Synthesis of Nitrile Equipped Unnatural Amino Acid Derivatives

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Reg. No. MS10107

A dissertation submitted for the partial fulfillment of BS-MS dual degree in science

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Knowledge City, Sector 81, SAS Nagar,

Manauli P.O., Punjab

April 2015

Certificate of Examination

This is to certify that the dissertation titled "*Synthesis of nitrile equipped unnatural amino acid derivatives*" submitted by Ms. Arya J. S. (Reg. No. MS10107) for the partial fulfillment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Assistant Professor	Assistant Professor	Assistant Professor
IISER Mohali	IISER Mohali	IISER Mohali (Supervisor)

Dated : April 24, 2015

Declaration

The work presented in this dissertation has been carried out by me under the guidance of **Dr. S. Arulananda Babu** at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussion. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Arya J. S.
(Candidate)

Dated : April 24,2015

In my capacity as the supervisor of the candidate's project work, I certify that the above statement by the candidate are true to the best of my knowledge.

Dr. S. Arulananda Babu
(Supervisor)

Acknowledgment

It is my privilege to express my sincere gratitude to my supervisor, **Dr. S. Arulananda Babu** for his invaluable discussion, guidance and supervision throughout the work. The confidence and dynamism with which he guided the work requires no elaboration. Additionally, I am very thankful to him for exposing me to this interesting field of research. Without his guidance, compiling the project report in this form would have been impossible.

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I am thankful to all my classmates and friends for their support throughout the MS project.

I am in short of words in expressing my heartful thanks to my family, always standing behind me with their love and support.

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Notations and Abbreviations

NMDA	*N*-Methyl-D-aspartate
AMPA	α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
GABA	Gamma amino butyric acid
NMDAR	*N*-Methyl-D-aspartate receptors
TLC	Thin layer chromatography
NMR	Nuclear magnetic resonance
δ	Chemical shift in ppm
ppm	Parts per million
MeCN	Acetonitrile
EtOAc	Ethyl acetate
IR	Infra-red
s	Singlet
d	Doublet
t	triplet
m	multiplet
br s	Broad singlet
dd	Doublet of doublet
dt	Doublet of triplet
td	Triplet of doublet
mp	Melting point
MS	Mass spectrometry
ESI	Electron Spray Ionization

Contents

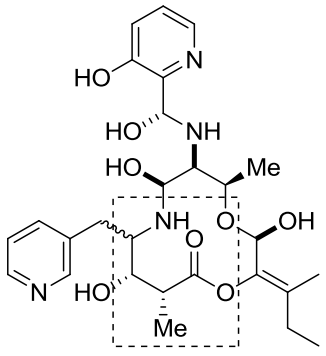
Abstract

An entry into synthesis of β-nitrile equipped unnatural amino acid derivatives via nucleophilic addition of bromoacetonitrile or 2-bromopropanenitrile to α-imino esters or diimino esters is reported. The synthesis of nitrile containing β-amino alcohol, aspartic acid, and β-nitrile equipped *N*-arylated amino acids was achieved from nitrile containing α-amino esters obtained from this work. Dipeptide composed of nitrile attached unnatural α-amino acid and (*R*)-phenylalanine was also synthesized.

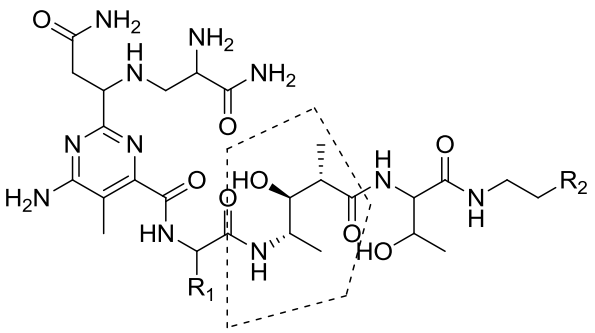
Chapter 1

Introduction

Amino acids are important for the living systems as building blocks of peptides, proteins and a number of natural products. Natural as well as unnatural amino acids serve as intermediates in biological synthesis and as a core of many natural products (Figure 1). Unnatural/Non-proteinogenic amino acids are those which are not found in the coding sequence of any of the organisms. Other than 23 amino acids (21 in eukaryotes), there are many amino acids and amino acid derivatives produced by different natural pathways. Some of these are noteworthy because of their bio-chemical role in the living system. For example, NMDA (*N*-Methyl-D-aspartate) receptors and AMPA (α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptors are two types of glutamate receptors (Figure 1 and 2).[1] In mammalian central nervous system, glutamate is the principle excitatory neurotransmitter. Gamma amino butyric acid (GABA, a derivative of glutamate), and serotonin and melatonin (derivatives of tryptophan) also act as neurotransmitters (Figure 1).[1] The slow component of glutamatergic synaptic current is mediated by NMDARs (*N*-Methyl-D-aspartate receptors, Figure 1 and 2).[2]



Pyridomycin[7]



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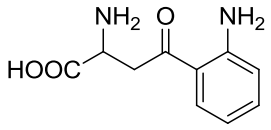
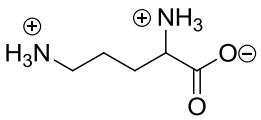
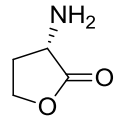


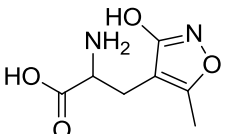
Kynurenine



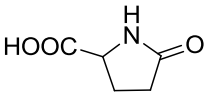
Ornithine



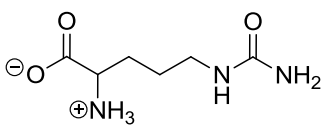
L-Homoserine Lactone



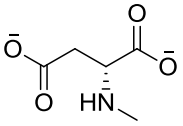
AMPA



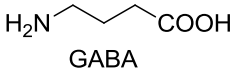
5-oxopyrrolidine-2-carboxylic acid



Citruline



N-methyl-D-aspartate



GABA

Figure 1. Representative examples for unnatural amino acids and natural products containing unnatural amino acid.

Kynurenine[3] is a metabolite of tryptophan amino acid and 5-Oxopyrrolidine-2-carboxilic acid[4] is a metabolite in the glutathione cycle (Figure 1). In the urea cycle citruline and ornithine are intermediates.[5]

Unnatural amino acids can also be used as starting materials for many chiral compounds. Likewise many functional or modified amino acids can be synthesized from unnatural amino acids available in Nature. Bio-based homoserine lactones (Figure 1) and organic acids have been synthesized by the hydrolysis of *O*-acyl homoserine produced by a luminescent bacteria *Vibrio fischeri* in the presence of an acid catalyst.[6]

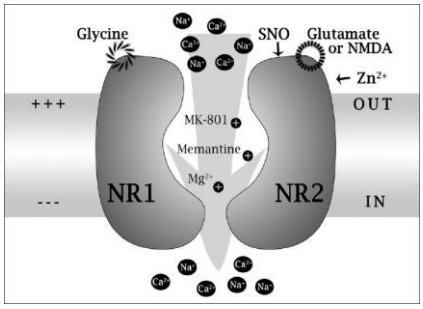


Figure 2. NMDA receptor ion channel. (Once glutamate or NMDA binds to the NMDAR, the ion channel opens and the ions get transmitted.)

In the field of medicinal chemistry, peptides play a very important role. Now a days peptide based antibiotics and therapeutics are becoming very common. In this scenario exploring peptide chemistry is interesting. However, the potential range of applications of these materials is limited by small number of proteinogenic amino acids. We can design the peptide of our desire by using corresponding amino acids (Figure 3A). As a result a great deal of work has been carried out in recent years aimed at the synthesis of un-natural amino acids that mimic the natural peptide sequences and there has been so much development in incorporating unnatural amino acids and peptides which has enhanced biological activity into protein via both *in-vitro*[8] (Figure 3B) and *in-vivo*[9,10] methods. About 100 unnatural amino acids have been successfully incorporated into different protein structure.[11] Other than the above mentioned fields, unnatural amino acids are used in prebiotic experiments[12] and also to explore the evolution of amino acids.[13] It is clear that all the amino acids that are prebiotically synthesised have not been ended up in protein sequencing and not all the sequencing amino acids were synthesised prebiotically (Figure 3C).[13] To understand the prebiotic organic chemistry and chemical evolution,

unnatural amino acids and different derivatives of naturally occurring amino acids are being used.

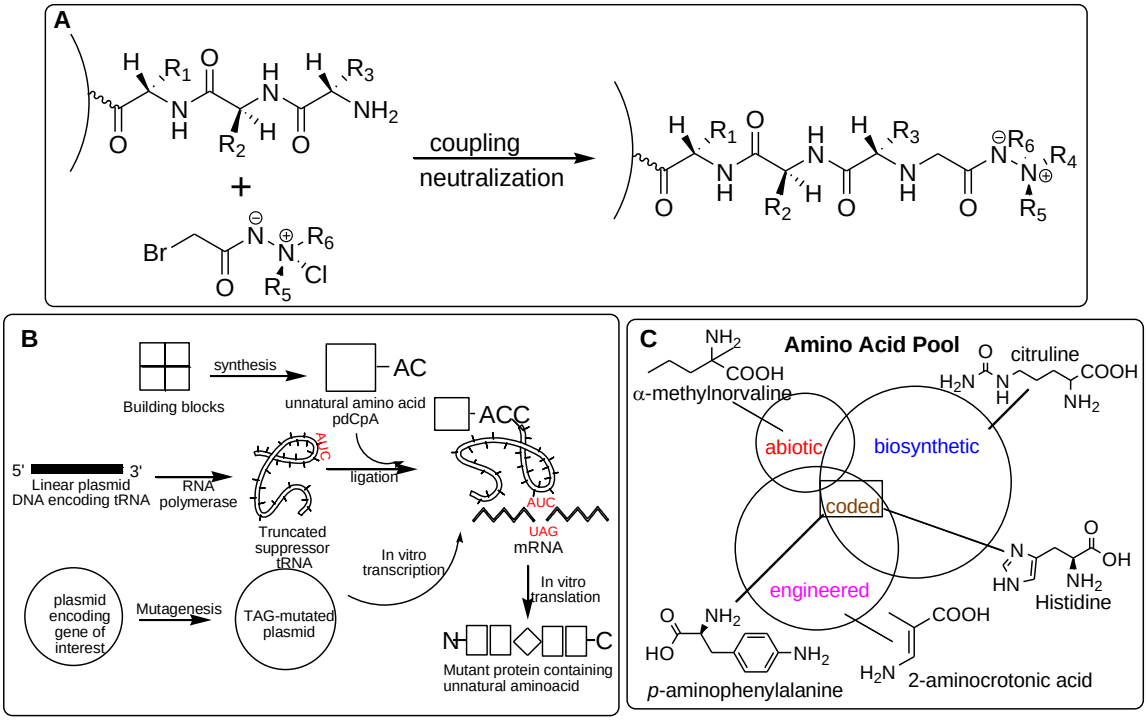


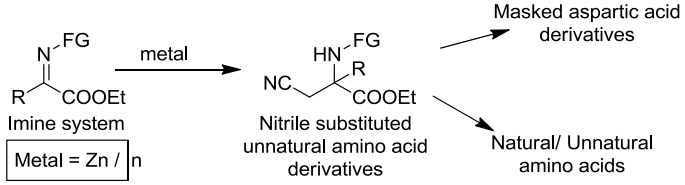
Figure 3. (A) Designing peptide of interest using unnatural amino acid derivatives. (B) In vitro incorporation of unnatural amino acid to create mutant protein. (C) Venn diagram showing different categories of amino acids.

