Development of a UV-Cleavable Protecting Group for Hydroxylamines, Synthesis of a StructurallyWide Variety of Hydroxamic Acids, and Identification of Histone Deacetylase Inhibitors

Mortensen, Kim Thollund; Qvortrup, Katrine; Nielsen, Thomas Eiland

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Ph.D. Thesis

Kim Thollund Mortensen

March 2017

Technical University of Denmark
Department of Chemistry

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Ph.D. Supervisor
Katrine Qvortrup, DTU
Ph.D. Co-supervisor
Thomas Eiland Nielsen, Novo Nordisk

Preface

The work presented in this thesis was carried out during my Ph.D. studies at Technical University of Denmark (DTU), the first year under the supervision of former Professor Thomas Eiland Nielsen (Director at Novo Nordisk), and subsequently under the supervision of Senior Researcher Katrine Qvortrup. A six-month external stay was carried out under the supervision of Assistant Professor Liang Yang and Research Director Professor Michael Givskov at the Singapore Centre for Environmental Life Sciences Engineering (SCELSE), and Professor Choon-Hong Tan at the Division of Chemistry and Biological Chemistry at the School of Physical and Mathematical Sciences, Nanyang Technological University (NTU).

The dissertation covers four projects and illustrates a multidisciplinary Ph.D. divided into three Chapters. In Chapter 2, the development of an UV-labile protecting group for hydroxylamines is presented: its use for the synthesis of *N*-alkylated hydroxamic acids and an orthogonality study. The synthesis of *N*-alkylated hydroxamic acids, is an extension of my master project, where the methodology was developed. Chapter 3 describes the development of a synthetic approach towards histone deacetylase inhibitors. The inhibitors are based on a sub-class selective substrate previously found. The histone deacetylase enzymes are important for the regulation of a variety of diseases, especially cancer. We wanted to use this information to transform the substrate into an inhibitor. The research covered in Chapter 4 was carried out in collaboration with the Yang and Tan group at NTU, Singapore, to synthesize itaconimides with novel quorum sensing inhibitor properties.

During my Ph.D. I have been involved in other projects, which I have decided not to include in this dissertation. The projects comprise of a synthesis of novel oligopeptides as efflux pump inhibitors, synthesis of pyrazolones, a total synthesis of Desferal, and the synthesis of 2-mercaptobenzothiazole building blocks for quorum sensing inhibitors.

Kim Thollund Mortensen Date

Abstract

Photo-cleavable protecting groups are highly applicable for the synthesis of structural complex and sensitive compounds, including biological important molecules. Herein, we present the development of a novel *O*-hydroxylamine photo-cleavable protecting group, based on the methyl-6-nitroveratryl moiety. We demonstrate the application of the protected hydroxylamine derivative for the synthesis of *N*-alkylated hydroxamic acids. We have shown that the construct is stable toward a diverse set of reaction conditions, as well as orthogonal with conventional protection groups. The *O*-protected hydroxylamine derivative was applied to synthesize a small collection of *N*-alkylated hydroxamic acids as inhibitors of the histone deacetylase enzymes, an important class of enzymes for the treatment of a range of diseases, most importantly cancer.

During my external stay at Nanyang Technological University, Singapore, I worked on a project with the aim of synthesizing compounds that target the quorum sensing network in *Pseudomonas aeruginosa*, important for the treatment of bacterial infections. The structure was based on a recent found hit compound, by our collaborators in Singapore, showing high activity.

Resumé

Lysfølsomme beskyttelsesgrupper er attraktive til syntese af komplekse, sensitive kemiske forbindelser, heriblandt biologisk interessante molekyler. Dette projekt præsenterer udviklingen af en ny *O*-hydroxylamin lyskløvbar beskyttelsesgruppe baseret på methyl-6-nitroveratryl gruppen. Vi demonstrerer anvendelsen af det beskyttede hydroxylamin derivat til dannelse af *N*-alkylerede hydroxamsyrer. Vi demonstrerer, at denne gruppe er stabil overfor forskellige reaktionsbetingelser, så vel som ortogonal overfor konventionelle beskyttelsesgrupper. *O*-Beskyttet hydroxylamin derivatet blev brug til at syntetisere en stofsamling af *N*-alkylererede hydroxamsyrer med det formål at identificere selektive histon deacetyltransferase enzymer inhibitorer. Histon deacetyltransferase en vigtig klasse af enzymer for behandlingen af en række lidelser, ikke mindst kræft.
Under mit udlandsophold på Nanyang Teknologiske Universitet, Singapore, arbejdede jeg på et projekt, hvor målet var at fremstille forbindelser, som inhiberer quorum sensing netværket i *Pseudomonas aeruginosa*. Sådanne stoffer er vigtige til behandling af bakterielle infektioner. Den strukturelle opbygning af inhibitorerne var baseret på en forbindelse, som vores samarbejdspartner for nylig identificerede.

Acknowledgment

To my primary supervisor, Katrine Qvortrup, thank you being a huge inspiration. Your ability to see new directions and get involved in non-familiarized research areas is most notable. This you also show in your eager to make new multi-collaborative projects. Thanks, for the daily conversations and guidance throughout the years.
I would like to thank former Professor Thomas Eiland Nielsen (Director at Novo Nordisk) for accepting me as a Ph.D. in his group of highly talented people. How Thomas was able to keep track of all his + 20 ongoing projects and continued to come up with new ideas and guide us, was truly impressive. Furthermore, his help and commitment during my external stay in Singapore is much appreciated.
A thanks should go to all the former members of the Nielsen group. The level of talented, research-driven people will be difficult to encounter again. A special thanks to Casper Lykke Hansen, Ph.D., Thomas Flagstad, Ph.D., Peng Wu, MD, and Tina Gustafsson, laboratory assistant, for the all the professional discussions, guidance and help over the years.
Furthermore, I would like to address a special thanks to Professor Michael Givskov and Assistant Professor Liang Yang for hosting me in Singapore and being a great help to the Danish Viking in warm, humid Singapore. I would also want to thanks Mingjun Yuan and July Fong for a great time in the labs and the other members of the Yang group for making my stay a pleasure and highly educational, especially Ph.D. student T. W. Keong.
I would like to thanks Professor Mads Hartvig Clausen for inviting me to his group meetings with fruitful discussions and ideas over the last 1.5 year and Professor David Tanner for his guidance and open-door policy.
A huge thanks to the NMR team, Charlotte Held Gotfredsen, Associated Professor, Casper Hoeck, Ph.D., Jens Duus, Professor, Kasper Enemark-Rasmussen, AC-TAP, and Anne Hector, lab technician, for always being helpful to set-up new experiments, vital inputs over the years, and making the NMR run smoothly.
A great thanks should go out to the whole technical staff of building 211, Philip Charlie Johansen, Brian Dideriksen, Emma Burnæs, Johanne Marie Nielsen and former staff member Brian Ekman-Gregersen for keeping the whole machinery running without any problems, they are truly appreciated.
Knud Højgaard, Oticon, and PA Fiskers Fond are all appreciated for supporting my external stay financially.
Also thanks to Daniel Madsen for proof-reading Chapter 3.

Finally, I would like to acknowledge friends and family for their support over the last 3.5 years. A special thanks should be addressed to Anders Mørk for his great help proof-reading this dissertation, and for always being there and recreational activities over the years, for which I hope there will be many more in the future.
I would like to thank Christine Kinnaert for her huge support through the last 1.5 year, her belief in me, forcing me out of the lab occasionally, and for proof-reading this dissertation. I would not have been able to perform at this level, without her help and support.
Finally, I would like to thank my mom and dad for their care and support and trying to understand what I am doing. Looking forward to spend much more time together in the future with the rest of the family and my little niece.

List of Abbreviation

Ac	acetyl
AHL	*N*-acyl homoserine lactone
All	allyl
Alloc	allyloxycarbonyl
AMC	7-amino-4-methylcoumarin
aq.	aqueous
ATP	adenosine triphosphate
BHL	*N*-butanoyl-*L*-homoserine lactone
Bn	benzyl
Boc	*tert*-butoxycarbonyl
br.	broad
BTC	bis-(trichloromethyl)carbonate
c	concentration
Cbz	benzyloxycarbonyl
CFDA	China Food and Drug Administration
COSY	correlation spectroscopy
CTCL	cutaneous T-cell lymphoma
d	doublet
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DCVC	dry column vacuum chromatography
DDQ	dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	*N*,*N*-diisobutylaluminium hydride
DIC	*N*,*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DHP	3,4-dihydro-2*H*-pyran
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deutrated dimethyl sulfoxide
DTU	Technical University of Denmark
equiv.	equivalent
ESI	electrospray ionization

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EWG	electron-withdrawing group
FDA	United States Food and Drug Administration
Fmoc	9-fluorenylmethoxycarbonyl
FT	fourier transform
gfp	green fluorescent protein
h	hour(s)
H2BC	heteronuclear 2 bond correlation
HDAC	histone deacetylase
HDACi	HDAC inhibitor
HDLP	histone deacetylase-like protein
HAT	histone acetyltransferases
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HFIP	hexafluoroisopropyl alcohol
HMBA	4-(hydroxymethyl)benzoic acid
HMBC	heteronuclear multiple bond correlation
HMDS	hexamethyldisilazane
HRMS	high resolution mass spectrometry
HOBt	hydroxybenzotriazole
HSQC	heteronuclear single quantum coherence
HSQC-TOCSY	heteronuclear single quantum coherence-total correlation spectroscopy
Hz	hertz
IC_{50}	half maximal inhibitory concentration
*i*Pr	isopropyl
IR	infrared spectroscopy
m	multiplet
Me	methyl
MEF2	myocyte enhancer factor-2
MeNV	methyl-6-nitroveratryl
MIC	minimum inhibitory concentration
MITR	MEF2-interacting transcription repressor
MOM	methoxymethyl

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mp.	melting point
MRSA	methicillin resistant *staphylococcus aureus*
Ms	mesyl
MSNT	1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4- triazole
NAD^+	nicotinamide adenine dinucleotide
N-CoR	nuclear receptor corepressor
NEM	*N*-ethylmorpholine
NGO	non-governmental organization
NMI	*N*-methylimidazole
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
NTU	Nanyang Technological University
OD_{600}	optical density at 600 nm
OSu	*O*-succinimide
p	pentet
P. aeruginosa	*Pseudomonas aeruginosa*
PEGA	polyethylene glycol polyacrylamide
PG	protecting group
Ph	phenyl
ppm	parts per million
PREP-HPLC	preparative high-performance liquid chromatography
PPTS	pyridinium *p*-toluenesulfonate
PTCL	peripheral T-cell lymphomas
PTM	post-translational modification
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	quartet
quant.	quantitative
QS	quorum sensing
QSi	QS inhibitor
rt	room temperature
s	singlet
SAHA	suberoylanilide hydroxamic acid

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sat.	saturated
SCELSE	Singapore Centre for Environmental Life Sciences Engineering
sept	septet
SMRT	silencing mediator for retinoid and thyroid receptor
SPS	solid-phase synthesis
UPLC-MS	ultra performance liquid chromatograph mass spectrometer
UPLC	ultra performance liquid chromatograph
t	triplet
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBTU	*O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate
*t*Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TSA	trichostatin A
UV	ultra violet
WHO	World Health Organization
ZBG	zinc-binding group
δ	chemical shift

Publications

Part of this Dissertation

- Mortensen, K. T.; Olsen, L. B.; Møller, K. J.; Rasmussen, M. B. R.; Møller, S. S.; Nielsen, T. E.; Qvortrup, K. Development of a Photo-labile Protecting Group for the release of *N*-Alkyl Hydroxamic Acids, a total synthesis of Deferoxamine B, manuscript in preparation

- Mortensen, K. T.; Madsen, D.; Qvortrup, K. High-Throughput Profiling of Substrates for Histone Deacetylase Enzymes and the Development of Sub-Class Selective Inhibitors, manuscript in preparation

- Mortensen, K. T.; Fong, J.; Santos, M. M. S.; Chua, S. L.; Qvortrup, K.; Yang, L.; Nielsen, T. E.; Givskov, M. Itaconimides as Novel Quorum Sensing Inhibitors of *Pseudomonas aeruginosa*, manuscript in preparation

Not a part of this Dissertation

- Fong, J.; Yuan, M.; Jakobsen, T. H.; Mortensen, K. T.; Santos, M. M.S. D.; Chua, S. L.; Yang, L.; Tan, C.; Nielsen, T. E.; Givskov, M. Disulfide Bond-containing Ajoene Analogues as Novel Quorum Sensing Inhibitors of *Pseudomonas aeruginosa*. *J. Med. Chem.* **2017**, *60*, 215–227.

- Mortensen, K. T.; Keong, T. W.; Chin, J.; Le Quement, S. T.; Qvortrup, K.; Yang, L.; Nielsen, T. E.; Givskov, M. Peptide-based Efflux Pump Inhibitors Potentiate Antibiotics against Gram-Negative Bacteria, manuscript in preparation

- Mikkelsen, R. J. T.; Mortensen, K. T.; Nielsen, T. E.; Qvortrup, K. Photolabile Linkers for Solid-Phase Organic Synthesis, manuscript in preparation

Included in Appendix

- Bang, C. G.; Jensen, J. F.; Court, E. O.; Olsen, L. B.; Siyum, S. G.; Mortensen, K. T.; Poulsen, T. S.; Berthelsen, J.; Givskov, M.; Qvortrup, K.; Nielsen,T. E. Solid-Phase Synthesis of Hydroxamic Acids and Identification of HDAC Inhibitors Using a Chemiluminescence-based Assay, submitted to ACS Combinatorial Science

Contents

Chapter 1

Introduction

Hydroxamates are an important class of compounds owing to their presence in numerous biologically active molecules. Therefore, the implication of chemical strategies for high-throughput generation of structurally diverse hydroxamic acids is self-evident. Hydroxamic acids represent the largest class of histone deacetylase inhibitors. These compounds are important because histone acetylation affects both chromatin packing and recruitment of transcription factors and thus affecting gene expression in the cell. Several potent histone deacetylase inhibitors have entered clinical trials for treatment of various types of cancer. Furthermore, individual histone deacetylase enzymes have also been linked to a variety of non-cancerous diseases, including neurodegenerative disorders, chronic pain, and cystic fibrosis, as well as affect learning and memory. Therefore, substrates for efficient and accurate profiling of histone deacetylase subtypes are highly desirable. The present dissertation aims to develop mild and reliable chemical strategies for high-throughput synthesis of a broad range of structurally diverse hydroxamic acids. While, hydroxamic acids may be obtained by the use of acid- or base-cleavable protecting groups, these strategies are not compatible with structurally elaborate hydroxamic acids. The application of strong acids and bases with these strategies limit the range of chemical transformations that are applicable for the synthesis of hydroxamic acids and other protecting group principles are necessary to provide a further dimension to the chemical diversity. Completely orthogonal protection systems are only offered with relatively few sets of cleavage conditions, photolysis being one of them. Furthermore, photolytic cleavage offers a chemoselective and mild method of cleavage, which is particularly attractive when synthesizing highly functionalized biologically active compounds, which may not be tolerant of more harsh conditions, such as acidic- or basic-labile or metal-catalyzed hydrogenation. A key goal of the project will be to develop a hydroxamic acid photo-cleavable protecting group for the synthesis of highly functionalized biologically relevant hydroxamic acids.

Chapter 2

Development of a Hydroxylamine Photo-labile Protecting Group for the Synthesis of Hydroxamic Acids

2.1 Introduction

Protecting groups (PGs) are a vital tool in organic chemistry, but also pose several disadvantages. In order to synthesize molecules with various functional groups, the ability to block and unblock reactivity in a controlled way is essential. However, two steps are added to the total step count, the protection and the deprotection. The protecting group will limit the type of chemistry that can be conducted on the scaffold, as conditions that will result in deprotection cannot be employed. Furthermore, the desired end product need to be stable towards the deprotection conditions.
UV-light removable protecting groups pose several advantages such as mild release of functionalized molecules and high orthogonality to most functional groups. The development of PGs which are cleaved upon UV-exposure, so-called photo-labile protecting group s, have been employed for several decades. The mild photolytic cleavage conditions have been utilized to protect anything from adenosine triphosphate (ATP) ("caged ATP")[2] to highly complex molecules showcased by the K. C. Nicolaou[3] synthesis of Calicheamicin-γ-1^I. In the Nielsen group and Qvortrup group, photo-labile protecting groups have been developed for the synthesis of bioactive molecules.
For an introduction to photo-labile protecting groups and an overview of the diverse photo-mediated deprotection strategies, a review written by Petr Klán is recommended.[4]
The *ortho*-nitrobenzyl group have attracted much attention in the last decades. We utilized this scaffold to produce an *O*-photo protected hydroxylamine for the generation of protected hydroxamates which liberates hydroxamic acids upon UV-light exposure. Hydroxamic acids have attracted much attention as inhibitors of metalloenzymes involved in the regulation of cancer. The biological importance of the hydroxamic acids are explained in greater detail in Chapter 3.

2.2 The Use of *ortho*-Nitrobenzyl as an Efficient Photo-labile Protecting Group

"Release" from *ortho*-nitrobenzyl compounds were reported already in the beginning of the 20th century.[5] This was further investigated by Barltrop and Schofield, who worked on the *ortho*-nitrobenzyl alcohol and showed the release of carboxylic acids under mild conditions from the corresponding esters.[6] However, the authors experienced low yields and the formation of a by-product. The photolytic by-product, *ortho*-nitroso benzaldehyde self-condensate which decreased the accessibility of the UV-light. The authors quickly realized that this could be prevented by introducing a substitution in the benzylic position, to afford the less reactive *ortho*-nitroso ketone by-product. As well the introduction of a carbamate group to selectively release amines proved to be advantageous.

Woodward employed the 6-nitroveratryloxycarbonyl (NVOC) and 2-nitrobenzyloxycarbonyl (NBOC), Figure 2.1, to protect and liberate amine functionalities.[7] However, the photo-release performed poorly. This was rationalized to be a consequence of the undesired imine formation between the nitroso benzaldehyde by-product and the amino group. To prevent this, again a substitution in the benzylic position showed to be beneficial. The introduction of two methoxy groups in the phenyl ring, **MeNV**, provided an improved energy absorption profile with a rapid deprotection.[8]

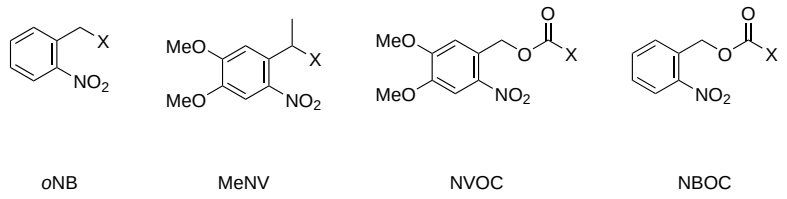
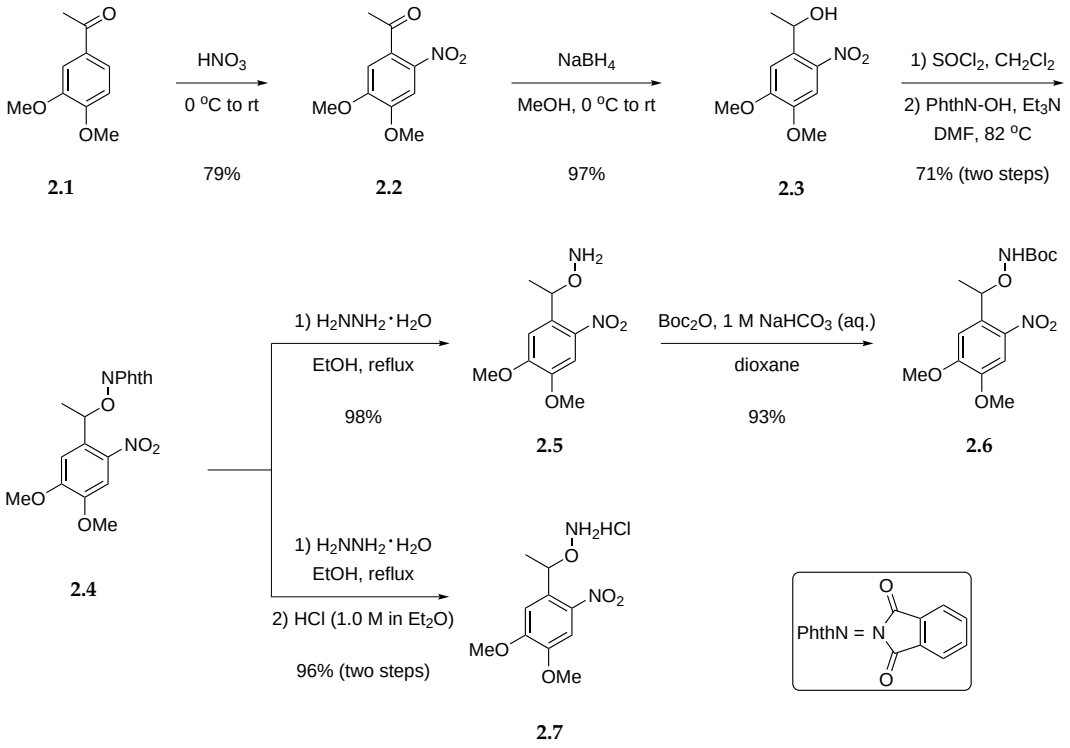


Figure 2.1: Literature reported UV-removable protecting groups

Knowledge from previous work gave inspiration for a protecting group useful in this work.[9–12] In this study a photo-protected hydroxylamine derivative was developed based upon **MeNV**, Figure 2.1. In my master project, we aimed to apply this photo-labile group for a synthesis of a broad range of hydroxamic acids. The aim was to synthesize and evaluate *N*-alkylated hydroxamic acids based on the construct of suberoylanilide hydroxamic

acid (SAHA) and furthermore, to use a photo-labile protecting group for the synthesis of diverse hydroxamic acids. Two papers were published last year by two different groups, one with the photo-protection of SAHA and the other with the synthesis of *N*-alkylated SAHA analogues.[13,14]

O-MeNV hydroxylamine **2.5** was previously developed in the Nielsen group.[12] The synthesis commenced from the commercially available 3'-4'-dimethoxyacetophenone **2.1**, Scheme 2.1. The nitration performed in high yield (79%) using HNO_3[15] to give **2.2**, which was further reduced to the alcohol **2.3** with $NaBH_4$ in excellent yield (97%). Chlorination of the resulting alcohol, and introduction of the hydroxylamine moiety was performed in high yield over two steps (72%). This afforded the *N*-hydroxylamine phthalimide **2.4** in a few steps without the need of flash column chromatography starting from a cheap starting material. The phthalimide group was removed by hydrazine to the free *O*-MeNV hydroxylamine **2.5**, or precipitated as its *O*-MeNV hydroxylamine hydrochloride salt **2.7**. Moreover, the Boc-protected *O*-MeNV hydroxylamine **2.6** could also be obtained in excellent yield (93%).

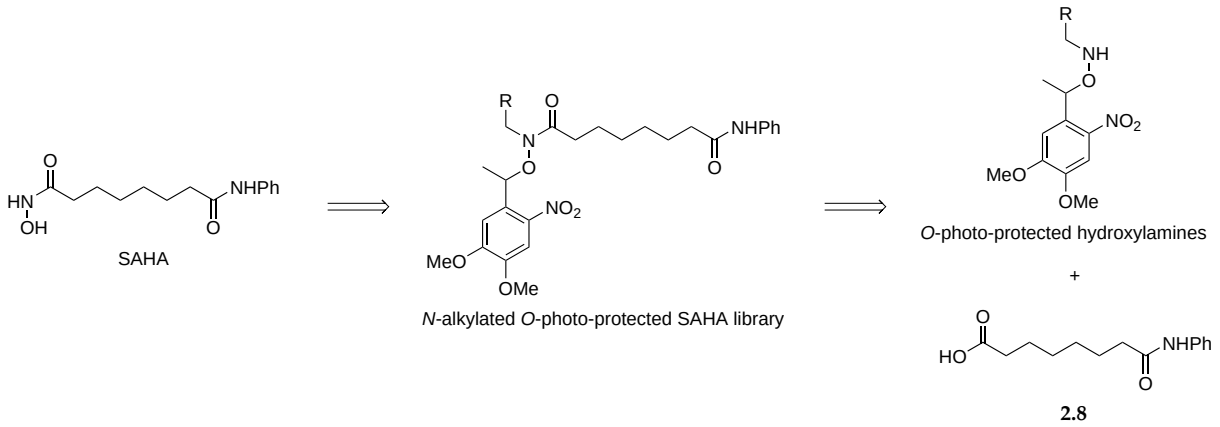


Scheme 2.1: Synthesis of *O*-hydroxylamine protecting group

During my Ph.D. I chose to precipitate the crude photo-protected *O*-hydroxylamine derivative **2.5** as its hydrochloride salt, which was easier to handle.

2.2.1 Synthesis of *N*-Alkylated Hydroxamic Acids

Hydroxamic acids are important bioactive compounds that has shown inhibition activity against histone deacetylase (HDAC) enzymes. Unregulated activity of these enzymes has been linked to various cancer types. Biological importance of HDACs and their role in the epigenetic machinery is described in Chapter 3. SAHA was the first United States Food and Drug Administration (FDA) approved hydroxamic acid for treatment of cutaneous T-cell lymphoma (CTCL). SAHA is recognized as a pan-inhibitor, which may be the reason for the observed adverse effects. We wanted to explore the effects of introducing *N*-alkylated hydroxamic acids, as we hoped it would result in higher selectivity profile. The synthetic strategy is illustrated in Scheme 2.2.



Scheme 2.2: Synthetic overview for *N*-alkylated hydroxamic acids

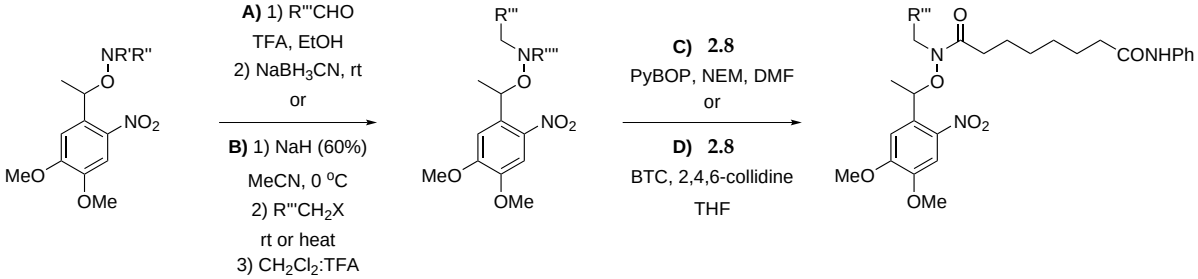
Suberanilic acid **2.8** was synthesized using a reported procedure by Mai *et al.*[16] To obtain the desired *N*-alkylated hydroxylamines, we first investigated the reductive alkylation approach using commercially available aldehydes and ketones. During my master project, I prepared mono-alkylated *O*-MeNV hydroxylamines by a one-pot approach. Oximes were *in situ* generated from the hydroxylamine **2.5** with aldehydes and ketones under acidic conditions and subsequently reduced with the addition of $NaBH_3CN$. The pH is crucial for selective mono-alkylation.[17]

The oxime formation and the following reduction of the oxime performed well with aliphatic aldehydes and ketones. However, we experienced that aryl oximes were difficult to reduce. When using benzaldehyde, we had to add several portions of $NaBH_3CN$ (5.0 equiv. in total) to obtain full conversion. Therefore, we changed the strategy for the synthesis of substituted *N*-benzylated hydroxylamines to use a direct alkylation strategy from *N*-Boc *O*-MeNV hydroxylamine **2.6**. Maria Birgitte Routhe Rasmussen and Kasper Juul Møller joined the group and helped me to extend the substrate scope of the *N*-alkylated SAHA analogues by a reductive alkylation approach with aliphatic aldehydes or a direct alkylation approach with substituted benzyl halides or *O*-mesylates (Ms). All the reactions are summarized in Table 2.1.

To obtain the *N*-alkylated hydroxamic acids, the *N*-alkylated *O*-MeNV hydroxylamines were acylated with suberanilic acid **2.8**. When the *O*-MeNV hydroxylamines were alkylated, if not a Me group, it was impossible to obtain efficient acylation with the standard

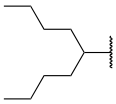
coupling reagent, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP). BTC (bis-(trichloromethyl)carbonate) has been described to facilitate reactions on hindered substrates or substrates where standard SPS coupling reagents were found to be inefficient.[18–20] BTC is also known under the name triphosgene, due to the conversion into three phosgene molecules. An overview of all the synthesized SAHA analogues are shown in Table 2.1.

Table 2.1: Overview of *N*-alkylated SAHA compound collection

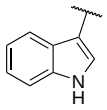
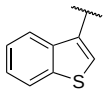
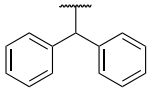


Entry	R′	R″	X	Alkylation	R‴ A or B	R⁗	Yield (%)	Acylation C or D	Yield (%)
1	H	H	-	-[a]	H	H	73	**C**	69
2	H	H	-	**A**[b]		H	86	**D**	77
3[c]	H	H · HCl	-	**A**[d]		H	71	**D**	54
4[c]	H	H · HCl	-	**A**[d,e]	F_3C	H	50	**D**	51
5	H	H	-	**A**		H	68	**D**	93
6[c]	H	H · HCl	-	**A**[d]		H	29	**D**	74
7[c]	H	H · HCl	-	**A**[d,e]		H	87	**D**	57
8[c]	H	H · HCl	-	**A**[d]	O	H	63	**D**	73
9[f]	Boc	H	Br	**A**	O	H	87	**D**	61
10[f]	Boc	H	Br	**A**	O O	H	69	**D**	70
11	H	H	-	**A**		H	67	**D**	88
12	H	H	-	**A**[b]		H	97	**D**	69

continued on next page

Entry	R′	R″	X	Alkylation	R‴ A or B	R⁗	Yield (%)	Acylation C or D	Yield (%)
13	H	H	-	**A**[b]		H	88	**D**	54
14	H	H	-	**A**		H	74	**D**	91
15	H	H	-	**A**		H	97	**D**	63
16[c]	H	H · HCl	-	**A**[d,e]		H	85	**D**	76
17[c]	H	H · HCl	-	**A**[d,e]		H	79	**D**	60
18[c]	H	H · HCl	-	**A**[d,e]		H	76	**D**	84
19[c]	H	H · HCl	-	**A**[d,e]		H	60	**D**	50
20	H	H	-	**A**		H	89	**D**	69
21[g]	Boc	H	Br	**B**		H · TFA	83	**D**	43
22[g]	Boc	H	Br	**B**		H · TFA	81	**D**	62
23[g]	Boc	H	OMs[h]	**B**		H · TFA	88	**D**	66
24[g]	Boc	H	OMs[i]	**B**		H · TFA	89	**D**	54
25[g]	Boc	H	Cl[j]	**B**		H · TFA	67	**D**	45

continued on next page

Entry	R′	R″	X	Alkylation	R‴ A or B	R⁗	Yield (%)	Acylation C or D	Yield (%)
26[g]	Boc	H	OMs[h]	**B**		H · TFA	81	**D**	74
27[g]	H	H	-	**A**		H	43	**D**	79
28	Boc	H	Br[h]	**B**		H · TFA	55[k]	**D**	26

Procedure **A** (reductive alkylation): aldehyde and **2.5** or **2.7** were mixed together in EtOH with TFA. Corresponding oxime was reduced with the addition of $NaBH_3CN$. Procedure **B** (direct alkylation): *N*-Boc *O*-MeNV hydroxylamine **2.6** was alkylated under NaH-conditions with an alkylating agent. Boc-Deprotection: Boc-protected hydroxylamines were dissolved in CH_2Cl_2 and added TFA. Procedure **C** (acylation): the *N*-methyl *O*-MeNV hydroxylamine was acylated with suberanilic acid **2.8** mediated by PyBOP and NEM in DMF. Procedure **D** (acylation): the *N*-alkylated *O*-MeNV hydroxylamines were acylated with suberanilic acid **2.8** mediated by BTC and 2,4,6-collidine in THF (a) oxime formation utilizing a literature procedure.[21] (b) oxime formation was performed under reflux (c) performed by Maria Birgitte Routhe Rasmussen (d) heat was applied to obtain full conversion to the oxime (e) oxime formation carried out at 50 °C (f) made in the collaboration with Katrine Qvortrup (g) performed by Kasper Juul Møller (h) from the commercially available alcohol and mesylated[22] (i) obtained by reduction of the commercially available aldehyde and reduced with $NaBH_4$ in EtOH and mesylated[22] (j) from the commercially available hydrochloride salt (k) refluxed for 3 days

We have previously observed that photolytic cleavage of this type of compounds result in a mixture of the hydroxamic acid and the corresponding amide (unpublished results). During my master thesis, I show that acceptable selectivity for the hydroxamic acid over the amide could be obtained with a solvent mixture of *i*PrOH:1 M HCl (aq.) (10:1). Using acidic conditions limits the substrate scope and therefore, the goal was to find non-acidic solvent mixtures affording hydroxamic acid selectivity during photolytic cleavage.

2.3 Investigation of the *O*-MeNV Hydroxylamine Protecting Group Stability

To demonstrate the compatibility of the photo-labile protecting group, we synthesized a library of compounds, Table 2.4 and Table 2.5, containing various conventionally used protecting groups, Table 2.3, and demonstrated selective removal of one protecting group over another.

Table 2.3: Applied conventional protecting groups

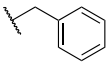
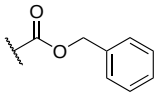
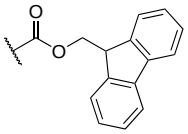
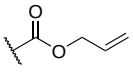
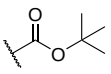
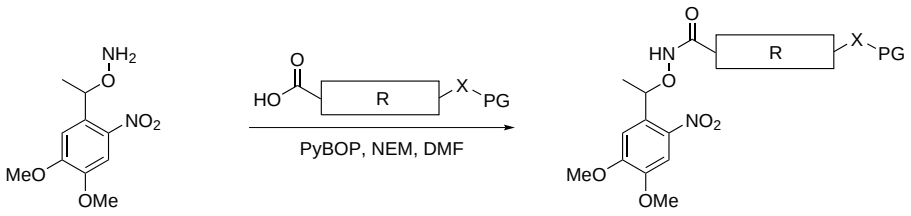
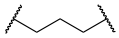
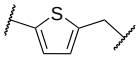
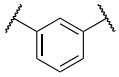
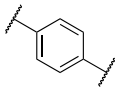
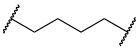
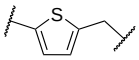
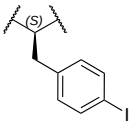
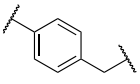
Entry	PG	Abbreviation	Structure
1	benzyl	Bn	
2	allyl	All	
3	methoxymethyl	MOM	
4	acetyl	Ac	
5	*tert*-butyldimethylsilyl	TBS	
6	tetrahydropyranyl	THP	
7	benzyloxycarbonyl	Cbz	
8	9-fluorenylmethoxycarbonyl	Fmoc	
9	allyloxycarbonyl	Alloc	
10	*tert*-butoxycarbonyl	Boc	

Table 2.4: Synthesis of hydroxamates containing conventional protecting groups

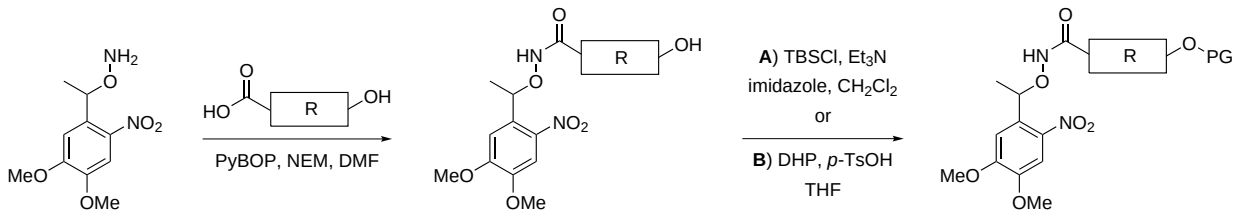


2.5

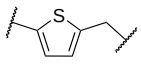
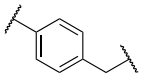
Entry	Carboxylic acid	R	X	PG	Product	Yield (%)
1	**2.14**		O	Bn	**2.15**	91
2	**2.16**		O	All	**2.17**	78
3	**2.18**		O	MOM	**2.19**	80
4	**2.20**		O	Ac	**2.21**	67
5	**2.22**		NH	Cbz	**2.23**	84
6	**2.24**		NH	Fmoc	**2.25**	73
7	**2.26**		NH	Alloc	**2.27**	
8	**2.28**		NH	Boc	**2.29**	95

To obtain the TBS and THP protected compounds, 5-(hydroxymethyl)thiophene-2-carboxylic acid and 4-(hydroxymethyl)benzoic acid (HMBA) were coupled to the protecting group **2.5** resulting in the hydroxy-functionalized hydroxamates. The hydroxy group was afterwards protected by TBSCl or 3,4-dihydro-2*H*-pyran (DHP), Table 2.5.

Table 2.5: Synthesis of TBS- and THP-containing hydroxamates



2.5

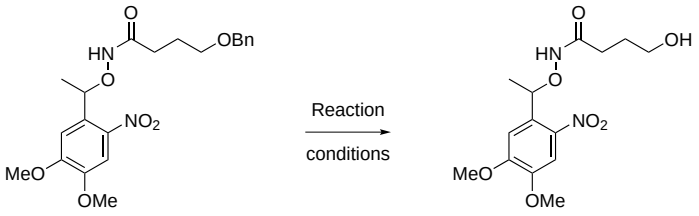
Entry	R	Alcohol	Yield	PG	Protected alcohol	Yield
1		**2.30**	67%	TBS	**2.31**	75%
2		**2.32**	27%	THP	**2.33**	51%

With all the compounds in hand, we investigated the orthogonality of the *O*-MeNV protected hydroxamates. Boc, THP, TBS, Fmoc, Ac, and MOM were removed by standard conditions, TFA in CH_2Cl_2, 10% HCl (aq.) in THF, TBAF in THF, 5% piperidine in DMF, K_2CO_3 in MeOH, and TFA in CH_2Cl_2, respectively. Alloc was found to be selectively removed by $Pd(PPh_3)_4$ and pyrrolidine in CH_2Cl_2.[23]

2.3.1 Selective Removal of Benzyl Ethers

Protection of hydroxy groups with a benzyl group is one of the most applied protecting groups in organic chemistry.[24] The tested deprotection for the testing of the removal of the benzyl ether protecting group are listed in Table 2.6.

Table 2.6: Overview of tested clevage conditions for Bn



2.15 **2.34**

Entry	Reaction conditions	Conversion (**2.15**:**2.34**)[a]
1[25]	TFA	100:00[b]
2[26]	1) TFA, Ac_2O	-[c]
	2) NaOMe, MeOH	
3[27]	$FeCl_3$, CH_2Cl_2, 0 °C	-[c]
4[28]	LiCl, DMF, 160 °C	-[c]
5[29]	$Pd(OH)_2/C$ (0.5 equiv.), *i*PrOH, reflux	100:00
6[30]	1) CSI, Na_2CO_3, CH_2Cl_2, -78 °C	-[c]
	2) NaOMe, MeOH	
7	$Pd(OH)_2/C$ (2.0 equiv.), EtOH:H_2O (9:1), reflux	00:100
8[31]	BCl_3, CH_2Cl_2, -78 °C	100:00
9[32]	DDQ, H_2O:CH_2Cl_2 (1:2)	00:100
10[33]	$AlCl_3$, $PhNMe_2$, CH_2Cl_2	100:00
11[34]	$PPh_3 \cdot HBr$, MeCN, reflux	-[c]
12[35]	*t*$BuNH_2$, *t*BuOH, K	-[c]
	10-crown-6, THF	
13	10% Pd/C (10 mol%), H_2, MeOH	-[d]
14	$MeSO_3H$, $CHCl_3$	-[c]
15	6 M HCl (aq.), reflux	-[c]
16[36]	catechol boron bromide	100:00[b]
17[37]	TMSI, thiourea, NMI, MeCN	-[c]

(a) observed by LCMS (b) **2.15** and impurities (c) complex reaction mixture without the desired product (d) chemoselective reduction of the nitro group was observed

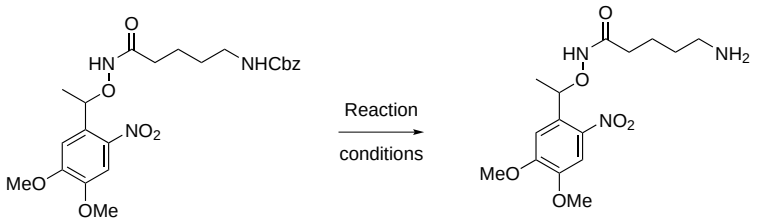
The photo-labile protecting group did not withstand several of the tested procedures. The use of Pearlman's catalyst was first shown to cleave benzyl ethers in 1981 by Hanessian and co-workers[38] under catalytic transfer hydrogenation with cyclohexene as a hydrogen donor.[39] Later, Prugh *et al.* showed that the reaction could proceed in the absence of a hydrogen donor.[40] In our case, adding small amounts of water to the reaction mixture turned

out to be highly advantageous and afforded the free hydroxy group chemoselectively (Entry 7). We also tested several non-hydrogenation conditions and found that oxidative cleavage by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded the formation of **2.34** (Entry 9).

2.3.2 Selective Removal of Benzyl Carbamate

Other well-explored protecting groups are based on the carbamates functionality, for example the Cbz group, which turned out to be more difficult to selectively remove. All the tested conditions are listed in Table 2.7.

Table 2.7: Overview of tested cleavage conditions for Cbz



2.23 **2.35**

Entry	Reaction conditions	Conversion (**2.23**:**2.35**)[a]
1[41]	$AlCl_3$, anisole	100:00
2	40% (wt:wt) KOH (aq.), MeOH	-[d]
3[42]	30% HBr, AcOH	-[b]
4	$Pd(OH)_2/C$ (1.0 equiv.), *i*PrOH, reflux	100:00
5[43]	*t*BuNH_2, MeOH, reflux	100:00
6[44]	TFA, reflux	00:100
7	10% Pd/C (10 mol%), H_2, MeOH	-[c]
8	$Pd(OH)_2/C$ (1 equiv), EtOH:H_2O (9:1), 90 °C	100:00
9	6.0 M KOH (aq.):EtOH (1:1), reflux	-[e]
10	10% Pd/C (10 mol%), NH_4COOH, MeOH	100:00
11	10% Pd/C (10 mol%), Et_3SiH, MeOH	-[c]
12[45]	6 M HCl (aq.), reflux	-[c]
13	6 M HCl (aq.)	-[f]
14	25% NH_4OH	100:00
15	10% Pd/C (10 mol%), H_2, EtOAc	-[c]
16	*i*PrOH, reflux	100:00
17	catechol boron bromide	-[c]

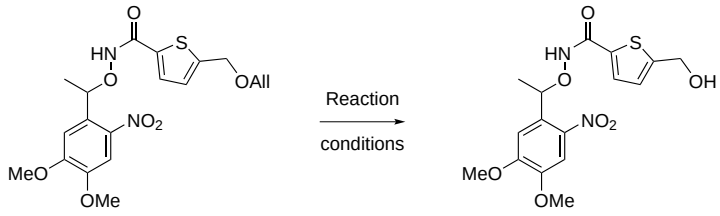
(a) observed by LCMS (c) complex reaction mixture without the desired product (d) methyl carbamate was observed (b) impure mixture of by-products and **2.35** (e) impure mixture of by-products and ethyl ester was observed (f) hydrolysis to the corresponding photo-protected hydroxylamine **2.5**

The Cbz group has been shown to be both acid- and base-labile. When we tried to use standard acidic conditions with 30% HBr in AcOH, the desired amine **2.35** was observed along with by-products. Changing to TFA at reflux led to fast deprotection to the desired product (Entry 6). Several basic conditions were tested. A transformation was observed with 40% (wt:wt) KOH (aq.) in MeOH (Entry 2). However, this turned out to be conversion to the corresponding methyl carbamate.

2.3.3 Selective Removal of Allyl Ether

Another ether protecting group is the All group. Isomerization and cleavage conditions were tested. However, no reaction was observed, Entries 1-2 Table 2.8. Conditions using $Pd(OH)_2/C$ was tested and resulted in selectively removal of the All group to afford the free hydroxy group, Entries 5 and 6.

Table 2.8: Overview of tested clevage conditions for All



2.17 **2.36**

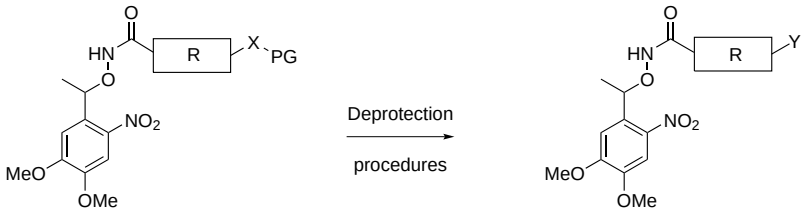
Entry	Reaction conditions	Conversion (**2.17**:**2.36**:)[a]
1[46]	*t*BuOK, DMSO, 100 °C H_2SO_4	100:00
2[46]	*t*BuOK, DMSO, 100 °C NaOH, $KMnO_4$	100:00
3	$Pd(PPh_3)_4$, pyrrolidine, CH_2Cl_2	100:00
4[47]	$Pd(PPh_3)_4$, K_2CO_3, MeOH	100:00
5[29]	$Pd(OH)_2/C$ (0.5 equiv.), *i*PrOH, reflux	27:73[b]
6	$Pd(OH)_2/C$ (1.1 equiv.), *i*PrOH, reflux	00:100[b]

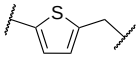
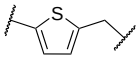
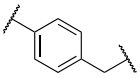
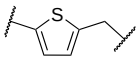
(a) observed by LCMS (b) no reaction was observed (c) complex reaction mixture

2.3.4 Up-scaling the Selective Removal of Conventional Protecting Groups

A few of the successful conditions were performed on a larger scale for NMR characterization, Table 2.9. To avoid the use of high-boiling DMF for the highly polar amine, Et_2NH in MeCN was used for Fmoc deprotection.[48] The investigation showed that the protecting group is stable towards a variety of reaction conditions.

Table 2.9: Large scale cleavage of selected conventional protecting groups

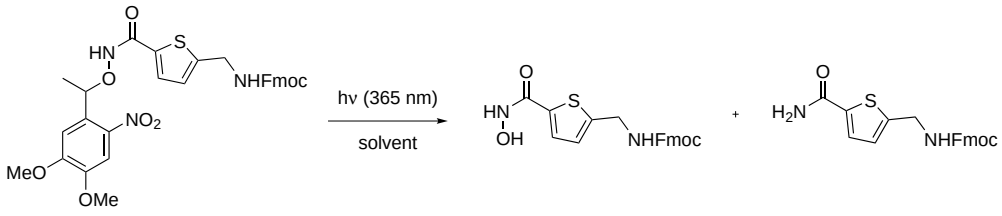


Entry	SM	R	X	Conditions	PG	Y	Product	Yield
1	**2.17**		O	All	$Pd(OH)_2/C$, *i*PrOH, reflux	OH	**2.37**	52%
2	**2.31**		O	TBS	TBAF, THF	OH	**2.38**	82%
3	**2.33**		O	THP	10% HCl (aq.), THF	OH	**2.39**	87%
4	**2.25**		NH	Fmoc	Et_2NH, MeCN	NH_2	**2.40**	quant.

2.3.5 Photolysis Study to Release a broad range of Hydroxamic Acids

To identify conditions that allowed for the selective photolytic release of hydroxamic acids, the hydroxamate **2.25** was used as a model compound. The previous established conditions, *i*PrOH:1 M HCl (aq.) (10:1), afforded acceptable selectivityof the *N*-alkylated hydroxamic acids over the amide, but the acidity of these conditions, limit the substrate scope, and therefore a study was conducted to find milder conditions, Table 2.10.

Table 2.10: Solvent screen for selective liberation of hydroxamic acid



2.25 **2.41** **2.42**

Entry[a]	Solvent	Concentration (nM)	Reaction time (h)	Conversion (**2.25**:**2.41**:**2.42**)[b]
1[c]	*i*PrOH	28.5	10.0	42:28:30
2[c]	*i*PrOH:H_2O (10:1)	28.5	10.0	48:26:26
3[c]	*i*PrOH:0.01 M HCl (aq.) (10:1)	28.5	50.5	00:52:48
4[c]	*i*PrOH:0.1 M HCl (aq.) (10:1)	28.5	5.0	29:36:35
5[c]	*i*PrOH:0.1 M HCl (aq.) (19:1)	28.5	26.0	21:49:30
6[c]	*i*PrOH:0.5 M HCl (aq.) (10:1)	28.5	10.0	13:45:42
7[49]	MeCN	14.2	7.5	18:42:42
8[50]	MeOH	4.0	9.5	00:78:22
9[51]	dioxane	10.0	3.5	00:36:64
10	THF	14.0	3.5	00:24:76
11	THF	28.5	5.0	00:25:75
12	THF	57.0	5.5	40:26:34
13[3]	THF:H_2O(10:1)	7.6	5.0	00:63:37
14	THF:H_2O(4:1)	8.0	6.0	7:79:14
15	THF:H_2O(1:1)	28.5	2.0	68:16:16
16	THF:*p*-TSOH $\cdot$ H_2O[d]	28.5	5.0	00:32:68
17	THF:0.01 M HCl (aq.) (10:1)	2.8	2.0	11:72:17
18[52]	mesitylene	28.5	19.0	44:00:56
19[52]	HFIP:mesitylene (1:1)[c]	28.5	5.0	42:58:00
20[52]	HFIP[e,f]	7.1	5.0	6:91:3
21[52]	HFIP[e,f]	14.0	5.5	19:78:3
22[52]	HFIP[e,f]	28.5	5.5	54:46:2

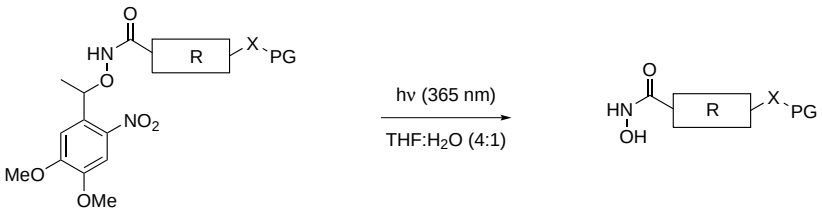
(a) all reactions conducted on 5 mg scale. Several impurities observed by LCMS could not be assigned (b) observed by LCMS (d) a couple of seeds (c) conducted on 10 mg scale (e) new significant peak appeared (f) highly volatile, over an extended time period HFIP evaporates

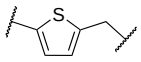
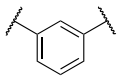
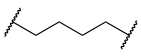
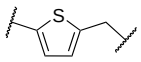
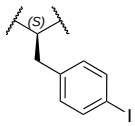
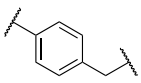
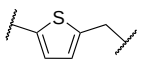
When HCl (aq.) was used in combination with *i*PrOH, a preference for the hydroxamic acid was observed (Entry 1-6 Table 2.10). We found that THF:H_2O gave high selectivity (Entry 14). Moreover, we found high selectivity with HFIP (Entry 20-22), but with the

formation of a side-product was observed.

As for the conventional protecting groups, the photolysis was scaled up from 5-10 mg to 50 mg scale reactions. A total of seven compounds were liberated by UV-radiation and due to the high polarity of the free hydroxamic acid, PREP-TLC was used for purification. Generally, excellent selectivity was obtained with acceptable yields, Table 2.11.

Table 2.11: Large scale liberation of hydroxamic acid



Entry	SM	R	X	PG	Product	Yield
1	**2.17**		O	All	**2.43**	31%
2	**2.19**		O	MOM	**2.44**	27%
3	**2.23**		NH	Cbz	**2.45**	19%
4	**2.25**		NH	Fmoc	**2.46**	32%
5	**2.27**		NH	Alloc	**2.47**	20%
6	**2.29**		NH	Boc	**2.48**	57%
7	**2.31**		O	TBS	**2.49**	34%

2.4 Conclusion

UV-cleavable groups have been used for a vast amount of functionalities over several decades. Their inherent mild cleavage conditions have made them useful in many applications, among these caging of ATP. During my master project, I developed a photolabile group, methyl-6-nitroveratryl, for *O*-protection of hydroxylamine allowing for the synthesis of *N*-alkylated SAHA analogues in great yields. In this project, we wanted to investigate the orthogonality of this *O*-hydroxylamine protecting group. Rewardingly, the newly developed protecting group showed high stability, therefore we were able to selectively remove a broad range of commonly used protecting groups. Furthermore, the protecting group could be removed without affecting the protecting groups. Pearlman's catalyst proved to be efficient for removal of both Bn, **2.15**, and All, **2.17**. Furthermore, conventional acid/base-labile protecting groups were removed by standard conditions. We also worked on developing suitable mild photolytic cleaving conditions. We demonstrated that this group allowed for the chemoselective photolytic release of either hydroxamates or carboxamates. The bi-detachable mode of the protecting group, may simply be controlled by the choice of solvent. Hydroxamic acids obtained by performing photolysis in THF:H_2O (4:1). Whereas, photolysis in mesitylene or THF enables the release of carbaxamides. Using the developed conditions we synthesized seven hydroxamic acids having a broad structural diversity in good yields (19-57%).

2.5 Experimental Section

2.5.1 General Methods

All solvents were of HPLC quality from SigmaAldrich, and all commercially available reagents were used without further purification. Dry THF, CH_2Cl_2, Et_2O, MeCN, toluene, DMSO and DMF were obtained from a PureSolv^{TM} MD-7 Solvent Purification System, Innovative Technology with Al_2O_3 as stationary phase. Dry alcoholic solvents and $CHCl_3$ were obtained by using preheated 3 Åmol sieves. Et_3N and pyridine was kept dry by storage over KOH pellets.

For TLC, Merck aluminium sheets covered with silica gel C-60 F_{254} were used and developed using UV-light or a suitable stain. Flash chromatography was performed using a glass column packed with Merck Geduran 60 silica gel (40-63 µm particles) as stationary phase. For dry column vacuum chromatography (DCVC)[53] the dry column was packed with Merck Geduran 60 silica gel (15-40 µm particles). All liquid phases are specified in experimental procedures.
PREP-TLC purification was performed with Merck silica gel 60 C-60 F_{254} (10-12 µm particles) plates with a coating thickness of 250 µM.

All novel compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, IR, R_f , and when appropriate UPLC-MS (ESI), optical rotation, and/pr melting point were measured. HRMS sample was obtained for a representative collection of novel compounds. For all known compounds on ^{1}H-NMR is listed with a reference to reported values.
NMR spectra were recorded on either a Varian Mercury spectrometer operating at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR or a Bruker Ascend spectrometer with a Prodigy cryoprobe operating at 400 MHz for ^{1}H-NMR and 101 MHz for ^{13}C-NMR. For all compounds containing the photo-labile protecting group, ^{13}C-NMR was performed with a delay of at least 3 seconds, compared to a standard 1.2 seconds, and 512-2048 scans due to low or no observed intensity. HSQC, HMBC, H2BC, $NOESY_{400}$, and HSQC-TOCSY experiments were used to verify the structures when ^{1}H-, ^{13}C-NMR, and COSY were inadequate. The specific deuterated solvent is stated for each compound. Chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz.

IR analysis was performed on a Bruker Alpha FT-IR spectrometer from 4000 cm^{-1} to 400 cm^{-1} of a neat sample.

Analytic UPLC-MS analysis was run on a Waters AQUITY UPLC system equipped with PDA and SQD electrospray MS detector. Column: Kinetex 1.7 µm XB-C18, 2.1 x 50mm. Column temperature: 50 °C. Flowrate: 0.6 mL/min. Solvent A: 0.1% HCOOH in H_2O, Solvent B: 0.1% HCOOH in MeCN. Gradient: 5% B to 100% B in 2.4 min, hold 0.1 min, total run time 2.6 min. Analytical LC-HRMS (ESI) analysis was performed on an Agilent 1100 RP-LC system equipped with a diode array detector using a Phenomenex Luna C-18 column (*d* 3 µm, 2.1 x 50 mm; column temp: 40 °C; flow: 0.4 mL/min). Eluents A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in MeCN) were used in a linear gradient (20% B to 100% B) in a total run time of 15 min. The LC system was coupled to a Micromass LCT orthogonal time-of-flight mass spectrometer equipped with a Lock Mass probe operating in positive electrospray mode.
Melting points were measured using a Stuart SMP30 melting point apparatus. Photo cleavage was performed in a custom made aluminium box with a Omnilux UV-lamp 400W E-40 irradiating blue light at 360 nm.
Optical rotation was measured on a Perkin-Elmer 341polarimeter (polarimeter cell, 1.0 mL, 100 mm), with a Na-lamp (589.3 nm, 22 °C).

2.5.2 Synthetic Procedures

General Procedure: Direct Alkylation

In an oven-dried round-bottomed flask containing a magnetic stirring bar, NaH (60% in oil) was portionwise added to a cold solution of *N*-Boc protected hydroxylamine **2.6** in MeCN (0.2 M) under an inert atmosphere. When gas evolution stopped, the solution was stirred for 10 min and the alkyl halide was added in one portion. The reaction mixture was stirred at reflux. The reaction mixture was cooled and quenched by the addition of cold H_2O. The aqueous solution was extracted with CH_2Cl_2 (3 x) and the combined organic organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The compound was purified by flash chromatography.

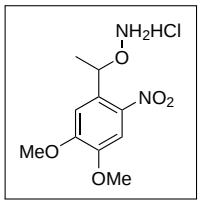
General Procedure: Coupling Reagent Acylation

In a round-bottomed flask containing a magnetic stirring bar, coupling reagent and base were added to a solution of carboxylic acid in DMF. The carboxylic acid was activated before hydroxylamine was added to the stirring solution. The reaction mixture was transferred to a separation funnel with H_2O and EtOAc. The organic layer was washed according to the description for each reaction and dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified either by flash chromatography or recrystallization.

General Procedure: Photo-Mediated Deprotection

In a small glass jar containing a magnetic stirring bar, photo-protected hydroxamic acids were dissolved in the listed cleavage mixture. A quartz lid was placed on top of the glass jar and stirred in a aluminium box under a UV-lamp at 365 nm. Due to elevated temperatures and long reaction time, additionally cleavage mixture was added. The desired hydroxamic acid was purified according to the description for each reaction.

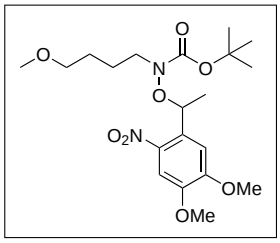
***O*-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)hydroxylamine hydrochloride salt (2.7)**



In a round-bottomed flask containing a magnetic stirring bar, $H_2NNH_2 \cdot H_2O$ (70%, 1.0 mL, 20.1 mmol, 1.2 equiv.) was added to a gray suspension of *N*-hydroxylamine phthalimide **2.4** (5.00 g, 13.4 mmol, 1.0 equiv.) in EtOH (120.0 mL) and stirred at reflux. When full conversion was observed (3 h), the reaction mixture was cooled, and the precipitate was removed by filtration. The solution was evaporated under reduced pressure. The residues were dissolved in EtOAc and precipitation occurred one more time. The precipitate was removed by filtration and the solution was evaporated under reduced pressure. The dark brown oil was dissolved in Et_2O (150.0 mL) and vigorously stirred during the addition of HCl (1.0 M in Et_2O, 33.6 mL, 33.6 mmol, 2.0 equiv.). To ensure full precipitation, the gray solution was stirred for 30 min further. The hydrochloride salt **2.7** was collected by filtration and washed with cold Et_2O and placed under vacuum to afford it as a gray solid (3.56 g, 95%). R_f = 0.78 (CH_2Cl_2:MeOH (19:1); UV); IR (neat) ν (cm^{-1}): 3145, 2938, 2848, 2791, 2619, 1619, 1590, 1544, 1520, 1456, 1335, 1274; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.85 (br. s, 3H, ONH_3Cl), 7.62 (s, 1H, Ar**H**), 7.18 (s, 1H, Ar**H**), 5.74 (q, *J* =

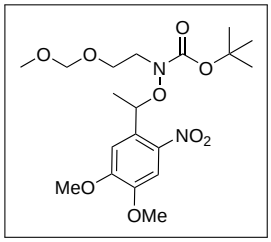
6.3 Hz, 1H, ArC**H**CH$_3$), 3.98 (s, 3H, OC**H$_3$**), 3.88 (s, 3H, OC**H$_3$**), 1.56 (d, *J* = 6.3 Hz, 3H, ArCHC**H$_3$**), ^{13}C-NMR (101 MHz, DMSO-*d$_6$*): δ 153.6, 148.1, 140.3, 130.3, 108.8, 107.8, 77.8, 56.23, 56.17, 21.3; MS (ESI) *m/z*: calcd. for $C_{10}H_{12}NO_4^+$ $[M + H]^+$ 210.1, found 210.0.

***tert*-Butyl (1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)(4-methoxybutyl)carbamate (2.9)**



According to the general procedure for direct alkylation, NaH (60% in oil, 79.4 mg, 1.99 mmol, 1.7 equiv.) was added to a cold solution of **2.6** (400 mg, 1.17 mmol, 1.0 equiv.) in MeCN (5.8 mL). 1-Bromo-4-methoxybutane (188 µL, 1.40 mmol, 1.2 equiv.) was added and stirred at reflux overnight. Incomplete conversion was observed by TLC and LCMS, additional NaH (60% in oil, 46.7 mg, 1.17 mmol, 1.0 equiv.) was added to the cooled reaction mixture followed by 1-bromo-4-methoxybutane (157 µL, 1.17 mmol, 1.0 equiv.) and left overnight. The title compound **2.9** was purified by aqueous work-up and flash chromatography (EtOAc:heptane 3:7) providing as a green oil (435 mg, 87%). Trace amount of CH_2Cl_2 was detected by H^1-NMR. R_f = 0.24 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 2977, 2935, 2867, 2830, 1702, 1581, 1457, 1335, 1271; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.53 (s, 1H, Ar**H**), 7.41 (s, 1H, Ar**H**), 5.63 (q, *J* = 6.4 Hz, 1H, ArC**H**CH$_3$), 4.02 (s, 3H, OC**H$_3$**), 3.94 (s, 3H, OC**H$_3$**), 3.47–3.21 (m, 7H, C**H$_3$**OC**H$_2$**CH$_2$CH$_2$C**H$_2$**NC(O)), 1.63–1.44 (m, 16H, 3 x C**H$_3$**, ArCHC**H$_3$**, CH$_3$OCH$_2$C**H$_2$**C**H$_2$**CH$_2$N); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 156.9, 153.5, 148.1, 140.6, 133.9, 109.7, 107.4, 81.7, 77.4, 72.4, 58.7, 56.6, 56.5, 50.0, 28.3, 27.0, 23.7, 21.7; MS (ESI) *m/z*: calcd. for $C_{20}H_{32}N_2NaO_8^+$ $[M + Na]^+$ 451.2, found 451.1.

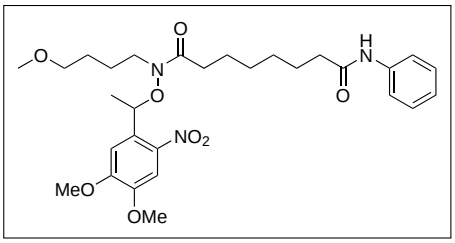
***tert*-Butyl (1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)(4-methoxybutyl)carbamate (2.10)**



According to the general procedure for direct alkylation, NaH (60% in oil, 79.4 mg, 1.99 mmol, 1.7 equiv.) was added to a cold solution of **2.6** (400 mg, 1.17 mmol, 1.0 equiv.) in MeCN (5.83 mL). 1-Bromo-2-(methoxymethoxy)ethane (163.7 µL, 1.40 mmol, 1.2 equiv.) was added and stirred at reflux overnight. Incomplete conversion was observed by TLC and LCMS, additional NaH (60% in oil) (46.7 mg, 1.17 mmol, 1.0 equiv.) was added to the cooled reaction mixture followed by 1-bromo-2-(methoxymethoxy)ethane (136 µL, 1.12 mmol, 1.0 equiv.) and refluxed overnight.

The title compound **2.10** was purified by aqueous work-up and flash chromatography (EtOAc:heptane 3:7) providing it as a green oil (349 mg, 69%). Trace amount of CH_2Cl_2 was detected by H^1-NMR. R_f = 0.24 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 2977, 2935, 2867, 2830, 1702, 1581, 1457, 1335, 1271; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.54 (s, 1H, Ar**H**), 7.46 (s, 1H, Ar**H**), 5.66 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 4.56 (s, 2H, $CH_3OC\mathbf{H_2}OCH_2$), 4.03 (s, 3H, $OC\mathbf{H_3}$), 3.94 (s, 3H, $OC\mathbf{H_3}$), 3.75–3.47 (m, 4H, $CH_3OCH_2OC\mathbf{H_2}C\mathbf{H_2}$NHC(O)O), 3.32 (s, 3H, $C\mathbf{H_3}OCH_2OCH_2$), 1.58 (d, *J* = 6.4 Hz, 3H, ArCHC$\mathbf{H_3}$), 1.44 (s, 9H, 3 x $C\mathbf{H_3}$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 157.3, 153.6, 148.0, 140.6, 134.0, 109.8, 107.4, 96.4, 81.8, 77.3 (overlap with $CDCl_3$), 63.4, 56.6, 56.5, 55.3, 50.3, 28.3, 21.7; MS (ESI) *m/z*: calcd. for $C_{19}H_{30}N_2NaO_9^+$ $[M + Na]^+$ 453.2, found 453.2.

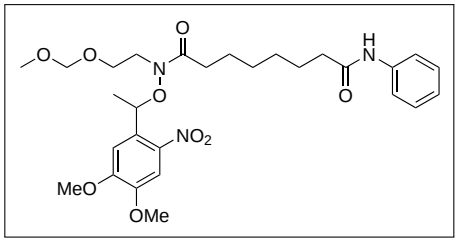
N_1-(1-(4,5-Dimethoxy-2-nitrophenyl)ethoxy)-*N_1*-(4-methoxybutyl)-*N_8*-phenyl-octanediamide (2.11)



According to the general procedure for Boc-deprotection, **2.9** (417 mg, 0.97 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (1:1) (9.8 mL) for 1.33 h. The crude TFA amine was placed under vacuum. According to the general procedure for BTC acylation, in a flame-dried round-bottomed flask, 2,4,6-collidine (900 µL, 6.81 mmol, 7 equiv.) was dropwise added over 2.0 h to a solution of BTC (202 mg, 0.68 mmol, 0.7 equiv.) and suberanilic acid **2.8** (315 mg, 1.27 mmol, 1.3 equiv.) in dry THF (24.5 mL) followed by the addition of the crude TFA amine in THF (5 mL) and stirred overnight. The organic phase was washed with H_2O (2 x 100 mL), sat. $NaHCO_3$ (aq.) (2 x 100 mL), 1 M HCl (aq.) (3 x 100 mL), and brine (2 x 100 mL). The title compound was purified by flash column chromatography (EtOAc:heptane 1:1) to afford **2.11** (330 mg, 61%) as a green semi solid. R_f = 0.08 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 3307, 2933, 2858, 1661, 1599, 1518, 1440, 1334, 1271; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1H, C(O)N**H**Ph), 7.59–7.54 (m, 3H, Ar**H**), 7.30–7.25 (m, 3H, Ar**H**), 7.01 (t, *J* = 7.4 Hz, 1H, Ar**H**), 5.44 (q, *J* = 6.5 Hz, 1H, ArC**H**CH_3), 3.94 (s, 3H, $OC\mathbf{H_3}$), 3.86 (s, 3H, $OC\mathbf{H_3}$), 3.56–3.49 (m, 1H, $CH_2C\mathbf{H'}H''NC(O)CH_2$), 3.21–3.16 (m, 5H, $C\mathbf{H_3}COCH_2CH_2$, $CH_3OC\mathbf{H_2}CH_2$), 3.05–2.97 (m, 1H, $H_2CH_2CH'\mathbf{H''}NC(O)CH_2$), 2.30–2.26 (m, 4H, $C\mathbf{H_2}CH_2$, $C\mathbf{H_2}C(O)$), 1.60–1.24 (m, 15H, $OCH_2C\mathbf{H_2}C\mathbf{H_2}CH'H''NC(O)$, $NC(O)CH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}CH_2C(O)NHPh$,

ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.3, 171.2, 152.9, 148.2, 140.7, 139.3, 130.6, 128.6, 122.9, 119.0, 109.7, 107.3, 77.5, 71.4, 57.7, 56.2, 56.1, 46.3 36.4, 31.9, 28.5, 28.4, 26.2, 25.0, 24.1, 23.3, 20.9; MS (ESI) *m/z*: calcd. for $C_{29}H_{42}N_3O_8^+$ $[M + H]^+$ 560.3, found 560.3.

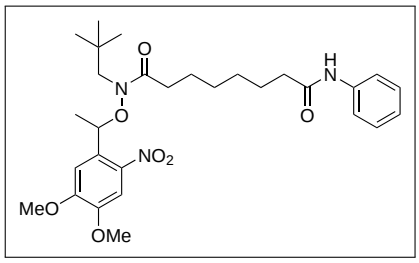
N$_1$-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-*N*$_1$-(2-(methoxymethoxy)ethyl)-*N*$_8$-phenyloctanediamide (2.12)



According to the general procedure for Boc-deprotection, **2.10** (337.4 mg, 0.78 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (9:11) (5.9 mL) for 1.0 h at 0 °C. The crude TFA amine was placed under vacuum.

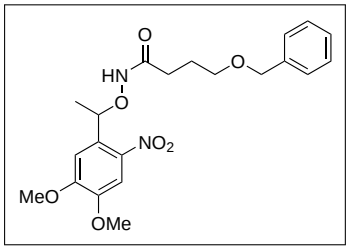
According to the general procedure for BTC acylation, in a flame-dried round-bottomed flask, 2,4,6-collidine (725 µL, 5.49 mmol, 7 equiv.) was dropwise added over 2.0 h to a solution of BTC (163 mg, 0.55 mmol, 0.7 equiv.) and suberanilic acid **2.8** (254 mg, 1.01 mmol, 1.3 equiv.) in dry THF (15.0 mL) followed by the addition of the crude TFA amine in THF (7 mL) and stirred overnight. The organic phase was washed with H_2O (2 x 100 mL), sat. $NaHCO_3$ (aq.) (2 x 100 mL), 1 M HCl (aq.) (3 x 100 mL), and brine (2 x 100 mL). The title compound was purified by flash column chromatography (EtOAc:heptane 1:1) to afford **2.12** 309 mg, 70%) as a green semi solid. R_f = 0.50 (EtOAc; UV); IR (neat) ν (cm^{-1}): 3308, 2933, 2857, 1661, 1599, 1518, 1440, 1334, 1271; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.82 (s, 1H, C(O)N**H**Ph), 7.59–7.54 (m, 3H, Ar**H**), 7.32–7.25 (m, 3H, Ar**H**), 7.02–6.99 (m, 1H, Ar**H**), 5.49 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 4.45 (s, 2H, $CH_3OC\mathbf{H_2}O$), 3.93 (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.69 (m, 1H, $OCH_2C\mathbf{H'}H''NC(O)$), 3.46 (m, 2H, $OC\mathbf{H_2}CH_2NC(O)$), 3.36–3.29 (overlap with H_2O) (m, 1H, $OCH_2CH'\mathbf{H''}NC(O)$), 2.32–2.26 (m, 4H, $NC(O)C\mathbf{H_2}CH_2$, $C\mathbf{H_2}C(O)NHPh$), 1.59–1.37 (m, 7H, ArCHC**H$_3$**, $NC(O)CH_2C\mathbf{H_2}CH_2CH_2C\mathbf{H_2}CH_2$), 1.29–1.19 (m, 4H, $NC(O)CH_2CH_2C\mathbf{H_2}C\mathbf{H_2}CH_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.6, 171.2, 152.9, 148.1, 140.7, 139.3, 130.6 (HMBC), 128.6, 122.9, 119.0, 109.8 107.2, 95.5, 77.4, 62.8, 56.2, 56.1, 54.5, 36.4, 31.9, 28.5, 28.3, 25.0, 24.1, 20.9; MS (ESI) *m/z*: calcd. for $C_{28}H_{40}N_3O_9^+$ $[M + H]^+$ 562.3, found 562.3.

N_1-(1-(4,5-Dimethoxy-2-nitrophenyl)ethoxy)-N_1-neopentyl-N_8-phenyloctanediamide (2.13)



According to the general procedure for BTC acylation, in a flame-dried round-bottomed flask, 2,4,6-collidine (592 µL, 4.48 mmol, 7 equiv.) was dropwise added over 2.75 h to a solution of BTC (133 mg, 0.45 mmol, 0.7 equiv.) and suberanilic acid **2.8** (192 mg, 0.77 mmol, 1.2 equiv.) in dry THF (12.0 mL) followed by the addition of **3.92** (200 mg, 064 mmol, 1.0 equiv.) in THF (4.0 mL) and stirred overnight. The organic phase was washed with H_2O (2 x 100 mL), sat. $NaHCO_3$ (aq.) (2 x 100 mL), 1 M HCl (aq.) (3 x 100 mL), and brine (2 x 100 mL). The title compound was purified by flash column chromatography (EtOAc:heptane 2:3) to afford **2.13** (317 mg, 91%) as a green semi solid. R_f = 0.12 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3307, 2935, 2861, 1660, 1599, 1518, 1462, 1334, 1272, 1218; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.82 (s, 1H, C(O)N**H**Ph), 7.58 (d, J = 7.9 Hz, 2H, Ar**H**), 7.52 (s, 1H, Ar**H**), 7.29–7.25 (m, 3H, Ar**H**), 7.01 (t, J = 7.4 Hz, 1H, Ar**H**), 5.51 (q, J = 6.4 Hz, 1H, ArC**H**CH_3), 3.92 (s, 3H, OC$\mathbf{H_3}$), 3.85 (s, 3H, OC$\mathbf{H_3}$), 3.43 (d, J = 14.8 Hz, 1H, CH_3CC**H'**H"NC(O)), 3.11 (d, J = 14.8 Hz, 1H, CH_3CCH'**H"**NC(O)), 2.29–2.20 (m, 4H, NC(O)C$\mathbf{H_2}$$CH_2CH_2CH_2CH_2C\mathbf{H_2}$C(O)N), 1.56–1.17 (m, 11H, NC(O)CH_2C$\mathbf{H_2}$C$\mathbf{H_2}$C$\mathbf{H_2}$C$\mathbf{H_2}$$CH_2$C(O)N, ArCHC$\mathbf{H_3}$), 0.85 (s, 9H, C(C$\mathbf{H_3}$)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 171.2, 152.7, 148.3, 141.1, 139.3, 128.6, 122.9, 120.9, 119.0, 110.2, 107.3, 75.4, 56.2, 56.1, 54.9, 36.3, 33.3, 28.4, 28.2, 25.0, 24.4, 20.4; MS (ESI) *m/z*: calcd. for $C_{29}H_{42}N_3O_7^+$ $[M + H]^+$ 544.3, found 544.4.

