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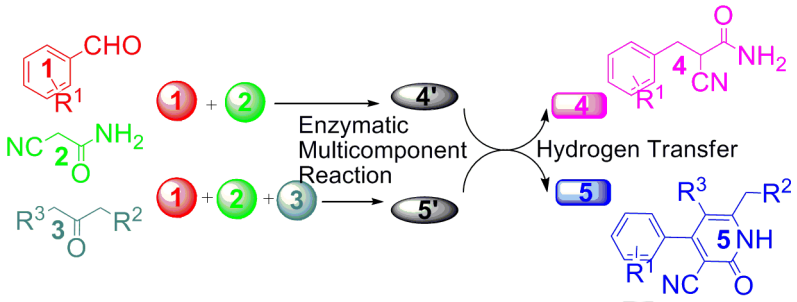

Enzymatic multicomponent reaction for simultaneous synthesis of two important scaffolds, pyridin-2-ones and α-alkylated nitriles

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Abstract

An efficient approach for the synthesis of highly substituted pyridin-2-one derivatives and α-alkylated nitriles through enzymatic multicomponent reaction (MCR) was developed. This MCR involved bio-mimetic reduction between 3,4-dihydropyridin-2-one and activated olefin, both of which were *in situ* generated in the Acylase Amano (AA)-catalyzed domino reaction starting from benzaldehyde, cyanoacetamide and ketone. A wide range of substituted benzaldehydes and ketones were accepted by this reaction. Both final products (pyridin-2-one derivatives and α-alkylated nitriles) were important skeletons and synthetic intermediates. The synthetic application of prepared α-alkylated nitrile was demonstrated by converting it into the corresponding α-alkyl-β^2-amino acid with high yield.

Keywords

Enzyme catalysis; Multicomponent reactions; Transfer hydrogenation; Pyridin-2-one; Alkylated nitrile

1. Introduction

As one representative of substituted six-membered lactams, compounds with pyridine-2-one scaffold have shown significant pharmaceutical activities such as antifungal[1], antibacterial[2] and antitumor[3]. Specifically, amrinone (**a**) and milrinone (**b**) in Fig. 1 have been found to display effective activities on therapy of myocardial infarction, and compound (**c**) has the activity of antihypertensive. Meanwhile, the pyridin-2-one ring system has successfully been applied in the natural synthesis as a building block[4]. Thus, development of convenient, efficient and mild strategy to establish these lactam cores is still interesting in the realm of synthetic organic chemistry. Although several synthetic methodologies have been reported[5], most of them have their own drawbacks such as harsh reaction conditions, low yields or complicated multistep routes. Similarly, α-alkylated nitriles are also important building blocks for synthesizing ketones and biologically active agents[6], the main methods for constructing α-alkylated nitriles are reactions of nitriles with alkyl halides (or alcohols or carbonyl compounds) using homogeneous inorganic bases such as NaH and $NaNH_2$[7] as catalysts. Toxicity of halogenated substrates, the use of homogeneous strong bases and metals are general drawbacks. With ever-increasing environmental concerns, developing new method to construct pyridine-2-one and α-alkylated nitrile derivatives still remains a significant challenge.

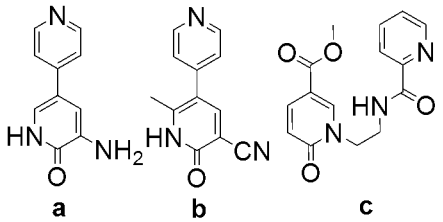
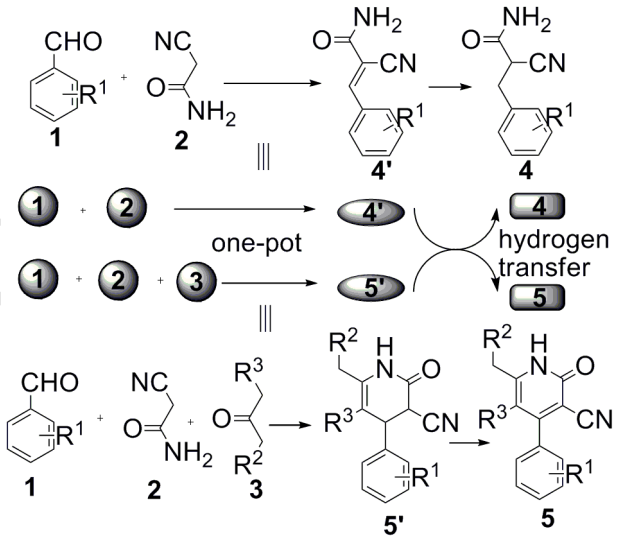


Fig. 1. Compounds with pyridin-2-one core

Since enzymes were discovered to maintain activity in organic solvents by Klibanov in the early 1980s[8], enzymes have been proved as efficient and green biocatalysts in organic synthesis due to their intrinsic advantages of high selectivity, mild reaction conditions, simple operation and environmentally benign behavior. In the past decade, a growing number of enzymes were found to be capable of catalyzing not only their ''natural'' reactions but also one or more alternative reactions, and this feature is called catalytic promiscuity[9]. Our group has found that the Michael and Markovnikov addition could be catalyzed by alkaline protease and acylase[10]. And recently we have discovered many enzymatic multicomponent reactions to synthesize heterocyclic compounds[11]. More and more new cases of enzymatically promiscuous reactions were reported by other groups[12], for example, Zhang[12b] provided a new strategy to synthesize spirooxindole derivatives via hydrolase, then Trypsin was founded to catalyze the formation of 4-thiazolidinones and 3,4-dihydropyrimidin-2-ones through three-component reaction[12e,12f], and Guan reported the first enzymatic aza-Diels-Alder Reaction[12a]. Though many works involved single-enzyme catalyzed multistep transformations were found, more explorations concerning new enzymatic domino reaction are still urgent in terms of the development of enzymology and industrial importance. Herein, we discovered an interesting reaction to simultaneously synthesize important pyridine-2-ones and α-alkylated nitriles via enzymatic multicomponent reaction involving *in situ* hydrogen transfer.

2. Results and discussion

Our initial idea was the synthesis of 3,4-dihydropyridin-2-one derivatives (**5'')** *via* a multicomponent reaction starting from benzaldehyde (**1)**, cyanoacetamide (**2)** and monoketone (**3)** catalyzed by enzyme (Scheme 1). Surprisingly, compounds **4** and **5** were obtained after flash chromatograph separation, which were very similar to the direct Knoevenagel condensation product (**4'**) and expected product of three components (**5'**). It seemed that hydrogen transfer process happened between compounds **4'** and **5'**.



Scheme 1. enzymatic one-pot multicomponent reaction involving *in situ* hydrogen transfer

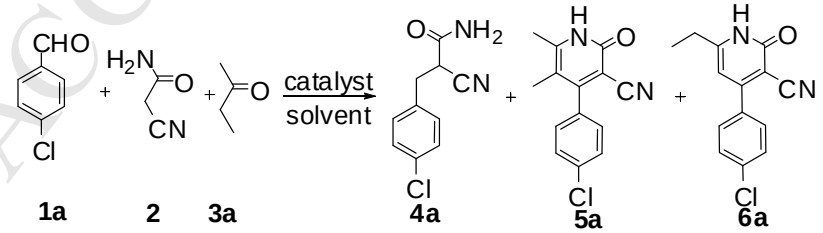
Considering the efficiency of this amusing phenomenon, we chose 4-chlorobenzaldehyde (**1a**),

cyanoacetamide (**2**) and butanone (**3a**) as the starting materials, catalyst screening was firstly performed in ethylene glycol (EG) at 50 ^{o}C. Among the selected commercial enzymes, Acylase Amano (AA) exhibited the best catalytic activity with 50% yield (Table 1, entry 1), 33% yield was obtained by D-aminoacylase (DA) (Table 1, entry 2). The moderate yields were mainly due to the left substrates. Butanone could form two pyridine-2-one products due to its different active positions. While other hydrolases, such as CAL-B (Table 1, entry 3), Lipase from *Candida rugosa*, Lipozyme from *Mucor miehei* and Amano Lipase PS (immobilized on diatomite), showed no activity toward the reaction (data not shown). Next, we investigated the effect of different solvents on the model reaction, probably because cyanoacetamide had low solubility in dioxane, toluene and acetonitrile, and no products were observed in these generally used solvents (data not shown). Fortunately, the reaction in diethylene glycol (Di-EG) gave the best yield of 71% at 50 ^{o}C after 48 h (Table 1, entry 9), and 50% yield in EG was gotten (Table 1, entry 1), while both DMF and DMSO provided the yields less than 20% (Table 1, entries 7 and 8). Determining Di-EG as the optimum solvent, we performed some control experiments to verify the specific catalytic effect of the enzyme on the initial three component reaction. No product was detected when the reaction underwent in the absence of enzyme (Table 1, entry 4). In addition, since AA is a zinc-dependent enzyme, EDTA (ethylene diamine tetraacetic acid) was used to denature the enzyme. We found that no reaction happened with EDTA-denatured AA or bovine serum albumin (BSA) (Table 1, entries 6 and 5), ruling out the possibility that amino acid of the protein surface or other impurities in AA could promote the initial three component reaction. On the other hand, these results also validated that the specific active site and the tertiary structure of AA was essential for the three component reaction.

It is noteworthy that the ratio of three starting materials has important influence on the output of the multicomponent reaction. When the ratio of compounds **1a**:**2**:**3a** was 0.5:0.75:0.5, the total yield reached to 80%. Further changing the ratio, no improvement of yield was obtained. Thus, we chose the molar ratio of substrate (**1**:**2**:**3**=0.5:0.75:0.5) for subsequent studies. Actually in our previous report, the interesting *in situ* hydrogen transfer process to form α-alkylated nitriles (**4**) and pyridine-2-ones (**5**) was not observed because of the much lower amount of aromatic aldehyde in the optimized feeding ratio of three starting materials (**1:2:3**=0.125:1:0.75), thus the hydrogen-accepting Knoevenagel product (**4'**) was not enough and only 3,4-dihydropyridin-2-ones (**5'**) was obtained[11b].

Table 1

Optimization of reaction conditions [a]



1a **2** **3a** **4a** **5a** **6a**

Entry	Catalyst	Solent	**4a** (μmol)[b]	**5a** (μmol)[b]	**6a** (μmol)[b]	Total yield(%)[d]
1	AA	EG	131	95	26	50
2	DA	EG	84	65	18	33
3	CAL-B	EG	n.d.	n.d.	n.d.	n.d.

4	-	Di-EG	n.d.	n.d.	n.d.	n.d.
5	BSA	Di-EG	Trace	trace	Trace	trace
6	AA[c]	Di-EG	Trace	trace	Trace	trace
7	AA	DMF	Trace	trace	Trace	trace
8	AA	DMSO	41	33	5	16
9	AA	Di-EG	175	124	58	71

[a] Reactions were carried out in EG (1 mL) with equimolar substrate (0.5 mmol) in the presence of 20 mg enzyme at 50 °C for 48h.

[b] Determined by HPLC.

[c] Denatured by EDTA in water for 12h at 100 °C.

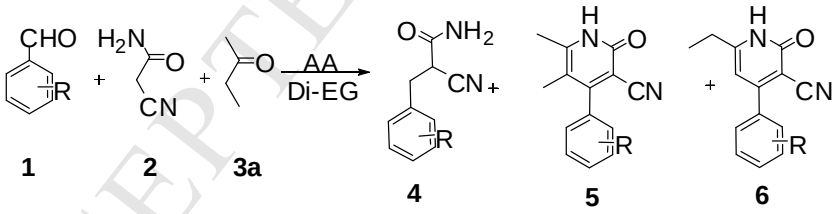
[d] Total yields of **4**+**5**+**6** based on the amount of aromatic aldehyde, calculating equation is Yield =[(Amount **4**+**5**+**6**) / 500] × 100%.

n.d.: Not determined.

With the optimal conditions in hand, we further examined the scope and limitation of the new enzymatic multicomponent reaction. It can be seen from Table 2 that a wide range of substituted benzaldehydes could successfully participate in the reaction. Generally, the enzyme exhibited better activity for benzaldehydes with electron-withdrawing substitutions than with electron-donating ones. For example, 4-(trifluoromethyl)benzaldehyde gave an excellent yield of 94% (Table 2, entry 1), while 4-isopropyl and 2-methoxybenzaldehyde only could get products in 70% yields (Table 2, entries 2 and 4). 3-Substituted benzaldehyde could also be accepted in the reaction (Table 2, entry 5).

Table 2

Examination of the substituent effect of the aromatic aldehyde [a]



1 **2** **3a** **4** **5** **6**

Entry	R	Product	Amount (μmol)[b]	Product	Amount (μmol)[b]	Product	Amount (μmol)[b]	Total yield(%)[c]
1	4-CF_3	**4b**	233	**5b**	156	**6b**	81	94
2	4-iPr	**4c**	172	**5c**	122	**6c**	56	70
3	4-F	**4d**	212	**5d**	155	**6d**	51	84
4	2-OCH_3	**4e**	171	**5e**	135	**6e**	37	69
5	3-Cl	**4f**	205	**5f**	155	**6f**	51	82

[a] Reactions were carried out in Di-EG (1 mL) with **1** (0.5 mmol), **2** (0.75 mmol) and **3** (0.5 mmol) in the presence of 20 mg AA at 50 °C for 48h.

[b] Determined by HPLC.

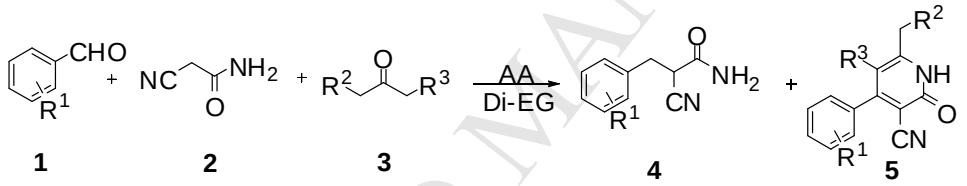
[c] Total yields of **4**+**5**+**6** based on the amount of aromatic aldehyde, calculating equation is Yield =[(Amount

4+**5**+**6**) / 500] ×100%

Then reactions of various ketones with different aldehydes were further tested. Both acetone and 3-pentanone could successfully participate in the reaction to form α-alkylated nitriles and pyridine-2-ones. But the yield declined sharply with the increase of chain length, from 70% to 32% (Table 3, entries 1 and 2), possibly because the activity of α-hydrogen in carbonyl compound reduced when increasing the chain length of ketones. Theoretically, 1,3-dicarbonyl compounds like methyl acetoacetate could also give the corresponding products. In our previous report,[11b] the amount of cyanoacetamide and methyl acetoacetate was excessive compared to aromatic aldehyde, and these substrates were simultaneously added to the reaction flask, giving the product of 3,4-dihydropyridin-2-one. Herein we first mixed aromatic aldehyde and cyanoacetamide together, when they fully changed to the Knoevenagel condensation product, half amount of methyl acetoacetate was introduced to the system. Finally, the expected α-alkylated nitrile and pyridine-2-one were obtained through slow transformation after 4 days (Table 3, entry 4).

Table 3

Variation of aldehyde and ketone [a]



1 **2** **3** **4** **5**

Entry	R^1	R^2	R^3	Product	Amount (μmol)[b]	Product	Amount (μmol)[b]	Total yield (%)[c]
1	4-iPr	H	H	**4c**	175	**5g**	176	70
2	4-iPr	CH_3	CH_3	**4c**	79	**5h**	83	32
3[d]	4-iPr	CH_3	CH_3	**4c**	84	**5h**	89	35
4[e]	p-Cl	H	$COOCH_3$	**4a**	158	**5i**	154	62

[a] Reactions were carried out in Di-EG (1 mL) with **1** (0.5 mmol), **2** (0.75 mmol) and **3** (0.5 mmol) in the presence of 20 mg AA at 50 °C for 48h.

[b] Determined by HPLC.

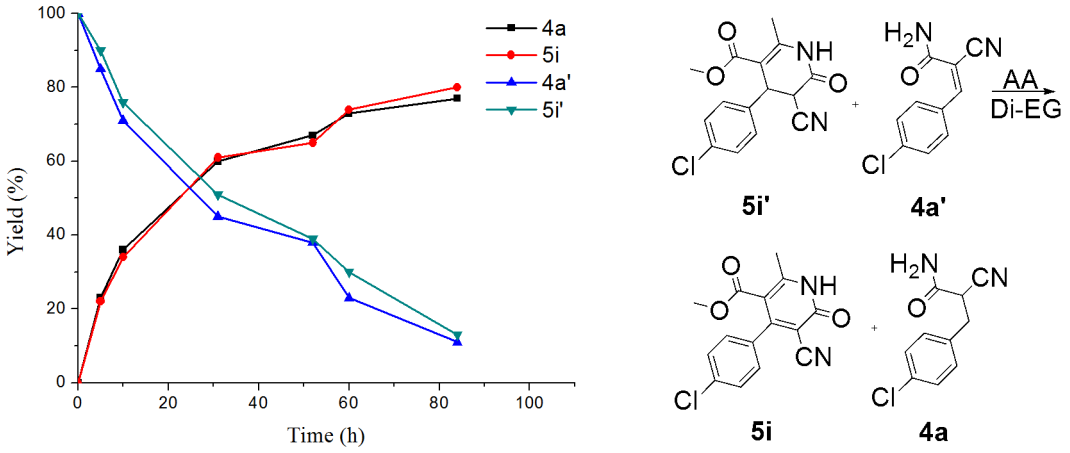
[c] Total yields of **4**+**5**+**6** based on the amount of aromatic aldehyde, calculating equation is Yield =[(Amount **4**+**5)** / 500] ×100%.

[d] Protected under nitrogen atmosphere.

[e] After 6h the reaction of **1** (0.5 mmol) and **2** (0.5 mmol) in the presence of 20 mg AA at 50 °C, 1 mL Di-EG, **3** (0.25 mmol) was added for 4 days

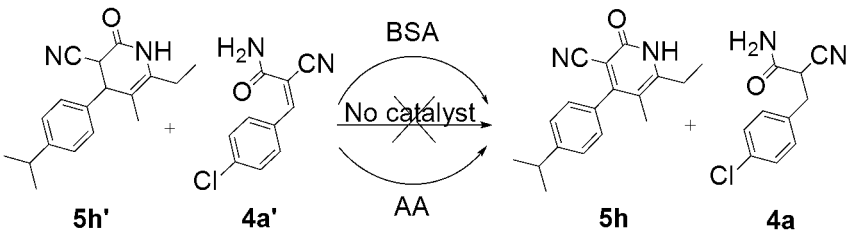
Basing on the above successful synthesis of two series of important compounds, i.e. pyridine-2-one derivatives and α-alkylated nitriles, we hope to confirm the existence of hydrogen transfer process. Firstly, we should determine whether the oxygen acts as oxidant in the system. Therefore, controlled reaction under the protection of nitrogen was performed, the yield was in accordance with that in air

(Table 3, entries 2 and 3). This result excluded the role of oxygen as oxidant in the reaction. Secondly, can the solvent be the hydrogen donor? When we only put Knoevenagel product and the enzyme in the solvent (Di-EG), no hydrogenation product was observed after two days, which excluded the possibility that the solvent acted as hydrogen donor. The amounts of pyridine-2-one derivatives should be equal to that of α-alkylated nitriles if hydrogen transfer process existed, which were confirmed by their respective amount in Table 1-3. In order to further verify the internal transfer hydrogenation process quantitatively, we tried to prepare two supposed intermediates, 3,4-dihydropyridin-2-one (Scheme 2, **5i'** and Scheme 3, **5h'**) and Knoevenagel condensation products (**4a'**). When we mixed **5i'** with **4a'** in Di-EG containing AA, two compounds (**5i** and **4a**) were successfully detected after chromatography as we expected. To have a better understanding for the reaction process, we monitored the transforming course of **5i'** and **4a'** quantitatively by HPLC, which was shown in Scheme 2. Gratifyingly, the amount of **5i** and **4a** increased as the reaction proceeded. Correspondingly, the substrates (**5i'** and **4a'**) gradually reduced. Same results were observed for **5h'** and **4a'**, which further confirmed the *in situ* hydrogen transfer process.



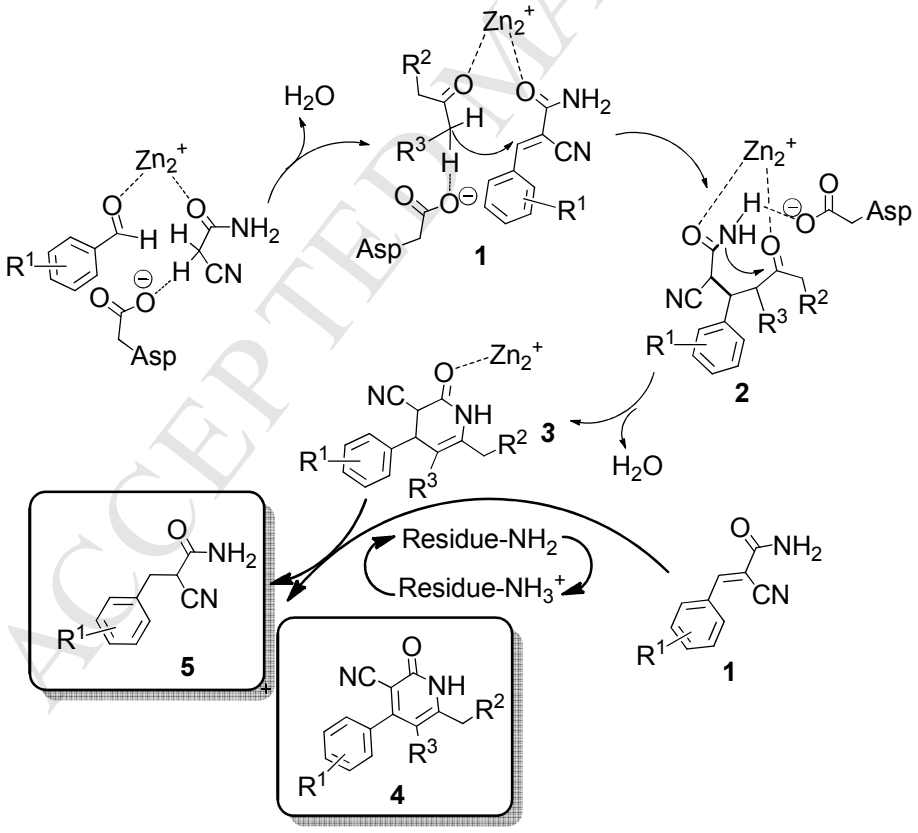
Scheme 2. Quantitatively monitoring the transforming process between two intermediates (**5i'** and **4a'**)

Another question needs to be considered is whether the *in situ* hydrogen transfer process happened in the active site of this enzyme. We mixed 0.2 mmol **5h'** and **4a'** in Di-EG for one day under the catalyst-free condition, or in the presence of BSA or AA (Scheme 3). No reaction occurred under the catalyst-free condition, and the catalysis of AA could provide the products with 90% yields (**5h** and **4a**). Interestingly the control reaction under the catalysis of BSA also gave the products in 70% yields. Then could amino acid catalyze this process? Aspartate (acidic), lysine (basic) and proline (neutral) as biocatalyst were investigated respectively, only lysine could promote the transformation with 71% yield, indicating the *in situ* hydrogen transfer process was possibly catalyzed by some surface basic amino acids of the enzyme.



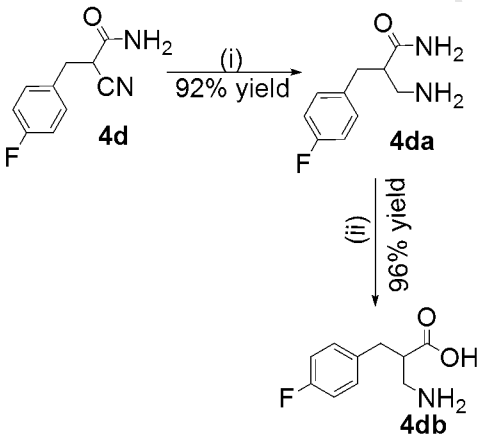
Scheme 3. Controlled experiments to explore the hydrogen transfer process

Thus, a tentatively proposed mechanism was illustrated on the basis of these data in Scheme 4. First, AA catalyzed the fast formation of Knoevenagel condensation reaction of aldehyde and cyanoacetamide to compound **1**. Then the zinc ion would coordinate with carbonyl group of cyanoacetamide and ketone to increase the nucleophilicity, Asp 366 of AA deprived one α-H of ketone, which occurred the Michael addition with compound **1** to produce intermediate **2**. Next, the electron-rich N-H performed the nucleophilic addition to the carbonyl group of ketone, at the same time, one molecular water was lost from intermediate **2** to form product **3**. Lysine catalyzed the transfer hydrogenation process as a hydrogen carrier, obtained proton from compound **3** and transferred to compound **1**. The final products pyridin-2-one derivatives **4** and α-alkylated nitriles **5** were obtained.



Scheme 4. The proposed mechanism of this multicomponent reaction.

In order to validate the utility of this strategy, firstly, we examined the reaction on tenfold amount of entry 3 in Table 2, total isolated yield was 69%. Then we synthesized the α-substituted β^2-amino acid from one product, α-alkylated nitrile *via* simple conversions. β-amino acids are key building blocks of natural products, β-peptides and pharmaceuticals[13]. Compounds with this skeleton usually exhibit antivirus and antibacterial activity[14]. So developing new method to construct β-amino acids is still of urgency. Our protocol is outlined in Scheme 5. Initially, Raney Ni was used as the catalyst for the hydrogenation of cyano group in the reductive product **4d** under hydrogen atmosphere, **4da** was obtained in high yield of 92%. Further simple treatment of **4da** with concentrated hydrochloric acid at room temperature for 3 h provided the target α-substituted β^2-amino acid (**4db**) in excellent yield of 96%. This successful demonstration of converting prepared α-alkylated nitrile into the corresponding α-alkyl-β^2-amino acid with high yield displayed the great potential of this enzymatic multicomponent reaction in organic synthesis.



