SYNTHETIC APPLICATIONS OF DIETHYL CYANOMALONATE

by

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Abstract

Diethyl cyanomalonate was prepared in high yield from ethyl cyanoacetate and ethyl chloroformate in refluxing acetone/anhydrous K_2CO_3 and converted to its salts by titration with alkali metal and tetraalkylammonium hydroxides. These stable, non-hygroscopic salts were soluble in a variety of organic solvents and were reactive towards alkylation by electrophiles under neutral conditions. Under optimized conditions (Bu_4N^+ salt, DMSO, 80°C), diethyl cyanomalonate was monoalkylated exclusively on carbon by a variety of 1° and 2° alkyl bromide and iodide electrophiles, as well as electrophiles containing base-sensitive or acidic groups. The highly functionalized alkylation products were isolated using a simple work up procedure.

GABA, an ω-amino acid and important neurotransmitter, was synthesized from diethyl cyanomalonate and ethyl bromoacetate via alkylation, decarboethoxylation, ester hydrolysis and nitrile reduction reactions. While selective deuterium incorporation was not achieved during the decarboethoxylation reaction, a selective nitrile-mediated, base-catalyzed deuterium exchange method was developed and used to prepare $[3,3\text{-}^2H_2]\text{-}3\text{-}$cyanopropanoate and $[4,4\text{-}^2H_2]\text{-}4\text{-}$cyanobutanoic acid. $[3,3\text{-}^2H_2]$GABA was obtained upon reduction of the former, and the latter was used to demonstrate a gas-phase McLafferty-type rearrangement of carboxylate ions.

Alkylation of diethyl cyanomalonate by halogenated esters and acids, followed by ester hydrolysis and monodecarboxylation provided a homologous series of 2-cyanodicarboxylic acids which, after nitrile reduction, provided a similar series of 2-(aminomethyl)dicarboxylic acids (β^2-amino acids). The two series of compounds inhibited enzymes of amino acid metabolism and also were valuable substrates for an ESI-MS study to elucidate the collision-induced fragmentation processes of substituted dicarboxylic acids.

List of Abbreviations and Symbols Used

bp	boiling point
br s	broad singlet
Bu_4N^+	tetrabutylammonium
^oC	degrees Celsius
d	doublet
DBU	diazabicyclo[5.4.0]undec-7-ene
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
ε	extinction coefficient
EI-MS	electron ionization mass spectrometry
eq	equivalent
ESI-MS	electrospray ionization mass spectrometry
Et	ethyl
EWG	electron withdrawing group
GABA	γ-aminobutyric acid (4-aminobutyric acid)
h	hour(s)
HMPA	hexamethylphosphoramide
HMPT	hexamethylphosphorous triamide
HRMS	high resolution mass spectrometry
Hz	Hertz
IR	infra-red

J	Coupling constant
LDA	lithium diisopropylamide
m	multiplet
M	moles per litre
Me	methyl
Me_4N^+	tetramethylammonium
MHz	megahertz
mg	milligram(s)
mL	millilitre(s)
mmol	millimole(s)
mp	melting point
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
q	quartet
R	alkyl group
s	singlet
t	triplet
*t*Bu	*tert*-butyl
THF	tetrahydrofuran
TMS	tetramethylsilane
UV	ultra-violet

Acknowledgements

Chapter 1: Introduction

One of the most important aspects of synthetic organic chemistry is the formation

of carbon-carbon single bonds.[1,2,3] Often this is accomplished by the alkylation of a

carbanion, a negatively charged carbon nucleophile in which the carbon atom formally

bears a unit of negative charge, by a carbon electrophile, such as an alkyl halide. Most

carbanions encountered in organic synthesis, such as enolates, contain at least one

adjoining electron-withdrawing group to help stabilize the negative charge.[1] The main

goal of this thesis is to demonstrate the synthetic utility of the diethyl cyanomalonate

carbanion (1-1A) in which the negative charge is stabilized by three electron-

withdrawing groups (Scheme 1.1).
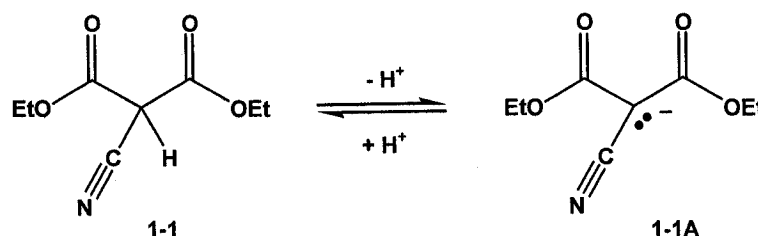
Scheme 1.1 Diethyl cyanomalonate (1-1) and its highly stabilized anion (1-1A).

1.1 Generation and Stabilization of Carbanions

Often carbanions are generated by the deprotonation of an acidic C-H. The

acidity of hydrogens in organic molecules is determined by their proximity to one or

more functional groups that stabilize the negative charge developed upon deprotonation.

Typically, the carbanions are best stabilized by conjugation with the polarized π system

of the electron-withdrawing group. Several examples of the effect of electron-

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withdrawing functional groups on C-H acidity are given in Figure 1.1. Note that pK_a values measured in water usually differ from those measured in DMSO because of the stabilization of anions by hydrogen bonding in water.[16]

Alkylation of carbanions occurs primarily through an S_N2 reaction mechanism,[1] so alkylation efficiency is maximized when reaction conditions and substrate structural features promote a bimolecular reaction mechanism. Therefore, the rate of reaction depends on the concentration of carbanion, so the formation of a high concentration of reactive carbanion in the reaction mixture is important.

The accessibility of carbanions for use in synthesis depends on the availability of suitable bases. For example, formation of carbanions stabilized by one electron withdrawing group generally requires specialized strong, non-nucleophilic bases, such as lithium diisopropyl amide (LDA), and nonprotic solvents to form a high concentration of reactive carbanion species in solution.[4,5,6,7]

On the other hand, carbon acids containing two electron-withdrawing groups are more easily deprotonated, and when two acidic hydrogen atoms are present, are referred to as "active methylene compounds".[8] The electron-withdrawing and delocalizing ability of the two geminal electron-withdrawing groups makes these compounds acidic enough to be deprotonated by common hydroxide, alkoxide or carbonate bases, ensuring a high concentration of reactive carbanion in the reaction mixture. In the past this has led to the greater use of the malonate ester anion (1-11, pK_a 13.3)[10] rather than the ethyl acetate anion (1-10, pK_a 24.5).[11] More recently, the phenylsulfonyl group has found use as the second stabilizing group for the generation of nitrile[36] and phosphonate[9] stabilized carbanions.

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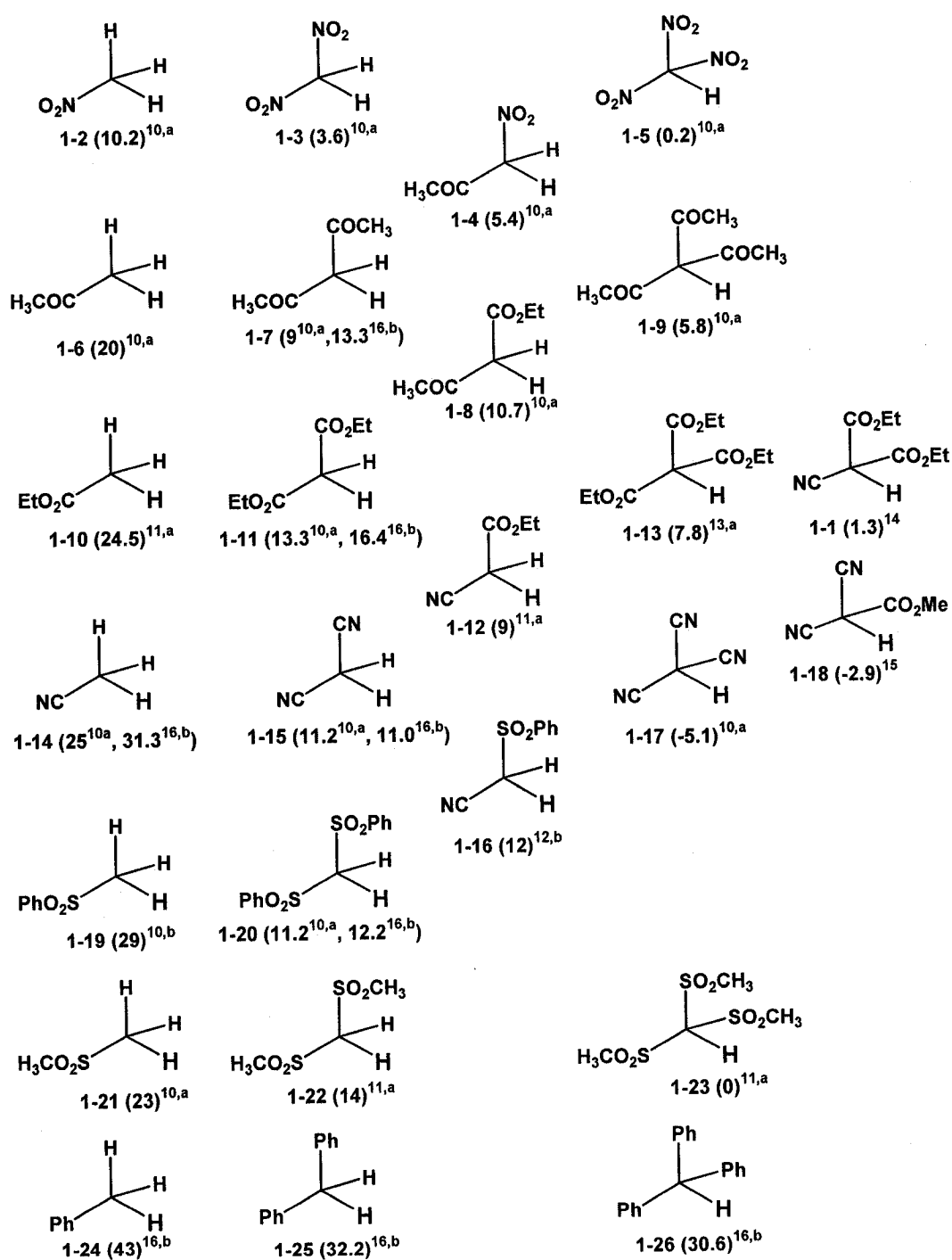
Figure 1.1 Effect of number and type of electron-withdrawing groups on C-H acidity as indicated by pK_a values (given in parentheses, H_2O[a], DMSO[b]).[10,11,12,13,14,15,16]

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1.2 Stabilized Carbanions in Synthesis

With the advent of strong amide bases such as LDA, alkylation of carbanions stabilized by one electron withdrawing group such as ketones (**1-6**), esters (**1-10**) and nitriles (**1-14**) has become commonplace in the modern organic laboratory.[5,7] However, the alkylation of active methylene compounds containing two or more stabilizing groups has been and continues to be a useful synthetic method in organic chemistry. The best known examples are the malonate (**1-11**) and acetoacetate (**1-8**) ester syntheses.[17]

In the malonate ester synthesis, the malonate ester (**1-11**) serves as a two-carbon homologating agent in the synthesis of carboxylic acids (Scheme 1.2). The overall procedure involves four distinct steps. Initially, ethoxide base is added to an ethanolic diethyl malonate solution, generating the reactive enolate nucleophile. Addition of an alkyl halide to the reaction mixture leads to the alkylated ester (**1-27**). Formation of the product carboxylic acid and the removal of the second activating group by decarboxylation are accomplished by heating in aqueous acid or base giving an α-substituted carboxylic acid (**1-29**).[18,19]

Recently, the malonate ester (**1-11**) synthesis has been applied to the syntheses of enzyme inhibitors,[20,21] an immunosuppressant analogue,[22] biologically active oxadiazoles,[23] and deuterated analogues of a beetle pheromone.[24] A sequential dialkylation of diethyl malonate with ethyl bromide and diiodocarbene led to a component of the polyketide natural product spiculoic acid A.[25] Dialkylation of malonate and cyanoacetate esters, followed by ring closing metathesis, has generated macrocyclic malonate derivatives that served as precursors to disubstituted glycines which were incorporated into peptides.[26] Alkylation of ethyl cyanoacetate (**1-12**) has generated an

intermediate in the synthesis of purine biosynthesis inhibitors[27] and bridged isoflavones.[28]

Malonate (**1-11**), cyanoacetate (**1-12**) and acetoacetate (**1-8**) esters have been alkylated in ionic liquids by a series of prenyl chlorides and bromides.[29]
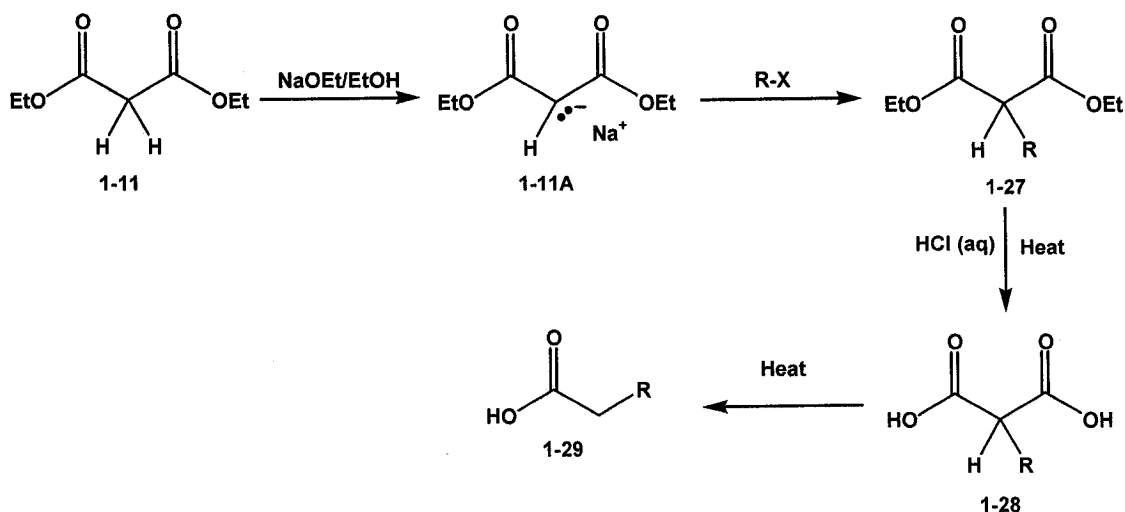
Scheme 1.2 Classical malonate ester synthesis.

The malonate ester synthesis also has been employed in nucleophilic aromatic substitution reactions, as an alternative to the Friedel-Crafts reaction.[30,31,33,34] Although the Friedel-Crafts reaction is one of the most important ways to alkylate an aromatic ring, it has several drawbacks, such as rearrangement of the alkylating agent, multiple alkylations of the aromatic ring, and insensitivity of aromatic rings containing electron-withdrawing groups to alkylation.[32]

In the example shown in Scheme 1.3, arylation of the malonate ester is followed by alkylation and didecarboalkoxylation to introduce a carbon substituent containing a variety of functional groups.[33] The α-aryl malonate (**1-31**) was prepared from dimethyl malonate (**1-11B**) and 3,4-dichloronitrobenzene (**1-30**) in THF with NaH as the base.

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Alkylation by a variety of alkyl halides and Michael acceptors generated several alkylated aromatic products (**1-32A-D**) which, after Krapcho decarboxylation,[60] gave the desired alkylated aromatic ring (**1-33**).
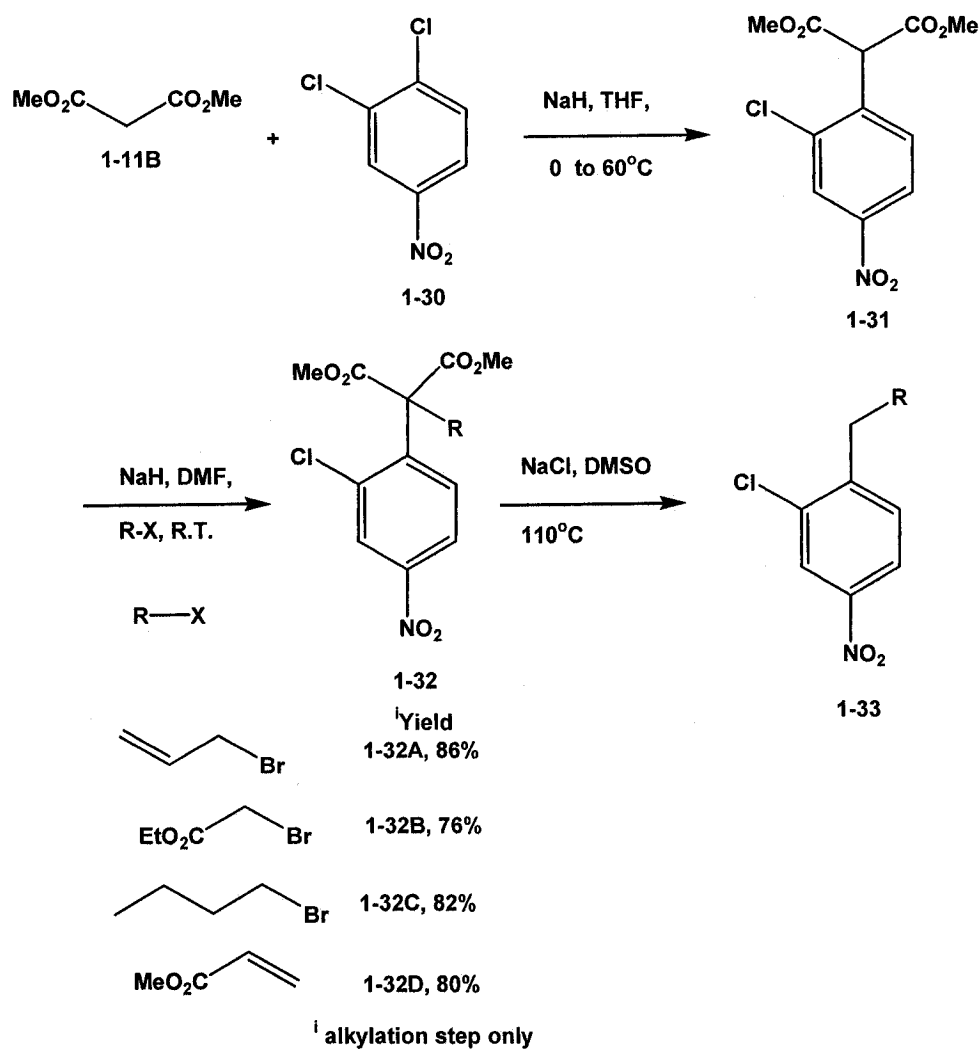
Scheme 1.3 α-Arylation and subsequent alkylation of dimethyl malonate.[33]

An arylation of ethyl cyanoacetate (**1-12**) with 2-iodo-6-methoxynaphthalene (**1-34**) was used in a synthesis of Naproxen, a nonsteroidal anti-inflammatory drug (Scheme

1.4).[34] Subsequent alkylation with methyl iodide gave ethyl 2-cyano-2-(6-methoxynaphth-2-yl) propionate (1-35) in 70% yield. After nitrile and ester hydrolysis, the desired monocarboxylic acid was obtained in 90% yield.
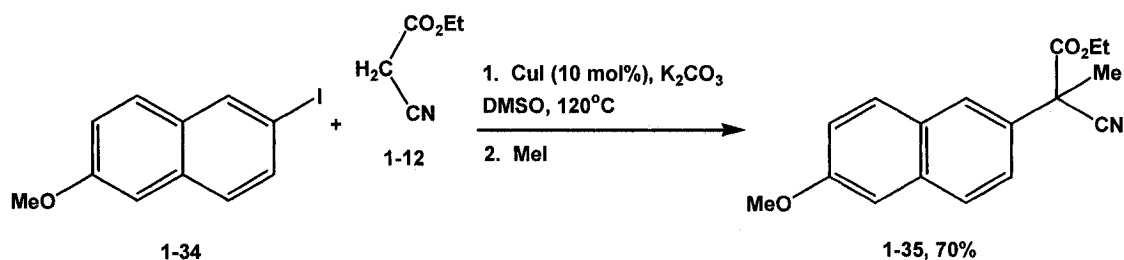
Scheme 1.4 α-Arylation and alkylation of ethyl cyanoacetate, giving a precursor to Naproxen.[34]

The increased delocalization of carbanions stabilized by two functional groups results in a decrease in nucleophilicity, thus requiring more vigorous reaction conditions for alkylation. However, the more highly stabilized carbanions demonstrate greater selectivity. This is especially important when the electrophile contains more than one reactive centre. For example, the stabilized anion of diethyl malonate (1-11) selectively displaces bromide in the presence of chlorine on a disubstituted alkyl halide.[35]

The enhanced selectivity of a stabilized phenylsulfonylacetonitrile anion (1-16) has been used in the synthesis of L-indospicine, a toxic arginine analogue found in certain plants.[36] Initially, direct alkylation of the acetonitrile anion (1-14A) by an amino acid triflate derivative (1-35) was attempted (Scheme 1.5). However, due to the basicity of the acetonitrile anion (pKa = 25),[10] the amide (pK$_a$ ≈ 17)[5] present in the glutamate derivative was deprotonated and cyclization occurred (1-37). Nucleophilic acyl substitution of the t-butyl ester by the acetonitrile anion was also observed (1-38).

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Therefore, the more stabilized, less basic ($pK_a = 12$)[37] and less nucleophilic

phenylsulfonylacetonitrile anion (**1-16A**) was used to avoid the unwanted side reactions

(Scheme 1.6). This less nucleophilic anion (**1-16A**) was successfully alkylated by an

iodo derivative of glutamate (**1-39**) in 82% yield. The phenylsulfonyl group was

removed in a subsequent step by reduction with Na/Hg in a methanolic disodium

hydrogen phosphate buffer at $0°C$.[36]

1-36, Desired Product (Not Observed)

1-37 + **1-38**

Observed Products
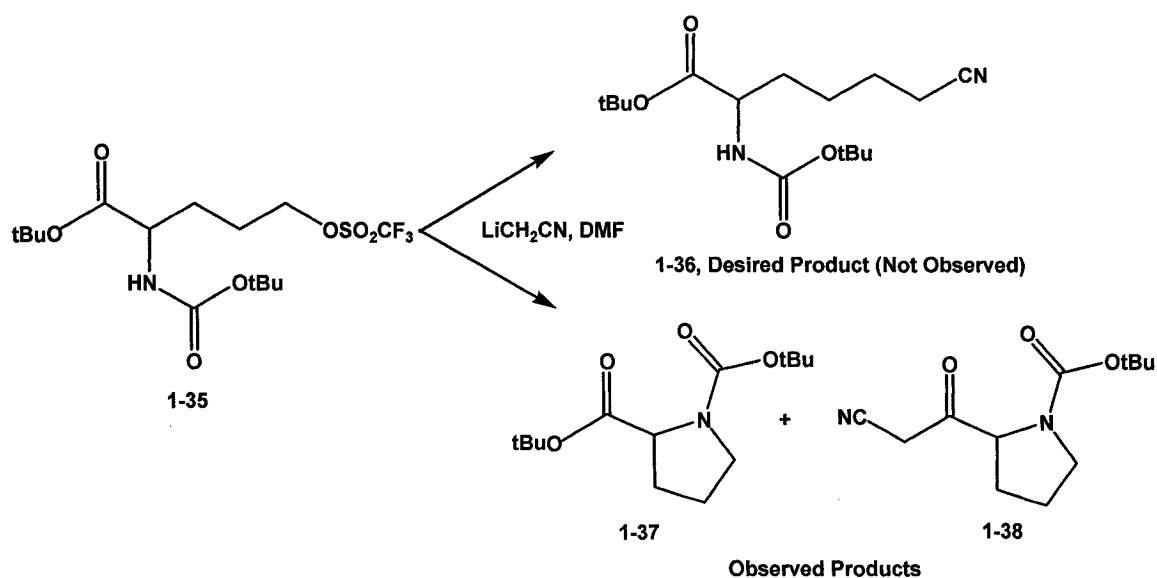
Scheme 1.5 Attempted alkylation of the acetonitrile anion (**1-12**) in the synthesis of L-indospicine.[36]
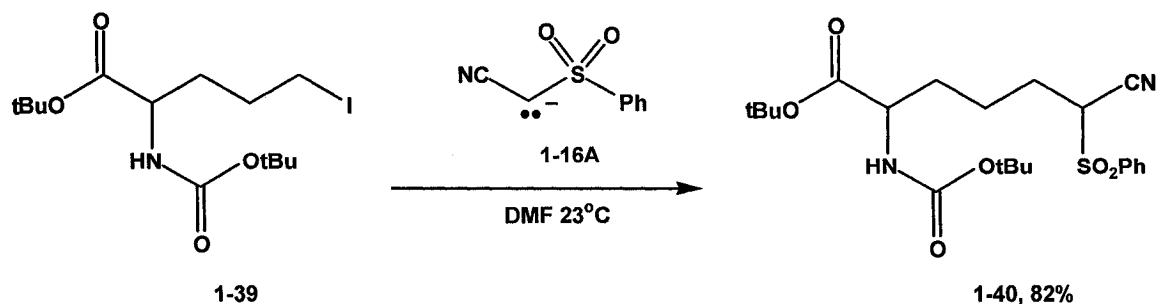
Scheme 1.6 Successful alkylation of a stabilized phenylsulfonylacetonitrile anion (**1-16A**) in the synthesis of L-indospicine.[36]

N-Methoxy-*N*-methylamides or Weinreb amides (**1-41**) are examples of active

methylene compounds that are synthetic equivalents of $^-CH_2CHO$ and $^-CH_2COR$ anions

(Scheme 1.7).[38] The Weinreb amides, however, are unstable under strongly basic

conditions and sulfone stabilizing groups have been used to generate more acidic

Weinreb amides.
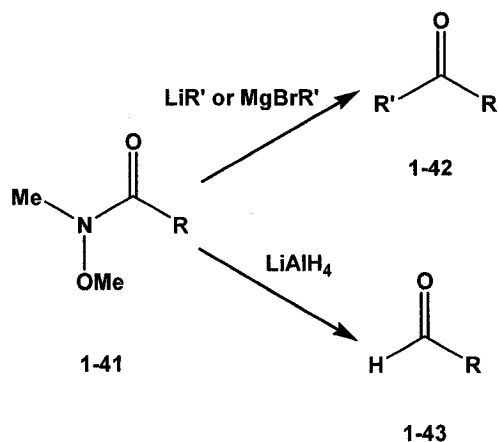
Scheme 1.7 Weinreb amides and the synthesis of ketones and aldehydes.[38]

For the synthesis of 2,3-dideoxy sugars,[39] alkylation of the stabilized nucleophile

generated from *N*-methoxy-*N*-methyl-2-phenylsufonylacetamide (**1-44**) was carried out

under mild conditions (K_2CO_3, DMF) avoiding strong base (Scheme 1.8). After removal

of the sulfonyl group, the functionalized Weinreb amide (**1-45**) was carried forward to the

synthetic target.
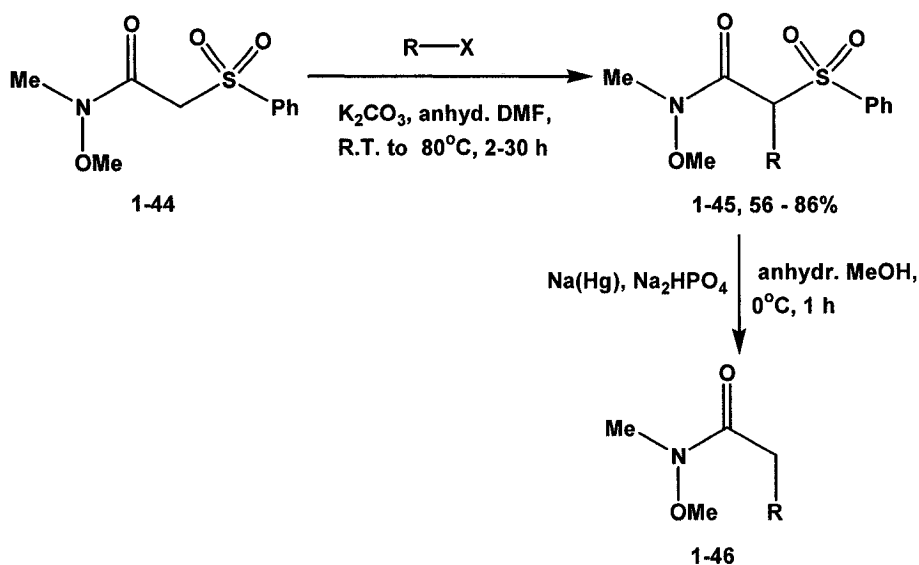
Scheme 1.8 Alkylation of N-methoxy-N-methyl-2-phenylsufonyl-acetamide under mild conditions generating a functionalized Weinreb amide.[39]

1.2.1 Dialkylation of Active Methylene Compounds

The success of the classical malonic ester synthesis[17] (Scheme 1.2) results from the relative acidities of the initial malonate ester (**1-11**), the alkylated malonate ester product (**1-28**) and the alcohol solvent.[7] For example, deprotonation of diethyl malonate (**1-11**, $pK_a = 13.3$)[10] using ethoxide ion in ethanol ($pK_a = 16$)[5] would generate a high concentration of the malonate ester anion. Upon alkylation of the α-carbon, the acidity of the remaining proton decreases by about two pK_a units,[18] yielding an alkylated diethyl malonate (**1-27**) with a pK_a value of approximately 15.5. Under conditions in which the amount of base is limited to one equivalent, the anion of the initial malonate ester will be present in the highest concentration, promoting the initial alkylation step. Only a small amount of the anion of the alkylated malonate ester would be present, minimizing the dialkylation side reaction.

Despite this, dialkylation is a common side reaction, particularly when reactive electrophiles or more acidic active methylene compounds are used. Active methylene compounds containing nitrile groups are more acidic than their diester counterparts (Figure 1.1); alkylation of ethyl cyanoacetate (**1-12**, pK_a 9)[11] using alkoxide bases leads to substantial dialkylation since the second α-hydrogen remains significantly more acidic ($pK_a \approx 11$) than the alcohol solvent (pK_a = 15-16),[5] even after introduction of an alkyl group.[18]

Recently, dialkylated ethyl cyanoacetates were required for the synthesis of octahydropyrimido[3,4-*a*]-*s*-triazine derivatives, which are potential antifungal agents.[40] The original plan used to obtain a dialkylated ethyl cyanoacetate with two different alkyl groups (**1-47B**) involved successive alkylation of ethyl cyanoacetate (**1-12**) in base using two different halides (Scheme 1.9). Despite efforts to modify the proportions of ethyl cyanoacetate (**1-12**), base and alkyl halide, the initial monoalkylated product (**1-48**) was never obtained cleanly. Instead, mixtures of monoalkylated (**1-48**) and dialkylated (**1-49**) ethyl cyanoacetates along with starting material (**1-12**) were obtained (Scheme 1.10).
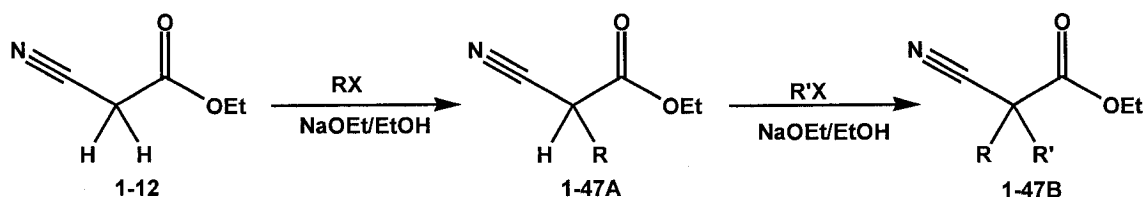
Scheme 1.9 Original scheme for the generation of dialkylated ethyl cyanoacetate with two different alkyl groups.[40]
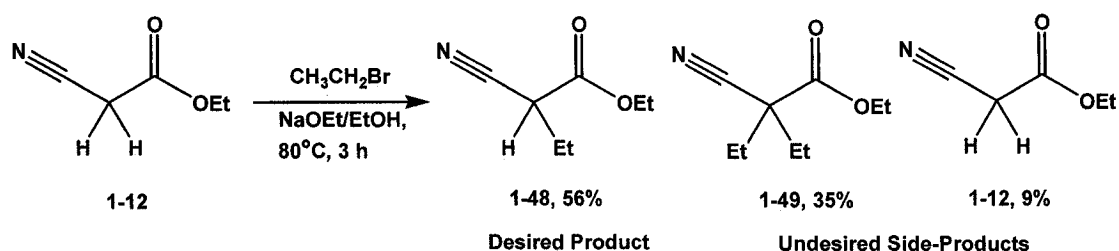
Scheme 1.10 Attempted monoalkylation of ethyl cyanoacetate resulted in a mixture of products.[40]

Previously in this laboratory,[41] several attempts were made to prepare dimethyl cyanosuccinate (**1-51A**) by alkylation of methyl cyanoacetate (**1-12B**) with methyl bromoacetate (**1-50**) in NaOMe/MeOH (Scheme 1.11). Despite variations in the proportion of reagents, the isolated product always consisted of a mixture of unreacted starting material (**1-12B**), the desired monoalkylated product (**1-51A**) and the dialkylated product (**1-51B**). A subsequent attempt to monoalkylate ethyl cyanoacetate with ethyl bromoacetate in acetone containing K_2CO_3 produced an equimolar amount of starting material and dialkylated product.
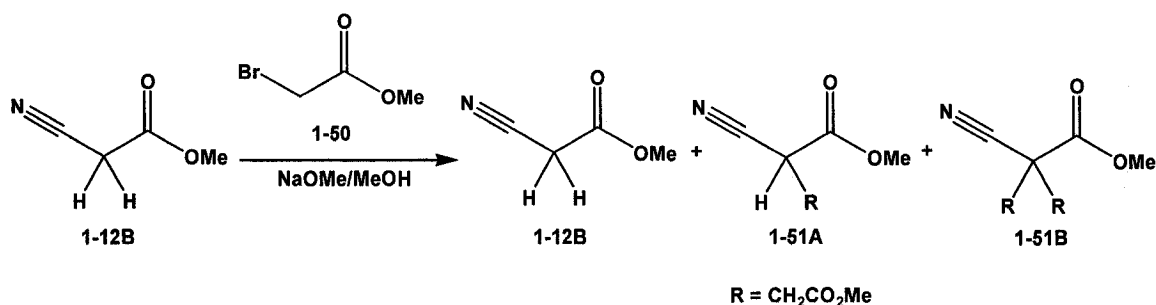
Scheme 1.11 Attempted monoalkylation of methyl cyanoacetate giving a mixture of products.[41]

Another example illustrating the problems involved with achieving monoalkylation of a relatively reactive active methylene compound is provided by the attempted synthesis of diethyl 2,6-dicyanopimelate (1-53) from ethyl cyanoacetate (1-12, 2 mmol/mmol electrophile) and 1,3-dibromopropane (1-52, Scheme 1.12).[43] Instead of the desired product, diethyl 2,6-dicyanopimelate, dialkylation occurred giving ethyl 1-cyanocyclobutanecarboxylate (1-54) as the major product. In a second attempt to promote the disubstitution of dibromopropane (1-52), a large excess of ethyl cyanoacetate was used. However, this adjustment gave only the ethyl cyanoacetate self-condensation product 1-55.

In two of these examples,[40,41] a low yield of the desired monoalkylated product in a mixture of unreacted starting material and side products was obtained. In the third example (Scheme 1.12),[43] the anticipated product was not formed. In each case, dialkylation was circumvented by adopting a modified approach.
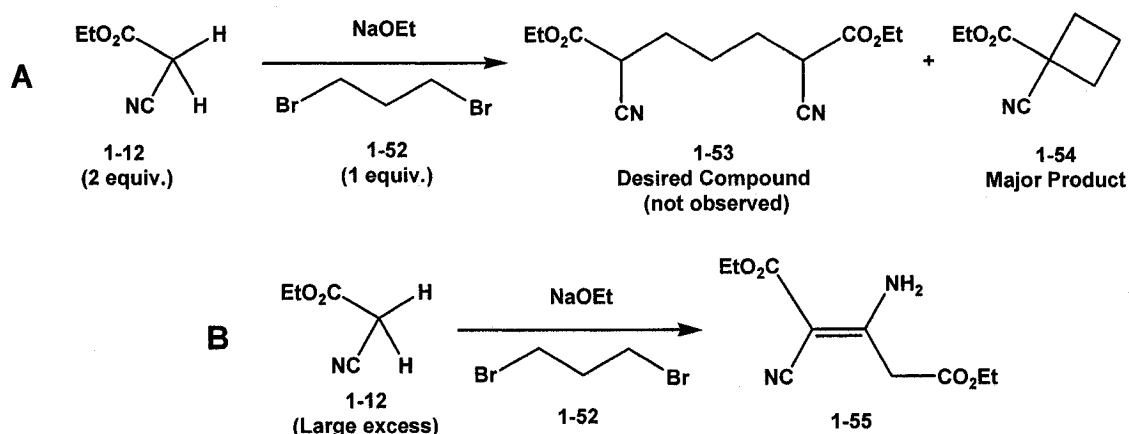
Scheme 1.12 Attempted synthesis of diethyl 2,6-dicyanopimelate (1-53) from ethyl cyanoacetate.[43]

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In the first example, presented in Scheme 1.10,[40] dialkylation was avoided

through the use of an α-chlorocarboxylic acid (**1-47C**), essentially a "pre-monoalkylated"

precursor to monoalkylated ethyl cyanoacetate (Scheme 1.13). Displacement of the

chloride by cyanide ion followed by esterification gave monoalkylated ethyl cyanoacetate

(**1-47A**). The desired dialkylated ethyl cyanoacetate (**1-47B**) was obtained following

alkylation. For the other two cases presented in Scheme 1.11 and Scheme 1.12, blocking

groups were devised to prevent dialkylation. These will be discussed in the next

subsection.

R	R'	Yield (%)
Bu	Et	83
Pr	Bu	22
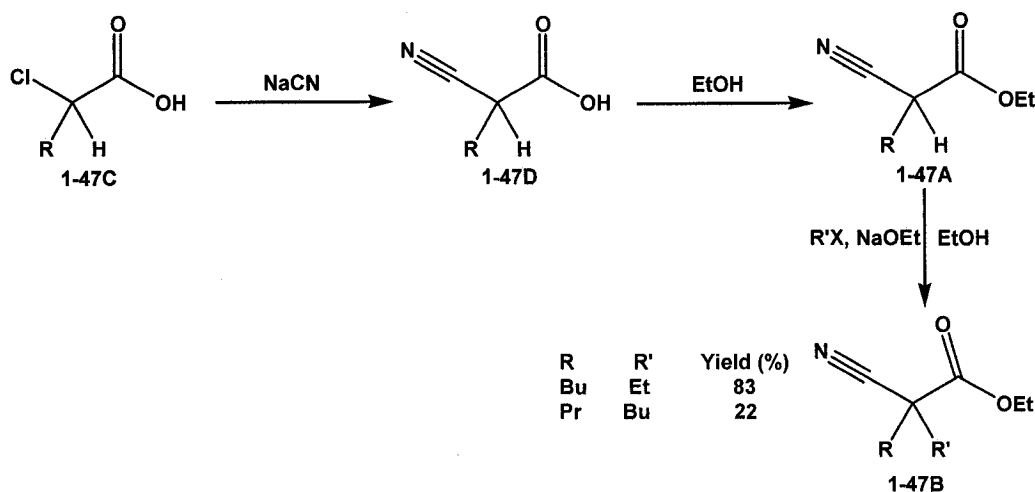
Scheme 1.13 Modified approach for the generation of dialkylated ethyl cyanoacetate with two different alkyl groups.[40]

1.2.2 Use of Blocking Groups to Prevent Dialkylation

The introduction of a protecting group on the α-carbon of active methylene

compounds, replacing the second acidic hydrogen, has proven to be effective in

preventing dialkylation. The blocking group can be removed after the desired alkylation

has occurred. Blocking groups such as allyl[42] and alkylidene[43] have been used

successfully to protect malonate (**1-11**) and cyanoacetate esters (**1-12**) respectively.

