Peptide fragment screening towards a new inhibitor of neutrophil elastase

By

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Undersökning av en ny metod att söka efter inhibitorer

Vad görs när immunsystemet, systemet byggt för att skydda oss, attackerar oss istället? I dagsläget finns inte så mycket man kan göra. Jag har därför undersökt en ny metod för att hitta ämnen som ska kunna hindra det trasiga immunsystemet från att skada!

Framsteg inom medicinen är bland de viktigaste som kan göras för mänsklighetens fortlevnad. Ett specifikt område inom medicin där det är relativt snålt med detta är immunsystemet. Men immunsystemet är väl bra som det är ju? Mja, visst, när allt funkar som det ska. Ibland slår immunsystemet slint och börjar attackera kroppen, då behövs det läkemedel som tillfälligt kan hindra, med ett finare ord, inhibera, därav ordet inhibitor, de delar som gör skada.

En viktig del av immunsystemet är de vita blodcellerna, de är kroppens försvarare mot olika främmande saker, exempelvis bakterier eller virus som vill bryta sig in i kroppen. Enzymet som valdes att testa vår metod på, det vill säga försöka hitta en inhibitor till, heter neutrofilelastas.

Enzymer är katalysatorer, dvs. påskyndare. Enzymer består av en kedja av aminosyror, där en aminosyra kan ses som byggstenen för enzymer. Tänk på aminosyrorna som pärlor på ett ihopknycklat pärlhalsband, och enzymet som det ihopknycklade halsbandet. Någonstans på detta halsband, finns ett aktivt centrum, där reaktioner skyndas på. En inhibitors syfte är att blockera aktiva centrumet så att enzymet inte kan öka hastigheten på reaktioner, vilket även kan betyda att reaktionen inte kommer ske alls.

Den vita blodcellen som heter neutrofil sprutar ut enzymet neutrofilelastaset när den hittar något i kroppen den inte gillar. Neutrofilelastaset tuggar då endast sönder den främmande saken, i alla fall vanligtvis. Två saker kan ske som gör neutrofilelastaset farligt för kroppen:

1. Kroppens ”stoppmekanism”, det som signalerar att jobbet är färdigt och det är dags att återgå till det normala, är ur funktion.
2. Den lokala koncentrationen av bakterie/virus etc. är låg.

När något av ovanstående händer kan neutrofilelastaset börja attackera kroppen istället, särskilt lungorna. Neutrofilelastas kan orsaka kroniskt obstruktiv lungsjukdom (KOL).

Under fem månaders tid testade jag därför en ny metod för att försöka få fram en inhibitor. Tyvärr hittades ingen inhibitor, varken till neutrofilelastaset eller trypsin som denna metod också undersöktes på. Däremot tror jag ändå på metoden trots att det inte lyckades med just dessa två protein.

Metoden gick ut på att massproducera aminosyrekedjor, dvs pärlhalsbandsbitar, med tre aminosyror per kedja. Mellan 15 till 24 unika aminosyrakedjor var lösta i 13 olika lösningar, vilket gav ca 200 kedjor totalt, och dessa testades med Weak Affinity Chromatography (WAC).

I WAC, något förenklat, har man två packade rör. Det ena röret är bara en kontroll och i det andra binder man fast sitt enzym. Man pumpar sen alla sina lösningar genom de båda rören. Man jämför sedan, med en referens som man vet sätter sig i enzymets aktiva centrum, hur länge kedjorna befunnit sig i rören. Om någon av kedjorna befinner sig längre tid i enzymröret än referensen har man potentiellt hittat startskottet till en ny inhibitor!

Preface

There are many people; family, friends, classmates, professors and my guidance counsellor, who have helped me to gain the knowledge needed to complete this master thesis, I thank you all!

As for the actual completion of my master thesis, there are a select few that made this master thesis possible.

To start with, I would like to extend my infinite gratitude to Dr Richard Johnsson, for his boundless patience, with both me and my constant questions, his pedagogic supervising and his challenging question which taught me a lot over the course of this master thesis project. I am forever indebted to you and endlessly appreciative of your support!

Secondly, I would also like to thank professor Ulf Nilsson for giving me the suggestion to do my master thesis at Red Glead Discovery AB. I am even more grateful that professor Nilsson also took time to be my supervisor. Credit is also due to professor Marie Wahlgren for being my examiner.

Thirdly, I would also like to thank Red Glead Discovery AB's Dr Thomas Brimert who proposed the idea of letting me do my master thesis at the company to the management. Meaning, of course, that I am also very thankful to Dr Johan Evenäs, the CEO of Red Glead Discovery AB, as well the rest of the management for allowing me to be here. I am also thankful to every employee at Red Glead Discovery AB who made me feel like a co-worker and always helped me when I needed it. Three of these employees stand out and deserve a special mention and these are Stella Persson, who prepared and performed the WAC runs for me, and Dr Maria Volkova & Joakim Larsson who always helped me out immediately, whenever I needed it. Thank you to all three of you, without you my master thesis would have never reached the same quality.

Last but not least, I would like to give thanks to Dr Bo Svensson at SARomics for helping me to find a suitable analogue to human neutrophil elastase.

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Abstract

The main purpose of this master thesis was to test a novel approach of finding leads for new pharmaceuticals, by using Solid Phase Peptide Synthesis (SPPS) in combination with Weak Affinity Chromatography (WAC). Put in a more concrete way, the objective was to find a lead for a better human neutrophil elastase inhibitor. The plan was to take advantage of the fact that the tripeptide Valine-Proline-Valine is a known binder to elastase, and synthesis peptide libraries of tripeptides and test the binding affinity using WAC. Unfortunately, the in-house method of binding the protein onto the WAC-column was not compatible with human elastase, so porcine elastase was used instead. A hit for a new lead was possibly discovered, but the data are inconclusive as the reference binder did not show any binding in the assay, raising the suspicious of denatured protein. To validate the method, the same setup, but with a different protein, porcine trypsin and reference binder Threonine-Arginine-Glutamine was also evaluated. According to the results tripeptides with Ac-X-Glutamine-Valine-NH_2 showed potential to have inhibitory effect, but the design of the additional libraries focusing on this sequence showed negative results. Any future experiments should test amino acids with saturated carbon side chains in position X, as all amino acids in this position for the first trypsin WAC run were of that type.

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Aim

The aim of this thesis is twofold. 1) To examine the hypothesis that peptides can be used as fragments in WAC should be tested and evaluated, something that has never been done before and 2) To find a new starting point in development of a new protein inhibitor for neutrophil elastase.

Abbreviations

3-Pal – (3-Pyridyl)-alanine

Aad – Aminoadipic acid

Abu – Aminobutyric acid

Ac – Acetyl

Aze – Azetidine-2-carboxylic acid

Dab –Diaminobutyric acid

DIC – N,N'-Diisopropylcarbodiimide

DIPEA – N-Ethyl-N-(propan-2-yl)propan-2-amine

DMF – Dimethylformamide

DMSO – Dimethyl sulfoxide

Fmoc – Fluorenylmethyloxycarbonyl (protecting group)

Har – Homoarginine

Hph – Homophenylalanine

Hyp – Hydroxyproline

iPrOH - Isopropanol

Nva – Norvaline

Nle – Norleucine

Orn – Ornithine

Oxyma – Ethyl (2Z)-2-cyano-2-hydroxyiminoacetate

Phg – Phenylglycine

Pip – Pipecolic acid (Homoproline)

SPPS – Solid Phase Peptide Synthesis

TFA – Trifluoroacetic acid

TIPS – Triisopropylsilane

WAC – Weak Affinity Chromatography

Background & Introduction

Biological background

The immune system is a highly complex and fascinating system, which protects its host, that is, when it works as it is intended. There are numerous cases when the immune system does harm instead of good.

An example of harm involves one type of white blood cells, more specifically neutrophils, which form a part of the innate immune system. Neutrophils are both the most abundant type of granulocytes, which get their names from the fact that they have granules in their cytoplasm, and the most abundant type of white blood cells in general [1].

Neutrophils are created in the bone marrow from stem cells, and when mature they are normally found in the bloodstream. They have three different ways of fighting antigens; the first is by phagocytosis, the second is by secreting anti-microbials and the third is by forming Neutrophil extracellular traps (NET) [2].

The enzyme neutrophil elastase (NE), which is stored in the azurophilic granules of neutrophils, is both secreted as anti-microbial on its own, but it is also an important anti-microbial role in NETs. Much like the acronym of NET hints, a net-like structure is secreted and formed by neutrophils, which thus trap the microbials, and these NETs also contain the enzyme in large concentrations to eliminate the microbials [2].

The risk of harm arises from the fact that the elastase has a very high affinity for membranes [3], meaning for example that when the microbial within the NET, i.e. locally, is in very low concentration the enzyme can start to attack host cell membranes instead. One specific area where NE has been known to cause damage is in the lungs, where it can cause Chronic Obstructive Pulmonary Disease (COPD). Although there are a few inhibitors naturally occurring in the body, that are called serpins (serine protease inhibitors) [4], it is of interest to create a NE inhibitor. One of the reasons for this is that the endogenous inhibitor cannot follow NE out of the blood stream because of their large size [3]. Regardless of the endogenous NE inhibitors, the need for a synthetic inhibitor is of relevance, especially for those with deficiency-diseases, like A1AD (alpha-1-antitrypsin deficiency), as people with these conditions lack the endogenous inhibitor.

Peptide chemistry background

This master thesis would not have been possible without the scientific achievements of Robert Bruce Merrifield who invented Solid Phase Peptide Synthesis (SPPS), and thus increased the speed of which peptide could be synthesized. For this discovery he received the Nobel Prize in chemistry 1984 [5].

Before the screening of the peptides could take place, they had to be synthesized. They were synthesized on a Biotage Alstra Initiator using SPPS, this machine can be seen in **Figure 1** below.

Figure 1. The Biotage Alstra Initiator, used for peptide synthesis.

The principles of SPPS are based on relatively straight-forward and repetitive chemistry. An overview of a general tripeptide synthesis can be seen in **Figure 2** below.

