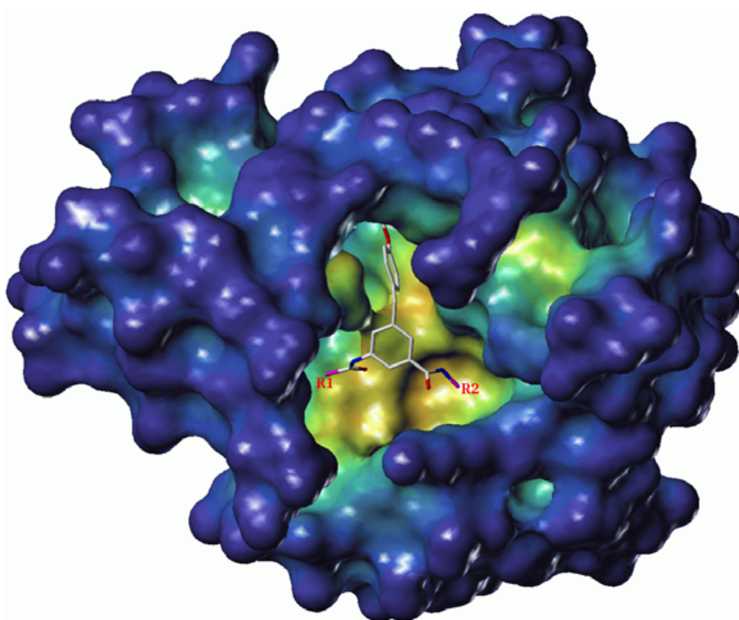
Synthesis of a Hepatitis C Virus NS3 Serine Protease Inhibitor with a Novel Peptidomimetic Scaffold

A Masters Degree Project in Medicinal Chemistry



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Abstract

Hepatitis C Virus (HCV) constitutes a global health problem with 170 million people infected worldwide. Of those infected with HCV, 80 % develop a chronic infection, which can result in cirrhosis and/or hepatocellular carcinoma. Today, HCV is treated with a combination therapy with interferon-α and ribivarin. Unfortunately, severe side effects and resistance among some HCV genotypes follow with this treatment. The need for a new therapy for HCV is therefore by all means urgent. Intense research aims at developing inhibitors to HCV NS3 protease inspired by the successful protease inhibitors for treatment of HIV. The inhibitors known today are based on the substrate and have consequently a peptide-like structure, which is impropriate for per oral administration. In this thesis a new type of peptidomimetic scaffold has been suggested based on the knowledge from the Angiotensin II project at the department. The aim was to reduce the peptidic character of the inhibitor and to produce a more drug-like substance. In the synthesis several important steps have been conducted using microwave mediated palladium-catalysed couplings. The advantage of microwave technology is the convenient temperature and pressure control together with the efficient heating which shorten the reaction times. The synthesised inhibitor shows moderate binding affinity, $K_i = 17.1 \pm 1.8$ μM, and is a promising starting point for optimisation of a new type of aromatic scaffold for HCV NS3 serine protease inhibitors.

Abbreviations and definitions

BI	Boehringer Ingelheim
Brine	Saturated NaCl-solution
DCM	Dichloromethane
DIEA	Diisopropylethylamine
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IRBM	Istituto di Ricerche di Biologia Molecolare P. Angeletti
IRES	Internal ribosome entry site
NMR	Nuclear mass resonance
NS	Non-structural
RDRP	RNA-dependent RNA polymerase
SVR	Sustained viral response
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography

Some information in the thesis is classified and removed from this version. Supplemental information about e.g. structures, NMR-data, MS-data and results of elementary analysis can be requested from Prof. A. Hallberg at the Division of Organic Pharmaceutical Chemistry, Department of Medicinal Chemistry, Uppsala University.

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1 Introduction

1.1 Epidemiology of Hepatitis C Virus

Hepatitis C Virus is today globally spread and WHO estimated in October 2000 that 170 million people worldwide are chronically infected with HCV. This number equals 3% of the population in the world![1] HCV is one of seven different hepatotropic viruses known today. The others are labelled A, B, D, E, F and G.[2] The HCV wasn't identified until 1989, previously it was known as "non A, non B Hepatitis". Together with the other four viruses A, B, D and E, the HCV accounts for a vast majority of viral hepatitis.[1]

HCV has different prevalence in different parts of the world. In the main parts of Africa, Europe, Southeast Asia and America the prevalence is below 2.5%. In the Western Pacific the prevalence varies between 2.5-4.9% and in the countries of Middle East the HCV infected represent 1% to more than 12% of the population.[4] There are distinct local variations with extreme high prevalence for example in Cameroon, Republic of the Congo, Central African Republic, Equatorial Guinea and Mongolia, all with more than 10% of the population infected.[3] The highest known prevalence is in Egypt with 24% of the population infected. The exceptionally high amount of infected in Egypt is due to a national paranteral antischistosomal therpy (PAT) campaign, in which inadequate sterilisation of injection equipment was common.[5]

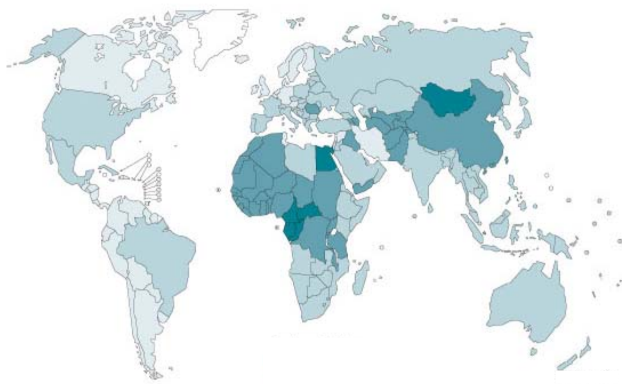


Figure 1. Prevalence of HCV in the world 2003. The darker sections indicates high prevalence.

1.1.1 Transmission

HCV is spread primarily via human blood by unscreened blood transfusions, inadequately sterilized needles, syringes etc.[1] Among intravenous drug users in developed countries 20-40% are being infected with HCV during their first year of intravenous drug abuse and after five years more than 90% are infected.[4] Spreading of HCV by sexual contacts may occur but are less frequent. Casual contact or coughing provides no risks of HCV spreading neither are food or water infectious. There is also a low risk (5%) for mother-to-infant transmission of HCV observed globally.[4]

1.2 The Hepatitis C Virus

HCV is a single-stranded, positive-sense RNA virus in the *flaviviraidae* family.[2] In this virus family you also find viruses as the Dengue virus and the animal pathogenic pestiviruses.[6] HCV encodes a single polyprotein of ~3010 amino acids[2] which is processed co- and post translationally into structural and nonstructural (NS) proteins.[6] Today there are about ten known products of this process. The HCV execute this process with help from a host cell signalases and two viral proteases.[6]

1.2.1 Genome

The genome of the HCV carries a single open reading frame (ORF) with highly conserved non-translated regions (NTR) in its 5' and 3' ends. The NTR:s forms stable secondary and tertiary structures. In the 5' end there is an internal ribosome entry site (IRES), which allows the RNA to bind to the ribosomes close to the start codon of the ORF. This connection maker enables translation of the ORF. In the 3' end there is a conserved region important for RNA-replication. When translated the polyprotein is processed and the substructures core (C), envelope protein 1 (E1), E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B are produced in order from 5'-3'.[6]