Scheme 5. Reagents and conditions: (i) Raney Ni (10% w/w), H_2 (1 atm), 2 M NH_3/MeOH, 50 ^oC, 20 h; (ii) aq HCl, rt, 3h

3. Conclusions

In summary, we have discovered an interesting process involving transfer hydrogenation between 3,4-dihydropyridin-2-one and activated olefin, both of them were *in situ* generated in an Acylase Amano (AA)-catalyzed multicomponent domino reaction starting from benzaldehyde, cyanoacetamide and ketone. A wide range of substituted benzaldehydes and ketones were accepted by this reaction. The resultant two final products (pyridin-2-one derivatives and α-alkylated nitriles) were both building blocks of a variety of biologically active agents. As a demonstration, these α-alkylated nitriles could be transformed to synthetically important α-substituted β^2-amino acids via simple conversions. The reaction process was verified in detail by some controlled experiments, separating intermediates and monitoring the transfer hydrogenation course between 3,4-dihydropyridin-2-one and activated olefin quantitatively. This novel single enzyme-catalyzed multicomponent reaction is attractive in terms of high atom economy, easy workup procedure, tolerance of various functional groups and mild reaction conditions, and will widen the application of enzyme in organic synthesis.

4. Material and methods

4.1 General information for reagents and analytical methods

D-aminoacylase from *E. coli* (10000 U/mg, 1 U is defined as enzyme quantity, which produces 1 mmol of D-Amino acid per 30 min) and Acylase 'Amano' (AA) from *Aspergillus oryzae* (≥ 30000 U/g, 1 U is defined as enzyme quantity, which produces 1 mmol of L-Amino acid per 30 min) were purchased from Amano Enzyme Inc (Japan). Lipase immobilized on acrylic resin from *C. antarctica* (10000 U/g, recombinant, expressed in *Aspergillus oryzae*) was purchased from Sigma (Steinheim, Germany). The ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 or 500 MHz spectrometer. Chemical shifts were expressed in parts per million and coupling constants (*J*) in hertz. Analytical HPLC was performed using an Agilent TC-C18 series (250×4.6 mm) and a UV detector (210 nm). The mobile phase was methanol and water. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Melting points were determined using XT-4 apparatus and were not corrected. All the known products were characterized by comparing the ^{1}H NMR data with those reported in the literature. The structures of new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS.

4.2 General procedure for the enzymatic multicomponent reaction

A mixture of aromatic aldehyde (0.5 mmol), cyanoacetamide (0.75 mmol), monoketone (0.5 mmol), and 20 mg AA in 1 mL Di-EG was shaken at 50 ^{o}C and 200 rpm for 48h. After completion of the reaction as monitored by TLC, the detecting solvent was petroleum ether/ethyl acetate mixture (1/2 v/v), then the mixture was loaded directly onto a silica gel column using petroleum ether/ethyl acetate mixture (1/1 v/v) as eluent to afford the desired product of α-alkylated nitriles and pyridin-2-ones, the α-alkylated nitriles was first obtained, importantly, it had very slight UV absorption at 254 nm, but it can be confirmed in the iodine jar.

4.3 Procedure for the scale-up synthesis of α-alkylated nitrile and pyridin-2-one

A mixture of 4-fluorobenzaldehyde (5 mmol), cyanoacetamide (7.5 mmol), butanone (5 mmol), and 150 mg AA in 10 mL Di-EG was shaken at 50 ^{o}C and 200 rpm for 72h. The mixture was loaded directly onto a silica gel column using petroleum ether/ethyl acetate mixture (1/1 v/v) as eluent to afford the desired product of **4d** (337 mg), **5d** (350mg) and **6d** (61 mg), the total isolated yield was 69 %.

4.4 Procedure for the synthesis of α-alkyl-β^2-amino acid

0.3 mmol compound **4d** and Raney Ni (10% w/w) were put into 10 mL NH_3/MeOH solvent (2 M), the mixture was stirred at 50 ^{o}C for 20 h under hydrogen atmosphere. After completion of the reaction, Raney Ni was filtered and the solvent was removed under reduced pressure, then the residual solid was washed with 2 mL petroleum ether/ethyl acetate mixture (1/1 v/v) twice. The obtained compound **4da** was put into 10 mL concentrated hydrochloric acid for 3 h at room temperature, the final product was gotten after removing solvent.

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Zheng, H.; Zhu, Q.; Zhang, P. F. *Adv. Synth. Catal.* **2011,** *353*, 371-375; (c) Lopez-Iglesias, M.; Busto, E.; Gotor, V.; Gotor-Fernandez, V. *Adv. Synth. Catal.* **2011,** *353*, 2345-2353; (d) Wu, M. Y.; Li, K.; He, T.; Feng, X. W.; Wang, N.; Wang, X. Y.; Yu, X. Q. *Tetrahedron* **2011,** *67*, 2681-2688; (e) Zheng, H.; Mei, Y. J.; Du, K.; Shi, Q. Y.; Zhang, P. F. *Catal. Lett.* **2013,** *143*, 298-301; (f) Xie, Z. B.; Wang, N.; Wu, W. X.; Le, Z. G.; Yu, X. Q. *J. Biotechnol.* **2014,** *170*, 1-5.

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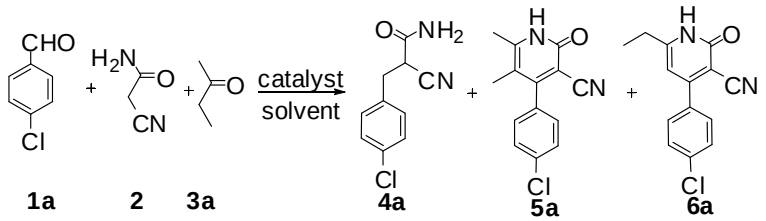
Table 1

Optimization of reaction conditions [a]



1a **2** **3a** **4a** **5a** **6a**

Entry	Catalyst	Solent	**4a** (μmol)[b]	**5a** (μmol)[b]	**6a** (μmol)[b]	Total yield(%)[d]
1	AA	EG	131	95	26	50
2	DA	EG	84	65	18	33
3	CAL-B	EG	n.d.	n.d.	n.d.	n.d.
4	-	Di-EG	n.d.	n.d.	n.d.	n.d.
5	BSA	Di-EG	Trace	trace	Trace	trace
6	AA[c]	Di-EG	Trace	trace	Trace	trace
7	AA	DMF	Trace	trace	Trace	trace
8	AA	DMSO	41	33	5	16
9	AA	Di-EG	175	124	58	71

[a] Reactions were carried out in EG (1 mL) with equimolar substrate (0.5 mmol) in the presence of 20 mg enzyme at 50 °C for 48h.

[b] Determined by HPLC.

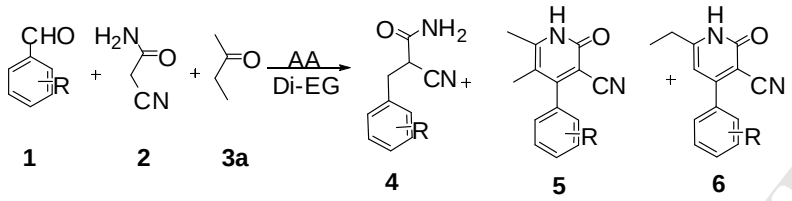
[c] Denatured by EDTA in water for 12h at 100 °C.

[d] Total yields of **4**+**5**+**6** based on the amount of aromatic aldehyde, calculating equation is Yield =[(Amount **4**+**5**+**6**) / 500] × 100%.

n.d.: Not determined.

Table 2

Examination of the substituent effect of the aromatic aldehyde [a]



1 **2** **3a** **4** **5** **6**

Entry	R	Product	Amount (μmol)[b]	Product	Amount (μmol)[b]	Product	Amount (μmol)[b]	Total yield(%)[c]
1	4-CF_3	**4b**	233	**5b**	156	**6b**	81	94
2	4-iPr	**4c**	172	**5c**	122	**6c**	56	70
3	4-F	**4d**	212	**5d**	155	**6d**	51	84
4	2-OCH_3	**4e**	171	**5e**	135	**6e**	37	69
5	3-Cl	**4f**	205	**5f**	155	**6f**	51	82

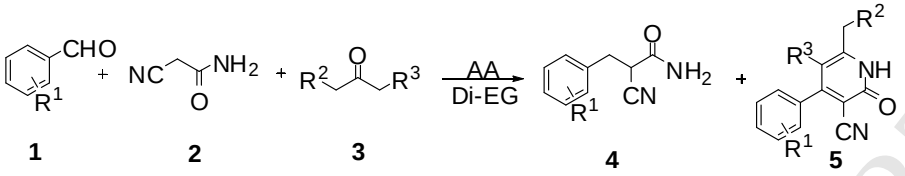
[a] Reactions were carried out in Di-EG (1 mL) with **1** (0.5 mmol), **2** (0.75 mmol) and **3** (0.5 mmol) in the presence of 20 mg AA at 50 ^{o}C for 48h.

[b] Determined by HPLC.

[c] Total yields of **4**+**5**+**6** based on the amount of aromatic aldehyde, calculating equation is Yield =[(Amount **4**+**5**+**6**) / 500] ×100%

Table 3

Variation of aldehyde and ketone [a]



1 **2** **3** **4** **5**

Entry	R^1	R^2	R^3	Product	Amount (μmol)[b]	Product	Amount (μmol)[b]	Total yield (%)[c]
1	4-iPr	H	H	**4c**	175	**5g**	176	70
2	4-iPr	CH_3	CH_3	**4c**	79	**5h**	83	32
3[d]	4-iPr	CH_3	CH_3	**4c**	84	**5h**	89	35
4[e]	p-Cl	H	$COOCH_3$	**4a**	158	**5i**	154	62

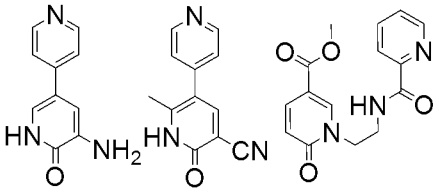
[a] Reactions were carried out in Di-EG (1 mL) with **1** (0.5 mmol), **2** (0.75 mmol) and **3** (0.5 mmol) in the presence of 20 mg AA at 50 °C for 48h.

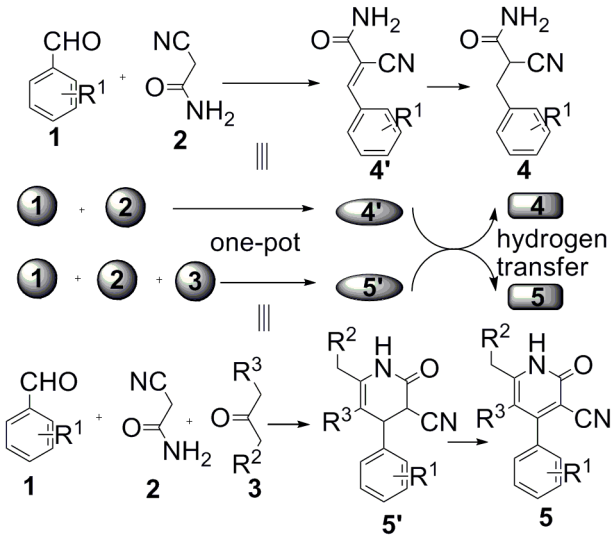
[b] Determined by HPLC.

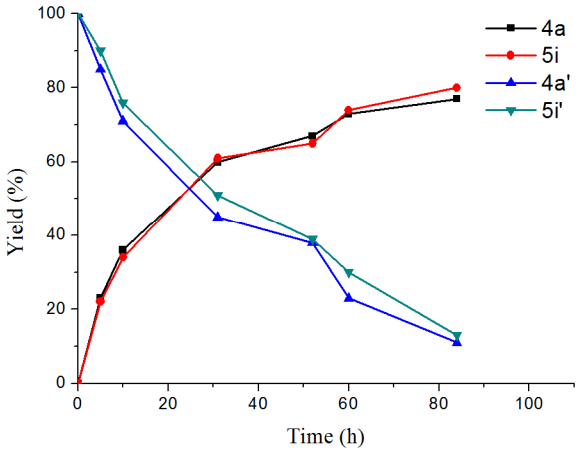
[c] Total yields of **4+5+6** based on the amount of aromatic aldehyde, calculating equation is Yield =[(Amount **4**+**5)** / 500] ×100%.

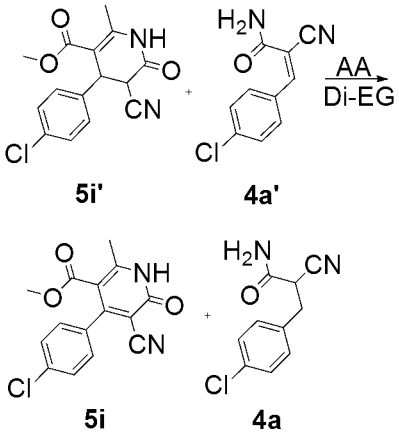
[d] Protected under nitrogen atmosphere.

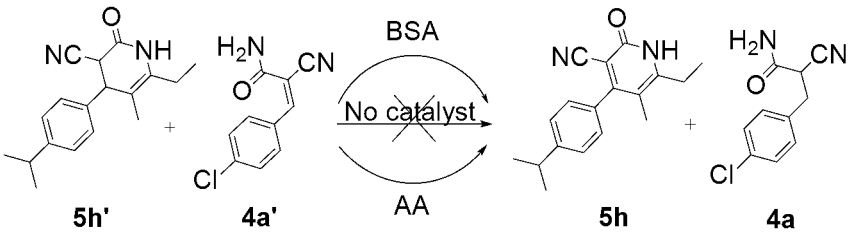
[e] After 6h the reaction of **1** (0.5 mmol) and **2** (0.5 mmol) in the presence of 20 mg AA at 50 °C, 1 mL Di-EG, **3** (0.25 mmol) was added for 4 days

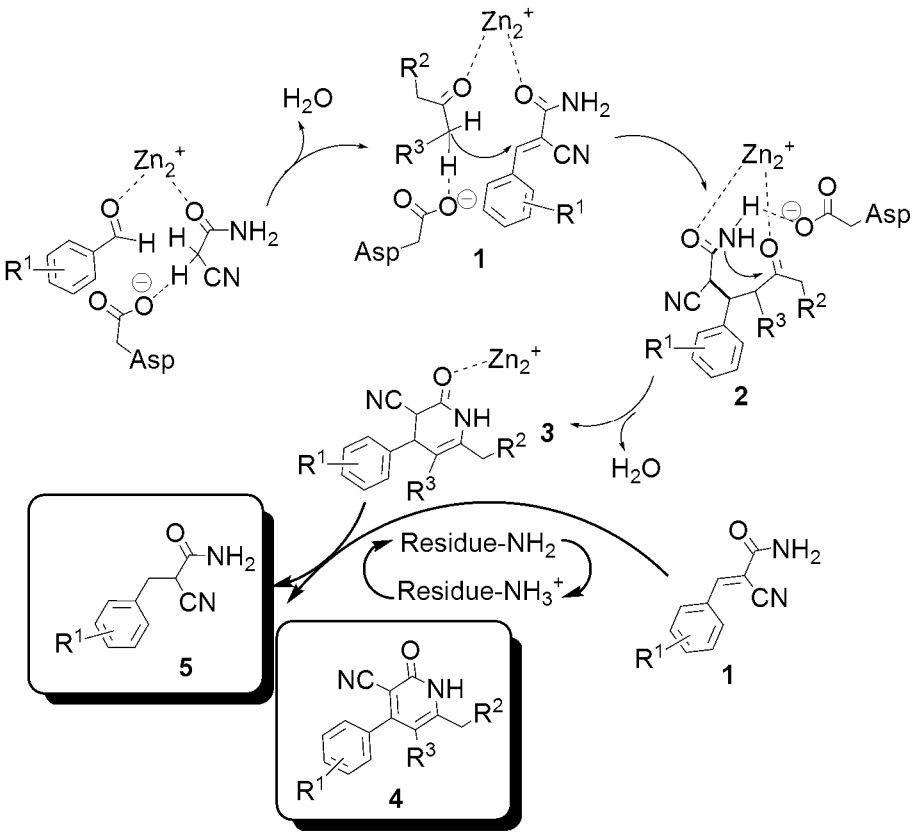


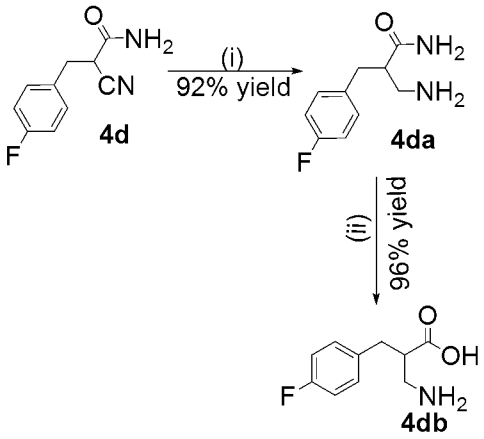












Enzymatic multicomponent reaction for simultaneous synthesis of two important scaffolds, pyridin-2-ones and α-alkylated nitriles

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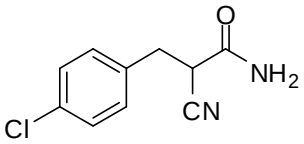
Supplementary Data

Table of Content

Supporting Information

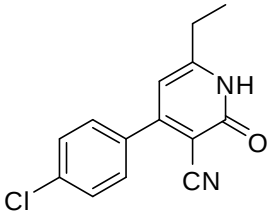
Characterizations for Compounds

4a: 3-(4-chlorophenyl)-2-cyanopropanamide



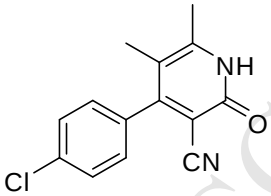
m.p. 180-181 °C; IR (neat): 3423, 3315, 3206, 2252, 1667, 1611; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.49 (s, 1H), 7.41-7.31 (m, 4H), 3.96-3.93 (m, 1H), 3.14 (dd, J = 13.6, 6.8 Hz, 1H), 3.16 (dd, J = 13.6, 8.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.3, 141.1, 137.0, 136.2, 136.2, 133.6, 133.6, 123.5, 39.6; HRMS (EI) calcd. for $C_{10}H_9N_2OCl$ [M^+]: 208.0403, found: 208.0403

6a: 4-(4-chlorophenyl)-6-ethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



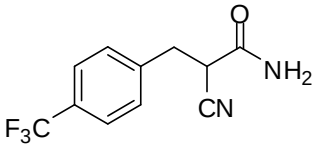
m.p. 260-261 °C; IR (neat): 2220, 1645, 1614; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (s, 1H), 7.66-7.60 (m, 4H), 6.34 (s, 1H), 2.58 (q, J = 7.6 Hz, 2H), 1.19 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.4, 159.1, 157.5, 135.2, 134.9, 129.9, 128.9, 116.4, 104.9, 97.7, 26.1, 12.7; HRMS (EI) calcd. for $C_{14}H_{11}N_2OCl$ [M^+]: 258.0560, found: 258.0562

5a: 4-(4-chlorophenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



m.p. 278-279 °C; IR (neat): 2223, 1652, 1604; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.64 (s, 1H), 7.60-7.33 (m, 4H), 2.30 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.9, 150.7, 150.7, 135.1, 133.9, 129.7, 128.8, 116.2, 111.3, 99.7, 18.0, 13.9; HRMS (EI) calcd. for $C_{14}H_{11}N_2OCl$ [M^+]: 258.0560, found: 258.0561

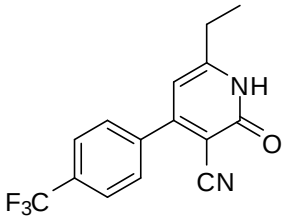
4b: 2-cyano-3-(4-(trifluoromethyl)phenyl)propanamide



m.p. 148-149 °C; IR (neat): 3361, 3193, 2248, 1677, 1627; ^{1}H

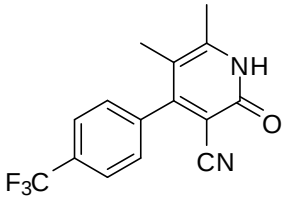
NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.71-7.51 (m, 5H), 4.01 (t, J = 7.6 Hz, 1H), 3.25 (dd, J = 13.8, 6.6 Hz, 1H), 3.16 (dd, J = 13.6, 8.4 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.2, 147.0, 135.2, 135.1, 133.1, 132.9, 130.6, 130.5, 128.4, 123.4, 40.0; HRMS (EI) calcd. for $C_{11}H_9N_2OF_3$ [M^+]: 242.0667, found: 242.0667

6b: 6-ethyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carbonitrile



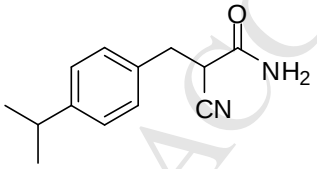
m.p. 200-201 ^{o}C; IR (neat): 2272, 1694, 1617; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 7.92-7.81 (m, 4H), 6.37 (s, 1H), 2.58 (q, J = 7.6 Hz, 2H), 1.18 (t, J = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.3, 158.9, 157.9, 140.2, 130.4, 130.2, 129.0, 126.5, 125.7, 125.7, 125.0, 122.8, 116.2, 105.0, 98.1, 26.1, 12.6; HRMS (EI) calcd. for $C_{15}H_{11}N_2OF_3$ [M^+]: 292.0823, found: 292.0827

5b:
5,6-dimethyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carbonitrile



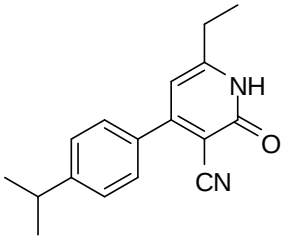
m.p. 264-265 ^{o}C; IR (neat): 2227, 1652, 1617; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 7.92-7.56 (m, 4H), 2.33 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.2, 159.8, 151.1, 140.4, 129.5, 129.3, 128.7, 125.7, 125.7, 125.0, 122.9, 116.1, 111.1, 99.5, 18.0, 13.9; HRMS (EI) calcd. for $C_{15}H_{11}N_2OF_3$ [M^+]: 292.0823, found: 292.0826

4c: 2-cyano-3-(4-isopropylphenyl)propanamide



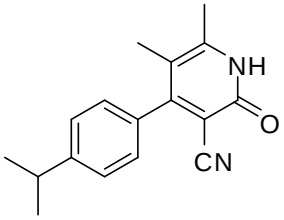
m.p. 150-151 ^{o}C; IR (neat): 3433, 3367, 3194, 2253, 1659, 1630; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7,48 (s, 1H), 7.20 (s, 4H), 3.93-3.89 (m, 1H), 3.11 (dd, J = 13.8, 6.6 Hz, 1H), 2.99 (dd, J = 13.6, 8.8 Hz, 1H), 2.89-2.83 (m, 1H), 1.19 (d, J = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.6, 152.3, 139.4, 134.1, 131.6, 123.7, 40.1, 38.3, 29.1; HRMS (EI) calcd. for $C_{13}H_{16}N_2O$ [M^+]: 216.1263, found: 216.1266

6c: 6-ethyl-4-(4-isopropylphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile



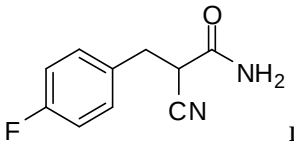
m.p. 280-281 °C; IR (neat): 2219, 1652, 1605; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (s, 1H), 7.57-7.41 (m, 4H), 6.33 (s, 1H), 3.00-2.93 (m, 1H), 2.58 (q, J = 7.4 Hz, 2H), 1.25-1.17 (m, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.6, 160.2, 157.0, 151.0, 133.6, 128.1, 126.7, 116.7, 105.0, 97.2, 33.3, 26.1, 23.6, 12.6; HRMS (EI) calcd. for $C_{17}H_{18}N_2O$ [M^+]: 266.1419, found: 266.1417

5c: 4-(4-isopropylphenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



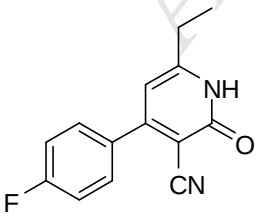
m.p. 290-291 °C; IR (neat): 2219, 1648, 1605; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 7.40-7.20 (m, 4H), 2.99-2.92 (m, 1H), 2.30 (s, 3H), 1.70 (s, 3H), 1.24 (d, J = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.8, 160.1, 150.3, 149.2, 133.7, 127.7, 126.5, 116.4, 111.4, 99.7, 33.2, 23.7, 18.0, 14.0; HRMS (EI) calcd. for $C_{17}H_{18}N_2O$ [M^+]: 266.1419, found: 266.1424

4d: 2-cyano-3-(4-fluorophenyl)propanamide



m.p. 149-150 °C; IR (neat): 3468, 3323, 3201, 2250, 1676, 1614; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (s, 1H), 7.49 (s, 1H), 7.35-7.14 (m, 4H), 3.95-3.91 (m, 1H), 3.14 (dd, J = 13.8, 7.0 Hz, 1H), 3.05 (dd, J = 13.8, 8.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.7, 162.8, 160.9, 133.6, 133.6, 131.5, 131.4, 118.8, 115.8, 115.6, 34.8; HRMS (EI) calcd. for $C_{10}H_9N_2OF$ [M^+]: 192.0699, found: 192.0703

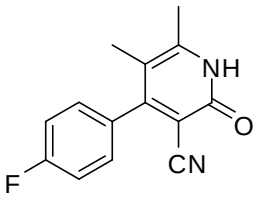
6d: 6-ethyl-4-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile



m.p. 128-129 °C; IR (neat): 2272, 1695, 1618; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 7.68-7.33 (m, 4H), 6.30 (s, 1H), 2.55 (q, J = 7.4 Hz, 2H), 1.16 (t,

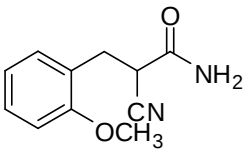
J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.2, 162.2, 161.4, 159.3, 157.3, 132.5, 132.5, 130.6, 130.5, 116.5, 115.9, 115.7, 105.0, 97.7, 26.1, 12.6; HRMS (EI) calcd. for $C_{14}H_{11}N_2OF$ [M^+]: 242.0855, found: 242.0856

