potent and selective Sirt2 inhibitors with IC_{50} values in the low μM range. These compounds are considered as a novel lead series in the development of Sirt2 inhibitors. To continue the work towards the development of potent and selective Sirt2 inhibitors the following aspects should be considered:

- Perform computational modeling in combination with enzymatic kinetic studies to investigate the binding site of the chroman-4-one and chromone based Sirt2 inhibitors.

- To further improve the physico-chemical properties of the chroman-4-one and chromone based Sirt2 inhibitors.

- To synthesize and evaluate more diverse analogs with regards to the various substituents of the chroman-4-one and chromone series and also to develop strategies to functionalize related scaffolds.

- To develop non-peptidic inhibitors of Sirt2 binding to the substrate binding site.

- To develop synthetic methods to obtain enantioselective chroman-4-one cyclizations.

8. Acknowledgements

Särskilda tack vill jag rikta till:

Kristina Luthman, min handledare för att du antog mig som doktorand. Tack för att du generöst delat med dig av dina erfarenheter och idéer. Tack också för den positiva atmosfär du bidrar med. Du är verkligen en person att se upp till och jag vill tacka för att jag fått förmånen att arbeta med dig.

Morten Grøtli, min biträdande handledare. Tack för ditt engagemang och din positiva anda.

Annika Friberg och **Nils** Pemberton för att jag fått ta del av era färdigheter i organisk kemi. **Krystle** da Silva Andersson, **Tina** Seifert, and **Karin** Engen, ni var hårt arbetande examensarbetare som vågade er på kromonprojekten. Tack för fina samarbeten!

Elina Jarho and **Maija** Lahtela-Kakkonen i Kuopio för intressanta samarbeten i Sirtprojektet. Tack för innehållsrika besök i Kuopio! Thanks to **Tarja** Kokkola and **Tiina** Suuronen for help with the biological testing.

Mate Erdelyi för din entusiasm med NMR studierna. **Göran** Hilmersson och **Tobias** Ankner för ett bra samariumsamarbete. **Marie** Rydén-Landergren för hjälp med VCD spektrat. **Erik** Wallén och **Kristian** Dahlén för intressanta kemidiskussioner. **Lars Kristian** Hansen för röntgenstrukturen.

Tina Seifert för ett bra samarbete! Du har bidragit med en mycket stor del av labarbetet till den här avhandlingen!

Per-Ola Norrby och **Marcus** Malo för tips till modellerandet. Tack Marcus för hjälp med framsidan.

Former and present group members in the medicinal chemistry group: **Christine** (**Bisse**), **Itedale**, **Kristina B**, **Mariell**, **David**, **Henrik**, **Anja**, **Chris**, **Peter**, and **Fariba**. For nice socializing coffee breaks, pea soup dinners, crab fish and Christmas parties.

Tobias Ankner och **Hans** Emtenäs för korrekturläsning av avhandlingen.

Mamma och **pappa** som stöttat och uppmuntrat mig under alla år jag funnits. **Anna** och **David** för ert stöd. **Monica** och **Carl-Johan** för er omtanke.

Noel och **Judith** för att ni gjort livet bara bättre. **Markus** för din kärlek.

9. Populärvetenskaplig sammanfattning

Peptider är involverade i viktiga fysiologiska processer i kroppen såsom reglering av blodtryck och smärtsignalöverföring samt kontroll av blodglukosnivåer. Peptider består av aminosyror som är sammanlänkade via amidbindningar vilket bidrar till hög vattenlöslighet och snabb enzymatisk nedbrytning i kroppen. Peptiders strukturella egenskaper medför en stor utmaning vid utvecklingen av peptidbaserade läkemedel. Det finns däremot strategier för att förbättra t.ex. stabiliteten hos peptider genom att ersätta amidbindningarna med stabila kemiska grupper. Man kallar sådana strukturer för peptidomimetika.

I den första av två delstudier användes neuropeptiden somatostatin som en modellpeptid där den del av peptiden som är viktig för aktiviteten byttes ut mot naturligt förekommande grundstrukturer (kromanoner och kromoner). De aminosyror som är viktiga för aktiviteten hos somatostatin introducerades på grundstrukturen med olika kemiska metoder. De nya föreningarna testades på somatostatinreceptorer och visade samma typ av aktivitet som somatostain. Därmed har vi visat att man kan härma somatostatins aktivitet med andra, mer stabila substanser.

I den andra delstudien användes kromanoner och kromoner i ett projekt som behandlar enzymet Sirt2. Sirt2 är involverat i olika åldersrelaterade sjukdomar som cancer, diabetes och neurodegenererande sjukdomar t. ex. Parkinsons och Alzheimers sjukdom. Enzymets specifika funktion är att påverka avläsningen av DNA och att påverka cellcykeln. Därför är Sirt2 speciellt intressant inom cancerforskning. Kromanon/kromonderivaten har visat sig selektivt kunna hämma Sirt2. Hur bra hämmningen blir är helt beroende på vilka grupper som introducerats på kromanon/kromonsystemet. Intressant nog har två syntetiserade kromanonbaserade Sirt2-inhibitorer också visats minska tillväxt av bröstcancer- och lungcancerceller.

Genom att använda substituerade kromanoner och kromoner har dessa studier visat att substanserna besitter olika effekter beroende på vilka substituenter som bundits till grundstrukturerna. Substanserna som inhiberar Sirt2 kan förhoppningsvis bidra till att öka förståelsen och kunskapen om enzymets funktion.

10. References and Notes

1. Fischer, E.; Fourneau, E., A derivative from glykocolls. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 2868-2877.
2. Duvigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G.; Gordon, S., The Synthesis of an Octapeptide Amide with the Hormonal Activity of Oxytocin. *J. Am. Chem. Soc.* **1953**, *75* (19), 4879-4880.
3. Duvigneaud, V.; Lawler, H. C.; Popenoe, E. A., Enzymatic Cleavage of Glycinamide from Vasopressin and a Proposed Structure for This Pressor-Antidiuretic Hormone of the Posterior Pituitary. *J. Am. Chem. Soc.* **1953**, *75* (19), 4880-4881.
4. Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R., Identification of 2 Related Pentapeptides from Brain with Potent Opiate Agonist Activity. *Nature* **1975**, *258* (5536), 577-579.
5. Sanger, F.; Thompson, E. O. P.; Kitai, R., Amide Groups of Insulin. *Biochem. J.* **1955**, *59* (3), 509-518.
6. Ryle, A. P.; Sanger, F.; Smith, L. F.; Kitai, R., Disulphide Bonds of Insulin. *Biochem. J.* **1955**, *60* (1-4), 541-556.
7. Brazeau, P.; Vale, W.; Burgus, R.; Ling, N.; Butcher, M.; Rivier, J.; Guillemi.R, Hypothalamic Polypeptide That Inhibits Secretion of Immunoreactive Pituitary Growth-Hormone. *Science* **1973**, *179* (4068), 77-79.
8. Tigerstedt, R.; Bergman, P. G., Niere und kreilauf. *Skand. Arch. Physiol.* **1898**, *8*, 223-227.
9. Rose, G. D.; Gierasch, L. M.; Smith, J. A., Turns in peptides and proteins. *Adv. Protein Chem.* **1985**, *37*, 1-109.
10. Venkatachalam, C., Stereochemical Criteria for Polypeptides and Proteins .V. Conformation of a System of 3 Linked Peptide Units. *Biopolymers* **1968**, *6* (10), 1425-1436.
11. Wilmot, C. M.; Thornton, J. M., Analysis and Prediction of the Different Types of Beta-Turn in Proteins. *J. Mol. Biol.* **1988**, *203* (1), 221-232.
12. Hutchinson, E. G.; Thornton, J. M., A Revised Set of Potentials for Beta-Turn Formation in Proteins. *Protein Sci.* **1994**, *3* (12), 2207-2216.
13. Rotondi, K. S.; Gierasch, L. M., Natural polypeptide scaffolds: beta-sheets, beta-turns, and beta-hairpins. *Biopolymers* **2006**, *84* (1), 13-22.
14. Hruby, V. J.; Balse, P. M., Conformational and topographical considerations in designing agonist peptidomimetics from peptide leads. *Curr. Med. Chem.* **2000**, *7* (9), 945-970.
15. Ripka, A. S.; Rich, D. H., Peptidomimetic design. *Curr. Op. Chem. Biol.* **1998**, *2* (4), 441-452.
16. Hanessian, S.; McNaughtonSmith, G.; Lombart, H. G.; Lubell, W. D., Design and synthesis of conformationally constrained amino acids as versatile scaffolds and peptide mimetics. *Tetrahedron* **1997**, *53* (38), 12789-12854.
17. Giannis, A., Peptidomimetics for Receptor Ligands Discovery, Development, and Medical Perspectives. *Angew. Chem. Int. Ed. Engl.* **1993**, *32* (9), 1244-1267.
18. Farmer, P. S.; Ariens, E. J., Speculations on the Design of Non-Peptidic Peptidomimetics. *Trends Pharmacol. Sci.* **1982**, *3* (9), 362-365.
19. Wermuth, G.; Ganellin, C. R.; Lindberg, P.; Mitscher, L. A., Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1998). *Pure Appl. Chem.* **1998**, *70* (5), 1129-1143.
20. Thornber, C. W., Isosterism and Molecular Modification in Drug Design. *Chem. Soc. Rev.* **1979**, *8* (4), 563-580.
21. Meanwell, N. A., Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54* (8), 2529-2591.
22. Ball, J. B.; Alewood, P. F., Conformational constraints: nonpeptide b-turn mimics. *J. Mol. Recog.* **1990**, *3* (2), 55-64.
23. Souers, A. J.; Ellman, J. A., beta-turn mimetic library synthesis: scaffolds and applications. *Tetrahedron* **2001**, *57* (35), 7431-7448.