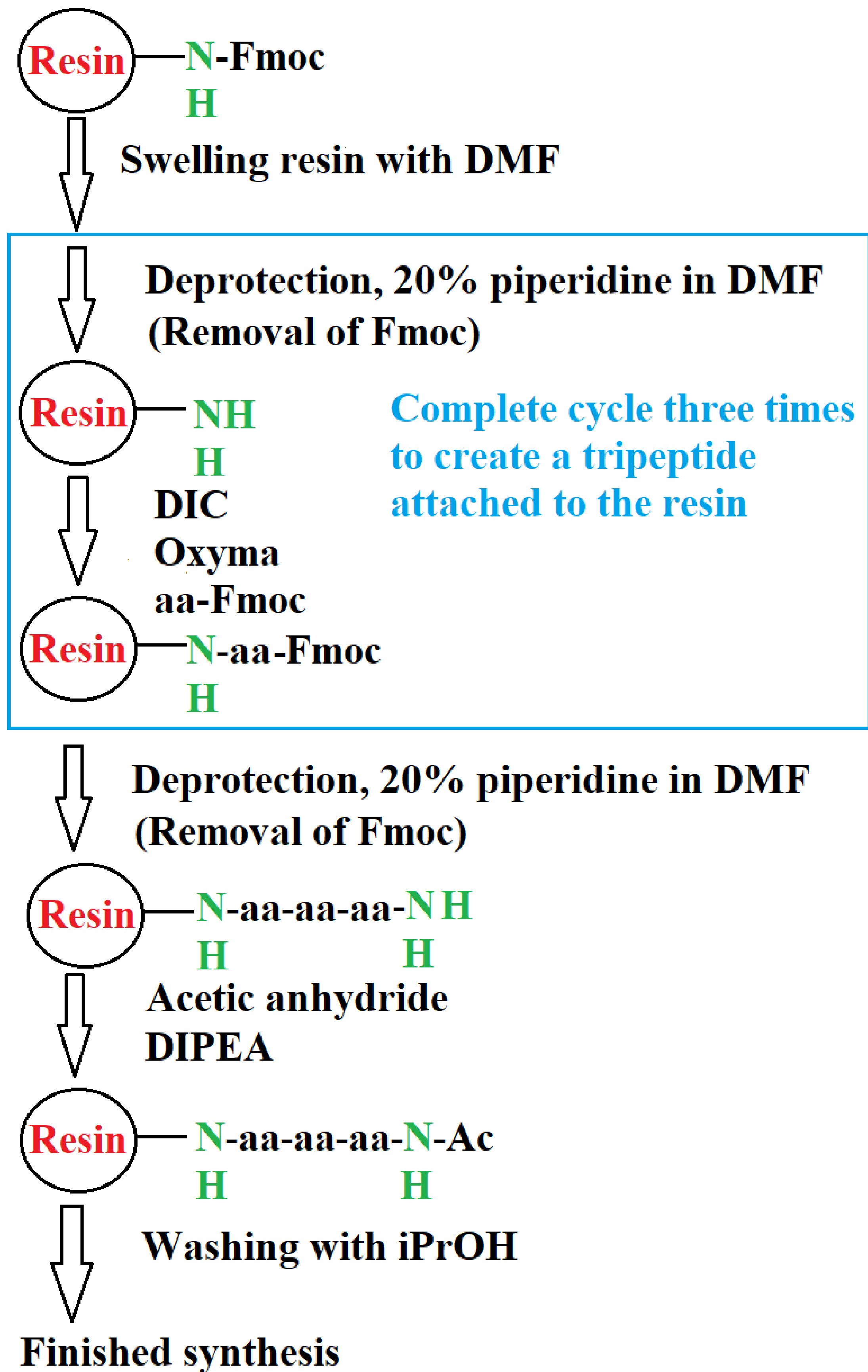

Figure 2. A general schematic of the peptide chemistry behind SPPS for a tripeptide on a Biotage Alstra Initiator.

The resin can be thought of as a small porous bead with many handles on which the amino acids can be attached, where most of the handles are not on the surface, but within the bead. These handles are composed of an amine attached to the resin via a linker, and an Fmoc group attached to protect the amine. In order to reach these handles, the resin is swelled using DMF. When the resin has been swelled, the Fmoc protecting group is removed using piperidine. When the Fmoc has been removed, the coupling reagents DIC and Oxyma as well as an Fmoc protected amino acid are added to the reaction vessel containing the resin. The reason to use Oxyma and not, for example hydroxybenzotriazole (HOBt), is because Oxyma is less explosive and is a better racemization-suppressant [6].

The amino acid reacts first with DIC to create the O-acylisourea. This intermediate is then attacked by Oxyma, to form the activated ester and the DIC is released as a urea. This new ester bond, between the amino acid and Oxyma, is then attacked by the amine attached to the resin and thus the first amino acid has been successfully coupled onto the resin. When the first amino acid has been attached, which will be the C-terminal of the tripeptide (i.e. it is synthesized backwards, as the common praxis of amino acid numbering in peptides is from the N-terminal to the C-terminal), the Fmoc on the N-terminal is removed using piperidine just like from the resin. The reaction mechanisms of attaching a threonine (Thr) to the resin with Fmoc on is illustrated in **Figure 3** below.

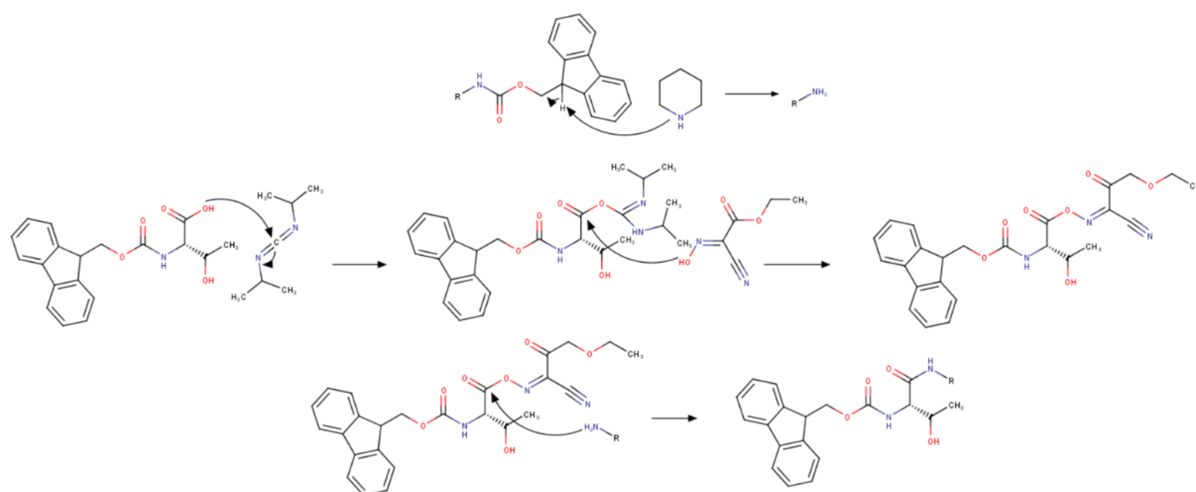

Figure 3. The reaction mechanism of attaching a Thr to the resin, R = resin.

When the Fmoc has been removed, it is possible to attach a new amino acid to the previous one that is attached to the resin. This is done following the same procedure, with DIC/Oxyma, as for attaching the first amino acid to the resin.

This cycle is then repeated until the whole tripeptide is attached to the resin. After the last amino acid is attached, and its Fmoc has been removed, the N-terminal is acetylated with acetic anhydride with help of the base DIPEA. There are two reasons to acetylate the N-terminal, one is to increase the serum stability of the tripeptide and the other is make it a better mimic of a longer peptide sequence.

The last step in the peptide synthesizer is to wash the resin with iPrOH, both to shrink the swollen resin but mainly to get rid of the solvent DMF.

After a completed synthesis, the peptides are dried in a vacuum desiccator overnight to remove the iPrOH. When the peptides have been dried, they are cleaved from the resin with TFA, any side-chain protecting group present are also cleaved with TFA at the same time. TIPS and water are added simultaneously to make sure these protecting group do not reattach to the tripeptide.

The resin is then filtered off from the peptide solution that is mainly TFA, and then evaporated to remove the TFA. The peptide is then re-dissolved and lyophilized overnight.

An illustration of the freeze-dried tripeptide powder can be seen in **Figure 4** below.

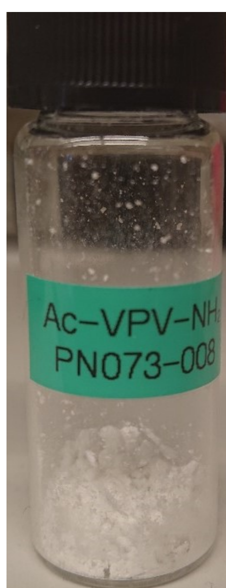

Figure 4. How the tripeptides, in this case the reference Valine-Proline-Valine, look after it has been synthesized, dried, evaporated and lyophilized.

This freeze-dried powder was finally dissolved in buffer before it was screened with WAC.

WAC background

The fundamentals of WAC are rather simple to understand. In layman's terms four things are required to do it. A reference chromatography column with no bound target material, and a target chromatography column where the target, in this case elastase, has been bound to the column. The third thing is the reference binder, i.e. something that is known to bind to the target. Lastly, molecules are needed that are to be compared with the reference binder to see if they bind better than the reference.

To achieve above-mentioned conditions, the stationary phase of the columns is made up of silica. On the target column the protein is immobilized on the silica and on the reference column the silanol groups are masked with ethanolamine. All the tripeptides as well as the reference compounds are run on both columns, and their retention time are measured in both systems. The binding is measured by comparing the retention time difference between the reference column and the target column, and the strength of the binding is then correlated with the known binders [7] [8].

Results & Discussion

WAC

The protein column was run twice to acquire a higher statistical precision on how well the tripeptides interact with the protein. The strength of the interaction is the essential part of finding an inhibitor, so it goes without saying that the highest possible precision is desirable.

Neutrophil elastase

The standard way to bind protein to the WAC column is through the lysines of the protein. However, the human neutrophil elastase does not contain any lysine and could hence not be bound in the same way [9]. Possibly, the N-terminal, which also contains a free amine, could be used, however it is buried within the protein's tertiary structure and thus not accessible. To find an alternative we looked at porcine neutrophil elastase which has a homology of 70% to human NE and contains three lysines. An illustration of the porcine NE superimposed on human NE can be seen in **Figure 5** below.

