Synthesis and Characterization of Acrylfentanyl Metabolites

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**Title**

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To have metabolites for analysis are very important as they are the main target in drug testing.

The method used to synthesize the metabolites is a five-step synthesis with an additional 6th step for the dihydroxy metabolite. The methods used in the synthesis includes protection of amine with tert-butyloxy carbonyl, reductive amination with sodium triaceto borohydride, alkylation and demethylation with boron tribromide. The methods used produced good results with high yields in nearly all steps.

**Nyckelord**

Acrylfentanyl, Metabolite, Synthesis
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Abbreviations

RMV    the Swedish National Board of Forensic Medicine
TLC    Thin layer chromatography
NMR    Nuclear magnetic resonance spectroscopy
LC     Liquid chromatography
MS     Mass spectroscopy
TFA    Trifluoroacetic acid
DCM    Dichloromethane
Boc    tert-butyloxycarbonyl
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Introduction

Aim
The aim with this thesis is to synthesize metabolites of acrylfentanyl in enough amounts and purity to allow the compounds to be used by RMV (The Swedish National Board of Forensic Medicine) to work out new analytical methods to identify metabolites of acrylfentanyl in urine.

Background
When someone takes a drug the molecule are changed in the liver to become more water solvable and more easily be excreted in urine. The changed molecule is called a metabolite as it has passed through the metabolism. Metabolites are present in the body longer than the original molecule and are also present in urine in higher amounts, thus making them good target molecules for drug testing. [1,2]

Fentanyl, including acrylfentanyl are synthetic opioids that have become more common over the last few years. [3–6] The first cases of acrylfentanyl usage in Sweden occurred in spring 2016 [5] and the drug was classified in Sweden as narcotic in August 2016 [7]. Even if the drug is classified it takes some time to fully investigate all metabolites and fully implement a new drug in routine analysis, and therefore metabolite studies are necessary. [2,7,8]

Fentanyl and Acrylfentanyl
Fentanyl and acrylfentanyl are examples of synthetic opioids that have been used by drug addicts. Fentanyl are used as an analgesic for terminal cancer patients but the analogues do not have any medicinal uses and are only used as narcotics. [2] Synthetic drugs are often widely used before they are classified as they are not illegal to use at that time, but because they are legal does not make them safe. [5] The only structural difference between fentanyl and acrylfentanyl is a double bond and their structures can be found in Figure 1.

![Figure 1. Structures of fentanyl to the left and acrylfentanyl to the right.](image)

Acrylfentanyl became classified as a narcotic in Sweden in August 2016. Although it had been known on internet forums since 2014, the discussion on Swedish forums started in January 2016, at the time when some other fentanyl analogues, including furanyl fentanyl and 4-MeO-butyrfentanyl, became illegal. The pattern when a newly classified drug is exchanged for a non-classified drug is not new and has been observed many times the last years. [4,5]

The first cases of Swedish acrylfentanyl intoxications was reported in March 2016 and during the following 6 months 30 cases of intoxication with acrylfentanyl were reported. Eight of these cases were analyzed and confirmed as part of a Swedish research project. There are some uncertainty in the number of reported cases as it is based on reports to the Swedish Poisons Information Centre. [4]
A study on metabolites of acrylfentanyl and other analogues of fentanyl have been performed where metabolites made by human liver cells in an in vitro experiment were compared with metabolites found in urine from overdose cases. Knowledge about metabolites is required to get reliable methods for analyzing them, both at forensic and clinical laboratories. [9] The number of studies on metabolites of acrylfentanyl and other fentanyl analogues are few, but the studies that have been done show that some analogues have the same metabolic pattern as fentanyl while others do not. [2,9]

Ethics and Social Relevance
Metabolites are an important component in the process to work out reliable analytical methods to use in determination of drug abuse. [2,9] To have reliable analytical methods are important for forensic laboratories as their results will influence the outcome if someone is taken to court for abuse of illegal substances. It is also important for clinical laboratories to have safe methods so that they can help a patient with overdose as fast and efficient as possible.

To make metabolites synthetically is a preferable method as it is easy to get a high amount of a pure product instead of using liver cells or urine samples as these methods require a lot of sample and separation before the desired product is achieved. To make metabolites synthetically also have the advantage of knowing the structure of the product before analysis. [8,9]
Synthesis
Target Molecules

Hydroxylation is a common metabolic pathway [1,2,9] and therefore two hydroxylated metabolites were chosen to be the target molecules in this thesis. The target molecules are one monohydroxylated metabolite and one dihydroxylated metabolite. Structures of the target molecules can be found in Figure 2.