Abiotic amino acids are those that might have formed before the life evolved (e.g. α-Methylnorvaline, Figure 3C). Citruline like unnatural amino acids are produced by natural biosynthetic pathway; biosynthetic and engineered amino acids are experimentally incorporated into proteins by bio-medical research projects (e.g. *p*-aminophenylalanine, Figure 3C); 20 standard amino acids have been coded for protein sequence. From this it is clear that the 20 coding amino acids are just a small subset of what is biologically or chemically possible.[13]

Due to the versatile use of unnatural amino acids in pharmaceuticals, synthetic/medicinal chemists have taken keen interest in developing new routes for the synthesis of unnatural amino acids having biological importance. Additionally, nitriles possess various applications in pharmaceuticals. 30 Nitrile containing pharmaceuticals have been marketed for various medical indications and also about 20 are under clinical developmental studies.[14] In some cases, it increases water solubility or decreases susceptibility to metabolism inside body or as a substrate for enzymes.[15] It has also been

postulated that nitriles played a very important role in prebiotic chemistry, which led to evolution of biomolecules.[16] As a result, we focussed our attention to develop a methodology for the synthesis of nitrile substituted unnatural α-amino acid derivatives (masked aspartic acid derivatives) that combine the importance of gifted nitrile group with that of unnatural amino acids.

Nucleophilic addition of carbon nucleophiles to imines to achieve C-C bond formation is an important tool to synthesize nitrogen containing biologically relevant molecules. Barbier type nucleophilic addition reaction has been known for many years and has been well investigated as these reactions can be carried out with relatively inexpensive metals like zinc, tin, bismuth etc.[17] Nucleophilic addition reaction of bromoacetonitrile to carbonyl compounds[18, 19, 20] and imine systems[21, 22, 23] has been already reported. Glyoxalate imines are well studied as starting material for the synthesis of natural/unnatural α-amino acids and its derivatives or related compounds via ene reaction[24] or cycloaddition[25] or nucleophilic addition of organometallic compounds.[26, 27, 28, 29, 30] We have also chosen metal mediated Barbier type nucleophilic addition reaction of bromoacetonitrile to glyoxalate imine systems to synthesize nitrile substituted unnatural amino acid derivatives. Barbier type nucleophilic addition to glyoxalate imines results in *N*-substituted α-amino acid derivatives under mild reaction conditions and in good yields.[31, 32]



Scheme 1. Theme of this work: Synthesis of nitrile substituted unnatural amino acid derivatives via Barbier-type reaction.

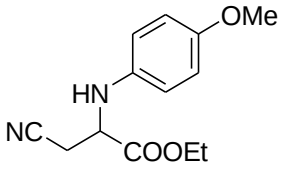
Chapter 2

Experimental

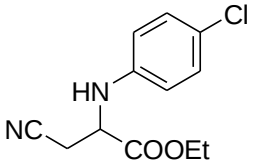
General. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz and 100 MHz spectrometers respectively with TMS as an internal or external standard. IR spectra were recorded as KBr pellets or thin films. Column chromatography was carried out on silica gel. Reactions were carried out in dry solvent under nitrogen atmosphere wherever required. Solutions were dried using anhydrous sodium sulfate. Reagents were added to the reaction flask with the help of syringe. Thin layer chromatography (TLC) was performed on silica plates or and components were visualized by observation under iodine. Isolated yields of all the products were reported (yields were not optimized). Ratios of diastereomers were determined from the ^{1}H and ^{13}C NMR spectra of crude reaction mixtures or after isolation.

Procedure A: Zn-mediated addition of bromoacetonitrile (2a) or 2-bromoprpanenitrile (2b) to α-imino esters (1). Zinc (1 mmol), bromoacetonitrile **2a** (0.75 mmol) or 2-bromopropanenitrile **2b** (0.75 mmol) and 2 mL of MeCN were stirred vigorously under nitrogen atmosphere for 15 min. To this mixture, α-imino ester **1** (0.25 mmol) was added and the complete mixture was allowed to stir for 12 h. After this period, the reaction mixture was quenched with 5 mL of water and transferred to a separating funnel and extracted with EtOAc (3x10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 Then the solvent was evaporated under vacuum. Purification of resulting crude reaction mixture by column chromatography on silica gel (EtOAc/Hexane) as eluent gave the product **3** (See table 1, 2, 4 and scheme 2 for individual entries).

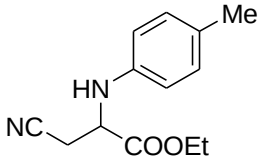
Procedure B: Zn-mediated addition of bromoacetonitrile (2a) to diimino esters (4). Zinc (2 mmol), bromoacetonitrile **2a** (1.5 mmol) and 2 mL of MeCN were stirred vigorously under nitrogen atmosphere for 15 min. To this mixture, diimino ester **4** (0.25 mmol) was added and the complete reaction mixture was allowed to stir for 12 h. After this period, the reaction mixture was quenched with 5 mL of water and transferred to a separating funnel and extracted with EtOAc (3x10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 Then the solvent was evaporated under vacuum. Purification of resulting crude reaction mixture by column chromatography on silica gel (EtOAc/Hexane) as eluent gave the product **5** (See table 3 for individual entries).



Ethyl 2-(4-methoxyphenylamino)-3-cyanopropanoate (3a): Following the general procedure A described above, **3a** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as colorless solid (46 mg, 74%): mp 80 ^{o}C; R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3408, 2930, 2205, 1734, 1512 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82 (d, J = 8.9 Hz, 2 H), 6.67 (d, J = 8.9 Hz, 2 H), 4.32-4.29 (m, 3 H), 3.77 (s, 3 H), 2.90(d, J = 5.6 Hz, 2 H), 1.33 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 153.6, 138.9, 116.4, 116.0, 115.0, 62.5, 55.7, 54.5, 21.9, 14.2; HRMS (ESI): calcd. for $C_{13}H_{17}N_2O_3$ $[M + H]^+$ 249.1239; found 249.1235.

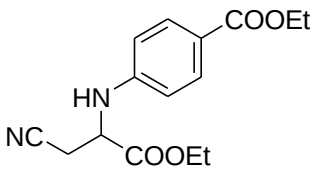


Ethyl 2-(4-chlorophenylamino)-3-cyanopropanoate (3b): Following the general procedure A described above, **3b** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as colorless solid (50 mg, 80%): mp 153-158 ^{o}C; R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3404, 2987, 2248, 1710, 1495 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (d, J = 8.9 Hz, 2 H), 6.60 (d, J = 8.9 Hz, 2 H), 4.60 (s, 1 H), 4.35-4.30 (m, 3 H), 2.90 (dd, J_1, J_2 = 5.6, 1.0 Hz, 2 H), 1.33 (t, J = 7.12 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 143.6, 129.5, 124.2, 116.2, 115.0, 62.7, 53.2, 21.7, 14.2; HRMS (ESI): calcd. for $C_{12}H_{14}ClN_2O_2$ $[M + H]^+$ 253.0744; found 253.0744.

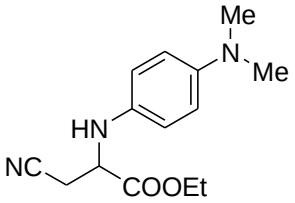


Ethyl 2-(*p*-tolylamino)-3-cyanopropanoate (3c): Following the general procedure A described above, **3c** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as brown coloured semisolid (41 mg, 70%): R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3400, 2923, 2252, 1735, 1516 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (d, J = 8.4 Hz , 2 H), 6.61 (d, J = 8.4 Hz, 2 H) 4.38-4.30 (m, 4

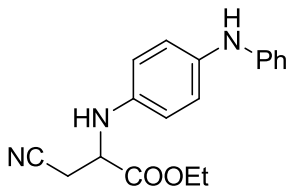
H), 2.92 (d, J = 5.3 Hz, 2 H), 2.28 (s, 3 H), 1.34 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 142.6, 130.1, 129.0, 116.3, 114.2, 62.5, 53.6, 21.8, 20.5, 14.2; HRMS (ESI): calcd. for $C_{13}H_{17}N_2O_2$ $[M + H]^+$ 233.129; found 233.1293.



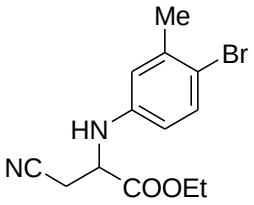
Ethyl 4-(1-(ethoxycarbonyl)-2-cyanoethylamino)benzoate (3d): Following the general procedure A described above, **3d** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as yellow coloured semisolid (39 mg, 54%): R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3399, 2984, 2252, 1736, 1523 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, J = 8.0 Hz, 2 H), 6.64 (d, J = 8.0 Hz, 2 H), 4.50 (d, J = 7.7 Hz, 1 H), 4.37-4.32 (m, 4 H), 2.97 (d, J = 5.3 Hz, 2 H), 1.40-1.36 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 166.5, 148.5, 131.7, 121.1, 115.8, 112.4, 63.0, 60.5, 52.3, 21.6, 14.4, 14.2; HRMS (ESI): calcd. for $C_{15}H_{19}N_2O_3$ $[M + H]^+$ 291.1345; found 291.1340.



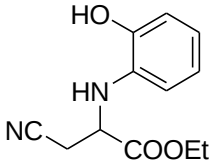
Ethyl 2-(4-(dimethylamino)phenylamino)-3-cyanopropanoate (3e): Following the general procedure A described above, **3e** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as black coloured semisolid (26 mg, 39%): R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3519, 3003, 1712, 1522, 1361 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (d, J = 9.0 Hz, 2 H), 6.69 (d, J = 9.0 Hz, 2 H), 4.32-4.28 (m, 3 H), 3.05-2.87 (m, 8 H), 1.33 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 145.5, 136.6, 116.5, 116.5, 115.3, 62.4, 54.9, 41.8, 21.9, 14.2; HRMS (ESI): calcd. for $C_{14}H_{20}N_3O_2$ $[M + H]^+$ 262.1556; found 262.1560.



Ethyl 3-cyano-2-((4-(phenylamino)phenyl)amino)propanoate (3f): Following the general procedure A described above, **3f** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as dark green sticky liquid. (50 mg, 65%): R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3431, 2923, 2194, 1633, 1516 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.28-7.21 (m, 2 H), 7.05 (d, J = 8.6 Hz, 2 H), 6.93-6.84 (m, 3 H), 6.67 (d, J = 8.6 Hz, 2 H), 5.5 (br s, 1 H), 4.37-4.32 (m, 3 H), 2.93 (d, J = 5.0 Hz, 2 H), 1.36 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 145.1, 140.4, 135.1, 129.3, 122.6, 119.5, 116.3, 115.6, 115.4, 62.6, 54.0, 21.9, 14.2; HRMS (ESI): calcd. for $C_{18}H_{20}N_3O_2$ $[M + H]^+$ 310.1556; found 310.1559.

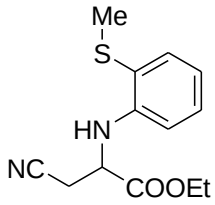


Ethyl 2-(4-bromo-3-methylphenylamino)-3-cyanopropanoate (3g): Following the general procedure A described above, **3g** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as colorless solid (44 mg, 57%): mp 82-84 ^oC; R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3431, 1634, 1476, 1369, 1214 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.33 (d, J = 8.6 Hz, 1 H), 6.57 (d, J = 2.8 Hz, 1 H), 6.38 (dd, J_1, J_2 = 8.6, 2.8, 1 H), 4.50 (br s,1 H), 4.38-4.28 (m, 3 H), 2.92 (d, J = 5.6 Hz, 2 H), 2.34 (s, 3 H), 1.35 (t, J = 7.16 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 144.2, 138.9, 133.0, 116.3, 116.1, 114.1, 112.8, 62.7, 53.1, 23.1, 21.7, 14.2; HRMS (ESI): calcd. for $C_{13}H_{16}BrN_2O_2$ $[M + H]^+$ 311.0395; found 311.0394.