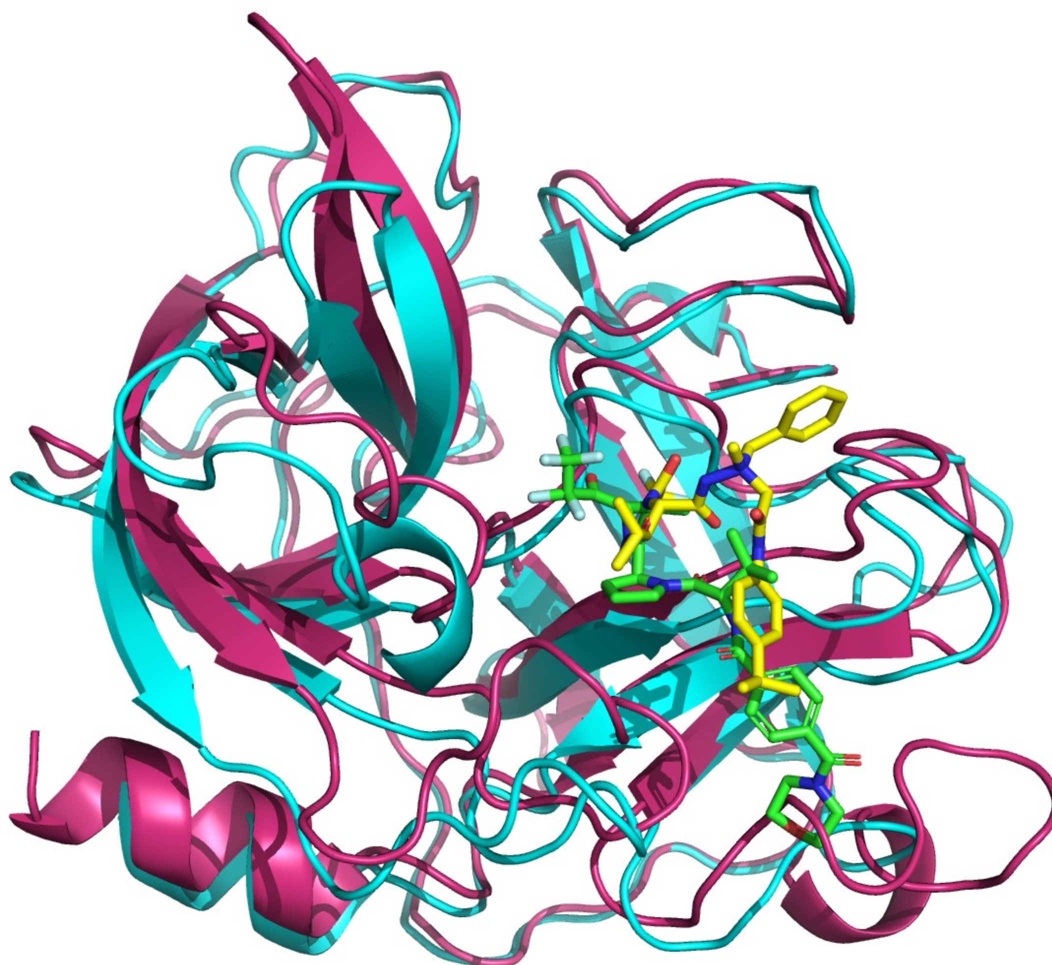

Figure 5. The alignment of porcine (purple) and human (cyan) NE with their respective inhibitor. Porcine NE inhibitor in yellow, human NE inhibitor in green. Created in the program PyMOL by Dr Bo Svensson (SARomics).

Two of the lysines seemed to be in the active site but one should be easily accessible for binding to the column.

The tripeptide Val-Pro-Val is a recognition sequence for neutrophil elastase [10]. Therefore, it was decided it would be the reference, meaning it was the retention time of this tripeptide that was used as comparison in WAC. Initially it was synthesized as H-Val-Pro-Val-NH_2. However, on a tripeptide the amino functionality has a large impact on the properties on the molecule rendering it difficult to purify, hence we decided to acetylate the N-terminal to yield Ac-Val-Pro-Val-NH_2. This tripeptide was thus used as the reference for the neutrophil elastase in WAC.

Two small molecules, 3-aminobenzotrifluoride and 1-acetamido-3-trifluoromethylbenzene, are also good reference molecules as they interact with the binding site of neutrophil elastase. The three reference binders can be seen in **Figure 6** below.

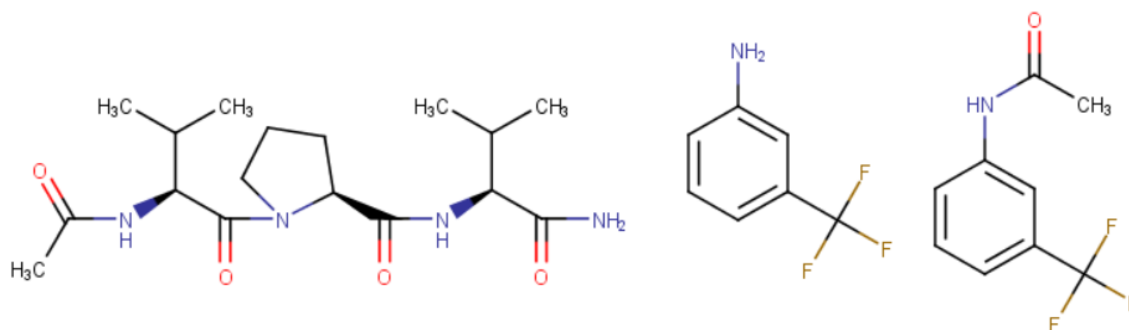

Figure 6. The structure of the acetylated tripeptide Ac-V-P-V-NH_2 (left) 3-aminobenzotrifluoride (middle) and 1-acetamido-3-fluoromethylbenzene (right).

All libraries were designed following a certain pattern to systematically try and to find a better inhibitor using similar tripeptides or amino acid traits.

We decided to keep the valine in position 3 constant and vary position 1 and 2 in the libraries. Lib014, Lib016 and Lib022 had saturated carbon chains of differing length on the side chain at aa_1, which is what valine has too. Since proline is a cyclic amino acid and is in the middle of the Val-Pro-Val sequence (i.e. position aa_2), two other cyclic amino acids (azetidine-2-carboxylic acid (Aze) & pipecolic acid (Pip)) as well as proline were tested in Lib014, Lib016 and Lib022 at position aa_2 in combination with the previously mentioned saturated carbon side chain amino acids. These three libraries also had three "exploring" amino acid each at position aa_2. By "exploring" amino acid it is meant that they do not really follow a certain system or trail of thought when they were added, but instead they are added to find unprecedented interactions and to increase the likelihood of a hit. The only thing that needed to be considered when adding these "exploring" amino acids was not to break the rule that tripeptides within the same library should differ in molar mass by at least 1 g/mol. This rule was there so that all tripeptides could be distinguished in WAC.

As previously mentioned, it was known that two aromatic substances are known binders to human neutrophil elastase, it seemed as a logical assumption to create tripeptides with cyclic and aromatic side chains. Thus, this was done with Lib018, with a few "explorers" at position aa_2.

Lib020 was created as a random library containing only low molecular weight usual amino acids.

The results of the WAC for neutrophil elastase were easily analyzed as every single tripeptide gave a response on both the reference and protein column (see Weak Affinity Chromatography

(WAC) results for neutrophil elastase). Sadly, very few tripeptides had an increased retention in the protein column, and only one had an increased retention that was statistically significant.

Ac-Val-Phg-Val-NH_2 was the only tripeptide with a statistically significant increased retention time, i.e. the only possible new lead, but the data was inconclusive as the reference binder did not show any binding in the assay, raising the suspicious of denatured protein. Meaning it was not deemed interesting for further investigation.

Trypsin

To validate the method, i.e. to use tripeptides in WAC, we switched to a different protein, porcine trypsin, that had a published tripeptide binder Ac-Thr-Arg-Glu-NH_2 [11]. The Ac-Thr-Arg-Glu-NH_2 tripeptide can be seen in **Figure 7** below.

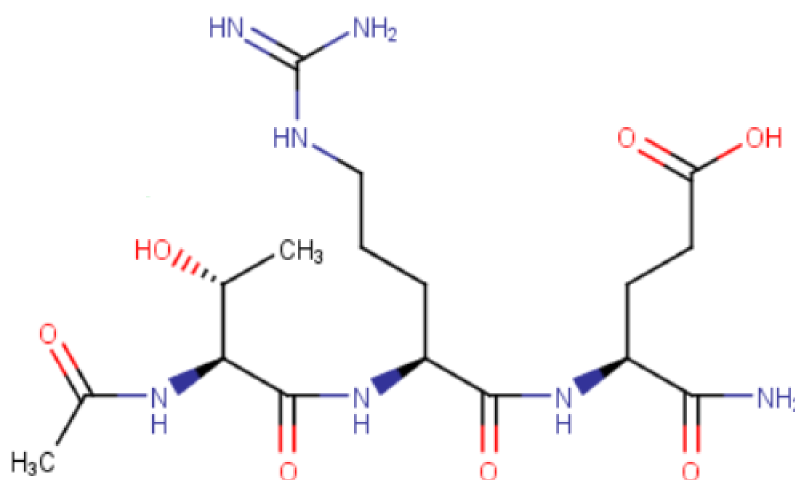

Figure 7. The Ac-Thr-Arg-Glu-NH_2 tripeptide

The libraries for the porcine trypsin WAC followed a simple concept. According to Saikhedkar et al. the tripeptide Ac-Thr-Arg-Glu-NH_2 was the most efficient tripeptide inhibitor against porcine trypsin in their study, but there were also other inhibitors that inhibited the porcine trypsin to lesser extent [11].

All the inhibitors in their study contained a threonine or proline at position aa_1, which was taking into consideration when choosing the amino acids for our libraries. In position aa_1 two different groups of four amino acids were used for the six libraries. In one group the side chain contained a thiol or an alcohol functional group, due to that threonine has an alcohol group on its side chain. In the other group the amino acid was either a proline or an amino acid with similar molar mass.

At position aa_2 arginine was the amino acid used in the more successful inhibitors, but there was also some inhibition when there was a lysine instead [11]. Hence, the two available amino acids containing guanidine, homoarginine (Har) and arginine, were tested as well as amino acids containing amines on the side chain. Specifically, diaminobutyric acid (Dab), lysine and ornithine (Orn) were also tested. Once again, it seemed interesting to test aromatic amino acids as well, so 3-pyridyl alanine (3-Pal) and homophenylalanine (Hph) were also tested in position

aa_2. 3-Pal was tested as its aromatic ring contains a nitrogen, meaning there is some similarity with the amines, and Hph was tested as an "exploring" because it is aromatic.

At position aa_3 two of the three amino acids, for the six different inhibitors, were glutamic acid and asparagine [11]. Therefore, it was deemed interesting to investigate amino acids with an acidic side chain and the corresponding side chain with an amide side chain. Meaning that in position aa_3 two different groups of three amino acids were used. One group contained the three acidic amino acids; aspartic acid (Asp), glutamic acid (Glu) and aminoadipic acid (Aad). In the other group the only two amide amino acids that were available were asparagine (Asn) and glutamine (Gln). It was desired to have at least one "exploring" amino acid in this position, besides Asn and Gln. Since we synthesized peptide amides, meaning that the C-terminal (aa_3) would be an amide, one interesting amino acid would be glycine, as it will generate one amide functionality instead of two as for Asn and Gln.

Unlike during the WAC run for neutrophil elastase and those libraries, the trypsin libraries were very hard to analyze as many tripeptides did not give a response on neither the reference column nor the protein column. Initially most of the tripeptides in these libraries did not give a response at their molar mass. Out of the six libraries only three (lib030, lib032 and lib034) gave responses at a satisfactory level to begin with.