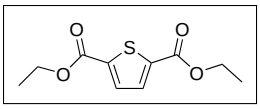
4-(Benzyloxy)-*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)butanamide (2.15)



According to the general procedure for coupling reagent acylation, PyBOP (645 mg, 1.23 mmol, 1.5 equiv.) and NEM (623 µL, 4.95 mmol, 6 equiv.) was added to a stirring solution of 4-benzyloxybutyric acid (205 µL, 1.16 mmol, 1.4 equiv.) in DMF (8.3 mL) and stirred for 10 min, before **2.5** (200 mg, 0.83 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine:H_2O (1:1) (5 x 100 mL), sat. Na_2SO_4 (aq.) (50 mL), and brine (10 mL). The hydroxamate product **2.15** was isolated by flash

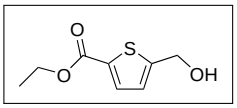
chromatography (EtOAc:heptane 7:3) as a light green solid (316 mg, 91%). R_f = 0.13 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3141, 2985, 2944, 2855, 2753, 1650, 1578, 1510, 1466, 1332, 1270, 1219, 1080; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.38 (s, 1H, Ar**H**), 7.34–7.23 (m, 5H, Ar**H**), 5.47 (q, *J* = 6.3 Hz, 1H, ArC**H**CH_3), 4.29 (s, 2H, OC$\mathbf{H_2}$Ar), 3.95 (s, 3H, OC$\mathbf{H_3}$), 3.82 (s, 3H, OC$\mathbf{H_3}$), 3.23–3.20 (t, *J* = 6.5 Hz, 2H, C**H**OCH_2Ph), 1.94–1.90 (t, *J* = 7.0 Hz, 2H, C(O)C$\mathbf{H_2}$$CH_2CH_2$O), 1.67–1.60 (p, *J* = 6.5 Hz, 2H, C(O)CH_2C$\mathbf{H_2}$$CH_2$O), 1.48 (d, *J* = 6.3 Hz, 3H, ArCHC$\mathbf{H_3}$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 168.8, 153.4, 147.7, 140.2, 138.5, 132.6, 128.2, 127.4, 127.3, 109.2, 107.4, 77.3, 71.8, 68.5, 56.04, 56.02, 28.9, 25.1, 21.0; MS (ESI) *m/z*: calcd. for $C_{21}H_{27}N_2O_7^+$ $[M + H]^+$ 419.2, found 419.3; HRMS (ESI) *m/z*: calcd. for $C_{21}H_{27}N_2O_7^+$ $[M + H]^+$ 419.1813, found 419.1817; mp. = 120.7–121.7 °C.

Diethyl 2,5-thiophenedicarboxylate



In a round-bottomed flask containing a magnetic stirring bar and equipped with a condenser, conc. H_2SO_4 (2.0 mL) was added to a stirring solution of 2,5-thiophenedicarboxylic acid (25.0 g, 145 mmol, 1.0 equiv.) in EtOH (500 mL) and heated at reflux overnight. Incomplete conversion was observed by TLC and LCMS, and therefore all volatiles were evaporated under reduced pressure and co-evaporated with toluene. The white precipitation was redissolved in EtOH (350 mL) and added H_3PO_4 (2.0 mL) followed by reflux for 2 days. The reaction mixture was cooled and all volatiles were removed under reduced pressure. The white solid was dissolved in EtOAc (500 mL) and the organic phase was washed with 2.0 M NaOH (aq.) (300 mL), brine (300 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford diethyl 2,5-thiophenedicarboxylate as a white crystalline solid, used without further purification (25.24 g, 76%). Spectral data in correspondence with reported literature values.[54] ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.79 (d, *J* = 0.4 Hz, 2H, Ar**H**), 4.32 (q, *J* = 7.1 Hz, 4H, 2 x C(O)C$\mathbf{H_2}$$CH_3$), 1.30 (t, *J* = 7.1 Hz, 6H, 2 x C(O)CH_2C$\mathbf{H_3}$);

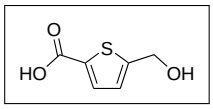
Ethyl 5-(hydroxymethyl)thiophene-2-carboxylate



In a round-bottomed flask containing a magnetic stirring bar, $NaBH_4$ (5.53 g, 142 mmol, 1.3 equiv.) was portion wise added to diethyl 2,5-thiophenedicarboxylate (25.0 g, 110 mmol, 1.0 eqiuv.) in EtOH

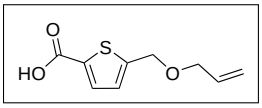
(300 mL) and heated at reflux. When the amount of thiophene-2,5-diyldimethanol started to increase, observed by LCMS (2.5 h), the reaction mixture was cooled to rt and slowly quenched by the addition of H_2O (100 mL). EtOH was removed under reduced pressure and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by by flash chromatography (EtOAc:heptane 1:9 $\rightarrow$ 1:4) afford ethyl 5-(hydroxymethyl)thiophene-2-carboxylate (12.9 g, 63 %) as an oil. Spectral data in correspondence with reported literature values.[54] ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.65 (d, J = 3.9 Hz, 1H, C(O)CC**H**CHC), 7.02 (m, 1H, C(O)CCHC**H**C), 5.73 (t, J = 5.8 Hz, 1H, CH_2O**H**), 4.67 (m, 2H, CC**H$_2$**OH), 4.26 (q, J = 7.1 Hz, 2H, C(O)C**H$_2$**CH_3), 1.28 (t, J = 7.1 Hz, 3H, C(O)CH_2C**H$_3$**);

5-(Hydroxymethyl)thiophene-2-carboxylic acid



To a round-bottomed flask containing a magnetic stirring bar, LiOH (416 mg, 17.3 mmol, 3 equiv.) was added to a stirring solution of ethyl 5-(hydroxymethyl)thiophene-2-carboxylate in EtOH:H_2O (2:1) (13.9 mL) and refluxed. When full conversion was observed by TLC and LCMS (75 min), the reaction mixture was cooled and evaporated under reduced pressure to almost dryness and extracted with Et_2O (50 mL). The aqueous phase was acidified to pH $<$ 3 with 1.0 M HCl (aq.) (30 mL) and extracted with Et_2O (3 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to collect 5-(hydroxymethyl)thiophene-2-carboxylic acid (1.36 g, 87%) as a off-white solid that was used without further purification. Spectral data in correspondence with reported literature values.[55] ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.92 (br. s, 1H, ArCOO**H**), 7.58 (d, J = 3.7 Hz, 1H, Ar**H**), 7.00 (d, J = 3.7 Hz, 1H, Ar**H**), 5.68 (m, 1H, CH_2O**H**), 4.66 (s, 2H, ArC**H$_2$**OH);

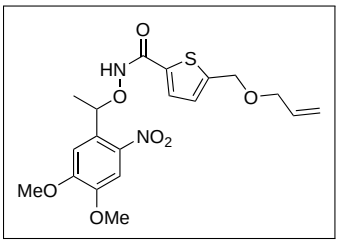
5-((Allyloxy)methyl)thiophene-2-carboxylic acid (2.16)



In a flame-dried round-bottomed flask containing a magnetic stirring bar, 5-(hydroxymethyl)thiophene-2-carboxylic acid (400 mg, 2.53 mmol, 1.0 equiv.) in DMF (8.4 mL) was slowly added to a cold stirring solution of NaH (60% in oil) (253 mg, 6.32 mmol, 2.5 equiv.) in DMF (2.1 mL) under argon atmosphere. AllBr (274 µL, 3.16 mmol, 1.25 equiv.) was added to the cold solu-

tion and stirred at rt. When full conversion was observed by TLC and LCMS (3.5 h), the reaction mixture was cooled and quenched by the addition of H_2O (5 mL). The aqueous phase was extracted with EtOAc (2 x 25 mL) and acidified to pH $<$ 3 with 1.0 M HCl (aq.). The acidic aqueous phase was extracted with EtOAc (3 x 25 mL). The combined organic phases were washed with 0.5 M HCl (aq.) (5 x 50 mL), brine (10 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Residual DMF was removed by wash a Et_2O solution with brine:H_2O (1:1) (6 x 30 mL), brine (10 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. This afforded a white solid (321 mg, 64%) that was used without further purification ^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.10 (br. s, 1H, COO**H**), 7.61 (d, *J* = 3.7 Hz, 1H, Ar**H**), 7.10 (d, *J* = 3.7 Hz, 1H, Ar**H**), 5.96–5.86 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.31–5.26 (m, 1H, $CH_2CHC\mathbf{H'}H''$), 5.20–5.16 (m, 1H, $CH_2CHCH'\mathbf{H''}$), 4.67 (s, 2H, $ArC\mathbf{H_2}OAll$), 4.03–4.01 (dq, *J* = 5.4, 1.6 Hz, 2H, OCH_2CHCH_2); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 162.8, 148.8, 134.7, 133.9, 133.0, 126.7, 116.9, 70.4, 66.1; MS (ESI) *m/z*: calcd. for $C_9H_{11}O_3S^+$ $[M + H]^+$ 199.0, found 199.0; HRMS (ESI) *m/z*: calcd. for $C_9H_{11}O_3S^+$ $[M + H]^+$ 199.0423, found 199.0422.

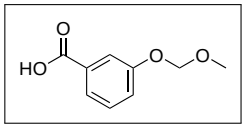
5-((Allyloxy)methyl)-*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)thiophene-2-carboxamide (2.17)



According to the general procedure for coupling reagent acylation, PyBOP (929 mg, 1.78 mmol, 1.1 equiv.) and NEM (1.22 µL, 9.73 mmol, 6 equiv.) was added to a stirring solution of **2.16** (322 mg, 1.62 mmol, 1.0 equiv.) in DMF (18.0 mL) and stirred for 20 min, before **2.5** (432 g, 1.78 mmol, 1.1 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine:H_2O (1:1) (5 x 100 mL). The hydroxamate product **2.17** was isolated by flash chromatography (EtOAc:heptane 3:7) as a yellow solid (537 mg, 78%). Trace amount of impurities were observed by ^{1}H-NMR. R_f = 0.38 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3177, 2995, 2977, 2850, 1620, 1580, 1513, 1456, 1328, 1268, 1219, 1168, 1012; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.50 (s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.48 (s, 1H, Ar**H**), 7.38–7.37 (m, 1H, Ar**H**), 7.00 (d, *J* = 3.7 Hz, 1H, Ar**H**), 5.93–5.84 (m, 1H, $CH_2C\mathbf{H}CH_2$), 5.66 (q, *J* = 6.4 Hz, 1H, $ArC\mathbf{H}CH_3$), 5.29–5.23 (dq, *J* = 17.3, 1.8 Hz 1H, $CH_2CHCH(E)\mathbf{H}$), 5.18–5.15 (dq, *J* = 10.4, 1.8, 1.4 Hz, 1H, $CH_2CHCH(Z)\mathbf{H}$), 4.61 (s, 2H $Ar\mathbf{H_2}O$), 3.99–3.97 (m, 5H,

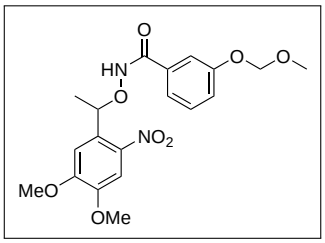
$OC\mathbf{H_2}CHCH_2$, $OC\mathbf{H_3}$), 3.84 (s, 3H, $OC\mathbf{H_3}$), 1.55 (d, J = 6.38 Hz, 3H, $ArCHC\mathbf{H_3}$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 159.6, 153.3, 147.7, 146.8, 140.2, 135.3, 134.7, 132.4, 128.4, 126.5, 116.9, 109.4, 107.4, 77.9, 70.3, 65.9, 56.1, 56.0, 21.1; MS (ESI) *m/z*: calcd. for $C_{19}H_{23}N_2O_7S^+$ $[M + H]^+$ 423.1, found 423.4; HRMS (ESI) *m/z*: calcd. for $C_{19}H_{23}N_2O_7S^+$ $[M + H]^+$ 423.1220, found 423.1222. mp. = 97.9-101 °C.

3-(Methoxymethoxy)benzoic acid (2.18)



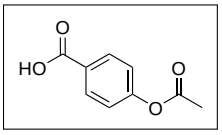
In a round-bottomed flask containing a magnetic stirring bar, 3.5 M $NaClO_2$ (aq.) (13.7 mL) was dropwise added over 40 min to a cold mixture of 3-(methoxymethoxy)benzaldehyde (1.0 mL, 6.87 mmol, 1.0 equiv.), 0.7 M NaH_2PO_4 (aq.) (24.5 mL), and 35% H_2O_2 (2.4 mL, 34.4 mmol, 5 equiv.) in MeCN (51.5 mL), to ensure reaction temperature below 10 °C. The reaction mixture was stirred at 0 °C for 1.5 h, followed by stirring at rt. When full conversion was observed by TLC and LCMS (4 h), the reaction mixture was cooled and carefully quenching by slow addition of Na_2SO_3 (6.06 g, 48.1 mmol, 7.0 equiv.). Sat. $NaHCO_3$ (aq.) was added to ensure basic pH before MeCN was removed under reduced pressure. The aqueous solution was carefully acidified by the addition of 1.0 M HCl (aq.) (pH ≈ 3-4) and extracted with EtOAc (3 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford the title compound as a white solid in (1.21 g, > 95%) that was used without further purification. Spectral data in correspondence with reported literature values.[56] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.00 (br. s, 1H, COO**H**), 7.59–7.55 (m, 2H, Ar**H**), 7.44–7.40 (m, 2H, Ar**H**), 7.28–7.25 (m, 1H, Ar**H**), 5.24 (s, 2H, $ArOC\mathbf{H_2}OCH_3$), 3.38 (s, 3H, $ArOCH_2OC\mathbf{H_3}$);

_N_-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-3-(methoxymethoxy)benzamide (2.19)



According to the general procedure for coupling reagent acylation, PyBOP (978 mg, 1.88 mmol, 1.3 equiv.) and NEM (1.09 µL, 8.67 mmol, 6 equiv.) was added to a stirring solution of **2.18** (316 mg, 1.73 mmol, 1.2 equiv.) in DMF (14.4 mL) and stirred for 15 min, before **2.5** (350 mg, 1.44 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine:H_2O (1:1) (5 x 100 mL) and sat. $NaHCO_3$ (aq.) (100mL). The hydroxamate product **2.19** was isolated by flash chromatography (EtOAc:heptane 2:3) as a light green solid (469 mg, 80%). Trace amount of EtOAc was observed by ^{1}H-NMR. R_f = 0.21 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3358, 3083, 3007, 2937, 2838, 1672, 1587, 1523, 1506, 1472, 1343, 1270, 1216, 1079; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.49 (s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.50 (s, 1H, Ar**H**), 7.35–7.31 (m, 1H, Ar**H**), 7.22–7.14 (m, 3H, Ar**H**), 5.65 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 5.19 (s, 2H, ArOC**H$_2$**OCH_3), 3.98 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 3.35 (s, 3H, OCH_2OC**H$_3$**), 1.56 (d, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 163.9, 156.7, 153.3, 147.7, 140.3, 133.5, 132.4, 129.7, 120.2, 119.3, 114.7, 109.4, 107.4, 93.9, 77.5, 56.1, 56.0, 55.6, 21.1; MS (ESI) *m/z*: calcd. for $C_{19}H_{23}N_2O_8^+$ $[M + H]^+$ 407.1, found 407.2; HRMS (ESI) *m/z*: calcd. for $C_{19}H_{23}N_2O_8^+$ $[M + H]^+$ 407.1449, found 407.1450. mp. = 138–140 °C.

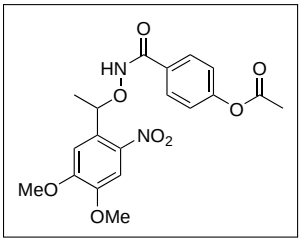
4-Acetoxybenzoic acid (2.20)



To a round-bottomed flask containing a magnetic stirring bar, H_3PO_4 (five drops) was added to a stirring suspension of 4-hydroxybenzoic acid (3.00 g, 21.7 mmol, 1.0 equiv.) in Ac_2O (6.00 ml, 63.6 mmol, 6 equiv.) and heated at 90 °C. When full conversion was observed by TLC and LCMS (20 min), the reaction mixture was cooled and poured slowly into cold H_2O (150 mL). The off-white precipitate was collected by filtration and washed with cold H_2O (20 mL). The precipitate was stirred with sat. $NaHCO_3$ (100 mL) until gas evolution ceased. The remaining precipitate was removed by filtration. To the filtrate was added 6 M HCl (aq.) until constant precipitation was obtained. The white solution was cooled, and the precipitate was collected by filtration and washed with cold H_2O (20 mL), until neutral pH. More product was observed in the acidic filtrate and therefore it was extracted with

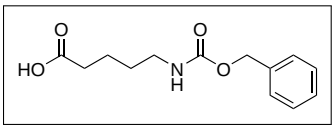
EtOAc (2 x 20 mL), followed by evaporation and the precipitation method repeated. The two crops were mixed to afford 4-acetoxybenzoic acid as white-off solid (2.77 g, 71%) that was used without further purification. Spectral data in correspondence with reported literature values.[57] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.01 (br. s, 1H, ArCOO**H**), 7.9 (m, 2H, Ar**H**), 7.25 (m, 2H, Ar**H**), 2.29 (s, 3H, C(O)CH_3).

4-((1-(2-Amino-4,5-dimethoxyphenyl)ethoxy)carbamoyl)phenyl acetate (2.21)



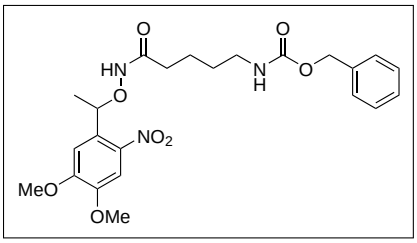
In a flame-dried Schlenk tube containing a magnetic stirring bar and equipped with a condenser, DMF (two drops) was added to a stirring solution of **2.21** (781 mg, 4.33 mmol, 3 equiv.) in $SOCl_2$ (2.11 ml, 28.9 mmol, 20 equiv.) under inert atmosphere and heated at 80 °C. When full conversion was observed by TLC and LCMS (1.0 h), excess $SOCl_2$ was removed under reduced pressure and the crude 4-acetoxybenzoyl chloride placed under vacuum for a couple of hours and placed under inert atmosphere. The crude intermediate was dissolved in Et_2O (3 mL) and to the white suspension was added K_2CO_3 (998 mg, 7.22 mmol, 5 equiv.) followed by **2.5** (350 mg, 1.45 mmol, 1.0 equiv.). When full conversion was observed by TLC and LCMS, the reaction mixture was quenched by the addition of H_2O (10 mL) and extracted with EtOAc (30 mL). The organic layer was washed with sat. $NaHCO_3$ (aq.) (20 mL), 1.0 M HCl (aq.) (2 x 20 mL), brine (20 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The hydroxamate product **2.21** was isolated by flash chromatography (EtOAc:heptane 4:6) as a brown oil (393 mg, 67%). Trace amount of impurities were observed by ^{1}H-NMR. R_f = 0.15 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3246, 2979, 2937, 2846, 1757, 1654, 1603, 1582, 1517, 1465, 1334, 1218; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H, **H**NC(O)), 7.64–7.61 (m, 2H, Ar**H**), 7.56 (s, 1H, Ar**H**), 7.50 (s, 1H, Ar**H**), 7.19–7.17 (m, 2H, Ar**H**), 5.65 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 3.98 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 2.26 (s, 3H, C(O)CH_3), 1.56 (d, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 168.9, 163.7, 153.3, 152.9, 147.7, 140.2, 132.4, 129.6, 128.7, 122.0, 109.4, 107.4, 77.6, 56.1, 56.0, 21.1, 20.8; MS (ESI) *m/z*: calcd. for $C_{19}H_{21}N_2O_8^+$ $[M + H]^+$ 405.1, found 405.2;

5-(((Benzyloxy)carbonyl)amino)pentanoic acid (2.22)



To a round-bottomed flask containing a magnetic stirring bar, CbzCl (2.23 mL, 17.1 mmol, 1.0 equiv.) was dropwise added to a cold solution of 5-aminopentanoic acid (2.00 g, 17.1 mmol. 1-0 equiv.) in 2.0 M NaOH (aq.) (17.1 mL) at 0 °C. pH was followed. The reaction mixture was stirred for 1 h further and further at rt for 12 h. The light green aqueous solution was extracted with Et_2O (3 x 25 mL) and acidified to pH $\approx$ 2 by the addition of 1.0 M HCl (aq.). The white precipitate was collected by filtration and washed with a small amount of cold H_2O and dried to afford **2.22** as a white solid (3.3006 g, 77%) that was used without further purification. Spectral data in correspondence with reported literature values.[58] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.00 (br. s, 1H, COO**H**), 7.38–7.24 (m, 6H, Ar**H**, CH_2N**H**Cbz), 5.00 (s, 2H, OC$\mathbf{H_2}$Ar), 3.01–2.96 (m, 2H, C$\mathbf{H_2}$,CH_2NHCbz), 2.22–2.18 (t, J = 7.2 Hz, 2H, COOHC$\mathbf{H_2}CH_2$), 1.52–1.36 (m, 4H, COOHCH_2C$\mathbf{H_2}$C$\mathbf{H_2}CH_2$NHCbz);

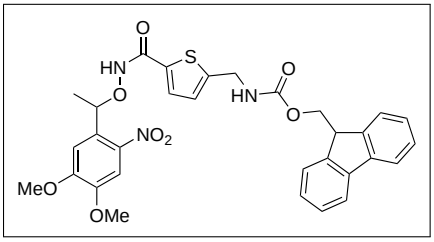
Benzyl (5-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-5-oxopentyl)carbamate (2.23)



According to the general procedure for coupling reagent acylation, PyBOP (1.86 g, 3.57 mmol, 1.3 equiv.) and NEM (2.07 µL, 16.5 mmol, 6 equiv.) was added to a stirring solution of **2.22** (828 mg, 3.30 mmol, 1.2 equiv.) in DMF (27.5 mL) and stirred for 20 min, before **2.5** (665 mg, 2.75 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine (5 x 150 mL). The product was isolated by flash chromatography (EtOAc:heptane 4:1) to afford **2.23** (1.10 mg, 84%) as a yellow solid foam. A trace amount of impurities was observed by ^{1}H-NMR. R_f = 0.36 (EtOAc; UV); IR (neat) ν (cm^{-1}): 3416, 3203, 2953, 2933, 2860, 1697, 1656, 1581, 1518, 1336; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.76 (br. s, 1H, **H**NC(O)CH_2), 7.56 (s, 1H, Ar**H**), 7.37–7.28 (m, 6H, Ar**H**), 7.18–7.15 (t, 1H, CH_2N**H**C(O)O), 5.45 (q, J = 6.3 Hz, 1H, ArC**H**CH_3), 4.99 (s, 2H, C$\mathbf{H_2}$Ar), 3.95 (s, 3H, OC$\mathbf{H_3}$), 3.84 (s, 3H, OC$\mathbf{H_3}$), 2.88 (m, 2H, C$\mathbf{H_2}$NHC(O)), 1.85–1.81 (m, 2H, NC(O)C$\mathbf{H_2}CH_2$), 1.48 (d, J = 6.3 Hz, 3H, ArCHC$\mathbf{H_3}$), 1.40–1.32 (m, 2H, NC(O)CH_2C$\mathbf{H_2}CH_2$), 1.24–1.17 (m, 2H, NC(O)CH_2CH_2C$\mathbf{H_2}$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 169.0, 156.0, 153.3, 147.7. 140.2, 137.3, 132.6, 128.3, 127.7, 109.3, 107.4, 77.3,

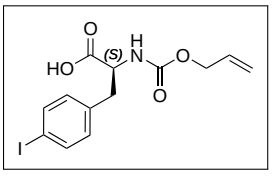
65.1, 56.1, 56.0, **C**H_2NHC(O)O missing under DMSO-d_6, 31.7, 28.6, 22.1, 21.0; MS (ESI) *m/z*: calcd. for $C_{23}H_{30}N_3O_8^+$ $[M + H]^+$ 476.2, found 476.3; HRMS (ESI) *m/z*: calcd. for $C_{23}H_{30}N_3O_8^+$ $[M + H]^+$ 476.2027, found 476.2035.

(9*H*-Fluoren-9-yl)methyl ((5-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)carbamoyl)-thiophen-2-yl)methyl)carbamate (2.25)



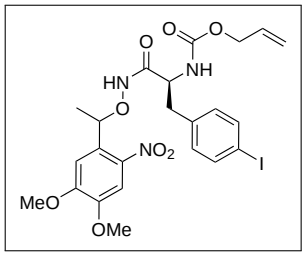
According to the general procedure for coupling reagent acylation, PyBOP (5.17 g, 1.78 mmol, 1.2 equiv.) and NEM (6.25 mL, 49.7 mmol, 6 equiv.) was added to a stirring solution of 5-(((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)methyl)thiophene-2-carboxylic acid (3.457 g, 9.11 mmol, 1.1 equiv.) in DMF (297.0 mL) and stirred for 15 min, before **2.5** (2.00 g, 8.28 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine (5 x 300 mL). The hydroxmate product was purified by crystallization from hot acetone and heptane. The mother liquor was evaporated to dryness and the residues were recrystallized. The two combined crops afforded **2.25** as a greyish solid in (3.65 g, 73% yield). R_f = 0.10 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3324, 3220, 3009, 2970, 2938, 2915, 2851, 1692, 1639, 1520, 1466, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.46 (s, 1H, **H**NC(O)), 8.00 (t, *J* = 6.0 Hz, 1H, ArCH$_2$N**H**C(O)O), 7.88 (d, *J* = 7.5 Hz, 2H, Ar**H**), 7.67 (d, *J* = 7.5 Hz, 2H, Ar**H**), 7.56 (s, 1H, Ar**H**), 7.49 (s, 1H, Ar**H**), 7.40 (t, *J* = 7.4 Hz, 2H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 6.87 (d, *J* = 3.5 Hz, 1H, Ar**H**), 5.61 (q, *J* = 6.5 Hz, 1H, ArC**H**CH$_3$), 4.34 (d, *J* = 6.8 Hz, 2H, C(O)OC**H$_2$**CH), 4.30 (d, *J* = 6.0 Hz, 2H, ArC**H$_2$**NH), 4.22 (t, *J* = 6.8 Hz, 1H, OCH$_2$C**H**), 3.97 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.55 (d, *J* = 6.5 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 159.6, 156.1, 153.3, 148.8, 147.7, 143.8, 140.7, 140.1, 137.4, 132.5, 128.9, 127.6, 127.0, 125.5, 125.1, 120.1, 109.4, 107.4, 77.9, 65.5, 56.1, 56.0, 46.7, Ar**C**H$_2$NHC(O)O under DMSO-d_6, 21.1; MS (ESI) *m/z*: calcd. for $C_{31}H_{30}N_3O_8S^+$ $[M + H]^+$ 604.2, found 604.4; HRMS (ESI) *m/z*: calcd. for $C_{31}H_{30}N_3O_8S^+$ $[M + H]^+$ 604.1748, found 604.1748; mp. = 174.6–177.4 °C.

(*S*)-2-(((Allyloxy)carbonyl)amino)-3-(4-iodophenyl)propanoic acid (2.26)



In a round-bottomed flask containing a magnetic stirring bar, AllocCl (805 µL, 7.56 mmol 2 equiv.) was added to a cold slurry of 4-iodo-*L*-phenylamine (1.10 g, 3.78 mmol, 1.0 equiv.) in THF (14.2 mL), followed by 0.4 M Na_2CO_3 (aq.) (14.2 mL) and stirred at rt. When full conversion was observed by TLC and LCMS (1.0 h), the reaction mixture was transferred to a separation funnel with Et_2O (100 mL) and $NaHCO_3$ (sat. aq.) (40 mL). The aqueous layer was acidified with 1.0 M HCl (aq.) (pH $<$ 1) and extracted with Et_2O (3 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. This provided **2.26** (1.01 g, 71%) as a white solid that was used without further purification. R_f = 0.40 (CH_2Cl_2:MeOH 19:1; UV); IR (neat) ν (cm^{-1}): 3313, 3131, 2979, 2940, 1694, 1214; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.78 (br. s, 1H, C(O)O**H**), 7.64–7.57 (m, 3H, OC(O)N**H**CH, 2 x Ar**H**), 7.08–7.06 (m, 2H, Ar**H**), 5.88–5.79 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.23–5.12 (m, 2H, $OCH_2CHC\mathbf{H_2}$), 4.45–4.35 (m, 2H, $OC\mathbf{H_2}CHCH_2$), 4.16–4.10 (m, 1H, HO(O)CC**H**NH), 3.04–2.98 (m, 1H, HO(O)CCHC**H'**H"Ar), 2.81–2.74 (m, 1H, HO(O)CCHCH'**H"**Ar); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 173.1, 155.8, 137.8, 136.9, 133.5, 131.6, 116.9, 92.3, 64.4, 55.2, 35.9; MS (ESI) *m/z*: calcd. for $C_{13}H_{15}INO_4^+$ $[M + H]^+$ 376.0, found 376.0; mp. = 97.8–101.5 °C.

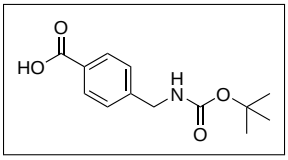
Allyl ((2*S*)-1-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-3-(4-iodophenyl)-1-oxopropan-2-yl)carbamate
(2.27)



According to the general procedure for coupling reagent acylation, PyBOP (878 mg, 1.69 mmol, 1.5 equiv.) and NEM (855 µL, 6.76 mmol, 6 equiv.) was added to a stirring solution of alloc-4-iodo-phenylalanine **2.26** (591 mg, 1.575 mmol, 1.4 equiv.) in DMF (12.4 mL) and stirred for 15 min, before **2.5** (273 mg, 1.13 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine (5 x 50 mL). The hydroxmate product **2.27** was purified by column chromatography. Trace amount of impurities were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical

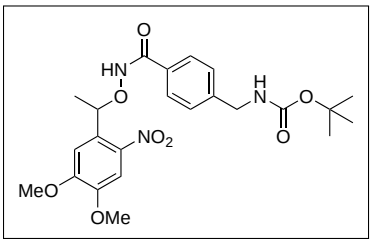
shifts are reported. Multiplicity for each diastereomer is reported if possible. Two compounds were observed by TLC. IR (neat) ν (cm^{-1}): 3295, 3201, 3090, 3012, 2978, 2933, 1687, 1663, 1583, 1518, 1462, 1335, 1274, 1219, 1175, 1083, 1006; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.13/11.10* (s, 1H, **H**NC(O)), 7.64–7.49 (m, 3H, Ar**H**), 7.37/7.32* (d, 1H, Ar**H**), 6.93–6.86 (m, 2H, Ar**H**), 5.86–5.75 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.54–5.39 (m, 1H, $ArC\mathbf{H}CH_3$), 5.22–5.11 (m, 2H, $OCH_2CHC\mathbf{H_2}$), 4.37–4.33 (m, 2H, $OC\mathbf{H_2}CHCH_2$), 3.96–3.85 (m, 7H, 2 x $OC\mathbf{H_3}$, HNC(O)C**H**NHC(O)), 2.63–2.57 (m, 2H, $HNC(O)CHCH_2Ar$), 1.47–1.43 (m, 3H, $ArCHC\mathbf{H_3}$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 168.2/168.1* 155.9, 153.9/153.8*, 148.1, 140.5, 137.7/137.5*, 137.25/137.19*, 133.9, 133.0/132.9* 131.8, 117.4, 109.7/109.6*, 107.9, 92.8, 77.7, 64.8, 56.55, 56.49, 54.1, 37.2/37.1*, 21.50/21.46*; MS (ESI) *m/z*: calcd. for $C_{23}H_{27}IN_3O_8^+$ $[M + H]^+$ 604.1, found 604.4; mp. = 148.5–150.0 °C.

4-(((*tert*-Butoxycarbonyl)amino)methyl)benzoic acid (2.28)



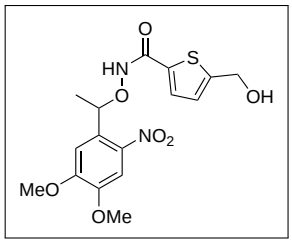
To a round-bottomed flask containing a magnetic stirring bar, 0.5 M NaOH (aq.) (16 mL) was added to a slurry of 4-(aminomethyl)benzoic acid (1.00 g, 6.62 mmol, 1.0 equiv.) in dioxane (16 mL) at rt. The clear solution was cooled and added Boc_2O (1.60 g, 7.32 mmol, 1.1 equiv.) and stirred rt overnight. When full conversion was observed by TLC and LCMS, dioxane was removed under reduced pressure. pH was adjusted to pH = 4 by the addition of 20% citric acid (aq.), and immediately extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. This afforded **2.28** as a white solid (2.265 g, quant.) which was used without further purification. Spectral data in correspondence with reported literature values.[59]. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.85 (br. s, 1H, C(O)O**H**), 7.89 (d, *J* = 8.2 Hz, 2H, Ar**H**), 7.47 (t, *J* = 6.1 Hz, 1H, $ArC\mathbf{H_2}NHC(O)O$), 7.34 (d, *J* = 8.2 Hz, 2H, Ar**H**), 4.19 (d, *J* = 6.1 Hz, 2H, $ArC\mathbf{H_2}NHC(O)O$), 1.39 (s, 9H, $C(C\mathbf{H_3})_3$);

tert-Butyl (4-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)carbamoyl)benzyl)carbamate (2.29)



According to the general procedure for coupling reagent acylation, PyBOP (978 mg, 1.88 mmol, 1.3 equiv.) and NEM (1.09 µL, 8.67 mmol, 6 equiv.) was added to a stirring solution of **2.28** (871 mg, 3.48 mmol, 2.4 equiv.) in DMF (14.5 mL) and stirred for 20 min, before **2.5** (350 mg, 1.44 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine (5 x 100 mL), sat. $NaHCO_3$ (aq.) (100 mL), and brine (10 mL). The hydroxamate product **2.29** was isolated by flash chromatography (EtOAc:heptane 1:1) as a yellow solid (651 mg, 95%). Trace amount of EtOAc was observed by ^{1}H-NMR. R_f = 0.61 (EtOAc; UV); IR (neat) ν (cm^{-1}): 3328, 3237, 3008, 2981, 2935, 2835, 1683, 1656, 1584, 1520, 1498, 1467, 1336, 1275, 1220, 1160, 1016, 793; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.45 (br. s, 1H, **H**NC(O)), 7.56–7.51 (m, 4H, Ar**H**), 7.42 (t, *J* = 6.0 Hz, 1H, CH_2N**H**C(O)O), 7.25 (d, *J* = 8.1 Hz, 2H, Ar**H**), 5.65 (q, *J* = 6.3 Hz, 1H, ArC**H**CH_3), 4.11 (q, *J* = 6.0 Hz, 2H, ArC**H$_2$**NHC(O)O), 3.98 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.56 (d, *J* = 6.3 Hz, 3H, ArCHC**H$_3$**), 1.37 (s, 9H, C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 164.4, 155.8, 153.3, 147.7, 144.1, 140.2, 132.5, 130.5, 127.1, 126.8, 109.4, 107.4, 77.9, 77.5, 56.11, 56.01, 43.1, 28.2, 21.1; MS (ESI) *m/z*: calcd. for $C_{23}H_{30}N_3O_8^+$ $[M + H]^+$ 476.2, found 476.2; mp. = 149–150 °C.

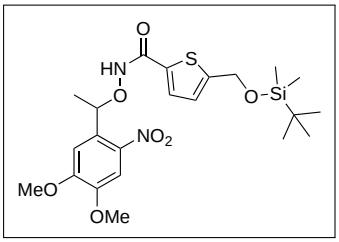
N-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-5-(hydroxymethyl)thiophene-2-carboxamide (2.30)



According to the general procedure for coupling reagent acylation, PyBOP (1.18 g, 2.26 mmol, 1.3 equiv.) and NEM (1.31 µL, 10.4 mmol, 6 equiv.) was added to a stirring solution of 5-(hydroxymethyl)thiophene-2-carboxylic acid (330 mg, 2.08 mmol, 1.2 equiv.) in DMF (17.4 mL) and stirred for 15 min, before **2.5** (421 mg, 1.74 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine:H_2O (1:1) (5 x 100 mL), sat. $NaHCO_3$ (aq.) (100 mL), and brine (10 mL). The hydroxamate product **2.30** was isolated by flash chromatography (EtOAc:heptane 7:3) as a yellow solid (445 mg, 67%).

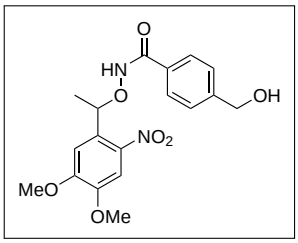
Trace amount of impurities were observed by ^{1}H-NMR. R_f = 0.46 (EtOAc; UV); IR (neat) ν (cm^{-1}): 3245, 3142, 3005, 2966, 2935, 1634, 1579, 1544, 1518, 1505, 1459, 1333, 1272, 1219, 1174; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.44 (s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.48 (s, 1H, Ar**H**), 7.35–7.34 (m, 1H, Ar**H**), 6.90 (d, J = 3.7 Hz, 1H, Ar**H**), 5.62–5.57 (m, 1H, ArC**H**CH$_3$), 4.60–4.59 (d, 2H ArC**H$_2$**OH), 3.97 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.55 (d, J = 6.4 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 160.0, 153.3, 152.3, 147.7, 140.2, 133.9, 132.4, 128.5, 124.1, 109.4, 107.4, 77.9, 58.4, 56.1, 56.0, 21.1; MS (ESI) *m/z*: calcd. for $C_{16}H_{19}N_2O_7S^+$ $[M + H]^+$ 383.1, found 383.1; HRMS (ESI) *m/z*: calcd. for $C_{16}H_{19}N_2O_7S^+$ $[M + H]^+$ 383.0907, found 383.0902. mp. = 176–177.1 °C.

5-(((*tert*-Butyldimethylsilyl)oxy)methyl)-*N*-(1-(4,5-dimethoxy-2-nitrophenyl)-ethoxy)thiophene-2-carboxamide (2.31)



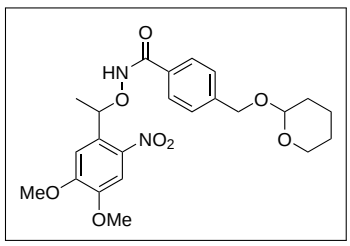
In a flame-dried round-bottomed flask containing a magnetic stirring bar, TBSCl (218 mg, 1.44 mmol, 1.3 equiv.) in CH_2Cl_2 (4.0 mL) was added to a stirring solution of **2.30** (425 mg, 1.11 mmol, 1.0 equiv.) in CH_2Cl_2 (12.0 mL) under inert atmosphere. The resulting solution was stirred for 10 min before imidazole (98 mg, 1.44 mmol, 1.3 equiv.) was added followed stirring overnight. The reaction mixture was concentrated under reduced pressure and purified directly by flash chromatography (heptane → EtOAc:heptane 1:4) providing **2.31** (412 mg, 75 %) as a light green solid. R_f = 0.37 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3154, 2952, 2930, 2855, 1674, 1581, 1511, 1462, 1332, 1272, 1219, 1077, 836; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.46 (s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.48 (s, 1H, Ar**H**), 7.36–7.35 (m, 1H, Ar**H**), 6.93–6.92 (d, J = 4.0 Hz, 1H, Ar**H**), 5.60 (q, J = 6.3 Hz, 1H, ArC**H**CH$_3$), 4.83 (s, 2H, ArC**H$_2$**O), 3.97 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.55 (d, J = 6.3 Hz, 3H, ArCHC**H$_3$**), 0.88 (s, 9H, SiC(C**H$_3$**)$_3$), 0.07 (s, 6H, OSi(C**H$_3$**)$_2$C); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 160.3, 153.8, 151.4, 148.2, 140.6, 134.8, 132.9, 129.0, 124.5, 109.9, 107.9, 78.4, 60.7, 56.6, 56.5, 26.1, 21.6, 18.4, -4.9; MS (ESI) *m/z*: calcd. for $C_{22}H_{33}N_2O_7SSi^+$ $[M + H]^+$ 497.2, found 497.2; HRMS (ESI) *m/z*: calcd. for $C_{22}H_{33}N_2O_7SSi^+$ $[M + H]^+$ 497.1772, found 497.1770. mp. = 106–107 °C.

N-(1-(2-amino-4,5-dimethoxyphenyl)ethoxy)-4-(hydroxymethyl)benzamide (2.32)



According to the general procedure for coupling reagent acylation, PyBOP (1.12 g, 2.15 mmol, 1.3 equiv.) and NEM (1.25 mL, 9.91 mmol, 6 equiv.) was added to a stirring solution of 4-(hydroxymethyl)benzoic acid (302 mg, 1.98 mmol, 1.2 equiv.) in DMF (16.5 mL) and stirred for 20 min, before **2.5** (400 mg, 1.65 mmol, 1.0 equiv.) was added and the reaction mixture was stirred overnight. The organic layer was washed with brine:H_2O (1:1) (5 x 100 mL). The hydroxamate product **2.32** (171 mg, 27%) was isolated by flash chromatography (EtOAc:heptane 7:3) as a light green solid. Trace amount of impurities were observed by ^{1}H-NMR. R_f = 0.39 (EtOAc; UV); IR (neat) ν (cm^{-1}): 3284, 3145, 2983, 2874, 1636, 1575, 1518, 1469, 1375, 1334, 1274, 1223, 1177, 1005; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.46 (br. s, 1H, **H**NC(O)), 7.56–7.52 (m, 4H, Ar**H**), 7.34 (d, J = 8.1 Hz, 2H, Ar**H**), 5.65 (q, J = 6.3 Hz, 1H, ArC**H**CH$_3$), 5.28 (t, J = 5.7 Hz, 1H, ArCH$_2$O**H**), 4.50 (d, J = 5.7 Hz, 2H, ArC**H$_2$**OH), 3.98 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.56 (d, J = 6.3 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 164.4, 153.3, 147.7, 146.5, 140.2, 132.6, 130.4, 126.9, 126.1, 109.4, 107.4, 77.5, 62.3, 56.1, 56.0, 21.1; MS (ESI) *m/z*: calcd. for $C_{18}H_{21}N_2O_7^+$ [M + H]$^+$ 377.1, found 377.2; mp. = 172.1–173.4 °C.

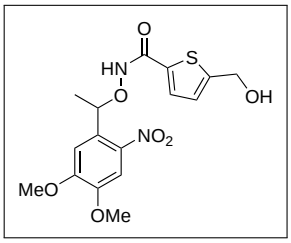
N-(1-(4,5-Dimethoxy-2-nitrophenyl)ethoxy)-4-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)benzamide (2.33)



In a flame-dried Schlenk tube containing a magnetic stirring bar, 3,4-dihydro-2*H*-pyran (435 µL, 4.76 mmol, 8 equiv.) and *p*-TsOH (11 mg, 0.059 mmol, 0.1 equiv.) was added to the alcohol (224 mg, 5.95 mmol, 1.0 equiv.) in THF (5 mL) under inert atmosphere and stirred overnight. The brown reaction mixture was evaporated to dryness and title compound was isolated directly by flash chromatography (EtOAc:heptane 4:6) (140 mg, 51%) as a light green foam. A trace amount of impurites were observed ^{1}H-NMR. R_f = 0.20 (EtOAc:heptane 1:1; UV); IR (neat) ν (cm^{-1}): 3202, 2939, 2868, 1648, 1576, 1517, 1463, 1333, 1271, 1218, 1173, 1120, 1013; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H, **H**NC(O)), 7.58–7.56 (m, 3H, Ar**H**), 7.51 (s, 1H, Ar**H**), 7.37 (d, J = 7.9 Hz, 2H, Ar**H**), 5.66 (q, J = 6.3 Hz, 1H, ArC**H**CH$_3$),

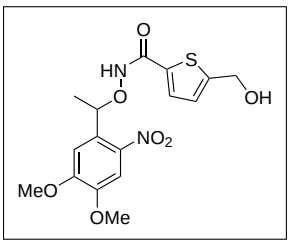
4.69–4.63 (m, 2H, ArC**H**'H''O,CH_2OC**H**O), 4.89–4.45 (m, 1H, ArCH'**H''**O), 3.98 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 3.78–3.72 (m, 1H, CH_2CH_2CHOC**H'''**H''''CH_2), 3.47–3.43 (m, 1H, CH_2CH_2CHOCH'''**H''''**CH_2), 1.75–1.46 (m, 9H, C**H$_2$**C**H$_2$**CHOCH_2C**H$_2$**, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 164.2, 153.3, 147.7, 142.2, 140.2, 132.5, 131.1, 127.3, 127.1, 109.4, 107.4, 97.3, 77.5, 67.4, 61.3, 56.1, 56.0, 30.1, 25.0, 21.1, 19.0; MS (ESI) *m/z*: calcd. for $C_{23}H_{29}N_2O_8^+$ $[M + H]^+$ 461.2, found 461.2.

N-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-5-(hydroxymethyl)thiophene-2-carboxamide (2.37)



In a flame-dried Schlenk tube containing a magnetic stirring bar equipped with a cold finger, $Pd(OH)_2/C$ (18.3 mg, 0.130 mmol, 1.1 equiv.) was added to a stirring solution of **2.17** (50 mg, 0.128 mmol, 1.0 equiv.) in *i*PrOH (1.2 mL) and refluxed overnight. The dark reaction mixture was filtered through a pad of Celite® and washed thoroughly with CH_2Cl_2 and concentrated under reduced pressure. Trace impurities were observed so the alcohol was purified by flash column chromatography (EtOAc:heptane 3:2) to afford **2.37** (32 mg, 71%) as a light green solid. Trace amount of impurities were observed by ^{1}H-NMR. Spectral data in correspondence with **2.30**. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.45 (br. s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.48 (s, 1H, Ar**H**), 7.34 (d, *J* = 3.5 Hz, 1H, Ar**H**), 6.90 (d, *J* = 3.5 Hz, 1H, Ar**H**), 5.64–5.58 (m, 2H, ArC**H**CH_3, CH_2O**H**), 4.60 (d, *J* = 5.6 Hz, 2H, ArC**H$_2$**OH), 3.97 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.55 (d, *J* = 6.2 Hz, 3H, ArCHC**H$_3$**); MS (ESI) *m/z*: calcd. for $C_{16}H_{19}N_2O_7S^+$ $[M + H]^+$ 383.1, found 383.2;

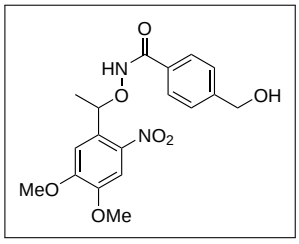
N-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-5-(hydroxymethyl)thiophene-2-carboxamide (2.38)



In a Schlenk tube containing a magnetic stirring bar, TBAF (1 M in THF, 55 µL, 0.0554 mmol, 1.1 equiv.) was dropwise added to a stirring solution of **2.31** (25 mg, 0.0554 mmol, 1.0 equiv.) in THF (200 µL). When full conversion was observed (8.0 h), the reaction mixture was evaporated and purified directly by PREP-TLC (CH_2Cl_2:MeOH 39:1). The silica was stirred in EtOAc before the

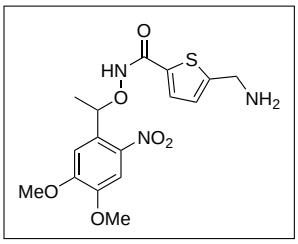
organic solution was collected by filtration and co-evaporated with CH_2Cl_2 to afford **2.38** as a light green solid (16 mg, 82%). Trace amount of EtOAc was observed by ^{1}H-NMR. Spectral data in correspondence with **2.30**. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.44 (br. s, 1H, **H**NC(O)), 7.56 (s, 1H, Ar**H**), 7.48 (s, 1H, Ar**H**), 7.34 (d, *J* = 3.4 Hz, 1H, Ar**H**), 6.91 (d, *J* = 3.4 Hz, 1H, Ar**H**), 5.62–5.58 (m, 2H, ArC**H**CH_3, CH_2O**H**), 4.60 (d, *J* = 5.9 Hz, 2H, ArC**H$_2$**OH), 3.97 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.55 (d, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**); MS (ESI) *m/z*: calcd. for $C_{16}H_{19}N_2O_7S^+$ $[M + H]^+$ 383.1, found 383.2;

***N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-4-(hydroxymethyl)benzamide (2.39)**



In a Schlenk tube containing a magnetic stirring bar, 10% HCl (aq.) (110 µL) was added to a stirring solution of **2.33** (25 mg, 0.0543 mmol, 1.0 equiv.) in THF (545 µL). When full conversion was observed (2.5 h), the reaction mixture was transferred to a separation funnel with EtOAc (5 mL) and H_2O. The organic layer was extracted with EtOAc (2 x 5 mL) and the combined organic phases were washed with H_2O (5 mL), dried over Na_2SO_4, filtered, concentrated *in vacuo*. **2.39** was purified by PREP-TLC (EtOAc:heptane 3:2), extracted from the silica with EtOAc, and twice co-evaporated with CH_2Cl_2 affording the alcohol as a yellow solid (18 mg, 87%). Trace amount of EtOAc and CH_2Cl_2 were observed by ^{1}H-NMR. Spectral data in correspondence with **2.32**.
^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.46 (br. s, 1H, **H**NC(O)), 7.56-7.52 (m, 4H, Ar**H**), 7.34 (d, *J* = 8.8 Hz, 2H, Ar**H**), 5.64 (q, *J* = 6.5 Hz, 1H, ArC**H**CH_3), 5.28 (t, *J* = 5.7 Hz, 1H, ArC**H$_2$**OH), 4.51 (d, *J* = 5.7 Hz, 2H, $ArCH_2$O**H**), 3.99 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 1.56 (d, *J* = 6.5 Hz, 3H, ArCHC**H$_3$**); MS (ESI) *m/z*: calcd. for $C_{18}H_{21}N_2O_7^+$ $[M + H]^+$ 377.1, found 377.2; HRMS (ESI) *m/z*: calcd. for $C_{18}H_{21}N_2O_7^+$ $[M + H]^+$ 377.1343, found 377.1354.

5-(aminomethyl)-*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)thiophene-2-carboxamide (2.40)

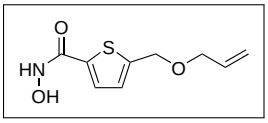


In a Schlenk tube containing a magnetic stirring bar, **2.25** (48 mg, 0.078 mmol, 1.0 equiv) was added to a stirring solution of MeCN:Et_2NH (10:1) (3.1 mL). When full conversion was observed (1.75 h), all volatiles were removed under reduced pressure and directly purified by flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH 17:3). **2.40** was obtained as a green oil (30 mg, quant.). Trace amount of impurities were observed by ^{1}H-NMR.
^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.56 (s, 1H, Ar**H**), 7.49 (s, 1H, Ar**H**), 7.35 (d, *J* = 3.4 Hz, 1H, Ar**H**), 6.88 (d, *J* = 3.4 Hz, 1H, Ar**H**), 5.60 (q, *J* = 6.3 Hz, 1H, ArC**H**CH_3), 3.97 (s, 3H, OC**H$_3$**), 3.86-3.84 (m, 5H, OC**H$_3$**, ArC**H$_2$**NH_2), 1.54 (d, *J* = 6.3 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 156.0, 154.4, 153.4, 147.7, 140.2, 133.4, 132.7, 128.6, 123.8, 109.5, 107.4, 77.8, 56.1, 56.0, 40.8, 21.1; MS (ESI) *m/z*: calcd. for $C_{16}H_{20}N_3O_6S^+$ $[M + H]^+$ 382.1, found 382.2; HRMS (ESI) *m/z*: calcd. for $C_{16}H_{20}N_3O_6S^+$ $[M + H]^+$ 382.1067, found 382.1095.

General Photo-Cleavage Procedure

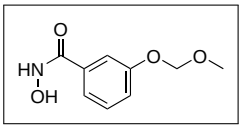
The MeNV-protected compound was dissolved in a solvent indicated under each compound in a small glass jar and a quartz lid was placed on top. The jar was placed under a UV-lamp at 365 nm inside a large customized aluminium box until full conversion was observed by LCMS. Solvent was added every 10-12 h to prevent drying-out. The solution was transferred to a separation funnel by the use of EtOAc and H_2O. The aqueous layer was extracted with EtOAc (3 x). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The hydroxamic acids were purified by PREP-TLC. The desired band was collected and the silica stirred in CH_2Cl_2:Et_3N (2:1) (3 mL) for 30 min before it was filtrated. The mother liquor was concentrated under reduced pressure and co-evaporated from toluene, filtrated and twice co-evaporated from CH_2Cl_2.

N-Hydroxy-5-((prop-1-en-1-yloxy)methyl)thiophene-2-carboxamide (2.43)



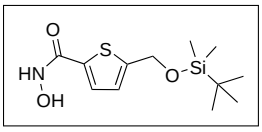
According to the general photo-cleavage procedure, **2.17** (50 mg, 0.118 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 44.5 h, extracted and purified by PREP-TLC (CH_2Cl_2:MeOH 9:1) to afford **2.43** as a brown oil (8 mg, 31%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.18 (s, 1H, **H**NC(O)), 9.10 (s, 1H, **H**ONHC(O)), 7.48 (s, 1H, Ar**H**), 7.04 (d, J = 3.6 Hz, 1H, Ar**H**), 5.95–5.86 (m, 1H, $OCH_2C\mathbf{H}CH_2$) 5.28 (m, 1H, $OCH_2CHC\mathbf{H'}H''$), 5.18 (m, 1H, $OCH_2CHCH'\mathbf{H''}$), 4.63 (s, 2H, $ArC\mathbf{H_2}O$), 4.01 (dt, J = 5.4, 1.5 Hz, 2H, $OC\mathbf{H_2}CHCH_2$; ^{13}C-NMR (101 MHz, DMSO-d_6): δ 159.5, 145.6, 136.9, 134.8, 127.2, 126.6, 116.9, 70.3, 66.0; MS (ESI) *m/z*: calcd. for $C_9H_{12}NO_3S^+$ $[M + H]^+$ 214.1, found 214.1;

N-hydroxy-3-(methoxymethoxy)benzamide (2.44)



According to the general photo-cleavage procedure, **2.19** (50 mg, 0.123 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 47.0 h, extracted with EtOAc (3 x 5 mL) and purified by PREP-TLC (CH_2Cl_2:MeOH 9:1) to afford **2.44** as a brown oil (7 mg, 27%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.20 (s, 1H, N**H**C(O)), 9.03 (s, 1H, **H**ONHC(O)), 7.39–7.36 (m, 3H, Ar**H**), 7.17–7.14 (m, 1H, Ar**H**), 5.22 (s, 2H, $OC\mathbf{H_2}OCH_3$), 3.38 (s, 3H, $OCH_2OC\mathbf{H_3}$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 163.8, 156.7, 134.2, 129.6, 120.1, 119.0, 114.6, 93.9, 55.6; MS (ESI) *m/z*: calcd. for $C_9H_{12}NO_4^+$ $[M + H]^+$ 198.1, found 198.1; HRMS (ESI) *m/z*: calcd. for $C_9H_{12}NO_4^+$ $[M + H]^+$ 198.0761, found 198.0766.

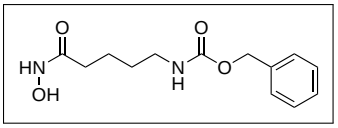
5-(((*tert*-Butyldimethylsilyl)oxy)methyl)-*N*-hydroxythiophene-2-carboxamide (2.49)



According to the general photo-cleavage procedure, **2.31** (50 mg, 0.101 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 47.0 h, extracted with EtOAc (3 x 5 mL) and purified by PREP-TLC (CH_2Cl_2:MeOH 39:1) to afford **2.49** as a brown film (10 mg, 34%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H, N**H**C(O)), 9.07 (s, 1H, **H**ONHC(O)), 7.49 (m, 1H,

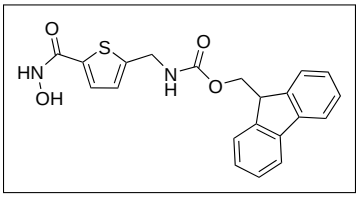
Ar**H**), 6.95 (d, *J* = 3.3 Hz, 1H, Ar**H**), 4.85 (s, 2H, ArC**H**$_2$O), 0.89 (s, 9H, SiC(C**H**$_3$)$_3$), 0.08 (s, 6H, OSi(C**H**$_3$)$_2$C); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 159.6, 149.7, 135.8, 127.2, 124.1, 60.2, 25.7, 17.9, -5.3; MS (ESI) *m/z*: calcd. for $C_{12}H_{22}NO_3SSi^+$ [M + H]$^+$ 288.1, found 288.1; HRMS (ESI) *m/z*: calcd. for $C_{12}H_{22}NO_3SSi^+$ [M + H]$^+$ 288.1084, found 288.1080.

Benzyl (5-(hydroxyamino)-5-oxopentyl)carbamate (2.45)



According to the general photo-cleavage procedure, **2.23** (50 mg, 0.105 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 47.0 h, extracted with EtOAc (3 x 5 mL) and purified by PREP-TLC (CH_2Cl_2:MeOH 39:1) to afford **2.45** as a brown film (5 mg, 19%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H, **H**NC(O)), 8.66 (s, 1H, **H**ONHC(O)), 7.38–7.24 (m, 1H, Ar**H**, CH_2N**H**C(O)CH_2Ph), 5.00 (s, 2H, C(O)OC**H**$_2$Ph), 2.99-2.94 (m, 2H, CH_2C**H**$_2$NHCbz), 1.93 (t, *J* = 7.2 Hz, 2H, HNC(O)C**H**$_2$), 1.51–1.34 (m, 4H, CH_2C**H**$_2$C**H**$_2$$CH_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 167.0, 156.1, 137.3, 128.4, 127.7, 65.1, under DMSO, 31.9, 29.0, 22.5; MS (ESI) *m/z*: calcd. for $C_{13}H_{19}N_2O_4^+$ [M + H]$^+$ 267.1, found 267.2. HRMS (ESI) *m/z*: calcd. for $C_{13}H_{19}N_2O_4^+$ [M + H]$^+$ 267.1339, found 267.1357.

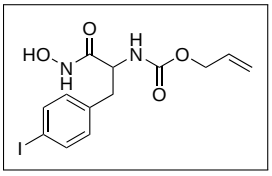
(9*H*-Fluoren-9-yl)methyl ((5-(hydroxycarbamoyl)thiophen-2-yl)methyl)carbamate (2.46)



According to the general photo-cleavage procedure, **2.25** (50 mg, 0.0828 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 24.0 h, extracted with EtOAc (3 x 5 mL) and purified by PREP-TLC (CH_2Cl_2:MeOH 39:1) to afford **2.46** as a brown film (11 mg, 32%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H, N**H**CO), 9.07 (s, 1H, **H**ONHCO), 8.02 (t, *J* = 5.8 Hz, 1H, CH_2N**H**C(O)O), 7.88 (d, *J* = 7.4 Hz, 2H, Ar**H**), 7.69 (d, *J* = 7.4 Hz, 2H, Ar**H**), 7.41 (d, *J* = 7.3 Hz, 2H, Ar**H**), 7.32 (m, 3H, Ar**H**), 6.90 (d, *J* = 3.0 Hz, 1H, Ar**H**), 4.36–4.33 (m, 4H, ArC**H**$_2$NHC(O)OC**H**$_2$CH), 4.25–4.21 (m, 1H, C(O)OCH$_2$C**H**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 159.6 156.2, 147.7, 143.8, 140.8, 127.7, 127.1, 125.5, 125.2, 120.1, 65.6, 46.7, 39.2 (overlap with DMSO-d_6); MS (ESI)

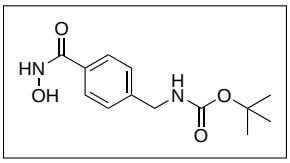
m/z: calcd. for $C_{21}H_{19}N_2O_4S^+$ [M + H]$^+$ 395.1, found 395.2. HRMS (ESI) *m/z*: calcd. for $C_{21}H_{19}N_2O_4S^+$ [M + H]$^+$ 395.1060, found 395.1058.

Allyl (1-(hydroxyamino)-3-(4-iodophenyl)-1-oxopropan-2-yl)carbamate (2.47)



According to the general photo-cleavage procedure, **2.27** (50 mg, 0.0834 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 38.0 h, extracted with EtOAc (3 x 5 mL) and purified by PREP-TLC (CH_2Cl_2 → CH_2Cl_2:MeOH 9:1) to afford **2.47** as a brown film (7 mg, 20%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.68 (s, 1H, **H**NC(O)), 8.88 (s, 1H, **H**ONHC(O)), 7.63–7.61 (m, 2H, Ar**H**), 7.53 (d, *J* = 8.2 Hz, 2H, Ar**H**), 7.06 (d, *J* = 8.8 Hz, 1H, CHN**H**C(O)O), 5.87–5.77 (m, 1H, C(O)OCH$_2$C**H**CH$_2$), 5.19 (m, 1H, CH$_2$CHC**H'**H''), 5.12 (m, 1H, CH$_2$CHCH'**H''**), 4.42–4.33 (m, 2H, C(O)OC**H$_2$**CHCH$_2$), 4.07–4.01 (m, 1H, HNC(O)C**H**NHC(O)O), 2.85–2.70 (m, 2H, HNC(O)CHC**H$_2$**Ar); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 167.9, 155.5, 137.8, 136.8, 133.5, 131.7, 116.9, 92.3, 64.3, 53.8, 37.1; MS (ESI) *m/z*: calcd. for $C_{13}H_{18}N_2O_4$ [M + H]$^+$ 266.1, found 266.2.

***tert*-Butyl (4-(hydroxycarbamoyl)benzyl)carbamate (2.48)**



According to the general photo-cleavage procedure, **2.29** (50 mg, 0.105 mmol, 1.0 equiv.) was placed under an UV-lamp and irradiated for 47.0 h, extracted with EtOAc (3 x 5 mL) and purified by PREP-TLC (CH_2Cl_2:MeOH 39:1) to afford **2.48** as a brown film (16 g, 57%). A trace amount of impurities and CH_2Cl_2 was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.16 (s, 1H, **H**NC(O)), 8.99 (s, 1H, **H**ONHC(O)), 7.69 (d, *J* = 7.9 Hz, 2H, Ar**H**), 7.44 (t, *J* = 5.5 Hz, 1H, ArCH$_2$N**H**C(O)O), 7.29 (d, *J* = 7.9 Hz, 2H, Ar**H**), 4.15 (d, *J* = 5.5 Hz, 2H, ArC**H$_2$**NHC(O)O), 1.39 (s, 9H, C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 164.2, 155.8, 143.5, 131.20 126.9, 126.8, 77.9, 43.1, 28.2; MS (ESI) *m/z*: calcd. for $C_{13}H_{19}N_2O_4^+$ [M + H]$^+$ 266.1, found 266.2. HRMS (ESI) *m/z*: calcd. for $C_{13}H_{19}N_2O_4^+$ [M + H]$^+$ 267.1339, found 267.1345.

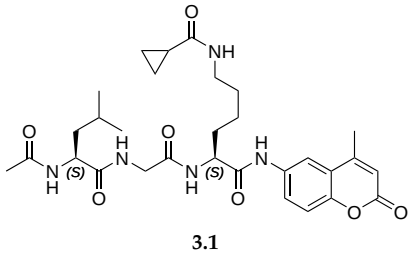
Chapter 3

A Guided Design of Selective HDAC Inhibitors Based on Substrate Profiling

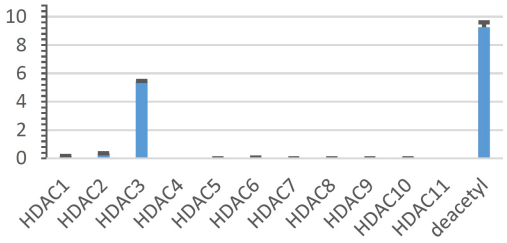
3.1 Introduction

Cancer remains one of the most fatal diseases in the developed parts of the world. Consequently, there is a massive focus on this area.[60] An early diagnose and treatment, are key factors for recovery. Therefore, the danish non-governmental organization (NGO), the Danish Cancer Society, works to raise public awareness of cancer by the use of information campaigns, but also supports through research grants.[61] In many cases, cancer is treated with highly potent compounds, most of which are non-selective towards cancer cells making them toxic. Better treatments are in the pipeline, one promising method is delivery of the drug specifically to cancer cells by various methods for targeted treatment with fewer adverse effects.

For decades, new compounds have been taken into clinical tests, but only a few of them have been approved to fill this unmet need. Our goal is to synthesize compounds that are sub-type selective for vital enzymes shown to be up-regulated in several cancer types. Based on a substrate profiling study performed previous in our group, a hit compound was identified as being selective for HDAC3 (**3.1**), Figure 3.1. We set out to investigate the possibility of converting this substrate into a sub-class selective inhibitor.



(a) Hit compound found through substrate profiling study



(b) Activity profile of the tester HDAC isozymes

Figure 3.1: HDAC3 selective substrate and activity profile

3.2 Histone Deacetylase

The genetic code of mammalian cells is built into DNA, which is primarily found in the nucleus of the cells. The nuclein was isolated by Friedrich Miescher in 1869,[62] but the structure of DNA was first characterized in 1953 by James Watson and Francis Crick.[63] Watson, Crick, and Wilkins received the Nobel Prize for Physiology in 1962. The human DNA is organized in a highly condensed structure called the chromosomes, Figure 3.2. DNA coils around histones, positively charged proteins with a high amount of lysine residues in the protruding histone *N*-amino tails. The ε-amino groups are protonated at physiological pH, hence form strong electrostatic bonds with the negatively charged phosphate group in the backbone of the coiled DNA. Histones are sites for post-translational modifications (PTMs) including phosphorylation, methylation, acetylation, biotinylation, etc. This has been well-described in some excellent papers [65–67]. PTMs alter DNA-histone interactions and binding of transcription factors.[68] Our primary focus is deacetylation, because the balance of chromatin acetylation-deacetylation is linked to various cancer types.[69]

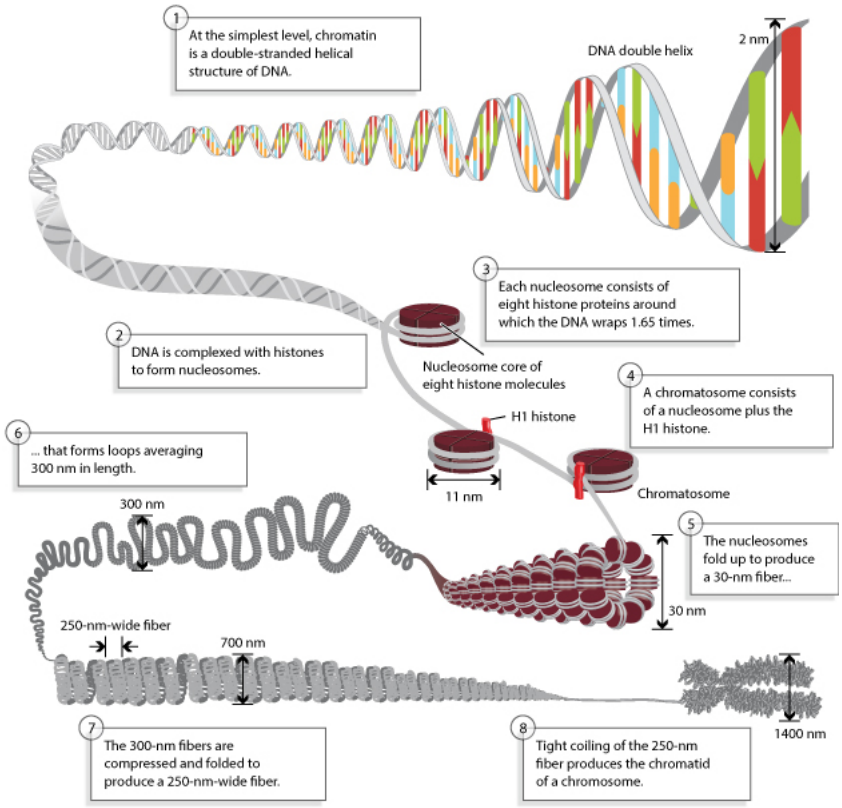


Figure 3.2: Overview of the chromosome and its component. Adapted from Annunziato.[64]

Two enzymes are responsible for this balance, a epigenetic writer (histone acetyltransferase, HAT) and an epigenetic eraser (HDAC). HAT are responsible for acetylation of lysine residues, whereas HDAC removes the attached acetyl group. Acetylation of lysine results in a neutral residue at physiological pH, which cannot bind strongly to the phosphate group of DNA, overall affording a less condensed structure/arrangement. The acetylation also operates as a recognizable tag for epigenetic readers with bromodomains to recruit replication factors.[70] Not only replication factors but also HAT contain a bromodomain to recognize acetylated lysine residues.[71] HDAC removal of the acetyl group restores the electrostatic interaction between protonated lysine-rich histone tails and negative phosphate in DNA. All the histone proteins can be acetylated, but the most important lysine residues are positioned on H3 and H4.[72] Recent studies show that, HDAC enzymes have proven them efficient not only to remove acetyl groups from various sub-

strates, but also small acyl groups, which we also will highlight later in this dissertation.

3.2.1 HDAC Isozymes

The mammalian genome consists of 18 HDAC isozymes,[73] which can be organized in four classes based on their yeast equivalent, with a further sub-class division for class II: class I (HDAC1, HDAC2, HDAC3, and HDAC8), class IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and class IIb (HDAC6 and HDAC10), class III accommodates the sirtuins family (Sirt1-7), and class IV (HDAC11). The class III enzymes are different from the other classes, due to the nicotinamide adenine dinucleotide (NAD^+)-dependent nature, whereas HDAC1-11 are zinc-dependent. As they distinctly differ from HDAC1-11, sirtuins will not be further described.
HDAC1 and HDAC2 have a high sequence identity (86%), and compared to HDAC1: HDAC3 and HDAC8 have 63% and 43%, respectively. In comparison HDAC10 have a sequence identity with HDAC7 and HDAC6 of 34% and 49% respectively, and for the three other class II enzymes (HDAC4, HDAC5, and HDAC9) have an equal 34% sequence identity.[74,75] The tissue distribution of HDAC in the body varies and they are found in many different organs as shown in Table 3.1.

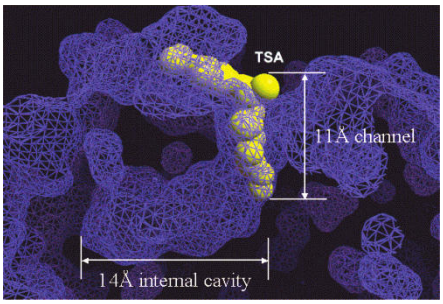
Table 3.1: Distribution HDAC isozymes throughout the body. Modified from Ruijter *et al.*[76]

Tissue	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10
Brain	+	+	+	-	-	+	+	+	+	+
Breast	-	-	+	-	-	+	+	-	+	+
Colon	+	+	-	-	-	+	-	-	+	+
Kidney	+	+	-	-	-	-	-	-	-	+
Ovary	+	+	+	-	-	-	+	+	+	+
Pancreas	+	+	+	+	-	-	+	-	+	-
Prostate	+	+	+	-	-	+	+	+	+	-
Heart	-	+	-	-	-	+	+	+	+	-

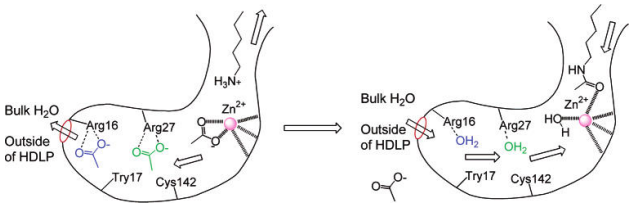
HDAC11 was not included in this study, but when it was first described in 2002 by Gao *et al.* high levels were found in heart, testicles, skeletal muscle, and kidney.[77] In 2007, Broide and co-workers found HDAC11 in the brain of rats.[78]

3.2.2 Active site Construction and Enzymatic Mechanism

In 1999, Finnin *et al.* postulated how the enzyme pocket and the active site in HDAC are organized, Figure 3.3 and Figure 3.4.[79] The authors showed the presence of an internal cavity positioned next to the active site in the enzymatic pocket, Figure 3.3a. In Figure 3.3b, the way of the incoming *N*-acetylated lysine and important residues in HDLP are sketched.



(a) Structure of the enzymatic pocket and internal cavity of histone deacetylase-like protein (HDLP) and with bound trichostatin A (TSA)



(b) Approach of the incoming acetylated lysine-residue and transport of the acetate out of the internal pocket

Figure 3.3: Illustration of HDLP internal cavity and construction of the pocket. Modified from Wang and co-workers.[80]

In the active site, a zinc atom is stabilized by two aspartic acids, one histidine, and a water molecule, Figure 3.4 (a).[79] In addition to the stabilizing residues, the active site contains two aspartic acids, two histidines, and one tyrosine residue. Aspartic acid and histidine forms a charge-relay system, that have been described for serine proteases.[81] The two charge-relay histidine-aspartic acid systems in combination with tyrosine and are important to facilitate deacetylation. One aspartic acid carboxylate hydrogen-binds to the nitrogen of histidine, increasing pK_a of the histidine. One of the charge-relay systems is buried deeper, in closer proximity to the zinc ion in the active site than the other one. Furthermore, the charge-relay system facilitates optimal spatial orientation of the substrate in the active site. After establishing the important residues, Finnin *et al.* proposed a mechanism for the hydrolysis of acetylated lysine residues, Figure 3.4. However, this mechanism is still debated and could differ from one HDAC to another.[82]

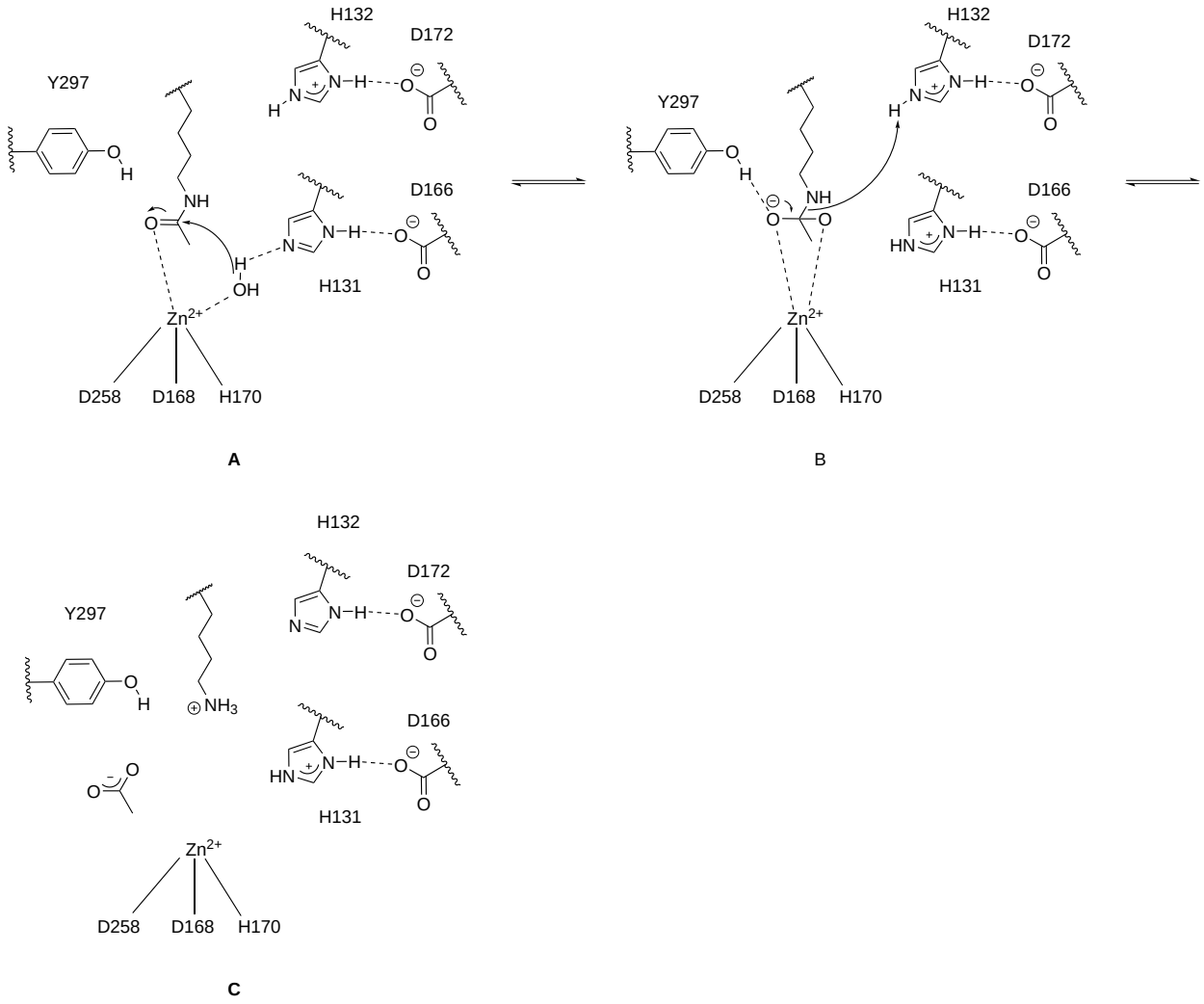


Figure 3.4: Mechanism of deacetylation of *N*-acetylated lysine residue. **A** Incoming acetyl group is attacked by the activated water molecule. **B** The tetrahedral intermediate is protonated and colapses. **C** Liberation of protonated lysine residue. Modified from Finnin *et al.*[79]

3.2.3 HDAC Isozyme Cellular Localization and Active Multi-Protein Complexes

Class I HDACs are primarily localized in the nucleus, although HDAC3 and HDAC8 can be found in the cytoplasm. HDAC sub-class IIa is located in the nucleus where DNA

replication factors are present. HDAC4 is relocated from the nucleus to cytoplasm by PTM, serine-phosphorylation, to expose a 14-3-3 binding domain to facilitate nucleocytoplasmic transport hereby regulating its accessibility to DNA.[83,84]

McKinsey *et al.*[85] described that HDAC5 contains multiple phosphorylation sites for calcium/calmodulin-dependent protein kinase (CaMK), and some others have been found recently.[86] This regulation of HDACs affect the enzymatic activity. Binding of 14-3-3 to HDAC4 and HDAC5 result in an inactivation of the two enzymes and are also incapable of bind to importin α, a transporter complex for nuclear uptake of proteins.[87,88] Verdal and co-workers proved that HDAC6 also contains a highly potent nuclear export sequence and was predominately found in the cytoplasm.[89] HDAC10 is also found in the nucleus and the cytoplasm.[90]

HDACs are often organized in multi-protein complexes in their active enzymatic form. HDAC8 displays enzymatic activity as a single protein, this is linked to the absence of a specific peptide sequence used for protein complex formation.[91] Not only HDAC4 but also HDAC5, HDAC7, and HDAC9 can interact with the HDAC3-silencing mediator for retinoid and thyroid receptor (SMRT)/nuclear receptor corepressor (N-CoR) complex, and have been suggested to serve as recruiters to deliver HDAC3 to act as a HDAC.[92] Besides a 14-3-3 binding site, class IIa also contain a MEF2-interacting transcription repressor (MITR) domain close to the 14-3-3 domain recognized by myocyte enhancer factor-2 (MEF2). MEF2 binds to DNA and acts as a transcription factor and is important in muscle differentiation.[93]

3.2.4 Regulation of Acetylation by HDAC and their Biology Influences

The 11 isozymes are involved in a comprehensive network of biological targets, and HDAC dysfunctional activity has been associated with a variety of diseases. As such, the use of HDACis have been shown to be efficient in the treatment of cancer as well as various non-cancer diseases such as HIV,[94,95] Alzheimer's disease,[96] Parkinson's dis-

ease,[97] inflammation,[98–100] long-term memory loss,[101] diabetes mellitus,[102] heart failure,[103] and Friedreich ataxia.[104]
HDACis have especially proved to be highly efficient against mature T-cell non-Hodgkin lymphomas, both against the aggressive peripheral T-cell lymphomas (PTCL)[105] and the primary skin lymphoma, CTCL.[106]
Therefore, HDACis have a broad application profile and due to the amount of compounds been advanced to clinical trials, more compounds are expected to be approved for treatment in the future. Hydroxamic acids strongly chelate to divalent ions, the reason why this functional group is the most applied for inhibition of HDAC enzymes. Although, there are several concerns regarding the use of hydroxamic acid, as it show low HDAC isozyme selectivity and poor pharmacokinetics, summarized by Kozikowski.[107] One of the most potent HDACi identified so far is TSA.[108] However, due to extensive mutagenesis, it has little use in clinical settings. This has been postulated to arise from the degradation of the hydroxamic acids to the corresponding isocyanates via the Lossen rearrangement.[107] Kozikowski has suggested that *N*-alkylated hydroxamic acids are not as prone to rearrange and therefore, may not afford mutagenesis. Many have looked into the use of other functional groups as HDACis, and in particular the *ortho*-aminoanilide group has been shown to be highly activity.