![Figure 2: Structures of the target molecules, monohydroxy to the left and dihydroxy to the right](image)

Synthetic Route

The synthetic route to obtain the target molecules was a five-step synthesis with an additional step for the synthesis of the dihydroxy metabolite. A schematic figure of the steps and the compounds are found in Scheme 1. The synthesis started with 4-piperidoene and the first step was to protect the nitrogen with the protection group tert-butyloxycarbonyl (Boc) so that the nitrogen does not undergo any unwanted alkylation or react with itself in the next steps. Boc is a common protection group with the advantages that it is stable towards bases and reduction [10].

The second step (compound 1 to compound 2) was to add aniline to the keto position using sodium triacetoxyborohydride as a reducing agent to get reductive amination. The use of this reducing agent have been investigated and found to be a good choice for cyclic ketones and it has mild reducing properties, thus allowing only the desired reduction to take place without risking to reduce the ketone. [11]

The third step (compound 2 to compound 3) was to acylate the aniline nitrogen with an acid chloride, in this case acryloyl chloride, to add the double bound that makes the difference between acrylfentanyl and fentanyl. The fourth step was to remove the protection group using trifluoroacetic acid [10] to allow the nitrogen to get alkylated.

The alkylation (compound 4 to compound 5, compound 4 to compound 7) was done as the fifth step to allow different metabolites to be made without repeating too many steps. The alkylation can also be made as the first step, instead of doing the protection step [12], although it is not recommended to do with phenols as they might interfere with other reactions. Compound 7, the monohydroxylated metabolite was made directly via alkylation of compound 4 but the dihydroxylated metabolite was first made as a dimethoxylated compound. To convert the methoxy groups to hydroxyl groups (compound 5 to compound 6, step 6) boron tribromide was used as the reagent. The use of boron tribromide have been described in literature with satisfying yields. [13]
Scheme 1. A schematic picture of the different steps in the synthesis. Compound 6 is made from compound 5 and compound 7 is made directly from compound 4.
Mechanisms

Mechanism suggestions for the different steps in the synthesis are found in Scheme 2 to 7. Scheme 2 shows the first step in the synthesis, the addition of the protecting group Boc to 4-piperidone. [10]

Scheme 3 shows the second step in the synthesis. This step is a reductive amination and this mechanism is based on an article from 1996. [11].

Scheme 4 shows the third step in the synthesis, which is an acylation.

Scheme 5 shows the forth step in the synthesis, this is the removal of the protecting group Boc with TFA [10].

Scheme 6 shows the alkylation, which follows a $S_N2$ mechanism. Depending on the R-group used in this step different compounds can be made. Both compound 5 and compound 7 are made from this step. For compound 5 the R-group is 3,4-dimethoxy benzene and for compound 7 the R-group is 4-phenol.

Scheme 7 shows the sixth step in the synthesis of compound 6, which is demethylation with boron tri bromide [13] to convert the dimethoxy groups of compound 5 to hydroxyl groups.

Scheme 2. Mechanism for the addition of the protecting group Boc to 4-piperidone
Scheme 3. Mechanism suggestion for reductive amination with aniline

Scheme 4. Mechanism suggestion for the acylation
Scheme 5. Mechanism suggestion for the removal of Boc with TFA

Scheme 6. Mechanism suggestion for the alkylation step in the synthesis. Different compounds can be made from this step, depending on the R-group used. For compound 5 a dimethoxy benzene is used as R and for compound 7 a phenol is used.
Repeated reactions

Compound 6

Scheme 7. Mechanism suggestion for the demethylation with boron tri bromide
Materials and Methods

Synthesis
The general principle of the synthesis was to start with the protection of the nitrogen on the piperidone using the protection group tert-butyloxy carbonyl, then aniline undergoes reductive amination with the aid of Na(OAc)₃BH, followed by acylation using acryloyl chloride, which is added to the free nitrogen and an amide is formed. The protection group is removed with trifluoroacetic acid and the no longer protected nitrogen is alkylated by 4-(2-bromoethyl)-1,2-dimethoxybenzene (compound 5) or 4-(2-bromoethyl)phenol (compound 7). To make compound 6 demethylation with boron tribromide was also performed.