It was later discovered that an error had occurred in the peptide synthesizer during the acetylation step, so many of the tripeptides were never acetylated. When this had been taken into account and the mass without the acetylation had been confirmed on a LCMS, responses were found for lib028 and lib036 after adjusting the mass. Further investigation revealed that a programing error had occurred, the Fmoc on the last amino acid had not been removed, with lib026. Although this was confirmed with LCMS most of the tripeptides in this library did not give of any response on the WAC even after adjusting the mass.

Since it only meant more data, and that most of the work was already done, the libraries from the neutrophil elastase were run on the trypsin column as well. Not only did all the tripeptides give a response again, but the four tripeptides that had Gln in position aa_2 and Val in position aa_3 (all from lib22) seemed to indicate an increased retention time on the trypsin column. Therefore, an additional two peptide libraries were synthesized trying different constellations with Gln and Asn in position aa_2, with four different amino acids in positions aa_1 and aa_3.

These two new libraries (lib038 and lib040) were run on the reference column and trypsin column as well.

Although all the tripeptides in these two libraries gave a response, none of them showed any significantly increased retention time, meaning no new lead for an inhibitor had been discovered.

Conclusion and future perspective

We set out to see if peptide fragments could be tested and evaluated with WAC, an unprecedented method, and we proved that it is possible. Especially with the neutrophil elastase where we had response from all the tested tripeptides. Meaning we fulfilled our first aim.

Moreover, thirteen different libraries had been examined as a fragment of potential inhibitors for two different enzymes. It can on the other hand still not be ruled out that the tripeptide Ac-Val-Phg-Val-NH_2 might have inhibitory effect on Neutrophil elastase and that the motif Ac-X-Q-V-NH_2 is an inhibitor for Trypsin.

Despite the second aim not being fulfilled, for today at least, there is a high belief that using peptide fragment screening on WAC will yield new inhibitors for proteins in the future.

It can easily be argued that the future of finding peptide fragment inhibitors is almost solely dependent on the technology of the synthesizer used, which would be a fair assumption. Since a variety of peptides is accessible by the fast and robust SPPS method, as well as being able to synthesize the unusual amino acids in addition to making and handling different usual amino acids, there is a bright future for peptides in fragment-based drug discovery.

It would be interesting to bind human neutrophil elastase (hNE) to a WAC column using another method that does not involve lysines and run these libraries again on hNE. It would also be interesting to test the sequence Ac-Val-Phg-Val-NH_2 to remove any doubt whether it has the potential to be an inhibitor or not for hNE and not the porcine analogous.

If these experiments would be repeated, I would also like to make sure that all tripeptides will be present in acetylated form and investigate why some tripeptides do not give a response.

Finally, in the first trypsin WAC run, the four potential hits, the sequences Ac-X-Gln-Val-NH_2, all contained a non-cyclic amino acid with a saturated carbon side chain, and these types were not tested in the second trypsin WAC run. Therefore, it would be interesting to investigate these types of amino acids in position aa_1 in the future.

Experimental part

Standard peptide synthesis

Peptides were synthesized on a Biotage Alstra Initiator. The main solvent was DMF, coupling reagents were DIC in 0.5 M DMF and OxymaPure, and Fmoc deprotection was performed with 20% piperidine in DMF. Amino acids were used in 4 equivalents at 0.4 M concentration and the coupling time was 60 min at room temperature for mixes containing Cys and His, 20 min at 45 °C for mixes containing Arg and Har, and 8 min at 70 °C for the rest of the mixes. After the final deprotection the peptide was washed with iPrOH before drying.

Standard release from resin and side-chain deprotection

The peptide was suspended in 10 mL of a TFA/H_2O/TIPS 95:3:2 (v:v:v) mixture and shaken at room temperature for at least 180 min if it contained Arg or Har, or at least 60 min otherwise. After the cleavage was done the resin was filtered off and the TFA solution was evaporated down. The residue was re-dissolved in dioxane or AcOH and lyophilized.

Ac-Val-Pro-Val-NH_2

Was synthesized on TentaGel HL RAM resin (676 mg, 0.38 mmol/g) and cleaved according to standard procedure. Confirmed by LCS, MS (ESI) calculated for $C_{17}H_{30}N_4O_4H$ $[M+H]^+$ 355.23, found 355.20. The crude peptide was purified by Flash chromatography on a C18 (SNAP Bio 25 g) column. An ^1H NMR was performed, and the following data was received: ^1H NMR (400 MHz, DMSO-d6) δ 8.03 (d, J = 8.5 Hz, 1H), 7.68 (d, J = 8.9 Hz, 1H), 7.32 (s, 1H), 6.98 (s, 1H), 4.42 (dd, J = 7.7, 4.1 Hz, 1H), 4.30 (t, J = 8.4 Hz, 1H), 4.07 (dd, J = 8.7, 6.5 Hz, 1H), 3.72 (s, 1H), 3.58 – 3.51 (m, 1H), 1.99 – 1.90 (m, 4H), 1.85-1.81 (m, 2H), 1.83 (s, 3H), 0.86 (dt, J = 15.6, 6.7 Hz, 12H).

Lib014 (Ac-aa_1-aa_2-Val-NH_2)

Was synthesized on TentaGel HL RAM resin (1106 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 20 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib014 at position aa_1 and aa_2 can be seen in **Table 1** below.

Table 1. The amino acids used in position aa_1 and aa_2 for Lib014

aa_1	aa_2
Gly	Pro
Ala	Cys
Abu	Thr
Nva	Trp
Nle	

Lib016 (Ac-aa_1-aa_2-Val-NH_2)

Was synthesized on TentaGel HL RAM resin (1105 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 20 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib016 at position aa_1 and aa_2 can be seen in **Table 2** below.

Table 2. The amino acids used in position aa_1 and aa_2 for Lib016

aa1	aa2
Gly	Aze
Ala	Ser
Abu	Phe
Nva	Tyr
Nle	

Lib018 (Ac-aa_1-aa_2-Val-NH_2)

Was synthesized on TentaGel HL RAM resin (1090 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 15 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib018 at position aa_1 and aa_2 can be seen in **Table 3** below.

Table 3. The amino acids used in position aa_1 and aa_2 for Lib018

aa_1	aa_2
Ser	Phe
Pro	Phg
Val	Hph
Phe	
Trp	

Lib020 (Ac-aa_1-aa_2-Val-NH_2)

Was synthesized on TentaGel HL RAM resin (657 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 16 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib020 at position aa_1 and aa_2 can be seen in **Table 4** below.

Table 4. The amino acids used in position aa_1 and aa_2 for Lib020

aa_1	aa_2
Leu	Thr
Gly	Ala
Ala	Val
Cys	Pro

Lib022 (Ac-aa_1-aa_2-Val-NH_2)

Was synthesized on TentaGel HL RAM resin (638 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 20 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib022 at position aa_1 and aa_2 can be seen in **Table 5** below.

Table 5. The amino acids used in position aa_1 and aa_2 for Lib022

aa_1	aa_2
Gly	Pip
Ala	Met
Abu	Gly
Nva	Gln
Nle	

Ac-Thr-Arg-Glu-NH_2

Was synthesized on TentaGel HL RAM resin (671 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the peptide was confirmed by LCMS, MS (ESI) calculated for $C_{17}H_{31}N_7O_7H$ $[M+H]^+$ 446.23, found at 445.90.

Lib026 (Ac-aa_1-aa_2-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (686 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 24 peptides in the mixture were not found LCMS initially. After an investigation it was revealed that the last Fmoc had not been removed and by adjusting the masses to this fact, all the 24 peptides presence were confirmed with LCMS. All the amino acids used in Lib026 at positions aa_1, aa_2, and aa_3 can be seen in **Table 6** below.

Table 6. The amino acids used in position aa_1, aa_2 and aa_3 for Lib026

aa_1	aa_2	aa_3
Thr	Arg	Asp
Tyr	Orn	Glu
Hyp		Aad
Cys		

Lib028 (Ac-aa_1-aa_2-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (691 mg, 0.38 mmol/g) and cleaved according to standard procedure. This library did not acetylate, but all the non-acetylated peptides were confirmed with LCMS. All the amino acids used in Lib028 at positions aa_1, aa_2, and aa_3 can be seen in **Table 7** below.

Table 7. The amino acids used in position aa_1, aa_2 and aa_3 for Lib028

aa_1	aa_2	aa_3
Thr	Har	Asp
Tyr	Dab	Glu
Hyp		Aad
Cys		

Lib030 (Ac-aa_1-aa_2-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (671 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 24 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib030 at positions aa_1, aa_2, and aa_3 can be seen in **Table 8** below.

Table 8. The amino acids used in position aa_1, aa_2 and aa_3 for Lib030

aa_1	aa_2	aa_3
Pro	Arg	Asp
Ser	Orn	Glu
Val		Aad
Met		

Lib032 (Ac-aa_1-aa_2-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (676 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 24 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib032 at positions aa_1, aa_2, and aa_3 can be seen in **Table 9** below.

Table 9. The amino acids used in position aa_1, aa_2 and aa_3 for Lib032

aa_1	aa_2	aa_3
Pro	Har	Asp
Ser	Hph	Glu
Val		Aad
Met		

Lib034 (Ac-aa_1-aa_2-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (671 mg, 0.38 mmol/g) and cleaved according to standard procedure. The presence of the 24 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib034 at positions aa_1, aa_2, and aa_3 can be seen in **Table 10** below.

Table 10. The amino acids used in position aa_1, aa_2 and aa_3 for Lib034

aa_1	aa_2	aa_3
Thr	Arg	Asn
Tyr	Lys	Gln
Hyp		Gly
Cys		

Lib036 (Ac-aa_1-aa_2-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (695 mg, 0.38 mmol/g) and cleaved according to standard procedure. This library did not acetylate, but all the non-acetylated peptides were confirmed with LCMS. All the amino acids used in Lib036 at positions aa_1, aa_2, and aa_3 can be seen in **Table 11** below.

Table 11. The amino acids used in position aa_1, aa_2 and aa_3 for Lib036

aa_1	aa_2	aa_3
Thr	Har	Asn
Tyr	3-Pal	Gln
Hyp		Gly
Cys		

Lib038 (Ac-aa_1-Gln-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (686 mg, 0.24 mmol/g) and cleaved according to standard procedure. The presence of the 16 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib038 at positions aa_1, aa_2, and aa_3 can be seen in **Table 12** below.

Table 12. The amino acids used in position aa_1 and aa_3 for Lib038

aa_1	aa_3
Pro	Asn
Thr	Tyr
Arg	Phe
Lys	Val

Lib040 (Ac-aa_1-Asn-aa_3-NH_2)

Was synthesized on TentaGel HL RAM resin (670 mg, 0.24 mmol/g) and cleaved according to standard procedure. The presence of the 16 peptides in the mixture was confirmed by LCMS. All the amino acids used in Lib040 at positions aa_1 and aa_3 can be seen in **Table 13** below.