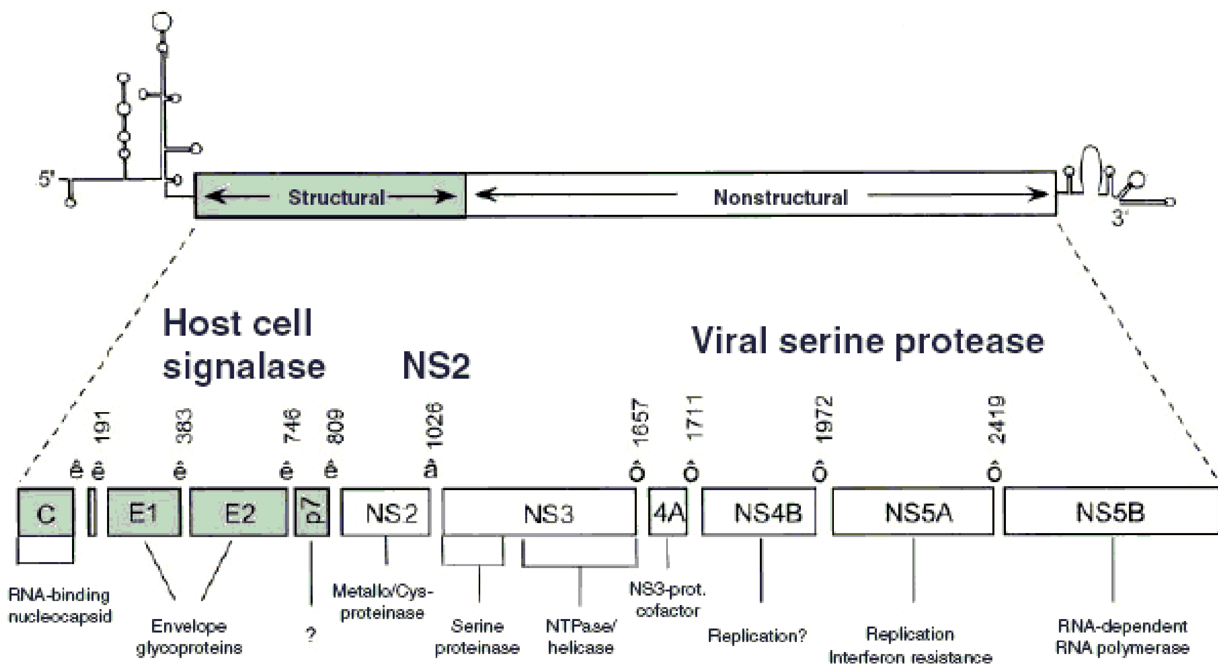


Figure 2. The genome of HCV with subfragments. The first five fragments are cleaved by cellular signalases, the cleavage between NS2 and NS3 is mediated by the virus-encoded proteins NS2/3 and remaining cleavage are performed by NS3.[7]

The different substructures have various tasks in the life of HCV. The core is a structural protein involved in cellular processes; the E1 and E2 are transmembrane proteins forming heterodimers, for example important for HCV's entry into the cell. NS2 together with the amino end of NS3 is responsible for cleavage between NS2 and NS3. The NS3 brings enzymatic activity in two forms. The amino terminal domain of NS3 forms a serine-type protease of great importance. This protease is responsible for all cleavages carboxy terminal of the NS3.[6] The carboxy terminal of NS3 carries an NTPase/helicase. The NS4A acts as a co-factor to NS3 protease, the NS5A seems to be involved in the preservation of HCV replication and the NS5B is the key to replication of the HCV RNA as it acts as a RNA-dependent RNA polymerase (RDRP). The function of p7 and NS4B are still unknown.[6] The NS3 is indirectly as important to the virus replication as NS5B. Without the capability of NS3 to cleave the polyprotein, it is not divided and there will consequently be no free NS5B and no, for the replication, essential RNA polymeras.[7]

1.2.2 Life cycle of HCV

The life cycle and replication process of HCV is not completely understood. Still, there is at least a hypothetical model of the replication. The HCV enters the cell with help from E1 and E2 and cell receptors. Different ideas about which receptors are involved are presented for example of Bartenschlager *et al*[6] and Szabó *et al*[2]. Endocytosis via CD 81 as well as via corresponding receptors to immunoglobulins has been presented as possible ways of cell entry.[6] When inside the cell the RNA is released into the cytoplasm. The IRES initiate the translation of the polyprotein, which is then processed to the ten independent proteins.

The proteins made after cleavage of the poly-protein creates a multi-protein complex, associated to intracellular membranes. The replication of the HCV RNA takes places in the region of the multi-protein complex with a NS5B RNA-dependent RNA polymeras as the most important actor. The NTPase/helicase from NS3

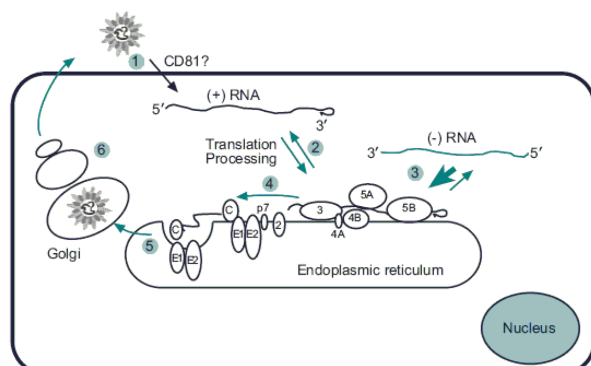


Figure 3. A proposed life cycle of HCV.[6]

is an other key player with its capacity of unwind stable RNA structures, thereby facilitating the synthesis of RNA.[6] The replication then starts with the HCV RNA being copied to a minus-stranded RNA, which is used to produce more plus-stranded RNA. The newly synthesised plus-stranded RNA can either be used to produce more minus-strands or be encapsidated into virus particles. The virus particles are then transported out of the cell via the Golgi.[6]

1.2.3 The infection of HCV

During an acute HCV infection only 20-30% of the infected persons develop symptoms.[4] On the other hand only about 20% of the infected can see a spontaneous clearance. The remaining 80% of the infected develop a chronic infection, 10-20% of these 80% develop cirrhosis and 1-5% with a chronic infection develops liver cancer within 20-30 years.[1]

How HCV causes liver injury, acute as well as chronic, is unknown but it is believed that immune response by the infected cell is involved in the process.[4] The risk of developing cirrhosis is depending of factors as alcohol abuse and age at time of infection. The importance of different HCV genotypes, co-infections (e.g. HBV or HIV) or gender are still in many aspects unknown.[4] Even though the development of cirrhosis is the main determinant of morbidity and mortality[8] one of the most serious consequences of a chronic HCV infection is the development of hepatocellular carcinoma (HCC).[4] The risk of developing HCC increases with age, duration of infection and cirrhosis.