5d: 4-(4-fluorophenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



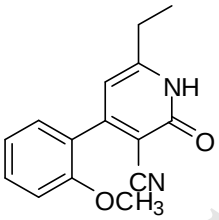
m.p. 253-254 ^{o}C; IR (neat): 2224, 1652, 1603; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 7.37-7.33 (m, 4H), 2.30 (s, 3H), 1.69 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.0, 162.0, 161.6, 160.7, 151.3, 133.3, 133.0, 130.8, 130.7, 117.0, 116.5, 116.3, 112.2, 100.6, 18.7, 14.6; HRMS (EI) calcd. for $C_{14}H_{11}N_2OF$ [M^+]: 242.0855, found: 242.0856

4e: 2-cyano-3-(2-methoxyphenyl)propanamide



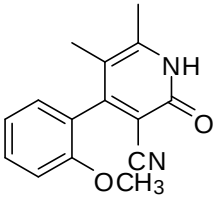
m.p. 116-117 ^{o}C; IR (neat): 3334, 3199, 2245, 1683, 1602; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.47 (s, 1H), 7.29-6.88 (m, 4H), 3.92-3.88 (m, 1H), 3.08 (dd, *J* = 13.6, 6.8 Hz, 1H), 3.02 (dd, *J* = 13.6, 9.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.3, 157.2, 130.4, 128.7, 124.5, 120.3, 118.5, 110.8, 55.4, 37.3, 30.2; HRMS (EI) calcd. for $C_{11}H_{12}N_2O_2$ [M^+]: 204.0899, found: 204.0901

6e: 6-ethyl-4-(2-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile



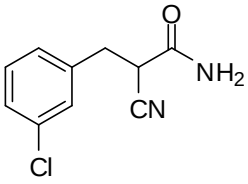
m.p. 249-250 ^{o}C; IR (neat): 2223, 1644, 1617; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.48-7.03 (m, 4H), 6.18 (s, 1H), 3.78 (s, 3H), 2.55 (q, *J* = 7.4 Hz, 2H), 1.16 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.7, 159.1, 157.0, 156.2, 131.9, 129.8, 125.8, 121.1, 116.7, 112.4, 106.6, 100.6, 56.1, 26.5, 13.1; HRMS (EI) calcd. for $C_{15}H_{14}N_2O_2$ [M^+]: 254.1055, found: 254.1057

5e: 4-(2-methoxyphenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



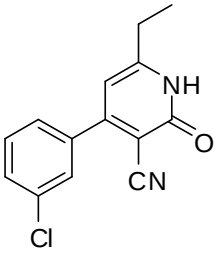
m.p. 262-263 ^{o}C; IR (neat): 2223, 1652, 1605; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 1H), 7.49-7.06 (m, 4H), 3.76 (s, 3H), 2.30 (s, 3H), 1.63 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.1, 159.7, 155.2, 149.7, 130.8, 128.8, 124.9, 120.7, 116.3, 112.2, 111.7, 100.4, 55.6, 17.9, 13.5; HRMS (EI) calcd. for $C_{15}H_{14}N_2O_2$ [M^+]: 254.1055, found: 254.1052

4f: 3-(3-chlorophenyl)-2-cyanopropanamide



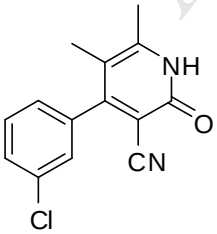
m.p. 115-116 ^{o}C; IR (neat): 3366, 3200, 2250, 1675, 1624; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.51 (s, 1H), 7.39-7.25 (m, 4H), 4.00-3.96 (m, 1H), 3.16 (dd, J = 13.6, 6.8 Hz, 1H), 3.07 (dd, J = 14.0, 8.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.0, 139.4, 132.9, 130.2, 128.8, 127.8, 127.1, 118.2, 34.6; HRMS (EI) calcd. for $C_{10}H_9N_2OCl$ [M^+]: 208.0403, found: 208.0402

6f: 4-(3-chlorophenyl)-6-ethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



m.p. 253-254 ^{o}C; IR (neat): 2220, 1640, 1616; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 7.68-7.58 (m, 4H), 6.37 (s, 1H), 2.58 (q, J = 7.6 Hz, 2H), 1.19 (t, J = 7.6 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.3, 158.7, 157.6, 138.1, 133.4, 130.7, 130.1, 127.7, 126.8, 116.3, 105.0, 97.9, 26.1, 12.7; HRMS (EI) calcd. for $C_{14}H_{11}N_2OCl$ [M^+]: 258.0560, found: 258.0563

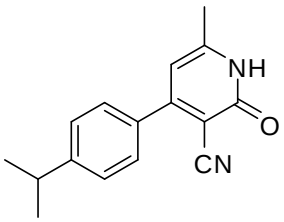
5f: 4-(3-chlorophenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



m.p. 249-250 ^{o}C; IR (neat): 2223, 1652, 1591; ^{1}H NMR (400 MHz,

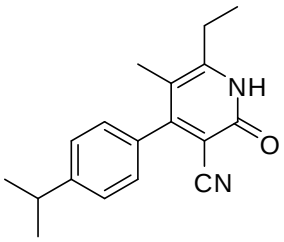
DMSO-d_6) δ 12.65 (s, 1H), 7.56-7.27 (m, 4H), 2.32 (s, 3H), 1.70 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.1, 159.9, 150.8, 138.3, 133.3, 130.7, 129.0, 127.4, 126.4, 116.1, 111.3, 99.7, 18.0, 13.9; HRMS (EI) calcd. for $C_{14}H_{11}N_2OCl$ [M^+]: 258.0560, found: 258.0564

5g: 4-(4-isopropylphenyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



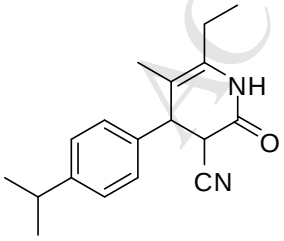
m.p. 265-266 ^{o}C; IR (neat): 2224, 1652, 1615; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 7.55-7.40 (m, 4H), 6.33 (s, 1H), 2.99-2.92 (m, 1H), 2.29 (s, 3H), 1.22 (d, J = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.6, 160.0, 151.9, 151.0, 133.5, 128.0, 126.7, 116.8, 106.5, 96.9, 33.3, 23.6, 19.1; HRMS (EI) calcd. for $C_{16}H_{16}N_2O$ [M^+]: 252.1263, found: 252.1263

5h: 6-ethyl-4-(4-isopropylphenyl)-5-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile



m.p. 254-255 ^{o}C; IR (neat): 2223, 1652, 1598; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H), 7.40-7.22 (m, 4H), 2.99-2.92 (m, 1H), 2.60 (q, J = 7.6 Hz, 2H), 1.74 (s, 3H), 1.24 (d, J = 6.8 Hz, 6H), 1.14 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.5, 160.2, 154.6, 149.1, 133.7, 127.7, 126.5, 116.3, 110.5, 100.3, 33.2, 24.6, 23.7, 13.5, 12.7; HRMS (EI) calcd. for $C_{18}H_{20}N_2O$ [M^+]: 280.1576, found: 280.1579

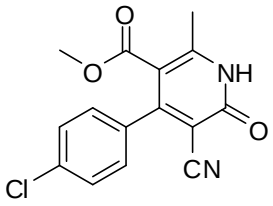
5h':
6-ethyl-4-(4-isopropylphenyl)-5-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile



m.p. 184-185 ^{o}C; IR (neat): 2253, 1699, 1682; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21, 8.13 (s, 1H), 7.27-7.08 (m, 4H), 4.14-3.72 (m, 1H), 3.60-3.56 (m, 1H), 2.93-2.86 (m, 1H), 2.33-2.21 (m, 2H), 1.70 (s, 3H), 1.30-1.23 (m, 6H), 1.18-1.11 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.6, 162.0, 149.0, 148.9, 134.6, 133.1, 132.7, 132.6, 128.1,

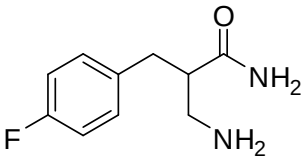
127.4, 127.4, 127.1, 116.5, 115.1, 110.4, 108.4, 48.0, 46.7, 41.6, 40.9, 33.8, 23.9, 23.9, 23.9, 23.4, 23.2, 16.2, 16.0, 12.3, 12.2; HRMS (EI) calcd. for $C_{18}H_{22}N_2O$ [M^+]: 282.1732, found: 282.1734

5i: Methyl 4-(4-chlorophenyl)-5-cyano-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate



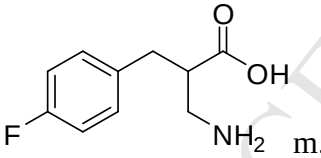
m.p. 265-266 ^oC; IR (neat): 2231, 1734, 1651; ^1H NMR (400 MHz, $CDCl_3$) δ 13.01 (bs, 1H), 7.58-7.34 (m, 4H), 3.40 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 159.7, 158.3, 153.6, 134.6, 134.3, 129.1, 128.6, 115.3, 111.6, 100.7, 52.1, 18.4; HRMS (EI) calcd. for $C_{15}H_{11}N_2O_3Cl$ [M^+]: 302.0458, found: 302.0459

4da: 3-amino-2-(4-fluorobenzyl)propanamide



m.p. 121-122 ^oC; IR (neat): 3355, 3298, 1670, 1606, 1510; ^1H NMR (400 MHz, DMSO-d_6) δ 7.31 (s, 1H), 7.21-7.07 (m, 4H), 6.75 (s, 1H), 2.74-2.42 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ175.6, 161.8, 159.4, 136.4, 130.5, 130.4, 114.7, 114.6, 50.7, 43.8, 34.5; HRMS (EI) calcd. for $C_{10}H_{13}FN_2O$ [M^+]: 196.1012, found: 196.1012

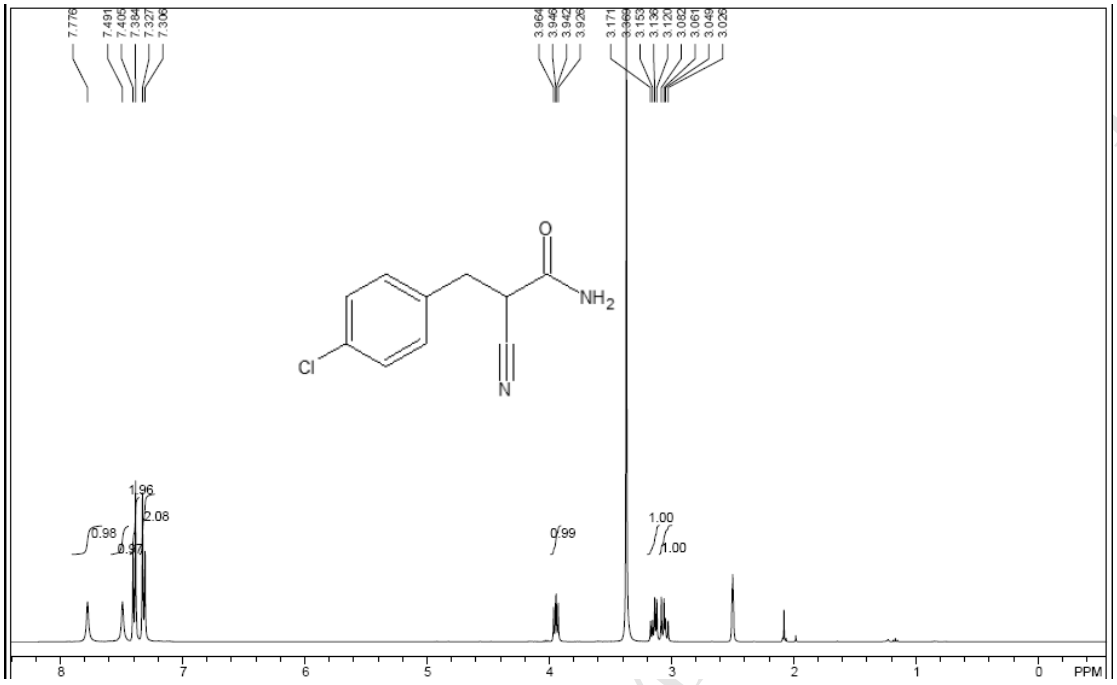
4db: 3-amino-2-(4-fluorobenzyl)propanoic acid

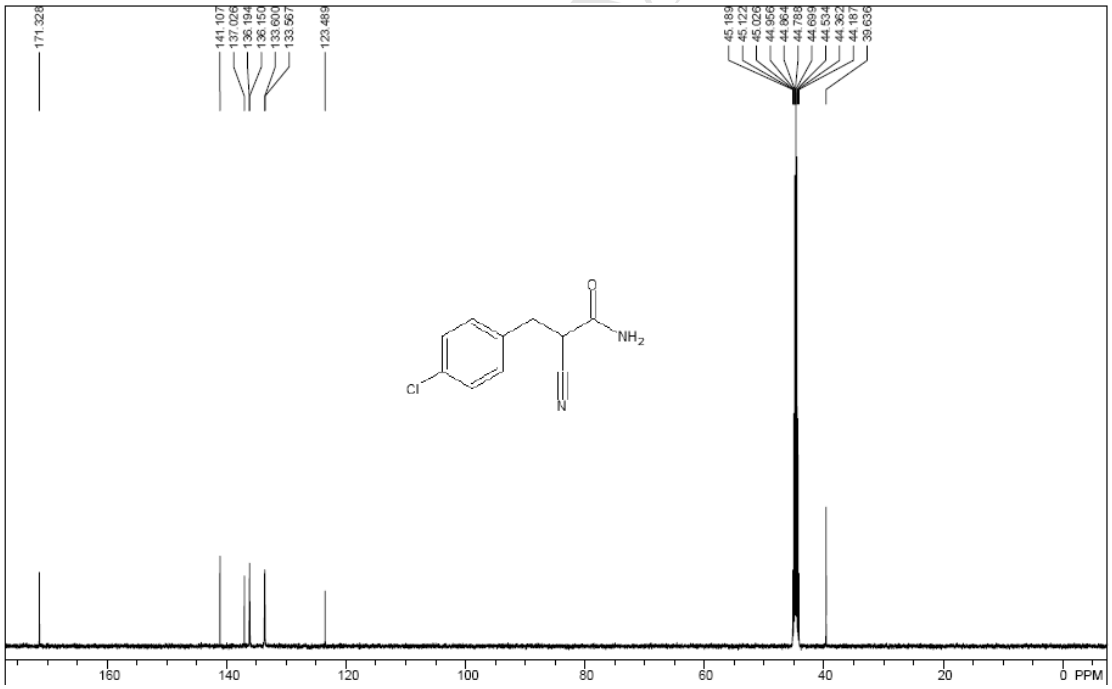


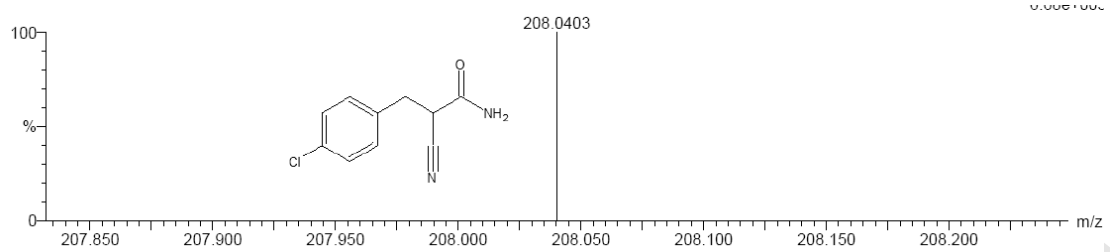
m.p. 242-243 ^oC; IR (neat): 3362, 3184, 1665, 1637, 1511; ^1H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 2H), 7.75 (s, 1H), 7.38-7.10 (m, 4H), 2.91-2.67 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ173.4, 162.1, 159.7, 134.6, 134.6, 130.9, 130.8, 115.0, 114.8, 44.4, 34.8; HRMS (EI) calcd. for $C_{10}H_{12}FNO_2$ [M^+]: 197.0852, found: 197.0849

Copies of ^{1}H NMR, ^{13}C NMR and HRMS of Compounds

4a: 3-(4-chlorophenyl)-2-cyanopropanamide

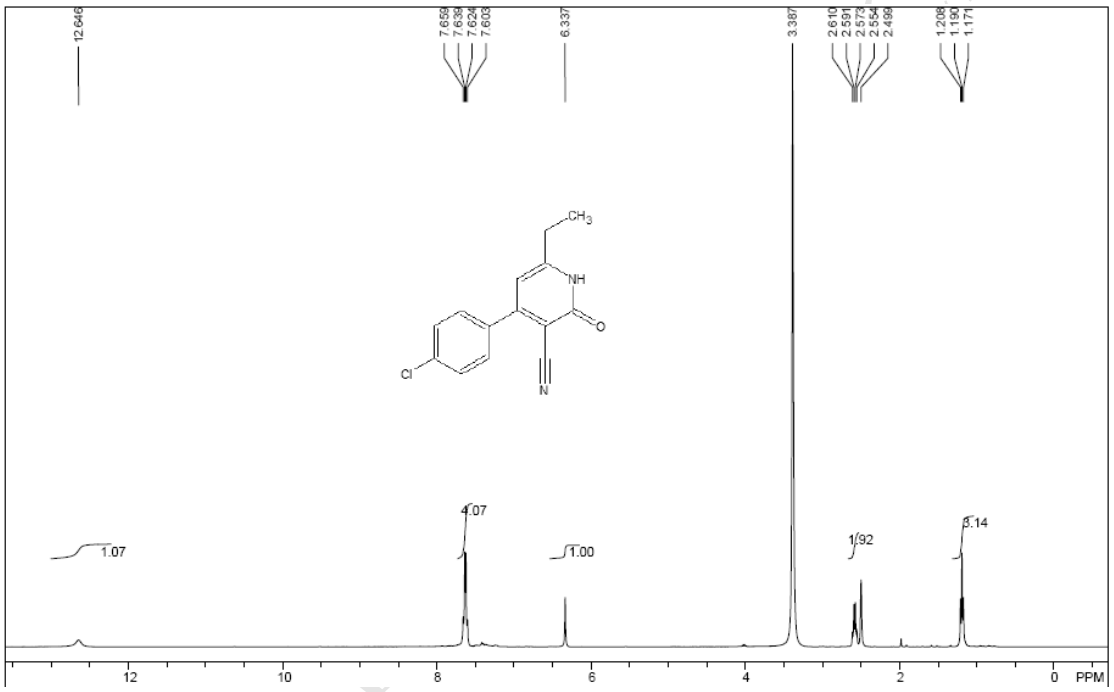


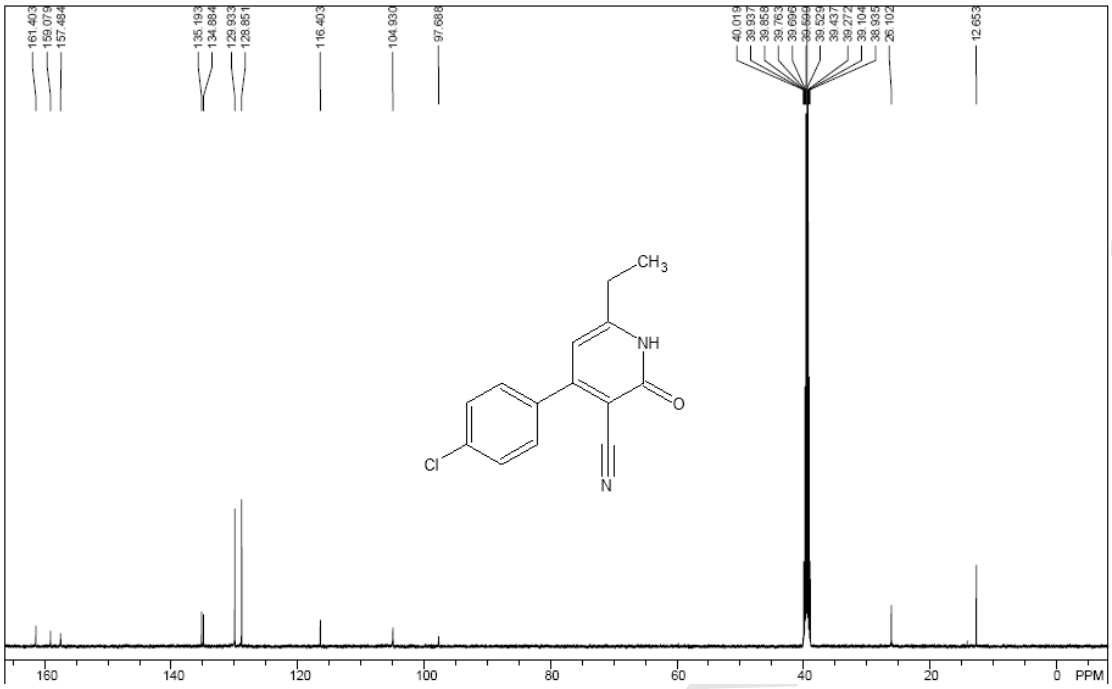


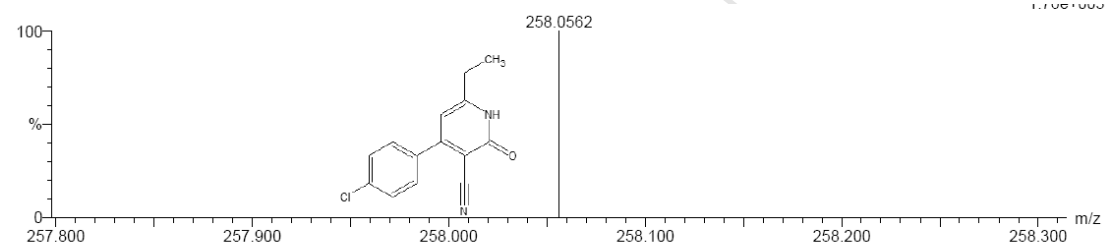


Minimum:				-1.5		
Maximum:		0.3	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
208.0403	208.0403	0.0	0.0	7.0	5549359.0	C10 H9 N2 O Cl

6a: 4-(4-chlorophenyl)-6-ethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile

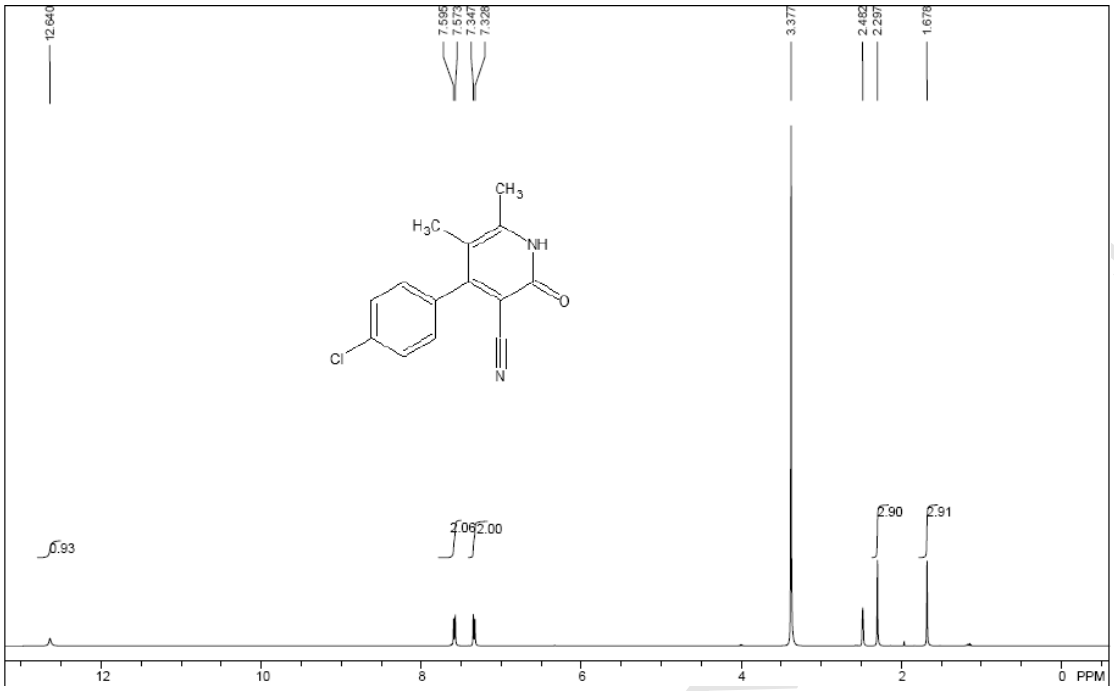


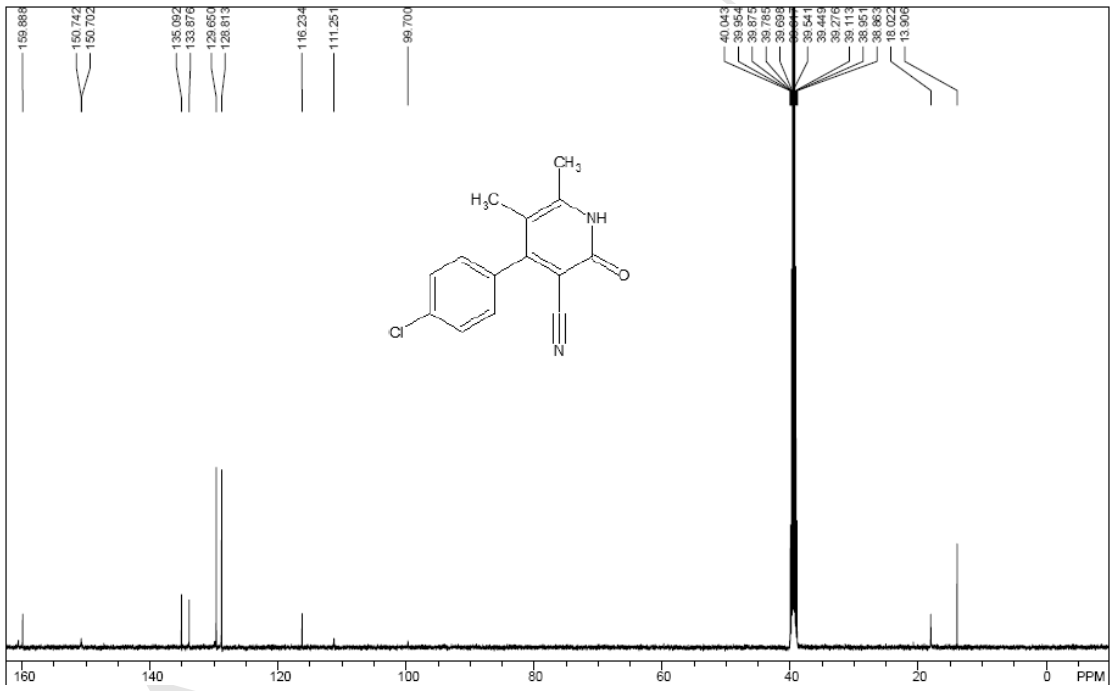


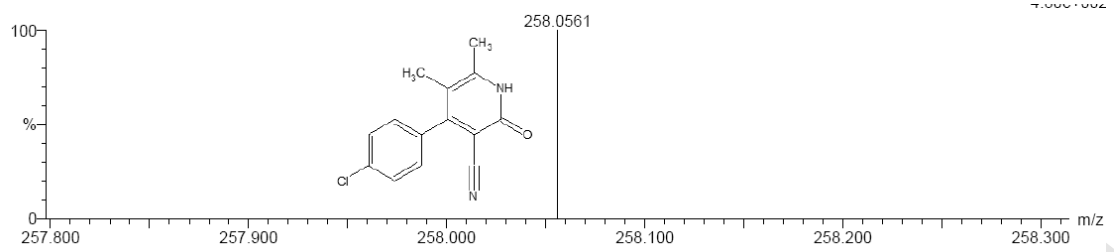


Minimum:				-1.5		
Maximum:		0.3	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
258.0562	258.0560	0.2	0.8	10.0	5546896.0	C14 H11 N2 O Cl