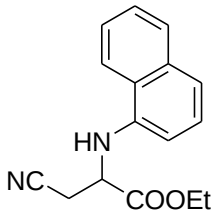


Ethyl 2-(2-hydroxyphenylamino)-3-cyanopropanoate (3h): Following the general procedure A described above, **3h** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as brown coloured solid (22 mg, 38%): R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3389, 2926, 2205, 1736, 1516 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.87-6.81 (m, 3 H), 6.72 (dd, J_1, J_2 = 7.4, 1.0 Hz, 1 H), 4.34-4.31 (m, 3 H), 2.93 (d, J = 5.6 Hz, 2 H), 1.35 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz,

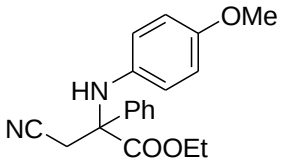
$CDCl_3$): δ 170.8, 145.7, 133.4, 121.4, 121.1, 116.4, 115.4, 115.2, 62.6, 54.4, 22.0, 14.1; HRMS (ESI): calcd. for $C_{12}H_{15}N_2O_3$ $[M + H]^+$ 235.1083; found 235.1082.



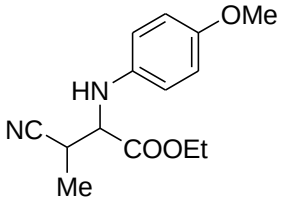
Ethyl 2-(2-(methylthio)phenylamino)-3-cyanopropanoate (3i): Following the general procedure A described above, **3i** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as light yellow coloured semisolid (40 mg, 60%); R_f (30% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3357, 2923, 1739, 1588, 1316 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.49 (dd, J_1, J_2 = 7.6, 1.6 Hz, 1 H), 7.23 (td, J_1, J_2 = 7.6, 1.6 Hz, 1 H), 6.79 (td, J_1, J_2 = 7.6, 1.2 Hz, 1 H), 6.57 (d, J = 8.0 Hz, 1 H), 5.85 (d, J = 8.2 Hz, 1 H), 4.49-4.44 (m, 1 H), 4.38-4.29 (m, 2 H), 2.98 (d, J = 5.7 Hz, 2 H), 2.38 (s, 3 H), 1.35 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 145.4, 135.0, 129.6, 121.9, 119.2, 116.1, 110.5, 62.6, 53.1, 21.7, 18.5, 14.2; HRMS (ESI): calcd. for $C_{13}H_{17}N_2O_2S$ $[M + H]^+$ 265.1011; found 265.1018.



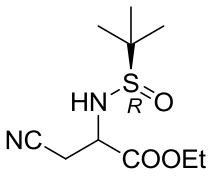
Ethyl 3-cyano-2-(naphthalen-1-ylamino)propanoate (3j): Following the general procedure A described above, **3j** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 30:70) as brown coloured solid (46 mg, 68%): mp 78-80 ^oC; R_f (30% EtOAc/Hexane) 0.60; IR (thin film): υ_{max} 3407, 2980, 2252, 1737, 1530 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98-7.97 (m,1 H), 7.84 (t, J = 9.5 Hz, 1 H), 7.55-7.52 (m, 2 H), 7.38 (d, J = 2.1 Hz, 2 H), 6.57 (dd, J_1, J_2 = 6.6, 1.6 Hz, 1 H), 5.30 (d, J = 6.6 Hz, 1 H), 4.60 (d, J = 6.2 Hz, 1 H), 4.41-4.35 (m, 2 H), 3.06 (d, J = 5.3 Hz, 2 H), 1.39 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 140.2, 134.5, 128.7, 126.3, 126.0, 125.5, 124.0, 120.2, 119.8, 116.3, 105.5, 62.8, 53.1, 21.5, 14.2; HRMS (ESI): calcd. for $C_{16}H_{17}N_2O_2$ $[M + H]^+$ 269.1290; found 269.1291.



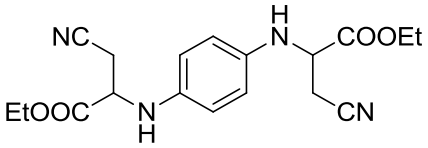
Ethyl 2-(4-methoxyphenylamino)-3-cyano-2-phenylpropanoate (3k): Following the general procedure A described above, **3k** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 40:70) yellow coloured solid (63 mg, 43%): R_f (40% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3377, 2925, 2248, 1730, 1669, 1511, 1447, 1240, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, J = 7.6 Hz, 2 H), 7.57-7.39 (m, 3 H), 6.73 (d, J = 8.8 Hz, 2 H), 6.56 (d, J = 8.8 Hz, 2 H), 4.83 (s, 1 H), 4.31-4.18 (m, 2 H), 3.74 (s, 3 H), 3.53 (d, J = 16.5 Hz, 1 H), 3.39 (d, J = 16.5 Hz, 1 H), 1.24 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 154.2, 138.5, 136.4, 129.1, 128.8, 126.2, 119.7, 116.6, 114.7, 65.8, 62.9, 55.5, 24.9, 13.9; HRMS (ESI): calcd. for $C_{19}H_{21}N_2O_3$ $[M + H]^+$ 325.1552; found 325.1560.



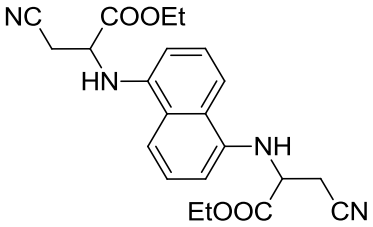
Ethyl 2-(4-methoxyphenylamino)-3-cyanobutanoate (3l): Following the general procedure A described above, **3l** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 60:40) as brown coloured semisolid (51 mg, 78%): R_f (60% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3364, 2925, 1736, 1514, 1239 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.82 (d, J = 9.0 Hz, 2 H), 6.73 (d, J = 9.0 Hz, 1 H), 6.68 (d, J = 9.0 Hz, 1 H), 4.30-4.25 (m, 2 H), 4.15 (t, J = 5.2, 2 H), 3.77 (s, 3 H), 3.23-3.19 (m, 1 H), 1.44 (d, J = 7.2, 3 H), 1.32 (t, J = 7.2, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.4, 153.8, 153.6, 139.7, 120.1, 120.0, 116.6, 116.0, 115.0, 115.0, 62.2, 62.2, 60.6, 60.0, 55.7, 55.7, 29.6, 29.2, 14.7, 14.2; HRMS (ESI): calcd. for $C_{14}H_{19}N_2O_3$ $[M + H]^+$ 263.1396; found 263.1390. This compound was isolated as a mixture of diastereomers. The ^1H and ^{13}C NMR data given for mixture of diastereomers.



Ethyl 3-cyano-2-((*R*)-1,1-dimethylethylsulfinamido)propanoate (3m): Following the general procedure A described above, **3m** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 60:40) as light yellow coloured liquid (22 mg, 36%): R_f (60% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3190, 2919, 1740, 1528, 1401 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.45 (d, J = 5.0 Hz, 1 H) 4.34-4.28 (m, 4 H), 2.89 (d, J = 5.3 Hz, 2 H), 1.35 (t, J = 7.1 Hz, 3 H), 1.31 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 115.8, 63.2, 56.7, 53.3, 23.2, 22.5, 14.1. This compound contains traces of the minor isomer.

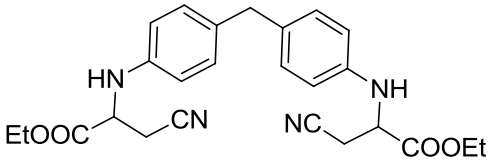


Diethyl 2,2'-(1,4-phenylenebis(azanediyl))bis(3-cyanopropanoate) (5a): Following the general procedure B described above, **5a** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 50:50) as brown coloured solid (13 mg, 15%): mp 102-104 ^oC; R_f (50% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3354, 2923, 2198, 1733, 1624 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.63 (s, 4 H), 4.32-4.28 (m, 6 H), 2.89 (dd, J = 5.4 Hz, 4 H), 1.33 (t, J = 7.2 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 138.6, 116.4, 62.5, 54.4, 29.7, 21.9, 14.2; HRMS (ESI): calcd. for $C_{18}H_{23}N_4O_4$ $[M + H]^+$ 359.1719; found 359.1718. The NH signal was not clearly visible in the ^1H NMR spectrum.

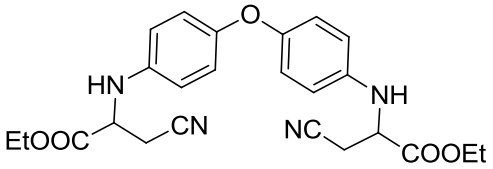


Diethyl 2,2'-(naphthalene-1,5-diylbis(azanediyl))bis(3-cyanopropanoate) (5b): Following the general procedure B described above, **5b** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 50:50) as black coloured sticky solid (71 mg, 70%): R_f (50% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3386, 2984, 2248, 1732, 1537 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.47 (d, J = 8.5 Hz, 2 H), 7.38 (t, J = 7.5 Hz, 2 H), 6.60 (d, J = 7.5 Hz, 2 H), 5.31 (s, 2 H), 4.58 (t, J = 5.1 Hz, 2 H), 4.42-4.34 (m, 4 H), 3.05 (d, J = 5.3 Hz, 4 H), 1.38 (t, J = 7.1 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ

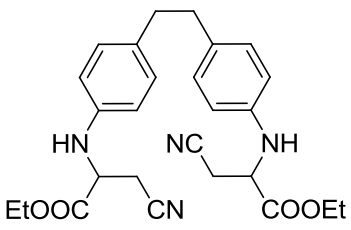
170.36, 140.7, 125.7, 124.9, 116.2, 111.7, 106.2, 62.8, 53.1, 21.5, 14.2; HRMS (ESI): calcd. for $C_{22}H_{25}N_4O_4$ $[M + H]^+$ 409.1876; found 409.1876.



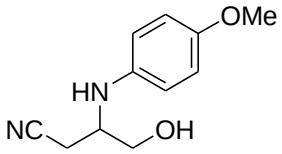
Diethyl 2,2'-((methylenebis(4,1-phenylene))bis(azanediyl))bis(3-cyanopropanoate) (5c): Following the general procedure B described above, **5c** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 50:50) as yellow coloured semisolid (26 mg, 23%): R_f (50% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3384, 2984, 2252, 1736, 1614 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.04 (d, J = 8.5 Hz, 4 H), 6.61 (d, J = 8.5 Hz, 4 H), 4.38-4.30 (m, 8 H), 3.80 (s, 2 H), 2.91 (d, J = 5.4 Hz, 4 H), 1.34 (t, J = 7.1 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 143.1, 132.7, 129.9, 116.3, 114.1, 62.6, 53.4, 40.1, 21.8, 14.2; HRMS (ESI): calcd. for $C_{25}H_{29}N_4O_4$ $[M + H]^+$ 449.2189; found 449.2187.



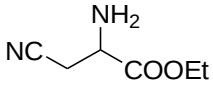
Diethyl 2,2'-((oxybis(4,1-phenylene))bis(azanediyl))bis(3-cyanopropanoate) (5d): Following the general procedure B described above, **5d** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 50:50) as brown coloured solid (82mg, 73%): mp 90-92 ^oC; R_f (50% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3386, 2923, 2252, 1736, 1498 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.87 (dd, J_1, J_2 = 8.6, 1.8 Hz, 4 H), 6.64 (dd, J_1, J_2 = 8.6, 1.1 Hz, 4 H), 4.35-4.29 (m, 6 H), 2.92 (d, J = 5.6 Hz, 4 H), 1.33 (t, J = 7.1 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 151.0, 140.8, 119.8, 116.4, 115.3, 62.6, 54.0, 21.8, 14.2; HRMS (ESI): calcd. for $C_{24}H_{27}N_4O_5$ $[M + H]^+$ 451.1981; found 451.1982.