24. Blankley, C. J.; Hodges, J. C.; Klutchko, S. R.; Himmelsbach, R. J.; Chucholowski, A.; Connolly, C. J.; Neergaard, S. J.; Vannieuwenhze, M. S.; Sebastian, A.; Quin, J.; Essenburg, A. D.; Cohen, D. M., Synthesis and Structure-Activity-Relationships of a Novel Series of Nonpeptide Angiotensin-Ii Receptor-Binding Inhibitors Specific for the At2 Subtype. *J. Med. Chem.* **1991**, *34* (11), 3248-3260.
25. DeLucca, G. V.; EricksonViitanen, S.; Lam, P. Y. S., Cyclic HIV protease inhibitors capable of displacing the active site structural water molecule. *Drug Discov. Today* **1997**, *2* (1), 6-18.
26. Belanger, P. C.; Dufresne, C., Exo-6-Benzyl-Exo-2-(Meta-Hydroxyphenyl)-1-Dimethylaminomethylbicyclo[2.2.2.]Octane - a Nonpeptide Mimic of Enkephalins. *Can. J. Chem.* **1986**, *64* (8), 1514-1520.
27. Pierce, K. L.; Premont, R. T.; Lefkowitz, R. J., Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **2002**, *3* (9), 639-650.
28. Congreve, M.; Langmead, C. J.; Mason, J. S.; Marshall, F. H., Progress in Structure Based Drug Design for G Protein-Coupled Receptors. *J. Med. Chem.* **2011**, *54* (13), 4283-4311.
29. Ji, T. H.; Grossmann, M.; Ji, I. H., G protein-coupled receptors I. Diversity of receptor-ligand interactions. *J. Chem. Biol. Chem.* **1998**, *273* (28), 17299-17302.
30. Rose, G. D.; Gierasch, L. M.; Smith, J. A., *Adv. Protein Chem.* **1985**, *37*, 1-109.
31. Gurrath, M., Peptide-binding G protein-coupled receptors: New opportunities for drug design. *Curr. Med. Chem.* **2001**, *8* (13), 1605-1648.
32. Brazeau, P.; Vale, W.; Burgus, R.; Guillemi.R, Isolation of Somatostatin (a Somatotropin-Release-Inhibiting-Factor) of Ovine Hypothalamic Origin. *Can. J. Biochem.* **1974**, *52* (11), 1067-1072.
33. Weckbecker, G.; Lewis, I.; Albert, R.; Schmid, H. A.; Hoyer, D.; Bruns, C., Opportunities in somatostatin research: Biological, chemical and therapeutic aspects. *Nat. Rev. Drug Discov.* **2003**, *2* (12), 999-1017.
34. Reisine, T.; Bell, G. I., Molecular biology of somatostatin receptors. *Endocr. Rev.* **1995**, *16*, 427-442.
35. Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R., Bioactive Conformation of Luteinizing-Hormone-Releasing Hormone - Evidence from a Conformationally Constrained Analog. *Science* **1980**, *210* (4470), 656-658.
36. Freidinger, R. M.; Perlow, D. S.; Randall, W. C.; Saperstein, R.; Arison, B. H.; Veber, D. F., Conformational Modifications of Cyclic Hexapeptide Somatostatin Analogs. *Int. J. Pept. Prot. Res.* **1984**, *23* (2), 142-150.
37. Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R., Conformationally Restricted Bicyclic Analogs of Somatostatin. *Proc. Natl. Acad. Sci.* **1978**, *75* (6), 2636-2640.
38. Janecka, A.; Zubrzycka, M.; Janecki, T., Somatostatin analogs. *J. Peptide Res.* **2001**, *58* (2), 91-107.
39. Veber, D. F.; Freidinger, R. M.; Perlow, D. S.; Paleveda, W. J.; Holly, F. W.; Strachan, R. G.; Nutt, R. F.; Arison, B. H.; Homnick, C.; Randall, W. C.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R., A Potent Cyclic Hexapeptide Analog of Somatostatin. *Nature* **1981**, *292* (5818), 55-58.
40. Freidinger, R. M., Design and synthesis of novel bioactive peptides and peptidomimeties. *J. Med. Chem.* **2003**, *46* (26), 5553-5566.
41. Bauer, W.; Briner, U.; Doepfner, W.; Haller, R.; Huguenin, R.; Marbach, P.; Petcher, T. J.; Pless, J., Sms 201-995 - a Very Potent and Selective Octapeptide Analog of Somatostatin with Prolonged Action. *Life. Sci.* **1982**, *31* (11), 1133-1140.
42. Marbach, P.; Briner, U.; Lemaire, M.; Schweitzer, A.; Terasaki, T., From Somatostatin to Sandostatin(R) - Pharmacodynamics and Pharmacokinetics. *Metab. Clin. Exp.* **1992**, *41* (9), 7-10.
43. Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengeler, P. A.; Hamley, P.; Smith, A. B.; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D., De-Novo Design and Synthesis of Somatostatin Nonpeptide Peptidomimetics Utilizing Beta-D-Glucose as a Novel Scaffolding. *J. Am. Chem. Soc.* **1993**, *115* (26), 12550-12568.
44. Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B.; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L., Nonpeptidal Peptidomimetics with a Beta-D-Glucose Scaffolding - a Partial Somatostatin Agonist