3.3 From a Pan-Inhibitor to Isozyme-Selective Compounds

The first reported HDACi was DMSO. Friend *et al.* found it to induce growth arrest and terminal differentiation in murine erythroleukemia cells (MELC). These results initiated the HDACi research field and furthermore lead to the synthesis of SAHA by P. A. Marks and R. Breslow, a compound which was approved by FDA in 2006 as an anticancer drug.[109,110] The generally accepted pharmacophore of HDACis composes of a cap group, a linker moiety, and a ZBG, see Figure 3.5.

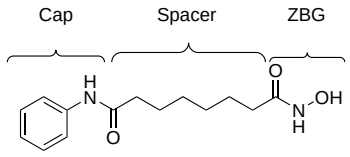


Figure 3.5: Well-acknowledged pharmacophore of HDAC inhibitors

Since the discovery of TSA and SAHA as potent HDACis, an enormous amount of research has been conducted within this field. However, so far only the four compounds, highlighted in Figure 3.6, have been granted FDA approval, SAHA and Romidepsin (Istodax, 2009) for CTCL[111,112], and Belinostat[113] (Beleodaq, 2014) for PTCL. Furthermore, Chidamide was approved against PTCL in 2014 by China Food and Drug Administration (CFDA).[114] In 2011, Romidepsin was also approved for PTCL by FDA in 2011.[115]

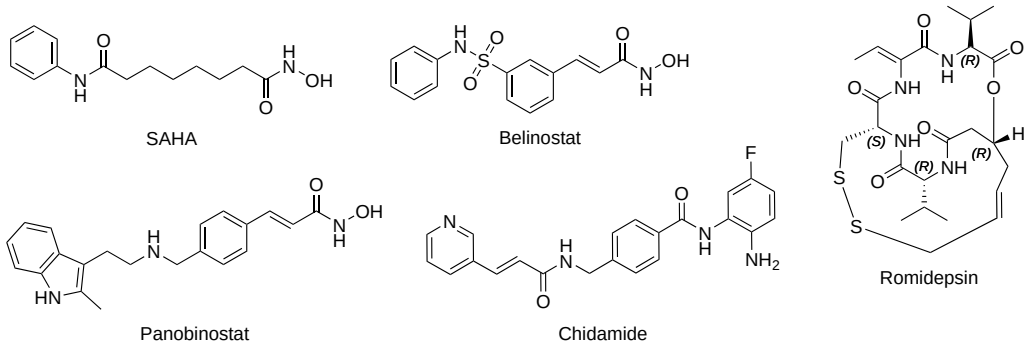
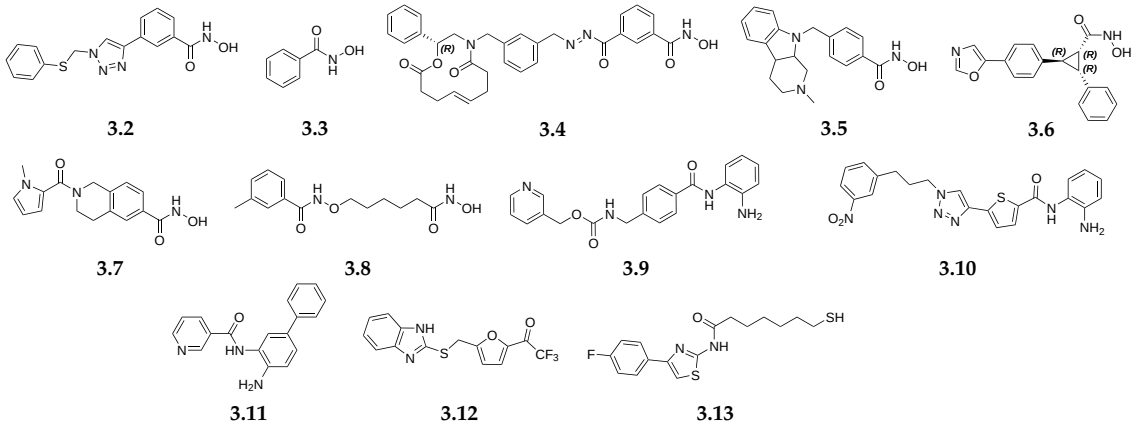


Figure 3.6: Clinical approved HDACi

SAHA, Belinostat, Romidepsin, and Panobinostat are all recognized as pan-inhibitors, and therefore there is a need to find sub-class selective HDACis.

Due to high degree of homology of the active site of HDAC isozymes, it is difficult to predict the structural requirements of a sub-class selective inhibitor. Only a few selective HDACi have until now been published, Table 3.2, and there is definitely a need for a better understanding to develop the next generation of HDACis.[82,116–122]

Table 3.2: Overview of chosen HDACis and their IC_{50} values



3.2 **3.3** **3.4** **3.5** **3.6**

3.7 **3.8** **3.9** **3.10**

3.11 **3.12** **3.13**

HDACi	Selectivity	Activity (IC_{50})	HDACi	Selectivity	Activity (IC_{50})
Hydroxamic acids					
3.2	HDAC8	70 nM[123]	**3.3**	HDAC6	115 nM[124]
3.4	HDAC8	23 nM[125]	Tubastatin A **3.5**	HDAC1	16.4 µM[126]
				HDAC6	4 nM[126]
3.6	HDAC4	20 nM[127]	**3.7**	HDAC6	36 nM[128]
	HDAC5	4 nM[127]		HDAC8	2.10 µM[128]
	HDAC7	30 nM[127]			
	HDAC9	40 nM[127]			
3.8	HDAC4	16.3 nM[129]			
	HDAC5	2.31 nM[129]			
	HDAC6	26.9 nM[129]			
o-Aminoanilide					
Entinostat **3.9**	HDAC1	0.51 µM[130]	**3.10**	HDAC3	0.26 µM[131]
	HDAC3	0.20 µM[132]			
3.11	HDAC1	48 nM[133]			
	HDAC2	0.9 µM[133]			
Trifluoromethyl ketone			Thiol		
3.12	HDAC1	>50 M[134]	**3.13**	HDAC1	36 nM[135]
	HDAC4	43 µM[134]		HDAC6	0.55 µM[135]
	HDAC8	1.8 µM[134]		HDAC9	57 nM[135]

3.4 High-Throughput In-Bead Screening of Substrates for HDAC Enzymes Profiling

We wanted to further investigate a recently developed in-bead strategy[9] to allow for HDAC substrate profiling studies useful for identification of isozyme selective compounds. A former master student, Daniel Madsen, applied the in-bead screening platform for the identification of isozyme selective HDAC substrates from a split-and-mix compound library with acylated lysine oligopeptides.

3.4.1 Introduction to In-Bead Screening Technology

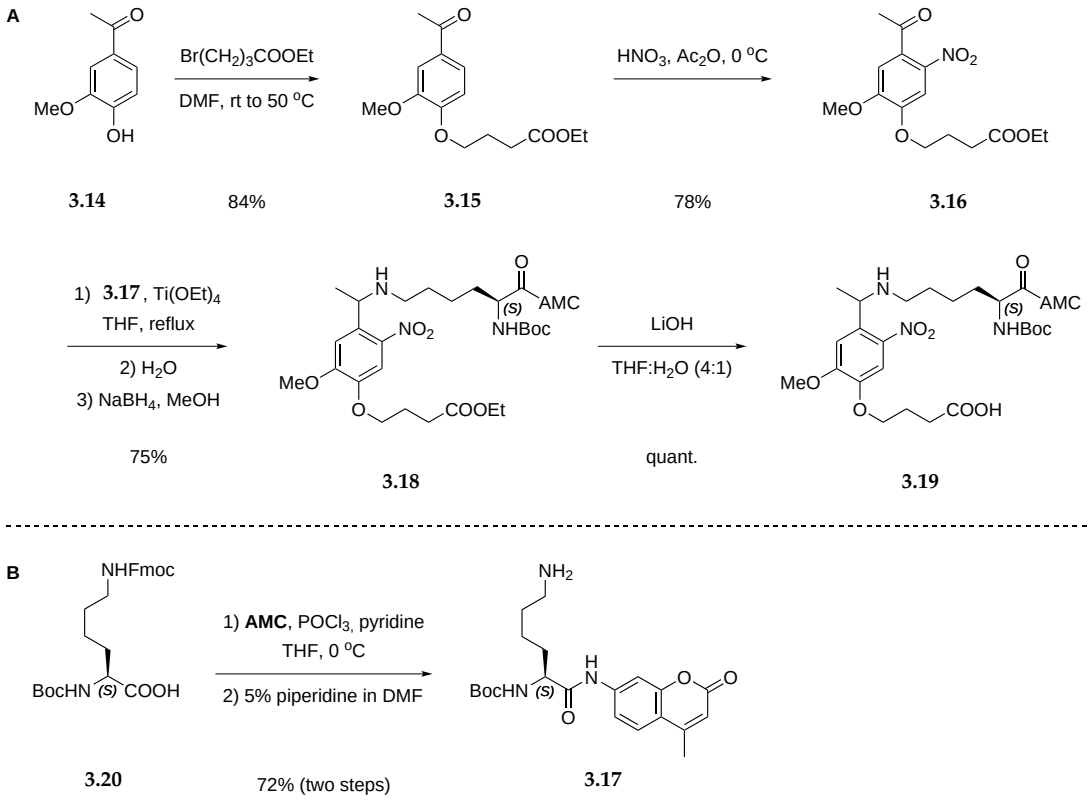
Several on-bead solid-supported screening papers have been published (references herein).[9] To diminish the influence by the solid-support backbone, Qvortrup and Nielsen rationalized that releasing compounds from the solid-support in an aqueous media, could retain the compounds inside the beads, due to the hydrophobic interior, hence the terminology in-bead. Daniel Madsen synthesized a large library of substrates for a profiling study of HDAC to find selective isozymes. The compounds where build on solid-support by standard SPS chemistry with a photo-labile linker. After photolytic release of the substrates, HDAC enzymes were added to the beads. If the acylated substrate was recognized by HDAC, the acyl group would be cleaved and liberate protonated lysine residue. Upon the addition of the endopeptidase, trypsin,[136,137] the protonated substrate was recognized by this enzyme and due to its nature, it will cleave the *C*-terminal of protonated residues. This will cleave a fluorophore from the *C*-terminal with a detectable change in fluorescence. The photolytic cleavage conditions prevents the use of chemical reagents, that could interfere with the enzymes-substrate interactions or the integrity of HDAC. For the solid-support polyethylene glycol polyacrylamide (PEGA) was chosen, due to its reported accommodation of larger proteins.[138]

3.4.2 Substrate Profiling of HDAC Enzymes

Potent lysine-containing substrates used for HDAC profiling have been summarized by the Olsen lab.[139] 7-amino-4-methylcoumarin (AMC) is a standard utilized fluorophore. The applied profiling substrates contain an Ac-Leu-Gly-Lys-AMC backbone, and the isozyme selectivity was found to depend on the nature of the acyl group.

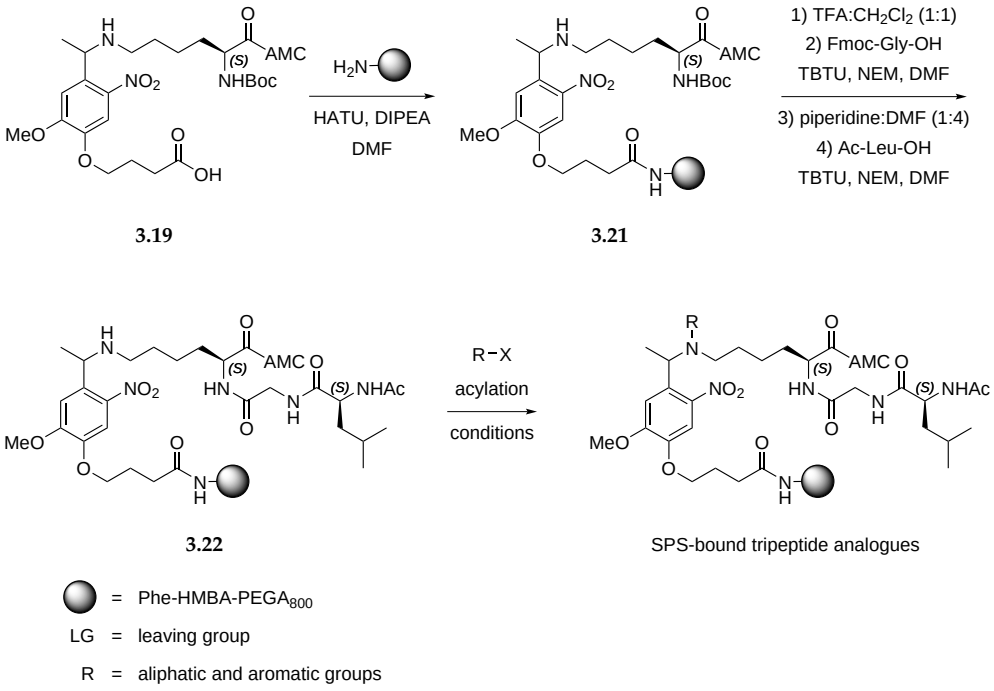
3.4.3 Development and Functionalization of an ε-Lysine Bound Photo-Labile Linker for High-Throughput Screening

To obtain potential sub-type selective HDAC substrates, we first synthesized a photo-labile construct **3.19** based on the Holmes photo-linker.[140]
Synthesis of the linker construct, commenced with an alkylation reaction of commercially available acetovanillone (**3.14**) with ethyl 4-bromobutanoate, **A** Scheme 3.1. After the attachment of this handle moiety for immobilization to the solid-support, nitration of **3.15** was achieved by HNO_3 and Ac_2O, affording **3.16** in high yield (78%). **3.17** was synthesized by a two-step procedure in high yields starting from commercially available Boc-Lys(Fmoc)-OH, **B** Scheme 3.1. The construct **3.17** was then be introduced by reductive alkylation affording **3.18** in high yields before quantitative hydrolysis of the ester with LiOH providing **3.19**. The reductive alkylation was performed with $Ti(OEt)_4$. During the hydrolysis of the ethyl ester partial hydrolysis of coumarin was observed, however re-lactonization was achieved by stirring the crude compound at neutral pH for 2 h followed by up-concentration under reduced pressure.



Scheme 3.1: **A** Synthetic route toward the lysine-AMC containing photo-labile linker. **B** Synthesis of AMC-containing lysine

Introduction of the base-labile HMBA linker to the immobilized construct allows for LCMS analysis of the proceeding reactions, Scheme 3.2. Phenylalanine was coupled to HMBA under 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4- triazole (MSNT) conditions. After Fmoc deprotection the photo-labile linker, **3.19**, was coupled to immobilized phenylalanine under *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HATU) conditions using only 1.1 equiv. of the photo-labile linker to obtain **3.21**. For the last steps, standard SPS Boc strategy was applied to obtain the ε-bound Ac-Leu-Gly-Lys tripetide, **3.22**.



Scheme 3.2: Synthesis of substrate scope. See Figure 3.7 for an overview of applied the R groups

As for the final step, ε-lysine was acylated. Decreased reactivity of the nitrogen[141] was observed for the *N*-alkylated hydroxylamine *O*-photo-labile protecting group. The secondary amine of **3.22** is probably better shielded due to the substituted benzylic position. Generally, large amounts of reagents were used, and in some cases, heat was required to obtain full conversion. In Figure 3.7, the different acyl groups used are shown.

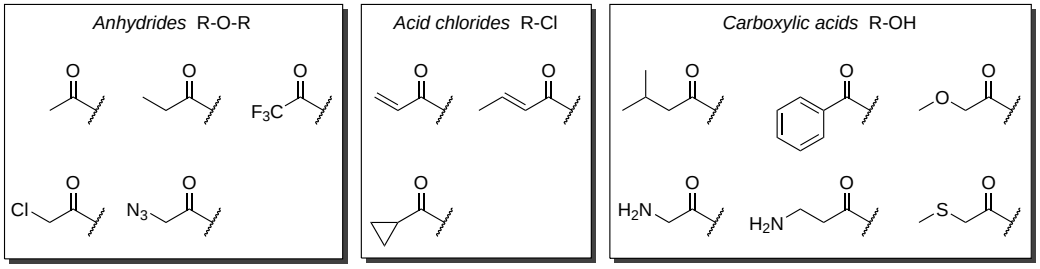


Figure 3.7: Applied acyl groups in the profiling study

Photo-mediated cleavage of the immobilized compounds was performed in excellent purity to release tripeptide substrates, Figure 3.8, ready for HDAC profiling studies.

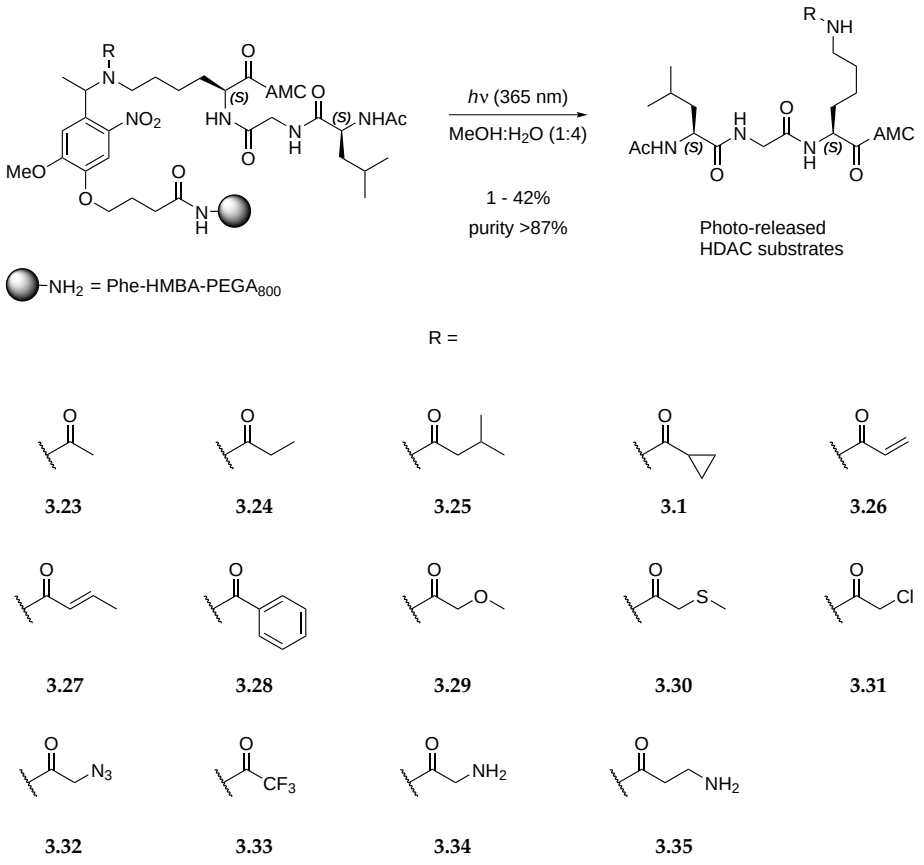


Figure 3.8: Liberation of acylated lysine-containing tripeptides

All substrates (Figure 3.8) were tested against the 11 HDACs. The cyclopropionamide **3.1** was a selective substrate for HDAC3. *Can we redesign the selective tripeptide substrate to generate a sub-type selective HDAC inhibitor* At this point I was involved in the project.

3.5 Guided Inhibitor Synthesis Strategy

With the discovery of the substrate **3.1**, we became interested in modulating the substrate into an inhibitor with isozyme-selectivity. To have a good zinc-chelator, we wanted to

introduce the hydroxamic acid functionality which is the strongest and most investigated ZBG. We planned a collection of hydroxamic acid functionalized tripeptides. AMC was exchanged to a aniline amide as this fluorophore is not needed for this assay type.
We wanted to investigate both the position of the hydroxamic acid carbonyl group and the length of the linker. Finally, a range of acyl groups were chosen. For this study we designed a set of inhibitors, seen in Figure 3.9.

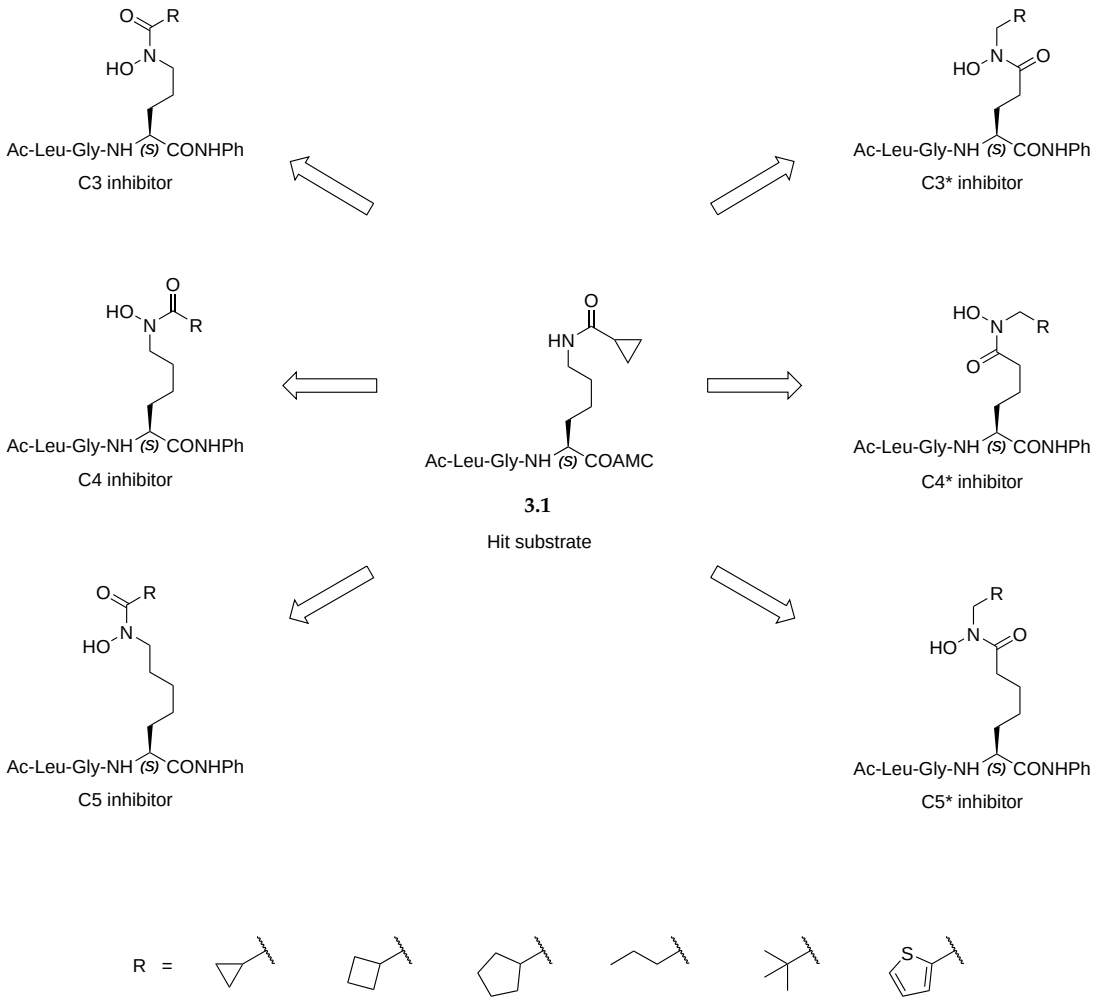
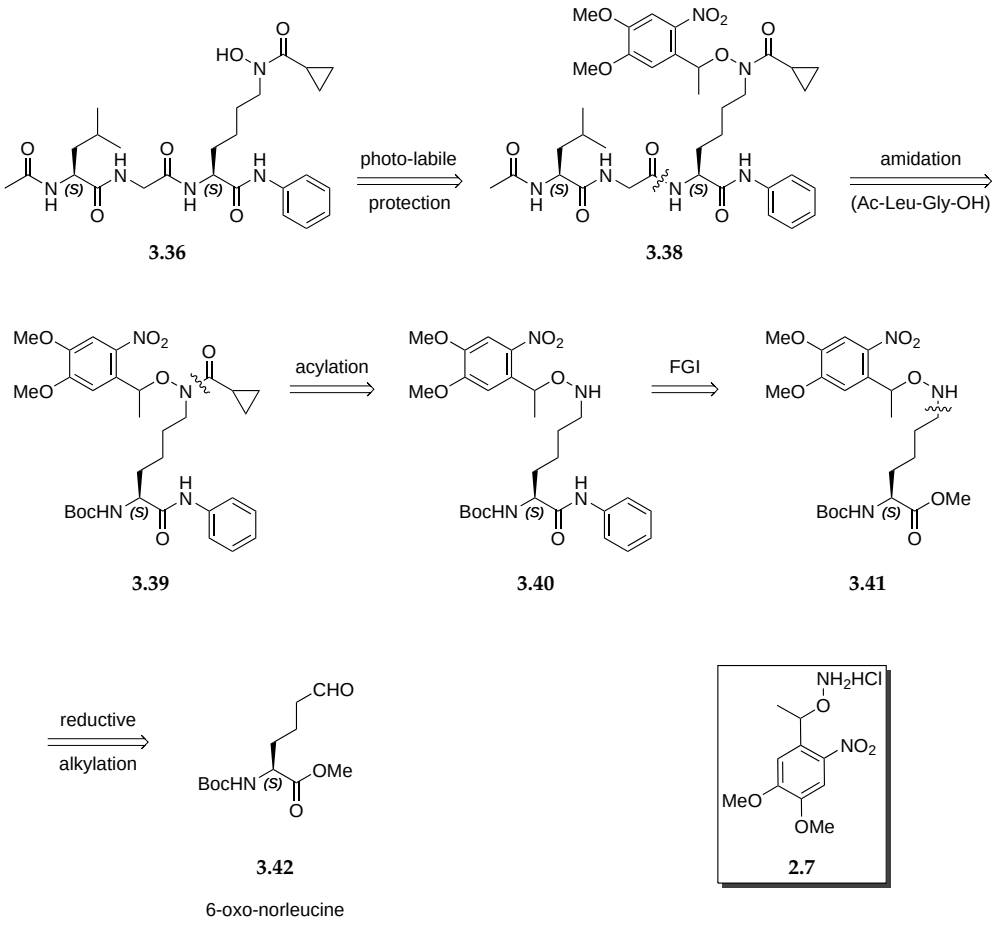


Figure 3.9: Overview of inhibitor scaffolds designed from the HDAC3 selective substrate, **3.1**

We aimed to synthesize one analogue for C3 and C3*, the cyclopropaneacetyl or *N*-cyclopropanemethyl, respectively. For C4 and C4*, all the analogues in Figure 3.9 was designed to resemble to the hit substrate **3.1**. The *tert*-butyl and 2-thiophene groups were excluded for the C5 and C5* analogues.

A retrosynthetic analysis of one of the proposed structures **3.36** is seen in Scheme 3.3. We planned to employ the newly developed hydroxylamine photo-labile protecting group due to its stability and the mild release conditions. The desired hydroxamic acid would

then originate from cleavage of the photo-protected hydroxamate **3.38**. We envisioned that **3.38** could be formed from coupling the dipeptide **3.37** with **3.39**. The derivatization of the scaffold, **3.40**, could be performed by acylation of the hydroxylamine, **3.39**. The compound **3.40** was rationalized to arise from 6-oxo-norleucine **3.42**, by coupling with the photo-labile protecting group **2.7**, followed by standard functional group interconversion.



Scheme 3.3: Synthetic overview of desired hydroxamic acids

This combinatorial approach provides easy accessibility to a diverse set of compounds as structural parameters such as the length of the *C*-terminal residue, the amide group on the *C*-terminal, the dipeptide, and the acyl group can easily be changed.

3.6 Investigation of a Synthetic Strategy for the Norleucine-Derived Key Building Block

Natural amino acids constitute an attractive starting material, due to their inherent stereocenter, ready availability, and low cost. Hereby, the stereocenter is set from the beginning of the synthesis, and therefore, reaction conditions that do not result in epimerization, are crucial. In the literature, synthesis of 6-oxo-norleucine **3.42** has not been reported, possibly because of the chance for spontaneous cyclization. However, some 6-oxo-norleucine derivatives were reported by Fanning *et al.*[142] and Padrón *et al.*[143] starting from aspartic acid **3.49**. The authors took advantage of a two-carbon elongation with the Wittig reagent, (triphenylphosphoranylidene)acetaldehyde. The Wittig reaction was first published in 1954 by Georg Wittig and Ulrich Schöllkopf.[144] Phosphonium ylides react with aldehydes or ketones to generate alkenes. Ylides are formed from deprotonation of the corresponding phosphonium salts. Stabilized ylides generally afford *E*-alkenes.[145]

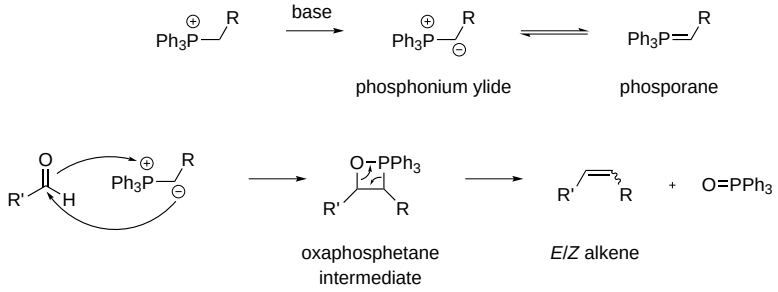


Figure 3.10: Mechanism of Wittg reaction

Changing the Wittig reagent provided a feasible approach to obtain all the desired amino acids, from one single key precursor, Figure 3.11.

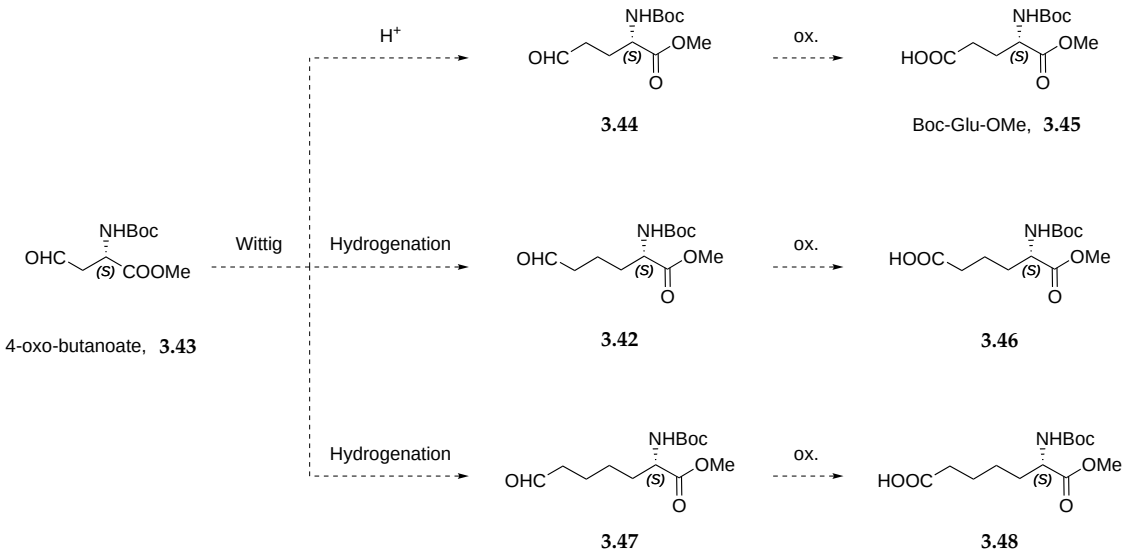
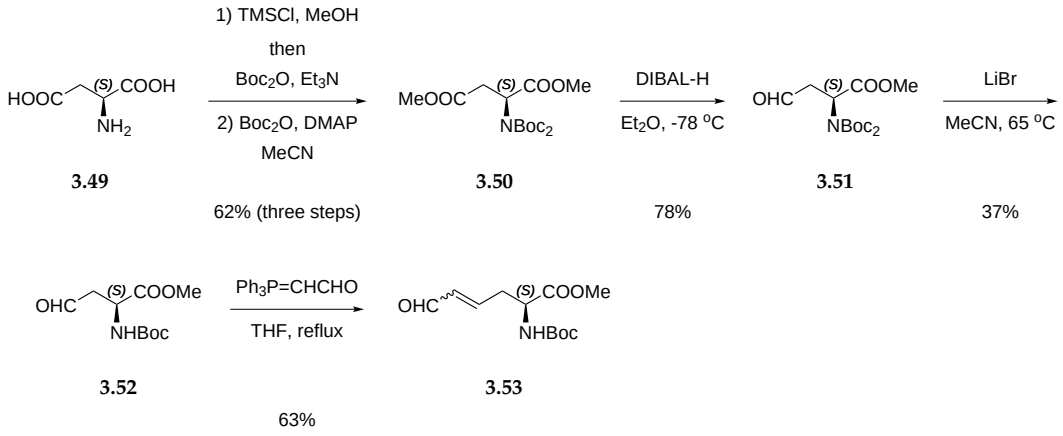


Figure 3.11: Synthetic strategy of important intermediates from the key precursor **3.43**

The Padrón procedure was applied to synthesize **3.50** from aspartic acid in a three-step approach.[143] 4.0 g of *L*-aspartic acid **3.49** was treated with TMSCl in MeOH, followed by Boc protection with Boc_2O and $NaHCO_3$ in MeOH, Scheme 3.4. Lastly, **3.50** was obtained by a 4-dimethylaminopyridine (DMAP) mediated Boc protection under dry conditions. Overall, **3.50** was afforded in 62% over three steps.



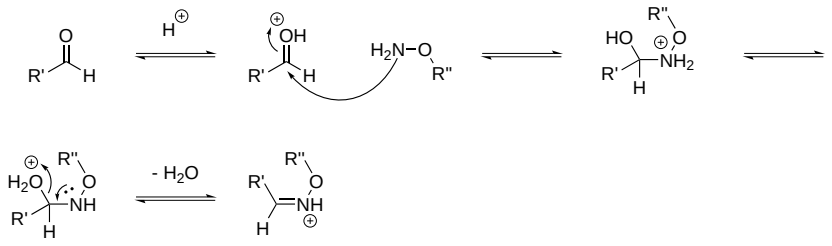
Scheme 3.4: Synthesis of the conjugated aldehyde from aspartic acid

The reaction also performed well on large scale, and specific rotation was found to be in accordance with the literature. As epimerization is a concern throughout the entire synthesis, optical rotation was recorded for all pure enantiomers in this dissertation. For novel compounds, optical rotation was recorded with a concentration of 1.00 in a suitable solvent.

Optimizing the pace of *N*,*N*-diisobutylaluminium hydride (DIBAL-H) (1.1 equiv., 1 M in hexanes) addition as well as stirring time was necessary for complete conversion of the side-chain methyl ester. Optimized conditions: addition of DIBAL-H to **3.50** at -78 °C over 30 min followed by 30 min stirring and subsequently quenched by the addition of H_2O. Generally, both the starting material and the corresponding alcohol were observed in low quantities and separation of **3.51** was difficult.

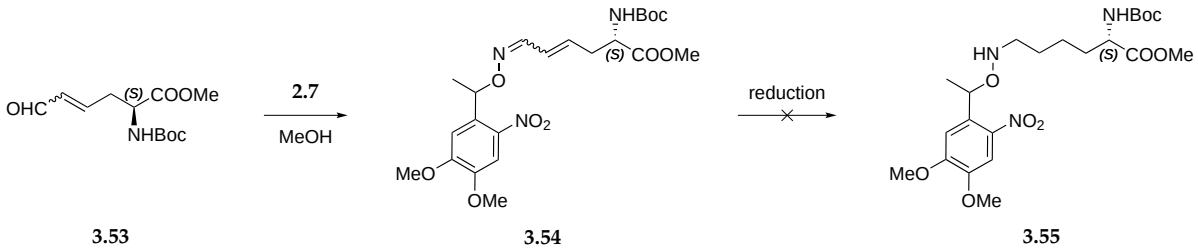
Fanning *et al.* experienced low yield for the Wittig reaction of **3.51**.[142] The expected steric hindrance of the Boc groups, was proven to be the case, as the corresponding mono-Boc compound **3.52** afforded excellent yield under Wittig conditions. The mono-deprotection was achieved by LiBr, a procedure developed in the Martín lab.[146] Unfortunately these conditions in our hands from 3.96 g of **3.51** afforded incomplete conversion and once more difficult separation. Low yield (37%) of **3.52** was observed. With **3.52** in hand, the Wittig reaction performed well under reflux to obtain 63% of the α,β-unsubstituted aldehyde **3.53**. Trace impurities were observed by NMR and the stereochemistry ratio of the double bond was found by ^{1}H-NMR to be (*Z*:*E*) 33:67.

Fanning *et al.*[142] observed cyclization of the saturated aldehyde. To prevent this, we chose to continue with the conjugated alkene. In Chapter 2, we performed reductive alkylations on our developed photo-protected hydroxylamine **2.5** to synthesize *N*-alkylated hydroxylamines. Aldehydes were reacted with the *O*-MeNV hydroxylamine hydrochloride salt **2.7** in EtOH to generate the oxime, which was then reduced with $NaBH_3CN$ (1.0 equiv.) under acidic conditions. We changed the solvent to dry MeOH, to prevent transesterification, and attempted neutral conditions, due to the risk of Boc-deprotection. However, acidic conditions have been reported to be crucial for mono-alkylation of oximes with the use of $NaBH_3CN$.[17] Full conversion to the oxime was observed within a couple of hours, however after prolonged reaction time, starting material was detected, probably a consequence of the reversible nature of oxime formation, Scheme 3.5. It was subsequently found that complete conversion to the oxime was achieved with pyridine.[147]



Scheme 3.5: Reversible oxime formation

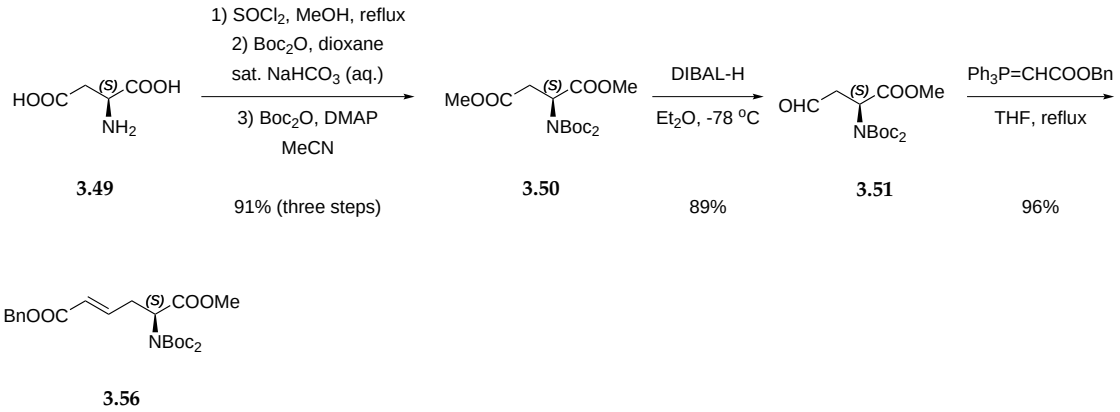
The crude oxime was cooled to 0 °C and $NaBH_3CN$ was added, Scheme 3.6. The conjugated oxime proved to be highly stable under neutral conditions, even after the addition of an excess of $NaBH_3CN$ (5 equiv.). After observing that neutral conditions was inefficient, we tried the previously developed reaction conditions using $NaBH_3CN$ (1.0 equiv.) and TFA (pH $<$ 3). This reaction afforded trace amounts of **3.55** but also the formation of an unidentified by-product. pH was checked continuously for 12 h and reducing agent was added in portions, a total of 22 equiv., without achieving complete reduction. Reduction of oximes has previously shown to be efficient using the borane-THF complex $BH_3 \cdot THF$, but also these conditions were unsuccessful.



Scheme 3.6: Coupling and attempted double reduction of the conjugated oxime

Useful reduction conditions of **3.54** were not found, and the synthetic strategy had to be changed. First of all, we had to avoid the conjugated alkene, and thereby reintroduce the di-Boc protection to prevent cyclization. These modifications also prevent the need for the low yield mono-Boc deprotection. We wanted to use Wittig reagents with a terminal oxygen-functionality that could be converted to the corresponding aldehyde and the carboxylic acid. Commercially available benzyl(triphenylphosphoranylidene)acetate[148] was found to be suitable reagent. We envisioned that reacting the aldehyde **3.51** with the stabilized phosphonium ylide, would result in the conjugated benzyl ester which could

be further derivatized to the corresponding saturated carboxylic acid or saturated benzyl ester. The latter once more would again be regioselectively reduced to the corresponding aldehyde. This approach would allows us to use a single precursor for the formation of the desired building blocks for both the C4 and C4* analogues.
Only small modifications gave even more efficient reactions starting from aspartic acid **3.49**: high yielding esterification was observed with $SOCl_2$ instead of TMSCl and an efficient Boc-protection was seen with dioxane and sat. $NaHCO_3$ (aq.). On a 5.0 g scale reaction, this afforded 12.3 g of **3.50** (91% yield) over three steps. Encouraged by this, we scaled-up to start with 20.0 g of aspartic acid **3.49**, which provided 49.8 g of the product (91%). DIBAL-H reduction afforded 14.7 g of **3.51** in excellent yield (89%). The two-carbon elongation of **3.51** performed well with commercially available benzyl(triphenylphosphoranylidene)acetate (1.5 equiv.) in refluxing THF to provide the conjugated benzyl ester **3.56** in 96% yield, Scheme 3.8. The Boc groups were not a steric issue, as mentioned earlier for the aldehyde substrate.



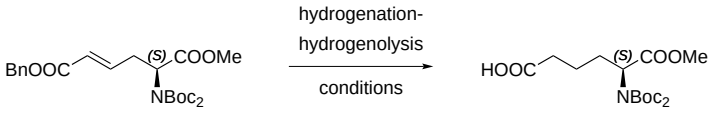
Scheme 3.7: Synthesis of the conjugated benzyl ester **3.56** from aspartic acid

An optimized and high yielding five-step procedure for the key intermediate **3.56** had been developed.

3.6.1 Chemoselective-Controlled Hydrogenation or Hydrogenation-Hydrogenolysis of the Key Conjugated Benzyl Ester Intermediate

To obtain the saturated carboxylic acid, hydrogenation of the alkene bond and hydrogenolysis of the benzyl ester **3.56** was desired. An overview of the tested reaction conditions is listed in Table 3.3.

Table 3.3: Screening of hydrogenation-hydrogenolysis conditions



3.56 **3.57**

Entry	Scale	Pd	Solvent	Additives	Atm.	Temp.	Time (h)	Yield[a]
1	25.4 mg	$Pd(OH)_2/C$ (20 mol%)[b]	EtOAc	-	H_2	rt	25.5	88[c,d]
2	25.0 mg	$Pd(OH)_2/C$ (20 mol%)[b]	EtOAc	-	H_2	reflux	10.0	87[c,e]
3	2.0 g	$Pd(OH)_2/C$ (20 mol%)[b]	EtOAc	-	H_2	reflux	15.0	79[c,f]
4	6.9 g	$Pd(OH)_2/C$ (40 mol%)[b]	EtOAc	-	H_2	reflux	10.2	85[c,g]
5	50.0 mg	Pd/C (20 wt%)[h]	MeOH	Et_3SiH (10 equiv.)	argon	rt	0.17	quant.[c,f,i]
6	50.0 mg	$Pd(OAc)_2$ (2.5 mol%)	MeOH	charcoal[j]	H_2	rt	14.0	94[c,e]
7	50.0 mg	$Pd(OAc)_2$ (5.0 mol%)	*i*PrOH	charcoal[j]	H_2	rt	14.5	98[c,e]
8	50.0 mg	$Pd(OAc)_2$ (5.0 mol%)	MeOH	charcoal[j]	H_2	rt	15.5	97[c,e]
9	50.0 mg	Pd/C (10 wt%)	MeOH	-	H_2	rt	15.75	93[c,e]
10	1.0 g	$Pd(OAc)_2$ (5.0 mol%)	MeOH	charcoal[j]	H_2	rt	16.0	quant.[c,k]

(a) Isolated yield (b) 20% Pd (c) filtrated through a pad of Celite® (d) hydrogenated product indicated by ^{1}H-NMR (e) trace amount of **3.56** (f) 8% 5-((Boc)amino)-6-methoxy-6-oxohexanoic acid was indicated by ^{1}H-NMR based on the Boc group (g) 50% 5-((Boc)amino)-6-methoxy-6-oxohexanoic acid was indicated by ^{1}H-NMR based on the Boc group (h) 10% Pd (i) Et_3SiH indicated by ^{1}H-NMR (j) $Pd(OAc)_2$:charcoal 10:90 (wt:wt) (k) CH_2Cl_2 and other minor impurities observed by ^{1}H-NMR

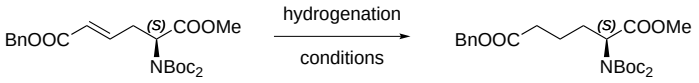
The first test reaction was carried out using Pearlman's catalyst (20% $Pd(OH)_2/C$)[149] in MeOH under a hydrogen atmosphere (Entry 1, Table 3.3). However, ^{1}H-NMR showed incomplete conversion. Increasing the temperature improved conversion (Entry 2). Upscaling to 2.0 g (Entry 3) afforded a small drop in yield with minor impurities observed by ^{1}H-NMR. Specific optical rotation was determined, $[a]_D^{22}$ - 31.6° (*c* 1.00, MeOH). The amount of $Pd(OH)_2/C$ was doubled to obtain full conversion on larger scale (Entry 4).

Unfortunately, this led to the formation of the mono-Boc protected side-product. ^{1}H-NMR indicated a 1:1 ratio

Pd/C is known to facilitate catalytic transfer hydrogenation with Et_3SiH as a hydrogen donor.[150] Changing to 20 wt% Pd/C from the reported conditions afforded **3.56** in quant. yield, however impurities of Et_3SiH were indicated by ^{1}H-NMR (Entry 5).
The combination of methanol vapors with oxygen pose a high safety risk due to the pyrophoric nature, not to mention having hydrogen-containing balloons in the fume hood. The Fouquet group has developed a safer method for hydrogenation-hydrogenolysis.[151,152] The group reported a hydrogenation-hydrogenolysis protocol with *in situ* formation of Pd/C from $Pd(OAc)_2$ and charcoal under hydrogen atmosphere (Entry 6-8). Utilizing these conditions provided high yield (Entry 6). MeOH and *i*PrOH are both well-tolerated solvents. Using a commercially available Pd/C gave similar yield (Entry 9). Up-scaling the *in situ* Pd/C-formation conditions on 1.0 g, gave the substituted carboxylic acid **3.57** in quantitative yield (Entry 10).

We next turned our attention to the hydrogenation of the α,β-unsaturated benzyl ester **3.56**, to afford the saturated benzyl ester (**3.58**), a precursor for the desired aldehyde for the synthesis of C4 analogues. Transition metal complexes have been reported to afford hydrogenation of the conjugated alkenes. In special cases, Pd/C or $Pd(OH)_2$ have been reported to afford the saturated benzyl ester, but these conditions have already proved to give the saturated carboxylic acid for our substrate, Table 3.3. One example of a useful metal complex for this type of reaction, is the Wilkinson's catalyst,[153] Table 3.4.

Table 3.4: Screening of hydrogenation conditions



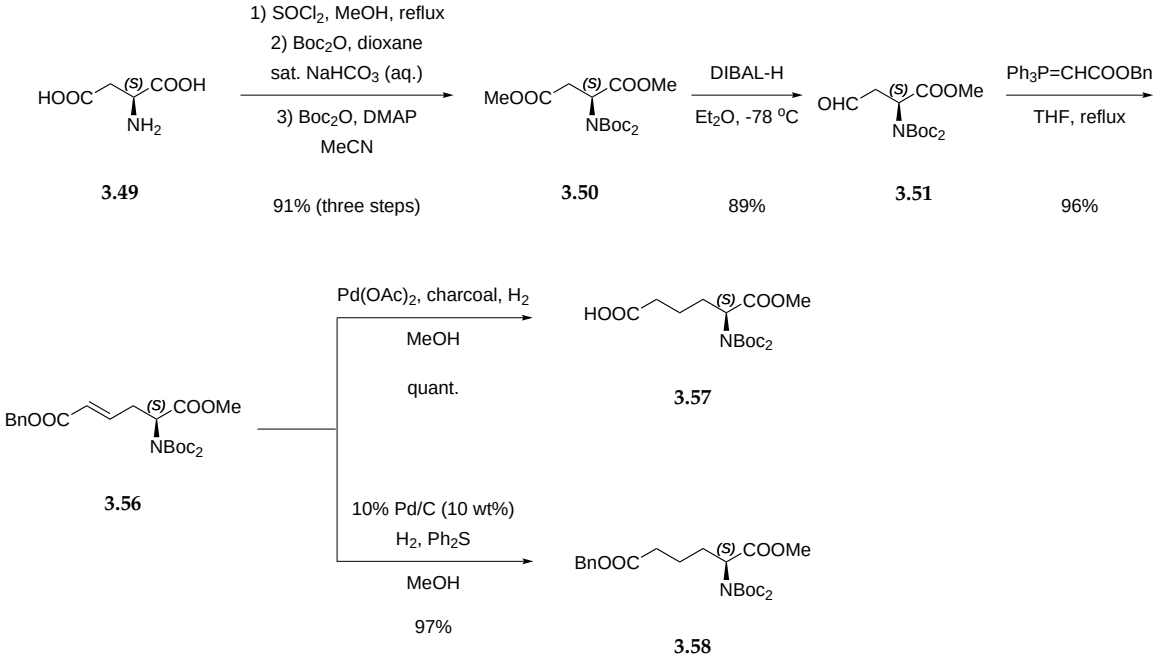
3.56 **3.58**

Entry	Scale	Reagent (mol%)	Solvent	Atm.	Temp.	Time (h)	Yield[a] (%)
1	20.0 mg	$RhCl(PPh_3)_3$ (23.0 mol%)	toluene[a]	H_2	50 °C	21.25	quant.[b]
2	0.5 g	$RhCl(PPh_3)_3$ (5.0 mol%)	toluene[a]	H_2	reflux	15.0	76[c,d]
3	1.0 g	$RhCl(PPh_3)_3$ (8.0 mol%)	toluene[a]	H_2	50 °C	15.0	80[c,d]
4	100.0 mg	10% Pd/C (10.0 wt%), Ph_2S (1.0 mol%)	MeOH	H_2	rt	40.0	99[d]
5	50.0 mg	$Pd(OAc)_2$ (2.5 mol%), Ph_2S (1.0 mol%) charcoal[g]	MeOH	H_2	rt	36.0	-[e]
6	1.01 g	10% Pd/C (10.0 wt%), Ph_2S (1.0 mol%)	MeOH	H_2	rt	36.0	98[g]
7	3.05 g	10% Pd/C (10.0 wt%), Ph_2S (1.0 mol%)	MeOH	H_2	rt	36.0	98[g]
8	5.51 g	10% Pd/C (10.0 wt%), Ph_2S (1.0 mol%)	MeOH	H_2	rt	40.0	94[g]
9	6.48 g	10% Pd/C (10.0 wt%), Ph_2S (1.0 mol%)	MeOH	H_2	rt	36.0	97[g]

(a) dry condition (b) purified by glass pipette column chromatography (c) purified by flash column chromatography (d) starting material observed after flash column chromatography (e) 94% recovered starting material (f) $Pd(OAc)_2$:charcoal 10:90 (wt:wt) (g) filtered trough a pad of Celite® and evaporated to dryness, this procedure was repeated once

Initially, the reaction with Wilkinson's catalyst showed excellent selectivity and quant. yield (Entry 1, Table 3.4). Using 23 mol% catalyst turned out to be crucial (Entry 2 and 3). The need for flash column chromatography, made this approach undesirable. Sajiki *et al.* decreased the Pd/C activity by the use of amine additives in order to increase the chemoselectivity (hydrogenation over hydrogenolysis)[154,155] and later sulfur-containing additives were found to facilitate chemoselective Pd-complexes.[156] Sulfur is notorious known to reduce the activity of palladium and other noble metals, such as platinum, by occupying catalytic sites.[157] One of the most successful "poisoned catalysts" applied in organic synthesis is the Lindlar catalyst developed in 1966. It contains a combination of $Pd/CaCO_3$ and lead, and further addition of quinoline can be advantageous.[158] Sajiki *et al.* show diphenyl sulfide to be a good additive for chemoselective hydrogenation of α,β-unsaturated benzyl esters.[156] Using these conditions (Entry 4, 6-9), worked well on our substrate resulting in a multi-gram hydrogenation of **3.56** to afford **3.58** in 97% yield (Entry 9).

We have established reaction conditions from commercially available aspartic acid **3.49**, to the key conjugated benzylic ester intermediate **3.56**, and by manipulating the reaction conditions, we were able to obtain either the saturated carboxylic acid **3.57** or benzyl ester **3.58**, see Scheme 3.8.



Scheme 3.8: Updated overview of the synthetic approach to the four-carbon carboxylic acid and benzyl ester

With a well-established approach in hand, the next step was the one-carbon elongation to synthesize the three-carbon aldehyde **3.60** from the corresponding two-carbon aldehyde precursor **3.51** by Wittig approach.

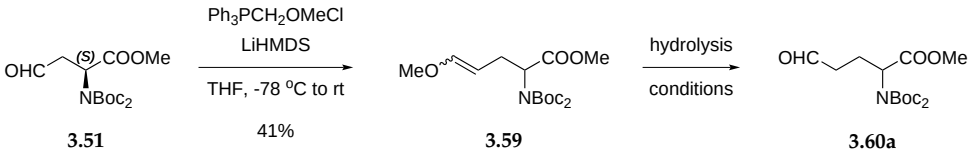
3.7 Synthesis of Natural and Unnatural Amino Acids from a Chiral Precursor

3.7.1 One-Carbon Elongation Mediated by Wittig reactions

One-carbon elongation of aldehydes can be achieved by using (methoxymethyl)triphenylphosphonium chloride, followed by hydrolysis of the resulting enol ether. This method was utilized in the attempt to to synthesize optical pure glutamate aldehyde **3.60**, Table 3.5.

From a modified literature procedure with (methoxymethyl)triphenylphosphonium chloride,[159] lithium hexamethyldisilazane (LiHMDS) was added to the phosphonium salt at -78 °C resulting in a dark red colored solution. This afforded enol ether **3.59** in 41% with an *E*:*Z* ratio of 81:19.

Table 3.5: Tested conditions of the one-carbon elongation



Entry	Scale	Reagent (equiv.)	Solvent	Temp.	Time (h)	Conversion[a]
1	50.0 mg	*p*-TsOH · H_2O (0.5)	acetone	rt	0.58	**3.59** [b]
2	50.0 mg	*p*-TsOH · H_2O (0.7)	acetone and H_2O	rt	2.0	**3.59** + **3.60a** [c,d]
3	82.7 mg	Cl_3CCOOH (5.0)	$CHCl_3$	reflux	1.75	**3.60a** [b,e]
4	82.7 mg	Amberlite 120[f], LiBr (9.0)	acetone	rt	22.25	-
5	82.7 mg	PPTS (1.5)	acetone	rt	21.25	**3.59** + **3.60a** [c]
6	0.58 g	*p*-TsOH · H_2O (0.5)	acetone	rt	0.83	**3.60a** [b,g]

(a) indicated by TLC (b) full conversion by TLC (c) incomplete conversion by TLC (d) longer reaction time afforded a highly polar compound (e) highly polar compound was observed (f) 26 mg (g) 65% yield isolated by flash column chromatography

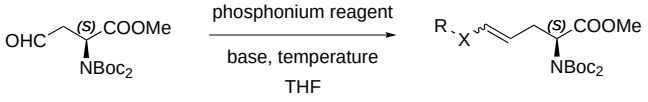
Several weak acids were explored in order to hydrolyze the enol ether, Table 3.5. *p*-TsOH · H_2O[160] provided the aldehyde selectively and fast (Entry 1). When additional water was added,[161] incomplete conversion was observed (Entry 2). The stronger acid, Cl_3CCOOH,[162] resulted in full conversion to a polar side-product, observed by TLC (En-

try 3). The acidic resin, Amberlite,[163] and the pyridinium *p*-toluenesulfonate (PPTS) gave incomplete conversion (Entry 4 and 5). Therefore, the *p*-TsOH $\cdot$ H_2O conditions (Entry 6) were up-scaled to afford **3.60a** in 65% yield. Unfortunately, it was not optically pure (observed by comparison with a sample previously prepared in the lab). This was probably a result of LiHMDS and an incomplete deprotonation of the ylide. Lithium is known to have good chelation abilities and therefore, may increase the α-proton acidity by chelation between the two Boc groups. This chelating effect was earlier used to facilitate mono-Boc deprotection by LiBr.[146] Improvement of the Wittig one-carbon extension by changing base, varying the reaction temperature profile, as well as increasing the phosphonium salt:base ratio did not lead to satisfying results.

3.7.2 Three-Carbon Elongation Mediated by Wittig reactions

We did not succeed to convert **3.51** into **3.60** by a one-carbon Wittig elongation procedure. The three-carbon Wittig elongation was investigated with the same precursor. The full overview is shown in Table 3.6.

Table 3.6: Wittig three-carbon elongation approach



3.51

Entry	Reagent (equiv.)	base (equiv.)	Temp. (°C)	Yield[a] (%)
1	$Ph_3PCH_2CH_2COOHBr$ (2.2)	NaHMDS (3.0)	rt $\rightarrow$ 0 °C $\rightarrow$ rt[b]	quant[c]
2	$Ph_3PCH_2CH_2COOBnBr$ (1.5)	NaHMDS (1.4)	rt	-[d]
3	$Ph_3PCH_2CH_2COOBnBr$ (1.8)	NaHMDS (1.6)	- 78 °C $\rightarrow$ 0 °C $\rightarrow$ reflux[b]	-[e]
4	$Ph_3PCH_2CH_2COBnBr$ (1.5)	NaHMDS (1.4)	- 78 °C $\rightarrow$ 0 °C $\rightarrow$ - 78 °C $\rightarrow$ 0 °C $\rightarrow$ rt[f]	85%

(a) Based on isolated yield (b) base added slowly over 35 min and stirred for an additional 35 min at rt. The aldehyde was added at 0 °C over 30 min and then allowed to reach rt (c) starting material, phosphonium salt, and Ph_3PO was detected by ^{1}H-NMR (d) ^{1}H-NMR indicated no product (e) inconclusive ^{1}H-NMR results (f) stirred at - 78 °C for 1 h after addition of NaHMDS, slowly warmed to 0 °C and cooled back to - 78 °C before adding the aldehyde. After the addition of the aldehyde, the reaction was stirred at 0 °C for 30 min before left overnight at rt

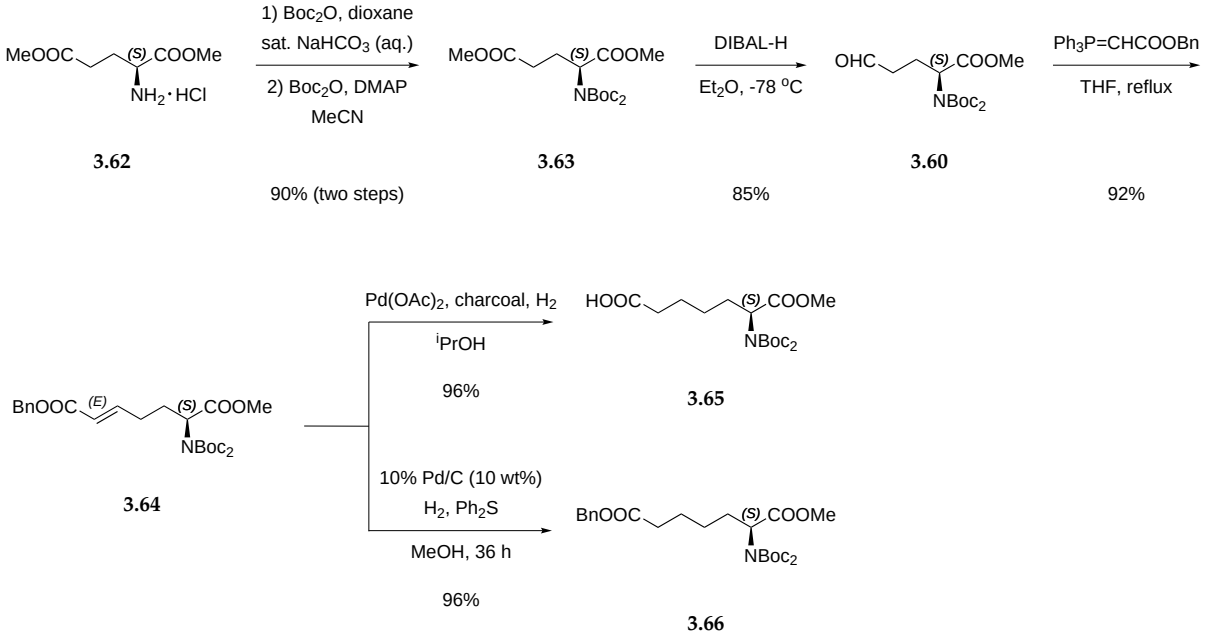
Starting from the commercially available (2-carboxyethyl)triphenylphosphonium bromide[164] and double deprotection using NaHMDS, followed by addition of **3.51**, Table 3.6,

led to only trace amount of the desired product. The corresponding benzyl ester phosphonium salt could be synthesized from benzyl 3-bromopropanoate **3.61** and Ph_3P in a one-pot fashion followed by deprotonation and addition of the aldehyde (Entry 2). We also attempted to prepare a large amount of the benzyl ester phosphonium salt[165] and then deprotonated the crude reagent followed by the addition of the aldehyde (Entry 3). Both of these reactions gave disappointing results. The α-proton of the ester may interfere during deprotection because of its acidity. We hypothesized, that the corresponding protected alcohol reagent would be a great alternative. Commercially available (3-benzyloxypropyl)triphenylphosphonium bromide was reacted with NaHMDS. This afforded the unsaturated benzyl ether in a high yield, Table 3.6. Unfortunately, the benzyl ether was found to be unreactive under hydrogenation-hydrogenolysis conditions. Inspired from the literature,[166] we tried to change the benzyl to a silyl protecting group.[167] However, the Wittig reaction was not successful at sub gram scale.

3.7.3 Synthesis of Key Building Blocks for Generation of C5 and C5* Analogues

With these disappointing results, the previously established reaction conditions was adapted to glutamic acid successfully. The approach also made it possible to obtain the three-carbon aldehyde **3.60** for the C3 analogue, and further functionalized to the corresponding saturated carboxylic acid **3.65** and the saturated benzyl ester **3.66** building blocks for the synthesis of the C5 and C5* analogues.
Starting from 15.0 g of dimethyl *L*-glutamate hydrochloride **3.62**, afforded 23.9 g (90%) of **3.63**, Scheme 3.9. The regioselective DIBAL-H reduction of **3.63** gave **3.60** in high yield (85%). To 21.1 g of **3.60** was added benzyl(triphenylphosphoranylidene)acetate , afforded 26.9 g (92%) of **3.64**. Hydrogenation of the α,β-saturated benzyl ester afforded high yield on multi-gram scale to the corresponding saturated benzyl ester **3.66** (8.5 g, 96%).
When applying the hydrogenation-hydrogenolysis protocol to synthesize the five-carbon saturated carboxylic acid **3.65**. NMR studies clearly indicated the formation of the methyl ester. The methyl ester formation was probably a consequence of the AcOH formation during the *in situ* Pd/C generation. *i*PrOH is less reactive in a nucleophilic substitution reaction, with decreased tendency of esterification. Changing to *i*PrOH resulted in a clean conversion to the saturated carboxylic acid **3.65** starting from 6.5 g of starting material to

the desired product in 96% yield. Using *i*PrOH instead of MeOH for the hydrogenation-hydrogenolysis of the four-carbon α,β-unsaturated benzyl ester **3.56** afforded the four-carbon saturated carboxylic acid **3.57** in excellent yield on multi-gram scale (4.3 g, 99% yield).

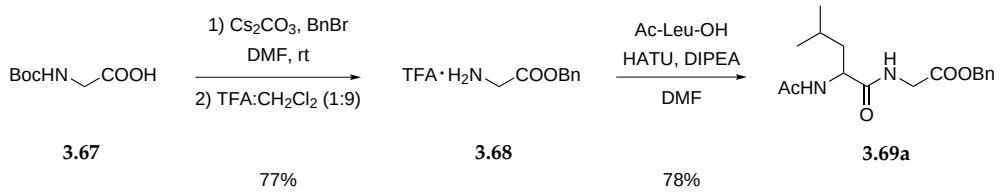


Scheme 3.9: Synthesis of the important five-carbon carboxylic acid and benzyl ester from dimethyl glutamate

3.8 Synthesis of Optically Pure Ac-Leu-Gly-OH (3.37)

3.37 has previously been reported by the Olsen lab.[168] *N,N'*-diisopropylcarbodiimide (DIC)/hydroxybenzotriazole (HOBt) coupling conditions of commercially available acetyl-*L*-leucine and methyl glycinate hydrochloride followed by hydrolysis. However, the risks related to anhydrous HOBt, made this procedure less favorable for the current synthesis. We envisioned that changing from the methyl ester to the benzyl ester would increase the lipophilicity of the compounds, thus giving easier purification.
The benzyl protecting group was introduced by a simple two-step approach[169] in good yields on multi-gram scale, **3.68** in Scheme 3.10. Standard HATU coupling conditions

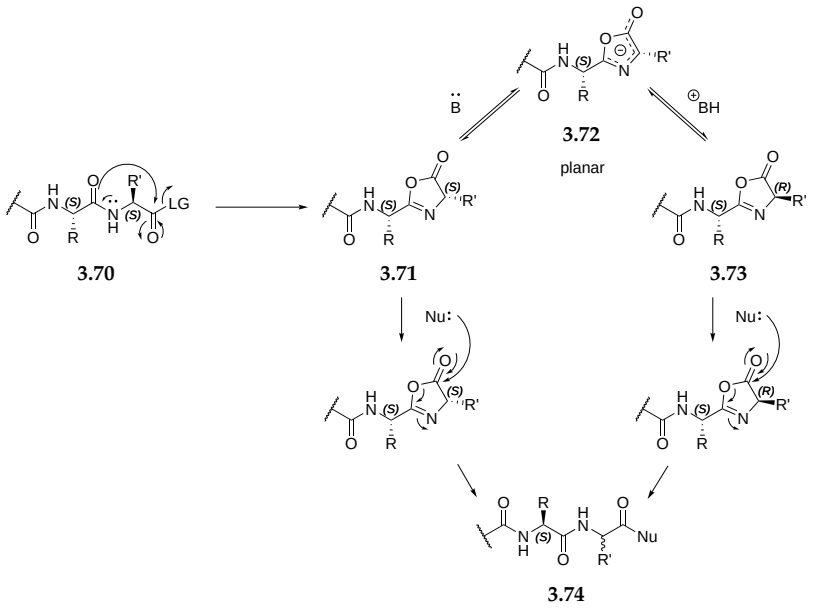
were found to afford the desired benzyl protected dipeptide in good yield (78%) on 10 g scale, **3.69a**.



Scheme 3.10: Synthesis of racemic Ac-Leu-Gly-OH

For the overall three-step sequence, only a single flash column chromatography purification was necessary starting from (*tert*-butoxycarbonyl)glycine **3.67**. HATU is regarded as a coupling reagent that afford low degree of epimerization.[170] Surprisingly, the optical rotation, α, of **3.69a** was observed to be +0.002 (*c* 1.00 in $CHCl_3$), which indicates racemization.

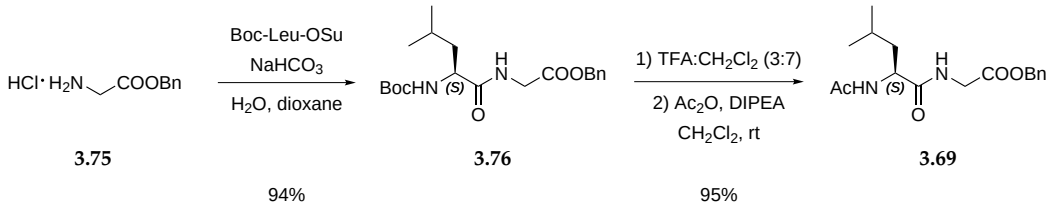
Racemization may take place in peptide synthesis if the *C*-terminal is activated, which result in the formation of a planar oxazolone anionic intermediate **3.72**, Scheme 3.11.



Scheme 3.11: Peptide couplings are prone to the oxazolone production that lead to a planar cyclic intermediate to induce racemization

S-Oxazolone **3.71** is formed upon cyclization and accompanied by the formation of a planar cyclic anionic intermediate under the basic reaction conditions, **3.72**. This intermediate can be protonated to afford either the *S*-Oxazolone (**3.71**) or the *R*-Oxazolone **3.73** that react with an incoming nucleophile with loss of chiral integrity.[170,171] A literature procedure was reported by Zhang and co-workers.[172] Herein, the authors synthesized *O*-succinimide (OSu)-activated amino acids by *N*,*N*′-dicyclohexylcarbodiimide (DCC)/HOSu coupling conditions, followed by stirring in dioxane and H_2O under basic conditions ($NaHCO_3$) with a free *N*-terminal amino acid. We chose to use the commercially available benzyl glycinate hydrochloride and with increased amount of $NaHCO_3$. To this solution, commercially available 2,5-dioxopyrrolidin-1-yl acetyl-*L*-leucinate (Boc-Leu-OSu) was added. On 100 mg scale, excellent yield (88%) was obtained of **3.76**, but most importantly, the observed optical rotation was - 0.265 with a calculated specific optical rotation of $[a]_D^{22}$ - 26.5° (*c* 1.00 in $CHCl_3$). The dipeptide has been reported in several papers, although none of them reports optical rotation, though in some cases the corresponding amine salt has been reported.[172–174] A small amount of CH_2Cl_2 was observed by ^{1}H-NMR due to the high viscosity of the substance. With the proper reaction condi-

tions in hand, the reaction was up-scaled and gave even better results on larger scale, Scheme 3.12.



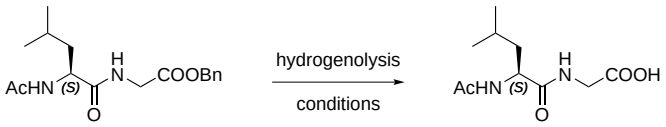
3.75 **3.76** **3.69**

94% 95%

Scheme 3.12: Synthesis of optical pure Ac-Leu-Gly-OH

Boc-removal and acetylation was inspired by the work of Huber *et al.*[175] and slightly modified to perform excellently. Starting from 26.4 g (95%) of **3.76**, afforded 21.2 g of the *N*-acetylated dipeptide **3.69**. A successful screening of hydrogenolysis conditions was performed, Table 3.7.

Table 3.7: Screening hydrogenolysis conditions



3.69 **3.37**

Entry	Scale	Pd source (equiv.)	Solvent	Time (h)	$[a]_D^{22}$ [a]	Yield[b] (%)
1	293 mg	$Pd(OAc)_2$, charcoal	EtOAc:*i*PrOH (1:1)	17.15	-51.2	quant.
2	142 mg	$Pd(OAc)_2$, charcoal	MeOH	15.5	-51.6	quant.[c]
3	103 mg	$Pd(OAc)_2$, charcoal	THF	15.0	-49.4	93
4	86 mg	Pd/C[d]	MeOH	14.0	-52.7	quant.
5	11.4 g	Pd/C[d]	MeOH	14.75	-54.6	98

(a) $[a]_D^{25}$ -50.0° (*c* 2.00, MeOH) (b) Purified by filtration through a pad of Celite® (c) trace amount of starting material, **3.69** (d) 10% Pd/C, 50% wet with H_2O, fluorochem product number 023236

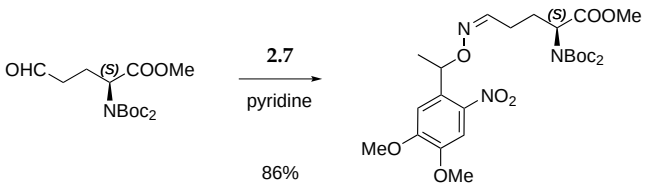
Hydrogenolysis in both MeOH and THF gave good conversion (Entry 2-4). Also commercially available wet 10% Pd/C afforded excellent yield (Entry 4 and 5).

3.9 Synthesis of C3, C4, and C5 Analogues

Daniel Madsen showed that the acyl group was vital for isozyme selectivity. As already described, a small collection of acyl groups were chosen based on his work and previous results in the group. Synthesis of the four- and five-carbon benzyl ester (**3.58** and **3.66**, respectively) resulted in excellent yields. The three-carbon aldehyde **3.60** was used as a model substrate to investigate reaction conditions.

3.9.1 Development of a Synthetic Procedure for the Generation of Photo-Protected δ-*N*-Hydroxy-*L*-Ornithine Derivatives

Aldehyde **3.60** was condensed with **2.7** in pyridine[176] in high yield, Scheme 3.13.