Table 13. The amino acids used in position aa_1 and aa_3 for Lib040

aa_1	aa_3
Pro	Asn
Thr	Tyr
Arg	Phe
Lys	Val

Immobilization of protein to WAC column

The preparations were done over the course of three days, following an in-house WAC-protocol. Two batches were prepared, one control column (reference column) and one column with protein. The first day the first step was to weigh out 150 mg diol silica, in two 15 mL Falcon-tubes. Each silica diol was washed with 10 mL of Milli-Q Water, and then centrifuged with 1000 rpm for 6 min. After the centrifugation the supernatant was removed.

Metaperiodic acid (250 mg) was weighed out in two vials and dissolved in 2.5 mL Milli-Q water. The acid was added to each batch to start a Malaprade oxidation on the diol, and then the two vials were put in the centrifuge for 2 hours.

The oxidation reaction was finished when the silica batches have been washed 3 times with 10 mL 0.2 M sodium phosphate buffer at pH 7 with a centrifugation with 1000 rpm for 6 min after each wash. The supernatant was removed after each wash.

The following steps were performed at 4 °C.

The protein stock was diluted to a volume of 2.5 mL in a 15 mL Falcon tube with immobilization buffer and was then mixed gently. For neutrophil elastase, 7.45 mg lyophilized neutrophil elastase from porcine pancreas was weighed up (from Sigma Aldrich, batch # SLBQ6560V) and diluted in 2.5 mL immobilization buffer. For trypsin, 9.7 mg trypsin from porcine pancreas was weighed up (from Sigma Aldrich, batch # SLBW1172) and first diluted in 0.5 mL milliQ water and then 2.0 mL immobilization buffer.se

The mixture was centrifuged at 4000 rpm for 6 min to remove any precipitated protein. 10 µL was transferred and mixed with 120 µL immobilization buffer (0.2 M sodium phosphate at pH 7). The absorbance of the previously mentioned mixture was measured three times at 280 nm, with the zero being pure immobilization buffer. The 130 µL solution was then added to the one of the silica slurry tubes and marked as protein.

The other silica slurry was marked as reference and 2.5 mL immobilization buffer as well as 17.5 µL ethanolamine was added. The final step of the first day was to add 25 mg $NaBH_3CN$ to both the reference column and the protein one, and then both columns were put on a gentle shaker overnight in a cool room (4 ^{0}C).

The ethanolamine reacts with the aldehyde to form an imine in the reference column, and with the help of $NaBH_3CH$ it is reduces to an amine, with an alcohol that is less reactive with substances being tested, at the end. This reaction is called reductive amination.

The reason to perform this reaction is to deactivate the reactive aldehydes on the silica, to prevent unwanted side reactions, which is also why it is only done in the reference column to start with. In the protein column the aldehydes are kept initially, because these are needed to bind the amines of the protein to the silica. This is also done by a reductive amination, but instead of ethanolamine it is usually the amine on the lysine side chain that take part in the reaction.

The morning of the second day the immobilization reaction of the protein was stopped by washing three times with 10 mL of immobilization buffer, centrifuging after each time at 1000 rpm for 6 minutes. The supernatant was pooled and collected from the protein marked tube. 2.5 mL immobilization buffer was added to the silica. The supernatant was centrifuged at 4000

rpm for 6 min to remove any remaining silica and the absorbance was measured at 280 nm three times to indirectly estimate the immobilization yield of the protein.

The remaining aldehyde groups (not bound to the protein because of steric hindrance or due to being in excess) in the protein marked tube, were deactivated with 17.5 µL ethanolamine to prevent previously mentioned reactive aldehydes from creating undesired side reactions.

The third and final day the material for the protein column was washed 3 times with 10 mL immobilization buffer, and centrifuge at 1000 rpm for 6 min after each wash. Lastly, 1 mL immobilization buffer was added. The slurry was stored on ice until the packing of the columns occurred, which was later that same day.

Before the WAC could be run, the reference and peptide libraries had to be dissolved, so 0.5 mL of each library, and the reference was prepared at a concentration of 50 µM in ammonium acetate buffer 20 mM pH 6.8 with 0.1% DMSO.

The dissolved peptides were run through the reference column once for 20 min, while they were run through the target column twice for 30 min.

Weak Affinity Chromatography (WAC) results for neutrophil elastase

The results from the WAC run for neutrophil elastase were put together in a table, and this can be seen in **Table 14** below.

Table 14. The results from the WAC for porcine neutrophil elastase. Where Mix refers to if it is a reference binder or which library it is from. Fragment ID is the peptide sequence from the N-terminal to the C-terminal, although not written all N-terminals are acetylated. $[M+H]^+$ is the molar mass of the protonated, and hence charged, peptide. The possible lead Ac-V-Phg-V-NH_2 is marked with green.

Mix	Fragment ID	$[M+H]^+$	Retention time, reference column (min)	Comment	Retention time, protein column 1 (min)	Retention time, protein column 2 (min)	Comment	Average retention time, protein column (min)	ΔRT (min)
Reference	V-P-V	355,3	0,598		0,671	0,669		0,67	0,0720
14	G-P-V	313,2	0,556		0,521	0,522		0,5215	-0,0345
14	A-P-V	327,2	0,573		0,618	0,610		0,614	0,0410
14	Abu-P-V	341,2	0,592		0,697	0,669		0,683	0,0910
14	Nva-P-V	355,3	0,626		0,736	0,770		0,753	0,1270
14	Nle-P-V	369,3	0,734	DP(0,503)	0,764	0,759		0,7615	0,0275
14	G-C-V	319,2	0,658		0,698	0,698		0,698	0,0400
14	A-C-V	333,2	0,656		0,736	0,743		0,7395	0,0835
14	Abu-C-V	347,2	0,677		0,803	0,821		0,812	0,1350
14	Nva-C-V	361,2	0,720		0,882	0,915		0,8985	0,1785
14	Nle-C-V	375,2	0,857		0,494	0,495	Prot1 DP(0,900) Prot2 DP(906)	0,4945	-0,3625
14	G-T-V	317,2	0,553		0,511	0,519		0,515	-0,0380
14	A-T-V	331,2	0,543		0,559	0,580		0,5695	0,0265
14	Abu-T-V	345,2	0,559		0,603	0,601		0,602	0,0430
14	Nva-T-V	359,3	0,593		0,639	0,640		0,6395	0,0465
14	Nle-T-V	373,3	0,638		0,606	0,609		0,6075	-0,0305
14	G-W-V	402,2	0,698		0,618	0,611		0,6145	-0,0835
14	A-W-V	416,3	0,737		0,632	0,645		0,6385	-0,0985
14	Abu-W-V	430,2	0,799		0,676	0,679		0,6775	-0,1215
14	Nva-W-V	444,3	1,033		0,706	0,699		0,7025	-0,3305
14	Nle-W-V	458,3	1,662		0,740	0,749		0,7445	-0,9175
16	G-Aze-V	299,2	0,554		0,519	0,521		0,52	-0,0340
16	A-Aze-V	313,2	0,563		0,571	0,556		0,5635	0,0005
16	Abu-Aze-V	327,2	0,598		0,637	0,639		0,638	0,0400
16	Nva-Aze-V	341,2	0,650		0,670	0,672		0,671	0,0210
16	Nle-Aze-V	355,2	0,856		0,711	0,707		0,709	-0,1470
16	G-S-V	303,2	0,553		0,519	0,520		0,5195	-0,0335
16	A-S-V	317,2	0,555		0,573	0,591		0,582	0,0270
16	Abu-S-V	331,2	0,549		0,645	0,640		0,6425	0,0935
16	Nva-S-V	345,2	0,590		0,697	0,697		0,697	0,1070
16	Nle-S-V	359,3	0,634		0,659	0,661		0,66	0,0260
16	G-F-V	363,2	0,610		0,627	0,621		0,624	0,0140

16	A-F-V	377,2	0,625		0,638	0,638		0,638	0,0130
16	Abu-F-V	391,2	0,647		0,647	0,653		0,65	0,0030
16	Nva-F-V	405,3	0,731		0,679	0,682		0,6805	-0,0505
16	Nle-F-V	419,3	1,008		0,691	0,690		0,6905	-0,3175
16	G-Y-V	379,2	0,581		0,607	0,574		0,5905	0,0095
16	A-Y-V	393,2	0,602		0,612	0,614		0,613	0,0110
16	Abu-Y-V	407,2	0,608		0,670	0,658		0,664	0,0560
16	Nva-Y-V	421,3	0,637		0,516	0,515	Prot1 DP(0,679) Prot 2 DP(0,687)	0,5155	-0,1215
16	Nle-Y-V	435,3	0,627	DP(0,710)	0,593	0,597		0,595	-0,0320
18	S-Phg-V	379,2	0,601		0,681	0,686		0,6835	0,0825
18	P-Phg-V	389,2	0,635		0,842	0,770		0,806	0,1710
18	V-Phg-V	391,2	0,639		1,368	1,369	Prot1 DP(0,731) Prot2 DP(0,742)	1,3685	0,7295
18	F-Phg-V	439,2	0,789	DP(1,031)	0,642	0,641	Prot1 DP(1,038) Prot2 DP(1,028)	0,6415	-0,1475
18	W-Phg-V	478,2	0,840	DP(1,629)	0,926	0,935	Prot1 DP(1,224) Prot2 DP(1,241)	0,9305	0,0905
18	S-Hph-V	407,2	0,716		0,635	0,635		0,635	-0,0810
18	P-Hph-V	417,2	0,790	DP(0,525)	0,671	0,671		0,671	-0,1190
18	V-Hph-V	419,3	0,836		0,923	0,933		0,928	0,0920
18	F-Hph-V	467,3	2,421		0,948	0,947		0,9475	-1,4735
18	W-Hph-V	506,3	6,013	DP(2,483)	1,232	1,227		1,2295	-4,7835
18	S-F-V	393,2	0,622		0,571	0,544	Prot1 DP(1,448) Prot2 DP(1,408)	0,5575	-0,0645
18	P-F-V	403,3	0,687		0,626	0,619	Prot1 DP(0,926) Prot2 DP(0,923)	0,6225	-0,0645
18	V-F-V	405,3	0,689		0,631	0,634		0,6325	-0,0565
18	F-F-V	453,3	1,349		0,687	0,691	Prot2 DP(1,425)	0,689	-0,6600
18	W-F-V	492,3	2,858	DP(1,387)	0,812	0,817		0,8145	-2,0435
20	LTV	373,3	0,627		0,502	0,507	Prot1 DP(0,504) Prot2 DP(0,501)	0,5045	-0,1225