Bearing a Close Structural Relationship to a Potent, Selective Substance-P Antagonist. *J. Am. Chem. Soc.* **1992**, *114* (23), 9217-9218.
45. Papageorgiou, C.; Borer, X., A non-peptide ligand for the somatostatin receptor having a benzodiazepinone structure. *Bioorg. Med. Chem. Lett.* **1996**, *6* (3), 267-272.
46. Smith, A. B.; Charnley, A. K.; Mesaros, E. F.; Kikuchi, O.; Wang, W. Y.; Benowitz, A.; Chu, C. L.; Feng, J. J.; Chen, K. H.; Lin, A.; Cheng, F. C.; Taylor, L.; Hirschmann, R., Design, synthesis, and binding affinities of pyrrolinone-based somatostatin mimetics. *Org. Lett.* **2005**, *7* (3), 399-402.
47. Mowery, B. P.; Prasad, V.; Kenesky, C. S.; Angeles, A. R.; Taylor, L. L.; Feng, J. J.; Chen, W. L.; Lin, A.; Cheng, F. C.; Smith, A. B.; Hirschmann, R., Catechol: A minimal scaffold for non-peptide peptidomimetics of the i+1 and i+2 positions of the beta-turn of somatostatin. *Org. Lett.* **2006**, *8* (20), 4397-4400.
48. Prasad, V.; Birzin, E. T.; McVaugh, C. T.; van Rijn, R. D.; Rohrer, S. P.; Chicchi, G.; Underwood, D. J.; Thornton, E. R.; Smith, A. B.; Hirschmann, R., Effects of Heterocyclic aromatic substituents on binding affinities at two distinct sites of somatostatin receptors. Correlation with the electrostatic potential of the substituents. *J. Med. Chem.* **2003**, *46* (10), 1858-1869.
49. Strahl, B. D.; Allis, C. D., The language of covalent histone modifications. *Nature* **2000**, *403* (6765), 41-45.
50. Kouzarides, T., Acetylation: a regulatory modification to rival phosphorylation? *Embo Journal* **2000**, *19* (6), 1176-1179.
51. Polevoda, B.; Sherman, F., The diversity of acetylated proteins. *Gen. Biol.* **2002**, *3* (5).
52. Frye, R. A., Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.* **1999**, *260* (1), 273-279.
53. Glozak, M. A.; Sengupta, N.; Zhang, X. H.; Seto, E., Acetylation and deacetylation of non-histone proteins. *Gene* **2005**, *363*, 15-23.
54. Imai, S.; Armstrong, C. M.; Kaeberlein, M.; Guarente, L., Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **2000**, *403* (6771), 795-800.
55. North, B. J.; Marshall, B. L.; Borra, M. T.; Denu, J. M.; Verdin, E., The human Sir2 ortholog, SIRT2, is an NAD(+)-dependent tubulin deacetylase. *Mol. Cell* **2003**, *11* (2), 437-444.
56. Michan, S.; Sinclair, D., Sirtuins in mammals: insights into their biological function. *Biochem. J.* **2007**, *404*, 1-13.
57. Frye, R. A., Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem. Biophys. Res. Commun.* **2000**, *273* (2), 793-798.
58. Haigis, M. C.; Sinclair, D. A., Mammalian Sirtuins: Biological Insights and Disease Relevance. *Ann. Rev. Pathol. Mech. Dis.* **2010**, *5*, 253-295.
59. Haigis, M. C.; Guarente, L. P., Mammalian sirtuins - emerging roles in physiology, aging, and calorie restriction. *Gen. Dev.* **2006**, *20* (21), 2913-2921.
60. Morimoto, J.; Hayashi, Y.; Suga, H., Discovery of Macrocyclic Peptides Armed with a Mechanism-Based Warhead: Isoform-Selective Inhibition of Human Deacetylase SIRT2. *Angew. Chem. Int. Ed. Engl.* **2012**, *51* (14), 3423-3427.
61. Smith, B. C.; Denu, J. M., Sir2 deacetylases exhibit nucleophilic participation of acetyl-lysine in NAD(+) cleavage. *J. Am. Chem. Soc.* **2007**, *129* (18), 5802-5803.
62. Sanders, B. D.; Jackson, B.; Marmorstein, R., Structural basis for sirtuin function: What we know and what we don't. *Biochim. Acta Biophys. Prot. Proteomic.* **2010**, *1804* (8), 1604-1616.
63. Sauve, A. A.; Wolberger, C.; Schramm, V. L.; Boeke, J. D., The biochemistry of sirtuins. *Ann. Rev. Biochem.* **2006**, *75*, 435-465.
64. Jackson, M. D.; Schmidt, M. T.; Oppenheimer, N. J.; Denu, J. M., Mechanism of nicotinamide inhibition and transglycosidation by Sir2 histone/protein deacetylases. *J. Chem. Biol. Chem.* **2003**, *278* (51), 50985-50998.
65. Feige, J. N.; Auwerx, J., Transcriptional targets of sirtuins in the coordination of mammalian physiology. *Curr. Op. Cell Biol.* **2008**, *20* (3), 303-309.

66. Finkel, T.; Deng, C. X.; Mostoslavsky, R., Recent progress in the biology and physiology of sirtuins. *Nature* **2009**, *460* (7255), 587-591.
67. Taylor, D. M.; Maxwell, M. M.; Luthi-Carter, R.; Kazantsev, A. G., Biological and Potential Therapeutic Roles of Sirtuin Deacetylases. *Cell. Mol. Life Sci.* **2008**, *65* (24), 4000-4018.
68. Cen, Y.; Youn, D. Y.; Sauve, A. A., Advances in Characterization of Human Sirtuin Isoforms: Chemistries, Targets and Therapeutic Applications. *Curr. Med. Chem.* **2011**, *18* (13), 1919-1935.
69. Lavu, S.; Boss, O.; Elliott, P. J.; Lambert, P. D., Sirtuins - novel therapeutic targets to treat age-associated diseases. *Nat. Rev. Drug Discov.* **2008**, *7* (10), 841-853.
70. Jordan, M. A.; Wilson, L., Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer* **2004**, *4* (4), 253-265.
71. Nogales, E., Structural insights into microtubule function. *Ann. Rev. Biochem.* **2000**, *69*, 277-302.
72. Dryden, S. C.; Nahhas, F. A.; Nowak, J. E.; Goustin, A. S.; Tainsky, M. A., Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol. Cell Biol.* **2003**, *23* (9), 3173-3185.
73. Outeiro, T. F.; Kontopoulos, E.; Altmann, S. M.; Kufareva, I.; Strathearn, K. E.; Amore, A. M.; Volk, C. B.; Maxwell, M. M.; Rochet, J. C.; McLean, P. J.; Young, A. B.; Abagyan, R.; Feany, M. B.; Hyman, B. T.; Kazantsev, A. G., Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science* **2007**, *317* (5837), 516-519.
74. Finnin, M. S.; Donigian, J. R.; Pavletich, N. P., Structure of the histone deacetylase SIRT2. *Nat. Struc. Biol.* **2001**, *8* (7), 621-625.
75. Bellamacina, C. R., Protein motifs .9. The nicotinamide dinucleotide binding motif: A comparison of nucleotide binding proteins. *FASEB J.* **1996**, *10* (11), 1257-1269.
76. Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L., Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J. Chem. Biol. Chem.* **2001**, *276* (42), 38837-38843.
77. Medda, F.; Russell, R. J. M.; Higgins, M.; McCarthy, A. R.; Campbell, J.; Slawin, A. M. Z.; Lane, D. P.; Lain, S.; Westwood, N. J., Novel Cambinol Analogs as Sirtuin Inhibitors: Synthesis, Biological Evaluation, and Rationalization of Activity. *J. Med. Chem.* **2009**, *52* (9), 2673-2682.
78. Schemies, J.; Uciechowska, U.; Sippl, W.; Jung, M., NAD(+)-Dependent Histone Deacetylases (Sirtuins) as Novel Therapeutic Targets. *Med. Res. Rev.* **2010**, *30* (6), 861-889.
79. Kiviranta, P. H.; Leppanen, J.; Rinne, V. M.; Suuronen, T.; Kyrylenko, O.; Kyrylenko, S.; Kuusisto, E.; Tervo, A. J.; Jarvinen, T.; Salminen, A.; Poso, A.; Wallen, E. A. A., N-(3-(4-hydroxyphenyl)-propenoyl)-amino acid tryptamides as SIRT2 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17* (9), 2448-2451.
80. Suzuki, T.; Khan, M. N. A.; Sawada, H.; Imai, E.; Itoh, Y.; Yamatsuta, K.; Tokuda, N.; Takeuchi, J.; Seko, T.; Nakagawa, H.; Miyata, N., Design, Synthesis, and Biological Activity of a Novel Series of Human Sirtuin-2-Selective Inhibitors. *J. Med. Chem.* **2012**, *55* (12), 5760-5773.
81. Kiviranta, P. H.; Suuronen, T.; Wallen, E. A. A.; Leppanen, J.; Tervonen, J.; Kyrylenko, S.; Salminen, A.; Poso, A.; Jarho, E. M., N-epsilon-Thioacetyl-Lysine-Containing Tri-, Tetra-, and Pentapeptides as SIRT1 and SIRT2 Inhibitors. *J. Med. Chem.* **2009**, *52* (7), 2153-2156.
82. Huhtiniemi, T.; Suuronen, T.; Lahtela-Kakkonen, M.; Bruijn, T.; Jaaskelainen, S.; Poso, A.; Salminen, A.; Leppanen, J.; Jarho, E., N-epsilon-Modified lysine containing inhibitors for SIRT1 and SIRT2. *Bioorg. Med. Chem.* **2010**, *18* (15), 5616-5625.
83. Inoue, T.; Nakayama, Y.; Yamada, H.; Li, Y. C.; Yamaguchi, S.; Osaki, M.; Kurimasa, A.; Hiratsuka, M.; Katoh, M.; Oshimura, M., SIRT2 downregulation confers resistance to microtubule inhibitors by prolonging chronic mitotic arrest. *Cell Cycle* **2009**, *8* (8), 1279-1291.
84. Inoue, T.; Hiratsuka, M.; Osaki, M.; Yamada, H.; Kishimoto, I.; Yamaguchi, S.; Nakano, S.; Katoh, M.; Ito, H.; Oshimura, M., SIRT2, a tubulin deacetylase, acts to block the entry to chromosome condensation in response to mitotic stress. *Oncogene* **2007**, *26* (7), 945-957.
85. Inoue, T.; Hiratsuka, M.; Osaki, M.; Oshimura, M., The molecular biology of mammalian SIRT proteins - SIRT2 in cell cycle regulation. *Cell Cycle* **2007**, *6* (9), 1011-1018.