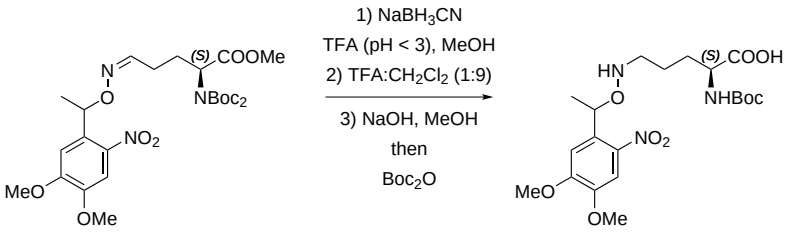


3.60 **3.77**

Scheme 3.13: Three-carbon oxime formation

After condensation to form **3.77**, we planned to reduce the oxime and hydrolyze the methyl ester to introduce the aniline amide, before adding the acyl groups. Previously, we illustrated that the secondary amine of the photo-labile protecting group is unreactive due to steric hindrance, Section 2.2.1. Hydrolysis of the di-Boc protected amino acids without epimerization was reported to be troublesome by Martín *et al.*[143] To prevent epimerization we removed the Boc groups before hydrolysis, inspired by the Martín lab. The oxime was dissolved in MeOH under dry conditions: cooled to °C before the addition of $NaBH_3CN$ (1.5 equiv.), followed by adjusting the pH < 3 with TFA, Scheme 3.14. After 1.5 h, nearly full conversion was observed without any undesired Boc-deprotection. After work-up, the Boc-groups were removed by the addition of TFA:CH_2Cl_2 (1:9). After full conversion observed by LCMS and TLC, co-evaporation from toluene and CH_2Cl_2 afforded the

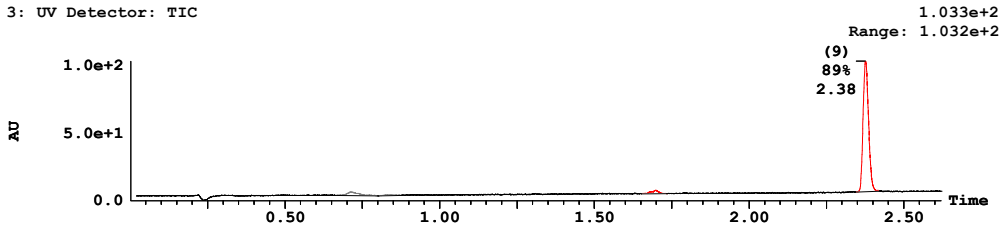
TFA salt. The methyl ester was hydrolyzed by the addition of aqueous NaOH (1.0 M) in MeOH and the amino group was afterwards Boc-protected by the addition of Boc_2O to afford the mono-Boc protected **3.78**.



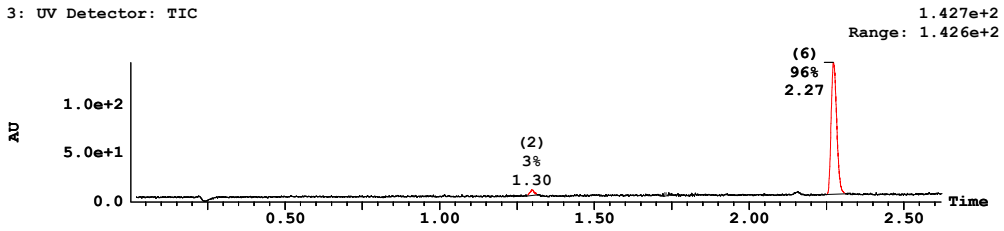
3.77 **3.78**

Scheme 3.14: Development of a four-step reaction sequence

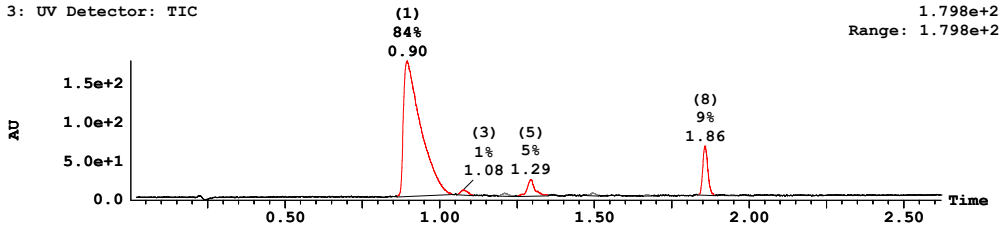
The reactions gave full conversion, all LCMS-spectra included in Figure 3.12. Small impurities were observed after multiple steps. To further optimize the conditions a few changes were implemented. The amount of $NaBH_3CN$ was increased to 2 equiv. for a faster reduction. To speed-up the Boc-deprotection step, the amount of acid was increased to 3:7 TFA:CH_2Cl_2. We also changed to LiOH (7 equiv.) to afford a clean and fast hydrolysis.



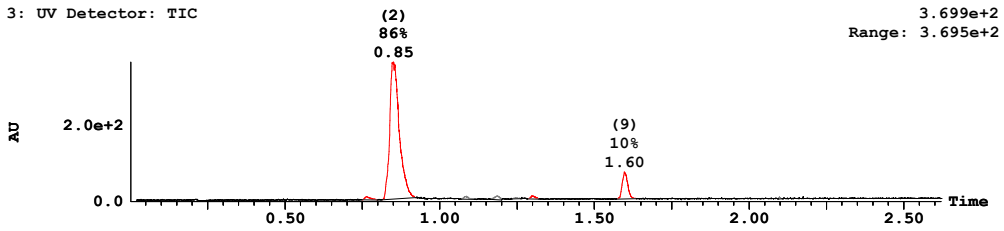
(a) Pure oxime



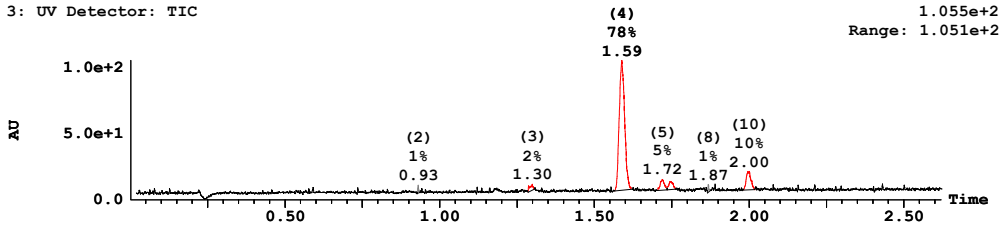
(b) Reduced oxime



(c) Boc-deprotection



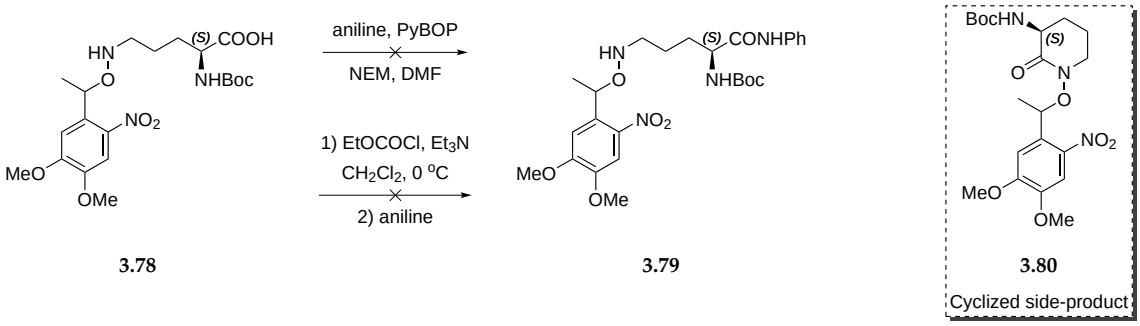
(d) Hydrolysis of the methyl ester



(e) Mono-Boc protected amino acid

Figure 3.12: Overview of LCMS spectra for the developed four-step sequence

To obtain the desired scaffold, amidation of the carboxylic acid was investigated. Two reaction conditions were tested, Scheme 3.15.



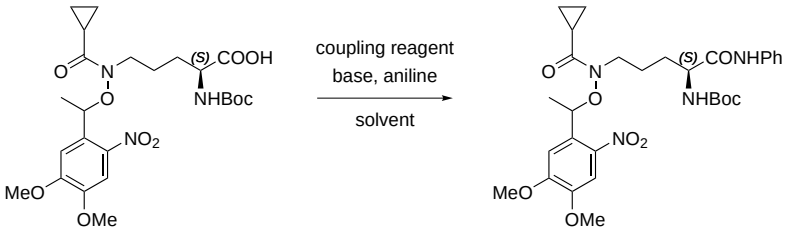
3.78 **3.79** **3.80**

Scheme 3.15: Screened condition for the synthesis of aniline amide and the produced cyclic side-product

No formation of the desired aniline amide **3.79** was formed, but full conversion to a side-product with the mass of the six-membered cyclization product was observed by LCMS. Although this was not the desired product, this observation is interesting as the cyclized 1-hydroxy-2-oxo-3-piperidinyl **3.80** is a key component for the family of siderophores (iron-chelating compounds), such as pseudobactin,[177] heterobactins,[178] and exochelin MN[179].

To obtain the desired product, the order of the events was changed: the oxime was first reduced, followed by acylation of the amino group, and then hydrolysis, Scheme 3.16. To synthesize the aniline amide, different amidation protocols were tested on a sample of **3.81**, Table 3.8.

Table 3.8: Screening of reaction conditions for the synthesis of the aniline amide intermediate

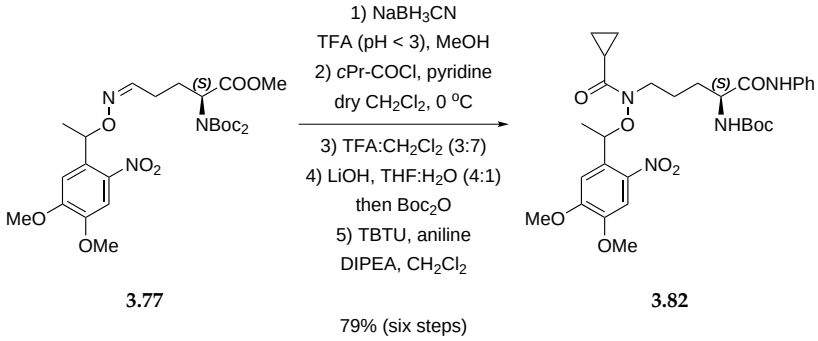


3.81 **3.82**

Entry	Scale	Coupling reagent (equiv.)	Base (equiv.)	Aniline (equiv.)	Solvent	Time (h)	Conversion[a] (**3.81**:**3.82**)
1	35 mg	HATU (1.0)	DIPEA (6)	1.1	DMF	5.50	17:83[b]
2	35 mg	PyBOP (1.0)	NEM (6)	1.1	DMF	5.50	00:100[c,d]
3	35 mg	TBTU[e] (1.0)	NEM (6)	1.1	DMF	5.50	00:100[d]
4	56 mg	TBTU (1.0)	DIPEA (6)	1.0	CH_2Cl_2 [f]	19.00	13:87
5	54 mg	DCC (1.1)	-	1.4	CH_2Cl_2 [f]	19.00	00:100[c]
6	147 mg	TBTU (1.0)	DIPEA (6)	1.0	CH_2Cl_2 [f]	25.00	17:83

(a) indicated by LCMS (b) impurities were observed (c) full conversion, but impurities from the coupling reagent was observed (d) a non-identified side-product was identified (e) *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uronium tetrafluoroborate (f) dry conditions

A range of coupling reagents, including HATU, TBTU, and PyBOP[170] were tested (Entry 1-3, Table 3.8). The coupling reagents were pre-activated with base and **3.81** for 15 min before addition of the aniline. Full conversion was observed for TBTU and PyBOP. A combination of TBTU and DIPEA in CH_2Cl_2[180] afforded **3.82** with lower conversion (Entry 4). Also a DCC coupling was tested.[181] Good conversion, but several impurities were observed by LCMS (Entry 5). The conditions described in Entry 4 were chosen as standard protocol for the amidation with aniline. With the TBTU-DIPEA conditions, **3.82** was synthesized in excellent yield over six steps on large scale (79%), Scheme 3.16.

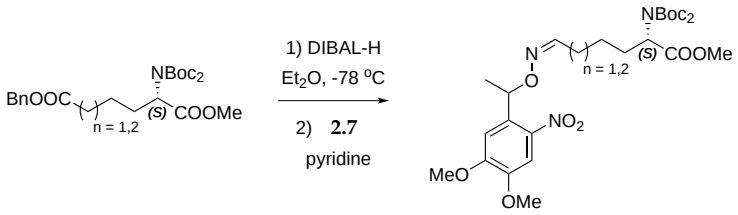


Scheme 3.16: Synthesis of C3 inhibitor intermediate in excellent yield via an improved multi-step synthesis sequence

3.9.2 Synthesis of Photo-Protected ε-*N*-Hydroxy-*L*-Lysine and δ-*N*-Hydroxy-*L*-Homolysine Derivatives

Next, the synthesis of **3.83** and **3.84** was investigated. The DIBAL-H reduction of the benzyl esters to the corresponding aldehydes was unsuccessful, and both the starting material and the alcohol were observed. Furthermore, the starting material proved difficult to separate from the product. Thus, the crude aldehydes were directly coupled with the hydroxylamine photo-labile protecting group **2.7** in pyridine to ease separation, Table 3.9.

Table 3.9: Synthesis of the four- and five-carbon oximes

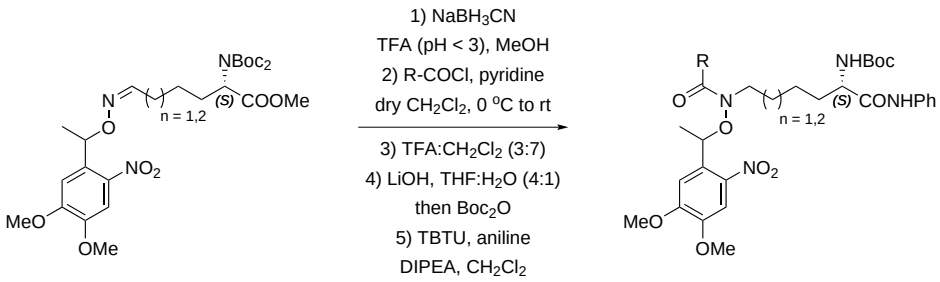


Entry	Benzyl ester	n	Oxime	Yield (%)
1	**3.58**	1	**3.83**	42–84
2	**3.66**	2	**3.84**	25–79

The four-carbon oxime **3.83** was synthesized in up to 84% yield on multi-gram scale (Entry 1, Table 3.9). For the five-carbon oxime **3.84** the highest obtained yield was 79% (Entry 2). The oximes were observed to be unstable, resulting in degradation over time. Degra-

dation was found to occur under purification by flash column chromatography. Due to time-restrictions the number of analogues were reduced, Table 3.10.

Table 3.10: Utilization of the improved seven-step sequence on the degraded oximes

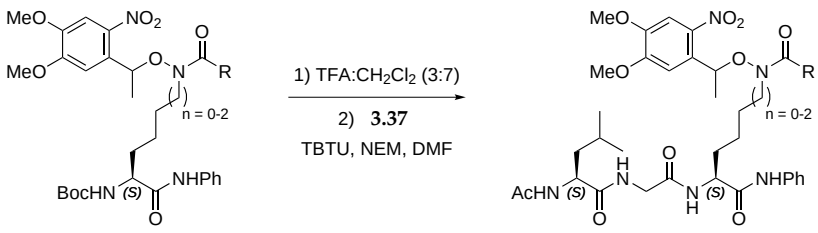


Entry	Oxime	n	R	Hydroxamate	Yield (%)
1	**3.83**	1		**3.39**	25
2	**3.83**	1		**3.85**	28
3	**3.84**	2		**3.86**	18

3.9.3 Synthesis of Photo-Protected C3, C4, and C5 Tripeptides

To finalize the synthesis of the C3, C4, and C5 analogues, we needed to couple the dipeptide **3.37** to the synthesized aniline amides, Table 3.11. We tested commonly applied SPS coupling reagents, TBTU, PyBOP, and HATU with their respective bases applied in the Qvortrup lab: NEM, NEM, and *N*,*N*-diisopropylethylamine (DIPEA), respectively. TBTU was found to be the best choice, see Table 3.11.

Table 3.11: Coupling of **3.37** with the C3, C4, and C5 inhibitor intermediater



Entry	Hydroxamate	n	R	Tripeptide	Yield (%)
1	**3.82**	0		**3.87**	88
2	**3.39**	1		**3.38**	86
3	**3.85**	1		**3.88**	82
4	**3.86**	2		**3.89**	82

When characterizing the starting materials and products, two sets of resonances for isolated protons, without neighboring protons, were observed. This trend was also reflected in the ^{13}C-NMR spectra, and for both instances, in a 1:1 ratio. For ^{1}H-NMR, this was frequently encountered for the aniline amide proton and or the methoxy group(s) resonances. However, the multiplicity of two could also arise from two overlapping singlet peaks, one resonance for each of the diastereomers, as a consequence of the racemic benzylic proton. To exclude conformational isomers, NMR temperature experiment serie was conducted on the synthesized tripeptide **3.38**, in DMSO-d_6, to investigate the occurrence of diastereomers. Five ^{1}H-NMR spectra were recording at different temperatures, ranging from 22 °C to 77 °C. The results are listed in Figure 3.13.
A upfield shift of the aniline amide proton, from approx. 9.8 ppm to 9.2 ppm, was clearly observed by ^{1}H-NMR, a similar trend of displacement was also observed for the three other amide protons. Over the temperature interval, no merging of the two resonance signals for the aniline amide proton was observed, indicating a mixture of diastereomers as suspected. Compounds where the two diastereomers can be differentiated by NMR is marked with a star (*) in the experimental section, 3.13.

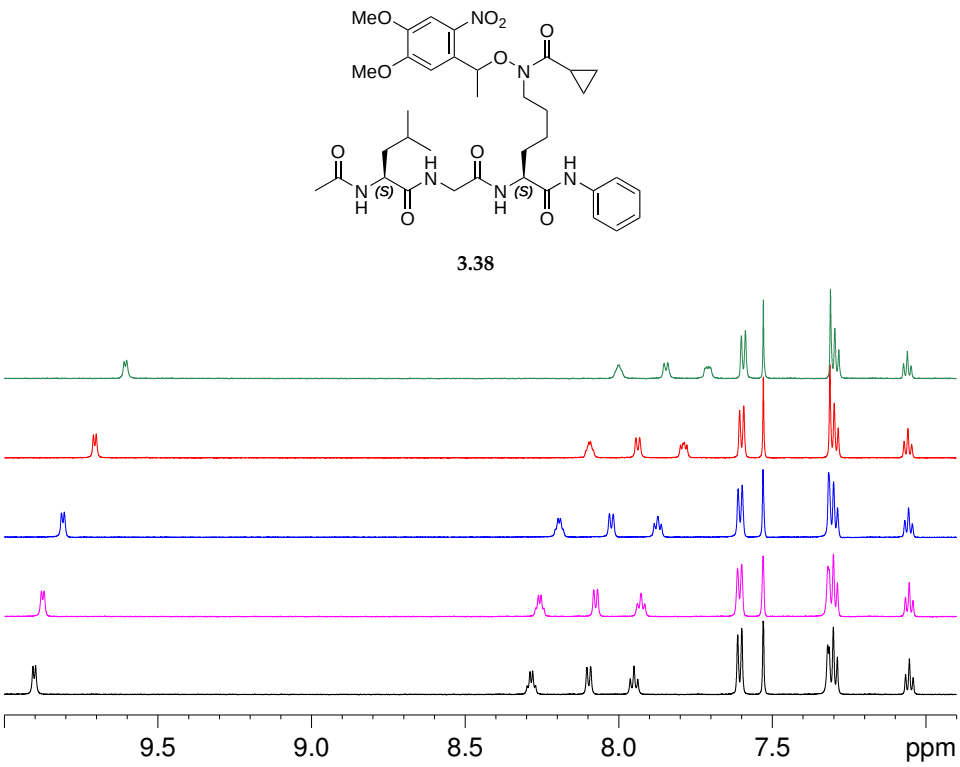


Figure 3.13: Selected expansion of ^{1}H-NMR spectra from heat experiments for the investigation of the diastereomeric nature of **3.38**. Black = 22 °C, purple = 25 °C, blue = 37 °C, red = 57 °C, green = 77 °C

3.10 Synthesis of C3*, C4*, and C5* Analogues

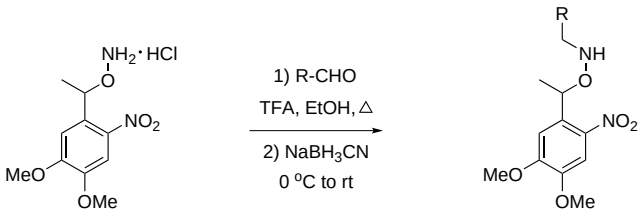
For the C3*, C4*, and C5* analogues, the photo-labile protecting group **2.7** was alkylated by a reductive alkylation approach with commercially available aldehydes and subsequently acylated with carboxylic acids.

3.10.1 Reductive Alkylation of Our Developed Hydroxylamine Photo-Labile Protecting Group

Compound **2.7** (1.0 equiv.) was stirred under dry acidic conditions with commercially available aldehydes (1.0 equiv.) in EtOH. The reaction mixture was heated for a short period of time to dissolve the amine salt, and after additional three hours at rt, full conversion to the corresponding oxime was observed by TLC and LCMS. The oximes were

further reduced to obtain mono-alkylated *O*-protected hydroxylamines, Table 3.12.

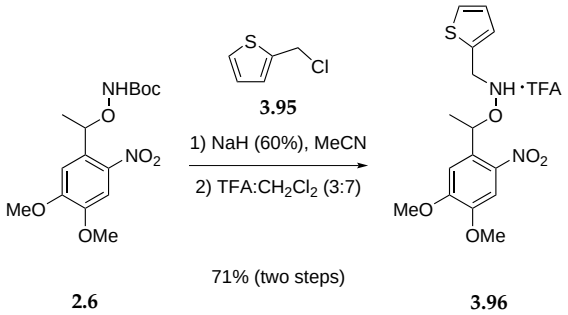
Table 3.12: Reductive alkylation of *O*-MeNV hydroxylamine



2.7

Entry	R	Hydroxylamines	Yield (%)
1		**3.90**	85
2		**3.91**	61
3		**3.92**	74
4		**3.93**	87
5		**3.94**	oxime stable

The aliphatic aldehydes worked well (Entry 1-4). The 2-methylthiophene substituted oxime was observed to be stable under the reductive conditions (Entry 5): after 12.5 equiv. of $NaBH_3CN$ only a trace amount of the reduced oxime was observed by LCMS.
The desired thiophene-containing compound **3.96**, was synthesized by alkylation of the Boc-protected photo-labile protecting group **2.6** with 2-(chloromethyl)thiophene[182] **3.95**, followed by acidic cleavage of the Boc group affording **3.96** in 71% yield over two steps, Scheme 3.17.

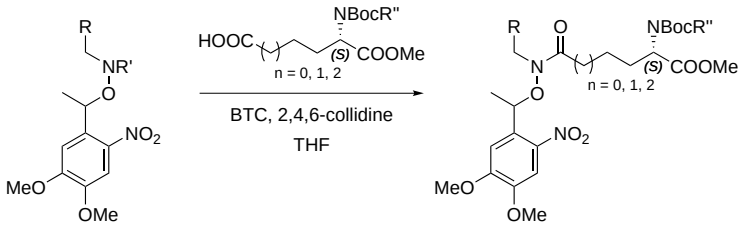


Scheme 3.17: Direct alkylation approach for the synthesis of the thiophen-2-ylmethyl alkylated hydroxylamine

3.10.2 BTC-Mediated Acylation of *N*-Alkylated Hydroxylamines

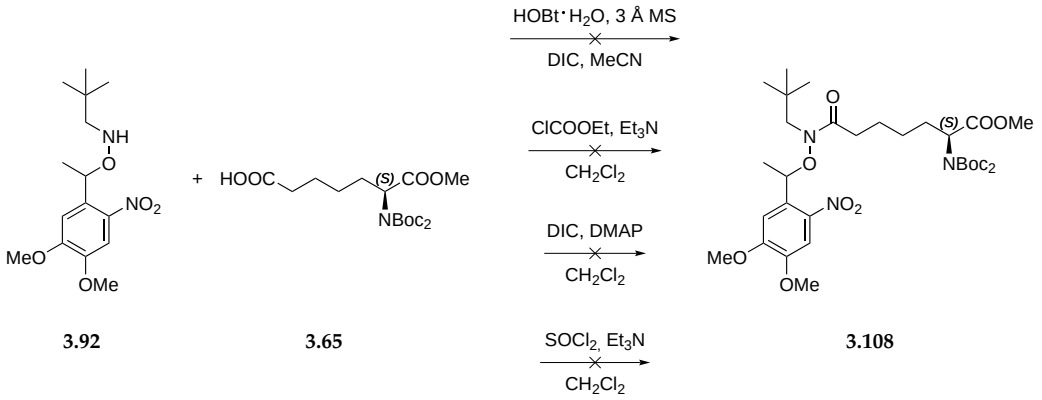
With the synthesized *N*-alkylated hydroxylamines in hand, the next step was to couple them with the saturated carboxylic acids. As already described in Section 2.2.1, whenever our developed hydroxylamine photo-labile protecting group is alkylated, TBTU, HATU, and PyBOP were inefficient. However, BTC was found to be an efficient acylation reagent. The *N*-alkylated hydroxylamines were coupled to commercially available methyl (*tert*-butoxycarbonyl)glycinate **3.45** and the prepared four- and five-carbon saturated carboxylic acids (**3.57** and **3.65**) (Entry 1-10 Table 3.13).
For all the reactions full conversion of the hydroxylamines were observed by TLC and LCMS, but a side-product was observed. However, it has not yet been possible to fully determine the structures, but clearly contained both the *N*-alkylated hydroxylamines and the carboxylic acid in a 2:1 ratio (Entry 7-10). For Entry 1, full conversion was also observed, unfortunately several impurities and the corresponding side-product were too observed for this entity.

Table 3.13: Acylation of *N*-alkylated hydroxylamines mediated by BTC-coupling conditions



Entry	Hydroxylamine	R	R′	R″	Carboxylic acid	n	Hydroxamate	Yield (%)
1	**3.90**		H	H	**3.45**	0	**3.97**	0
2	**3.91**		H	Boc	**3.57**	1	**3.98**	35
3	**3.93**		H	Boc	**3.57**	1	**3.99**	34
4	**3.100**		H	Boc	**3.57**	1	**3.101**	24
5	**3.92**		H	Boc	**3.57**	1	**3.102**	30
6	**3.96**		H · TFA	Boc	**3.57**	1	**3.103**	32
7	**3.90**		H	Boc	**3.65**	2	**3.104**	0
8	**3.91**		H	Boc	**3.65**	2	**3.105**	0
9	**3.100**		H	Boc	**3.65**	2	**3.106**	0
10	**3.100**		H	Boc	**3.65**	2	**3.107**	0

Coupling of the carboxylic acids to the racemic *N*-alkylated hydroxylamine result in the formation of two diastereomers as observed by NMR (denoted with a star (*) in the experimental section 3.13). The acylation reactions were performed in the final stage of my Ph.D. and time-limitations did not allow me to fully optimize these reactions, Scheme 3.18. Due to the disappointing results, four reaction conditions were investigated for the acylation of **3.92**. The reactions were performed on a 100 mg scale and analyzed by LCMS.

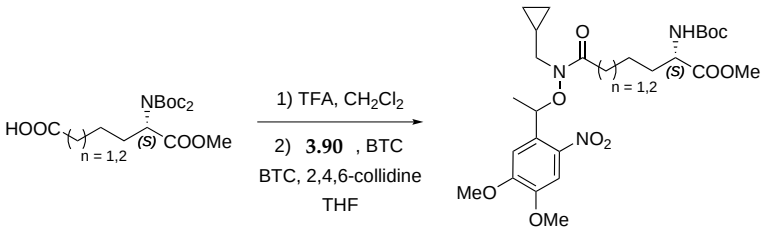


Scheme 3.18: Screened alternative coupling conditions to prevent side-product formation

$HOBt \cdot H_2O$ and **3.65** were stirred in dry MeCN for 15 min at rt with molecular sieves before **3.92** was added and the mixture was cooled to 0 °C followed by the addition of DIC.[183] Full conversion to the side-product was observed by LCMS. **3.65** was treated with ethyl chloroformate followed by the addition of **3.92**. Only trace amount of the desired compound **3.108** was detected by LCMS. The DIC[184] and $SOCl_2$[185] test reactions both resulted in formation of the side-product.

The last parameter we could change, was the number of Boc groups. The amount has previously been reported to give steric issues under other reaction conditions.[142] Earlier, we found that acylation with methyl (*tert*-butoxycarbonyl)glycinate **3.45** to the *N*-alkylated hydroxylamine worked well, but extending the aliphatic carbon chain was found to afford low - to no desired product. Another clear change, from the commercially available carboxylic acid to our synthesized saturated carboxylic acids, is the double Boc-protected amino group. Therefore, we investigated whether the double protection could be accountable for the unsuccessful reaction. Mono-deprotection[186] with 1.25 equiv. of TFA in CH_2Cl_2 for 30 min followed by evaporation and drying before standard BTC coupling conditions, proved to be efficient for a 150 mg scale reaction with low yield (36%). On larger scale the selective mono-deprotection was slow and the amount of TFA was changed to 1.4 equiv. Hereby, we isolated the hydroxamates **3.109** and **3.110** in 49% and 44% yield respectively, Table 3.14. Indeed, the desired products were obtained, but also trace amount of a unidentified side-products were observed for the optimized reaction conditions.

Table 3.14: Improved synthetic strategy to obtain the C4* and C5* inhibitors

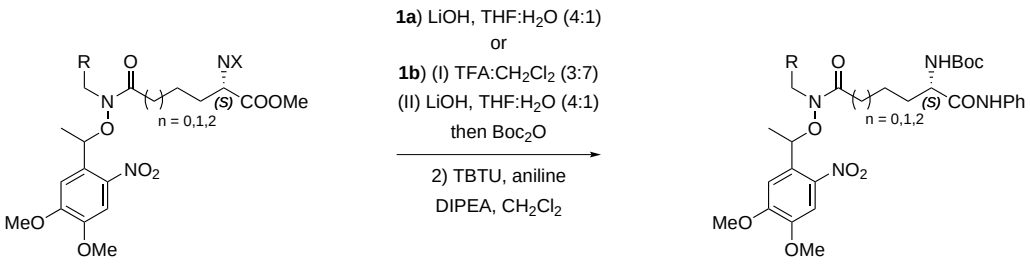


Entry	Carboxylic acid	n	Hydroxamate	Yield (%)
1	**3.57**	1	**3.109**	49
2	**3.65**	2	**3.110**	44

3.10.3 Synthesis of Photo-Protected γ-(hydroxycarbamoyl)-*L*-Norvaline, δ-(hydroxycarbamoyl)-*L*-Norleucine, and ε-(hydroxycarbamoyl)-*L*-Homoleucine Derivatives

The mono- and di-Boc protected amino acids were converted into the corresponding aniline amide, Table 3.15. The mono-Boc amino acids were successfully hydrolyzed with 3 equiv. of LiOH[187] (1a Table 3.15). For the remaining di-Boc protected compounds, the already developed hydrolysis sequence (1b Table 3.15) was applied.

Table 3.15: Synthesis of the aniline amides



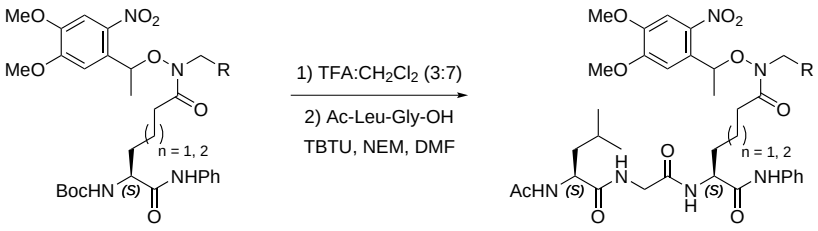
Entry	Methyl ester	n	X	R	Aniline amide	Steps	Yield (%)
1	**3.109**	1	HBoc		**3.111**	2	88
2	**3.101**	1	Boc_2		**3.112**	4	53
3	**3.98**	1	Boc_2		**3.113**	4	34
4	**3.102**	1	Boc_2		**3.114**	4	54
5	**3.99**	1	Boc_2		**3.115**	4	59
6	**3.103**	1	Boc_2		**3.116**	4	82
7	**3.110**	2	HBoc		**3.117**	2	89

The results clearly indicate that the reactions with the highest yield were the ones using the mono-Boc protected amino acids (Entry 1 and 7). Synthesizing compound **3.113** from **3.57** required five steps with an overall yield of 16%, whereas **3.111** was obtained in 43% yield over four steps starting from **3.57**.

3.10.4 Synthesis of Photo-Protected C4* and C5* Tripeptides

To complete the synthesis of the photo-protected analogues, the previously described dipeptide coupling conditions were employed, Table 3.16. Synthesis of the desired photo-protected C4* and C5* tripeptide analogues worked well in high yields (Entry 1-7).

Table 3.16: Synthesis of protected tripeptides

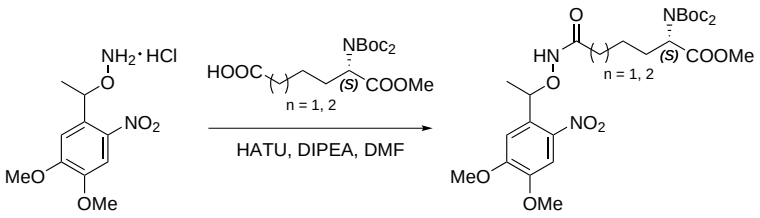


Entry	Hydroxamate	n	R	Tripeptide	Yield (%)
1	**3.111**	1		**3.118**	70
2	**3.112**	1		**3.119**	87
3	**3.113**	1		**3.120**	75
4	**3.114**	1		**3.121**	88
5	**3.115**	1		**3.122**	89
6	**3.116**	1		**3.123**	84
7	**3.117**	2		**3.124**	79

3.11 Non-Substituted Hydroxamic Acid

In earlier projects, we have had great success coupling our photo-removable protecting group with various acids promoted by PyBOP, to form the protected hydroxamates. However, PyBOP proved to be inefficient for these compounds. Rewardingly, changing to HATU gave full conversion on sub gram scale, Table 3.17.

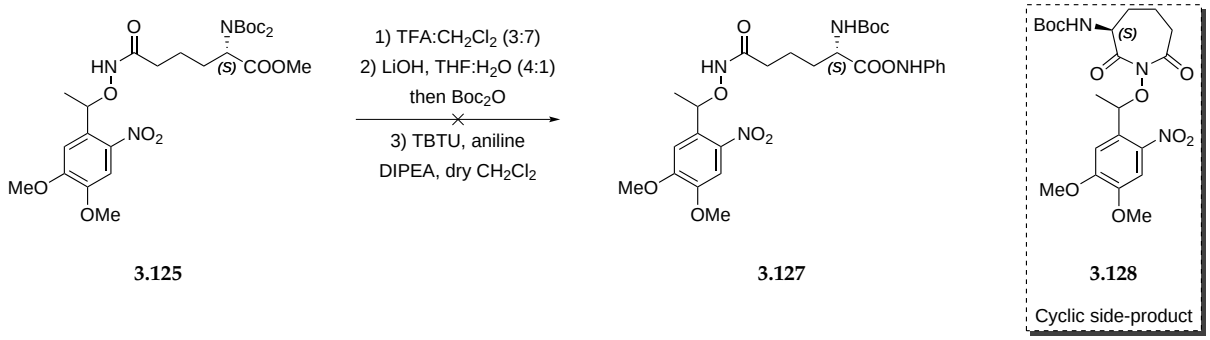
Table 3.17: Synthesis of O-protected hydroxamates



2.7

Entry	Carboxylic acid	n	Hydroxamate	Yield (%)
1	**3.57**	1	**3.125**	92
2	**3.65**	2	**3.126**	76

Deprotection of the Boc groups, hydrolysis, mono-Boc protection, TBTU-aniline coupling was tested on **3.125**, Scheme 3.19. Unfortunately, the amidation step failed due to the formation of the cyclized side-product, **3.128**, indicated by LCMS.

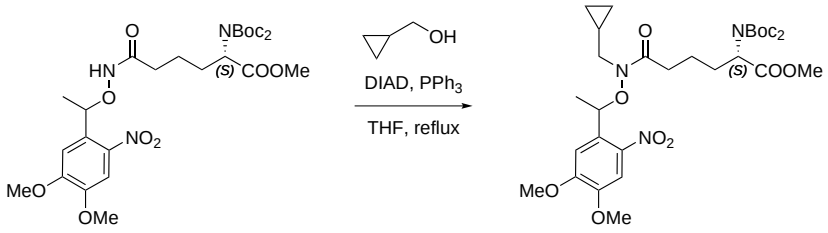


3.125 **3.127** **3.128**

Cyclic side-product

Scheme 3.19: Introduction of aniline amide

With the inefficient BTC-coupling conditions for the di-Boc protected compounds, HATU-mediated acylation of **2.7** followed by N-alkylation could be an efficient method to prevent the use of BTC. Mitsunobu alkylation conditions were tested with great success, full conversion was observed of **3.125** to the *N*-alkylate *O*-MeNV hydroxamate **3.129**, Scheme 3.20.



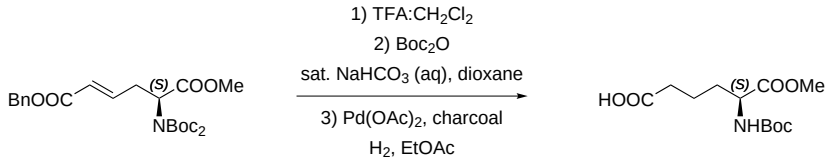
3.125 **3.129**

Scheme 3.20: Synthesis of *N*-alkylated *O*-MeNV hydroxamates via Mitsunobu conditions

Before this approach is pursued on large scale, eventual epimerization should be investigated.

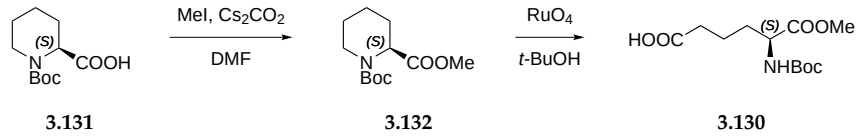
3.11.1 Alternative Synthesis of four- and five-Carbon Carboxylic Acids

Due to the influence of the Boc-protecting groups described earlier, Section 3.10.2 Page 95, and the precautions we had to take to prevent epimerization/racemization as a consequence of the increased α-proton acidity, along with the time-consuming synthesis, starting from aspartic acid or glutamic acid is not advantageous. Therefore, we will give a short introduction to some previously published alternative synthetic strategies here, for the formation of the four- and the five-carbon carboxylic acids, (*S*)-5-((*tert*-butoxycarbonyl)amino)-6-methoxy-6-oxohexanoic acid **3.130** and (*S*)-6-((*tert*-butoxycarbonyl)amino)-7-methoxy-7-oxoheptanoic acid **3.139**.
We also propose an alternative synthetic strategy for the mono-Boc protected saturated carboxylic acids. First, deprotection of the Boc groups would be conducted, followed by a mono-Boc protection, and the $Pd(OAc)_2$-mediated hydrogenation-hydrogenolysis affording the desired mono-Boc carboxylic acids, exemplified by the synthesis of **3.130** in Scheme 3.21. As reported by Fanning *et al.*, the conjugated alkene would hinder cyclization. Optimization of either the TFA-mediated mono-Boc deprotection or the LiBr approach could afford suitable reaction conditions.



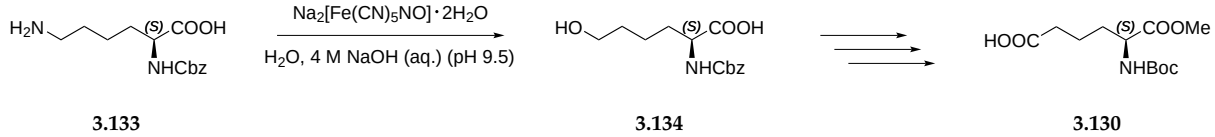
Scheme 3.21: Alternative synthesis of mono-Boc protected amino acids

Another method to synthesize **3.130** has been reported[188] by RuO_4-oxidation of *(S)*-methyl 1-Boc-piperidine-2-carboxylate **3.132** originating from the readily available Boc-*L*-pipecolic acid **3.131**[189], Scheme 3.22.



Scheme 3.22: Literature procedure for the synthesis of **3.130** via a RuO_4-oxidation approach

Yet another, but more time-consuming method involves the versatile diazotization reaction of commercially available Cbz-Lys-OH **3.133**, with sodium nitroprusside dihydrate ($Na_2[Fe(CN)_5NO] \cdot 2\,H_2O$)[190] to afford Cbz-protected *L*-α-aminoadipic acid **3.134**, Scheme 3.23.

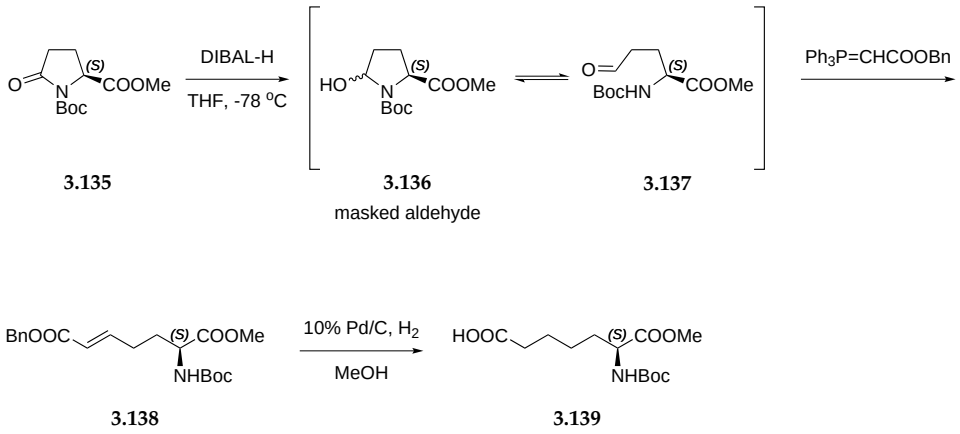


Scheme 3.23: Literature diazotization procedure for the synthesis of **3.130**

One disadvantage of this approach is that the alkyl diazo compounds are explosive, and *L*-α-aminoadipic acid is commercially available.

Synthesis of **3.139** has been reported in a patent[191] filed by Miao *et al.*, Scheme 3.24. Literature procedures[192–194] were applied to synthesize our desired carboxylic acid, starting from commercially available (*S*)-methyl-*N*-Boc-pyroglutamate (Boc-Pyr-OEt). In our case, the even cheaper Boc-Pyr-OMe (**3.135**) could be used. The cyclic amide is reduced

to the hemiaminal **3.136**, Scheme 3.24. In the patent, the authors reacted the masked aldehyde **3.137** with benzyl(triphenylphosphoranylidene)acetate, to obtain the α,β-unsubstituted benzyl ester **3.138**. In the final step, a hydrogenation-hydrogenolysis reaction was conducted in order to obtain the fully saturated mono-Boc protected carboxylic acid, **3.139**.

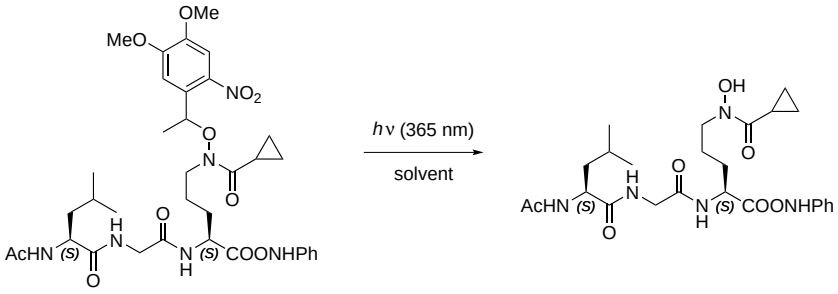


Scheme 3.24: Literature procedure for the synthesis of **3.139**

3.11.2 Screening of Photolytic Cleavage Conditions

Finally, the photo-mediated deprotection of our photo-labile protecting group was carried out. In Chapter 2, photo-cleavage of the photo-labile protecting group was investigated. For the first *N*-alkylated SAHA analogues a 1.0 M HCl (aq.):*i*PrOH (1:11) mixture was used to get a good ratio between the hydroxamic acid and the corresponding amide. For the non-substituted hydroxamates, we found that THF:H_2O (4:1) afforded the desired hydroxamic acid in high selectivity. Generally, *N*-alkylated hydroxamates require acidic conditions to give high selectivity. From the work with the photo-labile protecting group, several solvent systems afforded high selectivity, Table 2.10 20. **3.87** was chosen as a model substrate for this step to afford the hydroxamic acid **3.140**. It was not possible to see separation of the hydroxamic acid and the corresponding amide on MS, but the mass traces indicated selectivity.

Table 3.18: Solvent screen for selective hydroxamic acid liberation



3.87 **3.140**

Entry[a]	Solvent[b]	Ratio	Selectivity[c]
1	MeOH:H_2O	1:1	-
2	MeOH:1.0 M HCl (aq.)	4:1	-
3	HFIP		-
4[d]	HFIP		-
5	HFIP:THF	1:1	-
6	HFIP:H_2O	1:1	-
7	HFIP:TFA	4:1	-
8	HFIP:TFA	3:2	-
9	HFIP:H_2O	4:1	-
10	HFIP:1.0 M HCl (aq.)	4:1	-
11	HFIP:1.0 M $Fe(OAc)_3$ (aq.)	1:1	A
12	HFIP:MeCN	1:1	-
13	HFIP:MeCN:TFA	1:1:1	-
14	HFIP:MeCN	4:1	-
15	HFIP:1.0 M $FeCl_3 \cdot 7\,H_2O$ (aq.)	1:1	-
16	HFIP:MeCN:1.0 M $FeCl_3 \cdot 7\,H_2O$ (aq.)	1:1:1	-
17	HFIP:1.0 M $FeCl_3 \cdot 7\,H_2O$ (aq.)	9:1	A
18	MeCN:TFA	1:4	-
19	MeCN:TFA	1:1	-
20	MeCN:TFA	9:1	-
21	MeCN:H_2O	4:1	-

continued on next page

Entry[a]	Solvent[b]	Ratio	Selectivity[c]
22[d]	MeCN:1.0 M $FeCl_3 \cdot 7\,H_2O$ (aq.)	1:1	-
23	MeCN:1.0 M $FeCl_3 \cdot 7\,H_2O$ (aq.)	1:1	-[e]
24[13]	DMSO		-
25	DMSO:TFA	1:4	-
26	DMF		-
27	*i*PrOH		A
28	*i*PrOH:TFA	9:1	-
29	*i*PrOH:1.0 M HCl (aq.)	1:1	-
30	*i*PrOH:1.0 M HCl (aq.)	4:1	-
31	*i*PrOH:1.0 M HCl (aq.)	9:1	-
32	1.0 M HCl (aq.)		-
33	*i*PrOH:1.0 M $ZnSO_4 \cdot 7\,H_2O$ (aq.)	1:1	-
34	*i*PrOH:1.0 M $FeSO_4 \cdot 7\,H_2O$ (aq.)	1:1	-
35	*i*PrOH:1.0 M $ZnCl_2$ (aq.)	1:1	-
36	*i*PrOH:1.0 M $FeCl_3 \cdot 7\,H_2O$ (aq.)	1:1	-
37	*i*PrOH:1.0 M $Zn(OAc)_2 \cdot 2\,H_2O$ (aq.)	1:1	A
38	*i*PrOH:1.0 M $FeCl_2$ (aq.)	1:1	-
39	*i*PrOH:1.0 M $Fe(OAc)_2$ (aq.)	1:1	-
40	TFA		HA

(a) Conducted on 5 mg scale (b) operating concentration 21.6 mM if otherwise reported (c) based on the positive mass trace, HA = hydroxamic acid (**3.140**), A = amide, - no selectivity (d) 2.16 M (e) HA was degraded

Photolytic cleavage in TFA displayed high selectivity, but as our goal was to find mild conditions, more experiments are needed. Furthermore, we discovered, that Entry 7, 8, 13, 18, 19, 22, and 38 gave good selectivity based on the mass trace intensities. Although due to time-constraints, these conditions were not further investigated. Because we did not find proper conditions for the photolytic cleavage, we were not able, before writing this dissertation, to conduct biological screening of the inhibitors. But it is our future perspectives to further investigate the solvent dependency for the hydroxamic acid release and conduct a profiling study on these compounds in HDAC enzymes.

3.12 Conclusion

In a previous project in the Qvortrup group, a HDAC substrate profiling study was performed and identified a HDAC subclass-3 selective substrate. The aim of my work was to use this information and develop a subclass-3 selective inhibitor. HDACs are an important class of enzymes and HDAC inhibitors have shown to be important in the treatment of cancer as well as a range of non-cancer diseases. A selection of hydroxamic acid functionalized peptides was successfully synthesized, consisting of a leucine-glycine dipeptide coupled to a lysine analogue. A hydroxamic acid functionality was introduced, as this functionality has shown to result in good HDAC-inhibitor activity. Based on the hit substrate, a selection of *N*-hydroxamate groups was chosen. To synthesize the desired *O*-protected hydroxamate tripeptides, I developed a convergent synthetic route to key intermediates starting from commercially available aspartic- and glutamic acid. A preliminary test of photolytic cleavage conditions was conducted, which afforded the desired hydroxamic acids.

3.13 Experimental Section

General Methods

See Section 2.5.1 for general methods.

Due to instability of the oximes, **3.83** and **3.84** were characterized by LCMS alone.

General Procedure: Oxime Reduction

In a round-bottomed flask containing a magnetic stirring bar, the oxime was placed under vacuum for a couple of hours before a nitrogen atmosphere was introduced. The oxime was dissolved in MeOH (0.25 M) and cooled to 0 °C. To the cold stirring solution were added $NaBH_3CN$ (1.3-2.0 equiv.) and a few drops of TFA (pH $<$ 3) followed by stirring at rt. When full conversion was observed by LCMS, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (aq.) and MeOH was removed under reduced pressure. The aqueous phase was extracted with EtOAc and the organic phase was washed with sat. $NaHCO_3$ (aq.) (3 x), dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

General Procedure: Acid Chloride Acylation

In a round-bottomed flask containing a magnetic stirring bar, the amine was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. The hydroxylamine (1.0 equiv.) was dissolved in CH_2Cl_2 (0.22 M) and cooled to 0 °C. To the cold stirring solution were dropwise added pyridine (10.0 equiv.) and acid chloride (1.0 equiv.) and stirred at rt. When full conversion was observed by TLC and/or LCMS, the reaction mixture was concentrated under reduced pressure, and the residues were dissolved in sat. NH_4Cl (aq.) and washed with CH_2Cl_2 (3 x). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

General Procedure: Boc-Deprotection

In a round-bottomed flask containing a magnetic stirring bar, the Boc-protected amine was dissolved in TFA:CH_2Cl_2. When full conversion was observed by TLC and/or LCMS,

the reaction mixture was concentrated under reduced pressure, and co-evaporated twice from toluene and CH_2Cl_2.

General Procedure: One-Pot LiOH Hydrolysis and Boc-Protection

In a round-bottomed flask containing a magnetic stirring bar, LiOH (7.0 equiv.) was added to a stirring solution of the ester in THF:H_2O (4:1) (0.04 M). When full conversion to the carboxylic acid was observed by LCMS, Boc_2O was added to the stirring basic solution. When full Boc-protection was observed by TLC and/or LCMS, the reaction mixture was diluted with EtOAc by half of the reaction volume and acidified to pH $\approx$ 3-4 by the addition of 1.0 M HCl (aq.) and immediately extracted with EtOAc (3 x). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

General Procedure: LiOH Hydrolysis

In a round-bottomed flask containing a magnetic stirring bar, LiOH (3.0 equiv.) was added to a stirring solution of the ester in THF:H_2O (4:1) (0.04 M). When full conversion was observed by TLC and/or LCMS, the reaction mixture was diluted with EtOAc by half of the reaction volume and acidified to pH $\approx$ 3-4 by the addition of 1.0 M HCl (aq.) and immediately extracted with EtOAc (3 x). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

General Procedure for BTC Acylation

In a dry round-bottomed flask containing a magnetic stirring bar, BTC was added to a stirring solution of the carboxylic acid in THF (0.04 M) under nitrogen atmosphere. To the solution was dropwise added 2,4,6-collidine to form a milky suspension with a yellow shade over 1-2 h. *N*-Alkylated hydroxylamines were added to the milky mixture in one portion and stirred. When full conversion was observed by TLC and/or LCMS, the reaction mixture was quenched by the addition of H_2O and transferred to a separation funnel by the use of EtOAc. The organic phase was washed according to the description for each specific reactions. The non-acidic phases were combined and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The title compound was purified by flash column chromatography.

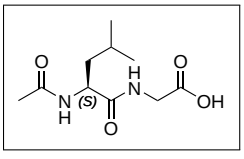
General TBTU-DIPEA Aniline Amidation[180]

A round-bottomed flask containing the Boc-protected carboxylic acid and a magnetic stirring bar was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. TBTU was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (0.07 M) and stirred. DIPEA and aniline were added to stirring solution. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc and washed with H_2O, sat. $NaHCO_3$ (aq.), 1.0 M HCl (aq.), and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The title compound was purified by flash column chromatography.

General Reductive Alkylation Procedure

To a dry round-bottomed flask containing a magnetic stirring bar, *O*-MeNV hydroxylamine hydrochloride salt **2.7** was dissolved in an alcoholic solution (0.25 M). The aldehyde (1.0 equiv.) was added to the solution followed by TFA (pH $<$ 3). The solution was heated for a short period of time to followed by stirring at rt under a nitrogen atmosphere. When full conversion was observed by TLC and/or LCMS, the solution was cooled to 0 °C and the oxime was reduced by the addition of $NaBH_3CN$. When full reduction was observed by TLC and/or LCMS, the reaction was quenched with H_2O and the alcohol was removed under reduced pressure. The residues were taken up in EtOAc and washed with sat. $NaHCO_3$ (aq.). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. This provided the crude *N*-alkylated *O*-MeNV hydroxylamine which was purified by flash column chromatography.

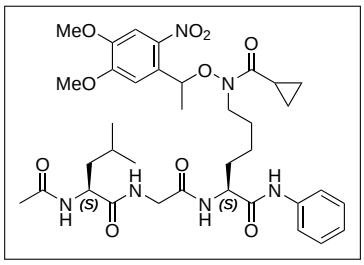
Acetyl-*L*-leucylglycine (3.37)



In a round-bottomed flask containing a magnetic stirring bar, benzyl acetyl-*L*-leucylglycinate (11.4 g, 35.7 mmol, 1.0 equiv.) was placed under vacuum for an hour before a nitrogen atmosphere was established and dissolved in MeOH (143 mL), and added 10% Pd/C (50% wetted in H_2O) (2.29 g, 10 wt%). Two vacuum/hydrogen cycles were performed before hydrogen was bubbled through the solution for 30 min. The reaction mixture was stirred under hydrogen atmosphere overnight. The reaction mixture was filtered through a pad

of Celite®, and washed thoroughly with CH_2Cl_2 and concentrated under reduced pressure. This afforded acetyl-*L*-leucylglycine as a off-white solid (8.1002 g, 91%). Spectral data in correspondence with reported literature values.[195] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.48 (br. s, 1H, C(O)O**H**), 8.19 (t, J = 5.9 Hz, 1H, C(O)N**H**CH_2C(O)), 7.98 (d, J = 8.4 Hz, 1H, CH_3C(O)N**H**CH), 4.34–4.29 (m, 1H, CH_3C(O)NHC**H**C(O)), 3.77–3.65 (m, 2H, NHC**H$_2$**C(O)), 1.83 (s, 3H, C**H$_3$**C(O)NH), 1.65–1.55 (m, 1H, $CHCH_2$C**H**$(CH_3)_2$), 1.49–1.37 (m, 2H, CHC**H$_2$**$CH(CH_3)_2$), 0.88–0.82 (m, 6H, $CH(CH_3)_2$); $[a]_D^{22}$ - 54.6°(c 2.00, EtOH), lit. $[a]_D^{17}$ - 50.0°(c 2.00, $CHCl_3$);[196] $[a]_D^{22}$ - 52.8°(c 1.00, EtOH).

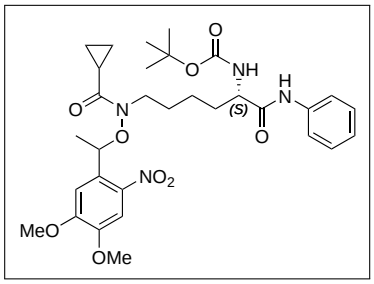
N-((*S*)-5-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-6-oxo-6-(phenylamino)hexyl)-*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)cyclopropanecarboxamide (3.38)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.39** (648 mg, 1.03 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7, 16.4 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours, before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (0.4305 g, 1.34 mmol, 1.3 equiv.) and NEM (908 µL, 7.21 mmol, 7.0 equiv.) was added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (285 mg, 1.24 mmol, 1.2 equiv.) in DMF (2.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (8.3 mL) was added and stirred overnight. Incomplete conversion was observed, and therefore a new batch of acetyl-*L*-leucylglycine **3.37**, TBTU, and NEM were prepared in DMF (2.0 mL) and after stirring for 15 min added to the reaction mixture followed by stirring overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.38** was isolated by flash column chromatography (CH_2Cl_2:MeOH 99:1 → 93:7) as a pale green semi-solid (657 mg, 86%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances

for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.24 (CH_2Cl_2:MeOH (19:1); UV); IR (neat) ν (cm^{-1}): ; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.88/9.87* (s, 1H, C(O)N**H**Ph), 8.27–8.24 (m, 1H, C(O)N**H**CH_2C(O)), 8.07 (d, *J* = 7.4 Hz, 1H, CH_3C(O)N**H**CH), 7.93 (dd, *J* = 7.7, 4.7 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.61–7.60 (m, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.56–5.51 (m, 1H, ArC**H**CH_3), 4.35–4.28 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.24–4.19 (m, 1H, H_3C(O)NHC**H**), 3.91/3.90* (s, 3H, OC**H$_3$**), 3.85/3.84* (s, 3H, OC**H$_3$**), 3.76–3.65 (m, 2H, C(O)NHC**H$_2$**C(O)), 3.61–3.52 (m, 1H, CH_2C**H'**H''NC(O)), 3.17–3.03 (m, 1H, CH_2CH'**H''**NC(O)), 2.10–2.01 (m, 1H, C(O)NC**H**$-CH_2CH_2-$), 1.83/1.82* (s, 3H, C**H$_3$**C(O)NH), 1.68–1.37 (m, 10H, CH_3C(O)NHCHC**H$_2$**C**H**, NHCHC**H$_2$**C**H$_2$**CH_2, ArCHC**H$_3$**), 1.27–1.09 (m, 2H, CH_2C**H$_2$**CH_2NC(O)), 0.88–0.61 (m, 10H, NC(O)CH$-$C**H$_2$C H$_2$** – , CH(C**H$_3$**)$_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.6, 172.8, 170.41/170.39*, 169.6, 168.8, 152.8, 148.2, 140.99/140.98*, 138.8, 130.1, 128.7, 123.4, 119.29/119.27*, 109.79/109.77*, 107.4, 76.9, 56.3 56.1, 53.3/53.2*, 51.4, 46.8, 42.0, 40.6, 31.6, 26.2/26.1*, 24.1, 22.9, 22.6/22.5*, 22.4, 21.6, 20.6, 10.6, 7.9, 7.7; MS (ESI) *m/z*: calcd. for $C_{36}H_{51}N_6O_{10}^+$ $[M + H]^+$ 727.4, found 727.8.

tert-Butyl ((2*S*)-6-(*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)cyclopropanecarboxamido)-1-oxo-1-(phenylamino)hexan-2-yl)carbamate (3.39)

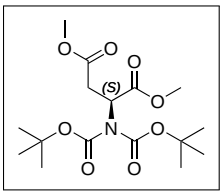


According to the general procedure for oxime reduction, $NaBH_3CN$ (1.12 g, 17.8 mmol, 2.0 equiv.) was added to a stirring cold solution of decomposed **3.83** (5.19 g, 8.89 mmol, 1.0 equiv.) in MeOH (35.6 mL) at 0 °C and pH was adjusted to pH < 3 with TFA. After 15 min, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (aq., 50 mL) and volatiles were removed under reduced pressure. The residues were dissolved in EtOAc (200 mL) and washed with sat. $NaHCO_3$ (aq., 3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mono *N*-alkylated hydroxylamine was placed under vacuum with a magnetic stirring bar for an hour before a nitrogen atmosphere was established. According to the general procedure for acid chloride acylation, pyridine (7.17 mL, 89.0 mmol, 10.0 equiv.) and cyclopropanecarbonyl chloride (924 mL, 8.89 mmol, 1.0 equiv.) were added to a cold solution of the *N*-alkylated

hydroxylamine in CH_2Cl_2 (46.7 mL) at 0 °C and stirred for 1.83 h. The concentrated reaction mixture was washed added sat. NH_4Cl (aq., 200 mL) and washed with CH_2Cl_2 (3 x 100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. According to the general procedure for Boc-deprotection, the di-Boc protected compound was stirred in TFA:CH_2Cl_2 (3:7, 135 mL) overnight. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (1.49 g, 62.3 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1, 222 mL). After 3.0 h, Boc_2O (3.88 g, 17.8 mmol, 2.0 equiv.) was added and stirred overnight. The reaction mixture was diluted with EtOAc (100 mL), and pH adjusted to $\approx$ 3-4 by the addition of 1.0 M HCl (aq., 43.0 mL), and extracted with EtOAc (2 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The Boc-protected carboxylic acid was purified by flash column chromatography (CH_2Cl_2:MeOH 99:1 $\rightarrow$ 19:1) and placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (2.07 g, 6.46 mmol, 1.0 equiv.) was added to a stirring solution of the *N*-Boc protected amino acid (3.48 g, 6.46 mmol, 1.0 equiv.) in CH_2Cl_2 (30.8 mL). After 10 min, DIPEA (6.74 mL, 38.8 mmol, 6.0 equiv.) and aniline (647.3 µL, 7.10 mmol, 1.1 equiv.) were added and stirred overnight. Volatiles were removed under reduced pressure and taken up in EtOAc (100 mL) and washed with H_2O (100 mL), sat. $NaHCO_3$ (aq., 100 mL), and 1.0 M HCl (aq., 2 x 100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 2:3 $\rightarrow$ 11:9) to provide the aniline amide **3.39** (2.80 g, 51%) as a pale green foam. A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.14 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3268, 2975, 2931, 1600, 1518, 1441, 1366, 1334, 1273; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90/9.89* (s, 1H, C(O)N**H**Ph), 7.58–7.53 (m, 3H, Ar**H**), 7.31–7.27 (m, 3H, Ar**H**), 7.05–7.02 (m, 1H, Ar**H**), 6.93–6.89 (m, 1H, CH_2CHN**H**C(O)O), 5.56–5.52 (m, 1H, ArC**H**CH_3), 4.05–3.97 (m, 1H, C(O)$CH_2CH_2CH_2$C**H**NBoc), 3.91/3.90* (s, 3H, OC**H$_3$**), 3.85/3.84* (s, 3H, OC**H$_3$**), 3.62–3.52 (m, 1H, C(O)NC**H'**H"CH_2CH_2), 3.17–3.02 (m, 1H, C(O)NCH'**H"**HCH_2CH_2), 2.11–2.02 (m, 1H, $-CH_2CH_2-$C**H**C(O)N), 1.62–1.15

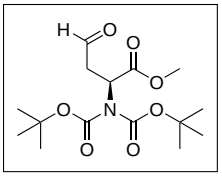
(m, 18H, C(O)NCH'H''C$\mathbf{H_2}$C$\mathbf{H_2}$C$\mathbf{H_2}$CHNHBoc, C(C$\mathbf{H_3}$)$_3$, ArCHC$\mathbf{H_3}$), 0.86–0.62 (m, 4H, −C$\mathbf{H_2}$C$\mathbf{H_2}$−CH(O)N); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.6 (observed by HMBC), 171.4, 155.4, 152.8, 148.2, 140.1, 139.0, 130.3, 128.7, 123.2, 119.2, 109.8, 107.4, 78.0, 77.9, 56.3, 56.11/56.10*, 54.9, 46.9 (observed by HSQC), 31.5, 28.2, 26.2/26.1*, 22.81/22.77*, 20.6, 10.4, 7.9, 7.6; MS (ESI) *m/z*: calcd. for $C_{31}H_{43}N_4O_9^+$ [M + H]$^+$ 615.3, found 615.8.

Dimethyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)butane-1,4-dioate (3.50)[143]



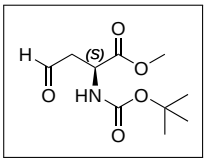
To an oven-dried round-bottomed flask containing a magnetic stirring bar and equipped with a condenser, $SOCl_2$ (13.2 mL, 0.180 mol, 1.2 equiv.) was added dropwise to a suspension of *L*-aspartic acid **3.49** (20.0 g, 0.150 mol, 1.0 equiv.) in MeOH (200 mL) at 0 °C under nitrogen atmosphere. The mixture was heated at reflux for 22 h. The reaction mixture was cooled to rt and the solvent was removed under reduced pressure. The crude white intermediated was dissolved in a solvent mixture of sat. $NaHCO_3$ (aq., 200 mL) and dioxane (200 mL). $(Boc)_2O$ (39.4 g, 0.180 mol, 1.2 equiv.) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was extracted with EtOAc (3 x 400 mL). The combined organic layer were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The round-bottomed flask was placed under vacuum for a couple of hours, before a nitrogen atmosphere was established. The crude white powder was dissolved in MeCN (250 mL). DMAP (3.67 g, 30.1 mmol, 0.2 equiv.) and $(Boc)_2O$ (55.8 g, 0.255 mol, 1.7 equiv.) were subsequently added and the reaction mixture stirred for overnight. The resulting red reaction mixture was evaporated to dryness. The residues were purified by DCVC (EtOAc:heptane 1:99 → 3:7) to afford **3.50** (49.7 g, 91%) as a white solid. Spectral data in correspondence with reported literature values.[143] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.46 (t, *J* = 6.8 Hz, 1H, CH'H''C**H**N), 3.72 (s, 3H, CHC(O)OC$\mathbf{H_3}$), 3.70 (s, 3H, C$\mathbf{H_3}$O(O)CCH'H''), 3.27–3.21 (m, 1H, C**H'**H''C(O)OCH$_3$), 2.76–2.70 (m, 1H, CH'**H''**C(O)OCH$_3$), 1.50 (s, 18H, 2 x C(C$\mathbf{H_3}$)$_3$); $[a]_D^{22}$ - 67.5°(*c* 2.00, $CHCl_3$); lit. $[a]_D^{25}$ - 61.0°(*c* 2.00, $CHCl_3$).

Methyl (*S*)-2-(di-*tert*-butoxycarbonylamino)-4-oxobutanoate (3.51)[143]



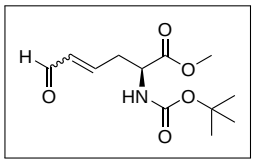
In an oven-dried round-bottomed flask containing a magnetic stirring bar, DIBAL-H (1 M in toluene, 10.8 mL, 9.13 mmol, 1.1 equiv.) was added dropwise over a period of 30 min to a solution of the methyl ester **3.50** (3.00 g, 8.30 mmol, 1.0 equiv.) in Et_2O (83.0 mL) at -78 °C under a nitrogen atmosphere. The reaction mixture was stirred for additional 30 min, before quenched by the addition of H_2O (1.05 mL, 58.1 mmol, 7.0 equiv.) followed by 30 min stirring. The solution was dried over $MgSO_4$ and filtered through a pad of Celite® and concentrated *in vacuo*. Six identical reactions were combined and purified by one flash column chromatography (EtOAc:Heptane 1:9 $\rightarrow$ 2:3) to afford the aldehyde **3.51** (14.7 g, 89%) as a clear oil. Spectral data in correspondence with reported literature values.[143] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.79 (s, 1H, O**H**CCH_2), 5.53 (t, *J* = 6.4 Hz, 1H, CH'H''C**H**N), 3.73 (s, 3H, COOC**H$_3$**), 3.45–3.38 (m, 1H, C**H'**HCHO), 2.86–2.80 (m, 1H, CH'**H''**CHO), 1.50 (s, 18H, 2 x C(C**H$_3$**)$_3$); $[a]_D^{22}$ - 56.9°(*c* 2.00, $CHCl_3$); lit. $[a]_D^{25}$ - 54.9°(*c* 2.00, $CHCl_3$).

Methyl (*S*)-2-(*tert*-butoxycarbonylamino)-4-oxobutanoate (3.52)[146]



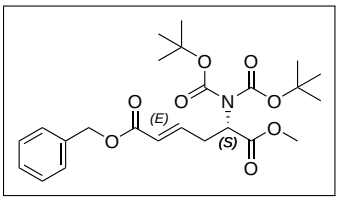
In an oven-dried round-bottomed flask containing a magnetic stirring bar, LiBr (3.12 g, 35.9 mmol, 3.0 equiv.) was added to a solution of di-Boc aldehyde **3.51** (3.96 g, 12.0 mmol, 1.0 equiv.) in MeCN (135 mL) under nitrogen atmosphere and refluxed overnight. Incomplete conversion was observed and the reaction mixture was concentrated under reduced pressure and directly purified by flash column chromatography (EtOAc:Heptane 3:7) to afford the mono-Boc protected aldehyde **3.52** (1.02 g, 37%) as an oil. A trace amount of impurities and **3.51** were observed by ^{1}H-NMR. Spectral data in correspondence with reported literature values.[146] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.59 (s, 1H, O**H**CCH_2), 7.32 (d, *J* = 8.1 Hz, 1H, CHN**H**C(O)), 4.51 (m, 1H, CH_2C**H**NHC(O)), 3.62 (s, 3H, C(O)OC**H$_3$**), 2.87–2.69 (m, 2H, OHCC**H$_2$**CH), 1.38 (s, 9H, C(CH_3)$_3$).

Methyl (*S,E*)-2-((*tert*-butoxycarbonyl)amino)-6-oxohex-4-enoate (3.53)



In an oven-dried round-bottomed flask containing a magnetic stirring bar equipped with a condenser, (triphenylphosphoranylidene)acetaldehyde (2.11 g, 6.93 mmol, 2.0 equiv.) was added to a solution of the mono-Boc protected aldehyde **3.52** (801 mg, 3.46 mmol, 1.0 equiv.) in THF (13.9 mL) and refluxed under nitrogen atmosphere for 26 h. The reaction mixture was concentrated under reduced pressure. The residues were purified by flash column chromatography (EtOAc:heptane 1:4 $\rightarrow$ 1:1) to afford the α/ β-conjugated aldehyde **3.53** as a yellow oil (596.4 mg, 63%). Triphenylphosphineoxide was observed by ^{1}H-NMR. *Z*:*E* ratio was found to be 33:67 by ^{1}H-NMR. A trace amount of impurities was observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.55–9.50 (s, 1H, **H**(O)CCH), 7.09–7.02 (m, 0.33H, H(O)CCHC**H**), 6.78–6.71 (m, 0.67H, H(O)CCHC**H**), 6.40–6.34 (m, 0.33H, H(O)CC**H**CH), 6.18–6.07 (m, 0.67H, H(O)CC**H**CH), 5.16–5.09 (m, 1H, CH_2CHN**H**C(O)O), 4.54–4.46 (m, 1H, CH_2C**H**NHC(O)O), 3.77–3.75 (s, 3H, C(O)OC**H$_3$**), 2.94–2.56 (m, 2H, C**H$_2$**CHNHC(O)O), 1.43 (s, 9H, C(CH_3)$_3$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 193.9/193.5, 172.1/171.8, 155.2, 151.5/151.4, 135.7 80.6, 52.9, 52.7/52.5, 36.6/36.2, 28.41/28.39.

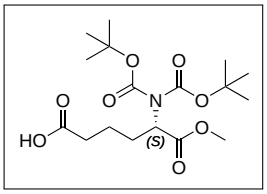
1-Benzyl 6-methyl *(S,E)*-5-(bis(*tert*-butoxycarbonyl)amino)hex-2-enedioate (3.56)



In an oven-dried round-bottomed flask containing a magnetic stirring bar and equipped with a condenser, benzyl(triphenylphosphoranylidene)acetate (19.36 g, 47.2 mmol, 1.5 equiv.) was added to a stirring solution of the di-Boc aldehyde **3.51** (10.42 g, 31.4 mmol, 1.0 equiv.) in THF (81.0 mL) and refluxed overnight under a nitrogen atmosphere. The reaction mixture was cooled to rt and diluted by the addition of sat. NH_4Cl (aq., 300 mL) and extracted with EtOAc (3 x 300 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (heptane $\rightarrow$ EtOAc:heptane 4:6) providing the four-carbon α,β-conjugated benzyl ester (14.06 g, 96%) as a clear oil. A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. R_f = 0.53 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 2988, 2937, 1746, 1719, 1699, 1658, 1478, 1138; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.36–7.31 (m, 5H, Ar**H**), 6.98–6.91 (m, 1H, BnO(O)CC**H**CHCH'H''), 5.94–5.90 (m, 1H, BnO(O)CCHC**H**CH'H''), 5.15 (s, 2H,

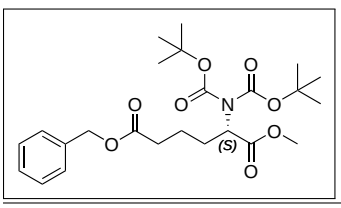
ArC**H**$_2$O), 5.03 (dd, *J* = 9.8, 5.0 Hz, 1H, CH'H''C**H**N), 3.73 (s, 3H, CHC(O)OC**H**$_3$), 3.04–2.97 (m, 1H, C**H'**H''C**H**N), 2.87–2.79 (m, 1H, CH'**H''**C**H**N), 1.46 (s, 18H, 2 x C(C**H**$_3$)$_3$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 170.4, 165.9, 151.8, 144.8, 136.0, 128.7, 128.4, 128.3, 124.0, 83.7, 66.3, 57.0 52.6, 33.2, 28.0; MS (ESI) *m/z*: calcd. for $C_{24}H_{33}NNaO_8^+$ [M + Na]$^+$ 486.2, found 486.8; HRMS (ESI) *m/z*: calcd. for $C_{24}H_{33}NNaO_8^+$ [M + Na]$^+$ 486.2098, found 486.2097. $[a]_D^{22}$ - 54.4°(*c* 1.00, $CHCl_3$).

(*S*)-5-(bis(*tert*-butoxycarbonyl)amino)-6-methoxy-6-oxohexanoic acid (3.57)



In a round-bottomed flask containing a magnetic stirring bar, α,β-conjugated benzyl ester **3.56** (5.35 g, 11.5 mmol, 1.0 equiv.) was placed under vacuum for an hour before a nitrogen atmosphere was established and dissolved in *i*PrOH (57.7 mL), and added $Pd(OAc)_2$ (64.8 mg, 0.288 mmol, 0.025 equiv.) and charcoal (584 mg, $Pd(OAc)_2$:charcoal 10:90 (wt:wt)). Two vacuum/hydrogen cycles were performed before hydrogen was bubbled through the solution over 45 min. The reaction mixture was stirred under hydrogen atmosphere overnight. The reaction mixture was filtered through a pad of Celite®, and washed thoroughly with CH_2Cl_2 and concentrated under reduced pressure. This afforded the carboxylic acid **3.58** as a clear oil (4.29 g, 99%). A trace of amount of CH_2Cl_2 was observed by ^{1}H-NMR. R_f = 0.16 (CH_2Cl_2:MeOH (95:5); $KMnO_4$); IR (neat) ν (cm^{-1}): 3215, 2949, 2888, 1740, 1703, 1136, 1113; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.04 (br. s, 1H, **H**O(O)C), 4.82 (dd, *J* = 10.0, 4.9 Hz, 1H, CH'H''C**HN**), 3.62 (s, 3H, C(O)OC**H**$_3$), 2.30–2.16 (m, 2H, C(O)C**H**$_2$CH$_2$), 2.02–1.91 (m, 1H, CH$_2$C**H'**H''CHN), 1.86–1.77 (m, 1H, CH$_2$CH'**H''**CHN), 1.48–1.40 (m, 20H, (O)CCH$_2$C**H**$_2$CH$_2$CH, 2 x C(C**H**$_3$)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.6, 171.0, 152.0, 83.0, 57.7, 52.5, 33.6, 29.1, 27.9, 21.5; MS (ESI) *m/z*: calcd. for $C_{17}H_{29}NNaO_8^+$ [M + Na]$^+$ 398.2, found 398.6; HRMS (ESI) *m/z*: calcd. for $C_{17}H_{29}NNaO_8^+$ [M + Na]$^+$ 398.1791, found 398.1797; $[a]_D^{22}$ - 33.6°(*c* 1.00, MeOH).