Uncommon reagents important for the synthesis, like acryloyl chloride, 4-(2-bromoethyl)-1,2-dimethoxybenzene and 4-(2-bromoethyl)phenol were bought from Sigma-Aldrich. Other chemicals used were mainly from Sigma-Aldrich as well but solvents were from a broad variety of companies, depending on availability in the laboratory.

Purification
To purify the synthesized compounds two main methods was used: silica column and preparative liquid chromatography (LC-prep). Both these methods use a column with a stationary phase and a mobile phase to separate compounds based on polarity. With a silica column the mobile phase is a nonpolar organic solvent and the stationary phase is polar silica particles, thus making polar compounds to interact with the column and nonpolar to travel with the mobile phase. LC columns come in a broad variety but the most common is to have a nonpolar stationary phase and a polar mobile phase, mainly based on water mixed with acetonitrile or methanol, this gives a reversed separation pattern were the polar compounds eluate first and the nonpolar last.

Flash Chromatography
Flash Chromatography was used to purify reaction mixtures to get the desired product. The column was prepared by mixing 100 ml silica powder with 100 ml mobile phase and applying the resulting slurry to a glass column. The most commonly used mobile phase was a mixture of 50% ethyl acetate and 50% heptane but 100% ethyl acetate was also used when compound 5 was purified. In that case the mixture was 99% ethyl acetate and 1% triethylamine to avoid interactions with the amine and the hydroxyl groups on the column to get the compound to eluate as concentrated as possible. Some methanol was also used in increasing amounts to speed up the elution.

Silica powder was bought from Sigma-Aldrich.

Preparative LC:
Preparative LC was also used to purify reaction mixtures, mainly for the target compounds as these were quite polar and easily eluted with a polar mobile phase.

Two different systems were used to purify with LC-prep. For both systems the mobile phases: A: 95% water, 5 % acetonitrile and 10 mmol ammonium acetate, B: 90% Acetonitrile, 10% water and 10 mmol ammonium acetate were used.
a. Waters LC-MS, column Xselect prep phenyl hexyl with dimensions 19*250 mm. The flow 25 ml/min and a mobile phase system starting at 20% B, gradient to 70% for 8 minutes and then a hold time 4 minutes.

b. Pretetch system, column waters xbridge prep C18 5µm with the dimensions 19*100 mm. The flow was 10 ml/min and the mobile phase system was starting at 15% B, gradient to 70% for 20 minutes and then a hold time for 10 minutes.

System b was used for the second purification of the dihydroxy metabolite and for purification of the second synthesis of compound 5, in all other cases system a was used for purification with preparative LC.

Analysis
To analyze the synthesized compounds three different methods were used. NMR (nuclear magnetic resonance) was used to identify the different compounds after purification. TLC (thin layer chromatography) was used to have an estimation of the number of compounds in a mixture and was mostly used to investigate if a reaction had finished or not. LC-MS (liquid chromatography with mass spectrometer detector) were used to analyze reaction mixtures when it was desired to know not only how many kinds of compounds it contained but also what kind of compound it was, particularly when the reaction could lead to undesired side products.

NMR
NMR was used to confirm that the synthesis had given the correct product and that the product was pure. To avoid disturbance from solvents used during the synthesis the sample was evaporated twice with chloroform before analysis. As solvent for NMR chloroform or methanol where all hydrogen had been exchanged for deuterium was used.

Hydrogen NMR was made at 300 MHz and carbon NMR was made at 75 MHz. The apparatus used was a Varian NMR spectrometer.

TLC
TLC was mainly used to know if a reaction mixture still contained starting material or not and to investigate if fractions from purification contained anything or not. The mobile phase used for TLC was a mixture of 50% ethyl acetate and 50 % heptane. The plates were detected under ultra violet light with a wavelength of 254 nm. Silica plates were bought from Sigma-Aldrich.

LC-MS
LC-MS was used to investigate reaction mixtures, mainly for the alkylation steps were the mixture was more likely to contain side products apart from the desired product and starting materials. This method gives a separation of the compounds in the mixture as well as an analysis of the compound, thus is it possible to identify where the compound is.