Rapoport circumvented multiple alkylations in the alkylation of malonate esters by

preparing a triester (**1-13**).[44]

In order to mask the second acidic hydrogen on ethyl cyanoacetate (**1-12**) and

therefore prevent the dialkylation described in Scheme 1.12, an alkylidene blocking

group was introduced.[43] Alkylation of the alkylidene derivative (**1-56**) of ethyl

cyanoacetate with dibromopropane (Scheme 1.14) successfully displaced both bromo

substituents, yielding the symmetrical product **1-57** (Scheme 1.14). Monoalkylation of

diethyl malonate (**1-11**) and malononitrile (**1-15**) by dibromopropane was also

demonstrated using an alkylidene blocking group. The alkylidene group was removed

under mild conditions by ozonolysis followed by alcoholysis to generate the acyclic

product **1-53** instead of the cyclic product **1-54** from the bifunctional electrophile

(Scheme 1.14).
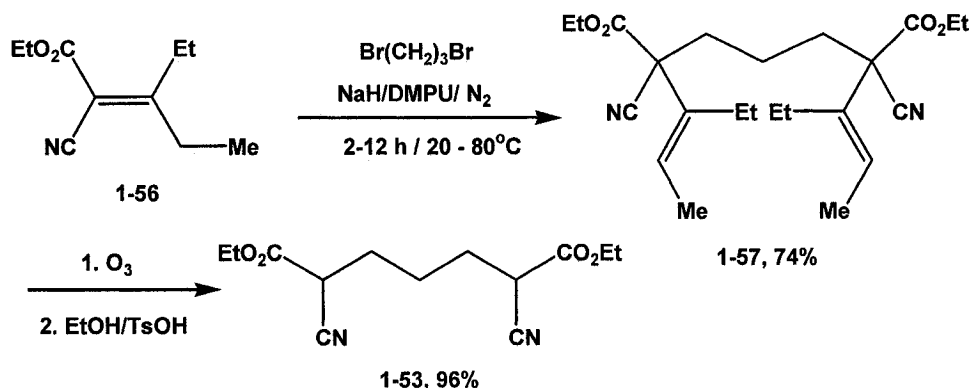
Scheme 1.14 Monoalkylation and deprotection of an alkylidenecyanoacetate (**1-56**) giving the desired diethyl 2,6-dicyanopimelate product.[43]

The allyl blocking group has been used for the monoalkylation reactions of malonate esters (Scheme 1.15).[42] Diethyl allylmalonate (**1-58**) was successfully alkylated with 1,5-diiodopentane. The allyl protecting group was removed by the addition of *i*-PrMgCl and Ti(O-*i*-Pr)$_4$ to the reaction mixture leaving the monoalkylated product **1-60**.

The addition of a third ester group to act as a blocking group has been used to prevent dialkylation reactions of malonate (**1-11**).[45] The sodium salt of triethyl methanetricarboxylate (**1-13A**) was successfully monoalkylated with 1,4-dibromobutane using this approach (Scheme 1.6), producing a malonate ester derivative with a displaceable group on the end of the alkyl chain.[44] After a second alkylation with benzyl pipecolate (**1-62**), hydrogenation and monodecarboxylation generated the desired monosubstituted malonate **1-64**.
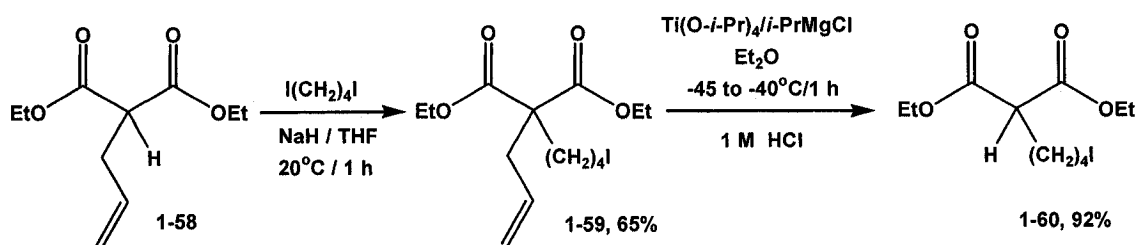
Scheme 1.15 Allyl used as a blocking group to prevent dialkylation of a malonate ester.[42]
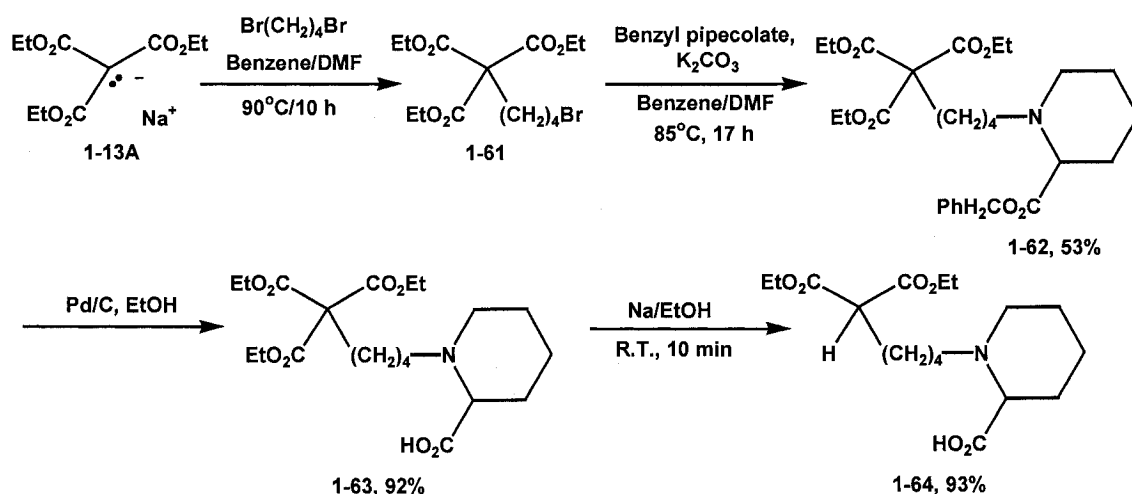
Scheme 1.16 Monoalkylation of sodium triethyl methanetricarboxylate.[44]

Methanetricarboxylic esters are synthesized from the metal salts of malonate esters and alkyl chloroformates.[45] In the previous example,[44] triethyl methanetricarboxylate (1-13) was used as its preformed, non-hygroscopic sodium salt (Scheme 1.17) that was alkylated without the addition of another base (Scheme 1.16).
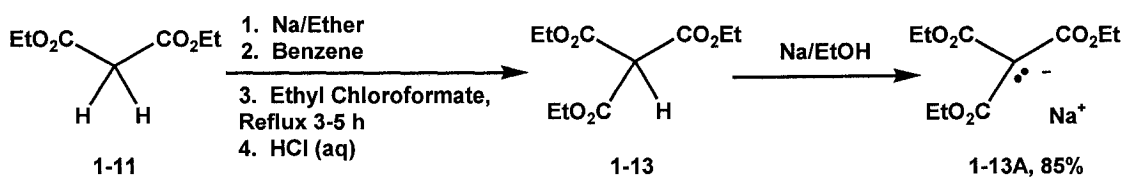
Scheme 1.17 Preparation of sodium triethyl methanetricarboxylate (1-13A) from diethyl malonate.[44]

1.3 Diethyl Cyanomalonate and Its Highly Stabilized Carbanion

As an extension of Rappoport's triester (1-13) alternative to malonate diesters (1-11),[44] the addition of an ester blocking group to ethyl cyanoacetate (1-12) to eliminate possible dialkylation would generate diethyl cyanomalonate (1-1). The small size and

linear shape of the nitrile group minimizes steric congestion allowing the tertiary carbanion of diethyl cyanomalonate (**1-1A**) to be better stabilized than that of the triester (**1-13**). As a result, diethyl cyanomalonate (**1-1**) is a stronger acid ($pK_a = 1.3$)[14] than Rappoport's triester (**1-13**, $pK_a = 7.8$)[13] and would be deprotonated easily using any common strong or weak base.

The potential synthetic utility of the diethyl cyanomalonate anion **1-1A** has been indicated by a few literature reports (summarized in the next subsection) of **1-1A** reacting as a carbon nucleophile under typical laboratory reaction conditions. The minimal steric interactions of the nitrile group most likely contribute to the nucleophilicity of **1-1A**.

The unusual combination of weakly basic and nucleophilic properties in **1-1A** suggests that this carbanion can be used in more neutral media rather than the strongly basic surroundings typically used for carbanion formation. In the alkylation of more basic carbanions, for example, the electrophile is limited to monofunctional alkyl halides to avoid unwanted side reactions. As illustrated in Scheme 1.14, alkylation reactions employing highly functionalized electrophiles containing moderately acidic hydrogens can be synthetically useful. Moreover, the use of metal ion salts of **1-1A** in alkylation reactions has been indicated by literature precedents (Subsection 1.3.2). The use of a salt in an alkylation reaction as shown in Scheme 1.16, for example, would provide conditions in which the weakly basic **1-1A** would be the strongest base present, creating an almost neutral environment for alkylation reactions.

1.3.1 Literature Precedents for the Alkylation of the Diethyl Cyanomalonate Anion

In 1946, the sodium salt of diethyl cyanomalonate (**1-1B**) was alkylated by an equimolar amount of methylgramine methiodide (**1-65**) in water at reflux temperature (Scheme 1.18).[46] The alkylated cyanomalonate **1-66** was treated with aqueous sodium hydroxide to hydrolyze the esters and the nitrile yielding the malonic acid product **1-67** after decarboxylation.

Diethyl cyanooctylcyanomalonate (**1-69**) was synthesized by treating diethyl cyanomalonate (**1-1**) in aqueous base with an equimolar amount of 1-bromooctane (**1-68**) in dimethyl digol (Bis(2-methoxyethyl)ether) (Scheme 1.19).[47] The octylcyanomalonate product **1-69** was isolated by extraction into benzene, and used as a reference compound to verify the structure of the same compound formed by a free-radical addition reaction. In the free-radical reaction (Scheme 1.20),[47,48] a mixture of 1-octene (**1-70**) and the radical initiator diisopropyl peroxydicarbonate was added to diethyl cyanomalonate ester (**1-1**) in hexane. The diethyl octylcyanomalonate product **1-69** was isolated in modest yield by distillation.
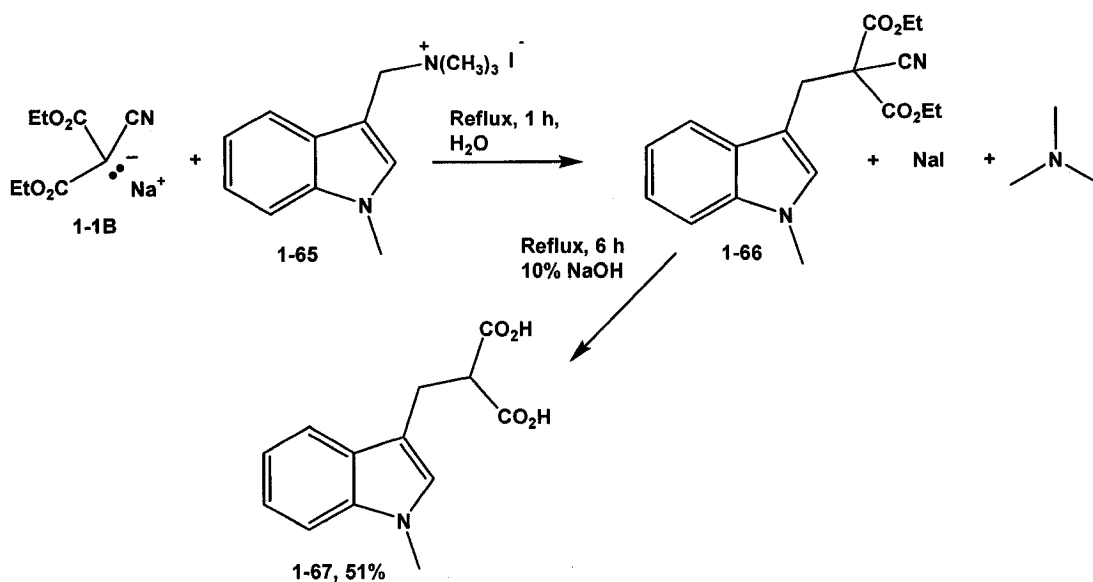
Scheme 1.18 Alkylation of diethyl cyanomalonate by methylgramine methiodide.[46]
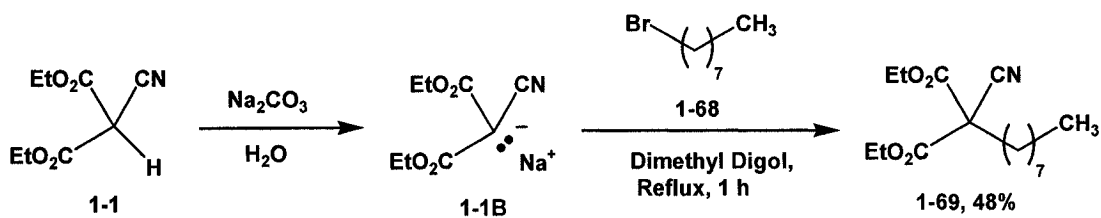
Scheme 1.19 Alkylation of diethyl cyanomalonate by 1-bromooctane.[47]
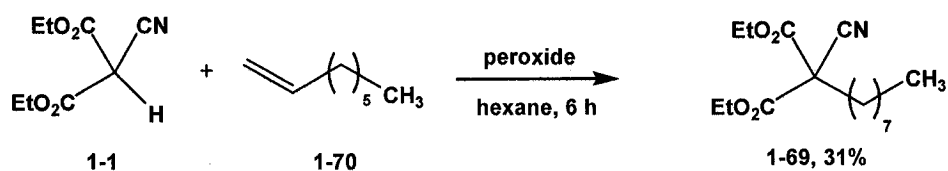
Scheme 1.20 Free radical addition of diethyl cyanomalonate to an olefin.[48]

The alkylation of the sodium salt of methyl *t*-butyl cyanomalonate (**1-71**) with

methyl iodide (Scheme 1.21) has been used as the first step in the synthesis of a β-lactam

ring.[49] The mixed ester was synthesized by reacting *t*-butyl cyanoacetate with sodium

hydride in ether to form the sodium salt which in turn was acylated using methyl

chloroformate. For construction of the β-lactam ring, the nitrile was converted to a

thioamide. Thioamides are often synthesized from nitriles by heating with hydrogen

sulfide in an alcoholic solution containing a catalytic base such as diethylamine (Scheme

1.21).[50] Due to the acidic nature of cyanomalonate, the synthesis of the thioamide was

unsuccessful in basic conditions,[51] but the alkylated cyanomalonate (**1-72**) was easily

converted to its thioamide (**1-73**) under basic conditions.
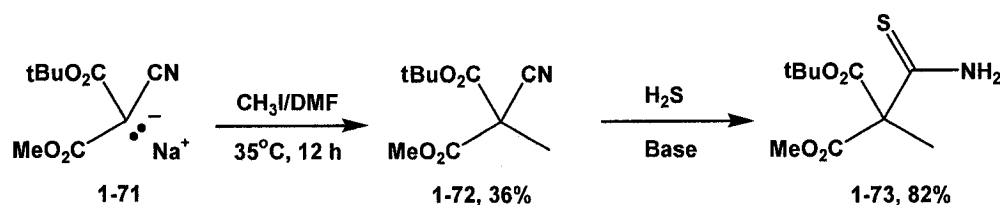
Scheme 1.21 Methylation of methyl *t*-butyl cyanomalonate and conversion to thioamide.[49]

Previously in this laboratory,[41] addition of methyl bromoacetate and potassium

iodide to a mixture of diethyl cyanomalonate (**1-1**) and potassium carbonate in refluxing

acetone (Scheme 1.22) gave ethyl methyl 2-carboethoxy-2-cyanobutanedioate (**1-74**), an

intermediate in the synthesis of 4-iodobutanenitrile.

1. K_2CO_3, Acetone,
Reflux, 15 min,

2. Methyl Bromoacetate, KI,
Reflux, 18 h

EtO   H CO_2Et N   **1-1**

EtO CO_2Me CO_2Et N   **1-74, 97%**
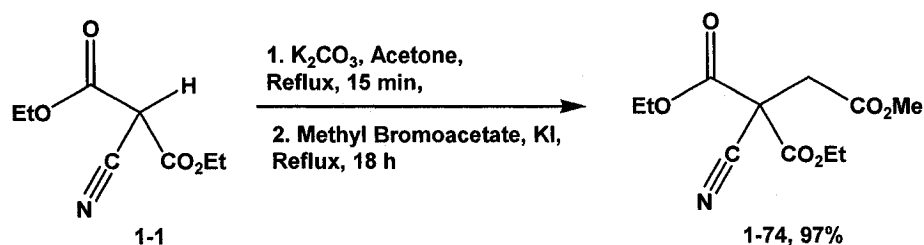
Scheme 1.22 Synthesis of ethyl methyl 2-carboethoxy-2-cyanobutanedioate by alkylation of diethyl cyanomalonate.[41]

Equimolar amounts of diethyl cyanomalonate and a fullerene derivative, $C_{60}F_{18}$ were stirred in toluene with DBU at room temperature for 10 min.[52] HPLC analysis of the reaction mixture gave three peaks, which were identified by mass spectrometry as $C_{60}F_{18}$, $C_{60}F_{17}[CCN(CO_2Et)_2]$ (25%) and $C_{60}F_{16}[CCN(CO_2Et)_2]_2$ (13%). Presumably, the mono and bis addition products were formed by displacement of fluoride by the diethyl cyanomalonate anion.

Michael addition was demonstrated for dimethyl cyanomalonate (**1-75**) and atropaldehyde (**1-76**) (Scheme 1.23).[53] The dimethyl cyanomalonate anion, generated *in situ* using triethylamine, attacked the terminal alkene carbon atom of atropaldehyde (**1-76**). This produced an enolate anion, which was trapped by trimethylchlorosilane to give the product 2,2-dicarbomethoxy-4-phenyl-5-trimethylsilyloxy-4-pentenonitrile (**1-77**).

MeO₂C   CN
       ⟩⟨
MeO₂C   H

1-75
(100 mmol)

+

1-76 structure (aldehyde with phenyl substituent)

1-76
(9.8 mmol)

N(CH₂CH₃)₃/ Si(CH₃)₃Cl
————————————————→
CCl₄, 40-47°C, 40 min

product structure 1-77

1-77, 99%
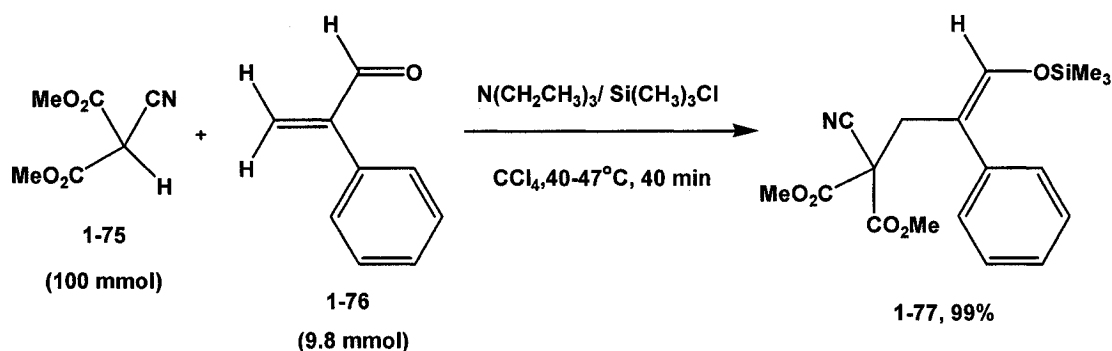
Scheme 1.23 Michael addition with dimethyl cyanomalonate.[53]

The diethyl cyanomalonate anion (**1-1A**) is also reactive towards carbon disulfide (**1-78**) and phenylisothiocyanate (**1-80**).[54] In a one-pot reaction (Scheme 1.24), the diethyl cyanomalonate anion initially reacted with carbon disulfide forming the dithiolate anion (**1-79**, not isolated) which was trapped by a series of electrophiles giving **1-80** which, in turn, underwent cyclization to give a variety of heterocyclic products (e.g., **1-81A** and **1-81B**). Similarly, cyclic products were obtained when diethyl cyanomalonate, phenylisothiocyanate and a series of electrophiles were subjected to the same reaction conditions.
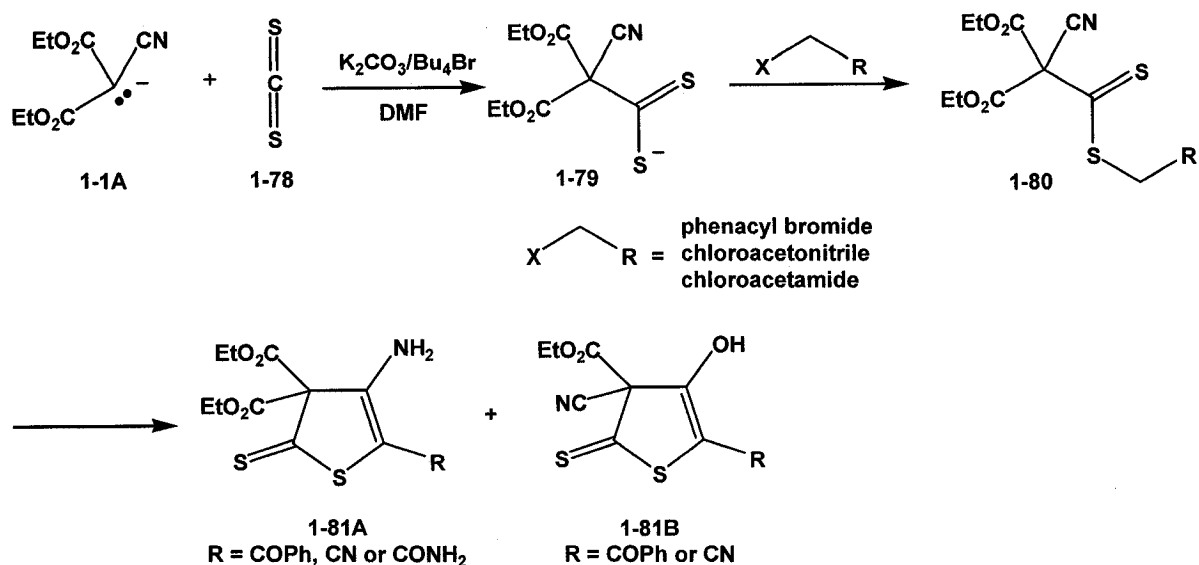
Scheme 1.24 Reaction of diethyl cyanomalonate anion with carbon disulfide.[54]

The examples presented in this subsection provide several precedents for the formation of the diethyl cyanomalonate anion (**1-1A**) in reaction mixtures using a variety of bases. In two examples, however, the diethyl cyanomalonate anion was introduced into reaction mixtures as its preformed sodium salt.

1.3.2 Literature Syntheses of Cyanomalonate Ester Salts

The preparations of several salts of cyanomalonate have been reported in the literature. The sodium, calcium and lead salts of cyanomalonate were synthesized by Haller from the appropriate metal carbonate in ethanol.[55] The sodium salt of diethyl cyanomalonate (**1-1B**) was prepared by titrating diethyl cyanomalonate (**1-1**) in a 50% aqueous solution of dimethoxyethane with sodium hydroxide,[56] whereas Witherell[41] added sodium ethoxide to a solution of diethyl cyanomalonate in ethanol. Sodium diethyl

cyanomalonate also has been prepared from phenyl cyanate and sodium diethyl malonate.[57]

The sodium salts of methyl *tert*-butyl cyanomalonate and dimethyl cyanomalonate were each isolated after the reaction of methyl chloroformate with sodium *tert*-butyl cyanoacetate and sodium methyl cyanoacetate, respectively.[49]

The potassium and diisopropylamine salts of diethyl cyanomalonate (**1-1**) and the potassium, diisopropylamine, triethylamine and tert-butylamine salts of dimethyl cyanomalonate were formed upon cleaving 3,7-di(m)ethoxy-4H,8H-benzo[1,2-c:4,5-c']diisoxazole-4,8-dione with the appropriate base.[58]

The potassium, methylammonium and ammonium salts of diethyl cyanomalonate (**1-1**) were formed via a sulfur extrusion reaction.[59] The potassium salt formed by treating diethyl thiocyanatomalonate with an equimolar amount of potassium acetate was isolated in 70% yield. Treatment of diethyl cyanomalonate with an aqueous ammonia solution produced the ammonium salt.[59] Similarly, addition of triethylamine or pyridine to diethyl cyanomalonate led to the formation of the triethylammonium and pyridinium salts.[41]

Witherell[41] also prepared sodium and potassium salts by adding alkali metal to dimethyl cyanomalonate (**1-75**) dissolved in methanol or 2-butanol. Tetrabutylammonium dimethyl cyanomalonate was prepared by mixing the ester with a solution of tetrabutylammonium hydroxide, and addition of triethylamine to dimethyl cyanomalonate gave the triethylammonium salt.

The salts of cyanomalonate esters are reported as crystalline solids with melting points greater than 250°C for alkali metal cations and less than 200°C for the ammonium or pyridinium counter ions. The salts are described as stable, nonhygroscopic compounds that are soluble in a range of organic solvents including polar, aprotic solvents that are suitable for alkylation reactions.

1.4 Potential Synthetic Applications of Diethyl Cyanomalonate

In the previous section, the advantages of using a weakly basic tertiary carbanion were presented along with literature precedents to support the diethyl cyanomalonate anion (**1-1A**) as a synthetically useful nucleophile, particularly for reactions under neutral conditions.

As a weakly basic synthetic equivalent of the anions of ethyl cyanoacetate (**1-12A**) and acetonitrile (**1-14A**) (Figure 1.2), the diethyl cyanomalonate anion (**1-1A**) would be a most useful synthetic precursor of a nitrogen-containing substructure within the synthetic target. Accordingly, the diethyl cyanomalonate anion (**1-1A**) is a potential source of "C_4N" (**1-82**), "C_3N" (**1-83**), or "C_2N" (**1-84**) subunits (Figure 1.3), depending on whether the ester functional groups are retained or removed after bond formation. The dotted line in each subunit indicates the position at which bond formation occurs to join the subunit to another part of the target molecule.

Diethyl Cyanomalonate
(1-1A)
$pK_a = 1.3$[14]

Ethyl Cyanoacetate
(1-12A)
$pK_a = 9$[11]

Acetonitrile
(1-14A)
$pK_a = 25$[10]
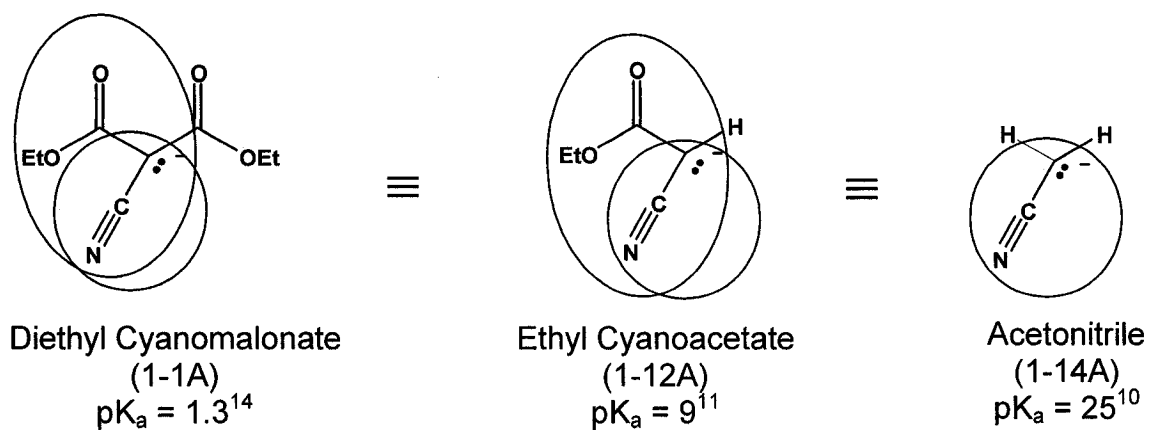
Figure 1.2 Diethyl Cyanomalonate anion as a synthon of ethyl cyanoacetate and acetonitrile.
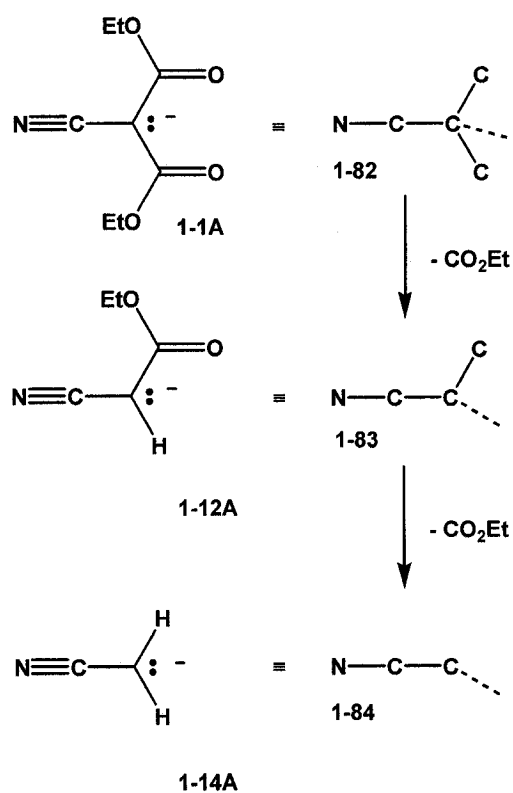
Figure 1.3 Relationship between the structural fragments derived from the anions of diethyl cyanomalonate, ethyl cyanoacetate and acetonitrile.

27

The close proximity of these functional groups in **1-1A** would facilitate the removal of the ester groups after alkylation by hydrolysis/decarboxylation or by Krapcho decarboethoxylation[60] under neutral conditions. In each case, bond cleavage is assisted by stabilization of negative charge on the α-carbon by the remaining functional group(s).

In addition to their role in stabilizing charge and blocking dialkylation, the functional groups in the diethyl cyanomalonate anion **(1-1A)** are available for transformation into a number of other functional groups. As indicated in the previous paragraph, an ester may be hydrolyzed to a carboxylic acid or removed altogether. Alternatively, an ester could be selectively reduced to an alcohol.[61] On the other hand, the nitrile group offers a broader range of possible transformations. Hydrolysis[62] to an amide or reduction[63,64,65] to an amine would retain the nitrogen, whereas reduction to an aldehyde or hydrolysis to a carboxylic acid would remove the nitrogen provided in the original nitrile, reducing the overall "atom economy" of the synthesis.

1.4.1 Structural Relationships Between Amino Acids and Diethyl Cyanomalonate

The structural relationship between β- and ω-amino acids **(1-85, 1-87)** and diethyl cyanomalonate **(1-1)** is highlighted in bold within the retrosynthetic analysis presented in Scheme 1.25. The proposed synthetic steps involve alkylation of the cyanomalonate anion **(1-1A)**, removal of one or both ester groups and nitrile reduction.

In the proposed synthesis of β-amino acids **(1-85)**, diethyl cyanomalonate **(1-1)** provides a C_3N subunit **(1-83)** and the substituent R derives from the electrophile used in the alkylation step. Similarly, diethyl cyanomalonate can provide a C_2N subunit **(1-84)** in the synthesis of ω-amino acids **(1-87)**. However, neither of the ester groups in diethyl

cyanomalonate are correctly positioned to become the carboxyl group of the ω-amino acid and this must be provided by the electrophile.

In the synthetic approach outlined in Scheme 1.25, the β-amino acid (**1-85**) core structure (in bold) is derived from cyanomalonate (**1-1**) allowing structural diversity to be introduced by varying the electrophile in the alkylation step. For the ω-amino acids (**1-87**), the $^+H_3NCH_2CH_2$ fragment (in bold) is derived from diethyl cyanomalonate (**1-1**), while the carboxyl group is supplied by the electrophile. By varying the electrophile, this route could provide a homologous series of ω-amino acids, including 4-aminobutyric acid (GABA, **1-87**, n = 1) as the shortest chain length.
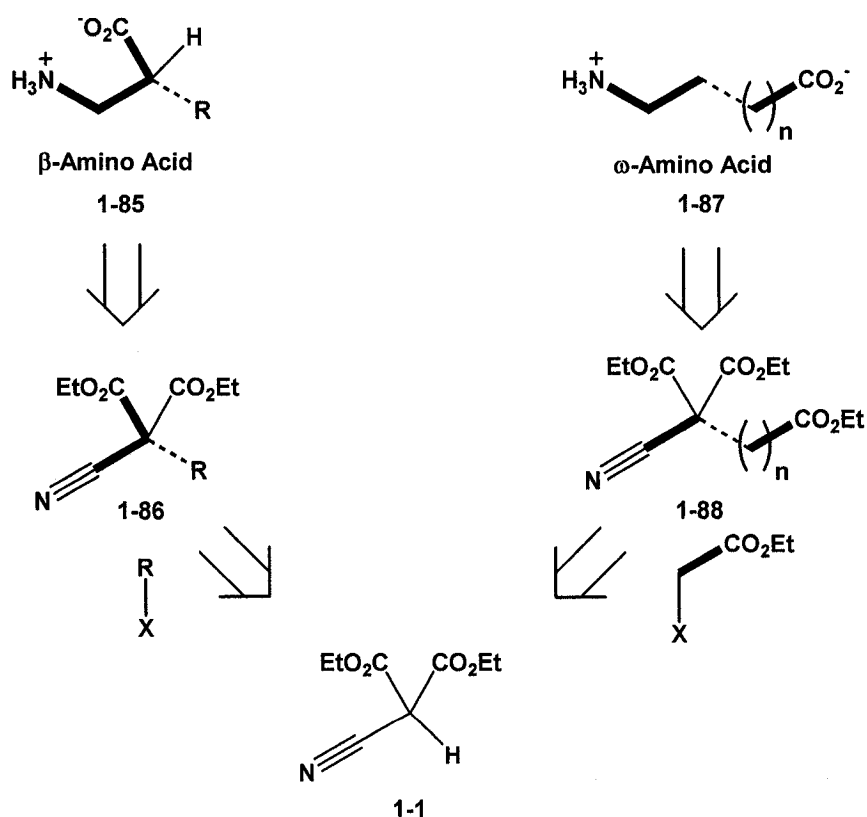
Scheme 1.25 Retrosynthetic analysis based on common structural subunits in amino acids and diethyl cyanomalonate (R = alkyl and X = leaving group).

The synthetic route outlined in Scheme 1.25 also can be adapted to provide

dialkylated β-amino acids (**1-85A**) and ω-1 substituted ω-amino acids (**1-87A**) as

illustrated in Scheme 1.26. In either case, following the initial alkylation of diethyl

cyanomalonate (**1-1**), removal of one of the geminal diesters, exposes the α-carbon for a

second alkylation which would generate a dialkylated ethyl cyanoacetate with two

different alkyl substituents (**1-47B, 1-90**). Note that each alkyl substituent is introduced

at a step in which only monoalkylation can take place. As a result, the problems

associated with dialkylation[40] (e.g., Scheme 1.10-1.12A) can be avoided.

While α-amino acids (**1-90**) are the most abundant amino acids in nature because

they form the building blocks of proteins, both β- and ω-amino acids (**1-85, 1-87**) have

essential roles. For example, GABA is an important neurotransmitter in the mammalian

nervous system[66] and β-alanine (**1-85**, R = H) is a structural component of coenzyme A.[67]

The β- and ω-amino acids (**1-85, 1-87**) differ from each other and α-amino acids

(**1-90**) in the position of the amino group relative to the carboxylate group. The better

known α-amino acids have both the amino and carboxylate functional groups bonded

directly to the same (α) carbon atom (Figure 1.4). In β-amino acids (**1-85**), the amino

and carboxyl groups are bonded to adjacent carbon atoms, and the side chain can be

attached to either of these α- and β-carbons, creating two structural subsets (Figure 1.4).

The synthesis of β-substituted β-amino acids (β^3-amino acids, **1-85B**) has been

investigated extensively, whereas only a few syntheses of α-substituted β-amino acids

(β^2-amino acids, **1-85**) have been accomplished.[68,69] The structural relationship of the

latter to diethyl cyanomalonate (Figure 1.4), however, suggests a novel synthetic route

(Scheme 1.25).
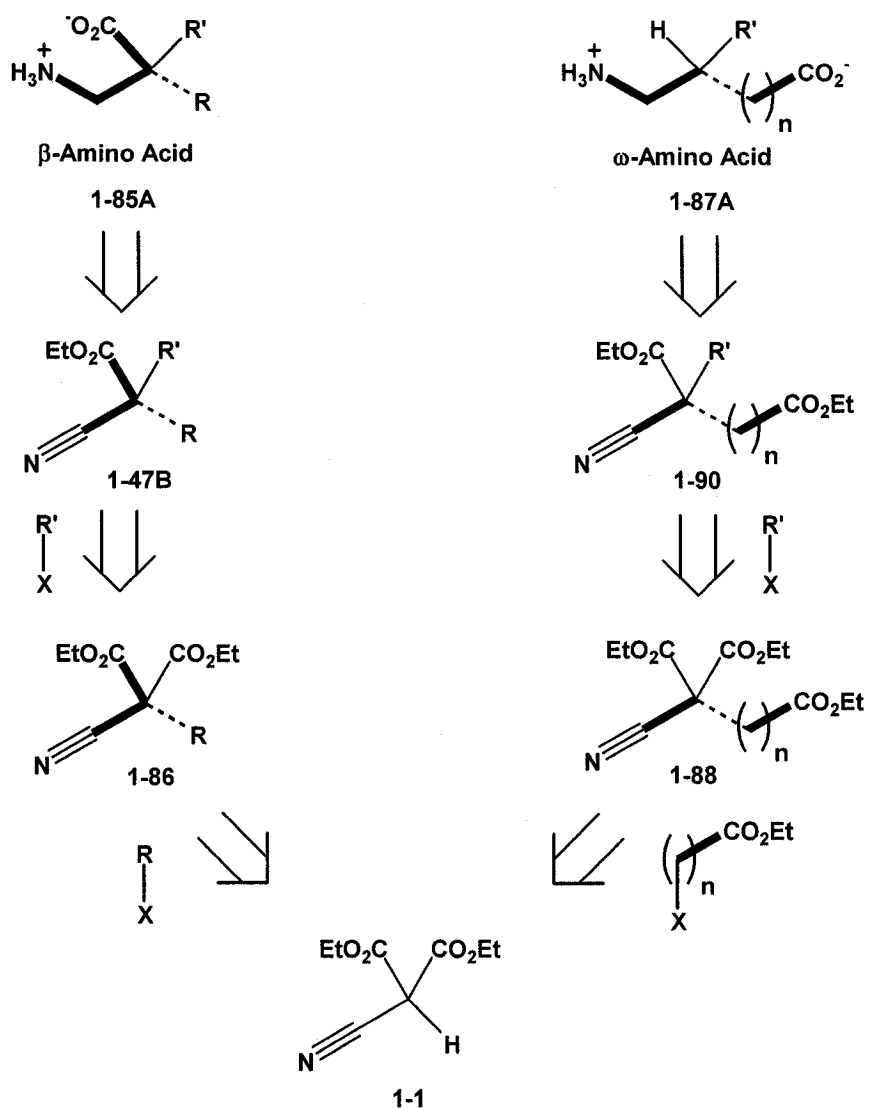
Scheme 1.26 Retrosynthetic analysis of disubstituted β-amino acids and ω-1 substituted ω-amino acids.
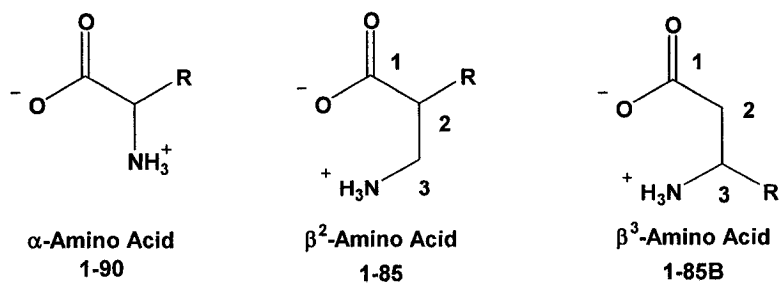
Figure 1.4 Structural comparison and nomenclature[68] of α- and β-amino acids.