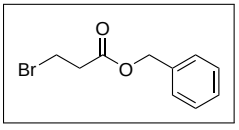
6-Benzyl 1-methyl *(S)*-2-(bis(*tert*-butoxycarbonyl)amino)hexanedioate (3.58)



In a round-bottomed flask containing a magnetic stirring bar, α,β-conjugated benzyl ester **3.56** (6.48 g, 17.8 mmol, 1.0 equiv.) was placed under vacuum for an hour before a nitrogen atmosphere was established and dissolved in MeOH (54.3 mL), fol-

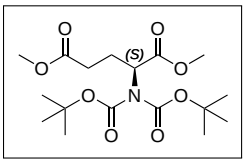
lowed by the addition of Ph_2S (29.8 µL, 0.178 mmol, 0.01 equiv.) and 10% Pd/C (648 mg, 10wt%). Two vacuum/hydrogen cycles were performed before hydrogen was bubbled through the solution over 30 min. The reaction mixture was stirred under hydrogen for 36 h. The reaction mixture was filtered twice through a pad of Celite®, and washed thoroughly with CH_2Cl_2 and concentrated under reduced pressure each time. This afforded the benzyl ester **3.58** as a clear oil (6.28 g, 97%). R_f = 0.56 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 2979, 2951, 1736, 1699, 1456, 1136; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.38–7.29 (m, 5H, Ar**H**), 5.11 (s, 2H, ArC**H$_2$**O), 4.87 (dd, *J* = 9.5, 5.1 Hz, 1H, CH'H''C**H**N), 3.70 (s, 3H, C(O)OC**H$_3$**), 2.47–2.33 (m, 2H, (O)CC**H$_2$**CH$_2$), 2.18–2.09 (m, 1H, C**H'**H''CHN), 1.99–1.89 (m, 2H, CH'**H''**CHN), 1.75–1.67 (m, 2H, (O)CCH$_2$C**H$_2$**CH$_2$CH), 1.48 (s, 18H, 2 x C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.1, 171.3, 152.2, 136.1, 128.7, 128.3 (two overlapping signals), 83.3, 66.3, 57.9, 52.3, 34.0, 29.5, 28.1, 21.8; HRMS (ESI) *m/z*: calcd. for $C_{14}H_{20}NO_4^+$ [M - 2 x Boc + 3 x H]$^+$ 266.1387, found ?; MS (ESI) *m/z*: calcd. for $C_{14}H_{20}NO_4^+$ [M - 2 x Boc + 3 x H]$^+$ 266.1, found 266.6; $[a]_D^{22}$ - 32.0°(*c* 1.00, $CHCl_3$).

Benzyl 3-bromopropanoate (3.61)[197]



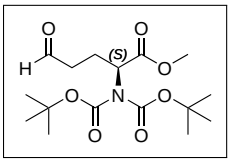
In a flame-dried round-bottomed flask containing a magnetic stirring bar and equipped with a Dean-Stark and a condenser, 3-bromopropionic acid (7.50 g, 49.0 mmol, 1.0 equiv.), BnOH (5.48 mL, 52.7 mmol, 1.1 equiv.), and *p*-TsOH (466 mg, 2.45 mmol, 0.05 equiv.) were stirred in toluene (52.7 mL) under a nitrogen atmosphere at reflux. When full conversion was observed by TLC and LCMS (6.5 h), volatiles were removed under reduced pressure, and the crude was directly purified by DCVC (heptane → EtOAc:heptane 5:95) to obtain benzyl 3-bromopropanoate as a clear oil (10.0 g, 84%). Spectral data in correspondence with reported literature values.[197] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.39–7.32 (m, 5H, Ar**H**), 5.15 (s, 2H, OC**H$_2$**Ar), 3.67 (t, *J* = 6.3 Hz, 2H, BrC**H$_2$**CH$_2$C(O)), 3.03 (t, *J* = 6.3 Hz, 2H, BrCH$_2$C**H$_2$**C(O)).

Dimethyl (2*S*)-2-(bis-*tert*-butoxycarbonylamino)pentane-1,5-dioate (3.63)[143]



In a round-bottomed flask containing a magnetic stirring bar, $(Boc)O_2$ (18.56 g, 85.0 mmol, 1.2 equiv.) was added to a solution of dimethyl *L*-glutamate hydrochloride **3.62** (15.0 g, 70.9 mmol, 1.0 equiv.) in a solvent mixture of sat. $NaHCO_3$ (aq., 94.0 mL) and dioxane (94.0 mL) and stirred overnight. The solution was extracted with EtOAc (3 x 300 mL). The combined organic layer were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mono-Boc protected intermediate was placed under vacuum for a couple of hours, before a nitrogen atmosphere was established. The crude oil was dissolved in MeCN (118 mL) followed by the addition of DMAP (1.73 g, 14.2 mmol, 0.2 equiv.) and $(Boc)_2O$ (26.3 g, 120 mmol, 1.7 equiv.) were added and stirred overnight. The resulting red reaction mixture was evaporated to dryness. The title compound (23.9 g, 90%) was obtained as a white solid by DCVC (heptane $\rightarrow$ EtOAc:Heptane 1:9). Spectral data in correspondence with reported literature values.[143] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.95–4.91 (dd, J = 9.4, 4.6 Hz, 1H, CH'H''C**H**N), 3.71 (s, 3H, C**H$_3$**O(O)CCH), 3.67 (s, 3H, C**H$_3$**O(O)CCH_2), 2.53–2.34 (m, 3H, $CH_3O(O)CCH_2$, C**H'**H''CHN), 2.23–2.14 (m, 1H, CH'**H''**CHN), 1.49 (s, 18H, 2 x $C(CH_3)_3$); $[a]_D^{22}$ - 39.0°(c 2.15, $CHCl_3$); lit. $[a]_D^{25}$ - 37.2°(c 2.15, $CHCl_3$).

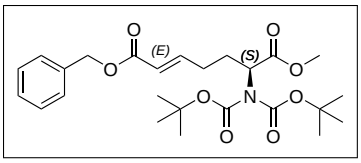
Methyl (*S*)-2-(di-*tert*-butoxycarbonylamino)-5-oxopentanoate (3.60)[143]



In an oven-dried round-bottomed flask containing a magnetic stirring bar, DIBAL-H (1.0 M in toluene, 8.79 mL, 8.79 mmol, 1.1 equiv.) was added dropwise over a period of 30 min to a solution of the di-Boc methyl ester (**3.63** (3.00 g, 7.99 mmol, 1.0 equiv.) in dry Et_2O (79.9 mL) at -78 °C. The reaction mixture was stirred for a further 30 min, before it was quenched by the addition of H_2O (1.00 mL, 55.9 mmol, 7 equiv.)followed by 30 min stirring. The solution was dried over $MgSO_4$ and filtered through a pad of Celite® and concentrated *in vacuo*. Nine identical reactions were combined and purified by one flash column chromatography (EtOAc:Heptane 1:9 $\rightarrow$ 2:3) to afford the di-Boc aldehyde **3.60** (21.1 g, 85%) as a clear oil, that forms a semi solid over time. Spectral data in correspondence with reported literature values.[143] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.77 (s, 1H, O**H**C), 4.88 (dd, J = 9.5, 5.1 Hz, 1H, CH'H''C**H**N), 3.72 (s, 3H, C(O)OC**H$_3$**), 2.63–2.45 (m, 3H, OHCC**H$_2$**C**H'**H''CH), 2.20–2.11 (m, 1H, CH'**H''**CH), 1.49 (s, 18H, 2 x C(C**H$_3$**)$_3$); $[a]_D^{22}$ - -38.0

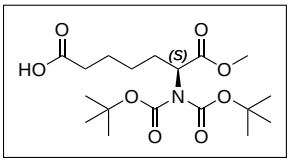
°(*c* 1.0, $CHCl_3$); lit. $[a]_D^{25}$ - 35.3°(*c* 2.3, $CHCl_3$).

1-Benzyl 7-methyl *(S,E)*-6-(di(*tert*-butoxycarbonyl)amino)hept-2-enedioate (3.64)



In an oven-dried round-bottomed flask containing a magnetic stirring bar, benzyl(triphenylphosphoranylidene)acetate (37.6 g, 91.6 mmol, 1.5 equiv.) was added to a stirring solution of di-Boc aldehyde **3.60** (21.1 g, 61.1 mmol, 1 equiv.) in THF (156.7 mL) and refluxed overnight under a nitrogen atmosphere. The reaction mixture was cooled to rt and diluted by the addition of sat. NH_4Cl (aq., 500 mL) and extracted with EtOAc (3 x 500 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (heptane → EtOAc:heptane 3:7) providing α,β-conjugated benzyl ester **3.64** (26.8 g, 92%) as a clear oil. R_f = 0.26 (EtOAc:heptane (1:4); UV); R (neat) ν (cm^{-1}): 2980, 2951, 1745, 1717, 1455, 1138; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.37–7.32 (m, 5H, Ar**H**), 7.00 (dt, *J* = 15.7, 6.5 Hz, 1H, O(O)CCHC(*E*)**H**CH_2), 5.90 (d, *J* = 15.7 Hz, 1H, O(O)CC(*E*)**H**$CHCH_2$), 5.17 (s, 2H, Ar$C\mathbf{H_2}$O), 4.86 (dd, *J* = 9.0, 4.1 Hz, 1H, CH'H''C**H**N), 3.71 (s, 3H, C(O)O$C\mathbf{H_3}$), 2.33–2.25 (m, 3H, $C\mathbf{H_2}C\mathbf{H'}$H''CHN), 2.10–2.02 (m, 1H, CH_2CH'**H''**CHN), 1.48 (s, 18H, 2 x C($C\mathbf{H_3}$)$_3$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 171.1, 166.3, 152.2, 148.4, 136.2, 128.7, 128.3, 128.3, 121.9, 83.5, 66.2, 57.6, 52.4, 29.1, 28.5, 28.1; MS (ESI) *m/z*: calcd. for $C_{25}H_{35}NNaO_8^+$ $[M + Na]^+$ 500.2, found 500.7; $[a]_D^{22}$ - 28.0°(*c* 1.0, $CHCl_3$).

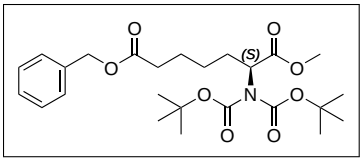
***(S)*-6-(bis(*tert*-butoxycarbonyl)amino)-7-methoxy-7-oxoheptanoic acid (3.65)**



In a round-bottomed flask containing a magnetic stirring bar, α,β-conjugated benzyl ester **3.64** (6.52 g, 13.7 mmol, 1.0 equiv.) was placed under vacuum for an hour before a nitrogen atmosphere was established and dissolved in *i*PrOH (68.3 mL), and added $Pd(OAc)_2$ (77.9 mg, 0.3470 mmol, 0.025 equiv.) and charcoal (690 mg, $Pd(OAc)_2$:charcoal 10:90 (wt:wt)). Two vacuum/hydrogen cycles were performed before hydrogen was bubbled through the solution over 45 min. The reaction mixture was stirred under a hydrogen atmosphere overnight. The reaction mixture was filtered through a pad of Celite®, and washed thoroughly with CH_2Cl_2 and concentrated under reduced pressure. This afforded the saturated carboxylic acid **3.58** as a clear oil (5.13 g, 96%). A trace amount

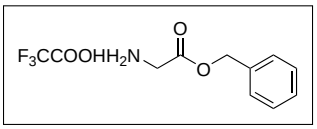
CH_2Cl_2 was observed by ^{1}H-NMR. R_f = 0.25 (CH_2Cl_2:MeOH (95:5); $KMnO_4$); IR (neat) ν (cm^{-1}): 3194, 2980, 2939, 1738, 1703, 1367, 1134; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.99 (br. s, 1H, **H**OOC), 4.81 (dd, *J* = 9.9, 5.0 Hz, 1H, CH'H''C**H**N), 3.62 (s, 3H, C(O)OC**H$_3$**), 2.18 (t, *J* = 7.3 Hz, 2H, CH'H''C**H**N), 2.01–1.92 (m, 1H, HO(O)CC**H$_2$**), 1.83–1.73 (m, 1H, CH_2CH'**H''**HCHN), 1.55–1.37 (m, 20H, 2 x C(C**H$_3$**)$_3$, HO(O)CCH_2C**H$_2$**), 1.31–1.20 (m, 2H, C**H$_2$**CH_2CHN); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.3, 170.6, 151.6, 82.5, 57.4, 52.0, 33.5, 29.0, 27.5, 25.0, 24.1; MS (ESI) *m/z*: calcd. for $C_{18}H_{31}NNaO_8^+$ $[M + Na]^+$ 412.2, found 412.6; $[a]_D^{22}$ - -31.0°(*c* 1.00, MeOH).

7-Benzyl 1-methyl *(S)*-2-(bis(*tert*-butoxycarbonyl)amino)heptanedioate (3.66)



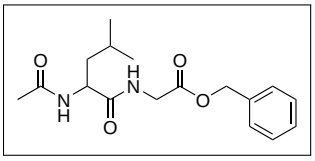
In a round-bottomed flask containing a magnetic stirring bar, α,β-conjugated benzyl ester **3.64** (8.89 g, 18.6 mmol, 1.0 equiv.) was placed under vacuum for two hour before a nitrogen atmosphere was established and dissolved in MeOH (74.4 mL) followed by the addition of Ph_2S (31.2 µL, 0.186 mmol, 0.01 equiv.) and 10% Pd/C (889 mg, 10wt%). Two vacuum/hydrogen cycles were performed before hydrogen was bubbled through the solution over 30 min. The reaction mixture was stirred under a hydrogen atmosphere for 36 h. The reaction mixture was filtered twice through a pad of Celite®, and washed thoroughly with CH_2Cl_2 and concentrated under reduced pressure each time. This afforded the benzyl ester **3.66** as a clear oil (8.54 g, 96%). R_f = 0.60 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 2979, 2937, 2871, 1736, 1699, 1456, 1133; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.38–7.30 (m, 5H, Ar**H**), 5.10 (s, 2H, ArC**H$_2$**O), 4.84 (dd, *J* = 9.4, 5.2 Hz, 1H, CH'H''C**H**N), 3.70 (s, 3H, C(O)OC**H$_3$**), 2.36 (t, *J* = 7.6 Hz, 2H, (O)CC**H$_2$**), 2.17–2.08 (m, 1H, C**H'**H''CHN), 1.93–1.83 (m, 1H, CH'**H''**CHN), 1.73–1.62 (m, 2H, CH_2C**H$_2$**CH_2CH'H''CH), 1.48 (s, 18H, 2 x C(CH_3)$_3$), 1.42–1.34 (m, 2H, CH_2CH_2C**H$_2$**CH'H''CHN); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.4, 171.4, 152.2, 136.2, 128.7, 128.3, 128.3, 83.2, 66.3, 58.1, 52.3, 34.3, 29.8, 28.1, 26.0 24.7; MS (ESI) *m/z*: calcd. for $C_{15}H_{22}NO_4^+$ $[M + 3 x H - 2 x Boc]^+$ 280.2, found 280.6; HRMS (ESI) *m/z*: calcd. for $C_{25}H_{37}NNaO_8^+$ $[M + Na]^+$ 502.2411, found 502.2427. $[a]_D^{22}$ - 28.0°(*c* 1.00, $CHCl_3$).

Benzyl glycine TFA salt (3.68)



In a flame-dried round-bottomed flask containing a magnetic stirring bar, Cs_2CO_3 (18.6 g, 57.1 mmol, 1.0 equiv.) was added to a stirring solution of (*tert*-butoxycarbonyl)glycine **3.67** (10.0 g, 57.1 mmol, 1.0 equiv.) in DMF (114 mL) at 0 °C and left for 60 min before BnBr (6.45 mL, 54.2 mmol, 0.95 equiv.) was added followed by a further 30 min at this temperature before leaving it overnight at rt. The reaction mixture was poured into H_2O (400 mL) and extracted with heptane (4 x 100 mL). Precipitation of (*S*)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid was observed during extraction and collected by filtration. The combined organic phases were stored in the fridge overnight and a second crop was collected. (*S*)-5-(Benzyloxy)-4-((tert-butoxycarbonyl)amino)-5-oxopentanoic acid (11.6673 g) was dissolved in TFA:CH_2Cl_2 (1:10) (227 mL). When full conversion was observed by TLC and LCMS (7 h), the reaction mixture was concentrated under reduced pressure, and co-evaporated from toluene and two times CH_2Cl_2. This afforded **3.68** as a off-white solid (11.6 g, 77%). R_f = 0.81 (CH_2Cl_2:MeOH (95:5); UV); IR (neat) ν (cm^{-1}): 3324, 3066, 2981, 2936, 1749, 1681, 1540, 1169; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.33 (s, 3H, $\mathbf{H_3}NCH_2$), 7.43–7.34 (s, 5H, Ar**H**), 5.24 (s, 2H, OC$\mathbf{H_2}$Ar), 3.90 (s, 2H, $H_3NC\mathbf{H_2}$); ^{13}C-NMR (101 MHz,): δ 167.6, 135.2, 128.5, 128.4, 128.2, 66.8, $H_3N\mathbf{C}H_2$ under DMSO-d_6); MS (ESI) *m/z*: calcd. for $C_9H_{12}NO_2^+$ [M + H - TFA]$^+$ 166.1, found 166.0;

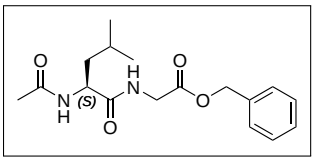
Benzyl acetyl-leucylglycinate (3.69a)



According to the general procedure for coupling reagent acylation, HATU (14.9 g, 39.4 mmol, 1.1 equiv.) and DIPEA (37.4 mL, 0.215 mol, 6.0 equiv.) were added to a stirring solution of *N*-acetyl-*L*-leucine (7.44 g, 43.0 mmol, 1.2 equiv.) in DMF (358 mL) and stirred for 25 min, before the crude TFA salt **3.68** was added and stirred overnight. The reaction mixture was diluted with EtOAc (500 mL) and H_2O (300 mL), and the organic layer was washed with brine (3 x 300 mL), sat. $NaHCO_3$ (aq., 300 mL), and brine (25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Racemic benzyl acetyl-leucylglycinate **3.69a** was isolated by flash column chromatography (CH_2Cl_2:MeOH 95:5) as a white solid (8.96 g, 78%). A trace amount of impurities were observed by ^{1}H-NMR. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.39–7.31 (m, 5H, Ar**H**),

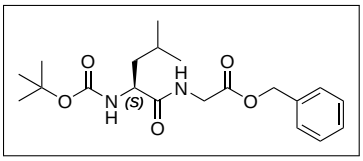
6.81 (t, *J* = 5.0 Hz, 1H, N**H**CH_2C(O)OBn), 6.09 (d, *J* = 8.2 Hz, 1H, CH_3C(O)N**H**CH), 5.16 (s, 2H, OC**H_2**Ar), 4.54–4.48 (m, 1H, CH_3C(O)NHC**H**C(O)), 4.11–3.98 (m, 2H, NHC**H_2**C(O)), 1.98 (s, 3H, **CH_3**C(O)NH), 1.72–1.59 (m, 2H, NHCHC**H'**H''$CH(CH_3)_2$, $CHCH_2$C**H**$(CH_3)_2$), 1.56–1.41 (m, 1H, CHCH'**H''**$CHCH_3$), 0.92 (t, *J* = 6.3 Hz, 6H, $CH(CH_3)_2$); $[a]_D^{22}$ + 0.002°(*c* 1.00, $CHCl_3$).

Benzyl acetyl-*L*-leucylglycinate (3.69)



According to the general procedure for Boc-deprotection, benzyl (*tert*-butoxycarbonyl)-*L*-leucylglycinate **3.76** (26.4 g, 69.6 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (279 mL). The TFA salt was placed under vacuum for a couple of hours before a nitrogen atmosphere was introduced and dissolved in CH_2Cl_2 (126 mL). Subsequently, DIPEA (36.4 mL, 0.209 mol, 3.0 equiv.) and Ac_2O (9.90 mL, 0.104 mol, 1.5 equiv.) were added and stirred overnight. The reaction mixture was concentrated under reduced pressure. The residues were taken up in EtOAc (500 mL), and washed with with 1.0 M HCl (aq., 2 x 250 mL), sat. $NaHCO_3$ (aq., 2 x 250 mL), and brine (250 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The acetylated dipeptide **3.69** was purified by flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH 98:2) as a off-white solid (21.2 g, 95%). Spectral data in correspondence with reported literature values.[174] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.39–7.31 (m, 5H, Ar**H**), 6.83 (t, *J* = 5.3 Hz, 1H, N**H**CH_2C(O)OBn), 6.10 (d, *J* = 8.2 Hz, 1H, CH_3C(O)N**H**CH), 5.16 (s, 2H, OC**H_2**Ar), 4.55–4.49 (m, 1H, CH_3C(O)NHC**H**C(O)), 4.11–3.98 (m, 2H, NHC**H_2**C(O)), 1.98 (s, 3H, C**H_3**C(O)NH), 1.72–1.60 (m, 2H, NHCHC**H'**H''$CH(CH_3)_2$, $CHCH_2$C**H**$(CH_3)_2$), 1.57–1.48 (m, 1H, CHCH'**H''**$CHCH_3$), 0.92 (t, *J* = 6.3 Hz, 6H, $CH(CH_3)_2$); $[a]_D^{22}$ - 56.5°(*c* 1.00, $CHCl_3$).

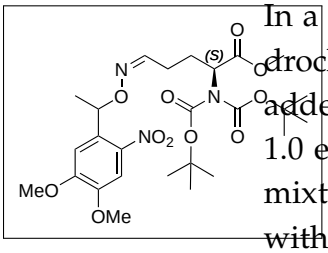
Benzyl (tert-butoxycarbonyl)-*L*-leucylglycinate (3.76)



In a round-bottomed flask containing a magnetic stirring bar, 2,5-dioxopyrrolidin-1-yl (*tert*-butoxycarbonyl)-*L*-leucinate (26.9 g, 81.8 mmol, 1.1 equiv.) was added to solution of benzyl glycinate hydrochloride (15.0 g, 74.4 mmol, 1.0 equiv.) and $NaHCO_3$ (13.7 g, 0.164 mmol, 2.2 equiv.) in H_2O:dioxane (3:7) (434 mL) and stirred overnight. The reaction mixture was extracted with EtOAc (3 x 500 mL). The com-

bined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were by flash column chromatography (EtOAc:heptane 2:8 $\rightarrow$ 4:6) to afford **3.76** it as a high viscosity clear oil (26.5 g, 94%). R_f = 0.38 (EtOAc:heptane (1:1); $KMnO_4$); IR (neat) ν (cm^{-1}): 3304, 3034, 2956, 2935, 1749, 1659, 1519, 1455, 1163; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.38–7.31 (s, 5H, Ar**H**), 6.75 (br. s, 1H, (O)CN**H**CH_2), 5.17 (s, 2H, OC**H$_2$**Ar), 4.94 (d, *J* = 8.2 Hz, 1H, O(O)CN**H**CH), 4.17 (m, 1H, O(O)CNHC**H**), 4.07 (dd, *J* = 5.4, 1.9 Hz, 2H, NHC**H$_2$**C(O)OCH_2), 1.69–1.66 (m, 2H, CHC**H**'H''$CHCH_3$, $CHCH_2$C**H**$(CH_3)_2$), 1.50–1.43 (m, 10H, CHCH'**H''**$CHCH_3$, $C(CH_3)_3$), 0.93 (m, 6H, $CH(CH_3)_2$); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.0, 169.7, 155.6, 135.3, 128.8, 128.7, 128.5, 80.3, 67.3, 53.0, 41.4, 28.4, 24.8, 23.1, 22.0; MS (ESI) *m/z*: calcd. for $C_{20}H_{31}N_2O_5^+$ $[M + H]^+$ 379.2, found 379.7; $[a]_D^{22}$ - 26.6°(*c* 1.00, $CHCl_3$).

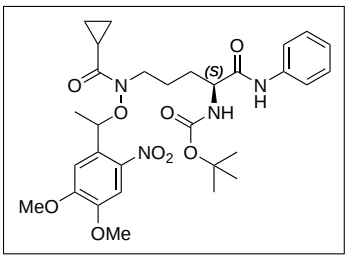
Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-5-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)imino)pentanoate (3.77)



In a round-bottomed flask containing a magnetic stirring bar, the hydrochloride hydroxylamine **2.7** (3.73 g, 13.4 mmol, 1.0 equiv.) was added to a stirring solution of the aldehyde **3.60** (4.62 g, 13.4 mmol, 1.0 equiv.) in pyridine (83.7 mL) and stirred overnight. The reaction mixture was concentrated under reduced pressure and co-evaporated with heptane. The residues were taken up in sat. NH_4Cl (aq., 250 mL) and extracted with EtOAc (3 x 250 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 1:9 $\rightarrow$ 1:3) to provide the oxime **3.77** (6.58 g, 86%) as a neon green oil. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.16 (EtOAc:Heptane (1:3); UV); IR (neat) ν (cm^{-1}): 2979, 2936, 1744, 1699, 1518, 1455, 1366, 1334, 1270; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.58–7.55* (m, 1.5H, Ar**H**, 0.5 x ONC**H**CH2), 7.02/6.95* (m, 1H, Ar**H**), 6.80–6.75* (m, 0.5H, 0.5 x ONC**H**CH2), 5.68–5.59 (m, 1H, ArC**H**CH_3), 4.92–4.87/4.79–4.74* (m, 1H, CH_2C**H**C(O)), 3.87–3.84 (m, 6H, 2 x OC**H$_3$**), 3.64/3.61–3.60 (m, 3H, C(O)OC**H$_3$**), 2.39–2.30 (m, 1H, C**H'**H''CHC(O)OC**H$_3$**), 2.26–1.72 (m, 3H, C**H$_2$**CH'**H''**CHC(O)OC**H$_3$**), 1.53–1.49 (m, 3H, ArCHC**H$_3$**), 1.42–1.37 (m, 18H, 2 x

C($C\mathbf{H_3}$)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ MS (ESI) *m/z*: calcd. for $C_{26}H_{39}N_3NaO_{11}^+$ [M + Na]$^+$ 592.2, found 592.8; HRMS (ESI) *m/z*: calcd. for $C_{26}H_{39}N_3NaO_{11}^+$ [M + Na]$^+$ 592.2477, found 592.2498.

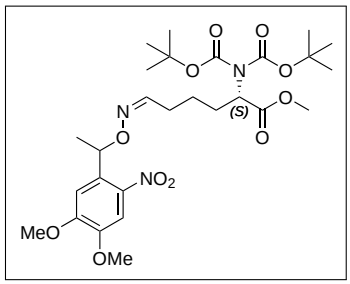
tert-Butyl ((2*S*)-5-(*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)cyclopropanecarboxamido)-1-oxo-1-(phenylamino)pentan-2-yl)carbamate (3.82)



According to the general procedure for oxime reduction, $NaBH_3CN$ (583 mg, 9.27 mmol, 2.0 equiv.) was added to a stirring cold solution of **3.77** (2.64 g, 4.64 mmol, 1.0 equiv.) in MeOH (18.5 mL) at 0 °C and subsequently pH was adjusted to pH $<$ 3 with TFA. After 25 min at rt, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (aq., 50 mL) and volatiles were removed under reduced pressure. The residues were dissolved in EtOAc (200 mL) and washed with sat. $NaHCO_3$ (aq.) (3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mono *N*-alkylated hydroxylamine was placed under vacuum with a magnetic stirring bar for an hour before a nitrogen atmosphere was established. According to the general procedure for acid chloride acylation, pyridine (3.73 mL, 46.4 mmol, 10.0 equiv.) and cyclopropanecarbonyl chloride (421 µL, 4.64 mmol, 1.0 equiv.) were added to a cold solution of the *N*-alkylated hydroxylamine in CH_2Cl_2 (21.2 mL) at 0 °C. After 1.5 h at rt, the concentrated reaction mixture was washed added sat. NH_4Cl (aq.) (100 mL) and washed with CH_2Cl_2 (3 x 100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. According to the general procedure for Boc-deprotection, the di-Boc protected compound was stirred in TFA:CH_2Cl_2 (3:7) (73.8 mL) overnight. The following day, volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (777 mg, 32.5 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1) (116 mL). After 2.5 h, Boc_2O (2.02 g, 9.72 mmol, 2.0 equiv.) was added. After 2.0 h, the reaction mixture was diluted with EtOAc (100 mL), and pH adjusted to ≈ 3-4 by the addition of 1.0 M HCl (aq., 31.0 mL), and extracted with EtOAc (2 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The Boc-protected carboxylic acid was purified by flash column chromatography

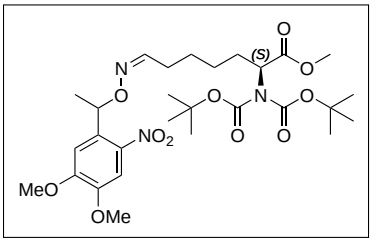
($CH_2Cl_2 \rightarrow CH_2Cl_2$:MeOH 47:3) and placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (1.67 g, 5.72 mmol, 1.1 equiv.) was added to a stirring solution of the *N*-protected amino acid in CH_2Cl_2 (24.6 mL). After 5 min, DIPEA (5.43 mL, 31.2 mmol, 6.7 equiv.) and aniline (521.0 µL, 5.72 mmol, 1.2 equiv.) were added and stirred overnight. The reaction mixture was concentrated under reduced pressure, and the residues were taken up in EtOAc (50 mL) and washed with H_2O (50 mL), sat. $NaHCO_3$ (aq., 50 mL), 1.0 M HCl (aq., 2 x 50 mL), and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 2:3 $\rightarrow$ 11:9) to provide **3.82** (2.20 g, 79%) as a green semi-sold. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.25 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3304, 2978, 2935, 2858, 1671, 1601, 1517, 1442, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO): δ 9.88 (s, 1H, C(O)N**H**Ph), 7.58–7.53 (m, 3H, Ar**H**), 7.32–7.27 (m, 3H, Ar**H**), 7.06–6.99 (m, 2H, Ar**H**, CHN**H**C(O)O), 5.55 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 4.03 (m, 1H, CH2C**H**NHC(O)O), 3.90/3.88* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.62–3.59 (m, 1H, C(O)NC**H'**H"CH_2), 3.20–3.12 (m, 1H, C(O)NCH'**H"**CH_2), 2.10–2.02 (m, 1H, $-CH_2CH_2-$C**H**C(O)), 1.62–1.50 (m, 7H, C(O)NCH'H"C**H$_2$**C**H$_2$**CHNH, ArCHC**H$_3$**), 1.37 (s, 9H, C(C**H$_3$**)$_3$); 0.80–0.61 (m, 4H, $-$C**H$_2$**C**H$_2$**$-$CHC(O)); ^{13}C-NMR (101 MHz, DMSO): δ 174.6 (observed by HMBC), 171.0, 155.4, 152.88/152.85*, 148.2, 140.9/140.8*, 138.9, 130.3, 128.7, 123.3, 119.1, 109.7, 107.38/107.35*, 78.1, 76.5, 56.25/56.20*, 56.1, 54.7, 46.6, 29.2, 28.2, 23.2, 20.65/20.58*, 10.5, 7.9, 7.7; MS (ESI) *m/z*: calcd. for $C_{30}H_{41}N_4O_9^+$ $[M + H]^+$ 601.3, found 601.8;

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)imino)hexanoate (3.83)



In an oven-dried round-bottomed flask containing a magnetic stirring bar, DIBAL-H (1 M in toluene, 5.27 mL, 5.27 mmol, 1.1 equiv.) was added dropwise over a period of 30 min to a solution of the benzyl ester **3.58** (2.23 g, 4.79 mmol, 1.0 equiv.) in Et_2O (47.9 mL) at -78 °C under a nitrogen atmosphere. The reaction mixture was stirred for additional 30 min, before quenched by the addition of H_2O (604 µL, 33.5 mmol, 7.0 equiv.) followed by 30 min stirring. The solution was dried over $MgSO_4$ and filtered through a pad of Celite® and concentrated *in vacuo*. Hydrochloride hydroxylamine **2.7** (1.34 g, 4.79 mmol, 1.0 equiv.) was added to the crude aldehyde in pyridine (29.9 mL) and stirred overnight. The reaction mixture was concentrated under reduced pressure and co-evaporated with heptane. The residues were taken up in sat. NH_4Cl (aq., 150 mL) and extracted with EtOAc (3 x 150 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 1:3 → 2:3) to provide the oxime **3.83** (5.01 g, 84%) as a neon green oil. MS (ESI) *m/z*: calcd. for $C_{27}H_{41}N_3NaO_{11}^+$ $[M + H]^+$ 606.3, found 606.9.

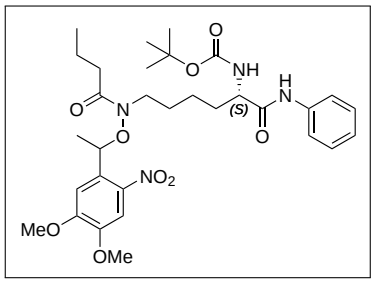
Methyl (2*S*)-2-((*tert*-butoxycarbonyl)amino)-7-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)imino)heptanoate (3.84)



In an oven-dried round-bottomed flask containing a magnetic stirring bar, DIBAL-H (1 M in toluene, 9.91 µL, 9.91 mmol, 1.1 equiv.) was added dropwise over a period of 30 min to a solution of the benzyl ester **3.66** (4.32 g, 9.01 mmol, 1.0 equiv.) in Et_2O (90.1 mL) at -78 °C under a nitrogen atmosphere. The reaction mixture was stirred for additional 30 min, before quenched by the addition of H_2O (1.14 mL, 63.1 mmol, 7.0 equiv.) followed by 30 min stirring. The solution was dried over $MgSO_4$ and filtered through a pad of Celite® and concentrated *in vacuo*. Hydrochloride hydroxylamine **2.7** (2.51 g, 9.02 mmol, 1.0 equiv.) was added to the crude aldehyde in pyridine (56.3 mL) and stirred overnight. The reaction mixture was concentrated under reduced pressure and co-evaporated with heptane.

The residues were taken up in sat. NH_4Cl (aq., 150 mL) and extracted with EtOAc (3 x 150 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 1:4 $\rightarrow$ 3:2) to provide the oxime **3.84** (4.23 g, 79%) as a neon green oil. MS (ESI) *m/z*: calcd. for $C_{28}H_{43}N_3NaO_{11}^+$ $[M + H]^+$ 620.3, found 620.9.

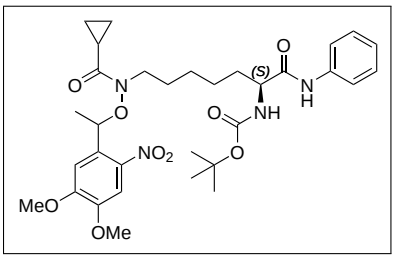
tert-*butyl* ((2*S*)-6-(*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)butyramido)-1-oxo-1-(phenylamino)hexan-2-yl)carbamate (3.85)



According to the general procedure for oxime reduction, $NaBH_3CN$ (474 mg, 7.54 mmol, 2.0 equiv.) was added to a stirring cold solution of decomposed **3.83** (2.20 g, 3.77 mmol, 1.0 equiv.) in MeOH (15.1 mL) at 0 °C and subsequently pH was adjusted to pH $<$ 3 with TFA. After 1.25 min at rt, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (aq., 100 mL) concentrated under reduced pressure. The residues were taken up in EtOAc (200 mL) and washed with sat. $NaHCO_3$ (aq.) (3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mono *N*-alkylated hydroxylamine was placed under vacuum with a magnetic stirring bar for an hour before a nitrogen atmosphere was established. According to the general procedure for acid chloride acylation, pyridine (3.04 mL, 37.7 mmol, 10.0 equiv.) and butyryl chloride (392 µL, 3.77 mmol, 1.0 equiv.) was added to a cold solution of the *N*-alkylated hydroxylamine in CH_2Cl_2 (17.2 mL) at 0 °C. After 1 h, the concentrated reaction mixture was washed added sat. NH_4Cl (aq.) (200 mL) and washed with CH_2Cl_2 (3 x 200 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. According to the general procedure for Boc-deprotection, the di-Boc protected compound was stirred in TFA:CH_2Cl_2 (3:7) (59.9 mL) for 3.0 h before volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (632 mg, 26.4 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1) (94.2 mL). The following day, Boc_2O (1.65 g, 7.54 mmol, 2.0 equiv.) was added. After 2.5 h, the reaction mixture was diluted with EtOAc, and pH adjusted to $\approx$ 3-4 by the addition of 1.0 M HCl (aq., 13.0 mL), and extracted with EtOAc (2 x 200 mL). The combined

organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The Boc-protected carboxylic acid was purified by flash column chromatography (CH_2Cl_2:MeOH 95:5 → 9:1) and placed under vacuum for a couple of hours before a nitrogen atmosphere was introduced. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (529 mg, 1.65 mmol, 1.0 equiv.) was added to a stirring solution of the *N*-protected amino acid (892 mg, 1.65 mmol, 1.0 equiv.) in CH_2Cl_2 (7.8 mL). After 10 min, DIPEA (1.72 mL, 9.88 mmol, 6.0 equiv.) and aniline (165.1 µL, 1.81 mmol, 1.1 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (50 mL) and washed with H_2O (50 mL), sat. $NaHCO_3$ (aq., 50 mL), 1.0 M HCl (aq., 2 x 50 mL), and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 3:7 → 11:9) to provide **3.85** (572 mg, 28%) as a neon yellow oil. A trace amount of EtOAc and CH_2Cl_2 were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.15 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3306, 2973, 2934, 2871, 1667, 1600, 1517, 1334, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90/9.89* (s, 1H, C(O)N**H**Ph), 7.58–7.52 (m, 3H, Ar**H**), 7.31–7.27 (m, 3H, Ar**H**), 7.05–7.02 (m, 1H, Ar**H**), 6.92 (m, 1H, CHN**H**C(O)O), 5.45/5.44* (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 4.03–3.96 (overlap with EtOAc) (m, 1H, CH_2C**H**NHC(O)O), 3.92/3.91* (s, 3H, OC**H$_3$**), 3.85/3.84* (s, 3H, OC**H$_3$**), 3.57–3.48 (m, 1H, C(O)NC**H'**H"CH_2), 3.09–2.97 (m, 1H, C(O)NCH'**H"**CH_2), 2.26–2.22 (m, 2H, CH_3CH_2C**H$_2$**C(O)N), 1.59–1.14 (m, 20H, CH_3C**H$_2$**CH_2, C**H$_2$**C**H$_2$**C**H$_2$**CHNH, C(C**H$_3$**)$_3$, ArCHC**H$_3$**), 0.80/0.79* (t, *J* = 7.4 Hz, 3H, C**H$_3$**CH_2); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.0 (observed by HMBC), 171.7, 155.9, 153.3, 148.6, 141.3/141.2*, 139.5, 131.2, 129.1, 123.6, 119.6, 110.2/110.1*, 107.8, 78.5, 77.5, 56.7 56.57/56.56*, 55.4 46.8, 34.3, 32.0, 28.6, 23.3/23.2*, 21.30/21.27*, 21.22, 18.1, 14.0; MS (ESI) *m/z*: calcd. for $C_{31}H_{45}N_4O_9^+$ $[M + H]^+$ 617.3, found 617.9; HRMS (ESI) *m/z*: calcd. for $C_{31}H_{45}N_4O_9^+$ $[M + H]^+$ 617.3181, found 617.3208.

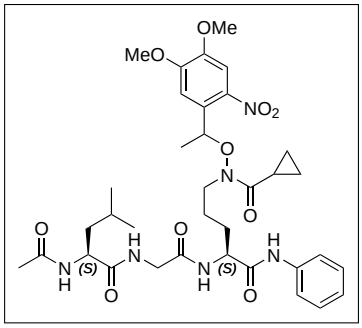
tert-Butyl ((2*S*)-7-(*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)cyclopropanecarboxamido)-1-oxo-1-(phenylamino)heptan-2-yl)carbamate (3.86)



According to the general procedure for oxime reduction, $NaBH_3CN$ (890 mg, 14.2 mmol, 2.0 equiv.) was added to a stirring cold solution of decomposed **3.84** (4.23 g, 7.1 mmol, 1.0 equiv.) in MeOH (28.3 mL) at 0 °C and subsequently pH was adjusted to pH $<$ 3 with TFA. After 20 min at rt, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (aq., 100 mL) concentrated under reduced pressure. The residues were taken up in EtOAc (200 mL) and washed with sat. $NaHCO_3$ (aq.) (3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mono *N*-alkylated hydroxylamine was placed under vacuum with a magnetic stirring bar for an hour before a nitrogen atmosphere was established. According to the general procedure for acid chloride acylation, pyridine (5.70 mL, 70.8 mmol, 10.0 equiv.) and cyclopropanecarbonyl chloride (643 µL, 7.08 mmol, 1.0 equiv.) was added to a cold solution of the *N*-alkylated hydroxylamine in CH_2Cl_2 (32.4 mL) at 0 °Cand stirred overnight at rt. The following day, the concentrated reaction mixture was washed added sat. NH_4Cl (aq.) (200 mL) and washed with CH_2Cl_2 (3 x 200 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. According to the general procedure for Boc-deprotection, the di-Boc protected compound was stirred in TFA:CH_2Cl_2 (3:7) (112.7 mL) for 2.5 h before volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (1.19 g, 49.6 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1) (177.0 mL). After 2.5 h, Boc_2O (3.09 g, 14.2 mmol, 2.0 equiv.) was added. After 2 h, full conversion was not observed, and therefore additional Boc_2O (500 mg, 2.29 mmol, 0.3 equiv.) was added and stirred overnight. The reaction mixture was diluted with EtOAc, and pH adjusted to $\approx$ 3-4 by the addition of 1.0 M HCl (aq.), and extracted with EtOAc (2 x 200 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The Boc-protected carboxylic acid was purified by flash column chromatography (CH_2Cl_2:MeOH 95:5 $\rightarrow$ 93:7) and placed under vacuum for a couple of hours before a nitrogen atmosphere was introduced. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (1.16 g, 3.61 mmol, 1.0 equiv.) was added to a stir-

ring solution of the *N*-protected amino acid (2.00 g, 3.61 mmol, 1.0 equiv.) in CH_2Cl_2 (17.1 mL). After 10 min, DIPEA (3.77 mL, 21.7 mmol, 6.0 equiv.) and aniline (362 µL, 3.89 mmol, 1.1 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (50 mL) and washed with H_2O (50 mL), sat. $NaHCO_3$ (aq., 50 mL), 1.0 M HCl (aq., 2 x 50 mL), and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 2:3 $\rightarrow$ 1:1) to provide **3.86** (696 mg, 18%) as a pale green foam. A trace amount of impurities and CH_2Cl_2 were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.21 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3305, 2978, 2935, 2859, 1667, 1600, 1518, 1441, 1366; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90 (s, 1H, C(O)N**H**Ph), 7.59–7.53 (m, 3H, Ar**H**), 7.32–7.26 (m, 3H, Ar**H**), 7.05–6.95 (m, 2H, Ar**H**, CHN**H**C(O)O), 5.53 (q, *J* = 6.5 Hz, 1H, ArC**H**CH_3), 4.04–3.98 (m, 1H, CH'H''C**H**NHC(O)O), 3.91/3.90* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.58–3.51 (m, 1H, C(O)NC**H'**H''CH_2), 3.14–3.04 (m, 1H, C(O)NCH'**H''**CH_2), 2.10–2.04 (m, 1H, $-CH_2CH_2-$C**H**C(O)), 1.63–1.04 (m, 20H, NCH_2C**H$_2$**C**H$_2$**C**H$_2$**C**H'H''**, C(C**H$_3$**)$_3$, ArCHC**H$_3$**), 0.82–0.63 (m, 4H, $-$C**H$_2$**C**H$_2$** – CHC(O)); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.4 (observed by HMBC), 171.4, 155.5, 152.8, 148.2, 140.97/140.95* (observed by HMBC), 139.3/139.0*, 130.4 (observed by HMBC), 128.7/128.6*, 123.2/123.0*, 119.2/118.9*, 109.8, 107.3, 78.0, 77.1, 56.3, 56.1, 55.0, 47.3 (observed by HSQC), 31.8, 28.2, 26.3, 25.8, 25.3, 20.1, 10.5, 7.9, 7.6; MS (ESI) *m/z*: calcd. for $C_{32}H_{45}N_4O_9^+$ $[M + H]^+$ 629.3, found 629.9; HRMS (ESI) *m/z*: calcd. for $C_{32}H_{45}N_4O_9^+$ $[M + H]^+$ 629.3181, found 629.3196.

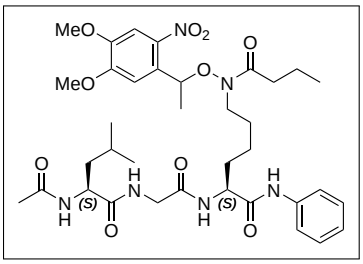
_N_-((_S_)-4-(2-((_S_)-2-acetamido-4-methylpentanamido)acetamido)-5-oxo-5-(phenylamino)pentyl)-_N_-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)cyclopropanecarboxamide (3.87)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.82** (665 mg, 1.11 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (17.6 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (462 mg, 1.44 mmol, 1.3 equiv.) and NEM (974 µL, 7.75 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (306 mg, 1.33 mmol, 1.2 equiv.) in DMF (2.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (9.1 mL) was added and stirred overnight. Incomplete conversion was observed, and therefore a new batch of acetyl-*L*-leucylglycine **3.37**, TBTU, and NEM were prepared in DMF (2.0 mL) and after stirring for 15 min added to the reaction mixture followed by stirring overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photoprotected tripeptide **3.87** was isolated by flash column chromatography (CH_2Cl_2:MeOH 99:1 $\rightarrow$ 23:2) as a light green semi-solid (500 mg, 84%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.20 (CH_2Cl_2:MeOH (19:1); UV); IR (neat) ν (cm^{-1}): 3304, 2978, 2935, 2858, 1671, 1601, 1517, 1442, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.86/9.85* (s, 1H, C(O)N**H**Ph), 8.25 (t, *J* = 5.6 Hz, 1H, C(O)N**H**CH_2C(O)), 8.06 (d, *J* = 7.4 Hz, 1H, CH_3C(O)N**H**CH), 8.01–7.97 (m, 1H, C(O)N**H**CHC(O)NHPh), 7.61–7.58 (m, 2H, Ar**H**), 7.53/7.52* (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.55 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 4.38–4.31 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, H_3C(O)NHC**H**), 3.89–3.85 (m, 6H, 2 x OC**H$_3$**), 3.75–3.56 (m, 3H, C(O)NHC**H$_2$**C(O), CH_2C**H'**H''NC(O)), 3.23–

3.07 (m, 1H, $CH_2CH'\mathbf{H''}NC(O)$), 2.11–2.02 (m, 1H, $NC(O)C\mathbf{H}-CH_2CH_2-$), 1.830/1.827* (s, 3H, $C\mathbf{H_3}C(O)NH$), 1.65–1.40 (m, 10H, $CH_3C(O)NHCHC\mathbf{H_2}C\mathbf{H}$, $NHCHC\mathbf{H_2}C\mathbf{H_2}CH_2$, $ArCHC\mathbf{H_3}$), 0.88–0.63 (m, 10H, $NC(O)CH-C\mathbf{H_2}C\mathbf{H_2}-$, $CH(C\mathbf{H_3})_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.5, 172.85/172.82*, 170.18/170.16*, 169.6, 168.8, 152.88/152.85*, 148.2, 140.8, 138.7, 130.2, 128.7, 123.5, 119.3, 109.7, 107.4, 77.1, 56.3/56.2*, 56.1, 53.1, 51.4, 46.5 (observed by HSQC) 42.0, 40.6, 29.3/29.2*, 24.1, 23.1/23.0*, 22.9, 22.4, 21.6, 20.65/20.59*, 10.5, 7.9, 7.7; MS (ESI) *m/z*: calcd. for $C_{35}H_{49}N_6O_{10}^+$ $[M + H]^+$ 713.4, found 713.8; HRMS (ESI) *m/z*: calcd. for $C_{35}H_{49}N_6O_{10}^+$ $[M + H]^+$ 713.3505, found 713.3516.

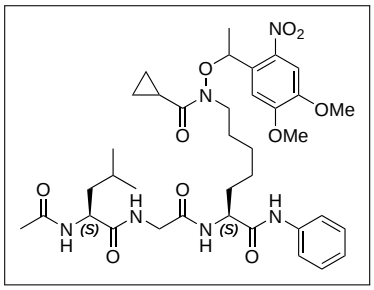
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-6-(*N*-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)butyramido)-*N*-phenylhexanamide (3.88)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.85** (519 mg, 0.84 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (13.4 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (351 mg, 1.09 mmol, 1.3 equiv.) and NEM (741 µL, 5.89 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (233 mg, 1.01 mmol, 1.2 equiv.) in DMF (2.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (6.4 mL) was added and stirred overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.89** was isolated by flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH 19:1) as a pale green foam (502 mg, 82%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.24 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.88/9.87* (s, 1H, $C(O)N\mathbf{H}Ph$), 8.28–8.24 (m, 1H, $C(O)N\mathbf{H}CH_2C(O)$), 8.07 (d, *J* =

7.4 Hz, 1H, $CH_3C(O)N\mathbf{H}CH$), 7.94/7.92* (d, J = 5.1 Hz, 1H, $C(O)N\mathbf{H}CHC(O)NHPh$), 7.60 (m, 2H, Ar**H**), 7.52 (s, 1H, Ar**H**), 7.31–7.27 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.47–5.41 (m, 1H, $ArC\mathbf{H}CH_3$), 4.35–4.28 (m, 1H, $C(O)NHC\mathbf{H}C(O)NHPh$), 4.24–4.19 (m, 1H, $H_3C(O)NHC\mathbf{H}$), 3.92/3.90 (s, 3H, $OC\mathbf{H_3}$), 3.845/3.843* (s, 3H, $OC\mathbf{H_3}$), 3.76–3.65 (m, 2H, $C(O)NHC\mathbf{H_2}C(O)$) 3.56–3.48 (m, 1H, $CH_2C\mathbf{H'}H''NC(O)$), 3.06–2.99 (m, 1H, $CH_2CH'\mathbf{H''}NC(O)$), 2.25–2.22 (m, 2H, $NC(O)C\mathbf{H_2}CH_2CH_3$), 1.833/1.827* (s, 3H, $C\mathbf{H_3}C(O)NH$), 1.66–1.37 (m, 12H, $CH_3C(O)NHCHC\mathbf{H_2}C\mathbf{H}$, $NHCHC\mathbf{H_2}CH_2C\mathbf{H_2}CH'H''$, $ArCHC\mathbf{H_3}$, $NC(O)CH_2C\mathbf{H_2}CH_3$), 1.23–1.12 (m, 2H, $NHCHCH_2C\mathbf{H_2}CH_2$), 0.88–0.77 (m, 9H, $CH(C\mathbf{H_3})_2$, $NC(O)CH_2CH_2C\mathbf{H_3}$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.0, 172.8, 170.39/170.36*, 169.6, 168.8, 152.8, 148.2, 140.8, 138.8, 130.6, 128.7, 123.4, 119.3, 109.7, 107.3, 77.0, 56.2, 56.1, 53.3/53.2*, 51.4, 46.5, 42.0, 40.5, 33.8, 31.6, 26.1, 24.1, 22.9, 22.5, 22.4, 21.6, 20.80/20.77*, 17.6, 13.6; MS (ESI) m/z: calcd. for $C_{36}H_{53}N_6O_{10}^+$ $[M + H]^+$ 729.4, found 729.8.

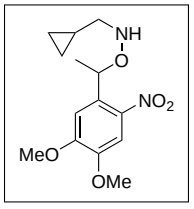
N-((*S*)-6-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-7-oxo-7-(phenylamino)heptyl)-N-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)cyclopropanecarboxamide (3.89)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.86** (685 g, 1.09 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (17.4 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours, before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (455 g, 1.42 mmol, 1.3 equiv.) and NEM (959 µL, 7.62 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (301 mg, 1.30 mmol, 1.2 equiv.) in DMF (2.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (8.9 mL) was added and stirred overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.89** was isolated by flash column chro-

matography (CH_2Cl_2 → CH_2Cl_2:MeOH 93:7) as a pale green foam (663 mg, 82%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.21 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.87 (s, 1H, C(O)N**H**Ph), 8.30–8.26 (m, 1H, C(O)N**H**CH_2C(O)), 8.08 (d, *J* = 7.1 Hz, 1H, CH_3C(O)N**H**CH), 7.91 (d, *J* = 7.8 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.61 (d, *J* = 8.2 Hz, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.06–7.03 (m, 1H, Ar**H**), 5.53 (q, *J* = 6.5 Hz, 1H, ArC**H**CH_3), 4.35–4.30 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.21 (m, 1H, CH_3C(O)NHC**H**), 3.91/3.90* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.76–3.65 (m, 2H, C(O)NHC**H$_2$**C(O)), 3.58–3.51 (m, 1H, CH_2C**H'**H''NC(O)), 3.14–3.04 (m, 1H, CH_2CH'**H''**NC(O)), 2.11–2.03 (m, 1H, −CH_2CH_2−C**H**C(O)N), 1.83 (m, 3H, C**H$_3$**C(O)NH), 1.67–1.09 (m, 14H, CH_3C(O)NHCHC**H$_2$**C**H**, NHCHC**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**, ArCHC**H$_3$**), 0.88–0.64 (m, 10H, CH(C**H$_3$**)$_2$, −C**H$_2$**C**H$_2$**−CHC(O)N); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.5, 172.8, 170.51/170.48*, 169.6, 168.8, 152.8, 148.2, 141.0, 138.8, 130.3, 128.7, 123.4, 119.3, 109.8, 107.3, 77.2, 56.3, 56.1, 53.4, 51.4, 47.0, 42.1, 40.5, 31.8, 26.3, 25.9, 25.10/25.06*, 24.1, 22.9, 22.4, 21.6, 20.6 10.5, 7.9, 7.6; MS (ESI) *m/z*: calcd. for $C_{36}H_{51}N_6O_{10}^+$ [M + H]$^+$ 727.4, found 727.8.

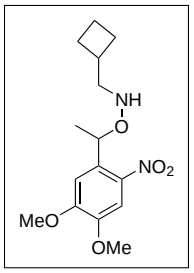
N-(Cyclopropylmethyl)-*O*-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)hydroxylamine (3.90)



According to the general procedure for reductive alkylation, cyclopropanecarboxaldehyde (670 µL, 8.97 mmol, 1.0 equiv.) and **2.7** (2.00 g, 8.97 mmol, 1 equiv.) in dry EtOH (36.0 mL) at rt afforded the corresponding oxime (1 h) that subsequently was reduced by the addition of $NaBH_3CN$ (733 mg, 11.7 mmol, 1.3 equiv.) (4 h). The residues were purified by a basic work-up and flash chromatography (EtOAc:heptane 1:3) providing *N*-alkylated hydroxylamine **3.90** (2.27 g, 85%) as a neon-green oil. A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. R_f = 0.21 (EtOAc:heptane (1:3); UV); IR (neat) ν (cm^{-1}): 3278, 3080, 3001, 2978, 2935, 2913, 2849, 1580, 1513, 1461, 1332, 1267; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.54 (s, 1H, Ar**H**), 7.17 (s, 1H, Ar**H**), 6.46 (t, *J* = 6.3 Hz, 1H, $CHCH_2$N**H**O), 5.22 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 3.94 (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 2.59 (t, *J* = 6.3

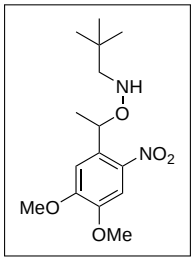
Hz, 2H, CHC**H$_2$**NH), 1.38 (d, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**), 0.88–0.78 (m, 1H, CH_2C**H**NH), 0.43–0.33 (m, 2H, −C**H'**H''C**H'**H'' −), 0.16–0.04 (m, 2H, −CH'**H''**CH'**H''** −); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 153.4, 147.2, 140.1, 135.7, 108.9, 107.3, 75.6, 56.2, 56.04, 56.01, 21.5, 9.0, 3.4, 3.1; MS (ESI) *m/z*: calcd. for $C_{14}H_{21}N_2O_5^+$ $[M + H]^+$ 297.1, found 297.0.

N-(cyclobutylmethyl)-*O*-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)hydroxylamine (3.91)



According to the general procedure for reductive alkylation, cyclobutanecarboxaldehyde (323 µL, 3.59 mmol, 1.0 equiv.) and **2.7** (1.00 g, 3.59 mmol, 1 equiv.) in dry EtOH (14.4 mL) at rt afforded the corresponding oxime (3 h) that subsequently was reduced by the addition of $NaBH_3CN$ (226 mg, 3.59 mmol, 1.0 equiv.) (1 h). The residues were purified by basic work-up and flash chromatography (EtOAc:heptane 2:3) providing *N*-alkylated hydroxylamine **3.91** (683 mg, 61%) as a neon-green oil. R_f = 0.21 (EtOAc:heptane (1:3); UV); IR (neat) ν (cm^{-1}): 3283, 2965, 2934, 2859, 1578, 1508, 1454, 1324, 1270; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.52 (s, 1H, Ar**H**), 7.14 (s, 1H, Ar**H**), 6.45 (t, *J* = 5.3 Hz, 1H, CHCH'H''N**H**), 5.09 (q, *J* = 6.4 Hz, 1H, ArC**H**CH_3), 3.93 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 2.85–2.79 (m, 1H, CHC**H'**H''NH), 2.73–2.66 (m, 1H, CHCH'**H''**NH), 2.40 (sept, *J* = 7.6 Hz, 1H, C**H**CH'H''NH), 1.98–1.89 (m, 2H, −C**H'''**H''''CH_2C**H''**H'''' −), 1.85–1.71 (m, 2H, − CH'''H''''C**H2**CH'''H''''-), 1.65–1.54 (m, 2H, −CH'''**H''''**CH_2CH'''**H''''** −), 1.35 (d, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 153.3, 147.2, 140.1, 135.6, 108.9, 107.2, 75.6, 57.2, 56.02, 56.01, 33.0, 26.03, 26.01, 21.4, 18.2; MS (ESI) *m/z*: calcd. for $C_{15}H_{23}N_2O_5^+$ $[M + H]^+$ 311.2, found 311.3.

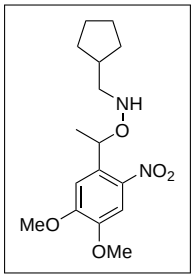
O-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)-*N*-neopentylhydroxylamine (3.92)



According to the general procedure for reductive alkylation, trimethylacetaldehyde (546 µL, 5.02 mmol, 1 equiv.) and **2.7** (1.40 g, 5.02 mmol, 1 equiv.) in dry EtOH (20.1 mL) at rt afforded the corresponding oxime (7 h) that subsequently was reduced by the addition of $NaBH_3CN$ (410 mg, 6.53 mmol, 1.3 equiv.) (1.5 g). The residues were purified by basic work-up and flash chromatography (EtOAc:heptane 3:7) providing *N*-alkylated hydroxylamine **3.92** (1.16 g, 74%) as a light yellow semi solid. R_f = 0.35 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3272, 3009, 2959, 2937, 2900, 2867, 1580, 1514, 1457, 1334,

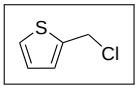
1268; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.53 (s, 1H, Ar**H**), 7.15 (s, 1H, Ar**H**), 6.47 (t, J = 6.8 Hz, 1H, CCH'H''N**H**), 5.17 (q, J = 6.4 Hz, 1H, ArC**H**CH$_3$), 3.93 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 2.64–2.52 (overlap with DMSO-d_6) (m, 2H, CC**H'H''**NH), 1.36 (d, J = 6.4 Hz, 3H, ArCHC**H$_3$**), 0.79 (s, 9H, C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 153.8, 147.7, 140.7, 135.8, 109.5, 107.7, 75.5, 63.3, 56.51, 56.47, 31.2, 28.4, 21.9; MS (ESI) *m/z*: calcd. for $C_{15}H_{25}N_2O_5^+$ $[M + H]^+$ 313.2, found 313.1.

N-(cyclopentylmethyl)-*O*-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)hydroxylamine (3.93)



According to the general procedure for reductive alkylation, cyclopentanecarboxaldehyde (307 µL, 2.87 mmol, 1 equiv.) and **2.7** (0.80 g, 2.87 mmol, 1.0 equiv.) in dry EtOH (11.5 mL) at rt afforded the corresponding oxime (4.5 h) that subsequently was reduced by the addition of $NaBH_3CN$ (235 mg, 3.73 mmol, 1.3 equiv.) (1.5 h). The residues were purified by basic work-up and flash chromatography (EtOAc:heptane 1:4) providing *N*-alkylated hydroxylamine **3.93** (811 mg, 87%) as a pale green semi solid. R_f = 0.19 (EtOAc:heptane (1:4); UV); IR (neat) ν (cm^{-1}): 3275, 3089, 2956, 2937, 2865, 1578, 1507, 1462, 1454, 1333, 1270; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.53 (s, 1H, Ar**H**), 7.15 (s, 1H, Ar**H**), 6.47 (t, J = 6.0 Hz, 1H, CHCH$_2$N**H**), 5.14 (q, J = 6.4 Hz, 1H, ArC**H**CH$_3$), 3.93 (s, 3H, OC**H$_3$**), 3.84 (s, 3H, OC**H$_3$**), 2.74–2.68 (m, 1H, CHC**H'**H''NH), 2.61–2.54 (m, 1H, CHCH'**H''**HNH), 1.95 (m, 1H, HNCH'H''C**H**), 1.63–1.56 (m, 2H, −C**H'''**H''''CH$_2$CH$_2$C**H'''**H'''' −), 1.52–1.39 (m, 4H, − CH'''H''''C**H2**C**H2**CH'''H'''' −), 1.37 (d, J = 6.4 Hz, 3H, ArCHC**H$_3$**), 1.14–1.05 (m, 2H, −CH'''**H''''**CH$_2$CH$_2$C**H''''** −); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 153.3, 147.2, 140.2, 135.6, 108.9, 107.2, 75.5, 56.8, 56.0 (two overlapping signals), 37.0, 30.43, 30.37, 24.73, 24.66, 21.4; MS (ESI) *m/z*: calcd. for $C_{16}H_{25}N_2O_5^+$ $[M + H]^+$ 325.2, found 325.2.

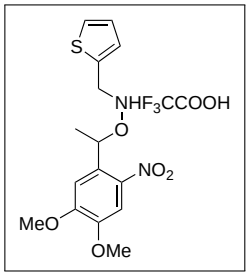
2-(Chloromethyl)thiophene (3.95)[198]



In an oven-dried round-bottomed flask containing a magnetic stirring bar, $SOCl_2$ (1.78 mL, 24.42 mmol, 2.0 equiv.) was slowly added to a cold solution of 2-thiophenemethanol (2.49 mL, 12.21 mmol, 1.0 equiv.) in CH_2Cl_2 (48.8 mL) at 0 °C and stirred overnight at rt under a nitrogen atmosphere. The following day, the reaction mixture was concentrated under reduced pressure. The residues were dissolved

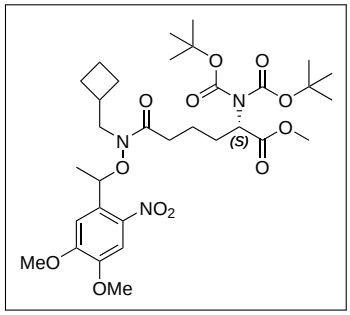
in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (aq., 3 x 100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The title compound (3.0536 g, 58%) was used without further purification. Spectral data in correspondence with reported literature values.[198] ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.32 (dd, *J* = 5.1 Hz, 1.2, 1H, Ar**H**), 7.10–7.08 (m, 1H, Ar**H**), 6.96 (dd, *J* = 5.1 Hz, 3.5, 1H, Ar**H**), 4.82 (s, 1H, ArC**H$_2$**Cl).

_O_-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)-_N_-(thiophen-2-ylmethyl)hydroxylamine TFA salt 3.96



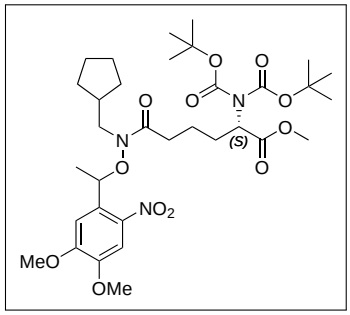
According to the general procedure for alkylation procedure, NaH (60% in oil) (596 mg, 14.9 mmol, 1.7 equiv.) was added to a cold solution of *N*-Boc protected hydroxylamine **2.6** (3.00 g, 8.76 mmol, 1.0 equiv.) in MeCN (43.7 mL) at 0 °C and stirred overnight at rt. The reaction mixture was quenched by the addition of H_2O (50 mL), and extracted with CH_2Cl_2 (3 x 150 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The *N*-Alkylated *N*-Boc-hydroxylamine was purified flash chromatography (EtOAc:heptane 1:4 → 2:3) to afforded 2.71 g (71%) of thiophene-2-methyl *N*-Boc hydroxylamine. According to the general procedure for Boc-deprotection, the alkylated Boc-hydroxylamine (2.58 g, 5.88 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (58.8 mL) for 3 h before volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2 to provide the TFA salt in quant. yield (2.6598 g). R_f = 0.38 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 2951, 2943, 2943, 2915, 2848, 1664, 1575, 1515, 1465, 1364, 1141; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.40 (br. s, 2H, ArCH$_2$N**H$_2$**), 7.54 (s, 1H, Ar**H**), 7.43–7.41 (dd, *J* = 5.0, 1.3 Hz, 1H, Ar**H**), 7.13 (s, 1H, Ar**H**), 7.00–6.95 (m, 2H, Ar**H**), 5.21 (q, *J* = 6.4 Hz, 1H, ArC**H**CH$_3$), 4.14 (q, *J* = 14.7, 5.3 Hz, 2H, ArC**H$_2$**NH2), 3.91 (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 1.36 (m, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 153.4, 147.4, 140.2, 140.0, 134.7, 126.6, 126.5, 125.7, 108.9, 107.3, 76.1, 56.04, 56.01, 49.7, 21.5; MS (ESI) *m/z*: calcd. for $C_{15}H_{19}N_2O_5S^+$ [M - TFA + H]$^+$ 339.1, found 399.5.

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-((cyclobutylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-6-oxohexanoate (3.98)



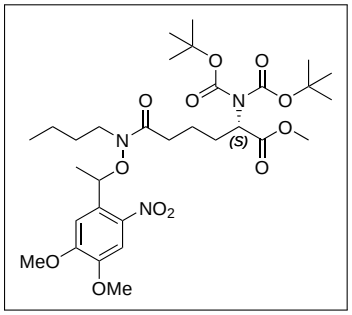
According to the general procedure for BTC acylation, 2,4,6-collidine (3.01 mL, 22.8 mmol, 7.0 equiv.) was dropwise added to a solution of BTC (676 mg, 2.28 mmol, 0.7 equiv.) and the carboxylic acid **3.57** (1.47 g, 3.91 mmol, 1.2 equiv.) in dry THF (61.4 mL) followed by the addition of the *N*-alkylated hydroxylamine **3.91** (1.01 g, 3.26 mmol, 1.0 equiv.) in THF (20.0 mL) and stirred overnight. The organic phase was washed with sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 2 x 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 1:9 $\rightarrow$ 7:13) to afford the hydroxamate **3.98** (756 mg, 35%) as a green oil. A trace amount of CH_2Cl_2 and impurities were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.26 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 2978, 2937, 2871, 1742, 1699, 1668, 1520, 1457, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.53 (s, 1H, Ar**H**), 7.28/7.27* (s, 1H, Ar**H**), 5.46–5.40 (m, 1H, ArC**H**CH_3), 4.84–4.75 (m, 1H, CH'H''C**H**N), 3.93 (s, 3H, OC**H$_3$**), 3.86 (s, 3H, OC**H$_3$**), 3.64–3.56 (m, 4H, CHC**H'**H''NC(O), C(O)OC**H$_3$**), 3.17–3.08 (m, 1H, CHCH'**H''**NC(O)), 2.52–2.48 (overlap with DMSO-d_6) (m, 1H, C**H**CH_2HNC(O)), 2.35–2.30 (m, 2H, C(O)C**H$_2$**CH_2CH'H''CHN), 1.99–1.55 (m, 9H, – C**H$_2$**C**H$_2$**C**H$_2$** – CH, ArCHC**H$_3$**), 1.48–1.34 (m, 20H, C(O)CH_2C**H$_2$**CH'H''CH, 2 x C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.1, 170.51/170.48*, 152.8, 151.6, 148.2, 140.8/140.7*, 130.5, 109.8, 107.3, 82.57/82.54*, 77.1, 57.3, 56.2, 56.1, 52.0, 51.5, 33.2, 31.6, 28.76/28.66*, 27.48/27.45*, 25.89/25.87*, 25.53/25.51*, 21.6, 20.7, 17.9; MS (ESI) *m/z*: calcd. for $C_{22}H_{34}N_3O_8^+$ [M - 2 x Boc + 3 x H]$^+$ 468.2, found 468.8.

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-((cyclopentylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-6-oxohexanoate (3.99)



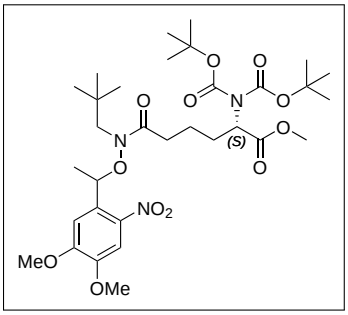
According to the general procedure for BTC acylation, 2,4,6-collidine (2.94 mL, 22.22 mmol, 7.0 equiv.) was dropwise added to a solution of BTC (660 mg, 2.22 mmol, 0.7 equiv.) and the carboxylic acid **3.57**(1.43 g, 3.84 mmol, 1.2 equiv.) in dry THF (59.4 mL) followed by the addition of the *N*-alkylated hydroxylamine **3.93** (1.03 g, 3.18 mmol, 1 equiv.) in THF (20.0 mL) and stirred overnight. The organic phase was washed with sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 2 x 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 1:9 $\rightarrow$ 9:11) to afford the hydroxamate **3.99** (743 mg, 34%) as a green oil. A trace amount of CH_2Cl_2 and impurities were observed by ^{1}H-NMR . A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.21 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 2977, 2949, 2870, 1744, 1699, 1519, 1456, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.53 (s, 1H, Ar**H**), 7.30/7.29* (s, 1H, Ar**H**), 5.47–5.41 (m, 1H, ArC**H**CH_3), 4.84–4.77 (m, 1H, CH_2C**H**N), 3.93 (s, 3H, OC$\mathbf{H_3}$), 3.86 (s, 3H, OC$\mathbf{H_3}$), 3.63 (s, 3H, C(O)OC$\mathbf{H_3}$), 3.53–3.46 (m, 1H, CHC**H'**H''NC(O)), 3.00–2.91 (m, 1H, CHCH'**H''**NC(O)), 2.37–2.33 (m, 2H, NC(O)C$\mathbf{H_2}CH_2$), 2.15–2.08 (m, 1H, C**H**CH_2HNC(O)), 1.99–1.89 (m, 1H, CH_2CH_2C**H'''**H'''CHN), 1.84–1.75 (m, 1H, CH_2CH_2CH'''**H''''**CHN), 1.61–1.41 (m, 29H, –C$\mathbf{H_2}$C**H''''**H''''C**H''''**H''''C$\mathbf{H_2}$ – CH, 2 x C(C$\mathbf{H_3}$)$_3$, ArCHC$\mathbf{H_3}$, NC(O)CH_2C-$\mathbf{H_2}$CHH''H'''CHN), 1.11–1.00 (m, 2H, CH–CH_2CH''''**H''''**CH''''**H''''**$CH_2$$^-$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.4, 170.5, 152.8, 151.63/151.60*, 148.2, 140.8/140.7*, 130.5, 109.8, 107.3, 82.57/82.53*, 77.5, 57.3, 56.2, 56.1, 52.0, 50.5 (observed by HSQC) 37.5, 31.5, 29.71/29.68*, 29.6, 28.8, 27.5, 24.4, 20.9, 20.8; MS (ESI) *m/z*: calcd. for $C_{23}H_{36}N_3O_8^+$ [M - 2 x Boc + 3 x H]$^+$ 482.2, found 482.8.

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-(butyl(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-6-oxohexanoate (3.101)



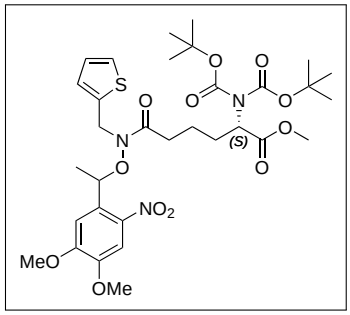
According to the general procedure for BTC acylation, 2,4,6-collidine (2.91 mL, 22.03 mmol, 7.0 equiv.) was dropwise added to a solution of BTC (654 mg, 2.20 mmol, 0.7 equiv.) and the carboxylic acid **3.57** (1.42 g, 3.78 mmol, 1.2 equiv.) in dry THF (58.7 mL) followed by the addition of the *N*-alkylated hydroxylamine **3.92** (939 mg, 3.15 mmol, 1.0 equiv.) in THF (20.0 mL) and stirred overnight. The organic phase was washed with sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 2 x 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 1:9 $\rightarrow$ 1:1) to afford the hydroxamate **3.101** (498 mg, 24%) as a brown oil. A trace amount of CH_2Cl_2 and impurities were observed by ^{1}H-NMR . A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.44 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 2798, 2955, 2936, 2873, 1744, 1699, 1667, 1520, 1336, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.54 (s, 1H, Ar**H**), 7.299/7-293* (s, 1H, Ar**H**), 5.46–5.40 (m, 1H, ArC**H**CH$_3$), 4.83–4.77 (m, 1H, CH$_2$C**H**N), 3.93 (s, 3H, OC**H$_3$**), 3.86 (s, 3H, OC**H$_3$**), 3.63 (s, 3H, C(O)OC**H$_3$**), 3.55–3.49 (m, 1H, CH$_2$C**H'**H''NC(O)), 3.03–2.93 (m, 1H, CH$_2$CH'**H''**NC(O)), 2.38–2.32 (m, 2H, NC(O)C**H$_2$**CH$_2$CH$_2$CH), 2.01–1.89 (m, 1H, NC(O)CH$_2$CH$_2$C**H'''**H''''CHN), 1.84–1.74 (m, 1H, NC(O)CH$_2$CH$_2$CH'''**H''''**CHN), 1.61–1.23 (m, 25H, 2 x C(C**H$_3$**)$_3$, ArCHC**H$_3$**, CH$_3$CH$_2$C**H$_2$**CH$_2$NC(O)CH$_2$C**H$_2$**CH'''H''''CHN), 1.15–1.02 (m, 2H, CH$_3$C**H$_2$**CH$_2$), 0.77 (m, 3H, C**H3**CH$_2$CH$_2$CH$_2$NC(O)); ^{13}C-NMR (101 MHz, DMSO-d_6): δ H'H''N**C**(O)CH$_2$ is missing, 170.5, 152.8, 151.6, 148.2, 140.7, 130.6 (observed by HMBC), 109.7, 107.3, 82.5, 77.5 (observed by HSQC), 57.3, 56.2, 56.1, 52.0, 46.3 (observed by HSQC), 31.5, 28.8, 28.4, 27.5, 20.9, 20.8/20.7*, 19.3, 13.5; MS (ESI) *m/z*: calcd. for $C_{21}H_{34}N_3O_8^+$ [M - 2 x Boc + 3 x H]$^+$ 456.2, found 456.8.