The system used was a Waters LC-MS with a C18 3.5 µm Waters X bridge column, 4.6*50 mm. The mobile phase A: 95% water, 5 % acetonitrile and 10 mmol ammonium acetate; B: 90% Acetonitrile, 10% water and 10 mmol ammonium acetate. Gradient elution was used with the starting conditions 90% A and 10 % B, gradient to 50:50 for 1 min and hold time for 4 minutes.
Results and Discussion

All compounds were synthesized as intended, except the resynthesis of compound 6 when something new happened and compound 8 was formed. Enough amounts were made of the final products to allow RMV to use them for analysis and the final products had acceptable purity. Yield in mg and percentage of all synthesized compounds can be found in Table 1. The big difference in mass between compound 4 (2260 mg) and compound 5 (55 mg) is because only a part of the synthesized compound 4 was used for the reaction.

Table 1. Comparison of the yield and purity of the different compounds, compound 4 occurred as a mixture of compound 4 and TFA. Compound 5 and 7 are made with the alkylation step.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (mg)</th>
<th>Yield (%)</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>1310 mg</td>
<td>100%</td>
<td>Pure, according to NMR</td>
</tr>
<tr>
<td>Compound 2</td>
<td>1370 mg</td>
<td>76%</td>
<td>Pure after purification</td>
</tr>
<tr>
<td>Compound 3</td>
<td>1490 mg</td>
<td>90%</td>
<td>Pure after purification</td>
</tr>
<tr>
<td>Compound 4</td>
<td>2260 mg oil</td>
<td>100%</td>
<td>Full conversion, no NMR made.</td>
</tr>
<tr>
<td>Compound 5</td>
<td>55 mg</td>
<td>27%</td>
<td>Pure after purification</td>
</tr>
<tr>
<td>Compound 6</td>
<td>27 mg</td>
<td>53%</td>
<td>Impure due to stability problems</td>
</tr>
<tr>
<td>Compound 7</td>
<td>3.5 mg</td>
<td>11%</td>
<td>Pure after purification</td>
</tr>
<tr>
<td>Compound 8 (brominated)</td>
<td>15 mg</td>
<td>55%</td>
<td>Pure but compound underwent new side reaction.</td>
</tr>
</tbody>
</table>

Discussion on the Synthesis

The reactions from synthesis of compound 1 to synthesis of compound 4 have high yields, all over 75%, and high purity. For compound 3 extra purification was used to get the pure compound but this did not affect the yield much. Compound 1 and 4 have an assumed yield of 100%, for compound 1 the sample was not completely dry and therefore got a higher mass than possible, for compound 4 the product was an oily mixture of compound 4 and TFA, therefore the yield was regarded to be 100%.

In general the demethylation step (compound 6) has an acceptable yield about 53 to 55% but the alkylation steps have a low yields and will be more thoroughly discussed in a separate section.

Compound 5:
The low yield is partly related to some loss of product during the synthesis when a beaker tilted too much and some product was lost to the fume hood. Another problem is that the mass of the flask used for evaporation of the compound was not noticed before the evaporation and when the mass was to be calculated the mass of the flask before the synthesis was used, depending on how well the flask was cleaned and the cesium carbonate removed these masses might vary. The yield can also have been affected as some miss calculations happened to give the wrong molar ratio between compound 4 and 4-(2-bromoethyl)-1,2-dimethoxybenzene.

Resynthesis of Compound 5:
To make more of compound 6 the synthesis of compound five was done a second time. That time the synthesis went as intended but with surprisingly low yield, as the same method as in the first synthesis was used and no beakers fell this time. The low yield could be due to the purification method used, as some of the product could have been stuck in the column. During the purification one pink fraction
occurred even if none of the compounds should be pink and this was regarded as contaminations from the column. If other products might get stuck in the column it could have happened with this one as well. The low yield could also arise from the fact that a very big flask was used to evaporate the solvent after purification and the change in mass after addition of each fraction is very little compared to the mass of the flask.

**Compound 6:**
The demethylation went without any problems and the reaction mixture was purified with LC-prep in three rounds. The first round went best and had one pure fraction and two contaminated fractions. The second and third round did not have any pure fractions, they only had the contaminated fractions.

The best of the contaminated fractions was saved and later purified again, unfortunately without much difference in the purity.

The lack of pure fraction in the second and third run could be because the mixture had been left over night in an acidic solution, something that might have caused the acryloyl group to be deacylated. This is likely to have happened because the major product in the impure fractions had a mass of 311 g/mol, which is the mass of the product without the acryloyl group. A mechanism suggestion for this degradation can be found in Scheme 8.

