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DET HELSEVITENSKAPELIGE FAKULTET
INSTITUTT FOR FARMASI

ALDOL CONDENSATION WITH 2,5-DIKETOPIPERAZINES

FAR-3901

Thesis for the degree Master of Pharmacy

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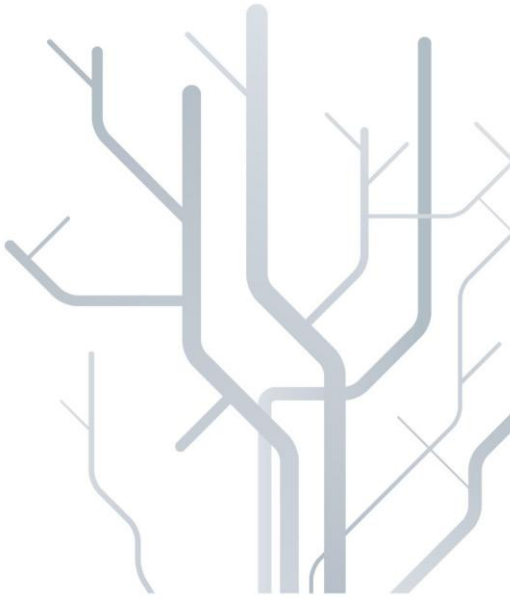

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God bless

May 2013

Murwanashyaka Juvenal

Symbols and abbreviations

$(Ac)_2$DKP - 1,4-diacetylpiperazine-2,5-dione

DBN- 1,5-Diazabicyclo[4.3.0]non-5-ene

DBU - 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCM – Dichloromethane

DIPEA - N,N-Diisopropylethylamine

DKPs – 2,5-diketopiperazines

DMA – Dimethylacetamide

DMAP – 4-Dimethylaminopyridine

DMF – Dimethylformamide

DMSO – Dimethyl sulfoxide

GC-MS - Gas chromatography–mass spectrometry

HMDS - Bis(trimethylsilyl)amine

HRMS – High resolution mass spectrometry

IR spectroscopy - Infrared spectroscopy

LiHMDS – Lithium bis(trimethylsilyl)amide

NMR - Nuclear magnetic resonance

TAPC - 4,4′-Cyclohexylidenebis[*N*,*N*-bis(4-methylphenyl)benzenamine]

TBD - 1,5,7-Triazabicyclo[4.4.0]dec-5-ene

TEA – Triethylamine

TLC - Thin layer chromatography

TMG – 1,1,3,3-Tetramethylguanidine

$Zn(tmp)_2$) - Bis(2,2,6,6-tetramethylpiperidinyl)zinc

1. Aim of the project

2,5-diketopiperazines (DKPs) are naturally occurring cyclopeptides obtained by the condensation of two amino acids. They are found alone or among other natural products produced by fungi, bacteria, the plant kingdom and mammals[1]. This family of molecules is attractive in drug discovery as they are stable to proteolysis while capable of mimicking linear peptides, and they can bind to a wide range of receptors due to the nature of the diketopiperazine ring structure[1]. Despite its simplicity, the ring can have up to four hydrogen donating and accepting groups. Diversity can be introduced at up to six positions and stereochemistry at up to four positions, giving an infinite possibility of derivatives (figure 1). So far, the only product on the pharmaceutical marked containing a 2,5-diketopiperazine core is tadalafil (cialis, adcira), licensed for the treatment of erectile dysfunction, prostate hypertrophy and pulmonary hypertension[2].

Barettin (figure 1) is a 2,5-diketopiperazine isolated from the marine sponge *Geodia baretti*[3]. Much of the existing work on this compound has been the exploration of its antifouling properties, and the attempt to synthesize derivatives[4]. But in recent time, biochemical assays performed at MabCent-SFI have shown this compound to also have anti-oxidant and anti-inflammatory properties (Karianne F. Lind, *private communication*). Due to possible medicinal and commercial applications, it is the main focus of our group to synthesize a library of barettin derivatives for bioactivity screening.

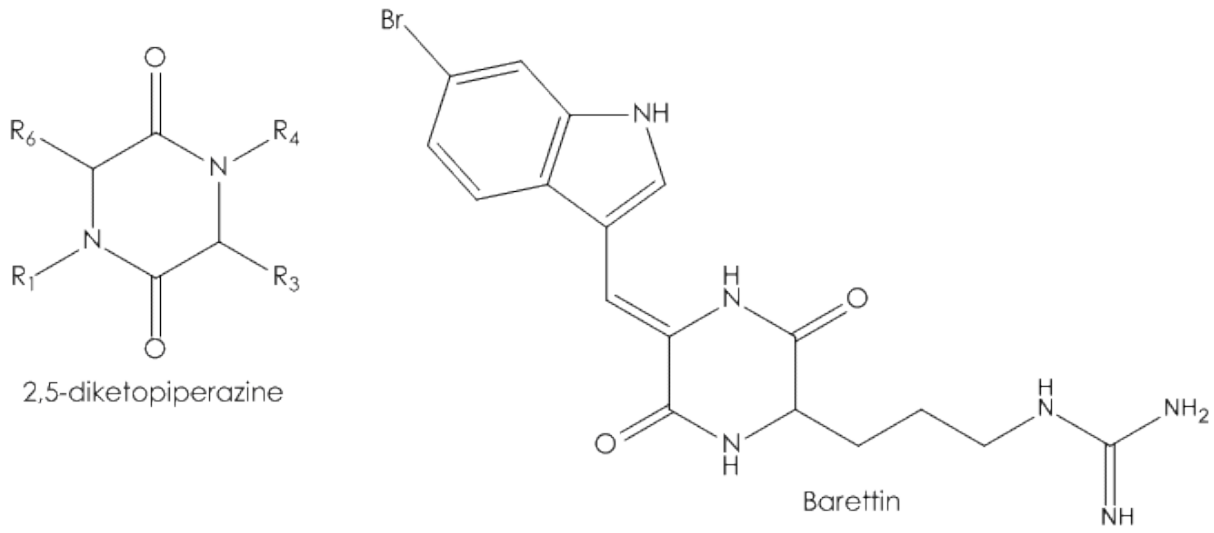
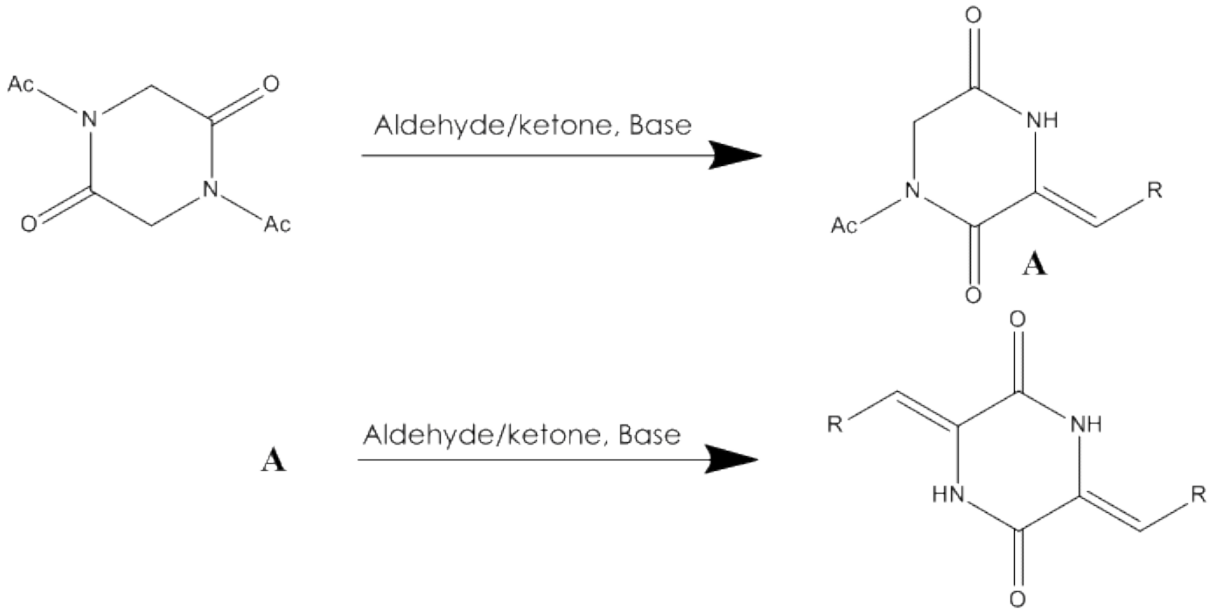


Figure1: The structure of 2,5-diketopiperazines and Barettin.

One of the possible ways for obtaining barettin derivatives is by aldol condensation of aldehydes or ketones with a cheap 2,5-diketopiperazine starting material[5]. This approach is attractive because of its simplicity and the fact that a cheap starting material, glycine anhydride, is commercially available. This method has been limited to aldehydes only[6].

In this method, basic conditions are used to induce the condensation of 1,4-diacetylpiperazine-2,5-dione ($(Ac)_2$DKP) with both aliphatic and aromatic aldehydes (scheme 1). Only three bases have been tested for this reaction; triethylamine (TEA)[6-7], potassium tert-butoxide[5] and cesium carbonate[8]. The aim of this project, as summarized below, is to improve on this method by mapping the effect of various bases on the reaction of $(Ac)_2$DKP with a standard aldehydes, benzaldehyde, and then apply the best conditions to additional aldehydes and ketones.

- Map the effect of various bases on the monocondensation of $(Ac)_2$DKP with benzaldehyde (section 3.2.1).
- Apply the best bases to monocondensation with pentanal and indole-3-carboxaldehyde (section 3.2.2).
- Apply the best conditions to monocondensation with ketones (section 3.3).



Scheme 1: The aim of this project is to improve on the method for the synthesis of A.

2 Literature review on 2,5-diketopiperazines

2.1 Bioactivity of a few 2,5-diketopiperazines

There are numerous natural products and compounds available from combinatorial libraries containing the 2,5-diketopiperazine core template which by different mechanisms show diverse and interesting biologic properties. The literature regarding the medicinal chemistry of 2,5-diketopiperazines is vast, and only a few compounds will be briefly presented. For a comprehensive review on the subject, the reader should refer to the review article[1].

Tadalafil

Tadalafil (figure 2) is the only 2,5-diketopiperazine on the pharmaceutical marked. It is a PDE5 inhibitor and is licensed for the treatment of erectile dysfunction and pulmonary hypertension under the brand names Cialis and Adcira[2]. Surprisingly, tadalafil (IC_{50} = 5 nM) was the product of structure activity relationship studies, and the lead compound, ethyl β-carboline-3-carboxylate (IC_{50} = 800 nM),

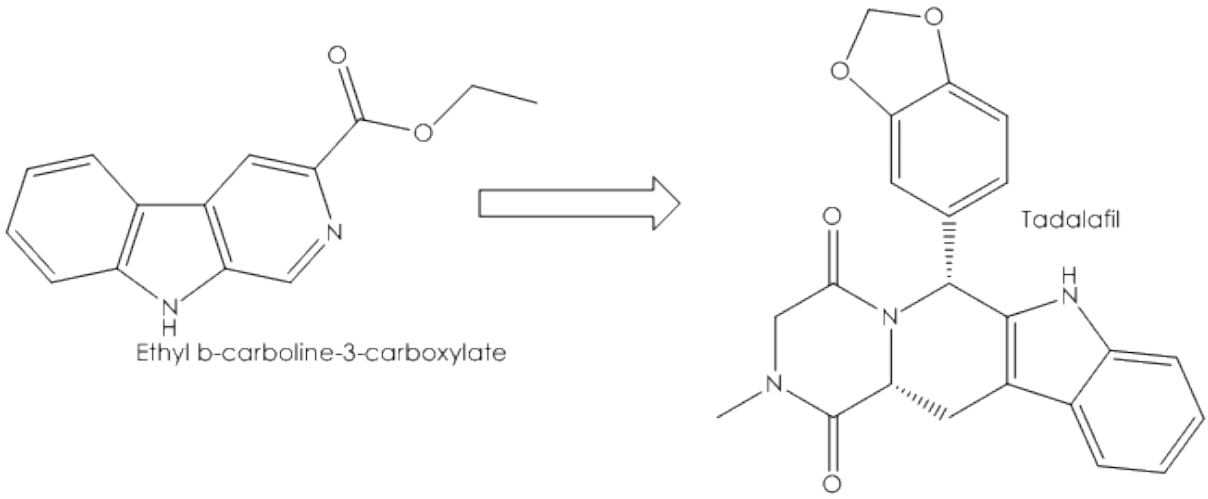
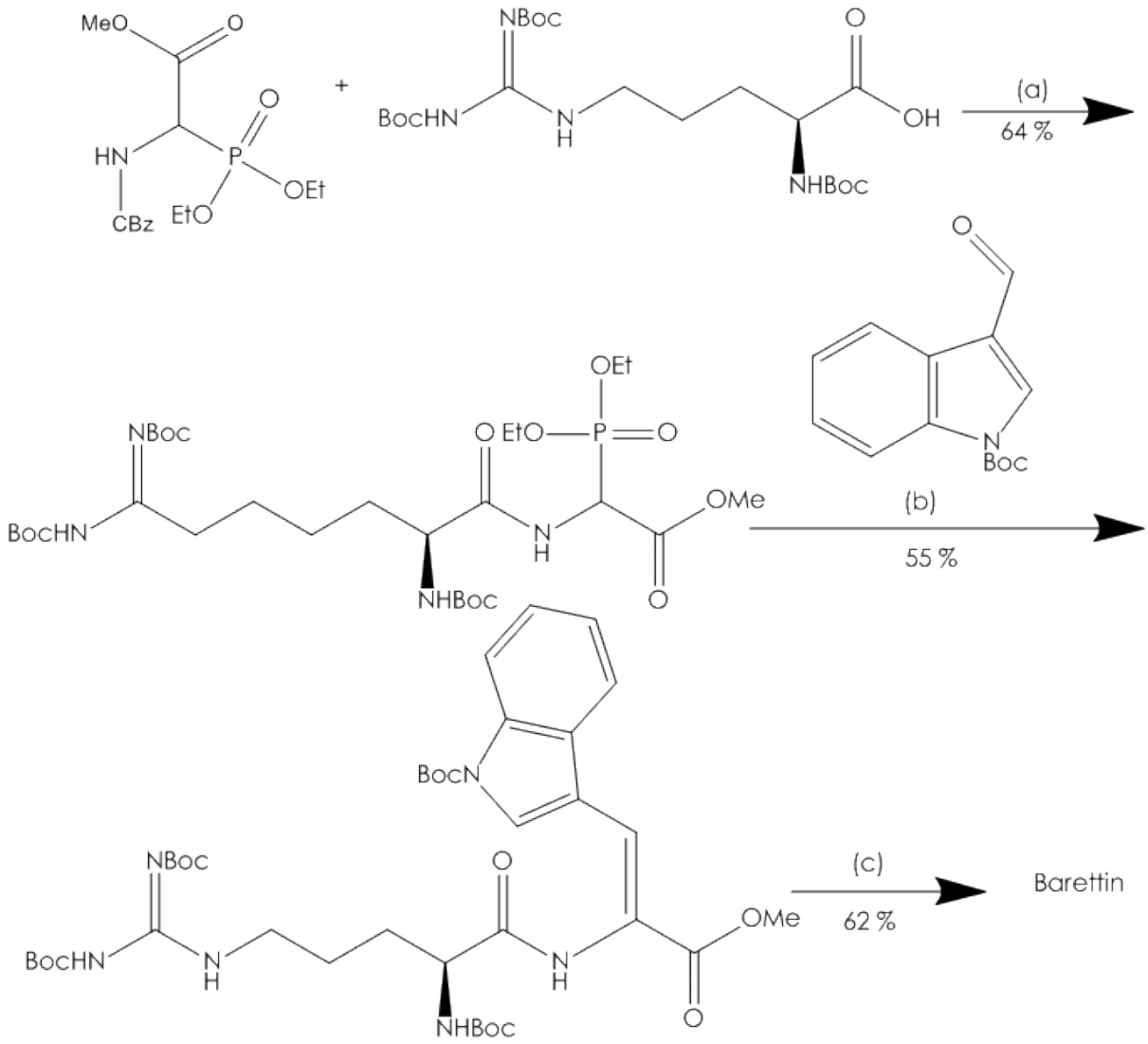


Figure 2: The structure of Tadalafil and ethyl β-carboline-3-carboxylate

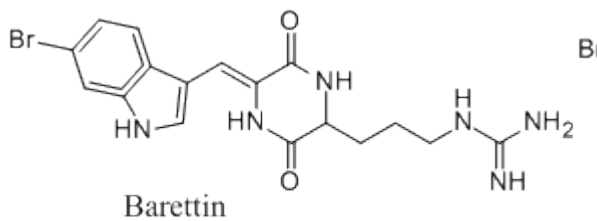
Barettin

Barettin and its saturated derivative dihydrobarettin, were first isolated by a Swedish group in 1986[3] from the cold water sponge *Geodia Baretti*, and shown to have antifouling properties[9]. At the time of isolation, in 1986, using of existing analytical techniques, it was deduced that barettin had a 6-bromoindole moiety bound to a 2,5-diketopiperazine core. It was in 2002 that the true structure of barettin was proposed[10] and later confirmed by total synthesis (scheme 2)[11].

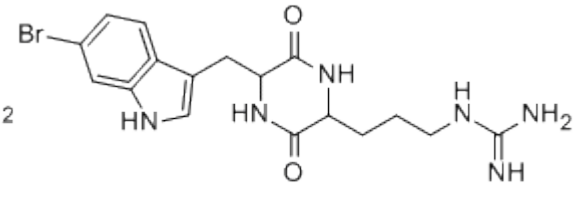


Scheme 2: (a) (i) H_2, Pd/C, EtOH, 4.5 h. (ii) **6**, EDCI, HOBt, DIEA, CH_2Cl_2, rt 18 h. (b) DBU, CH_2Cl_2, −78° to rt 20 h. (c) (i) TFA, CH_2Cl_2, rt 12 h. (ii) 0.1 M HOAc/1-BuOH, NMM, reflux 6 h.

Geodia Baretti is an organism with poor motility and ability to escape predators and fouling organisms. Despite this, the organism has a clean and undamaged surface, which is believed to be due to the release of the two antifouling indole alkaloids barettin and dihydrobarettin in the ambient water[9]. Surprisingly, the saturated derivative (figure 3) alone has no inhibitory properties, but together, the two compounds act in synergy to lower larval settlement[12] . It is believed that Barettin works in reversible manner, and possibly by interactions with serotonin receptors[13].



Barettin



Dihydrobarettin

Figure 3: The structure of barettin and its saturated derivative dihydrobarettin

Recent work on barettin by Karianne F. Lind and colleagues at MabCent-SFI (*private communication*), shows that barettin has both antioxidant and anti-inflammatory properties in biochemical assays, but show no activity in intracellular antioxidant assays. The lack of intracellular activity is assumed to be due to poor uptake. In our group, this problem is approached by substituting the guanidine group with less polar bioisosteres, and by substituting the indole group with other bulky hydrophobic groups as shown in figure 4.

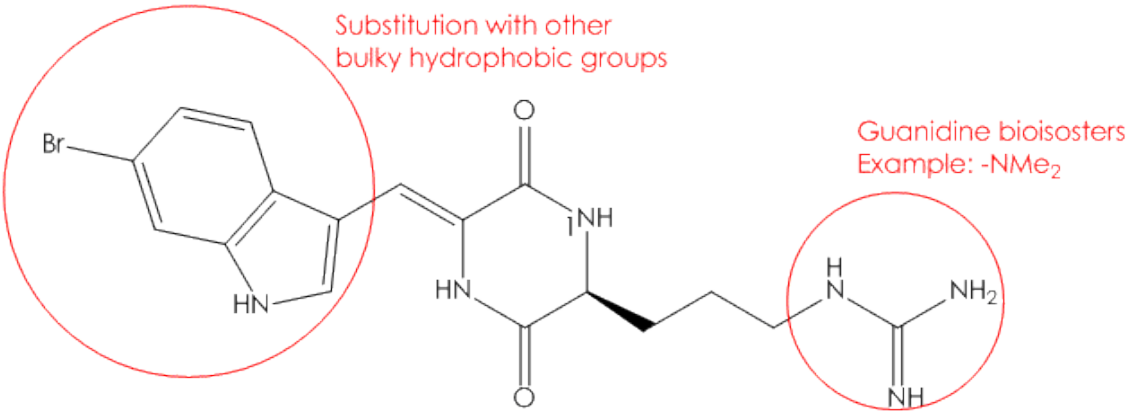


Figure 4: The approach of our group towards barettin derivatives with antioxidant and anti-inflammatory properties.

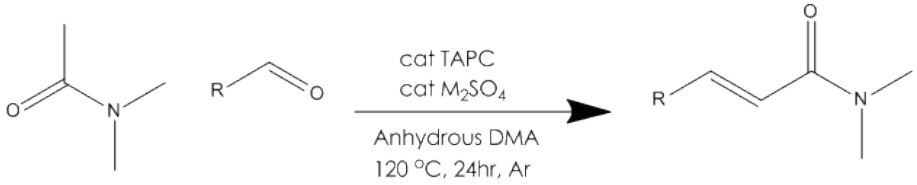
2.2 Present work on aldol condensation with amides

Aldol chemistry is a well established field and there has been published extensive work on the subject and related reactions such as knoevenagel condensation, Perkin reaction, Claisen condensation etc. On the other hand, aldol type reactions with amides are a less explored, with the few existing articles being limited only to reactions with aldehydes. Catalysis of this reaction is frequently hampered and is of significant challenge due to the high thermodynamic stability and the low acidity of the α-CH of amides.

To the best of my knowledge; at present day, it seems that there is only one published article[14], where direct aldol reaction of aromatic aldehydes and in situ generated amide enolates is reported. Several simple secondary amides are deprotonated with Bis(2,2,6,6-tetramethylpiperidinyl)zinc($Zn(tmp)_2$) and react smoothly with aromatic aldehydes in good yields.

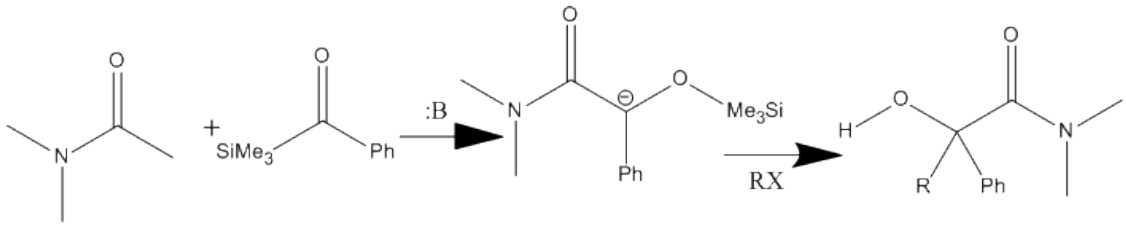
One can assume that one challenge with a conventional aldol approach is the reversibility of the aldol reaction due to the unstabilized aldol product. One group solved this problem[15] by using N-bocamide where the boc moiety rearranges to stabilize the resulting hydroxyl anion forcing the reaction towards product formation. This approach though, is mostly limited to aromatic aldehydes and gives modest yields for aliphatic aldehydes.

In a recently published article[16], the authors pursue a more radical approach of utilizing the combined properties of a 4,4'-Cyclohexylidenebis[*N*,*N*-bis(4-methylphenyl)benzenamine] (TAPC) based phosphorous/SO4- catalysis in which the two species act synergically to accelerate the reaction (scheme 3). Although these reaction conditions are new and promising, they are so far only limited to aromatic aldehydes and leave not much hope for generalization to all aldehydes and further application to ketones.



Scheme 3: Condition for reacting dimethylacetamide with aldehydes, R being aromatic or aliphatic

A promising approach to derivatization of amides comes not from reaction with aldehydes or ketones, but with acylsilanes[17]. As illustrated in scheme 4, this approach offers a high degree of substitution due to the possible diversity of the reactants ranging from alkyl halides to aldehydes, ketones and similar. This is the only method that assimilates ketones, making it of possible interest for our project if the planned approach is to fail.



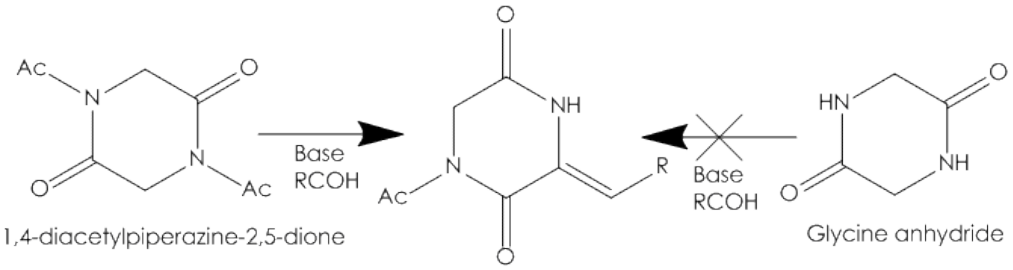
Scheme 4 : illustrating derivatization of amides with acylsilanes. RX can be allyl/alkyl halide, benzaldehyde, acetone or similar.

2.3 Present work on aldol condensation with 2,5-diketopiperazines

Aldol condensation as a method of obtaining 3,6-substituted 2,5-diketopiperazine is attractive due to its simplicity and the fact that a possible starting material, glycine anhydride, is commercially available and reasonably cheap. This approach is cost-effective as it requires few synthesis steps; in addition to that, this method has been well studied as will become clear later.

2.3.1 Experimental conditions and limitations

It attractive to think that one can do a direct aldol condensation with glycine anhydride. This fails, most likely because the N hydrogens are more acidic than the a-carbon hydrogens, which hinders enolate formation. For a successful aldol reaction, it necessary to use a activated starting material as first illustrated in 1967[18] where perkin reaction type conditions were used to condense 3-isopropylidene-2,5-piperazinedione with benzaldehyde. After these results, the general starting material came to be 1,4-diacetylpiperazine-2,5-dione ($(Ac)_2DKP$) (scheme 5)[6-7].

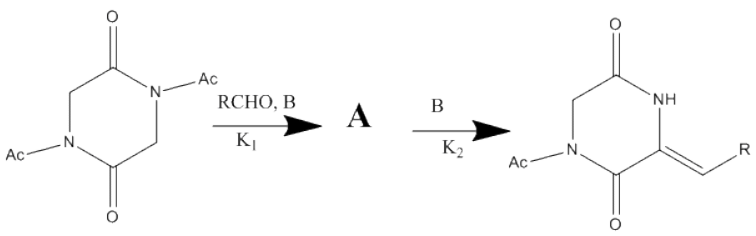


Scheme 5: Shows that only the activated $(Ac)_2DKP$ reacts and not glycine anhydride.

The original pioneers of this field, Gallina and Liberatori, in 1973[6-7] illustared that it is possible to condense $(Ac)_2DKP$ with aromatic aldehydes in the presence of triethylamine (TEA) and dimethylformamide (DMF) as a solvent to obtain 1-acetyl-3-arylidenepiperazine-2,5-diones in good yield. Unfortunately, with these reaction conditions, they failed to induce a reaction with aliphatic aldehydes even at elevated temperatures. It is only when they changed the base to the stronger and more bulky potassium tert-butoxide that both aromatic and aliphatic aldehydes reacted smoothly, though application to ketones or double condensation with aliphatic aldehydes failed[6].

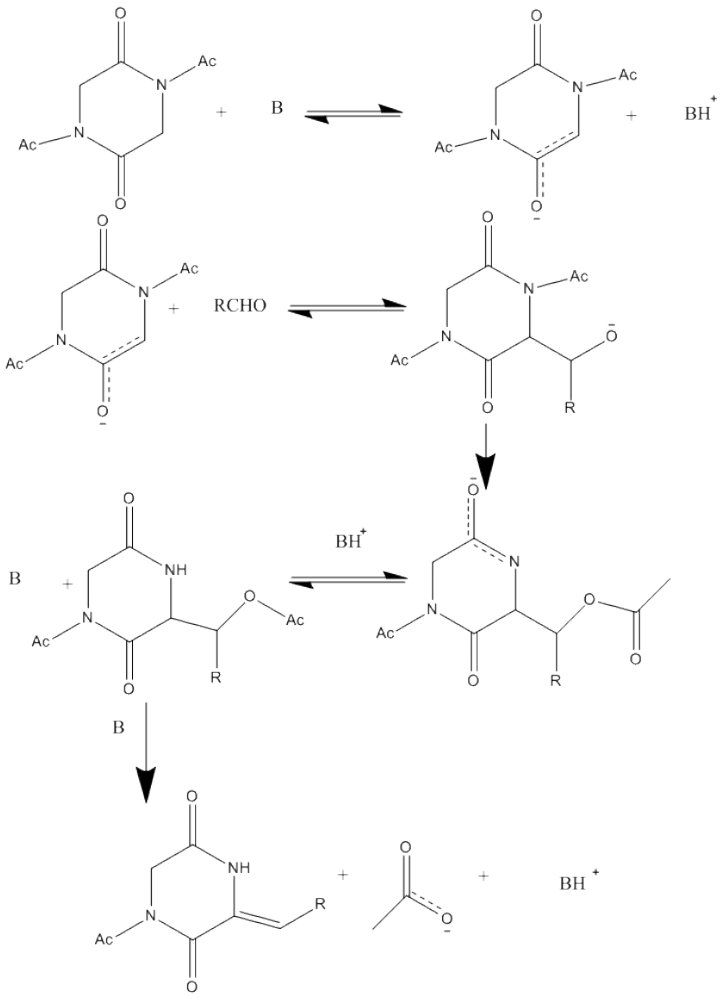
In 2004[5], it was shown that by simply changing the reaction solvent to dichloromethane (DCM), work-up is simplified and the yield improves in monocondensation with unsubstituted and highly oxygenated benzaldehydes, as well as other electron rich aromatic. The most impressive improvement in this field has been changing the base to cesium carbonate[8], as this method offers simpler reaction setup and workup, although the reaction time is longer and the method has so far only been reported to work for aromatic aldehydes.

2.3.2 Reaction Mechanism and stereochemistry



Scheme 6: Illustrating the general reaction through an intermediate A

The finding by Shemyakin[19] that the hydrogenolysis of $(Ac)_2$DKP gives bis O-acetylserine anhydride set forward the idea that this type of reaction proceeds according to scheme 6, where the intermediate **A** contains an acetate function. This allowed Gallina and Liberatori[6] to hypothesize that this type of reaction follows a mechanism shown in scheme 7. N-acetylating glycine anhydride enhances the acidity of the vicinal methylene protons and allows for a kinetically favored intramolecular acyl transfer to which the success of the reaction should be attributed.

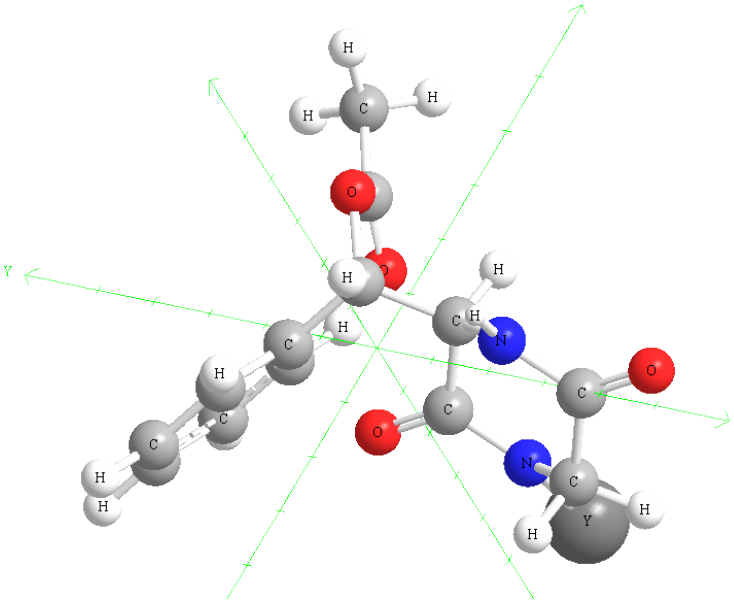


Scheme 7: Illustration of the reaction mechanism in aldol condensation of $(Ac)_2$DKP with aldehydes

By going back to scheme 3, we can see that the intermediate A can have two possible diastereomers, all with equal probability for formation. For elimination to occur, the transition state must follow a coplanar alignment of the carbon orbital of the leaving group and the departing hydrogen. For parallel orbital alignment to occur, the molecule can adopt an anti-coplanar or syn-coplanar conformation with 180 or 0^o separation, respectively, of the leaving group and the departing hydrogen.

Normally, we would expect to find a 50/50 distribution of E and Z product, since there would be two elimination transition states available. What is actually seen is the exclusive preference for the Z geometry for reaction with aromatic aldehydes.