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-((1-(4,5-dimethoxy-2-nitrophenyl)-ethoxy)(neopentyl)amino)-6-oxohexanoate (3.102)



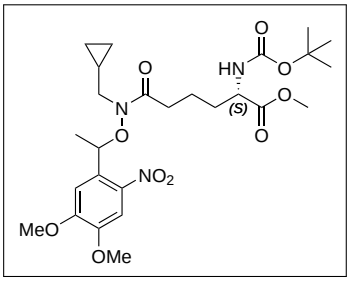
According to the general procedure for BTC acylation, 2,4,6-collidine (2.53 mL, 19.1 mmol, 7.0 equiv.) was dropwise added to a solution of BTC (565 mg, 1.90 mmol, 0.7 equiv.) and the carboxylic acid **3.57** (1.23 g, 3.27 mmol, 1.2 equiv.) in dry THF (58.0 mL) followed by the addition of the *N*-alkylated hydroxylamine **3.92** (850 mg, 2.72 mmol, 1 equiv.) in THF (20.0 mL) and stirred overnight. The organic phase was washed with sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 2 x 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 1:9 $\rightarrow$ 9:11) to afford the hydroxamate **3.102** (552 mg, 30%) as a green oil. A trace amount of CH_2Cl_2 and impurities were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.16 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 2977, 2953, 2872, 1743, 1699, 1520, 1458, 1366, 1273; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.52 (s, 1H, Ar**H**), 7.25 (s, 1H, Ar**H**), 5.54–5.48 (m, 1H, ArC**H**CH_3), 4.84–4.74 (m, 1H, CH_2C**H**N), 3.92 (s, 3H, OC$\mathbf{H_3}$), 3.86 (s, 3H, OC$\mathbf{H_3}$), 3.62 (s, 3H, C(O)OC$\mathbf{H_3}$), 3.46 (m, 1H, CC**H'**H''NC(O)), 3.07 (m, 1H, CCH'**H''**NC(O)), 2.38–2.16 (d, 2H, C(O)C$\mathbf{H_2}$$CH_2$), 2.00–1.73 (m, 2H, C(O)CH_2CH_2C$\mathbf{H_2}$CHN), 1.59 (d, J = 6.1 Hz, 3H, ArCHC$\mathbf{H_3}$), 1.48–1.35 (m, 20H, 2 x OC(C$\mathbf{H_3}$)$_3$, C(O)CH_2C$\mathbf{H_2}$$CH_2$CHN), 0.84 (m, 9H, CH_2C(C$\mathbf{H_3}$)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.1, 170.45/170.45*, 152.7, 151.63/151.60*, 148.3, 141.1/141.0*, 129.3, 110.2, 107.3, 82.5, 75.6, 57.28/57.25*, 56.14, 56.10/56.08*, 54.3 (observed by HSQC), 52.0, 33.1, 31.5, 28.2, 27.5, 27.4, 21.1, 20.4; MS (ESI) *m/z*: calcd. for $C_{21}H_{34}N_3O_8^+$ [M - 2 x Boc + 3 x H]$^+$ 456.2, found 456.8.

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-((1-(4,5-dimethoxy-2-nitrophenyl)-ethoxy)(thiophen-2-ylmethyl)amino)-6-oxohexanoate (3.103)



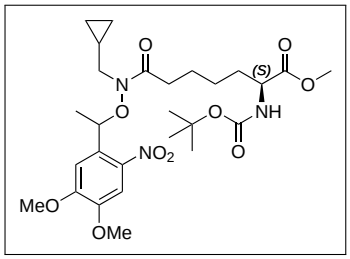
According to the general procedure for BTC acylation, 2,4,6-collidine (3.22 mL, 24.4 mmol, 8.0 equiv.) was dropwise added to a solution of BTC (634 mg, 2.14 mmol, 0.7 equiv.) and carboxylic acid **3.57** (1.37 g, 3.04 mmol, 1.2 equiv.) in dry THF (56.3 mL) followed by the addition of the *N*-alkylated hydroxylamine **3.96** (1.37 g, 3.04 mmol, 1.0 equiv.) in THF (20.0 mL) and stirred overnight. The organic phase was washed with sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 2 x 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 1:4 $\rightarrow$ 7:13) to afford the hydroxamate **3.103** (683 mg, 32%) as a brown oil. A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.19 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 2978, 2937, 2873, 1743, 1697, 1519, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.52 (s, 1H, Ar**H**), 7.38 (m, 1H, Ar**H**), 7.222/7.216* (s, 1H, Ar**H**), 6.91–6.88 (m, 1H, Ar**H**), 6.82–6.78 (m, 1H, Ar**H**), 5.58–5.52 (m, 1H, ArC**H**CH$_3$), 4.88–4.75 (m, 2H, ArC**H'**H''NC(O), C(O)CH$_2$CH$_2$CH'''H''''C**H**N), 4.52–4.45 (m, 1H, ArCH'**H''**NC(O)), 3.86 (d, 6H, 2 x OC**H$_3$**), 3.63 (s, 3H, C(O)OC**H$_3$**), 2.44–2.33 (m, 2H, NC(O)C**H$_2$**CH$_2$CH'''H''''), 1.96–1.89 (m, 1H, NC(O)CH$_2$CH$_2$C**H'''**H''''CHN), 1.84–1.74 (m, 1H, NC(O)CH$_2$CH$_2$CH'''**H''''**CHN), 1.60 (d, *J* = 6.4 Hz, 3H, ArCHC**H$_3$**), 1.48–1.37 (m, 20H, NC(O)CH$_2$C**H$_2$**CH$_2$CH$_2$NBoc$_2$, 6 x C**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.8, 170.48/170.46*, 152.9, 151.60/151.58*, 148.1, 140.4, 138.6, 130.7, 126.63/126.60*, 126.4, 125.8, 109.7, 107.4, 82.5, 78.0, 57.3, 56.1, 52.0, 46.2, 31.7/31.6*, 28.7, 27.5, 21.1, 20.6/20.5*; MS (ESI) *m/z*: calcd. for $C_{22}H_{30}N_3O_8S^+$ [M - 2 x Boc + 3 x H]$^+$ 496.2, found 496.7. HRMS (ESI) *m/z*: calcd. for $C_{32}H_{45}N_3NaO_{12}S^+$ [M + Na]$^+$ 718.2616, found 718.2626;

Methyl (2*S*)-2-((*tert*-butoxycarbonyl)amino)-6-((cyclopropylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-6-oxohexanoate (3.109)



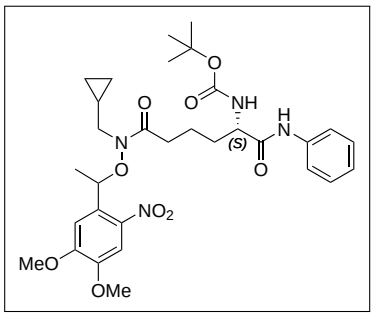
According to the general procedure for Boc-deprotection, TFA (403 µL, 5.27 mmol, 2.6 equiv.) was added to carboxylic acid **3.57** (1.41 g, 3.77 mmol, 1.3 equiv.) and stirred in CH_2Cl_2 (17.1 mL) for 3.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for BTC acylation, 2,4,6-collidine (2.68 mL, 20.3 mmol, 7.0 equiv.) was dropwise added to a solution of BTC (604 mg, 2.04 mmol, 0.7 equiv.) and the crude mono-Boc carboxylic acid in THF (50.0 mL) followed by the addition of *N*-alkylated hydroxylamine **3.90** (858 mg, 2.90 mmol, 1.0 equiv.) in THF (20.0 mL) and stirred for 3.0 h. The organic phase was washed with H_2O (100 mL), sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 3:7 $\rightarrow$ 9:11) to afford the hydroxamate **3.109** (783 mg, 49%) as a pale green oil. A trace amount of impurities, CH_2Cl_2 and, EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.51 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3346, 2976, 2936, 2853, 1743, 1710, 1663, 1517, 1440, 1335, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.54 (s, 1H, Ar**H**), 7.323/7.316* (s, 1H, Ar**H**), 7.23 (m, 1H, CHN**H**C(O)O), 5.50–5.46 (m, 1H, ArC**H**CH$_3$), 3.95–3.89 (m, 4H, OC**H$_3$**, CH$_2$CH$_2$C**H**NHC(O)O), 3.86 (s, 3H, OC**H$_3$**), 3.61 (s, 3H, C(O)OC**H$_3$**), 3.36–3.29 (overlap with H_2O) (m, 1H, $-CH_2CH_2-CHC\mathbf{H'}H''NC(O)$), 3.13–3.06 (m, 1H, $-CH_2CH_2-CHCH'\mathbf{H''}NC(O)$), 2.36–2.29 (m, 2H, C(O)C**H$_2$**CH$_2$CH$_2$CHNHBoc), 1.67–1.32 (m, 16H, C(O)CH$_2$C**H$_2$**C**H$_2$**, ArCHC**H$_3$**, OC(C**H$_3$**)$_3$), 0.99–0.85 (m, 1H, $-CH_2CH_2-C\mathbf{H}C$), 0.40–0.36 (m, 2H, $-C\mathbf{H'''}H''''C\mathbf{H'''}H''''-CH$), 0.16–0.13 (m, 2H, $-CH'''\mathbf{H''''}CH'''\mathbf{H''''}-CHCH_2NC(O)$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.6, 173.1, 155.6/155.5*, 152.8, 148.1, 140.7, 130.7, 109.8, 107.3, 78.2, 77.3, 56.2, 56.1, 53.23/53.20*, 51.7, 51.4 (observed by HSQC), 31.5/31.4*, 30.04/29.97*, 28.2, 20.81, 20.79, 9.2, 3.5, 3.2; MS (ESI) *m/z*: calcd. for $C_{26}H_{40}N_3O_{10}^+$ $[M + H]^+$ 554.3, found 554.7.

methyl (2*S*)-2-((*tert*-butoxycarbonyl)amino)-7-((cyclopropylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-7-oxoheptanoate (3.110)



According to the general procedure for Boc-deprotection, TFA (496 µL, 6.49 mmol, 2.6 equiv.) was added to the carboxylic acid **3.65** (1.80 g, 4.63 mmol, 1.3 equiv.) and stirred in CH_2Cl_2 (21.1 mL) for 3.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours, before a nitrogen atmosphere was established. According to the general procedure for BTC acylation, 2,4,6-collidine (3.30 mL, 24.9 mmol, 7.0 equiv.) was dropwise added to a solution of BTC (740 mg, 2.50 mmol, 0.7 equiv.) and the crude mono-Boc carboxylic acid in dry THF (70.0 mL) followed by the addition of *N*-alkylated hydroxylamine **3.90** (1.06 g, 3.57 mmol, 1.0 equiv.) in THF (19.1 mL) and stirred for 3.0 h. The organic phase was washed with H_2O (100 mL), sat. $NaHCO_3$ (aq., 100 mL), 1.0 M HCl (aq., 100 mL), and brine (100 mL). The residues were purified by flash column chromatography (EtOAc:heptane 1:3 → 2:3) to afford the hydroxamate **3.110** (898 mg, 44%) as a neon-green oil. A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.49 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3344, 2976, 2937, 2864, 1743, 1711, 1662, 1517, 1456, 1335, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.53 (s, 1H, Ar**H**), 7.32 (s, 1H, Ar**H**), 7.20/7.19* (d, *J* = 7.7, Hz, 1H, CHN**H**C(O)O), 5.51–5.46 (m, 1H, ArC**H**CH$_3$), 3.94–3.86 (m, 7H, 2 x OC**H$_3$**, CH_2CH_2C**H**NC(O)O), 3.61 (s, 3H, C(O)OC**H$_3$**), 3.34–3.29 (m, 1H, $-CH_2CH_2-CHC$**H'**H''NC(O)), 3.14–3.07 (m, 1H, $-CH_2CH_2-CHCH'$**H''**NC(O)), 2.31–2.28 (m, 2H, NC(O)C**H$_2$**CH_2), 1.64–1.19 (m, 16H, C(O)CH_2C**H$_2$C H$_2$**CHNHC(O)O, ArCHC**H$_3$**, C(C**H$_3$**)$_3$), 0.99–0.89 (m, 1H, $-CH_2CH_2-C$**H**CH_2), 0.40–0.36 (m, 2H, $-C$**H'''**H''''C**H'''**H''''$-CHCH_2$NC(O)), 0.16–0.13 (m, 2H, $-CH'''$**H''''**CH'''**H''''**$-CHCH_2$NC(O)); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 173.4 (observed by HMBC), 173.2, 155.5, 152.8, 148.2, 140.8, 130.4, 109.9, 107.3, 78.2, 77.2, 56.2, 56.1, 53.4, 51.7, 51.3, 31.9, 30.5, 28.2, 25.2, 23.8, 20.8, 9.2, 3.5, 3.2; MS (ESI) *m/z*: calcd. for $C_{27}H_{42}N_3O_{10}^+$ [M + H]$^+$ 567.3, found 567.7; HRMS (ESI) *m/z*: calcd. for $C_{27}H_{42}N_3O_{10}^+$ [M + H]$^+$ 568.2865, found 568.2870.

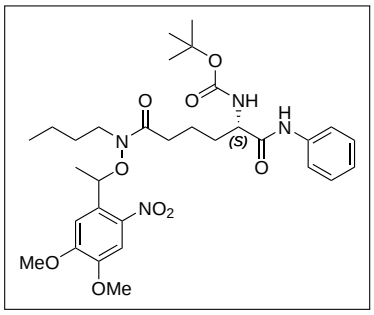
tert-Butyl ((2*S*)-6-((cyclopropylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-amino)-1,6-dioxo-1-(phenylamino)hexan-2-yl)carbamate (3.111)



According to the general procedure for LiOH hydrolysis, LiOH (96.2 mg, 4.02 mmol, 3.0 equiv.) was added to the methyl ester **3.109** (741 mg, 1.34 mmol, 1.0 equiv.) in THF:H_2O (4:1) (33.5 mL). After 2.15 h, the reaction mixture was diluted with EtOAc and acidified to pH ≈ 3-4 by the addition of 1.0 M HCl (aq., 5.4 mL) and immediately the aqueous phase was extracted with EtOAc (3 x 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo* and left under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (497 mg, 1.55 mmol, 1.0 equiv.) was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (12.6 mL). After 10 min, DIPEA (1.40 mL, 8.03 mmol, 6.0 equiv.) and aniline (134 µL, 1.47 mmol, 1.1 equiv.) were added and stirred overnight. Full conversion was not observed after 23.5 h, and therefore additionally DIPEA (699 µL, 4.02 mmol, 1.5 equiv.) was added and stirred for 15 min before TBTU (215 mg, 0.70 mmol, 0.5 equiv.) and aniline (67.1 µL, 0.74 mmol, 0.55 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), 1.0 M HCl (aq., 25 mL), and brine (25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The desired product was purified by flash column chromatography (EtOAc:heptane 3:7 → 9:11) to provide the aniline amide **3.111** (720 mg, 88%) as a light yellow foam. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.07 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3291, 2977, 2934, 2871, 1667, 1600, 1518, 1334, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92 (s, 1H, C(O)N**H**Ph), 7.60–7.53 (m, 3H, Ar**H**), 7.32–7.26 (m, 3H, Ar**H**), 7.06–6.98 (m, 2H, Ar**H**, CHN**H**C(O)O), 5.50–5.46 (m, 1H, ArC**H**CH_3), 4.09–4.02 (m, 1H, C(O)$CH_2CH_2CH_2$C**H**NHC(O)O), 3.93/3.92* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.36–3.30 (m, 1H, –CH‴'H‴"CH‴'H‴" – CHC**H**'H"N),

3.11–3.02 (m, 1H, –CHH‴'H‴"CH‴'H‴" – CHCH'**H"**N), 2.42–2.30 (m, 2H, C(O)C**H$_2$**CH$_2$), 1.68-1.48 (m, 7H, C(O)CH$_2$C**H$_2$**C**H$_2$**CH, ArCHC**H$_3$**), 1.38 (m, 9H, C(C**H$_3$**)$_3$), 0.97–0.89 (m, 9H, – CH‴'H‴"CH‴'H‴" – C**H**CH$_2$), 0.38–0.33 (m, 2H, –C**H‴'**H‴"C**H‴'**H‴"–CH), 0.16–0.10 (m, 2H, –CH‴'**H‴"**CH‴'**H‴"**–CH); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.7, 171.2 155.4, 152.8, 148.1, 140.70/140.67*, 139.3/139.0*, 130.7, 128.7/128.6*, 123.2/122.9*, 119.2/118.9*, 109.8, 107.3, 78.1, 77.1 (observed by HSQC), 56.23/56.22*, 56.1, 55.0/54.9*, 51.4, 31.9/31.8*, 31.4, 28.2, 21.1/21.0*, 20.83/20.76*, 9.2, 3.5/3.4*, 3.22/3.20*; MS (ESI) *m/z*: calcd. for $C_{27}H_{42}N_3O_{10}^+$ $[M + H]^+$ 567.3, found 567.7.

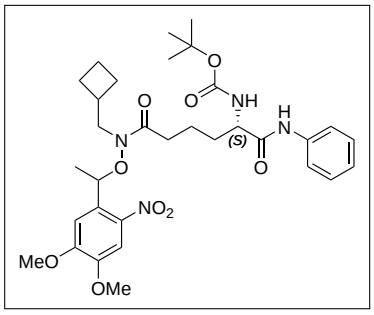
tert-Butyl ((2*S*)-6-(butyl(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-1,6-dioxo-1-(phenylamino)hexan-2-yl)carbamate (3.112)



According to the general procedure for Boc-deprotection, the Boc-protected methyl ester **3.101** (498 mg, 0.76 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (12.1 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (127 mg, 5.32 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1, 19.0 mL). After 2.0 h, Boc_2O (390 mg, 1.79 mmol, 2.4 equiv.) was added and stirred for 2.0 h. The reaction mixture was diluted with EtOAc (20 mL), and pH adjusted to ≈ 3-4 by the addition of 1.0 M HCl (aq., 5.0 mL), and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The carboxylic acid was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (244 mg, 0.76 mmol, 1.0 equiv.) was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (6.0 mL). After 10 min, DIPEA (793 µL, 4.56 mmol, 6.0 equiv.) and aniline (76.2 µL, 0.84 mmol, 1.1 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 2 x 50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography

(EtOAc:heptane 1:1 → 9:11) to provide the hydroxamate **3.112** (247 mg, 53%) as a pale green oil. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.38 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 3305, 2959, 2934, 2872, 1668, 1601, 1517, 1442, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92 (br. s, 1H, C(O)N**H**Ph), 7.60–7.58 (m, 2H, Ar**H**), 7.53 (m, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.06–6.98 (m, 2H, Ar**H**, CHN**H**C(O)O), 5.45–5.40 (m, 1H, ArC**H**CH_3), 4.09–3.99 (m, 1H, CH_2C**H**NHC(O)O), 3.923/3.920* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.56–3.49 (m, 1H, CH_2C**H'**H"NC(O)), 3.00–2.87 (m, 1H, CH_2CH'**H"**NC(O)), 2.40–2.29 (m, 2H, NC(O)C**H$_2$**CH_2CH_2), 1.68–1.48 (m, 7H, NC(O)CH_2C**H$_2$**C**H$_2$**CHN, ArCHC**H$_3$**), 1.42–1.24 (m, 11H, CH_3CH_2C**H$_2$**CH_2NC(O), C(C**H$_3$**)$_3$), 1.13–1.02 (m, 2H, CH_3C**H$_2$**), 0.76 (m, 3H, C**H$_3$**CH_2); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 171.2, 155.4, 152.9, 148.2, 140.71/140.68*, 139.0, 130.6, 128.7, 123.2, 119.2, 109.7, 107.3, 78.1, 77.5 (observed by HSQC), 56.2, 56.1, 54.9, 46.3 (observed by HSQC), 31.74/31.69*, 31.5, 28.4, 28.2, 20.9, 20.85/20.77*, 19.3 13.5; MS (ESI) *m/z*: calcd. for $C_{31}H_{45}N_4O_9^+$ $[M + H]^+$ 617.3, found 617.8.

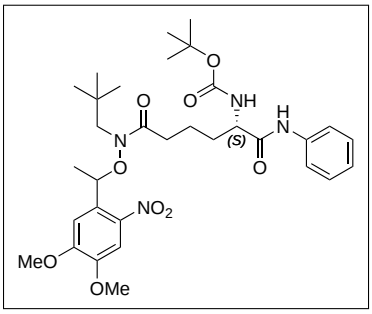
tert-Butyl ((2*S*)-6-((cyclobutylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)amino)-1,6-dioxo-1-(phenylamino)hexan-2-yl)carbamate (3.113)



According to the general procedure for Boc-deprotection, the Boc-protected methyl ester **3.98** (756 mg, 1.15 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (18.3 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (194 mg, 8.10 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1, 28.8 mL). After 1.5 h, Boc_2O (0.5033 mg, 2.31 mmol, 2.0 equiv.) was added and stirred for 2.0 h. The reaction mixture was diluted with EtOAc (25 mL), and pH adjusted to ≈ 3-4 by the addition of 1.0 M HCl (aq., 8.1 mL), and extracted with EtOAc (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The carboxylic acid was placed under vacuum for a couple of hours before

a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (407 mg, 1.27 mmol, 1.1 equiv.) was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (5.5 mL). After 10 min, DIPEA (1.20 mL, 6.92 mmol, 6.0 equiv.) and aniline (126 µL, 1.38 mmol, 1.2 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 2 x 25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 3:7 $\rightarrow$ 7:3) to provide the aniline amide **3.113** (241 mg, 34%) as a pale green oil. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.07 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3306, 2976, 2935, 2865, 1668, 1601, 1518, 1441, 1334, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92 (br. s, 1H, C(O)N**H**Ph), 7.59–7.57 (m, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.26 (m, 3H, Ar**H**), 7.06–7.02 (m, 1H, Ar**H**), 6.98 (d, *J* = 7.8 Hz, 1H, CHN**H**C(O)O), 5.45–5.40 (m, 1H, ArC**H**CH$_3$), 4.09–4.00 (m, 1H, CH$_2$C**H**NH), 3.919/3.917* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.64–3.57 (m, 1H, CHC**H'**H''NC(O)), 3.13–3.04 (m, 1H, CHCH'**H''**NC(O)), 2.53–2.44 (overlap with DMSO-d_6) (m, 1H, C**H**CH$_2$NC(O)), 2.37–2.28 (m, 2H, C(O)C**H$_2$**CH$_2$CH$_2$), 1.91–1.45 (m, 13H, C(O)CH$_2$C**H$_2$**C**H$_2$**CH, $-$C**H$_2$**C**H$_2$**C**H$_2$** $-$ CH, ArCHC**H$_3$**), 1.31 (m, 9H, C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH$_2$ missing, 171.2, 155.4, 152.8, 148.1, 140.72/140.67*, 139.0, 130.4, 128.7, 123.2, 119.2, 109.8, 107.3, 78.1, 76.8, 56.2, 56.1, 54.9, 51.6 (observed by HSQC), 33.2, 31.8/31.7*, 31.5, 28.2, 25.9/25.8*, 25.5, 21.1/21.0*, 20.8/20.7*, 17.8; MS (ESI) *m/z*: calcd. for $C_{32}H_{45}N_4O_9^+$ [M + H]$^+$ 629.3, found 629.8.

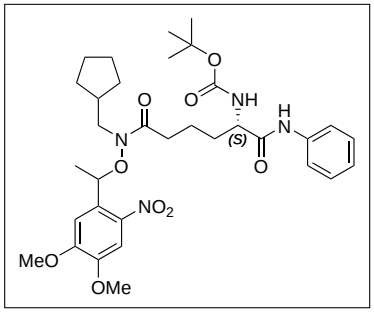
tert-Butyl ((2*S*)-6-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)(neopentyl)amino)-1,6-dioxo-1-(phenylamino)hexan-2-yl)carbamate (3.114)



According to the general procedure for Boc-deprotection, the Boc-protected methyl ester **3.102** (552 mg, 0.82 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (13.1 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (140 g, 5.84 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1, 20.6 mL). After 1.5 h, Boc_2O (360 mg, 1.65 mmol, 2.0 equiv.) was added and stirred for 2.0 h. The reaction mixture was diluted with EtOAc (25 mL), and pH adjusted to $\approx$ 3-4 by the addition of 1.0 M HCl (aq., 9.0 mL), and extracted with EtOAc (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The carboxylic acid was placed under vacuum for a couple of hours before nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (291 mg, 0.91 mmol, 1.1 equiv.) was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (3.9 mL). After 10 min, DIPEA (860 µL, 4.94 mmol, 6.0 equiv.) and aniline (90.1 µL, 0.99 mmol, 1.2 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic phase was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 2 x 25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 3:7 $\rightarrow$ 7:3) to provide the aniline amide **3.114** (219 g, 54%) as a pale green oil. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.09 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3301, 2956, 2937, 2870, 1668, 1601, 1519, 1442, 1366, 1273; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90 (br. s, 1H, C(O)N**H**Ph), 7.58 (m, 2H, Ar**H**), 7.52 (m, 1H, Ar**H**), 7.32–7.24 (m, 3H, Ar**H**), 7.06–7.02 (m, 1H, Ar**H**), 6.98 (m, 1H, CHN**H**C(O)O), 5.53–5.48 (m, 1H, ArC**H**CH$_3$), 4.09–4.00 (m, 1H, CH$_2$C**H**NHC(O)O), 3.915/3.911* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.52–3.46

(m, 1H, $(CH_3)_3CC\mathbf{H'}H''NC(O)$), 3.05–2.97 (m, 1H, $(CH_3)_3CCH'\mathbf{H''}NC(O)$), 2.43–2.21 (m, 2H, $NC(O)C\mathbf{H_2}CH_2CH_2$), 1.64–1.48 (m, 7H, $NC(O)CH_2C\mathbf{H_2}C\mathbf{H_2}CHN$, $ArCHC\mathbf{H_3}$), 1.37 (s, 9H, $OC(C\mathbf{H_3})_3$), 0.83 (s, 9H, $CH_2C(C\mathbf{H_3})_3$), ^{13}C-NMR (101 MHz, DMSO-d_6): δ 173.2 171.20/171.18*, 155.4, 152.7, 148.3, 141.0, 139.0, 129.1, 128.7, 123.2, 119.2, 110.2, 107.3, 78.1, 56.2, 56.1, 54.9, 54.3, 33.3, 31.6, 31.5, 28.2, 28.0, 21.3, 20.4; MS (ESI) *m/z*: calcd. for $C_{32}H_{47}N_4O_9^+$ $[M + H]^+$ 631.3, found 631.8.

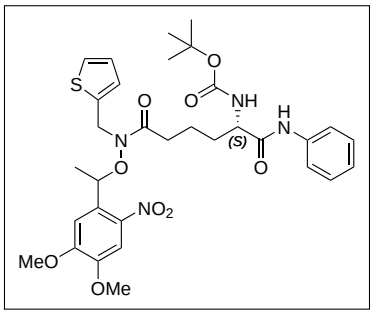
tert-Butyl ((2*S*)-6-((cyclopentylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-amino)-1,6-dioxo-1-(phenylamino)hexan-2-yl)carbamate (3.115)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.99** (743 g, 1.13 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (18.0 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (191 mg, 7.98 mmol, 7.1 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1, 28.3 mL). After 1.5 h, Boc_2O (495 mg, 2.27 mmol, 2.0 equiv.) was added and stirred for 2.0 h. The reaction mixture was diluted with EtOAc (25 mL), and pH adjusted to ≈ 3-4 by the addition of 1.0 M HCl (aq., 8.8 mL), and extracted with EtOAc (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The carboxylic acid was placed under vacuum for a couple of hours before nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (364 mg, 1.13 mmol, 1.1 equiv.) was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (5.4 mL). After 10 min, DIPEA (1.18 mL, 6.80 mmol, 6.0 equiv.) and aniline (114 µL, 1.25 mmol, 1.2 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 2 x 25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 3:7 → 6:4) to provide the aniline amide **3.115** (415 mg, 59%) as a pale green oil. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastere-

omeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.07 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3304, 2939, 2867, 1668, 1601, 1518, 1442, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92 (br. s, 1H, C(O)N**H**Ph), 7.58 (m, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.06–6.98 (m, 2H, Ar**H**, CHN**H**C(O)), 5.46–5.41 (m, 1H, ArC**H**CH_3), 4.10–4.02 (m, 1H, CH_2C**H**NHC(O)O), 3.92/3.91* (s, 3H, OC**H$_3$**), 3.86 (s, 3H, OC**H$_3$**), 3.53–3.47 (m, 1H, CHC**H'**H''NC(O)), 2.98–2.85 (m, 1H, CHCH'**H''**NC(O)), 2.41–2.30 (m, 2H, NC(O)C**H$_2$**CH_2), 2.14–2.06 (m, 1H, – CH'''H''''CH_2CH_2CH'''H'''' – C**H**), 1.67–1.23 (m, 22H, C**H$_2$**C**H$_2$**CHN, ArCHC**H$_3$**, C(C**H$_3$**)$_3$, – C**H'''**H''''C**H$_2$**C**H$_2$**C**H'''**H'''' – CH), 1.10–0.98 (m, 2H, – CH'''**H''''**CH_2CH_2CH'''**H''''** – CH), ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.0, 171.2, 155.4, 152.8, 148.2, 140.75/140.71*, 139.0, 130.4, 128.7, 123.2, 119.2, 109.8, 107.3, 78.1, 77.2, 56.2, 56.1, 54.9, 50.6, 37.5, 31.8/31.7*, 31.5, 29.6, 28.2, 24.4, 21.1, 20.8; MS (ESI) *m/z*: calcd. for $C_{33}H_{47}N_4O_9^+$ $[M + H]^+$ 643.3, found 643.8.

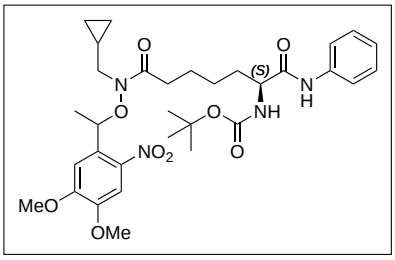
tert-butyl ((2*S*)-6-((1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)(thiophen-2-ylmethyl)-amino)-1,6-dioxo-1-(phenylamino)hexan-2-yl)carbamate (3.116)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.103** (683 g, 0.98 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (15.6 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. According to the general procedure for one-pot LiOH hydrolysis and Boc-protection, LiOH (164 mg, 6.87 mmol, 7.0 equiv.) was added to a stirring solution of the crude TFA salt in THF:H_2O (4:1, 24.5 mL). After 1.5 h, Boc_2O (428 mg, 1.96 mmol, 2.0 equiv.) was added and stirred for 2.0 h. The reaction mixture was diluted with EtOAc (25 mL), and pH adjusted to ≈ 3-4 by the addition of 1.0 M HCl (aq., 6.1 mL), and extracted with EtOAc (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The carboxylic acid was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (347 g, 1.08 mmol, 1.1 equiv.) was added to a stirring solution of the *N*-protected

amino acid in CH_2Cl_2 (4.6 mL). After 10 min, DIPEA (1.02 mL, 5.89 mmol, 6.0 equiv.) and aniline (107 µL, 1.18 mmol, 1.2 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 2 x 25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 3:7 $\rightarrow$ 7:3) to provide the aniline amide **3.116** (528 mg, 82%) as a pale green oil. A trace amount of CH_2Cl_2 and EtOAc were observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.07 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3307, 2976, 2934, 2852, 1667, 1600, 1517, 1441, 1334, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92 (s, 1H, C(O)N**H**Ph), 7.59 (d, *J* = 8.1 Hz, 2H, Ar**H**), 7.52 (s, 1H, Ar**H**), 7.35–7.28 (m, 3H, Ar**H**), 7.20 (s, 1H, Ar**H**), 7.06–7.02 (m, 1H, Ar**H**), 6.99 (d, *J* = 7.8 Hz, 1H, CHN**H**C(O)O), 6.89–6.86 (m, 1H, Ar**H**), 6.78 (m, 1H, Ar**H**), 5.55/5.54* (q, *J* = 6.5 Hz, 1H, ArC**H**CH$_3$), 4.87/4.86* (d, *J* = 16.3 Hz, 1H, C**H'**H''NC(O)), 4.47–4.40 (m, 1H, CH'**H''**NC(O)), 4.09–4.02 (m, 1H, CH$_2$C**H**NHc(O)O), 3.85 (s, 6H, 2 x OC**H$_3$**), 2.45–2.36 (m, 2H, C(O)C**H$_2$**CH$_2$), 1.66–1.49 (m, 7H, CH$_2$C**H$_2$**C**H$_2$**CHN, ArCHC**H$_3$**), 1.38 (m, 9H, CC(**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.9, 171.2, 155.4, 152.9, 148.1, 140.4/140.3*, 139.0, 138.6, 130.7, 128.7, 126.59/126.55*, 126.4, 125.80/125.79*, 123.2, 119.2 109.7, 107.4, 78.1, 78.0 (observed by HSQC), 56.11, 56.09, 54.9, 45.8, 31.9/31.8*, 31.42/31.40*, 28.2, 21.1/21.0*, 20.9/20.8*; MS (ESI) *m/z*: calcd. for $C_{32}H_{41}N_4O_9S^+$ $[M + H]^+$ 657.3, found 657.8.

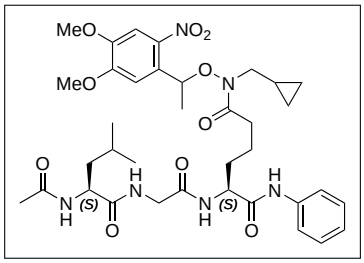
tert-Butyl ((2*S*)-7-((cyclopropylmethyl)(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-amino)-1,7-dioxo-1-(phenylamino)heptan-2-yl)carbamate (3.117)



According to the general procedure for LiOH hydrolysis, LiOH (111 mg, 4.64 mmol, 3.0 equiv.) was added to the methyl ester **3.110** (878 mg, 1.55 mmol, 1.0 equiv.) in THF:H_2O (4:1) (38.7 mL). After 2.15 h, the reaction mixture was diluted with EtOAc and acidified to pH $\approx$ 3-4 by the addition of 1.0 M HCl (aq., 5.4 mL) and immediately the aqueous phase was extracted with EtOAc (3 x 25 mL). The combined organic

phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo* and left under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for TBTU-DIPEA aniline amidation, TBTU (0.4967 g, 1.55 mmol, 1.0 equiv.) was added to a stirring solution of the carboxylic acid in CH_2Cl_2 (14.6 mL). After 10 min, DIPEA (1.62 mL, 9.28 mmol, 6.0 equiv.) and aniline (155 µL, 1.70 mmol, 1.1 equiv.) were added and stirred overnight. Incomplete conversion was observed (23.5 h), therefore additionally DIPEA (808 µL, 4.64 mmol, 3.0 equiv.) was added and stirred for 15 min, before TBTU (248 mg, 0.77 mmol, 0.5 equiv.) and aniline (77.5 µL, 0.85 mmol, 0.55 equiv.) were added and stirred overnight. The reaction mixture was evaporated under reduced pressure, and the organic layer was dissolved in EtOAc (25 mL) and washed with H_2O (25 mL), sat. $NaHCO_3$ (aq., 25 mL), 1.0 M HCl (aq., 25 mL), and brine (25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residues were purified by flash column chromatography (EtOAc:heptane 3:7 $\rightarrow$ 9:11) to provide the aniline amide **3.111** (864 g, 89%) as a pale yellow foam. A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.07 (EtOAc:heptane (3:7); UV); IR (neat) ν (cm^{-1}): 3304, 2977, 2935, 2861, 1667, 1601, 1518, 1441, 1334, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.93/9.92* (s, 1H, C(O)N**H**Ph), 7.60–7.58 (m, 2H, Ar**H**), 7.53/7.52* (s, 1H, Ar**H**), 7.31–7.27 (m, 3H, Ar**H**), 7.05–7.01 (m, 1H, Ar**H**), 6.97–6.94 (m, 1H, CHN**H**C(O)O), 5.50–5.46 (q, *J* = 7.8 Hz, 1H, ArC**H**CH$_3$), 4.06–4.01 (m, 1H, CH$_2$C**H**NHC(O)O), 3.92 (s, 3H, OC**H$_3$**), 3.854/3.848* (s, 3H, OC**H$_3$**), 3.34–3.29 (m, 1H, – CH‴H‴CH‴H‴ – CHC**H’**H″N), 3.12–3.05 (m, 1H, – CH‴H‴CH‴H‴ – CHCH′**H″**N), 2.34–2.31 (m, 2H, C(O)C**H$_2$**CH$_2$), 1.60–1.23 (m, 18H, CH$_2$C**H$_2$**C**H$_2$**C**H$_2$**CHN, ArCHC**H$_3$**, C(C**H$_3$**)$_3$), 0.97–0.88 (m, 1H, – CH‴H‴CH‴H‴ – C**H**CH$_2$), 0.37–0.36 (m, 2H, –C**H‴**H‴C**H‴**H‴ – CHCH$_2$), 0.15–0.11 (m, 2H, –CH‴**H⁗**CH‴**H⁗** – CHCH$_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 174.8, 171.4, 155.5, 152.8, 148.1, 140.7, 139.0, 130.7, 128.7, 123.2, 119.1, 109.8, 107.3, 78.0, 77.1, 56.2, 56.1, 54.9. 51.6, 32.0 31.7, 28.2, 25.2, 23.9, 20.8, 9.2, 3.5, 3.2; MS (ESI) *m/z*: calcd. for $C_{27}H_{42}N_3O_{10}^+$ $[M + H]^+$ 567.3, found 567.7.

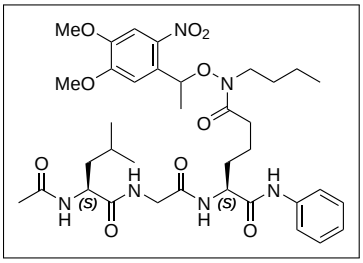
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_6-(cyclopropylmethyl)-N_6-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_1-phenylhexanediamide (3.118)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.111** (708 mg, 1.15 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (18.6 mL) for 1.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (481 g, 1.50 mmol, 1.3 equiv.) and NEM (1.014 mL, 8.06 mmol, 7.0 equiv.) was added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (318 mg, 1.38 mmol, 1.2 equiv.) in DMF (3.0 mL) and stirred for 20 min, before the crude TFA salt in DMF (8.5 mL) was added and stirred overnight. Incomplete conversion was observed, and therefore a new batch of acetyl-*L*-leucylglycine **3.37**, TBTU, and NEM were prepared in DMF (2.0 mL) and after stirring for 15 min added to the reaction mixture followed by stirring overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.118** was isolated by flash column chromatography (CH_2Cl_2 $\rightarrow$ CH_2Cl_2:MeOH 47:3) as a pale yellow foam (583 mg, 70%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.18 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.89 (s, 1H, C(O)N**H**Ph), 8.28 (t, *J* = 5.7 Hz, 1H, C(O)N**H**CH_2C(O)), 8.09 (d, *J* = 7.4 Hz, 1H, CH_3C(O)N**H**CH), 7.96 (d, *J* = 7.6 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.61 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.50–5.45 (m, 1H, ArC**H**CH_3), 4.39–4.33 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, H_3C(O)NHC**H**), 3.92 (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.77–3.67 (m, 2H, C(O)NHC**H$_2$**C(O)), 3.35–3.29 (overlap with H_2O) (m, 1H, NC**H'**H''CH – CH'''H''''CH'''H'''' –), 3.11–3.04 (m, 1H, NCH'**H''**CH – CH'''H''''CH'''H'''' –), 2.41–2.33 (m, 2H, NHCHCH_2CH_2C**H$_2$**C(O)N), 1.84/1.82 (s, 3H, C**H$_3$**C(O)NH), 1.77–1.42 (m, 10H,

$CH_3C(O)NHCHC\mathbf{H_2}C\mathbf{H}$, $NHCHC\mathbf{H_2}C\mathbf{H_2}CH_2$, $ArCHC\mathbf{H_3}$), 0.97–0.82 (m, 7H, $CH(C\mathbf{H_3})_2$, $NCH_2C\mathbf{H}$ – CH‴'H‴"CH‴'H‴" –), 0.36–0.35 (m, 2H, CH–C**H‴'**H‴"C**H‴'**H‴"–C), 0.14–0.12 (m, 2H, CH–CH‴'**H‴"**CH‴'**H‴"**–C); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.39/170.36*, 169.6, 168.9/168.8*, 152.8, 148.1, 140.7, 138.8, 130.6, 128.7, 123.4, 119.3, 109.8, 107.3, 77.0, 56.2, 56.1, 53.43/53.37*, 51.44, 51.38, 42.0, 40.5, 31.9, 31.5, 24.2, 22.9, 22.4, 21.6, 20.8, 20.7, 9.2, 3.50/3.46*, 3.23/3.22*;
MS (ESI) *m/z*: calcd. for $C_{36}H_{51}N_6O_{10}^+$ $[M + H]^+$ 727.4, found 727.8; HRMS (ESI) *m/z*: calcd. for $C_{36}H_{51}N_6O_{10}^+$ $[M + H]^+$ 727.3661, found 727.3665.

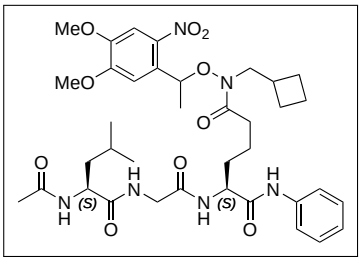
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_6-butyl-N_6-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_1-phenylhexanediamide (3.119)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.112** (233 mg, 0.38 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (6.0 mL) for 1.5 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (159 mg, 0.49 mmol, 1.3 equiv.) and NEM (333 µL, 2.65 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (106 mg, 0.46 mmol, 1.2 equiv.) in DMF (1.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (2.8 mL) was added and stirred overnight. Incomplete conversion was observed, and therefore a new batch of acetyl-*L*-leucylglycine **3.37**, TBTU, and NEM were prepared in DMF (2.0 mL) and after stirring for 15 min added to the reaction mixture followed by stirring overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.119** was isolated by flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH 93:7) as a pale green semi-solid (239 mg, 87%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts

are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.17 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90 (br. s, 1H, C(O)N**H**Ph), 8.29 (t, *J* = 5.8 Hz, 1H, C(O)N**H**CH_2C(O)), 8.08 (d, *J* = 7.4 Hz, 1H, CH_3C(O)N**H**CH), 7.96 (d, *J* = 8.0 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.63–7.61 (m, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.42/5.41* (q, *J* = 5.8 Hz, 1H, ArC**H**CH_3), 4.39–4.33 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, H_3C(O)NHC**H**), 3.921/3.920* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.77–3.67 (m, 2H, C(O)NHC**H$_2$**C(O)), 3.57–3.49 (m, 1H, C(O)NC**H'**H"CH_2), 3.00–2.89 (m, 1H, C(O)NCH'**H"**CH_2), 2.40–2.32 (m, 2H, NHCHCH_2CH_2C**H$_2$**C(O)N), 1.84/1.83* (s, 3H, C**H$_3$**C(O)NH), 1.77–1.23 (m, 12H, CH_3C(O)NHCHC**H$_2$**C**H**, NHCHC**H$_2$**C**H$_2$**CH_2, ArCHC**H$_3$**, C**H$_2$**CH_2CH_3), 1.14–1.03 (m, 2H, H_2C**H$_2$**CH_3), 0.89–0.73 (m, 9H, CH_2**CH$_3$**, CH(C**H$_3$**)$_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.39/170.37*, 169.7, 168.9/168.8*, 152.87/152.86*, 148.2, 140.69/140.66*, 138.8, 130.7, 128.7, 123.4, 119.3, 109.7, 107.3, 77.5, 56.2, 56.1, 53.41/53.36*, 51.4, 46.4, 42.1, 40.5, 31.72/31.67*, 31.5, 28.42/28.39*, 24.2, 22.9, 22.4, 21.6, 20.9/20.8*, 20.70/20.68*, 19.3, 13.5; MS (ESI) *m/z*: calcd. for $C_{36}H_{53}N_6O_{10}^+$ [M + H]$^+$ 729.4, found 729.9.

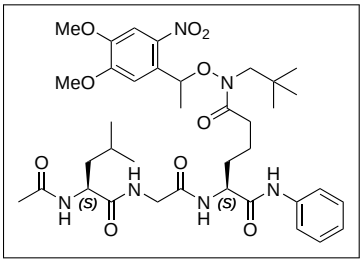
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_6-(cyclobutylmethyl)-N_6-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_1-phenylhexanediamide (3.120)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.113** (280 mg, 0.45 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (7.1 mL) for 1.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (186 mg, 0.58 mmol, 1.3 equiv.) and NEM (392 µL, 3.12 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (124 mg, 0.53 mmol, 1.2 equiv.) in DMF (2.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (2.45 mL) was added and stirred overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The

combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.120** was isolated by flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH 47:3) as a pale green foam (247 g, 75%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.24 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.89 (s, 1H, C(O)N**H**Ph), 8.28 (t, *J* = 5.8 Hz, 1H, C(O)N**H**CH_2C(O)), 8.09 (d, *J* = 7.3 Hz, 1H, CH_3C(O)N**H**CH), 7.97/7.96 (d, *J* = 7.8 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.62–7.60 (m, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.27 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.45–5.40 (m, 1H, ArC**H**CH_3), 4.39–4.32 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, CH_3C(O)NHC**H**), 3.92 (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.77–3.67 (m, 2H, C(O)NHC**H$_2$**C(O)), 3.61 (dd, *J* = 14.5, 8.0 Hz, 1H, NC**H**'H"CH−$CH_2CH_2CH_2$−), 3.13–3.04 (m, 1H, NCH'**H"**CH−$CH_2CH_2CH_2$−), 2.52–2.44 (overlap with DMSO-d_6) (m, 1H, NCH_2C**H**−$CH_2CH_2CH_2$−), 2.37–2.29 (m, 2H, $CHCH_2CH_2$C**H$_2$**C(O)N), 1.90–1.40 (m, 18H, CH_3C(O)NHC**H$_2$**C**H**, CHC**H$_2$**C**H$_2$**CH2C(O)N, ArCHC**H$_3$**, −C**H$_2$**C**H$_2$**C**H$_2$**−, C**H$_3$**C(O)NHCH), 0.88–0.83 (m, 6H, CH(C**H$_3$**)$_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.4, 169.7, 168.9/168.8*, 152.8, 148.1, 140.7, 138.8, 130.4, 128.7, 123.4, 119.3, 109.8, 107.3, 77.0, 56.2, 56.1, 53.4/53.3*, 51.89, 51.86 (observed by HSQC), 42.0, 40.5, 33.2, 31.8, 31.5, 25.88/25.86*, 25.5, 24.2, 22.9, 22.4, 21.6, 20.8, 20.7, 17.8; MS (ESI) *m/z*: calcd. for $C_{37}H_{53}N_6O_{10}^+$ $[M + H]^+$ 741.4, found 741.9.

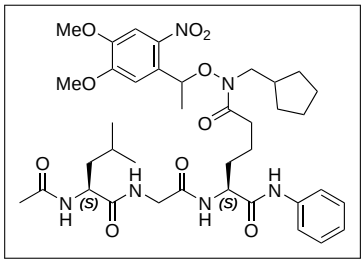
((2*S*))-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_6-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_6-neopentyl-N_1-phenylhexanediamide (3.121)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.114** (271 mg, 0.43 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (6.9 mL) for 1.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (180 mg, 0.57 mmol, 1.3

equiv.) and NEM (379 µL, 3.01 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (119 mg, 0.52 mmol, 1.2 equiv.) in DMF (2.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (2.30 mL) was added and stirred overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with Brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.121** was isolated by flash column chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$:MeOH 93:7) as a pale green foam (283 g, 88%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.20 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H, C(O)N**H**Ph), 8.28–8.26 (m, 1H, C(O)N**H**CH_2C(O)), 8.07 (d, *J* = 7.5 Hz, 1H, CH_3C(O)N**H**CH), 7.97 (d, *J* = 7.6 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.61 (d, *J* = 8.1 Hz, 2H, Ar**H**), 7.52 (s, 1H, Ar**H**), 7.32–7.25 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.53–5.48 (m, 1H, ArC**H**CH_3), 4.39–4.32 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, CH_3C(O)NHC**H**), 3.912/3.909* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.77–3.67 (m, 2H, NHC**H$_2$**C(O)), 3.50–3.46 (m, 1H, NC**H'**H''CH−$CH_2CH_2CH_2$−), 3.05–2.99 (m, 1H, NCH'**H''**CH−$CH_2CH_2CH_2$−), 2.43–2.21 (m, 2H, $CHCH_2CH_2$C**H$_2$**C(O)N), 1.84/1.83* (s, 3H, C**H$_3$**C(O)N), 1.74–1.39 (m, 10H, CH_3C(O)NHCHC**H$_2$**C**H**, NHCHC**H$_2$**C**H$_2$**CH_2, ArCHC**H$_3$**), 0.88–0.82 (m, 15H, C(C**H$_3$**)$_3$, CH(C**H$_3$**)$_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.4/170.3*, 169.6, 168.9/168.8*, 152.7, 148.3, 141.0, 138.8, 129.1, 128.7, 123.4, 119.3, 110.2/110.1*, 107.3, 75.6, 56.2, 56.1, 54.3 (observed by HSQC) 53.4/53.3*, 51.4, 42.0, 40.5, 33.2, 31.58, 31.56, 28.2, 24.1, 22.9, 22.4, 21.6, 21.0, 20.4; MS (ESI) *m/z*: calcd. for $C_{37}H_{55}N_6O_{10}^+$ [M + H]$^+$ 743.4, found 743.8; HRMS (ESI) *m/z*: calcd. for $C_{37}H_{55}N_6O_{10}^+$ [M + H]$^+$ 743.3974, found 743.3989.

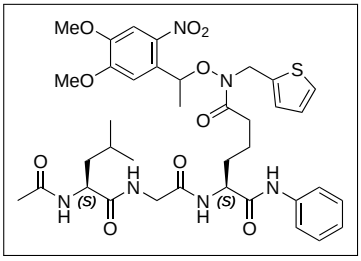
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_6-(cyclopentylmethyl)-N_6-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_1-phenylhexanediamide (3.122)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.115** (407 mg, 0.63 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (10.1 mL) for 1.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (265 mg, 0.82 mmol, 1.3 equiv.) and NEM (557 µL, 4.43 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (175 mg, 0.76 mmol, 1.2 equiv.) in DMF (3.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (6.33 mL) was added and stirred overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photoprotected tripeptide **3.122** was isolated by flash column chromatography (CH_2Cl_2:MeOH 99:1 $\rightarrow$ 93:7) as a pale green semi-solid (427 mg, 89%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.23 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.89 (s, 1H, C(O)N**H**Ph), 8.28 (t, *J* = 5.8 Hz, 1H, C(O)N**H**CH_2C(O)), 8.08 (d, *J* = 7.2 Hz, 1H, CH_3C(O)N**H**CH), 7.97/7.96* (d, *J* = 7.7 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.62–7.60 (m, 2H, Ar**H**), 7.53 (s, 1H, Ar**H**), 7.32–7.28 (m, 3H, Ar**H**), 7.07–7.03 (m, 1H, Ar**H**), 5.46–5.41 (m, 1H, ArC**H**CH_3), 4.39–4.33 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, CH_3C(O)NHC**H**), 3.92/3.92* (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.77–3.67 (m, 2H, C(O)NHC**H$_2$**C(O)), 3.49–3.47 (dd, *J* = 14.2, 8.4 Hz, 1H, C(O)NC**H'**H''CH – CH'''H''''CH_2CH_2CH'''H'''' –), 2.97–2.86 (m, 1H, C(O)NCH'**H''**CH – CH'''H''''CH_2CH_2CH'''H'''' –), 2.40–2.31 (m, 2H, $CHCH_2CH_2$C**H2**C(O)N), 2.13–2.05 (m, 1H, C**H** – CH'''H''''CH_2CH_2CH'''H'''' –), 1.84/1.83* (s, 3H, CH_3C(O)NH), 1.77–1.37 (m, 16H, CH_3C(O)NHCHC**H$_2$**C**H**, NHCHC**H$_2$**C**H$_2$**CH_2, ArCHC**H$_3$**, CH – C**H'''**H''''C**H$_2$**C**H$_2$**CH'''**H''''** –), 1.09–0.97 (m, 2H,

CH−CH‴**H‴‴**CH_2CH_2CH‴**H‴‴** −), 0.87 (d, *J* = 6.5 Hz, 3H, CHC**H$_3$**), 0.83 (d, *J* = 6.5 Hz, 3H, CHC**H$_3$**); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.39/170.38*, 169.7, 168.9/168.8*, 152.8, 148.2, 140.72/140.69*, 138.8, 130.6, 128.7, 123.4, 119.3, 109.8, 107.3, 77.2, 56.2, 56.1, 53.44/53.37*, 51.4, 50.5 (observed by HSQC) 42.1, 40.5, 37.5, 31.8/31.7*, 31.6/31.5*, 29.69/29.67* 24.4, 24.2, 22.9, 22.4, 21.6, 20.9/20.8*; MS (ESI) *m/z*: calcd. for $C_{38}H_{55}N_6O_{10}^+$ $[M + H]^+$ 755.4, found 755.9.

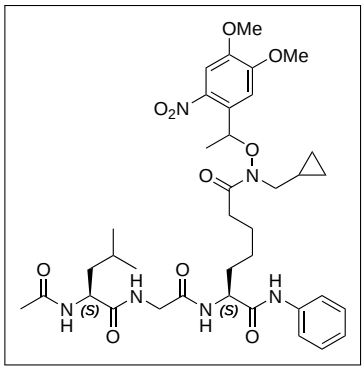
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_6-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_1-phenyl-N_6-(thiophen-2-ylmethyl)hexanediamide (3.123)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide **3.116** (510 mg, 0.78 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (12.3 mL) for 1.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (325 mg, 1.01 mmol, 1.3 equiv.) and NEM (684 µL, 5.44 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (216 mg, 0.94 mmol, 1.2 equiv.) in DMF (3.0 mL) and stirred for 15 min, before the crude TFA salt in DMF (4.33 mL) was added and stirred overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.123** was isolated by flash column chromatography (CH_2Cl_2:MeOH 99:1 → 93:7) as a brown semi-solid (500 mg, 84%). A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.19 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90 (s, 1H, C(O)N**H**Ph), 8.28 (t, *J* = 5.8 Hz, 1H, C(O)N**H**CH_2C(O)), 8.084/8.080* (d, *J* = 7.5 Hz, 1H, CH_3C(O)N**H**CH), 7.96 (d, 1H, C(O)N**H**CHC(O)NHPh), 7.63–7.61 (m, 2H, Ar**H**), 7.51 (s, 1H, Ar**H**), 7.35–7.28 (m, 3H, Ar**H**), 7.21/7.20* (s, 1H, Ar**H**), 7.07–

7.03 (m, 1H, Ar**H**), 6.89–6.86 (m, 1H, Ar**H**), 6.79–6.77 (m, 1H, Ar**H**), 5.57–5.52 (m, 1H, ArC**H**CH_3), 4.86 (d, *J* = 16.3 Hz, 1H, C(O)NC**H'**H"Ar), 4.48–4.32 (m, 2H, C(O)NCH'**H"**Ar, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, CH_3C(O)NHC**H**), 3.86 (s, 6H, 2 x O**C**$\mathbf{H_3}$), 3.77–3.67 (m, 2H, C(O)NHC$\mathbf{H_2}$C(O)), 2.46–2.33 (m, 2H, $CHCH_2CH_2C\mathbf{H_2}$C(O)N), 1.83–1.41 (m, 13H, C$\mathbf{H_3}$C(O)NHCHC$\mathbf{H_2}$C**H**, NHCHC$\mathbf{H_2}$C$\mathbf{H_2}CH_2$, ArCHC$\mathbf{H_3}$), 0.88–0.82 (m, 6H, CH(C$\mathbf{H_3}$)$_2$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.37/170.35*, 169.7, 168.9/168.8*, 152.93/152.92*, 148.1, 140.33/140.29*, 138.8, 138.6, 130.7, 128.7, 126.6/126.5*, 126.4, 125.8, 123.4, 119.3, 109.7, 107.4, 78.0, 56.12, 56.09, 53.43/53.37*, 51.4, 45.9, 42.0, 40.5, 31.9/31.8*, 31.5, 24.2, 22.9, 22.43/22.42*, 21.6, 21.1/21.0*, 20.6/20.5*; MS (ESI) *m/z*: calcd. for $C_{37}H_{49}N_6O_{10}S^+$ $[M + H]^+$ 769.3, found 769.8.

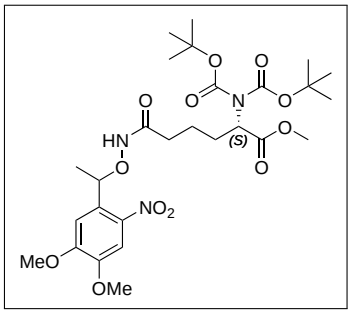
(2*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-N_7-(cyclopropylmethyl)-N_7-(1-(4,5-dimethoxy-2-nitrophenyl)ethoxy)-N_1-phenylheptanediamide (3.124)



According to the general procedure for Boc-deprotection, the Boc-protected aniline amide**3.117** (850 mg, 1.35 mmol, 1.0 equiv.) was stirred in TFA:CH_2Cl_2 (3:7) (21.5 mL) for 1.0 h. Volatiles were removed under reduced pressure and co-evaporated from toluene and CH_2Cl_2. The round-bottomed flask was placed under vacuum for a couple of hours before a nitrogen atmosphere was established. According to the general procedure for coupling reagent acylation, TBTU (565 mg, 1.76 mmol, 1.3 equiv.) and NEM (1.19 mL, 9.47 mmol, 7.0 equiv.) were added to a stirring solution of acetyl-*L*-leucylglycine **3.37** (374 mg, 1.62 mmol, 1.2 equiv.) in DMF (3.0 mL) and stirred for 20 min, before the crude TFA salt in DMF (10.5 mL) was added and stirred overnight. Incomplete conversion was observed, and therefore a new batch of acetyl-*L*-leucylglycine **3.37**, TBTU, and NEM were prepared in DMF (2.0 mL) and after stirring for 15 min added to the reaction mixture followed by stirring overnight. The reaction mixture was diluted with EtOAc (25 mL), and the organic layer was washed with brine (5 x 25 mL), sat. $NaHCO_3$ (aq., 25 mL), and 1.0 M HCl (aq., 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The photo-protected tripeptide **3.124** was isolated by flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH 93:7) as a light yellow foam (790 mg, 79%).

A trace amount of CH_2Cl_2 was observed by ^{1}H-NMR. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.24 (CH_2Cl_2:MeOH (19:1); UV); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92/9.91* (s, 1H, C(O)N**H**Ph), 8.29–8.26 (m, 1H, C(O)N**H**CH_2C(O)), 8.08 (d, J = 7.6 Hz, 1H, CH_3C(O)N**H**CH), 7.93 (d, J = 7.8 Hz, 1H, C(O)N**H**CHC(O)NHPh), 7.62 (d, J = 8.0 Hz, 2H, Ar**H**), 7.53/7.53* (s, 1H, Ar**H**), 7.31–7.27 (m, 3H, Ar**H**), 7.06–7.03 (m, 1H, Ar**H**), 5.48 (d, J = 6.5 Hz, 1H, ArC**H**CH_3), 4.38–4.33 (m, 1H, C(O)NHC**H**C(O)NHPh), 4.25–4.19 (m, 1H, H_3C(O)NHC**H**), 3.91 (s, 3H, OC**H$_3$**), 3.853/3.847* (s, 3H, OC**H$_3$**), 3.77–3.66 (m, 2H, C(O)NHC**H$_2$**C(O)) 3.34–3.28 (m, 1H, C(O)NC**H'**H''CH), 3.11–3.04 (m, 1H, C(O)NCH'**H''**CH), 2.34 (m, 2H, CH_2CH_2C**H$_2$**C(O)N), 1.84 (s, 3H, C**H$_3$**C(O)NH), 1.76–1.55 (m, 6H, CH_3C(O)NHCHCH_2C**H**, NHCHC**H$_2$**CH_2, ArCHC**H$_3$**), 1.51–1.41 (m, 4H, CH_3C(O)NHCHC**H$_2$**CH, CH_2C**H$_2$**CH_2C(O)), 1.36–1.21 (m, 2H, NHCHCH_2C**H$_2$**CH_2), 0.93–0.82 (m, 7H, CH(C**H$_3$**)$_2$, C(O)NCH2C**H**), 0.36–0.34 (m, 2H, −C**H'''**H''''C**H'''**H'''' −CHC(O)N), 0.16–0.09 (m, 2H, −CH'''**H''''**CH'''**H''''** −CHC(O)N); ^{13}C-NMR (101 MHz, DMSO-d_6): δ N**C**(O)CH_2 missing, 172.8, 170.5, 169.6, 168.8, 152.8, 148.1, 140.7, 138.9, 130.6, 128.7, 123.4, 119.3, 109.8, 107.3, 77.1, 56.2, 56.1, 53.2, 51.4, 51.3 (observed by HSQC) 42.1, 40.5, 32.0, 31.7, 25.0, 24.2, 23.94/23.90*, 22.9, 22.4, 21.6, 20.8, 9.2, 3.5, 3.2; MS (ESI) *m/z*: calcd. for $C_{37}H_{53}N_6O_{10}^+$ $[M + H]^+$ 741.4, found 741.8.

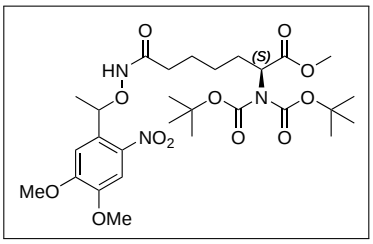
Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-((1-(4,5-dimethoxy-2-nitrophenyl)-ethoxy)amino)-6-oxohexanoate (3.125)



According to the general procedure for coupling reagent acylation, HATU (1.48 g, 3.89 mmol, 1.2 equiv.) and DIPEA (3.93 mL, 22.6 mmol, 7.0 equiv.) were added to a stirring solution of the carboxylic acid **3.57** (1.33 g, 3.55 mmol, 1.1 equiv.) in DMF (32.3 mL) and stirred for 15 min, before the hydroxylamine hydrochloride **2.7** (900 mg, 3.23 mmol, 1.0 equiv.) was added and stirred for 6 h. The organic layer was washed with brine (6 x 100 mL). The hydroxamate **3.125** (1.78 g, 92%) was isolated by flash column chromatography (EtOAc:heptane 1:1 → 3:2) as a pale green foam. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances

for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.25 (EtOAc:heptane (1:1); UV); IR (neat) ν (cm^{-1}): 3241, 2978, 2937, 1743, 1697, 1518, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.772/10.764* (s, 1H, **H**NC(O)), 7.57/7.56* (s, 1H, Ar**H**), 7.37/7.37* (s, 1H, Ar**H**), 5.45 (q, *J* = 6.3 Hz, 1H, ArC**H**CH$_3$), 4.74 (dd, *J* = 9.9, 4.9 Hz, 1H, CH'H"CH2C**H**N), 3.95 (s, 3H, OC**H$_3$**), 3.85 (s, 3H, OC**H$_3$**), 3.61 (s, 1H, C(O)OC**H$_3$**), 1.88–1.77 (m, 3H, NC(O)C**H$_2$**CH$_2$C**H'**H"CHN), 1.74–1.64 (m, 1H, CH'**H"**CHN), 1.47 (d, *J* = 6.3 Hz, 3H, ArCHC**H$_3$**), 1.46–1.31 (m, 20H, NC(O)CH$_2$C**H$_2$**CH$_2$, 2 x C(C**H$_3$**)$_3$); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 170.4, 169.0, 153.4, 151.6, 147.7, 140.1/140.0*, 132.6, 109.2, 107.4, 82.5, 77.4, 57.2, 56.03, 56.01, 52.0, 31.7, 28.9, 27.4, 21.5, 21.0; MS (ESI) *m/z*: calcd. for $C_{27}H_{41}N_3NaO_{12}^+$ [M + Na]$^+$ 622.3, found 622.7; HRMS (ESI) *m/z*: calcd. for $C_{27}H_{41}N_3NaO_{12}^+$ [M + Na]$^+$ 622.2582, found 622.258.

Methyl (2*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-7-((1-(4,5-dimethoxy-2-nitrophenyl)-ethoxy)amino)-7-oxoheptanoate (3.126)



According to the general procedure for coupling reagent acylation, HATU (1.25 g, 3.29 mmol, 1.2 equiv.) and DIPEA (3.34 mL, 19.2 mmol, 7.0 equiv.) were added to a stirring solution of the carboxylic acid **3.65** (1.74 mg, 3.01 mmol, 1.1 equiv.) in DMF (27.4 mL) and stirred for 20 min, before the hydroxylamine hydrochloride **2.7** (765 mg, 2.74 mmol, 1.0 equiv.) was added and stirred overnight. The organic layer was washed with brine:H_2O (1:1, 6 x 100 mL). The hydroxamate **3.126** (1.28 g, 76%) was isolated by flash column chromatography (EtOAc:heptane 2:3 → 13:7) as a pale green foam. A diastereomeric mixture of 1:1 ratio was observed in the NMR spectra. Two resonances for nuclei denoted with a star(*) were observed and both chemical shifts are reported. Multiplicity for each diastereomer is reported if possible. R_f = 0.24 (EtOAc:heptane (2:3); UV); IR (neat) ν (cm^{-1}): 3228, 2978, 2937, 2868, 1743, 1697, 1518, 1457, 1366, 1272; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.73 (s, 1H, **H**NC(O)), 7.56/7.55* (s, 1H, Ar**H**), 7.36 (s, 1H, Ar**H**), 5.47–5.41 (m, 1H, ArC**H**CH$_3$), 4.74–4.69 (m, 1H, CH$_2$C**H**N), 3.95 (s, 3H, OC**H$_3$**), 3.849/3.856* (s, 3H, OC**H$_3$**), 3.62 (s, 1H, C(O)OC**H$_3$**), 1.92–1.77 (m, 3H, NC(O)C**H$_2$**CH$_2$CH'**H"**CHN), 1.72–1.62 (m, 1H, CH$_2$CH'**H"**CHN), 1.48–1.31 (m, 23H, NC(O)CH$_2$C**H$_2$**CH$_2$, 2 x C(C**H$_3$**)$_3$, ArCHC**H$_3$**), 1.13–

1.02 (m, 2H, $CH_2C\mathbf{H_2}CH_2CHN$), ^{13}C-NMR (101 MHz, DMSO-d_6): δ 170.5, 169.0, 153.4, 151.6, 147.7, 140.22/140.19*. 132.6, 109.24/109.20* 107.4/107.3*, 82.5, 77.3, 57.3, 56.03, 55.99, 52.0, 31.9, 28.9, 27.5, 24.8, 24.5, 21.0; MS (ESI) *m/z*: calcd. for $C_{28}H_{43}N_3NaO_{12}^+$ [M + Na]$^+$ 636.3, found 636.8.

Chapter 4

Itaconimides as Novel Quorum Sensing Inhibitors of *Pseudomonas aeruginosa*

During my external stay, I was given the opportunity to go to Singapore and work with Assistant Professor Liang Yang at SCELSE and Professor Choon-Hong Tan at the Division of Chemistry and Biological Chemistry at NTU.

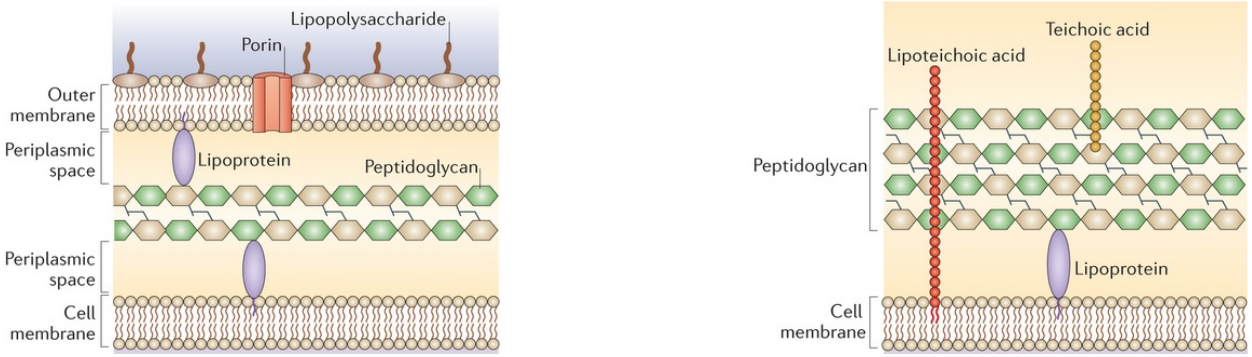
4.1 Cell Biology, Quorum Sensing, and the Battle against Antibiotic Resistant Bacteria

Sir Alexander Fleming discovered penicillin in 1928, and later, Professor Ernst Boris Chain and Sir Howard Walter Florey developed a useful large scale synthesis of the compound. Together, in 1945, Flemming, Chain, and Florey received the Nobel Prize for their achievements.[199] In his Nobel lecture, Sir Alexander Fleming addressed several issues: "*And we are not at the end of the penicillin story. Perhaps we are only just at the beginning. We are in a chemical age and penicillin may be changed by the chemists so that all its disadvantages may be removed and a newer and a better derivative may be produced.... There may be a danger, though, in underdosage. It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body.... Moral: If you use penicillin, use enough*"[200]

Today, we face the consequence of misused antibiotics, as uncontrolled usage has resulted in a large amount of bacteria becoming resistant toward common antibiotics. In South European countries, antibiotics can be bought without a prescription, often resulting in over- or misuse due to lack of council.[201,202] In the Northern countries, such as Denmark, antibiotics can only be bought with a prescription, lowering the risk of improper use.[203]

In 1990, Woese *et al.* revised the taxonomic classification of all living cells and subdivided the cells into three domains in the phylogenetic tree of life: Bacteria, Archaea, and

Eukaryota.[204] Archaea and Bacteria consists of prokaryote cells, whereas Eukaryota, as the name implies, is eukaryote cells. Bacteria can also be divided into two independent classes. In 1884, a Danish bacteriologist, Hans Christian Joachim Gram, found that different bacteria had a significant color variation when subjected to the Crystal violet dye. He classified the bacteria as Gram-negative or Gram-positive.
Gram-negative bacteria are composed of three membranes: an outer membrane, a peptidoglycan cell wall, and an inner membrane, Figure 4.1. Whereas Gram-positive bacteria does not have the outer membrane. This membrane acts as an outer barrier, and is one of the reasons why, Gram-negative bacteria display a higher degree of resistance and are difficult to target. The outer membrane is composed of two layers consisting of phospholipids and lipopolysaccharide, respectively.[205,206]



(a) Composition of Gram-negative cells

(b) Composition of Gram-positive cells

Figure 4.1: Illustrating the differences between Gram-negative and Gram-postive cells. Adapted from Brown *et al.*[206]

For decades bacteria were believed to be organized as colonies in a free-floating (planktonic) state, but it has later been shown that under 0.1% of the total microbial biomass is found in this planktonic state.[207] The bacteria tend to aggregate into communities and produce a protecting layer, called the biofilm.[208] Healy *et al.* describe that biofilm formation renders the bacteria more than 500 times less susceptible to antibiotics.[207] Biofilms are a problem in the medical treatment, as they tend to appear on the surfaces with a weakened immune defense, and in particular on foreign objects, such as implants. The lungs of cystic fibrosis patients often show high biofilm formation.[209] Development of highly resistant strains have been observed. Recently, an American female was infected with resistant *Klebsiella pneumoniae*, and showed resistance towards 26 antibiotics,[210] even

against carbapenem, which is considered to be one of the "last resort" drugs.[211] In another case, a strain of *Escherichia coli* showed resistance towards carbapenem and another last resort antibiotics, colistin.[212]
Livestock also show signs of severe antibiotic resistance. The poor regulation of antibiotics in livestock and the constant pressure for the farmers to gain more revenue per animal is to blame of this. An article in the Guardian reported that around two thirds of all Danish pig farms are infected with the feared antibiotic resistance staphylococcus bacteria, namely methicillin resistant *Staphylococcus aureus* (MRSA) CC398 strain. In British supermarkets it was found that 9% of the tested pork meat was infected with CC398, among those 89% of the meat came from Danish farms. If this food is handled in the right way, there is little risk of the strain being transferred to humans, but working with the animals pose a higher risk of infection, as prolonged exposure heightens the risk of mutations.[213]

Antibiotics are classified as bactericides, which means they kill the bacteria.[214] The bacteria develops resistance in order to survive. Therefore, it is important to develop antibiotics that targets mechanisms with a lower tendency to develop resistance. Another approach for targeting bacteria, is antipathogenic treatment.[215] Biofilm need a well-organized signal network to enable cell-to-cell communication within the bacteria, so-called quorum sensing (QS).[216] The course of the biofilm formation, developing, and dispersion into the extracellular matrix are shown in Figure 4.2.

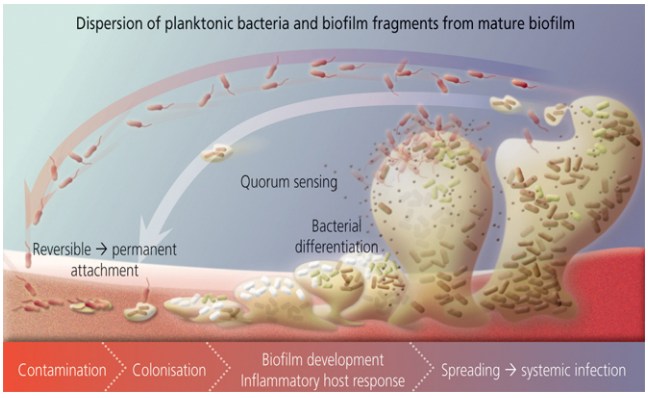
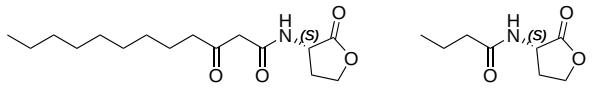


Figure 4.2: Biofilm formation, development, and the dispersion of bacteria into the extracellular matrix. Adapted from [217]

The quorum sensing apparatus monitors the bacteria growth by producing and releasing small signal molecules called autoinducers. The concentration of these molecules in the environment is proportional to the bacteria growth. When biofilms with high-density of bacteria cells are obtained, a procedure induces the signal molecules to bind to their activated cognate receptors which expresses various virulence factors.[218]
P. aeruginosa is a opportunistic pathogen that is found in chronic cystic fibrosis infections, one of the most investigated pathogens displaying intrinsic multidrug resistance. It exhibits a well-established biofilm formation, and has a broad tolerance profile.[209,219] Furthermore, WHO has recently placed *P. aeruginosa* in the top three of the 12 most critical antibiotic-resistance bacteria on its global priority list with urgent demand for new therapies.[220]
Gram-negative bacteria employ *N*-acyl homoserine lactones (AHLs) as autoinducers.
AHLs are structurally different between the Gram-negative bacteria, in both the length and the substitution pattern of the acyl chain. The *las* system employs two proteins for its mode of action, Figure 4.4. LasI, an autoinducer synthase that catalyzes the synthesis of *N*-(3-oxo-dodecanoyl)-*L*-homoserine lactone (OdDHL), Figure 4.3, whereas lasR is a transcriptional regulatory protein, controlling the expression of various virulence factors, LasB elastase, LasA protease, and basic protease.
For the *rhl* system, RhlI is an autoinducer synthase, mediating the synthesis of *N*-butanoyl-*L*-homoserine lactone (BHL), Figure 4.3. Furthermore, RhlR activates the transcription of other virulence factors, the rhamnolipid biosynthesis genes (*rhlAB*).[214,221]



OdDHL BHL

Figure 4.3: Autoinducers applied by the *las* quorum sensing network

The communication process is sketched in Figure 4.4.[219,221,222]

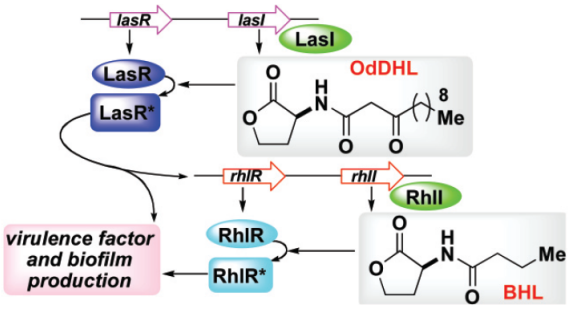


Figure 4.4: LasR and RhlR bind autoinducers to get activated as the corresponding LasR* and RhlR*. The Las system regulates the RhlR system. Modified from Glansdorp *et al.*[223]

Small molecules that induces a disruption of the quorum sensing communication network, also known as quorum quenchers, have been heavily investigated over the years, due to their low resistance tendency. It is believed that mutations will not be able to communicate with the rest of the group in the biofilm. Not only has there been a strong interest in the development of inhibitors (antagonists) against the quorum sensing system. Also, quorum sensing agonists (superagonists) has been studied.[224–226] These mimic a high-density state and prematurely induce dispersion of the biofilm, so the immune system is more likely to destroy the pathogens.
Modifying the acyl chain of homoserine lactone, has been the preferred choice, but several non-AHL synthesis programs have also been published. We will not describe all of the published inhibitors, but several good papers and reviews have already been mentioned in this dissertation. For more information see references [225, 227–229]. Quorum sensing inhibitors (QSis) have been found in food sources, two examples are ajoene from garlic[230] and iberin from horseradish[231], Figure 4.5.