There have also been problems with the NMRs of compound 6, the first NMR was not dry and efforts was made to make it dryer but for every time the NMR looked worse instead of better. This indicates that the product is unstable over time and likely heat sensitive as well because the evaporations made to make the product dry took place with increasing heating to remove as much solvent as possible.

Because of the low yield and unstable product the synthesis of compound 6 was repeated. LC-MS analysis showed that the product had become brominated, NMR was performed to find the placement of the bromination. The NMR showed that the bromination had occurred at the double bond and not at the aromatic residues. It looked like the addition occurred in an anti Markovnikov pattern, something

![Scheme 8. Mechanism suggestion for degradation of compound 6 in acidic solution, the blue and green arrow shows two possible reaction steps, corresponding products are shown in the same colour.](image)
that might not be as unexpected as one might think, as long as some oxidizing agent is present [14]. The demethylation reaction took place under nitrogen atmosphere, this should mean that no oxygen was present but a big flask was used and during to lack of time all solvent had not been completely removed. Because of this some water or other solvent that could act oxidizing might be present and it is also possible that not all oxygen had been removed. Another important thing to notice about the synthesis is that the solvent was evaporated before the reaction was quenched with methanol and this could also have had impact on the result. A figure of the proposed structure can be found in Figure 3.

![Proposed structure of brominated compound (compound 8)](image)

**Figure 3. Proposed structure of brominated compound (compound 8)**

**Compound 7:**
The low yield is mainly due to the fact that the molar ratio between compound 4 and 4-(2-bromoethyl)-phenol was 1:2.5 instead of 1:1 as it should be. The wrong molar ratio made it easier for the unwanted dialkylation reaction to take place and this gave much less of the correct product than it should had. The NMR did not contain the correct amount of hydrogen, this can be due to problems with the integration.

**Comments on alkylation steps**
During the alkylation cesium carbonate was used as a base. Most of the cesium carbonate was filtered of before evaporation but some remained dissolved in the solvent. After evaporation the cesium carbonate left in the flask became solid and very hard to remove from the flask during cleaning. The only way to get rid of it was to use strong acid.

Sometimes it was difficult to know how much product that actually had been made, mainly when it was little product and the flask was big. The scales at the lab had accuracy at 1 mg and when the products mass only is a few mg it is very difficult to know for sure how much it is. It is also a big uncertainty when a big flask is used and the mass of the product is a very small percentage of the flasks mass. 15 mg product in a flask with a mass of 57110 mg is only 0.026 % and then variations in measurements on the scale have very big impact on how much product that is detected. This problem occurred for compound 6 to 7, including both resynthesizes and all of them are cases where the mass of the product is less than 50 mg.

Another problem with the alkylation step is to get the molar ratio between compound 4 and 4-(2-bromoethyl)-1,2-dimethoxybenzene or 4-(2-bromoethyl)phenol correct. The molar ration should be 1:1 to get a good reaction but two times the calculations went wrong and the ratio as well. It is also hard to know exactly how much of compound 4 that has been used as it exist as a mixture with TFA and the
ratio is assumed to be 1 compound 4 to 3 TFA. Because no analysis of the mixture has been done the ratio could be another and the mixture could also contain other substances that will affect the ratio.

Finally the yield can also have been affected by the molar ratio between base and compound 4. With neutral starting material will the reaction need one molar equivalent base but with positive compound 4, as will be the case when it occurs as a mixture with TFA, two equivalents will be needed. This is to make sure compound 4 is neutral in the start of the reaction, otherwise it will not have any nucleophilic properties. When the synthesis was made, enough base to neutralize TFA was added in addition to the molar equivalent for neutral reaction but no base was added to neutralize compound 4. This might have resulted in less nucleophile available for the reaction and also affected the yield.

Stability
If the NMR spectra for the first synthesis of compound 6 are compared, see Figure 4, it is clear that they became worse with time. The last NMR has more peaks and more split peaks compared to the first NMR. This indicates that the product has a low stability over time and is heat sensitive, as many evaporations were made with the goal to remove more solvent and get better NMR.

![Figure 4. Comparison with NMR over time, the left NMR is from 12 April and the right is from 18 April.](image)

Ethics and Society
One problem is that the same procedure that has been used in this project to make metabolites of acrylfentanyl can be used to make the active drug. Some chemical reagents are hard to buy for personal use and the risk for misuse of this report are regarded as minor, compared with the benefit that the society can gain from it. If it is easier for laboratories to detect illegal substances, more people can get correct treatment at the hospital and drug dealers can become prosecuted.