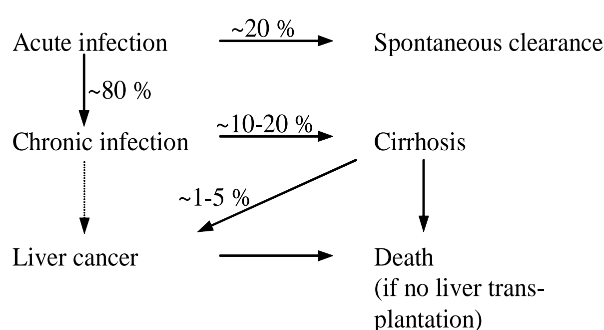


Figure 4. The outcome of HCV infections.[1]

1.3 Treatment of HCV

There are six different major genotypes, and about 100 subtypes, of HCV with varying presence in different parts of the world. Genotype 1-3 are spread worldwide, genotypes 4-5 are found mainly in Africa and genotype 6 is found in Asia.[4] Note that in the Western world genotype 1 is pre-dominate (60-90% of infected) and genotype 4 dominate in Egypt.[8]

The combination therapy with interferon-α and ribavirin, which is the most effective therapy known today, neutralize the virus after 6 months in 40-50% of the infection cases with genotype 1 and in 80% of the infection cases with genotype 2 and 3.[8] Infections of HCV genotype 4 is, as genotype 1, relatively resistant to the interferon-α/ribavirin combination therapy.[4]

The treatment with interferon-α and ribavirin has significant side effects and are quite expensive.[8] The side effects mainly derive from ribavirin as cough, shortness of breath, insomnia and haemolytic anaemia. Despite these drawbacks and the fact that ribavirin is teratogenic and requires frequent dose modifications, the combination therapy is at least twice as effective as the mono therapy, if you compare the sustained viral responses (SVR). Even from the economic point of view the combination therapy is preferable preventing future costs, which else should occur in connection with chronic liver diseases.[9] As mentioned above the success of the combination therapy varies due to the genotype of the infection. The best respond come from genotype 2 and 3 with SVR of ~65% after a 24 or 48 week treatment. A SVR rate of 29% is observed after a 48-weeks treat-ment of a genotype 1 infection.[9] Still, the therapies known today are not optimal and new approaches are needed.

The HCV virus has a high rate of mutations and for example can be noted a mutation rate in the RDRP region at $5x10^{-3}$/stite per year.[7] This fact together with the high genetic diversity and different genotypes in different parts of the world makes developing a worldwide useful antiviral agent a great challenge. To be successful against HCV you probable will have to use combinations therapies with antiviral agents designed to bind

to and to disable the functionality of functional proteins with conserved genetic regions.[7]

1.4 Why NS3 serine protease as a drug target?

Protease inhibitors have recently proven successful in treating severe illnesses as for example HIV. Based on this, protease inhibitors seems to be a promising target in drug discovery towards other viral diseases.[10]

The NS3 segment of the polyprotein is considered to be a useful drug target. Locarnini *et al* discusses three different potential antiviral targets. An inhibition towards NS3 protease would prevent the processing of the polypeptide, blocking the NS3 helicase/NTPase would disable the viral RNA replication and inhibition of the IRES at the 5' end and the UTR at the 3' end of the genome would affect the regulation of viral RNA elements.[7]

The interaction between NS3 and NS4A (which act as a co-factor to the NS3 serine protease), and the zinc-binding domain seem to be good targets for protease inhibition.[7] An other approach is to build a molecule mimicing the natural peptide ligand, a peptidomimetic where the cleavable amide bond is replaced with a non-cleavable isostere and at the same time reduce the peptidic structure of the molecule.[10] To inhibit the NS3 serine protease could be a good strategy for treatment of HCV.

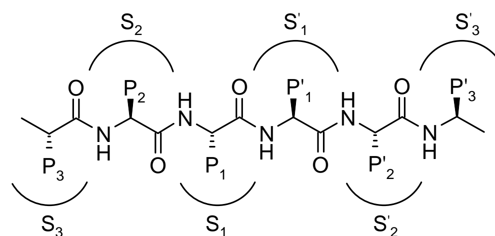


Figure 5. Traditional nomenclature for peptide sites.[10]

1.4.1 NS3 serine protease

There are four types of proteases; aspartic, serine, cysteine and metalloproteases. HCV NS3 protease is identified as a serine-type protease.[10] To describe the sites in the protease the standard nomenclature from Schrechter and Berger is used *(see Figure 5).* This nomenclature is based on the bond cleaved by the protease, the scissile bond. On the non-prime side of the cleavage site, positions P_3 to P_1 are found and P_1' to P_3' are found on the prime side. These residues match the corresponding sub sites S_3-S_1 and S_1'-S_3' in the protease.[10]

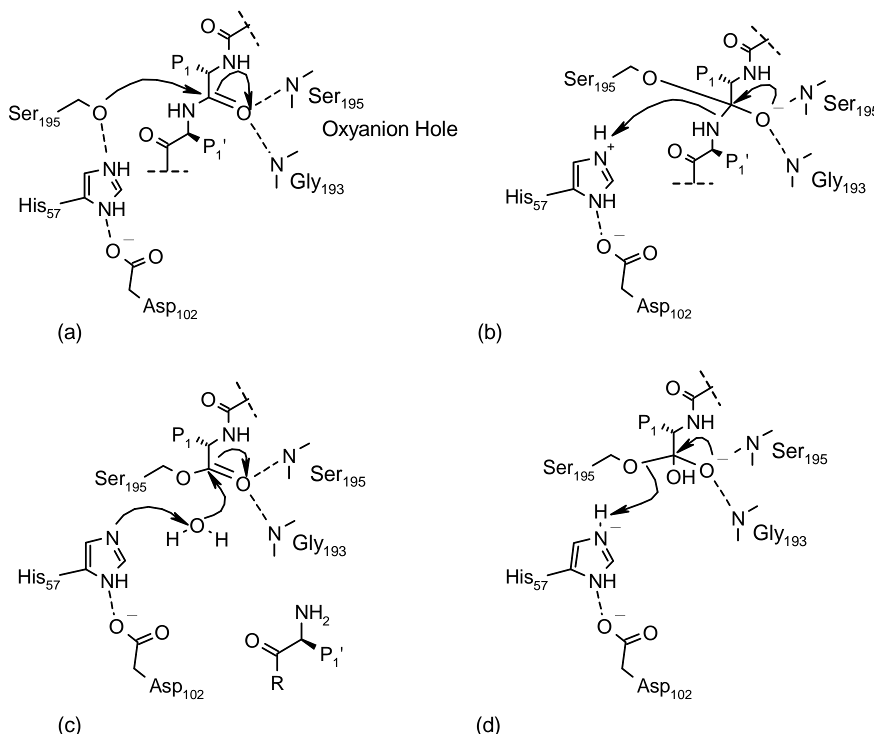


Figure 6. General catalytic mechanisms for serine proteases.[10]

The serine proteases can be divided in the subclasses trypsin-like, elastase-like and chymotrypsin-like due to the preferred P_1- residue. The trypsin-like ones have positively charged amino acids (aa), the elastase-like have small hydrophobic residues and finally the chymotrypsin-like have large hydrophobic residues.[10] The HCV NS3 serine protease is chymo-trypsin-like.[11]