86. Okada, H.; Mak, T. W., Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat. Rev. Cancer* **2004**, *4* (8), 592-603.
87. Wang, F.; Nguyen, M.; Qin, F. X. F.; Tong, Q., SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell* **2007**, *6* (4), 505-514.
88. Li, Y. Z.; Matsumori, H.; Nakayama, Y.; Osaki, M.; Kojima, H.; Kurimasa, A.; Ito, H.; Mori, S.; Katoh, M.; Oshimura, M.; Inoue, T., SIRT2 down-regulation in HeLa can induce p53 accumulation via p38 MAPK activation-dependent p300 decrease, eventually leading to apoptosis. *Genes to Cells* **2011**, *16* (1), 34-45.
89. North, B. J.; Verdin, E., Mitotic regulation of SIRT2 by cyclin-dependent kinase 1-dependent phosphorylation. *J. Chem. Biol. Chem.* **2007**, *282* (27), 19546-19555.
90. Peck, B.; Chen, C. Y.; Ho, K. K.; Di Fruscia, P.; Myatt, S. S.; Coombes, R. C.; Fuchter, M. J.; Hsiao, C. D.; Lam, E. W. F., SIRT Inhibitors Induce Cell Death and p53 Acetylation through Targeting Both SIRT1 and SIRT2. *Mol. Cancer Ther.* **2010**, *9* (4), 844-855.
91. He, X.; Nie, H.; Hong, Y. Y.; Sheng, C. B.; Xia, W. L.; Ying, W. H., SIRT2 activity is required for the survival of C6 glioma cells. *Biochem. Biophys. Res. Commun.* **2012**, *417* (1), 468-472.
92. Saengchantara, S. T.; Wallace, T. W., Chromanols, Chromanones, and Chromones. *Nat. Prod. Rep.* **1986**, *3* (5), 465-475.
93. Cottiglia, F.; Dhanapal, B.; Sticher, O.; Heilmann, J., New chromanone acids with antibacterial activity from Calophyllum brasiliense. *J. Nat. Prod.* **2004**, *67* (4), 537-541.
94. Edwards, A. M.; Howell, J. B. L., The chromones: history, chemistry and clinical development. A tribute to the work of Dr R. E. C. Altounyan. *Clin. Exp. Allergy* **2000**, *30* (6), 756-774.
95. Sharma, S. K.; Kumar, S.; Chand, K.; Kathuria, A.; Gupta, A.; Jain, R., An Update on Natural Occurrence and Biological Activity of Chromones. *Curr. Med. Chem.* **2011**, *18* (25), 3825-3852.
96. Middleton, E.; Kandaswami, C.; Theoharides, T. C., The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharm. Rev.* **2000**, *52* (4), 673-751.
97. Biddle, M. M.; Lin, M.; Scheidt, K. A., Catalytic enantioselective synthesis of flavanones and chromanones. *J. Am. Chem. Soc.* **2007**, *129* (13), 3830-3831.
98. Hodgetts, K. J.; Maragkou, K. I.; Wallace, T. W.; Wootton, R. C. R., Conjugate addition to 3-arylsulfinylchromones as a synthetic route to homochiral 2-substituted chromanones: scope and limitations. *Tetrahedron* **2001**, *57* (31), 6793-6804.
99. Heim, K. E.; Tagliaferro, A. R.; Bobilya, D. J., Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* **2002**, *13* (10), 572-584.
100. Lee, H.; Lee, K.; Jung, J. K.; Cho, J.; Theodorakis, E. A., Synthesis and evaluation of 6-hydroxy-7-methoxy-4chromanone-and chroman-2-carboxamides as antioxidants. *Bioorg. Med. Chem. Lett.* **2005**, *15* (11), 2745-2748.
101. Yu, D. L.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H., Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Med. Res. Rev.* **2003**, *23* (3), 322-345.
102. Chandler, I. M.; Mcintyre, C. R.; Simpson, T. J., Structural Revision and Synthesis of Ll-D253-Alpha and Related Chromanone Fungal Metabolites. *J. Chem. Soc. Perkin Trans. 1* **1992**, (18), 2271-2284.
103. Nguyen, T. B.; Lozach, O.; Surpateanu, G.; Wang, Q.; Retailleau, P.; Iorga, B. I.; Meijer, L.; Gueritte, F., Synthesis, Biological Evaluation, and Molecular Modeling of Natural and Unnatural Flavonoidal Alkaloids, Inhibitors of Kinases. *J. Med. Chem.* **2012**, *55* (6), 2811-2819.
104. Dyrager, C.; Mollers, L. N.; Kjall, L. K.; Alao, J. P.; Diner, P.; Wallner, F. K.; Sunnerhagen, P.; Grotli, M., Design, Synthesis, and Biological Evaluation of Chromone-Based p38 MAP Kinase Inhibitors. *J. Med. Chem.* **2011**, *54* (20), 7427-7431.
105. Evans, B. E.; Rittle, K. E.; Bock, M. G.; Dipardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J., Methods for Drug Discovery - Development of Potent, Selective, Orally Effective Cholecystokinin Antagonists. *J. Med. Chem.* **1988**, *31* (12), 2235-2246.
106. Horton, D. A.; Bourne, G. T.; Smythe, M. L., The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem. Rev.* **2003**, *103* (3), 893-930.