5a: 4-(4-chlorophenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile

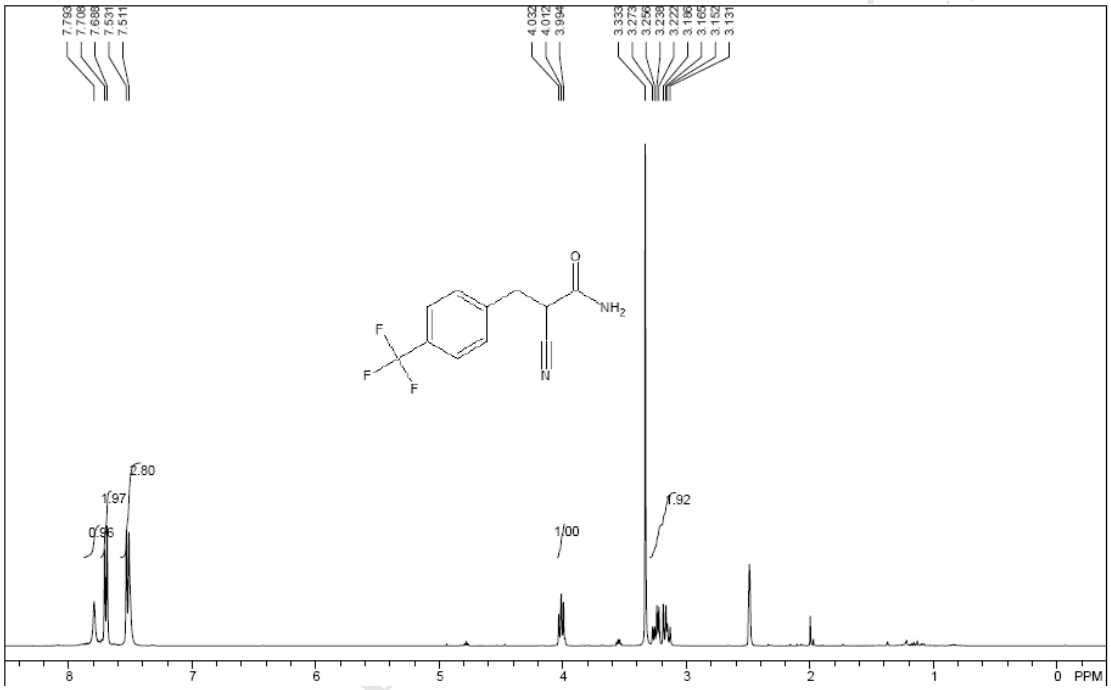

12.640
7.595
7.573
7.347
7.328
3.377
2.482
2.297
1.678
0.93
2.06
2.00
2.90
2.91
12
10
8
6
4
2
0
PPM

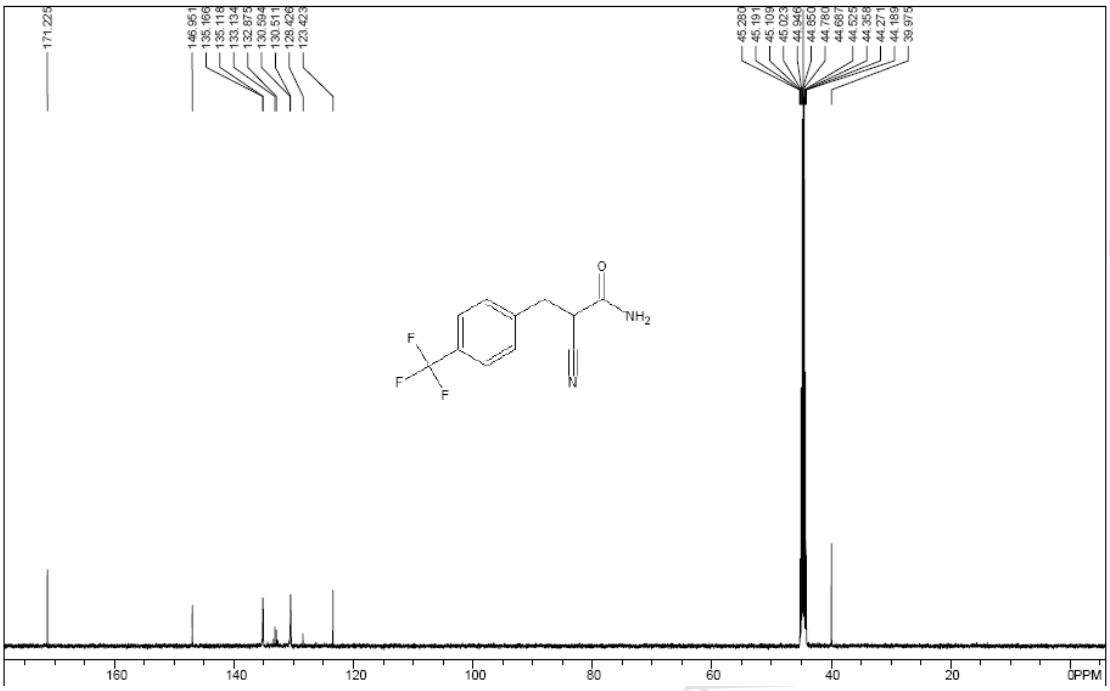

159.888
150.742
150.702
135.092
133.876
129.650
128.813
116.234
111.251
99.700
40.043
39.954
39.875
39.785
39.698
39.541
39.449
39.276
39.113
38.951
38.863
18.022
13.906
160
140
120
100
80
60
40
20
0
PPM

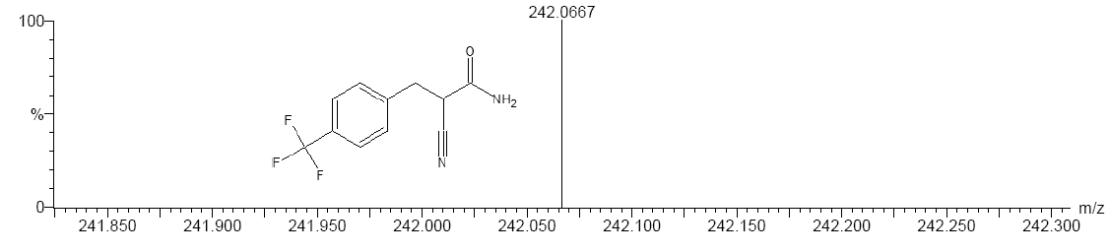


Minimum:				-1.5		
Maximum:		0.3	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
258.0561	258.0560	0.1	0.4	10.0	5546258.0	C14 H11 N2 O Cl

4b: 2-cyano-3-(4-(trifluoromethyl)phenyl)propanamide

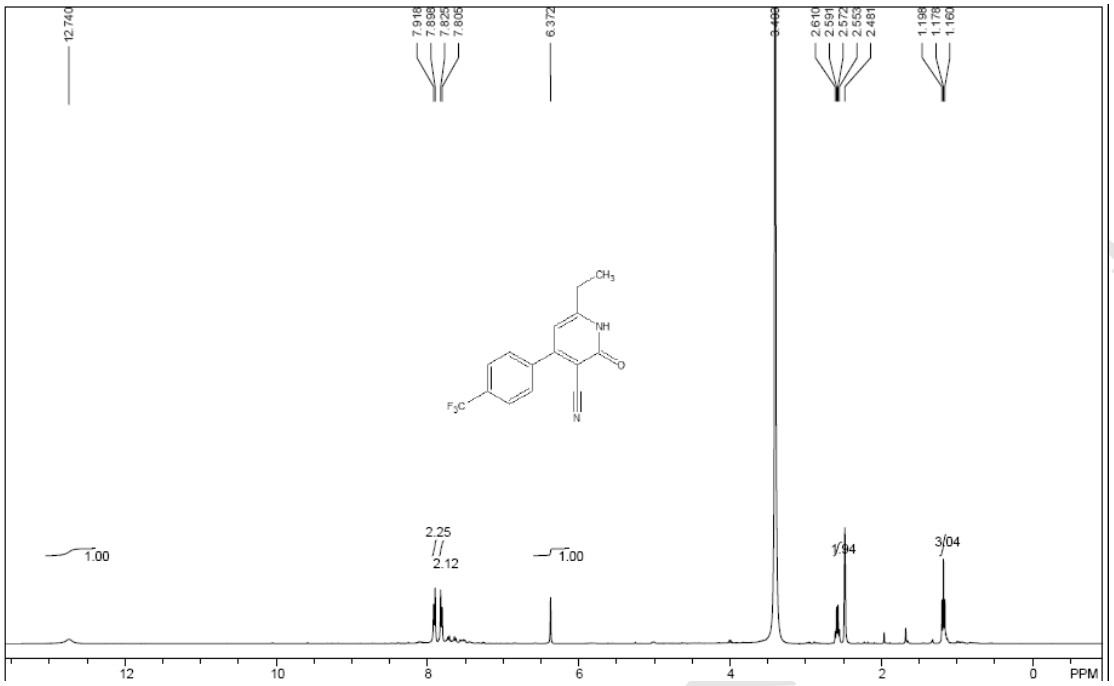






Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
242.0667	242.0667	0.0	0.0	7.0	5546494.5	C11 H9 N2 O F3

6b: 6-ethyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carbonitrile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7.918
7.898
7.825
7.805
6.372
3.488
2.610
2.591
2.572
2.553
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1.198
1.179
1.160
CH3
NH
O
F3C
N
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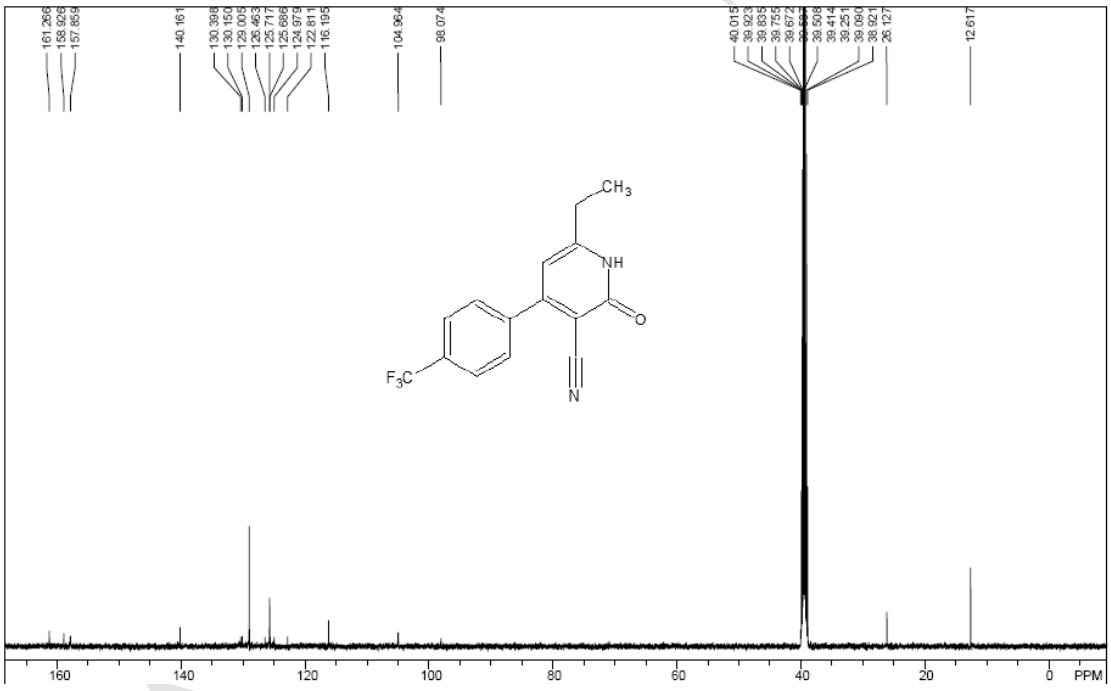
2.25
2.12
1.00
1.94
3.04
12
10
8
6
4
2
0
PPM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140.161
130.398
130.150
129.005
126.463
125.717
125.666
124.979
122.611
116.195
104.964
98.074
40.015
39.923
39.835
39.755
39.672
39.508
39.414
39.251
39.090
38.921
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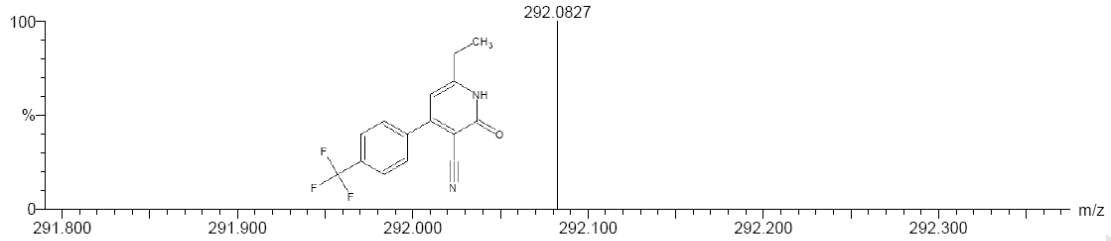
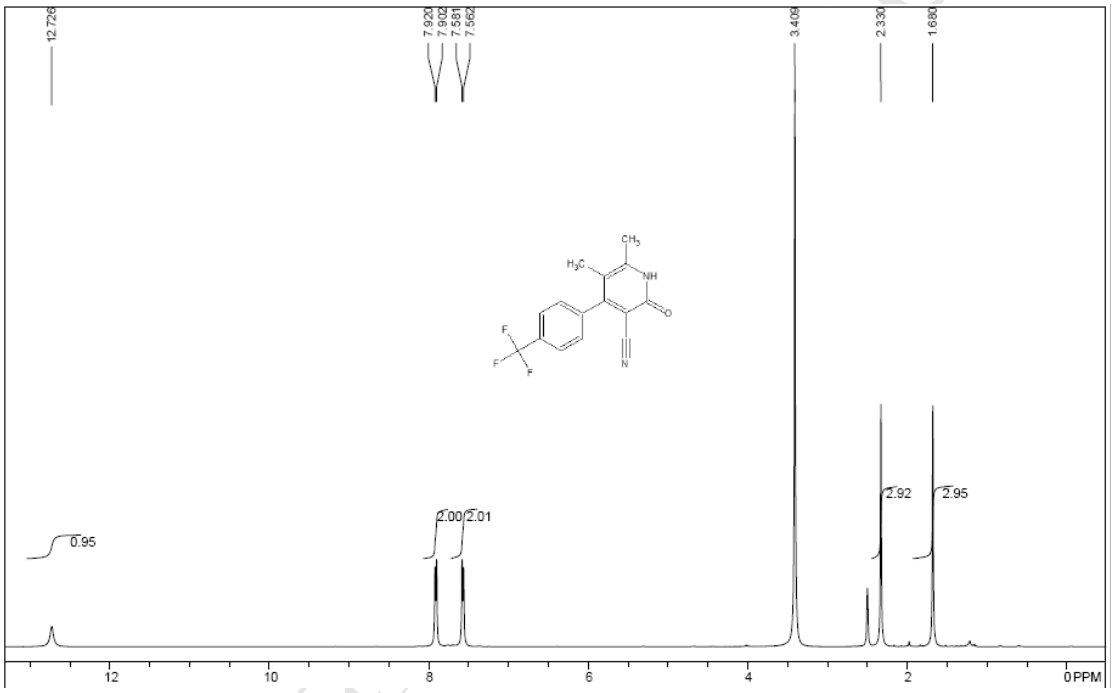
CH3
NH
O
F3C
N
160
140
120
100
80
60
40
20
0
PPM

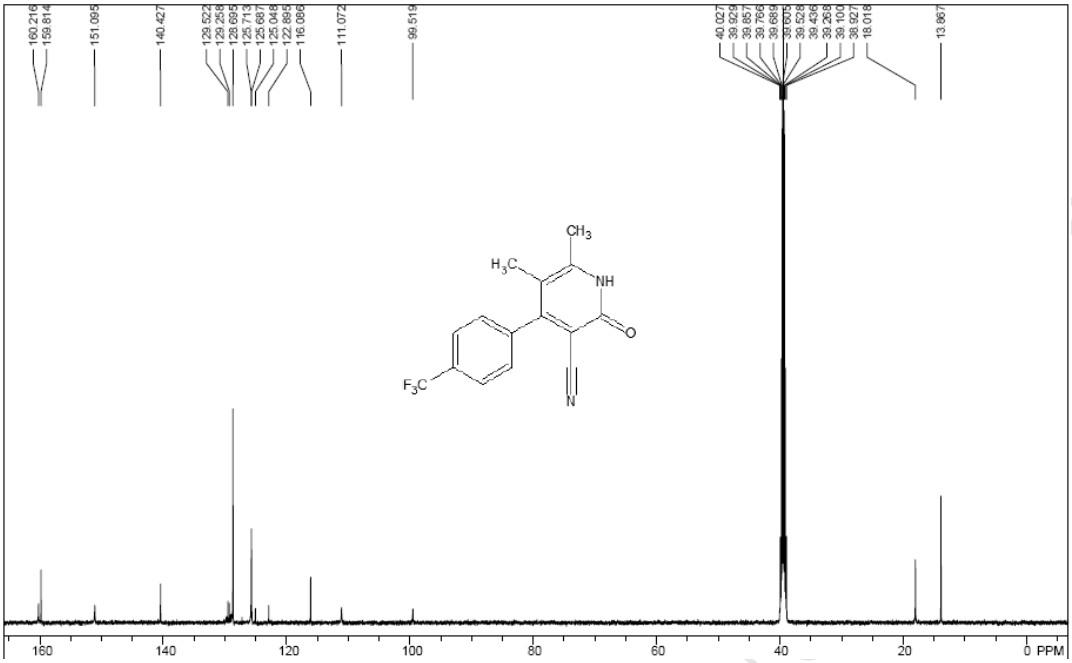


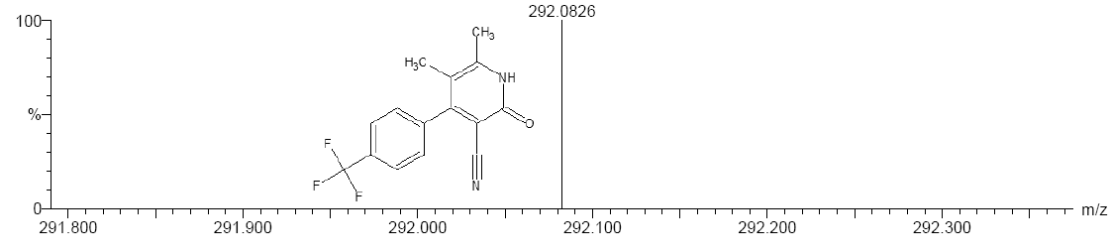
Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
292.0827	292.0823	0.4	1.4	10.0	5548104.5	C15 H11 N2 O F3

5b:

5,6-dimethyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carbonitrile

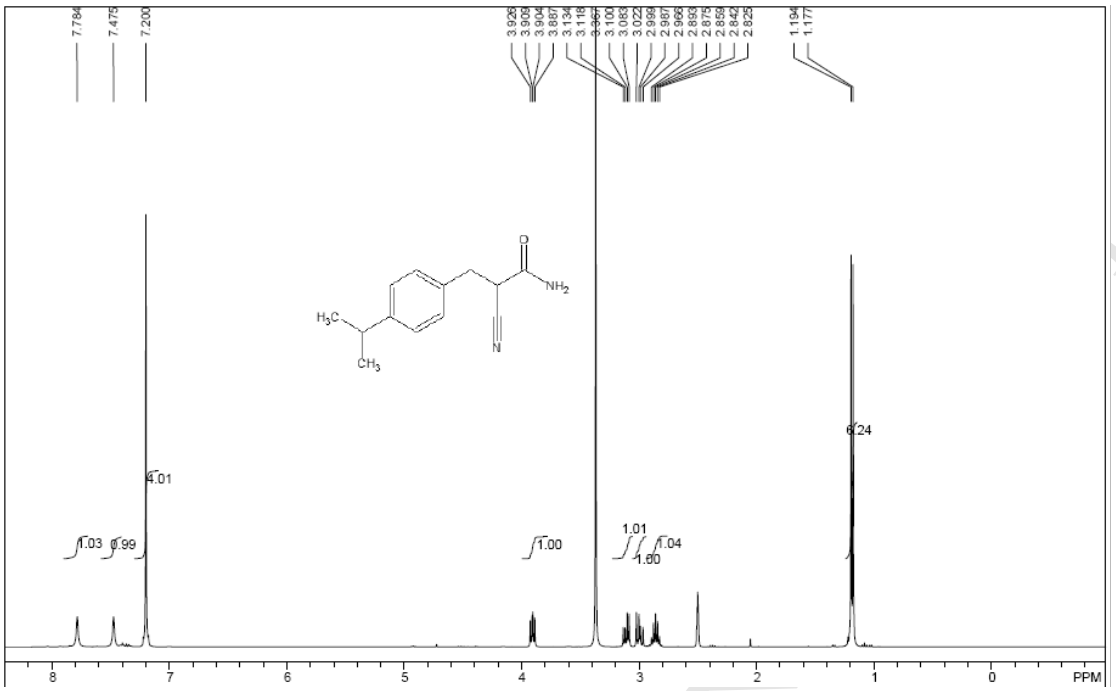






Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
292.0826	292.0823	0.3	1.0	10.0	5546441.5	C15 H11 N2 O F3