The action of serine protease is displayed in Figure 6. Based on chymotrypsin numbering the active site consists of a catalytic triade of Ser195, His57 and Asp102 and an oxyanion hole, which is formed by the backbone NH groups of Ser195 and Gly193. (a) The substrate binds to the active site and exposes the carbonyl group of the scissile amide bound to nucleofilic attack by the Ser195 hydroxyl, enhanced by hydrogen bond to His57. This action creates the first hemiacetal tetrahedral intermediate, which is stabilized by hydrogen bonding in the oxyanion hole. In (b) proton transfer from the charged His57 to the amine of the tetrahedral intermediate leads to expulsion of the C-terminal product as leaving group and gives a covalent bonded acyl complex. Water attacks (c) the acyl complex and a second tetrahedral intermediate is formed, which in (d) collapses via an acid-assisted catalysis by the charged His57. Ser195 is regenerated and the carboxylic acid, the N-terminal product, is cleaved.[10]

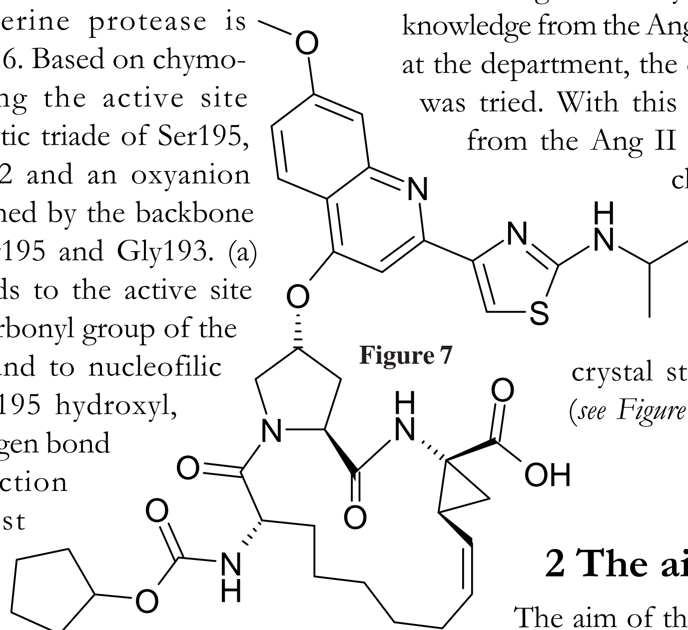


Figure 7

1.4.2 HCV NS3 serine protease inhibitors

After initial attempts with electrophilic inhibitors the focus has changed to product-based inhibitors[12] corresponding to the P_6-P_1 position, e.g. Asp-Asp-Ile-Val-Pro-Cys.[13] Further development yielded less peptidelike structures exemplified by the first NS3 seine protease inhibitor to reach clinical trials, the BILN 2061 (*see Figure 7*).[13]

There has also been work done on the prime side, primary by IRBM, but so far the strict prime side inhibitors have less potency than the inhibitors on the nonprime side.[12]

1.4.3 Modelling on NS3

Based on modelling made at the department, on the full length NS3 crystal structure and with the knowledge from the Angiotensin II (Ang II) project at the department, the docking of a new scaffold was tried. With this novel scaffold, originally from the Ang II project and with the side chains inspired from known HCV NS3 serine protease inhibitors, the alignment of the target molecule into the crystal structure seems promising (*see Figure 8*).

2 The aim of the thesis

The aim of this thesis was to synthesise and evaluate a new HCV NS3 serine protease inhibitor with a novel peptidomimetic scaffold. Based on an aromatic structure a new type of scaffold was synthesised. Side chains, proven to be efficient fragments from known HCV NS3 serine protease inhibitors, were attached to create the novel less peptide like inhibitor *(see figure 9)*.

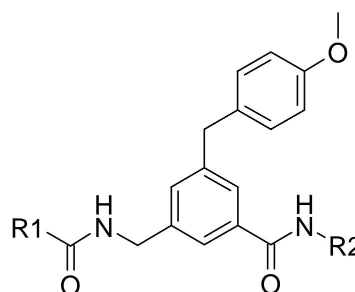


Figure 9. The target molecule.

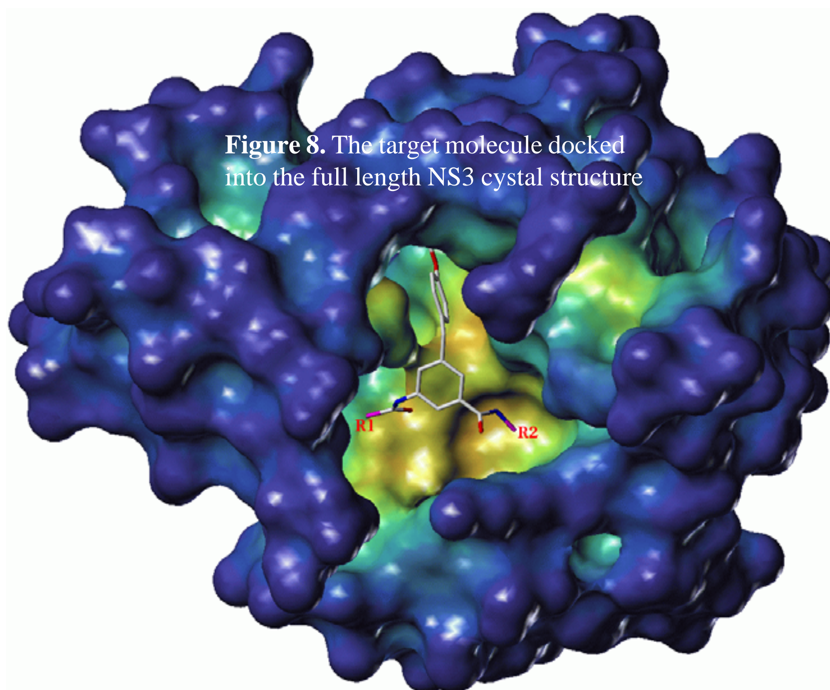
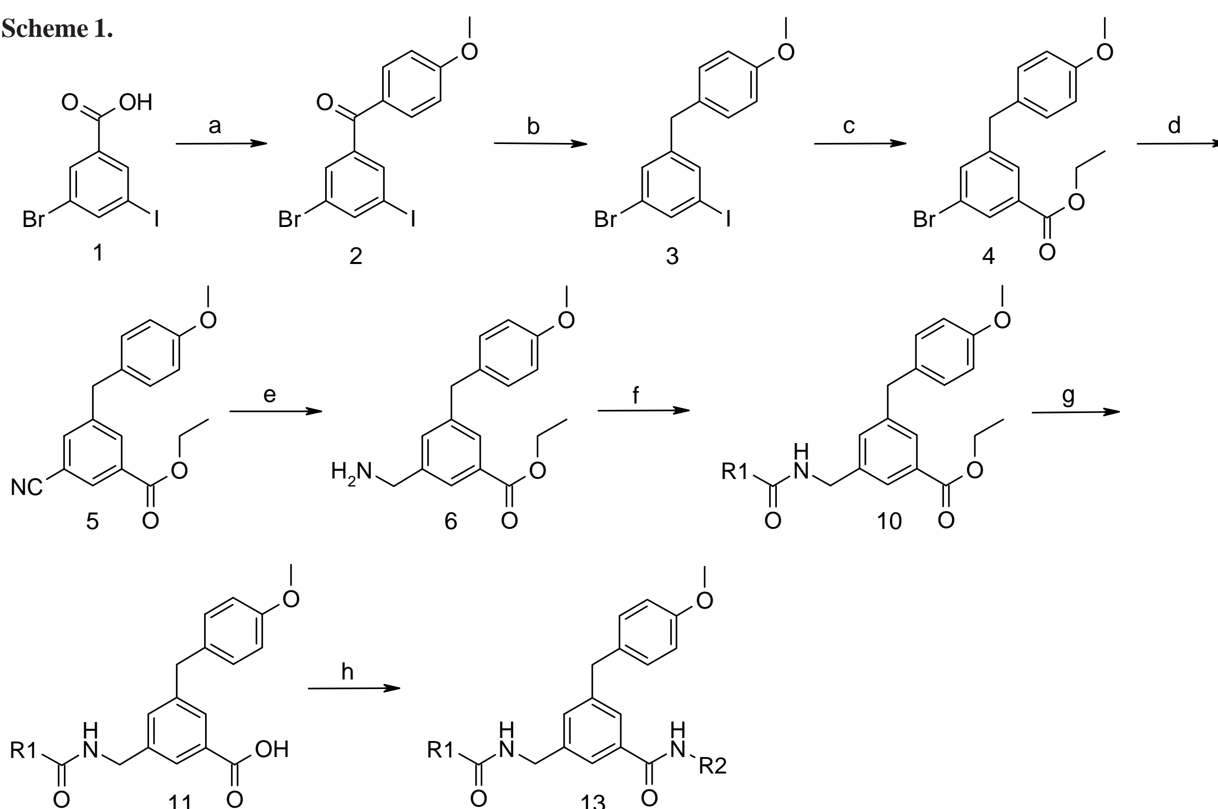