In nature, β-amino acids (**1-85**) are components in several natural products that have antibiotic, antifungal and cytotoxic properties.[69] For example, the β-lactam ring structure present in β-lactam antibiotics, such as penicillin (**1-91**) and cephalosporin (**1-92**), is the most recognizable, medicinally important, non-peptide, β-amino acid substructure (Figure 1.5), and probably one of the most studied β-amino acids, (2R,3S)-N-benzoyl-3-phenylisoserine, is a component of the chemotherapeutic drug paclitaxel (**1-93**).[69]

1-91

Penicillin substrucure

1-92

Cephalosporin substrucure

1-93
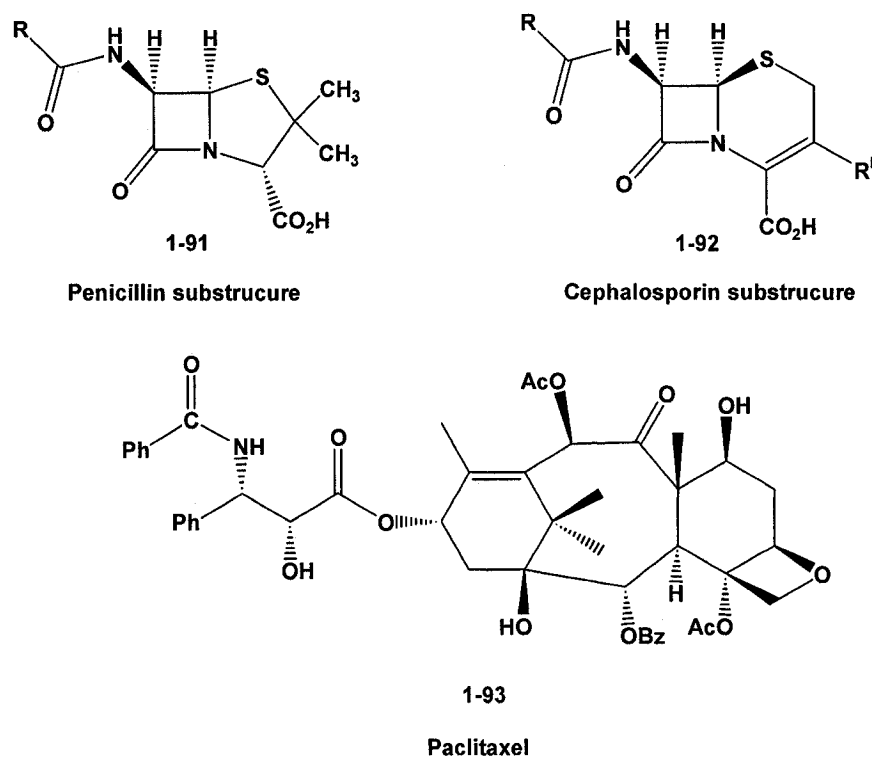
Paclitaxel

Figure 1.5 β-Lactam antibiotics and Paclitaxel (a chemotherapeutic drug) containing a β-amino acid substructure.

More specifically, β^2-amino acids exist in nature in several forms. In mammals, the free amino acid (R)-β-aminoisobutyric acid[68] is a metabolite of thymine and its enantiomer, (S)-β-aminoisobutyric acid, is a metabolite of valine (**1-95**, Figure 1.6). β-Aminoisobutyric acid has been found in invertebrates and in plants. The dipeptide γ-glutamyl-β-aminoisobutyric acid (**1-96**) has been found in plants and in bovine brain (Figure 1.6). β^2-Amino acids also have been found as structural components of a number of nonpeptidic natural products, such as the well known ergot alkaloid lysergic acid (**1-97**).[68]

1-95
β-Aminoisobutyric acid

1-96
γ-Glutamyl-β-isobutyric acid

1-97
Lysergic Acid
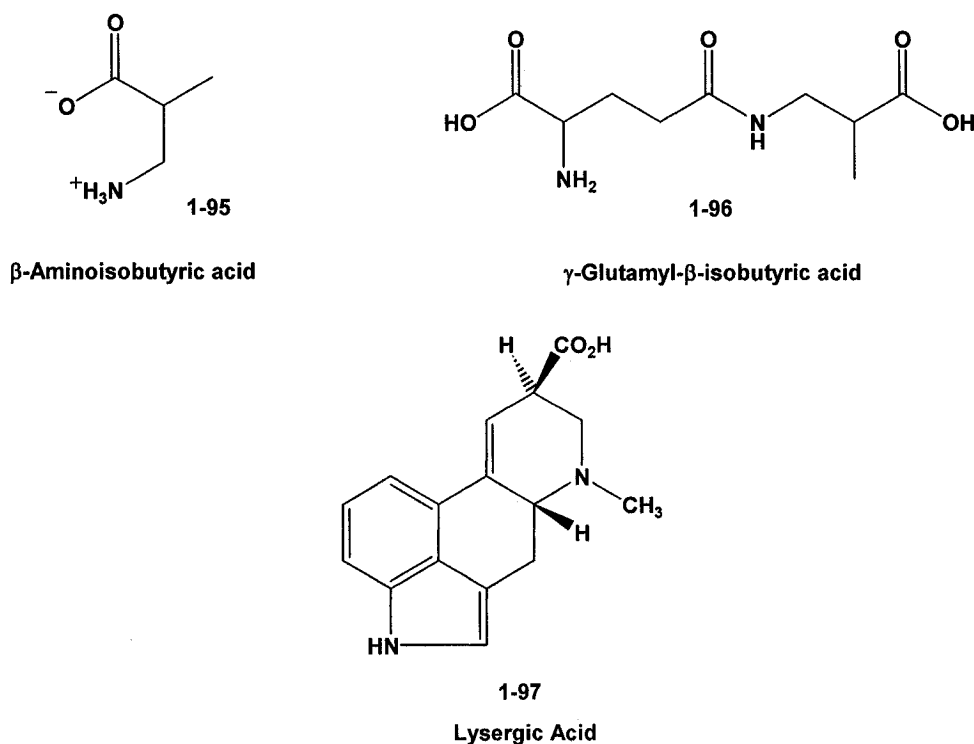
Figure 1.6 Examples of β^2-amino acids found in nature.[68]

1.5 Thesis Goals

The main goal of this thesis is to demonstrate the synthetic utility of the highly stabilized carbanion of diethyl cyanomalonate (**1-1A**). As a highly stabilized tertiary carbanion, **1-1A** offers the advantages of only undergoing monoalkylation reactions and low basicity. A few literature reports support the synthetic potential of the diethyl cyanomalonate anion but, to demonstrate this potential, the reactivity of the diethyl cyanomalonate anion toward alkylation by a range of electrophiles under neutral conditions must be explored (Chapter 2). The common structural subunits in the highly functionalized, alkylated diethyl cyanomalonate esters and β- and ω-amino acids indicate a substrate-product relationship to be utilized as a novel synthesis of amino acids. Appropriate targets are an unreported deuterium isotopomer of GABA (Chapter 3), an ω-amino acid, and a series of dicarboxylic β-amino acids as potential enzyme inhibitors and substrates for mass spectrometry studies (Chapter 5).

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Chapter 2: Preparation and Alkylation of Diethyl Cyanomalonate

2.1 Introduction

The literature review presented in Chapter 1 provided a few examples[1,2,3,4,5,6,7] of synthetic reactions in which the diethyl cyanomalonate anion was utilized as a nucleophile in alkylation reactions. Unlike the frequently used carbanion of ethyl cyanoacetate, the tertiary carbanion of diethyl cyanomalonate can only undergo monoalkylation, providing a complementary tool for the synthetic chemist.

The weakly basic nature of the diethyl cyanomalonate anion ($pK_a = 1.3$)[8] is unusual for a synthetically useful nucleophile, converting the usually basic medium for alkylation to a more neutral environment and removing limitations on the structural complexity of electrophiles.

In this chapter, the scope of the reactivity of the diethyl cyanomalonate anion in alkylation reactions is explored by varying the counter ion, solvent and the structure of the electrophile. These alkylated diethyl cyanomalonate products are needed as precursors to synthetic targets described later in this thesis. However, in order to investigate its synthetic utility, diethyl cyanomalonate must first be synthesized.

2.1.1 Literature Syntheses of Cyanomalonate Esters

While cyanomalonate esters are not available from commercial sources, several syntheses have been described in the literature. The first recorded synthesis of diethyl cyanomalonate (**1-1**) was in 1882 by Haller, who mixed the sodium salt of diethyl malonate (**1-11B**) with cyanogen chloride in ethanol (Scheme 2.1).[9]
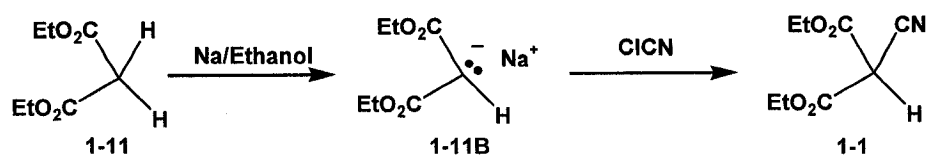
Scheme 2.1 Haller's initial synthesis of diethyl cyanomalonate.[9]

A second synthesis of diethyl cyanomalonate (**1-1**) described by Haller in 1887

involved the addition of ethyl chloroformate to an ethanolic solution of sodium diethyl

cyanoacetate.[10] The reddish colour of the oil after work-up was attributed to traces of

iron. A subsequent investigation has shown that diethyl cyanomalonate forms soluble,

stable complexes with iron in most organic solvents and is useful for the

spectrophotometric determination of iron(III) salts.[8] Cadogan used this method (1:1

sodium ethyl cyanoacetate: ethyl chloroformate) for the synthesis of diethyl

cyanomalonate (**1-1**) and attained a 44% yield (Scheme 2.2).[2] Diethyl cyanomalonate (**1-**

1) also has been prepared from ethyl cyanoacetate (**1-12**) and ethyl chloroformate using

reduced azobenzene as an electrogenerated base.[11]
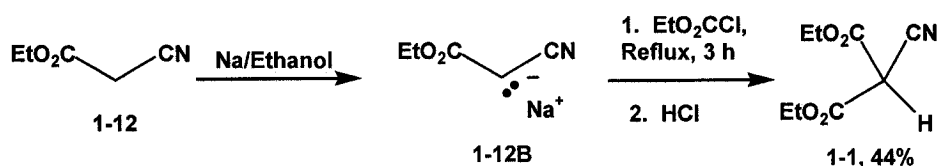
Scheme 2.2 Synthesis of cyanomalonate from ethyl cyanoacetate and ethyl
chloroformate.[2]

After a 1:1 mixture of ethyl cyanoacetate and ethyl chloroformate was heated at

50°C with potassium carbonate and tetrabutylammonium bromide in benzene for 4 h,[7]

the potassium salt of diethyl cyanomalonate may have been isolated instead of diethyl

cyanomalonate itself, as the reported melting point of 305°C is similar to the melting

point of the potassium diethyl cyanomalonate reported earlier[13] (286-287°C) and also in

this thesis (287- 289°C).

Cyanomalonate was used to assist the identification of ethyl 5-ethoxyisoxazole-4-

carboxylate (2-1).[12] When this compound was refluxed with sodium ethoxide, diethyl

cyanomalonate (1-1) was formed by a characteristic isoxazole bond cleavage (Scheme

2.3).
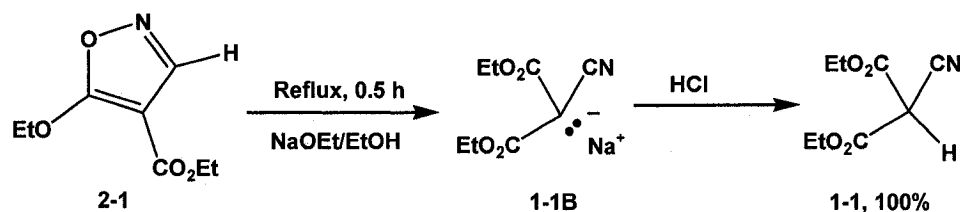
Scheme 2.3 Diethyl cyanomalonate from ethyl 5-ethoxyisoxazole-4-carboxylate.[12]

Diethyl cyanomalonate also was synthesized from diethyl thiocyanatomalonate

(2-2) by treatment with an equimolar amount of base (Scheme 2.4). This reaction

described by Kambe and Hayashi[13] involved migration of the cyano group from the

sulfur to the α-carbanion with extrusion of elemental sulfur. Three different bases were

used: potassium acetate, aqueous methylamine and aqueous ammonia. Each salt was

treated with dilute hydrochloric acid to give diethyl cyanomalonate (1-1).

41
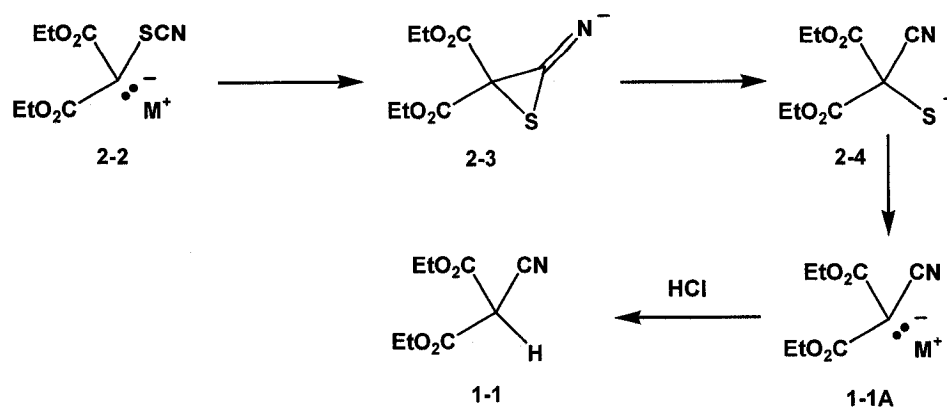
Scheme 2.4 Synthesis of diethyl cyanomalonate by sulfur extrusion.[13]

2.2 Preparation and Characterization of Diethyl Cyanomalonate and its Salts

2.2.1 Preparation of Diethyl Cyanomalonate

Using Witherell's[4] procedure adapted from that of Cadogan,[2] diethyl

cyanomalonate (**1-1**) was prepared by refluxing a mixture of ethyl cyanoacetate (**1-12**, 27

mmol), ethyl chloroformate (**2-5**, 103 mmol) and anhydrous potassium carbonate (87

mmol) for 6 h in acetone (30 mL) (Scheme 2.5). After acidification and workup of the

reaction mixture, diethyl cyanomalonate (**1-1**), a crude orange oil, was obtained

reproducibly in high yield (90-95%). The use of excess base in this method avoided

protonation of the ethyl cyanoacetate anion (**1-1A**) by diethyl cyanomalonate, which may

have contributed to the low yields (44%) when Cadogan[2] used a 1:1 mixture of sodium

ethyl cyanoacetate and ethyl chloroformate. However, distillation of the crude product

often led to only a 60-65% recovery.
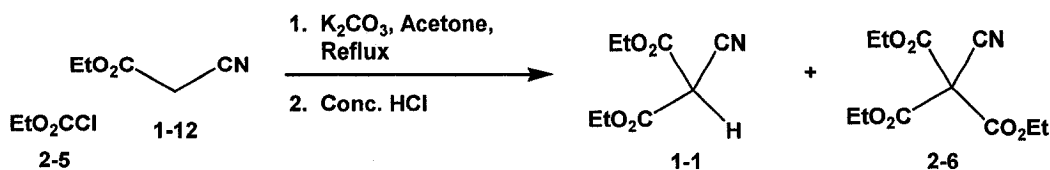
Scheme 2.5 Reaction of ethyl cyanoacetate and ethyl chloroformate.

When the reaction was carried out at a larger scale (54 mmol), the isolated oil often contained of up to 40% ethyl cyanoacetate (**1-12**), which was easily identified by a strong signal at δ_H 3.52 in the ^{1}H NMR spectrum. Initially, isolation of unreacted ethyl cyanoacetate was attributed to the presence of water in the reaction mixture, which would quench the reaction by protonation of the ethyl cyanoacetate anion (pK_a = 9).[14]

In order to reduce the amount of water in the reaction mixture, the acetone was stirred overnight with anhydrous potassium carbonate and filtered prior to use. This drying procedure virtually eliminated the presence of ethyl cyanoacetate (**1-12**) signals in the ^{1}H NMR spectrum of the isolated product. However, the signal at δ_H 4.59 in the ^{1}H NMR spectrum of the product, corresponding to the α-proton of diethyl cyanomalonate (**1-1**), had decreased in intensity, indicating that diethyl cyanomalonate was not the only product.

Under these reaction conditions, ethyl chloroformate (**2-5**) may have functioned not only as an acylating agent, but also as a drying agent, reacting with the water present initially in the acetone. The reaction of ethyl chloroformate with water would give ethanol, carbon dioxide and HCl, none of which would be detected after workup. However, Witherell also reported that the yield of diethyl cyanomalonate (**1-1**) depended on the amount of ethyl chloroformate (**2-5**) used in the reaction,[4] and concluded that 3.8

mmol of ethyl chloroformate per mmol of ethyl cyanoacetate (**1-12**) were required to obtain diethyl cyanomalonate in good yield.[4] If less than 3.8 mmol of ethyl chloroformate per mmol of ethyl cyanoacetate were used, a significant amount of unreacted ethyl cyanoacetate was observed as a singlet at δ_H 3.52 in the ^{1}H NMR spectrum of the product. When larger quantities of ethyl chloroformate were used by Witherell (e.g. 5.6 mmol),[4] a second product was isolated and identified by NMR spectroscopy as diethyl 2-cyano-2-carboxyethylpropanedioate (**2-6**).

Therefore, by drying the acetone prior to use, the water which would have otherwise reacted with ethyl chloroformate (**2-5**) was removed, thus leaving an excess of ethyl chloroformate present in the reaction mixture to react with the diethyl cyanomalonate anion (**1-1A**) giving the triacylated species, diethyl 2-cyano-2-carboxyethylpropanedioate (**2-6**).

To confirm the formation of the triester, diethyl 2-cyano-2-carboxyethylpropanedioate (**2-6**) was synthesized by refluxing a mixture of ethyl cyanoacetate, potassium carbonate and ethyl chloroformate (10 mmol/mmol of ethyl cyanoacetate) in anhydrous acetone (Scheme 2.5). Residual diethyl cyanomalonate was easily removed by a bicarbonate wash of an ethereal solution of the product. The signals in the ^{1}H and ^{13}C NMR spectra of diethyl 2-cyano-2-carboxyethylpropanedioate were the same as those observed by Witherell and to those of a side product observed when the acetone had been dried overnight. EI-MS revealed a molecular ion at $m/z = 257$, corresponding to $C_{11}H_{15}NO_6$, the molecular formula expected for diethyl 2-cyano-2-carboxyethylpropanedioate (**2-6**). The same compound was also isolated by distillation as the high boiling fraction (b.p. >130°C) from previous diethyl cyanomalonate syntheses

which used dried acetone and 3.8 mmol of ethyl chloroformate per mmol of ethyl cyanoacetate.

In order to minimize diethyl 2-cyano-2-carboxyethylpropanedioate (**2-6**) or ethyl cyanoacetate (**1-12**) in the isolated diethyl cyanomalonate product (**1-1**), the amount of ethyl chloroformate (**2-5**) was reduced to two equivalents, the acetone was dried over potassium carbonate and the workup procedure was also modified. A dichloromethane extraction of the basic reaction mixture was included to remove neutral compounds. Dichloromethane was chosen as the extraction solvent because the potassium salt of diethyl cyanomalonate was found to be highly insoluble in this solvent. This extraction step also removed a significant amount of yellow colour, which formed during the course of the reaction, from the aqueous layer. Together, the modified reaction conditions and workup procedure yielded diethyl cyanomalonate (**1-1**) cleanly and with a reproducible high yield: 89% (crude), and 91% recovery upon distillation.

Two other factors were important to attain a high yield of diethyl cyanomalonate. The reaction mixture had to be vigorously and continuously stirred to ensure thorough mixing of the reagents. If the stirring had stopped, a large amount of unreacted ethyl cyanoacetate was detected in ^{1}H NMR spectrum of the product. The use of a magnetic stir bar for agitation limited the scale of the reaction to about 60 mmol. The second factor involved the consistency of the potassium carbonate. The reaction proceeded well (> 85% yield), if the potassium carbonate was very powdery, but poorer yields (< 60%) resulted when granular potassium carbonate was used.

2.2.2 Preparation of Diethyl Cyanomalonate Salts

Titration of the cyanomalonate ester with hydroxide ion in methanol or ethanol and evaporation of the solvent yielded the corresponding salts as stable, non-hygroscopic solids that could be stored at room temperature. The formation of stable, non-hygroscopic salts has also been reported for the related methanetricarboxylic acid esters.[15] The salts were readily purified by recrystallization. The alkali metal salts had melting points above 200°C, but all salts (except Cs) had significant solubilities in a range of organic solvents (Table 2.1), making them potential substrates for alkylation reactions in organic solvents (Section 2.3).

Table 2.1 Solubility of diethyl cyanomalonate salts in polar, aprotic solvents.

Cation	Solubility (mmol/mL) in Each Solvent			
	Acetone	Acetonitrile	DMF	DMSO
Li$^+$ (1-1C)	0.07	0.10	0.95	1.21
Na$^+$(1-1B)	0.23	0.30	0.85	0.70
K$^+$(1-1D)	0.32	0.14	1.59	0.41
Cs$^+$ (1-1E)	0.03	0.08	1.07	0.95
Me$_4$N$^+$ (1-1F)	0.20	0.97	0.84	0.54
Bu$_4$N$^+$ (1-1G)	1.37	1.56	0.92	1.20

2.2.3 Characterization of Diethyl Cyanomalonate

The ^{1}H NMR spectrum of distilled diethyl cyanomalonate (1-1, Figure 2.1A), consisted of six signals, three major and three minor. The major signals included the expected ester triplet (δ_H 1.34) and quartet (δ_H 4.34) and the CH singlet at δ_H 4.59. Minor signals, at approximately 10% intensity of the major signals, included a triplet at

δ_H 1.40, a quartet at δ_H 4.42 and a very broad signal at δ_H 13.2. The ^{13}C NMR spectrum

consisted of five major signals at δ_C 13.5, 44.5, 63.8, 111.5 and 160.6 and five minor

signals (10%) at δ_C 13.9, 60.3, 64.2, 113.6, 176.0 (Figure 2.1B).
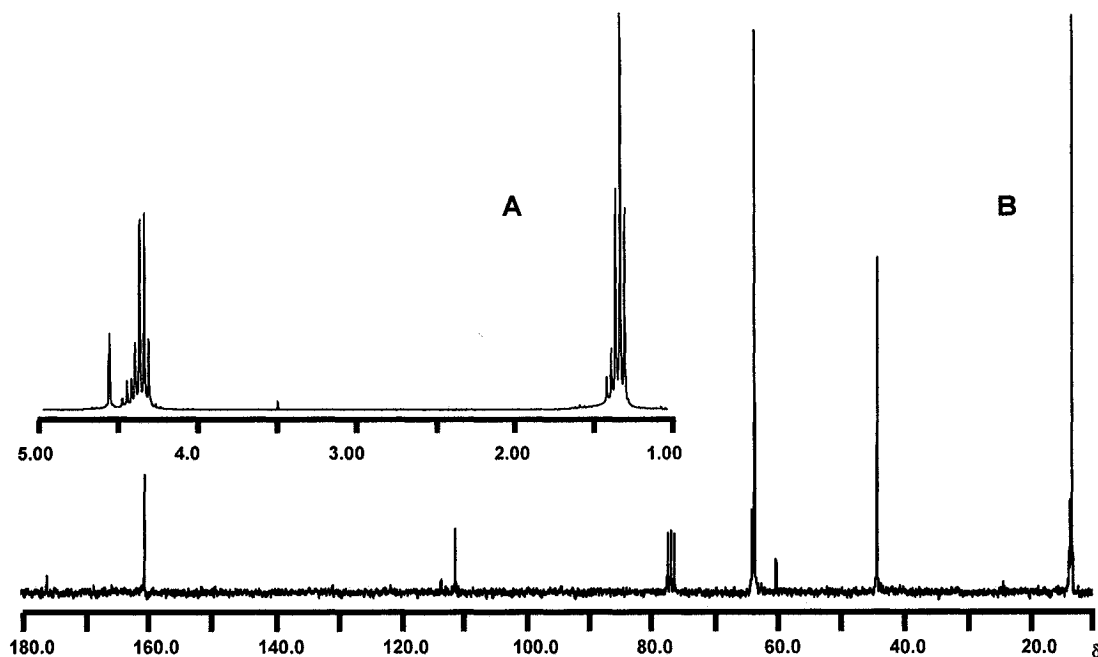
Figure 2.1 ^{1}H NMR (A) and ^{13}C NMR (B) spectra of distilled diethyl cyanomalonate in chloroform-d_1.

An obvious possibility for the minor signals observed in the NMR spectra was

contamination of the distilled diethyl cyanomalonate by unreacted starting materials

(ethyl cyanoacetate (**1-12**) and ethyl chloroformate (**2-5**)) and/or diethyl 2-cyano-2-

carboxyethylpropanedioate (**2-6**) formed as a side product. The chemical shifts and

splitting patterns of these potential contaminants are similar to those of diethyl

cyanomalonate (Table 2.2).

Table 2.2 ^{1}H and ^{13}C NMR chemical shifts for diethyl cyanomalonate and related compounds (CDCl$_3$).

Group	Diethyl Cyanomalonate				Ethyl Cyanoacetate		Ethyl Chloroformate		Triacylated Product	
	Major		Minor							
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
-CO$_2$-	-	160.6	-	176.0	-	163.1	-	150.4	-	159.6
CH$_2$-	4.34	63.8	4.42	64.3	4.26	62.8	4.38	68.4	4.40	64.9
-CH$_3$	1.34	13.5	1.40	13.9	1.37	13.9	1.39	13.8	1.31	13.8
α-H, C	4.59	44.5	13.2	60.3	3.52	24.7	-	-	-	61.7
CN	-	111.5	-	113.6	-	113.3	-	-	-	111.3

A sample of diethyl cyanomalonate dissolved in chloroform-d_1 was spiked with ethyl cyanoacetate (**1-12**, 10%). The ^{1}H NMR signals for ethyl cyanoacetate did not correspond to either the major or minor signals from the diethyl cyanomalonate. The CH$_2$ ester signals for ethyl cyanoacetate appear upfield from both the major and minor diethyl cyanomalonate signals. Also, the α-hydrogens of ethyl cyanoacetate produce an intense CH$_2$ singlet at δ$_H$ 3.52 compared with the diethyl cyanomalonate CH singlet observed at δ$_H$ 4.59. Also, the ^{13}C NMR spectrum of diethyl cyanomalonate did not have a signal at δ$_C$ 24.7, corresponding to the α carbon of ethyl cyanoacetate.

The chemical shifts of the ^{13}C NMR signals of ethyl chloroformate (**2-5**) were sufficiently unique to eliminate it as a source of the minor signals. Also it would be less likely for ethyl chloroformate to appear in the product, since it would most likely be hydrolyzed during the acidic workup of diethyl cyanomalonate.

It was also possible that diethyl 2-cyano-2-carboxyethylpropanedioate (**2-6**) could be responsible for the minor signals observed in the ^{1}H and ^{13}C NMR spectra of distilled diethyl cyanomalonate. ^{1}H and ^{13}C NMR spectra were recorded on a mixture consisting of the triacylated compound and diethyl cyanomalonate. The CH_2 ester signals of the triacylated product (**2-6**) overlapped almost exactly with the minor component signals of diethyl cyanomalonate in the ^{1}H NMR spectrum. However, the ^{1}H NMR ester CH_3 signal and the ^{13}C NMR resonances of diethyl 2-cyano-2-carboxyethyl propanedioate (**2-6**), especially the carboxylate (δ_C 159.6) and nitrile (δ_C 111.3) carbons, were distinct from the resonances in the diethyl cyanomalonate spectrum eliminating the triacylated compound as the source of the minor signals in the diethyl cyanomalonate NMR spectra. Also, the modified workup procedure involving extraction of the basic reaction mixture with dichloromethane would have removed any neutral compounds such as diethyl 2-cyano-2-carboxyethylpropanedioate from the final product.

If the minor signals observed in the NMR spectra of diethyl cyanomalonate (**1-1**) had originated from another contaminant, the presence of another compound was not evident in the NMR spectra of the diethyl cyanomalonate salts. The ^{1}H and ^{13}C NMR spectra of the salt showed only one set of signals. Morever, the ^{1}H and ^{13}C NMR spectra (CDCl$_3$) of diethyl cyanomalonate prepared by acidifying either the sodium or tetrabutylammonium salts purified by recrystallization, still had the major and minor signals present, suggesting that the minor component may be formed from diethyl cyanomalonate.

2.2.4 Tautomerism in Diethyl Cyanomalonate

After eliminating contaminants, tautomerization of diethyl cyanomalonate was considered (Figure 2.2) as the origin of the minor signals in the ^{1}H and ^{13}C NMR spectra in chloroform. For β-dicarbonyl compounds such as β-ketoesters and β-diketones, the amount of the enol form is usually significant due to stabilization by intramolecular hydrogen bonding.[16,17,18,19] For example in carbon tetrachloride, acetylacetone is about 95% enol, while ethyl acetoacetate is about 40% enol.[18] In general, carboxylic acid derivatives, such as esters, exist primarily in the keto form due to the electron-donating effect of the oxygen atom which provides greater stabilization than enolization.[20,21] Compounds structurally related to diethyl cyanomalonate such as di(m)ethyl malonate,[20,22] ethyl cyanoacetate,[20] methyl dicyanoacetate[23] and triethylmethanetricarboxylate[22] have only been observed as the keto form. However, the tautomerization of diethyl cyanomalonate in acetonitrile has been suggested from IR studies.[24] Indeed, two nitrile bands (2262, 2225 cm^{-1}) were observed when the infra-red spectrum (neat) of diethyl cyanomalonate was acquired.

Careful examination of the ^{1}H NMR spectrum of diethyl cyanomalonate in chloroform also yielded a very broad signal at δ_H 13.2, which is in the chemical shift range of a β-dicarbonyl enolic proton.[25] Overall, the minor NMR signals suggest that about 10% of diethyl cyanomalonate exists as the enol in chloroform.
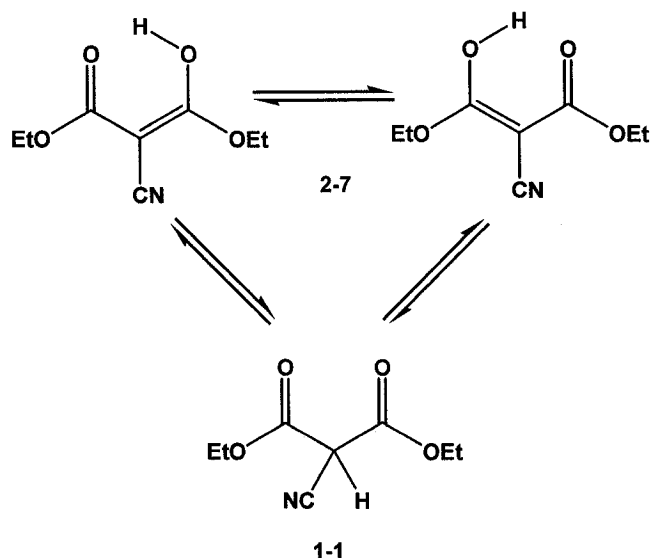
Figure 2.2 Tautomerization of diethyl cyanomalonate.

The enol content of β-dicarbonyl compounds is strongly dependent upon solvent polarity.[16,17,18] Polar solvents tend to reduce the measured enol content by disrupting the internal hydrogen bonding of the enol or by preferentially stabilizing the more polar keto form. For example, acetylacetone is about 97% enol in benzene, but only 13% enol in water.[18] Therefore, in less polar solvents the enol form, which is considered to be less polar than the keto tautomer,[16] is more significant. When the ^{1}H NMR spectrum was recorded on a diethyl cyanomalonate sample dissolved in more polar solvents such as methanol-d_4, or acetonitrile-d_3, only one set of ester signals was observed, indicating a decrease in enol content.

UV spectroscopy is also a good indicator of enol content since UV absorbance increases with the concentration of enol content, so the extinction coefficient should be larger when measured in a non-polar solvent.[26] The UV spectra of diethyl cyanomalonate showed variations in both λ_{max} and the extinction coefficient (log ε) according to the

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polarity of the solvent (Table 2.3). Both the wavelength of (248 to 256 nm) and the extinction coefficient (log ε 2.97 to 3.23) of λ_{max} increased in going from a polar solvent (acetonitrile) to a non-polar solvent (hexane). The increase in extinction coefficient with decreasing solvent polarity has been observed in several aliphatic β-diketones.[27,28]

Table 2.3 UV spectral data obtained for diethyl cyanomalonate in various solvents.

Solvent	λ_{max} (nm)	log ε
Acetonitrile	248	2.97
Dioxane	252	2.42
Hexane	255	3.23

A much larger extinction coefficient was measured when the UV spectra of diethyl cyanomalonate were recorded in methanol and water. In fact, the UV spectrum of diethyl cyanomalonate in water is similar to that of the sodium salt in water indicating that the carbon acid ionizes to a large extent (Figure 2.3), and these protic solvents are not suitable for assessing enol content.
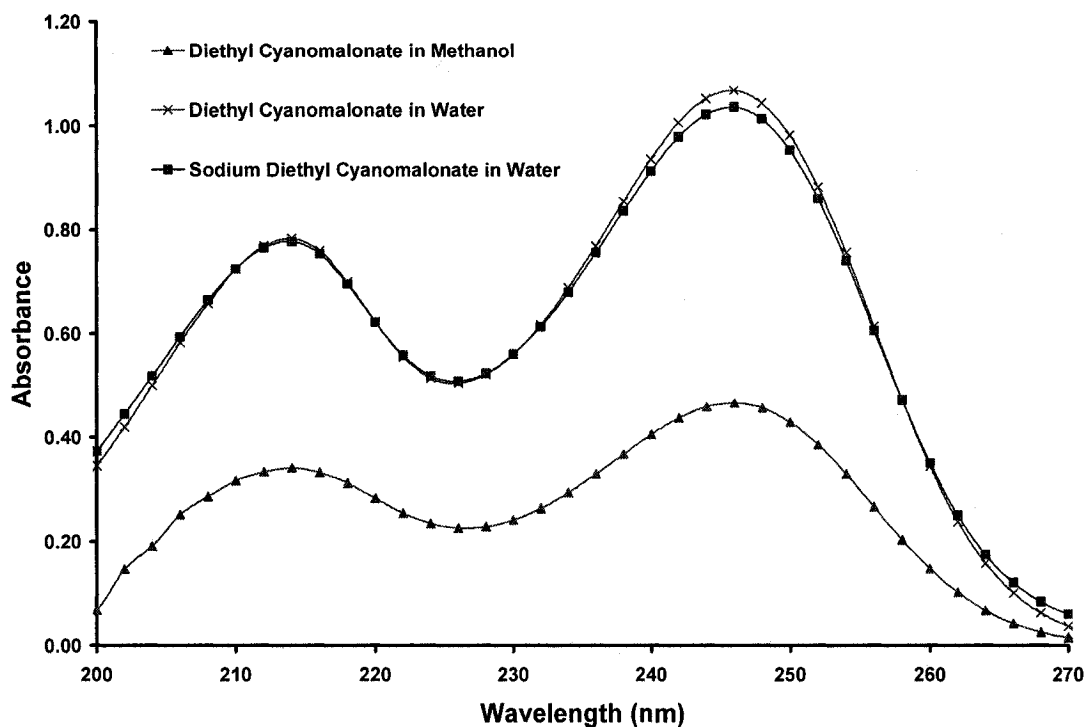
Figure 2.3 UV spectrum of diethyl cyanomalonate showing extent of ionization in water and methanol.

2.3   Alkylation of the Diethyl Cyanomalonate Anion

Due to the acidity of diethyl cyanomalonate (pK_a = 1.3),[8] any common strong or weak base will generate high concentrations of the diethyl cyanomalonate anion for alkylation reactions. Typically, amine and carbonate bases were used in the few examples from the literature describing the alkylation of cyanomalonate (Chapter 1).[1,2,3,4,5,6,7] The diethyl cyanomalonate salts (**1-1B-G**), however, are far more stable than diethyl cyanomalonate (**1-1**), which decomposes to ethyl cyanoacetate (**1-12**) after a few months at room temperature. On the other hand, the salts can be stored in a sample vial at room temperature for several years without any evidence of decomposition. The salts, which are soluble in a variety of organic solvents (Table 2.1), offer greater potential as

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substrates for alkylation reactions. In particular, the use of the preformed, weakly basic

diethyl cyanomalonate anion allows alkylation to be carried out under almost neutral

conditions using electrophiles that would react with bases.

2.3.1 Development of Alkylation Reaction Conditions

The reaction of a diethyl cyanomalonate salt with a single reactive electrophile

(benzyl bromide) was followed directly by ^{1}H NMR spectroscopy. Typically, the diethyl

cyanomalonate salt (1-1A) and benzyl bromide (0.1 mmol each) were dissolved in a

deuterated solvent (0.75 mL) and heated at 50°C (Scheme 2.6). The choice of 0.1 mmol

cyanomalonate salt per 0.75 mL solvent (0.13 mmol/mL) was based on initial solubility

studies of cyanomalonate salts. Cesium diethyl cyanomalonate (1-1E), however, was

insoluble in acetone and acetonitrile (Table 2.1), so alkylation experiments could not be

conducted for this salt in these solvents.
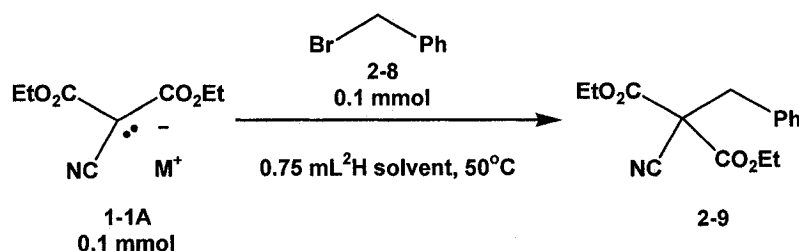
Scheme 2.6 Alkylation of diethyl cyanomalonate by benzyl bromide.

A typical set of ^{1}H NMR spectra collected from an alkylation experiment is

shown in Figure 2.4. The progress of cyanomalonate alkylation was monitored by

following the disappearance the -CH$_2$- ester resonance (δ_H 4.40) of the diethyl

cyanomalonate anion and the CH_2 resonance (δ_H 5.15) of benzyl bromide, along with the

appearance of the resonances for alkylated product diethyl 2-cyano-2-

benzylpropanedioate (2-9, $-CH_2-$ ester signal at δ_H 4.70 and CH_2-Ph at δ_H 3.85). The

percent conversion of starting material to alkylated product was determined by

integrating the ester $-CH_2-$ signals for both the diethyl cyanomalonate salt and the

alkylated product at each time period. In subsequent larger scale reactions, the product 2-

9 was isolated and characterized spectroscopically to verify the ^1H NMR assignments

used to interpret these time course experiments.
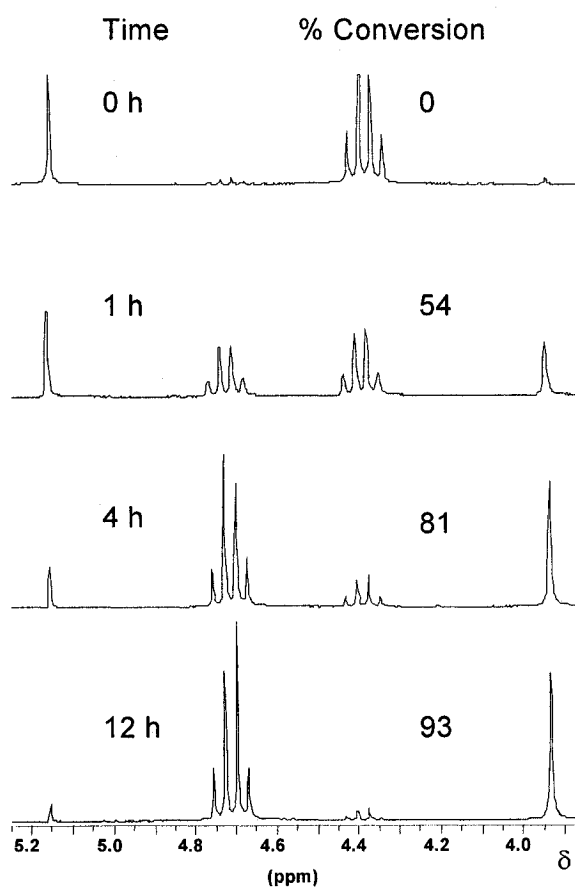
Figure 2.4 Partial ^1H NMR spectra collected during the alkylation of Na^+ diethyl
cyanomalonate with benzyl bromide (DMSO, 50°C).