Further Studies
Further studies on this subject could be to try to optimize the alkylation step to get higher yields, but also to make more metabolites for possible incorporation in analytical methods. Another thing that could be interesting to investigate further is the bromination that occurred at the resynthesis of compound 6, can it be reproduced or was it coincidence that it happened, are there any conditions that should be avoided to make sure this does not happen unintended? It would also be interesting to analyze side products obtained in the different steps as they might give knowledge about how to improve different steps.
Conclusions

Compound 6 was unstable in the first synthesis and become brominated the second time, still it was made in mg scale and the brominated compound had enough purity to allow it to be used for potential implementation in routine analysis. Some of the first synthesis was pure but it might have become degraded and contaminated over time.

Compound 7 was made in mg scale and it had high enough purity to be allowed to be used for potential implementation in routine analysis.

The yields of the alkylations were low, mainly due to erroneous calculations of the amounts of reagents that should be used.

Except for the alkylation, the synthetic route used in the thesis was good and gave satisfying results.
Experimental

tert-butyl 4-oxopiperidine-1-carboxylate (1):
4-piperidone (1.01 g, 6.6 mmol) was dissolved in a mixture of 10 ml deionized water and 10 ml THF. 0.28 g (7.0 mmol) NaOH and 1.435 g (6.6 mmol) ditertbutyl dicarbonate (Boc) was added. The reaction was left to stir over night (23h). TLC was preformed to confirm finished reaction (mobile phase Ethylacetate:Heptane 1:1). The reaction mixture was washed thriee times with 20 ml diethyl ether and then the organic phases were combined and washed with brine once. The solvent was evaporated to give compound 1 (1.31 g, 100 % yield) and NMR analysis was made. \(^1\)H-NMR (CDCl\(_3\) 300 MHz) \(\delta\): 3.74 (t J=6.6 Hz 4H), 2.46 (t J=6.6 Hz 4H), 1.51 (s 9H). \(^13\)C-NMR (CDCl\(_3\) 75 MHz) \(\delta\): 206.7, 153.7, 79.4, 40.4, 27.8, 27.7.

tert-butyl 4-anilinopiperidine-1-carboxylate (2):
Aniline (600 µl, 6.6 mmol) was dissolved in 30 ml DCM (Dichloromethane) over ice bath. 780 µl (6.6 mmol) acetic acid and 1.31 g (6.6 mmol) of compound 1 was added. 2.09 g (9.9 mmol) Na(OAc)\(_2\)BH was added carefully in portions with stirring. The reaction mixture was left to slowly gain room temperature while the ice melted and to react overnight. The next day the reaction was checked with TLC (mobile phase Ethylacetate:Heptane 1:1). When the reaction was finished 20 ml methanol was added and the mixture was washed with NaHCO\(_3\) (aq) until the aqueous phase was no longer acidic. This required 50 ml NaHCO\(_3\), 15 ml brine, 15 ml H2O and 15 ml NaHCO\(_3\) again before the aqueous phase finally was basic. The solvent was evaporated and product dissolved in as little DCM as possible before purification with flash chromatography (mobile phase Ethylacetate:Heptane 1:1). The fractions were checked with TLC (mobile phase Ethylacetate:Heptane 1:1), the purest fractions were evaporated directly and those that were contaminated with aniline were evaporated in a separate flask, then rinsed with some heptane to remove the aniline. An NMR sample was made and all the pure fractions were evaporated together to give compound 2 (1.37 g, 76 % yield). \(^1\)H-NMR (CDCl\(_3\) 300 MHz) \(\delta\): 7.22-7.13 (m 2H), 6.75-6.67 (m 1H), 6.65-6.58 (m 2H), 4.05 (bd 2H), 3.61 (bs 1H), 3.41 (m 1H), 2.91 (t J=13 Hz 2H), 2.02 (d J=13 Hz 2H), 1.50 (s 9H), 1.41-1.25 (m 3H). \(^13\)C-NMR (CDCl\(_3\) 75 MHz) \(\delta\): 154.8, 146.7, 129.4, 117.5, 113.4, 79.5, 40.4, 27.8, 32.3, 28.5