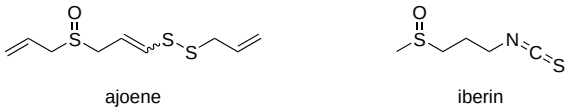
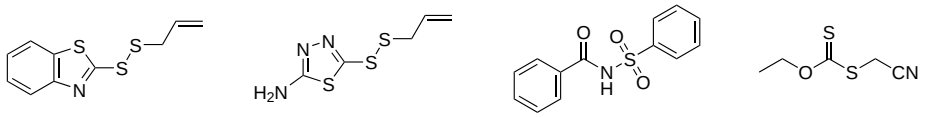


Figure 4.5: Naturally occuring QSis found in extracts from garlic and horseraddish

4.2 Hit Compound and Covalent Modifiers

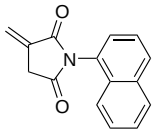
July Fong from the Yang group found six hit compounds that displayed activity against the quorum sensing network in *P. aeruginosa*. The most promising inhibitor was the 2-mercaptobenzothiazole disulfide allyl **4.1**, Figure 4.6, with an IC_{50} of 0.98 µM against a green fluorescent protein (gfp) tagged *P. aeruginosa* reporter strain,[214] PAO1-*lasB-gfp*[1]. The incorporation of the gfp tag makes it possible to detect the amount of produced LasB and thereby to detect LasR activity. A decrease in fluorescence, compared to a reference sample, show inhibition of OdDHL formation, and hereby restricted formation of gfp-tagged lasB.[219] LasB is a metalloprotease which acts by destroying the surrounding environment which liberates nutrition important for the growth of biofilm, as well as for initiating biofilm formation.[232]



4.1 **4.2** **4.3** **4.4**

Figure 4.6: Four hit compounds found to be active against quorum sensing. Adapted from Fong *et al.*[1]

The goal of my project, was to synthesize analogues of the *N*-napthyl itaconimide **4.5a**, Figure 4.7, to evaluate their quorum sensing properties. July found that **4.5a** has an IC_{50} value of 4.16 µM against PAO1-*lasB-gfp*. The scaffold is advantageous due to the easy accessibility to analogues, furthermore they can be synthesized at low costs.



4.5a

Figure 4.7: The fifth hit compound found in a screen of less than 500 compounds performed by J. Fong.

Itaconimides have been used to make polymers[233] and in 1999 a patent was filed on the synthesis and biological screening of itaconimides and citraconimides for activity against

mycobacteria.[234] The reported compounds demonstrated minimum inhibitory concentration (MIC) values of $\leq$ 20 µg/mL in a *M. tuberculosis* strain. Both the itaconimide- and citraconimide *N*-octyl analogues was synthesized and tested in the patent.

Itaconimide and citraconimide contain a α,β-conjugated double bond, and due to their Michael acceptor nature, these compounds could act as covalent modifiers. Covalent modifiers, reversible or irreversible, have been one of the most influential classes of drugs over time.[235] Singh *et al.* described, that three of the ten most selling drugs in America in 2009 were covalent inhibitors. Over time, a total of 39 covalent drugs have been approved by FDA, two well-known drugs are aspirin and β-lactams, such as penicillin,[236] Figure 4.8. A short timeline of covalent modifiers is illustrated in Figure 4.8.

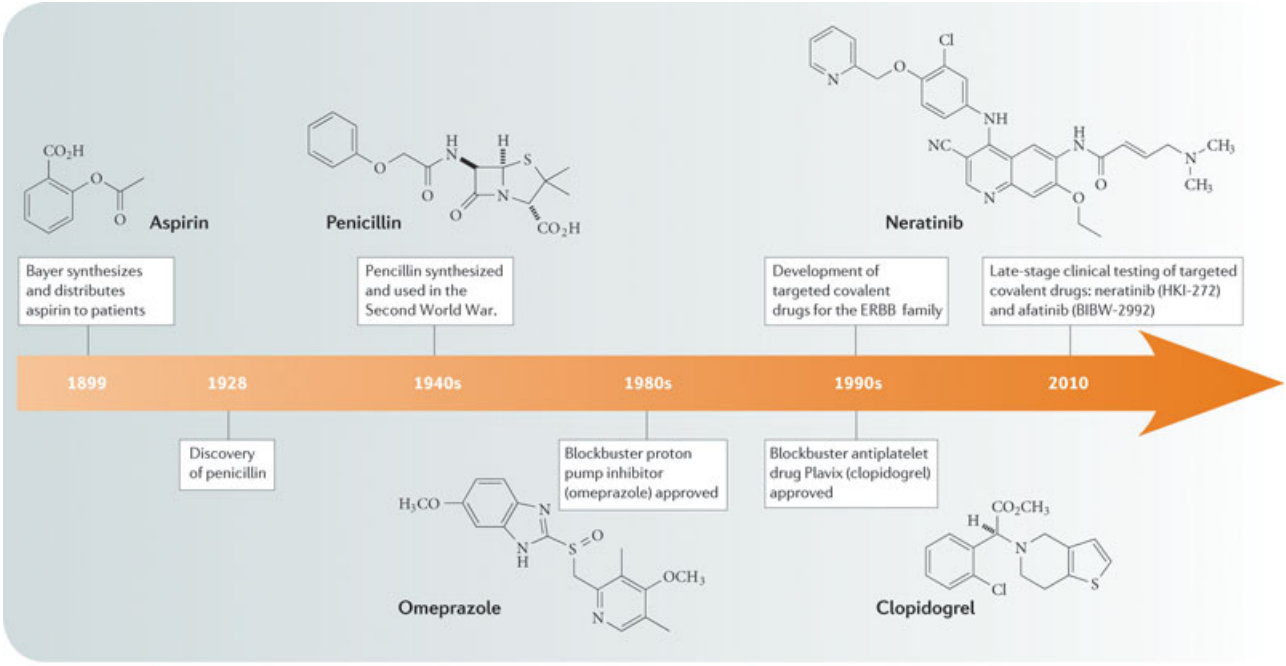


Figure 4.8: A selection of known covalent modifier and some of the recent approved. Afatinib, not drawn in the figure, was approved by FDA in 2013,[237] and neratinib is likely to be approved in 2017[238]. Adapted from Whitty *et al.*[235]

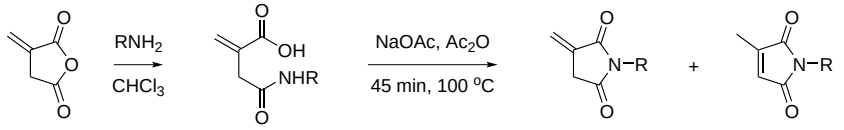
Omeprazole and clopidogrel becomes active *in vivo* by metabolic modifications.[236,239] Neratinib contains an acrylamide group, which is one of the most applied moieties,[240] that

are shown to react with cystein residues via a Michael addition/1,4-addition of the β-position.

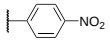
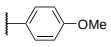
4.3 Synthesis of Quorum Sensing Inhibitors

The general procedure used for synthesis of itaconimide analogues, was originally published by Cava *et al.* in the 1960s for the synthesis of *N*-phenylmaleimide.[241] A primary amine (1.0 equiv.) is added to itaconic anhydride **4.6** in $CHCl_3$ which forms the corresponding α-itaconamic acid,[242] Table 4.1. In most cases, the precipitate was collected by filtration. For reactions with no precipitate or low yields, all volatiles were removed under reduced pressure. The α-itaconamic acid was added Ac_2O (3.5 equiv.) and NaOAc (0.5 equiv.) followed by stirring at elevated temperature. The imidization reactions afforded both the desired itaconimide and the citraconimide. The latter side-product probably arise from an isomerization reaction of the itaconimides. The citraconimides and the itaconimides was separated, and both included in the screening. All the synthesized compounds are listed in Table 4.1.

Table 4.1: Synthesized itaconimides and citraconimides



4.6 a Itaconimide b Citraconimide

Entry	R	a (%) [a]	b (%) [a]	Entry	R	a (%) [a]	b (%) [a]
1		**4.5a** (50)	**4.5b** (14)	11		**4.16a** (61)	**4.16b** (9)
2		**4.7a** (37)	**4.7b** (-)[b]	12		**4.17a** (37)	**4.17b** (20)
3		**4.8a** (17)	**4.8b** (-)[b]	13		**4.18a** (40)	**4.18b** (19)
4		**4.9a** (10)	**4.9b** (-)[b]	14		**4.19a** (-)[b]	**4.19b** (-) [b]
5		**4.10a** (47)	**4.10b** (-)[b]	15		**4.20a** (8)	**4.20b** (14)
6		**4.11a** (49)	**4.11b** (29)	16		**4.21a** (16)	**4.21b** (-)[b]
7		**4.12a** (-)[c]	**4.12b** (-)[d]	17		**4.22a** (13)	**4.22b** (10)
8		**4.13a** (31)	**4.13b** (40)	18		**4.23a** (4)	**4.23b** (12)
9		**4.14a** (24)	**4.14b** (14)	19		**4.24a** (2)	**4.24b** (11)
10		**4.15a** (35)	**4.15a** (15)				

a) Isolated yields b) not able to isolate c) a sample was available from the Tan lab d) not available

Generally, low yields were observed, and in some cases, it was not possible to isolate the citraconimide side-products.

For the biological screening, the compounds were tested in duplicates at three different

concentrations: 50 µM, 25 µM, and 12.5 µM. Each compound was tested for its effect on the bacteria growth, indicated by the optical density at 600 nm (OD_{600}), OD-AVE corresponds to the average value of the duplicates. Compounds causing decreased amounts of bacteria, display bactericidal activity and were discarded.

N-ortho-chlorophenyl **4.13a** and *N*-octyl **4.21a** showed more active than the original hit compound, **4.5a**. Due to these findings, the project was divided into two groups: *N*-aliphatic itaconimides and *N*-arylated itaconimides. All the inhibition curves of the itaconimides and the citraconimides are included in experimental section, Section 4.5.

To investigate the effect of aliphatic *N*-alkyl chain, we synthesized the hexyl, decyl, dodecyl, and tetradecyl analogues, Figure 4.9. Indeed, a correlation between the aliphatic chain length and the activity was found: tetradecyl (**4.24a**) > dodecyl (**4.23a**) > decyl (**4.22a**) > octyl (**4.21a**), Figure 4.9. We found that **4.24a** was the most active of all the synthesized compounds, Figure 4.9. At 50 µM, **4.24a** almost eradicated quorum sensing. However, antibiotic activity was observed by the OD_{600} measurement at 50 µM. This effect was significantly decreased at 12.5 µM.

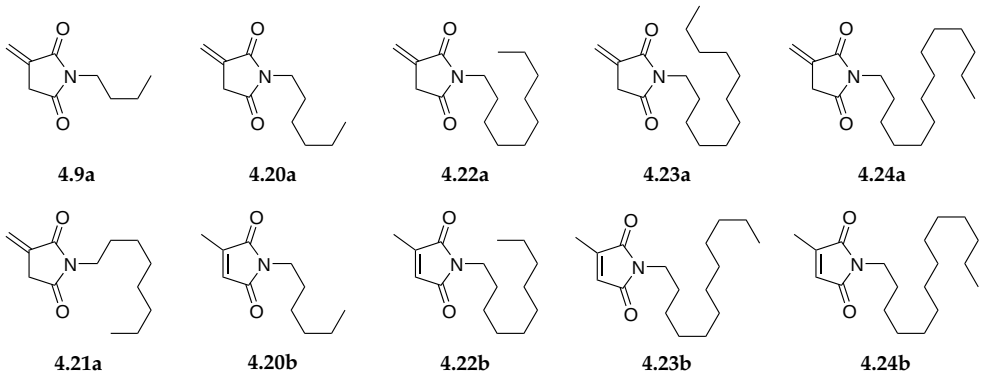


Figure 4.9: Synthesis of selected *N*-aliphatic itaconimides

The second part of this project involved the synthesis of *N*-arylated itaconimides with focus on using anilines functionalized with electron-withdrawing groups (EWGs), Figure Figure 4.10. This could potentially afford more reactive Michael acceptors. The syn-

thesized compounds are listed in Figure 4.10. The bromo substituted analogue **4.17a** showed highest activity. Brom is less electronegative than chlor. This means that the conjugated *exo*-cyclic alkene analogue **4.17a** is less electrophilic compared to the chloro analogue **4.13a**. This could indicate that the activity is not only affected by inductive effects. It was observed that both the *meta*-chloro **4.15a** and the *para*-chlor **4.16a** analogues had poorer activity. The decreased reactivity of **4.16a** could be a consequence of a chlorocarbonyl repulsion, constraining the spacial rotation around the N-C bond. We synthesized and tested the *ortho*-fluorophenyl analogue **4.18a** (not included in Figure 4.10), but remarkably a poor inhibition profile was observed, see Section 4.5, which emphasizes the statement that the inductive effect is not the only actor to afford strong inhibitors. Unfortunately, we did not synthesize the *para*-iodophenyl analogue, which could have given us more information about the electronegativity-dependency profile.

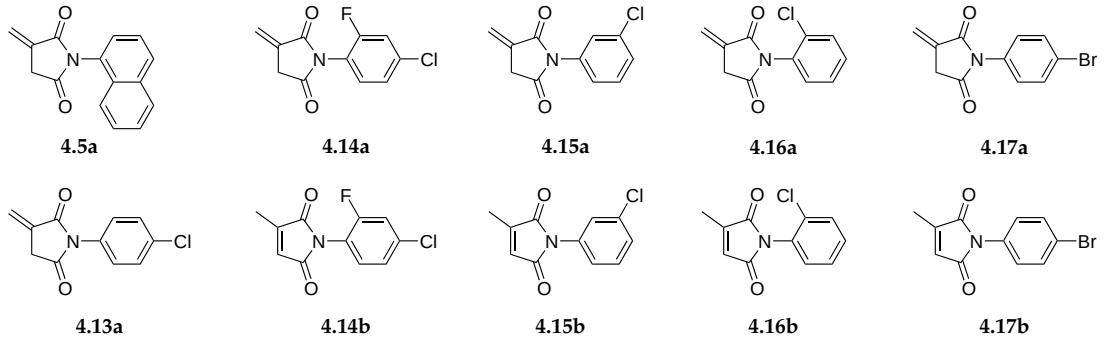


Figure 4.10: Synthesis of selected *N*-arylated itaconimides

Finally, the most active compounds were tested against each other for the inhibition of *P. aeruginosa*, Figure 4.11. We clearly observed that the tetradecyl compound **4.24a** was the most active of all the synthesized compounds.

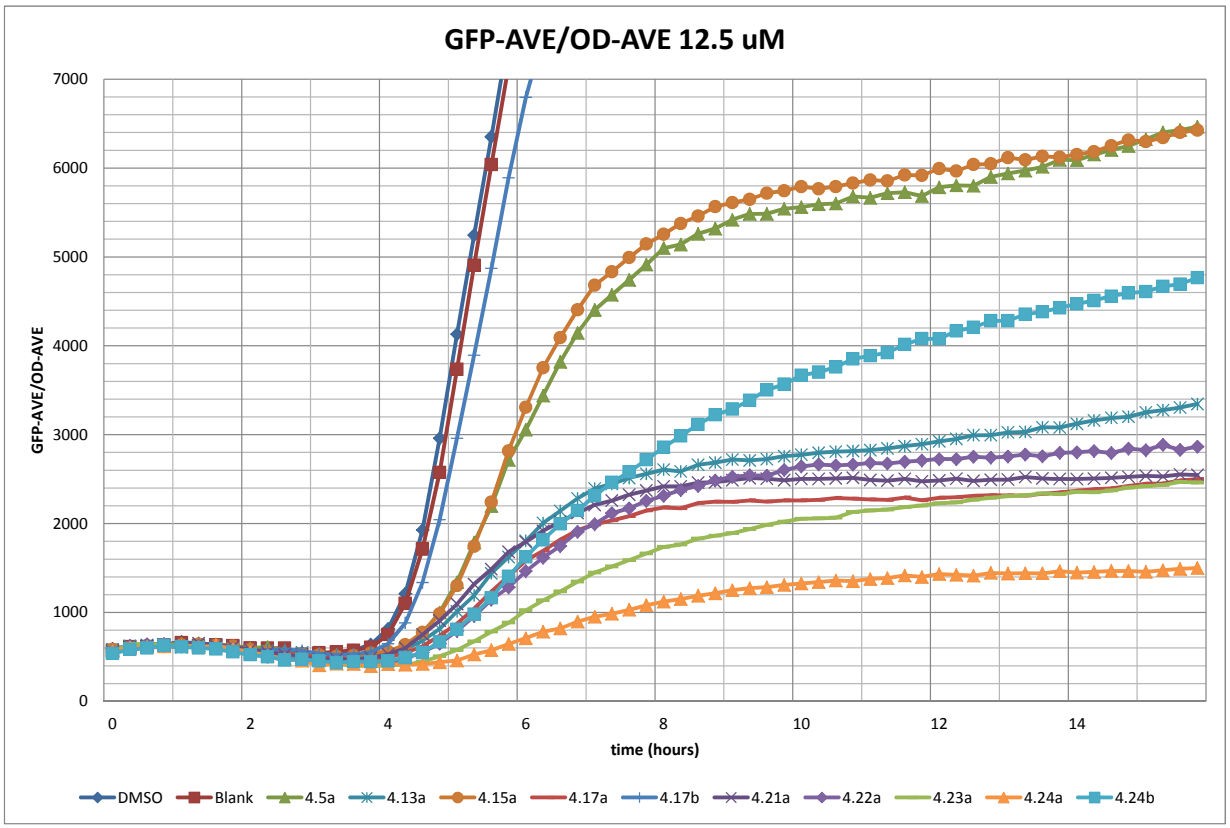


Figure 4.11: A screen including the most active compounds synthesized to find the most potent inhibitor

4.4 Conclusion

With the synthesis of **4.13a** and **4.21a** we found compounds with higher activity than the original hit compound **4.5a**. All inhibition curves are included in the Section 4.5. As highlighted earlier, QSis often have a long aliphatic chain that resembles the natural produced autoinducer. The EWG properties would afford a stronger Michael acceptor. However, the *para*-fluor analogue **4.18a** displayed decreased activity than the *para*-bromophenyl arylated itaconimide **4.17a**. It could imply that the fluorine analogue made the Michael acceptor too reactive, resulting in unspecific reactivity. The *N*-tetradecyl analogue **4.24a** was identified as the most active compound in this study. Fong recently found that the IC_{50} values for the *para*-bromophenyl itaconimide **4.17a** and the *N*-tetradecyl itaconimide **4.24a** against PAO1-*lasB*-*gfp* are 3.40 µM and 0.21 µM, respectively. These are very promis-

ing results. The tetradecyl analogue display almost 20 fold higher reactivity than the napthyl analogue and shows almost five-fold increased activity compared to the the recently published 2-mercaptobenzothiazole disulfide allyl **4.1** QSi by July Fong. More biological testing is currently being conducted in Singapore to establish its mode of action.

4.5 Experimental Section

4.5.1 General Methods

NMR spectra were recorded on BrukerAvance III-400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. The specific deuterated solvent is stated for each compound. Chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz.

Mass spectrometric data were recorded on a ThermoFinnigan PolarisQ MS/ThermoFinnigan LCQ Fleet MS. The values were reported in the unit of mass to charge ratio (m/z).
For TLC, Merck C-60 F_{254} silica gel plates were used and developed using UV-light or a suitable stain. Flash chromatography was performed using a glass column packed with Merck 60 silica gel (40-63 µm particles) as stationary phase. All liquid phases are specified in experimental procedures.

Melting points were measured using a Stuart SMP30 melting point apparatus.

4.5.2 *P. aeruginosa* QS Inhibition Assay[1,243]

Compounds were prepared in 96-well microtiter plate (Nunc, Denmark) at concentration of 10 mM in 100% biograde DMSO. Test compounds were then mixed with ABTGC medium and serial diluted to give a final concentration of 20 µM in the first well. An overnight culture of PAO1-*lasB-gfp* strain (grown in LB medium at 37 °C, 200 rpm) was diluted in ABTGC medium to a final optical density at 600 nm (OD_{600}) of 0.02 (2.5×10^8 CFU/mL). An equal amount of the bacterial suspension was added to the wells to reach final inhibitor concentration of 10 µM. DMSO control (0.1% final concentration) and blank control were used. The microtiter plate was incubated at 37 °Cin Tecan Infinate 200 Pro plate reader (Tecan Group Ltd., Männedorf, Switzerland) to measure the cell density (OD_{600}) and GFP fluorescence (excitation at 485 nm, emission at 535 nm) with 15 min intervals for at least 12 h. The inhibition assay for all test compounds and controls were done in duplicate manner. Originally, a compound, G10, was found to be active against *P. aeruginosa*, in the beginning this compound was used as a benchmark.

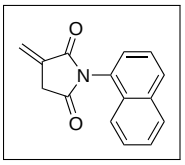
4.5.3 General Procedure: Synthesis of Imides

In a round-bottomed flask containing a magnetic stirring bar, the primary amine was slowly added to a vigorously stirring solution of itaconic anhydride **4.6** (1.0 equiv.) in $CHCl_3$ (0.1–0.2 M) and stirred for 1 h. In most cases, precipitation was observed within minutes. The carboxylic amide intermediate was collected by filtration and washed with a minimum $CHCl_3$. When the intermediate did not precipitate, all volatiles were removed under reduced pressure, and used without further purification. In a round-bottomed flask equipped with a plastic stopper, Ac_2O (3.5 equiv.) and NaOAc (0.5 equiv) was added to the intermediate and stirred for 45 min at 100 °C. The dark solution was cooled to rt and poured into ice cold H_2O and extracted with EtOAc (3 x). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residues were purified immediately by flash chromatography. During the imide-formation isomerization occurred, and for some of the reactions it was possible to isolate this compound.

3-Methylene-1-(naphthalen-1-yl)pyrrolidine-2,5-dione (4.5a) and 3-methyl-1-(naphthalen-1-yl)-1*H*-pyrrole-2,5-dione (4.5b)

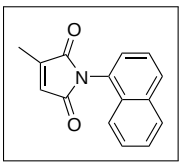
According to the general procedure for synthesis of imides, 1-naphthylamine (639 mg, 4.46 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (500 mg, 4.46 mmol, 1.0 equiv.) in $CHCl_3$ (42.8 mL) afforded a pale pink solid that was treated with Ac_2O (1.48 mL, 15.6 mmol, 3.5 equiv.) and NaOAc (146 mg, 1.78 mmol, 0.4 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:4).

3-Methylene-1-(naphthalen-1-yl)pyrrolidine-2,5-dione (4.5a)



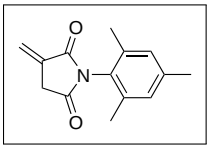
528 mg, 50%; pale yellow solid; R_f = 0.11 (EtOAc:hexane 1:4; UV); IR (neat) ν (cm^{-1}): 3069, 3051, 2969, 2921, 1710, 1662, 1597, 1468; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.99–7.92 (m, 2H, Ar**H**), 7.60–7.50 (m, 4H, Ar**H**), 7.38 (dd, *J* = 7.3, 1.0 Hz, 1H, Ar**H**), 6.53 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.81 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.67 (m, 2H, C(O)C**H$_2$**C(CH_2)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.3, 169.0, 134.6, 133.3, 130.3, 129.4, 128.8, 128.7, 127.4, 126.8, 126.4, 125.5, 122.2, 122.1, 34.4;

3-Methyl-1-(naphthalen-1-yl)-1*H*-pyrrole-2,5-dione (4.5b)



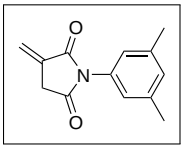
151 mg, 14%; yellow solid; R_f = 0.16 (EtOAc:hexane 1:4; UV); IR (neat) ν (cm^{-1}): 3091, 3059, 3013, 2960, 1702, 1637, 1597, 1465, 1374; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.96–7.91 (m, 2H, Ar**H**), 7.57–7.50 (m, 4H, Ar**H**), 7.36 (dd, *J* = 7.3, 1.3 Hz, 1H, Ar**H**), 6.60 (q, *J* = 1.6 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.25 (d, *J* = 1.6 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 171.3, 170.2, 146.3, 134.6, 130.6, 129.9, 128.7, 128.2, 127.9, 127.2, 127.0, 126.7, 125.5, 122.5, 11.5;

1-Mesityl-3-methylenepyrrolidine-2,5-dione (4.7a)



According to the general procedure for synthesis of imides, 2,4,6-trimethylaniline (376 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded a white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). The residues were purified by flash chromatography (EtOAc:hexane 1:3) to afford **4.7a** (226 mg, 37%) as a light brown solid. R_f = 0.19 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3095, 2984, 2952, 2919, 2858, 1707, 1660, 1485, 1371; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.98 (s, 2H, Ar**H**), 6.47 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.74 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.55 (t, *J* = 2.3 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)), 2.31 (s, 3H, ArC**H$_3$**), 2.07 (s, 6H, 2 x ArC**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.8, 168.5, 139.5, 135.3, 133.2, 129.4, 127.5, 121.6, 34.1, 21.1, 17.8;

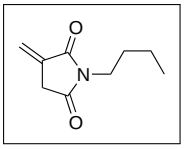
1-(3,5-Dimethylphenyl)-3-methylenepyrrolidine-2,5-dione (4.8a)



According to the general procedure for synthesis of imides, 3,5-dimethylaniline (334 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (301 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (25.6 mL) afforded a pale brown solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The cooled reaction mixture was poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Flash chromatography (EtOAc:hexane 1:4) provided of **4.8a** (99 mg, 17%) as a pale orange solid. R_f = 0.17 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 2998, 2958, 2922, 1706,

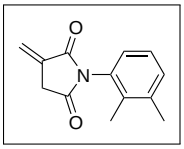
1664, 1595, 1469, 1382; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.04 (s, 1H, Ar**H**), 6.91 (m, 2H, Ar**H**), 6.46 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.72 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.49 (t, *J* = 2.3 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)), 2.35 (s, 6H, 2 x ArC**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.2, 168.8, 139.2, 133.2, 131.7, 130.8, 124.3, 121.7, 34.1, 21.4;

1-Butyl-3-methylenepyrrolidine-2,5-dione (4.9a)



According to the general procedure for synthesis of imides, *n*-butylamine (265 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (301 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded after evaporation a white residue that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Flash chromatography (EtOAc:hexane 1:3) provided **4.9a** (47 mg, 10%) as a red oil. IR (neat) ν (cm^{-1}): 2959, 2935, 2873, 1698, 1663; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.33 (t, *J* = 2.4 Hz, 1H, CCH**H**), 5.61 (t, *J* = 2.4 Hz, 1H, CC**H**H), 3.57 (t, *J* = 7.5 Hz, 2H, NC**H$_2$**CH_2), 3.30 (t, *J* = 2.4 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)), 1.61–1.54 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.37–1.28 (m, 2H, NCH_2CH_2C**H$_2$**CH_3), 0.92 (m, 3H, $NCH_2CH_2CH_2$C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 174.0, 169.7, 133.5, 120.5, 38.8, 33.9, 29.9, 20.2, 13.7;

1-(2,3-Dimethylphenyl)-3-methylenepyrrolidine-2,5-dione (4.10a)



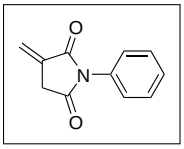
According to the general procedure for synthesis of imides, 2,3-dimethylaniline (327 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (25.6 mL) afforded a pink solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purification by flash column chromatography (EtOAc:hexane 1:3) gave **4.10a** (286 mg, 47%) as a light yellow solid. R_f = 0.14 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3088, 3005, 2983, 2953, 2923, 1706, 1662, 1601, 1469, 1373; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.26-7.19 (m, 2H, Ar**H**), 6.95 (d, *J* = 7.4 Hz, 1H, Ar**H**), 6.47 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.74 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.54 (m, 2H, C(O)C**H$_2$**C(CH_2)C(O)), 2.33 (s, 3H, ArC**H$_3$**), 2.01 (s, 3H, ArC**H$_3$**); ^{13}C-NMR

(101 MHz, $CDCl_3$): δ 173.1, 168.8, 138.6, 134.3, 133.6, 131.3, 131.0, 126.5, 125.6, 121.8, 34.2, 20.5, 14.5;

3-Methylene-1-phenylpyrrolidine-2,5-dione (4.11a) and 3-methyl-1-phenyl-1*H*-pyrrole-2,5-dione (4.11b)

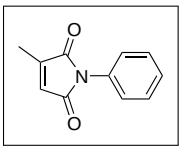
According to the general procedure for synthesis of imides, aniline (244 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (301 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded a white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3).

3-Methylene-1-phenylpyrrolidine-2,5-dione (4.11a)



245 mg, 49%; off white solid; R_f = 0.12 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3096, 3069, 2992, 2957, 1701, 1661, 1593, 1496; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.52–7.47 (m, 2H, Ar**H**), 7.43–7.39 (m, 1H, Ar**H**), 7.35–7.32 (m, 2H, Ar**H**), 6.48 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.75 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.52 (t, *J* = 2.3 Hz, 2H, C(O)C$\mathbf{H_2}$C(CH_2)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.0, 168.6, 133.1, 132.0, 129.3, 128.8, 126.5, 121.9, 34.1;

3-Methyl-1-phenyl-1*H*-pyrrole-2,5-dione (4.11b)



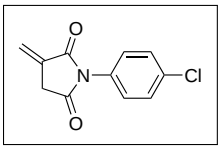
147 mg, 29%; pale yellow solid; R_f = 0.29 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3086, 3072, 2926, 1693, 1639, 1594, 1395; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.48–7.44 (m, 2H, Ar**H**), 7.37–7.33 (m, 3H, Ar**H**), 6.48 (q, *J* = 1.7 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.18 (d, *J* = 1.7 Hz, 3H, C(O)CHC(C$\mathbf{H_3}$)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 170.8, 169.7, 145.9, 131.8, 129.2, 127.9, 127.6, 126.1, 11.3;

1-(4-Chlorophenyl)-3-methylenepyrrolidine-2,5-dione (4.13a) and 1-(4-chlorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.13b)

According to the general procedure for synthesis of imides, 4-chloroaniline (341 mg, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$

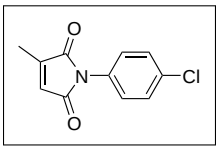
(12.8 mL) afforded a white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3).

1-(4-Chlorophenyl)-3-methylenepyrrolidine-2,5-dione (4.13a)



182 mg, 31%; off-white solid; R_f = 0.1 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3095, 3063, 1705, 1665, 1493; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.48–7.44 (m, 2H, Ar**H**), 7.33–7.30 (m, 2H, Ar**H**), 6.49 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.76 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.51 (t, *J* = 2.3 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.6, 168.3, 134.6, 132.8, 130.4, 129.5, 127.7, 122.3, 34.1;

1-(4-Chlorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.13b)

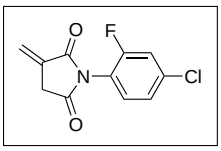


237 mg, 40%; pale yellow solid R_f = 0.24 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3083, 2928, 1696, 1644, 1495, 1400; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.44–7.41 (m, 2H, Ar**H**), 7.33–7.30 (m, 2H, Ar**H**), 6.49 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.17 (t, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 170.4, 169.4, 146.1, 133.5, 130.3, 129.4, 127.7, 127.1, 11.3;

1-(4-Chloro-2-fluorophenyl)-3-methylenepyrrolidine-2,5-dione (4.14a) and 1-(4-chloro-2-fluorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.14b)

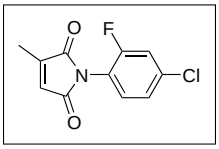
According to the general procedure for synthesis of imides, 4-chloro-2-fluoroaniline (297 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afford a white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3).

1-(4-Chloro-2-fluorophenyl)-3-methylenepyrrolidine-2,5-dione (4.14a)



157 mg, 24%; off-white solid; R_f = 0.13 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3119 3065, 2925, 1713, 1663, 1586, 1498; ^{1}H-NMR (400 MHz, DMSO): δ 7.29–7.20 (m, 3H, Ar**H**), 6.49 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.77 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.72–3.53 (m, 2H, C(O)C**H$_2$**C(CH_2)C(O))); ^{13}C-NMR (101 MHz, DMSO): δ 171.9, 167.7, 157.4 (d, J_{CF} = 256.3 Hz), 136.5 (d, J_{CF} = 10.0 Hz), 132.9, 131.15, 131.14, 125.4 (d, J_{CF} = 3.9 Hz), 122.8, 117.9 (d, J_{CF} = 23.1 Hz), 34.32;

1-(4-Chloro-2-fluorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.14b)

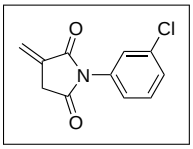


87 mg, 14 % off-white solid IR (neat) ν (cm^{-1}): 3102, 2993, 2957, 2923, 1709, 1641, 1585, 1500, 1388; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.27–7.18 (m, 3H, Ar**H**), 6.53–6.52 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.18 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 169.7, 168.5, 157.7 (d, J_{CF} = 255.9 Hz), 146.7, 135.7 (d, J_{CF} = 9.3 Hz), 130.49, 130.48, 128.2 125.2 (d, J_{CF} = 3.9 Hz), 117.7 (d, J_{CF} = 23.1 Hz), 11.4;

1-(3-Chlorophenyl)-3-methylenepyrrolidine-2,5-dione (4.15a) and 1-(3-chlorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.15b)

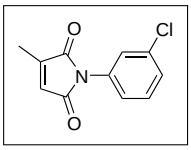
According to the general procedure for synthesis of imides, 3-chloroaniline (283 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded a off-white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated for 45 min. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3).

1-(3-Chlorophenyl)-3-methylenepyrrolidine-2,5-dione (4.15a)



206 mg, 35%; off-white solid; R_f = 0.1 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3119, 3079, 3011, 2922, 1706, 1659, 1593, 1477, 1381; ^{1}H-NMR (400 MHz, DMSO): δ 7.44–7.37 (m, 3H, Ar**H**), 7.28–7.26 (m, 1H, Ar**H**), 6.49 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.77 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.51 (t, *J* = 2.3 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)); ^{13}C-NMR (101 MHz, DMSO): δ 172.5, 168.2, 134.8, 132.1, 132.7, 130.2, 129.0, 126.8, 124.7, 122.4, 34.1;

1-(3-Chlorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.15b)

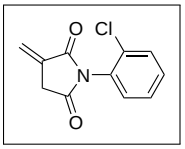


90 mg, 15%; off-white foam; R_f = 0.24 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3093, 2963, 2925, 1706, 1642, 1594, 1478, 1388; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.41–7.27 (m, 4H, Ar**H**), 6.49 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.18 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 170.3, 169.2, 146.1, 134.7, 132.9, 130.1, 127.9, 127.7, 126.0, 123.9, 11.3;

1-(2-Chlorophenyl)-3-methylenepyrrolidine-2,5-dione (4.16a) and 1-(2-chlorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.16b)

According to the general procedure for synthesis of imides, 2-chloroaniline (282 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded a off-white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3).

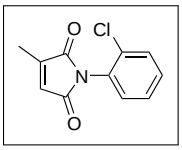
1-(2-Chlorophenyl)-3-methylenepyrrolidine-2,5-dione (4.16a)



362 mg, 61%; off-white solid; R_f = 0.13 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3101, 2960, 2932, 1710, 1662, 1483; ^{1}H-NMR (400 MHz, DMSO): δ 7.57–7.53 (m, 1H, Ar**H**), 7.44–7.37 (m, 2H, Ar**H**), 7.28–7.23 (m, 1H, Ar**H**), 6.48 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.76 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.63–3.48

(m, 2H, C(O)C**H$_2$**C(CH$_2$)C(O)); ^{13}C-NMR (101 MHz, DMSO): δ 172.3, 168.0, 133.2, 132.5, 131.0, 130.7, 130.1, 130.0, 128.0, 122.4, 34.4;

1-(2-Chlorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.16b)

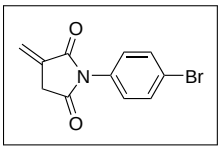


55 mg, 9%; light yellow solid; R$_f$ = 0.28 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3095, 3070, 3042, 1702, 1642, 1488, 1392; ^{1}H-NMR (400 MHz, DMSO): δ 7.54–7.52 (m, 1H, Ar**H**), 7.42–7.35 (m, 2H, Ar**H**), 7.27–7.24 (m, 3H, Ar**H**), 6.51 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH$_3$)C(O)), 2.19 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)); ^{13}C-NMR (101 MHz, DMSO): δ Peak ν(F1) [ppm] Intensity [abs] Annotation 170.1, 169.0, 146.4, 133.3, 130.8, 130.7, 130.5, 129.6, 128.0, 127.8, 11.4;

1-(4-Bromophenyl)-3-methylenepyrrolidine-2,5-dione (4.17a) and 1-(4-bromophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.17b)

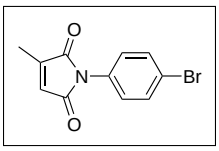
According to the general procedure for synthesis of imides, 4-bromoaniline (460 mg, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded a white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3).

1-(4-Bromophenyl)-3-methylenepyrrolidine-2,5-dione (4.17a)



267 mg, 37%; off-white solid; R$_f$ = 0.09 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3093, 3062, 2994, 1705, 1665, 1490; ^{1}H-NMR (400 MHz, DMSO): δ7.62–7.59 (m, 2H, Ar**H**), 7.27–7.23 (m, 2H, Ar**H**), 6.48 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.76 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.54 (t, *J* = 2.3 Hz, 2H, C(O)C**H$_2$**C(CH$_2$)C(O))); ^{13}C-NMR (101 MHz, DMSO): δ172.6, 168.2, 132.8, 132.5, 130.9, 128.0, 122.6, 122.3, 34.1;

1-(4-Bromophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.17b)

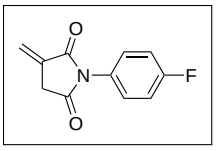


142 mg, 20%; light yellow solid; R_f = 0.28 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3087, 2962, 2923, 2843, 1697, 1646, 1592, 1491, 1375; ^{1}H-NMR (400 MHz, DMSO): δ7.59–7.56 (m, 2H, Ar**H**), 7.28–7.24 (m, 2H, Ar**H**), 6.48 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.17 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)); ^{13}C-NMR (101 MHz, DMSO): δ170.4, 169.3, 146.1, 132.4, 130.9, 127.7, 127.3, 121.4, 11.3;

1-(4-Fluorophenyl)-3-methylenepyrrolidine-2,5-dione (4.18a) and 1-(4-fluorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.18b)

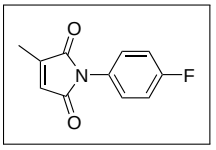
According to the general procedure for synthesis of imides, 4-fluoroaniline (254 µL, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded a white solid that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purified by flash chromatography (EtOAc:hexane 1:3). affording 1-(4-fluorophenyl)-3-methylenepyrrolidine-2,5-dione **4.18a** as a off white solid () and 1-(4-fluorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione **4.18b** as a white solid.

1-(4-Fluorophenyl)-3-methylenepyrrolidine-2,5-dione (4.18a)



222 mg, 40%; off-white solid; R_f = 0.08 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3067, 2962, 2928, 1703, 1664, 1602, 1511, 1386; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.36–7.31 (m, 2H, Ar**H**), 7.20–7.14 (m, 2H, Ar**H**), 6.48 (t, *J* = 2.4 Hz, 1H, CCH**H**), 5.75 (t, *J* = 2.4 Hz, 1H, CC**H**H), 3.51 (t, *J* = 2.4 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O))); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.9, 168.5, 162.3 (J_{CF} = 248.6 Hz), 132.9, 128.4 (J_{CF} = 8.6 Hz), 127.9 (J_{CF} = 3.25 Hz), 122.2, 116.3 (J_{CF} = 23.0 Hz), 34.0;

1-(4-Fluorophenyl)-3-methyl-1*H*-pyrrole-2,5-dione (4.18b)



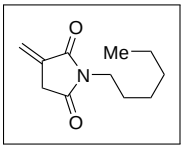
107 mg, 19%; off-white solid; R_f = 0.24 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 3093, 3078, 2997, 2931, 1698, 1640, 1602, 1513, 1405; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.34–7.29 (m, 2H, Ar**H**), 7.17–7.11 (m, 2H, Ar**H**), 6.48 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 2.17 (d, *J* = 1.8 Hz, 3H,

C(O)CHC(C**H$_3$**)C(O)), ^{13}C-NMR (101 MHz, $CDCl_3$): δ 170.7, 169.6, 161.8 (d, J_{CF} = 247.6 Hz), 146.01, 127.86 (d, J_{CF} = 8.5 Hz), 127.70 (d, J_{CF} = 3.1 Hz), 127.61, 116.18 (d, J_{CF} = 22.7 Hz), 11.3;

1-Hexyl-3-methylenepyrrolidine-2,5-dione (4.20a) and
1-hexyl-3-methyl-1*H*-pyrrole-2,5-dione (4.20b)

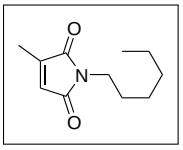
According to the general procedure for synthesis of imides, hexylamine (1.95 mL, 8.92 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (1.00 g, 8.92 mmol, 1.0 equiv.) in $CHCl_3$ (42.8 mL) afforded after evaporation a white residue that was treated with Ac_2O (3.0 mL, 31.2 mmol, 3.5 equiv.) and NaOAc (366 mg, 4,46 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). Purified by flash chromatography (hexane → 1:7 EtOAc:Hexane).

1-Hexyl-3-methylenepyrrolidine-2,5-dione (4.20a)



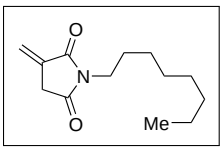
145 mg, 8%; red oil; R_f = 0.13 (EtOAc:hexane 1:7; UV); IR (neat) ν (cm^{-1}): 2930, 2859, 1701, 1663, 1391; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.32 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.60 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.55 (t, *J* = 7.5 Hz, 2H, NC**H$_2$**CH_2), 3.29 (t, *J* = 2.3 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)), 1.61-1.54 (m, 2H, NCH_2C**H$_2$**), 1.30-1.27 (m, 6H, C**H$_2$**C**H$_2$**C**H$_2$**), 0.86 (t, *J* = 6.8 Hz, 3H, C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ174.0, 169.7, 133.5, 120.4, 39.0, 33.9, 31.4, 27.8, 26.6, 22.6, 14.1;

1-Hexyl-3-methyl-1*H*-pyrrole-2,5-dione (4.20b)



246 mg, 14%; red oil; R_f = 0.34 (EtOAc:Heptane (1:7); UV); IR (neat) ν (cm^{-1}): 3100, 2956, 2930, 2859, 1702, 1643, 1376; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.29 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 3.46 (t, *J* = 7.3 Hz, 2H, NC**H$_2$**CH_2), 2.06 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)), 1.58–1.51 (m, 2H, NCH_2C**H$_2$**), 1.28-1.24 (m, 6H, C**H$_2$**C**H$_2$**C**H$_2$**); 0.86 (t, *J* = 6.7 Hz, 3H, C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.1, 171.1, 145.6, 127.3, 38.1, 31.4, 28.7, 26.5, 22.6, 14.1, 11.1;

3-Methylene-1-octylpyrrolidine-2,5-dione (4.21a)

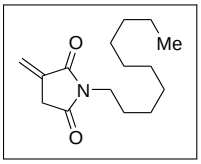


According to the general procedure for synthesis of imides, octylamine (442 mg, 2.68 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (300 mg, 2.68 mmol, 1.0 equiv.) in $CHCl_3$ (12.8 mL) afforded after evaporation a white residue that was treated with Ac_2O (886 µL, 9.37 mmol, 3.5 equiv.) and NaOAc (110 mg, 1.34 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). Purification by flash chromatography (1:3 EtOAc:Hexane) provided **4.21a** (98 mg, 16%) as a off white solid. R_f = 0.27 (EtOAc:hexane 1:3; UV); IR (neat) ν (cm^{-1}): 2925, 2855, 1703, 1664. 1367; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.33 (t, *J* = 2.3 Hz, 1H, CCH**H**), 5.61 (t, *J* = 2.3 Hz, 1H, CC**H**H), 3.56 (t, *J* = 7.5 Hz, 2H, NC**H₂**CH_2), 3.30 (t, *J* = 2.3 Hz, 2H, C(O)C**H₂**C(CH_2)C(O)), 1.59 (p, *J* = 7.3 Hz, 2H, NCH_2C**H₂**), 1.29–1.25 (m, 10H, C**H₂**C**H₂**C**H₂**C**H₂**C**H₂**), 0.87 (t, *J* = 6.7 Hz, 3H, CH_2C**H₃**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 174.0, 169.7, 133.5, 120.5, 39.1, 33.9, 31.9, 29.35, 27.9, 27.0, 22.8, 14.2;

1-Decyl-3-methylenepyrrolidine-2,5-dione (4.22a) and
1-decyl-3-methyl-1*H*-pyrrole-2,5-dione (4.22b)

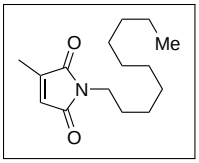
According to the general procedure for synthesis of imides, decylamine (1.78 mL, 8.92 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (1.00 g, 8.92 mmol, 1.0 equiv.) in $CHCl_3$ (42.8 mL) afforded after evaporation a white residue that was treated with Ac_2O (2.95 mL, 31.2 mmol, 3.5 equiv.) and NaOAc (366 mg, 4,46 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). Purified by flash chromatography (hexane → EtOAc:hexane 1:9).

1-Decyl-3-methylenepyrrolidine-2,5-dione (4.22a)



287 mg, 13%; red oil; R_f = 0.11 (EtOAc:hexane 1:9; UV); IR (neat) ν (cm^{-1}): 3093, 2913, 2847, 1697, 1662, 1390; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.33 (t, *J* = 2.5 Hz , 1H, CCH**H**), 5.60 (t, *J* = 2.0 Hz , 1H, CC**H**H), 3.56 (t, *J* = 7.5 Hz , 2H, NC**H₂**CH_2), 3.30 (t, *J* = 2.2 Hz , 2H, C(O)C**H₂**C(CH_2)C(O)), 1.62–1.55 (m, 2H, NCH_2C**H₂**), 1.28–1.24 (m, 14H, C**H₂**C**H₂**C**H₂**C**H₂**C**H₂**C**H₂**C**H₂**), 0.87 (t, *J* = 6.9 Hz, 3H, CH_2C**H₃**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 174.0, 169.7, 133.5, 120.4, 39.1, 33.9, 32.0, 29.63, 29.59, 29.4, 29.3, 27.9, 27.0, 22.8, 14.2;

1-Decyl-3-methyl-1*H*-pyrrole-2,5-dione (4.22b)

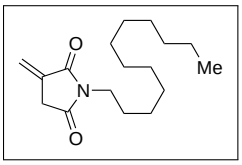


226 mg, 10%; pink semi-solid; R_f = 0.30 (EtOAc:hexane 1:9; UV); IR (neat) ν (cm^{-1}): 2923, 2854, 1704, 1643, 1375; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.29 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 3.47 (t, *J* = 7.4 Hz, 2H, NC**H$_2$**CH_2), 2.06 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)), 1.59–1.52 (m, 2H, NCH_2C**H$_2$**), 1.27–1.24 (m, 14H, C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**), 0.87 (t, *J* = 6.9 Hz, 3H, CH_2C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.1, 171.1, 145.6, 127.3, 38.1, 32.0, 29.64, 29.61, 29.4, 29.3, 28.7, 26.9, 22.8, 14.2, 11.1;

1-Dodecyl-3-methylenepyrrolidine-2,5-dione (4.23a) and 1-dodecyl-3-methyl-1*H*-pyrrole-2,5-dione (4.23b)

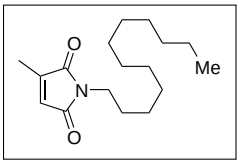
According to the general procedure for synthesis of imides, dodecylamine (2.05 mL, 8.92 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (1.00 g, 8.92 mmol, 1.0 equiv.) in $CHCl_3$ (64.2 mL) afforded a white solid that was treated with Ac_2O (2.95 mL, 31.2 mmol, 3.5 equiv.) and NaOAc (366 mg, 4,46 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). Purified by flash chromatography (EtOAc:hexane 1:9).

1-Dodecyl-3-methylenepyrrolidine-2,5-dione (4.23a)



92 mg, 4% dark red semi-solid; R_f = 0.28 (EtOAc:hexane 1:9; UV); IR (neat) ν (cm^{-1}): 3093, 2914, 2847, 1695, 1663, 1344; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.33 (t, *J* = 2.2 Hz, 1H, CCH**H**), 5.61 (t, *J* = 2.2 Hz, 1H, CC**H**H), 3.56 (t, *J* = 7.5 Hz, 2H, NC**H$_2$**CH_2), 3.30 (t, *J* = 2.2 Hz, 2H, C(O)C**H$_2$**C(CH_2)C(O)), 1.62-1.55 (m, 2H, NCH_2C**H$_2$**), 1.28-1.24 (m, 18H, C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**), 0.87 (t, *J* = 6.9 Hz, 3H, CH_2C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 174.0, 170.0, 133.5, 120.5, 39.1, 33.9, 32.1, 29.76, 29.75, 29.7, 29.6, 29.5, 29.3, 27.9, 27.0, 22.8, 14.2;

1-Dodecyl-3-methyl-1*H*-pyrrole-2,5-dione (4.23b)

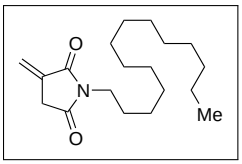


295 mg, 12%; pink semi-solid; R_f = 0.29 (EtOAc:hexane 1:9; UV); IR (neat) ν (cm^{-1}): 3081, 2915, 2848, 1685, 1638, 1376; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.29 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 3.47 (t, *J* = 7.3 Hz, 2H, NC**H$_2$**CH_2), 2.07 (d, *J* = 1.8 Hz, 3H, C(O)CHC(C**H$_3$**)C(O)), 1.59–1.52 (m, 2H, NCH_2C**H$_2$**), 1.27–1.24 (m, 18H, C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**), 0.87 (t, *J* = 1.8 Hz, 3H CH_2C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.0 171.0 145.4 127.2 38.0 32.0 29.61, 29.55, 29.5, 29.3, 29.1, 28.6, 26.8, 22.7, 14.1, 11.0;

3-Methylene-1-tetradecylpyrrolidine-2,5-dione (4.24a) and 3-methyl-1-tetradecyl-1*H*-pyrrole-2,5-dione (4.24b)

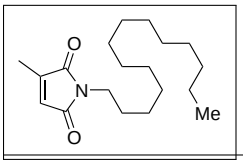
According to the general procedure for synthesis of imides, tetradecylamine (1.90 g, 8.92 mmol, 1.0 equiv.) and itaconic anhydride **4.6** (1.00 g, 8.92 mmol, 1.0 equiv.) in $CHCl_3$ (68.2 mL) afforded at white white solid that was treated with Ac_2O (2.95 mL, 31.2 mmol, 3.5 equiv.) and NaOAc (366 mg, 4,46 mmol, 0.5 equiv.) and heated at 100 °C. The dark reaction mixture was cooled and poured into ice cold H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). Purified by flash chromatography (hexane → EtOAc:hexane 1:9).

3-Methylene-1-tetradecylpyrrolidine-2,5-dione (4.24a)



63 mg, 2%; light brown solid; IR (neat) ν (cm^{-1}): ; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.33 (t, *J* = 2.5 Hz , 1H, CCH**H**), 5.61 (t, *J* = 2.0 Hz , 1H, CC**H**H), 3.56 (t, *J* = 7.5 Hz , 2H, NC**H$_2$**CH_2), 3.30 (t, *J* = 2.2 Hz , 2H, C(O)C**H$_2$**C(CH_2)C(O)), 1.61–1.55 (m 2H, NCH_2C**H$_2$**), 1.29–1.24 (m 22H C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**), 0.88 (t, *J* = 6.8 Hz, 3H CH_2C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 174.0, 170.0, 133.5, 120.4, 39.1, 33.9, 32.0, 29.82, 29.79, 29.76, 29.7, 29.6, 29.6, 29.3, 27.9, 27.0, 22.8, 14.2;

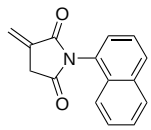
3-Methyl-1-tetradecyl-1*H*-pyrrole-2,5-dione (4.24b)



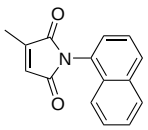
316 mg, 11%; light pink solid; IR (neat) ν (cm^{-1}): 3081, 2914, 2848, 1683, 1638, 1376; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.29 (q, *J* = 1.8 Hz, 1H, C(O)C**H**C(CH_3)C(O)), 3.47 (t, *J* = 7.3 Hz, 2H, NC**H$_2$**CH_2), 2.07 (d, *J* = 1.8

Hz, 3H, C(O)CHC(C**H$_3$**)C(O)), 1.57–1.54 (m, 2H, NCH$_2$C**H$_2$**), 1.29–1.24 (m, 22H, C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**C**H$_2$**), 0.87 (t, *J* = 6.8 Hz, 3H, C**H$_3$**); ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.0, 171.0, 145.5, 127.2, 38.0, 31.9, 29.7, 29.64, 29.62, 29.55, 29.5, 29.3, 29.1, 28.6, 26.8, 22.7, 14.1, 11.0;

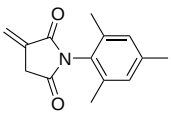
P. aeruginosa QS Inhibition Curves



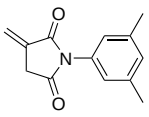
4.5a



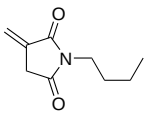
4.5b



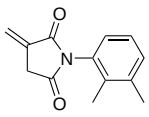
4.7a



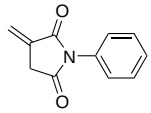
4.8a



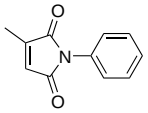
4.9a



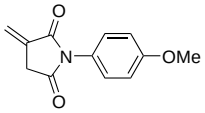
4.10a



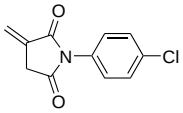
4.11a



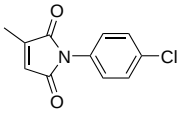
4.11b



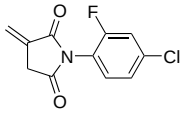
4.12a



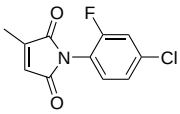
4.13a



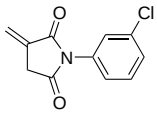
4.13b



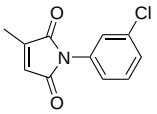
4.14a



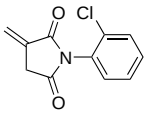
4.14b



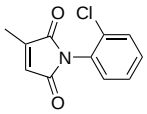
4.15a



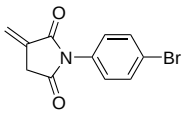
4.15b



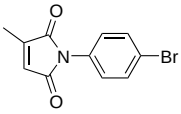
4.16a



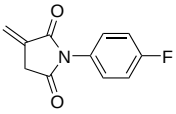
4.16b



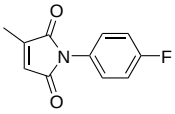
4.17a



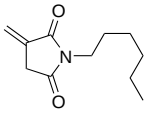
4.17b



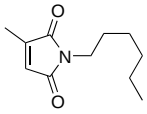
4.18a



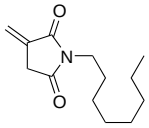
4.18b



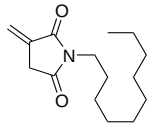
4.20a



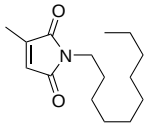
4.20b



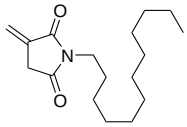
4.21a



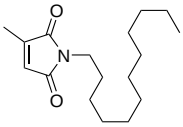
4.22a



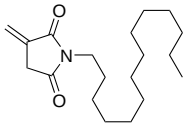
4.22b



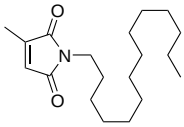
4.23a



4.23b

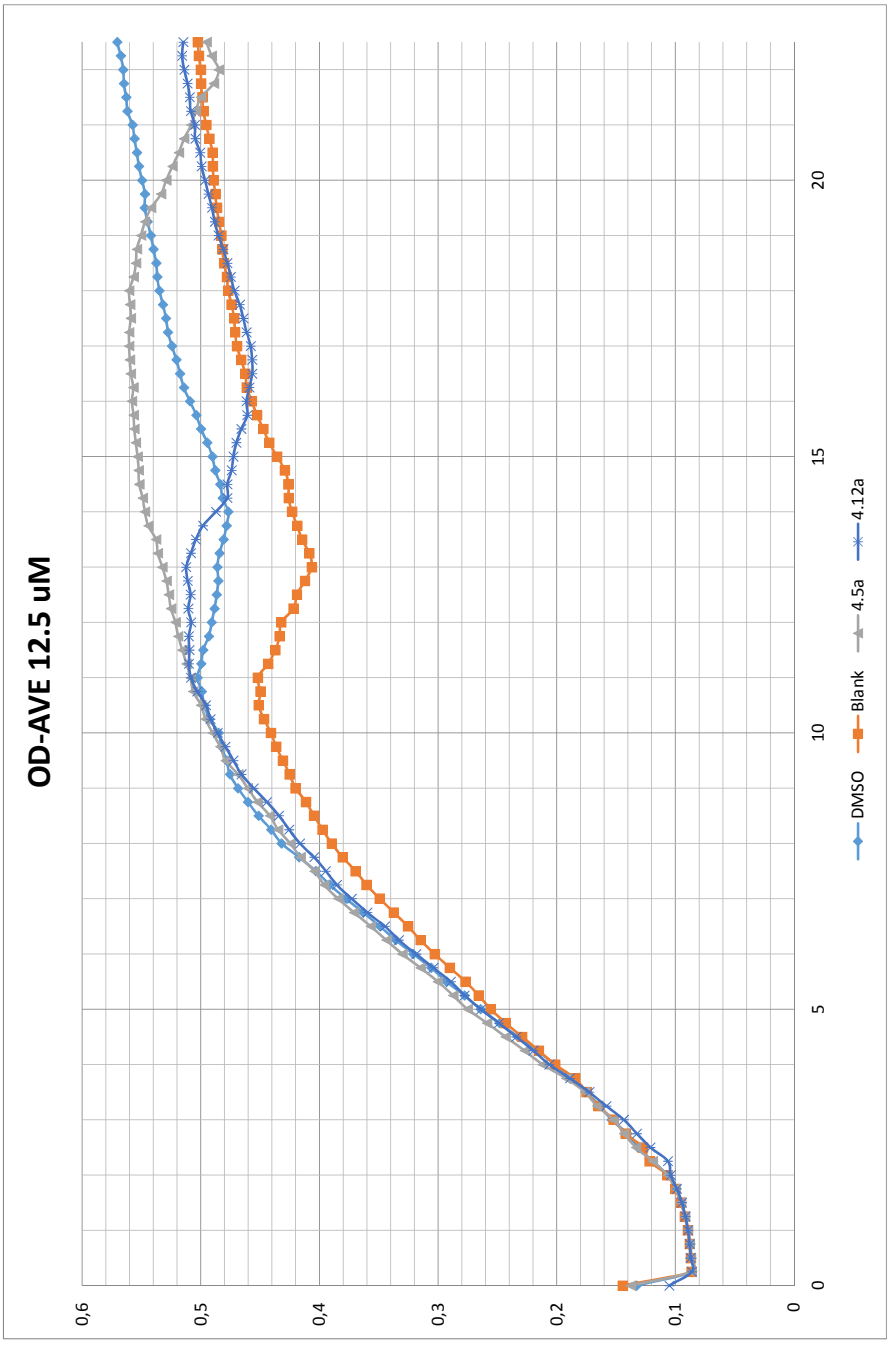


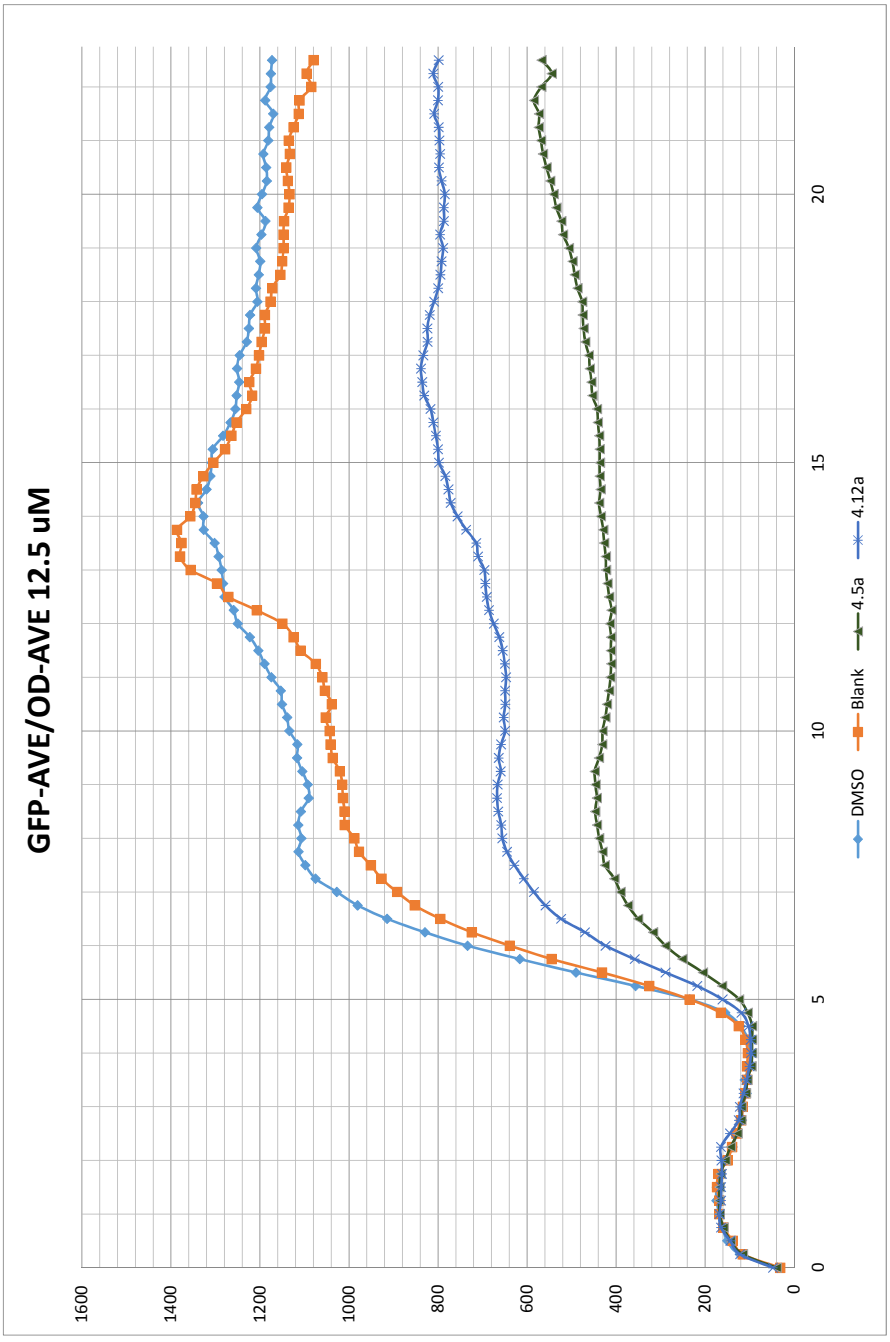
4.24a

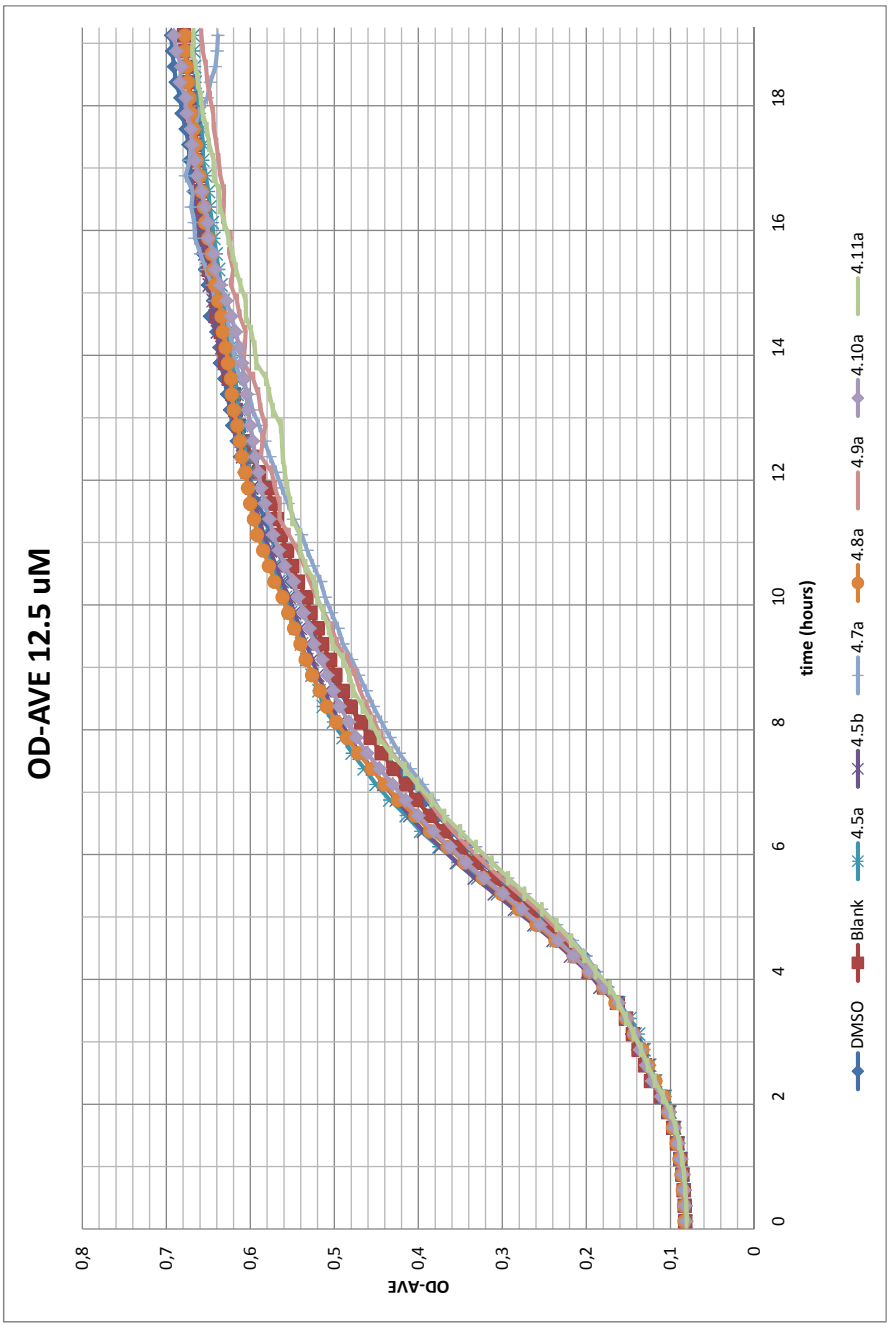


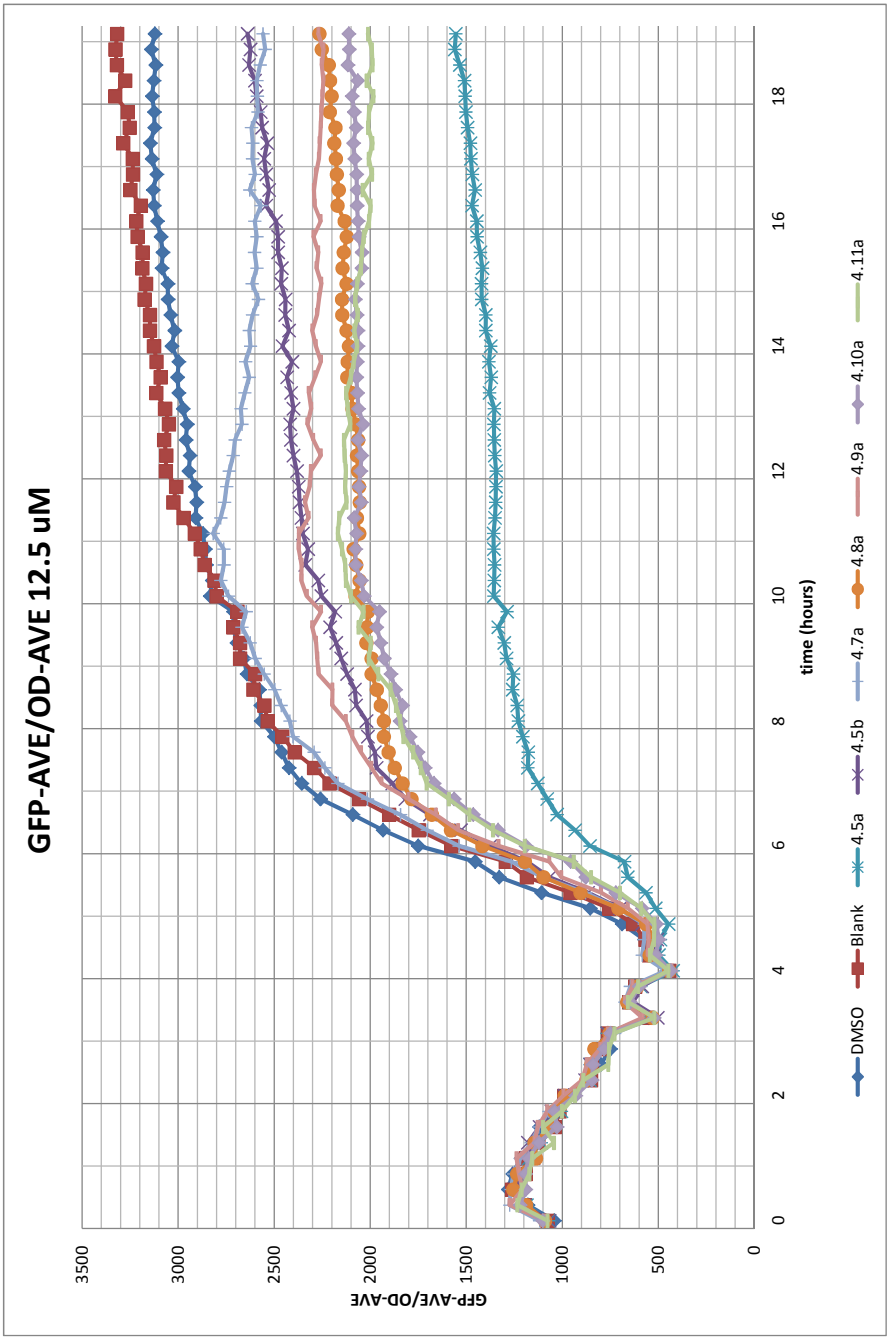
4.24b

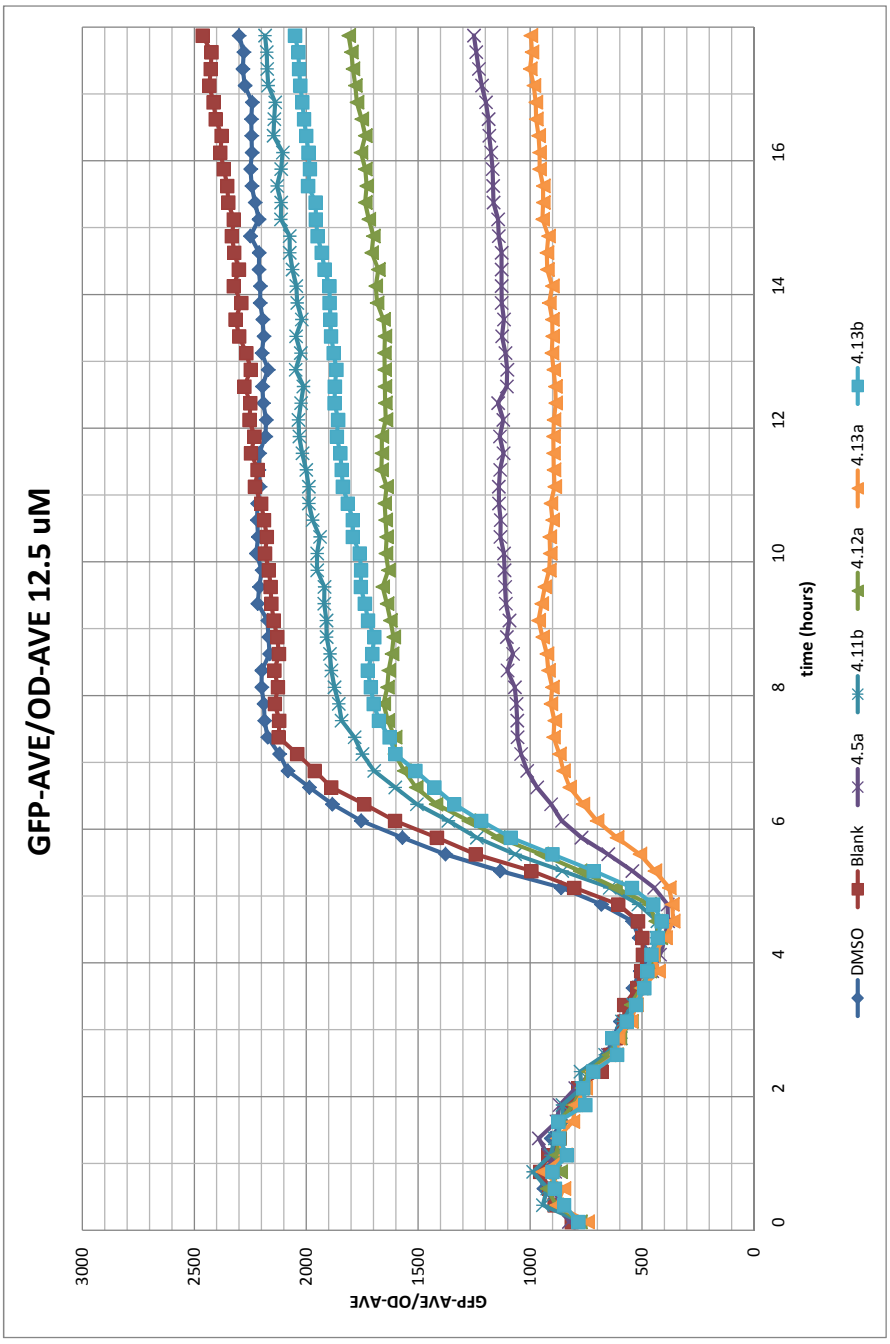
Appendix B: Inhibition curves

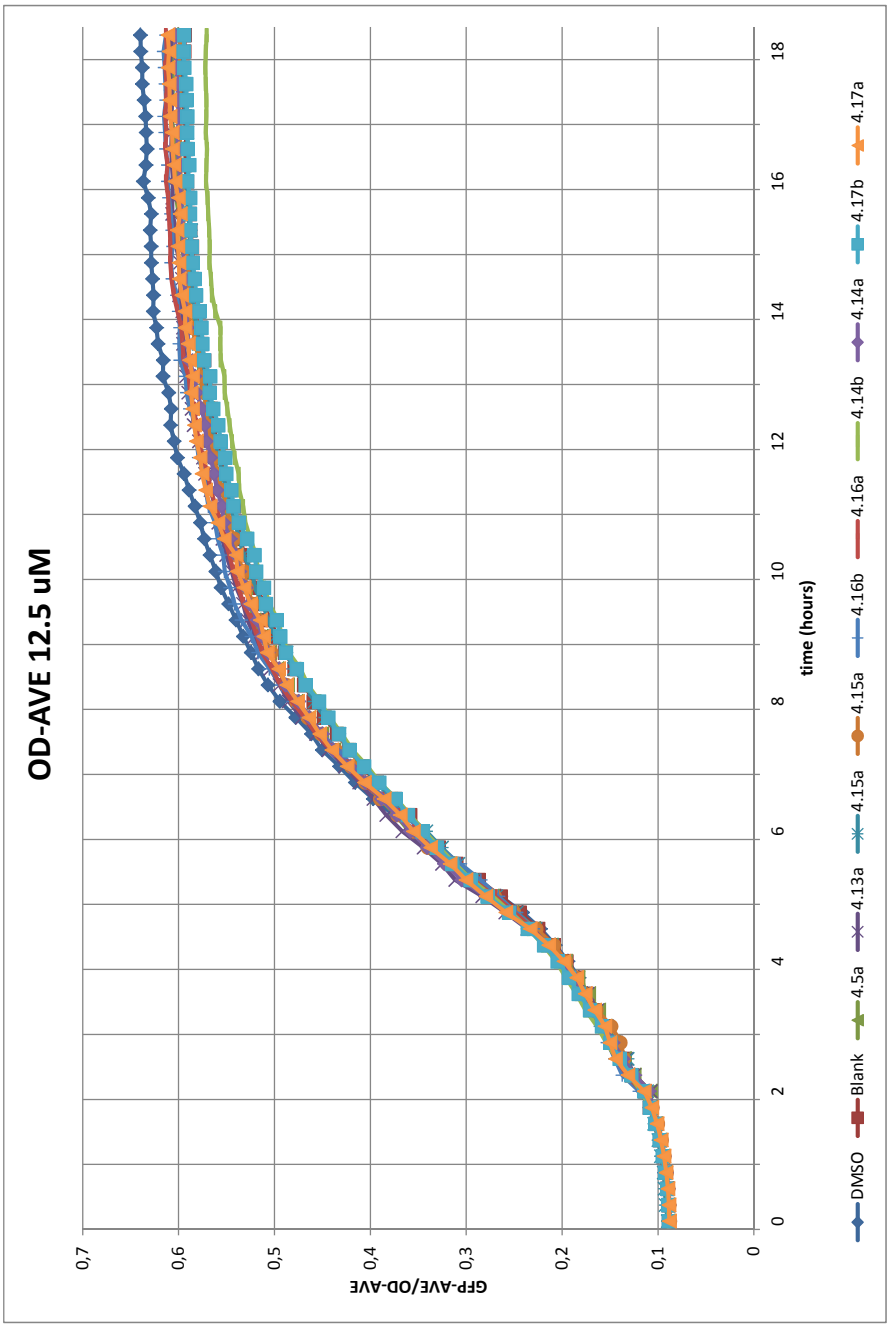


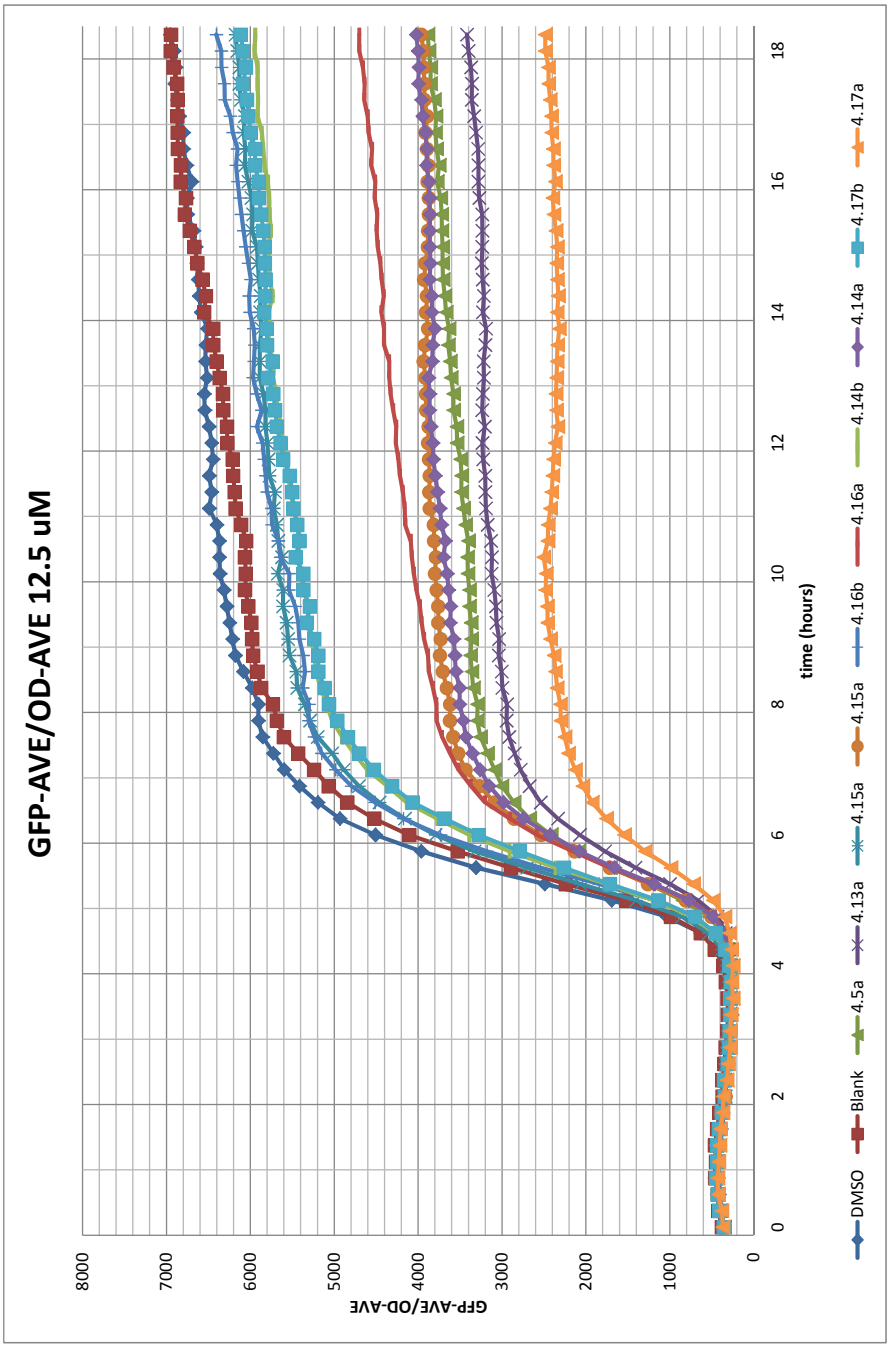

GFP-AVE/OD-AVE 12.5 uM
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
DMSO
Blank
4.5a
4.12a