2.3.1.1 Effect of Counter Ion and Solvent on Alkylation

The plots of percent conversion against time (Figure 2.5) for the alkylation of the various diethyl cyanomalonate salts in acetone show marked differences in reaction rates. Large rate differences were also obtained when the experiments were repeated in acetonitrile and DMF (Table 2.4). In contrast, similar rates were observed for all salts in DMSO. Overall for the diethyl cyanomalonate anion, solvent effects were minimized when the tetrabutylammonium cation, a large bulky counter ion, was used, and the effects of the counter ion were minimized when DMSO, a polar, aprotic solvent, was used. Although similar rates of alkylation were observed for all salts in DMSO or in all solvents for the tetraalkylammonium salts, the optimal conditions for alkylation of the diethyl cyanomalonate anion by benzyl bromide were achieved by using the tetrabutylammonium counter ion in DMSO.

The reactivity trends observed for alkylation of the diethyl cyanomalonate anion (Figure 2.4 and Table 2.4) are consistent with the idea that conditions which separate the cation from the enolate leave a "bare enolate" that is more reactive towards alkylation by an electrophile. In general, larger alkali metal cations are less associated with enolates, leaving them more accessible to alkylation by electrophiles.[29,30,31] For example, the rate of the intramolecular alkylation of diethyl 5-bromopentylmalonate was depressed as the concentrations of added lithium, sodium and potassium ions was increased.[29] The amount of rate retardation depended on the metal ion (lithium > sodium > potassium), but a similar increase in the concentration of a tetraethylammonium salt did not affect the rate of alkylation. In a separate investigation, the alkylation rate of ethyl acetoacetate salts by

ethyl tosylate in HMPT increased as the size of the alkali metal counter ion increased (Li^+

$< Na^+ < K^+ < Cs^+$).[31]
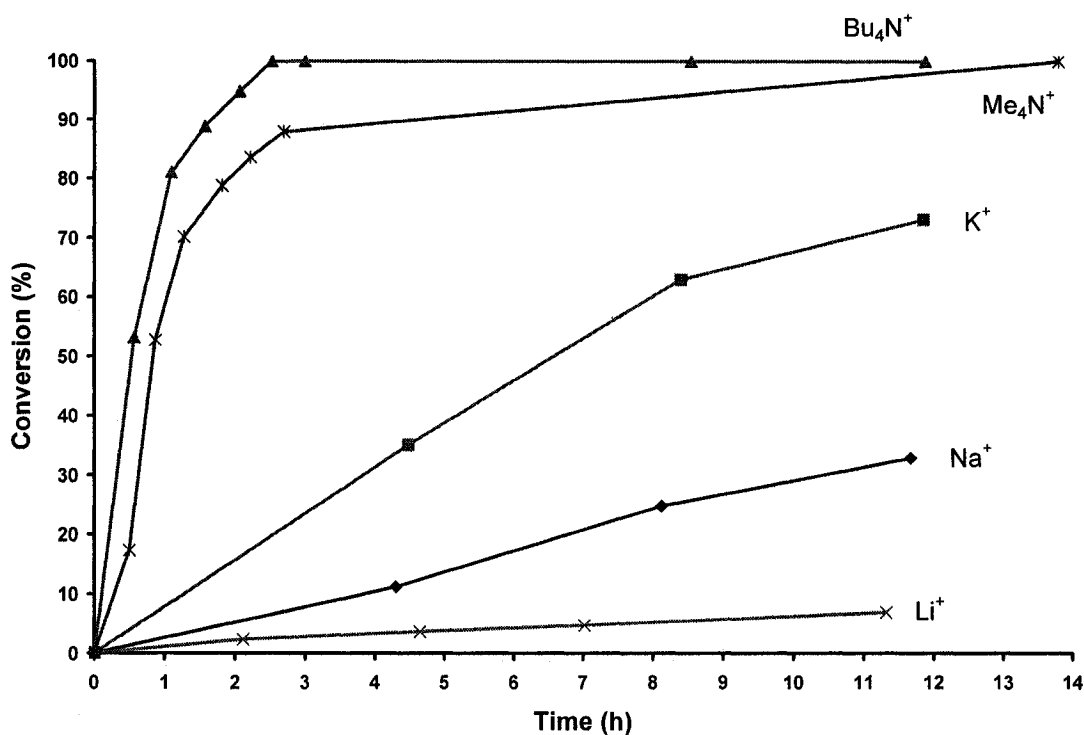
Figure 2.5 Time dependent alkylation of different diethyl cyanomalonate salts by benzyl bromide in acetone at $50°C$.

Table 2.4 Alkylation of the diethyl cyanomalonate anion (**1-1A**).

Counter Ion	% Conversion at 2 h in Each Solvent			
	Acetone	Acetonitrile	DMF	DMSO
Li^+	2	4	14	54
Na^+	5	16	25	67
K^+	16	46	60	80
Cs^+	N/A	N/A	78	84
Me_4N^+	73	76	86	88
Bu_4N^+	93	96	100	100

N/A = not applicable (Cs^+ insoluble)

Separation between the enolate and its counter ion is greatest when polar aprotic solvents, such as DMSO, DMF and THF are used.[32,33,34] These solvents function to specifically solvate the cation, leaving the enolate relatively unsolvated and more accessible to alkylation. For example, a series of experiments investigated the effects of the addition of DMF, DMSO and THF upon the alkylation of sodium diethyl *n*-butylmalonate by *n*-butyl bromide at 25°C in benzene.[33] DMSO and DMF were the most effective solvents as the alkylation rates in both were approximately 250 times that observed in benzene alone. For the alkylation of diethyl cyanomalonate salts, the alkylation rates were greatest in DMSO and DMF as well. Use of the most reactive combination of counter ion and solvent would be important when cyanomalonate was alkylated by less reactive electrophiles

Similarly, complexation of the counter ion by a crown ether, separates the cation from the enolate, increasing the reactivity of the cyanomalonate ester anion under otherwise relatively unreactive conditions.[34,35] To probe further the effect of separating the cation on the reactivity of the diethyl cyanomalonate anion, a crown ether was added to the sodium salt in acetone (i.e., relatively unreactive conditions). As indicated by the percent conversion at 12 h (Table 2.5), higher alkylation rates were achieved when increasing amounts of *cis*-dicyclohexano-18-crown-6-ether were included in the reaction mixture.

Table 2.5 Effect of crown ether (*cis*-dicyclohexano-18-crown-6-ether) on the alkylation of sodium diethyl cyanomalonate by benzyl bromide (50°C, acetone).

Crown Ether Equivalents	% Conversion at 12 h
0	33
0.2	48
0.5	68

Similar rate increases were observed for the alkylation rate of sodium diethyl *n*-butylmalonate by *n*-butyl bromide in both benzene and THF when the concentration of a crown ether, dicyclohexyl-18-crown-6 ether, was increased.[34] The electrostatic attraction between the ether oxygens and the cations impart powerful cation solvating abilities to the polydentate crown ethers, effectively separating the cation from the nucleophile. Tetraalkylammonium salts with bulky alkyl substituents, particularly the tetrabutylammonium ions, also provide large anion-cation separations, leading to high alkylation rates.[36] For the alkali metal salts of diethyl cyanomalonate in each solvent, the alkylation rate increased as the size of the counter ion increased ($Li^+ < Na^+ < K^+ < Cs^+$), but faster rates were always measured for the tetraalkylammonium salts (Me_4N^+, Bu_4N^+).

2.3.1.2 Diethyl Cyanomalonate: Correlation of Reactivity and ^{13}C NMR Chemical Shift

In several instances, ^{13}C NMR spectroscopy has been used as a measure of the variations of the electron density in carbanions in response to changes in solvation and counter ions.[37,38,39,40] Typically, the shift of the ^{13}C NMR signal of a carbon atom to

lower frequency upon a change in solvent or counter ion indicates an increase in electron density.[41]

The ^{13}C chemical shift of the α-carbon in the cyanomalonate anion (Table 2.6) showed a consistent progression to lower frequency as the size of the alkali metal counter ion increased, and appeared at lower frequency in the tetraalkylammonium salts. The variation of electron density reflected by the chemical shift of the α-carbon is consistent with the order of reactivity determined for the salts in four solvents (Figure 2.5 and Table 2.4). In addition, the resonance frequency of the α-carbon was lower in DMSO than in acetone (Table 2.6). The chemical shift difference was greater for the lithium salt (Δδ = 2.08 ppm) than the tetrabutylammonium salt (Δδ = 1.43 ppm). The electron density differences indicated by the chemical shift differences between DMSO and acetone correlate with the greater reactivity observed in DMSO (Table 2.4) and the larger Δδ in acetone is consistent with the large solvent effect on the reactivity of the lithium salt.

While the correlations pointed out in the above paragraph are based on small chemical shift differences, similar small chemical differences have been reported for salts of the enolate of phenylacetone.[41] For example, a 3.5 ppm difference was determined for the lithium (δ_C 95.3) and the potassium (δ_C 91.8) enolates. Again, the greater electron density at the α-carbon in the potassium enolate correlated with its greater reactivity. Also, the chemical shift of the α-carbon in the lithium enolate in the relatively polar solvent DME (δ_C 95.3) was lower in frequency (Δδ = 2.5 ppm) than in the relatively nonpolar solvent diethyl ether (δ_C 97.8). The greater reactivity of lithium phenylacetone in DME is consistent with the electron density changes indicated by the chemical shift differences.

Table 2.6 ^{13}C NMR chemical shifts for the diethyl cyanomalonate salts obtained in DMSO and acetone.

Cation	C:⁻		C=O		CN	
	DMSO	Acetone	DMSO	Acetone	DMSO	Acetone
Li$^+$	56.07	58.15	170.10	173.07	121.67	120.94
Na$^+$	55.34	57.16	168.67	171.28	123.02	122.61
K$^+$	55.01	56.72	167.69	170.23	123.96	123.47
Cs$^+$	54.89	-	167.37	-	124.35	-
Me$_4$N$^+$	54.75	-	167.11	-	124.52	-
Bu$_4$N$^+$	54.72	56.15	167.06	168.76	124.44	125.09

The chemical shift and reactivity differences noted above are consistent with variation in the separation of the counter ion from the enolate. Tightly associated counter ions (e.g., Li$^+$) coordinated to the oxygen atoms would draw electron density away from the α-carbon, deshielding it and reducing its reactivity. Changes that separate the cation from the enolate, such as an increase in size or high solvent polarity, would increase electron density at the α-carbon, resulting in the shift of ^{13}C resonances to lower frequency and greater nucleophilicity at the α-carbon.

2.3.1.3   Effect of Ester Composition on Alkylation

Reactivities of the diethyl and dimethyl cyanomalonate anions toward benzyl bromide alkylation were also followed by ^{1}H NMR spectroscopy (Table 2.7). The dimethyl and diethyl esters of the tetrabutylammonium cyanomalonate salt were alkylated in acetone, acetonitrile and DMSO, and the sodium salts of the two diesters

were studied in DMSO. In each solvent, the rate of alkylation was higher for the ethyl ester, suggesting that the cyanomalonate anion may be less associated with the counter ion when larger alkyl groups are present.

Table 2.7 Comparison of diethyl and dimethyl cyanomalonate alkylation by benzyl bromide (50°C).

		% Conversion at 2 h	
Solvent	Counter Ion	Methyl Ester	Ethyl Ester
Acetone	Bu_4N^+	63	95
Acetonitrile	Bu_4N^+	71	95
DMSO	Na^+	62	68
DMSO	Bu_4N^+	88	100

2.3.1.4 Preliminary Survey of Electrophiles

A preliminary study was conducted in which tetrabutylammonium diethyl cyanomalonate (1-1G) was reacted with a range of electrophiles. Overall the relative rates demonstrated that the structure of the electrophile had a large effect on the rate of the alkylation reaction (Table 2.8). The allyl and benzyl bromides, ethyl bromoacetate and methyl iodide showed greatest reactivity, whereas allyl chloride, ethyl chloroacetate and the two secondary alkyl halides were slow or unreactive.

As expected for S_N2 reactions, the alkylation rates correlated with the expected ability of the halide ions to function as leaving groups. As such, the bromides were more reactive than the chlorides (compare the benzyl and allyl halides as well as ethyl bromoacetate vs. ethyl chloroacetate). The selective reactivity displayed by the diethyl

cyanomalonate anion was further demonstrated by its reaction with 1-chloro-5-iodopentane.[42] Only the iodide was displaced, giving an alkylated cyanomalonate species with a chloride as a displaceable group suitable for further reactions.

Table 2.8 Reactivity of various electrophiles with tetrabutylammonium diethyl cyanomalonate in DMSO.

Electrophile	% Conversion at 2 h		Electrophile	% Conversion at 2 h	
	50°C	80°C		50°C	80°C
Benzyl bromide[a]	88	-	Iodoethane[a]	16	53
Benzyl chloride[a]	6	55	Ethyl bromoacetate[a]	44	-
Allyl bromide[a]	88	-	Ethyl chloroacetate[a]	no reaction	-
Allyl chloride[a]	no reaction	-	2-Bromopropane[a]	no reaction	-
Bromoacetonitrile[a]	23	-	Cyclohexyl iodide	no reaction	-
Iodomethane[a]	71	-			

[a]Full characterization of products carried out in association with preparative scale reactions described in the next section.

The alkyl chlorides showed little or no reactivity under these conditions at 50°C (Table 2.8). The addition of sodium iodide resulted in faster alkylation of sodium diethyl cyanomalonate with benzyl chloride in acetone at 50°C. For example, 8 h was required for 12% conversion with benzyl chloride alone, but 12% conversion was reached in only 2 h when sodium iodide (0.5 mmol/mmol benzyl chloride) was added. Iodide ion, a good nucleophile, displaces chloride ion from benzyl chloride and then acts as a good leaving group when displaced by the diethyl cyanomalonate anion, thereby increasing the rate of reaction.

In general, prolonged heating of the reaction mixture resulted in the observation of side products in the NMR spectrum. Use of iodides, however, reduced the alkylation time and the formation of side products, but halide ions generated by the nucleophilic substitution reaction could in turn act as nucleophiles promoting decarboethoxylation of the alkylated cyanomalonate and the formation of unwanted decarboethoxylated by-products.[43] At 100°C with benzyl bromide, side product formation was more pronounced giving a mixture of products by ^{1}H NMR spectroscopy. Therefore, 80°C appeared to be the maximum temperature at which the alkylation reactions could be carried out without a significant amount of side reactions occurring.

2.3.2 Preparative Scale Alkylation Reactions

Using the general conditions (DMSO, 80°C) developed from the results of the preliminary alkylation reaction experiments described in the previous section, tetrabutylammonium diethyl cyanomalonate was alkylated by a variety of electrophiles on a preparative scale (2 mmol). The concentration of the reagents was increased to 1 mmol/mL to take advantage of the solubility of the tetrabutylammonium salt in DMSO and each product was isolated from the reaction mixture and characterized (Subsection 2.3.3).

In general, the work-up of diethyl cyanomalonate alkylation reactions was straightforward, involving the addition of water and extraction into ether; no chromatography was required. If insufficient water was used in the work up, signals corresponding to DMSO or even tetrabutylammonium ion would be observed in the isolated product. If residual DMSO was observed in the ^{1}H NMR spectra of the isolated

product, it was easily removed by performing a back-extraction of the ether layer. Ethyl cyanoacetate, if present, was removed by washing the ether layer with 1 M NaOH. If side products, usually due to excessive heating, or unreacted alkyl halide were observed, adjustments were made to the reaction time or an excess of tetrabutylammonium diethyl cyanomalonate was used.

The results of the preparative scale reactions are summarized in the next two subsections. With the other reaction variables fixed, the reaction time was varied depending on the electrophile studied. Alkylation of tetrabutylammonium diethyl cyanomalonate with more reactive electrophiles gave high yields with reaction times ≤ 2 h. From the preliminary alkylation studies carried out at 50°C, chlorides were essentially unreactive, so preparative scale reactions were carried out using bromides or iodides. Iodides were easily synthesized from commercially available bromides using the Finkelstein reaction.[44]

2.3.2.1 Alkylation by Alkyl Halides

Due to the high reactivity of iodides towards substitution, alkylation of tetrabutylammonium diethyl cyanomalonate (1-1G) with iodomethane or iodoethane was straightforward with a clean product obtained in high yield after 2 h at 80°C (Table 2.9). Tetrabutylammonium diethyl cyanomalonate was also successfully alkylated by 2° alkyl halides, 2-iodopropane and two substituted benzyl bromides.

Initially the alkylation of tetrabutylammonium diethyl cyanomalonate by benzyl bromide, the standard electrophile in the prelimary alkylation reactions, was carried out for 2 h. Alkylation was successful, but ^{1}H NMR signals corresponding to side products

were observed in the spectrum. Alkylation, however, was complete by 0.5 h due to the

high reactivity of benzyl bromide, and fewer side products were present in the isolated

product. Similar short reaction times were optimal for isolating alkylated cyanomalonate

from reactions with reactive bromides such as allyl bromide, ethyl bromoacetate (Chapter

3) and bromoacetonitrile.

Table 2.9 Alkylation reactions of cyanomalonate by alkyl halides (R-X, in DMSO at 80°C).
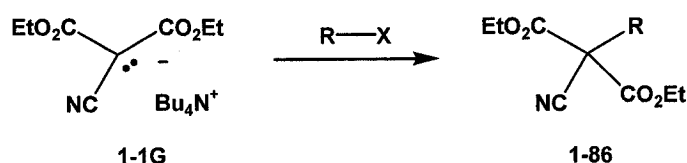
R	X	Time (h)	Yield (%)[a]
CH_3	I	2	81
CH_2CH_3	I	2	92
$CH_2CH_2CH_2CH_3$	Br	6	87
$CH_2CH_2CH_2CH_3$	I	2	86
CH_2Ph	Br	0.5	95
$CH_2CH=CH_2$	Br	0.5	74
CH_2CN	Br	0.17	50
CH_2CO_2Et[b]	Br	0.75	88
$CH_2CH_2CO_2Et$[c,d]	I	1.5	81
$CH_2CH_2CH_2CO_2Et$[c,d]	I	1.5	84
$CH(CH_3)_2$	I	2	67
$CH(CO_2CH_3)Ph$	Br	2	70
$CH(CH_3)Ph$	Br	2	76

[a]Isolated yield. [b]Synthesis described in Chapter 3, [c]Chapter 4. [d]Synthesized from commercially available bromides using the Finkelstein reaction.[44]

1-Bromobutane was chosen as a standard electrophile to develop general reaction conditions for alkyl bromides containing functional groups later used in the synthesis of γ- (Chapter 3) and β-amino acids (Chapter 4). The alkylation of tetrabutylammonium diethyl cyanomalonate (**1-1G**) was carried out at 4, 6 and 8 h to determine the optimal time for alkylation. At 4 h, the reaction was incomplete as signals corresponding to 1-bromobutane were observed in the ^{1}H NMR spectrum of the isolated product. After 8 h, the alkylation reaction was complete, as no ^{1}H NMR signals corresponding to 1-bromobutane were observed. However, signals not corresponding to the desired product or starting materials were observed in the ^{1}H NMR spectrum, indicating that side products had formed upon prolonged heating. The ideal time appeared to be 6 h, as a clean product was obtained (87% yield) containing neither 1-bromobutane nor side products.

Use of 1-iodobutane reduced the reaction time from 6 h to 2 h. The alkylated cyanomalonate was obtained as a clean product with a yield (86%) nearly identical to that obtained from 1-bromobutane. It was also found that prolonged heating of the 1-iodobutane reaction mixture did not give side products as readily as the bromide. No side products were detected by NMR at 4, 6 or even 8 h, even though the reaction was complete by 2 h.

2.3.2.2 Alkylation of Diethyl Cyanomalonate by Base-Sensitive Electrophiles

The synthetic utility of carbanion alkylation reactions is limited to electrophiles containing functional groups that are less acidic than the carbanion itself. For example in the synthesis of an L-indospicine precursor presented in Chapter 1,[45] the attempted

alkylation of the acetonitrile anion was unsuccessful due to the relatively high basicity of

the acetonitrile anion (pKa = 25)[46] compared to the amide (pK$_a \approx$ 17)[47] present in the

electrophile. The highly stabilized diethyl cyanomalonate anion with its low pK$_a$ value

(1.3)[8] has the potential to be alkylated by acidic or base sensitive electrophiles.

A potential side reaction resulting from the desired alkylation of a relatively basic

nucleophile is elimination (Scheme 2.7). Using alkylation of ethyl 3-iodopropanoate as

an example, if a nucleophile (X⁻) is too basic, alkylation (2-22, Scheme 2.7A) also may

be accompanied by elimination of HI (Scheme 2.7B), giving ethyl acrylate (2-23) as a

side product. The nucleophile (X⁻) may be unreactive towards Michael addition with the

ethyl acrylate (2-23) side product or be quenched by the very acidic HI formed in the

reaction mixture, resulting in low yields of the desired product (2-22). However, this

elimination side reaction was avoided when the highly stabilized diethyl cyanomalonate

anion (1-1A) was successfully alkylated by ethyl 3-iodopropanoate with an 81% isolated

yield (Table 2.9), showing no sign of the ethyl acrylate elimination side product in the

NMR spectrum.
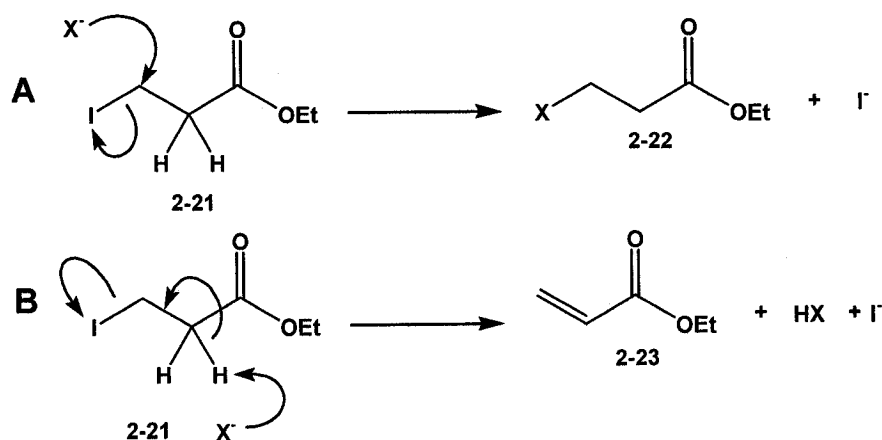
Scheme 2.7 A: Desired alkylation of nucleophile (X⁻) B: Potential side reaction of HI
elimination.

The diethyl cyanomalonate anion was successfully alkylated by α-bromo-*p*-toluic

acid and several ω-iodocarboxylic acids (Table 2.10). Alkylation of diethyl

cyanomalonate by α-bromo-*p*-toluic acid was chosen initially due to the high reactivity

of the similar electrophile, benzyl bromide, ensuring a short reaction time to minimize

side product formation. With the ω-iodocarboxylic acids, longer reaction times were used

and moderate, but lower yields were obtained.

Tetrabutylammonium diethyl cyanomalonate underwent alkylation with

iodoacetic acid and bromoacetic acid within 0.5 h at 80°C. The yields were similar (40-

50%) to those obtained for the longer chain ω-iodocarboxylic acids (Table 2.10) but, in

each case, the ^{1}H NMR spectrum of the isolated oil showed about 10% side products in

addition to the desired product. Prolonged heating (1-2 h) of the reaction mixture at 80°C

increased the amount of side products as shown by the appearance of extra signals in the

^{1}H NMR spectrum (CDCl$_3$) of the isolated oil, most notably two singlets at δ_H 7.19 and

8.17.

The effect of temperature and by-product formation was investigated by heating

tetrabutylammonium diethyl cyanomalonate and iodoacetic acid at 50°C for 19 h. Only

one alkylated product was isolated in a low yield (10%). However, this compound was

not the expected nitrile, but its corresponding amide. The ^{1}H NMR spectrum in DMSO-

d_6 showed the two singlets at δ_H 7.93 and 8.10 observed in the original reactions. The ^{13}C

NMR spectrum was more diagnostic, and it lacked the characteristic nitrile signal and

showed three signals at δ_C 167.3, 168.1 and 172.7, indicating the presence of three

different types of carbonyl groups in the molecule. The addition of water to the nitrile

would be catalyzed by acid, becoming a problematic side reaction in the presence of the

stronger bromo- and iodoacetic acids ($pK_a \approx 3$).[48] In the reactions utilizing the weaker ω-iodocarboxylic acids ($pK_a \approx 4$-5),[49] the rate of nitrile hydrolysis is slower, allowing alkylated diethyl cyanomalonates to be isolated in moderate yields.

Table 2.10 Alkylation reactions of cyanomalonate by acidic alkyl halides (R-X, in DMSO at 80°C).
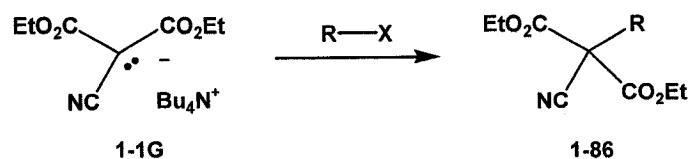
R	X	Time (h)	Yield (%)[a]
$(CH_2)_2CO_2Et^c$	I	1.5	81
CH_2PhCO_2H	Br	0.5	77
$CH_2CO_2H^c$	Br	0.5	44
CH_2CO_2H	I	0.5	53
$(CH_2)_2CO_2H$	I	2	45
$(CH_2)_3CO_2H^c$	I	2	22
$(CH_2)_4CO_2H^{b,c}$	I	2	48

[a]Isolated yield. [b]Synthesis described in Chapter 4. [c]Synthesized from commercially available bromides using the Finkelstein[44] reaction.

In each of these reactions, however, the very weakly basic cyanomalonate anion ($pK_a = 1.3$)[8] avoided protonation by the carboxylic acid groups ($pK_a \approx 4$)[49] present in the electrophiles and was successfully alkylated (Table 2.10). With alkylation by carboxylic acid containing electrophiles established, the diethyl cyanomalonate anion has been demonstrated to remain nucleophilic when the electrophiles contain acidic groups. If the pK_a is greater than approximately 4, then the nitrile group remains intact and the desired

alkylated product was isolated. Even for more acidic compounds, removal of water from the reaction medium would eliminate hydrolysis of the nitrile.

2.3.3 Characterization of Alkylated Diethyl Cyanomalonate

All alkylated diethyl cyanomalonates isolated from reaction mixtures were characterized spectroscopically. Each ^{13}C NMR spectrum showed the resonances at characteristic chemical shifts for the ester and nitrile carbons and the IR spectrum showed a distinctive IR band for the nitrile. Also, some of the alkylated diethyl cyanomalonate esters have been stored for years at room temperature and have not shown any signs of decomposition by NMR spectroscopy.

For several alkylated diethyl cyanomalonates, including diethyl 2-cyano-2-methylpropanedioate, diethyl 2-cyano-2-ethylpropanedioate and diethyl 2-cyano-2-allylpropanedioate, only the [M+H]$^+$ ion was observed by EI-MS, one mass unit higher than the expected molecular ion (M$^{+\bullet}$). However, this is not unusual for aliphatic nitriles which readily form M+H$^+$ ions under EI conditions.[50]

2.3.3.1 C-Alkylation of the Diethyl Cyanomalonate Anion

Carbanions, such as enolates are ambident ions, since the negative charge formed upon deprotonation of the α-carbon is delocalized onto a heteroatom such as oxygen.[51] Therefore, both carbon and oxygen can possibly act as nucleophiles, giving C-alkylated and O-alkylated species, respectively. When one of the electron-withdrawing groups is a ketone, as in ethyl acetoacetate, the enolate form is dominant, making the ketone oxygen sufficiently nucleophilic for O-alkylation to occur. Alkylation at oxygen

occurs most readily when a "bare" enolate is present, that is when good cation solvating

solvents, crown ethers or large cations are used.[51] Generally, O-alkylation is not

observed in diester enolates where the oxygen bonded to the carbonyl carbon stabilizes

the keto form by electron donation.[21] As a result, the keto form of the ester dominates,

making the ester carbonyl oxygen less nucleophilic resulting in alkylation occurring

primarily on the carbon.

The ratio of carbon to oxygen alkylation is also affected by the nature of the

alkylating agent according to the hard-soft acid base (HSAB) principle.[51] Alkyl halides

such as methyl iodide have softer leaving groups and preferentially react with the softer

carbon nucleophile. Harder electrophiles such as alkyl sulfonates preferentially alkylate

the harder oxygen nucleophile. For example, the amount of C-alkylation of ethyl

acetoacetate increased as the hardness of the leaving group decreased ($CH_3CH_2OTs <$

$CH_3CH_2Cl < CH_3CH_2Br < CH_3CH_2I$).[52]

The products isolated from diethyl cyanomalonate alkylation reactions show

NMR signals consistent with the formation of one product. For example, in the

alkylation of diethyl cyanomalonate by benzyl bromide, shown in the series of ^1H NMR

spectra (acetone-d_6) in Figure 2.4, only one new -CH_2- signal at δ_H 3.85 increases in

intensity as the reaction time progresses. In the ^1H and ^{13}C NMR spectra ($CDCl_3$) of the

isolated product (**2-9**, Table 2.11), the -CH_2- signal appears at δ_H 3.48 (s) and δ_C 39.8.

The ^1H and ^{13}C NMR chemical shifts observed in the spectra of alkylated diethyl

cyanomalonate esters (e.g., Table 2.11) are consistent with the chemical shifts observed

for a -CH_2- group bonded to two carbon atoms resulting from C-alkylation.[53] If O-

alkylation had occurred, the ^1H and ^{13}C NMR spectra would contain chemical shifts for a

-CH$_2$O group at approximately $\delta_H \approx 5$ and $\delta_C \approx 60$,[53] appearing at higher frequency, near

the ester -CH$_2$- signals.

Since only one set of signals appears in each NMR spectrum and there is no

evidence for a -CH$_2$-O chemical shift (aside from the ester groups), diethyl

cyanomalonate is alkylated exclusively at the α-carbon under the reaction conditions

used in this investigation. This result is consistent with the lack of O-alkylation observed

for diesters and the fact that diethyl cyanomalonate is a highly stabilized "soft

nucleophile" that readily reacts at carbon with "soft electrophiles" such as iodides and

bromide.

Table 2.11 Comparison of the chemical shifts of the β-CH$_2$ group of several alkylated diethyl cyanomalonates (CDCl$_3$).

1-86
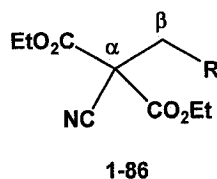
R	Chemical Shift β -CH$_2$- (ppm)	
	δ_H	δ_C
CH$_3$	2.25	28.1
Ph	3.48	39.8
CH=CH$_2$	2.92	38.5
CO$_2$Et	3.27	38.5

2.4 Summary

Diethyl cyanomalonate was synthesized reproducibly and in high yield. The diethyl cyanomalonate salts, easily formed by titration, are soluble in a variety of organic solvents and are stable; they have been stored at room temperature for years without any decomposition detectable by ^{1}H NMR spectroscopy. The diethyl cyanomalonate anion was cleanly alkylated by primary and secondary alkyl halide electrophiles in DMSO at 80°C. Prolonged reaction times or higher temperatures led to the accumulation of side products. Alkylation in the presence of free carboxyl groups in the electrophile was achieved because the cyanomalonate anion is a weak base. Also, only C-alkylation of diethyl cyanomalonate was observed, an important consideration for the alkylation of enolates. Work up of the alkylation reaction mixtures was simple, involving the addition of water and extraction into ether. The application of alkylated cyanomalonates in amino acid synthesis is presented in Chapters 3 and 4 of this thesis.

2.5 Experimental

2.5.1 General Procedures

^{1}H and ^{13}C NMR spectra were acquired on a Bruker/Techmag AC-250 MHz NMR spectrometer operating at 250.13 and 62.9 MHz, respectively or a Bruker Avance 500 MHz NMR spectrometer operating at 500.13 and 125.77 MHz, respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS, $\delta = 0$). ^{1}H NMR spectra of samples in chloroform-d were calibrated to the TMS signal, while residual solvent signals were used to calibrate spectra run in acetonitrile-d_3 (δ_H 1.94), DMSO-d_6 (δ_H 2.54), methanol-d_4 (δ_H 3.31) and D$_2$O (δ_H 4.79). ^{13}C NMR spectra were

calibrated using the central line of the signal produced by the following solvents: acetonitrile-d_3 (δ_C 1.32), chloroform-d (δ_C 77.16) and DMSO-d_6 (δ_C 39.50). All coupling constants (J) are reported in Hz and the splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (d.d.), exchangeable proton (exch.) and broad (br.). Ultraviolet spectra were recorded on a Hewlett-Packard 8452A UV-Vis spectrometer using 1 cm quartz cuvettes. Infrared spectra were recorded on a Bomem Michelson instrument. Solid samples were prepared as KBr discs or in Nujol while liquid samples were run neat. Electron ionization mass spectra (EI-MS) and high resolution mass spectra (HRMS) for accurate mass determinations were obtained on a Consolidated Electrodynamics Corporation Analytical and Control Division double focusing mass spectrometer in positive ion mode. Electrospray ionization mass spectra (ESI-MS) were collected on a Finnigan LCQ Duo ion trap. Melting points (uncorrected) were determined with a Gallenkamp melting point apparatus in open capillary tubes.

2.5.2 Preparation of Diethyl Cyanomalonate and its Salts

Diethyl Cyanomalonate (Diethyl 2-Cyanopropanedioate, 1-1): Ethyl cyanoacetate (5.70 mL, 54 mmol) was added to a stirred mixture of K_2CO_3 (24 g, 190 mmol, pre-treated by heating at 130°C for 16 h) and acetone (70 mL, dried over K_2CO_3 for 16 h). After 5 min, ethyl chloroformate (10.2 mL, 108 mmol) was added and the reaction mixture was heated at reflux for 6 h. The mixture was allowed to cool to room temperature, water (100 mL) was added and the acetone was removed *in vacuo*. Additional water (50 mL) was added and the basic solution was extracted with dichloromethane (3 x 20 mL). The aqueous layer was acidified to pH < 0 with

concentrated HCl (30 mL) and extracted with diethyl ether (4 x 25 mL). The ether layers

were combined, dried over anhydrous $MgSO_4$, and evaporated *in vacuo* to yield a crude

orange oil (8.86 g, 89%). The crude products from three parallel reactions (8.72 g, 7.74 g,

8.86 g) were combined (25.32 g) and 22.5 g of the product was vacuum distilled (b.p.

115-116°C) yielding diethyl cyanomalonate as a clear, colourless oil (20.5 g, 91%

recovery). IR (Neat) cm^{-1}: 2262, 2225 (CN), 1751 (C=O). UV (acetonitrile 54 μM) λ_{max}

= 248 nm, log ε = 2.97, λ_2 = 208 nm, log ε = 2.84. UV (dioxane 54 μM) λ_{max} = 252 nm,

log ε = 2.42. UV (hexane 54 μM) λ_{max} = 256 nm, log ε = 3.23, λ_2 = 200 nm, log ε = 2.86.

UV (methanol 54 μM) λ_{max} = 246 nm, log ε = 3.93, λ_2 = 214 nm, log ε = 3.80. UV (water

56 μM) λ_{max} = 246 nm, log ε = 4.28, λ_2 = 214 nm, log ε = 4.14. Major tautomer: ^{1}H

NMR (CDCl$_3$) δ 1.34 (t, J = 7), 4.34 (q, J = 7), 4.59 (s). ^{13}C NMR (CDCl$_3$) δ 160.6,

111.5, 63.8, 44.5, 13.5. Minor tautomer: ^{1}H NMR (CDCl$_3$) δ 1.40 (t, J = 7), 4.42 (q, J =

7), 13.20 (br. s). ^{13}C NMR (CDCl$_3$) δ 176.0, 113.6, 64.2, 60.3, 13.9. ^{1}H NMR

(Acetonitrile-d_3) δ 1.29 (t, J = 7, 6H), 4.30 (q, J = 7, 4H), 4.81 (s, 0.7H). ^{1}H NMR

(Methanol-d_4) δ 1.31 (t, J = 7, 6H), 4.31 (q, J = 7, 4H), 4.73 (s, 0.4H). ESI$^-$-MS 184

(100), 66 (12). CID (29%) of *m/z* 184: *m/z* 156 (10), 66 (100).

Diethyl 2-cyano-2-carboxyethyl propanedioate (2-6): Anhydrous potassium carbonate

(4.84 g, 35.0 mmol, pre-treated by heating at 130°C for 16 h) was added to anhydrous

acetone (15 mL, dried over K_2CO_3 for 16 h) and the mixture was stirred for 15 min.

Ethyl cyanoacetate (1.06 mL, 10.0 mmol) was added followed after 5 min by ethyl

chloroformate (9.56 mL, 100 mmol). The reaction mixture was refluxed with stirring for

6 h, allowed to cool, water (100 mL) was added and solution was acidified with

6 h, allowed to cool, water (100 mL) was added and solution was acidified with concentrated HCl (10 mL) to a pH < 0. The product was extracted with diethyl ether (3 x 25 mL). The ether layers were combined, dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. A portion (1.51g) of the crude product (2.51 g, 98%) was dissolved in ether (50 mL) and washed with saturated sodium bicarbonate (3 x 25 mL). The ether layer was dried over anhydrous $MgSO_4$ and upon evaporation *in vacuo*, diethyl 2-cyano-2-carboxyethyl propanedioate was obtained as a colourless oil (1.06 g, 70%). IR (Neat) cm^{-1}: 2267 (CN), 1753 (C=O). ^1H NMR ($CDCl_3$) δ 1.37 (t, J = 7, 3H), 4.40 (q, J = 7, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.7, 64.9, 111.3, 159.6. MS (70 eV): 257 ($M^{+\bullet}$,10), 185 (9), 156 (10), 139 (14), 128 (18), 43 (15), 28 (100). HRMS (70 eV) calculated for $C_{11}H_{15}NO_6$ = 257.0899 amu, found = 257.0881 ± 0.0008 amu.

2.5.2.1 Diethyl Cyanomalonate Salts

The diethyl cyanomalonate ester salts were prepared by titrating an alcoholic solution of diethyl cyanomalonate with an alcoholic or aqueous solution of alkali metal or tetraalkylammonium hydroxide. The progress of the titration was followed using a pH meter and stopped at the equivalence point of approximately pH 8. The solvent was removed *in vacuo* and the crude salts were recrystallized. All NMR spectra of the diethyl cyanomalonate salts were acquired at the same concentration (0.1 mmol/0.75 mL).

Lithium Diethyl Cyanomalonate (1-1C): Diethyl cyanomalonate (3.30 g, 18.0 mmol) in 95% ethanol (300 mL) was titrated with ethanolic LiOH (0.24 M) to yield, after recrystallization from acetonitrile (21 mL), lithium diethyl cyanomalonate as a colourless,

crystalline solid (1.68 g, 49%). m.p. 227-229°C. UV (50 μM, acetonitrile) λ_{max} = 248 nm,

logε = 4.22, λ_2 = 212 nm, logε = 4.04. IR (KBr disc) cm^{-1}: 2207 (CN), 1677 (C=O). ^{1}H

NMR (DMSO-d_6) δ 1.18 (t, J = 7, 6H), 4.02 (q, J = 7, 4H). ^{13}C NMR (DMSO-d_6) δ 14.8,

56.1, 58.0, 121.7, 170.1. ESI⁻-MS 184 (100), 66 (8). CID (32%) of m/z 184: m/z 156 (8),

66 (100).