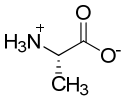
107. Welsch, M. E.; Snyder, S. A.; Stockwell, B. R., Privileged scaffolds for library design and drug discovery. *Curr. Op. Chem. Biol.* **2010**, *14* (3), 347-361.
108. http://www.fass.se/
109. Shen, H. C., Asymmetric synthesis of chiral chromans. *Tetrahedron* **2009**, *65* (20), 3931-3952.
110. Crozier, A.; Jaganath, I. B.; Clifford, M. N., Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* **2009**, *26* (8), 1001-1043.
111. Baker, W., Molecular rearrangement of some o-acyloxyacetophenones and the mechanism of the production of 3-acylchromones. *J. Chem. Soc.* **1933**, 1381-1389.
112. Mahal, H. S.; Venkataraman, K., Synthetical experiments in the chromone group Part XIV The action of sodamide on l-acyloxy-2-acetonaphthones. *J. Chem. Soc.* **1934**, 1767-1769.
113. Wallén, E. A. A.; Dahlén, K.; Grotli, M.; Luthman, K., Synthesis of 3-aminomethyl-2-aryl-8-bromo-6-chlorochromones. *Org. Lett.* **2007**, *9* (3), 389-391.
114. Dahlén, K.; Wallén, E. A. A.; Grotli, M.; Luthman, K., Synthesis of 2,3,6,8-tetrasubstituted chromone scaffolds. *J. Org. Chem.* **2006**, *71* (18), 6863-6871.
115. Zhou, C. X.; Dubrovsky, A. V.; Larock, R. C., Diversity-oriented synthesis of 3-iodochromones and heteroatom analogues via ICl-induced cyclization. *J. Org. Chem.* **2006**, *71* (4), 1626-1632.
116. Hölte, H. D.; Sippl, W.; Rognan, D.; Folkers, G., *Molecular Modeling, Basic Principles and Applications*. 2nd ed.; WILEY-VCH Verlag GmbH&Co: Weinheim, 2003.
117. Bostrom, J.; Norrby, P. O.; Liljefors, T., Conformational energy penalties of protein-bound ligands. *J. Comput. -Aided Mol. Design* **1998**, *12* (4), 383-396.
118. Kabbe, H. J.; Widdig, A., Synthesis and Reactions of 4-Chromanones. *Angew. Chem. Int. Ed. Engl.* **1982**, *21* (4), 247-256.
119. Kelly, S. E.; Vanderplas, B. C., An Alternative to the Kabbe Condensation for the Synthesis of Chromanones from Enolizable Aldehydes and Ketones. *J. Org. Chem.* **1991**, *56* (3), 1325-1327.
120. Chandrasekhar, S.; Vijeender, K.; Reddy, K. V., New synthesis of flavanones catalyzed by L-proline. *Tetrahedron Lett.* **2005**, *46* (41), 6991-6993.
121. MacroModel, version 8.0, Schrodinger, LCC, New York, NY, 2007.
122. Jaguar, version 7.9, Schrodinger, LCC, New York, NY, 2007.
123. Ferrali, M.; Bambagioni, S.; Ceccanti, A.; Donati, D.; Giorgi, G.; Fontani, M.; Laschi, F.; Zanello, P.; Casolaro, M.; Pietrangelo, A., Design, synthesis, and physicochemical and biological characterization of a new iron chelator of the family of hydroxychromenes. *J. Med. Chem.* **2002**, *45* (26), 5776-5785.
124. Jacobsen, M. F.; Turks, M.; Hazell, R.; Skrydstrup, T., SmI2-mediated cyclizations of derivatized beta-lactams for the highly diastereoselective construction of functionalized prolines. *J. Org. Chem.* **2002**, *67* (8), 2411-2417.
125. Namy, J. L.; Girard, P.; Kagan, H. B., New Preparation of Some Divalent Lanthanide Iodides and Their Usefulness in Organic-Synthesis. *New J. Chem.* **1977**, *1* (1), 5-7.
126. Girard, P.; Namy, J. L.; Kagan, H. B., Divalent Lanthanide Derivatives in Organic-Synthesis .1. Mild Preparation of Smi2 and Ybi2 and Their Use as Reducing or Coupling Agents. *J. Am. Chem. Soc.* **1980**, *102* (8), 2693-2698.
127. Kagan, H. B., Twenty-five years of organic chemistry with diiodosamarium: an overview. *Tetrahedron* **2003**, *59* (52), 10351-10372.
128. Nicolaou, K. C.; Ellery, S. P.; Chen, J. S., Samarium Diiodide Mediated Reactions in Total Synthesis. *Angew. Chem. Int. Ed. Engl.* **2009**, *48* (39), 7140-7165.
129. Molander, G. A.; Etter, J. B., Relative Asymmetric Induction in Formation of Substituted Cyclopentanols Via Intramolecular Carbonyl Additions. *Synth. Commun.* **1987**, *17* (8), 901-912.
130. Namy, J. L.; Souppe, J.; Kagan, H. B., Efficient Formation of Pinacols from Aldehydes or Ketones Mediated by Samarium Diiodide. *Tetrahedron Lett.* **1983**, *24* (8), 765-766.
131. Dahlén, A.; Hilmersson, G., Instantaneous SmI_2-H_2O-mediated reduction of dialkyl ketones induced by amines in THF. *Tetrahedron Lett.* **2002**, *43* (40), 7197-7200.
132. Ankner, T.; Hilmersson, G., Instantaneous SmI_2/H_2O/amine mediated reduction of nitroalkanes and alpha,beta-unsaturated nitroalkenes. *Tetrahedron Lett.* **2007**, *48* (32), 5707-5710.