If we can accept that this reaction follows the same mechanism as the Perkin reaction, then we can use Zimmerman`s conclusion[20] to explain this observation. We know that the transition state normally favors an anti-coplanar conformation because this leads a more stable transition state due to less steric hindrance. In this case though, we have to remember that the amide carbonyl is involved in stabilization of the electron pair and accompanying negative charge in the transition state. The effect of steric interaction with a cis group with the amide carbonyl will tend to force the carbonyl group involved in delocalization out of the plane of the incipient double bond with consequent poor orbital overlap. Zimmerman argues that energy increase of the transition state as a result of complete delocalization of the electron pair on carbon is much higher than the energy increase from the normal unfavorable steric interaction. Since the driving force is overlap control, both diastereomers in figure 5 will lead to the same product.



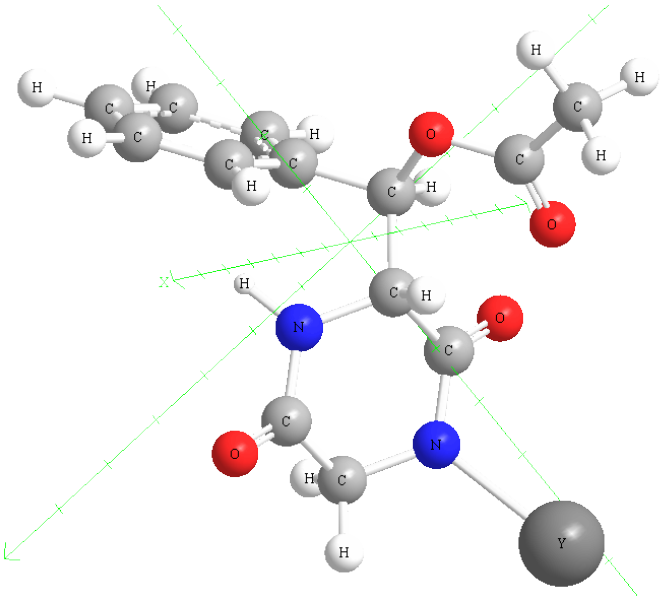
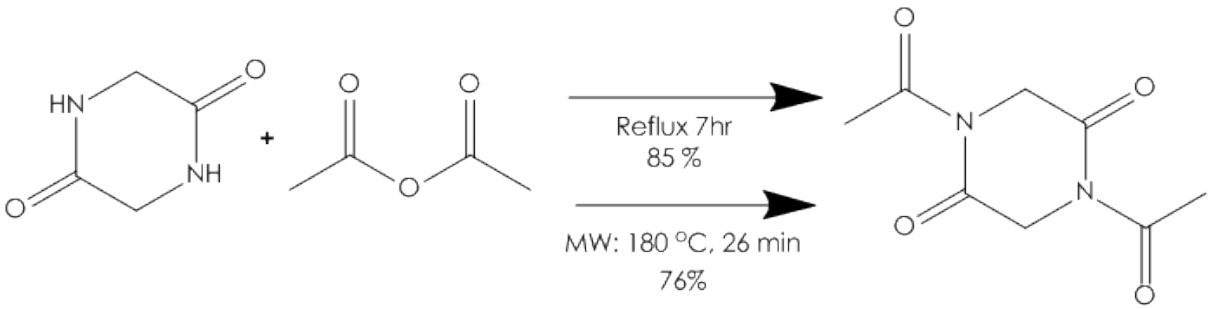


Figure 5: Two possible intermediates from reaction with benzaldehyde. The upper drawing is S/R and the other R/R. Both have syn-coplanar conformation, but only R/R (lower drawing) has best orbital overlap. S/R must eliminate with an anti-coplanar conformation.

3 RESULTS AND DISCUSSION

3.1 Synthesis of 1,4-diacetylpiperazine-2,5-dione

In order to begin our studies, it was necessary to have a method for obtaining the starting material, $(Ac)_2$DKP, pure and in large quantities. To the best of our knowledge, there are two published methods for synthesizing this compound (scheme 8). In one of the methods [21], glycine anhydride and excess acetic anhydride are refluxed neat for seven hours, giving brown solid in 85 % yield after work-up. In the other method[22], microwave heating is used to reduce the reaction time to less than a half hour, giving 76 % of white solid after column chromatography. The first method is more practical but is hampered by the brown impurity that can`t even be removed with activated charcoal. Due to the importance of having a pure starting material as the effect of the impurity is unpredictable on later reactions, effort was put in improving on the first method.



Scheme 8: Illustrates the two published methods for synthesizing of $(Ac)_2$DKP.

From a theoretical point of view, there are three possible routes to synthesizing the desired compound using acetic anhydride as the acetylating agent. The reaction can be run under neutral, basic or acidic conditions.

The use of neutral conditions has been explored as mentioned earlier. The use of basic conditions have been reported for a similar compound, the cyclic dipeptide c(Gly-L-Ala)[23]. The method uses TEA in acetonitrile as the base and 4-dimethylaminopyridine (DMAP) as the catalyst. The reported method was attractive because the reaction is run at room temperature and gives good yields (85 %).

When these conditions were applied to the Acetylation of glycine anhydride, brown discoloration of the product was observed when the reaction was conducted at room and reflux temperatures. In addition, the reaction was slow, taking more than two days for completion at reflux temperatures. Using the same work-up as with c(Gly-L-Ala)[23] gave poor yields (11 %) due to the high solubility of $(Ac)_2$DKP in water, though white solid was obtained.

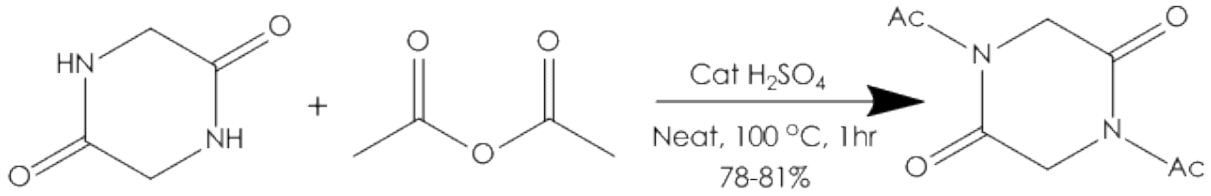
Several methods exists for acylation of amides with anhydrides promoted by acids[24], but no published method that applies this approach to the synthesis of $(Ac)_2$DKP has been found. Acid catalyzed Acetylation was attractive because with an acid catalysis, mild conditions may be used which in turn may reduce the brown discoloration.

Sulfuric acid promoted acylation of glycine anhydride with acetic anhydride failed to proceed below 100 ^{0}C with microwave or conventional heating. The optimal reaction temperature proved to be100 ^{0}C with both microwave and conventional heating, reducing the reaction time to 1 hour. Brown discoloration still occurred.

It was assumed that the brown discoloration were the result of dispersed particles as the result of polymerization of the starting materials or product. Attempts to remove the dispersed particles by filtration of the crude reaction mixture through celite failed. When the reaction mixture was diluted with ethyl acetate prior to filtration, it was observed that brown materials were retained by celite, giving light yellow particles after solvent evaporation.

By testing various recrystallization solvents, 78-81% yield (table 1) was obtained upon recrystallization from excess isopropanol instead of ethyl acetate –ether as in the original paper[21b]. It was crucial for a successful recrystallization that the solvent was used in large quantities so that the recrystallization process started with colorless and not yellow crystals.

Table 1: % yields by using different synthesis strategies towards $(Ac)_2$DKP



Entry	Conditions	Solvent	T (°C)	Time (hr)	Yield (%)	Purity
1	Neutral	Neat	120	7	85	Brown
2	Neutral (MW)	Neat	180	0,5	76	Colorless
3	TEA-DMAP	CH_3CN	Reflux	48	11	White
4	Cat H_2SO_4	Neat	100	1	78-81	Colorless

Entry 1[23] and 2[22] are published methods

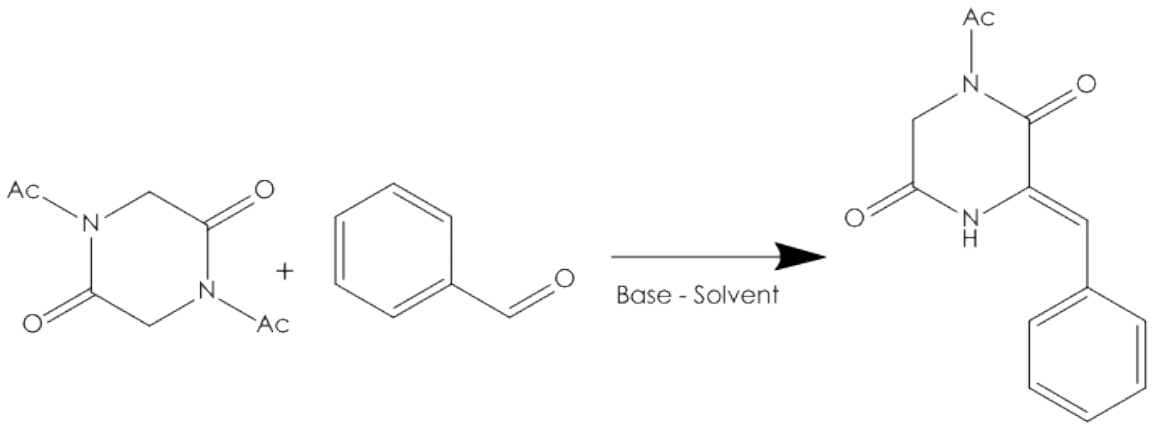
3.2 Aldol condensation of 1,4-diacetylpiperaazine-2,5-dione with aldehydes in basic media

3.2.1 Reactions with benzaldehyde

As mentioned in the literature section, three bases have previously been explored for this type of reaction, triethylamine[6-7], potassium tert-butoxide[5] and cesium carbonate[8]. TEA being a weak base requires high temperatures. Cesium carbonate is to prefer for practical reasons, although it has only been tested for reactions with aromatic aldehydes and the reaction must be run in degassed DMF and under argon to avoid possible oxidation of the activated a-carbon in the presence of cesium carbonate[25].

In this first part of the project, it was chosen to study the aldol condensation of $(Ac)_2$DKP with benzaldehyde in the presence of various bases to determine the optimal base for this type of reaction, and apply it to reaction with other aldehydes. It was chosen to mainly focus on the reaction outcome in response to increasing basicity. Going back to the reaction mechanism(section 2.3.2), it is important to notice that one of the products is acetic acid, and for this reason, any base stronger than the conjugate base of acetic acid cannot be used in catalytic amount. However, nucleophilic bases cannot be used due to the resulting cleavage of the N-acetate groups. Our results are summarized in table 2. Only one isomer is observed.

Table2: Results from reactions of $(Ac)_2$DKP with benzaldehyde in the presence of various bases



Entry	Base	pKa	Solvent	T (°C)	time (hr)	% Yield
1	NaOAc		AcOH DMF	RT RT	24 24	NR NR
2	$MgBr_2$*Et_2O/ TEA		DCM	RT	24	Nd
3	K_3PO_4		DMF CH_3CN	RT RT	24 24	66 26
4	Cs_2CO_3		DMF	RT	24 (2)	83 (76)
5	KO^{t}BU-^{t}BuOH		DCM	RT 0	4(4) 4	37 (99) 67
6	DBU	24.33[a]	DCM	RT	4	78
7	DBN	23.79[a]	DCM	RT	4	88
8	TMG	23.3[a]	DCM	0	4	Nd
9	TBD	25.96[a]	DCM	0	4	Nd
10	LiHMDS	30[b]	THF	-78	4	Nd

Definition: RT: Room temperature; NR: No reaction; Nd: not determined;
a: pKa in acetonitrile[26]; b: pka in dimethyl sulfoxide
The figures In parentheses are those used/obtained in the published methods.

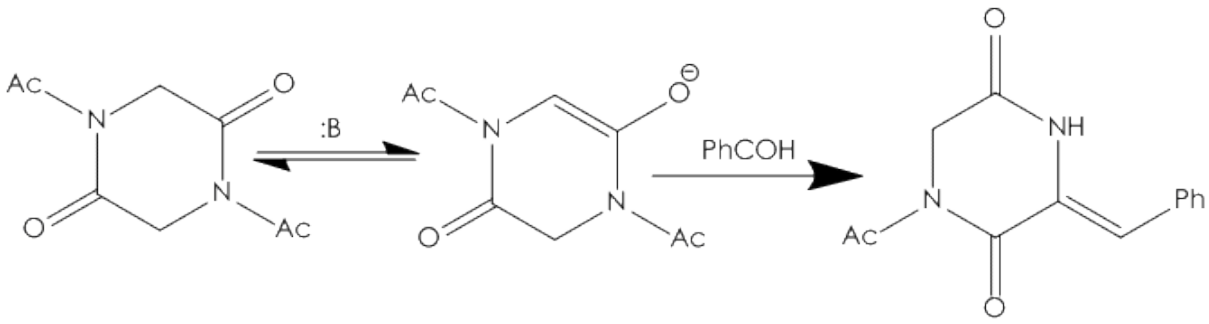
Cesium carbonate, Tribasic potassium phosphate , Sodium acetate

Since good results have been reported for cesium carbonate in condensation with aromatic aldehydes (table 2, entry 4), this reaction was first verified, and gave a little higher yield (83 %, table 2, entry 4) than reported (76 %, table 2, entry 4).

After this, two other inorganic bases with different basicity, sodium acetate and tribasic patasium phosphate, were tested. Sodium acetate has been shown to very efficiently catalyze the Claisen-Schmidt reaction[27], but high reaction temperatures are required, while potassium phosphate catalyzes the nitroaldol reaction very well at room temperature[28].

Sodium acetate in glacial acetic or DMF fails to induce the reaction of (Ac)2DKP and benzaldehyde at room temperature. Potassium phosphate on the other hand gave good results (66 %, table 2, entry 3) when the reaction was run in DMF but gave only modest results (26%, table 2, entry 3) in acetonitrile. The reaction with potassium

phosphate was slower than that of cesium carbonate, and higher than reported yield (table 2) may have been obtained if the reaction had run for longer time (two or three days). Weak bases like sodium acetate, cesium carbonate and potassium phosphate do not deprotonate $(Ac)_2$DKP irreversibly but leads instead to a reversible equilibrium. Since the rate of this reaction must depend on the concentration of the enolate anion present in the reaction mixture at any time, the reactivity must in turn depend on the basicity of the base used, with sodium acetate giving the poorest results and cesium carbonate being just perfect.



Scheme 5: Illustration of a possible reaction path, reactivity increases in the order NaOAc << K3PO4 < Cs2CO3.

Magnesium bromide ethyl etherate – Triethylamine

From the original experiments of Gallina and Liberatori, we know that heating is required when TEA is used as the base. A promising article[29] showed that it is possible to combine TEA with the lewis acid magnesium bromide ethyl etherate , and this system efficiently catalyzes the condensation of N-methylpiperidin-4-one with aromatic aldehydes at room temperature. One can assume that these reactions proceeds to the initial formation of a magnesium enolate intermediate, as most aldol reactions mediated by magnesium ions follow this mechanism[30].

When these conditions were applied to the reaction of $(Ac)_2$DKP and benzaldehyde (table 2, entry 2), it proceeded slower than the reaction with cesium carbonate. Both starting materials could be detected on GC-MS after 24 hours. It proved difficult to determine the yield from this reaction, as the crude and recrystallized sample failed to give good interpretable ^{1}HNMR spectra. The reason for the bad NMR spectra obtained may be attributed to residual metal ions, but these should have been removed during the aqueous work-up. The crude yield was determined to be 76% and much work was not put on purifying this sample as it was obvious that, although initially promising, magnesium bromide ethyl etherate – TEA was not superior to cesium carbonate.

Potassium tert-butoxide, DBU, DBN, TMG, TBD (figure 6)

Given the trend shown in scheme 5, one would expect stronger bases to give higher yields as they increase the enolate concentration present in the reaction mixture at any time. Previous works have indeed shown that using potassium tert-butoxide dissolved in tert-butanol gives very good yields, 99%[5]. Application of this method

gave much lower than expected yield (37 %, table 2, entry 5). The yield was improved (67 %, table 2, entry 5) by running the reaction at 0 ^{0}C.This indicated that perhaps the starting material or product may be unstable in the presence of strong bases.

When the strong guanidine based bases 1,1,3,3-Tetramethylguanidine (TMG) and 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD) were tested, much stronger instability was observed, as very much benzaldehyde relative to the product could be seen both on GC-MS and ^{1}HNMR even when the reactions were conducted at 0 ^{o}C. For this reason, purification of the crude product from reaction with these two bases was not pursued.

The amidine based bases 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (78 %, table 2, entry 6) and 1,5-Diazabicyclo[4.3.0]non-5-ene (DBN) (88 %, table 2, entry 7), gave good results even when the reactions were conducted at room temperature. When the pKa (table 2) values of the amidine and guanidine bases are compared, we see that the instability of $(Ac)_2$DKP is not proportional to the basicity.

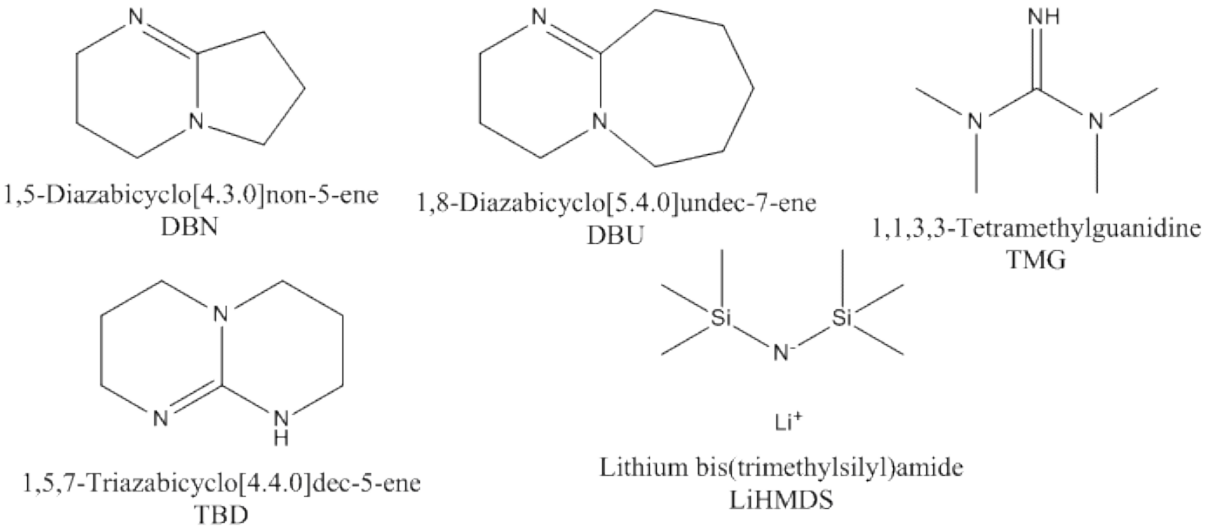


Figure 6: Structure of the various superbases

Lithium bis(trimethylsilyl)amide (figure 6)

It was desirable to test lithium bis(trimethylsilyl)amide (LiHMDS), as reactions with this base are run at very low temperature and it different from its organic counterparts. ^{1}HNMR spectra from the crude product of this reaction could not be interpreted due to the presence of multiple signals. From GC-MS it could be seen that the sample was composed of multiple products with different retention times but same m/z value (figure 7). They have different mass spectrum, indicating that they are different. The other strong bases like potassium tert-butoxide and TBD did not show any additional products on GC-MS.

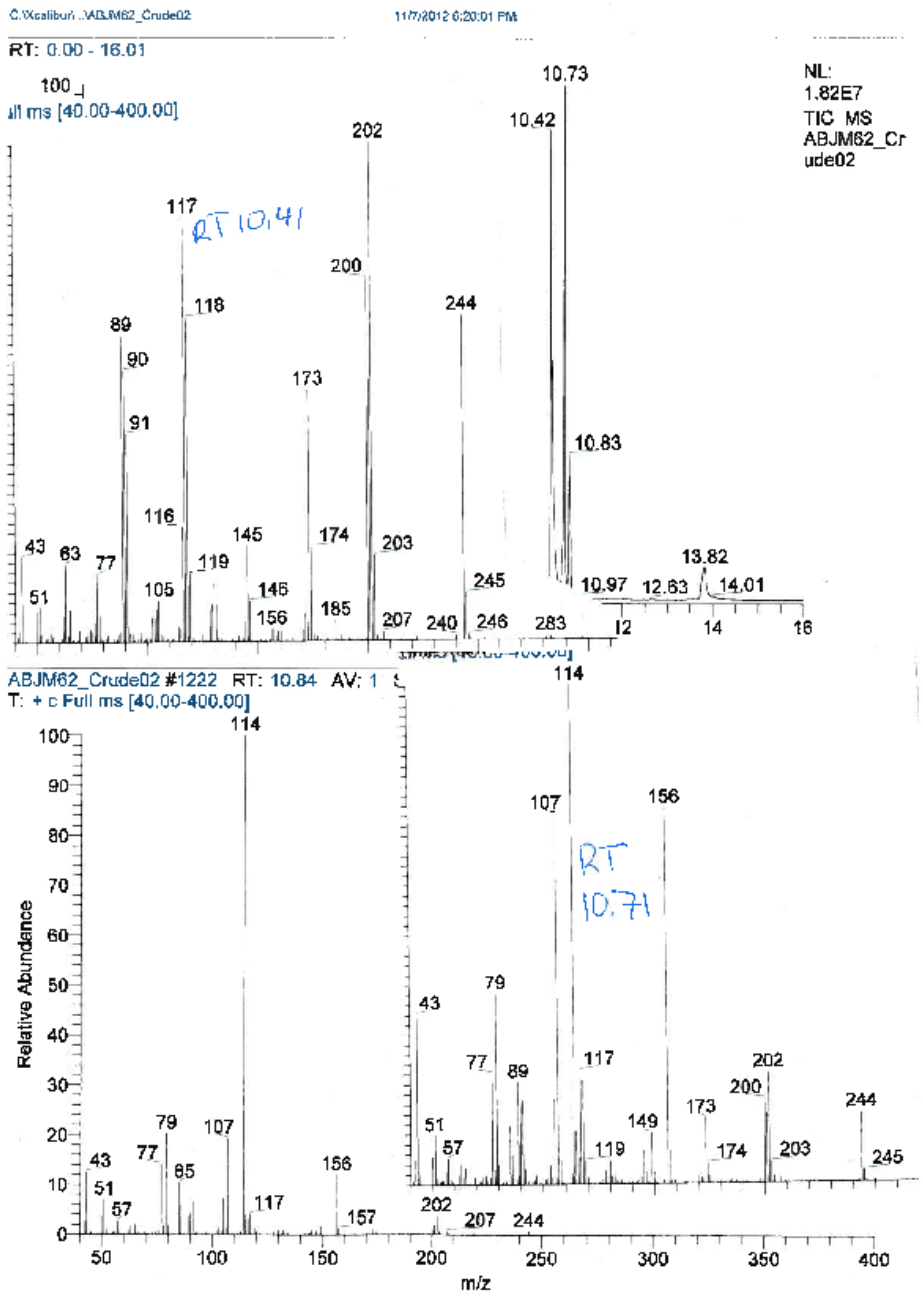
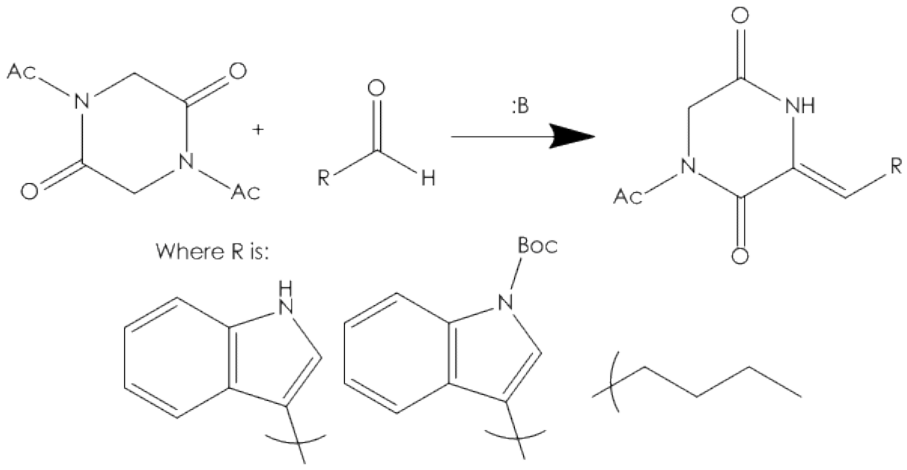


Figure 7: Shows that all three products with RT 10.83, 10.73, 10.42 have m/z 244.

3.2.2 Reactions with indole-3-carboxaldehyde and pentanal

As can seen in table 2, cesium carbonate, potassium tert-butoxide, DBN and DBU are the bases that give the best results, and these are the bases that were applied to reactions with indole-3-carboxaldehyde and pentanal as summarized in table 3. Again just one isomer is seen, expect for reaction with indole-3-carboxaldehyde where it is difficult to determine.

Table 3: Results from reactions of $(Ac)_2$DKP with various aldehydes in the presence of various bases



Reaction with		Indole-3-carboxaldehyde	N-Boc-indole-3-carboxaldehyde	Pentanal
Entry	Base	Yield (%)	Yield (%)	Yield (%)
1	Cs_2CO_3	45	36	NR
2	KO^tBU	NR	23	31
3	DBN	NR	43 (84)	24
4	DBU	NR	45 (89)	57(80)

All reactions were conducted at room temperature in dry DCM, but Cs2CO3 was run in DMF
Definition: NR: No desired reaction occured
for pentanal: Yield in parentheses is when 2 eqv pentanal is used
for boc-indole-3-carboxaldehyde: Yields are after recrystallization from toluene, and yields in parentheses are of crude products.

Indole-3-carboxaldehyde

To the best of our knowledge, there is no published article that reports the direct condensation of $(Ac)_2$DKP and indole-3-carboxaldehyde to yield the product dipodazine. In the reported method for the synthesis of dipodazine[31], $(Ac)_2$DKP is reacted with the N-phenylsulfonyl protected indole-3-carboxaldehyde (Cs2CO3, 77% yield) to later remove the protecting group to give the desired compound.

It was found that reacting $(Ac)_2$DKP with unprotected indole-3-carboxaldehyde worked fairly well for cesium carbonate (45 %, table 3, entry 1), but failed when stronger bases (KO^tBU, DBU, DBN) were used. It is possible to assume that reactions with strong bases fail because they favor deprotonation of indole-3-carboxaldehyde which increases the chance for unpredictable side reactions. Attempts with e equivalent base provided no improvements. Instead, reactions were performed with N-Boc protected indole-3-carboxaldehyde. Moderate yields (45-36 %, table 3) were obtained. This can be attributed to the large size of the reacting aldehyde.

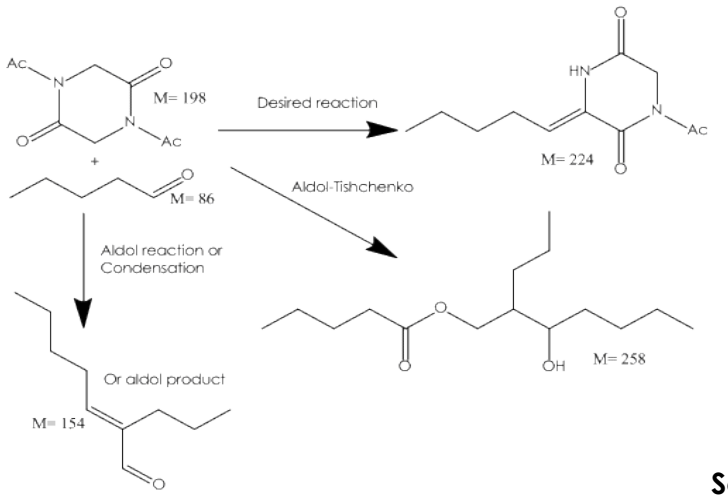
Pentanal

As mentioned previously, cesium carbonate has only been tested for aromatic aldehydes. When it was tested for pentanal, the reaction failed to produce any product as only the starting materials could be detected on GC-MS. The failure of cesium carbonate to induce condensation of $(Ac)_2$DKP and pentanal is very puzzling as aliphatic aldehydes are very electrophilic, although probably a bit less electrophilic than their aromatic counterparts.

When strong bases were tested on pentanal, low yields were obtained (57-31 %, table 3). On GC-MS, two compounds were detected in addition to the desired product (figure 8). One of the products was from self condensation of pentanal (RT 5,36, figure 8) and had much lower intensity than the desired product (RT 9,27, figure 8). The other (MW 154.25 g/mol, RT 8,36, figure 8) is unknown as it was difficult to determine the true low resolution mass of this compound.

The mass spectrum of the compound with RT 8,36 showed the fragments m/z197 and m/z which may come from the 2,5-diketopiperazine structure, but it was difficult to determine whether it was a product of a reaction between (Ac)2DKP and pentanal or a product from the aldol-Tishchenko reaction(scheme 6)[32] of pentanal with itself which would the molecular weight of 258,40 g/mol.

As visible in table 2, doubling the pentanal concentration drastically increased the yield, which meant that the low yield previously observed may have been due to side reaction of pentanal, or that pentanal reacts slow and competes with a side reaction of $(Ac)_2$DKP in the form of rearrangement, decomposition or an unconsidered reaction.



Scheme 6: Some of the possible products in the condensation of $(Ac)_2$DKP with pentanal

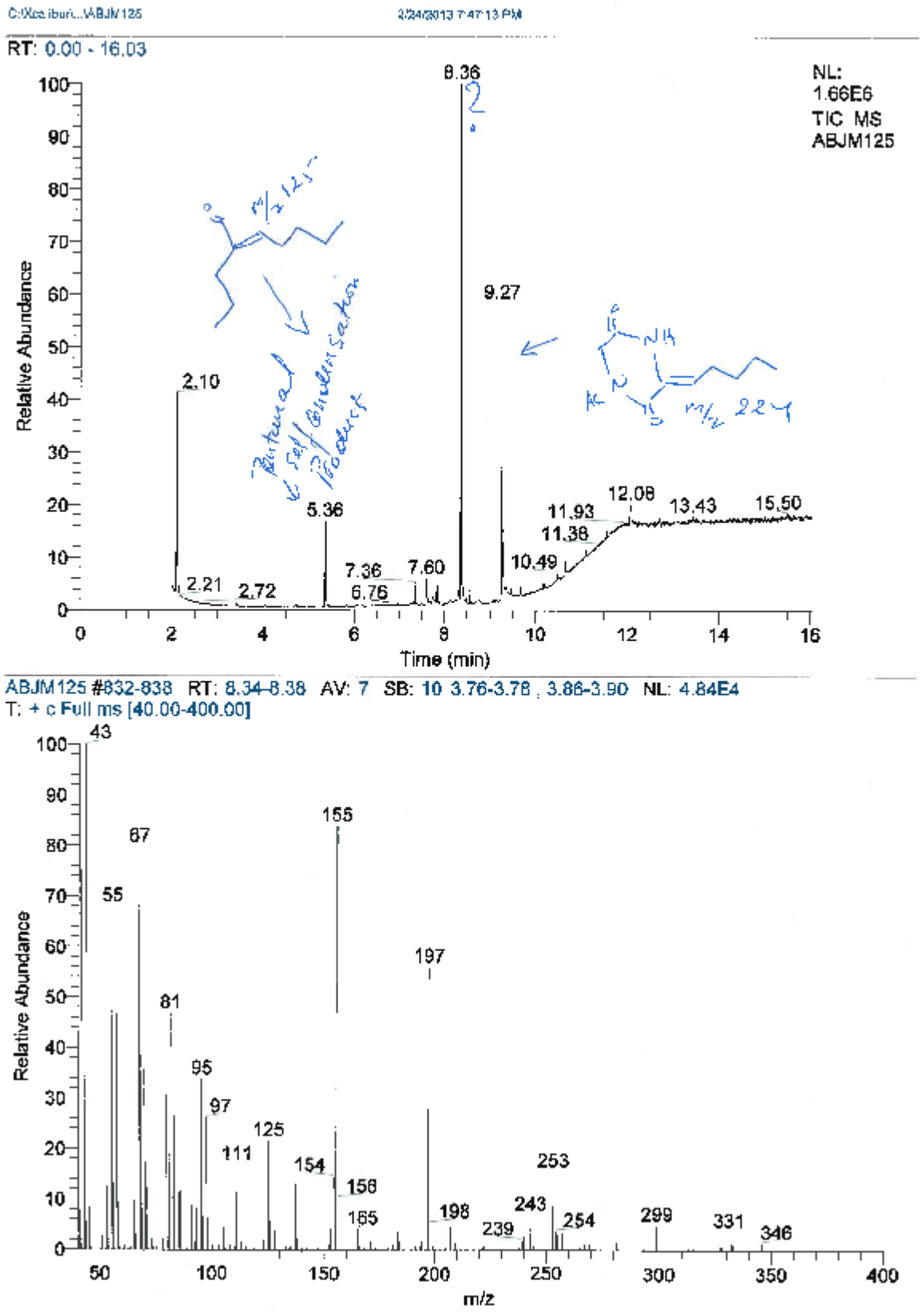


Figure 8: GC-MS spectra of a crude product from reaction of $(Ac)_2$DKP with pentanal.