Sodium Diethyl Cyanomalonate (1-1B): Diethyl cyanomalonate (4.29 g, 23.0 mmol) in

95% ethanol (250 mL) was titrated with ethanolic NaOH (0.13 M) to yield, after

recrystallization from acetonitrile (50 mL), sodium diethyl cyanomalonate as a

colourless, crystalline solid (1.44 g, 30%). m.p. 204-205°C. UV (50 μM, acetonitrile)

λ_{max} = 246 nm, logε = 4.25, λ_2 = 214 nm, logε = 4.11. IR (KBr disc) cm^{-1}: 2196 (CN),

1681(C=O). ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 7, 6H), 3.97 (q, J = 7, 4H). ^{13}C NMR

(DMSO-d_6) δ 14.9, 55.3, 57.2, 123.0, 168.7. ESI⁻-MS 184 (100), 66 (7). CID (32%) of

m/z 184: m/z 156 (15), 66 (100).

Potassium Diethyl Cyanomalonate (1-1D): Diethyl cyanomalonate (3.60 g, 20.0

mmol) in 95% ethanol (240 mL) was titrated with ethanolic KOH (0.10 M) to yield, after

recrystallization from acetonitrile (80 mL), potassium diethyl cyanomalonate as a

colourless crystalline solid (2.19 g, 50%). m.p. 287-289°C (lit. m.p. 286-287°C,[13]

>300[54]). UV (50 μM, acetonitrile) λ_{max} = 246 nm, logε = 4.26, λ_2 = 216 nm, logε = 4.14.

IR (KBr disc) cm^{-1}: 2187(CN), 1692 (C=O). ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 7, 6H),

3.92 (q, J = 7, 4H). ^{13}C NMR (DMSO-d_6) δ 15.0, 55.0, 56.7, 124.0, 167.7. ESI⁻-MS 184

(100), 66 (5). CID (32%) of m/z 184: m/z 156 (13), 66 (100).

Cesium Diethyl Cyanomalonate (1-1E): Diethyl cyanomalonate (4.80 g, 26.0 mmol) in 95% ethanol (260 mL) was titrated with ethanolic CsOH (0.14 M) to yield, after recrystallization from acetonitrile (50 mL), cesium diethyl cyanomalonate as a colourless crystalline solid (4.07 g, 49%). m.p. 234-235°C. UV (50 μM, acetonitrile) λ_{max} = 246 nm, logε = 4.21, λ_2 = 214 nm, logε = 4.09. IR (KBr disc) cm^{-1}: 2181 (CN), 1686 (C=O). ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J = 7, 6H), 3.90 (q, J = 7, 4H). ^{13}C NMR (DMSO-d_6) δ 15.1, 54.9, 56.6, 124.4, 167.4. ESI⁻-MS 184 (100), 66 (3). CID (32%) of m/z 184: m/z 156 (13), 66 (100).

Tetramethylammonium Diethyl Cyanomalonate (1-1F): Diethyl cyanomalonate (9.00 g, 49.0 mmol) in 95% ethanol (200 mL) was titrated with a 10% aqueous tetramethylammonium hydroxide solution to yield, after recrystallization from acetonitrile (20 mL), tetramethylammonium diethyl cyanomalonate as a colourless crystalline solid (6.19 g, 49%). m.p. 117-119°C. UV (50 μM, acetonitrile) λ_{max} = 246 nm, logε = 4.22, λ_2 = 216 nm, logε = 4.11. IR (KBr disc) cm^{-1}: 2188 (CN), 1692 (C=O). ^{1}H NMR (DMSO-d_6) δ 1.08 (t, J = 7, 6H), 3.10 (s, 12H), 3.86 (q, J = 7, 4H). ^{13}C NMR (DMSO-d_6) δ 15.0, 54.4 (t, J = 4), 54.8, 56.4, 124.5, 167.1. ESI⁻-MS 184 (100), 66 (3). CID (29%) of m/z 184: m/z 156 (11), 66 (100).

Tetrabutylammonium Diethyl Cyanomalonate (1-1G): Diethyl cyanomalonate (21.00 g, 113.0 mmol) in methanol (150 mL) was titrated with a 1.0 M tetrabutylammonium hydroxide solution in methanol until a pH of 8.0 was reached. The methanol was

removed *in vacuo* and the crude solid was recrystallized from ethyl acetate (40 mL) to yield tetrabutylammonium diethyl cyanomalonate as a colourless crystalline solid (44.5 g, 92%). m.p. 74-75°C. UV (50 μM, acetonitrile) λ_{max} = 246 nm, log ε = 4.22, λ_2 = 216 nm, log ε = 4.11. IR (KBr): 2173 (CN), 1707 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 0.96 (t, J = 7, 12H), 1.12 (t, J = 7, 6H), 1.35 (sextet, J = 7, 8H), 1.50-1.70 (m, 8H), 3.10-3.30 (m, 8H), 3.89 (q, J = 7, 4H). ^{13}C NMR (DMSO-d_6) δ 13.4, 15.0, 19.2, 23.0, 54.7, 56.3, 57.5 (t, J = 3), 124.4, 167.1. ^{1}H NMR (Acetone-d_6) δ 0.97 (t, J = 7, 12H), 1.12 (t, J = 7, 6H), 1.42 (sextet, J = 8, 8H), 1.74-1.86 (m, 8H), 3.41-3.48 (m, 8H), 3.93 (q, J = 7, 4H). ^{13}C NMR (Acetone-d_6) δ 13.9, 15.6, 20.3, 24.4, 56.2, 57.2, 59.2 (t, J = 3), 125.1, 168.7. ESI$^+$-MS: CID (39%) of *m/z* 242: 186 (100), 184 (45), 142 (85). ESI$^-$-MS: CID (30%) of *m/z* 184: *m/z* 156 (8), 94 (5) 66 (100).

2.5.2.2 Solubility Determination of Diethyl Cyanomalonate Salts

The solubilities of the diethyl cyanomalonate salts were determined at room temperature (20°C). The appropriate diethyl cyanomalonate salt was added to 2 mL of solvent until the mixture appeared cloudy. The mixture was filtered to remove undissolved salt and 1 mL of the solution was removed and dispensed into a pre-weighed round bottom flask. The solvent was removed *in vacuo* and the mass of the residue was determined.

2.5.3 Alkylation of Cyanomalonate

2.5.3.1 Alkylation Rate Experiments

For each trial, the appropriate cyanomalonate salt (0.100 mmol) was weighed into a sample vial and dissolved in the appropriate deuterated solvent (0.75 mL). Benzyl bromide (12 µL, 0.10 mmol) was added to the salt solution via syringe. The reaction mixture was mixed thoroughly and 0.70 mL was dispensed into an NMR tube. The reaction mixtures were maintained at constant temperature in a block heater and ^{1}H NMR spectra were recorded at periodic intervals, typically every 0.5 h for faster reactions and every 4 h for slower reactions. At each time interval, the ester ($-CH_2-$) quartets of the starting cyanomalonate salt and alkylated product were integrated from the ^{1}H NMR spectrum so a percent conversion of cyanomalonate salt to alkylated cyanomalonate product could be calculated. The percent conversion of reactant to product was calculated by dividing the area of the product ester ($-CH_2-$) quartet by the combined areas of the reactant and product ester ($-CH_2-$) quartets and multiplying by 100. The percent conversion of reactant to product was plotted against time generating a rate plot that could be used to determine the effect of counter ion or solvent on the rate of alkylation of diethyl cyanomalonate by benzyl bromide. For crown ether studies, *cis*-dicyclohexano-18-crown-6 ether, was added to the reaction mixture of sodium diethyl cyanomalonate ester and benzyl bromide in acetone in 0.2 or 0.5 molar equivalents and heated at 50°C. For the electrophile survey, an electrophile (0.1 mmol) was added to a reaction mixture consisting of tetrabutylammonium diethyl cyanomalonate ester salt in DMSO and heated at 50°C.

2.5.3.2 Preparative Scale Alkylation of Diethyl Cyanomalonate with Alkyl Halides

Equimolar amounts of tetrabutylammonium diethyl cyanomalonate and alkyl halide were dissolved in DMSO (1 mmol tetrabutylammonium salt/1 mL DMSO) and heated at 80°C. After cooling to room temperature, water (50 mL) was added and the product was extracted into diethyl ether (4 x 25 mL). The combined ether extracts were back extracted with water (2 x 20 mL) to remove residual DMSO, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* giving an alkylated diethyl cyanomalonate as the product.

Diethyl 2-cyano-2-methylpropanedioate (2-10): Tetrabutylammonium diethyl cyanomalonate (0.853 g, 2.00 mmol) and methyl iodide (0.125 mL, 2.00 mmol) in DMSO (2 mL) were heated at 80°C for 2 h, giving diethyl 2-cyano-2-methylpropanedioate as an oil (0.324 g, 81%). IR (neat) cm^{-1}: 2252 (CN), 1748 (C=O). ^1H NMR (CDCl$_3$) δ 1.34 (t, J = 8, 6H), 1.84 (s, 3H), 4.34 (q, J = 8, 4H). ^{13}C NMR (CDCl$_3$) δ 14.0, 20.8, 50.0, 64.1, 116.0, 164.4. EIMS (70 eV) 200 (MH$^+$, 0.4), 154 (58), 127 (70), 99 (100), 98 (12), 82 (41), 71 (20). HRMS (70 eV): calculated for $C_9H_{14}NO_4$ = 200.0924 amu, found = 200.0924 ± 0.0008 amu.

Diethyl 2-cyano-2-ethylpropanedioate (2-11): Tetrabutylammonium diethyl cyanomalonate (0.853 g, 2.00 mmol) and ethyl iodide (0.160 mL, 2.00 mmol) in DMSO (2 mL) were heated at 80°C for 2 h, giving diethyl 2-cyano-2-ethylpropanedioate as an oil (0.302 g, 92%). IR (neat) cm^{-1}: 2251 (CN), 1748 (C=O). ^1H NMR (CDCl$_3$) δ 1.13 (t, J = 8, 3H), 1.33 (t, J = 8, 6H), 2.25 (q, J = 8, 2H), 4.32 (q, J = 8, 4H). ^{13}C NMR (CDCl$_3$) δ

9.7, 14.0, 28.1, 56.4, 63.9, 115.1, 163.9. EIMS (70 eV) 214 (MH$^+$, 9), 170 (11), 154 (50), 143 (15), 142 (24), 126 (50), 124 (15), 112 (11), 99 (11), 98 (100), 97 (34), 69 (38), 68 (49), 66 (22), 65 (6), 64 (17), 55 (17), 54 (23), 53 (30), 52 (92), 51 (14). HRMS (70 eV): calculated for $C_{10}H_{16}NO_4$ = 214.1079 amu, found = 214.1084 ± 0.0008 amu.

Diethyl 2-cyano-2-butylpropanedioate (2-12): Tetrabutylammonium diethyl cyanomalonate (0.853 g, 2.00 mmol) and *n*-butyl iodide (0.246 mL, 2.00 mmol) in DMSO (2 mL) were heated at 80°C for 2 h, giving diethyl 2-cyano-2-*n*-butylpropanedioate as an oil (0.417 g, 86%). IR (neat) cm^{-1}: 2251 (CN), 1750 (C=O). ^{1}H NMR (CDCl$_3$) δ 0.94 (t, J = 7, 3H), 1.33 (t, J = 7, 6H), 1.44 (m, 4H), 2.17 (m, 2H), 4.32 (q, J = 7, 4H). ^{13}C NMR (CDCl$_3$) δ 14.0, 22.4, 27.4, 29.0, 34.0, 55.6, 63.9, 115.3, 163.9. EIMS (70 eV) 242 (MH$^+$, 12), 128 (22), 96 (71), 80 (35), 55 (40), 42 (38). HRMS (70 eV): calculated for $C_{12}H_{19}NO_4$ = 241.1314 amu, found = 241.1318 ± 0.0008 amu.

Diethyl 2-cyano-2-isopropylpropanedioate (2-13): Tetrabutylammonium diethyl cyanomalonate (0.853 g, 2.00 mmol) and 2-iodopropane (0.197 mL, 2.00 mmol) in DMSO (2 mL) were heated at 80°C for 2 h giving diethyl 2-cyano-2-isopropylpropanedioate as an oil (0.303 g, 67%). IR (neat) cm^{-1}: 2252 (CN), 1750 (C=O). ^{1}H NMR (CDCl$_3$) δ 1.12 (d, J = 7, 6H), 1.33 (t, J = 7, 6H), 2.78 (sep., J = 7, 1H), 4.32 (q, J = 7, 4H). ^{13}C NMR (CDCl$_3$) δ 13.9, 18.4, 33.6, 62.1, 63.7, 113.9, 163.3. EIMS (70 eV) 228 (MH$^+$, 0.6), 182 (15), 168 (43), 157 (15), 140 (100), 126 (28), 112 (40), 99 (5).

Diethyl 2-cyano-2-benzylpropanedioate (2-9): Tetrabutylammonium diethyl

cyanomalonate (0.853 g, 2.00 mmol) and benzyl bromide (0.238 mL, 2.00 mmol) in

DMSO (2 mL) were heated at 80°C for 0.5 h, giving diethyl 2-cyano-2-

benzylpropanedioate (0.520 g, 95%). IR (neat) cm^{-1}: 3090-3034 (sp^2 CH), 2252 (CN),

1750 (C=O). ^{1}H NMR (CDCl$_3$) δ 1.24 (t, J = 8, 6H), 3.48 (s, 2H), 4.26 (q, J = 8, 4H),

7.31 (s, 5H). ^{13}C NMR (CDCl$_3$) δ 14.0, 39.8, 57.0, 64.1, 115.0, 128.4, 128.8, 130.2,

133.2, 163.7. EIMS (70 eV) 275 ($M^{+\bullet}$, 0.1), 174 (1), 156 (2), 130 (3), 128 (2), 91 (37),

84 (22), 49 (20), 35 (41), 29 (100). HRMS (70 eV): calculated for $C_{15}H_{17}NO_4$ =

275.1157 amu, found = 275.1160 ± 0.0008 amu.

Diethyl 2-cyano-2-allylpropanedioate (2-14): Tetrabutylammonium diethyl

cyanomalonate (0.853 g, 2.00 mmol) and allyl bromide (0.173 mL, 2.00 mmol) in DMSO

(2 mL) were heated at 80°C for 0.5 h, giving diethyl 2-cyano-2-allylpropanedioate as an

oil (0.332 g, 74%). IR (neat) cm^{-1}: 3087 (sp^2 CH), 2254 (CN), 1750 (C=O). ^{1}H NMR

(CDCl$_3$) δ 1.33 (t, J = 7, 6H), 2.92 (d, J = 7, 2H), 4.32 (q, J = 7, 4H), 5.26-5.36 (m, 2H),

5.78-5.91 (m, 1H). ^{13}C NMR (CDCl$_3$) δ 14.0, 38.5, 55.3, 64.0, 114.8, 121.9, 129.6, 163.3.

EIMS (70 eV) 226 (MH^+, 0.04), 43 (4), 42 (2), 39 (6), 29(100). HRMS (70 eV):

calculated for $C_{11}H_{15}NO_4$ = 225.1001 amu, found = 225.1003 ± 0.0008 amu.

Diethyl 2-cyano-2-(2-ethylbenzene)propanedioate (2-15): Tetrabutylammonium

diethyl cyanomalonate (0.853 g, 2.00 mmol) and 1-bromoethylbenzene (0.273 mL, 2.00

mmol) in DMSO (2 mL) were heated at 80°C for 2 h, yielding diethyl 2-cyano-2-(2-

ethylbenzene)propanedioate as an oil (0.44 g, 76%). IR (neat) cm^{-1} 3089-3033 (sp^2 CH),

2250 (CN), 1752 (C=O). ^{1}H NMR (CDCl$_3$) δ 0.95 (t, J = 7, 3H), 1.35 (t, J = 7, 3H), 1.55

(d, J = 7, 3H), 3.91 (d of q, 2H), 4.37 (q, J = 7, 2H), 7.26-7.34 (m, 5H). ^{13}C NMR

(CDCl$_3$) δ 13.8, 14.2, 18.2, 44.2, 62.5, 63.8, 64.3, 114.8, 128.5, 128.7, 128.8, 139.0,

162.0, 163.2. EIMS (70 eV) 289 (M$^{+•}$, 8), 115 (25), 106 (52), 104 (73), 77 (58), 28 (100).

HRMS (70 eV): calculated for C$_{16}$H$_{19}$NO$_4$ = 289.1314 amu, found = 289.1306 ± 0.0008

amu.

Ethyl methyl 2-carboxyethyl-2-cyano-3-phenylbutanedioate (2-16):

Tetrabutylammonium diethyl cyanomalonate (1.28 g, 3.00 mmol) and methyl-2-bromo-2-

phenylacetate (methyl α-bromophenylacetate) (0.315 mL, 2.00 mmol) in DMSO (3 mL)

were heated at 80°C for 2 h, yielding a colourless solid (0.467 g, 70%). Recrystallization

in methanol gave ethyl, methyl 2-carboxyethyl-2-cyano-3-phenylbutanedioate as a

colourless crystalline solid (0.256 g, 38%). m.p. 82-83°C. IR (Nujol) cm^{-1} 3065-3034 (sp^2

CH), 2255, 2202 (CN), 1742 (C=O). ^{1}H NMR (CDCl$_3$) δ 1.06 (t, J = 7, 3H), 1.35 (t, J =

7, 3H), 3.73 (s, 3H), 4.10 (q, J = 7, 2H), 4.37 (q, J = 7, 2H), 4.74 (s, 1H), 7.37 (s, 5H).

^{13}C NMR (CDCl$_3$) δ 13.6, 13.8, 53.0, 54.1, 59.2, 64.3, 64.8, 113.9, 129.1, 129.2, 129.4,

131.6, 162.3, 162.8, 170.2. EIMS (70 eV) 333 (M$^{+•}$, 38), 302 (24), 301 (90), 229 (41),

228 (100), 219 (20), 174 (21), 156 (52), 149 (81). HRMS (70 eV): calculated for

C$_{17}$H$_{19}$NO$_6$ = 333.1212 amu, found = 333.1213 ± 0.0008 amu.

Ethyl 2-carboxyethyl-2,3-dicyanopropanoate (2-17): Tetrabutylammonium diethyl

cyanomalonate (0.853 g, 2.00 mmol) and bromoacetonitrile (1.39 mL, 2.00 mmol) in

DMSO (2 mL) were heated at 80°C for 10 min, yielding ethyl 2-carboxyethyl-2,3-

dicyanopropanoate as an oil (0.225 g, 50%). IR (neat) cm^{-1}: 2262 (CN), 1756 (C=O). ^{1}H

NMR (CDCl$_3$) δ 1.37 (t, J = 7 6H), 3.26 (s, 2H), 4.41 (q, J = 7, 4H). ^{13}C NMR (CDCl$_3$) δ

13.8, 23.3, 51.7, 65.4, 112.7, 113.8, 161.2. EIMS (70 eV) 224 (M$^{+\bullet}$, 1), 152 (34), 151

(16), 124 (85.9), 123 (73), 119 (25), 107 (28), 96 (100), 80 (47), 54 (22). HRMS (70

eV): calculated for C$_{10}$H$_{12}$N$_2$O$_4$ = 224.0797 amu, found = 224.0793 ± 0.0008 amu.

2.5.3.3 Alkylation by Base-Sensitive Electrophiles

Diethyl 2-((4-carboxyphenyl)methyl)-2-cyanopropanedioate (2-18):

Tetrabutylammonium diethyl cyanomalonate (0.853 g, 2.00 mmol) and α-bromo-p-toluic

acid (0.430 g, 2.00 mmol) in DMSO (2 mL) were heated at 80°C for 0.5 h. After cooling

to room temperature, water (20 mL) was added and the product was extracted into diethyl

ether (5 x 10 mL). The ether extract was dried over anhydrous MgSO$_4$ and evaporated *in*

vacuo to yield diethyl 2-((4-carboxyphenyl)methyl)-2-cyanopropanedioate as a white

powder (0.491 g, 77%). m.p. 107-109°C. IR (neat) cm^{-1}: 2254 (CN), 1742 (C=O). ^{1}H

NMR (acetone-d_6) δ 1.31 (t, J = 7, 6H), 3.62 (s, 2H), 4.36 (q, J = 7, 4H), 7.49 (d, J = 8,

2H), 8.03 (d, J = 8, 2H), 13.01 (br. s, 1H). ^{1}H NMR (DMSO-d_6) δ 1.23 (t, J = 7, 6H),

3.43 (s, 2H), 4.29 (q, J = 7, 4H), 7.43 (d, J = 8, 2H), 7.96 (d, J = 8, 2H), 13.06 (br. s, 1H).

^{13}C NMR (DMSO-d_6) δ 13.6, 56.6, 64.1, 114.7, 129.5, 130.2, 130.5, 138.4, 162.8, 167.0.

EIMS (70 eV) 319 (M$^{+\bullet}$, 2), 246 (26), 218 (15), 200 (32), 156 (15), 135 (59), 128 (13),

107 (25), 77 (22), 43 (14), 29 (100). HRMS (70 eV): calculated for C$_{16}$H$_{17}$NO$_6$ =

319.1056 amu, found = 319.1048 ± 0.0008 amu. ESI⁻MS m/z 318 [M-H]⁻; CID (22%) of

m/z 318 m/z 274 (100), 246 (21).

Ethyl 2-carboxyethyl-2-cyanopentanedioic acid (2-19): Tetrabutylammonium diethyl

cyanomalonate (0.853 g, 2.00 mmol) and 3-iodopropanoic acid (0.400 g, 2.00 mmol)

were dissolved in 2 mL of DMSO. The mixture was maintained at 80°C in a heating

block for 2 h. The reaction was allowed to cool to room temperature, water (25 mL) was

added and the product was extracted into diethyl ether (4 x 20 mL). The ether layers were

combined and extracted (4 x 20 mL) with saturated sodium bicarbonate. The sodium

bicarbonate layer was acidified with conc. HCl and extracted with diethyl ether (4 x 25

mL). The ether extract was dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to

yield ethyl 2-carboxyethyl-2-cyanopentanedioic acid as an oil (0.230 g, 45%). ^{1}H NMR

($CDCl_3$) δ 1.29 (t, J = 8, 3H), 2.53 (m, 4H), 4.28 (q, J = 8, 4H), 9.52 (s, 1H). ^{13}C NMR

($CDCl_3$) δ 13.7, 28.6, 29.6, 54.2, 64.2, 114.3, 163.1, 171.1.

1-Ethyl 2-carboxyethyl-2-cyanohexanedioic acid (2-20): Two parallel reactions each

consisting of tetrabutylammonium diethyl cyanomalonate (2.13 g, 5.00 mmol) and 4-

iodobutanoic acid (1.04 g, 4.85 mmol) dissolved in DMSO (5 mL) were heated for 2 h at

80°C. The reaction mixture was allowed to cool to room temperature, 150 mL of water

was added and the mixture was extracted with ether (4 x 50 mL). The ether layers were

combined, dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* yielding

diethyl 2-carboxyethyl-2-cyanohexanedioic acid as an oil (0.57 g, 22%). ^{1}H NMR

($CDCl_3$) δ 1.34 (t, J = 7, 6H), 1.82 (m, 2H), 2.25 (m, 2H), 2.47 (t, J = 7, 2H), 4.33 (q, J =

7, 4H). ^{13}C NMR ($CDCl_3$) δ 13.8, 33.0, 33.1, 20.2, 55.1, 64.0, 114.7, 163.4, 178.0.

extracted with ether (4 x 10 mL). The ether layers were combined, dried over anhydrous MgSO$_4$ and the solvent was removed *in vacuo* yielding diethyl 2-carboxyethyl-2-cyanohexanedioic acid as an oil (0.57 g, 22%). ^{1}H NMR (CDCl$_3$) δ 1.34 (t), 3.31 (s), 4.35 (q), 9.52 (br. s). Side products ^{1}H NMR (CDCl$_3$) δ 1.2-1.4, 3.4-3.55, 4.2-4.4, 7.28 (s), 8.20.

Amide Side Product (2-25): Tetrabutylammonium diethyl cyanomalonate (2.13 g, 5.00 mmol) and iodoacetic acid (0.930 g, 5.00 mmol) dissolved in DMSO (5 mL) were heated for 19 h at 50°C. The reaction mixture was allowed to cool to room temperature, 25 mL of water was added and the mixture was extracted with ether (4 x 25 mL). The ether layers were combined and treated with charcoal and filtered through Celite. The filtrate was dried with CaCl$_2$ and evaporated *in vacuo* giving a brown solid. Recrystallization of solid from acetonitrile yielded the amide side product as a white powder (0.119 g). ^{1}H NMR (DMSO-d_6) δ 1.62 (t, $J = 7$, 6H), 3.50 (s, 2H), 4.60 (q, $J = 7$, 4H), 7.93 (s, 1H), 8.10 (s, 1H), 12.92 (br. s, 1H). ^{13}C NMR (DMSO-d_6) δ 14.9, 38.7, 63.1, 63.8, 167.3, 168.1, 172.7.

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Chapter 3: Synthesis of 4-Aminobutanoic acid (GABA)

3.1 Introduction

4-Aminobutanoic acid (4-aminobutyric acid, GABA, Figure 3.1, **3-1**), the most

widespread inhibitory neurotransmitter in the mammalian central nervous system, is

responsible for synaptic inhibition in the vertebrate central nervous system, thereby

playing an important role in neurological function and disease.[1]

3-1
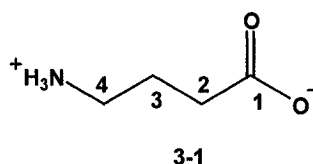
Figure 3.1 4-Aminobutanoic Acid (GABA).

Abnormal levels of GABA (**3-1**) have been linked to several neurological diseases

and its detection and quantification continues to be an important focus.[2] As a result,

deuterium labelled GABA is particularly sought after as an internal standard for the

analysis of GABA from human plasma and cerebrospinal fluid by mass spectrometry.[2,3,4]

Isotopically labelled samples of GABA also have been used as precursors in drug

synthesis,[5] substrates for stereochemical and mechanistic enzyme studies[6] and as samples

for NMR molecular dynamics studies.[7]

In Chapter 1, the structural analogy between ω-amino acids, including GABA,

and alkylated diethyl cyanomalonate was described within a retrosynthetic scheme for the

synthesis of ω-amino acids from diethyl cyanomalonate (Scheme 1.24). For the synthesis

of GABA (Scheme 3.1), alkylation of diethyl cyanomalonate anion (**1-1A**) by ethyl

bromoacetate (**3-3**) would be followed by a short sequence of transformations, including

Krapcho[8] decarboethoxylation, ester hydrolysis and nitrile reduction,[9] to complete the

synthesis of GABA (3-1).
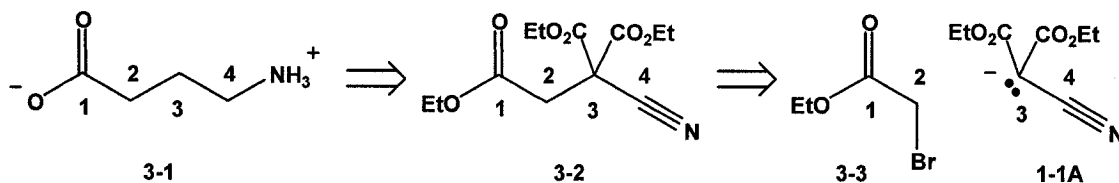
Scheme 3.1 Retrosynthetic analysis showing disconnection between C-2 and C-3 of GABA.

In the published syntheses of several deuterium and tritium labelled GABA

isotopomers, either reduction or exchange reactions have been used to introduce

hydrogen isotopes into a synthetic intermediate or GABA directly. Alternatively,

incorporation of a hydrogen isotope after carbon-carbon bond cleavage has been done

enzymatically using glutamate decarboxylase.[10] The association of isotope incorporation

with a bond cleavage reaction offers the potential for a one-step incorporation of high

levels of isotopic label from readily available D_2O. In the synthesis of GABA outlined in

Scheme 3.1, the Krapcho[8] decarboethoxylation step offers a similar opportunity for the

introduction of a hydrogen isotope. As indicated by the review of existing methods for

the preparation of labelled GABA in the next section, a synthesis from diethyl

cyanomalonate would be novel and would yield $[3,3-^2H_2]$GABA (**3-40**), an isotopomer

not available via published routes.

3.2 Literature Syntheses of Labelled GABA

A combination of chemical and enzymatic approaches developed to synthesize various isotopomers of GABA, including the commercially available [2,2-^{2}H$_2$]GABA,[2] [2,2,3,3-^{3}H$_2$]GABA[11,12,13,14] and [2,2,3,3,4,4-^{2}H$_6$]GABA,[6] are described in detail in the following subsection.

3.2.1 [2,2-^{2}H$_2$]GABA

Exchange at C-2 (the most labile hydrogens, pK$_a$ = 32)[15] yielding [2,2-^{2}H$_2$]GABA (3-4) has been accomplished by heating GABA in a sealed tube containing D$_2$O and either DCl[4,5,6,7] or NaOD[3] (Scheme 3.2, Table 3.1). Multiple exchange reactions each requiring up to several days at high temperature were necessary to obtain high (>95%) levels of isotope incorporation.
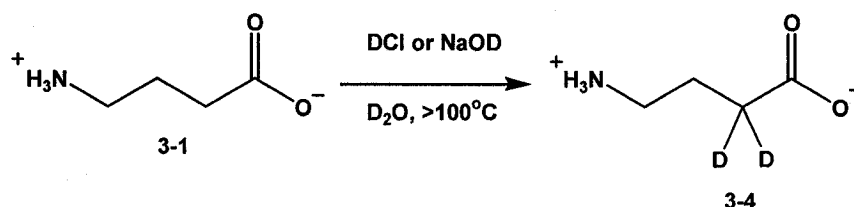
Scheme 3.2 Exchange reactions yielding [2,2-^{2}H$_2$]GABA (Refer to Table 3.1 for specific conditions).

Table 3.1 Exchange reaction conditions for the preparation of [2,2-^{2}H$_2$]GABA (3-5).

Ref.	Conditions	Exchanges (#)	% Yield	% ^{2}H
5	9% DCl, D$_2$O, 120°C, 3.5 d	3	100	93% ^{2}H$_2$, 7% ^{2}H$_1$ (MS)
6	9% DCl, D$_2$O, 120°C, 4 d	3	-*	96-97%
7	DCl, 100°C, 5 d	1	-*	-*
4	8% DCl, D$_2$O, 130°C, 12 d	3	-*	-*
11	5 M DCl, D$_2$O, 120°C, 2 d	1	-*	"quantitative"
3	20% NaOD, 120°C, 4 h	1	-*	-*

*no data reported

3.2.2 [2,2,3,3-^{3}H$_4$]GABA

The catalytic reduction of a triple bond with ^{3}H$_2$ as the source of label has been used in each of the reported syntheses of [2,2,3,3-^{3}H$_4$]GABA (3-8). Accordingly, methyl 4-phthalimido-2-butynoate (3-7) was prepared by sequential alkylation of the phthalimide ion (3-6) and methoxycarbonylation and then reduced using ^{3}H$_2$/Pd/C (Scheme 3.3).[16] Acid hydrolysis yielded [2,2,3,3-^{3}H$_4$]GABA (3-8) with a specific activity of 109 Ci/mmol. Each ^{3}H atom gives 29 Ci/mmol,[17] so 100% substitution by four ^{3}H atoms would give 116 Ci/mmol. Therefore, 109 Ci/mmol indicates that about 94% of the C-2/C-3 protons were replaced by ^{3}H. ^{3}H NMR spectroscopy showed 40% ^{3}H at C-2 and 60% ^{3}H at position C-3.
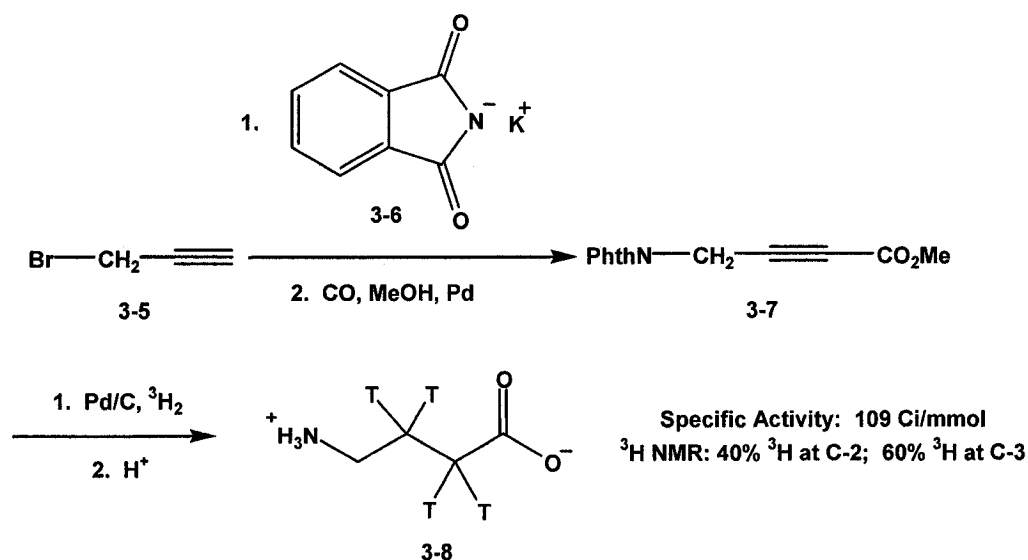
Scheme 3.3 Synthesis of [2,2,3,3-^{3}H$_4$]GABA (Phth = phthaloyl).[16]

A similar strategy was employed for the syntheses of [2,2,3,3-^{3}H$_4$]GABA (3-8) and the corresponding E and Z [1,2-^{3}H$_2$]-4-aminobut-2-enoic acids (Scheme 3.4).[18] The carbon skeleton of GABA was provided by methyl 4-phthalimidobut-2-ynol (3-9).

Oxidation and esterification of the alcohol yielded the substrate for reduction using

RhCl(PPh$_3$) and ^{3}H$_2$ gas. The mixture of reduced products (% determined by ^{1}H NMR

integration) was treated with acid to remove the ester and phthaloyl groups, and, after a

lengthy workup, [2,2,3,3-^{3}H$_4$]GABA (3-8), (E)-[1,2-^{3}H$_2$]-4-aminobut-2-enoic acid (3-14)

and (Z)-[1,2-^{3}H$_2$]-4-aminobut-2-enoic acid (3-13) were separated by HPLC. In the

labelled GABA product, an isotopic distribution of 38% ^{3}H$_4$, 28% ^{3}H$_3$, 21% ^{3}H$_2$, 6% ^{3}H$_1$

and 6% ^{1}H was determined from isotope peaks shown in the CI mass spectrum for the

[M+H$^+$-H$_2$O] ion. A specific activity of 83.3 Ci/mmol was calculated from the relative

intensities of the peaks assigned to tritiated ions in the mass spectrum.
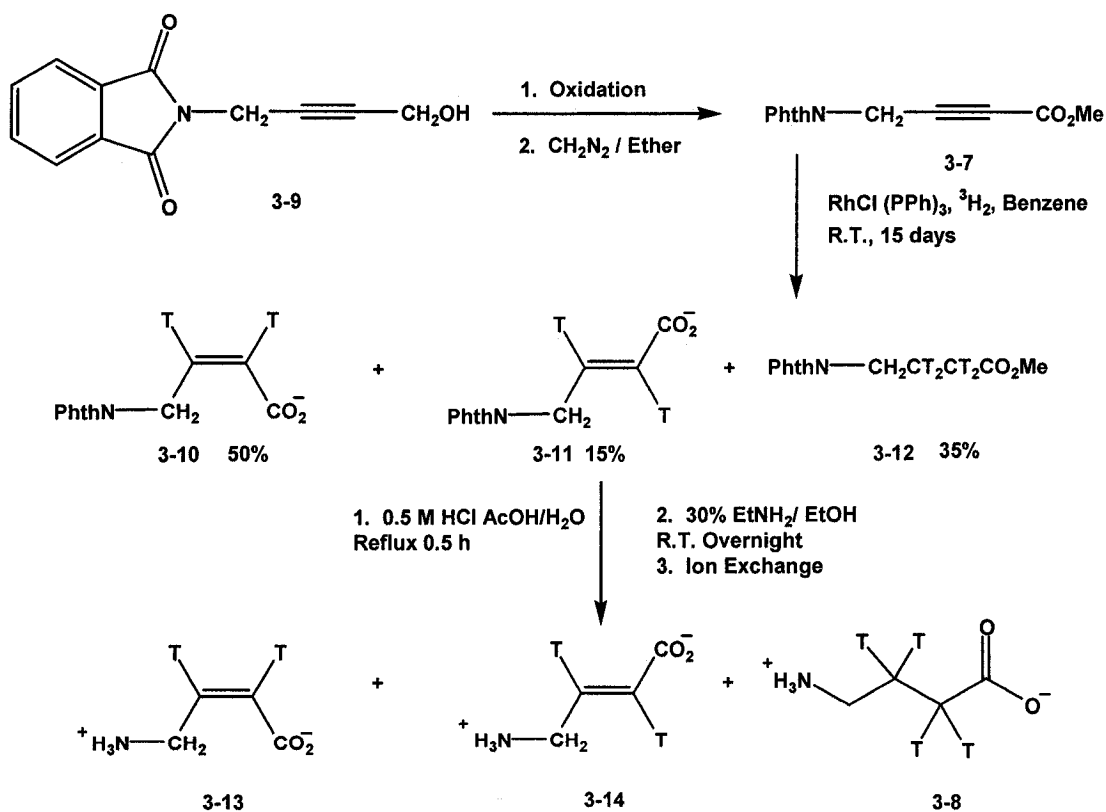
Scheme 3.4 Synthesis of [2,2,3,3-^{3}H$_4$]GABA, (E)-[1,2-^{3}H$_2$]-4-aminobut-2-enoic acid and
(Z)-[1,2-^{3}H$_2$]-4-aminobut-2-enoic acid.[18]

The third synthesis of $[2,2,3,3\text{-}^3H_4]$GABA (**3-8**, Scheme 3.5) used a Pd catalyzed

reduction of a *bis* trimethylsilyl derivative of 4-aminotetrolic acid with $^3H_2$.[19] The

trimethylsilyl (TMS) derivative (**3-16**) was synthesized in order to increase the solubility

of 4-aminotetrolic acid (**3-15**) in the reduction medium and to mask labile hydrogen

atoms on the amino and carboxyl groups. $[2,2,3,3\text{-}^3H_4]$GABA (**3-8**) was obtained with a

specific activity of 97.3 Ci/mmol, indicating 84% ^3H at C-2/C-3.

H_2N ⎯⎯⎯CO_2H

3-15

N,O-bis(trimethylsilyl)
acetamide
⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯→
Dioxane, R.T., 1 h

TMS⎯NH ⎯⎯⎯CO_2TMS

3-16

1. 10% Pd/O,
$^3H_2$, R.T., 3 h
⎯⎯⎯⎯⎯⎯⎯⎯⎯→
2. HCl, Chrom.

^+H_3N — (T,T) — (T,T) — CO_2^-

3-8

97.3 Ci/mmol
84% ^3H at C-2/C-3
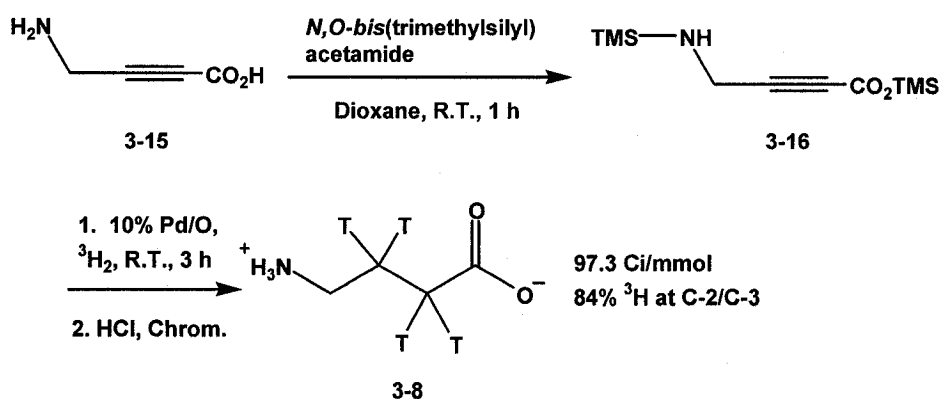
Scheme 3.5 Synthesis of $[2,2,3,3\text{-}^3H_4]$GABA.[19]

3.2.3 $[3\text{-}^3H]$GABA

An indirect procedure based on the exchange reactions leading to $[2,2\text{-}^2H_2]$GABA

(**3-5**) was used to obtain $[3\text{-}^3H]$GABA by heating commercially available $[2,3\text{-}^3H]$GABA

in H_2O under acidic conditions.[11] Under these conditions, the tritium atoms on C-2

exchanged with protons in the aqueous solution while the less acidic tritium atoms on C-3

remained giving a product with 48% radiochemical yield, implying that half the tritium

was exchanged out of the original sample.