133. Ankner, T.; Hilmersson, G., SmI_2/H_2O/amine promoted reductive cleavage of benzyl-heteroatom bonds: optimization and mechanism. *Tetrahedron* **2009**, *65* (52), 10856-10862.
134. Furstner, A., Recent Advancements in the Reformatsky Reaction. *Synthesis* **1989**, (8), 571-590.
135. Dahlén, A.; Hilmersson, G., Samarium(II) iodide mediated reductions - Influence of various additives. *Eur. J. Inorg. Chem.* **2004**, (17), 3393-3403.
136. Kahne, D.; Collum, D. B., Kinetic Cyanations of Ketone Enolates. *Tetrahedron Lett.* **1981**, *22* (50), 5011-5014.
137. The flavanone was reacted with $CuBr_2$ in $EtOAc/CH_2Cl_2$ under reflux for 2 h and gave 3-bromo flavanone **35a** in 73% yield and a cis/trans ratio of 65:35.
138. Osby, J. O.; Heinzman, S. W.; Ganem, B., Studies on the Mechanism of Transition-Metal-Assisted Sodium-Borohydride and Lithium Aluminum-Hydride Reductions. *J. Am. Chem. Soc.* **1986**, *108* (1), 67-72.
139. Brown, H. C.; Choi, Y. M.; Narasimhan, S., Selective Reductions .29. A Simple Technique to Achieve and Enhanced Rate of Reduction of Representative Organic-Compounds by Borane-Dimethyl Sulfide. *J. Org. Chem.* **1982**, *47* (16), 3153-3163.
140. Clayton, S. E.; Guinot, S. G. R.; Hepworth, J. D.; Wainwright, M., Extended conjugation in di- and tri-arylmethane dyes. Part 4. Steric and electronic effects in analogues of Malachite Green containing a 2H-1-benzopyran unit. *J. Chem. Soc. Perkin Trans. 2* **2000**, (2), 263-269.
141. Boger, D. L.; Yohannes, D., Selectively Protected L-Dopa Derivatives - Application of the Benzylic Hydroperoxide Rearrangement. *J. Org. Chem.* **1987**, *52* (23), 5283-5286.
142. Krenitsky, P. J.; Boger, D. L., Synthesis of the (S,S,S)-diastereomer of the 15-membered biaryl ring system of RP 66453. *Tetrahedron Lett.* **2003**, *44* (21), 4019-4022.
143. Dyrager, C.; Friberg, A.; Dahlen, K.; Friden-Saxin, M.; Borjesson, K.; Wilhelmsson, L. M.; Smedh, M.; Grotli, M.; Luthman, K., 2,6,8-Trisubstituted 3-Hydroxychromone Derivatives as Fluorophores for Live-Cell Imaging. *Chem. Eur. J.* **2009**, *15* (37), 9417-9423.
144. Sonogashira, K.; Tohda, Y.; Hagihara, N., Convenient Synthesis of Acetylenes - Catalytic Substitutions of Acetylenic Hydrogen with Bromoalkenes, Iodoarenes, and Bromopyridines. *Tetrahedron Lett.* **1975**, (50), 4467-4470.
145. Chinchilla, R.; Najera, C., The sonogashira reaction: A booming methodology in synthetic organic chemistry. *Chem. Rev.* **2007**, *107* (3), 874-922.
146. Angeles, A. R.; Neagu, I.; Birzin, E. T.; Thornton, E. R.; Smith, A. B.; Hirschmann, R., Synthesis and binding affinities of novel SRIF-mimicking beta-D-glucosides satisfying the requirement for a pi-cloud at C1. *Org. Lett.* **2005**, *7* (6), 1121-1124.
147. http://www.euroscreen.com/
148. Reubi, J. C., New Specific Radioligand for One Subpopulation of Brain Somatostatin Receptors. *Life. Sci.* **1985**, *36* (19), 1829-1836.
149. http://www.enzolifesciences.com/
150. Dahlen, K.; Grotli, M.; Luthman, K., A scaffold approach to 3,6,8-trisubstituted flavones. *Synlett* **2006**, (6), 897-900.
151. Freedman, T. B.; Cao, X. L.; Dukor, R. K.; Nafie, L. A., Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism. *Chirality* **2003**, *15* (9), 743-758.
152. The computational calculations were performed at AstraZeneca R&D, Mölndal, Sweden.
153. Mcdougal, P. G.; Rico, J. G.; Oh, Y. I.; Condon, B. D., A Convenient Procedure for the Monosilylation of Symmetrical 1,N-Diols. *J. Org. Chem.* **1986**, *51* (17), 3388-3390.
154. This compound was kindly recieved from Dr. Erik Wallén at the Division of pharmaceutical chemistry, University of Helsinki.
155. http://www.millipore.com/
156. Papazisis, K. T.; Geromichalos, G. D.; Dimitriadis, K. A.; Kortsaris, A. H., Optimization of the sulforhodamine B colorimetric assay. *J. Immunol. Methods* **1997**, *208* (2), 151-158.
157. Verkade, J. M. M.; van Hemert, L. J. C.; Quaedflieg, P. J. L. M.; Rutjes, F. P. J. T., Organocatalysed asymmetric Mannich reactions. *Chem. Soc. Rev.* **2008**, *37* (1), 29-41.

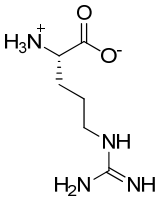
158. Gaunt, M. J.; Johansson, C. C. C.; McNally, A.; Vo, N. T., Enantioselective organocatalysis. *Drug Discov. Today* **2007**, *12* (1-2), 8-27.
159. Dalko, P. I.; Moisan, L., Enantioselective organocatalysis. *Angew. Chem. In. Ed. Engl.* **2001**, *40* (20), 3726-3748.
160. Axén, A.; Grennberg, H.; Gogoll, A., One-pot Synthesis of a 1,3,7,9-Tetraazacyclododecane Derivative and an Investigation of its Complexation Properties. *J. Chem. Res.* **1998**, 712-713.
161. Reacting 5'-bromo-3'-chloro-2'-hydroxyacetophenone and acetoaldehyde with DIPA in EtOH at 170 ^{o}C in MW for 1 h gave the 2-methyl chroman-4-one **80** in 28% yield.
162. Banks, J. L.; Beard, H. S.; Cao, Y. X.; Cho, A. E.; Damm, W.; Farid, R.; Felts, A. K.; Halgren, T. A.; Mainz, D. T.; Maple, J. R.; Murphy, R.; Philipp, D. M.; Repasky, M. P.; Zhang, L. Y.; Berne, B. J.; Friesner, R. A.; Gallicchio, E.; Levy, R. M., Integrated modeling program, applied chemical theory (IMPACT). *J. Comp. Chem.* **2005**, *26* (16), 1752-1780.
163. Cicero, D. O.; Barbato, G.; Bazzo, R., Nmr Analysis of Molecular Flexibility in Solution - a New Method for the Study of Complex Distributions of Rapidly Exchanging Conformations - Application to a 13-Residue Peptide with an 8-Residue Loop. *J. Am. Chem. Soc.* **1995**, *117* (3), 1027-1033.

Appendix

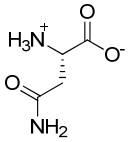
A. Essential amino acids found in proteins



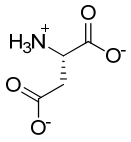
Alanine (Ala, A)



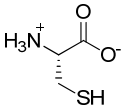
Arginine (Arg, R)



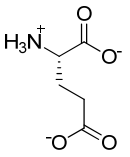
Aspargine (Asn, N)



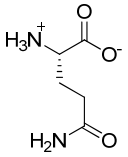
Aspartate (Asp, D)



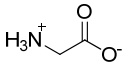
Cysteine (Cys, C)



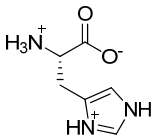
Glutamate (Glu, E)



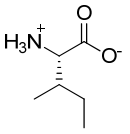
Glutamine (Gln, Q)



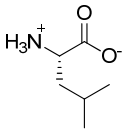
Glycine (Gly, G)



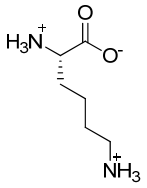
Histidine (His, H)



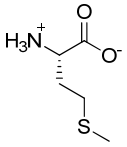
Isoleucine (Ile, I)



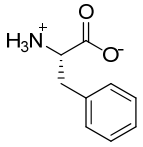
Leucine (Leu, L)



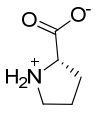
Lysine (Lys, K)



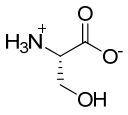
Methionine (Met, M)



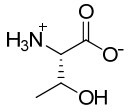
Phenylalanine (Phe, F)



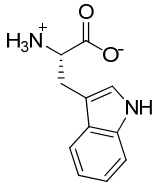
Proline (Pro, P)



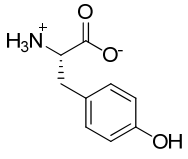
Serine (Ser, S)



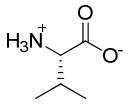
Threonine (Thr, T)



Tryptophan (Trp, W)

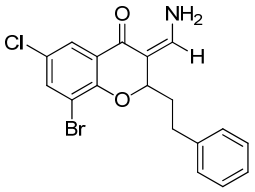


Tyrosine (Tyr, Y)

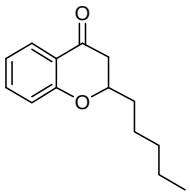


Valine (Val, V)

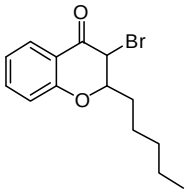
B. Experimental procedures not included in papers I-VI