Diethyl 2,2'-((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl))bis(3-cyanopropanoate) (5e): Following the general procedure B described above, **5e** was obtained after purification by silica gel column chromatography (EtOAc:Hexane = 50:50) as yellow coloured sticky solid (74 mg, 64%): R_f (50% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3380, 2977, 2191, 1736, 1520 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.04 (d, J = 8.4 Hz, 4 H) 6.61 (d, J = 8.5 Hz, 4 H), 4.39-4.31 (m, 8 H), 2.92 (d, J = 5.4 Hz, 4 H), 2.79 (s, 4 H), 1.34 (t, J = 7.2 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 143.0, 133.0, 129.6, 116.3, 114.0, 62.6, 53.5, 37.2, 21.8, 14.2; HRMS (ESI): calcd. for $C_{26}H_{31}N_4O_4$ $[M + H]^+$ 463.2345; found 463.2336.

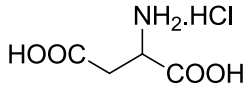


3-(4-Methoxyphenylamino)-4-hydroxybutanenitrile (6): 3a (0.2 mmol) was dissolved in 5 mL of EtOH and cooled to 0 ^oC. To this cooled solution, $NaBH_4$ (1.4 mmol) was added and was allowed to stir for 12 h. After this period, the reaction mixture was transferred to a separating funnel and extracted with EtOAc (3x10 mL). The combined organic layers were dried over anhydrous Na_2SO_4. Then the solvent was evaporated under vacuum. Purification of resulting crude reaction mixture by column chromatography on silica gel (EtOAc:Hexane = 60:40) as eluent gave the product **6** as brown coloured semisolid R_f (60% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3374, 2922, 1597, 1512, 1384 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 (br s, 1 H), 6.82 (d, J = 8.9 Hz, 2 H), 6.70 (d, J = 8.9 Hz, 2 H), 4.27 (s, 1 H), 3.90 (dd, J_1, J_2 = 8.6, 7.8 Hz,1 H) 3.77 (s, 3 H), 3.43 (dd, J_1, J_2 = 9.4, 6.8 Hz, 2 H) 2.77-2.73 (m, 1 H), 2.06-1.95 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.1, 152.8, 141.6, 115.0, 114.9, 55.8, 55.2, 39.2, 31.4; HRMS (ESI): calcd. for $C_{26}H_{31}N_4O_4$ $[M + H]^+$ 207.1134; found 207.1139.

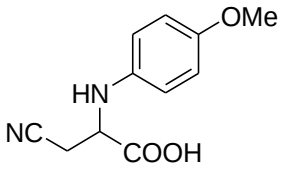


Ethyl 2-amino-3-cyanopropanoate (7): To the β-nitrile *N*-aryl α-amino ester **3a** (0.2 mmol) in MeCN (2 mL), aqueous solution of $(NH_4)_2S_2O_8$ (0.4 mmol) and ammonium ceric (III) nitrate (0.02 mmol) were added at 0 ^oC. The resulting reaction mixture was stirred for 3 h at 30 ^oC under open condition. After this period, the reaction mixture was

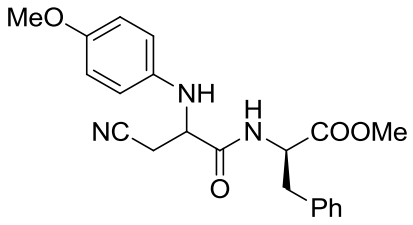
washed with DCM (3 mL) and treated with Na_2CO_3 to obtain pH 7. Then the product was extracted using DCM (5x1 mL). The combined organic layers were dried over anhydrous Na_2SO_4. Then the solvent was evaporated under vacuum that gave the product **7** as brown colour semisolid. IR (thin film): υ_{max} 3373, 2921, 1737, 1627, 1378 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.26-4.24(m, 2 H), 3.79 (dd, J_1, J_2 = 7.1, 5.2 Hz, 1 H), 2.84-2.69 (m, 2 H), 1.84 (s, 2 H), 1.32 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 117.0, 62.1, 51.2, 23.8, 14.1; HRMS (ESI): calcd. for $C_{26}H_{31}N_4O_4$ $[M + H]^+$ 143.0821; found 143.0818.



2-Aminosuccinic acid hydrochloride (8): To the crude Ethyl 2-amino-3-cyanopropanoate (**7**), 3 mL of conc. HCl (10 M) was added and allowed to stir for 3 h under open condition at 90 ^oC. After the reaction time, HCl was removed under vacuum that gave the product as colourless solid. IR (thin film): υ_{max} 3386, 1722, 1655, 1403 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.26 (t, J = 5.7 Hz, 1 H), 3.01 (t, J = 6.1 Hz, 2 H), 1.85 (s, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 171.0, 49.2, 33.6.



2-(4-Methoxyphenylamino)-3-cyanopropanoic acid (9): The compound **3a** (0.4 mmol) was dissolved in a mixture of THF/MeOH/H_2O = 3/1/1 (5 mL), and LiOH (1.2 mmol) was added at 0 ^oC. The mixture was allowed to stir for 12 h and then acidified with 1N aqueous HCl to pH = 2 and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4. Then the solvent was evaporated under vacuum that gave the product **9** as colourless solid (84 mg, 95%). mp 159 ^oC; IR (thin film): υ_{max} 3372, 2916, 1577, 1513, 1386 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.74 (d, J = 9.0 Hz, 2 H), 6.65 (d, J = 9.0 Hz, 2 H), 4.31 (dd, J_1, J_2 = 7.4, 5.7 Hz, 1 H), 3.65 (s, 3 H), 3.01-2.85 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 152.1, 141.3, 118.8, 115.0, 114.7, 55.7, 53.5, 21.2.



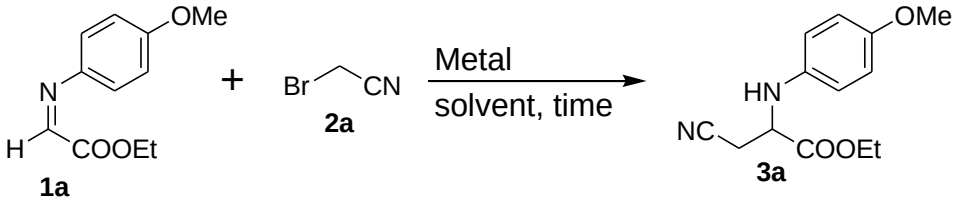
(2*R*)-Methyl 2-(3-cyano-2-((4-methoxyphenyl)amino)propanamido)-3-phenylpropanoate (10): The compound **9** (0.4 mmol) was dissolved in dry DCM (5 mL) and Phe-OMe.HCl (0.8 mmol), EDCI (0.8 mmol), HOBt.xH_2O (0.8 mmol), DIPEA (0.8 mmol) were added and the reaction mixture was allowed to stir at room temperature. After 12 h, the reaction mixture was washed with water and the organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and purification of resulting crude reaction mixture by column chromatography on silica gel (EtOAc:Hexane = 70:30) gave the product **10** as coloured semisolid (105 mg, 69%): R_f (60% EtOAc/Hexane) 0.50; IR (thin film): υ_{max} 3335, 2926, 1741, 1655, 1512 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 (d, J = 6.3 Hz, 2 H), 7.21 (d, J = 6.3 Hz, 1 H), 7.07 (d, J = 7.3 Hz, 1 H), 6.81 (dd, J_1, J_2 = 8.5, 5.5 Hz, 2 H), 6.61 (t, J = 8.5 Hz, 2 H), 4.90 (dd, J_1, J_2 = 13.2, 6.3 Hz, 1 H), 4.09-3.97 (m, 1 H), 3.80 (s, 3 H), 3.75 (s, 3 H), 3.23-3.08 (m, 2 H), 2.91 (dd, J_1, J_2 = 14.48, 5.2 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 169.7, 169.5, 154.2, 154.1, 138.5, 138.2, 135.5, 135.4, 129.2, 129.1, 128.7, 127.3, 127.2, 116.8, 116.7, 116.3, 115.0, 115.0, 56.5, 56.3, 55.7, 55.6, 53.2, 53.0, 52.5, 37.7, 37.7, 20.7, 20.1; HRMS (ESI): calcd. for $C_{21}H_{24}N_3O_4$ $[M + H]^+$ 382.1767; found 382.1765. This compound was isolated as a mixture of diastereomers. The ^1H and ^{13}C NMR data given for mixture of diastereomers.

Chapter 3

Results and Discussion

At the outset, we carried out optimization studies to get the best reaction conditions for the addition of bromoacetonitrile (**2a**) to α-imino ester (**1a**). A mixture of Zn dust, dry THF and bromoacetonitrile (**2a**) were allowed to stir for 15 minutes and α-imino ester (**1a**) was added into this. Then, the reaction mixture was stirred at 30 ^{o}C for 12 h, which yielded the product **3a** in 48% yield (Table 1, entry 1). Then the reaction was carried out at 60 ^{o}C, in which the yield got reduced to 29% (Table 1, entry 2).

Table 1. Nucleophilic Barbier type addition of bromoacetonitrile (**2a**) to **1a.**

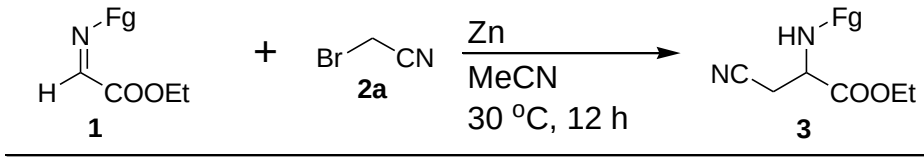


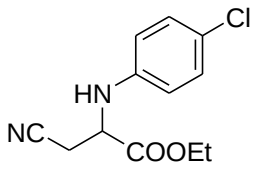
Entry	Metal (mmol)	Solvent (mL)	Time (h)	**3a**; yield (%)[a]
1	Zn (0.5)	THF (3)	24	48
2	Zn (0.5)	THF (3)	12	29[b]
3	Zn (1)	THF (3)	24	55[c]
4	Zn (1)	THF (3)	6	<5[d]
5	In (0.5)	THF (3)	12	37
6	In (0.5)	THF (3)	12	44[b]
7	Zn (0.5)	DMF (1)	24	63
8	Zn (0.5)	DMF (1)	24	30[c]
9	In (0.5)	DMF (1)	24	73
10	Zn (1)	1,4-Dioxane (3)	24	39
11	**Zn (1)**	**MeCN (3)**	**12**	**75**
12	Zn (1)	MeCN (3)	6	72
13	Zn (1)	MeCN (3)	12	24[e]
14	Zn (1)	MeCN (3)	12	64[f]
15	Zn (0.5)	MeCN (3)	12	37[g]
16	Zn (1)	MeCN (3)	12	67[h]
17	In (1)	MeCN (3)	12	24
18	Sn (1)	MeCN (3)	12	0
19	Bi (1)	MeCN (3)	12	0

[a] Reaction was carried out using **1a** (0.25 mmol) and **2a** (0.75; 3 equiv.) at 30 ^{o}C. Isolated yields are given.[b] Reaction was carried out at 60 ^{o}C. [c] TMSCl (0.5 mmol; 1 equiv.) was added. [d] glacial acetic acid (1 mmol; 4 equiv.). [e] Reaction was carried out at 80 ^{o}C. [f]Reaction was carried out at 5 ^{o}C. [g] 2a (2 equiv., 0.5 mmol) was used. [h] Reaction was carried out using iodo acetonitrile (2b; 0.75 mmol; 3 equiv.).