3.2.3 Stereochemistry of aldol condensation

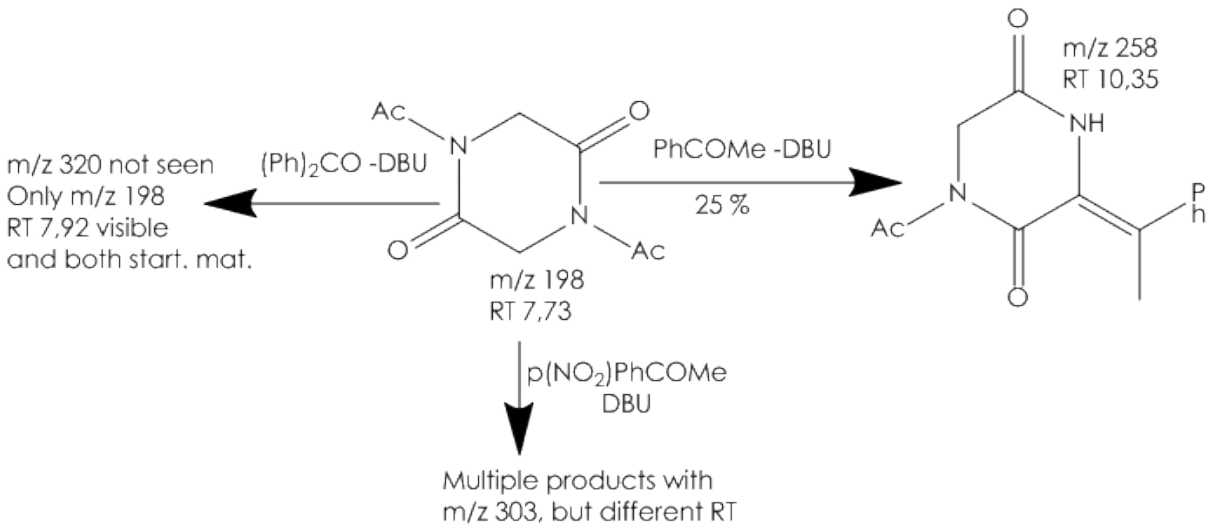
When stereochemistry is concerned, we expect this type of reaction to give E products most of the time[20]. The ^{1}HNMR spectrum of products obtained from reaction with all the aldehydes studied so far seem to support this idea, though this cannot be said for sure for products obtained from reaction with N-boc-indole-3-carboxaldehyde as recrystallization was used to purify these products and it is impossible to predict the behavior of the two possible isomers in the recrystallization solvent. Gallina and Liberatorie report obtaining a small amount of the Z isomer in reaction with aliphatic aldehydes[6], but this was not confirmed by us with the 1HNMR spectra's obtained, though on GC-MS a small amount of a product with a bit higher retention time but same m/z value as the desired was seen. A conclusion cannot be made from this data though, because the other isomer may have been the product of rearrangement due to the high temperature in the GC column.

3.3 Aldol condensation of 2,5-diketopiperazines with ketones

From experiments with benzaldehyde, indole-3-carboxaldehyde and pentanal, it was clear that DBU was the best base for monocondensation of $(Ac)_2$DKP with both aliphatic and aromatic aldehydes, as long as they do not contain an unprotected acidic group like for example indole-3-carboxaldehyde.

DBU was used to induce condensation of $(Ac)_2$DKP with benzophenone and acetophenone, respectively. No product was observed for benzophenone. For acetophenone the desired product was obtained in 25 % yield. An undesired byproduct with similar m/z but different retention time (RT 7.93, figure 9) was detected on GC-MS in both cases. The same byproduct was previously seen observed in the reaction of $(Ac)_2$DKP with benzaldehyde in the presence of TBD (section 3.2.1). When the more electrophilic p-nitroacetophenone was tested with DBU, multiple products with the same m/z value as the desired product were seen on GC-MS. This observation was paralleled by a comparable observation in the reaction of $(Ac)_2$DKP with benzaldehyde in the presence of LiHMDS (section 3.2.1).

These observations suggest that $(Ac)_2$DKP is unstable in the presence of strong bases. Part of this instability takes the form of rearrangement into one or several structural isomers that under certain conditions are stable enough to react and form products (scheme 7).



Scheme 7: Reaction of $(Ac)_2$DKP with ketones in the presence of DBU.

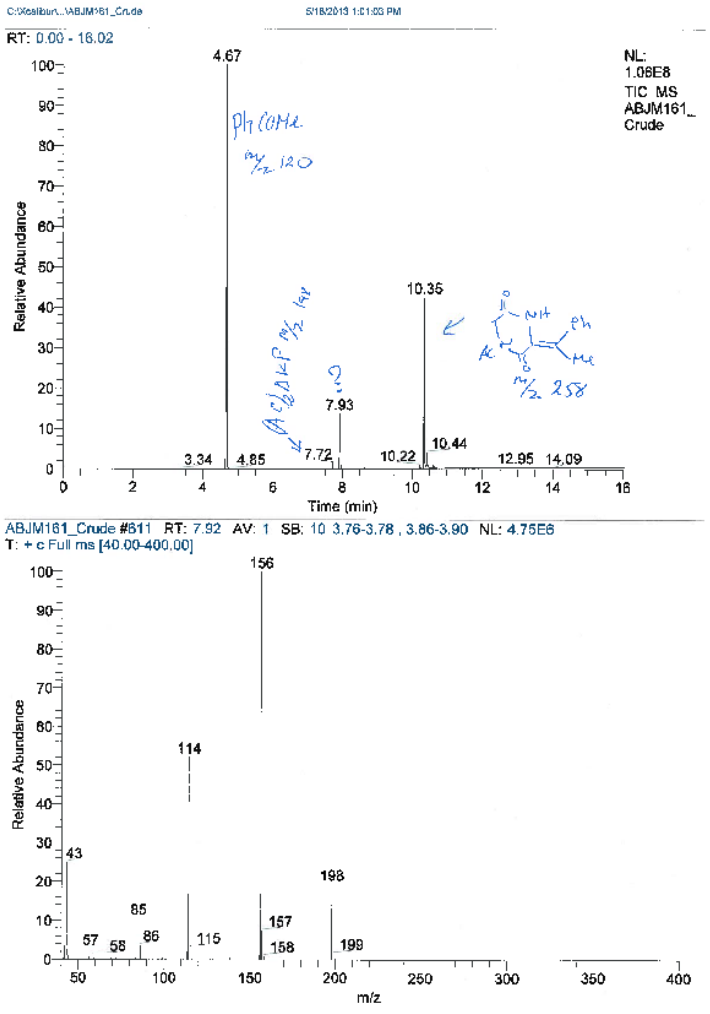
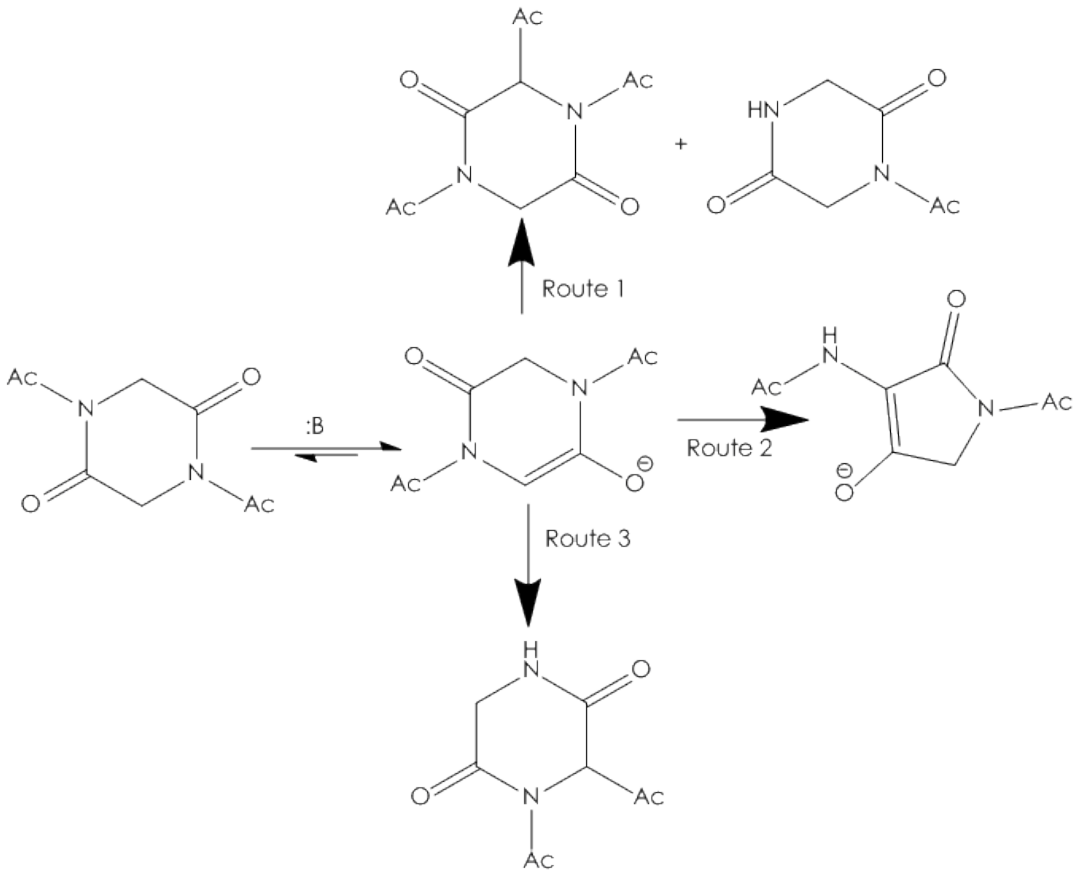


Figure 9: GC-MS spectra of the crude product from reaction of $(Ac)_2$DKP with Acetophenone. The possible structural isomer (RT 7.93, m/z 198) is visible.

One published article reports base induced rearrangement of 2,5-diketopiperazines[22]. This article reports that in the presence of a strong base, N-Boc and N-benzoyl protected 2,5-diketopiperazines rearrange by ring contraction (scheme 8, route 2), while $(Ac)_2$DKP rearranges to a tris-acetylated 2,5-diketopiperazine (scheme 8, route 1. The group attributed the later to an intermolecular acetyl migration that simultaneously must generate 1-acetylpiperazine-2,5-dione.

One possible cause of the multiple products with the same m/z value observed in the reaction of benzaldehyde and p-nitroacetophenone, is most likely the result of the rearrangement of $(Ac)_2$DKP into one or several structural isomers that in some cases are stable long enough to react and give products. Some of the possible rearrangements of $(Ac)_2$DKP are the transannular rearrangement[22] or N-C acetyl migration[33] as shown in scheme 8. The structural isomers may in turn undergo aldol condensation to lead to isomeric product mixtures. It was not possible to separate these products on thin layer chromatography (TLC).



Scheme 7: Illustrates some of the possible rearrangements of $(Ac)_2$DKP. Route 1 is reported, route is reported for N-Boc and N-Bz 2,5-diketopiperazines, while route 3 is an assumption.

One key observation at this point was that, when using DBU to promote aldol condensation with $(Ac)_2$DKP, they yield will always depend on the reactivity of the aldehydes or ketone. Since most ketones are normally less electrophilic than aldehydes, a different approach was necessary if to achieve aldol condensation with this family of compounds. Three possible approaches forward were to use a more stable starting material by varying the N-substituent on glycine anhydride, deprotonate $(Ac)_2$DKP using soft enolization (scheme x), or to work with the trimethyl silyl enol ether. Both approaches were explored.

3.3.1 The N-substituent effect

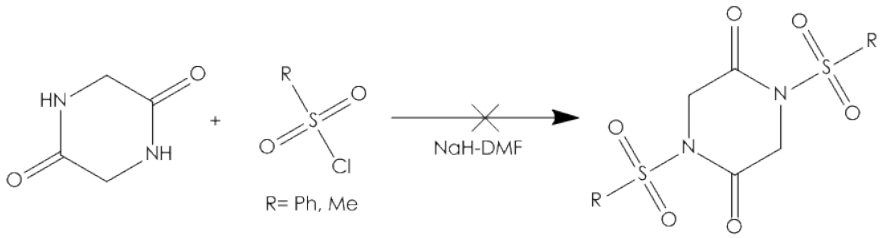
At this point, we desired to increase the stability of the reacting 2,5-diketopiperazine by varying the N-substituent. From the article on transannular rearrangement of activated 2,5-diketopiperazines[22], it may be generalized that most N-acyl and carbamate substituent's will show some kind of instability as seen with $(Ac)_2$DKP and 1,4-di-Boc-piperazine-2,5-dione. For this reason, no N-acyl and N-carbamate substituents were tested. The substituents studied are summarized in table 4.

Table 4: Summuary of the stability and reactivity of various *N*-substituents

Entry	N-substituent	Stability	Reactivity
1	Acyl	Assumed to be unstable	-
2	Carbamate	Assumed to be unstable	-
3	Sulfonyl	Failed Synthesis	-
4	Alkyl	Stable	No reaction

The substituents that would provide a starting material that closely resembles $(Ac)_2$DKP with respect to delocalization of the electron pair on nitrogen, are the sulfonyl derivatives. Attempt to synthesize 1,4-benzenesulfonylpiperazine-2,5-dione or 1,4-dimethylsulfonylpiperazine-2,5-dione by using sodium hydride in DMF failed(scheme 8)[34]. This is perhaps because these compounds are unstable in the presence of strong bases. Both starting materials and the desired product were not visible on ^{1}HNMR.

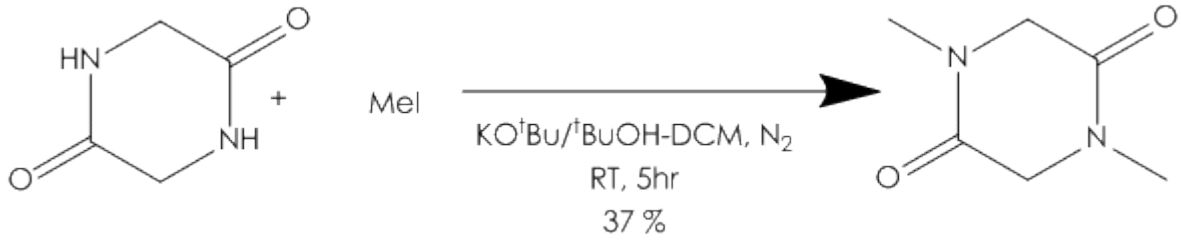
A method for the synthesis of N-sulfonyl DKPs has been reported[35], but was not considered further because it is time consuming, and because it was assumed that the N-sulfonylated DKPs are likely to be unstable. Weaker bases than sodium hydride were not tested to fully confirm this assumption because of the short time frame of this project.



Scheme 8: Failed synthesis of bis(N-sulfonyl)piperazine-2,5-dione using Sodium hydride in DMF

N-alkyl substiuents do not contain very electrophilic center and therefore, have a lower risk for rearrangement and decomposition compared to N-acyl, N-carbamate and N-sulfonyl DKPs. The simplest of these compounds, 1,4-dimethylpiperazine-2,5-dione could not be synthesized according to a reported method (glycine anhydride, Me_2SO_4, NaH, DMF, RT, overnight)[36].

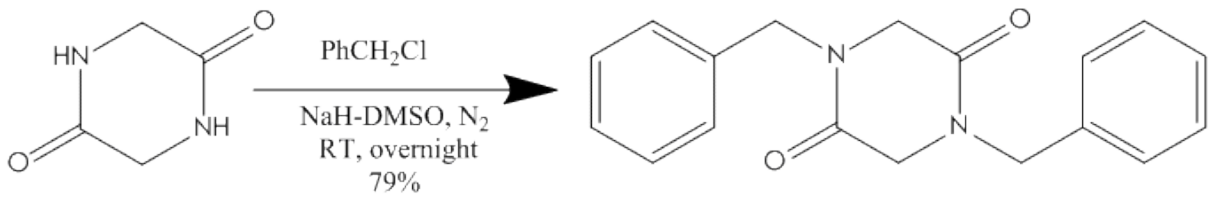
The reported method could not be used due to the lack of equipment to evaporate DMF. Because 1,4-dimethylpiperazine-2,5-dione is so water soluble, any method would have to avoid aqueous work-up. Back-extraction can`t be used either because this compound is poorly soluble in most volatile organic solvents. It was possible to synthesize this compound in 37 % yield according to scheme 7. In this way, non reacted potassium tert-butoxide was neutralized with a little acetic acid, and the potassium iodide and potassium acetate removed by filtration. Due to poor solubility of the target compound in DCM, it was not possible to synthesize it in practical quantities by scale-up.



Scheme 9: This method cannot be scaled up due to the poor solubility of the product in DCM.

The synthesis of this compound through catalytic hydrogenation at atmospheric pressure with Pd/C[37] of the easily synthesized 1,4-bis(hydroxymethyl)piperazine-2,5-dione[38] also failed. Synthesis of the methylol proceeded very well (97 %, glycine anhydride, paraformaldehyde, H_2O, reflux, 3hr), but hydrogenation of this compound at atmospheric pressure in various solvents and at various temperatures failed.

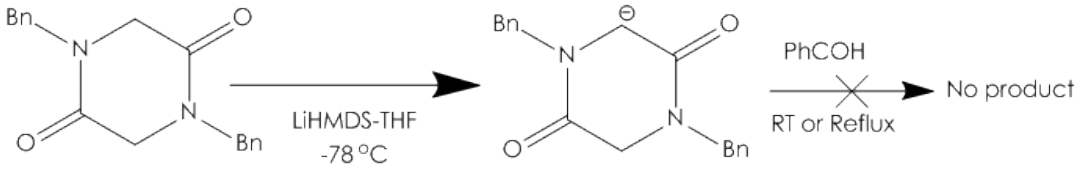
The less polar 1,4-dibenzylpiperazine-2,5-dione was easily prepared in 79 % yield according to reported method as shown in scheme 10[39].



Scheme 10: Preparation of 1,4-dibenzylpiperazine-2,5-dione

Attempt to condense 1,4-dibenzylpiperazine-2,5-dione with benzaldehyde failed to give the desired product using LiHMDS in THF at room temperature or reflux. This compound proved to be very stable as it was visible on ^{1}HNMR.

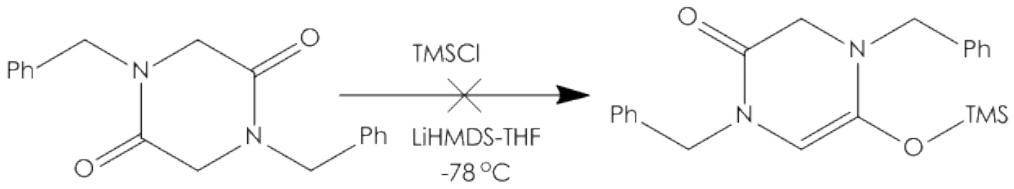
Due to hybrid structure of amide amides, it can be assumed that deprotonation of 1,4-dibenzylpiperazine-2,5-dione does not give the expect enolate, but instead the electrons are localized on carbon, possibly making this a poor nucleophile as shown in scheme 11.



Scheme 11: The reaction of 1,4-dibenzylpiperazine-2,5-dione with benzaldehyde fails at room temperature or reflux, also when Buthyl lithium (BuLi) is used.

Another reason for failure of this reaction may be because the aldol reaction is reversible, and when there isn`t group migration to stabilize the aldol product as with $(Ac)_2$DKP, product formation is not favored.

Attempt to stabilize the aldol product by using the the trimethylsilyl enol ether was not possible, because 1,4-dibenzylpiperazine-2,5-dione failed to react with trimethylsilyl chloride using known procedures (scheme 12)[40]. 1HNMR analysis of the reaction mixture without any workup, showed only the presence of both starting materials.

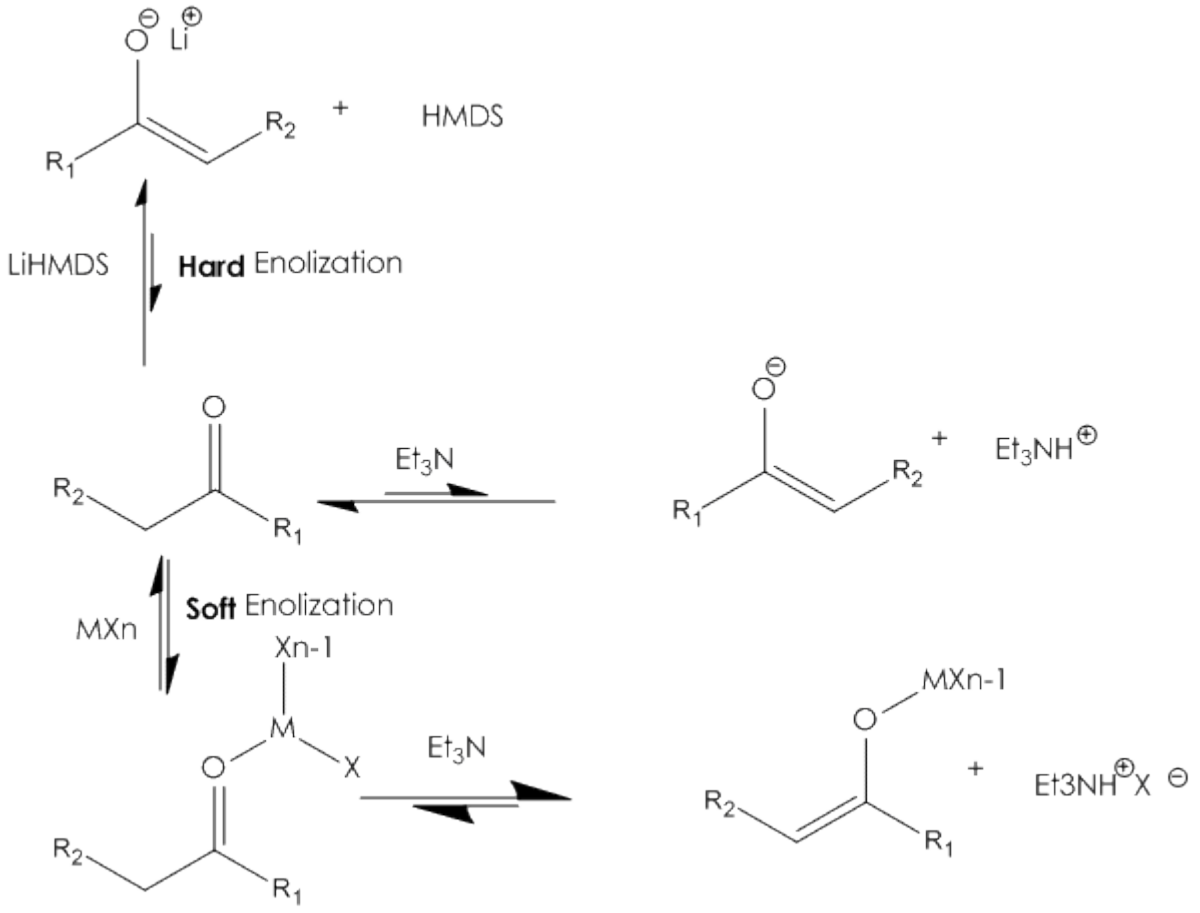


Scheme 12: Failed synthesis of the Trimethylsilyl enot ether of 1,4-dibenzylpiperazine-2,5-dione

3.3.2 The application of soft enolization and Silyl enol ethers

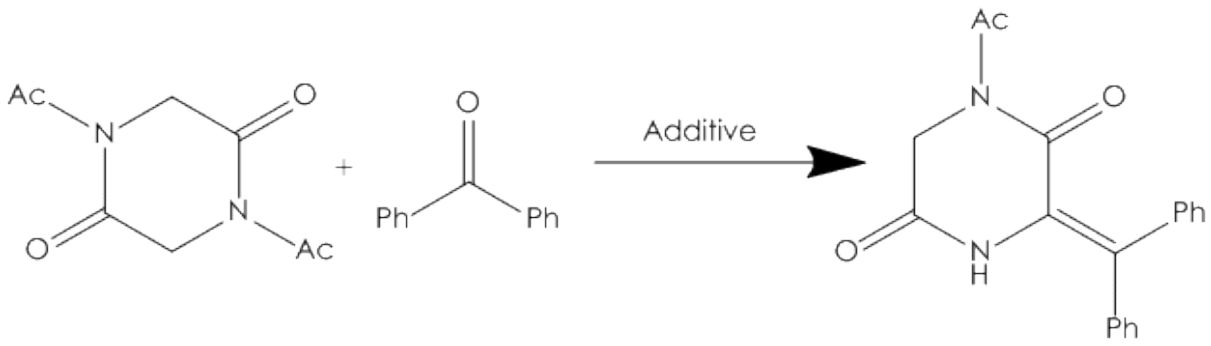
Deprotonation of a carbonyl compound with a strong base like LiHMDS allows complete deprotonation, while the use of a weak base like TEA usually is insufficient to produce useful quantities of the desired enolate intermediate. In the presence of a Lewis acid, deprotonation in the presence of a weak base is possible, making the generation of metal enolate under mild conditions possible (scheme 13)[30, 41]. At this point, benzophenone was chosen to be the model electrophile, because a successful procedure for benzophenone could easily be adapted to other ketones too. The results obtained with this approach are summarized in table 5.

There several reported soft enolization of oxazolidinone and thiazolidinethione using titanium chloride or magnesium bromide in the presence of a weak base like TEA or N,N-Diisopropylethylamine (DIPEA)[42 42b 43]. Since $(Ac)_2$DKP is an imide and shares some similarities with the mentioned compounds, it was assumed that soft enolization may work for $(Ac)_2$DKP too.



Scheme 13: Hard vs Soft enolization. M is usually boron, magnesium, titanium or tin.

Table 5: The application of soft enolization to aldol type condensation of $(Ac)_2$DKP with benzophenone.

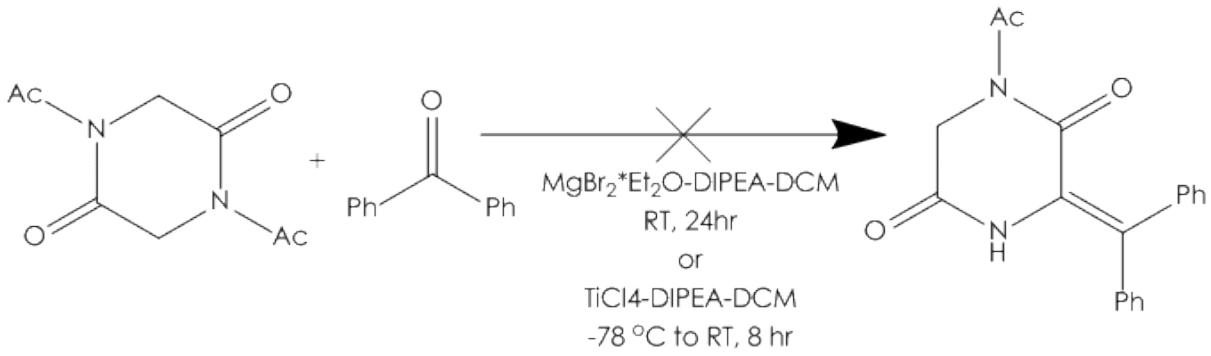


Additive	Solvent	T (^{0}C)	Time(hr)	Stability	% Yield
DBU	DCM	RT		unstable	NR
$MgBr_2$*Et_2O/DIPEA	DCM TCE	≥130	24	unstable at high T	NR
$MgBr_2$*Et_2O/DBU	DCM	RT	24	unstable	NR
TiCl4/DIPEA	DCM	-78 to RT	8	Stable	NR
$MgBr_2$*Et_2O/TMSCl/DBU	DCM	RT	24	Stable	NR
CuCl2/TMSCl/DBU	DCM	RT	24	Stable	NR
$Pd(OAc)_2$/TMSCl/DBU	DCM	RT	24	Stable	NR
BF_3*Et_2O/TMSCl/DBU	DCM	RT	24	Stable	NR
$AlCl_3$/TMSCl/DBU	DCM	RT	24	Stable	NR
$TiCl_4$/TMSCl/DBU	DCM	RT	24	Stable	NR
$SnCl_2$/TMSCl/DBU	DCM	RT	24	Stable	NR
$AlCl_3$/TMSCl/DBU	THF	Reflux	24	Stable	NR

Both TMSCl and the base and Lewis acids were used in more than 1eqv, expect for palladium acetate, cupper chloride and tin(II) chloride that were used in 0,2eqv.

From section 3.2.1, it is clear that aldol condensation of $(Ac)_2$DP and benzaldehyde in the presence of magnesium bromide etherate and TEA proceeds at slow rate. When the new reaction was run in the presence of *N*,*N*-Diisopropylethylamine (DIPEA) and magnesium bromide etherate, no product was obtained at any temperature, though $(Ac)_2$DKP became more unstable as the temperature increased. Changing the base to DBU had no effect, except that with this base $(Ac)_2$DKP is unstable even at room temperature.

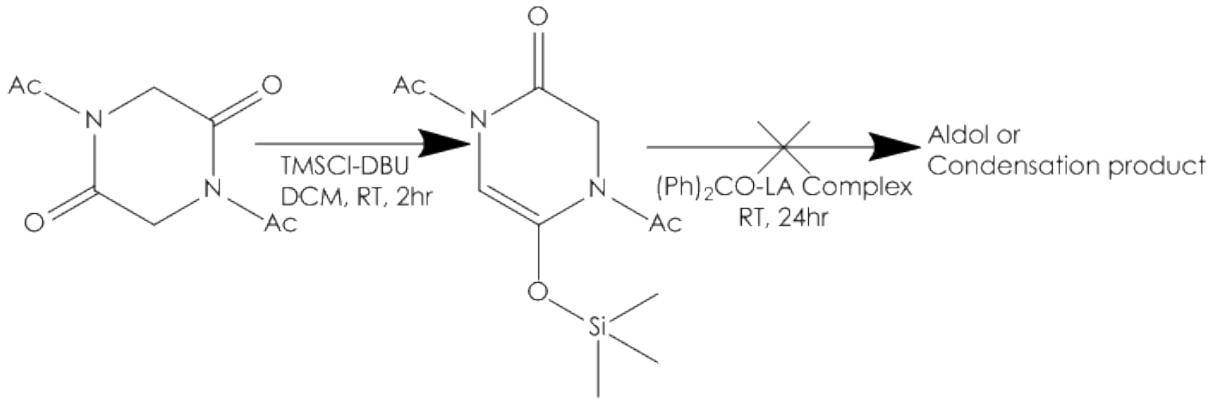
It was believed that magnesium bromide etherate could both act as a Lewis acid to activate benzophenone and aid in the formation of a possibly stable magnesium enolate. The titanium enolate proved to be stable at room temperature but failed to induce a reaction with both benzophenone and the less sterically hindered acetophenone at room temperature (scheme x).



Scheme 14: Magnesium and titanium enolate fail to induce the reaction, though they are stable at room temperature.

It was assumed that, if the trimethylsilyl (TMS) enol ether of $(Ac)_2$DKP could be made, then possibly, a Lewis acid could be used to activate both benzophenone and the TMS enolate[30, 32]. It was not possible to isolate the TMS enolate, but it could be made by stirring with TMSCl and DBU at room temperature for two hours, and then reacted with the activated benzophenone without isolation.

As can be seen form table x, attempts to induce this reaction with both weak (palladium acetate, cupper chloride) and strong lewis acid (Titanium tetrachloride) at room temperature or higher failed. It is clear that the TMS enolate of $(Ac)_2$DKP is formed as $(Ac)_2$DKP would otherwise be unstable in the presence of DBU. Failure of this reaction therefore lies in step 2 (scheme 13) for reasons that are not completely clear.



Scheme 15: Failed condensation of the TMS enol ether of $(Ac)_2$DKP and benzophenone.

4 Conclusion and future outlook

We were able to produce pure 1,4-Diacetlpiperazine-2,5-dione in 78-81 % yield. Improvement on the original method was in two areas. The use of acid catalysis to shorten the reaction time from seven hours to one hour, and filtration of the reaction mixture through celite prior to recrystallization from isopropanol to remove the brown discoloration.