(*Z*)-3-(Aminomethylene)-8-bromo-6-chloro-2-phenethylchoman-4-one (*Z*-36). 8-Bromo-6-chloro-3-cyano-2-phenethylchromone (**1c**) (25 mg, 0.06 mmol) was dissolved in anhydrous THF (2 ml) and cooled to -78 ºC. DIBAL-H (0.06 ml, 0.06 mmol, 1 M in CH_2Cl_2) was added dropwise to the reaction mixture. After 1 h at -78 ºC a further portion of DIBAL-H (0.06 ml, 0.06 mmol, 1 M in CH_2Cl_2) was added. After 2 h the reaction was quenched with NH_4Cl (sat., aq.) followed by the addition of EtOAc. The aqueous phase was extracted three times with EtOAc and the combined organic phases were washed once with H_2O and twice with brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was finally removed under vacuum. Purification by flash chromatography using EtOAc:heptane (2:8→4:6) gave ***Z*-36** (17 mg, 66%) as a beige solid. Mp 106–108 ºC; ^{1}H NMR ($CDCl_3$) δ 9.26 (br d, *J* = 10.3 Hz, 1H), 7.82 (d, *J* = 2.6 Hz, 1H), 7.63 (d, *J* = 2.6 Hz, 1H), 7.34–7.17 (m, 5H), 6.86–6.80 (m, 1H), 5.25 (br s, 1H), 4.88 (m, 1H), 2.88–2.76 (m, 2H), 2.30–2.19 (m, 1H), 1.87–1.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 180.7, 153.1, 147.3, 140.9, 136.5, 128.6, 128.5, 126.7, 126.1, 125.5, 125.2, 112.3, 102.7, 79.0, 37.4, 31.6; HRMS (ESI-LC/MS) $[M+H]^+$: Calcd for $C_{18}H_{16}BrClNO_2$: 392.0053 Found: 392.0053.

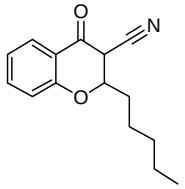


2-Pentylchroman-4-one (16). 2-Pentylchroman-4-one **16** was synthesized from 2-hydroxyacetophenone (2.00 ml, 16.62 mmol) according to the general procedure described in Paper I with the following modifications: The reaction was run for 70 min at 170 ºC in a microwave cavity. The solvent was evaporated and CH_2Cl_2 was added prior the work up. The crude product was purified by flash chromatography using EtOAc:heptane (5:95) as eluent to obtain **16** (1.33 g, 37%) as a pale yellow, viscous liquid. ^{1}H NMR ($CDCl_3$) δ 7.85 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.48-7.41 (m, 1H), 7.02-6.91 (m, 2H), 4.49–4.35 (m, 1H), 2.72-2.60 (m, 2H), 1.94-1.78 (m, 1H), 1.76-1.62 (m, 1H), 1.61-1.18 (m, 6H), 0.90 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 192.5, 161.6, 135.8, 126.8, 121.0, 120.9, 117.8, 77.8, 42.9, 34.8, 31.5, 24.5, 22.4, 13.9; Anal. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31 Found: C, 77.33; H, 8.40.

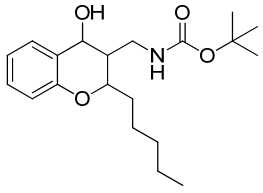


3-Bromo-2-pentylchroman-4-one (16a). 3-Bromo-2-pentylchroman-4-one **16a** was synthesized from **16** (1.21 g, 5.54 mmol) according to the general procedure described in Paper I. The purification by flash chromatography using toluene:heptane (6:4) gave **16a** (1.43 g, 86%) as a slightly yellow viscous liquid in a *syn:anti* ratio of 78:22. ^{1}H NMR ($CDCl_3$) δ 7.96-7.88 (m, 1H), 7.57-7.48 (m, 1H), 7.12-6.97 (m, 2H), 4.62-4.54 (m, *anti*, 0.22H), 4.51 (d, *J* = 6.5 Hz, *anti*, 0.22H), 4.38 (d, *J* = 1.7 Hz, *syn*, 0.78H), 4.17-4.11 (m, *syn*, 0.78H), 2.13-1.99 (m, *syn*, 0.78H), 1.92-1.72 (m, *syn*, *anti*, 1.22H), 1.64-1.25 (m, 6H), 0.92 (t, *J* = 7.0 Hz, 3H);

^{13}C NMR ($CDCl_3$) δ 186.2 (*syn*), 185.6 (*anti*), 160.6 (*syn*), 159.2 (*anti*), 136.7 (*anti*), 136.5 (*syn*), 128.2 (*syn*), 127.9 (*anti*), 122.0 (*syn*), 121.8 (*anti*), 118.6 (*anti*), 118.1 (*syn*), 118.0 (*anti*), 117.8 (*syn*), 81.7 (*anti*), 78.4 (*syn*), 50.7 (*syn*), 50.1 (*anti*), 32.6 (*syn*), 31.8 (*anti*), 31.4 (*syn*), 31.2 (*anti*), 24.7 (*anti*), 24.1 (*syn*), 22.40 (*anti*), 22.36 (*anti*), 13.9 (*syn*, *anti*); HRMS (ESI-LC/MS) $[M+H]^+$: Calcd for $C_{14}H_{18}BrO_2$: 297.0490 Found: 297.0505.

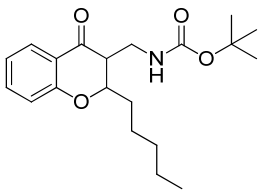


3-Cyano-2-pentylchroman-4-one (16c). 3-Cyano-2-pentylchroman-4-one **16c** was synthesized from **16b** (0.62 g, 2.10 mmol) according to procedures described in Papers I and II. Purification by flash chromatography using EtOAc:heptane (1:9) gave **16c** (0.38 g, 75%) in a *syn:anti* ratio of 18:82 as a colorless oil that crystallized over time. ^{1}H NMR δ 7.95-7.89 (m, 1H), 7.61-7.53 (m, 1H), 7.14-7.00 (m, 2H), 4.59-4.47 (m, *syn*, *anti*, 1H), 3.85 (d, J = 12.0 Hz, *anti*, 0.82H), 3.71 (d, J = 3.1 Hz, *syn*, 0.18H), 2.18-1.92 (m, *syn*, *anti*, 1.82H), 1.91-1.79 (m, *syn*, 0.18H), 1.77-1.29 (m, 6H), 0.97-0.88 (m, 3H); ^{13}C-NMR δ 182.9 (*anti*), 182.3 (*syn*), 160.8 (*anti*), 160.7 (*syn*), 137.5 (*syn*), 137.2 (*anti*), 128.0 (*syn*), 127.8 (*anti*), 122.5 (*anti*), 122.4 (*syn*), 118.9 (*anti*), 118.8 (*syn*), 118.3 (*syn*), 118.0 (*anti*), 113.6 (*anti*), 113.0 (*syn*), 78.5 (*anti*), 77.6 (*syn*), 45.0 (*anti*), 43.1 (*syn*), 33.6 (*anti*), 31.8 (*syn*), 31.3 (*anti*), 31.2 (*syn*), 24.6 (*syn*), 24.0 (*anti*), 22.44 (*anti*), 22.39 (*syn*), 13.94 (*anti*), 13.89 (*syn*); HRMS (FT-ICR-MS) $[M+H]^+$: Calcd for $C_{15}H_{18}NO_2$: 244.1338 Found: 244.1345.