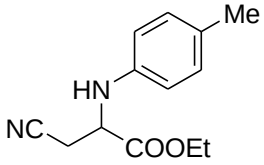
Next we carried out the reaction by adding TMSCl and glacial acetic acid as an additive in which **3a** was formed in 55% and 5% yields, respectively (Table 1, entry 3 and 4). Then, the reaction of α-imino ester (**1a**), bromoacetonitrile (**2a**) and indium in THF was carried out at 30 ^{o}C, which gave **3a** in 37% yield (Table 1, entry 5). Next, the reaction was carried out at 60 ^{o}C in which the product **3a** was yielded in 44% (Table 1, entry 6). Further, the reaction was done in DMF using Zn dust at 30 ^{o}C, which gave the product **3a** in 63% yield (Table 1, entry 7). Then, we carried out the reaction by adding TMSCl as additive at 30 ^{o}C in which the product **3a** was yielded in 30% (Table 1, entry 8). Then, we tried the reaction with indium as metal which yielded **3a** in 73% (Table 1, entry 9). Further, the reaction of α-imino ester (**1a**), bromoacetonitrile (**2a**) and Zn dust in 1,4-dioxane at 30 ^{o}C was carried out in which the product **3a** was produced in 39% yield (Table 1, entry 10).

Table 2. Zn-mediated Barbier type nucleophilic addition of **2a** to *N*-aryl α-imino esters.

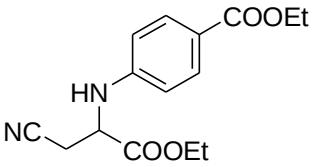




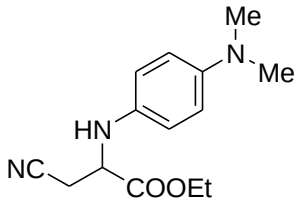
3b; 80%



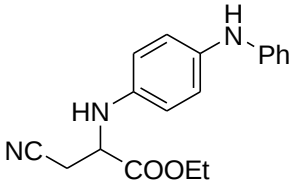
3c; 70%



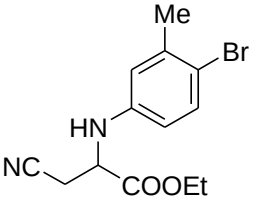
3d; 57%



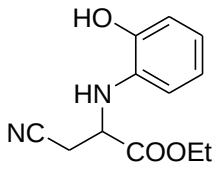
3e; 39%



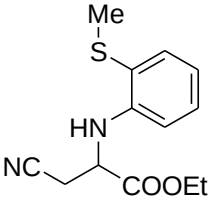
3f; 65%



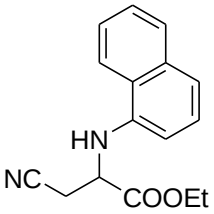
3g; 57%



3h; 38%

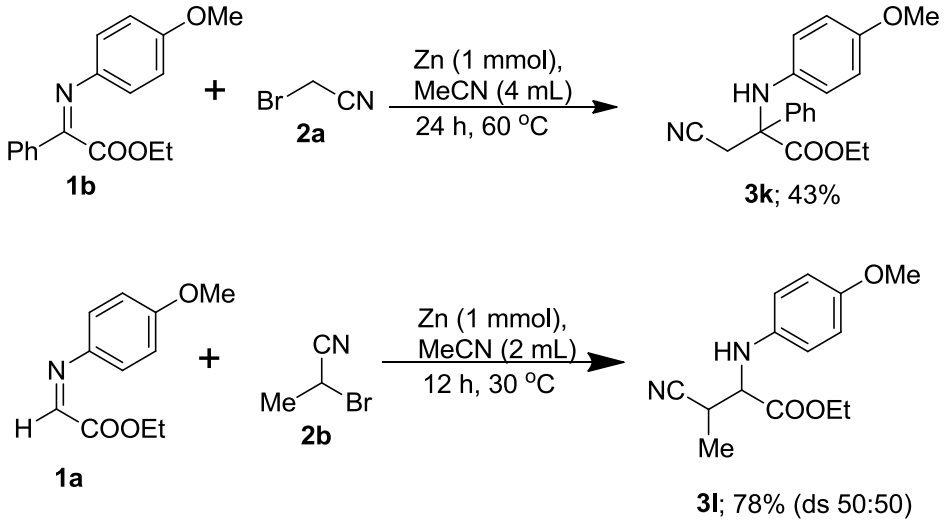


3i; 60%



3j; 68%

Successively, we carried out the reaction of **1a** with **2a** using Zn dust in MeCN at 30 ^{o}C for 12 h, which afforded the product **3a** in 75% yield (Table 1, entry 11). Then, we reduced the reaction time to 6 h in which **3a** was yielded in 72% (Table 1, entry 12). Further, we carried out the reaction at 80 ^{o}C, in which the yield got reduced to 24% (Table 1, entry 13). Then, the reaction was carried out at 5 ^{o}C in which the product **3a** was formed in 64% yield (Table 1, entry 14). Next, we carried out the reaction by using 2 equivalent of bromoacetonitrile (**2a**) in which the yield got reduced to 37% yield (Table 1, entry 15). The reaction of **1a** with iodoacetonitrile in the presence of Zn dust in MeCN was also carried out at 30 ^{o}C for 12 h gave the product **3a** in 67% yield (Table 1, entry 16). The reaction of **1a** with **2a** using In as metal in MeCN at 30 ^{o}C for 12 h yielded the product **3a** in 24% (Table 1, entry 17). Employing other metals like, Sn and Bi, did not afford **3a** (Table 1, entry 18 and 19).



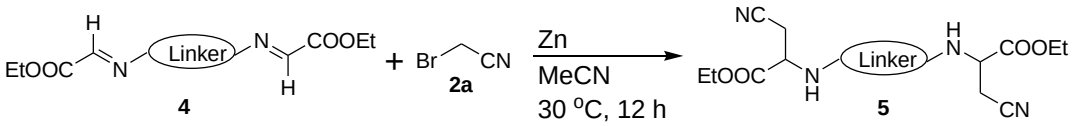
Scheme 2. Nucleophilic addition of **2a** with α-ketimino ester (**1b**); addition of 2-bromopropanenitrile **2b** with **1a**.

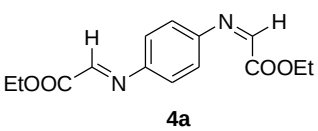
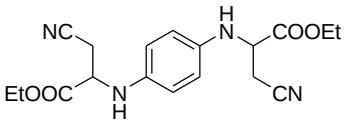
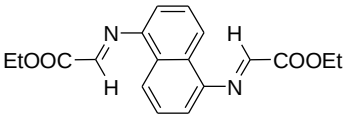
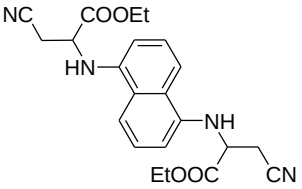
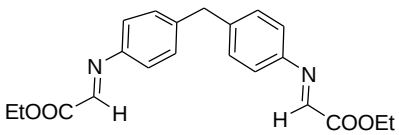
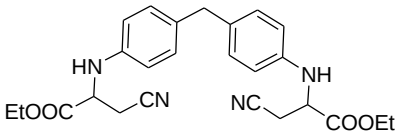
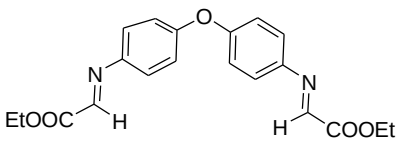
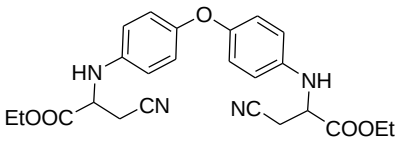
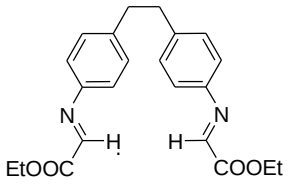
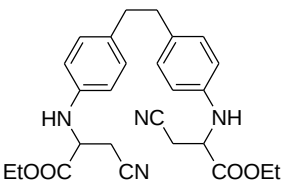
The scope and generality of Zn-mediated Barbier type addition reaction of bromoacetonitrile **2a** with other *N*-aryl α-imino esters (**1**) synthesized from different substituted anilines is shown in Table 2. Zn-mediated Barbier type addition reaction of bromoacetonitrile (**2a**) with various α-imino esters (**1**) in MeCN resulted in *N*-substituted β-nitrile α-amino acid derivatives **3b**–**3j** (Table 2).

To examinine the generality of the reaction towards α-ketimino ester, reaction was carried out with ethyl 2-(4-methoxyphenylimino)-2-phenylacetate (**1b**). Zn-mediated nucleophilic addition of bromoacetonitrile **2a** with α-ketimino ester afforded **3k** in 43%

yield (Scheme 2). To explore the stereoselective addition reaction and to obtain the product with good diastereoselectivity, we performed the reaction of 2-bromopropanenitrile **2b** with α-imino ester (**1a**). Zn-mediated Barbier type addition of **2b** with **1a**, which afforded **3l** in 78% yield without any diastereoselectivity (*dr* 50:50, Scheme 2).

Table 3. Nucleophilic addition of **2a** to diimino esters (**4**).



Entry	Substrate	Product	**5**; yield (%)[a]
1	 **4a**		**5a**; 15
2	 **4b**		**5b**; 70
3	 **4c**		**5c**; 23
4	 **4d**		**5d**; 73
5	 **4e**		**5e**; 64

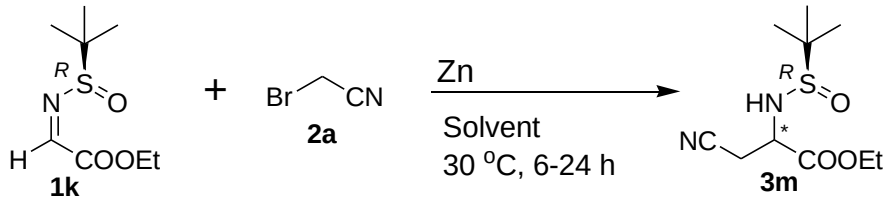
[a] isolated yields are given.

Next, we extended the generality of this method by using di–imino esters **4a**–**4e** as the starting materials as discussed in Table 3. The Zn-mediated reaction of various di–imino

esters **4** with bromoacetonitrile **2a** in MeCN at 30 ^{o}C for 12 h resulted in the formation of nitrile substituted bis–amino acid derivatives **5a**–**5e** (Table 3).

We further used chiral imino ester **1k** to get chiral β-nitrile substituted α-amino acid derivatives (Table 4). The reaction of chiral imino ester **1k** with bromoacetonitrile **2a** was carried out at 30 ^{o}C for 12 h using Zn metal in MeCN yielding **3m** in 28% yield with *dr* 83:19 (Table 4, entry 1). Then, the reaction was carried out in THF or DCE or EtOH or DMF to check whether better selectivity or yield can be achieved, however, our trials were not fruitful (Table 4, entry 2-5).

Table 4. Zn-mediated nucleophilic addition of **2a** to chiral sulfinyl imine (**1k**).