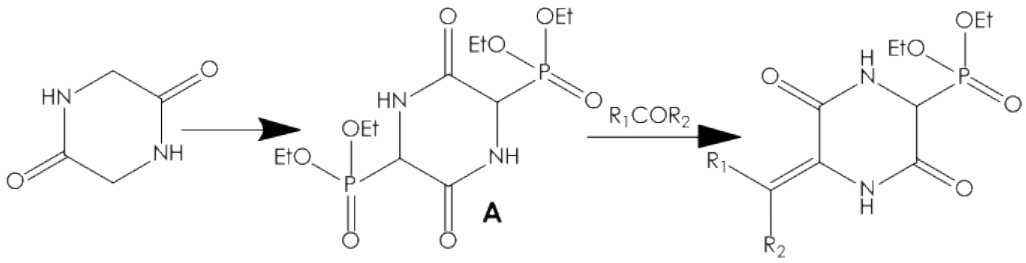
Cesium carbonate was found to catalyze very well the condensation of 1,4-Diacetlpiperazine-2,5-dione with aromatic aldehydes, but failed to induce a reaction with aliphatic aldehydes. For aldol condensation with aliphatic and aromatic aldehydes, DBU was found to the optimal base, but is not compatible with aldehydes containing acidic hydrogen like with indole-3-carboxaldehyde.

The use of DBU to induce aldol condensation with benzophenone failed, but a 25 % yield was obtained for acetophenone. The poor reactivity seen here is cause by the instability of 1,4-Diacetylpiperazine-2,5-dione and possibly the resulting products under strongly basic conditions. Under basic conditions, 1,4-Diacetylpiperazine-2,5-dione rearranges into one or several structural isomers, which in some cases react to form products.

Attempts to solve the instability problem by varying the N-substituent on glycine anhydride failed. The most stable starting material, 1,4-dibenzylpiperazine-2,5-dione failed to give a product even with benzaldehyde.

The application of soft enolization using magnesium bromide ethyl etherate and titanium chloride with DIPEA failed. The activation of the TMS silyl enol ether of 1,4-Diacetlpiperazine-2,5-dione with various lewis acid to induce a reaction with benzophenone also failed, although it was observed to be base stable.

In order to achieve aldol condensation with ketones, it is necessary to have a starting material that forms a stable enolate under strongly basic conditions. It is well known that phosphonate-stabilized enolates react well with ketones, and there are even some reports of induced condensation of phosphonate-stabilized enolates of unprotected amide type with ketones [44] similar to what`s shown in the scheme below. It plausible that aldol condensation with ketones may be successful with such a starting material because It is more likely to be base stable. N-alkyl protection of A may be necessary.



Scheme16: Proposal for using the starting material A to achieve aldol condensation with ketones. It is possible that the N-methyl substituted derivative is more reactive.

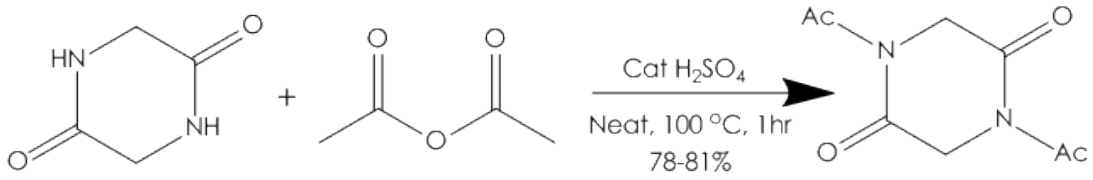
5 EXPERIMENTAL

General Experimental Methods

All reactions involving moisture sensitive reagents were conducted under a dry nitrogen or argon atmosphere with anhydrous solvents and glassware dried overnight at 140 ^{0}C. Dry solvents and hydroscopic reagents were transferred via a syringe and introduced into reaction vessel through rubber septa. Dry solvents were obtained from a dry solvent dispensing system. The reaction products were concentrated on a buchi rotary evaporator then on a vacuum pump. Column Chromatography was performed on silica gel (35-70 mesh) purchased from Merck. Reaction progression was followed using Thermo Scientific Xcalibur GC-MS (TriPlus RSH Autosampler, Trace GC Ultra, ITQ 1100). High resolution mass spectra were recorded on a LTQ Orbitrap XL in a positive or negative Electron Spray Ionization mode; with samples run in acetonitrile or methanol with or without addition of formic acid depending on the sample. IR spectra were recorded on a Varian 7000e FT-IR spectrometer. Nuclear Magnetic Resonance (NMR) spectra were collected on a Varian Mercury 400 MHz instrument at room temperature in the indicated solvents. The peak positions are reported with chemical shifts (δ) in ppm referenced to tetramethylsilane (0 ppm). The following abbreviations are used: singlet (s), doublet (d), triplet (t) and multiplet (m).

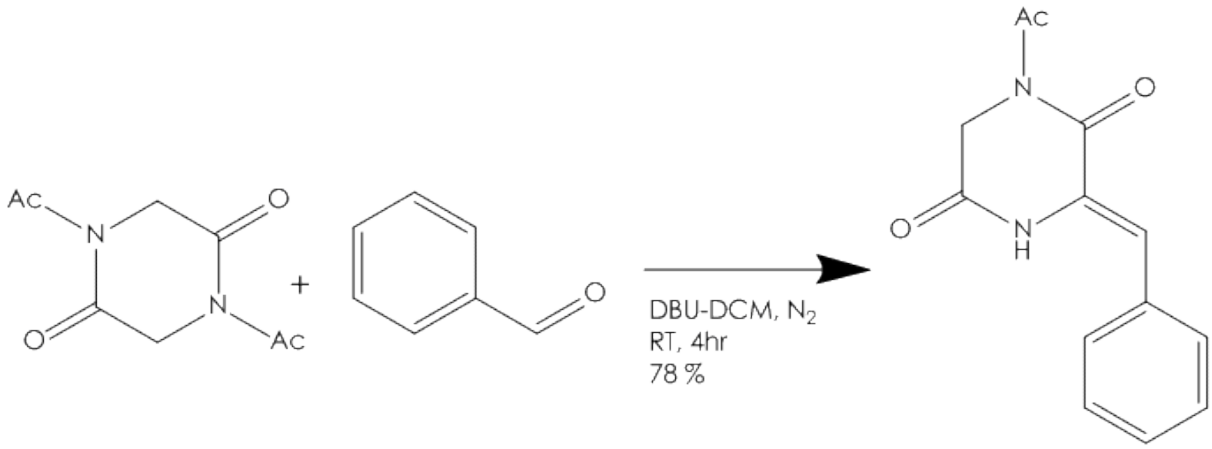
The following compounds were synthesized according to literature (Appendix for analytical data): Synthesis of Tert-butyl 3-formyl-1H-indole-1-carboxylate (boc-indole-3-carboxaldehyde)[45]; 1,4-dibenzylpiperazine-2,5-dione[39]; 1,4-Bis(hydroxymethyl)piperazine-2,5-dione[38]

<u>Synthesis of 1,4-diacetylpiperazine-2,5-dione ($(Ac)_2$DKP)</u>



4,012g of glycine anhydride is suspended in 22 ml of acetic anhydride in a 100ml round-bottom flask equipped with a reflux condenser. Two drops of sulfuric acid are added and the reaction mixture is refluxed for 1 hour to afford a red solution. The red solution is diluted with Ethyl acetate and filtered through a think pad of celite to afford a light yellow solution. The solvents are evaporated, and the crude light yellow product (96,3 % yield) is recrystallized from excess isopropanol to afford colorless crystals. It is important that the process of recrystallization starts with colorless crystals and not yellow.
Yield: 78-81 % colorless crystals
GC-MS (EI): RT 7.75 m/z 198
^{1}H NMR ($DCCl_3$): δ 4.57 (s, 4H), 2.56 (6H, s)
^{13}C NMR ($DCCl_3$): δ 170.70, 165.82, 47.15, 26.72
IR (cm^{-1}): 1700, 1432, 1358, 1305, 1261, 1225, 1179, 1129. 1079, 1040, 975, 947, 814, 690, 621, 597, 566

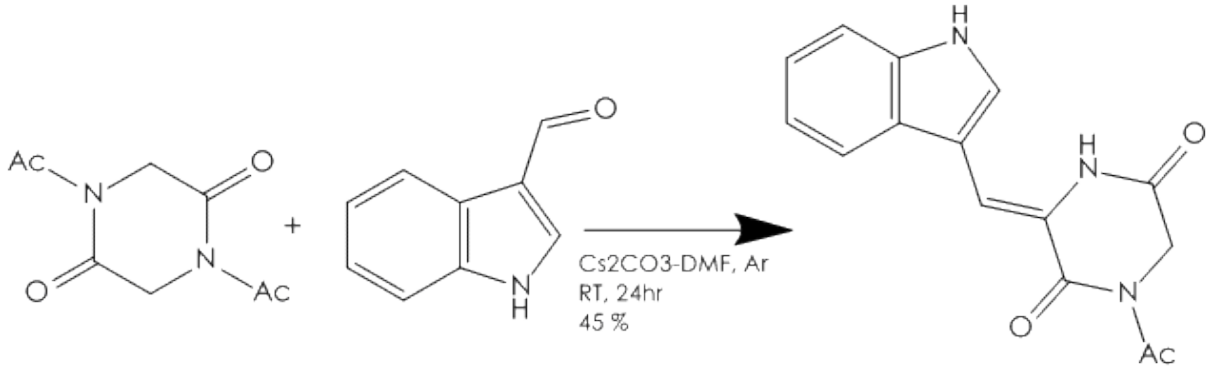
The condensation of $(Ac)_2$DKP with benzaldehyde



- Reaction with cesium carbonate was conducted according to reported method[8], with the reaction time increased to 24 hours.
- Reaction with potassium tert-butoxide was conducted according to reported[5] but at 0 °C
- Reaction with DBN was conducted as with DBU

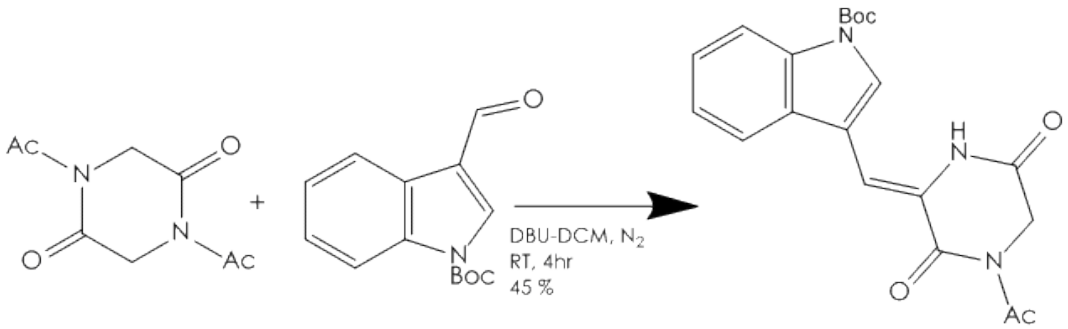
1,4-Diacetylpiperazine-2,5-dione (0,150g) and benzaldehyde (0,100g) are dissolved in 10ml dry DCM in a 25 ml dry round-bottom flask equipped with a nitrogen balloon. Then DBU (1,4ml) is added drop wise, and the resulting yellow solution is stirred at room temperature for 4 hours. The reaction mixture is washed with 10% citric acid (aq) (x3), brine, dried over sodium sulfate, and concentrated in vacuum.
Yield: 0,145g (78,43 %) pale yellow solid
Found HRMS (Negative ESI): 243.0776 (calculated for $C_{13}H_{11}N_2O_3$ 243.0775)
GC-MS (CI): RT 10.43 m/z 245
^{1}H NMR ($DCCl_3$): δ 8.02 (s, 1H), 7.48-7.37 (m, 5H), 7.18 (s, 1H), 4.50 (s, 2H), 2,65 (s, 3H)
^{13}C NMR ($DCCl_3$): δ 172.46, 162.70, 159,96, 132.49, 129.55, 129.37, 128.53, 125.67, 119.93, 46.09, 27.20
IR (cm^{-1}): 3272, 1681, 1632, 1496, 1430, 1361, 1255, 1226, 1209, 1103, 1037, 997, 937, 873, 838, 770, 717, 692, 656, 585, 558

The condensation of $(Ac)_2$DKP with indole-3-carboxaldehyde



1,4-Diacetylpiperazine-2,5-dione (0,099g) and indole-3-carboxaldehyde (0,079g) are dissolved in 2ml DMF. The solution is repeatedly evacuated in short time to remove oxygen and flushed with argon. Cs2CO3 (0,223g) is added the evacuation-flushing process repeated. The reaction mixture is stirred at room temperature for 24 hours. The reaction mixture is diluted with water, and the resulting precipitate is washed several times with water and cold ether to give 0,065g (45,22%) brown yellow solid.
Found HRMS (Positive ESI): 284.1034 (calculated for $C_{15}H_{14}N_3O_3$ 284.1030)
^{1}H NMR (DMSO-d_6): δ 10.34 (s, 1H), 9.99 (s, 1H), 8.34 (m, 5H), 8.21 (s, 1H), 7.70 (m, 3H), 7.37 (h, 6H), 7.15 (s, 2H), 6.99 (d, 1H), 6.85 (s, 1H), 4.39 (s, 2H), 4.06 (s, 2H), 2.74 (m, 7H) too many signals
^{13}C NMR (DMSO-d_6): δ see appendices
IR (cm^{-1}): 3290, 3131, 3042, 2912, 1677, 1631, 1553, 1455, 1412, 1379, 1358, 1322, 1218, 1148, 1079, 1017, 940, 856, 798, 749, 705, 672, 618, 577, 543

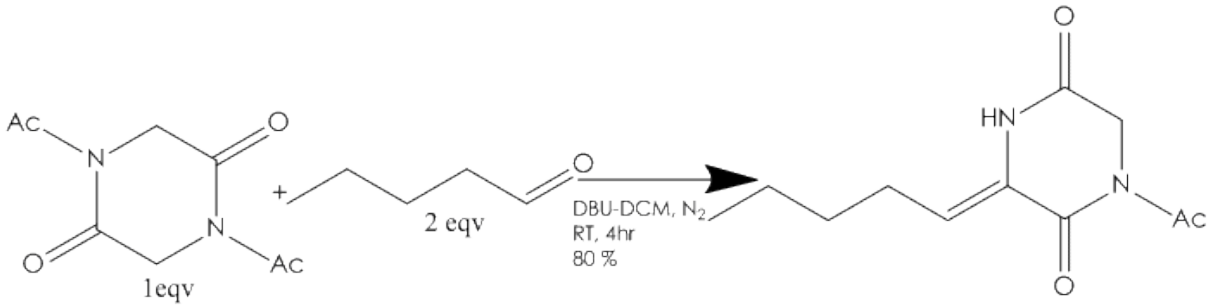
The condensation of $(Ac)_2$DKP with N-boc-indole-3-carboxaldehyde



1,4-Diacetylpiperazine-2,5-dione (0,200g) and N-Bocindole-3-carboxaldehyde (0,246g) are dissolved in 9 ml dry DCM in a 25 ml dry round-bottom flask equipped with a nitrogen balloon. Then DBU (0.17ml) is added drop wise, and the reaction mixture is stirred at room temperature overnight. The reaction mixture is washed with 10% citric acid (aq) (x3), brine and dried over sodium sulfate. The crude product is recrystallized from toluene to yield 0.175g (45.23 %) yellow crystals.
Found HRMS (Negative ESI):382.1404 (calculated for $C_{20}H_{20}N_3O_5$ 382.1408)
^{1}H NMR ($DCCl_3$): δ 8.53 (s, 1H), 8.13-8.11 (d, 1H), 7.93 (s, 1H), 7.67-7.65 (d, 1H), 7.41-7.31 (m, 3H), 4.49 (s, 2H), 2.66 (s, 3H), 1.70 (s, 9H)

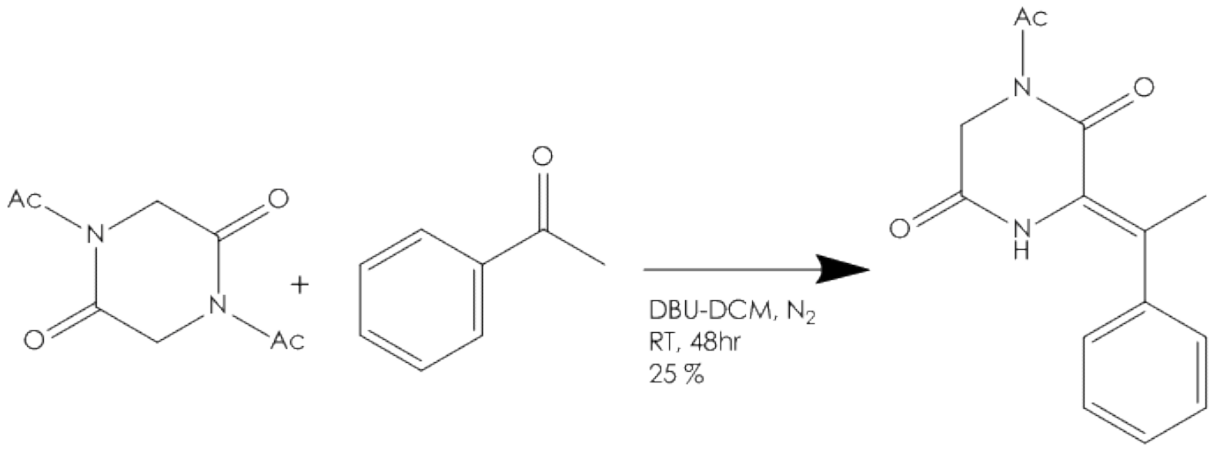
^{13}C NMR ($DCCl_3$): δ 163.33, 160.01, 134.97, 129.20, 125.62, 125.21, 123.51, 119.21, 115.48, 113.17, 111.19, 85.16, 76.71, 46.15, 28.13, 27.20
IR (cm^{-1}): 3217, 3120, 1736, 1682, 1626, 1553, 1478, 1453, 1409, 1372, 1306, 1252, 1227, 1152, 1091, 1020, 882, 852, 748, 656, 572, 529

The condensation of $(Ac)_2$DKP with Pentanal



1,4-Diacetylpiperazine-2,5-dione (0.206g) and pentanal (0.24ml) are dissolved in 8 ml dry DCM in a 25ml dry round-bottom flask equipped with a nitrogen balloon. Then DBU (0.17ml) is added drop wise, and the reaction mixture is stirred at room temperature for 6 hours. The reaction mixture is washed with 10% citric acid (aq) (x3), brine and dried over sodium sulfate. The crude product is purified by column chromatography on silica gel using
EtOAc−Pentane (7:3) + 2% TEA as an eluant to give 0.202g (79.56 %) of white solid.
Found HRMS (Negative ESI): 223.1090 (calculated for $C_{11}H_{15}N_2O_3$ 223.1088)
GC-MS (EI): RT 9.27 m/z 224
^{1}H NMR ($DCCl_3$): δ 9.49 (s, 1H), 6.28 (t, 1H), 4.35 (s, 2H), 2.54 (s, 3H), 2.20 (m, 2H), 1.43 (m, 2H), 1.33 (m, 2H), 0,87 (t, 3H)
^{13}C NMR ($DCCl_3$): δ172.58, 164.35, 159.95, 126.17, 125.52, 45.89, 30.44, 27.07, 25.76, 22.32, 13,73
IR (cm^{-1}): 3191, 3076, 2963, 2932, 2873, 1676, 1636, 1446, 1412, 1367, 1327, 1258, 1223, 1131, 1099, 1067, 1041, 1003, 982, 899, 810, 742, 721, 652, 601, 559, 478

Condensation of $(Ac)_2$DKP with Acetophenone



In a two necked 25ml round-bottom flask equipped with an Argon balloon, 0,201g 1,4-diacetylpiperazine-2,5-dione, 0,12ml acetophenone and 0,18ml DBU is dissolved in 10ml dry DCM. The solution is stirred at room temperature for 20hr. The reaction mixture is diluted with DCM, washed with 10% citric acid (aq) x3, brine, and dried over Na_2SO_4. The solvents are evaporated to afford a yellow oil (0.144g) .The crude product is purified by column chromatography on deactivated silica gel using Ethyl acetate -Pentane (3:9)+ 1% TEA to give 0,067g of white solid, 25,58%
GC-MS (EI): RT 10.35 m/z 258
^{1}H NMR ($DCCl_3$): δ7.19-7.49 (m, 5H), 4.42 (s, 2H), 2.64 (s, 3H), 2,52 (s, 3H) (N-H missing)
^{13}H NMR ($DCCl_3$): δ 172.34, 163.29, 161.36, 139.61, 137.41, 129.74, 128.86, 126.91, 121.80, 76.69, 45.70, 27.12, 22.08
IR (cm^{-1}): 3236, 1696, 1619, 1425, 1396, 1360, 1315, 1280, 1236, 1207, 1120, 1031, 983, 825, 775, 727, 705, 625, 591, 571, 547, 529

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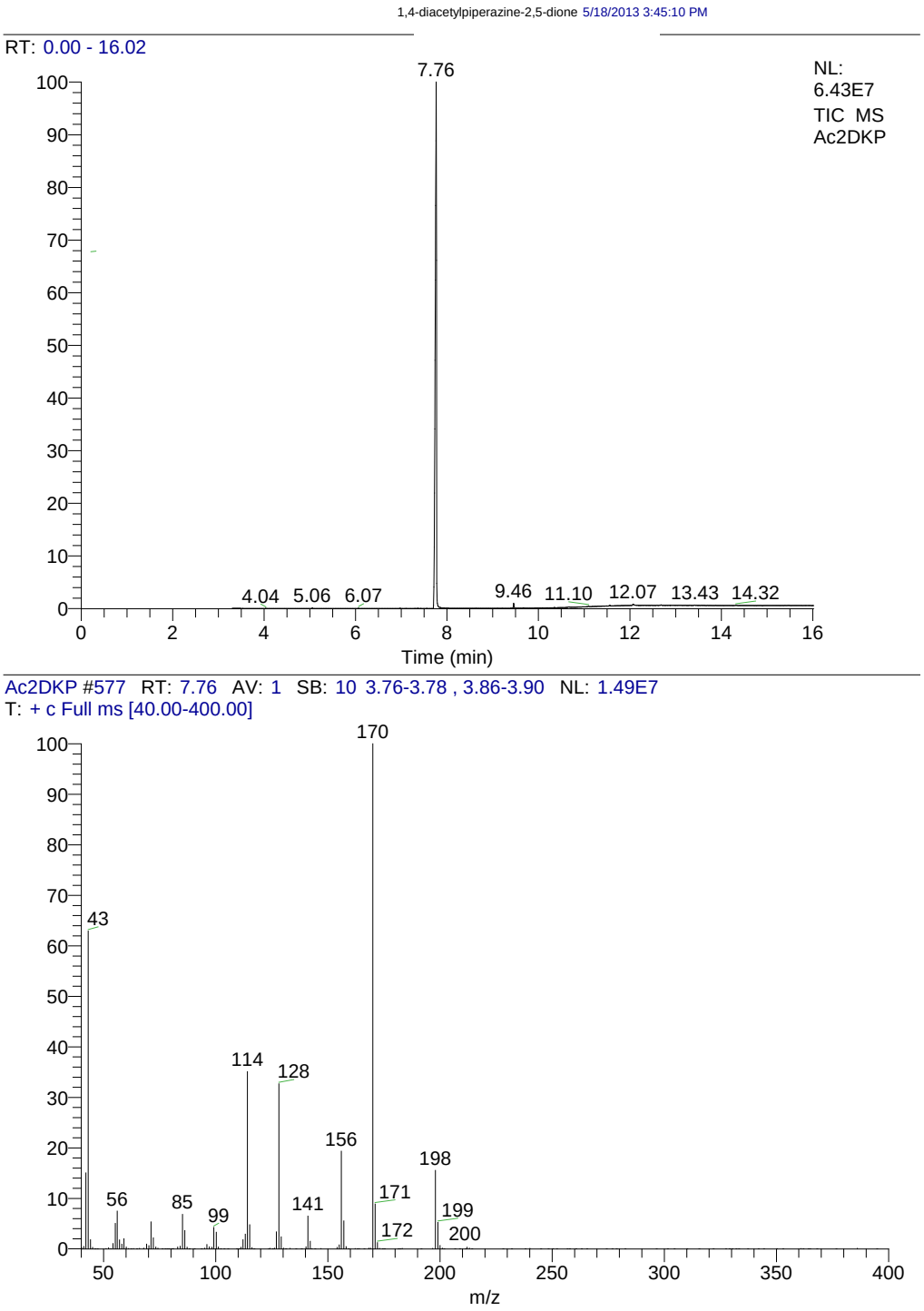
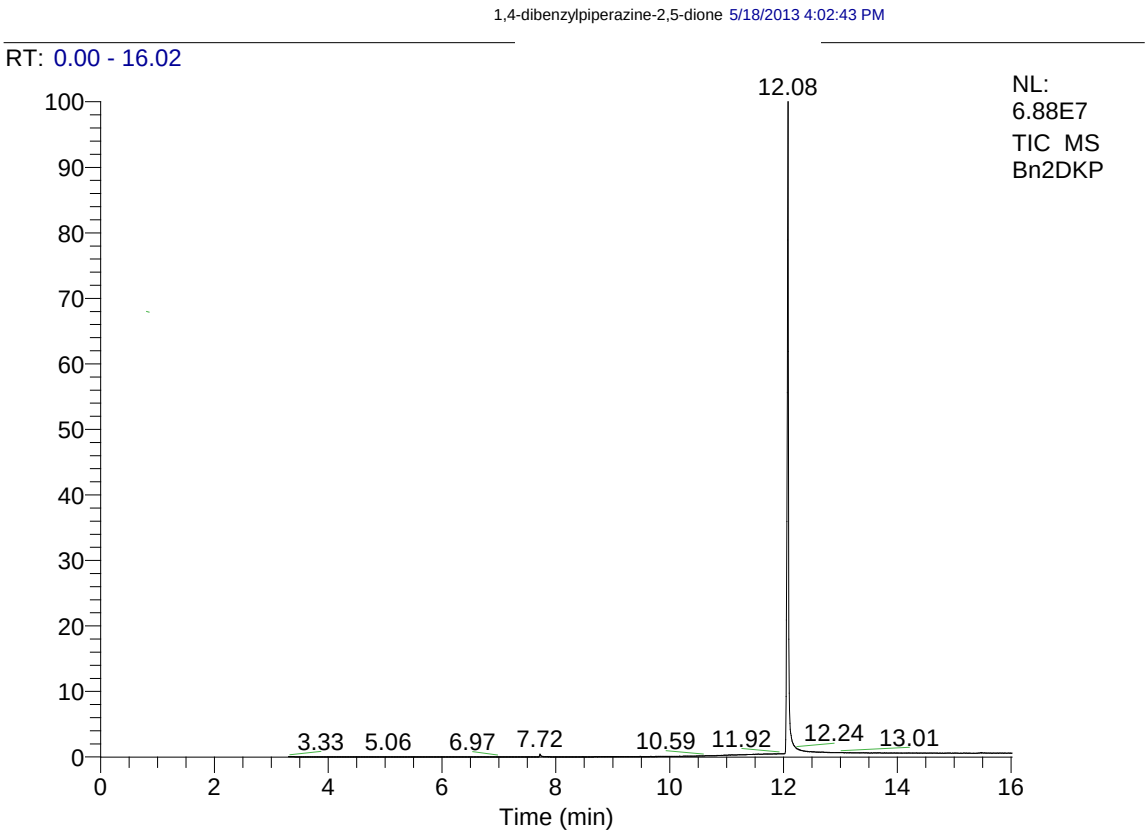
7. Appendices