Figure 8. The target molecule docked into the full length NS3 cystal structure

3. Results and discussion

Scheme 1.



Reagents: a) i) $SOCl_2$, 85 °C, 1 h ii) $AlCl_3$, Anisole, DCM, 20 °C, 16 h b) Et_3SiH, TFA, Triflic acid, DCM, 50 °C, 6 h, c) $Mo(CO)_6$, Pd/C, DIEA, DMAP, EtOH, Dioxane, 150 °C, 15 min, microwave heating, d) $Zn(CN)_2$, $Pd_2(dba)_3$, P(*o*-tolyl), DMF, 180 °C, 10 min, microwave heating, e) H_2, Pd/C, EtOH, f) R_1-COOH, HBTU, DIEA, DCM, 20 °C, 55 min, g) LiOH, MeOH, THF, H_2O, 20 °C, 22 h, h) R_2-NH_2 ,HATU, DIEA, DMAP, DBU, DCM, 20 °C, 23 h.

3.1 Chemistry

Compound **2** was synthesised from 3-bromo-5-iodo-benzoic acid, **1**, which was refluxed 1 h at 85 °C with thionylchloride to create the acid chloride. The acid chloride was then subjected to a classic Friedel-Crafts acylation with $AlCl_3$ as a lewis acid. Compound **2** was isolated through crystallisation in a good isolated yield of 69%.

The reduction of **2** to generate **3** was performed with Et_3SiH as hydride source and with catalytic amounts of triflic acid. The reaction was performed in DCM with TFA.[14,15] The reaction mixture was refluxed for 6 h at 50 °C and compound **3** was isolated in yield of 87%.

The carbonylation of **3** was performed using microwave flash heating. As catalyst Pd/C was used based on the protocol from Georgsson *et al.*[16] $Mo(CO)_6$ as a solid carbon monoxide source provided a more convenient method than the traditional one with carbon monoxide gas. With the use of microwave irradiation to 150 °C for 15 min (*see figure 10*) and with EtOH as alcohol ester **4** was isolated in a yield of 81%. The conditions used differs slightly from Georgsson *et al* regarding $Mo(CO)_6$ (1.0 equiv instead of 0.5 equiv), DIEA (3.0 equiv instead of 2.0 equiv) and DMAP (1.0 equiv instead of 2.0 equiv). Attempts to synthesise the methyl ester using microwave irradiation to 130 °C for 15 min failed probably due to vaporisation of the methanol. Notable is also the chemo selectivity of this process. The iodide is much more reactive, compared to bromide, which allows the use of a Pd-catalyst without any phosphine ligands. A consequence of the difference in reactivity between the two halides is that substitution only occurs at the iodide-position. The iodo-

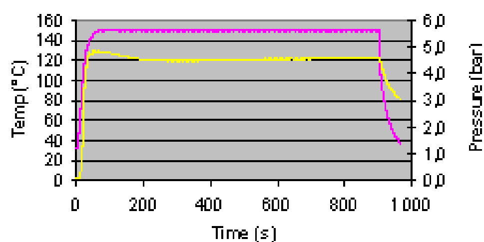


Figure 10. Graphic displaying temperature (yellow) and pressure (purple) in reaction vessel during microwave heating.

dehalogenated start material is the only side-product that was recorded.

The DMAP was used as an additive to promote the nucleofilic attack from the alcohol via an activated amide formed with DMAP.[16] The DIEA act as a base, neutralising the HI produced in the reaction (*see Figure 11*).

In a alkoxy carbonylation the aryl halide is added to the Pd(0) complex by oxidative addition. One of the ligands is thereafter exchanged to CO and migration occurs. The alcohol or DMAP performs a nucleofilic attack and the halogen is eliminated. By reductive elimination the carboxyl is set free and the catalytic cycle is closed.[17]

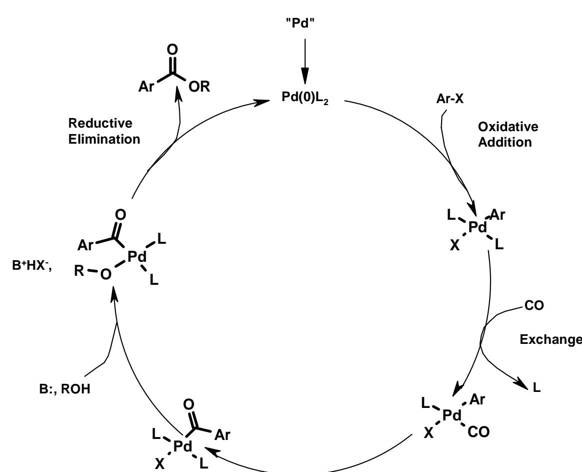


Figure 11. Mechanism for a alkoxy carbonylation.