4c: 2-cyano-3-(4-isopropylphenyl)propanamide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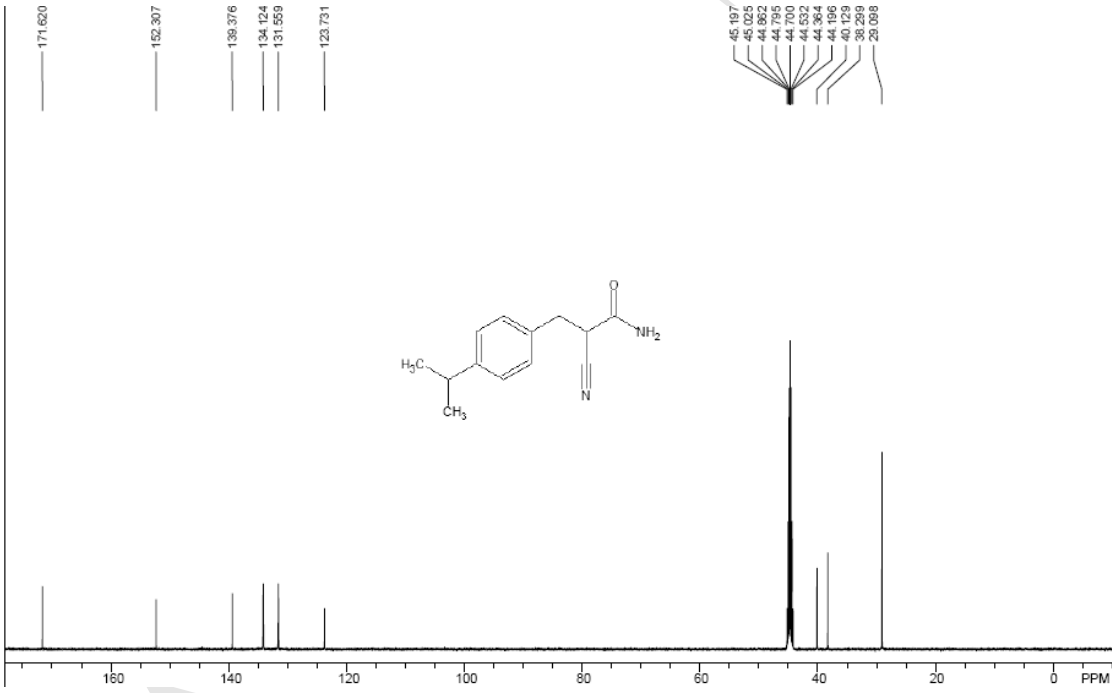
3.904
3.887
3.134
3.118
3.100
3.083
3.022
2.999
2.987
2.966
2.893
2.875
2.859
2.842
2.825
1.194
1.177
O
NH2
H3C
CH3
N
6.24
4.01
1.03
0.99
1.00
1.01
1.00
1.04
8
7
6
5
4
3
2
1
0
PPM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134.124
131.559
123.731
45.197
45.025
44.862
44.795
44.700
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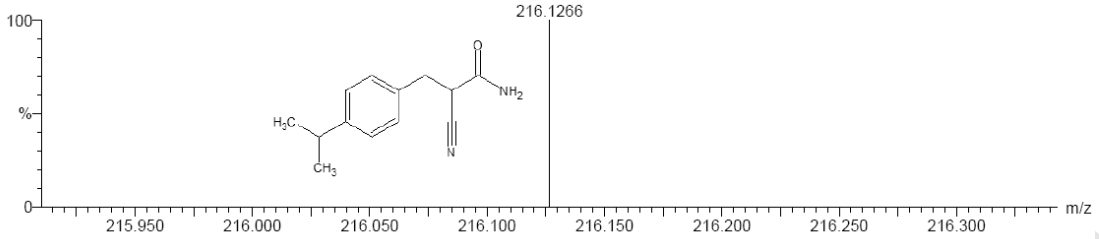
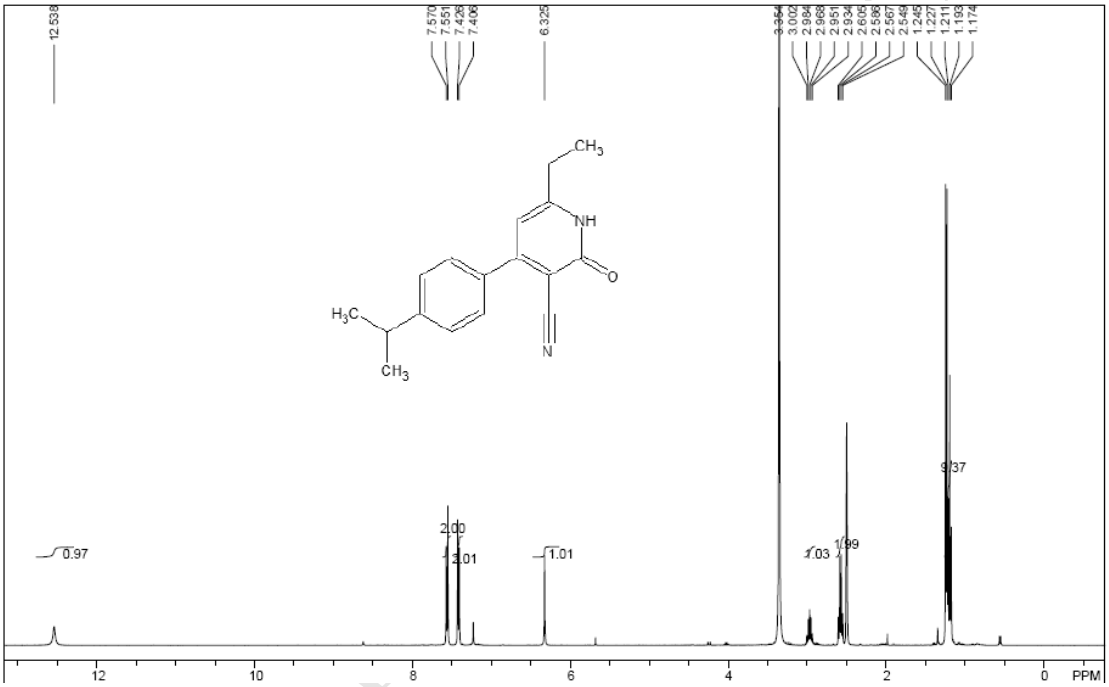
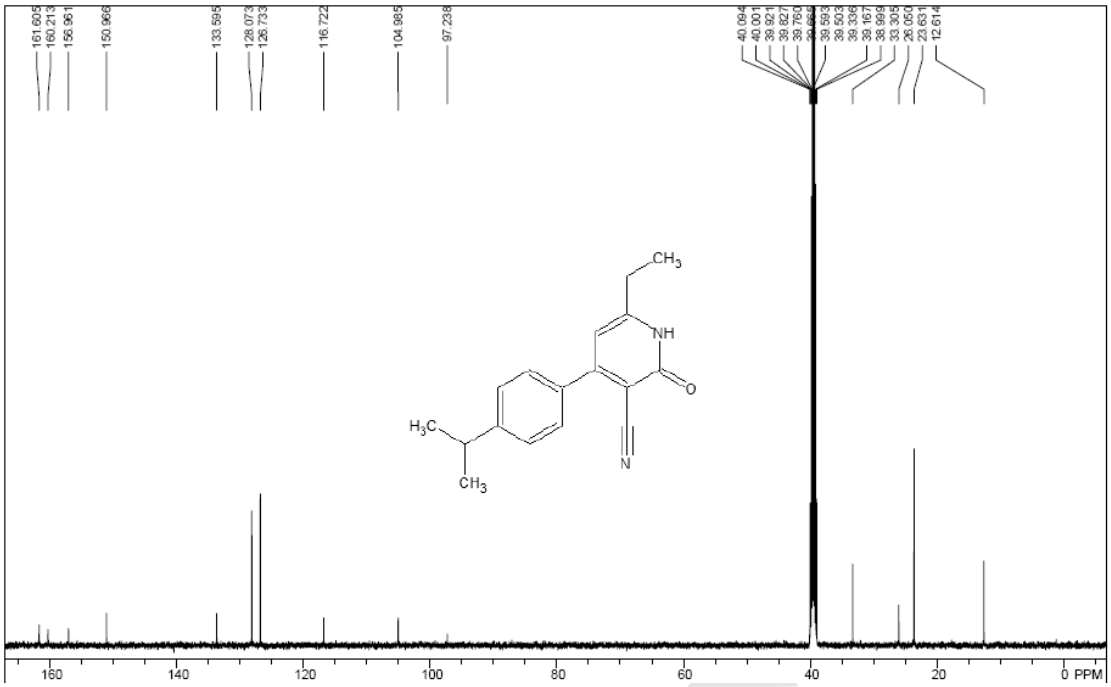
44.364
44.196
40.129
38.299
29.098
O
NH2
H3C
CH3
N
160
140
120
100
80
60
40
20
0
PPM

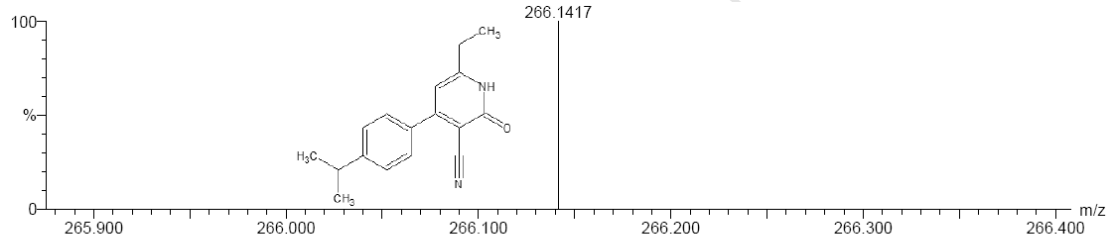


Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
216.1266	216.1263	0.3	1.4	7.0	5548285.5	C13 H16 N2 O

6c: 6-ethyl-4-(4-isopropylphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

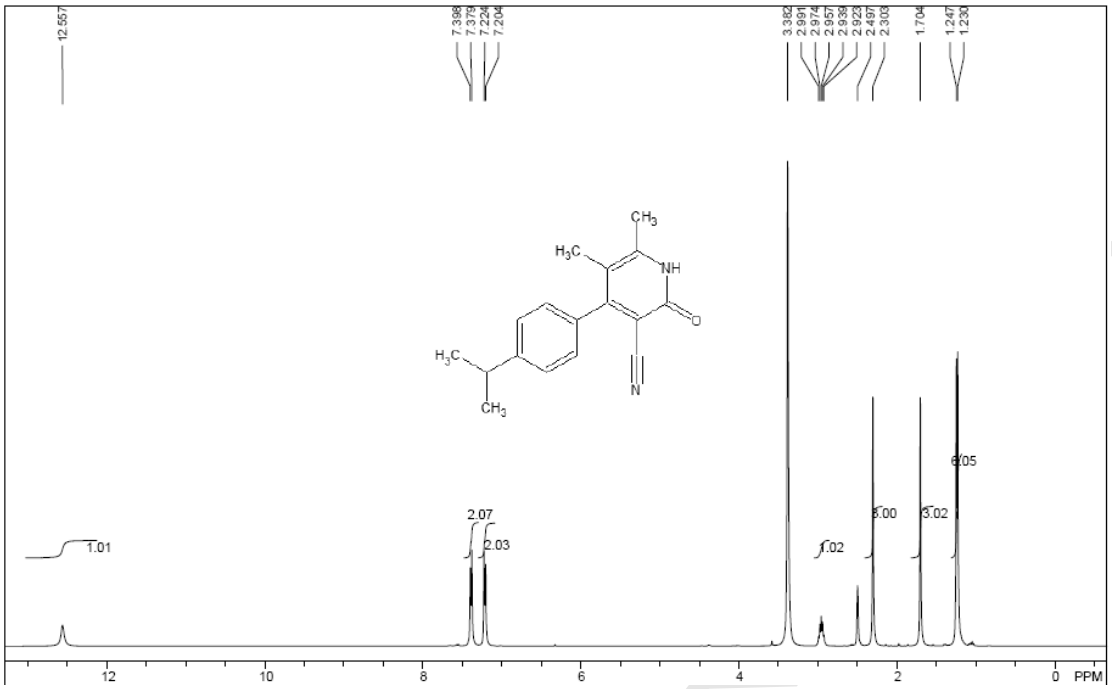






Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
266.1417	266.1419	-0.2	-0.8	10.0	5550697.0	C17 H18 N2 O

5c: 4-(4-isopropylphenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile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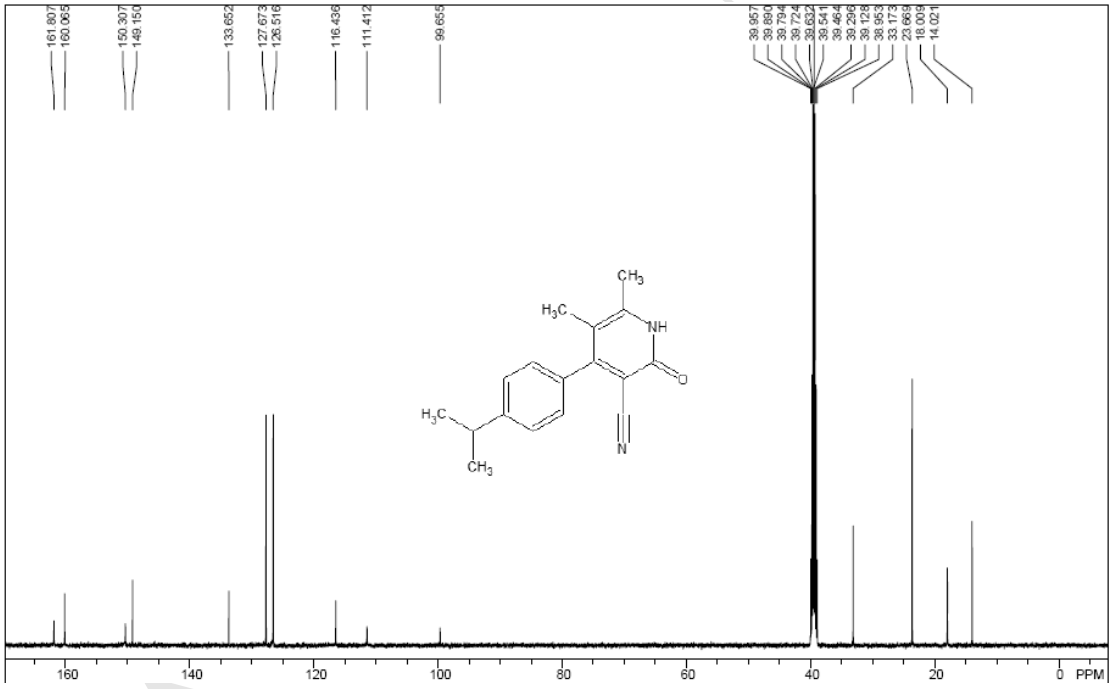
7.204
3.382
3.991
2.974
2.957
2.939
2.923
2.497
2.303
1.704
1.247
1.230
1.01
2.07
2.03
1.02
3.00
3.02
6.05
12
10
8
6
4
2
0 PPM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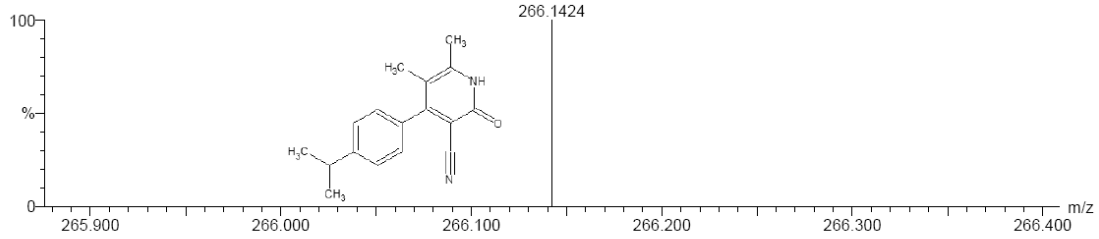
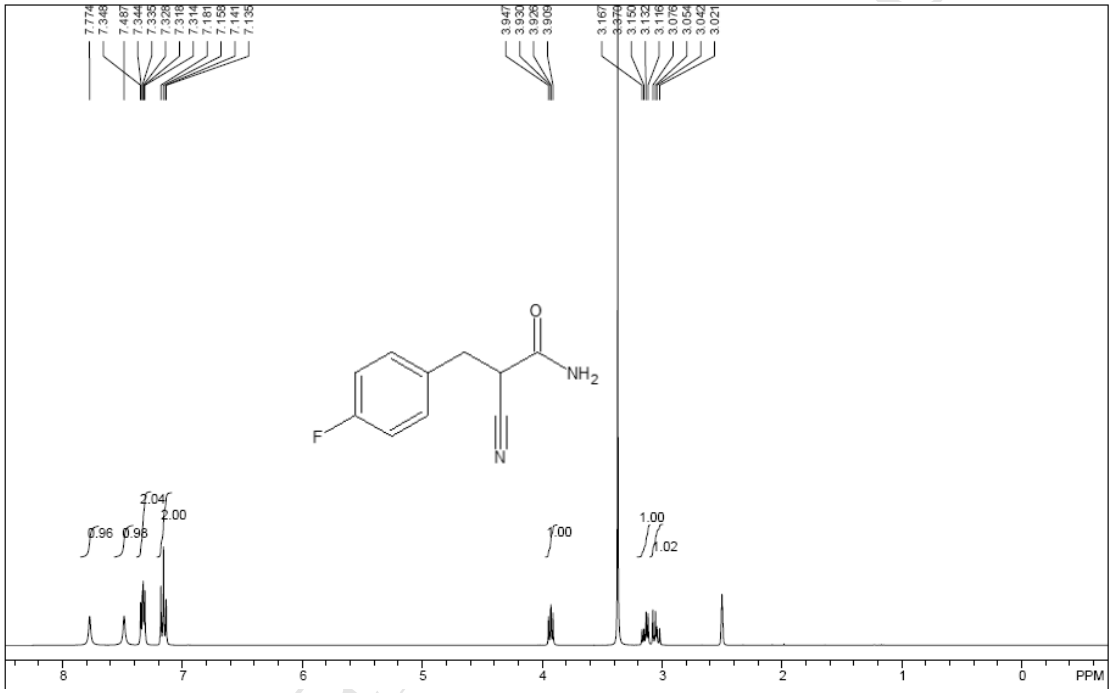
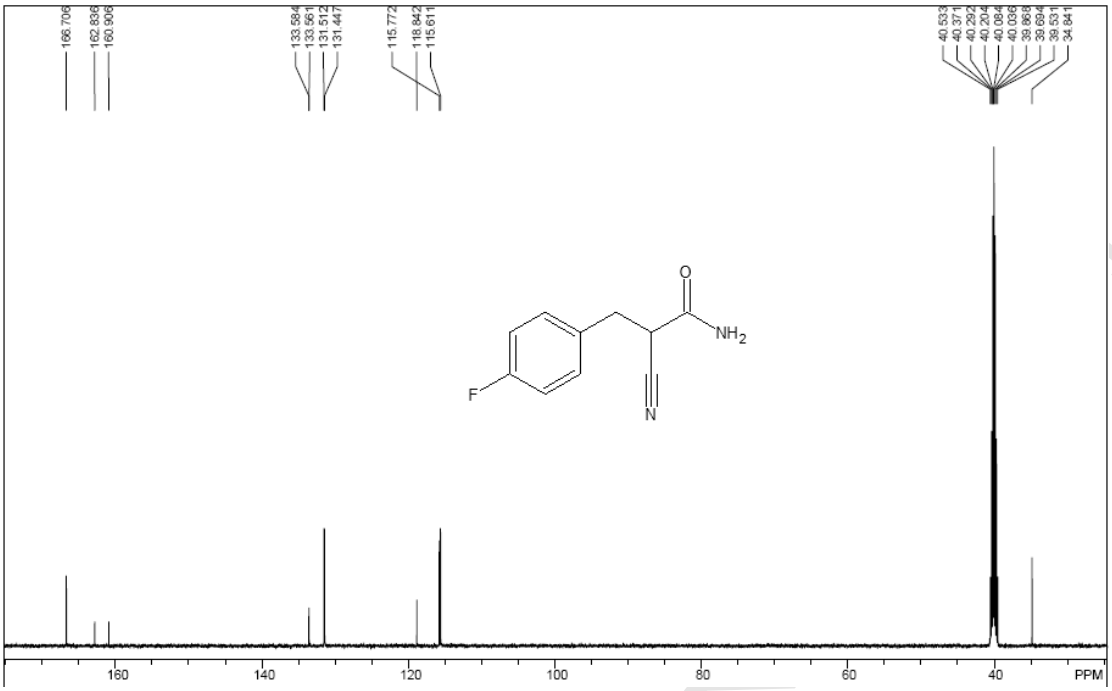
111.412
99.655
39.957
39.890
39.794
39.724
39.632
39.541
39.454
39.296
39.128
38.953
33.173
23.669
18.009
14.021
160
140
120
100
80
60
40
20
0 PPM

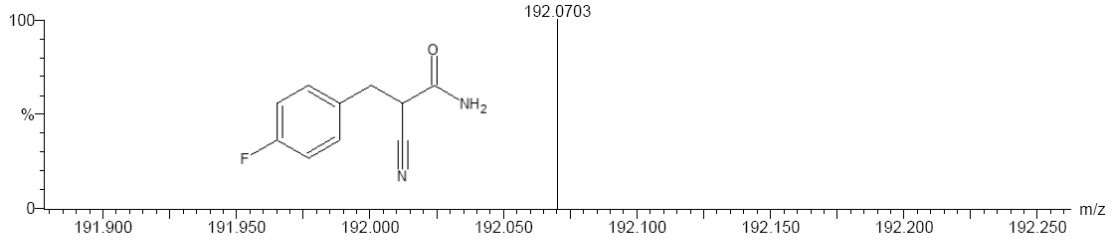


Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
266.1424	266.1419	0.5	1.9	10.0	5549204.0	C17 H18 N2 O

4d: 2-cyano-3-(4-fluorophenyl)propanamide

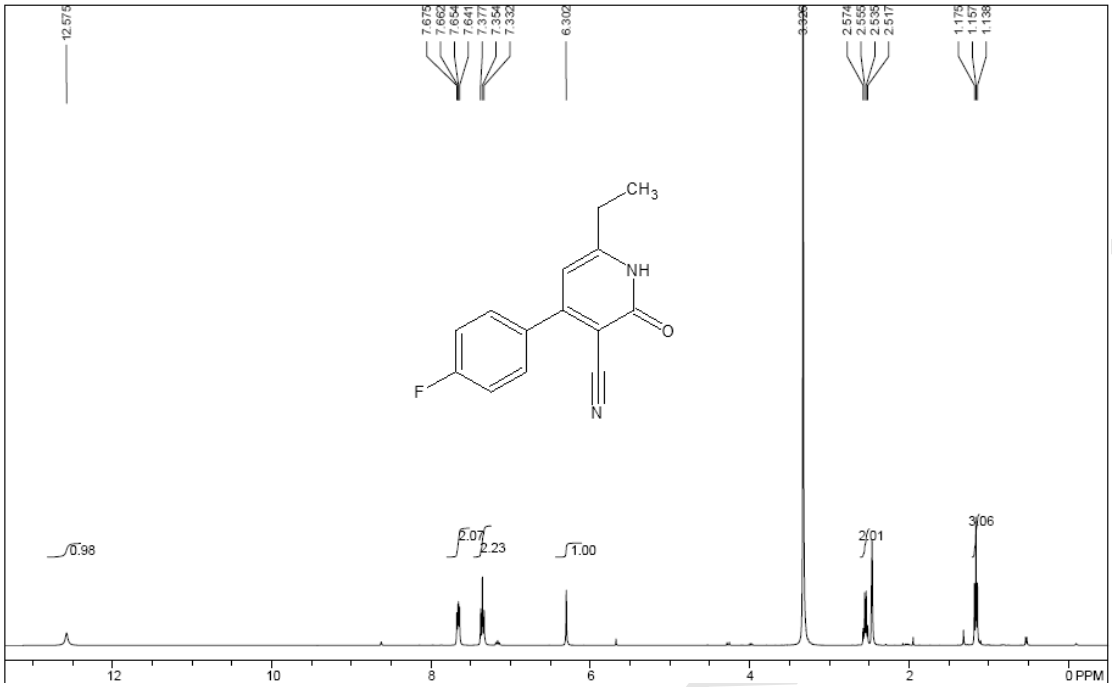






Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
192.0703	192.0699	0.4	2.1	7.0	5548234.0	C10 H9 N2 O F

6d: 6-ethyl-4-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile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7.332
6.302
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2.574
2.556
2.535
2.517
1.175
1.157
1.138
CH3
NH
O
F
N
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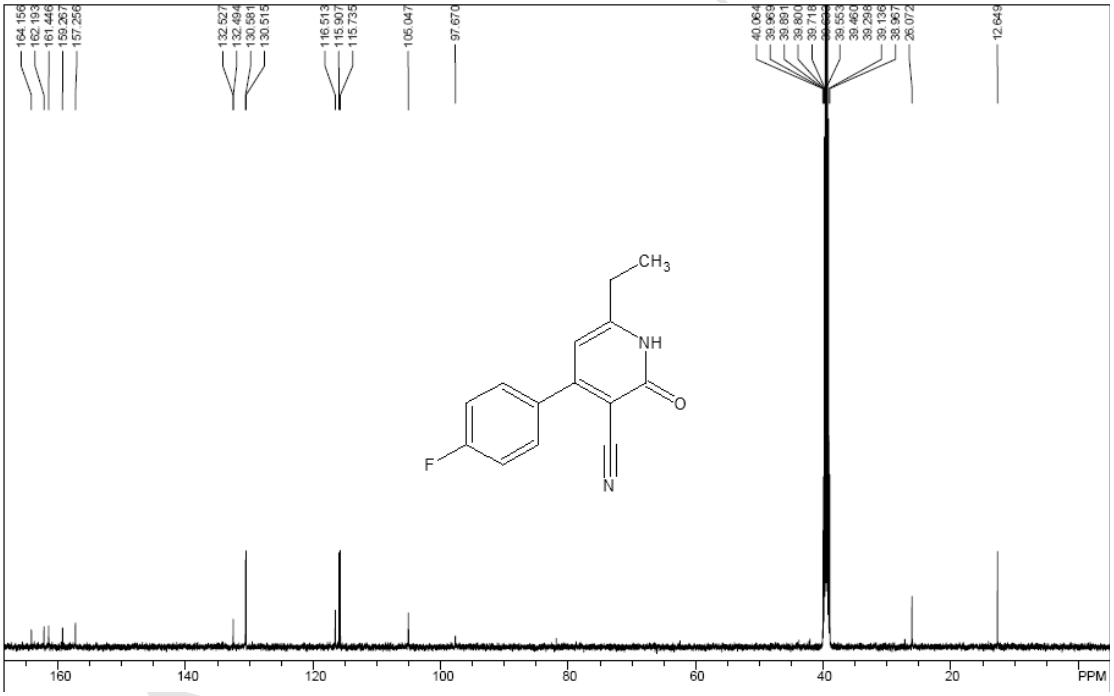
2.07
2.23
1.00
2.01
3.06
12
10
8
6
4
2
0 PPM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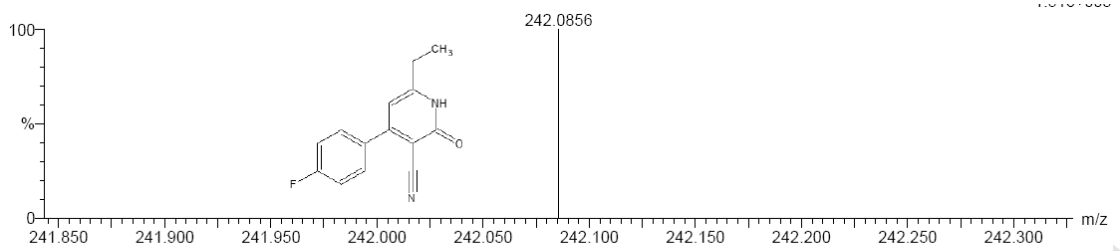
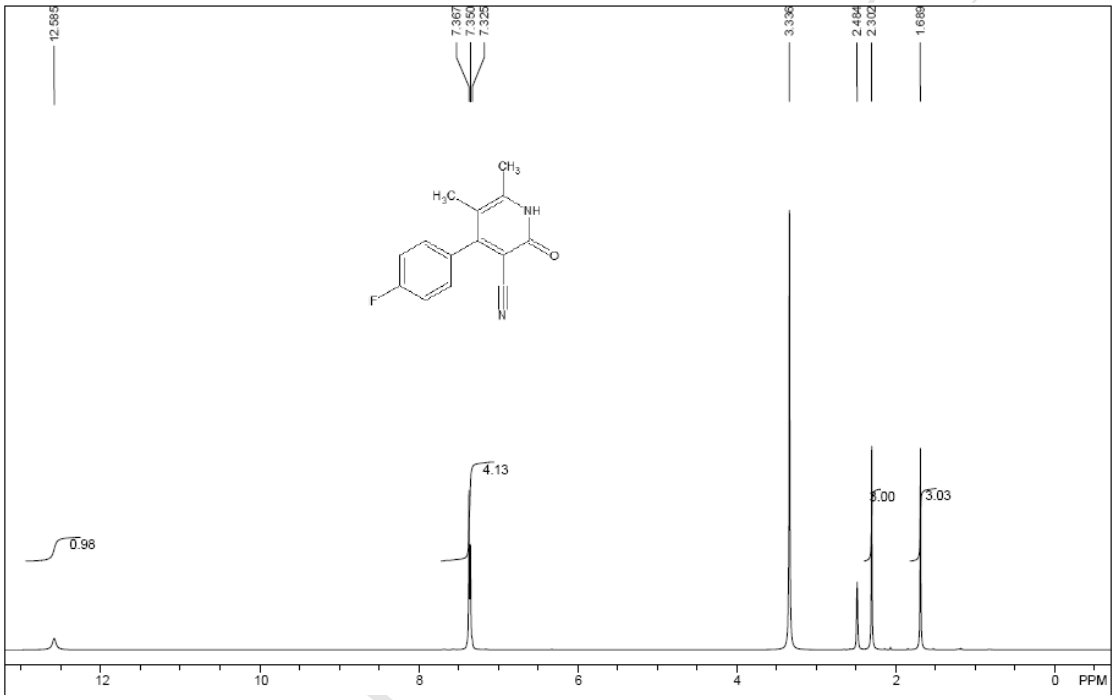
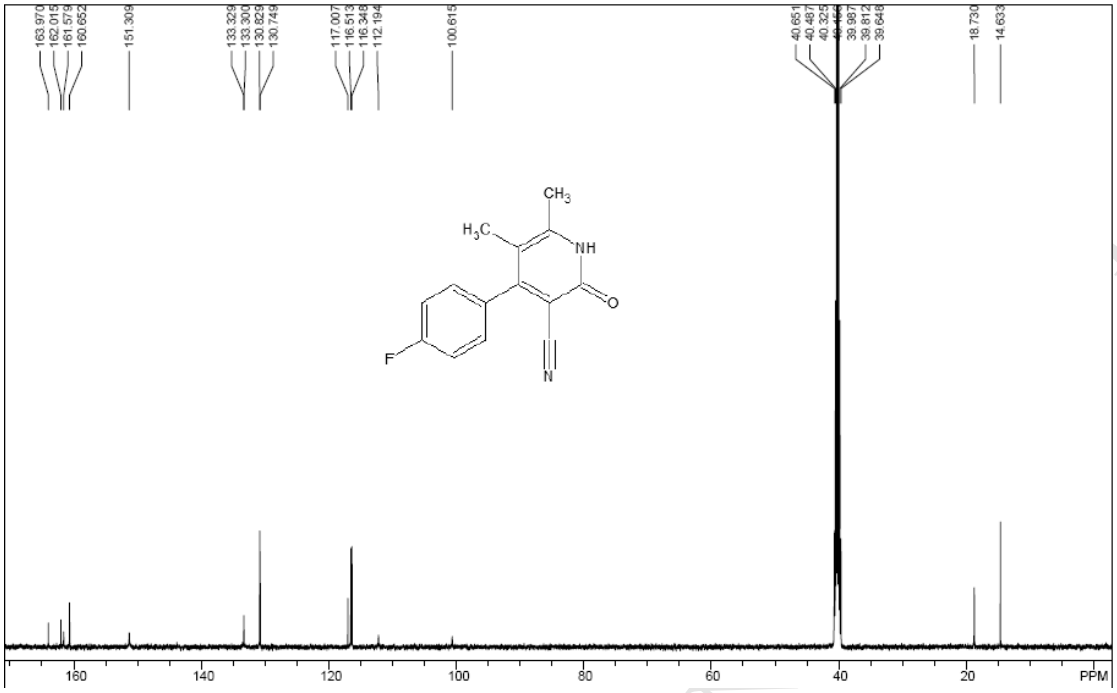
132.494
130.581
130.515
116.513
115.907
115.735
105.047
97.670
40.064
39.969
39.891
39.800
39.718
39.553
39.460
39.298
39.136
38.967
26.072
12.649
CH3
NH
O
F
N
160
140
120
100
80
60
40
20
PPM