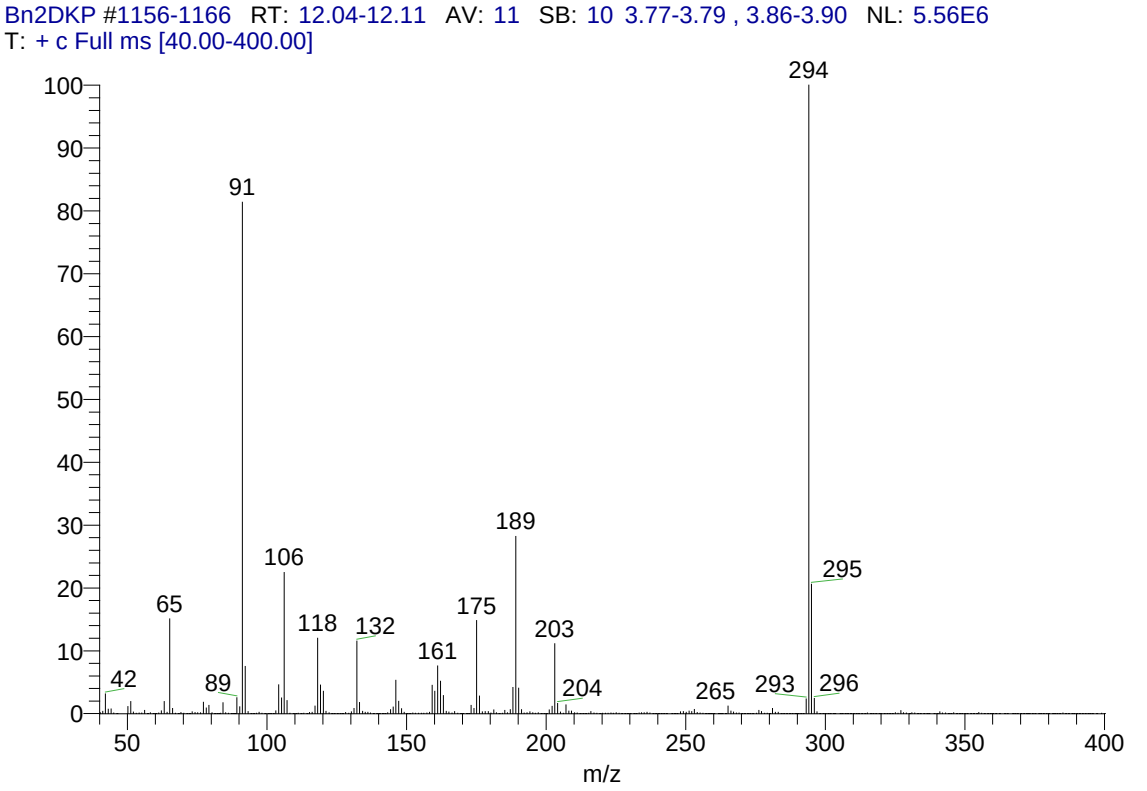
7.1 GC-MS Spectra

1,4-Diacetylpiperazine-2,5-dione

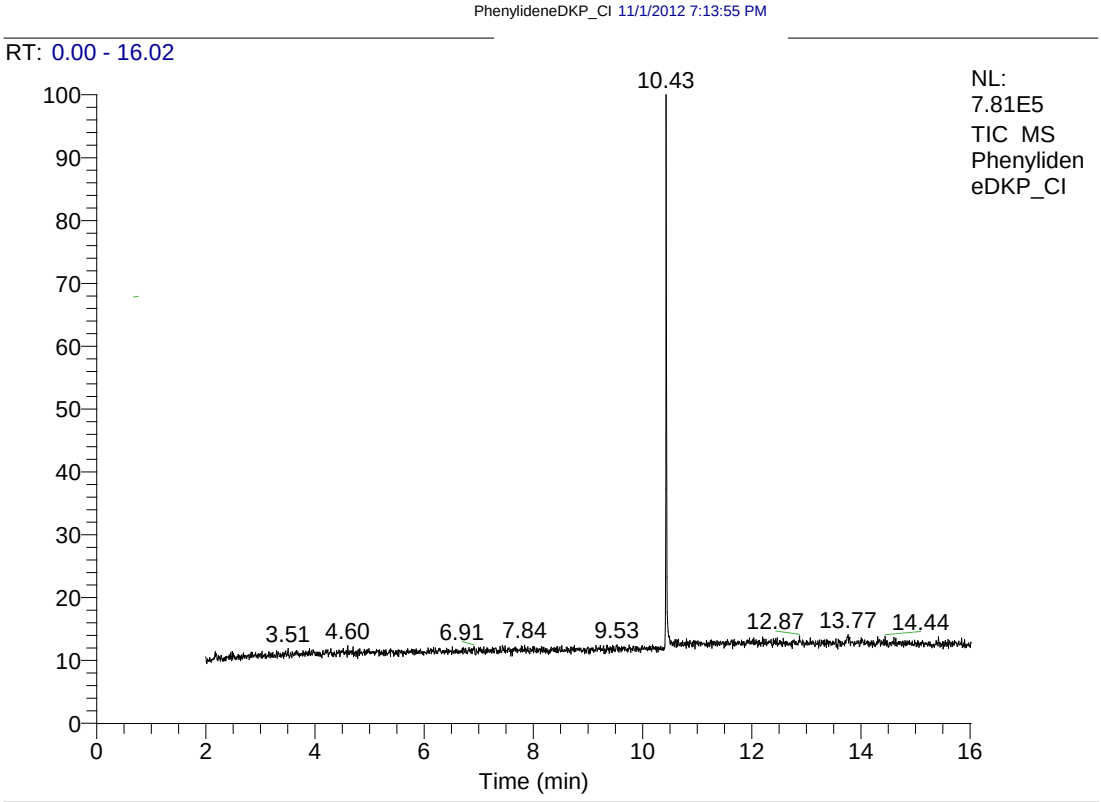


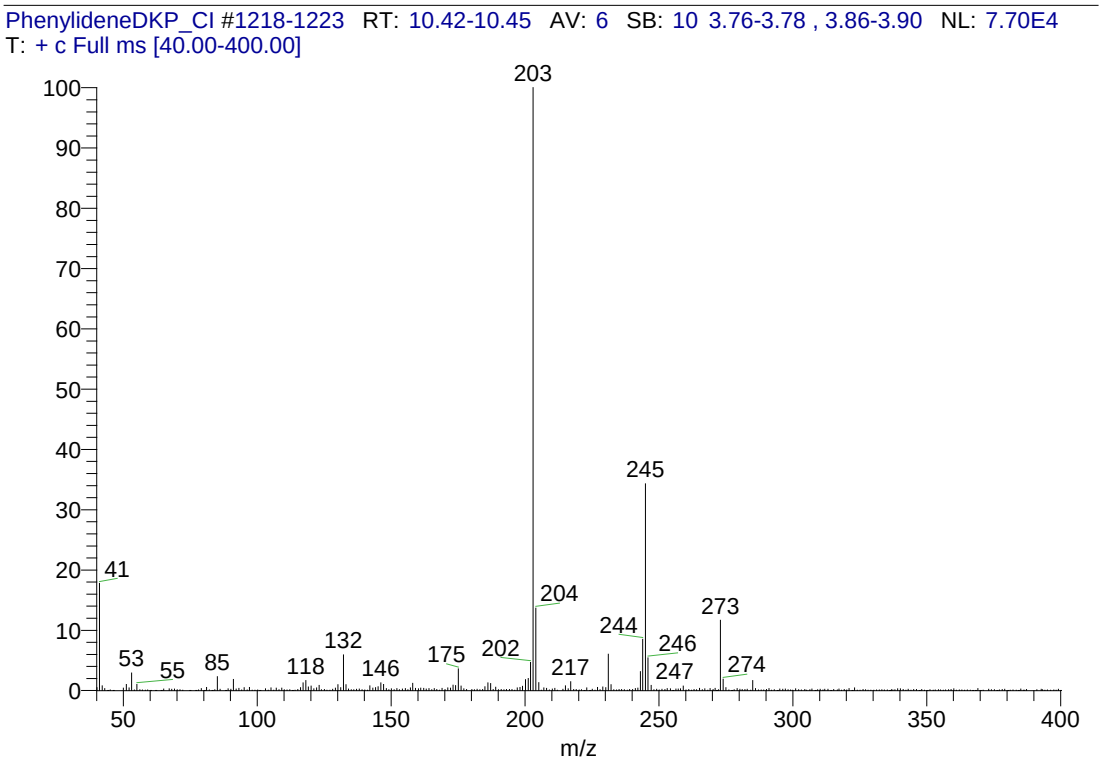
1,4-Dibenzylpiperazine-2,5-dione



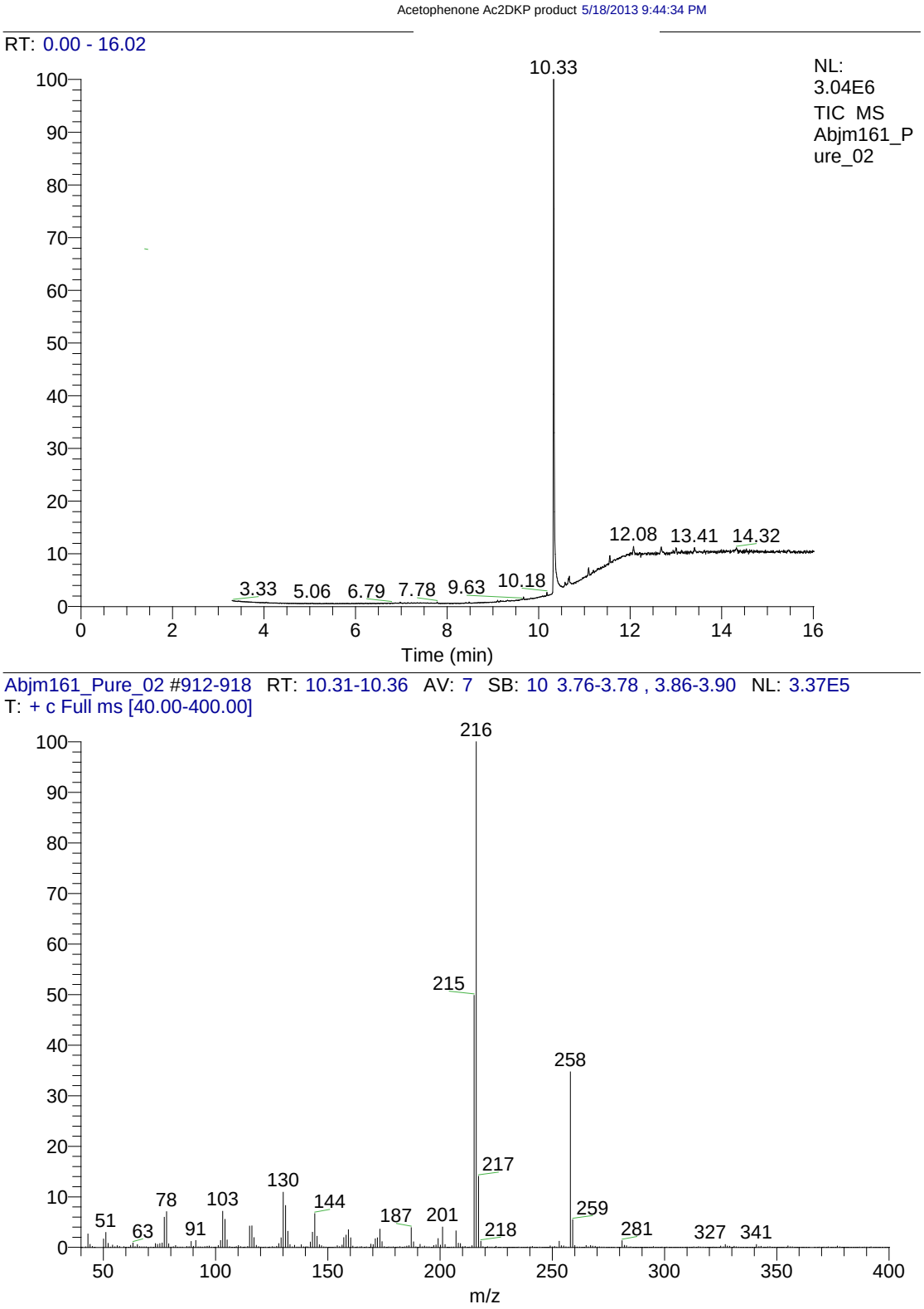


1-Acetyl-3-benzylidenepiperazine-2,5-dione



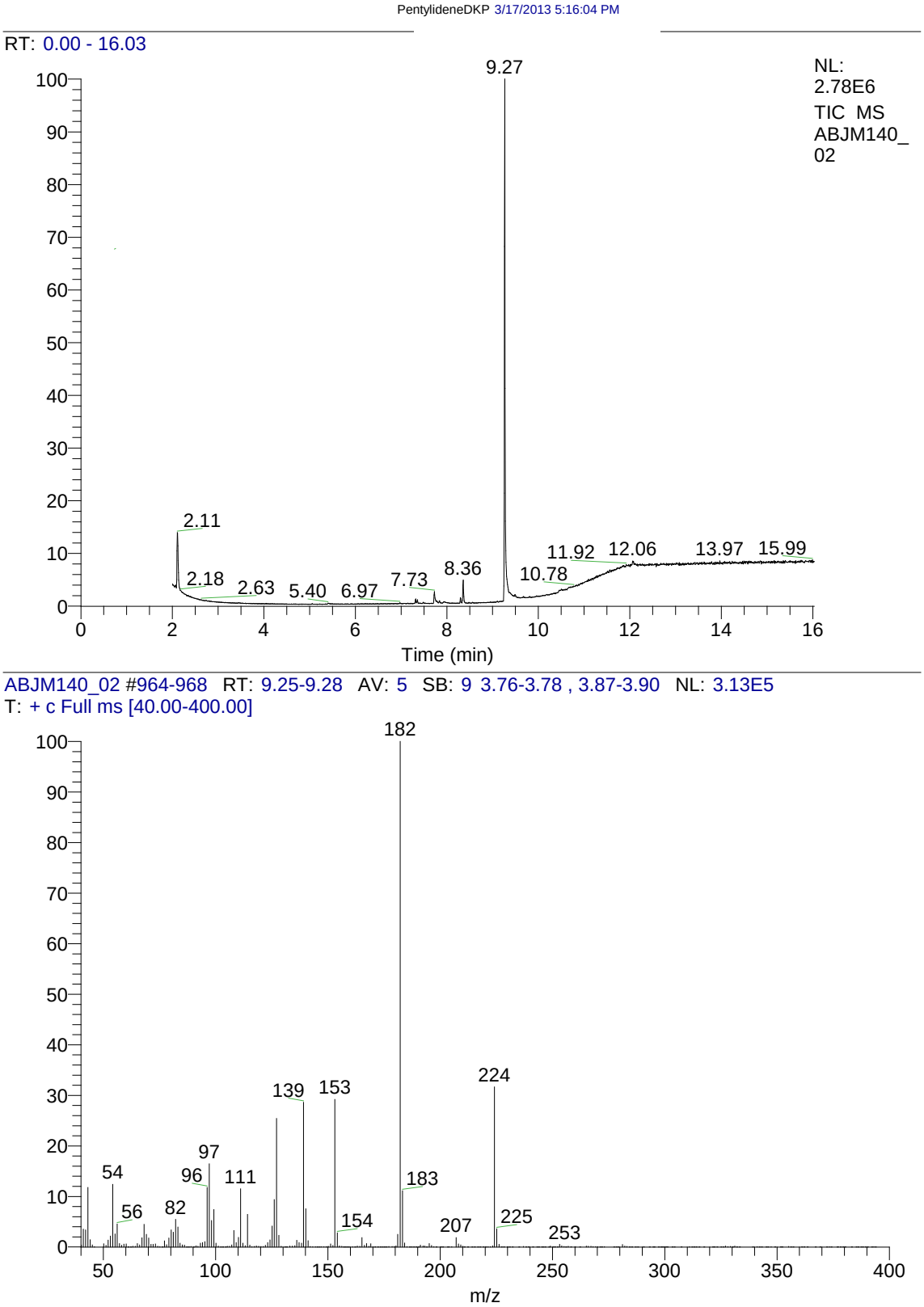


1-Acetyl-3-methyl-3-benzylidenepiperazine-2,5-dione



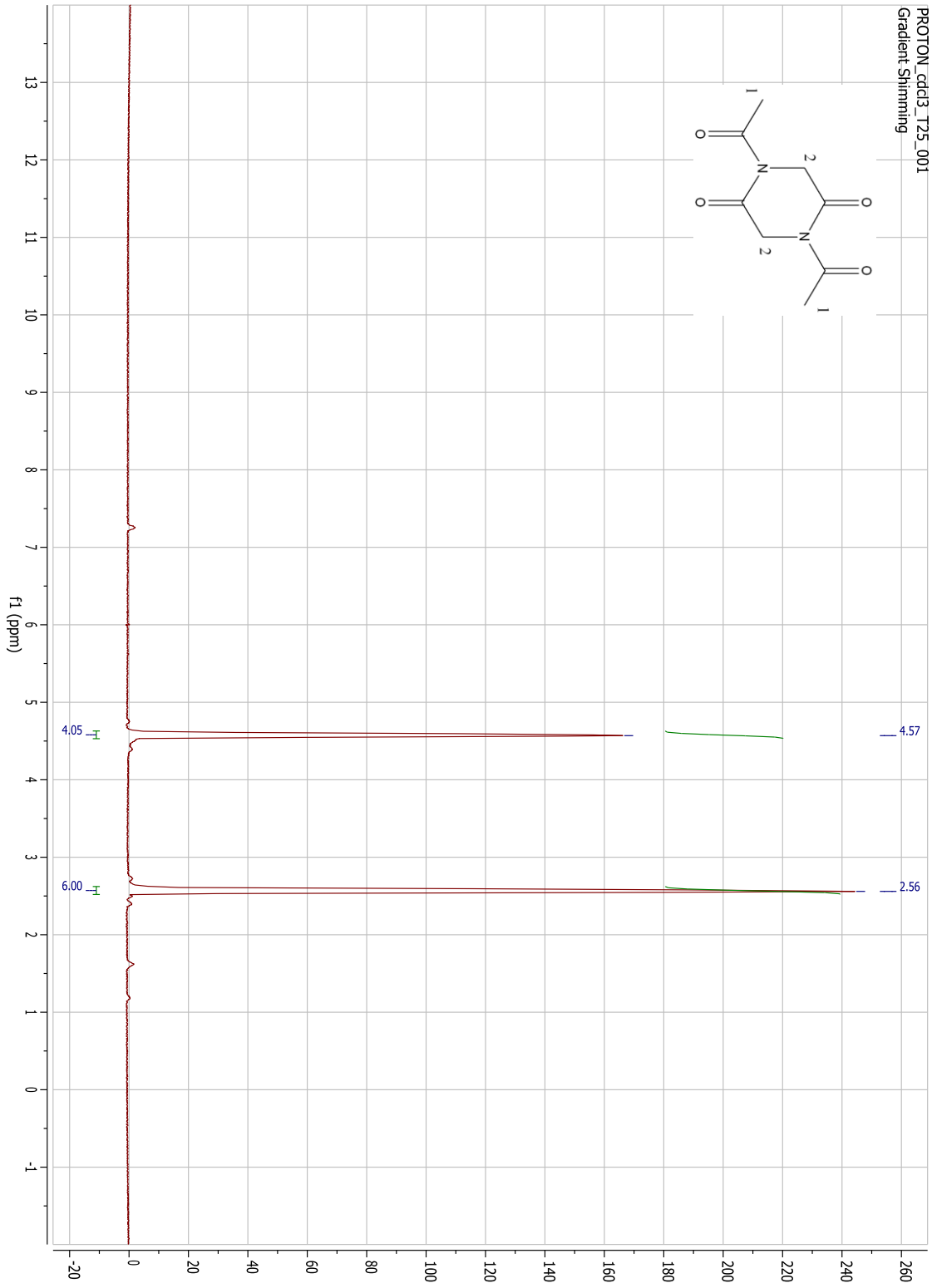
1-Acetyl-3-pentylidenepiperazine-2,5-dione

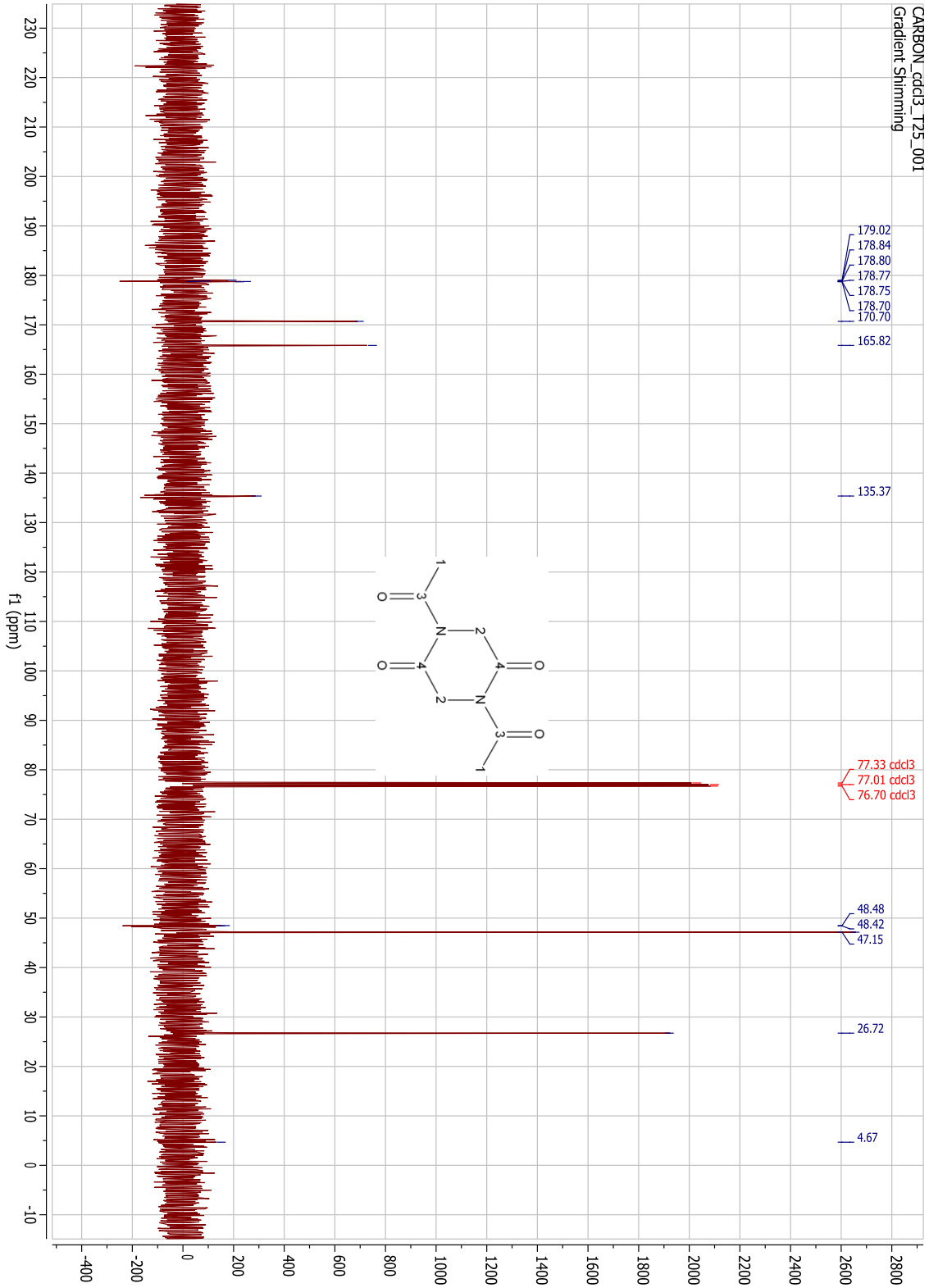
The extra signals on the gas chromatogram are contamination from the instrument



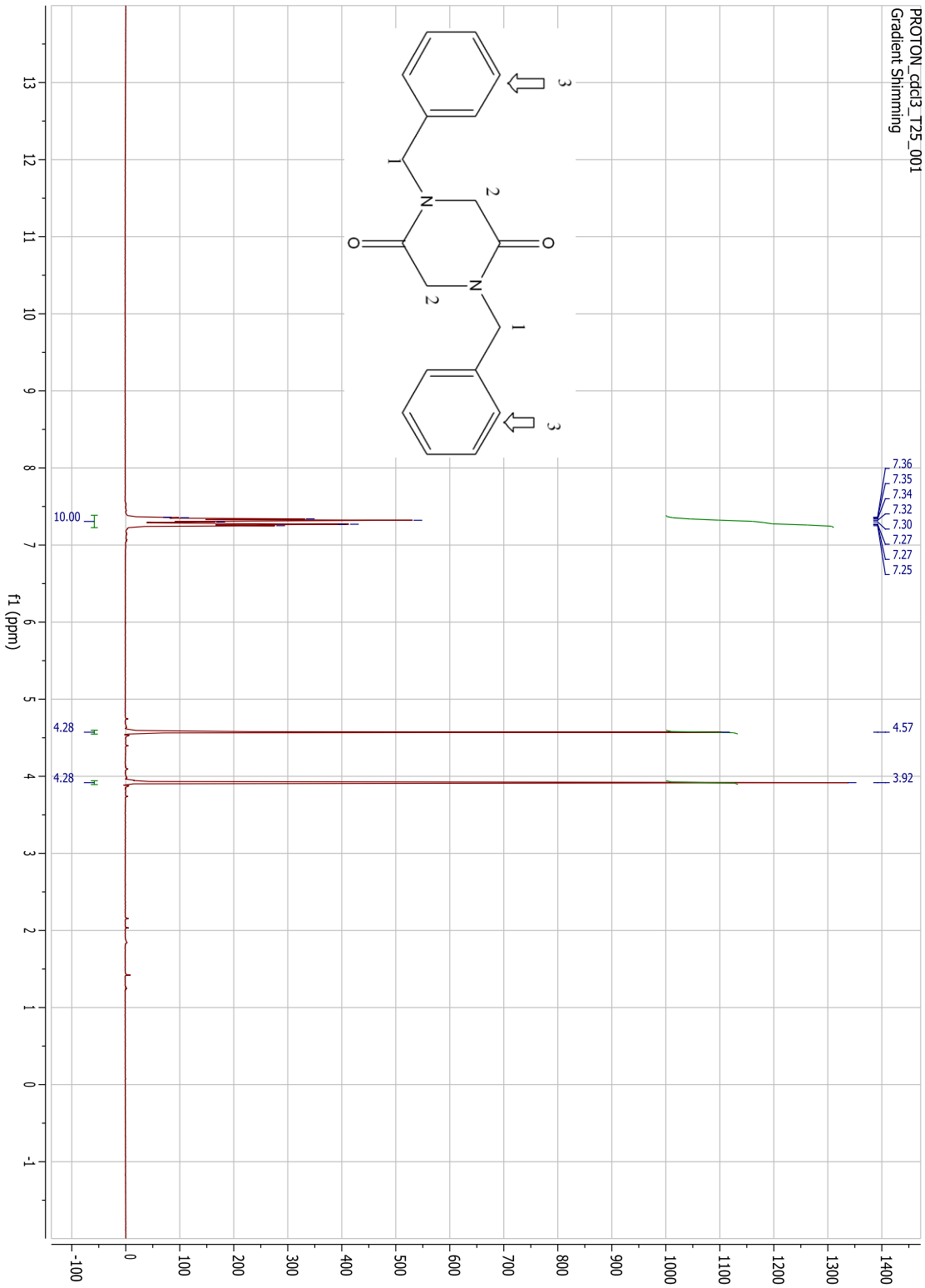
7.2 NMR Spectra

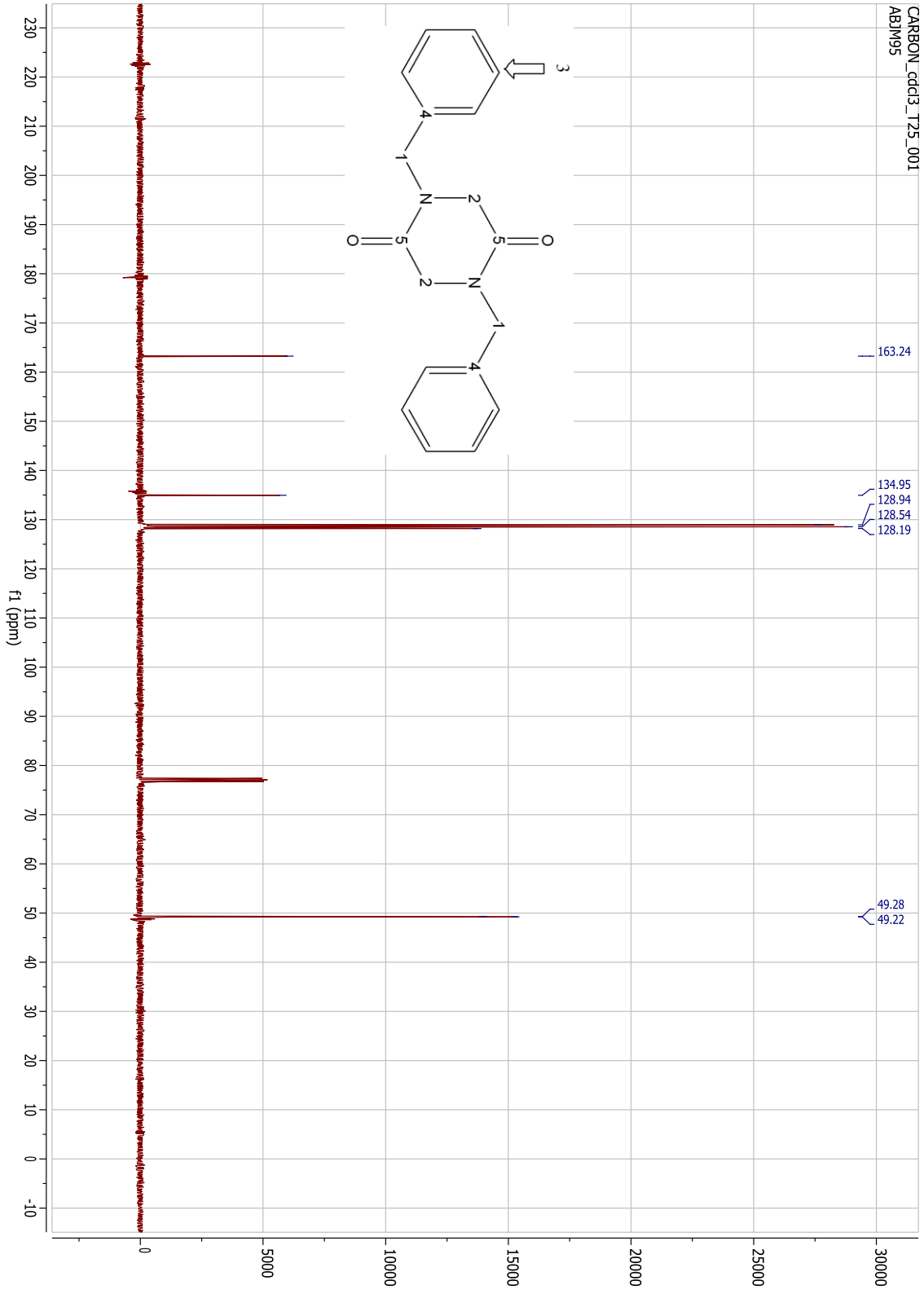
1,4-Diacetylpiperazine-2,5-dione



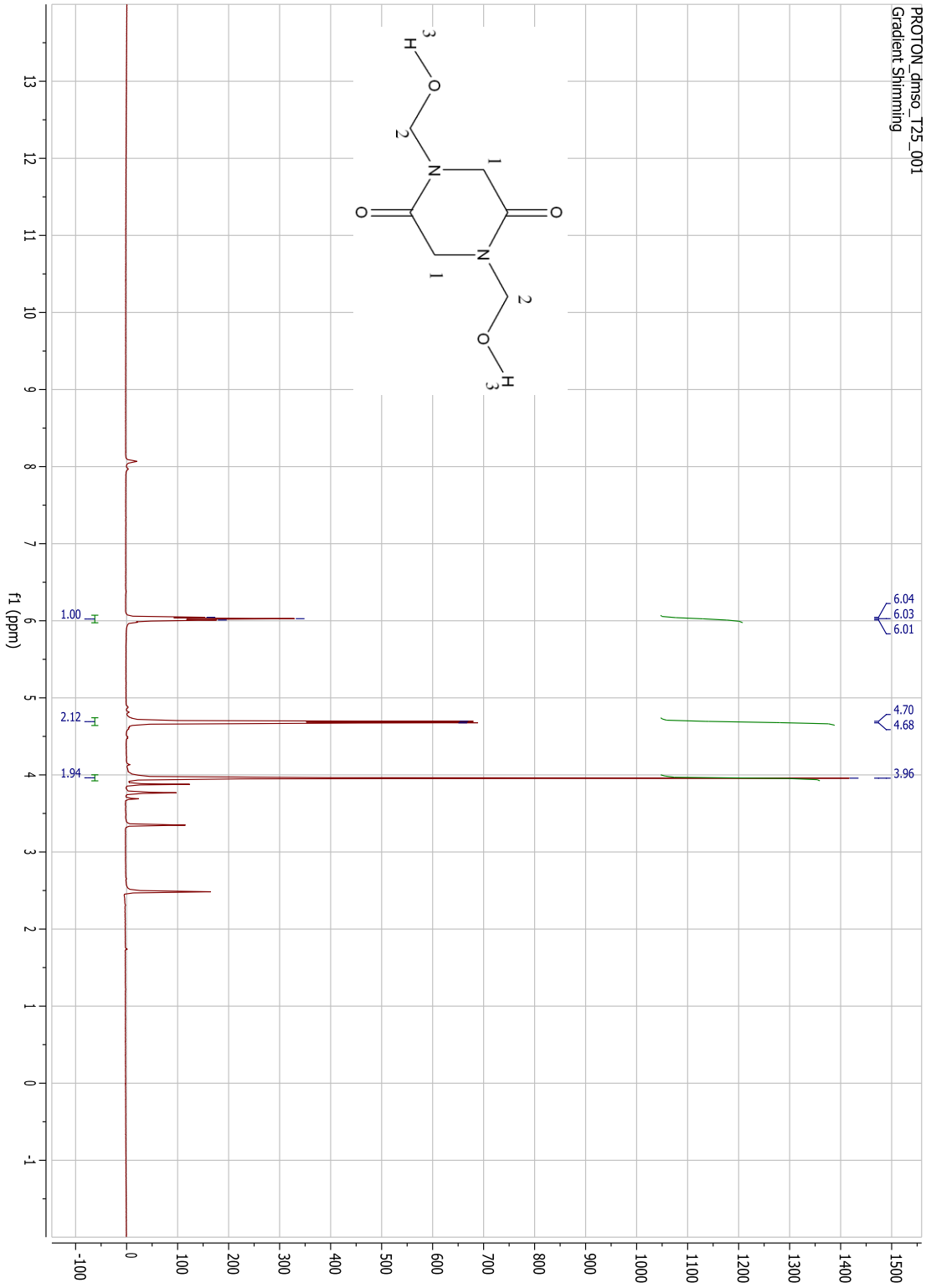

CARBON_cdcl3_T25_001
Gradient Shimming
f1 (ppm)
179.02
178.84
178.80
178.77
178.75
178.70
170.70
165.82
135.37
77.33 cdcl3
77.01 cdcl3
76.70 cdcl3
48.48
48.42
47.15
26.72
4.67