To achieve compound **5**, a palladium catalysed cyanationenhanced by microwave flash heating was used based on the protocol from Alterman *et al.*[18] In the number of attempts needed to find useful conditions, different palladium precatalysts, with or without P(*o*-tolyl)$_3$ as ligand and various cyanide sources were evaluated. The palladium sources were $Pd_2(dba)_3$ from various batches and Herrmanns palladacycle, the cyanide sources were $Zn(CN)_2$ from different batches and $K_4Fe(CN)_6$ in different concentrations. When the concentration of $Zn(CN)_2$ was lowered from 1 equiv to 0.6 equiv and with 0.02 equiv $Pd_2(dba)_3$, 0.08 equiv P(*o*-tolyl)$_3$, using DMF as solvent a yield of 81% was isolated after microwave irradiation to 180 °C for 10 min. The reduced amount of $Zn(CN)_2$ is supported of the reported successful reactions by Maligres *et al* and by Tschaen *et al.* [19,20] The modifications made which

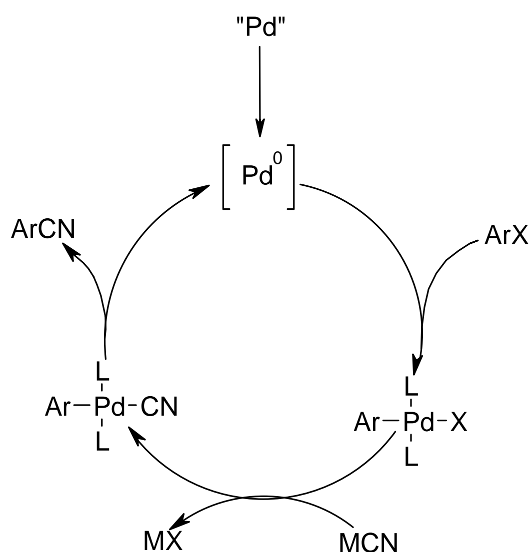


Figure 12. Proposed mechanism of the palladium-catalysed cyanation of aryl-X.[21]

resulted in the successful method was, beside the altered cyanide concentration, the change in procedure in which the solved $Pd_2(dba)_3$, P(*o*-tolyl)$_3$ and **4** was mixed and left in room temperature for about 5-10 min before $Zn(CN)_2$ was added.
The altered procedure and the lower CN^- concentration lowered the risk of poisoning the catalyst with CN^-, which ought to be the reason of the many unsuccessful attempts.[21]

Above *(see figure 12)* is a proposed mechanism of the palladium-catalysed cyanation of aryl-X as described by Sundermaier *et al*[21] is shown in figure 12. The catalytic cycle starts with the oxidative addition of the aryl halide to a Pd^0-ligand complex. The halide is then substituted with nitrile through transmetallation.

The intermediate arylpalladium(II) cyanide complex has not been isolated. The transmetallation is immediately followed by a reductive elimination in which the aryl nitril is produced. This step concludes the catalytic cycle and Pd^0 is recreated. It is thought that the transmetallation and reductive elimination are much faster processes than the oxidative addition.[21] An alternative to lower the amount of $Zn(CN)_2$ could have been to add TMEDA which prevent deactivation of the Pd^0 complex.[21]

The reduction of **5** to the benzylic amine **6** was performed using a classic hydrogenation with Pd /C as catalyst under H_2 atmosphere for 55 min. The isolated yield was 67%.

The HATU based amide coupling is mediated in two steps, activation and coupling *(see Figure 13)*. In the activation step HATU reacts with the deprotonated carboxylic group forming an active intermediate.
In the coupling step the created intermediate reacts with the amino component to form the amide. The first step is regulated of the structural feature of the carboxylic component. In the second step the rate is regulated of characteristics of the amino group and of the coupling reagent.[23]

R_1-COOH was coupled with **6** through an amide coupling with HBTU as coupling reagent. The structure of HATU and HBTU as well as the mechanism of the HATU and the HBTU mediated amide couplings are quite similar (*see Figure 14*).

1.5 equiv of R_1-COOH was used to ensure coupling of all **6**. The reaction was monitored by LC-MS and no remaining starting material was left after 65 min. After extractions and concentration the crude product was subjected to purification on preparative LC-MS. After a first

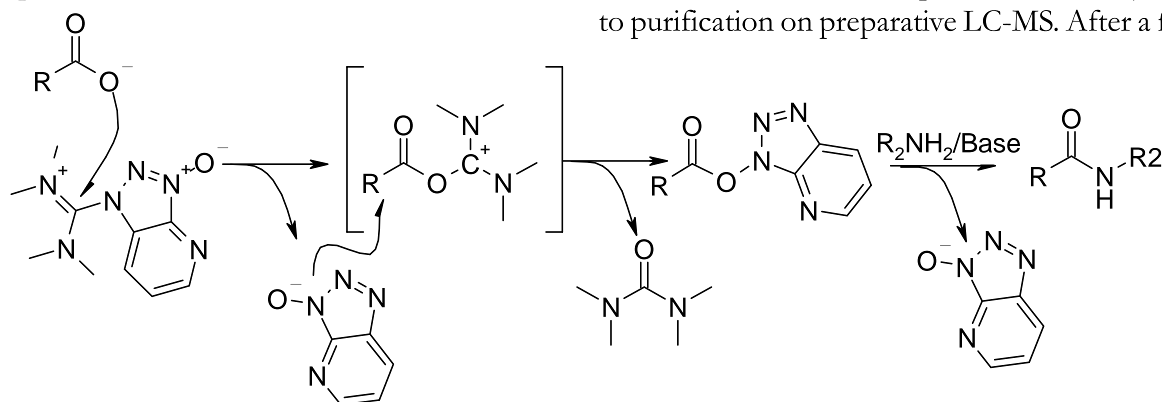


Figure 13. Mechanism of the HATU-mediated amide coupling.[23]

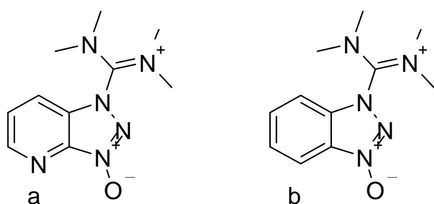


Figure 14. The structures of a) HATU and b) HBTU

separation two main fractions were collected, one with pure and one with slightly impure product. The impure product was subject to extensive purification by LC-MS using various gradients, flows and gradient times etc. according to table 1 *(Not included in this version)*. Pure fractions were pooled to yield 68 mg and used fore analysis and reference sample. Thereafter the pure and slightly impure product was pooled and used for synthesis of compound **13**.

To finalise the target molecule, an amide coupling between the synthesised fragment and R_2-NH_2 was performed after removal of the protecting group on on the synthesised molecule. Deprotection of **10** was performed the same way as the deprotection of **8**, yielding compound **11**. The coupling of **11** and R_2-NH_2 was performed based on a the HATU protocol. Based on the amount of **11**, 1.2 equiv HATU, 4.0 equiv DIEA, DMAP and DBU was used to react 0.5 equiv R_2-NH_2. The crude product was subject to purification on preparative LC-MS. The isolated yield was 51% of the target inhibitor, **13**.

3.2 Biological results

In the enzymatic assay comprised of the full length NS3 the synthesised inhibitor showed moderate potency, K_i= 17.1 ± 1.8 µM.

3.3 Conclusions

A successful way to synthesise the target molecule compound **13** with good yields was elaborated based on the described methods. A protocol for the use of $Pd_2(dba)_3$ and $Zn(CN)_2$ with microwave flash heating in the nitrile coupling was optimised. Results from the enzymatic assay showed moderate binding affinity. The target molecule is a promising lead compound for developing of a new type of aromatic scaffold for HCV NS3 serine protease inhibitors.