20	GTV	317,2	0,538		0,897	0,898	Prot1 DP(0,525) Prot2 DP(0,508)	0,8975	0,3595
20	ATV	331,2	0,543		0,570	0,584		0,577	0,0340
20	CTV	363,2	0,671		0,822	0,816		0,819	0,1480
20	LAV	343,3	0,620		0,695	0,695		0,695	0,0750
20	GAV	287,2	0,547		0,513	0,510		0,5115	-0,0355
20	AAV	301,2	0,546		0,603	0,602		0,6025	0,0565
20	CAV	333,2	0,675		0,911	0,913		0,912	0,2370
20	LVV	371,3	0,645		0,503	0,505	Prot1 DP(0,631) Prot2 DP(0,634)	0,504	-0,1410
20	GVV	315,2	0,559		0,516	0,521		0,5185	-0,0405
20	AVV	329,2	0,575		0,610	0,617		0,6135	0,0385
20	CVV	361,2	0,701		0,866	0,879		0,8725	0,1715
20	LPV	369,3	0,715		0,717	0,718		0,7175	0,0025
20	GPV	313,2	0,570		0,523	0,521		0,522	-0,0480
20	APV	327,2	0,601		0,637	0,647		0,642	0,0410
20	CPV	359,2	0,712		0,962	0,975	Prot1 DP(0,633) Prot2 DP(0,634)	0,9685	0,2565
22	GPipV	327,2	0,608		0,521	0,522		0,5215	-0,0865
22	APipV	341,2	0,542		0,608	0,564		0,586	0,0440
22	AbuPipV	355,2	0,556		0,687	0,679		0,683	0,1270
22	NvaPipV	369,2	0,542		0,557	0,561	Prot2 DP(0,689)	0,559	0,0170
22	NlePipV	383,3	0,540		0,617	0,618		0,6175	0,0775
22	GMV	347,2	0,575		0,529	0,526		0,5275	-0,0475
22	AMV	361,2	0,588		0,640	0,646		0,643	0,0550
22	AbuMV	375,2	0,607		0,766	0,764		0,765	0,1580
22	NvaMV	389,2	0,626		0,911	0,918		0,9145	0,2885
22	NleMV	403,3	0,725		0,795	0,796		0,7955	0,0705
22	GGV	273,2	0,524		0,529	0,522	Prot1 DP(0,896) Prot2 DP(0,903)	0,5255	0,0015
22	AGV	287,2	0,544		0,519	0,516	Prot1 DP(0,777) Prot2 DP(0,790)	0,5175	-0,0265
22	AbuGV	301,2	0,551		0,580	0,598		0,589	0,0380
22	NvaGV	315,2	0,581		0,609	0,608		0,6085	0,0275
22	NleGV	329,2	0,620		0,631	0,625		0,628	0,0080
22	GQV	344,2	0,587		0,644	0,643		0,6435	0,0565

22	AQV	358,2	0,592		0,764	0,763		0,7635	0,1715
22	AbuQV	372,2	0,628		0,916	0,915	Prot1 DP (0,647) Prot2 DP(0,648)	0,9155	0,2875
22	NvaQV	386,2	0,719		0,792	0,791	Prot2 DP(0,517)	0,7915	0,0725
22	NleQV	400,2	0,636		0,683	0,666		0,6745	0,0385
Reference	1-Acetamido-3-trifluoromethylbenzene	203,1	0,766		0,637	0,637		0,637	-0,1290
Reference	3-Aminobenzotriflouride	161,0	0,652		0,651	0,653		0,652	0,0000

Weak Affinity Chromatography (WAC) results for porcine trypsin

The results from the WAC run for trypsin were put together in a table, and the libraries from neutrophil elastase were run on the trypsin column too. This can be seen in **Table 15** below.

Table 15. The results from the WAC for porcine trypsin. Where Mix refers to if it is a reference binder or which library it is from. Fragment ID is the peptide sequence from the N-terminal to the C-terminal, although not written all N-terminals are acetylated. $[M+H]^+$ is the molar mass of the protonated, and hence charged, peptide. The five possible leads Ac-X-Q-V-NH_2 (X=Gly, Ala, Abu, Nva, Nle) are marked with green. The reference binder Ac-T-R-E-NH_2 did not give off a response, as it very likely was cleaved, the cleaved reference Ac-T-R-OH was found and gave a response. This is marked with yellow.

Mix	Fragment ID	$[M+H]^+$	Retention time, reference column (min)	Comment	Retention time, protein column 1 (min)	Retention time, protein column 2 (min)	Comment	Average retention time, protein column (min)	ΔRT (min)
Ref lib24	T-R	318,2	0,507		0,548	0,551		0,5	0,0425
14	G-P-V	313,2	0,556		0,551	0,566		0,6	0,0025
14	G-T-V	317,2	0,553		0,629	0,543		0,6	0,0330
14	G-C-V	319,2	0,658		0,616	0,652		0,6	-0,0240
14	A-P-V	327,2	0,573		0,532	0,549		0,5	-0,0325
14	A-T-V	331,2	0,543		0,525	0,530		0,5	-0,0155
14	A-C-V	333,2	0,656		0,644	0,647		0,6	-0,0105
14	Abu-P-V	341,2	0,592		0,566	0,539		0,6	-0,0395
14	Abu-T-V	345,2	0,559		0,543	0,524		0,5	-0,0255
14	Abu-C-V	347,2	0,677		0,631	0,658		0,6	-0,0325
14	Nva-P-V	355,3	0,626		0,573	0,563		0,6	-0,0580
14	Nva-T-V	359,3	0,593		0,554	0,530		0,5	-0,0510
14	Nva-C-V	361,2	0,720		0,65	0,677		0,7	-0,0565
14	Nle-P-V	369,3	0,734		0,566	0,578		0,6	-0,1620
14	Nle-T-V	373,3	0,638		0,547	0,553		0,6	-0,0880
14	Nle-C-V	375,2	0,857		0,676	0,710		0,7	-0,1640
14	G-W-V	402,2	0,698		0,588	0,590		0,6	-0,1090
14	A-W-V	416,3	0,737		0,596	0,596		0,6	-0,1410
14	Abu-W-V	430,2	0,799		0,606	0,600		0,6	-0,1960
14	Nva-W-V	444,3	1,033		0,624	0,636		0,6	-0,4030
14	Nle-W-V	458,3	1,662		0,687	0,681		0,7	-0,9780
16	G-Aze-V	299,2	0,554		0,555	0,554		0,6	0,0005
16	G-S-V	303,2	0,553		0,541	0,555		0,5	-0,0050
16	A-Aze-V	313,2	0,563		0,541	0,535		0,5	-0,0250
16	A-S-V	317,2	0,555		0,530	0,541		0,5	-0,0195
16	Abu-Aze-V	327,2	0,598		0,564	0,553		0,6	-0,0395
16	Abu-S-V	331,2	0,549		0,537	0,546		0,5	-0,0075
16	Nva-Aze-V	341,2	0,650		0,548	0,560		0,6	-0,0960
16	Nva-S-V	345,2	0,590		0,541	0,566		0,6	-0,0365
16	Nle-Aze-V	355,2	0,856		0,589	0,581		0,6	-0,2710
16	Nle-S-V	359,3	0,634		0,551	0,546		0,5	-0,0855
16	G-F-V	363,2	0,610		0,541	0,544		0,5	-0,0675
16	A-F-V	377,2	0,625		0,554	0,555		0,6	-0,0705
16	G-Y-V	379,2	0,581		0,538	0,546		0,5	-0,0390

16	Abu-F-V	391,2	0,647		0,582	0,583		0,6	-0,0645
16	A-Y-V	393,2	0,602		0,550	0,550		0,6	-0,0520
16	Nva-F-V	405,3	0,731		0,595	0,592		0,6	-0,1375
16	Abu-Y-V	407,2	0,608		0,589	0,573		0,6	-0,0270
16	Nle-F-V	419,3	1,008		0,607	0,606		0,6	-0,4015
16	Nva-Y-V	421,3	0,637		0,585	0,581		0,6	-0,0540
16	Nle-Y-V	435,3	0,627		0,586	0,585		0,6	-0,0415
18	S-Phg-V	379,2	0,601		0,553	0,552		0,6	-0,0485
18	P-Phg-V	389,2	0,635		0,589	0,586		0,6	-0,0475
18	V-Phg-V	391,2	0,639		0,582	0,580		0,6	-0,0580
18	S-F-V	393,2	0,622		0,547	0,558		0,6	-0,0695
18	P-F-V	403,3	0,687		0,594	0,594		0,6	-0,0930
18	V-F-V	405,3	0,689		0,591	0,578		0,6	-0,1045
18	S-Hph-V	407,2	0,716		0,581	0,575		0,6	-0,1380
18	P-Hph-V	417,2	0,790		0,615	0,611		0,6	-0,1770
18	V-Hph-V	419,3	0,836		0,598	0,600		0,6	-0,2370
18	F-Phg-V	439,2	0,789		0,599	0,598		0,6	-0,1905
18	F-F-V	453,3	1,349		0,627	0,637		0,6	-0,7170
18	F-Hph-V	467,3	2,421		0,685	0,679		0,7	-1,7390
18	W-Phg-V	478,2	0,840		0,691	0,641		0,7	-0,1740
18	W-F-V	492,3	2,858		0,740	0,740		0,7	-2,1180
18	W-Hph-V	506,3	6,013		0,912	0,886		0,9	-5,1140
20	GAV	287,2	0,547		0,551	0,533		0,5	-0,0050
20	AAV	301,2	0,546		0,551	0,551		0,6	0,0050
20	GPV	313,2	0,570		0,546	0,550		0,5	-0,0220
20	GVV	315,2	0,559		0,533	0,538		0,5	-0,0235
20	GTV	317,2	0,538		0,553	0,558	DP P2 0,721	0,6	0,0175
20	APV	327,2	0,601		0,555	0,560		0,6	-0,0435
20	AVV	329,2	0,575		0,545	0,547		0,5	-0,0290
20	ATV	331,2	0,543		0,531	0,532		0,5	-0,0115
20	CAV	333,2	0,675		0,678	0,688		0,7	0,0080
20	LAV	343,3	0,620		0,556	0,551		0,6	-0,0665
20	CPV	359,2	0,712		0,513	0,511	DP P2 0,725	0,5	-0,2000
20	CVV	361,2	0,701		0,695	0,724		0,7	0,0085
20	CTV	363,2	0,671		0,673	0,696		0,7	0,0135
20	LPV	369,3	0,715		0,565	0,577		0,6	-0,1440
20	LVV	371,3	0,645		0,589	0,589		0,6	-0,0560
20	LTV	373,3	0,627		0,547	0,566		0,6	-0,0705
22	GGV	273,2	0,524		0,572	0,578		0,6	0,0510
22	AGV	287,2	0,544		0,543	0,543		0,5	-0,0010
22	AbuGV	301,2	0,551		0,537	0,539		0,5	-0,0130
22	NvaGV	315,2	0,581		0,567	0,557		0,6	-0,0190
22	GPipV	327,2	0,608		0,587	0,566		0,6	-0,0315
22	NleGV	329,2	0,620		0,572	0,566		0,6	-0,0510
22	APipV	341,2	0,542		0,556	0,527		0,5	-0,0005