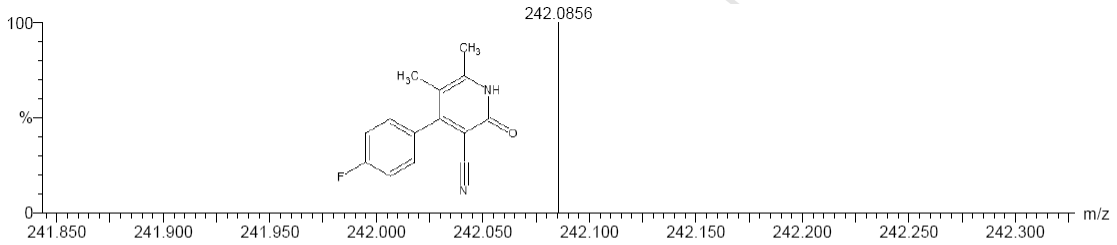


Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
242.0856	242.0855	0.1	0.4	10.0	5546758.0	C14 H11 N2 O F

5d: 4-(4-fluorophenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile

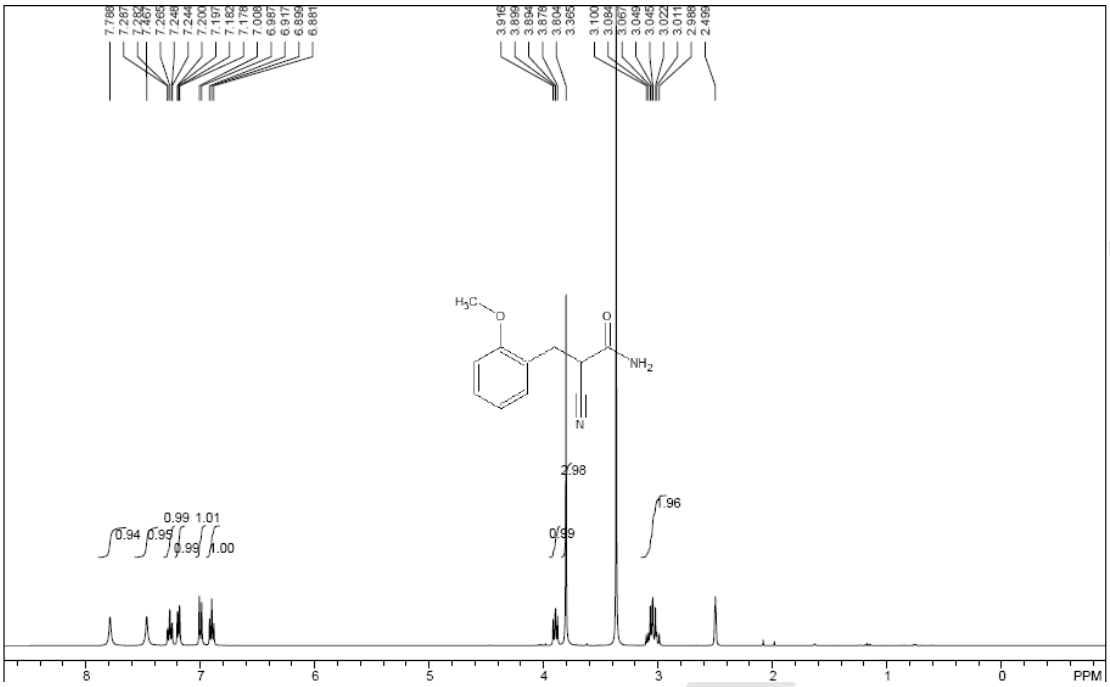






Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
242.0856	242.0855	0.1	0.4	10.0	5548168.0	C14 H11 N2 O F

4e: 2-cyano-3-(2-methoxyphenyl)propanamide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7.244
7.200
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7.182
7.178
7.008
6.987
6.917
6.899
6.881
3.916
3.899
3.894
3.878
3.804
3.365
3.100
3.084
3.067
3.049
3.045
3.022
3.011
2.988
2.499
H3C
O
O
NH2
N
2.98
1.96
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0.94
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0.99
1.01
1.00
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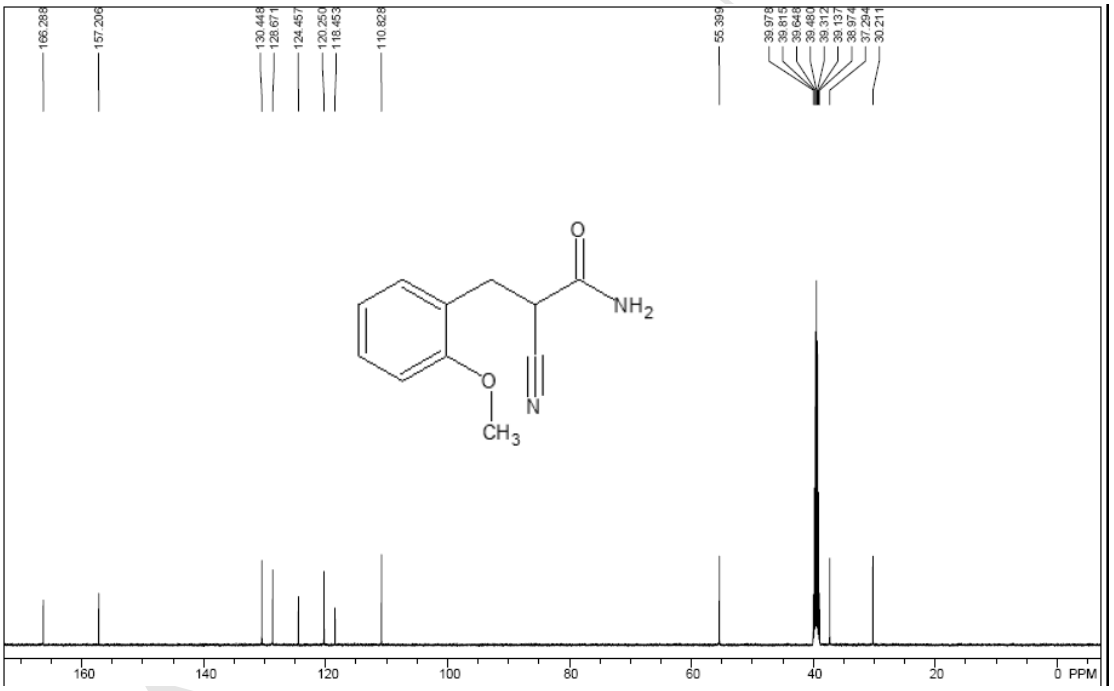
7
6
5
4
3
2
1
0
PPM


166.288
157.206
130.448
128.671
124.457
120.250
118.453
110.828
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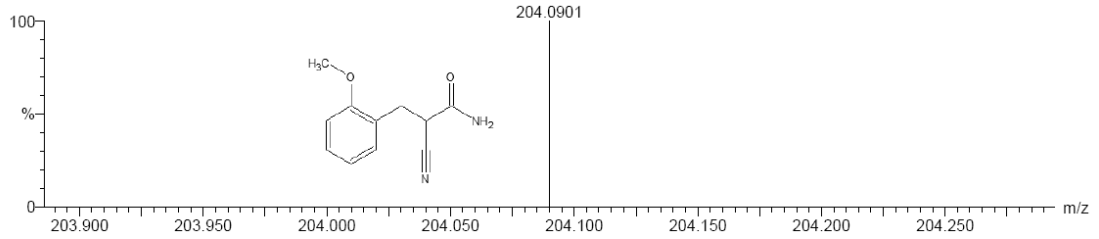
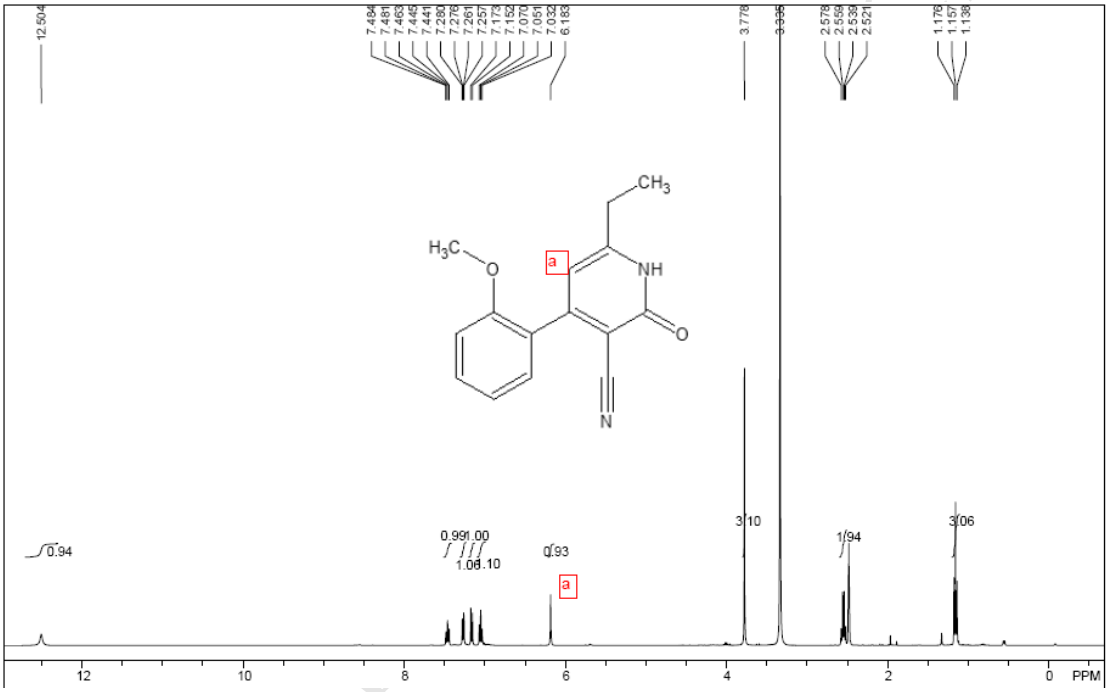
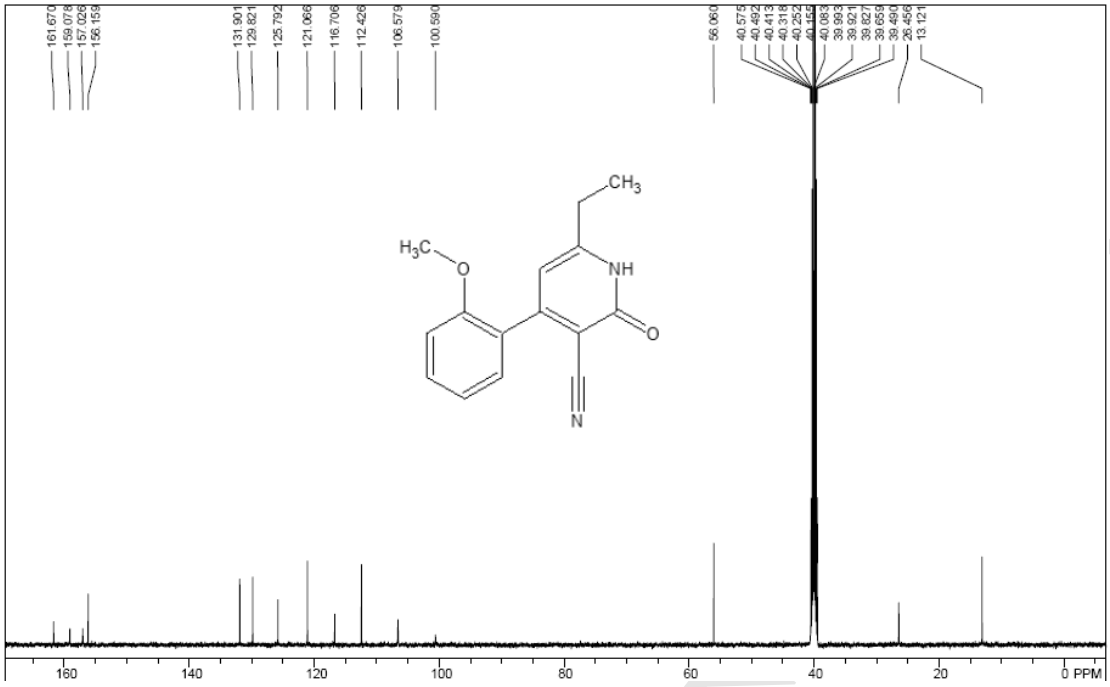
39.978
39.815
39.648
39.480
39.312
39.137
38.974
37.294
30.211
O
NH2
N
O
CH3
160
140
120
100
80
60
40
20
0
PPM

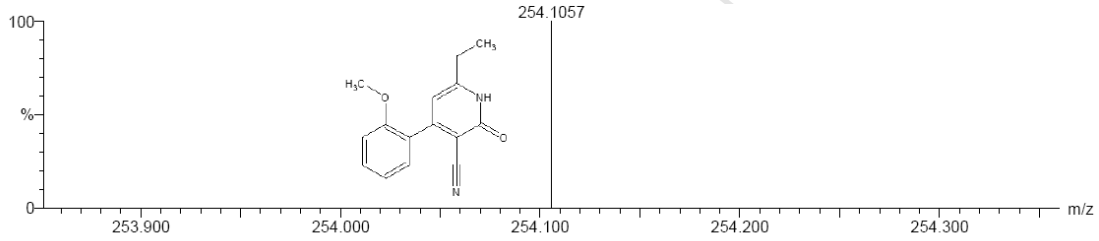


Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
204.0901	204.0899	0.2	1.0	7.0	5547212.0	C11 H12 N2 O2

6e: 6-ethyl-4-(2-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

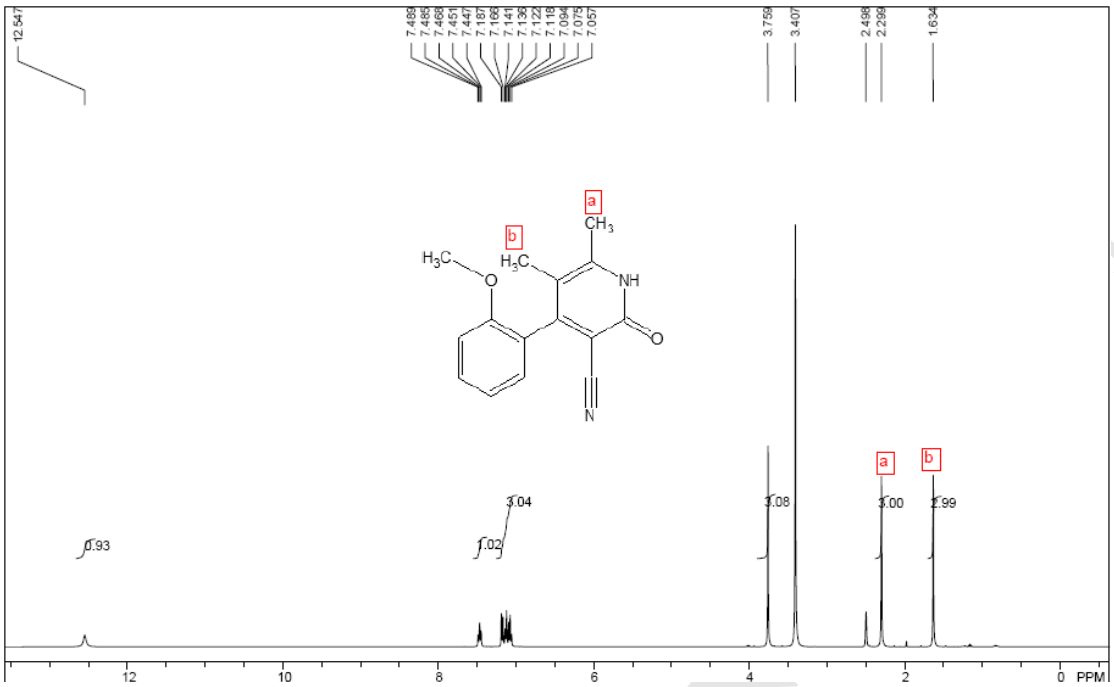






Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
254.1057	254.1055	0.2	0.8	10.0	5547643.0	C15 H14 N2 O2

5e: 4-(2-methoxyphenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile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7.485
7.468
7.451
7.447
7.187
7.166
7.141
7.122
7.118
7.094
7.075
7.057
3.759
3.407
2.498
2.299
1.634
a
b
CH3
H3C
H3C
O
NH
O
N
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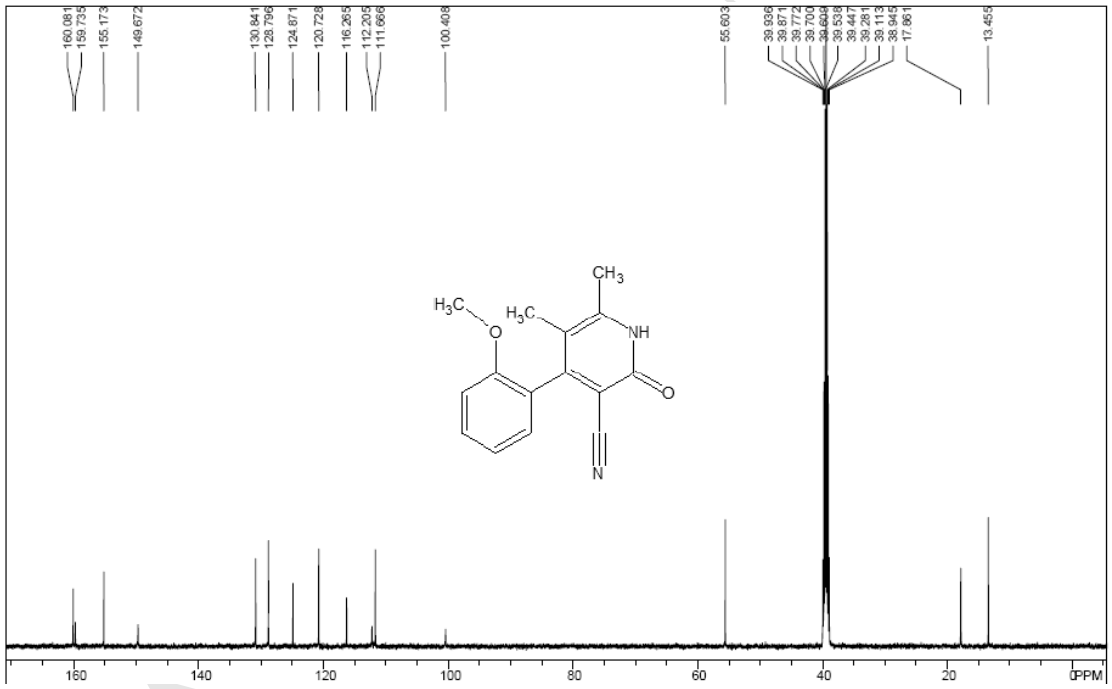
1.02
3.04
3.08
3.00
1.99
12
10
8
6
4
2
0 PPM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130.841
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112.205
111.668
100.408
55.603
39.936
39.871
39.772
39.700
39.604
39.538
39.447
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39.113
38.945
17.861
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CH3
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H3C
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NH
O
N
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140
120
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60
40
20
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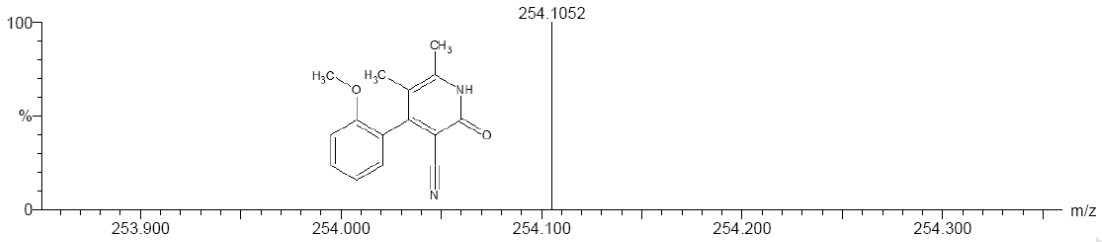
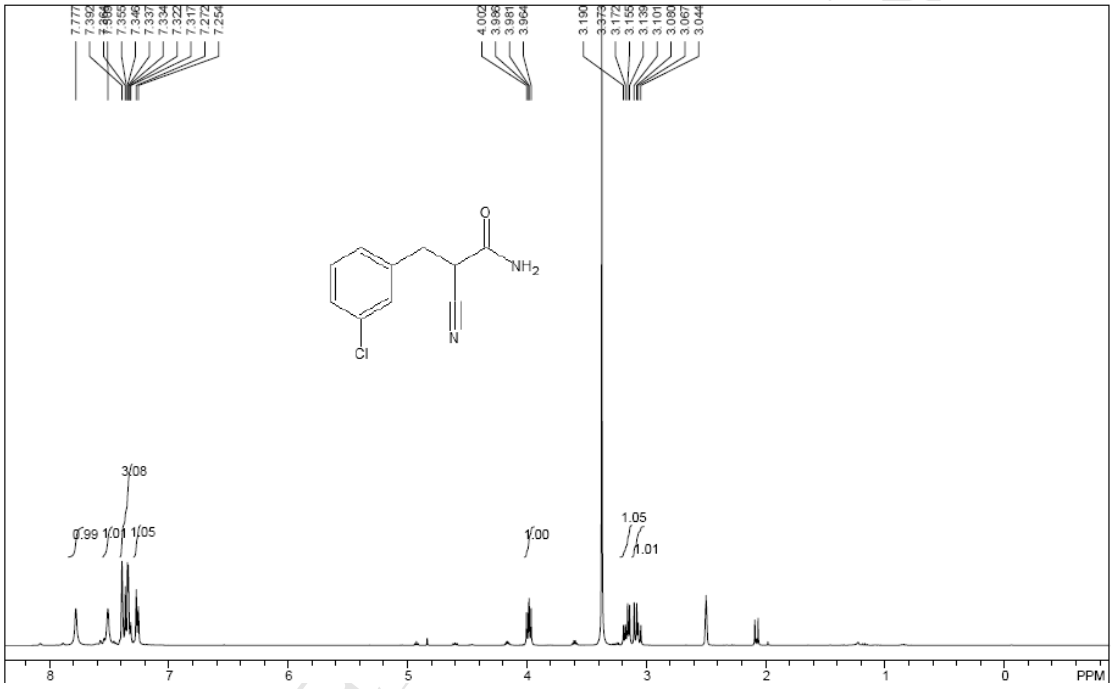
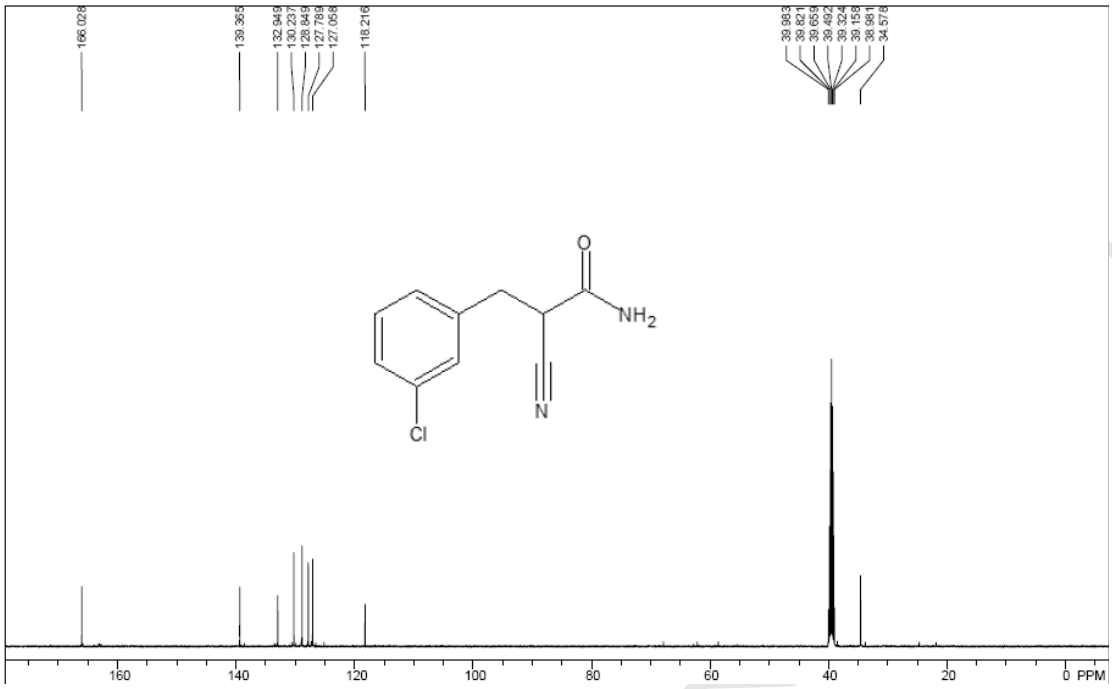
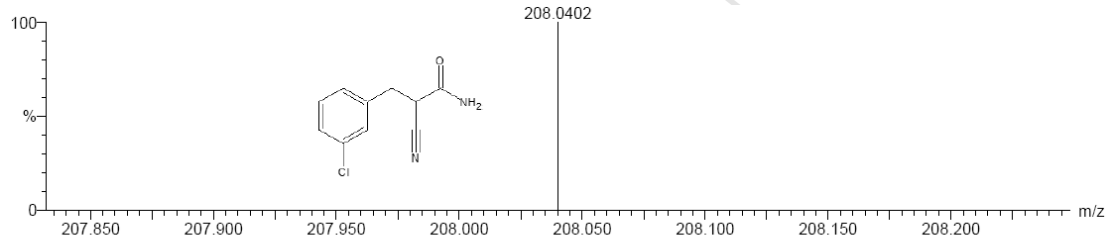


Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
254.1052	254.1055	-0.3	-1.2	10.0	5546635.5	C15 H14 N2 O2

4f: 3-(3-chlorophenyl)-2-cyanopropanamide

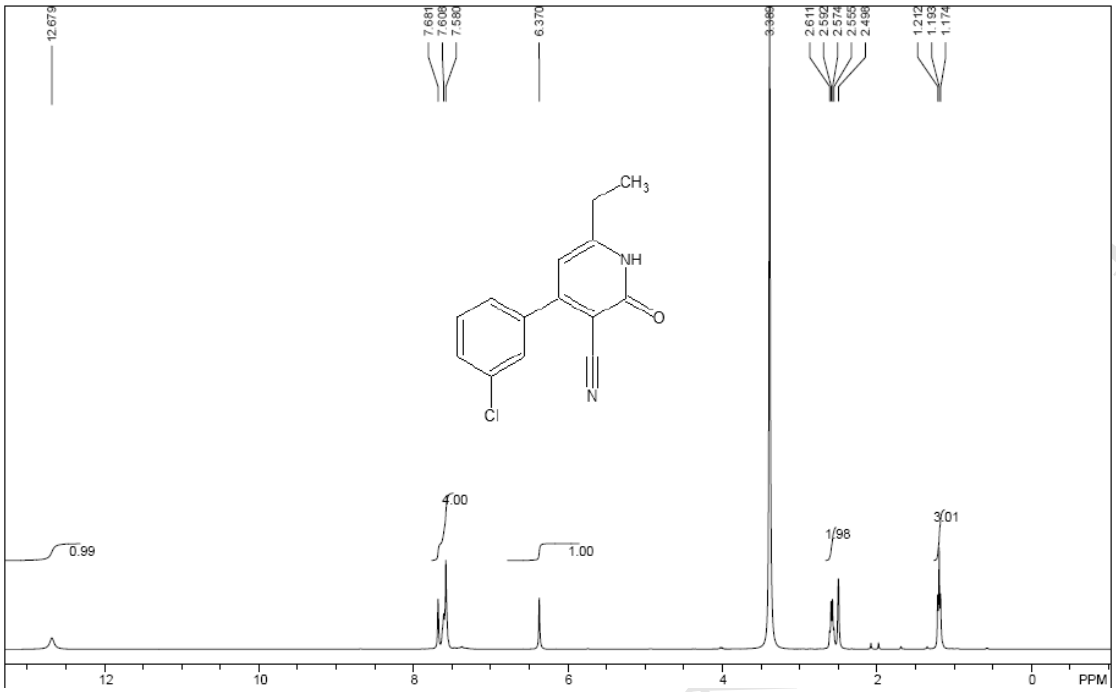






Minimum:				-1.5		
Maximum:		0.2	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
208.0402	208.0403	-0.1	-0.5	7.0	5548780.5	C10 H9 N2 O Cl

6f: 4-(3-chlorophenyl)-6-ethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile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2.592
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2.555
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1.193
1.174
CH3
NH
O
N
Cl
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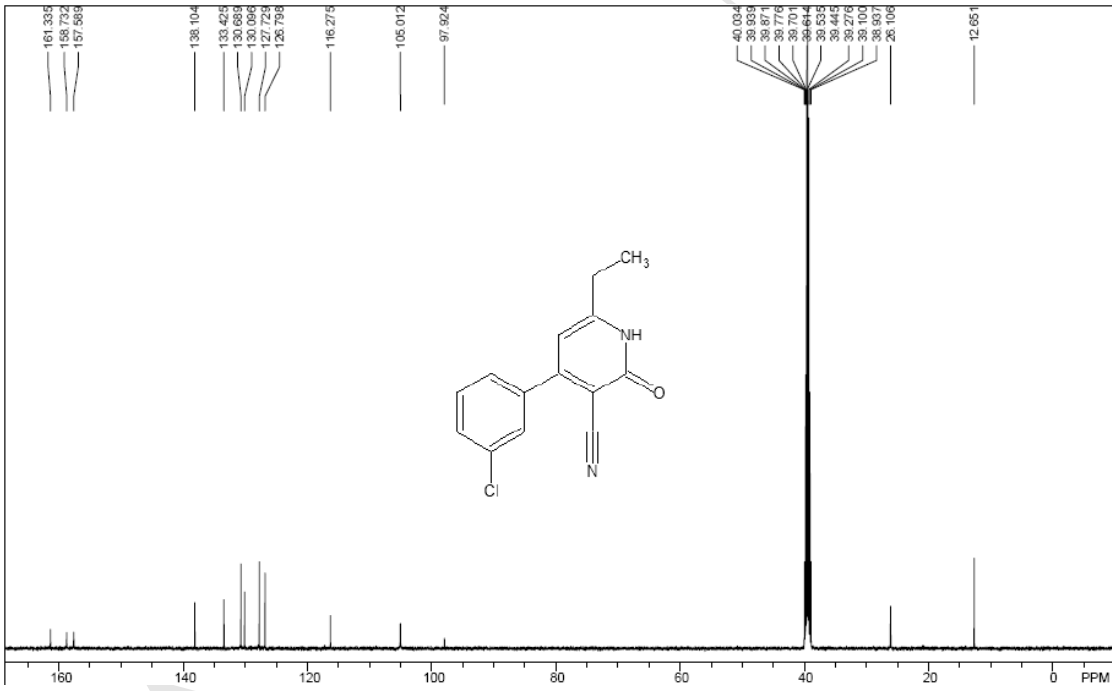
1.00
1.98
3.01
12
10
8
6
4
2
0
PPM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127.729
126.798
116.275
105.012
97.924
40.094
39.939
39.871
39.776
39.701
39.614
39.535
39.445
39.276
39.100
38.937
26.106
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CH3
NH
O
N
Cl
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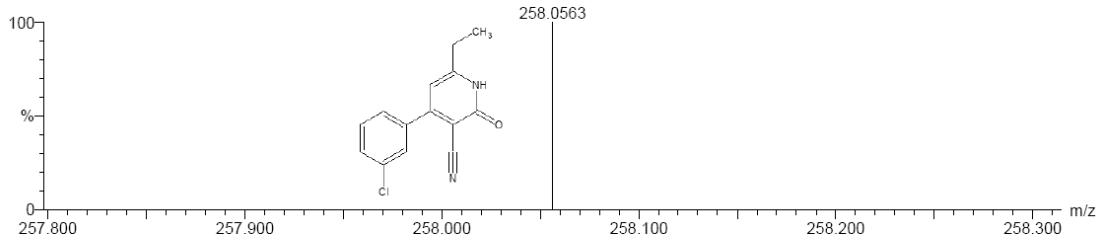
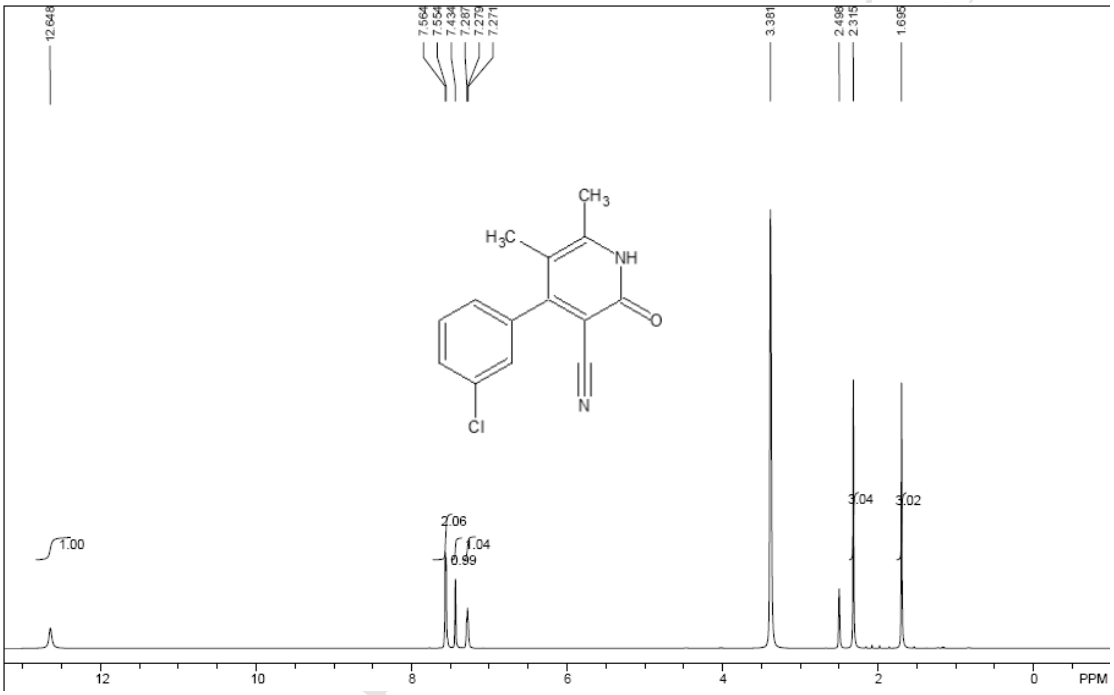
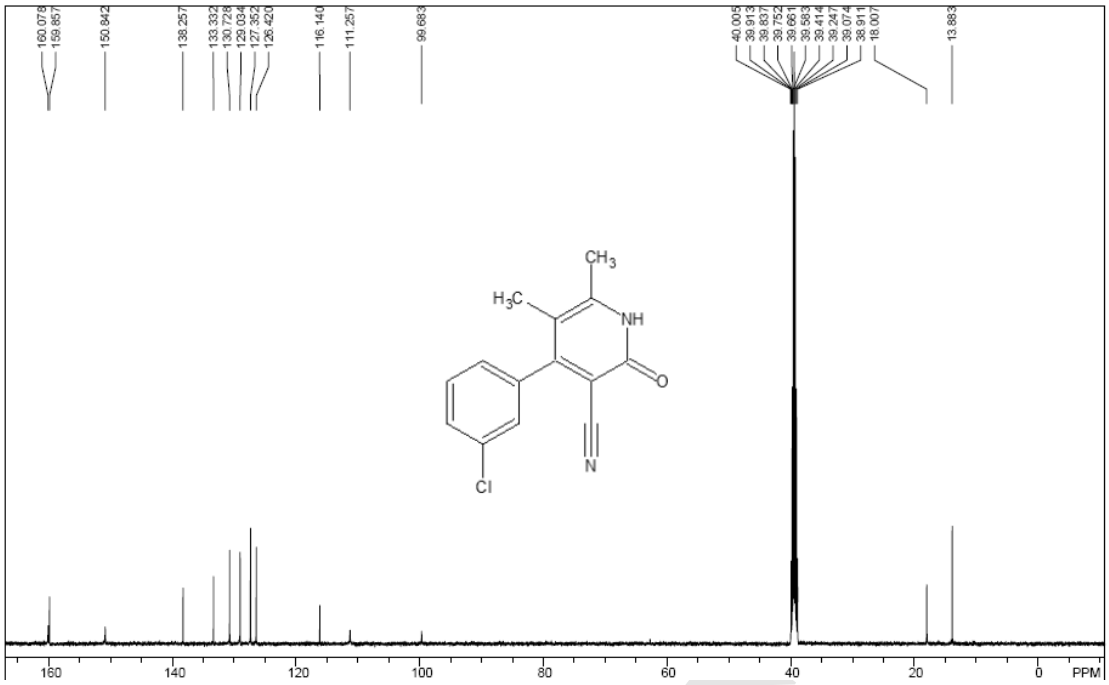
120
100
80
60
40
20
0
PPM

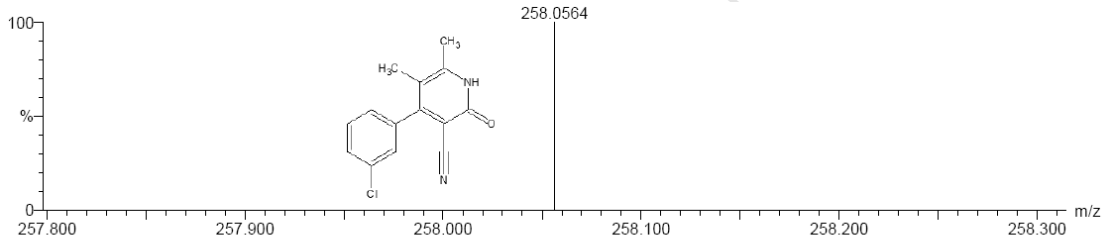


Minimum:				-1.5		
Maximum:		0.7	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
258.0563	258.0560	0.3	1.2	10.0	5546915.5	C14 H11 N2 O Cl

5f: 4-(3-chlorophenyl)-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile

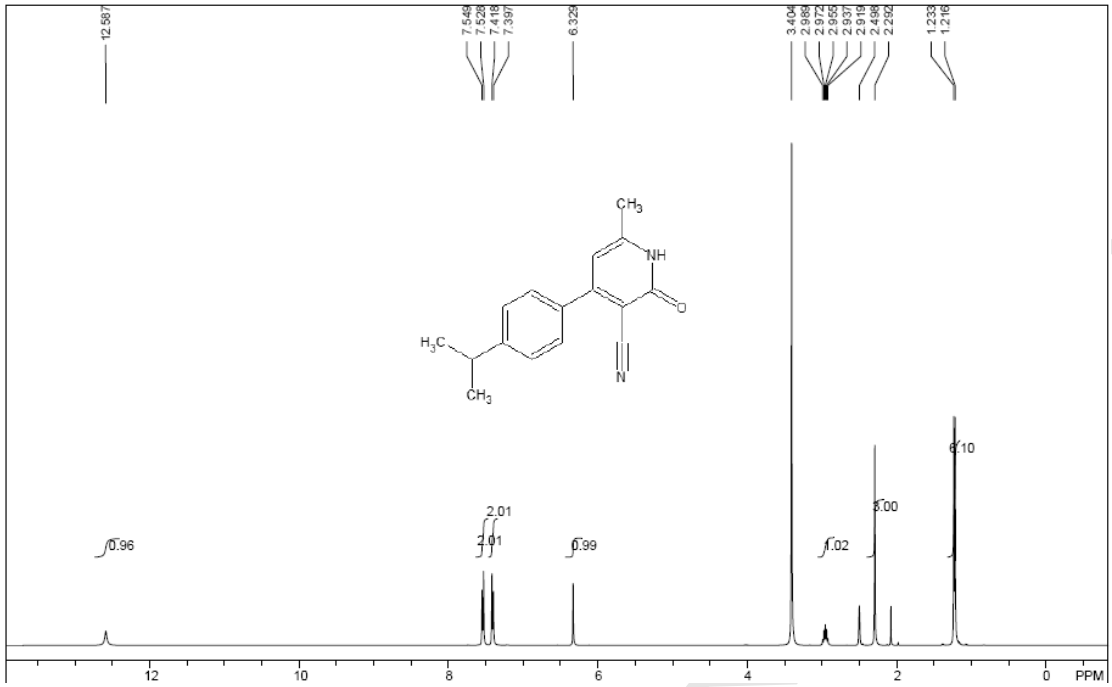






Minimum:				-1.5		
Maximum:		0.7	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
258.0564	258.0560	0.4	1.6	10.0	5546354.5	C14 H11 N2 O Cl

5g: 4-(4-isopropylphenyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile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2.292
1.233
1.216
0.96
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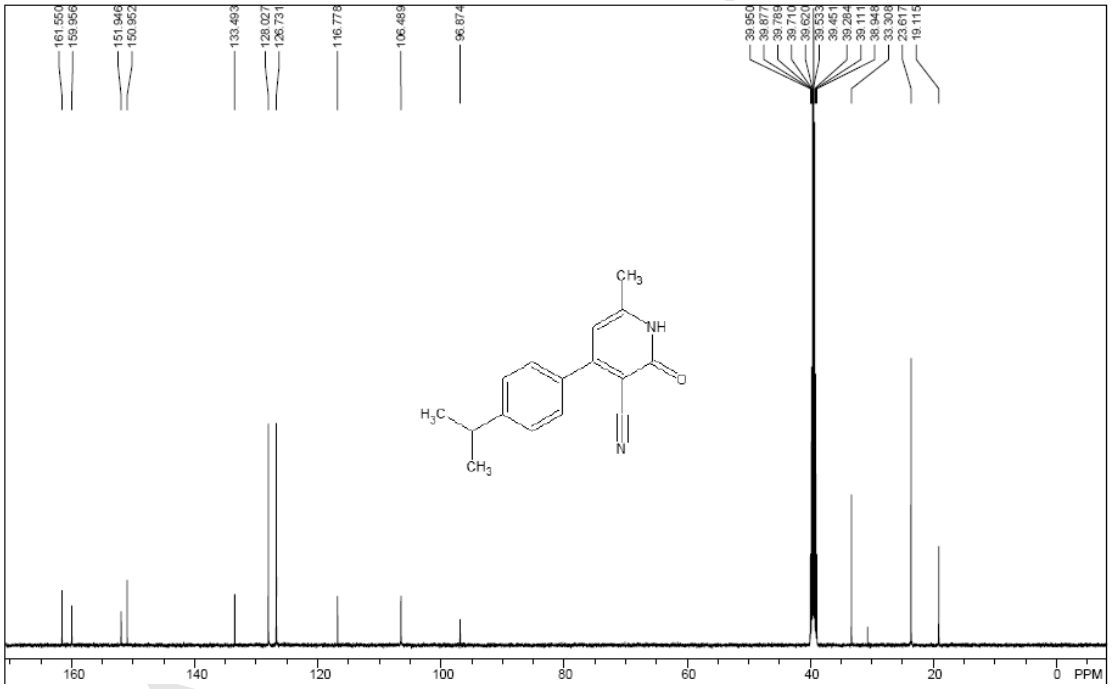
2.01
0.99
1.02
3.00
6.10
12
10
8
6
4
2
0
PPM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106.489
96.874
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39.710
39.620
39.533
39.451
39.284
39.111
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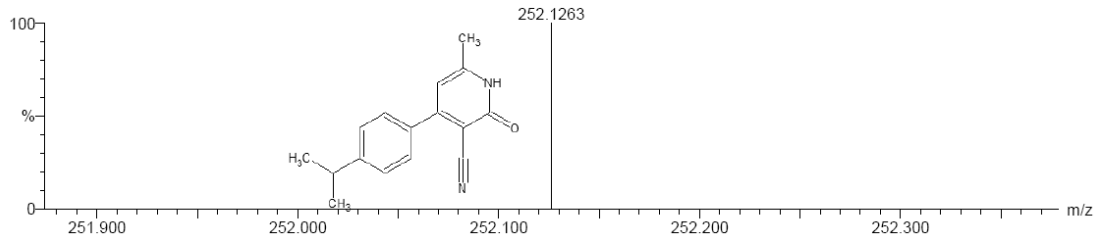
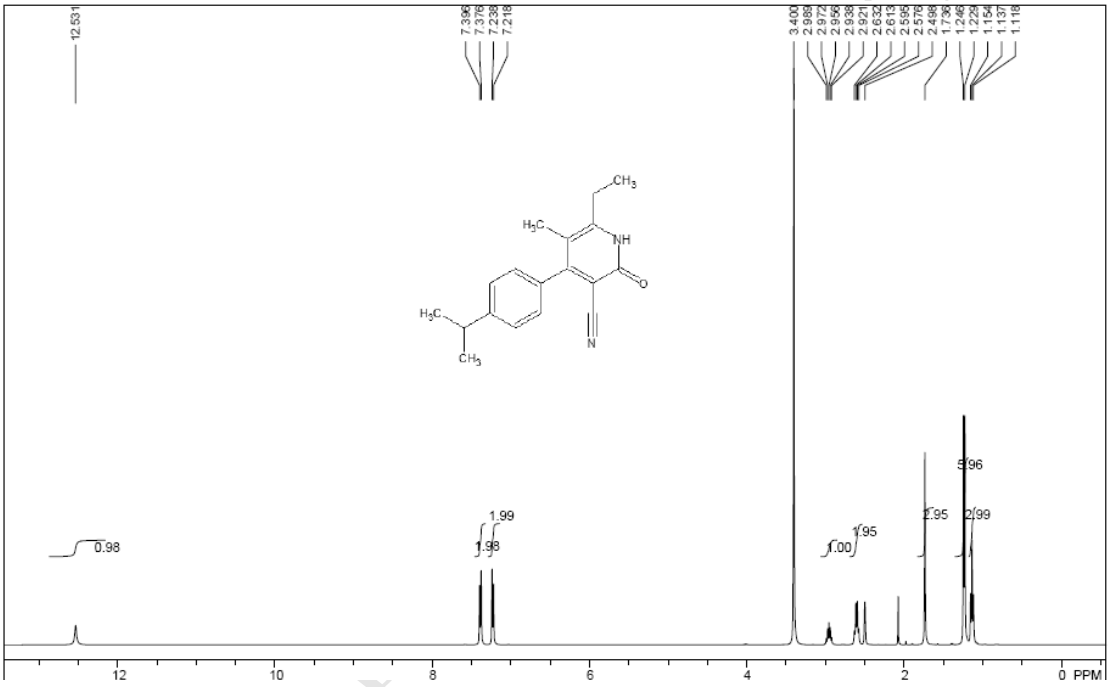
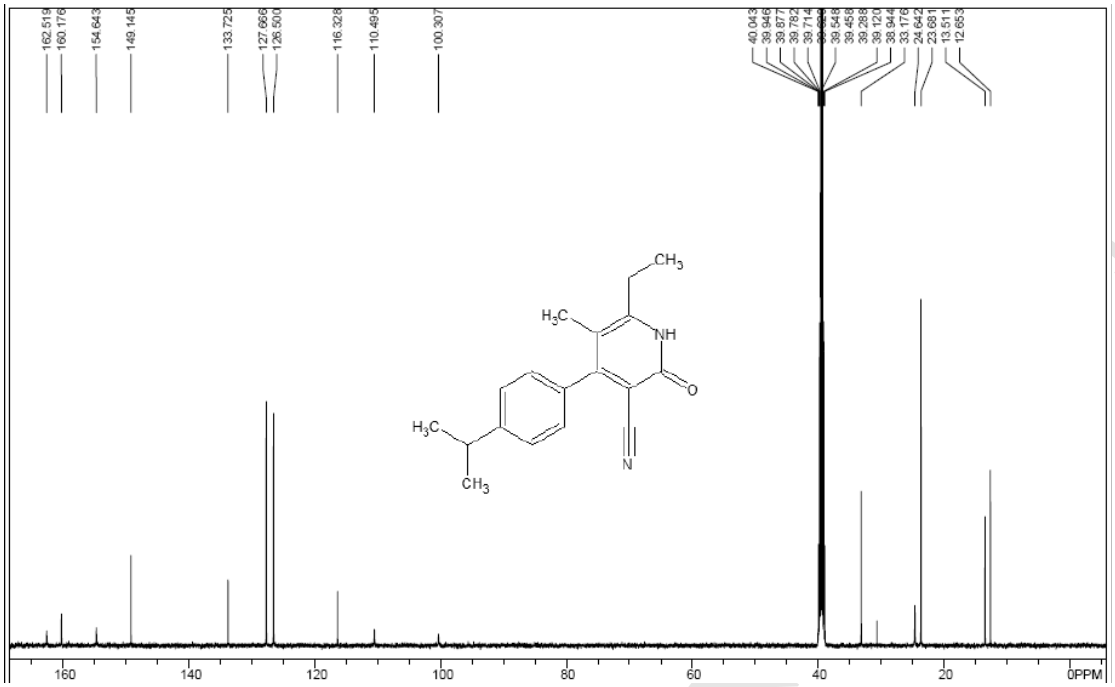
33.308
23.617
19.115
160
140
120
100
80
60
40
20
0
PPM