3.2.4 [4,4-^{2}H$_2$]GABA

A synthesis of [4,4-^{2}H$_2$]GABA[5,6] (3-22) has been reported (Scheme 3.6) where the carbon skeleton of GABA was provided by cyanide and ethyl 3-bromopropanoate (3-17), and the nitrogen was provided by potassium phthalimide. Deuterium was introduced in the first step by reducing ethyl 3-bromopropanoate to the alcohol with LiAlD$_4$. Nucleophilic substitution using potassium cyanide displaced bromide ion and treatment of the alcohol (3-19) with NBS converted the alcohol to [2,2-^{2}H$_2$]-1-bromo-3-cyanopropane (3-20). Reaction of the bromide with potassium phthalimide gave [3,3-^{2}H$_2$]-3-phthalimido-n-propyl cyanide (3-21) which was hydrolyzed under acidic conditions to [4,4-^{2}H$_2$]GABA (3-22) containing 98% ^{2}H$_2$ by mass spectrometry.
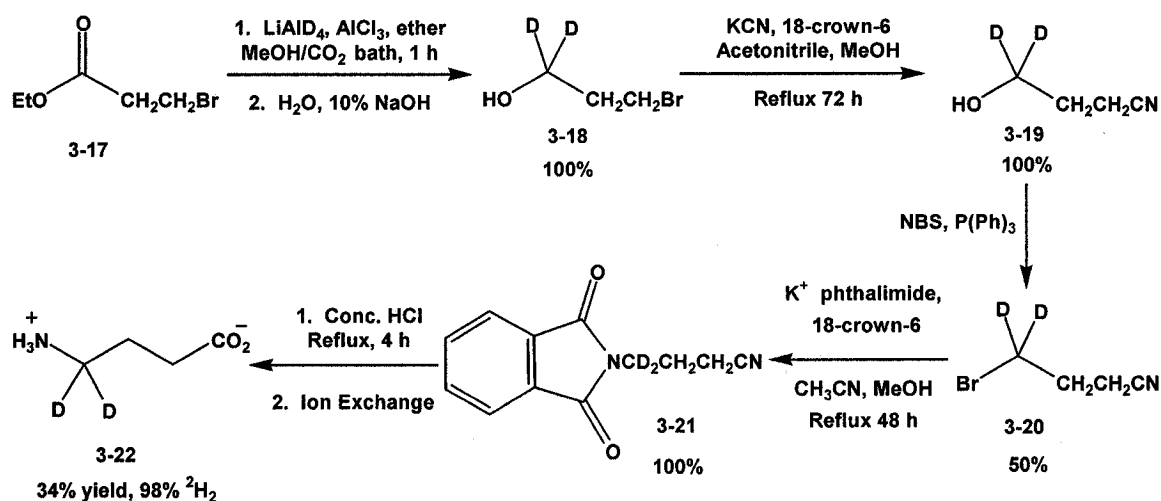
Scheme 3.6 Synthesis of [4,4-^{2}H$_2$]GABA.[5]

3.2.5 [2,2,3,3,4,4-^{2}H$_6$]GABA

The synthesis[20] of [2,2,3,3,4,4-^{2}H$_6$]GABA (**3-26**) introduced deuterium via two independent steps (Scheme 3.7). In the first step, [3,3,4,4-^{2}H$_4$]succinimide (**3-24**) was obtained from a very lengthy exchange reaction (30 days) under basic conditions. In the second step, reduction of one of the amide carbonyl groups with LiAlD$_4$ gave [2,2,3,3,4,4-^{2}H$_6$]pyrrolidinone (**3-25**). [2,2,3,3,4,4-^{2}H$_6$]GABA (**3-26**) containing 77% ^{2}H$_6$ and 15% ^{2}H$_5$ was obtained by amide hydrolysis in D$_2$O to avoid loss of deuterium.
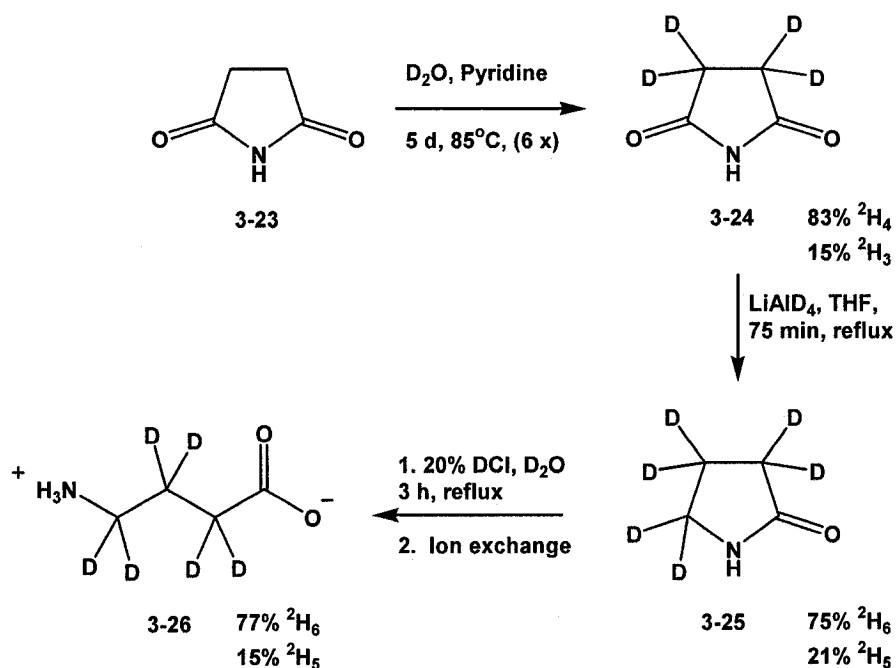
Scheme 3.7 Synthesis of [2,2,3,3,4,4-^{2}H$_6$]GABA.[20]

3.2.6 (*R*)-[4-^{2}H]- and (*S*)-[4-^{2}H]GABA

Glutamate decarboxylase is a pyridoxal phosphate dependent enzyme that catalyses the decarboxylation of glutamic acid giving GABA as the product.[10] It was shown that decarboxylation of (2*S*)-glutamic acid (**3-27**) catalyzed by glutamate

99

decarboxylase in D_2O resulted in $(4R)$-[4-^{2}H]GABA (Scheme 3.8, **3-28**).[10,21] Its

enantiomer, $(4S)$-[4-^{2}H]GABA (**3-30**) has been prepared by using glutamate

decarboxylase to decarboxylate $(2S)$-[2-^{2}H] glutamic acid in H_2O (Scheme 3.8, **3-29**).[22,23]

Commercially available (R,S)-[2,4,4-$^2H_3$] glutamic acid has also been decarboxylated by

glutamate decarboxylase to give $(4S)$-[2,2,4-$^2H_3$]GABA.[26] These chirally labelled GABA

molecules have been used as precursors for the synthesis of amino acids[24,25,26] and other

compounds[27,28] that have found application in stereochemical studies of enzyme

catalyzed reactions.
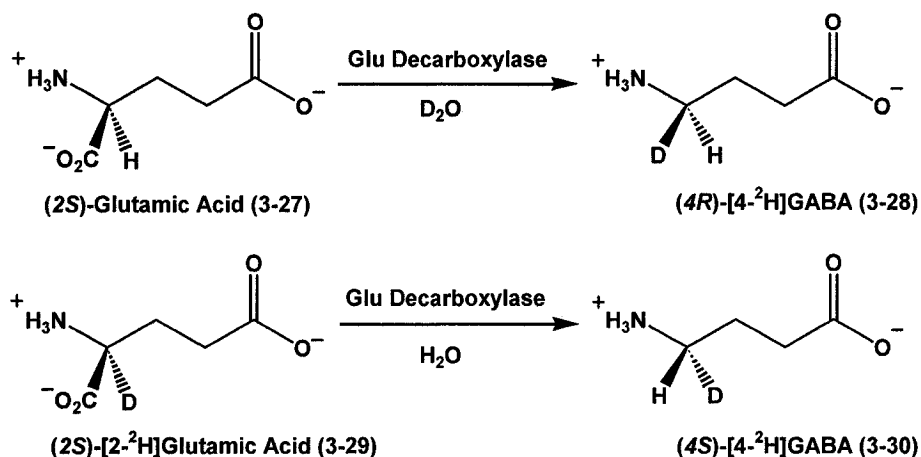
Scheme 3.8 Synthesis of chirally labelled GABA using glutamate decarboxylase.[14,21,22,23]

3.2.7 $(3S)$-[3-^{2}H]GABA, $(3S)$-[3-^{3}H]GABA and $(3R)$-[3-^{3}H]GABA

Syntheses of $(3S)$-[3-^{2}H]GABA, $(3S)$-[3-^{3}H]GABA (**3-34**) and $(3R)$-[3-^{3}H]GABA

(**3-39**) have been reported (Scheme 3.9).[29] The chirality of the final product depended on

the step in which ^3H_2O or ^2H_2O was added. For $(3S)$-[3-^{3}H]GABA and $(3S)$-[3-

^{2}H]GABA, tritium and deuterium, respectively, were introduced prior to decarboxylation

during the conversion of *threo*-D_s isocitrate ($2R,3S$-isocitrate, **3-31**) to α-ketoglutarate (3-

32) catalyzed by the enzyme isocitrate dehydrogenase (Scheme 3.9). For (3*R*)-[3-

^{3}H]GABA, tritium was introduced during the conversion of *cis*-aconitate (3-35) to *threo*-

D$_s$ isocitrate (3-36) catalyzed by the enzyme aconitase (Scheme 3.10).
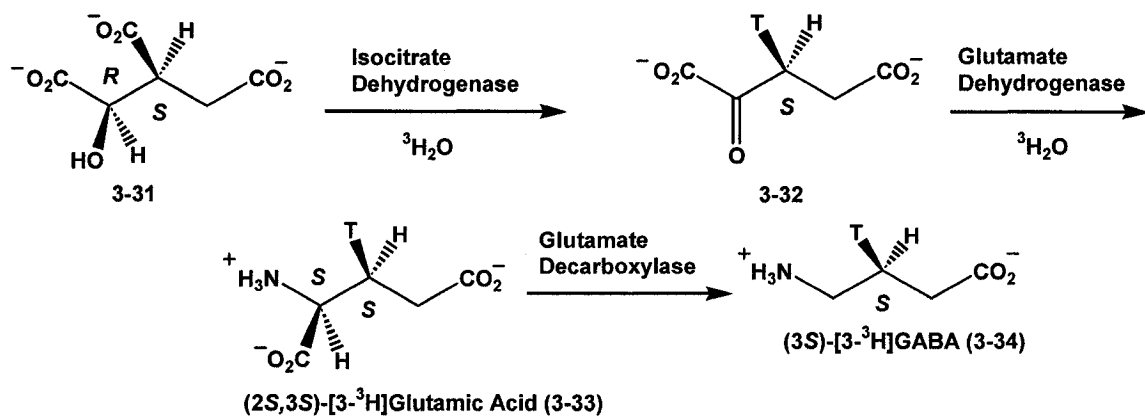
Scheme 3.9 Synthesis of (3*S*)-[3-^{3}H]GABA.[29]
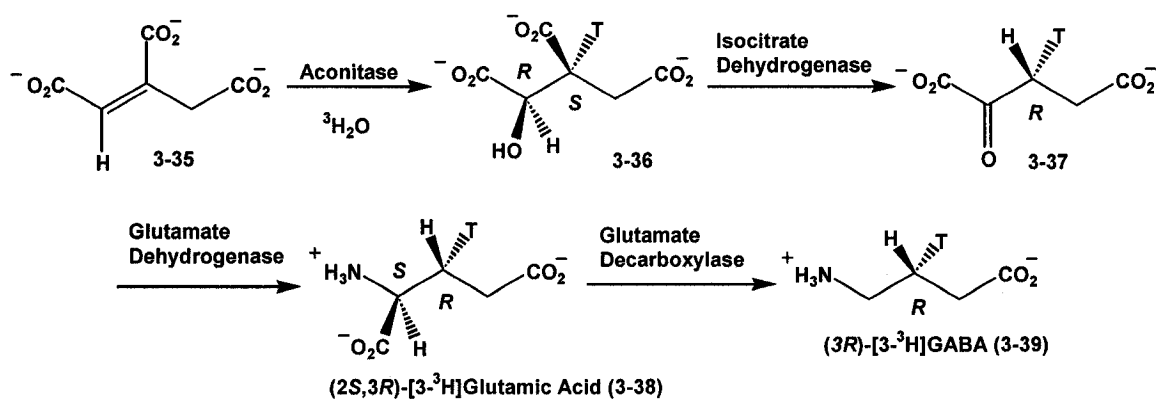
Scheme 3.10 Synthesis of (3*R*)-[3-^{3}H]GABA.[29]

3.3   Attempted Synthesis of [3,3-^{2}H$_2$]GABA From an Alkylated Cyanomalonate

A clear omission from the literature is a straightforward preparation of

specifically labelled [3,3-^{2}H$_2$]GABA (3-40). The focus of the remainder of this chapter is

the synthesis of [3,3-^{2}H$_2$]GABA.

A retrosynthetic scheme linking [3,3-^{2}H$_2$]GABA (3-40) to the diethyl

cyanomalonate anion (1-1A) is presented in Scheme 3.11. The key step in the sequence

is the proposed introduction of deuterium by heating diethyl 2-carboxyethyl-2-

cyanobutanedioate (3-2) under Krapcho[8] conditions in the presence of D$_2$O. In principle,

more efficient incorporation of deuterium would be expected from this reaction. Overall,

the geminal diester groups would be replaced with deuterium, giving ethyl [3,3-^{2}H$_2$]-3-

cyanopropanoate (3-41) and specifically labelled [3,3-^{2}H$_2$]GABA (3-40) would be

obtained following ester hydrolysis and nitrile reduction (Scheme 3.11).[9]
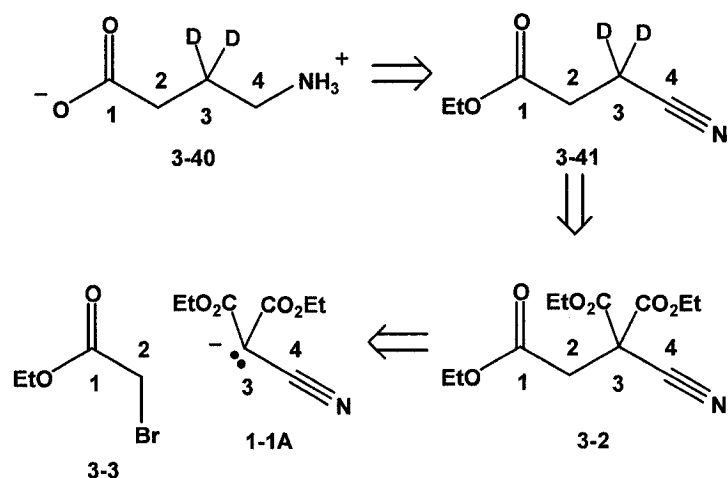
Scheme 3.11 Retrosynthetic analysis of [3,3-^{2}H$_2$]GABA.

The only literature precedent for introducing deuterium by the Krapcho reaction[30] is the decarboethoxylation of diethyl ethylmalonate in D_2O and DMSO. Ethyl butyrate was obtained with 80-90% $^2H_2$, but no further experimental details were provided.

As an alternative, deuterium exchange adjacent to a nitrile during acid-catalysed ester hydrolysis and thermal decarboxylation has been documented in the literature (Scheme 3.12).[31] [2-^{2}H]-2-Cyanopropanoic acid (**3-43**) was obtained after acid-catalyzed ester hydrolysis of ethyl-2-cyanopropanoate (**3-42**) was carried out in D_2O. Thermal decarboxylation, followed by nitrile reduction, gave [2,2-$^2H_2$]propylamine (**3-45**) with an overall deuterium incorporation of 96.5% as determined by mass spectrometry.

EtO$_2$C — CN
CH$_3$

1. D$_2$O, CH$_3$CO$_2$D, DCl Heat
2. D$_2$O, Heat

DO$_2$C — CN
H$_3$C   D

Heat

CN
D   D

LiAlH$_4$

NH$_2$
D   D

3-42   3-43   3-44   3-45
                        53% yield   54% yield
                                    93% d_2, 7% d_1
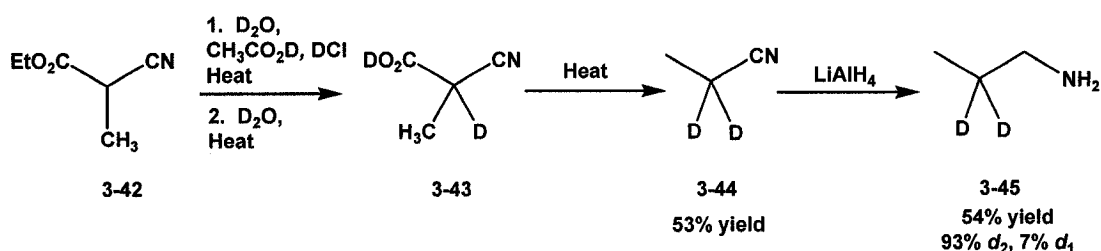
Scheme 3.12 Synthesis of [2,2-$^2H_2$]propylamine.[31]

3.3.1 Synthesis of GABA from Diethyl Cyanomalonate

Before undertaking deuterium labeling studies, the feasibility of the synthetic approach outlined in Scheme 1.24 and Scheme 3.1 was established by synthesizing GABA (**3-1**) from diethyl cyanomalonate (**1-1**, Scheme 3.13).
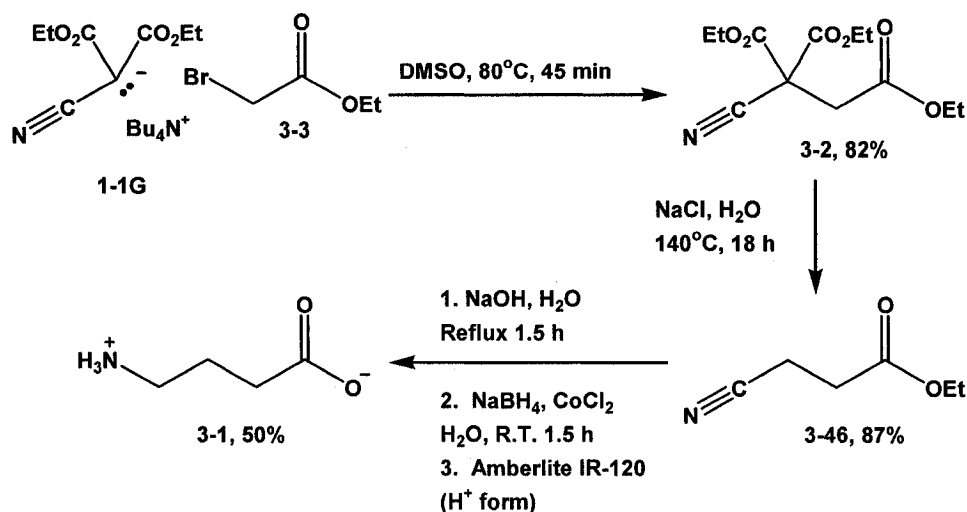
Scheme 3.13 Preparation of GABA from diethyl cyanomalonate.

Alkylation of tetrabutylammonium diethyl cyanomalonate (**1-1G**) was carried out

under optimized conditions (80°C, DMSO) developed from experiments described in

Chapter 2 of this thesis. Diethyl 2-carboxyethyl-2-cyanobutanedioate (**3-2**) was obtained

in high yield (82%) after only 45 min of heating and a simple workup (addition of water

and extraction into ether). As with all diethyl cyanomalonate alkylation reactions,

residual DMSO could be easily removed from the solution of the product in ether by a

back-extraction with water.

Progress of the Krapcho[8] didecarboethoxylation of diethyl 2-carboxyethyl-2-

cyanobutanedioate (**3-2**) at 140°C in DMSO-d_6 was followed by ^{1}H NMR spectroscopy at

2 h intervals. Decarboethoxylation was complete in 16 h; the final reaction mixture

consisted of ethyl 3-cyanopropanoate (**3-46**, δ_H 1.21(t), 2.62-2.72 (AA'BB'), 4.12 (q)) and

ethanol (δ_H 1.06 (t) and 3.07 (q)). This procedure, conveniently carried out overnight and

with a simple workup consisting of the addition of water and extraction into ether, gave

pure ethyl 3-cyanopropanoate in high yield (87%). By increasing the reaction

temperature to 180°C, complete didecarboethoxylation of diethyl 2-carboxyethyl-2-cyanobutanedioate (**3-2**) could be achieved in less time (only 0.5 h) in 71% yield.

Alkylation and didecarboethoxylation were also carried out as a one pot synthesis. Tetrabutylammonium diethyl cyanomalonate (**1-1A**) was alkylated by ethyl bromoacetate (**3-3**) in DMSO at 80°C giving diethyl 2-carboxyethyl-2-cyanobutanedioate (**3-2**), which was not isolated. After alkylation, water and NaCl were added, and the reaction mixture was heated to 180°C for 0.5 h. After work up, pure ethyl 3-cyanopropanoate (**3-46**) was obtained with a similar yield (69%) to that which was obtained via the two step procedure.

Following alkylation to form the carbon backbone and decarboethoxylation, ethyl 3-cyanopropanoate was subjected to a one-pot ester hydrolysis and nitrile reduction to give GABA (**3-1**) as a product. Hydrolysis of the ester was carried out by heating at reflux for 1.5 h with only a 10% excess of NaOH. The small excess of NaOH was used to minimize nitrile hydrolysis. Nitrile reduction[9,32] was achieved by carefully adding $NaBH_4/CoCl_2$ directly to the ester hydrolysis reaction mixture. GABA (**3-1**) was obtained as a colourless white powder following ion-exchange chromatography and freeze-drying. Overall, this short sequence of steps provided GABA with an overall yield of 36% (from Bu_4N^+ diethyl cyanomalonate).

3.3.2 Spectroscopic Characterization and Assessment of Deuterium Incorporation

The ^1H NMR spectrum of GABA (**3-1**) in D_2O gave three distinct signals: a quintet at δ_H 1.87 (2H) corresponding to H-3, a triplet at δ_H 2.26 (2H) corresponding to H-2 and a second triplet at δ_H 2.99 (2H) corresponding to H-4.[33] Integration of these well

separated signals and changes to the coupling patterns were used to determine the location and percent incorporation of deuterium into GABA. The ^{13}C NMR spectrum (D$_2$O) of unlabelled GABA gave four signals at δ_C 182.2 (C-1), 39.5 (C-2), 23.8 (C-3) and 34.6 (C-4).[34]

Positive ion electrospray mass spectrometry (ESI$^+$MS) of GABA (3-1) showed a peak at m/z 104 corresponding to the [M+H]$^+$ ion. Relative intensities of the isotope peaks in the [M+H]$^+$ cluster were used to estimate the relative abundance of d_0, d_1, d_2 and d_3 isotopomers. Collision induced dissociation (CID) of the [M+H]$^+$ ion derived from GABA gave two fragment ions resulting from loss of water (- 18 u) and loss of ammonia (- 17 u).

3.3.3 Introduction of Deuterium via Krapcho Decarboethoxylation

The feasibility of using the Krapcho[8] decarboethoxylation of diethyl 2-carboxyethyl-2-cyanobutanedioate (3-2) as a means of obtaining ethyl 3-cyanopropanoate (3-46) labelled specifically with deuterium on C-3 was evaluated in a series of trial experiments monitored by ^{1}H NMR spectroscopy (Table 3.2).

While the individual ^{1}H NMR signals (CDCl$_3$) for H-2 and H-3 of ethyl 3-cyanopropanoate (3-46) were not resolved in the AA'BB' spin system at δ_H 2.62-2.72, integration of this signal relative to the ester CH$_2$ signal (δ_H 4.20) was used to determine the total incorporation of deuterium. Ideally, incorporation of deuterium only on C-3 would be indicated by a 50% decrease in the intensity of the signal at δ_H 2.62-2.72.

Table 3.2 Introduction of deuterium into ethyl 3-cyanopropanoate via the Krapcho reaction.[i]

Expt. #	Scale (mmol)	Time (h)	Temp (°C)	NaCl (mmol)	DMSO (mL)		D_2O (mmol)	^2H (%)[iii]	Yield (%)
					d_0	d_6			
1	0.75	16	140	0.188	0.75	-	22.50	81	83
2	0.75	16	140	0.188	-	0.75	22.50	78	83
3	0.75	24	140	0.188	0.75	-	22.50	76	63
4	0.75	24	140	0.188	-	0.75	22.50	78	63
5	2.00	2	180	0.500	2.00	-	60.00	72	59
6	2.00	18	180	0.500	2.00	-	60.00	68	40
7	0.75	0.5	180	0.188	-	0.75	22.50	78	-[ii]
8	0.75	2	180	0.188	-	0.75	22.50	80	-[ii]
9	0.75	17	180	0.188	-	0.75	22.50	90	43

[i]Note that the Krapcho decarboethoxylation of diethyl 2-carboxyethyl-2-cyanobutanedioate is complete by 16 h at 140°C and by 0.5 h at 180°C.
[ii]Not isolated
[iii]Percent d_4

In the initial experiment (Table 3.2, Expt. 1), a solution of diethyl 2-carboxyethyl-2-cyanobutanedioate (3-2) in DMSO and D_2O was heated at 140°C for 16 h, the minimum time determined for complete decarboethoxylation. ^1H NMR analysis of the isolated ethyl 3-cyanopropanoate product revealed 81% deuterium. The deuterium incorporation of > 50% indicated that deuterium must have been present at C-2 in addition to that expected at C-3. The location of deuterium in ethyl 3-cyanopropanoate was confirmed by ^{13}C NMR spectroscopy; the signal at δ_C 13.0 (C-3, characteristic chemical shift of a carbon α to a nitrile)[46] was absent and the intensity of the signal at δ_C 30.0 (C-2, characteristic chemical shift of a carbon α to an ester)[46] was reduced

significantly, indicating complete exchange on C-3 and partial exchange on C-2. Given a total deuterium incorporation of 81% and complete exchange at C-2, then about 60% of the H-2 protons exchanged during the Krapcho reaction.

The effects of temperature, time and solvent on deuterium incorporation were examined (Table 3.2) but, in each case, the level of deuterium measured in the ethyl 3-cyanopropanoate product was approximately 80%. For example, neither replacing DMSO with DMSO-d_6 (Table 3.2, Expt. 2) nor increasing the reaction time (Table 3.2, Expts. 3 and 4) had a significant effect on the amount of deuterium incorporated into ethyl 3-cyanopropanoate. Similar deuterium incorporations were obtained when the Krapcho reaction was carried out for various times at higher temperatures (Table 3.2, Expts. 5-9).

The lack of increased deuterium incorporation after prolonged heating (i.e., after decarboethoxylation was complete) suggests that exchange does not occur readily at C-2 of the product ethyl 3-cyanopropanoate. The equivalent hydrogens, however, would be more acidic in the starting material, diethyl 2-carboxyethyl-2-cyanobutanedioate (3-2), because of induction provided by the two β-esters and the nitrile on C-2 in addition to resonance stabilization by the C-4 ester. Thus, it is likely that the H-D exchange occurs in the starting material (3-2) or the monodecarboethoxylated product (3-47 and 3-50) at the elevated temperatures of the Krapcho reaction before didecarboethoxylation is complete (Scheme 3.14).

EtO$_2$C CO$_2$Et 3 2 C≡N H H **3-2** DMSO, D$_2$O 140°C-180°C

D CO$_2$Et 3 2 C≡N H H **3-47** DMSO, D$_2$O 140°C-180°C

D D 2 3 C≡N H H **3-41**

D$_2$O

EtO$_2$C CO$_2$Et 3 2 C≡N H D **3-48**

D CO$_2$Et 3 2 C≡N H D **3-49**

D D 2 3 C≡N H D **3-50**

D$_2$O   D$_2$O

EtO$_2$C CO$_2$Et 3 2 C≡N D D **3-51**

D CO$_2$Et 3 2 C≡N D D **3-52**

D D 2 3 C≡N D D **3-53**
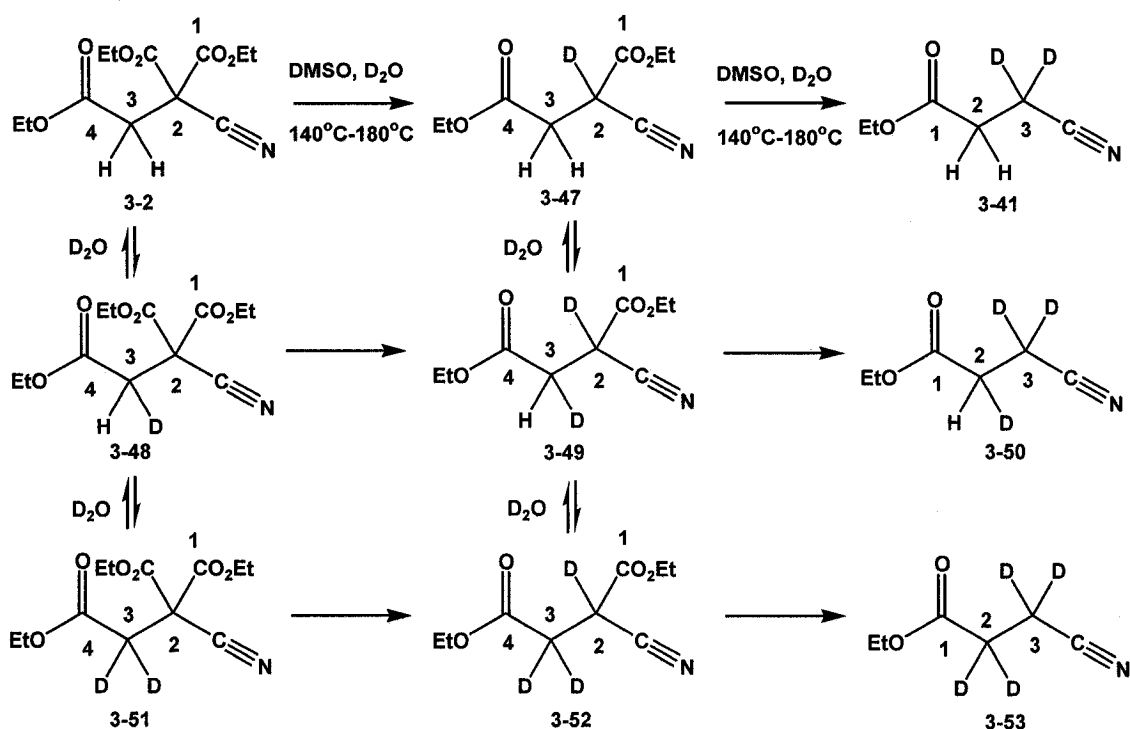
Scheme 3.14 Incorporation of deuterium at C-2 and C-3 of ethyl 3-cyanopropanoate.

As a result of the experiments summarized in Table 3.2, selective replacement of the geminal ester groups in diethyl 2-carboxyethyl-2-cyanobutanedioate (**3-2**) with deuterium under Krapcho conditions seemed unlikely. When higher temperatures and longer heating times were used (Table 3.2, Expt. 9), ethyl 3-cyanopropanoate containing a slightly higher deuterium content of (90% by ^{1}H NMR analysis) was isolated in low yield.

While the Krapcho reaction of diethyl 2-carboxyethyl-2-cyanobutanedioate (**3-2**) did not prove to be a suitable route to products highly and selectively enriched in deuterium, an alternative method for introducing deuterium was indicated when a sample of ethyl [2,2,3,3-^{2}H$_4$]-3-cyanopropanoate (**3-53**) containing 86% deuterium on C-2/C-3 was converted to GABA by ester hydrolysis in base and nitrile reduction.[32] Positive ion

ESI-MS demonstrated that the GABA product (**3-54**) was mainly dideuterated (4% d_3, 60% d_2, 30% d_1 and 6% d_0), and ^{1}H NMR analysis indicated that 80% of the deuterium label was located predominantly on C-2. These results showed that most of the deuterium label α to the nitrile on C-3 was lost, while the deuterium label α to the carboxyl group on C-2 was, for the most part, retained. This significant exchange of the deuterium atoms adjacent to the nitrile (C-3) must have occurred during the hydrolysis of the ester under basic conditions. Literature precedents for nitrile-mediated exchange are described in the next subsection.

3.3.4 Literature Examples of α-Deuterated Nitriles Prepared by Exchange

There are several literature examples of nitrile-mediated proton-deuterium exchange using hydroxide ion as a base. In the initial report,[35] the α-deuterated nitriles, [2,2,2-^{2}H$_3$]acetonitrile (**3-58**), [2,2-^{2}H$_2$]propanenitrile (**3-44**), [2,2-^{2}H$_2$]butanenitrile (**3-59**) and [2-^{2}H]acrylonitrile (**3-61**) (deuterium incorporation 93-96%) were prepared by heating with calcium deuteroxide at reflux in D$_2$O (Scheme 3.15).

D$_2$O, Ca(OD)$_2$

3-55, n = 0: reflux, 12 h
3-66, n = 1: 120°C, 12 h
3-77, n = 2: 120°C, 12 h

3-58, n = 0: 24% yield, 93.4% d_3, 4.4% d_2
3-44, n = 1: 85% yield
3-59, n = 2: yield not reported

D$_2$O, Ca(OD)$_2$

reflux, 6 h

3-60

3-61, 60% yield, 96% d_1
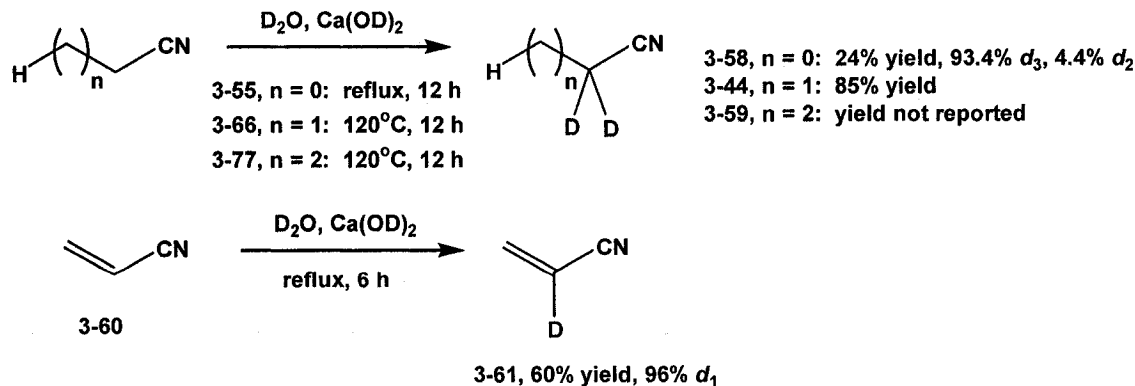
Scheme 3.15 Preparation of α-deuterated nitriles in refluxing D$_2$O and hydroxide ion.[35]

[2-^{2}H]-2-Phenylpropanoic acid (**3-64**) was prepared by heating the unlabelled

nitrile with sodium deuteroxide solution and benzene at reflux (Scheme 3.16).[36] After the

reflux was complete, the aqueous layer was removed, fresh deuteroxide solution was

added and the reflux was resumed. This process was repeated until 99.4% d_1 was

achieved as determined by ^{1}H NMR spectroscopy. In order to ensure that the deuterium

label was not washed out, the nitrile was hydrolyzed by refluxing with sodium

deuteroxide giving the corresponding acid with 109.4% deuterium incorporation,

indicating possible exchange with the protons on C-3 as well.
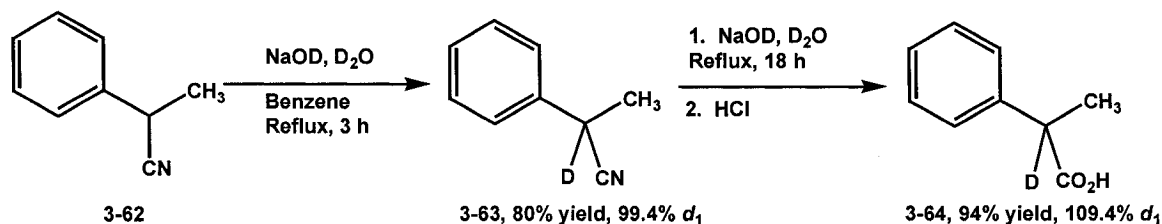
Scheme 3.16 Synthesis of [2-^{2}H]-2-phenylpropanoic acid.[36]

[2,2,3,3-^{2}H$_4$]Succinonitrile (**3-66**) was prepared using 0.1 M KOD in D$_2$O at room

temperature for 4 h with a reported 99% deuterium incorporation by ^{1}H NMR

spectroscopy (Scheme 3.17).[37]
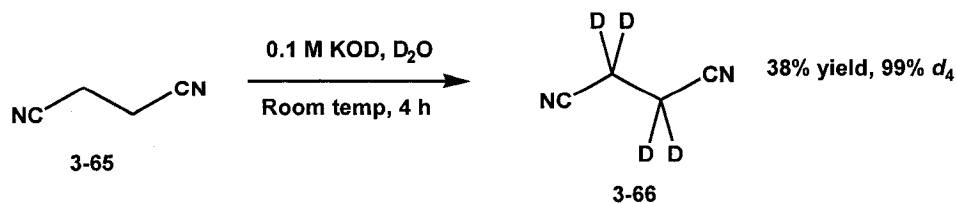
Scheme 3.17 Synthesis of [2,2,3,3-^{2}H$_4$]succinonitrile.[37]

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[2,2,3,3-^{2}H$_4$]Succinonitrile (**3-66**) also has been prepared by heating the

unlabelled dinitrile (**3-65**) in D$_2$O at reflux (Scheme 3.18).[38] The addition of base was

not mentioned. No proton signals were observed in the ^{1}H NMR spectrum, indicating a

high level of exchange. Reduction gave the corresponding amine, [2,2,3,3-^{2}H$_4$]putrescine

(**3-67**) with >99% deuterium incorporation as determined from the ^{1}H NMR spectra of

the dihydrochloride salt and the di(phenylaminothiocarbonyl) derivative.
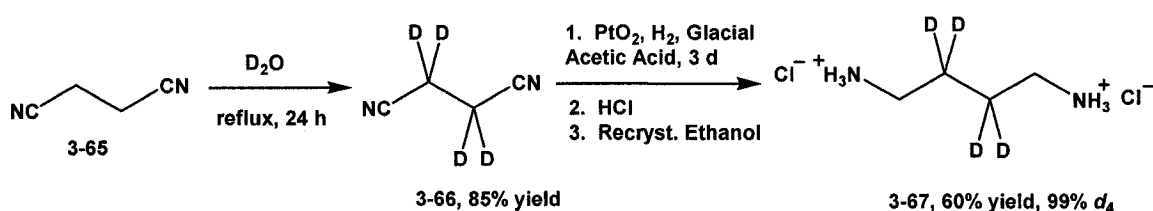
Scheme 3.18 Synthesis of [2,2,3,3-^{2}H$_4$]putrescine from labelled succinonitrile.[38]

In each case, a high level of deuterium incorporation is possible utilizing hydroxide

ions to promote nitrile-mediated exchange. Also, as was seen in the synthesis of

[2,2,3,3-^{2}H$_4$]putrescine (**3-67**),[38] the deuterium label was not lost upon reduction of the

nitrile.