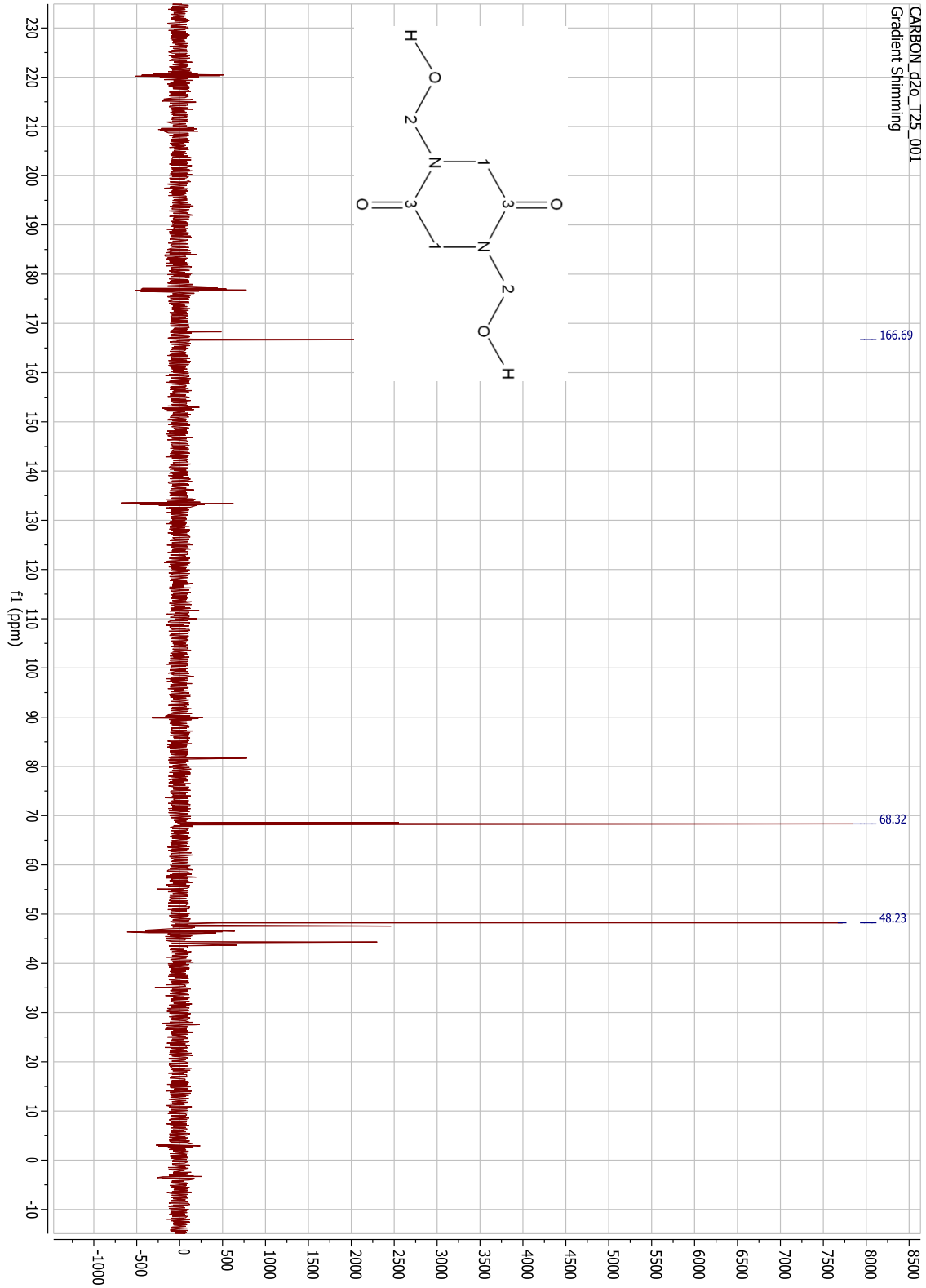
1,4-Dibenzylpiperazine-2,5-dione



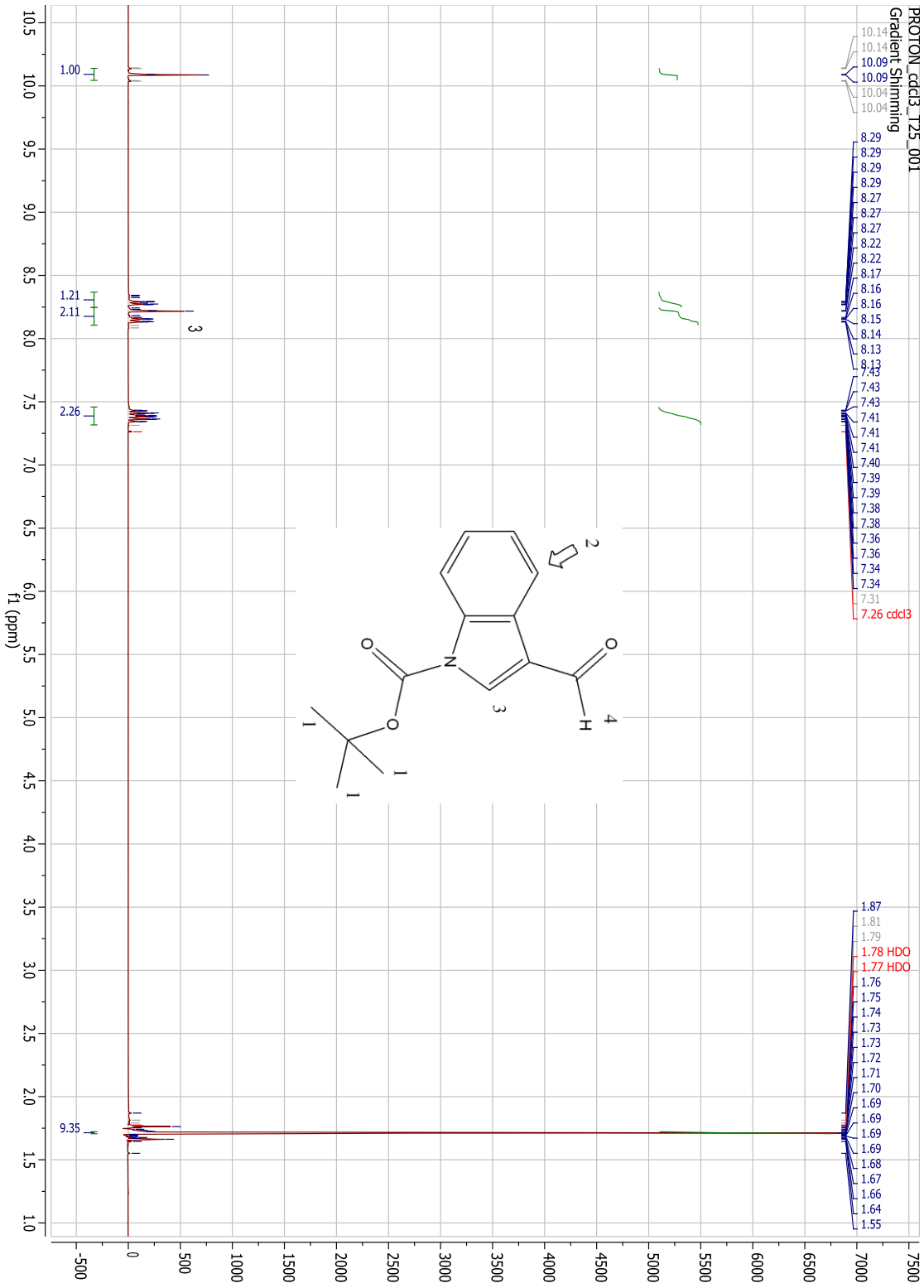

CARBON_cdcl3_T25_001
ABJM95
163.24
134.95
128.94
128.54
128.19
49.28
49.22
f1 (ppm)

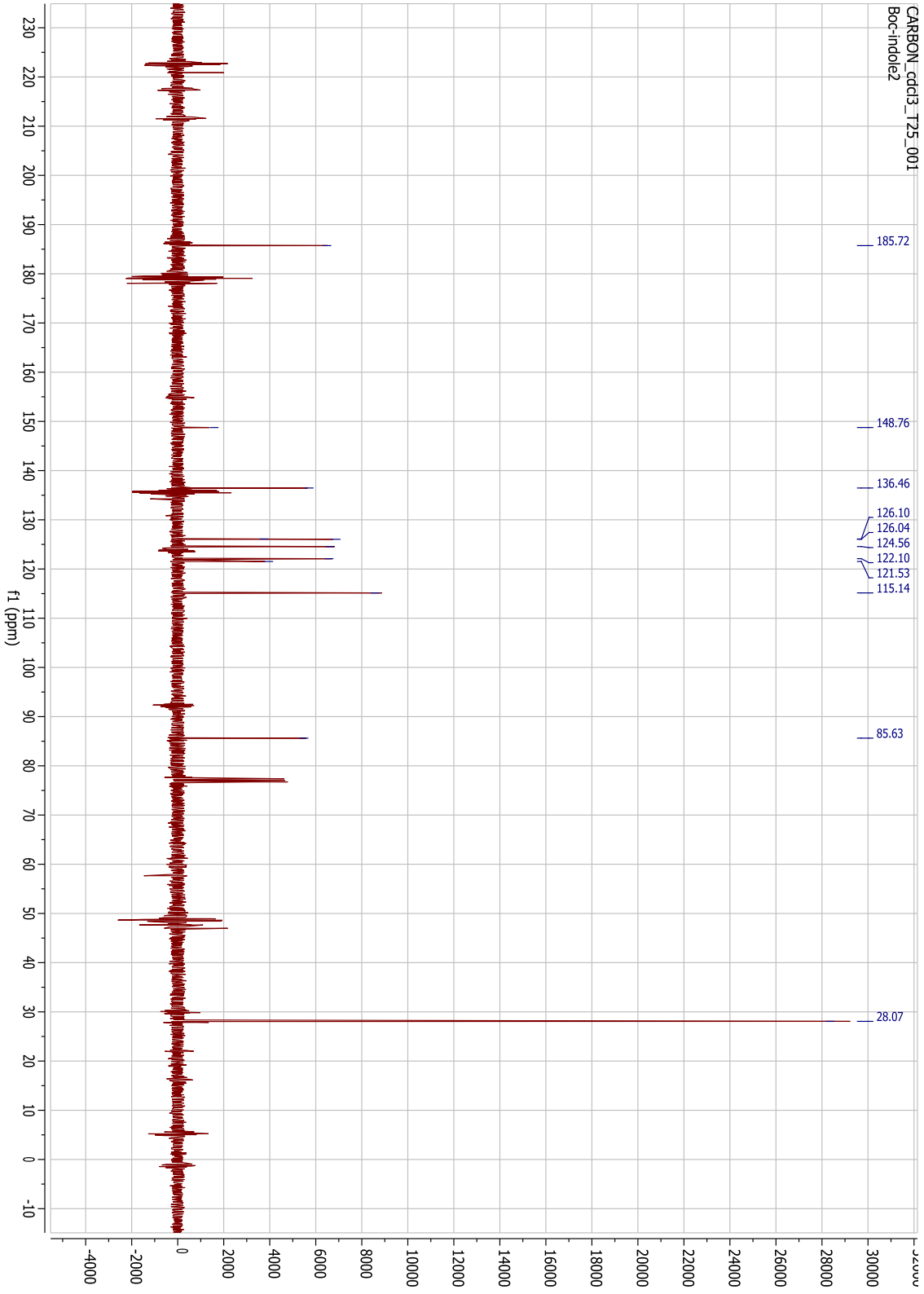
1,4-Bis(hydroxymethyl)piperazine-2,5-dione



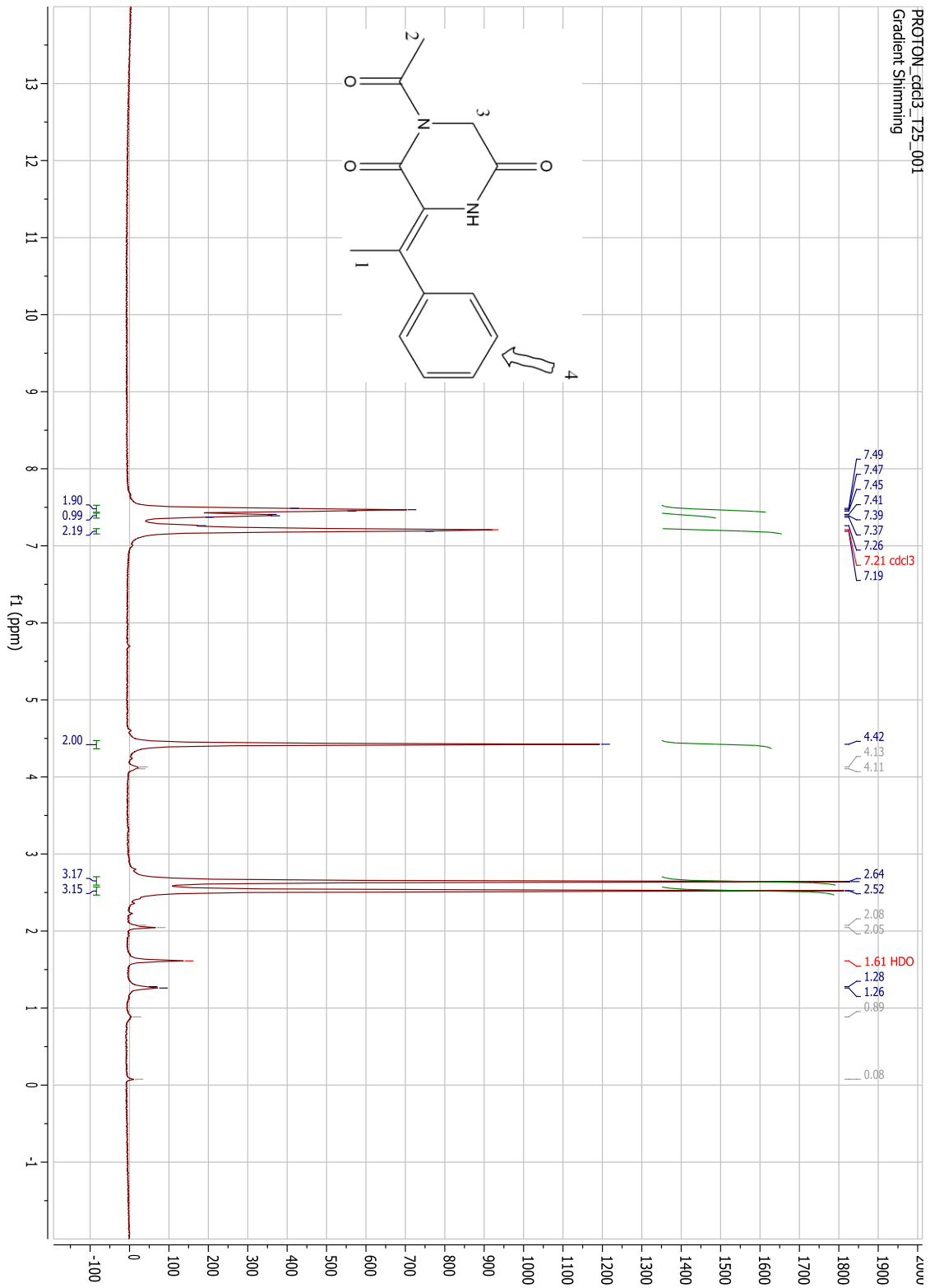

CARBON_d2o_T25_001
Gradient Shimming
166.69
68.32
48.23
f1 (ppm)

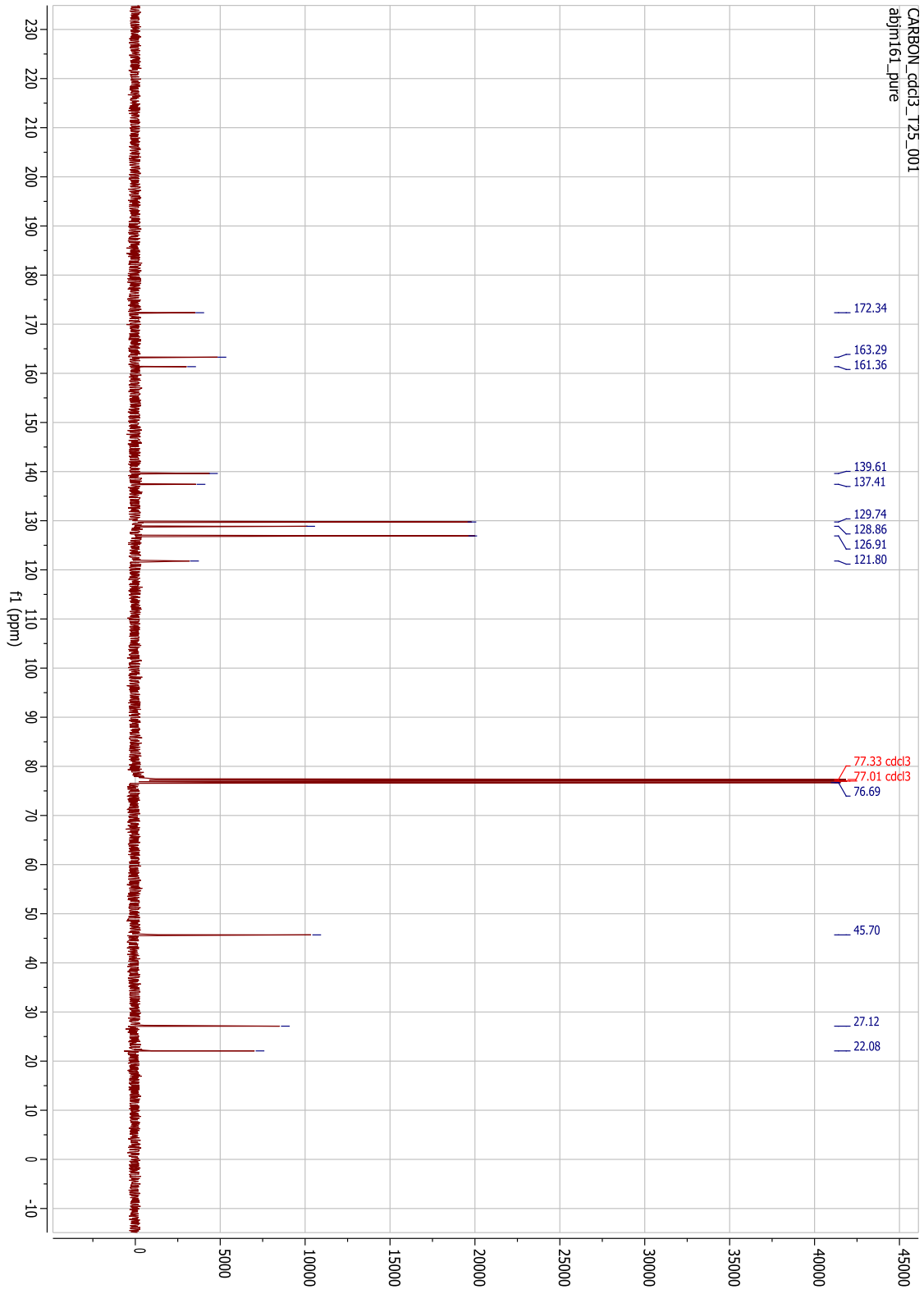
N-BocIndole-3-carboxaldehyde



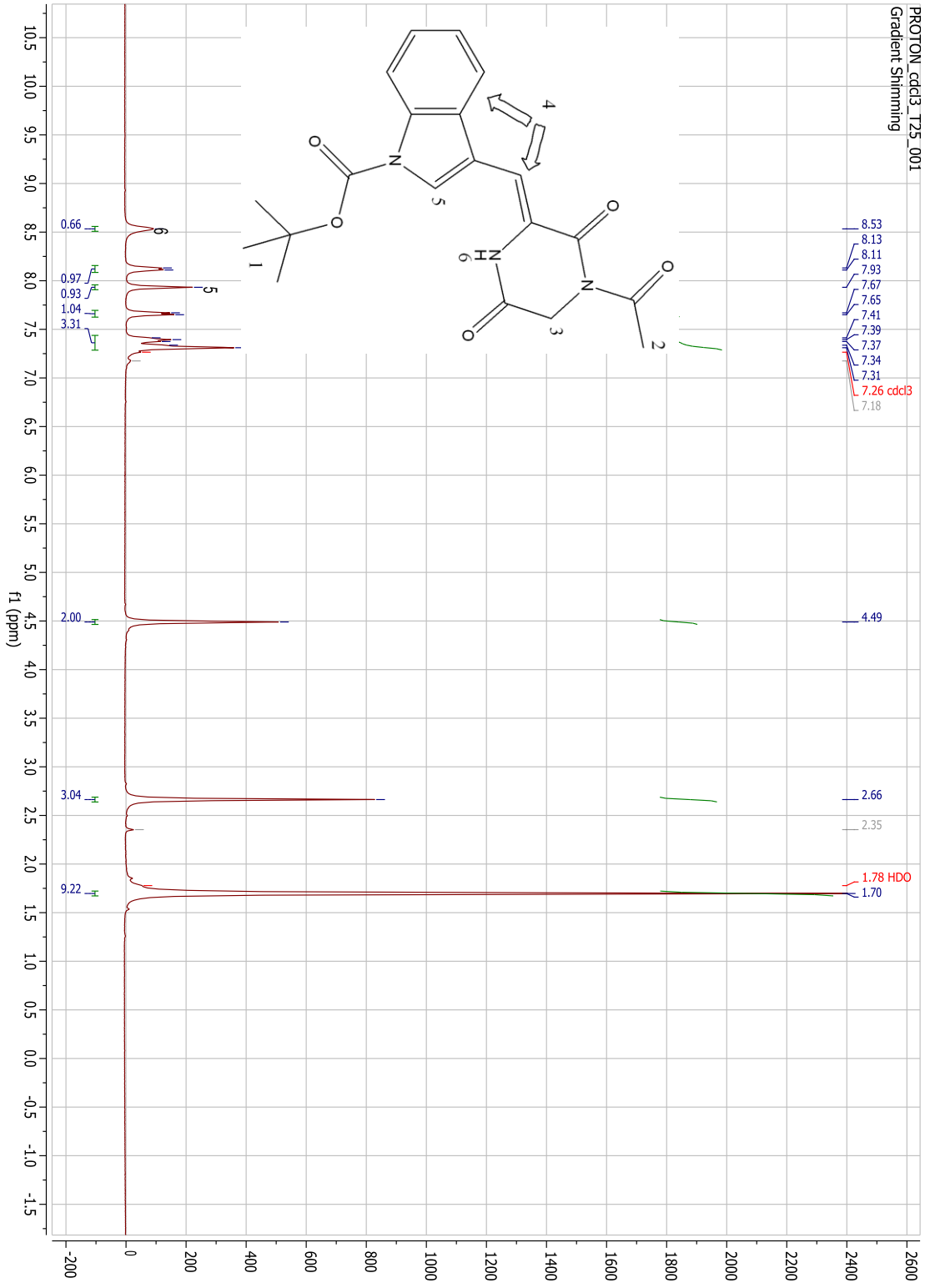

CARBON_cdcl3_T25_001
Boc-indole2
185.72
148.76
136.46
126.10
126.04
124.56
122.10
121.53
115.14
85.63
28.07
f1 (ppm)

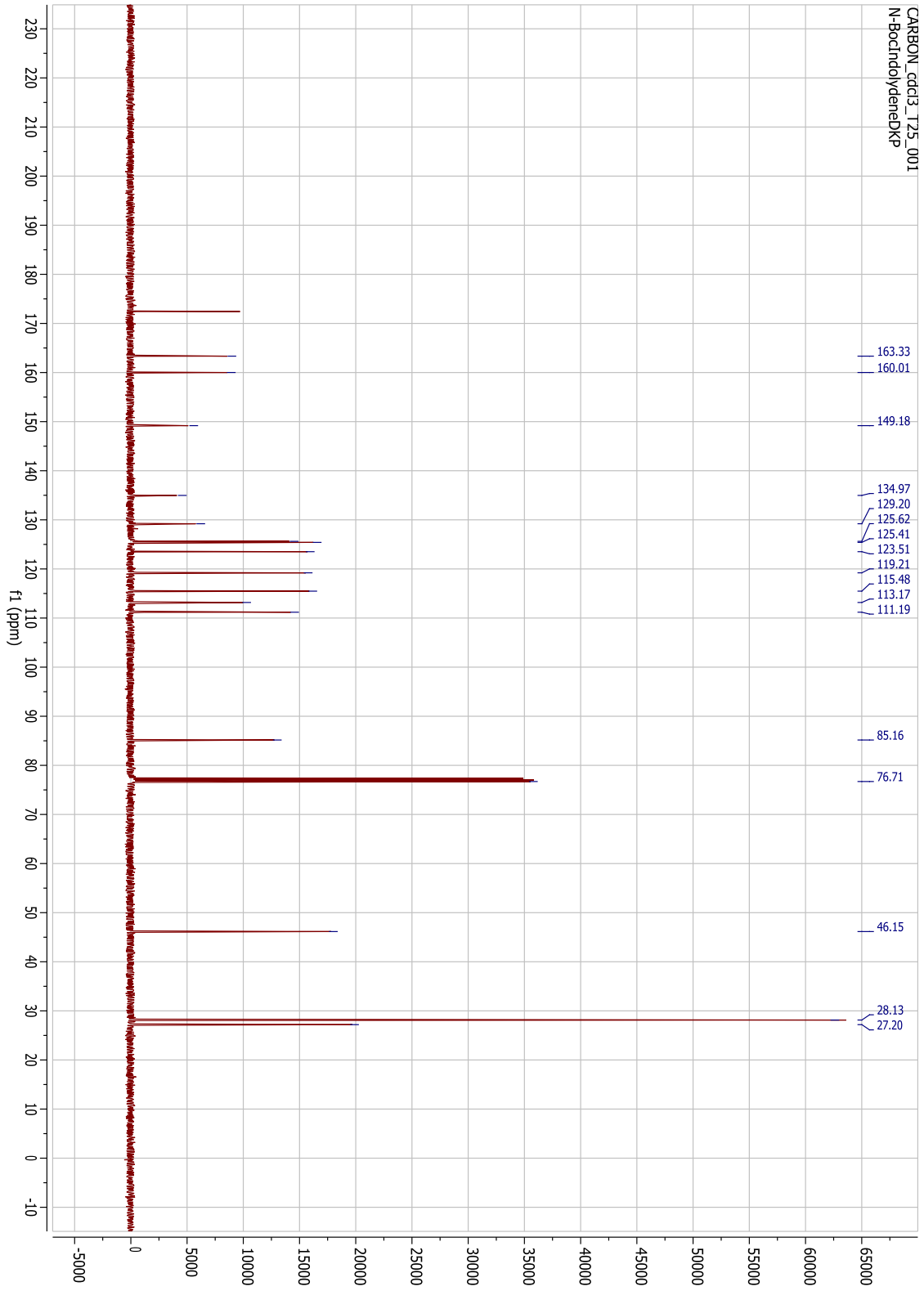
1-Acetyl-3-methyl-3-benzylidenepiperazine-2,5-dione



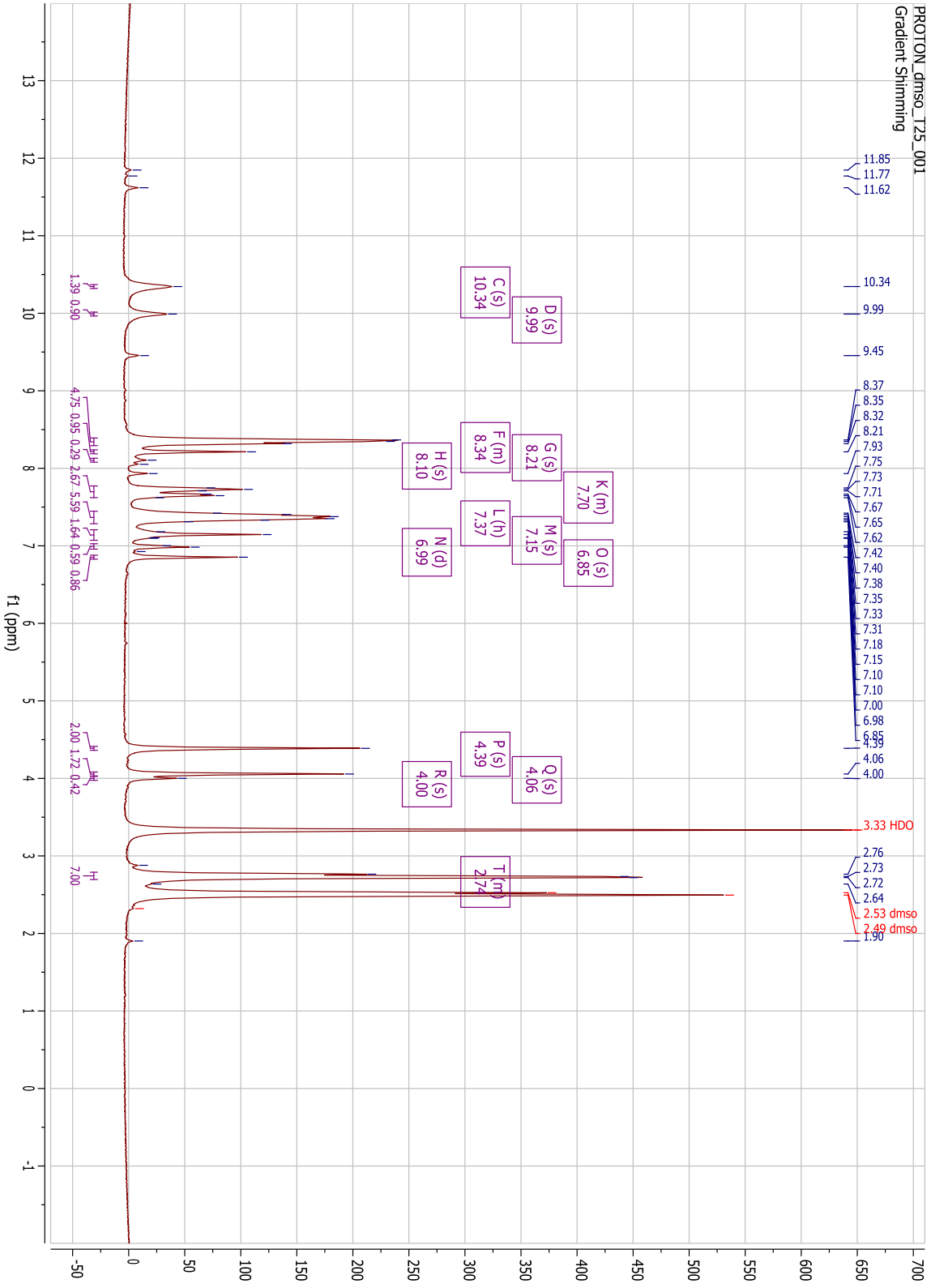

CARBON_cdcl3_T25_001
abjm161_pure
172.34
163.29
161.36
139.61
137.41
129.74
128.86
126.91
121.80
77.33 cdcl3
77.01 cdcl3
76.69
45.70
27.12
22.08
f1 (ppm)

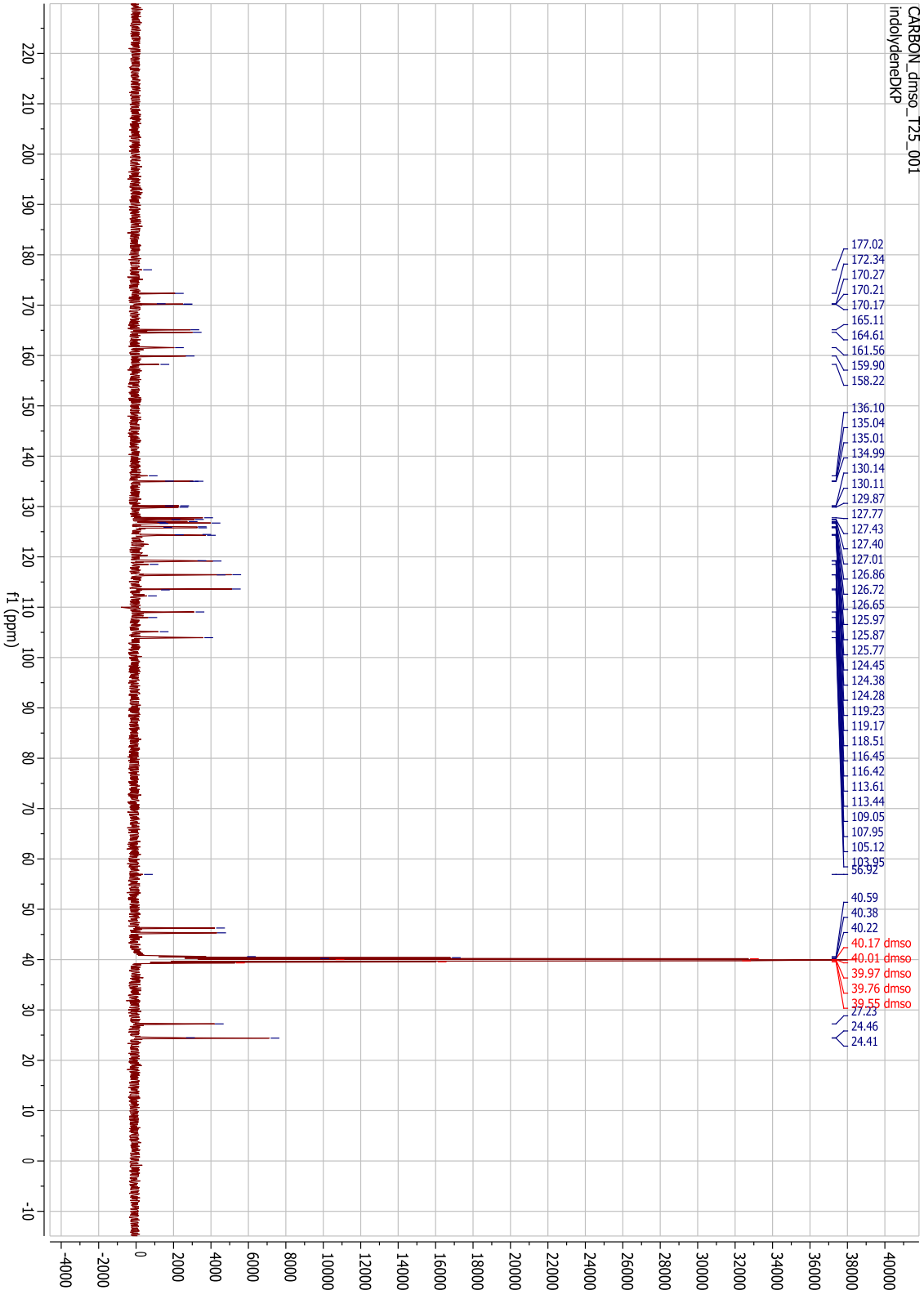
1-Acetyl-3-(N-Boc)Indolidenepiperazine-2,5-dione