tert-butyl 4-[acryloyl(phenyl)amino]piperidine-1-carboxylate (3):
Compound 2 (1.37g, 5.0 mmol) was put on ice with little DCM. 1.75 ml (10 mmol) DiPEA was added as well as some more DCM (in total 30 ml). 0.815 ml (10 mmol) Acryloyl chloride was added and the reaction was left to react on melting ice. After 2 h the reaction was monitored with TLC (mobile phase Ethylacetate:Heptane 1:1) and found to be finished. The mixture was transferred to a separation funnel and washed with 3*30 ml water, 2*15 ml brine and 1*20 ml NaHCO\(_3\) (aq) Solvents were evaporated and an NMR sample was made. The NMR showed some extra peaks and extra purification was decided to be necessary. Purification with silica gel (mobile phase Ethylacetate:Heptane 1:1). Fractions were investigated with TLC (mobile phase Ethylacetate:Heptane 1:1) and an NMR sample was made on pure fractions, two almost pure fractions were added after NMR. The solvents were evaporated to give compound 3 (1.49 g, 90 % yield) \(^1\)H-NMR (CDCl\(_3\) 300 MHz) \(\delta\): 7.24-7.22 (m 3H), 6.92-6.89 (m 2H), 6.14 (dd 1H), 5.66-5.57 (m 1H), 5.28-5.24 (m 1H), 4.64-4.62 (m 1H), 3.96 (d J=12 Hz 2H), 2.62 (t J=13 Hz 2H), 1.64 (d J=12 Hz 2H), 1.20 (s 9H), 1.17-1.05 (m 2H). \(^13\)C-NMR (CDCl\(_3\)75 MHz) \(\delta\): 164.8, 154.1, 137.8, 130.1, 129.1, 128.7, 128.7, 128.2, 127.1, 79.1, 52.4, 30.1, 28.0
N-phenyl-N-(piperidin-4-yl)prop-2-enamide (4): Compound 3 (1.49 g, 4.5 mmol) was dissolved in 20 ml DCM and 5 ml TFA was added. The reaction was left to stir for 2 h. TLC to the check reaction were performed (mobile phase Ethylacetate:Heptane 1:1). No starting material was left and the solvent was evaporated. Product occurred as an oily mixture with TFA, the oil was evaporated with CHCl3 a few times to try to remove as much TFA as possible and give compound 4. Yield: 2.26 g oil, because the product was an oily mixture, full conversion, 100% yield, was assumed. The ratio of TFA:Compound 4 was calculated to 3:1. No NMR was made.

4-(2-{4-[acryloyl(phenyl)amino]piperidin-1-yl}ethyl)benzene-1,2-dimethoxy (5): TFA/Compound 4 mixture (300 mg, 0.524 mmol compound 4), 0.212 g (0.865 mmol) 4-(2-bromoethyl)-1,2-dimethoxybenzene and 0.769 g (2.36 mmol) Cs₂CO₃ was dissolved in 10 ml acetonitrile and left to react at 65 °C overnight. The reaction mixture was investigated with LC-MS to find out if the reaction was finished. Filtrated using vacuum filtration to remove the cesium carbonate, evaporation to remove solvent, product dissolved in DCM and purified with flash chromatography. Mobile phase ethyl acetate with 1 % triethylamine. For every 100 ml mobile phase that had been used 1 ml methanol was added to the next batch of mobile phase to have a better elution of the product. The pure fractions was pooled together and evaporated to give compound 5 (55 mg, 27 % yield). The product was analyzed with NMR. ¹H-NMR (CDCl₃ 300 MHz) δ: 7.41-7.35 (m 3H), 7.10-7.07 (m 2H), 6.77-6.74 (m 1H), 6.70-6.66 (m 2H), 6.32 (dd 1H), 5.85-5.76 (m 1H), 5.44 (dd 1H), 3.81 (s 6H), 3.00 (d J=12 Hz 2H), 2.69-2.64 (m 2H), 2.55-2.50 (m 2H), 2.21-2.14 (m 3H), 1.87-1.83 (m 2H), 1.53-1.45 (m 2H). ¹³C-NMR (CDCl₃ 75 MHz) δ: 165.3, 148.8, 147.3, 138.2, 132.9, 129.3, 128.4, 127.3, 120.5, 112.0, 111.3, 60.5, 55.9, 55.8, 53.0, 33.4, 30.4.