4. Experimental section

4.1 General procedures

^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX 400 at 400 MHz and 100.5 MHz respectively or on a JEOL JNM-EX 270 at 270 MHz and 67.9 MHz respectively. Chemical shifts were reported as d values (ppm) by the solvent signal ($CHCl_3$) d 7.26 and d 77.0, by (CH_3OH) d 3.31 and 49.00 or by (($CH_3)_2CO$) d 2.05 and 29.84. GC was performed on a Varian 3900 equipped with a capillary column CP-Sil 8 CB lowbleed/MS using a 70-305 °C temperature gradient. The MS spectra were recorded on a Varian Saturn 2100T with an electron impact (70 eV) ionisation. Analytical LC-MS was performed on a Gilson HPLC system with a Chromolith Performance RP-18C column (4.6 × 100 mm) at a flow of 4 mL/min connected to a Finnigan AQA quadrapole mass spectrometer with electrospray ionisation (10 eV). Preparative LC-MS was performed on a Gilson HLPC system with a Zorbax SB-C8 column, 5 µm, 21.2 × 150 mm (Agilent technologies) connected to a Finnigan AQA quadrapole mass spectrometer with electrospray ionisation (10 eV). Thin-layer chromatography (TLC) was performed using aluminium sheets precoated with silica gel 60 F_{254} (0.2 mm, E. Merck). The spots were identified using UV-detection at 254 nm. Microwave heating was performed in an EmrysOptimizer™ single mode cavity with controlled irradiation at 2450 MHz. Reaction temperature and pressure were determined using built-in, on-line IR- and pressure sensors. The reactions were performed in Smith Vial™ (2-5 mL) under air with magnetic stirring. The reaction was cooled with compressed air. All chemicals were purchased from commercial suppliers and used directly without further purifications. Elementary analyses were performed by Analytiche Laboratorien AG, Germany.

4.2 Experiments

3-Bromo-5-Iodo-4'-metoxybenzophenone (2)
3-bromo-5-iodo-benzoic acid, 1, (11.17 g, 34.14 mmol) was dissolved in thionylchloride (59 mL) and refluxed at 85 °C for 1 h. Remaining thionylchloride was evaporated and the product

was dissolved in DCM and concentrated under reduced pressure. The acid chloride was dissolved in dry DCM and anisole (11 mL, 0.10 mol) was added. $AlCl_3$ (9.01 g, 68.26 mmol) was added during 30 min. The reaction mixture was stirred over night at room temperature equipped with a drying tube. The reaction mixture was poured into a mixture of 1M HCl (100 mL) and ice (100 mL) and extracted three times with DCM. The organic layers were pooled and washed twice with water and once with brine. According to TLC there was some product in the water phase after the first set of extractions. This water phase was extracted twice with EtOAc. The organic phases were pooled, washed twice with brine, dried with $MgSO_4$ and evaporated under reduced pressure. The crude product was crystallized in EtOH, filtrated and placed under vacuum over night. The isolated yield was 69% (9.79 g, 23,48 mmol). MS *m/z* (70 eV) 418 (M^+). ^{1}H NMR (400 MHz, $CHCl_3$) δ: 8.04 (m, 1H), 7.97 (m, 1H), 7.81 (m, 3H), 6.98 (m, 2H) 3.9 (s, 3H); ^{13}C NMR (100,5 MHz, $CHCl_3$) δ: 192.2 (CO), 163.8 (C), 142.6 (CH), 141.5 (C), 136.9 (CH), 132.6 (2 × CH), 131.7 (CH), 128.9 (C), 113.9 (2 ´ CH), 94.2 (C), 55.6 (CH_3).

1-bromo-3-iodo-5-(4-methoxybenzyl)-benzene (3)

2 (9.55 g, 22.90 mmol) was dissolved in DCM (149 mL) in a three-necked round bottomed flask. Reaction mixture was cooled on an ice-bath and TFA (25.16 g, 0.22 mol) and triflic acid (0.087 g, 0.58 mmol) were added. Et_3SiH (8.03 g, 69.2 mmol) was added drop-wise. The mixture was refluxed for 6 h at 50 °C. The reaction was followed by TLC every hour using DCM : isohexan (1:1) as mobile phase. The reaction mixture was cooled to room temperature and then washed three times with 1 M NaOH and once with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The isolated yield of **3** was 87% (8.18 g, 20.26 mmol). MS *m/z* (70 eV) 404 (M^+). ^{1}H NMR (400 MHz, $CHCl_3$) δ: 7.70 (m, 1H), 7.45 (m, 1H), 7.25 (m, 1H), 7,07 (m, 2H), 6.85 (m, 2H), 3.83 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (100,5 MHz, $CHCl_3$) δ: 158.4 (C), 145.8 (C), 137.3 (CH), 136.4 (CH), 131.27 (C), 131.26 (CH), 129.9 (2 × CH), 122.9 (C), 114.13 (2 × CH), 94.5 (C), 55.3 (CH_3), 40.2 (CH_2).

Ethyl-3-bromo-5-(4-methoxybenzyl)-benzoate (4)

A solution of **3** (0.51 g, 1.27 mmol) and DMAP (0.15 g, 1.25 mmol) in dioxane (3.68 mL) was added to a Smith Process VialÔ (2-5 mL) charged with Pd/C (10%) (0.067 g, 0.063 mmol) and $Mo(CO)_6$ (0.33 g, 1.26 mmol). EtOH (723 μL) was added followed by DIEA (648 μl, 3.72 mmol) and the vial was sealed with Crymper seal. The mixture was irradiated by microwaves to 150 °C for 900 s. The reaction mixture was filtered (milliQ), added to 1M HCl (40 mL) and extracted three times with EtOAc. The organic layers were pooled and washed with water and brine, dried over $MgSO_4$ and evaporated under reduced pressure. The product was subjected to purification on silica column chromatography with 40% isohexan in DCM as mobile phase. The isolated yield of **4** was 59% (0.26 g 0.75 mmol). MS *m/z* (70 eV) 350 (M^+). ^{1}H NMR (400 MHz, $CHCl_3$) δ: 8.00 (dd, J= 1.5, 1.8 Hz, 1H), 7.81 (dd, J= 1.5, 1.7 Hz, 1H), 7.47 (dd, J= 1.7, 1.8 Hz, 1H), 7.09 (m, 2H), 6.85 (m, 2H), 4.36 (q, J= 7.1 Hz, 2H), 3.92 (s, 2H), 3.78 (s, 3H), 1.38 (t, J= 7.1 Hz, 3H); ^{13}C NMR (100,5 MHz, $CHCl_3$) δ: 165.4 (CO), 158.3 (C), 144.1 (C), 136.0 (CH), 132.4 (C), 131.7 (C), 130.2 (CH), 129.9 (2 × CH), 128.6 (CH), 122.4 (C), 114.1 (2 × CH), 61.4 (CH_2), 55.3 (CH_3), 40.5 (CH_2), 14.3 (CH_3). Anal. Calcd for $C_{17}H_{17}BrO_3$: C, 58.47; H, 4.91. Found: C, 58.33; H, 4,90

Ethyl-3-cyano-5-(4-methoxybenzyl)-benzoate (5)