In other instances of exchange α to a nitrile, diazabicyclo[5.4.0]undec-7-ene (**3-**

68A, DBU) has been employed as a base (Scheme 3.19). DBU is a water soluble

amidine base (pK$_a$ of the conjugate acid about 11.5-13.5).[39] Due to its bicyclic, hindered

structure, it is less nucleophilic than other bases, including hydroxide ion, and has been

used in elimination reactions where substitution might occur as an undesirable side

reaction.[40] There are several literature examples where DBU has been used to catalyze

exchange of protons adjacent to other functional groups. For example, a DBU/THF/D$_2$O

mixture was used to label a series of aryl methyl sulfones giving incorporations of 90-99% deuterium.[41]

Scheme 3.19 Diazabicyclo[5.4.0]undec-7-ene (DBU).

A series of dinitriles, including succinonitrile (**3-65**), glutaronitrile (**3-69**) and adiponitrile (**3-70**), were labelled α to the nitrile by refluxing in $D_2O/1,4$-dioxane with 10% DBU as the base (Scheme 3.20).[42] After repetitive exchanges, the dinitriles were reduced to their corresponding diamines, [2,2,3,3-$^2H_4$]putrescine (**3-67**), [2,2,4,4-$^2H_4$]cadaverine (**3-73**) and [2,2,5,5-$^2H_4$]-1,6-diaminohexane (**3-74**), and isolated as dihydrochloride salts. Deuterium incorporations of 98%, 96% and 93% ($\pm$ 2%) $^2H_4$ were reported. Not only was a high deuterium level achieved using DBU catalyzed exchange, but no loss of deuterium was observed upon reduction of the nitrile.

In a second report using DBU,[43] [2,2,3,3-$^2H_4$]succinonitrile (**3-66**) was prepared by refluxing succinonitrile (**3-65**) in $D_2O/1,4$-dioxane and DBU for 24 h (Scheme 3.21). The reported deuterium incorporation of 98% was achieved after multiple exchange reactions.
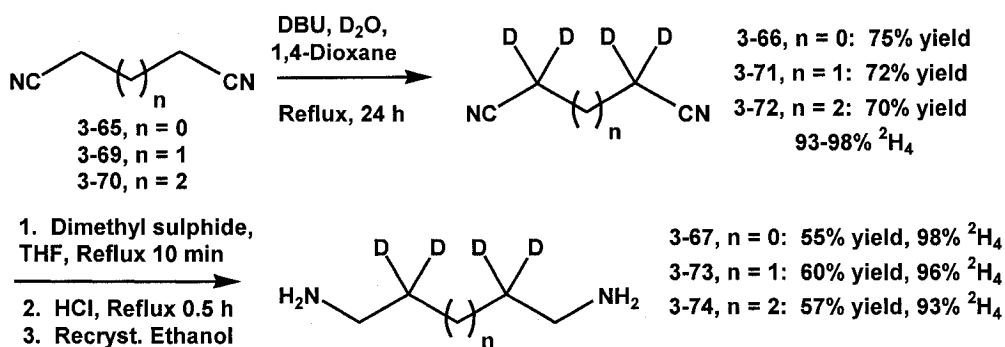
Scheme 3.20 Preparation of deuterium labelled α,ω-diamines.[42]
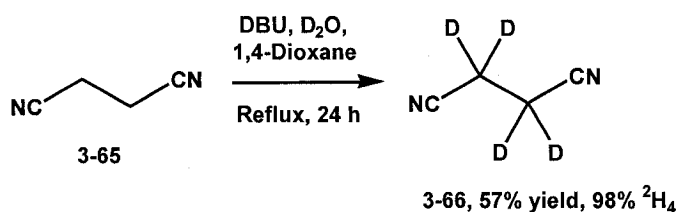
Scheme 3.21 Preparation of $[2,2,3,3\text{-}^2H_4]$succinonitrile.[43]

The high levels of deuterium achieved in the products suggested that nitrile-mediated exchange is a promising method for the introduction of deuterium in an alternative synthesis of $[3,3\text{-}^2H_2]$GABA (**3-40**).

3.4 Synthesis of $[3,3\text{-}^2H_2]$GABA by Nitrile-Mediated Hydrogen-Deuterium Exchange

3.4.1 Retrosynthetic Analysis of $[3,3\text{-}^2H_2]$GABA via Nitrile-Mediated Exchange

As shown in Scheme 3.22, the nitrile functional group could serve both as a precursor of the amino group in GABA (**3-1**) and as an electron-withdrawing group to assist the exchange of α-hydrogens. The feasibility of the hydrolysis and reduction steps proposed in Scheme 3.22 has been demonstrated earlier in this chapter. While the

synthesis of ethyl 3-cyanopropanoate (3-46) from diethyl cyanomalonate (1-1) also has

been accomplished in this thesis, a more direct route from commercially available ethyl

3-bromopropanoate (3-17) and cyanide (3-76) was devised. The challenge in

implementing the route outlined in Scheme 3.22 rests with the selective introduction of

label in a substrate containing two electron-withdrawing groups.
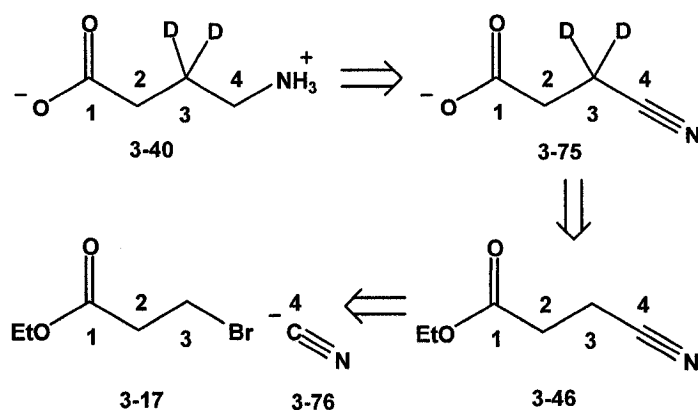
Scheme 3.22 Retrosynthetic analysis of [3,3-^{2}H$_2$]GABA based on nitrile-mediated introduction of deuterium.

3.4.2   Synthesis of GABA from Ethyl 3-Bromopropanoate

The feasibility of the synthetic route proposed in the previous subsection was

established by synthesizing GABA (3-1) via a three step reaction sequence (Scheme

3.23). In the first step, cyanide displacement of bromide from commercially available

ethyl 3-bromopropanoate (3-17) in DMSO gave ethyl 3-cyanopropanoate (3-46).[44] Low

yields of ethyl 3-cyanopropanoate contaminated with a significant amount of starting

material obtained using KCN were attributed to the low solubility of the salt in DMSO.

NaCN is soluble in DMSO, however, and use of NaCN gave both a homogeneous

reaction mixture and better results.

The nitrile ester was hydrolyzed in refluxing aqueous NaOH. In general, a total

volume of 25 mL was used for reactions < 10 mmol while a 50 mL volume was used for

reactions between 10-20 mmol. Hydrolysis reactions that were too concentrated (i.e. 10-

20 mmol/25 mL) resulted in undesirable nitrile hydrolysis products. After acidification

using HCl, 3-cyanopropanoic acid (3-77) was isolated by extraction and reduced using

NaBH$_4$/CoCl$_2$. Ion exchange chromatography of the reaction mixture provided GABA in

47% overall yield (Scheme 3.23).
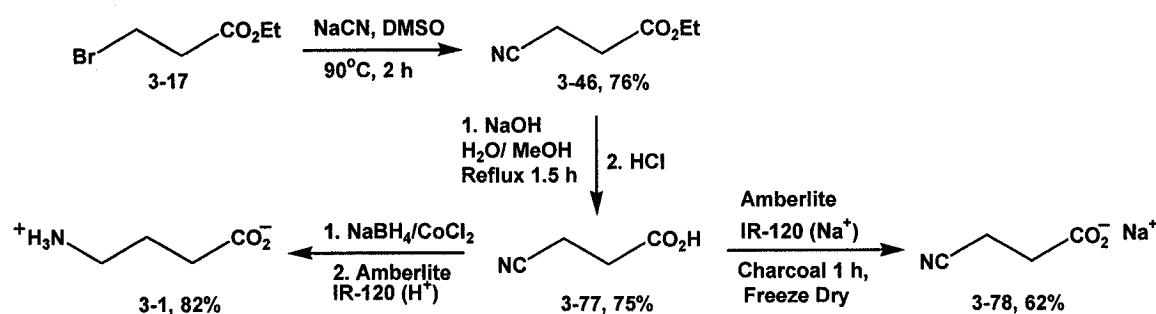
Scheme 3.23 Preparation of GABA and sodium 3-cyanopropanoate from ethyl 3-bromopropanoate.

In the synthesis of labelled GABA (Subsection 3.4.6), sodium 3-cyanopropanoate

(3-78) was shown to be a valuable intermediate. It was formed by applying an aqueous

solution of 3-cyanopropanoic acid (3-77) to an Amberlite IR-120 ion exchange column

(Na$^+$ form) (Scheme 3.23). Sodium 3-cyanopropanoate formed by this method, rather

than simply freeze-drying directly after ester hydrolysis, was free of excess NaOH used

in the hydrolysis step. The presence of NaOH in sodium 3-cyanopropanoate might have

complicated future exchange experiments.

3.4.3 Introduction of Deuterium During Ester Hydrolysis

In the synthesis of labelled GABA (3-54) from ethyl $[2,2,3,3-^2H_4]-3-$ cyanopropanoate (3-53, subsection 3.3.3), the loss of deuterium on position C-3 α to the nitrile upon ester hydrolysis in H_2O, suggested that ester hydrolysis carried out in D_2O could selectively introduce deuterium as shown in Scheme 3.22, and this approach was investigated.

After ester hydrolysis of ethyl 3-cyanopropanoate (3-46) in refluxing D_2O and NaOH, the sample was freeze-dried to remove D_2O and redissolved in water. Reduction of the nitrile[32] yielded deuterated GABA (34%). The ^1H NMR signals corresponding to H-2 and H-4 of GABA each integrated to almost equal values, while the ^1H NMR signal corresponding to H-3 was almost baseline, indicating about 97% deuterium incorporation. Analysis of GABA by ESI^+MS, however, indicated a mixture of isotopic species (6% d_4, 24% d_3, 52% d_2, 13% d_1 and 4% d_0) consistent with about 90% exchange at C-3.

The presence of d_3 and d_4 signals in the ESI^+MS of labelled GABA indicated that exchange was not completely selective as suggested by ^1H NMR. The approximately 30% exchange at C-2 (i.e., α to the ester) most likely occurred before hydrolysis of ethyl 3-cyanopropanoate. The similar pK_a values for hydrogen atoms α to ester $(pK_a = 25.6)^{45}$ or nitrile $(pK_a = 25)^{45}$ groups support this interpretation. Protons α to a carboxylate anion, however, are less acidic $(pK_a = 32)^{15}$ and would be expected to exchange less readily. Therefore, sodium 3-cyanopropanoate (3-78) was utilized in the exchange reactions described in the following subsection.

3.4.4 Hydrogen-Deuterium Exchange in 3-Cyanopropanoate

3.4.4.1 Method Development

In a preliminary experiment to assess the selectivity of exchange, sodium 3-cyanopropanoate (**3-78**) and a substoichiometric amount of NaOH (0.4 equiv.) were dissolved in D_2O and heated at $100°C$ for 0.5 h. After freeze-drying the exchange reaction mixture, the residue was dissolved in water and the nitrile was reduced using $NaBH_4/CoCl_2$. No signal corresponding to H-3 was observed in the ^1H NMR spectrum of GABA isolated from this exchange experiment and the other two signals corresponding to H-2 and H-4 appeared as singlets, integrating to similar values. Highly selective exchange (3% d_3, 80% d_2 and 15% d_1) was indicated by ESI^+MS analysis of GABA. Therefore, use of the sodium salt appeared promising and was investigated in greater detail to find the optimum conditions for a high (>98%) and selective incorporation of deuterium.

In subsequent experiments, the base-catalyzed exchange of protons in 3-cyanopropanoate with D_2O was monitored by ^1H NMR analysis of the reaction mixtures. In all reaction mixtures, chemical shifts were referenced to an internal standard, *tert*-butanol (δ_H 1.27). The AA'BB' protons in 3-cyanopropanoate (**3-78**) appeared as two distinct ^1H NMR multiplets at δ_H 2.53-2.59 and 2.65-2.72. These resonances could not be distinguished by differences in long-range coupling since each ^1H NMR signal correlated to both the nitrile and carboxyl carbons in the HMBC spectrum. In the HSQC spectrum, ^1H NMR signals at δ_H 2.53-2.59 and 2.65-2.72 correlated with the ^{13}C NMR signals at δ_C 32.1 and 13.7, respectively. The chemical shift of the ^{13}C NMR signal at δ_C 13.7 is characteristic of a carbon α to a nitrile,[46] so the signal at δ_H 2.65-2.72 was

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assigned to the protons α to the nitrile (H-3) and the ^{1}H NMR signal at δ_H 2.53-2.59 to

the protons α to the carboxyl group (H-2) (Figure 3.2).

Typically, in the D_2O exchange experiments, the multiplet at δ_H 2.65-2.72 (H-3)

decreased in intensity, and the pattern at δ_H 2.53-2.59 (H-2) simplified into a singlet

(Figure 3.3), indicating exchange α to the nitrile in 3-cyanopropanoate (3-78). The ^{1}H

NMR spectrum of GABA (3-40) obtained by reducing these deuterated 3-

cyanopropanoate (3-79) samples displayed only two singlets (Figure 3.2), verifying the

incorporation of deuterium at C-3 of both GABA and 3-cyanopropanoate.
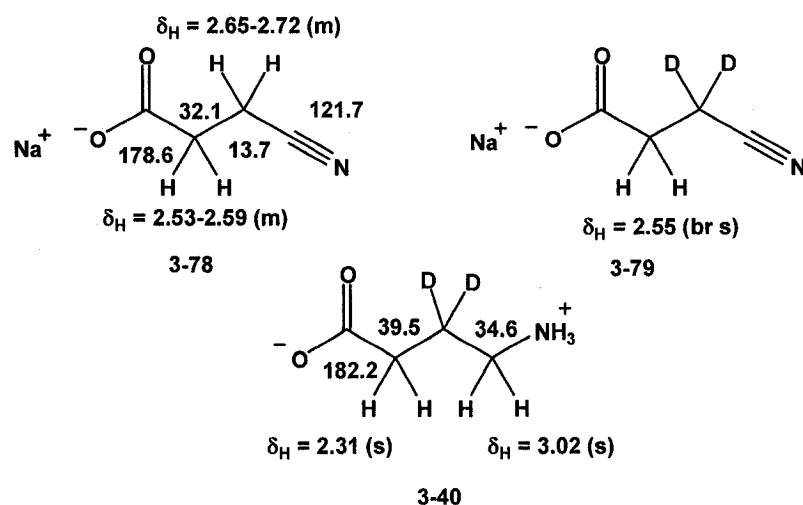
Figure 3.2 Assignment of NMR signals for sodium 3-cyanopropanoate (3-78), sodium [3,3-^{2}H$_2$]-3-cyanopropanoate (3-79) and [3,3-^{2}H$_2$]GABA (3-40).

In addition to the changes in the initial AA'BB' pattern indicating exchange

(previous section), ^{1}H NMR analysis of sodium 3-cyanopropanoate (3-78) heated in D_2O

and NaOH (50°C, 0.5 equiv.) revealed the formation of four other signals between δ_H

2.40-2.50 (Figure 3.3). The two sharp singlets at δ_H 2.48 and 2.43 appeared and grew in

intensity during the initial stages when H-D exchange was most rapid (0-6 h). The other

broader singlets at δ_H 2.47 and 2.42 became more intense after significant H-D exchange had occurred in 3-cyanopropanoate (> 4 h). At 36 h (spectrum not shown), the signal at δ_H 2.48 was no longer detected and the signals at δ_H 2.43 and 2.42 were the most intense in the spectrum.
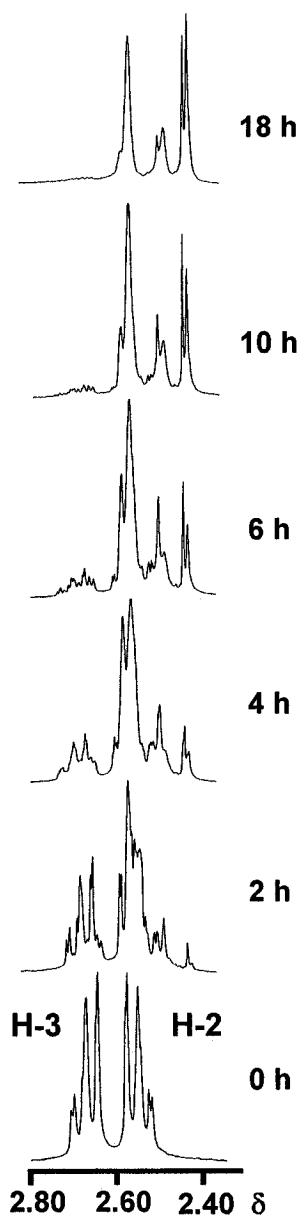
Figure 3.3 ^{1}H NMR spectra showing exchange progress and side product formation (0.5 mmol sodium 3-cyanopropanoate, 0.25 mmol NaOH, 1 mL D_2O, 50°C).

The variation of the relative intensities of the four signals indicates that they arise

by the formation of four different species in the reaction mixture. The most likely

candidate for side product formation is base promoted hydrolysis of the nitrile.

Hydrolysis of 3-cyanopropanoate (**3-78**) would lead initially to the amide, succinamate

(**3-80**), which could in turn hydrolyze to give succinate (**3-81**) (Scheme 3.24). Exchange

of either one or two hydrogens with deuterium in sodium 3-cyanopropanoate or in either

of the proposed side products would give deuterated substances as well, producing a

complicated mixture of at least nine different species. The assignment of signals in the ^{1}H

NMR spectra seen in Figure 3.3 was accomplished by using standard samples, varying

reaction conditions and ESI-MS to assess deuterium incorporations.
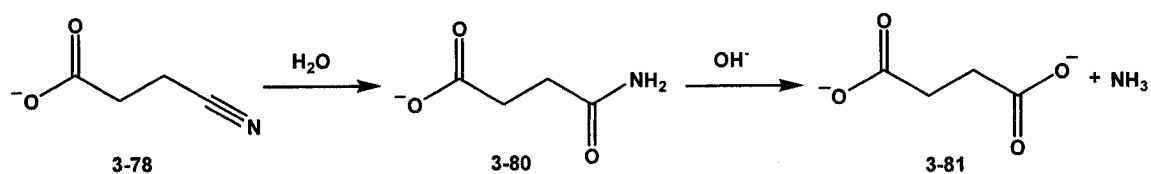
Scheme 3.24 Formation of succinamate (**3.80**) and succinate (**3.81**) by base-promoted
hydrolysis of 3-cyanopropanoate (**3.78**).

The ^{1}H NMR spectrum of authentic sodium succinate (**3-81**) acquired in aqueous

base (D_2O, 0.5 equiv. NaOH) showed a sharp singlet (δ_H 2.43, Figure 3.4B) at a lower

frequency than the AA'BB' signals of 3-cyanopropanoate (Figure 3.4A). The succinate

anion gave a [M-H]$^-$ peak at m/z = 117 in the negative ion ESI-MS. CID of m/z = 117

resulted in a minor peak at m/z = 99 (27), corresponding to the loss of water and a major

peak at m/z = 73 (100), corresponding to the loss of carbon dioxide.[47] The ^{1}H NMR

spectrum of authentic succinamic acid in D_2O and 1.5 equivalents of NaOH gave an

AA'BB' pattern at δ_H 2.48-2.52, in between the signals expected for 3-cyanopropanoate

and succinate (Figure 3.4D). The succinamate anion gave a [M-H]$^-$ peak at $m/z = 116$ in the ESI-MS with the CID of $m/z = 116$ only showing one fragment ion at $m/z = 98$, corresponding to the loss of water.

Sodium 3-cyanopropanoate (**3-78**) was heated in H_2O (50°C, 18 h) with sodium hydroxide (0.5 equiv.) to help confirm the identity of the proposed side products while avoiding complications arising from deuterium exchange. The ^1H NMR spectrum of the reaction mixture after 18 h consisted of signals for 3-cyanopropanoate at δ_H 2.53-2.59 and 2.65-2.72 and two new signals at δ_H 2.48-2.52 and δ_H 2.43 (Figure 3.4C) which were identical in chemical shift and similar in appearance to those observed for authentic succinamate (Figure 3.4D) and succinate (Figure 3.4B), respectively.

Negative ion ESI-MS analysis was performed on the worked up reaction mixture to confirm the identity of the side products. The mass spectrum (Figure 3.5A) showed the expected [M-H]$^-$ peak for 3-cyanopropanoate at m/z 98 and two additional peaks at m/z 116 and 117, corresponding to the m/z values for the [M-H]$^-$ ions of succinamate and succinate. CID of the m/z 116 (Figure 3.5B) produced a single ion at m/z 98 (- H_2O), identical to that observed upon CID of the succinamate [M-H]$^-$ ion (m/z 116) from a standard sample. CID of the ion at m/z 117 (Figure 3.5C), formed a major ion at m/z 73 (100%), corresponding to the loss of carbon dioxide (-44 u) and a less abundant ion at m/z 99 (35%), corresponding to the loss of water (-18 u). The CID spectrum was identical to that observed upon CID of the succinate [M-H]$^-$ ion (m/z 117) from a standard sample.[47] Therefore, nitrile hydrolysis was confirmed as the source of the side products observed in the NMR and mass spectra.
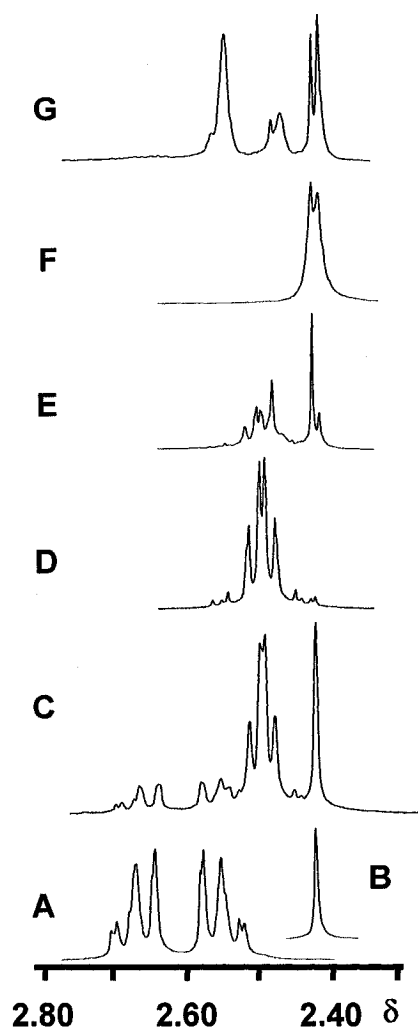
Figure 3.4 ^{1}H NMR spectra (D$_2$O) of (A) sodium 3-cyanopropanoate (0.5 equiv. NaOH), (B) sodium succinate (0.5 equiv. NaOH), (C) partially hydrolyzed sodium 3-cyanopropanoate (18 h, 0.5 equiv. NaOH, 50°C, H$_2$O), (D) succinamic acid (1.5 equiv. NaOH), (E) partially hydrolyzed sample of sodium succinamate (48 h, 0.5 equiv. NaOH, 100°C, D$_2$O), (F) hydrolyzed sample of succinamic acid (16 h, 3 equiv. NaOH., 100°C, D$_2$O), (G) partially hydrolyzed sodium 3-cyanopropanoate (18 h, 0.5 equiv. NaOH, 50°C, D$_2$O).

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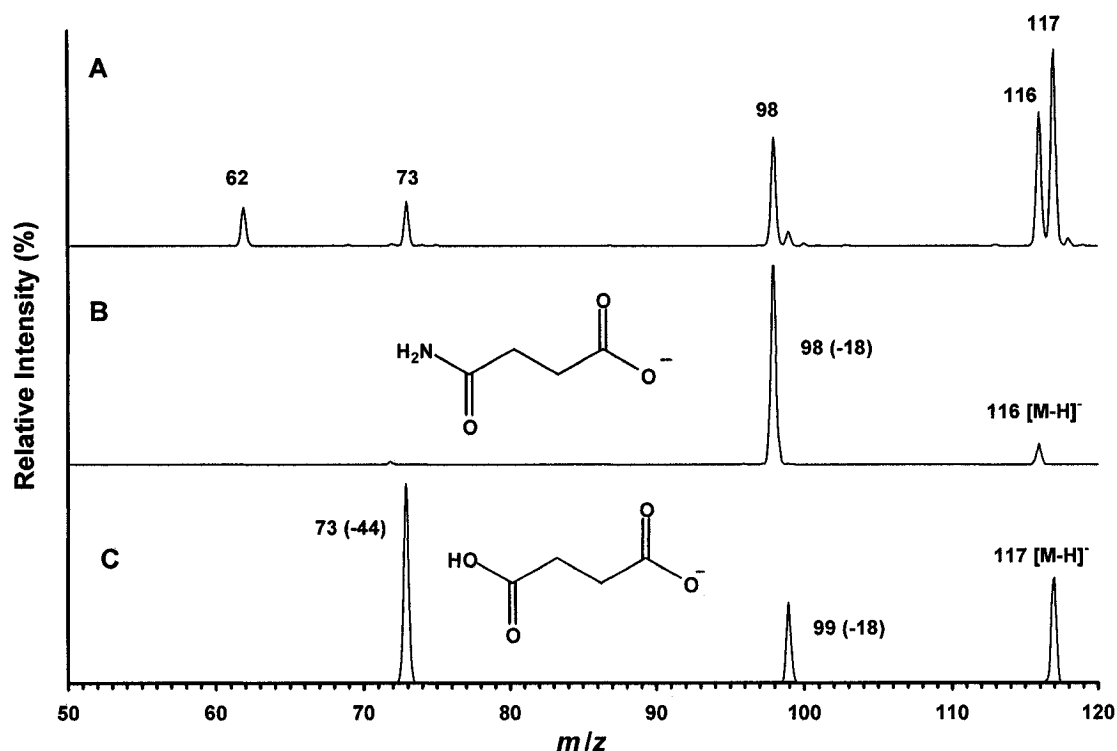
Figure 3.5 Sodium 3-cyanopropanoate hydrolysis reaction mixture (0.5 equiv. NaOH, H_2O, 50°C, 18 h): Negative ion ESI-MS (A); CID spectrum of the m/z 116 ion (B, succinamate); and CID spectrum of the m/z 117 ion (C, succinate).

With succinamate (**3-80**) and succinate (**3-81**) confirmed as the side products formed during the heating of 3-cyanopropanoate (**3-78**) in base, several experiments were carried out to probe the identity of deuterated side products and the extent of exchange. To probe deuterium exchange in sodium succinate (**3-80**), a standard sample was heated in D_2O under more vigorous conditions (0.5 equiv. NaOH, 100°C, 18 h) than the initial experiment (Figure **3.3**). However, the succinate singlet (δ_H 2.43) remained unchanged and the ESI-MS showed insignificant levels of deuterium (< 2% d_1). Therefore, neither deuterium exchange nor decomposition of succinate occurs under the reaction conditions.

The potential deuterium exchange in succinamate (**3-80**) was examined by heating authentic succinamic acid with three equivalents of sodium hydroxide in D_2O (16 h,

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100°C) in order to promote complete hydrolysis of the amide. The ^1H NMR spectrum (Figure 3.4F) showed the complete disappearance of the succinamate AA'BB' pattern at δ_H 2.48-2.52 and the appearance of signals at δ_H 2.42 and 2.43. Negative ion ESI-MS analysis showed peaks at m/z 117 (65), 118 (85) and 119 (100) and a group of similar peaks with relative intensities at m/z 73 (58), 74 (76) and 75 (85). CID of each of the ions at m/z 117, 118 and 119 each gave a mass spectrum showing loss of carbon dioxide (-44 u) as the major fragmentation process and a minor process corresponding to loss of water (-18 u) from the m/z 117 ion and losses of water (-18 u) and HDO (-19 u) from the ions at m/z 118 and 119. The relative intensities of the fragment ion peaks were consistent with those observed for the succinate ion.[47] Therefore, under basic conditions, a significant amount of exchange occurred between succinamate and D_2O, before amide hydrolysis yielded succinate, with about 40% [$^2H_2$]succinate present.

With the ^1H NMR signal at δ_H 2.43 previously assigned as unlabelled succinate (3-81), the broader signal at δ_H 2.42 in Figure 3.4F was attributed to the deuterated succinate. The 0.01 ppm chemical shift difference between the ^1H NMR signals for unlabelled (δ_H 2.43) and labelled (δ_H 2.42) succinate species is consistent with the known deuterium isotope shift to lower frequency observed for protons vicinal to deuterium atoms.[48] The expected slightly different chemical shifts for d_1 and d_2 succinate as well as H-D coupling[49] can account for the broad resonance assigned to deuterated succinate.

In order to futher assess exchange in succinamate (3-80), a sample of succinamic acid was heated (100°C, 48 h) in D_2O with only 1.5 equivalents of sodium hydroxide (Figure 3.4E). In addition to the AA'BB' signals of unreacted succinamate (δ_H 2.52-2.48), singlets at δ_H 2.42 (^2H succinate), 2.43 (unlabelled succinate) and 2.48 were observed in

the ^{1}H NMR spectra. ESI MS of this partially hydrolyzed, succinamic acid sample

showed a major peak for the [M-H]$^-$ ion at m/z 116 (100%), along with a minor peak at

m/z 117 corresponding to either unlabelled succinate ion or the [^{2}H]succinamate ion.

Upon CID of the m/z 117 ion, fragment ions were observed at m/z 99 (loss of H_2O,

100%) and m/z 73 (loss of CO_2, 88%), as expected for the [M-H]$^-$ ion of succinic acid.

However, the intensity of the peak at m/z 99 was much larger than that expected for loss

of water from succinate, indicating a significant contribution from [^{2}H]succinamate. CID

of the m/z 116 ion led to loss of water (m/z 98) as the only fragmentation process,

consistent with the fragmentation of authentic succinamate. Therefore, the ^{1}H NMR

singlet at δ_H 2.48, observed both in the succinamate samples and 3-cyanopropanoate

samples heated in D_2O and NaOH, was attributed to deuterated succinamate, further

evidence that exchange occurs adjacent to an amide under basic conditions.

The ^{1}H NMR spectrum for an exchanged sample of sodium 3-cyanopropanoate

(18 h, 0.5 equiv. NaOH, 50°C, D_2O) with prominent side product signals is shown in

Figure 3.4G. The mass spectrum (Figure 3.6A) of a 3-cyanopropanoate sample heated

under the same conditions showed peaks corresponding to d_0, d_1 and d_2 labelled 3-

cyanopropanoate at m/z 98, 99 and 100, respectively, along with peaks for the hydrolysis

side products at m/z 116, 117, 118 and 119. The peak at m/z 119 corresponds to

[^{2}H$_2$]succinate. The peak at m/z 116 corresponds to the [M-H]$^-$ ion of unlabelled

succinamate and CID of m/z 116 resulted in the characteristic loss of water as the only

observed fragmentation (Figure 3.6B). CID of the peak at m/z 117 produced three

fragment ions at m/z 99 (100), 98 (24) and 73 (56), corresponding to the loss of H_2O,

HOD and carbon dioxide (Figure 3.6C). The loss of carbon dioxide upon CID of the m/z

117 ion indicates that unlabelled succinate is present in the reaction mixture. The intense peak corresponding to loss of water shows that the m/z 117 peak has a major contribution from [^{2}H]succinamate. CID of the peak at m/z 118 gave three fragment ions at m/z 100 (100), 99 (86) and 74 (43), corresponding to the loss of H_2O, HOD and carbon dioxide (Figure 3.6D) indicating that [^{2}H$_2$]succinamate and [^{2}H]succinate contribute to the peak at m/z 118. Therefore, ESI-MS of the sodium 3-cyanopropanoate exchange reaction mixture shows that d_0, d_1 and d_2 isotopomers of succinamate and succinate are present.

Additionally, when sodium 3-cyanopropanoate (**3-78**) was heated with two equivalents of NaOH in D_2O (16 h, 100°C), the ^{1}H NMR spectrum showed a broad singlet at δ_H 2.42. Negative ion ESI-MS showed peaks at m/z 117 (5), 118 (18), 119 (100) and 120 (23) and another set of peaks at m/z 73 (2), 74 (5), 75 (28) and 76 (5). The fragmentation behaviour observed upon CID of m/z 119 was that seen for succinate. In this case, [^{2}H$_2$]succinate accounted for 81% of the mixture of succinate isotopomers, showing that for 3-cyanopropanoate, exchange is faster than hydrolysis.
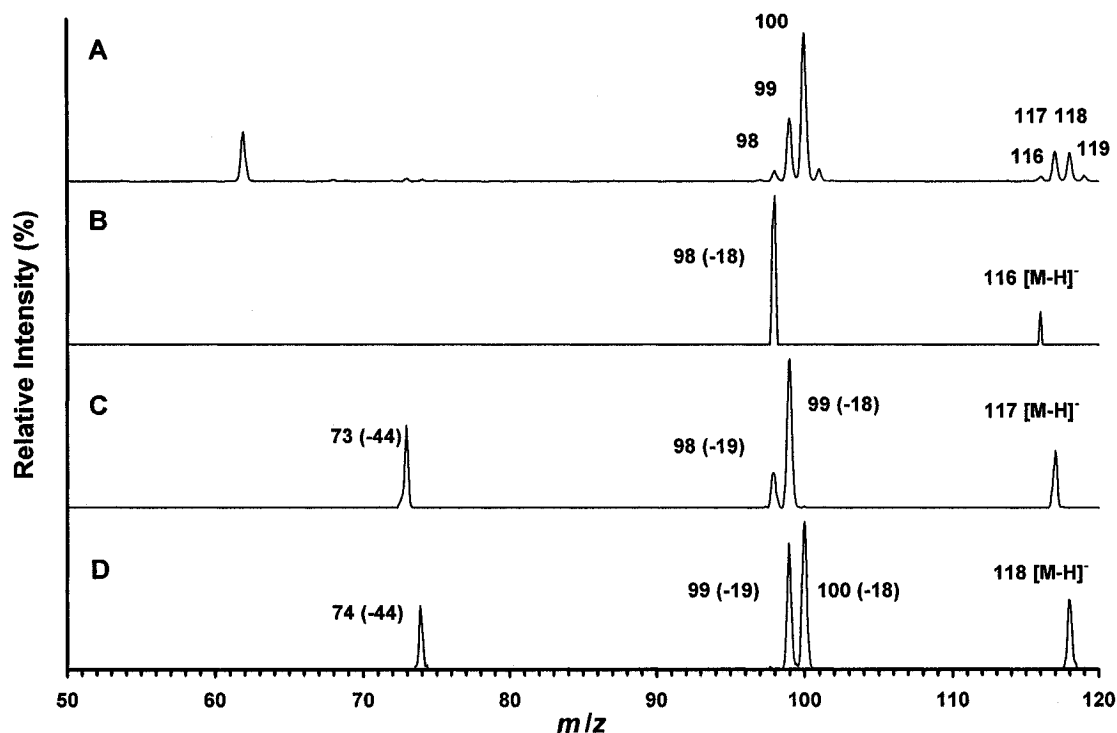
Figure 3.6 Sodium 3-cyanopropanoate exchange reaction mixture (18 h, D₂O, 0.5 equiv. NaOH): Negative ion ESI-MS (A); CID spectrum of the m/z 116 ion (B); CID spectrum of the m/z 117 ion (C); CID spectrum of the m/z 118 ion (D).

In summary, the side product ^{1}H NMR signals at δ_H 2.42, 2.43, 2.52-2.48 and 2.48 formed by heating 3-cyanopropanoate (**3-78**) with NaOH in D₂O (Figure 3.4G) were identified as [^{2}H]succinate, succinate (**3-81**), succinamate (**3-80**) and [^{2}H]succinamate, respectively. CID of the ESI-MS peaks observed between m/z 116-119 and MS/MS analysis confirmed the identity of the side products as d_0, d_1 and d_2 labelled succinamate and succinate. In addition to illustrating the potential complication of side product formation, these experiments generally showed that nitrile-mediated exchange was complete when only a portion of the original nitrile had hydrolyzed. These results suggest that the reaction conditions could be modified to minimize side product formation while achieving a high level of deuterium exchange.

3.4.5 Investigation of Bases

Under the conditions (0.5 equiv. NaOH, D_2O, 50°C) described in the previous section (Figure 3.4G), approximately 18 h were required to achieve a high level of exchange (~ 96%) in 3-cyanopropanoate (**3-78**). However, the formation of the deuterated product was accompanied by the reaction of 3-cyanopropanoate with hydroxide ion, yielding succinamate (**3-80**) and succinate (**3-81**) side products. In this subsection, a series of experiments are described to minimize side product formation while maintaining a high level of deuterium incorporation.

Without base, neither exchange nor nitrile hydrolysis was observed by ^1H NMR spectroscopy when sodium 3-cyanopropanoate (**3-78**) was heated in D_2O (100°C, 6 days). When only 0.05 mmol sodium hydroxide/mmol substrate was used as the base catalyst in the exchange reaction, only 5% deuterium incorporation was measured by ^1H NMR spectroscopy after 24 h at 50°C. Upon heating the same sample for another 48 h at 70°C, the deuterium incorporation increased to 15%, but 10% decomposition of the nitrile was evident. The low incorporation of deuterium indicates that the rate of exchange is also slow at low concentrations of hydroxide ion. At room temperature in the presence of 0.5 mmol NaOH/mmol substrate, exchange was slow (only 38% after 48 h) and side product formation (19%) was evident. At 70°C and with the same amount of NaOH, both faster exchange (91%, 6.5 h) and more side products (77%) were observed.

Using potassium carbonate under the conditions that led to a high level of deuterium incorporation using sodium hydroxide (50°C, D_2O, 0.5 mmol base/mmol substrate), only minimal deuterium incorporation (<5%) into 3-cyanopropanoate (**3-78**) was achieved after 24 h of heating. Heating the same sample at 70°C for an additional 48

h led to only 46% exchange. Whereas, heating a new sample of sodium 3-cyanopropanoate in D_2O with potassium carbonate at 100°C for 17 h (Figure 3.7) produced a high level of exchange (95% ^2H at C-3 by ^1H NMR and 89% $^2H_2$ by ESI⁻MS). The rate of deuterium exchange and final level of deuterium incorporation were similar to those obtained for sodium hydroxide after 18 h at 50°C (Figure 3.7), but were achieved with less decomposition (38% vs. 54%). The level of deuterium labelling was verified by reducing this $[3,3-^2H_2]$-3-cyanopropanoate sample to GABA (94% deuterium at C-3 by ^1H NMR and 92% $^2H_2$ by ESI⁺MS).

Increasing the amount of potassium carbonate increased the rate of the exchange reaction: two mmol of potassium carbonate/mmol substrate produced 93% exchange after 1.5 h with 12% decomposition, while four mmol of potassium carbonate/mmol substrate gave 92% exchange after only 0.5 h with only a marginal increase of side products to 15%.