22	GQV	344,2	0,587		0,554	0,547	DP P1 0,923 DP P2 0,885	0,6	-0,0365
22	GMV	347,2	0,575		0,546	0,540		0,5	-0,0320
22	AbuPipV	355,2	0,556		0,635	0,626		0,6	0,0745
22	AQV	358,2	0,592		0,559	0,548	DP P1 1,007 DP P2 1,064	0,6	-0,0385
22	AMV	361,2	0,588		0,555	0,573		0,6	-0,0240
22	NvaPipV	369,2	0,542		0,543	0,542		0,5	0,0005
22	AbuQV	372,2	0,628		0,570	0,569		0,6	-0,0585
22	AbuMV	375,2	0,607		0,559	0,549		0,6	-0,0530
22	NlePipV	383,3	0,540		0,540	0,546		0,5	0,0030
22	NvaQV	386,2	0,719		0,586	0,574		0,6	-0,1390
22	NvaMV	389,2	0,626		0,572	0,574		0,6	-0,0530
22	NleQV	400,2	0,636		0,555	0,561	DP P1 0,724 DP2 0,722	0,6	-0,0780
22	NleMV	403,3	0,725		-	-		#DIV/0!	#DIV/0!
24	TRE	446,3	-		0,562	0,556	5 um båda	0,6	#VALUE!
26	TOrnD	390,2	0,404		-	-		#DIV/0!	#DIV/0!
26	COrnD	392,2	-		-	-		#DIV/0!	#DIV/0!
26	HypOrnD	402,2	-		-	-		#DIV/0!	#DIV/0!
26	TOrnE	404,2	-		-	-		#DIV/0!	#DIV/0!
26	COrnE	406,2	-		-	-		#DIV/0!	#DIV/0!
26	HypOrnE	416,2	-		-	-		#DIV/0!	#DIV/0!
26	TOrnAad	418,2	-		-	-		#DIV/0!	#DIV/0!
26	COrnAad	420,2	-		-	-		#DIV/0!	#DIV/0!
26	HypOrnAad	430,2	-		-	-		#DIV/0!	#DIV/0!
26	TRD	432,4	-		-	-		#DIV/0!	#DIV/0!
26	CRD	434,5	-		-	-		#DIV/0!	#DIV/0!
26	HypRD	444,2	-		-	-		#DIV/0!	#DIV/0!
26	TRE	446,5	-		-	-		#DIV/0!	#DIV/0!
26	CRE	448,5	-		-	-		#DIV/0!	#DIV/0!
26	YOrnD	452,2	-		-	-		#DIV/0!	#DIV/0!
26	HypRE	458,2	-		-	-		#DIV/0!	#DIV/0!
26	TRAad	460,2	-		-	-		#DIV/0!	#DIV/0!
26	CRAad	462,2	-		-	-		#DIV/0!	#DIV/0!
26	YOrnE	466,2	-		-	-		#DIV/0!	#DIV/0!
26	HypRAad	472,2	-		-	-		#DIV/0!	#DIV/0!
26	YOrnAad	480,2	-		-	-		#DIV/0!	#DIV/0!
26	YRD	494,5	-		-	-		#DIV/0!	#DIV/0!
26	YRE	508,5	-		-	-		#DIV/0!	#DIV/0!
26	YRAad	522,3	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	TOrnD	570,2	3,767		0,793	0,794		0,8	-2,9735
26+Fmoc	COrnD	572,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	HypOrnD	582,2	2,277		0,712	0,714		0,7	-1,5640
26+Fmoc	TOrnE	584,2	4,107		0,788	0,778		0,8	-3,3240

26+Fmoc	COrnE	586,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	HypOrnE	596,2	2,425		0,716	0,713		0,7	-1,7105
26+Fmoc	TOrnAad	598,2	4,716		0,807	0,793		0,8	-3,9160
26+Fmoc	COrnAad	600,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	HypOrnAad	610,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	TRD	612,4	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	CRD	614,5	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	HypRD	624,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	TRE	626,5	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	CRE	628,5	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	YOrnD	632,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	HypRE	638,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	TRAad	640,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	CRAad	642,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	YOrnE	646,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	HypRAad	652,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	YOrnAad	660,2	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	YRD	674,5	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	YRE	688,5	-		-	-		#DIV/0!	#DIV/0!
26+Fmoc	YRAad	702,3	-		-	-		#DIV/0!	#DIV/0!
28	TDabD	376,2	-		-	-		#DIV/0!	#DIV/0!
28	CDabD	378,1	-		-	-		#DIV/0!	#DIV/0!
28	HypDabD	388,2	-		-	-		#DIV/0!	#DIV/0!
28	TDabE	390,2	0,422		-	-		#DIV/0!	#DIV/0!
28	CDabE	392,2	-		-	-		#DIV/0!	#DIV/0!
28	HypDabE	402,2	-		-	-		#DIV/0!	#DIV/0!
28	TDabAad	404,2	0,514		0,552	0,546		0,5	0,0350
28	CDabAad	406,2	-		-	-		#DIV/0!	#DIV/0!
28	HypDabAad	416,2	0,510		0,558	0,568		0,6	0,0530
28	YDabD	438,2	-		-	-		#DIV/0!	#DIV/0!
28	THarD	446,2	-		-	-		#DIV/0!	#DIV/0!
28	CHarD	448,2	-		-	-		#DIV/0!	#DIV/0!
28	YDabE	452,2	-		-	-		#DIV/0!	#DIV/0!
28	HypHarD	458,2	-		-	-		#DIV/0!	#DIV/0!
28	THarE	460,2	-		-	-		#DIV/0!	#DIV/0!
28	CHarE	462,2	-		-	-		#DIV/0!	#DIV/0!
28	YDabAad	466,2	0,552		0,607	0,570		0,6	0,0365
28	HypHarE	472,2	-		-	-		#DIV/0!	#DIV/0!
28	THarAad	474,3	-		-	-		#DIV/0!	#DIV/0!
28	CHarAad	476,2	-		-	-		#DIV/0!	#DIV/0!
28	HypHarAad	486,3	-		-	-		#DIV/0!	#DIV/0!
28	YHarD	508,2	-		-	-		#DIV/0!	#DIV/0!
28	YHarE	522,3	-		-	-		#DIV/0!	#DIV/0!
28	YHarAad	536,3	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	TDabD	334,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	CDabD	336,1	-		-	-		#DIV/0!	#DIV/0!

28 No Ac	HypDabD	346,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	TDabE	348,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	CDabE	350,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	HypDabE	360,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	TDabAad	362,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	CDabAad	364,2	1,276		-	-		#DIV/0!	#DIV/0!
28 No Ac	HypDabAad	374,2	0,408		-	-		#DIV/0!	#DIV/0!
28 No Ac	YDabD	396,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	THarD	404,2	0,514		0,552	0,546		0,5	0,0350
28 No Ac	CHarD	406,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	YDabE	410,2	0,494		-	-		#DIV/0!	#DIV/0!
28 No Ac	HypHarD	416,2	0,510		0,558	0,568		0,6	0,0530
28 No Ac	THarE	418,2	0,523		0,547	0,562		0,6	0,0315
28 No Ac	CHarE	420,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	YDabAad	424,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	HypHarE	430,2	0,531		0,573	0,557		0,6	0,0340
28 No Ac	THarAad	432,3	0,638		0,597	0,585		0,6	-0,0470
28 No Ac	CHarAad	434,2	-		-	-		#DIV/0!	#DIV/0!
28 No Ac	HypHarAad	444,3	0,552		0,592	0,629		0,6	0,0585
28 No Ac	YHarD	466,2	0,552		0,607	0,570		0,6	0,0365
28 No Ac	YHarE	480,3	0,530		0,618	0,611		0,6	0,0845
28 No Ac	YHarAad	494,3	0,612		0,634	0,607		0,6	0,0085
30	SOrnD	376,2	0,544		0,533	0,516		0,5	-0,0195
30	POrnD	386,2	0,535		0,532	0,545		0,5	0,0035
30	VOrnD	388,2	0,549		0,525	0,534		0,5	-0,0195
30	SOrnE	390,2	0,372		0,529	0,54		0,5	0,1625
30	POrnE	400,2	0,542		0,532	0,546		0,5	-0,0030
30	VOrnE	402,2	0,538		0,54	0,547		0,5	0,0055
30	SOrnAad	404,2	0,543		0,548	0,531		0,5	-0,0035
30	POrnAad	414,2	0,559		0,552	0,554		0,6	-0,0060
30	VOrnAad	416,2	0,558		0,548	0,534		0,5	-0,0170
30	SRD	418,2	0,548		0,528	0,530		0,5	-0,0190
30	MOrnD	420,2	0,547		0,542	0,525		0,5	-0,0135
30	PRD	428,2	0,549		0,535	0,538		0,5	-0,0125
30	VRD	430,3	0,548		0,537	0,526		0,5	-0,0165
30	SRE	432,2	0,55		0,53	0,536		0,5	-0,0170
30	MOrnE	434,2	0,561		0,543	0,53		0,5	-0,0245
30	PRE	442,3	0,532		0,528	0,536		0,5	0,0000
30	VRE	444,3	0,537		0,549	0,544		0,5	0,0095
30	SRAad	446,2	0,539		-	-		#DIV/0!	#DIV/0!
30	MOrnAad	448,2	0,55		0,539	0,536		0,5	-0,0125
30	PRAad	456,2	0,602		0,533	0,537		0,5	-0,0670
30	VRAad	458,3	0,543		0,535	0,536		0,5	-0,0075
30	MRD	462,2	0,55		0,581	0,557		0,6	0,0190
30	MRE	476,3	0,553		0,547	0,54		0,5	-0,0095
30	MRAad	490,2	0,567		-	-		#DIV/0!	#DIV/0!