4 (0.20 g, 0.56 mmol) was dissolved in DMF (2.00 mL) and added to a solution of $Pd_2(dba)_3$ (0.014 g, 0.12 μmol) and P(*o*-tolyl) (0.014 g, 0.460 μmol) in DMF (2.00 mL). The mixture was left at room temperature for 5 minutes before it was transferred to a Smith Process Vial™ (2-5 mL) loaded with $Zn(CN)_2$ (0.040 g, 0.34 mmol). The vial was sealed with Crymper seal and was irradiated by microwaves to 180 °C for 600 s. The reaction mixture was added to 2 M NH_4OH (ca 100 mL) and extracted three times with EtOAc. The organic layers were pooled and washed twice

with water and once with brine, dried over K_2CO_3 and concentrated under reduced pressure. The product was subjected to purification on silica column chromatography with 40% isohexan in DCM as eluent. The isolated yield of 5 was 81% (0.14 g, 0.46 mmol). MS *m/z* (10 eV) 591 (2 × M + H^+). ^{1}H NMR (400 MHz, $CHCl_3$) δ: 8.16 (dd, J= 1.5, 1.7 Hz 1H), 8.09 (dd, J= 1.7, 1.8 Hz, 1H), 7.58 (dd, J= 1.5, 1.8 Hz 1H), 7.08 (m, 2H), 6.86 (m, 2H), 4.39 (q, J= 7.1 Hz, 2H), 4.00 (s, 2H), 3.80 (s, 3H), 1.40 (t, J= 7.1 Hz 3H); ^{13}C NMR (100,5 MHz, $CHCl_3$) δ: 164.7 (CO), 158.5 (C), 143.7 (C), 135.9 (CH), 134.0 (CH), 131.8 (C), 130.91 (CH), 130.8 (C), 129.9(2 × CH), 118.0 (CN), 114.3 (2 × CH), 112.9 (C), 61.7 (CH_2), 55.3 (CH_3), 40.4 (CH_2), 14.20 (CH_3). Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.2; H, 5.8; N, 4.74. Found: C, 73,34; H, 5,82; N, 4,72.

Ethyl-3-aminomethyl-5-(4-methoxybenzyl)-benzoate (6)

5 (0.14 g, 0.47 mmol) was dissolved in EtOH (7.0 mL) and MeOH (3.0 mL) in a round flask. Pd/C (0.050 g, 0.47 mmol) was added and mixed with the solution. The flask was sealed with a septum, purged with nitrogen and thereafter with hydrogen and left stirring for 55 min. The reaction was monitored with LC-MS. The reaction mixture was filtrated and evaporated. The reaction mixture was dissolved in EtOAc and extracted three times with 1 M HCl. 1 M NaOH was added to the pooled water phases to pH 9 and thereafter extracted three times with EtOAc. The organic layers were pooled, concentrated under reduced pressure and dried under vacuum over night. The isolated yield was 67% (0.09 g, 0.30 mmol). MS *m/z* (10 eV) 300 (M + H^+). ^{1}H NMR (400 MHz, $CHCl_3$) δ: 7.83 (m, 1H), 7.77 (m, 1H), 7.32 (m, 1H), 7.10 (m, 2H), 6.83 (m, 2H), 4.36 (q, J= 7.1 Hz, 2H), 3.95 (s, 2H), 3.89 (s, 2H), 3.78 (s, 3H), 1.76 (s, 2 NH), 1.38 (t, J= 7.1 Hz, 3H); ^{13}C NMR (67.9 MHz, $CHCl_3$) δ: 166.7 (CO), 158.0 (C), 143.6 (C), 142.2 (C), 132.6 (C), 132.1 (CH), 130.9 (C), 129.8 (2 × CH), 128.5 (CH), 125.6 (CH), 113.9 (2 × CH), 61.0 (CH_2), 55.2 (CH_3), 45.9 (CH_2), 40.8 (CH_2), 14.3(CH_3). Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.2; H, 7.1; N, 4.7. Found: C, 73,61; H, 7,11; N, 4,20.

Compound 10

6 (0.30 g, 0.97 mmol) was dissolved in DCM (26.0 mL) and added to R_1-COOH in a 50 mL round bottomed flask with stirring bar. HBTU (0.46 g, 1.20 mmol) and DIEA (699 µl, 4.01 mmol) were added to the reaction mixture at room temperature. After 65 min LC-MS showed product and no remaining starting material. The reaction mixture was washed twice with 5% citric acid and twice with $NaHCO_3$ (*aq*). The water phase after the first washes with citric acid had remaining product, verified on LS-MS, and was thereby pooled and extracted once with DCM. There was still product remaining in the aqueuos phase. The organic phases were pooled and washed once with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The citric phase was then extracted another three times with EtOAc. LS-MS indicated no product in the water phase. The organic layer was dried with Na_2SO_4, pooled with the organic layer from the first extraction and concentrated under reduced pressure. Purification on silica with 10% EtOAc in toluene as eluent, was unsuccessful. The column was washed with EtOAc, all organic fractions were pooled and evaporated. The product it was dissolved in MeCN (3.5 mL), filtered, H_2O (1 mL) was added and solution was purified on preparative LC-MS (*See table 1, P1*). *(Table 1 is not included in this version.)* Fractions were checked on analytical LC-MS and the ones with pure product was pooled and concentrated to eliminate MeCN. The solution was extracted three times with EtOAc, washed once with water and once with brine before it was dried over Na_2SO_4 and evaporated under reduced pressure. The product was dissolved in MeOH an evaporated again. The other fractions containing impure product was treated as the pure ones. This purification resulted in 0.02 g pure product and 0.24 g impure product. Extensive purification by LC-MS according to table 1 was performed. The purification resulted in a total amount 0.068 g pure product and 0.097 g impure product.

Compound 13

Deprotection of 10

Compound **10** was dissolved in THF (6 mL). MeOH (1.0 mL) and H_2O (1.0 mL) were added to the mixture together with stirring bar. LiOH

(0.03 g, 1.28 mmol) was dissolved in H_2O (2.0 mL) and slowly added to the reaction mixture. The reaction mixture was left in room temperature over night. The reaction was finished after 22 h, verified on LC-MS. HCl (1 M) was added to the reaction mixture to pH 4. The reaction mixture was then extracted three times with EtOAc and washed once with H_2O and once with brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, dissolved in MeOH and evaporated again. The product was left under vacuum over night.

Coupling of 11 and R_2-NH_2

11 was dissolved in DMF (5.00 mL) and added to a round bottle flask with HATU (0.11 g, 0.30 mmol) and a stirring bar. DIEA (0.170 mL, 0.98 mmol) was added slowly to the reaction mixture. The reaction mixture was stirred for one hour in room temperature. R_2-NH_2 was dissolved in DMF (2.00 mL), DMAP (0.12 g, 0.98 mmol) and DBU (0.146 mL, 0.98 mmol) and was added to the solution. The reaction mixture was left stirring in room temperature over night. Control on LC-MS after 23 h indicated no starting material, R_2-NH_2. EtOAc (ca 50 mL) was added and the reaction mixture was washed twice with NaOAc-buffer (pH 4), once with $NaHCO_3$ (*aq*), once with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Left under vacuum over night. The crude product was dissolved in MeCN (ca 3 mL) and H_2O (0.5 mL), filtered and purified on preparative LC-MS with MeCN gradient 30-90% under 45 min and a flow of 10 mL/min. Pure fractions were pooled and concentrated. EtOAc was added and the layers were separated. The aqueous phase was extracted with EtOAc. Subsequently water and brine was added to the first organic phase, which was then extracted with EtOAc. All the organic phases were pooled, washed twice with brine and dried over Na_2SO_4. The product was concentrated under reduced pressure, dissolved in MeOH and concentrated again. Left under vacuum over night. The isolated yield of **13** was 51%.

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