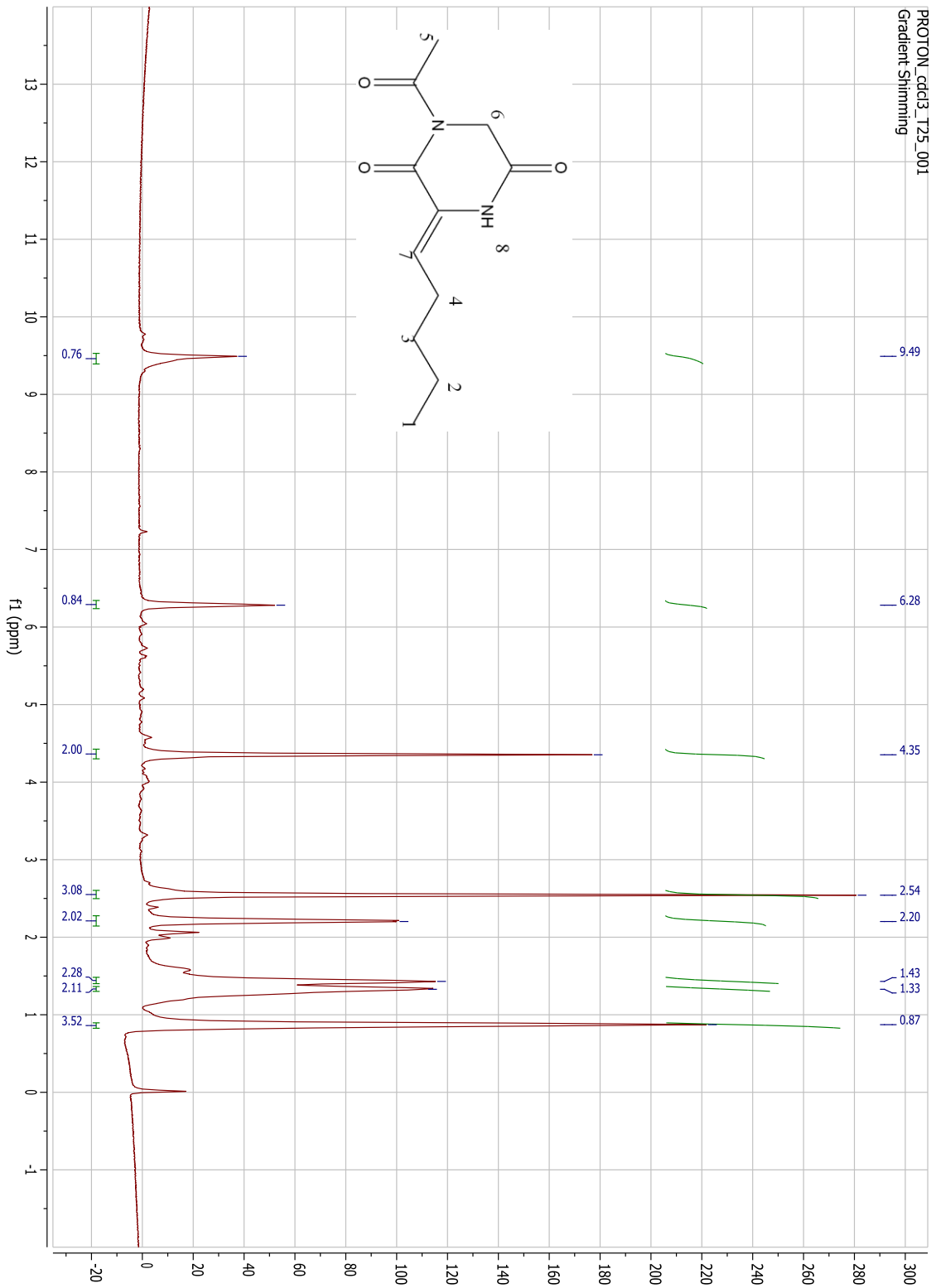

CARBON_cdcl3_TZ5_001
N-BocIndolydeneDKP
163.33
160.01
149.18
134.97
129.20
125.62
125.41
123.51
119.21
115.48
113.17
111.19
85.16
76.71
46.15
28.13
27.20
f1 (ppm)

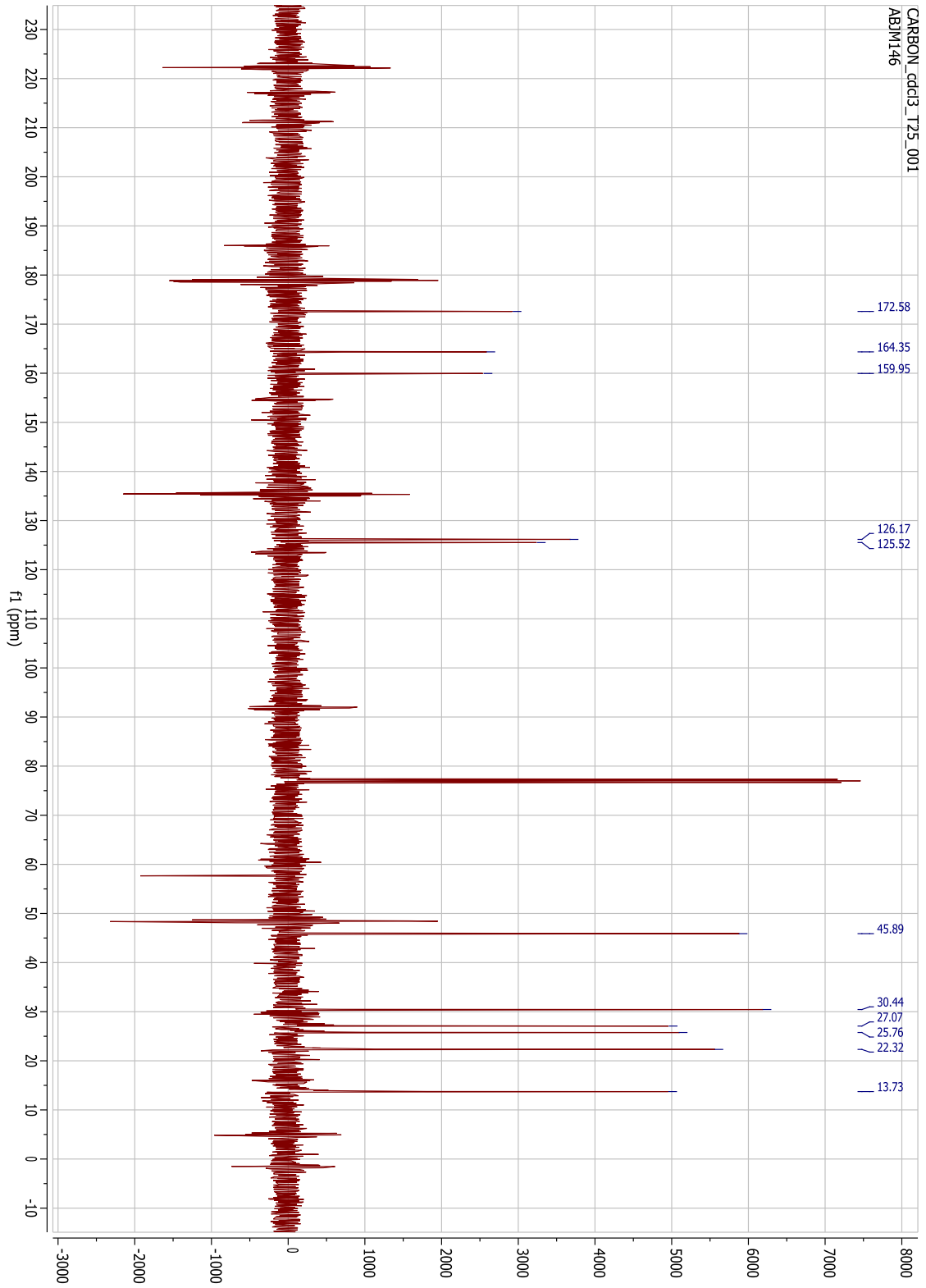
1-Acetyl-3-indolidenepiperazine-2,5-dione



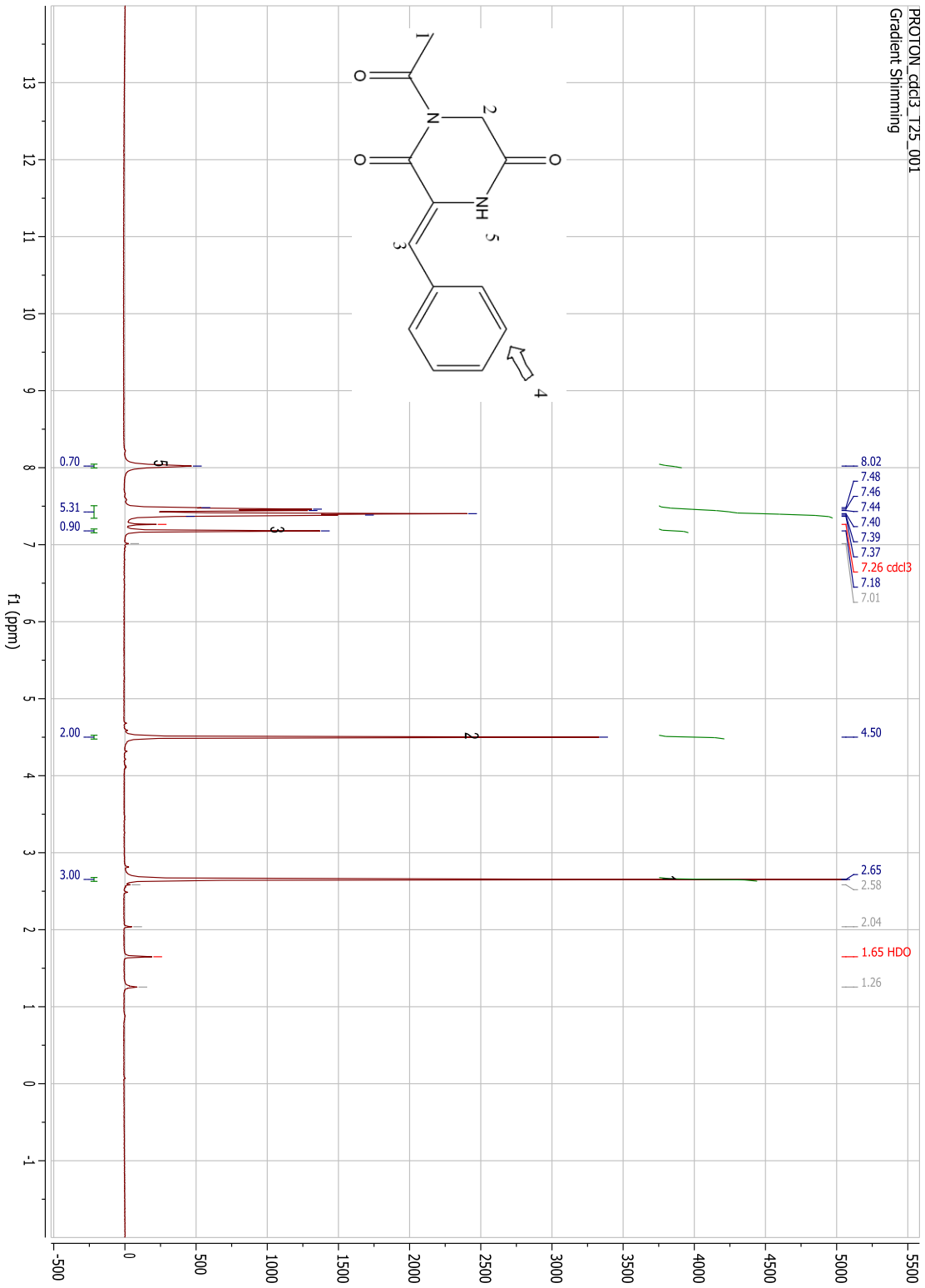

CARBON_dmso_T25_001
indolydeneDKP
f1 (ppm)
177.02
172.34
170.27
170.21
170.17
165.11
164.61
161.56
159.90
158.22
136.10
135.04
135.01
134.99
130.14
130.11
129.87
127.77
127.43
127.40
127.01
126.86
126.72
126.65
125.97
125.87
125.77
124.45
124.38
124.28
119.23
119.17
118.51
116.45
116.42
113.61
113.44
109.05
107.95
105.12
103.95
56.92
40.59
40.38
40.22
40.17 dmso
40.01 dmso
39.97 dmso
39.76 dmso
39.55 dmso
27.23
24.46
24.41

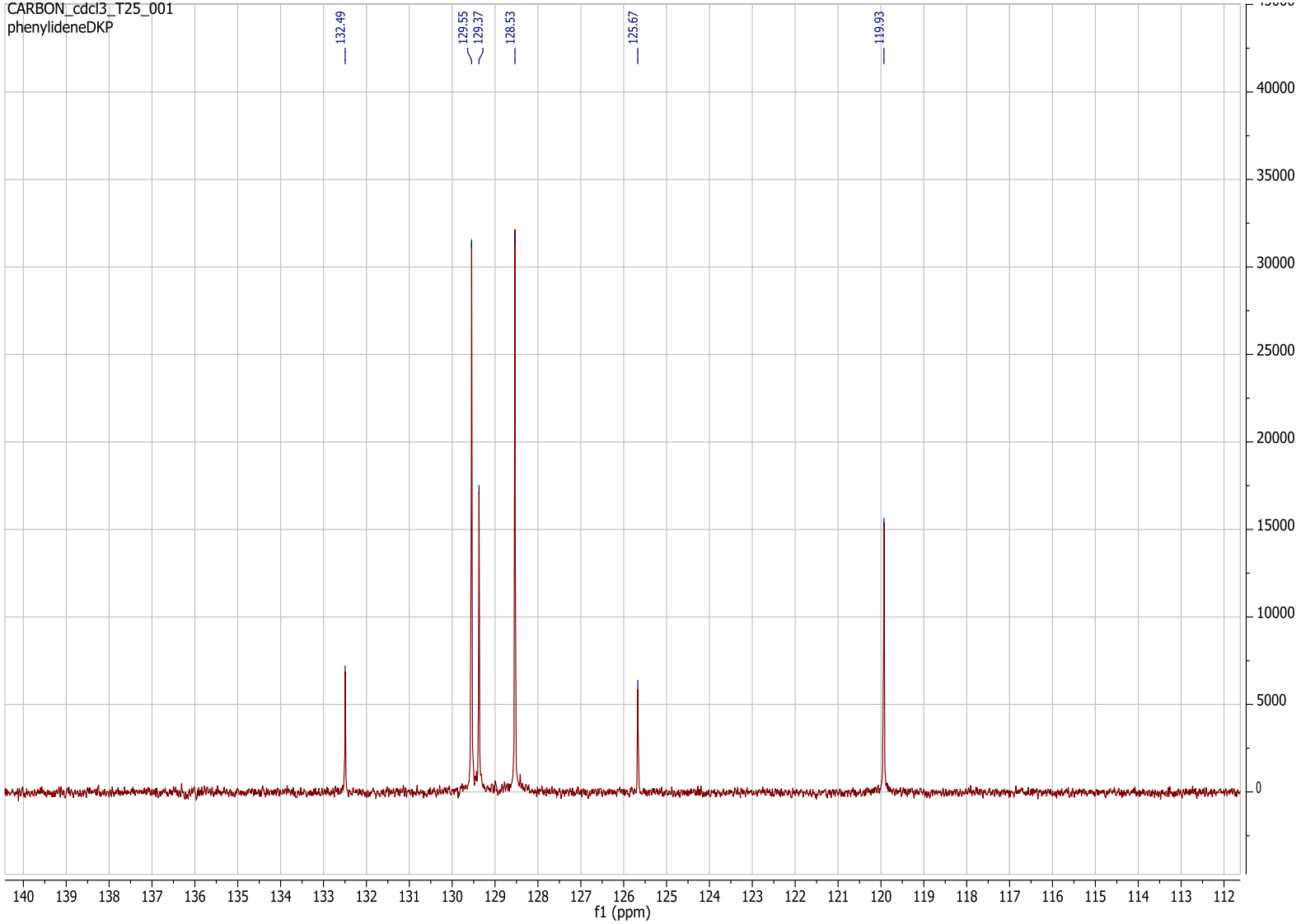
1-Acetyl-3-pentylidenepiperazine-2,5-dione




CARBON_cdcl3_T25_001
ABJM146
172.58
164.35
159.95
126.17
125.52
45.89
30.44
27.07
25.76
22.32
13.73
f1 (ppm)

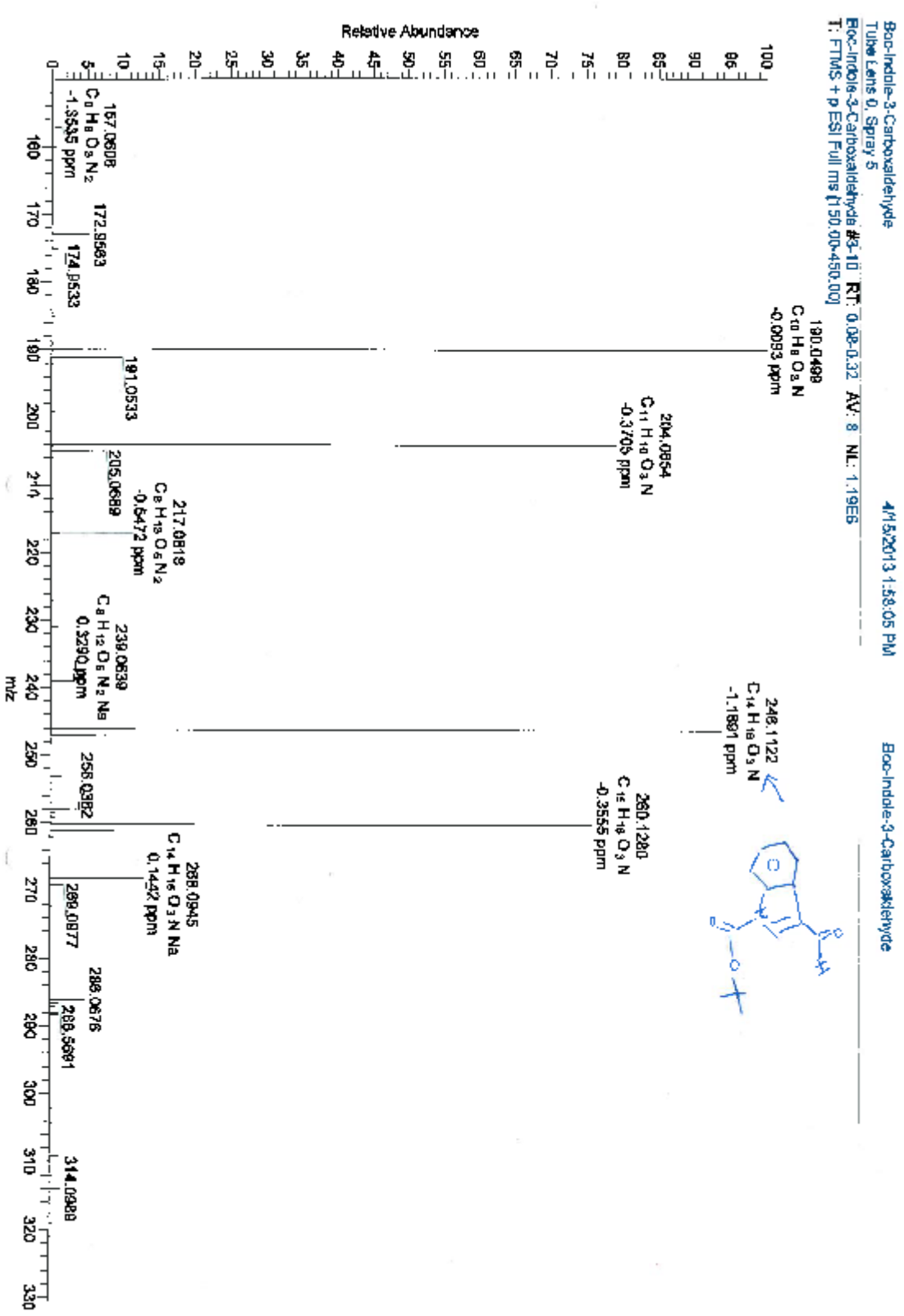
1-Acetyl-3-benzylidenepiperazine-2,5-dione



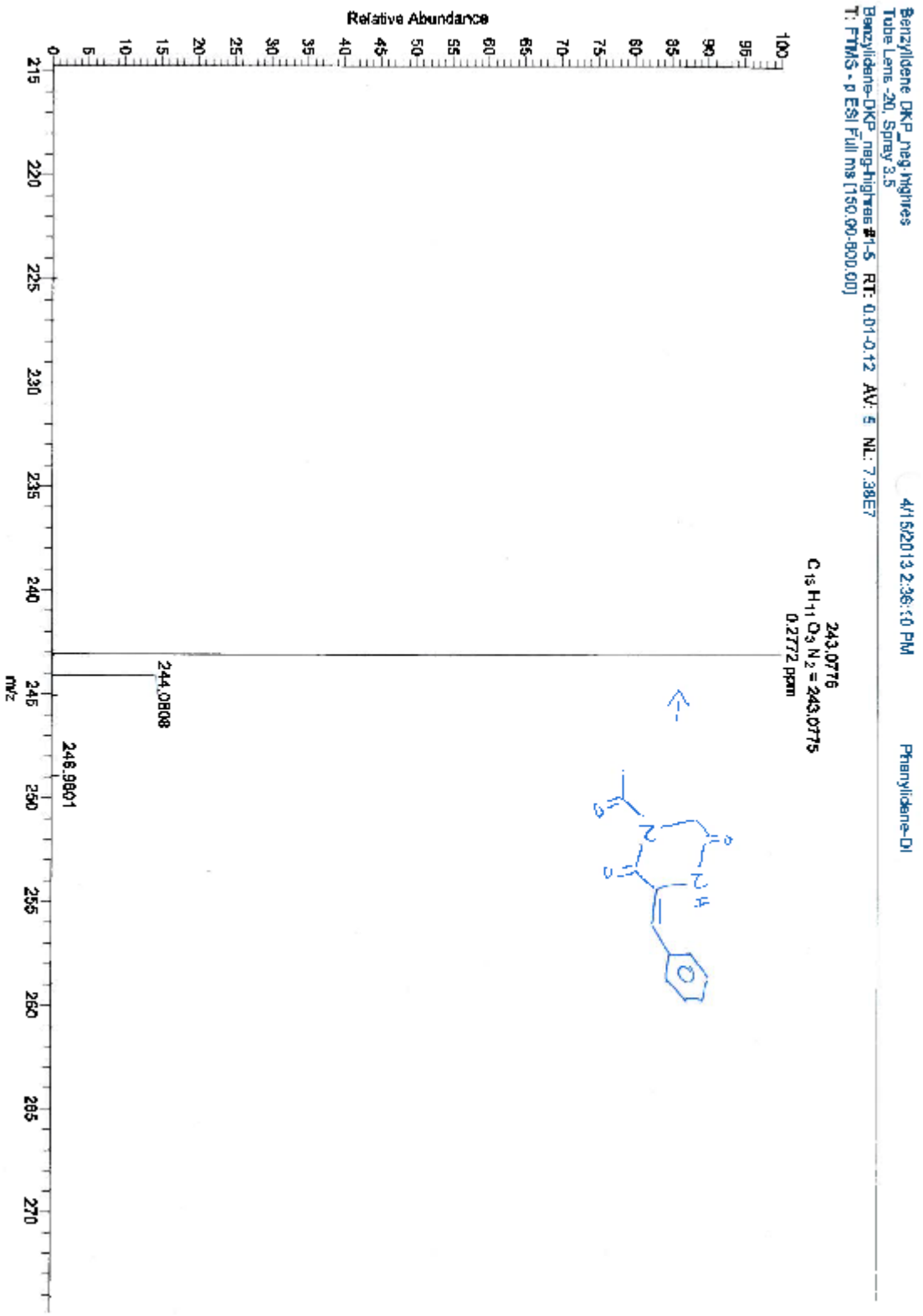

CARBON_cdcl3_T25_001
phenylideneDKP
132.49
129.55
129.37
128.53
125.67
119.93
40000
35000
30000
25000
20000
15000
10000
5000
0
140 139 138 137 136 135 134 133 132 131 130 129 128 127 126 125 124 123 122 121 120 119 118 117 116 115 114 113 112
f1 (ppm)

7.3 HRMS Spectra

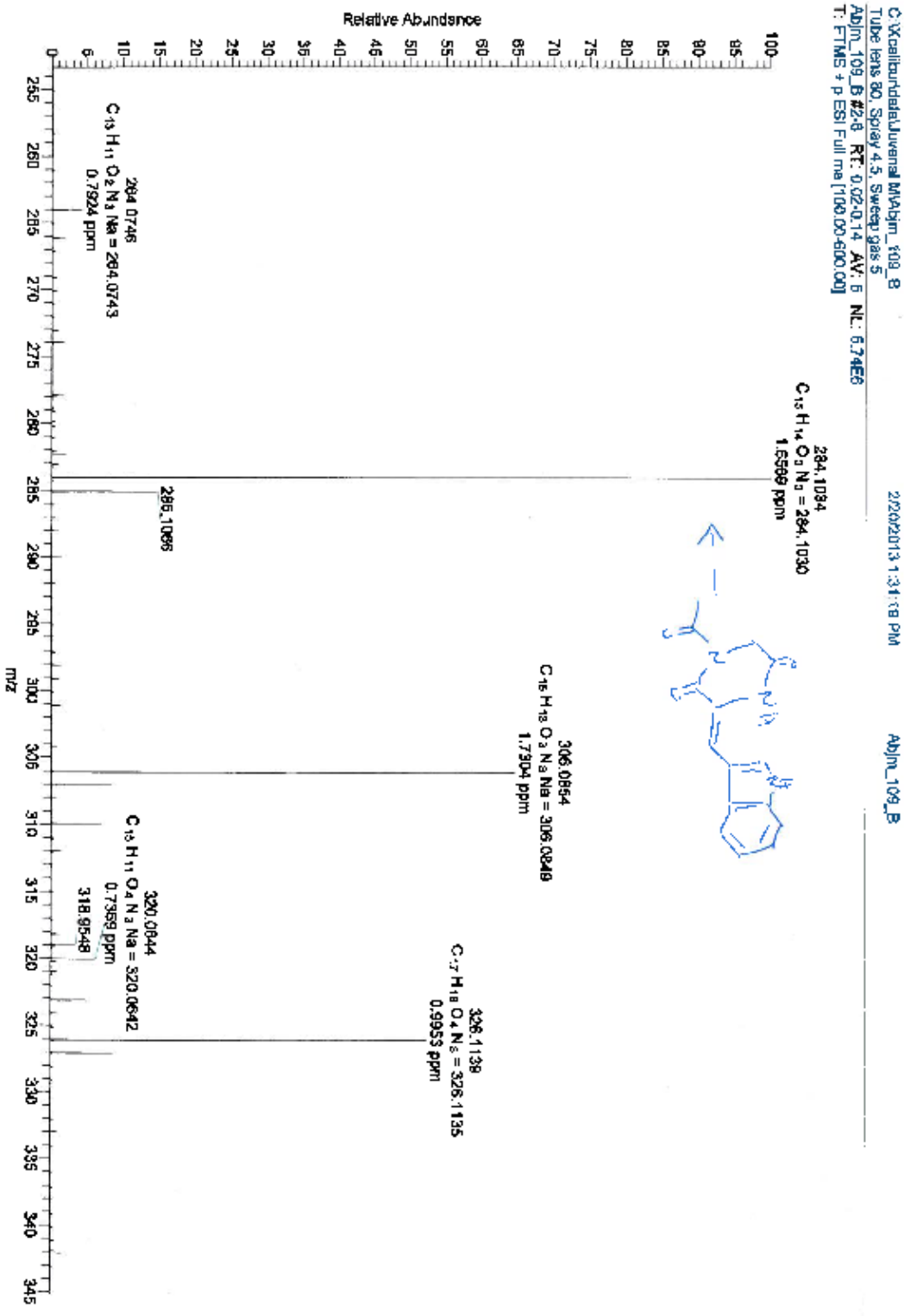
N-BocIndole-3-carboxaldehyde



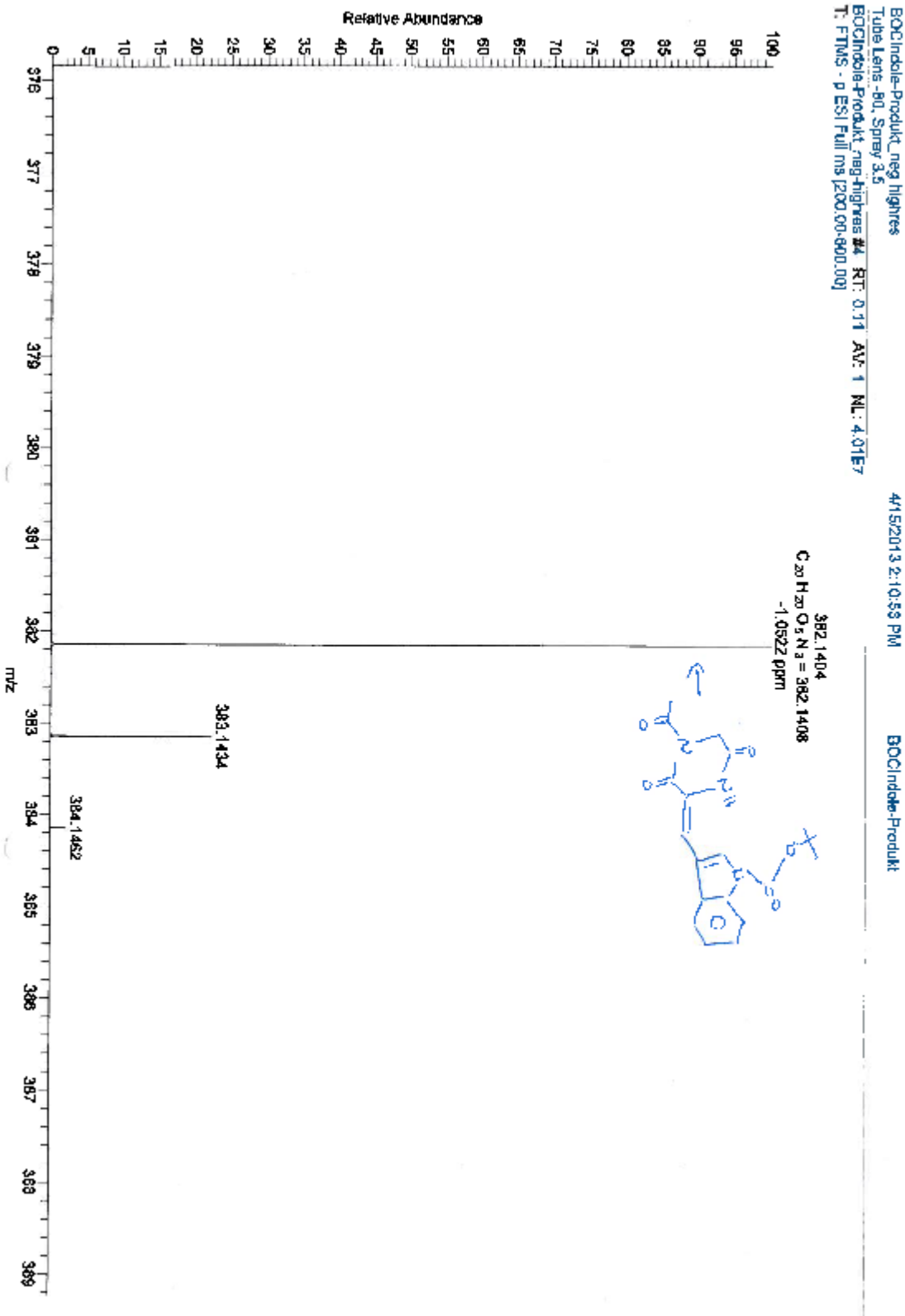
1-Acetyl-3-benzylidenepiperazine-2,5-dione