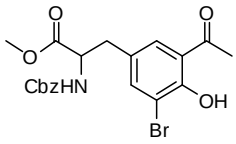


3-*tert*-Butoxycarbonylaminomethyl-4-hydroxy-2-pentylchroman (37). A solution of **16c** (50 mg, 0.2 mmol) in EtOH (99.5%, 10 ml) was hydrogenated using an H-Cube® apparatus (1 ml/min, 10% Pd/C) under 30 bar at 20 °C. The reaction mixture was concentrated under reduced pressure and the alcohol derivative was obtained in quantitative yield according to ^{1}H NMR spectroscopy on the crude product. The crude product (38 g, 0.15 mmol) was dissolved in MeOH/THF (1:1, 2.5 mL) and hydrogenated (1 ml/min, Raney Ni) under 10 bar at 25 °C. The solvent was removed by reduced pressure and the crude primary amine was directly used in next synthesis step without further purification. To a stirred solution of the primary amine (33 mg, 0.13 mmol) in THF (10 ml) TEA (22 μl, 0.16 mmol) and di-*tert*-butyl dicarbonate (35 mg, 0.16 mmol) were added. The reaction mixture was stirred for 20 h at ambient temperature. The solvent was evaporated and water and EtOAc were added. The aqueous phase was extracted five times with EtOAc and the combined organic phases were washed three times with H_2O and brine. Finally, the organic phase was dried over $MgSO_4$, filtered and evaporated to afford the Boc-protected amine as a slightly yellow solid. Purification by flash chromatography using 10% EtOAc:hexane gave pure **37** (30 mg, 41% over three steps) as a single isomer and as a white solid. ^{1}H NMR δ 7.52 (d, J = 7.9 Hz, 1H), 7.10 (t, J = 7.9 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 6.72 (d, J = 8.2 Hz, 1H), 5.03-4.93 (m, 1H), 4.88-4.79 (m, 1H), 4.59 (d, J = 10.3 Hz, 1H),

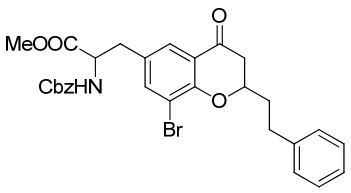
4.23-4.15 (m, 1H), 3.76-3.61 (m, 1H), 3.35-3.25 (m, 1H), 2.14-2.04 (m, 1H), 1.97-1.85 (m, 1H), 1.45-1.29 (m, 5H), 1.18 (s, 9H), 0.95–0.87 (m, 3H); ^{13}C NMR δ 157.2, 154.1, 128.3, 127.9, 125.9, 121.1, 115.8, 79.9, 77.7, 66.6, 41.5, 35.3, 32.4, 31.6, 28.1, 25.6, 22.6, 14.0; HRMS (ESI-LC/MS) $[M+H]^+$: Calcd for $C_{20}H_{32}NO_4$: 350.2331 Found: 350.2330.



3-*tert*-Butoxycarbonylaminomethyl-2-pentylchroman-4-one (38). Hydroxychroman **37** (25 mg, 0.08 mmol) was dissolved in CH_2Cl_2 (4 ml). The solution was transferred to a vial with activated molecular sieves (0.1 g, 3 A). Acetonitrile (0.4 ml, 10%) and *N*-methyl morpholine *N*-oxide (13 mg, 0.11 mmol) were added followed by TPAP (5 mg, 0.01 mmol, 20 mol%). The black reaction mixture was stirred at 25 °C for 6 h. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and the suspension was filtered through Celite. The solvent was removed under reduced pressure. Purification by flash chromatography using 10% EtOAc:hexane gave **38** (17 mg, 68%) as a yellow oil in a *syn*:*anti* ratio of 75:25. ^{1}H NMR δ 7.87-7.81 (m, 1H), 7.52-7.44 (m, 1H), 7.05-6.92 (m, 2H), 5.18-5.02 (m, *anti*, 0.25H), 5.01-4.88 (m,, *syn*, 0.75H), 4.60-4.49 (m, 1.5H), 4.41-4.30 (m, *anti*, 0.25H), 3.76-3.39 (m, 2H), 3.30-3.17 (m, 1H), 2.97-2.85 (m, 1H), 2.78-2.66 (m, 0.25H), 2.06-1.73 (m, 2H), 1.70-1.17 (m, 13H), 0.97-0.78 (m, 3H); ^{13}C NMR δ 194.7, 161.1, 160.5, 155.9, 155.8, 155.8, 136.3, 136.2, 127.0, 126.9, 121.2, 121.1, 120.4, 120.2, 118.0, 117.9, 79.6, 79.5, 79.3, 50.6, 49.8, 37.0, 36.4, 32.4, 31.4, 31.3, 29.7, 29.6, 28.3, 25.3, 24.3, 22.6, 22.4, 14.0, 13.9; HRMS (ESI-LC/MS) $[M+H]^+$: Calcd for $C_{20}H_{30}NO_4$: 348.2175 Found: 348.2175.



Methyl 3-(3-acetyl-5-bromo-hydroxyphenyl)-2-(benzyloxycarbonylamino)propanoate (42). NBS (0.17 g, 0.96 mmol) was dissolved in MeCN (2 mL) and cooled to 0 °C. Compound **41** (0.36 g, 0.96 mmol) was dissolved in MeCN (6 mL) and was added to the NBS solution. The mixture was allowed to reach rt and was stirred overnight. The reaction was quenched with water and EtOAc. The phases were separated and the organic phase was washed with water and brine, dried over $MgSO_4$, filtered and the solvent was finally removed under vacuum. Purification by flash chromatography 15→20% EtOAc:toluene gave **42** (0.36 g, 82%) as a white solid. Mp 151-152 °C; ^{1}H NMR δ 12.8 (s, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.45 (bs, 1H), 7.39-7.27 (m, 5H), 5.39 (br d, J = 8.0 Hz, 1H), 5.14 (d, J = 12.2 Hz, 1H), 5.04 (d, J = 12.2 Hz, 1H), 4.64 (q, J = 6.6 Hz, 1H), 3.75 (s, 3H), 3.13 (dd, J = 5.5, 14.1 Hz, 1H), 2.98 (dd, J = 6.5, 14.1 Hz, 1H), 2.53 (s, 3H); ^{13}C NMR δ 204.0, 171.5, 157.9, 155.5, 140.2, 135.9, 130.4, 128.5, 128.3, 128.0, 127.3, 120.1, 111.9, 67.1, 54.6, 52.5, 37.1, 26.6; HRMS (Q-TOF-MS) $[M+2K+H]^+$: Calcd for $C_{20}H_{21}BrK_2NO_6$: 527.9826 Found: 528.1637.



Methyl 2-benzyloxycarbonylamino-3-(8-bromo-4-oxo-2-phenethylchroman-6-yl)propanoate (43). Compound **42** (0.10 g, 0,23 mmol), 3-phenylpropanal (33 mg, 0.25 mmol) and DIPA (0.025 g, 35 uL, 0.25 mmol) in MeOH (2.5 mL) were mixed according to the procedure described in Paper I. Purification by flash chromatography 10→40% EtOAc:heptane gave **43** (92 mg, 72%) as a yellow oil. ^{1}H NMR δ 7.58 (t, *J* = 2.4 Hz, 1H), 7.53 (s, 1H), 7.42-7.24 (m, 9H), 7.24-7.18 (m, 1H), 5.33 (br d, *J* = 7.7 Hz, 1H), 5.17-5.04 (m, 2H), 4.62 (q, *J* = 5.9 Hz, 1H), 4.49-4.38 (m, 1H), 3.75 (s, 3H), 3.18-2.85 (m, 4H), 2.78-2.61 (m, 2H), 2.35-2.19 (m, 1H), 2.06-1.85 (m, 1H); ^{13}C NMR δ 191.2, 171.5, 156.9, 140.6, 128.6, 128.5, 128.2, 128.0, 126.2, 121.9, 77.2, 67.1, 52.6, 42.6, 36.4, 30.9; HRMS (Q-TOF-MS) [M-CO_2Me+H_2O]: Calcd for $C_{27}H_{28}BrNO_5$: 525.1151 Found: 525.1210.