4-(2-{4-[acryloyl(phenyl)amino]piperidin-1-yl}ethyl)benzene-1,2-diol (6): Compound 5 (55 mg, 0.139 mmol) was dissolved in 4 ml DCM under nitrogen atmosphere over ice bath. 0.56 ml (0.56 mmol) BBr₃ 1 M solution was added and the reaction was left to stir over night under nitrogen atmosphere. The reaction was monitored with LC-MS, the reaction was regarded as finished and some methanol was added to quench the reaction. The solvent was evaporated and the residue was dissolved in methanol before purification with preparative LC. The pure fraction was evaporated and some mostly pure factions were also combined and evaporated to give compound 6. (27 mg, 53 % yield) NMR was made. ¹H-NMR (CD₂OD 300 MHz) δ: 7.08-7.04 (m 3H), 6.69-6.66 (m 1H), 6.60-6.59 (m 1H), 6.49-6.45 (m 1H), 6.28-6.21 (m 1H), 5.84-5.75 (m 1H), 5.48-5.44 (m 1H), 3.70-3.66 (m 1H), 3.07 (d J=11 Hz 3H), 2.59 (s 5H), 2.42-2.40 (m 1H), 2.33-2.26 (m 3H), 1.87-1.84 (m 2H), 1.58-1.46 (m 2H).

4-(2-{4-[acryloyl(phenyl)amino]piperidin-1-yl}ethyl)phenol (7): TFA/compound 4 mixture ( 50 mg, 0.093 mmol compound 4), 0.045 g (0.224 mmol) 4-(2-bromoethyl)phenol and 0.321 g (0.985 mmol) cesium carbonate was dissolved in 5 ml Acetonitrile and left to react at 65 °C overnight. The reaction was monitored with LC-MS. Cesium carbonate was removed with vacuum filtration and the solvent was evaporated. The residue was dissolved in methanol and purified with preparative LC. Pure fraction was freeze-dried to give compound 7 (3.5 mg, 11% yield). An NMR sample was made. ¹H-NMR (CD₂OD 300 MHz) δ: 7.53-7.48 (m 3H), 7.23-7.20 (m 2H), 7.01-6.99 (m 2H), 6.71-6.68 (m 2H), 6.28 (dd 1H), 5.90-5.84 (m 1H), 5.52 (dd 1H), 6.71-6.65 (m 1H), 3.22 (dd 2H), 2.71 (s 4H), 2.52-2.43 (m 2H), 2.00-1.93 (m 1H), 1.60-1.55 (m 2H). ¹³C-NMR (CD₂OD 75 MHz) δ: 167.5, 157.1, 139.1, 131.6, 130.7, 130.6, 130.3, 130.1, 128.3, 116.3, 111.4, 60.9, 53.7, 53.6, 32.5, 30.4
Resynthesis of Compound 5:
4-(2-bromoethyl)-1,2-dimethoxybenzene (0.107 g, 0.436 mmol), 0.642 g (1.97 mmol) cesium carbonate and 259 mg mixture of compound 4/TFA (0.453 mmol compound 4) was dissolved in 6 ml acetonitrile. After 2 h the reaction was monitored with LC-MS and considered to be finished. The cesium carbonate was filtered off with vacuum filtration and the solvent was evaporated. The residue was dissolved in methanol and filtered before purification with LC-prep. 6 fractions was pure and these were evaporated to give compound 5 (24 mg, 13 % yield). No NMR was made due to lack of time.

Resynthesis of Compound 6:
Compound 5 (resynthesis) (24 mg, 0.061 mmol) was dissolved in 5 ml dry DCM over ice under nitrogen atmosphere and 0.25 ml 1M (0.25 mmol) solution of BBr₃ was added. The reaction was left to react overnight. The next day the solvent was evaporated. Methanol was added to quench the reaction and evaporated before the residue was dissolved in a small amount of methanol before purification with LC-prep. Evaporation to remove solvent and make NMR. The NMR looked like the bromine had been added to the double bond of the acryl group. Pure fractions evaporated to give compound 8 (brominated) (15 mg, 55% yield). ¹H-NMR (CD₃OD 300 MHz) δ: 7.54-7.50 (m 3H), 7.26-7.25 (m 2H), 6.68-6.49 (m 3H), 4.79-4.70 (m 2H), 3.58-3.53 (m 2H), 3.25 (s 2H), 2.79-2.69 (m 5H), 2.57-3.53 (m 4H), 1.93 (s 2H), 1.64-1.56 (m 2H) No carbon NMR available.
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References