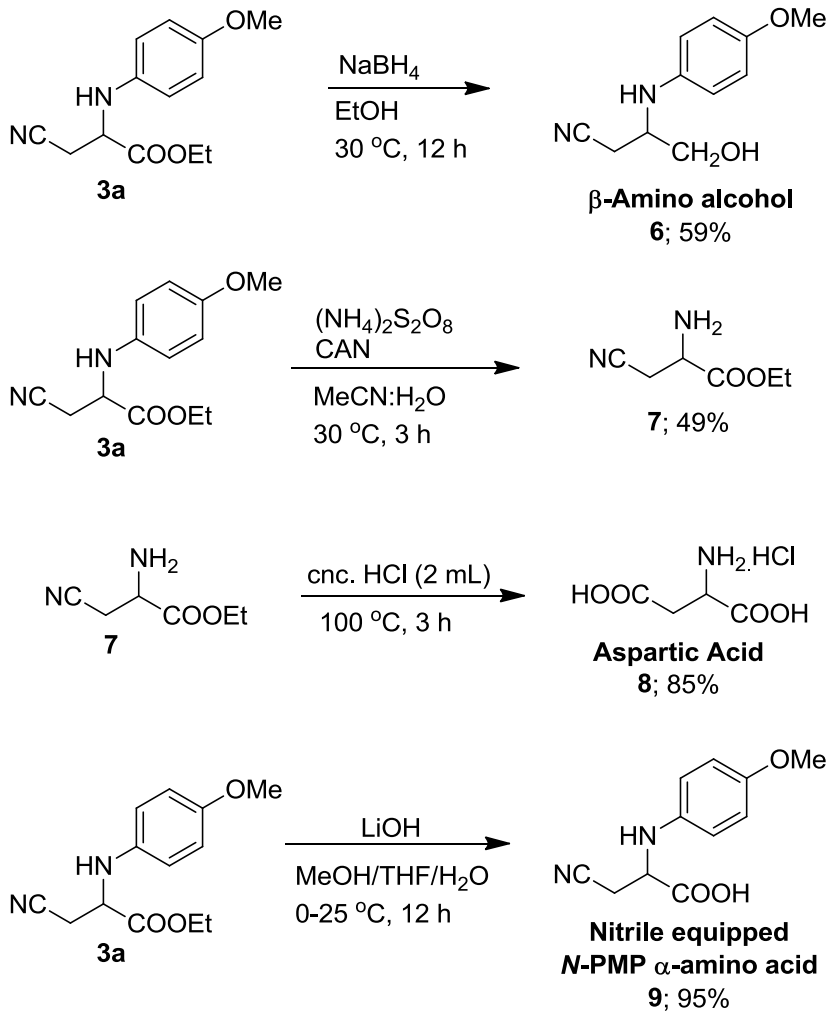
Expt.no:	Catalyst/additive	Solvent (mL)	Time (h)	3m; yield (%)[a] (dr)
1	------	MeCN (3)	12	28 (83:17)
2	------	THF (3)	12	38 (75:25)
3	------	DCE (2)	24	no rxn
4	------	EtOH (2)	20	no rxn
5	------	DMF (0.5)	20	12 (70:30)
6	CH_3COOH (1 mmol)	MeCN (2)	20	36 (70:30)
7	$InCl_3$ (10 mol%)	MeCN (2)	32	28 (70:30)
8	$HfCl_4$ (10 mol%)	MeCN (2)	12	31 (70:30)
9	LiBr (10 mol%)	MeCN (2)	24	33 (50:50)
10	AgCl (10 mol%)	MeCN (2)	12	34 (70:30)
11	I_2 (0.25 mmol)	MeCN (2)	12	20 (70:30)

[a] isolated yields are given.

Further ,we did the reactions by adding various catalysts or additives to improve the yield and selectivity. We carried out the Zn-mediated nucleophilic addition reaction of **1k** with

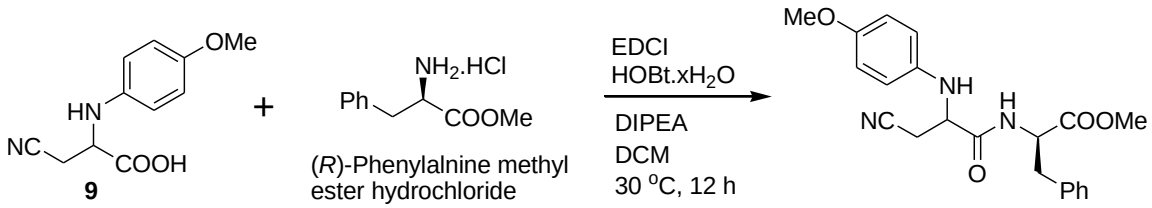
2a in MeCN by adding acetic acid as an additive which also gave **3m** in moderate yield and selectivity (Table 4, entry 6). Next, we carried out the reaction of **1k** with **2a** using Zn dust in MeCN by adding $InCl_3$ as catalyst, which gave **3m** in poor yield and moderate selectivity (Table 4, entry 7). Then, we used some other catalysts, such as, $HfCl_4$ or LiBr or AgCl and additive I_2. All these reactions gave **3m** in poor or moderate yield and selectivity (Table 4, entry 8-11).

Then, we focused on exploring some synthetic utilities of the products obtained in this methodology. At first, we did the reduction of **3a** using $NaBH_4$, which gave β-amino alcohol **6** in 59% yield (Scheme 3). Then, we did the PMP deprotection and yielded β-nitrile amino ester **7** which on further hydrolysis gave DL aspartic acid hydrochloride **8** in 85% yield (Scheme 3). Next, we tried the selective hydrolysis of the ester group present in **3a**. The selective hydrolysis of nitrile substituted *N*-protected α-amino esters yielded nitrile substituted *N*-protected α-amino acid **9** in very good yield (Scheme 3).



Scheme 3. Functional group transformations.

Peptide formation is of the important fundamental reaction and amino acids are important synthetic building blocks for the peptide synthesis methodology. To explore the utility of the amino acid synthesized from our work in peptide chemistry, we carried out the coupling reaction of the nitrile substituted *N*-protected amino acid **9** with (*R*)-phenylalanine methyl ester hydrochloride which gave the dipeptide **10** in good yield with *dr* 50:50 (Scheme 4).



Scheme 4. Coupling reaction of **9** with (*R*)-phenylalanine methyl ester hydrochloride.

Summary

In summary, synthesis of nitrile equipped unnatural amino acid derivatives were achieved via nucleophilic addition of bromoacetonitrile to α-imino esters. This methodology was applied to prepare nitrile substituted bis amino acid derivatives. Dipeptide of the nitrile equipped unnatural amino acid with (*R*)-Phenylalanine was also synthesized. Further works are under progress in our lab with regard to the addition of bromoacetonitrile to chiral α-imino esters.

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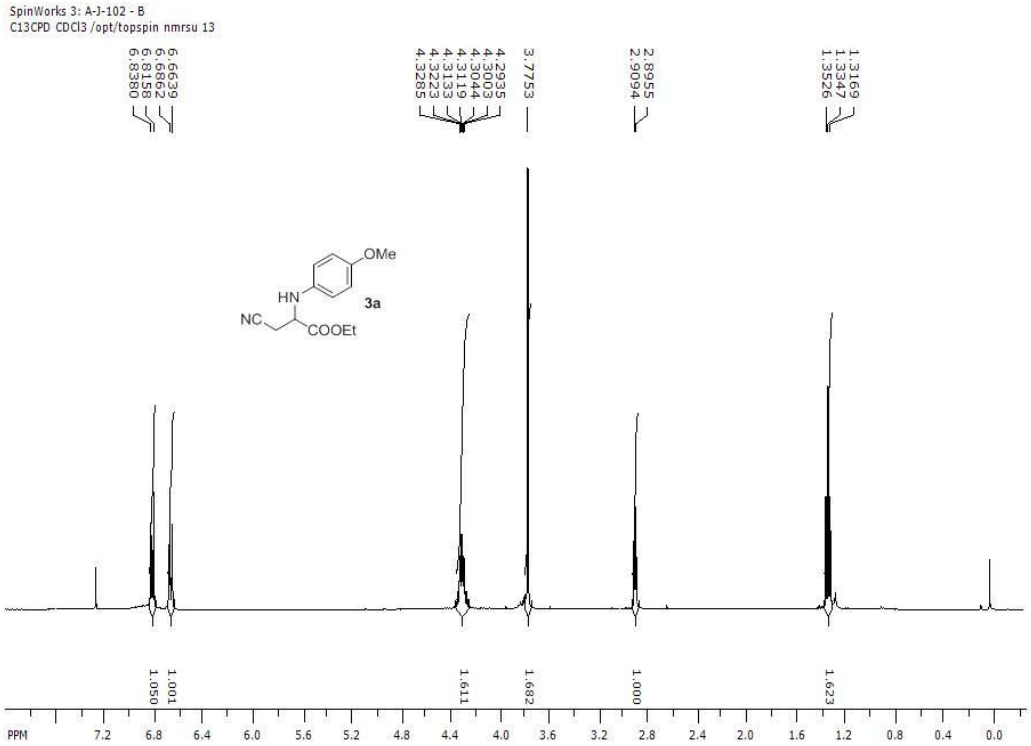
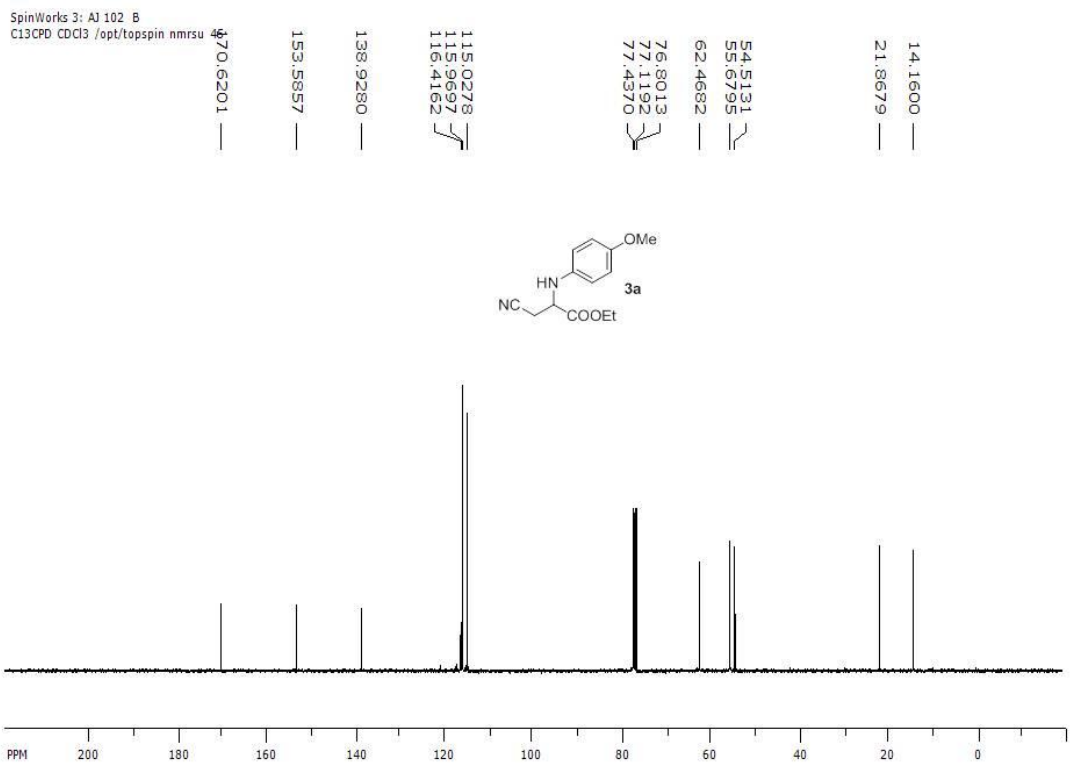
Appendix

NMR Spectra of Representative Compounds





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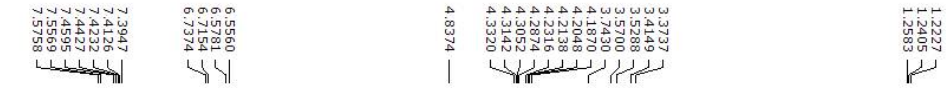