32	SHarD	432,2	0,547		0,559	0,543		0,6	0,0040
32	SHphD	439,2	-		-	-		#DIV/0!	#DIV/0!
32	PHarD	442,2	0,543		0,578	0,571		0,6	0,0315
32	VHarD	444,2	0,938	DP 0,559	0,58	0,583		0,6	-0,3565
32	SHarE	446,2	0,997		0,571	0,594		0,6	-0,4145
32	PHphD	449,2	0,974		0,606	0,599		0,6	-0,3715
32	VHphD	451,2	0,808		0,566	0,583		0,6	-0,2335
32	SHphE	453,2	-		-	-		#DIV/0!	#DIV/0!
32	PHarE	456,2	0,527		0,575	0,571		0,6	0,0460
32	VHarE	458,3	0,547		0,569	0,571		0,6	0,0230
32	SHarAad	460,2	0,555		0,54	0,555		0,5	-0,0075
32	PHphE	463,2	0,999		0,581	0,59		0,6	-0,4135
32	VHphE	465,2	1,003		0,562	0,587		0,6	-0,4285
32	SHphAad	467,2	1,051		0,586	0,600		0,6	-0,4580
32	PHarAad	470,3	0,589		0,58	0,583		0,6	-0,0075
32	VHarAad	472,3	0,573		0,576	0,582		0,6	0,0060
32	MHarD	476,2	0,563		0,561	0,574		0,6	0,0045
32	PHphAad	477,2	-		0,566	0,592		0,6	#VALUE!
32	VHphAad	479,2	1,151		0,574	0,585		0,6	-0,5715
32	MHphD	483,2	0,968		0,591	0,598		0,6	-0,3735
32	MHarE	490,2	0,579		0,565	0,574		0,6	-0,0095
32	MHphE	497,2	0,794	DP 1,175	0,598	0,592		0,6	-0,1990
32	MHarAad	504,3	0,578		0,572	0,574		0,6	-0,0050
32	MHphAad	511,2	0,796		0,736	0,57		0,7	-0,1430
34	TKG	346,2	0,502		0,51	0,568		0,5	0,0370
34	CKG	348,2	0,556		0,539	0,549		0,5	-0,0120
34	HypKG	358,2	0,500		0,588	0,525		0,6	0,0565
34	TRG	374,2	0,536		0,563	0,53		0,5	0,0105
34	CRG	376,2	0,556		0,56	0,551		0,6	-0,0005
34	HypRG	386,2	0,527		0,533	0,533		0,5	0,0060
34	TKN	403,3	0,506		-	-		#DIV/0!	#DIV/0!
34	CKN	405,2	0,535		-	-		#DIV/0!	#DIV/0!
34	YKG	408,2	0,505		0,549	0,547		0,5	0,0430
34	HypKN	415,2	0,497		-	-		#DIV/0!	#DIV/0!
34	TKQ	417,3	0,477		-	-		#DIV/0!	#DIV/0!
34	CKQ	419,2	-		-	-		#DIV/0!	#DIV/0!
34	HypKQ	429,2	0,488		-	-		#DIV/0!	#DIV/0!
34	TRN	431,3	0,509		0,53	0,539		0,5	0,0255
34	CRN	433,2	0,498		-	0,533		0,5	0,0350
34	YRG	436,3	0,55		0,544	0,55		0,5	-0,0030
34	HypRN	443,2	0,5		0,537	0,536		0,5	0,0365
34	TRQ	445,3	0,441		-	-		#DIV/0!	#DIV/0!
34	CRQ	447,2	0,500		0,532	0,535		0,5	0,0335
34	HypRQ	457,2	0,504		0,531	0,535		0,5	0,0290
34	YKN	465,3	0,514		-	-		#DIV/0!	#DIV/0!
34	YKQ	479,3	0,509		-	-		#DIV/0!	#DIV/0!

34	YRN	493,3	0,515		-	-		#DIV/0!	#DIV/0!
34	YRQ	507,3	0,540		-	-		#DIV/0!	#DIV/0!
36	T3PalG	366,2	-		-	-		#DIV/0!	#DIV/0!
36	C3PalG	368,1	0,497		-	-		#DIV/0!	#DIV/0!
36	Hyp3PalG	378,2	-		-	-		#DIV/0!	#DIV/0!
36	THarG	388,2	0,402		-	-		#DIV/0!	#DIV/0!
36	CHarG	390,2	0,406		-	-		#DIV/0!	#DIV/0!
36	HypHarG	400,2	-		-	-		#DIV/0!	#DIV/0!
36	T3PalN	423,2	-		-	-		#DIV/0!	#DIV/0!
36	C3PalN	425,2	0,504		-	-		#DIV/0!	#DIV/0!
36	Y3PalG	428,2	-		-	-		#DIV/0!	#DIV/0!
36	Hyp3PalN	435,2	-		-	-		#DIV/0!	#DIV/0!
36	T3PalQ	437,2	0,504		-	-		#DIV/0!	#DIV/0!
36	C3PalQ	439,2	0,5		-	-		#DIV/0!	#DIV/0!
36	THarN	445,2	0,51		0,547	0,678		0,6	0,1025
36	CHarN	447,2	-		-	-		#DIV/0!	#DIV/0!
36	Hyp3PalQ	449,2	-		-	-		#DIV/0!	#DIV/0!
36	YHarG	450,2	-		-	-		#DIV/0!	#DIV/0!
36	HypHarN	457,2	0,505		0,622	0,658		0,6	0,1350
36	THarQ	459,3	0,405		-	-		#DIV/0!	#DIV/0!
36	CHarQ	461,2	-		-	-		#DIV/0!	#DIV/0!
36	HypHarQ	471,3	-		-	-		#DIV/0!	#DIV/0!
36	Y3PalN	485,2	-		-	-		#DIV/0!	#DIV/0!
36	Y3PalQ	499,2	-		-	-		#DIV/0!	#DIV/0!
36	YHarN	507,3	-		-	-		#DIV/0!	#DIV/0!
36	YHarQ	521,3	-		-	-		#DIV/0!	#DIV/0!
36 No Ac	T3PalG	324,2	0,604		0,591	0,59		0,6	-0,0135
36 No Ac	C3PalG	326,1	-		-	-		#DIV/0!	#DIV/0!
36 No Ac	Hyp3PalG	336,2	0,53		0,621	0,624		0,6	0,0925
36 No Ac	THarG	346,2	0,514		0,545	0,551		0,5	0,0340
36 No Ac	CHarG	348,2	-		-	-		#DIV/0!	#DIV/0!
36 No Ac	HypHarG	358,2	0,509		0,63	0,606		0,6	0,1090
36 No Ac	T3PalN	381,2	0,509		0,574	0,56		0,6	0,0580
36 No Ac	C3PalN	383,2	-		-	-		#DIV/0!	#DIV/0!
36 No Ac	Y3PalG	386,2	0,574		0,675	0,731		0,7	0,1290
36 No Ac	Hyp3PalN	393,2	0,531		0,614	0,593		0,6	0,0725
36 No Ac	T3PalQ	395,2	0,532		0,595	0,589		0,6	0,0600
36 No Ac	C3PalQ	397,2	-		-	-		#DIV/0!	#DIV/0!
36 No Ac	THarN	403,2	0,512		0,55	0,658		0,6	0,0920
36 No Ac	CHarN	405,2	-		-	-		#DIV/0!	#DIV/0!
36 No Ac	Hyp3PalQ	407,2	0,549		0,605	0,687		0,6	0,0970
36 No Ac	YHarG	408,2	0,54		0,67	0,663		0,7	0,1265
36 No Ac	HypHarN	415,2	0,508		0,589	0,617		0,6	0,0950
36 No Ac	THarQ	417,3	0,514		0,655	0,555		0,6	0,0910
36 No Ac	CHarQ	419,2	0,504		-	-		#DIV/0!	#DIV/0!
36 No Ac	HypHarQ	429,3	0,513		0,638	0,613		0,6	0,1125

36 No Ac	Y3PalN	443,2	0,554		0,618	0,631		0,6	0,0705
36 No Ac	Y3PalQ	457,2	0,505		0,622	0,658		0,6	0,1350
36 No Ac	YHarN	465,3	0,54		0,663	0,647		0,7	0,1150
36 No Ac	YHarQ	479,3	0,536		0,672	0,677		0,7	0,1385
38	PQV	384,2	0,564		0,551	0,551		0,6	-0,0130
38	TQV	388,2	0,553		0,542	0,544		0,5	-0,0100
38	PQN	399,2	0,548		0,539	0,535		0,5	-0,0110
38	TQN	403,2	0,541		0,542	0,517		0,5	-0,0115
38	KQV	415,3	0,501		0,570	0,544		0,6	0,0560
38	KQN	430,3	0,455		0,539	0,529		0,5	0,0790
38	PQF	432,2	0,648		0,587	0,593		0,6	-0,0580
38	TQF	436,2	0,596		0,558	0,559		0,6	-0,0375
38	RQV	443,3	0,568		0,588	0,593		0,6	0,0225
38	PQY	448,2	0,604		0,572	0,577		0,6	-0,0295
38	TQY	452,2	0,563		0,542	0,543		0,5	-0,0205
38	RQN	458,3	0,558		0,543	0,548		0,5	-0,0125
38	KQF	463,3	0,521		0,578	0,577		0,6	0,0565
38	KQY	479,3	0,504		0,576	0,537		0,6	0,0525
38	RQF	491,3	0,649		0,610	0,598		0,6	-0,0450
38	RQY	507,3	0,598		0,603	0,578		0,6	-0,0075
40	PNV	370,2	0,557		0,544	0,543		0,5	-0,0135
40	TNV	374,2	0,554		0,548	0,536		0,5	-0,0120
40	PNN	385,2	0,565		0,543	0,550		0,5	-0,0185
40	TNN	389,2	0,531		0,543	0,535		0,5	0,0080
40	KNV	401,3	0,498		0,545	0,561		0,6	0,0550
40	KNN	416,2	0,510		0,529	0,552		0,5	0,0305
40	PNF	418,2	0,646		0,577	0,587		0,6	-0,0640
40	TNF	422,2	0,589		0,558	0,556		0,6	-0,0320
40	RNV	429,3	0,583		0,573	0,594		0,6	0,0005
40	PNY	434,2	0,597		0,559	0,570		0,6	-0,0325
40	TNY	438,2	0,520		0,539	0,549		0,5	0,0240
40	RNN	444,3	0,543		0,547	0,550		0,5	0,0055
40	KNF	449,3	0,522		0,576	0,574		0,6	0,0530
40	KNY	465,3	0,625		0,558	0,556		0,6	-0,0680
40	RNF	477,3	0,651		0,596	0,600		0,6	-0,0530
40	RNY	493,3	0,592		0,610	0,590		0,6	0,0080

Parameters of the WAC runs

The different parameters used in the two WAC runs can be seen in **Table 16** below.

Table 16. The parameters of the two WAC-runs.

LC set-up	
Instrument	Titan LC/MS
Detection	UV-220/254 and MS
Mobile phase	Ammonium acetate buffer 20 mM pH 6.8
Ref. column	Column ID 180629
No. of Mixes	13
Run time	20 (Ref.) or 30 min (Prot.) per mix
Injection volume	10 µL
Mix cmpd conc.	50 µM in ammonium acetate buffer 20 mM pH 6.8 with 0.1% DMSO

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