OD-AVE 12.5 uM
OD-AVE
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
2
4
6
8
10
12
14
16
18
time (hours)
DMSO
Blank
4.5a
4.5b
4.7a
4.8a
4.9a
4.10a
4.11a


GFP-AVE/OD-AVE 12.5 uM
GFP-AVE/OD-AVE
3500
3000
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
time (hours)
DMSO
Blank
4.5a
4.5b
4.7a
4.8a
4.9a
4.10a
4.11a


GFP-AVE/OD-AVE 12.5 uM
GFP-AVE/OD-AVE
3000
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
time (hours)
DMSO
Blank
4.5a
4.11b
4.12a
4.13a
4.13b


OD-AVE 12.5 uM
GFP-AVE/OD-AVE
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
time (hours)
0
2
4
6
8
10
12
14
16
18
DMSO
Blank
4.5a
4.13a
4.15a
4.15a
4.15a
4.16b
4.16a
4.14b
4.14a
4.17b
4.17a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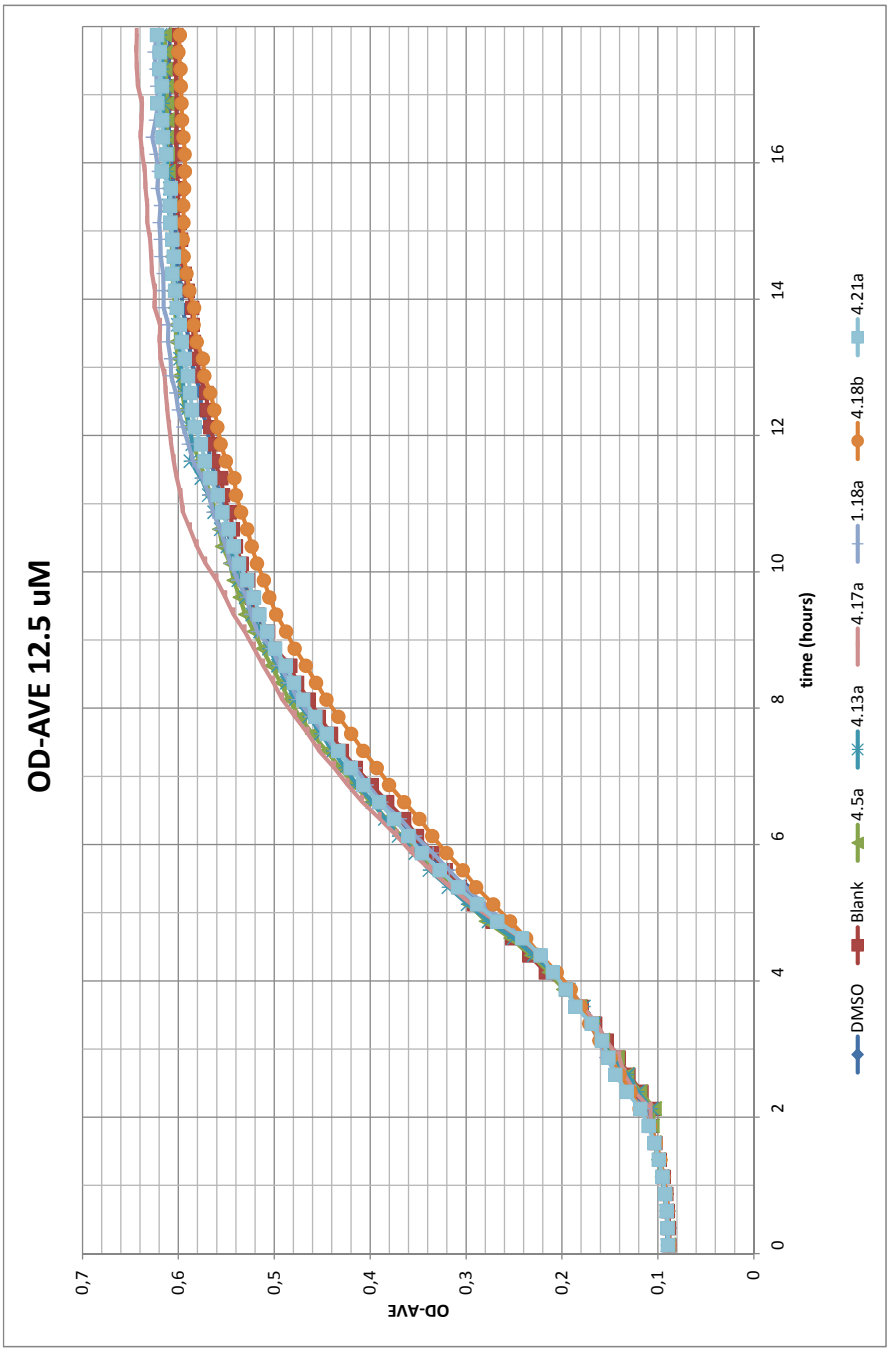
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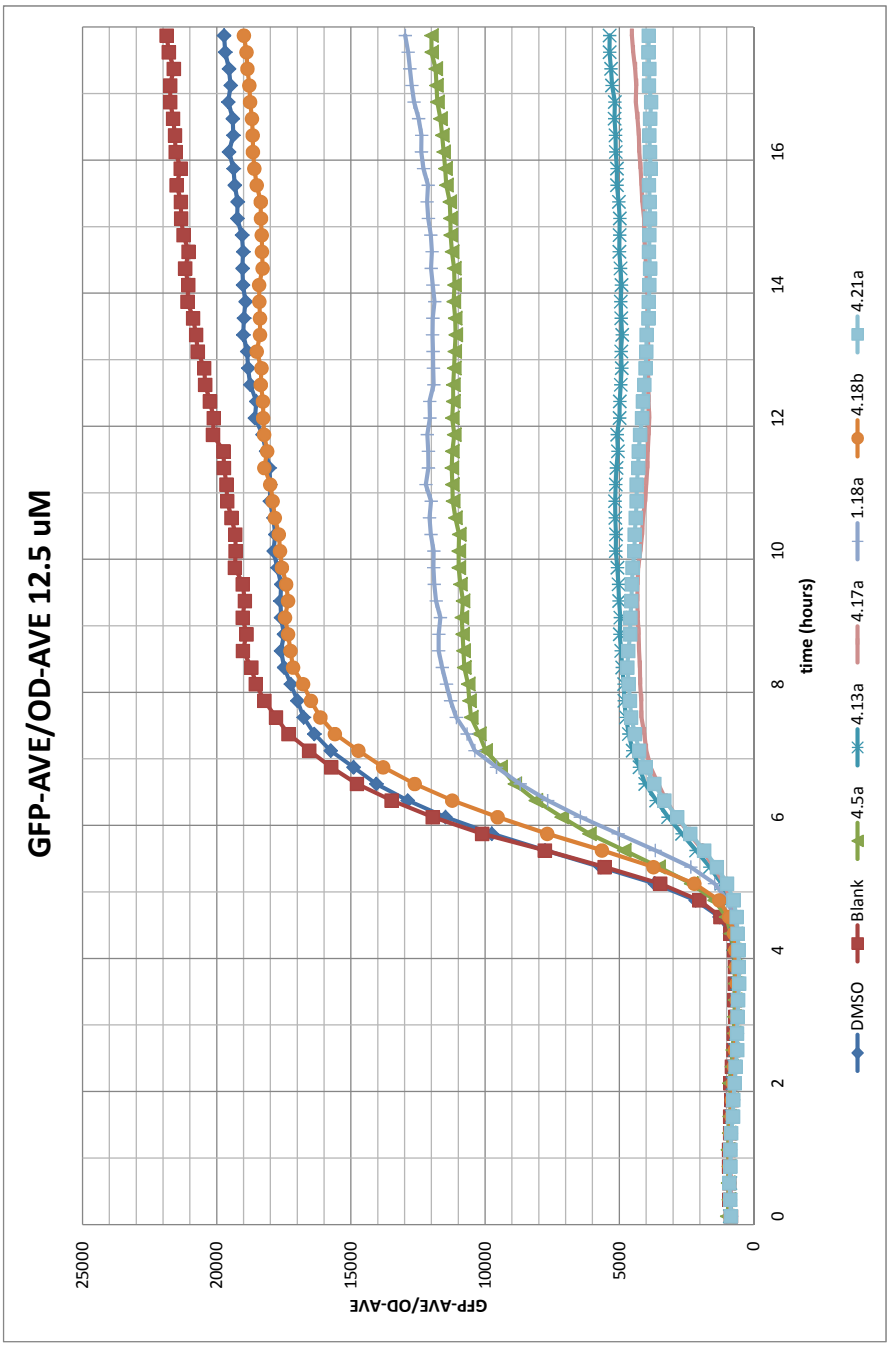
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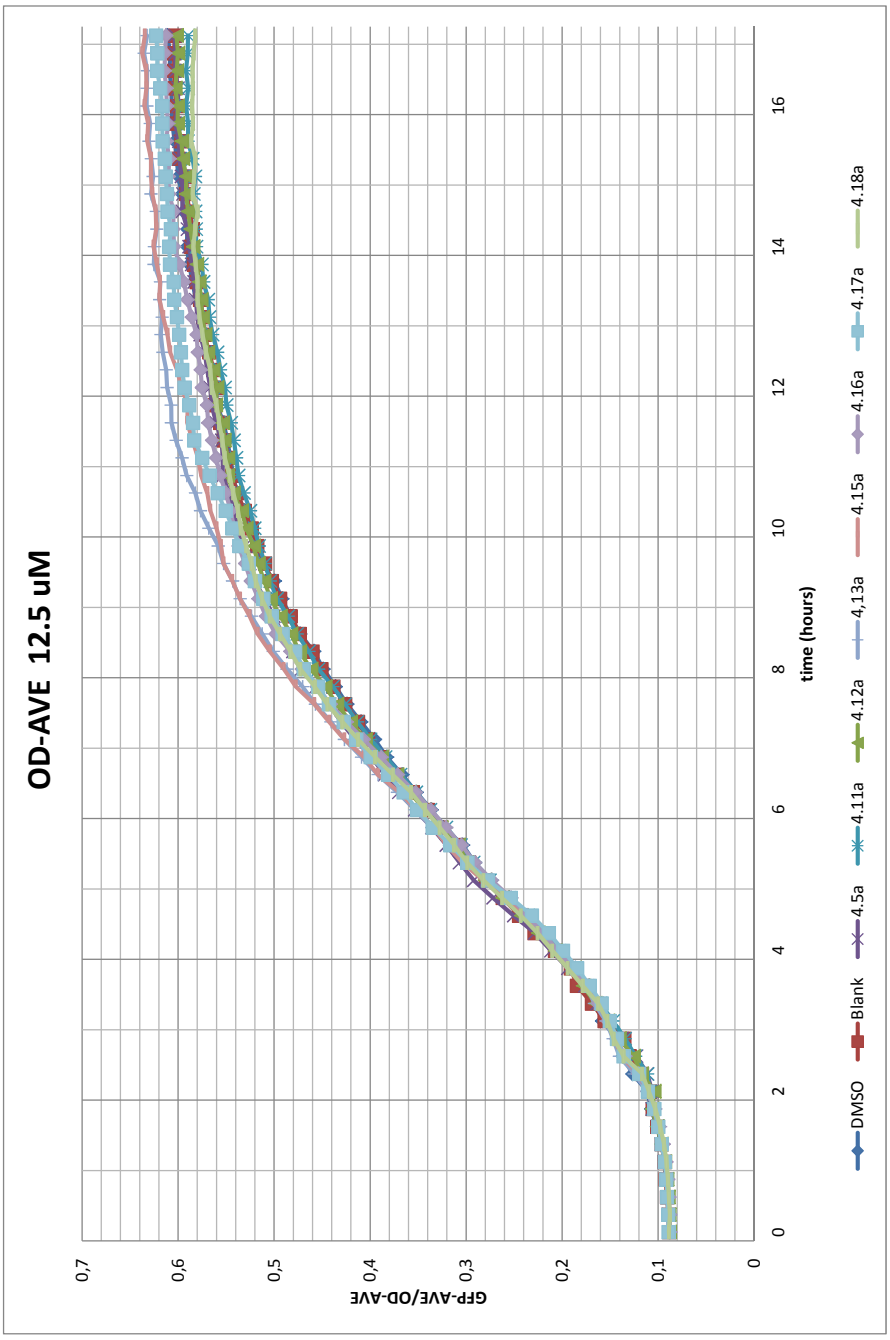
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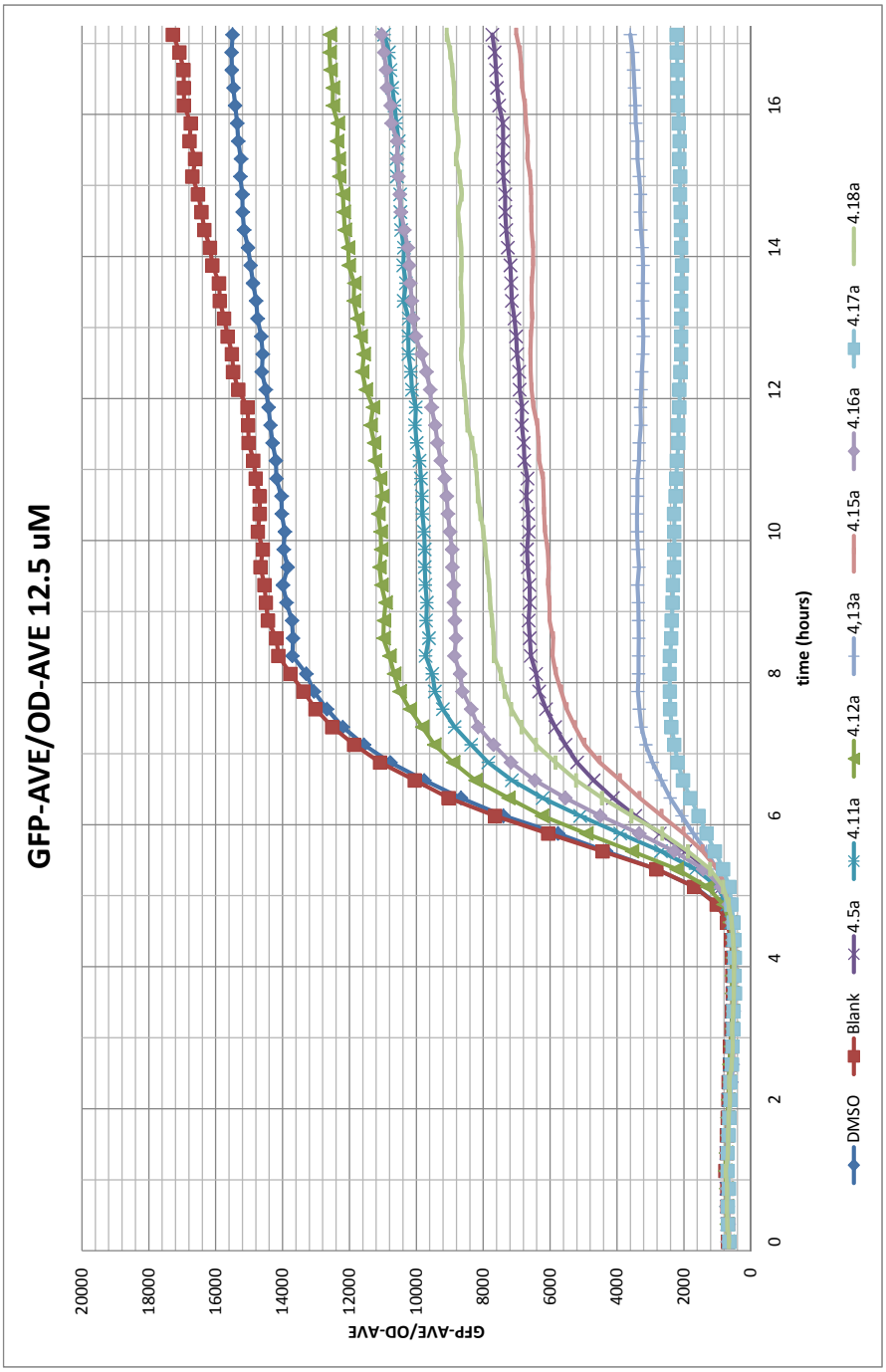
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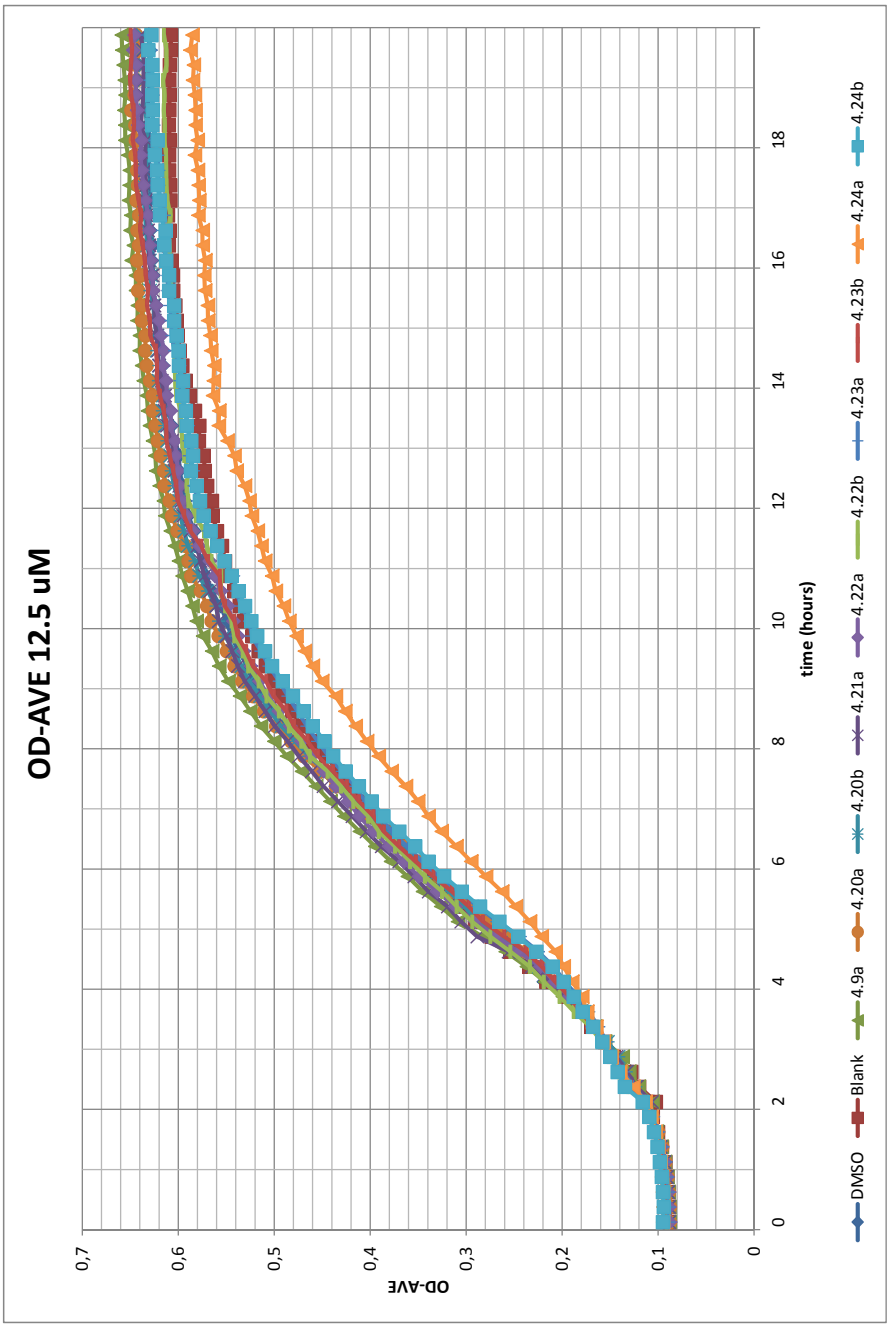
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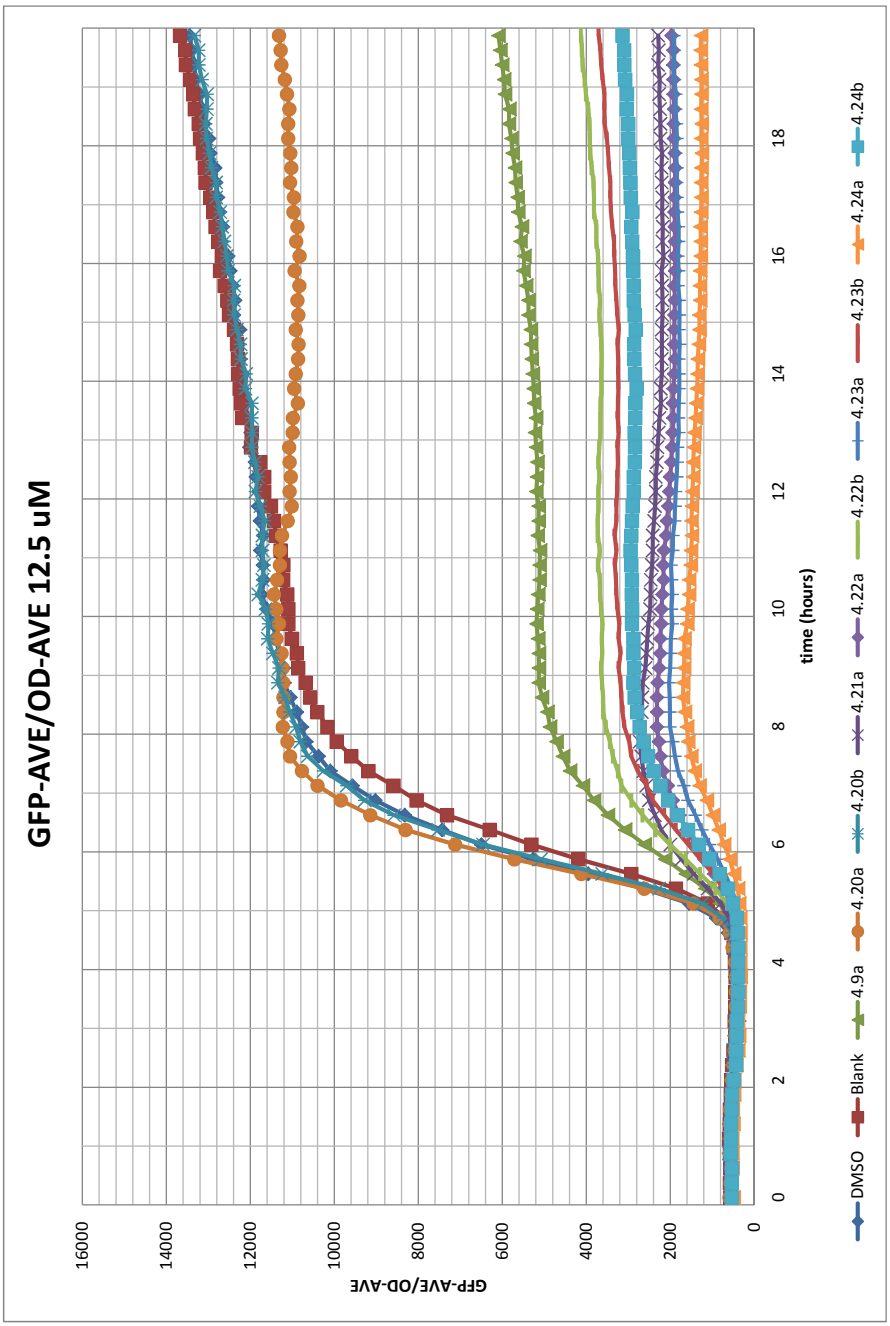
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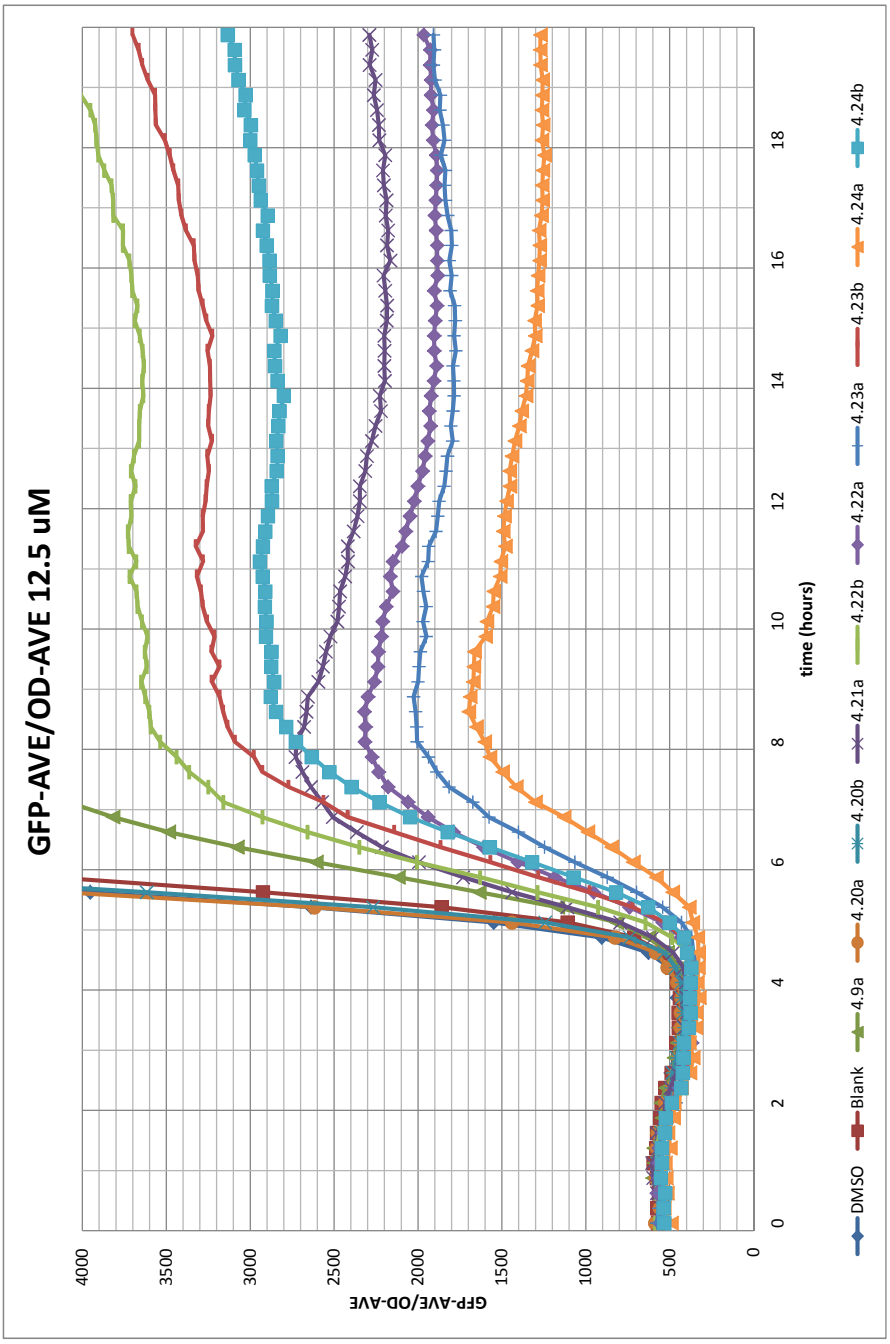
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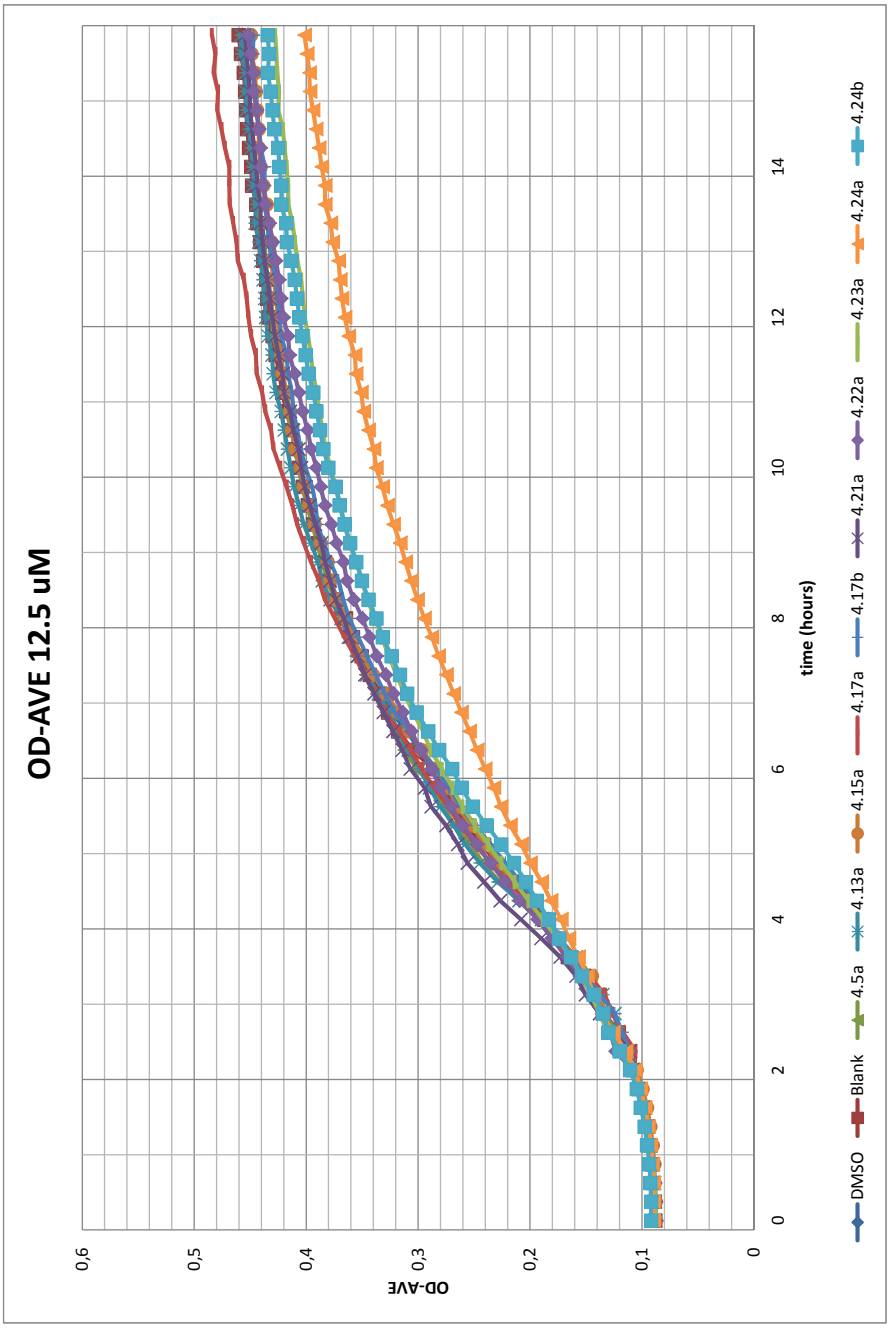
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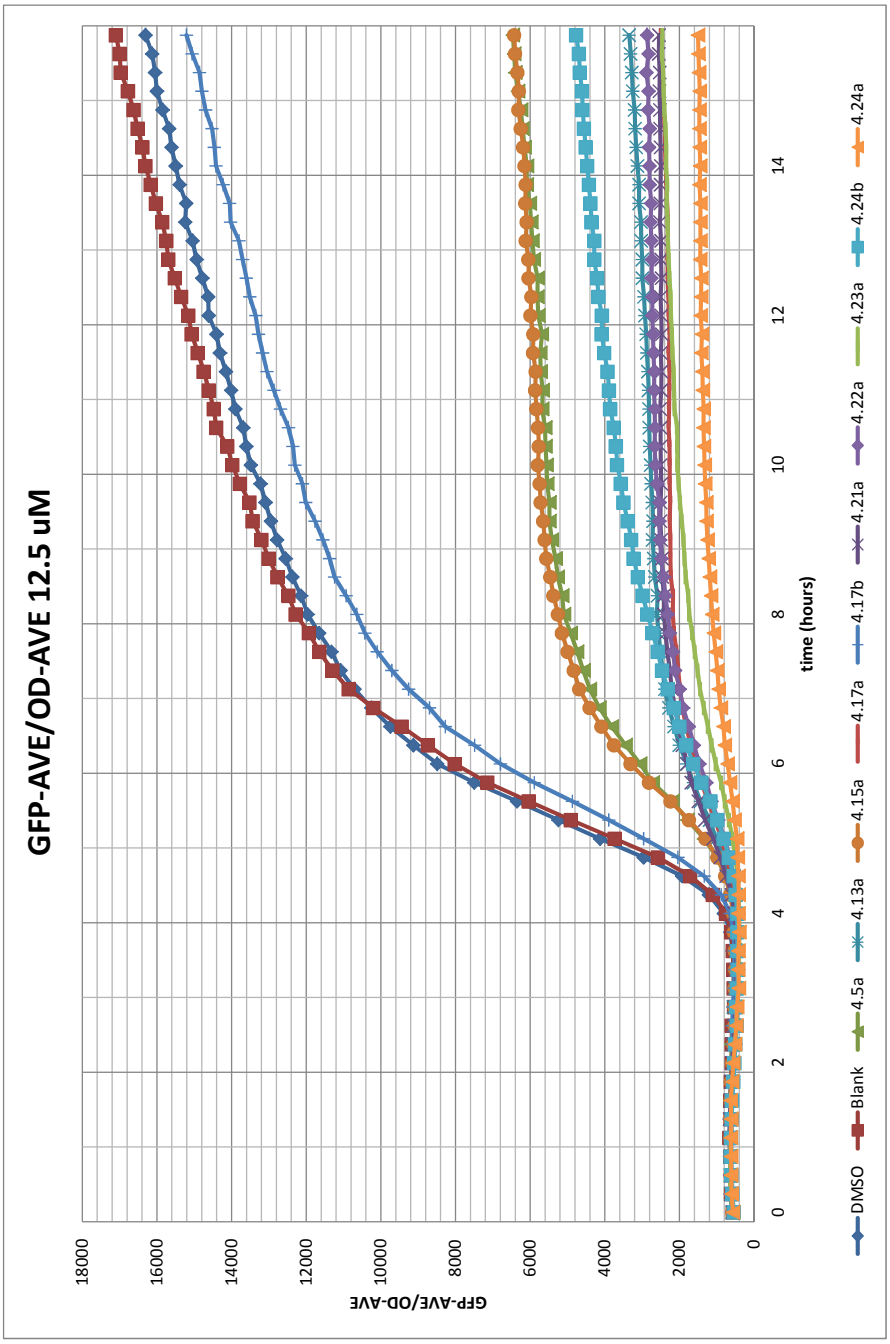
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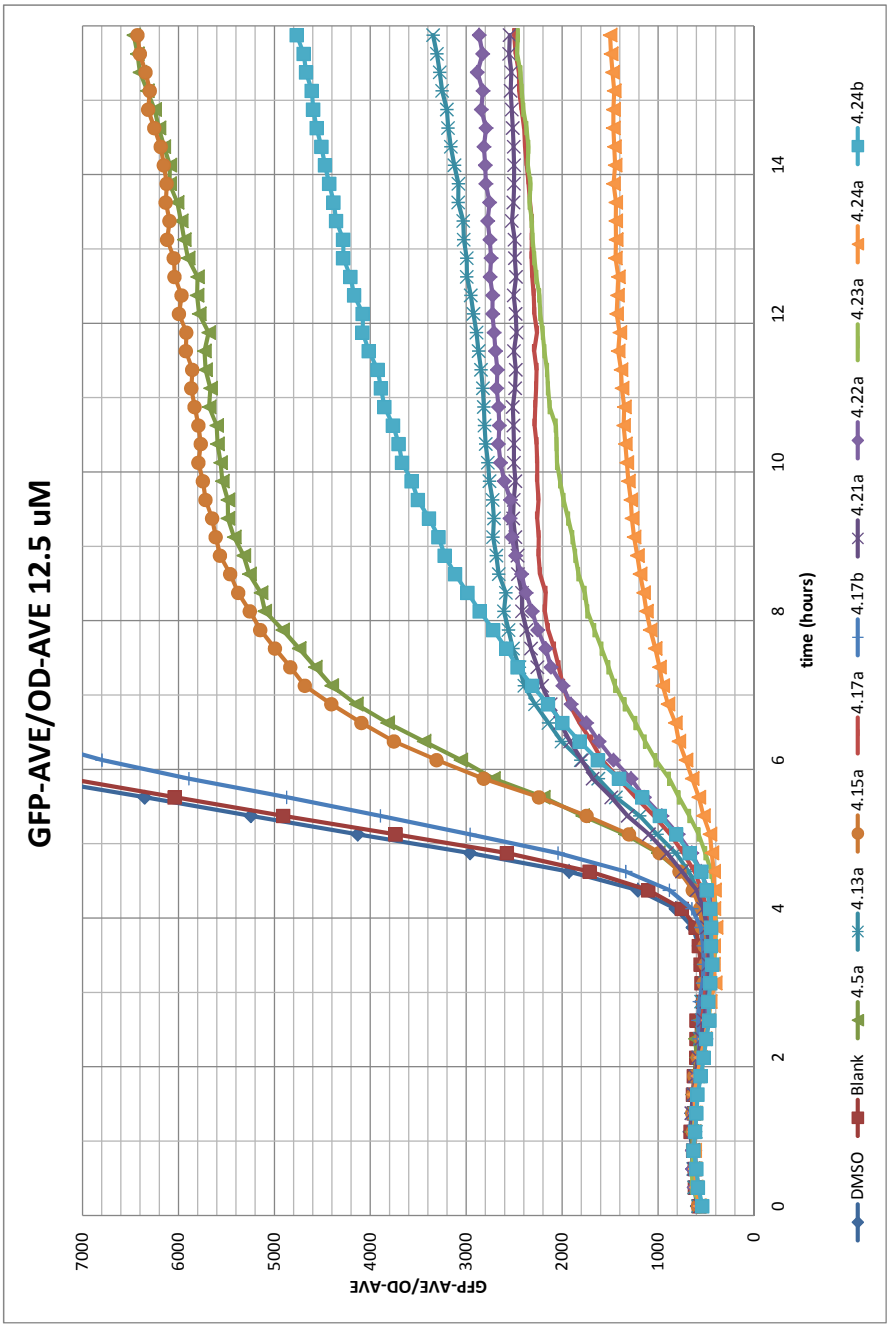
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Appendix

Appendix A: Publication

Bang, C. G.; Jensen, J. F.; Court, E. O.; Olsen, L. B.; Siyum, S. G.; Mortensen, K. T.; Poulsen, T. S.; Berthelsen, J.; Givskov, M.; Qvortrup, K.; Nielsen,T. E. Solid-Phase Synthesis of Hydroxamic Acids and Identification of HDAC Inhibitors Using a Chemiluminescence-based Assay, submitted to ACS Combinatorial Science

Solid-Phase Synthesis of Hydroxamic Acids and Identification of HDAC Inhibitors Using a Chemi-luminescence-based Assay.

Claus G. Bang,[a] Jakob F. Jensen,[a] Emil O´Hanlon Court,[a] Lasse B. Olsen,[a] Saba Gebremedhin Siyum,[a] Kim T. Mortensen,[a] Tine S. Poulsen,[b] Jens Berthelsen,[b] Michael Givskov,[b],[c] Katrine Qvortrup[a] and Thomas E. Nielsen[a],[b],[c]*

[a]Department of Chemistry, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark, [b] Department of Immunology and Microbiology, University of Copenhagen, DK-2200 Copenhagen N, Denmark, [c]Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore 637551, Singapore

KEYWORDS. Solid-phase synthesis, non-integral linker, hydroxamic acid, HDAC inhibitor.

ABSTRACT

We herein present broadly useful and readily available non-integral hydroxylamine linkers for the routine solid-phase synthesis of hydroxamic acids. The developed protocols enable the

efficient synthesis and release of a wide range of hydroxamic acids from various resins, relying on high control and flexibility with respect to reagents and synthetic processes. A trityl-based hydroxylamine linker was used to synthesize a library of peptide hydroxamic acids. The inhibitory effects of the compounds were examined for seven HDAC enzyme subtypes using a chemiluminescence-based assay.

Introduction

Hydroxamic acids belong to an important class of compounds with a plethora of biological properties, including antibacterial, antifungal and anticancer activity (Fig. 1).[1,2] The ability of hydroxamates to chelate metal ions, such as Fe^{3+} and Zn^{2+}, has been widely explored in biology and medicine, The siderophore desferrioxamene B (Desferal, **1**) is used medically to treat iron overdose, which can arise either due to multiple blood transfusions or an underlying genetic blood disease.[3] A large number of recent studies have dealt with hydroxamic acids as potent inhibitors of Zn^{2+}-containing enzymes, such as matrix metallo-proteinases (MMPs), and histone deacetylases (HDACs). HDAC inhibitors represent a promising class of anticancer agents, with two hydroxam acid-based inhibitors now approved for cutaneous and peripheral T-cell lymphoma, respectively. Recognizing the diverse anti-cancer activities of Vorinostat (SAHA, **3**)[4] and Belonistat (**4**)[5], huge efforts have been made to explore hydroxamic acids for the treatment of various cancers. In addition to promising anticancer effects, HDAC inhibitors have also been suggested for the treatment of a range of other diseases, such as internal fibrosis, autoimmune, inflammatory diseases, metabolic disorders and many more. However, disruption of multiple pathways by these inhibitors, and the lack of specificity by these inhibitors to a target

enzyme, may contribute to the cytotoxicity observed in many clinical trials. Therefore, there is an increasing need to develop more potent and isoform-selective HDAC inhibitors. Following the observation of clinical benefits of HDAC inhibitors in various diseases, there has been a substantial refocus on the organic chemistry and biomedical properties of the hydroxamic acid class of compounds.

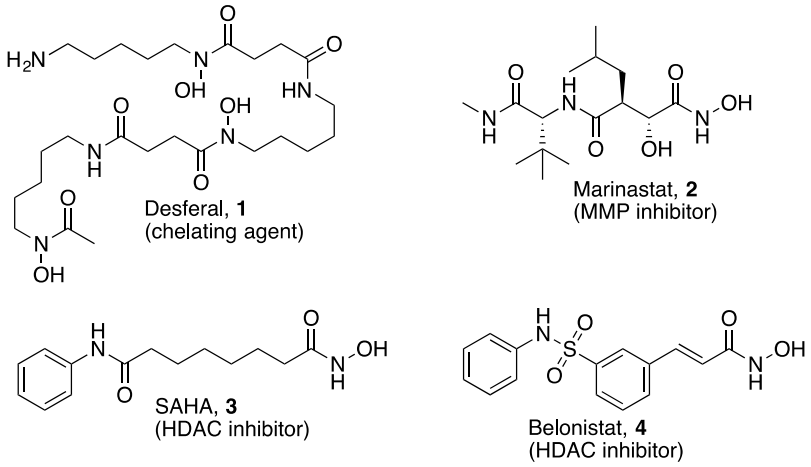


Figure 1. Clinically important hydroxamic acids.

Discussions.

Although a high number of potent hydroxamic acids have been identified in drug and probe discovery efforts,[5] only few general methods for the parallel and combinatorial synthesis of hydroxamic acids have been reported. There have been notable reports describing the solid-phase synthesis of hydroxamic acids,[6] e.g. hydroxylamine derivatives, being either *N*-tethered to MBHA,[7] and Tentagel resins,[8] or *O*-tethered to Wang,[9] Sasrin,[10] and trityl resins,[11] have served as the starting point for the synthesis of a range of hydroxamic acids. Alternatively, a reported

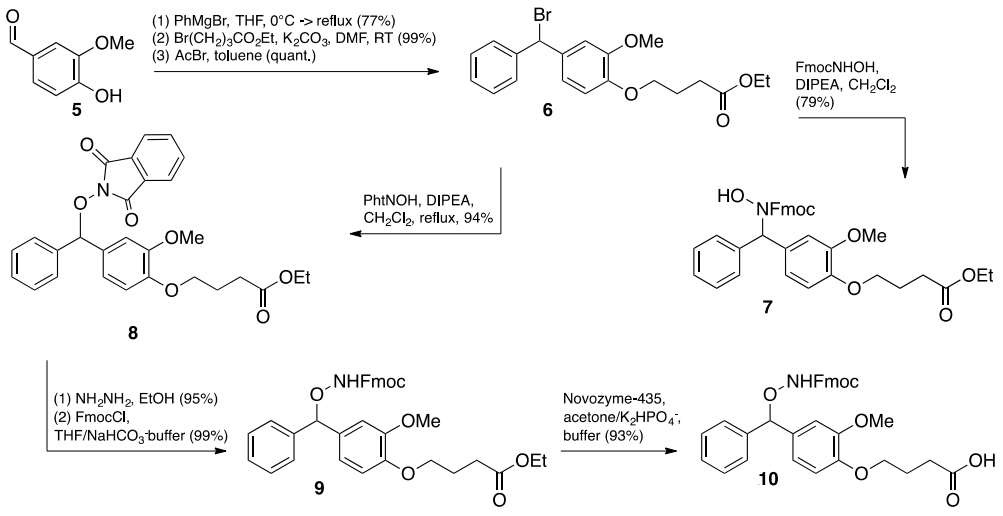
stepwise method relies on the hydrolysis of ester-bound compounds, followed by attachment of the formed carboxylic acids to a hydroxylamine resin using a peptide coupling agent, before final cleavage to generate hydroxamic acids.[12] The solid-phase synthesis of hydroxamic acids via direct hydroxyaminolysis of ester-linked substrates has also been reported.[13]

The use of non-integral linkers is usually preferred because these provide control and flexibility for the synthetic process,[14] and exact degree of loading and functionalization may easily be controlled and assessed. Along these lines, any functionalized solid support can be used with such linkers. When the linker is attached through an amide or ester bond, the quality of the starting resin can be assured by controlling the purity of the linker. When the first building block is attached to the linker through a more demanding bond, this may advantageously be formed in solution before attaching the linker construct to the solid support.[15] The majority of linkers used in solid-phase synthesis are thus of the non-integral type.

Results

As part of ongoing efforts to synthesize hydroxamic acids, we set out to develop a non-integral hydroxylamine linker for the solid-phase synthesis and release of hydroxamic acids. Important criteria comprise the applicability to protocols that effectively may lead to a wide variety of structurally diverse hydroxamic acids, notably with high control and flexibility for the synthetic process, on any kind of solid support. Initially, the *p*-methoxybenhydryl-linker **10** was investigated for the solid-phase synthesis of hydroxamic acids. The *p*-methoxybenhydryl-linker system was first reported by Walter[16] and Brown,[17] for the preparation of primary and secondary amides, respectively.

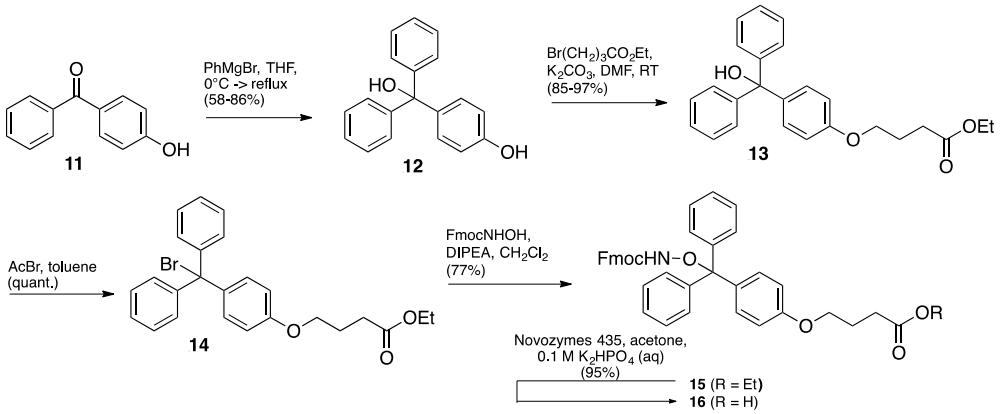
To construct linker **10**, phenylmagnesium bromide was added to vanillin, followed by alkylation with 4-bromobutanoate. Subsequent treatment with AcBr gave the bromide **6** in high yield. However, much to our surprise, reaction with FmocNHOH in the presence of DIPEA gave the *N*-alkylated derivative **7** as the sole product. Chan *et al.*[18] had previously reported a related synthetic sequence using Fmoc-NR-OH (R = alkyl chain), where *O*-alkylation was forced. To synthesize the desired *O*-alkylated hydroxylamine-linker **10**, bromide **6** was treated with *N*-hydroxyphthalimide, followed by removal of the phthalimide with hydrazine. Fmoc-protection followed by treatment with Novozyme-435 led to the desired linker **10**. Using standard reagents for solid-phase peptide synthesis, the linker was immobilized and synthetically elaborated on an amino-functionalized PEGA resin. Hydroxamic acids were released when the resin was treated with CH_2Cl_2/TFA (1:1) for 24 hours. The linker resists 20% TFA (aq) for 1 hour, and is thereby compatible with removal of highly acid-labile protecting groups. However, fluctuating yields and purities were generally observed with this linker system, often as a result of hydroxamate hydrolysis during periods of prolonged acidic treatment.



Scheme 1. Synthesis of p-methoxybenhydryl-based linker **10** for the solid-phase synthesis and release of hydroxamic acids

In search of a more acid-labile construct, we became interested in a linker based on the trityl-group (linker **16**). The trityl-group was pioneered in linkers for solid-phase synthesis by Leznoff[19] and Fréchet.[20] Cleavage hereby becomes more straightforward, relying on either dilute TFA or HBr.[20] More detailed studies indicated that anhydrous 0.3 M HCl (dioxane) was sufficient for cleavage. [19] Since then, trityl linker constructs have been generated for the release of a variety of functionalized molecules from solid support, particularly in integral linker constructs, e.g. peptides,[21] hydroxamic acids,[18] amines, acids, thiols,[22] and as a non-integral linker through 4-carboxy derivatives.[23]

Starting with 4-hydroxybenzophenone **11**, addition of phenylmagnesium bromide[24] gave trityl alcohol **12**, which was alkylated with ethyl 4-bromobutanoate at the phenolic position to provide intermediate trityl ester **13** in high yield. Bromination with acetylbromide in toluene followed by treatment with Fmoc-NHOH in CH_2Cl_2 in the presence of DIPEA generated Fmoc-protected hydroxylamine ester **15**. In this case, only *O*-substitution was observed with a sterically demanding alkylating reagent. Exposing ester **15** to hydrolysis with Novozyme-435 completed the synthesis of the trityl linker **16**.

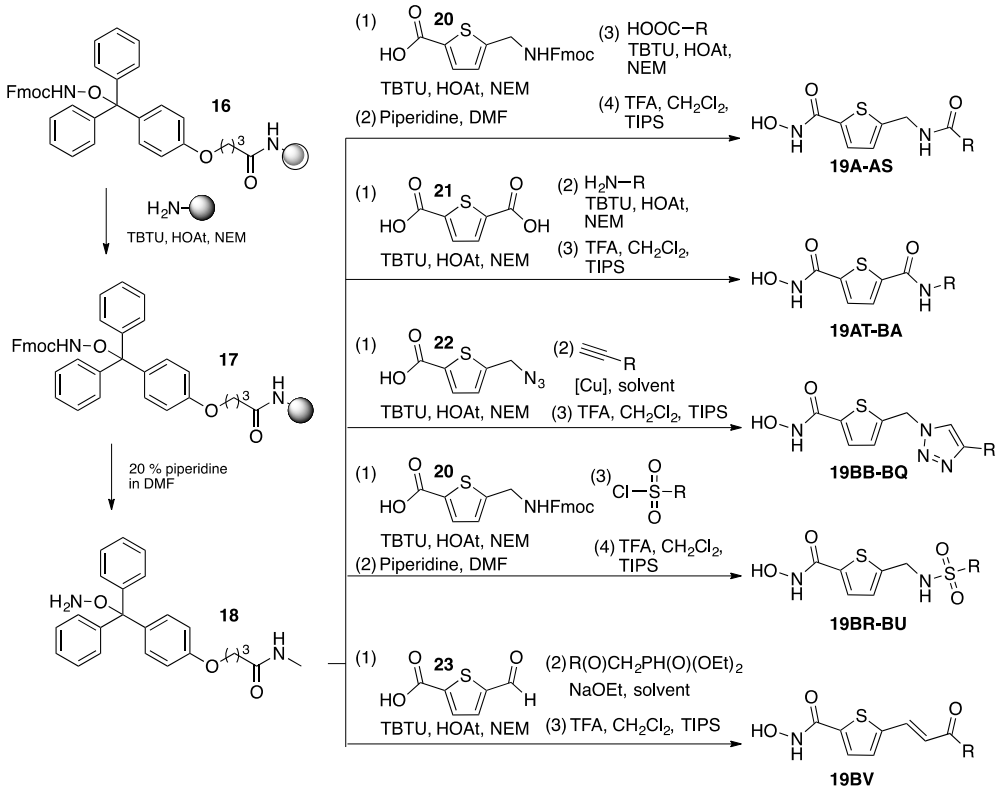


Scheme 2. Synthesis of trityl-based linker **16** for the solid-phase synthesis and release of hydroxamic acids

Using standard reagents for solid-phase peptide synthesis, the linker was easily immobilized and synthetically elaborated on an amino-functionalized PEGA resin (Table 1). Rewardingly, hydroxamic acids were cleanly released when cleavage was performed with TFA/CH_2Cl_2 (1:10) in the presence of 2% (*v*/*v*) TIPS (Triisopropylsilane) for 5 min. In comparison, the commercially available amino-functionalised polystyrene resin functionalized with the hydroxylamine linker **16** was tested under the same conditions. Here, hydroxamic acids were cleanly released after 5 min. of treatment with 1% TFA (CH_2Cl_2). The reduced reactivity of linker **16** at the PEGA resin is explained by a reduced proton activity in the polyethylene glycol polyacrylamide-functionalised resin (PEGA). The PEG based resins serve as proton sponges.

For preliminary biological screening of small-molecule and peptide hydroxamic acids, we generated a library of potential HDAC inhibitors (**19 A–BV**; Table 1). In previous studies, we have identified the thiophene-group as a promising sub-structure in HDAC-inhibitors, generally leading to inhibitors of good activity and selectivity.[25] Based on these results, further

investigation into thiophene-hydroxamic acids was chosen as the focus of this study. The library was synthesized using 4 different thiophene spacer-groups **20**,[26] **21**, **22**, **23** which was used in combination with various terminating moieties (Scheme 3).



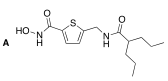
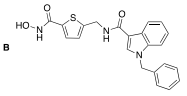
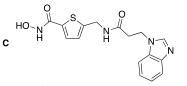
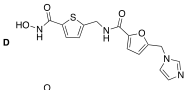
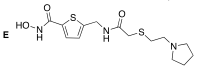
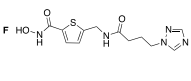
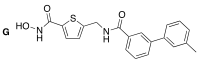
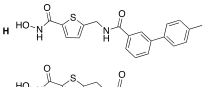
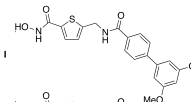
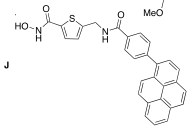
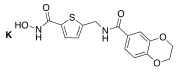
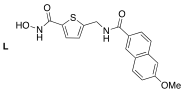
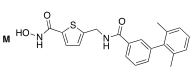
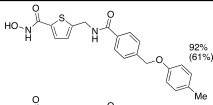
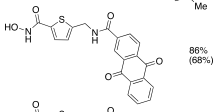
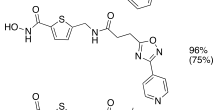
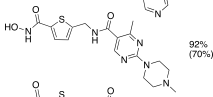
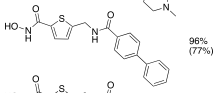
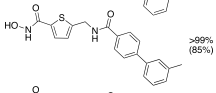
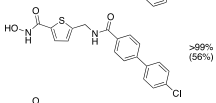
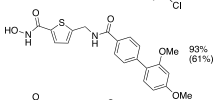
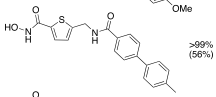
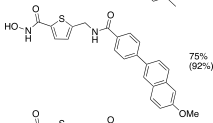
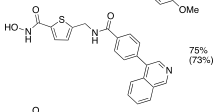
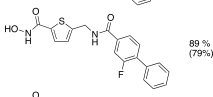
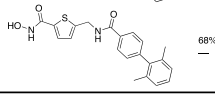
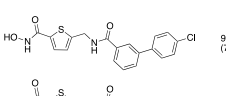
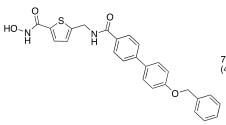
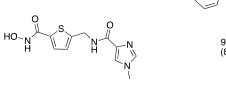
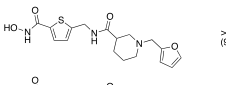
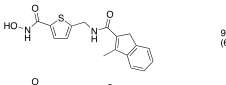
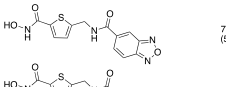
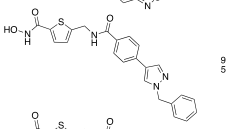
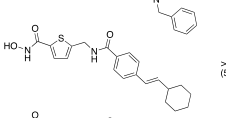
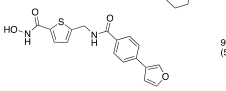
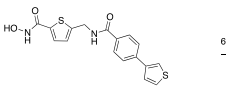
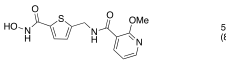
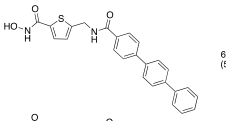
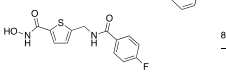
Scheme 3. Application of a trityl-based linker for the solid-phase synthesis and release of thiophene hydroxamic acids.

Much to our delight the synthesized hydroxamic acids **19A–BV** showed activity against HDAC subtype enzymes as well as a marked isoform-selectivity, thus indicating the potential of the compound collection to identify important binding interactions through stereostructure–

activity studies. Specifically, 11 compounds (**19N**, **19R**, **19S**, **19AB**, **19AD**, **19AF**, **19AJ**, **19AN**, **19AX**, **19BK**, **19Bl**) were found to selectively inhibit HDAC6. HDAC6 itself plays a role in degradation and clearance of misfolded proteins, as well as cell protection in response to environmental stress by interaction with stress granules.[27] Thus, the multiple functions of HDAC6 modulate many cellular pathways, which are relevant to tumor cell biology and malignant behavior. On the basis of these functions, HDAC6 appears to be one of the most attractive targets among HDACs for cancer treatment.

Conclusions.

In summary, we have developed two broadly useful non-integral hydroxylamine linkers, based on a trityl and a biphenyl group, respectively. The developed protocols enable the efficient synthesis and release of a wide variety of hydroxamic acids, and show high control and flexibility for the synthetic process, including solid phase composition. A proof-of-concept collection of 72 skeletally diverse hydroxamic acids compounds was prepared. The inhibitory effects of the compounds were examined against 7 HDAC enzyme subtypes using a Chemiluminescence-based assay.

Entry	Substrate	Purity (yield)	IC50 (μM
A		95% (78%)	HDAC1: 3.896 HDAC2: 5.898 HDAC3: 1.763 HDAC6: 0.292 HDAC8: 1.031 HDAC10: 27.956 HDAC11: 7.893
B		84% (81%)	HDAC1: 0.284 HDAC2: 0.391 HDAC3: 0.167 HDAC6: 0.008 HDAC8: 0.090 HDAC10: 0.246 HDAC11: 0.613
C		87% (72%)	HDAC1: 4.862 HDAC2: 6.218 HDAC3: 1.993 HDAC6: 0.164 HDAC8: 0.419 HDAC10: 20.895 HDAC11: 10.182
D		68% (61%)	HDAC1: 0.526 HDAC2: 0.487 HDAC3: 0.365 HDAC6: 0.054 HDAC8: 0.155 HDAC10: 1.227 HDAC11: 1.675
E		>99% (84%)	HDAC1: 3.968 HDAC2: 4.746 HDAC3: 1.642 HDAC6: 0.390 HDAC8: 0.737 HDAC10: 10.645 HDAC11: 8.343
F		87% (91%)	HDAC1: 10.004 HDAC2: 17.223 HDAC3: 7.306 HDAC6: 0.659 HDAC8: 2.096 HDAC10: 17.105 HDAC11: 15.156
G		>99% (48%)	HDAC1: 1.023 HDAC2: 1.121 HDAC3: 0.435 HDAC6: 0.110 HDAC8: 0.817 HDAC10: 1.538 HDAC11: 3.566
H		>99% (90%)	HDAC1: 0.193 HDAC2: 0.251 HDAC3: 0.080 HDAC6: 0.026 HDAC8: 0.147 HDAC10: 0.232 HDAC11: 0.429
I		95% (45%)	HDAC1: 0.463 HDAC2: 0.460 HDAC3: 0.405 HDAC6: 0.202 HDAC8: 0.293 HDAC10: 0.563 HDAC11: 0.722
J		69% —	HDAC1: 2.807 HDAC2: 4.057 HDAC3: 1.391 HDAC6: 0.248 HDAC8: 0.748 HDAC10: 9.859 HDAC11: 2.575
K		90% (87%)	HDAC1: 0.533 HDAC2: 0.792 HDAC3: 0.254 HDAC6: 0.031 HDAC8: 0.166 HDAC10: 0.913 HDAC11: 1.475
L		95% (74%)	HDAC1: 0.065 HDAC2: 0.106 HDAC3: 0.062 HDAC6: 0.010 HDAC8: 0.052 HDAC10: 0.092 HDAC11: 0.194
M		67% —	HDAC1: 3.501 HDAC2: 4.399 HDAC3: 1.102 HDAC6: 0.317 HDAC8: 0.528 HDAC10: 15.581 HDAC11: 4.773
N		92% (61%)	HDAC1: 0.670 HDAC2: 1.035 HDAC3: 0.323 HDAC6: 0.012 HDAC8: 0.377 HDAC10: 0.757 HDAC11: 0.801
O		86% (68%)	HDAC1: 0.065 HDAC2: 0.103 HDAC3: 0.045 HDAC6: 0.027 HDAC8: 0.127 HDAC10: 0.101 HDAC11: 0.233
P		96% (75%)	HDAC1: 4.370 HDAC2: 5.901 HDAC3: 3.780 HDAC6: 0.517 HDAC8: 0.530 HDAC10: 20.414 HDAC11: 11.390
Q		92% (70%)	HDAC1: 2.124 HDAC2: 2.281 HDAC3: 0.866 HDAC6: 0.094 HDAC8: 0.380 HDAC10: 4.421 HDAC11: 4.254
R		96% (77%)	HDAC1: 0.283 HDAC2: 0.324 HDAC3: 0.270 HDAC6: 0.070 HDAC8: 0.347 HDAC10: 0.484 HDAC11: 0.724
S		>99% (85%)	HDAC1: 0.253 HDAC2: 0.288 HDAC3: 0.240 HDAC6: 0.051 HDAC8: 0.186 HDAC10: 0.327 HDAC11: 0.587
T		>99% (56%)	HDAC1: 0.265 HDAC2: 0.383 HDAC3: 0.237 HDAC6: 0.053 HDAC8: 0.231 HDAC10: 0.502 HDAC11: 0.501
U		93% (61%)	HDAC1: 0.304 HDAC2: 0.311 HDAC3: 0.427 HDAC6: 0.033 HDAC8: 0.198 HDAC10: 0.560 HDAC11: 0.635
V		>99% (56%)	HDAC1: 0.165 HDAC2: 0.202 HDAC3: 0.146 HDAC6: 0.040 HDAC8: 0.132 HDAC10: 0.247 HDAC11: 0.396
W		75% (92%)	HDAC1: 2.042 HDAC2: 3.201 HDAC3: 1.950 HDAC6: 0.291 HDAC8: 1.023 HDAC10: 11.597 HDAC11: 2.774
X		75% (73%)	HDAC1: 0.583 HDAC2: 0.822 HDAC3: 0.350 HDAC6: 0.079 HDAC8: 0.388 HDAC10: 0.773 HDAC11: 0.952
Z		89 % (79%)	HDAC1: 0.129 HDAC2: 0.205 HDAC3: 0.106 HDAC6: 0.040 HDAC8: 0.138 HDAC10: 0.192 HDAC11: 0.251
AA		68% —	HDAC1: 3.062 HDAC2: 4.360 HDAC3: 1.631 HDAC6: 0.103 HDAC8: 0.552 HDAC10: 16.847 HDAC11: 3.809
AB		91% (77%)	HDAC1: 0.412 HDAC2: 0.553 HDAC3: 0.154 HDAC6: 0.047 HDAC8: 0.432 HDAC10: 0.581 HDAC11: 0.643
AC		75% (49%)	HDAC1: 5.292 HDAC2: 9.189 HDAC3: 3.124 HDAC6: 0.187 HDAC8: 0.783 HDAC10: 36.285 HDAC11: 4.877
AD		96% (80%)	HDAC1: 1.432 HDAC2: 2.272 HDAC3: 0.688 HDAC6: 0.077 HDAC8: 0.507 HDAC10: 4.442 HDAC11: 5.349
AE		>99% (96%)	HDAC1: 3.582 HDAC2: 4.469 HDAC3: 1.398 HDAC6: 0.321 HDAC8: 0.500 HDAC10: 10.444 HDAC11: 8.295
AF		90% (64%)	HDAC1: 1.357 HDAC2: 1.951 HDAC3: 0.599 HDAC6: 0.068 HDAC8: 0.146 HDAC10: 2.685 HDAC11: 2.963
AG		71% (58%)	HDAC1: 15.467 HDAC2: 19.300 HDAC3: 8.993 HDAC6: 0.520 HDAC8: 1.961 HDAC10: 36.819 HDAC11: 30.396
AH		96% 58%	HDAC1: 0.373 HDAC2: 0.490 HDAC3: 0.346 HDAC6: 0.037 HDAC8: 0.205 HDAC10: 0.632 HDAC11: 0.777
AI		>99% (57%)	HDAC1: 1.812 HDAC2: 2.591 HDAC3: 1.024 HDAC6: 0.187 HDAC8: 0.660 HDAC10: 6.291 HDAC11: 2.701
AJ		94% (55%)	HDAC1: 0.482 HDAC2: 0.779 HDAC3: 0.498 HDAC6: 0.048 HDAC8: 0.250 HDAC10: 0.840 HDAC11: 1.221
AK		67% —	HDAC1: 0.626 HDAC2: 0.614 HDAC3: 0.479 HDAC6: 0.054 HDAC8: 0.271 HDAC10: 0.712 HDAC11: 0.928
AL		58% (80%)	HDAC1: 2.213 HDAC2: 2.449 HDAC3: 0.633 HDAC6: 0.033 HDAC8: 0.223 HDAC10: 5.584 HDAC11: 4.500
AM		66% (50%)	HDAC1: 4.867 HDAC2: 7.264 HDAC3: 3.023 HDAC6: 0.457 HDAC8: 1.905 HDAC10: 21.816 HDAC11: 7.507
AN		84% —	HDAC1: 1.297 HDAC2: 2.260 HDAC3: 0.614 HDAC6: 0.050 HDAC8: 0.205 HDAC10: 2.448 HDAC11: 4.270

[a]Crude purities, [b]All compounds were purified by prepHPLC before biological testing

Table 1. Chemical and biological data for hydroxamic acids.

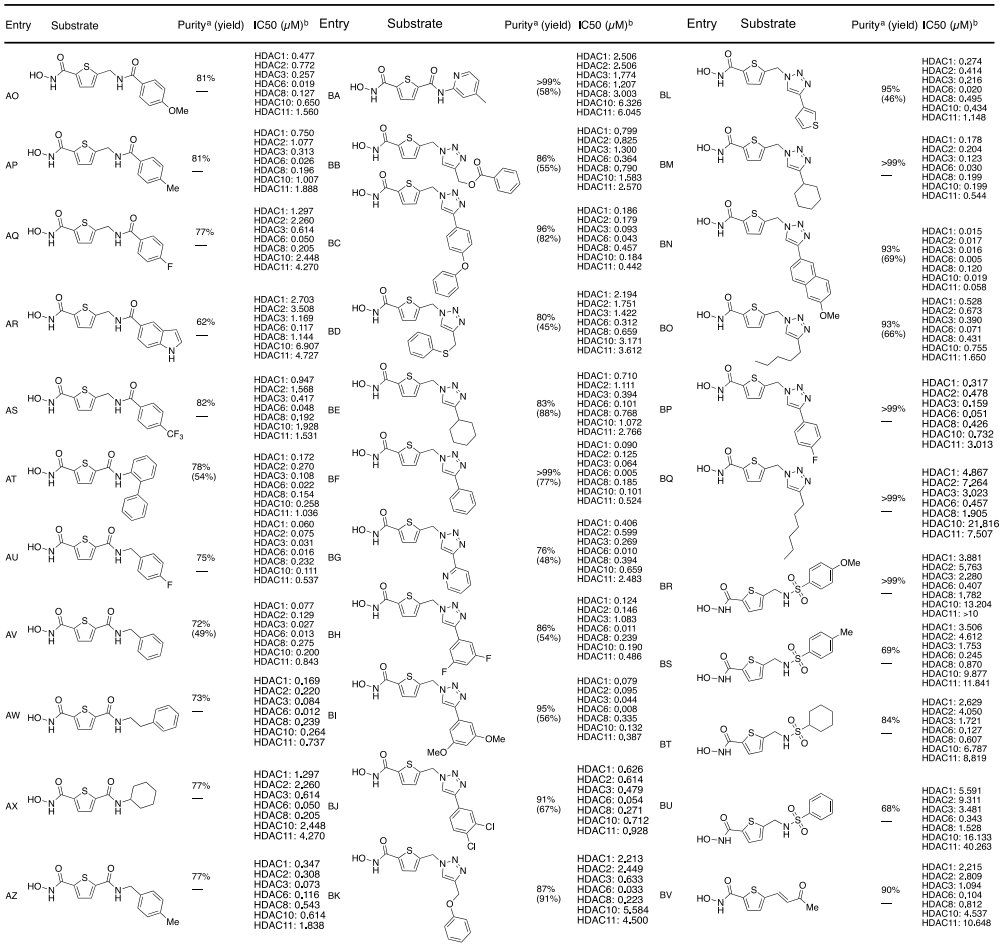


Table 1 continued

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