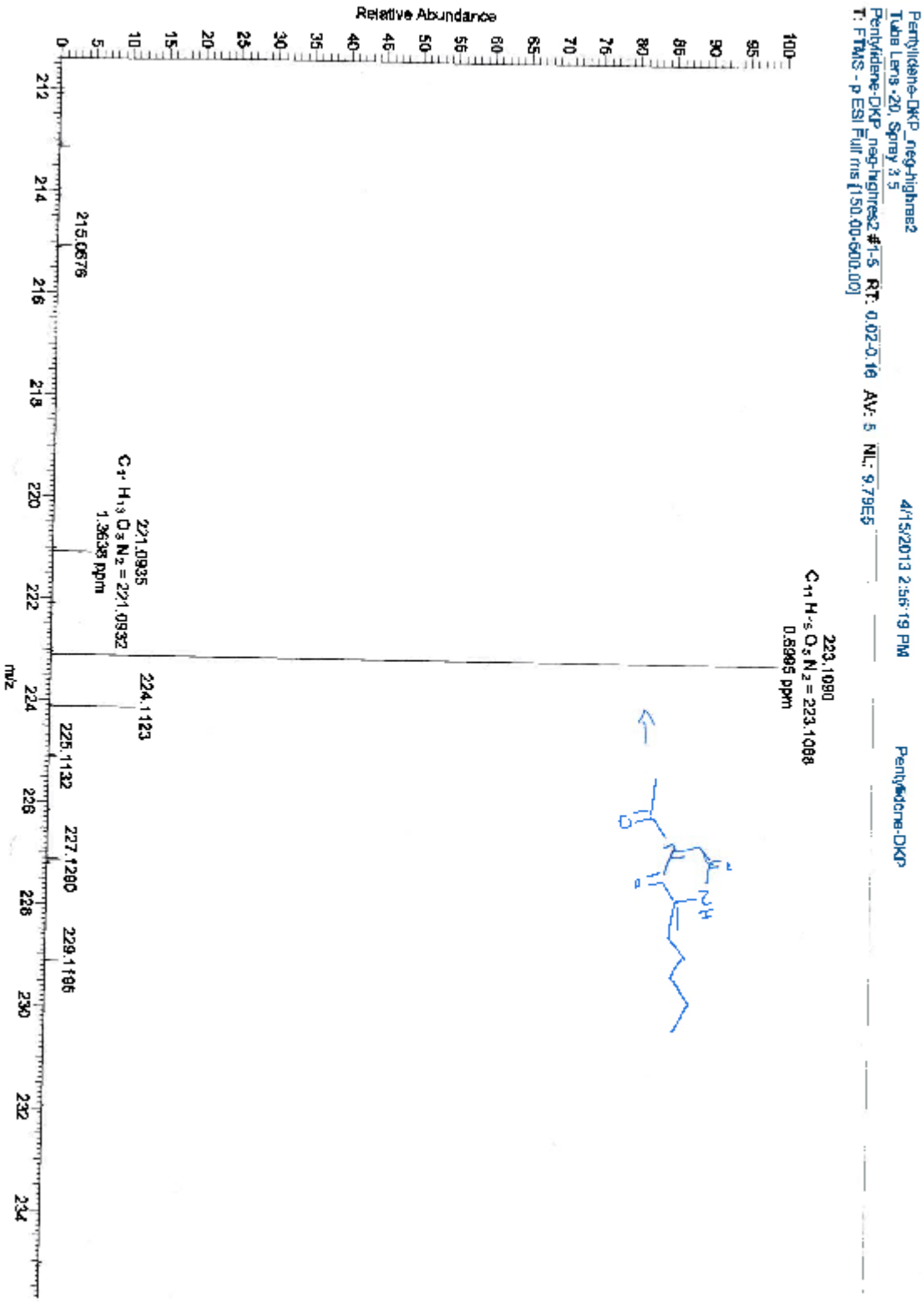
1-Acetyl-3-indolidenepiperazine-2,5-dione



1-Acetyl-3-(N-Boc)Indolidenepiperazine-2,5-dione

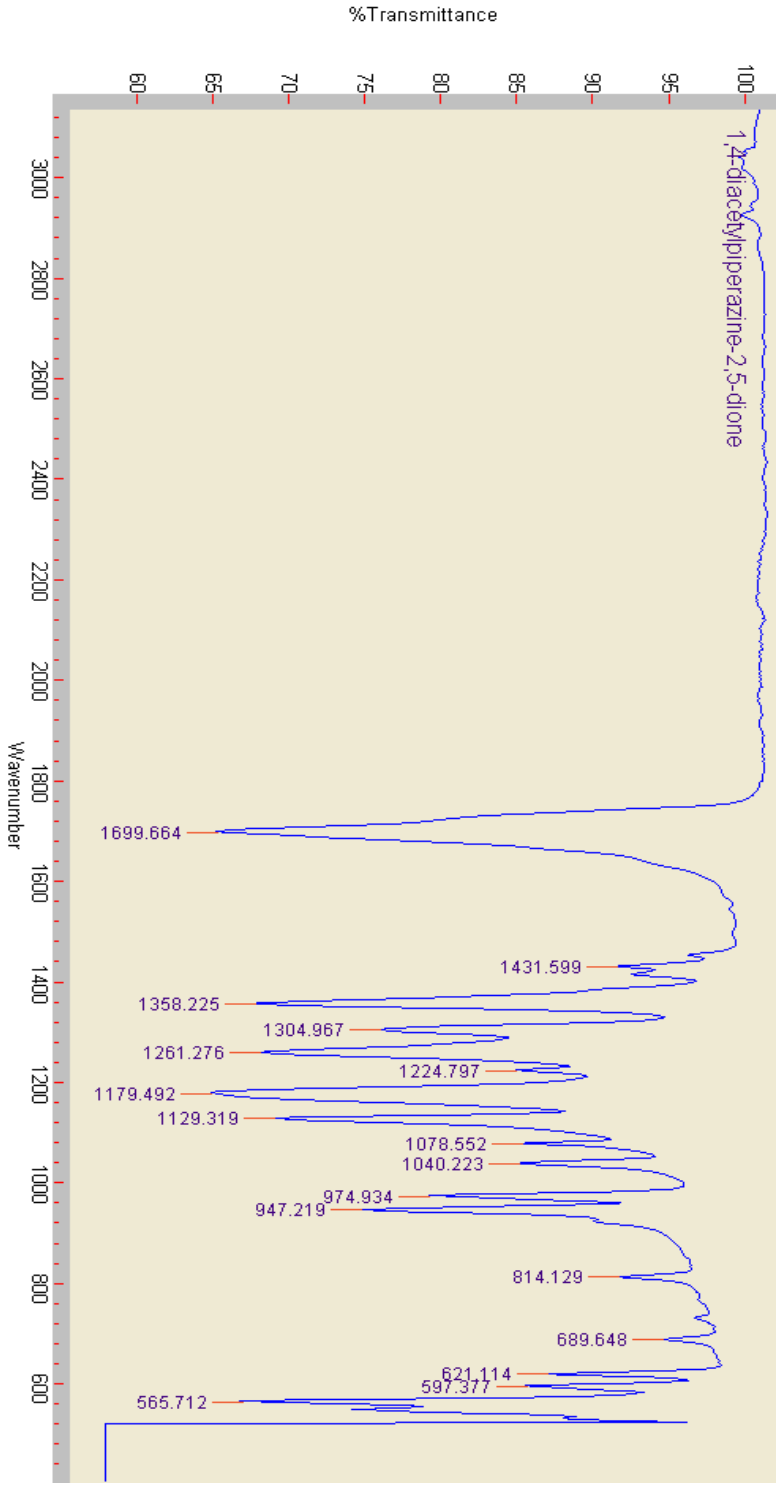


1-Acetyl-3-pentylidenepiperazine-2,5-dione

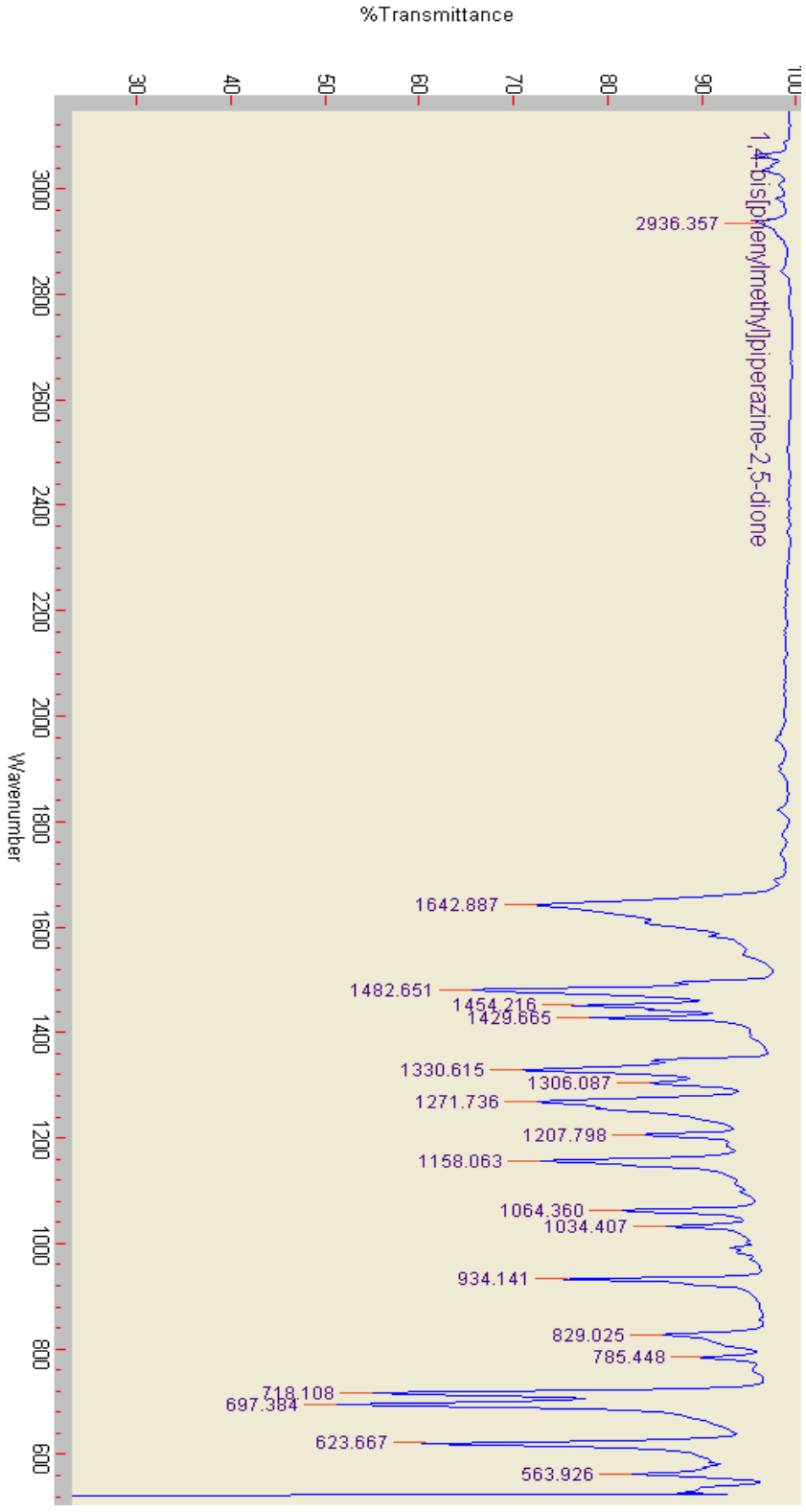


7.4 IR spectra

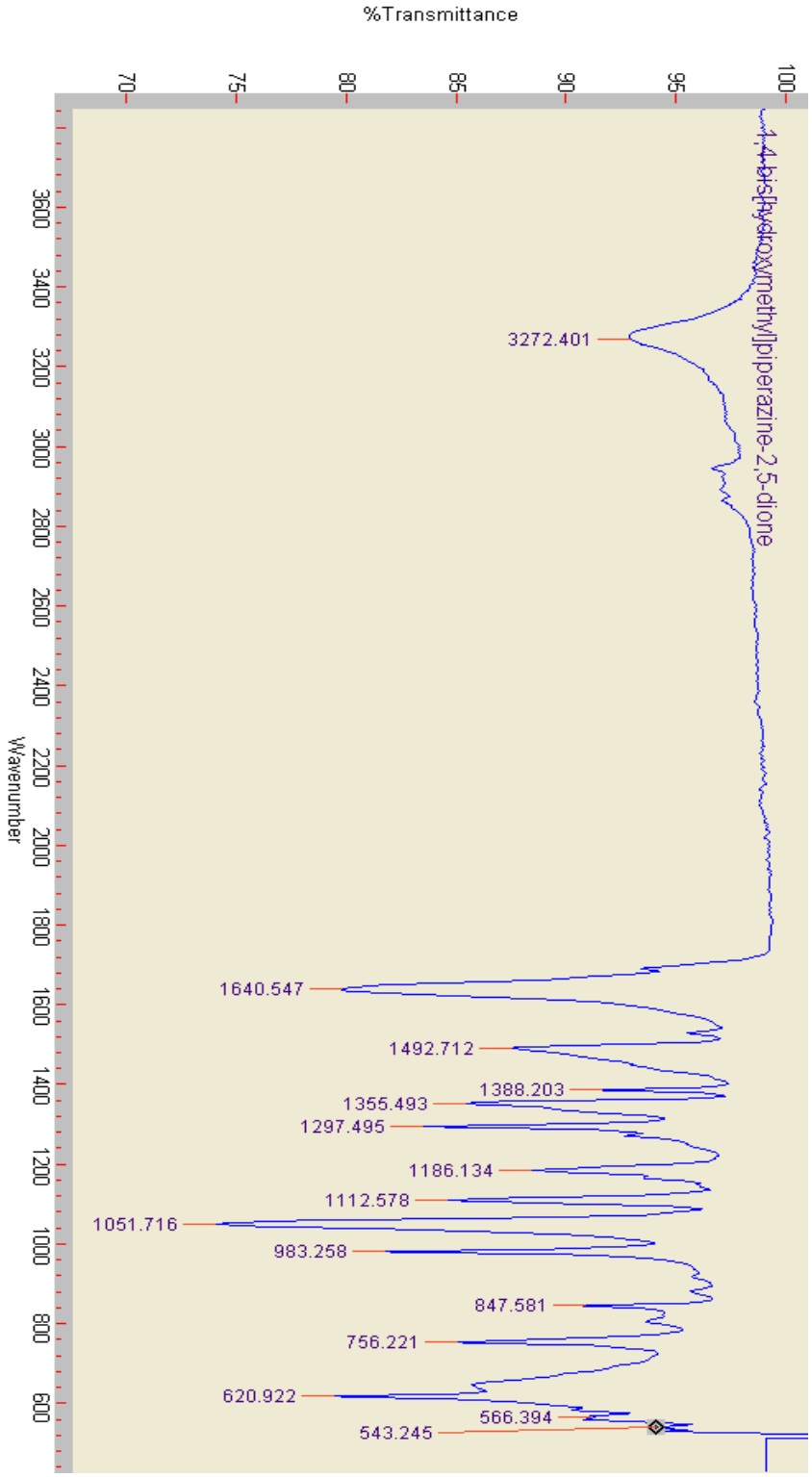
1,4-Diacetylpiperazine-2,5-dione



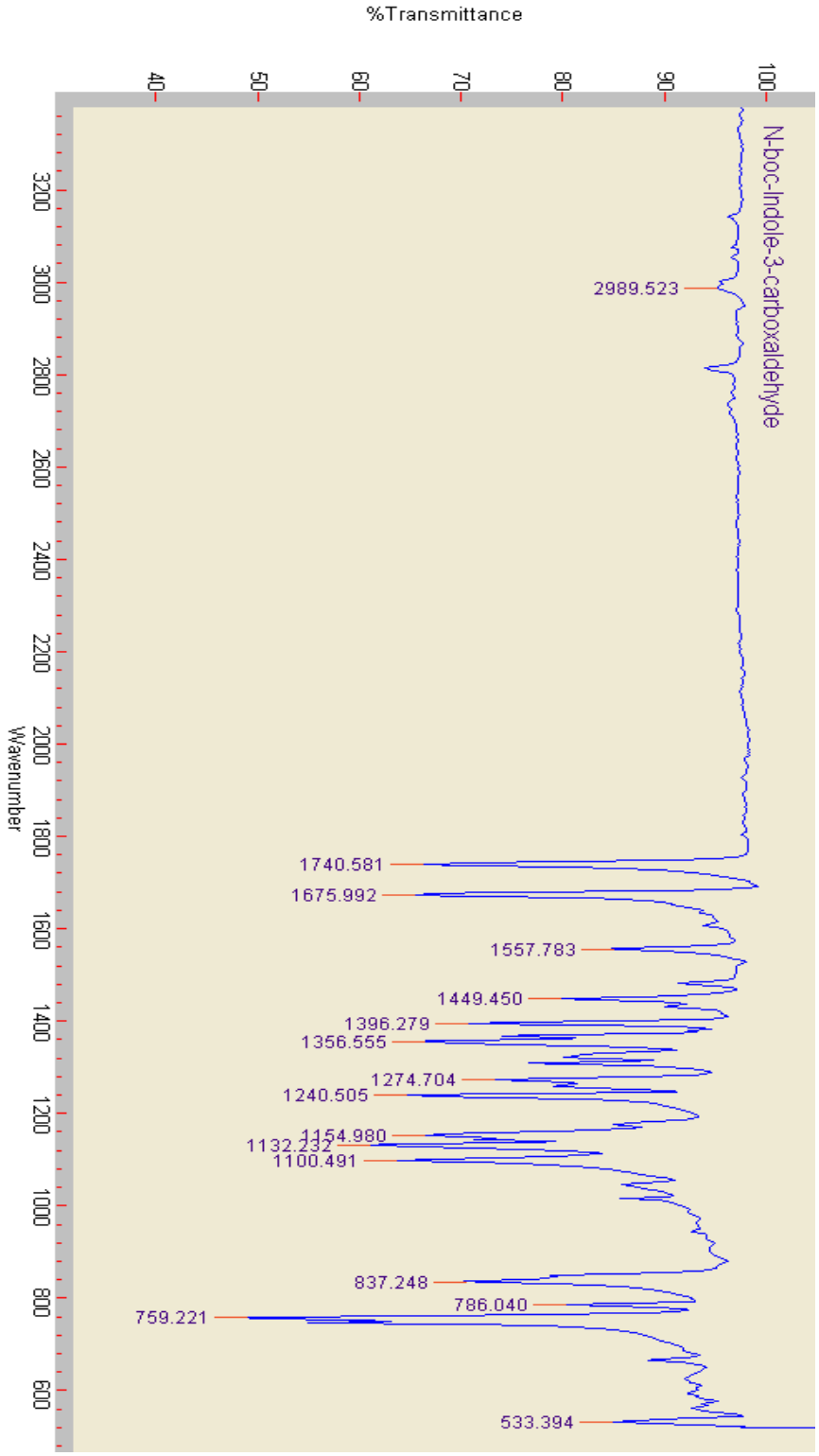
1,4-Dibenzylpiperazine-2,5-dione



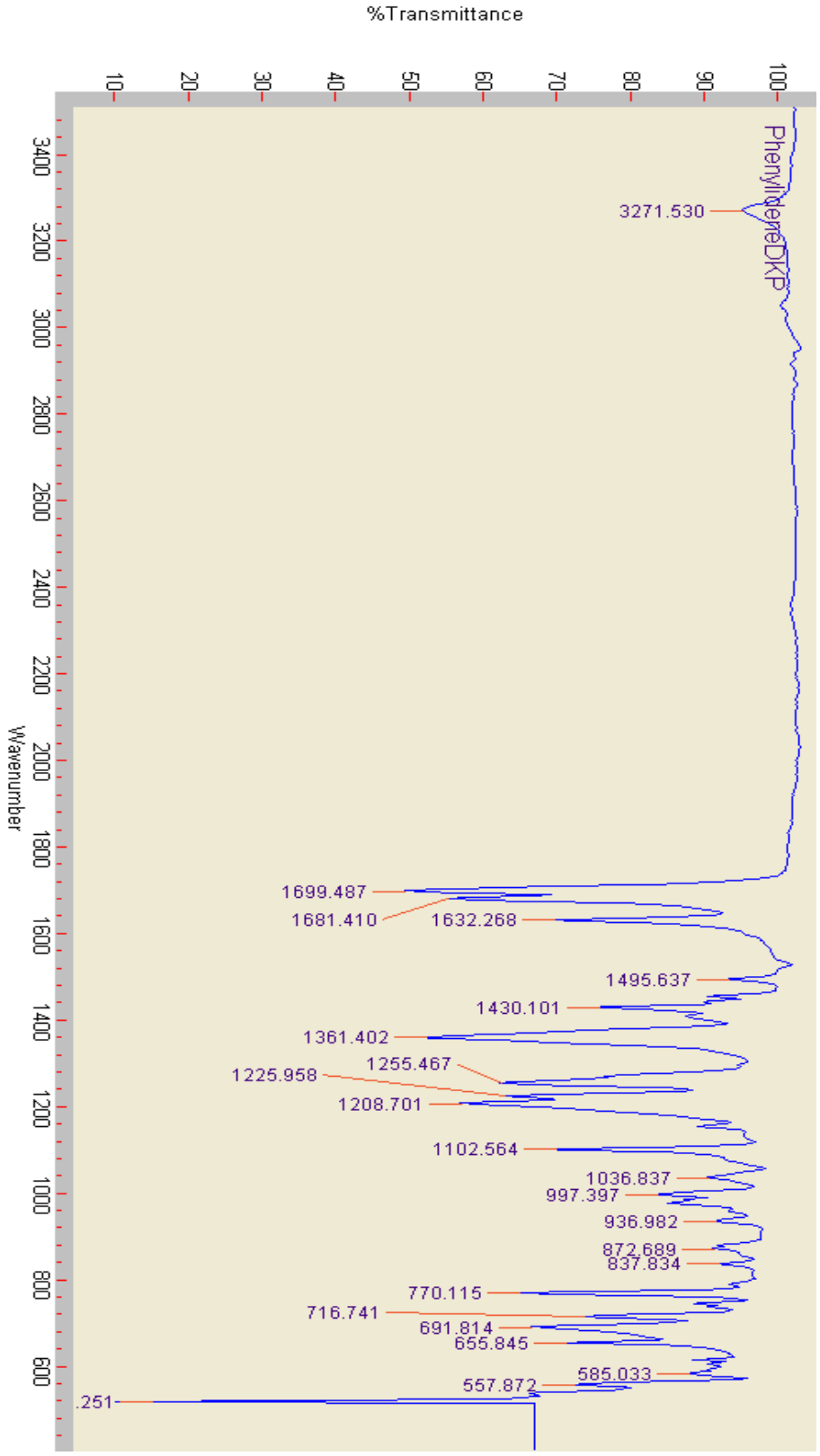
1,4-Bis(hydroxymethyl)piperazine-2,5-dione



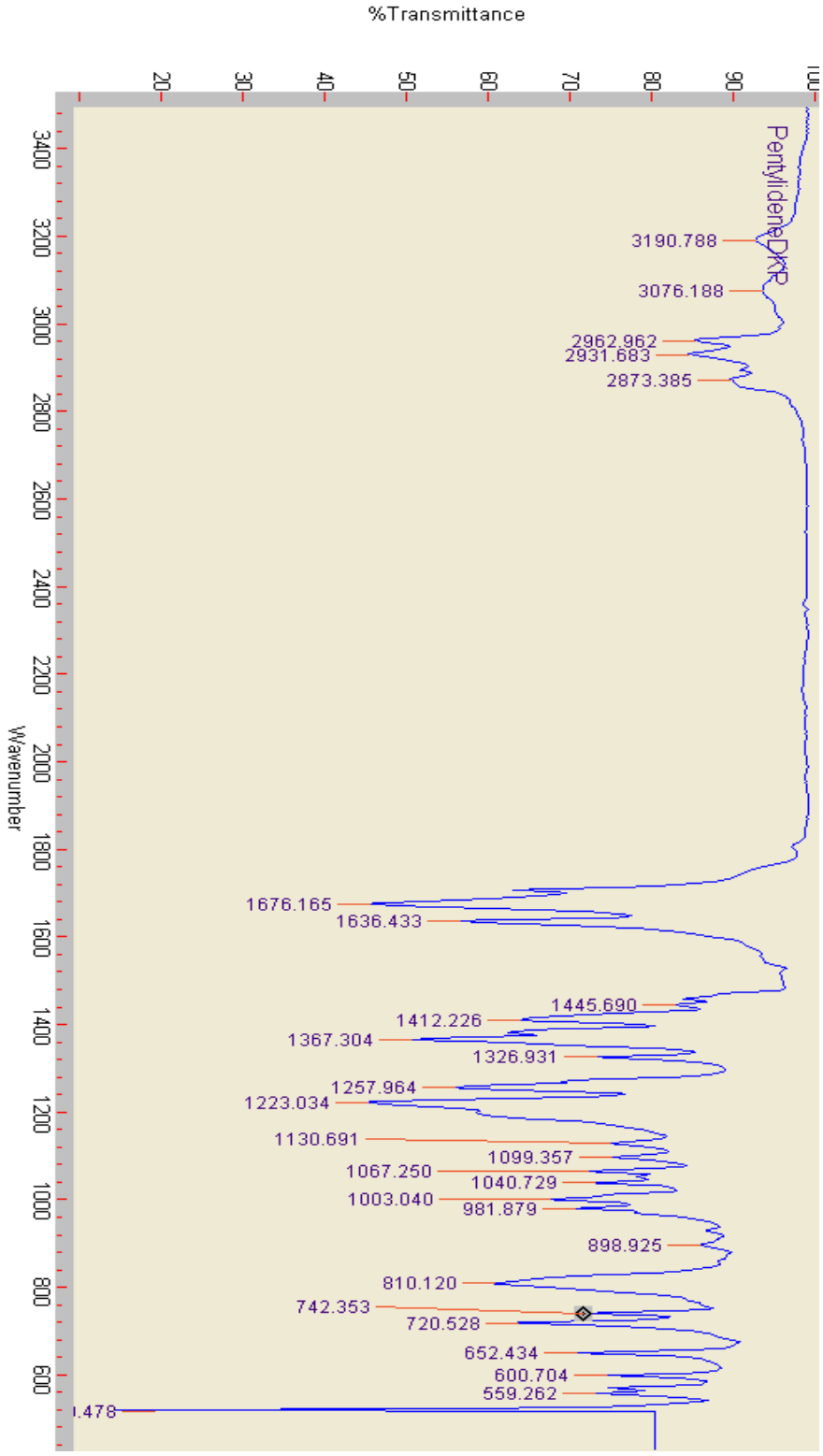
N-BocIndole-3-carboxaldehyde



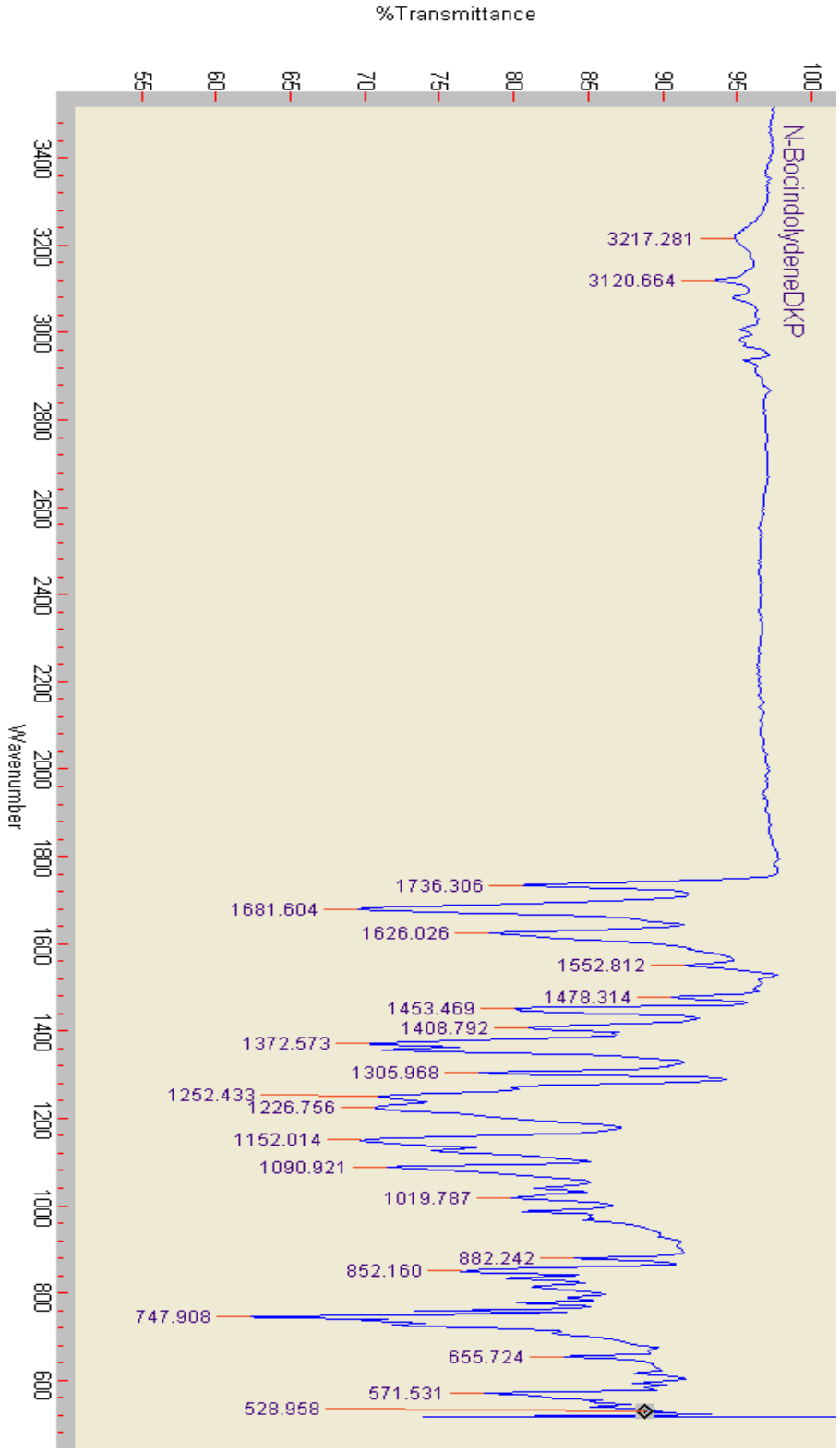
1-Acetyl-3-benzylidenepiperazine-2,5-dione



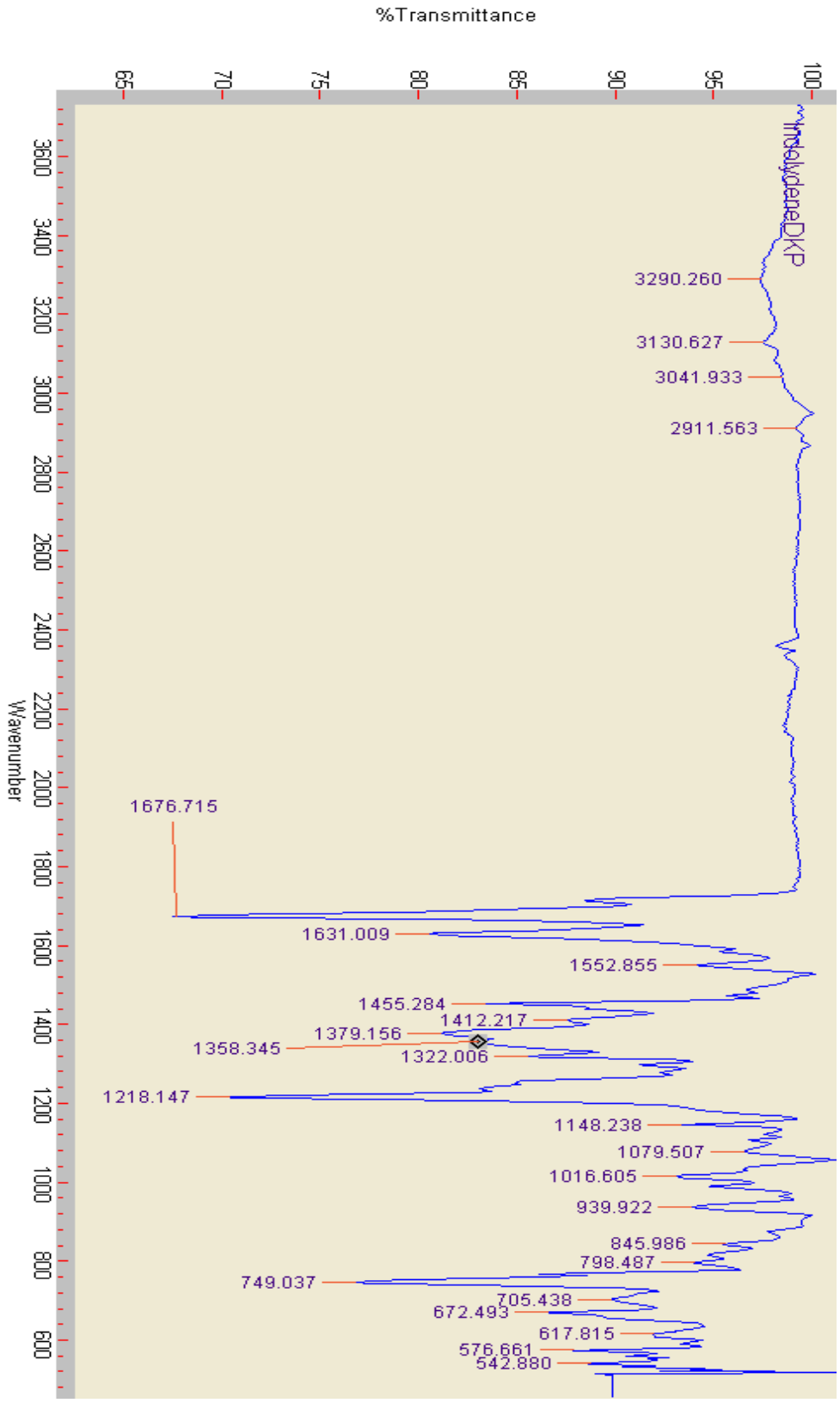
1-Acetyl-3-pentylidenepiperazine-2,5-dione



1-Acetyl-3-(N-Boc)Indolidenepiperazine-2,5-dione



1-Acetyl-3-indolidenepiperazine-2,5-dione



1-Acetyl-3-methyl-3-benzylidenepiperazine-2,5-dione