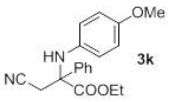

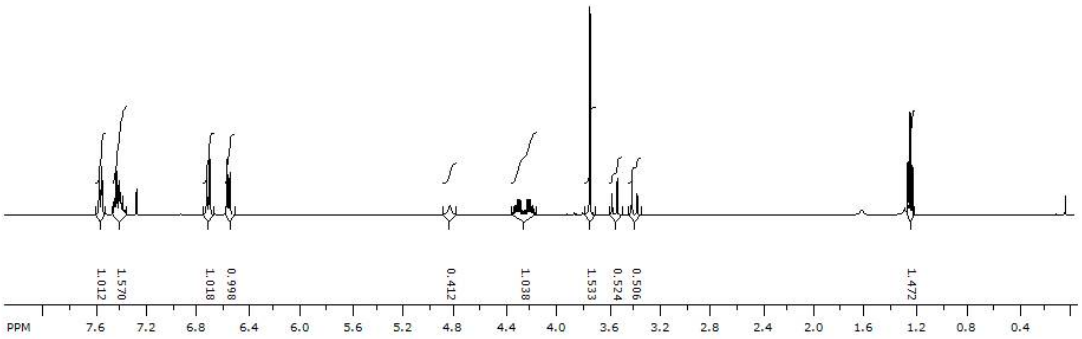

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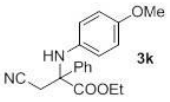

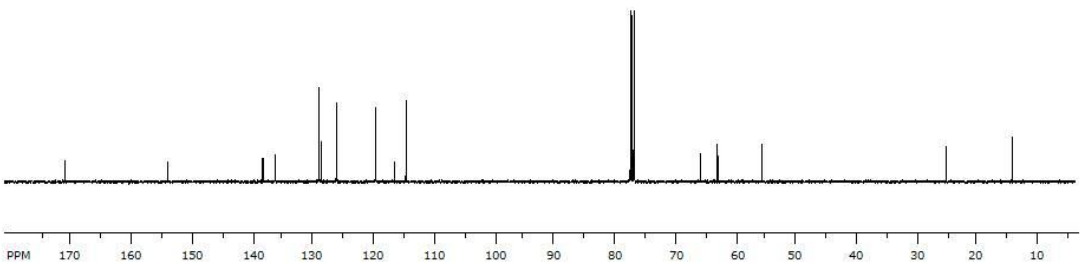

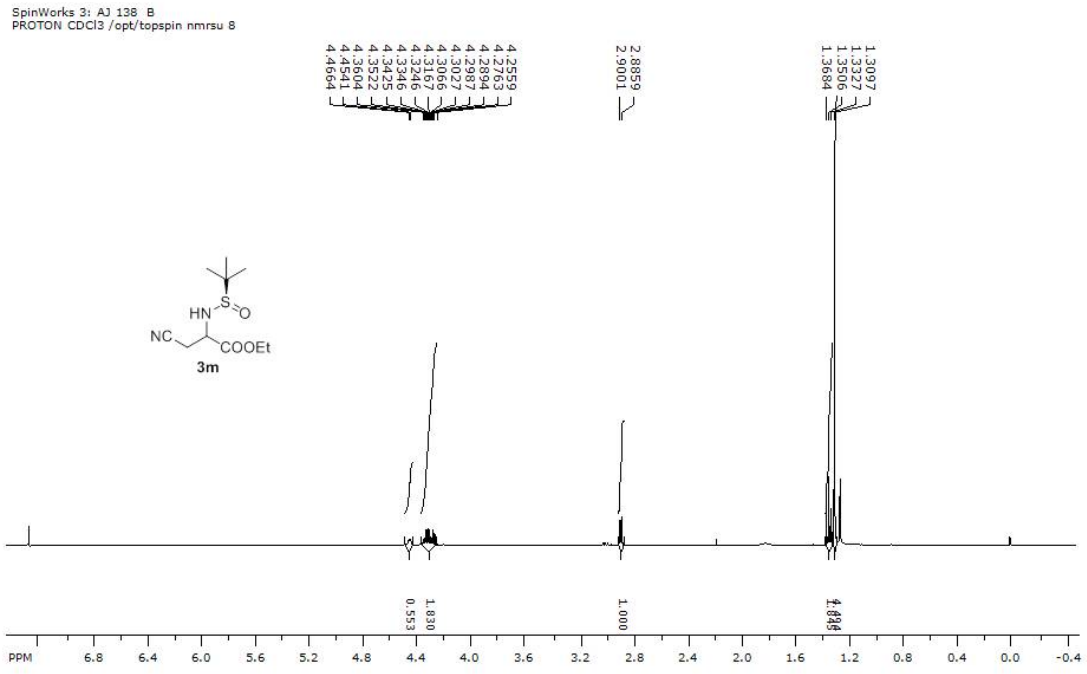
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4.3522
4.3425
4.3346
4.3246
4.3167
4.3066
4.3027
4.2987
4.2894
4.2763
4.2559
2.9001
2.8859
1.3684
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1.3097
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S
O
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COOEt
3m
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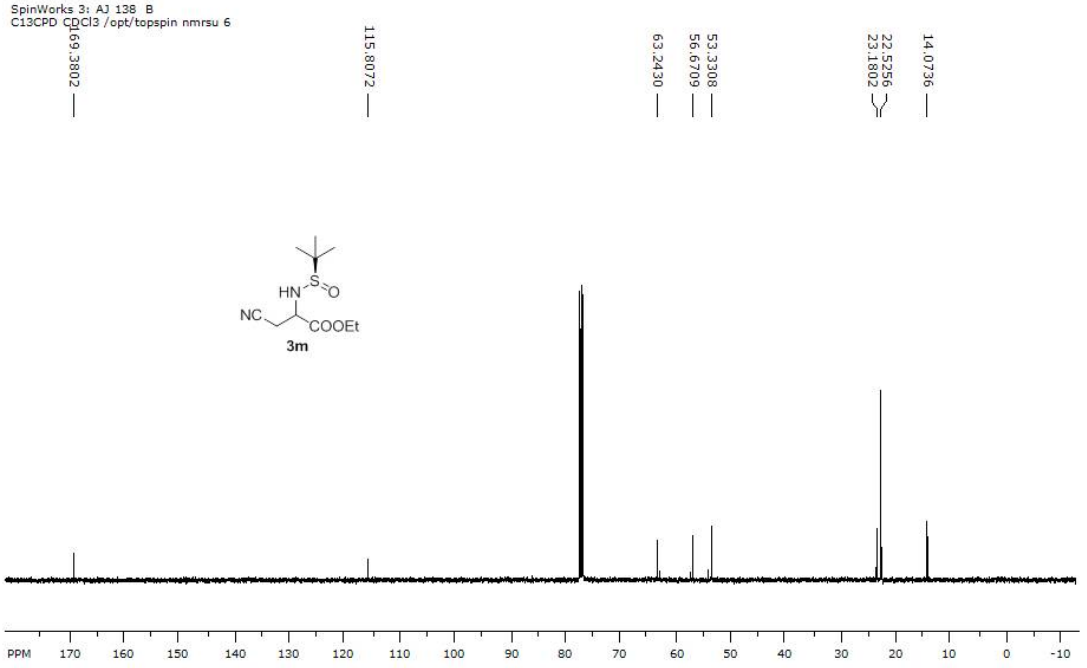
1.830
1.000
PPM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115.8072
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22.5256
14.0736
HN
S
O
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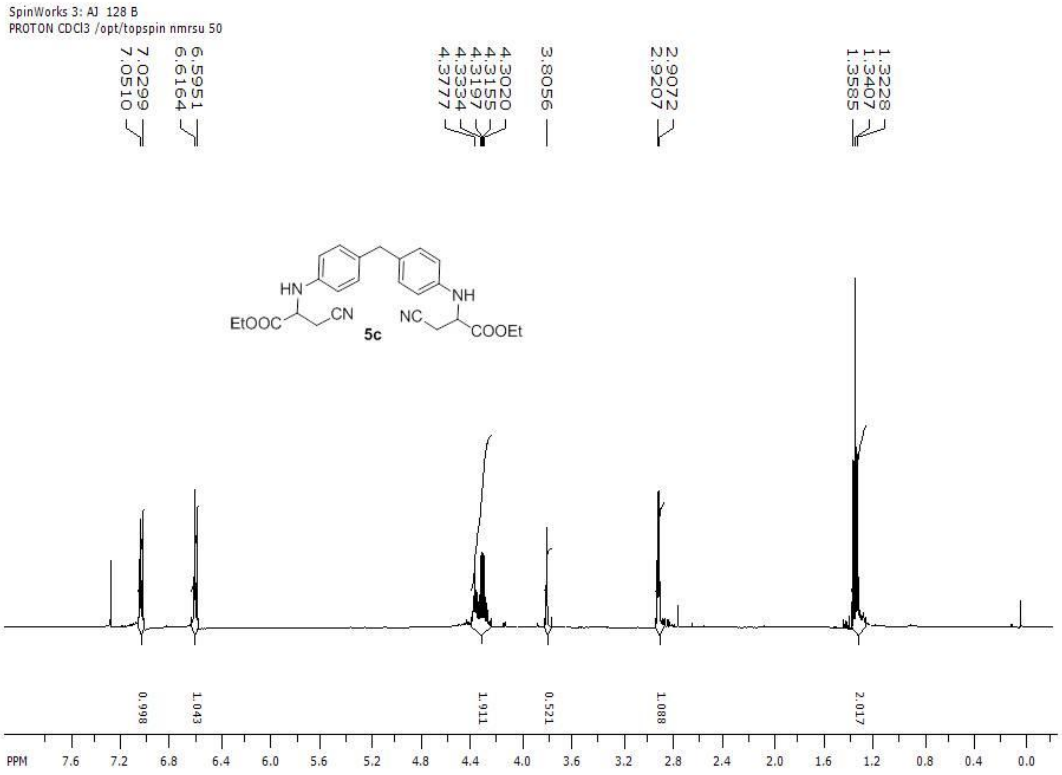
COOEt
3m
PPM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4.3334
4.3197
4.3155
4.3020
3.8056
2.9072
2.9207
1.3228
1.3407
1.3585
HN
NH
EtOOC
CN
NC
COOEt
5c
0.998
1.043
1.911
0.521
1.088
2.017
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
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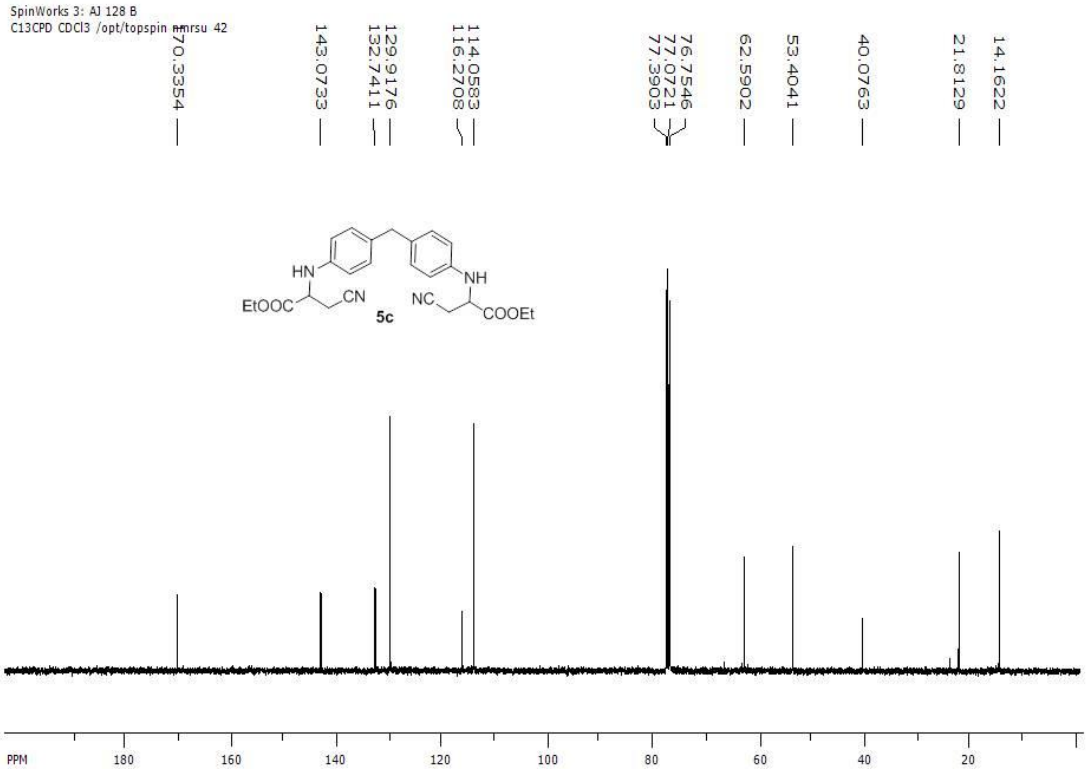
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129.9176
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77.0721
77.3903
62.5902
53.4041
40.0763
21.8129
14.1622
HN
NH
EtOOC
CN
NC
COOEt
5c
PPM
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160
140
120
100
80
60
40
20