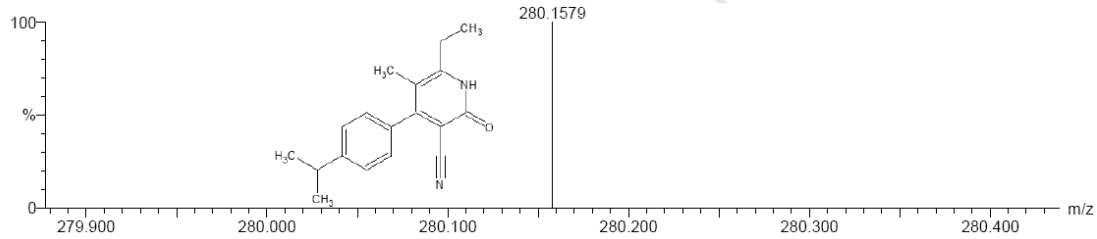


Minimum:			-1.5			
Maximum:		0.3	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
252.1263	252.1263	0.0	0.0	10.0	5550867.5	C16 H16 N2 O

5h: 6-ethyl-4-(4-isopropylphenyl)-5-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile

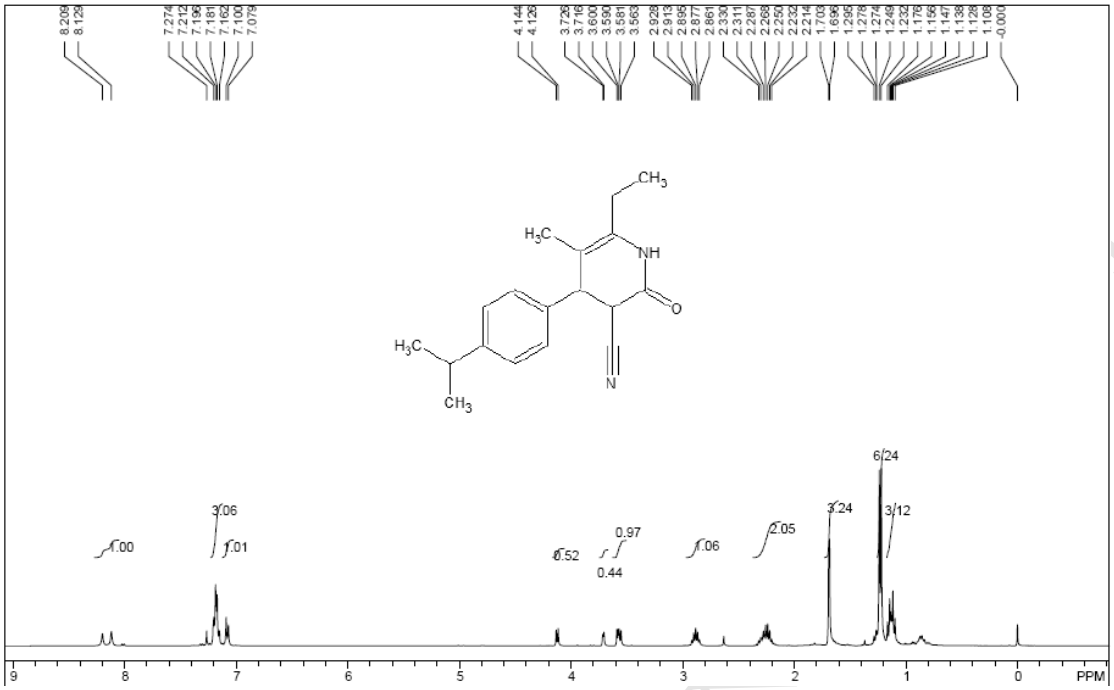






Minimum:				-1.5		
Maximum:		2.0	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
280.1579	280.1576	0.3	1.1	10.0	5548159.0	C18 H20 N2 O

5h':
6-ethyl-4-(4-isopropylphenyl)-5-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile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3.600
3.590
3.581
3.563
2.928
2.913
2.895
2.877
2.861
2.330
2.311
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2.268
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2.232
2.214
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1.232
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1.147
1.138
1.128
1.108
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H3C
NH
O
H3C
CH3
N
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1.01
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5
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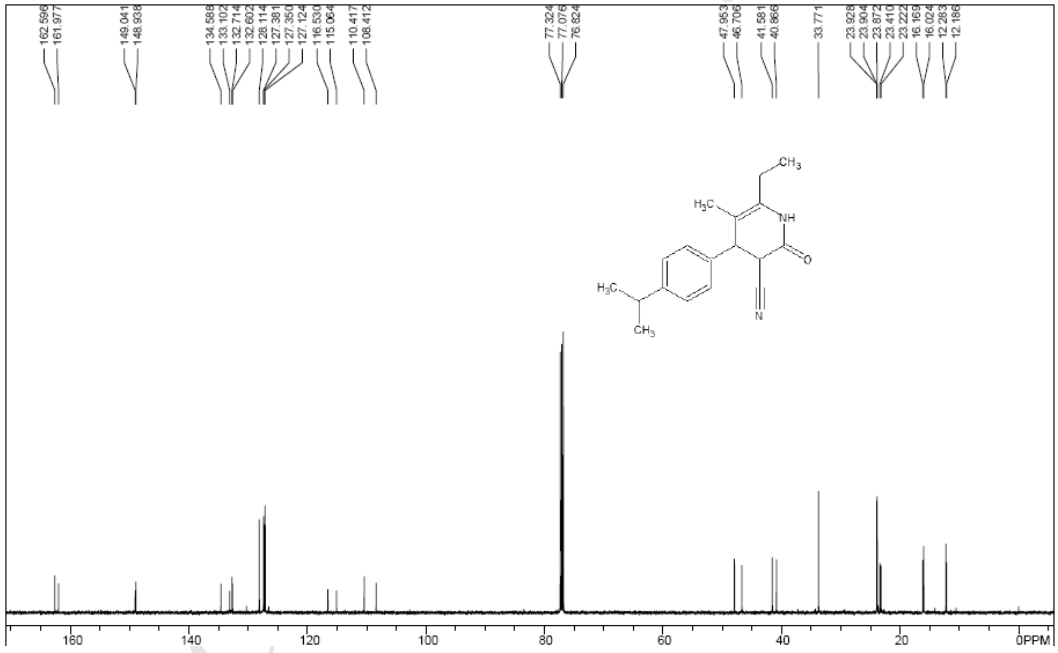
PPM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77.074
76.824
47.953
46.706
41.581
40.866
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23.904
23.872
23.410
23.222
16.169
16.024
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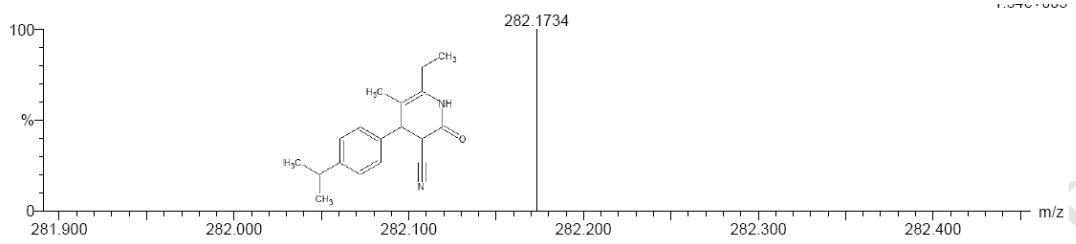
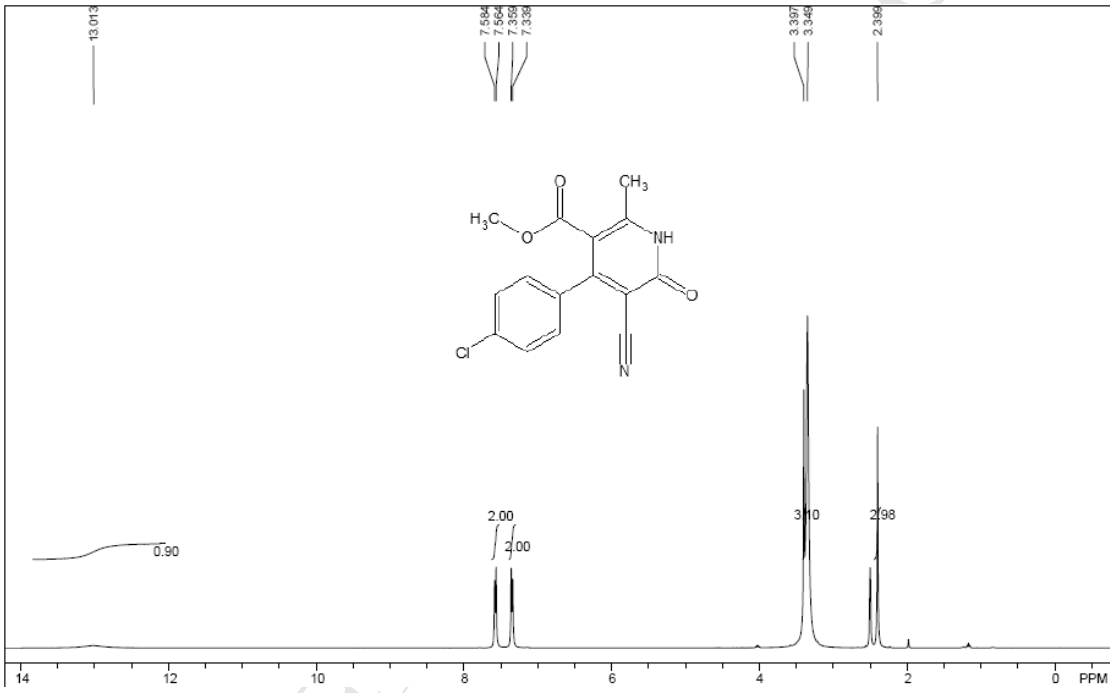
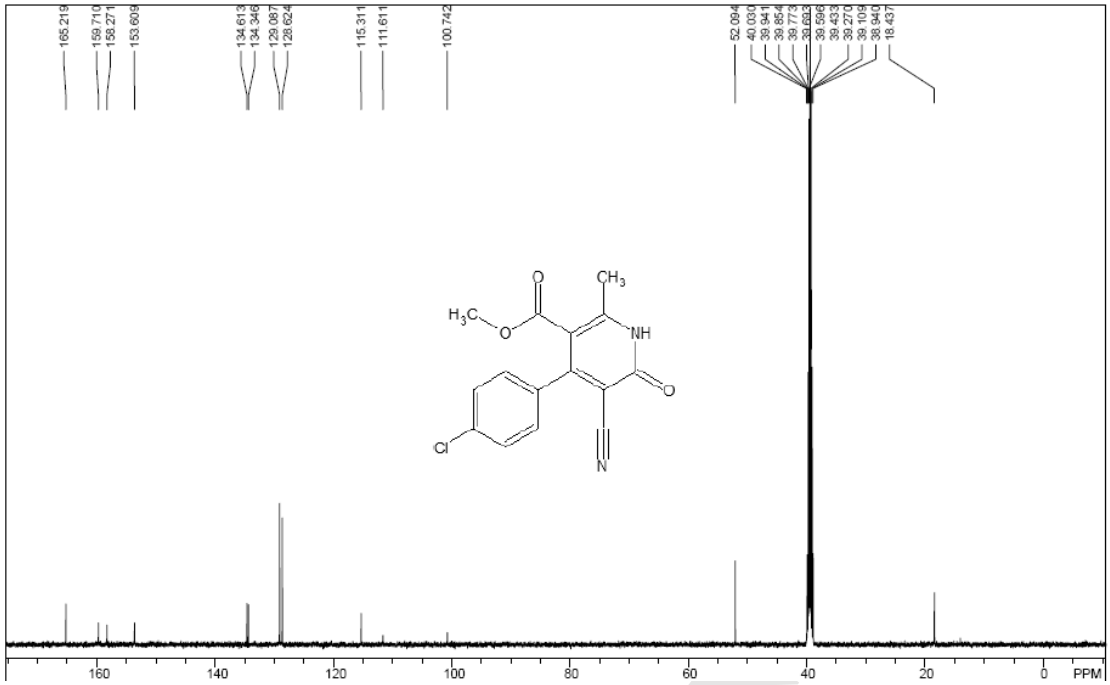


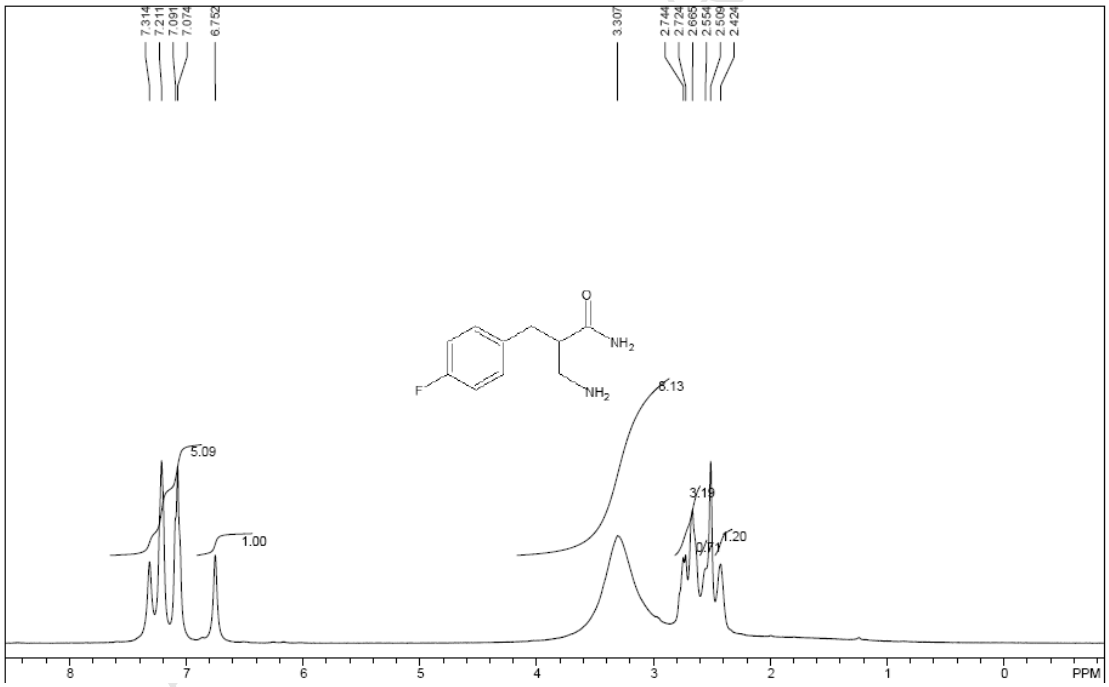
Minimum:				-1.5		
Maximum:		0.5	0.8	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
282.1734	282.1732	0.2	0.7	9.0	5546680.0	C18 H22 N2 O

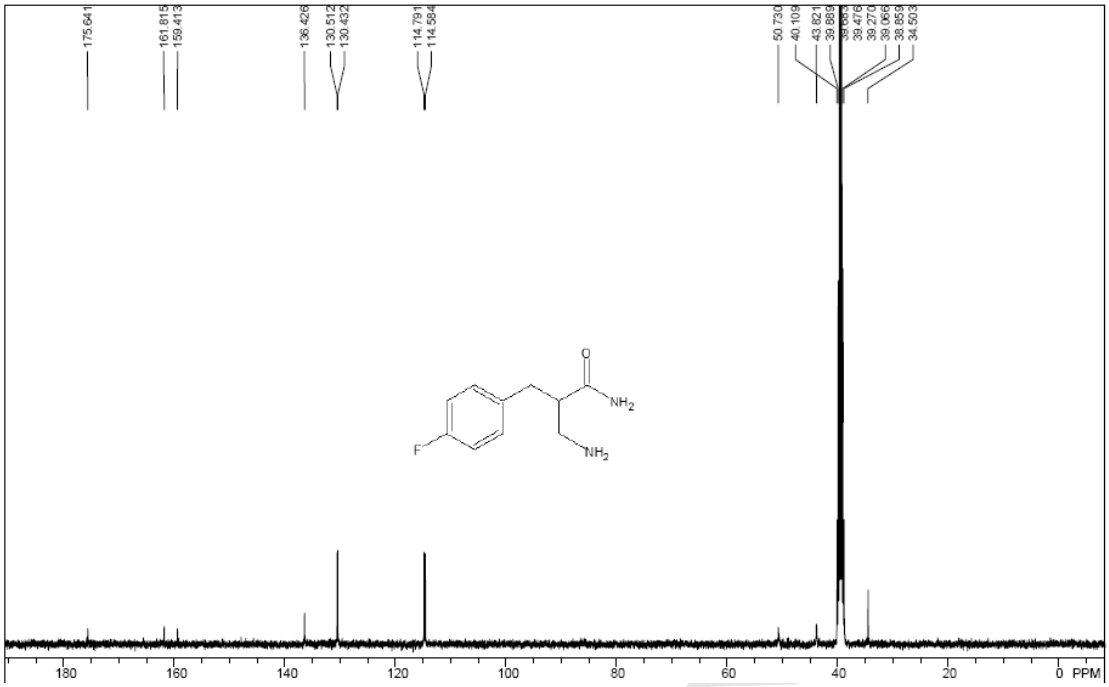
5i: Methyl 4-(4-chlorophenyl)-5-cyano-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate

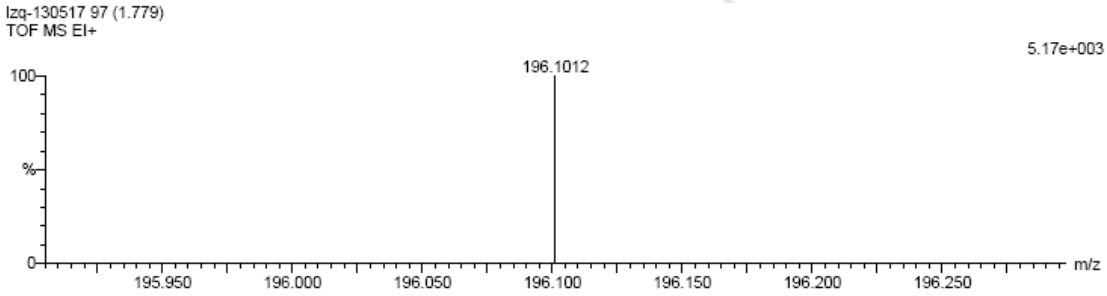




4da: 3-amino-2-(4-fluorobenzyl)propanamide

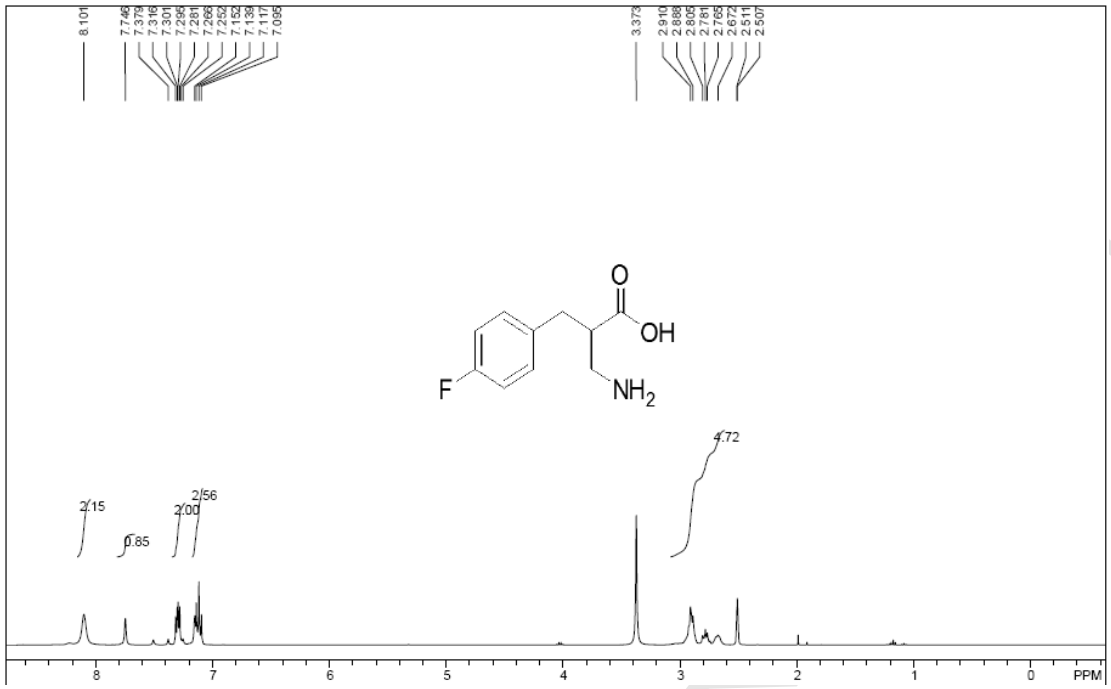






Minimum:				-1.5		
Maximum:		1.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
196.1012	196.1012	0.0	0.0	5.0	5548565.5	C10 H13 N2 O F

4db: 3-amino-2-(4-fluorobenzyl)propanoic acid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2.910
2.888
2.805
2.781
2.765
2.672
2.511
2.507
O
OH
F
NH2
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2.00
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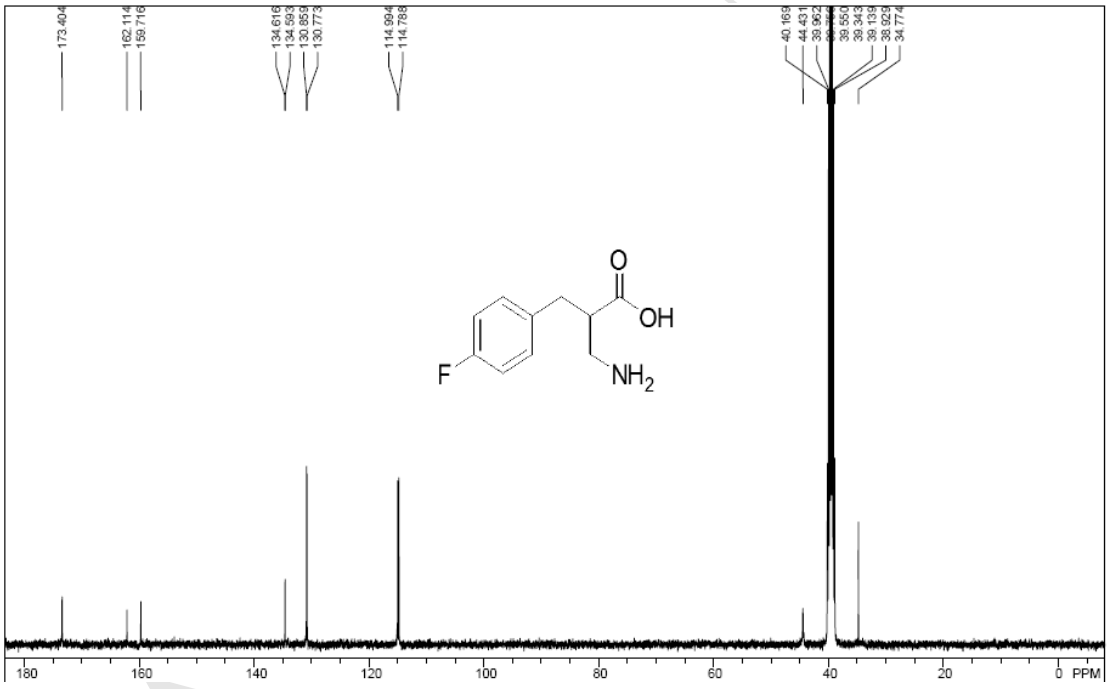
7
6
5
4
3
2
1
0
PPM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39.343
39.139
38.929
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OH
F
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160
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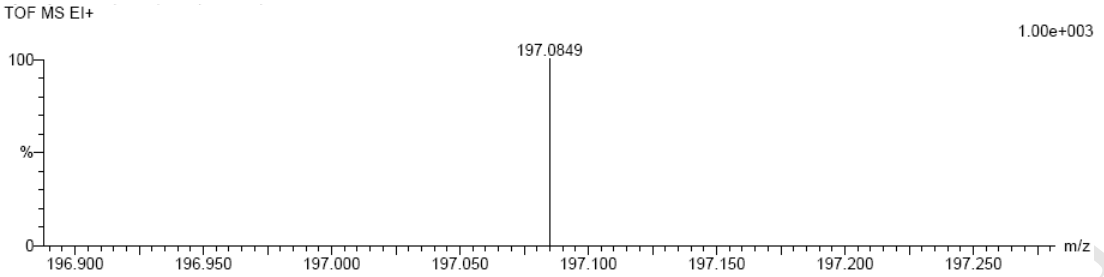
120
100
80
60
40
20
0
PPM



Minimum:				-1.5		
Maximum:		0.5	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
197.0849	197.0852	-0.3	-1.5	5.0	5546484.5	C10 H12 N O2 F