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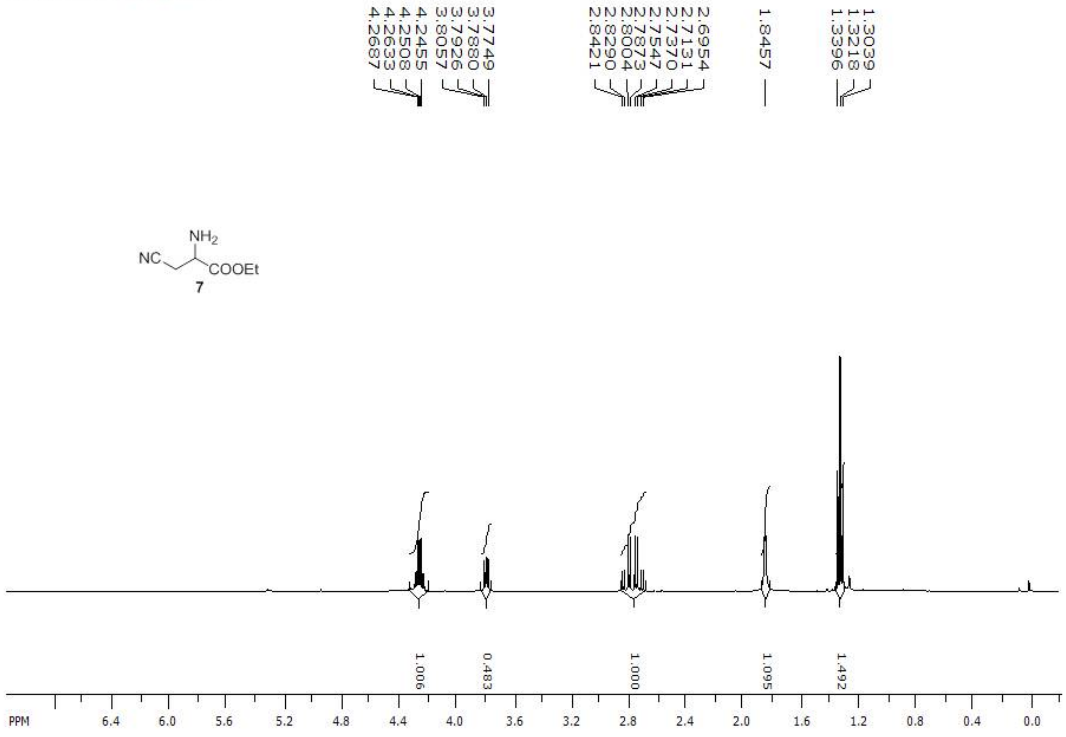

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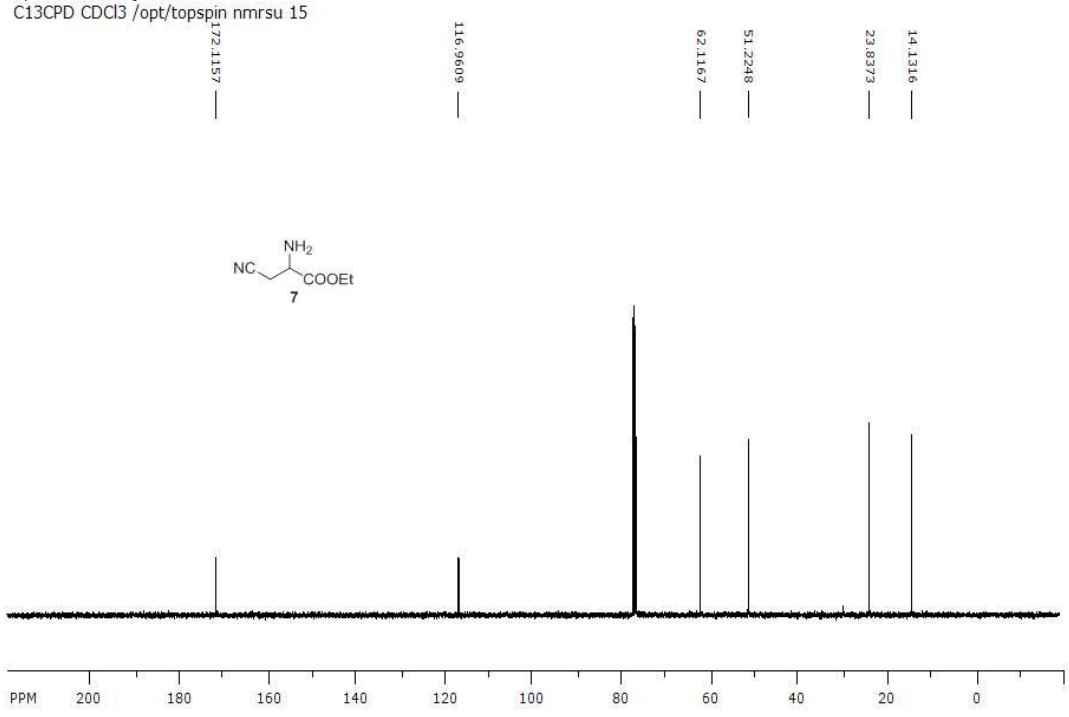

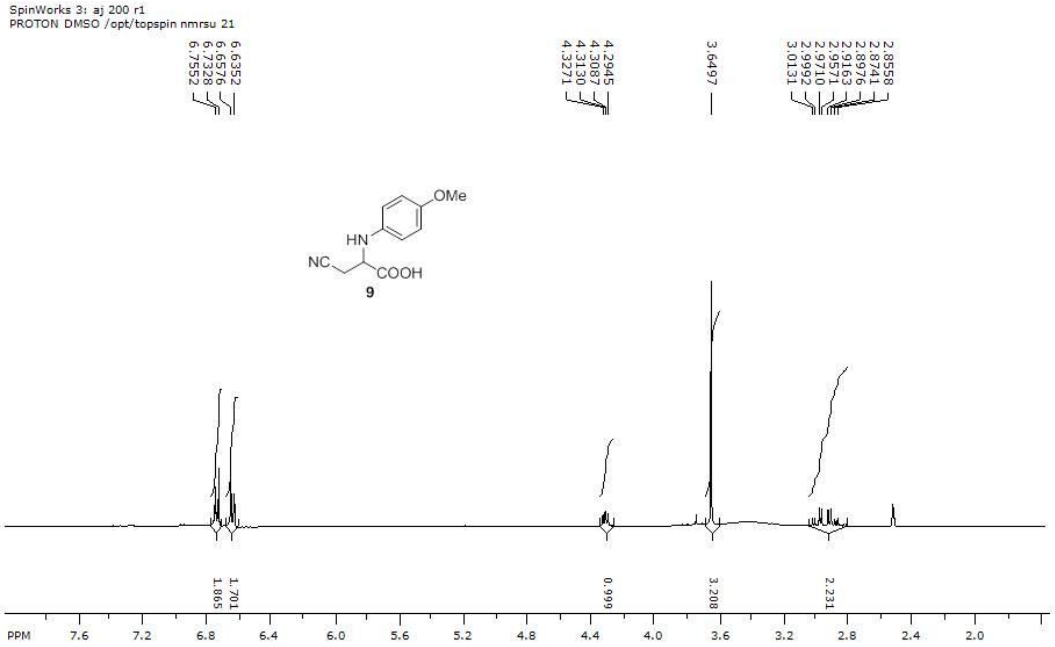
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4.3087
4.2945
3.6497
3.0131
2.9992
2.9710
2.9563
2.8976
2.8741
2.8558
OMe
HN
NC
COOH
9
1.865
1.701
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PPM
7.6
7.2
6.8
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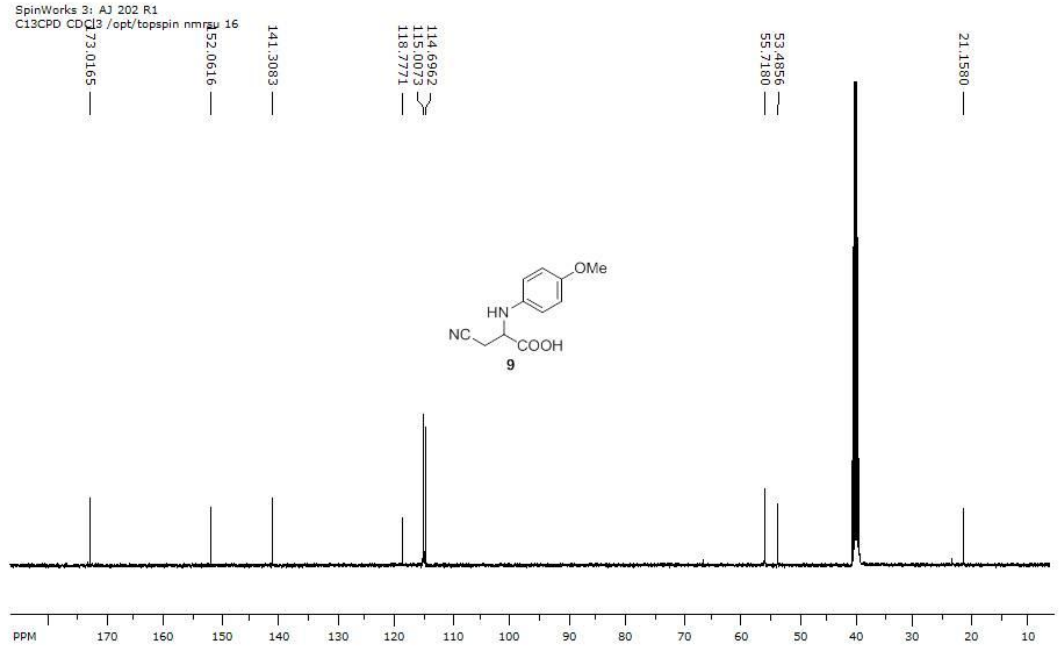
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53.4856
21.1580
OMe
HN
NC
COOH
9
PPM
170
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150
140
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100
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80
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60
50
40
30
20
10