The amount of deuterium incorporation into 3-cyanopropanoate (3-78) appeared to level off at about 95% using either sodium hydroxide or potassium carbonate to catalyze exchange. One or more additional exchanges in D_2O would be required, therefore, to reach the desired >98% deuterium level. However, after only two exchanges using four mmol of potassium carbonate/mmol of substrate, for example, the product would consist of approximately 30% unwanted hydrolysis side products. Despite an improvement over using sodium hydroxide, exchange with potassium carbonate still produced an unacceptable level of side products. Therefore, two nitrogen bases, triethylamine and DBU were examined to determine if a high level of exchange could be obtained with reduced amounts of side products.
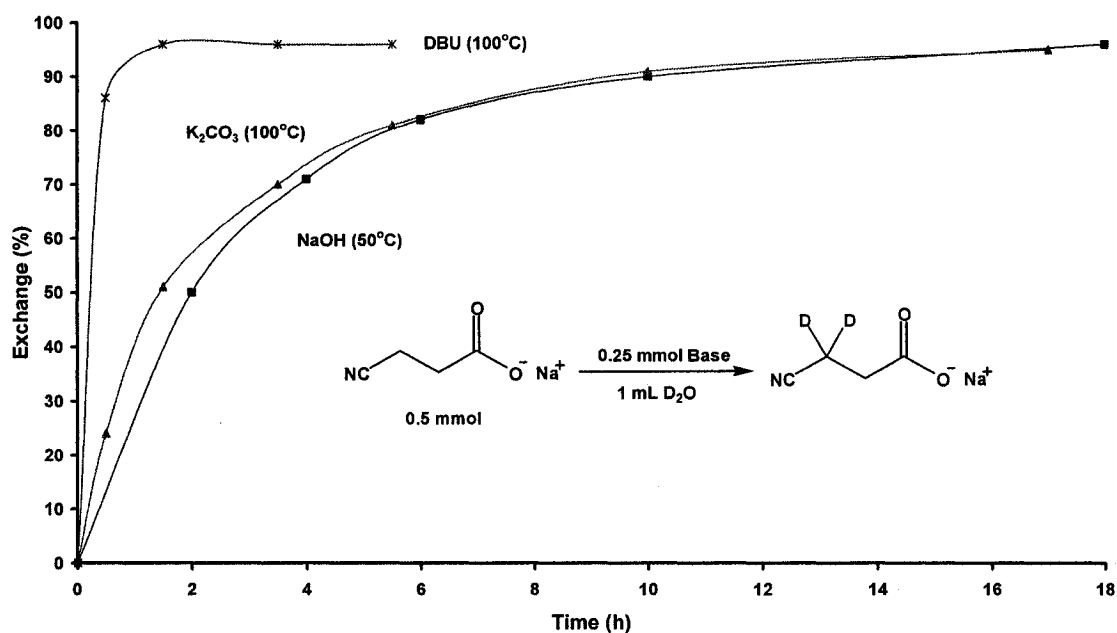
Figure 3.7 Comparison of deuterium incorporation into 3-cyanopropanoate using sodium hydroxide, potassium carbonate and DBU (0.5 mmol base/mmol substrate).

Heating sodium 3-cyanopropanoate (**3-78**) with triethylamine (0.5 mmol base/mmol substrate) at 50°C resulted in very little exchange (6% after 24 h), increasing to 19% after an additional 24 h at 70°C and then to 29% after a further 17 h at 100°C. A reaction mixture saturated with triethylamine (10 mmol base/mmol substrate) gave only 63% deuterium incorporation after 48 h at 100°C. Triethylamine was therefore an unsuitable base for catalyzing high levels of deuterium incorporation in a short time.

Exchange experiments examining the effectiveness of DBU were carried out in a D_2O/1,4-dioxane mixture (0.78 mL/0.22 mL)[42] following the same procedure as described earlier (0.5 mmol base/mmol substrate). However, a ^1H NMR signal from DBU (δ_H 2.45-2.49) overlapped with signals from sodium 3-cyanopropanoate, thus interfering with the determination of deuterium incorporation and side product formation.

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Acidification of the reaction mixture and extraction into ethyl acetate effectively separated 3-cyanopropanoic acid and the side products from DBU. When dissolved in D_2O, the ^1H NMR signals of 3-cyanopropanoic acid and the side products overlapped, but addition of base (NaOH or K_2CO_3) led to resolved signals with chemical shifts identical to those obtained for the sodium hydroxide and potassium carbonate reaction mixtures.

At 100°C in the $D_2O/1$,4-dioxane/DBU mixture, exchange at C-3 of 3-cyanopropanonate (**3-78**) was rapid, reaching 95% incorporation after 1.5 h with only 7% side product formation (Figure 3.7 and Figure 3.8). Under these conditions the concentration of the base DBU was not depleted by the formation of side products, maintaining a high rate of exchange up to about 95% incorporation of deuterium. Thus, the exchange reaction using DBU was superior to the other bases examined, combining high deuterium incorporation in a relatively short period of time with very low side product formation. This is clearly illustrated in Figure 3.9, which shows the ^1H NMR spectra of two sodium 3-cyanopropanoate samples heated with either sodium hydroxide (Figure 3.9A) or DBU (Figure 3.9B), each with about 95% incorporation of deuterium. Using DBU catalyzed exchange, a high deuterium content (>98%) might be possible using multiple exchanges in D_2O without the large build up of side products. This approach was explored in the next subsection on a larger scale to prepare [3,3-$^2H_2$]GABA.

3.5 h (96% ^{2}H, 11% S.P.)

1.5 h (97% ^{2}H, 7% S.P.)

0.5 h (86% ^{2}H, 4% S.P.)

0 h

2.80   2.60   2.40 δ
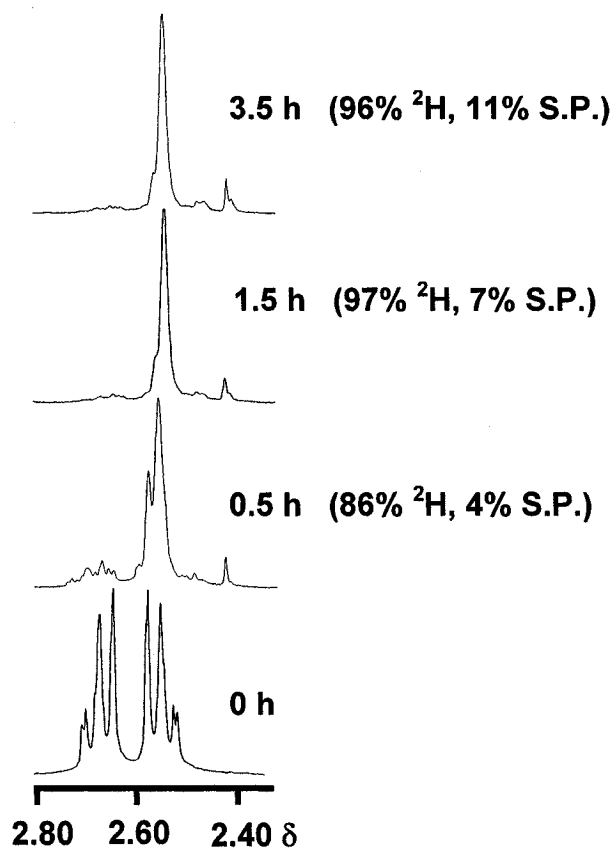
Figure 3.8 ^{1}H NMR spectra showing the DBU catalyzed exchange at C-3 of sodium 3-cyanopropanoate and side product formation (0.5 mmol DBU/mmol substrate, D$_2$O, 100°C). (S.P. = side product)
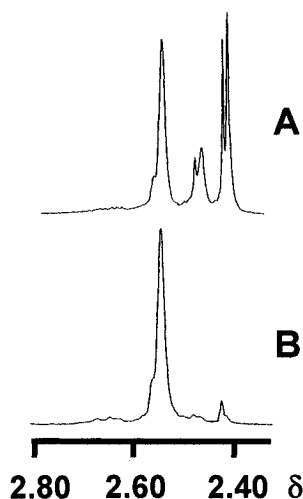
Figure 3.9 Comparison of side product formation at about 95% deuterium incorporation (0.5 mmol base/mmol substrate) between (A) sodium hydroxide (50°C, 18 h) and (B) DBU (100°C, 1.5 h).

3.4.6 Synthesis of [3,3-^{2}H$_2$]GABA

The results of three preparative scale exchange reactions (4.34 mmol) carried out using the DBU/1,4-dioxane/D$_2$O system are summarized in Table 3.3. A sample (0.2 mL) was taken after each round of exchange and worked up to give 3-cyanopropanoic acid. About 1-2 mg was removed for ESI-MS analysis and the remainder was mixed with potassium carbonate to resolve the signals in the ^{1}H NMR spectrum. The levels of deuterium incorporation obtained for each sample (Table 3.3) by ^{1}H NMR and ESI-MS are in good agreement, but slightly higher incorporations were consistently measured by ^{1}H NMR spectroscopy. Similar levels of deuterium were determined from the analyses of GABA, verifying that there is no loss of isotope during nitrile reduction when 3-cyanopropanoate is converted to GABA.

Table 3.3 Progress of deuterium labelling of 3-cyanopropanoate (**3-78**) and subsequent reduction to [3,3-^{2}H$_2$]GABA (**3-40**) followed by ^{1}H NMR and ESI-MS.

Expt.	Method	1st Exchange		2nd Exchange		3rd Exchange		Total % Dec.	GABA	
		Time (h)	%^{2}H	Time (h)	%^{2}H	Time (h)	%^{2}H		%^{2}H	Yield
1	NMR	1.5	96	1.5	96	-	-	19	94	53
	ESI MS		90		93		-		90	
2	NMR	2	95	2	97	2*	100	17	98	55
	ESI MS		92		92		98		96	
3	NMR	2	96	2*	100	-	-	21	98	44
	ESI MS		92		99		-		98	

* indicates addition of more DBU

Initially sodium 3-cyanopropanoate (**3-78**) was heated in D$_2$O with untreated DBU and 1,4-dioxane that had been stored over molecular sieves for 18 h (Table 3.3, Expt. 1). Deuterium incorporation was measured as 96% by ^{1}H NMR and 90% by ESI-MS. The sample was freeze-dried and a second exchange was carried out following the addition of fresh D$_2$O and 1,4-dioxane. The second exchange increased the deuterium content slightly, giving 3-cyanopropanoate with 96% and 93% deuterium by ^{1}H NMR and ESI-MS, respectively. After two consecutive exchange reactions, reduction of sodium [3,3-^{2}H$_2$]-3-cyanopropanoate using NaBH$_4$/CoCl$_2$ gave [3,3-^{2}H$_2$]GABA with only 90-94% deuterium incorporation, the maximum level observed during the initial labelling experiments described in the previous subsection.

Perhaps the reagents used in the reaction were not completely dry, serving to limit the final percent deuterium content to 94%. A broad band around 3000 cm^{-1} in the IR spectrum of DBU indicated that water was present. Distillation of DBU onto molecular sieves decreased this signal, while treatment with CaH$_2$ followed by distillation onto molecular sieves decreased this signal significantly. 1,4-Dioxane had been previously

stored over molecular sieves, but it was further dried by heating at reflux with sodium followed by distillation onto molecular sieves. The IR spectrum for untreated 1,4-dioxane showed a broad H_2O band around 3000 cm^{-1} which was greatly reduced when stored over sieves and virtually eliminated when storage over molecular sieves was combined with the sodium reflux.

Using dried 1,4-dioxane and DBU, sodium 3-cyanopropanoate (**3-78**) was heated at 100°C for 2 h and freeze-dried; additional D_2O and 1,4-dioxane were added, and the mixture was heated again for 2 h (Table 3.3, Expt. **2**). Deuterium incorporation into [3,3-$^2H_2$]-3-cyanopropanoate (**3-79**) was 92% following the first exchange. No increase in deuterium content (92%) was observed after the second exchange despite additional steps taken to ensure the dryness of the reagents. Perhaps DBU was lost during the freeze drying process, so a third exchange was carried out on the same sample where more DBU (0.25 equiv.) was added to the reaction mixture along with fresh D_2O and 1,4-dioxane. ESI-MS analysis of [3,3-$^2H_2$]-3-cyanopropanoate (**3-79**) gave 98% deuterium. After $NaBH_4/CoCl_2$ reduction, [3,3-$^2H_2$]GABA (**3-40**) was obtained with 96% deuterium incorporation. Therefore, the addition of DBU was required at each exchange step to promote further deuterium incorporation.

A final exchange experiment (Table 3.3, Expt. **3**) involving two consecutive exchanges each with the addition of D_2O, 1,4-dioxane and DBU gave [3,3-$^2H_2$]-3-cyanopropanoate (**3-79**) with 92% deuterium after the first exchange and 99% deuterium after the second exchange with 21% decomposition (Scheme 3.25 and Figure 3.10). The increased deuterium incorporation as a result of the second exchange can be seen in the ^1H NMR and mass spectra shown in Figure 3.10. [3,3-$^2H_2$]GABA (**3-40**, 98% ^2H) was

obtained in 44% yield following reduction of [3,3-$^2H_2$]-3-cyanopropanoate (**3-79**) with

NaBH$_4$/CoCl$_2$.
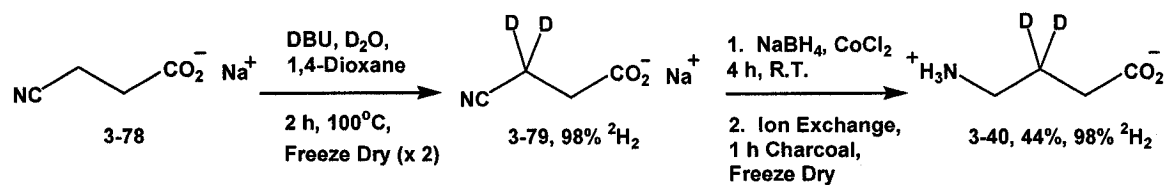
Scheme 3.25 Preparation of [3,3-$^2H_2$]GABA (**3-40**).
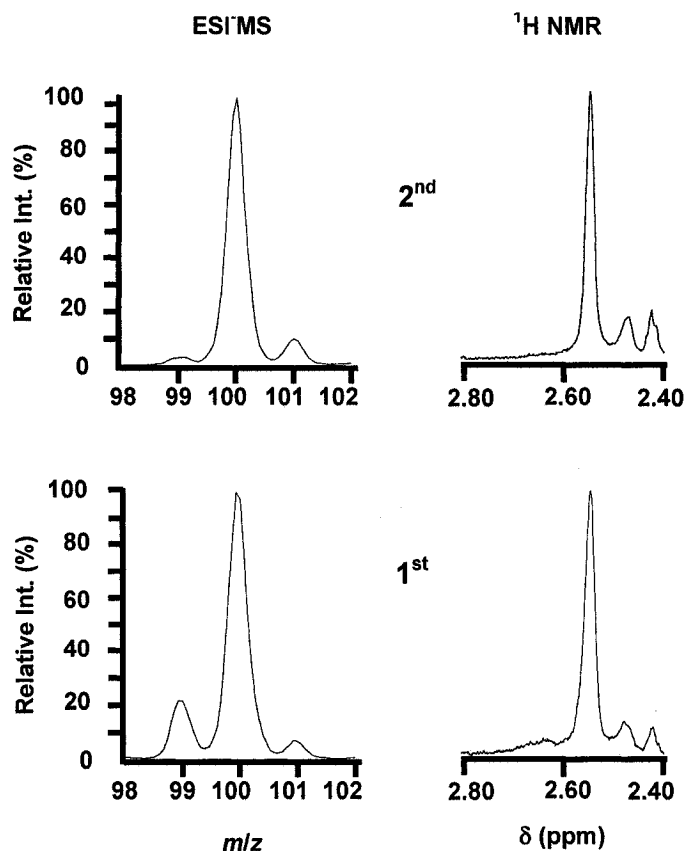
Figure 3.10 ESI-MS and ^1H NMR analysis of [3,3-$^2H_2$]-3-cyanopropanoate (**3-79**) after the 1st and 2nd exchanges catalyzed by DBU. (D$_2$O, 1,4-dioxane and 0.5 mmol DBU/mmol substrate, 100°C, 2 h).

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3.4.7 Characterization of [3,3-^{2}H$_2$]GABA

A comparison of the ^{1}H NMR spectra of unlabelled GABA (3-1) and specifically labelled [3,3-^{2}H$_2$]GABA (3-40) is shown in Figure 3.11. The ^{1}H NMR spectrum of [3,3-^{2}H$_2$]GABA consisted of two singlets at δ_H 2.38 (H-2) and 3.10 (H-4) and the absence of the quintet for H-3 (δ_H 1.88). The ^{13}C NMR spectrum consisted of three signals at δ_C 181.7 (C-1), 39.4 (C-2), 34.4 (C-4) and a low intensity resonance at δ_C 22.9-23.8 showing the coupling between deuterium and C-3. The NMR spectra for [3,3-^{2}H$_2$]GABA clearly illustrate the high specificity of the deuterium exchange at C-3. Integration of the residual signal from H-3 gave an overall deuterium incorporation of 98%.

The isotopic breakdown from the positive ion ESI-MS (Quattro) gave 0% d_0, 4% d_1, 94% d_2 and 2% d_3, showing very little over-incorporation of deuterium. The deuterium incorporation from the mass spectrum added to 98%, assuming d_1, d_2 and two deuterium atoms from d_3 were located at C-3. This total corresponds nicely with the result obtained by ^{1}H NMR spectroscopy.
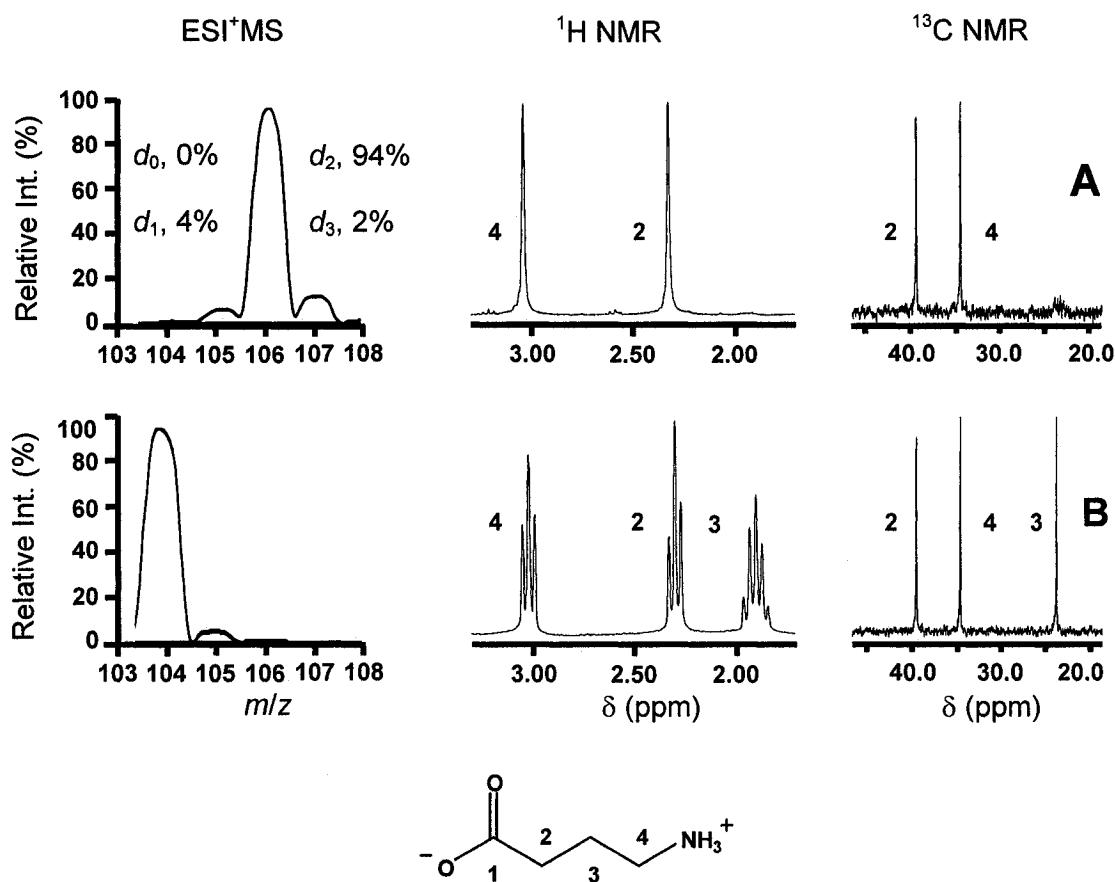
Figure 3.11 Comparison of ^1H NMR and mass spectra for $[3,3\text{-}^2H_2]$GABA (**A**) and unlabelled GABA (**B**).

3.5 Summary

Two approaches for the synthesis of $[3,3\text{-}^2H_2]$GABA (**3-40**) were presented. In the first, Krapcho[8] decarboethoxylation of diethyl 2-carboxyethyl-2-cyanobutanedioate (**3-2**) in D_2O generated deuterium labelled ethyl 3-cyanopropanoate (**3-53**), but specific labelling of C-3 was not accomplished. Under these reaction conditions, deuterium was present on both C-2 and C-3. While a high level of deuterium incorporation onto C-2/C-3 was achieved by heating ethyl 3-cyanopropanoate (**3-46**) in D_2O and DMSO-d_6 under Krapcho conditions, deuterium was lost from C-3 during the subsequent ester hydrolysis

of ethyl [2,2,3,3-^{2}H$_4$]-3-cyanopropanoate (**3-53**). Nitrile reduction gave [2,2-^{2}H$_2$]GABA

(**3-54**), an isotopomer readily available directly from GABA by exchange (Section 3.2.1).

The observation of the loss of deuterium adjacent to the nitrile of ethyl [2,2,3,3-

^{2}H$_4$]-3-cyanopropanoate (**3-53**) during ester hydrolysis led to the successful use of base-

catalyzed exchange for the synthesis of [3,3-^{2}H$_2$]GABA (**3-40**), an isotopomer that has

not been reported in the literature. Using DBU as a base, a high level of deuterium

labelling (98%) was selectively introduced α to a nitrile at C-3 of 3-cyanopropanoate (**3-

78**) with both minimal formation of side products and minimal exchange of hydrogen α

to the carboxyl group. This procedure was also applied in the synthesis of [4,4-^{2}H$_2$]-4-

cyanobutanoic acid for the mass spectrometry study presented in Chapter 5.

3.6   Experimental

3.6.1   General Methods

Refer to Chapter 2 for general methods. ESI mass spectra were collected on a

ThermoFinnigan LCQ duo ion trap or a Micromass Quattro triple quadrupole mass

spectrometer. The mass spectrometer operating conditions are given in Chapter 5,

Section 5.7.6. Deuterium oxide was used as obtained from the manufacturer (Aldrich,

99.9% ^{2}H) without any further purification. 1,4-Dioxane was heated at reflux over excess

sodium metal for 6 h, distilled and stored over 4 Å molecular sieves for 18 h before use.[50]

Diazabicyclo[5.4.0]undec-7-ene (DBU) was dried by stirring with CaH$_2$ for 18 h,

followed by vacuum distillation and storage over 4 Å molecular sieves for 18 h before

use.[51,52] Sodium 3-cyanopropanoate was kept under vacuum in a desiccator for 24 h.

3.6.2 Synthesis of GABA and [2,2-²H₂]GABA from Diethyl Cyanomalonate

3.6.2.1 Alkylation of Diethyl Cyanomalonate

Diethyl 2-carboxyethyl-2-cyanobutanedioate (3-2): Five parallel reaction mixtures, each consisting of a solution of tetrabutylammonium diethyl cyanomalonate (2.13 g, 5.00 mmol) and ethyl bromoacetate (0.554 mL, 5.00 mmol) in DMSO (5 mL), were heated at 80°C for 45 min. After cooling to room temperature, the reaction mixtures were combined, water (150 mL) was added and the product was extracted into diethyl ether (4 x 50 mL). The combined ether extracts were dried over MgSO₄ and evaporated *in vacuo*, yielding diethyl 2-carboxyethyl-2-cyanobutanedioate as an oil (5.56 g, 82%). ¹H NMR (CDCl₃) δ 1.29 (t, J = 7, 3H), 1.35 (t, J = 7, 6H), 3.27 (s, 2H), 4.22 (q, J = 7, 2H), 4.35 (q, J = 7, 4H). ¹³C NMR (CDCl₃) δ 13.8, 13.8, 38.5, 51.7, 61.8, 64.3, 114.6, 162.8, 167.9. EIMS (70 eV) 271.3 (1), 225.4(3), 152.4 (10), 124.3 (12), 97.2 (23), 42.7 (18), 28.4 (100). High Resolution MS (70 eV): calculated for $C_{12}H_{17}NO_6$ = 271.1056 amu, found = 271.1043 ± 0.0008 amu.

3.6.2.2 Krapcho Decarboethoxylation

Ethyl 3-Cyanopropanoate (3-46): Two parallel reaction mixtures, each consisting of a solution of diethyl 2-carboxyethyl-2-cyanobutanedioate (1.36 g, 5.00 mmol), water (2.70 mL, 150 mmol) and sodium chloride (0.0731 g, 1.25 mmol) in DMSO (5 mL), were heated at 140°C for 18 h. The reaction mixture was allowed to cool to room temperature, water was added (100 mL) and the product was extracted into ether (4 x 50 mL). The combined ether layers were dried over MgSO₄ and the solvent was removed *in vacuo*, giving ethyl 3-cyanopropanoate as an oil (1.10 g, 87%). ¹H NMR (CDCl₃) δ 1.29 (t, J =

7, 3H), 2.62-2.72 (m, 4H), 4.20 (q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ 13.2, 14.3, 30.2, 61.6, 118.8, 170.3.

Ethyl 3-Cyanopropanoate Labelling Experiments: Diethyl 2-carboxyethyl-2-cyanobutanedioate, NaCl (0.25 mmol/mmol reactant) and H$_2$O or D$_2$O (30 mmol/mmol of reactant) were added to DMSO or DMSO-d_6 (1 mL/mmol reactant) and heated at 140°C or 180°C in a sealed culture tube. Following a period of 16 h to 24 h, the reaction mixture was allowed to cool to room temperature. A ^{1}H NMR spectrum was recorded directly on the reaction mixture or on the isolated product following work-up. For the 0.75 mmol scale reactions, water (15 mL) was added and the aqueous layer was extracted with ether (4 x 20 mL). The ether layers were combined, washed with water (2 x 10 mL) and dried over MgSO$_4$. For the 2 mmol scale reactions, water (50 mL) was added and the aqueous layer was extracted with ether (4 x 25 mL). The ether layers were combined, washed with water (1 x 25 mL) and dried over MgSO$_4$. Ethyl 3-cyanopropanoate was obtained as an oil after removal of solvent *in vacuo*.

Ethyl [2,2,3,3-^{2}H$_4$]-3-Cyanopropanoate (3-53): Diethyl 2-carboxyethyl-2-cyanobutanedioate (0.542 g, 2.00 mmol), D$_2$O (1.08 mL, 60.0 mmol) and sodium chloride (0.0293 g, 0.500 mmol) were heated in DMSO-d_6 (2 mL) at 180°C for 14 h. The reaction mixture was allowed to cool to room temperature, saturated brine was added (50 mL) and the aqueous layer was extracted with ether (2 x 25 mL). The ether layers were combined and washed with brine (25 mL) which was back extracted with ether (25 mL). The ether layers were combined, treated with charcoal for 1 h and filtered through Celite.

After drying over $MgSO_4$, the solvent was removed *in vacuo* giving ethyl [2,2,3,3-$^2H_4$]-3-cyanopropanoate as an oil (0.18 g, 62%). ^1H NMR ($CDCl_3$) δ 1.29 (t, J = 7, 3H), 2.67 (m, 0.58 H), 4.20 (q, J = 7, 2H). ^{13}C NMR ($CDCl_3$) δ 14.2, 61.5, 118.6, 170.1.

3.6.2.3 Nitrile Reduction

GABA (3-1): Ethyl 3-cyanopropanoate (3-46, 0.258 g, 2.03 mmol) and NaOH (1.27 g, 3.20 mmol) were heated at reflux in water (15 mL) for 1.5 h and then allowed to cool to room temperature. $CoCl_2·6H_2O$ (0.966 g, 4.06 mmol) was added to the aqueous solution followed by the addition of solid $NaBH_4$ (0.768 g, 20.3 mmol) in small portions. After stirring at room temperature for 4 h, water (100 mL) was added and the pH was adjusted to 4.5 using conc. HCl. The mixture was filtered, and the filtrate was applied to an Amberlite IR-120 ion exchange column (30 cm x 2 cm, H^+ form). The column was eluted with 0.5 M aqueous ammonia and 200 mL fractions were collected. Fractions 3-8 were concentrated *in vacuo* and treated with activated charcoal for 1 h. The charcoal was removed by filtration through Celite and the filtrate was freeze dried giving GABA as white powder (0.10 g, 50%). ^1H NMR (D_2O) δ 1.88 (quint., J = 8, 2H), 2.28 (t, J = 8, 2H), 3.00 (t, J = 8, 2H). ^{13}C NMR (D_2O) δ 23.8, 34.6, 39.5, 182.2. ESI^+MS *m/z* 105 (5), 104 ($[M+H]^+$, 100), 87 (13), 86 (13). CID (17%) of *m/z* 104: *m/z* 87 (100), 86 (56).

[2,2-$^2H_2$]GABA (3-54): Ethyl [2,2,3,3-$^2H_4$]-3-cyanopropanoate (3-53, 0.18 g, 1.4 mmol) and NaOH (0.120 g, 3.01 mmol) were heated at reflux in water (15 mL) for 1.5 h, after which the reaction mixture was allowed to cool to room temperature. Reduction with $CoCl_2·6H_2O$ (0.654 g, 2.75 mmol) and $NaBH_4$ (0.52 g, 13.7 mmol) followed by ion

exchange chromatography gave [2,2-^{2}H$_2$]GABA as a white powder (0.07 g, 48%). ^{1}H

NMR (D$_2$O) δ 1.88 (m, 2H), 2.28 (m, 0.41H), 3.00 (m, 2H). ESI$^+$MS *m/z* 106 (49), 105

(24), 104 (4). CID (16%) of *m/z* 106: *m/z* 89 (65), 88 (36). CID (16%) of *m/z* 105: *m/z* 88

(55), 87 (35).

3.6.3   Synthesis of GABA and [3,3-^{2}H$_2$]GABA from Ethyl 3-Bromopropanoate

3.6.3.1   Cyanide Displacement and Ester Hydrolysis

Ethyl 3-cyanopropanoate[44] (3-46):   Four parallel reaction mixtures, each consisting of a

solution of ethyl 3-bromopropanoate (0.905 g, 5.00 mmol) and NaCN (0.490 g, 10.0

mmol) in DMSO (8 mL) were heated at 90°C for 2 h. After cooling to room temperature,

water (100 mL) was added to the combined reaction mixtures, which then were extracted

with ether (4 x 50 mL). The combined ether layers were washed with water (2 x 20 mL),

and the aqueous washes were back extracted with ether (2 x 25 mL). The combined ether

layers were dried over MgSO$_4$ and evaporated *in vacuo*, yielding ethyl 3-

cyanopropanoate as an oil (1.92 g, 76%). ^{1}H NMR and ^{13}C NMR spectra were identical

to those reported in Section 3.6.2.

3-Cyanopropanoic Acid (3-77):   A mixture of ethyl 3-cyanopropanoate (**3-46**, 1.92 g,

15.1 mmol) and NaOH (0.664 g, 16.6 mmol) in water (45 mL) and methanol (5 mL) was

heated at reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature;

water (50 mL) was added and the aqueous solution was washed with ether (2 x 20 mL).

The aqueous layer was acidified with concentrated HCl (red pH paper) and extracted with

ethyl acetate (4 x 50 mL). The ethyl acetate layers were combined, dried over MgSO$_4$

and evaporated *in vacuo*, yielding 3-cyanopropanoic acid as a colourless solid (1.12 g, 75%). m.p. 44-45°C (lit.[53] m.p. 50°C). ^{1}H NMR (CDCl$_3$) δ 2.63-2.70 (m, 2H), 2.74-2.80 (m, 2H), 9.06 (br. s, 1H). ^{13}C NMR (CDCl$_3$) δ 13.1, 30.1, 118.5, 175.7. ^{1}H NMR (Acetonitrile-d_3) δ 2.51-2.65 (m, 4H), 8.11 (br. s, 1H). ^{13}C NMR (Acetonitrile-d_3) δ 13.4, 30.1, 120.3, 172.9. ESI'MS *m/z* 98 ([M-H]$^-$, 100). CID (23%) of *m/z* 98: *m/z* 71 (100).

Sodium 3-Cyanopropanoate (3-78): 3-Cyanopropanoic acid (**3-77**, 1.12 g, 11.3 mmol) was dissolved in water (50 mL) and applied to an Amberlite IR-120 column (30 cm x 2 cm, Na$^+$ form) which was eluted with water. The first 1000 mL of eluate were collected and concentrated *in vacuo*. The concentrate (100 mL) was treated with charcoal for 1 h and filtered through Celite. The filtrate was freeze dried to yield sodium 3-cyanopropanoate as a colourless solid (1.13 g, 82%). m.p. 85-87°C. IR (Nujol, cm^{-1}) 2250, 1593. ^{1}H NMR (D$_2$O) δ 2.56-2.62 (m, 2H), 2.68-2.74 (m, 2H). ^{1}H NMR (D$_2$O, 500 MHz) δ 2.60 (t, J = 7, 2H), 2.70 (t, J = 7, 2H). ^{13}C NMR (D$_2$O, 125 MHz) δ 13.7, 32.1, 121.7, 178.6.

3.6.3.2 Nitrile-Mediated, Base-Catalyzed Deuterium Exchange

A mixture of sodium 3-cyanopropanoate (**3-78**, 60.5 mg, 0.500 mmol), *tert*-butanol (2.4 μL, 0.025 mmol) and a base (NaOH, K$_2$CO$_3$ or triethylamine) in 1 mL of D$_2$O was placed in a tightly capped tube and heated. The progress of exchange was monitored at intervals by ^{1}H NMR spectroscopy.

Signals from DBU, however, obscured the substrate resonances in the ^{1}H NMR spectrum. For the DBU-catalyzed exchange reactions, sodium 3-cyanopropanoate

(0.0605 g, 0.5 mmol) and DBU (0.037 mL, 0.25 mmol) were heated in a D_2O (0.78 mL)/1,4-dioxane (0.22 mL) mixture. For analysis, the solvent was removed *in vacuo*, the residue was dissolved in water (5 mL) and acidified with conc. HCl. The product was extracted into ethyl acetate (4 x 5 mL). The ethyl acetate layers were combined, dried over $MgSO_4$ and evaporated *in vacuo*. Each sample was redissolved in D_2O (1 mL) containing NaOH (1.5 mmol/mmol substrate) and *tert*-butanol (2.4 μL, 0.025 mmol), and the ^1H NMR spectrum was recorded.

The ^1H NMR signals corresponding to H-3 (δ_H 2.64-2.70) and H-2 (δ_H 2.52-2.58) of sodium 3-cyanopropanoate, succinamate (δ_H 2.46-2.50) and succinate (δ_H 2.40-2.43) were integrated seperately. *Tert*-butanol (δ_H 1.27) was used as standard to calibrate chemical shifts. The H-2 signal for sodium 3-cyanopropanoate was designated as 2H. The percent exchange was calculated by dividing the integral obtained for H-3 by two, subtracting from one and multiplying by 100. The percent decomposition was determined by adding the integrals for the side products and dividing by the total sum of the integrals for H-2 and the side products and multiplying by 100.

Samples for ESI-MS were prepared by freeze-drying the reaction mixture, dissolving in 5 mL of water and acidifying with conc. HCl. The acidified samples were extracted with ethyl acetate (4 x 5 mL) which was dried by $MgSO_4$ and removed *in vacuo*. Approximately 1 mg of residue was dissolved in 1 mL of MeOH to generate a sample for ESI-MS. Unlabelled 3-cyanopropanoic acid produced an [M-H]⁻ peak at *m/z* 98 in the mass spectrum. After an exchange reaction, the relative intensities of isotope peaks in the [M-H]⁻ cluster was used to estimate the relative abundance of d_0, d_1, d_2, d_3 seen at *m/z* 98, 99, 100 and 101, respectively.

[3,3-^{2}H$_2$]-3-Cyanopropanoate (3-79): DBU (0.324 mL, 2.17 mmol) was added to a solution of sodium 3-cyanopropanoate (0.430 g, 4.34 mmol) in D$_2$O (6.8 mL)/1,4-dioxane (1.9 mL), and the mixture was heated at 100°C for 2 h. The reaction mixture was allowed to cool to room temperature. A 0.2 mL sample was removed for analysis and the remainder was freeze dried. For the second exchange reaction, the same quantities of D$_2$O, 1,4-dioxane and DBU were added to the original freeze-dried reaction mixture and heated at 100°C for an additional 2 h. The reaction mixture was allowed to cool to room temperature, a 0.2 mL sample was removed for analysis and the reaction mixture was freeze-dried.

The 0.2 mL samples, containing 3-cyanopropanoate, that were removed for analysis were dissolved in H$_2$O (2 mL), acidified with a drop of conc. HCl and extracted with ethyl acetate (4 x 5 mL). The ethyl acetate layers were combined, dried over MgSO$_4$ and the solvent was removed *in vacuo* giving [3,3-^{2}H$_2$]-3-cyanopropanoic acid as a solid which was analyzed by ^{1}H NMR spectroscopy (D$_2$O, K$_2$CO$_3$) and negative ion ESI-MS.

1st Exchange: After the first exchange reaction and workup of the 0.2 mL sample, [3,3-^{2}H$_2$]-3-cyanopropanoic acid was obtained as a solid (1.7 mg). ^{1}H NMR (D$_2$O, K$_2$CO$_3$) δ 2.55 (s, 2H), 2.62-2.64 (m, 0.07), Side products 2.40-2.43, 2.45-2.48 (m, 0.20H). ESI-MS *m/z* 119 (5), 118 (10), 117 (15), 101 (8), 100 (100), 99 (22). CID (23%) of *m/z* 100: *m/z* 73.

2nd Exchange: After the second exchange and workup of the 0.2 mL sample, [3,3-^{2}H$_2$]-3-cyanopropanoic acid was obtained as a solid (4.6 mg). ^{1}H NMR (D$_2$O, K$_2$CO$_3$) δ 2.54 (s, 2H), Side products 2.40-2.43 (m, 0.28H), 2.45-2.48 (m, 0.26H). ESI'MS *m/z* 119 (5), 118 (5), 117 (4), 101 (10), 100 (100), 99 (4). CID (23%) of *m/z* 100: *m/z* 73.

3.6.3.3   Nitrile Reduction

GABA (3-1): CoCl$_2$·6H$_2$O (2.31 g, 9.70 mmol) was added to an aqueous solution (10 mL) of 3-cyanopropanoic acid **(3-78,** 0.48 g, 4.9 mmol), followed by the addition of solid NaBH$_4$ (1.83 g, 48.5 mmol) in small portions and the mixture was stirred at room temperature for 4 h. Water (100 mL) was added and the pH was adjusted to 4.5 using conc. HCl. The mixture was filtered, and the filtrate was applied to an Amberlite IR-120 ion exchange column (30 cm x 2 cm, H$^+$ form). The column was eluted with 0.5 M aqueous ammonia and 200 mL fractions were collected. Fractions 6-14 were concentrated *in vacuo* and treated with activated charcoal for 1 h. The charcoal was removed by filtration through Celite and the filtrate was freeze dried giving GABA as white powder (0.41 g, 82%). The ^{1}H NMR spectrum was identical to that obtained in Section 3.6.2.

[3,3-^{2}H$_2$]GABA (3-40): The freeze-dried reaction mixture containing sodium [3,3-^{2}H$_2$]-3-cyanopropanoate **(3-79)** was dissolved in water (10 mL) and reduced using CoCl$_2$·6H$_2$O (2.06 g, 8.68 mmol) and NaBH$_4$ (1.64 g, 43.4 mmol) and following ion exchange chromatography gave [3,3-^{2}H$_2$]GABA as a colourless solid (0.16 g, 44%). ^{1}H NMR (D$_2$O) δ 1.85-1.95 (m, 0.07H), 2.38 (s, 2H), 3.10 (s, 2H). ^{13}C NMR (D$_2$O) 22.9-

23.8 (m), 34.4, 39.4, 181.7. ESI⁺MS (LCQ) *m/z* 129 (8), 128 (33), 127 (15), 126 (13) 107

(15), 106 (100), 105 (10), 89 (36), 88 (42). ESI⁺MS *m/z* 107 (7), 106 (100), 105 (4).

3.7   References

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Chapter 4: Synthesis of 2-(Aminomethyl)dicarboxylic Acids

4.1 Introduction

The derivation of the β^2-amino acid (**1-85**) structure from an alkylated diethyl cyanomalonate (**1-86**) is described in Section 1.4.1 of Chapter 1. In this chapter, the previous retrosynthetic approach was implemented to generate a homologous series of 2-(aminomethyl)dicarboxylic acids or β^2-amino acids with acidic side chains as potential enzyme inhibitors and as substrates for ESI-MS studies (Chapter 5).

Alkylation of the diethyl cyanomalonate anion (**1-1A**) by a halogenated ester (**4-2**) was deemed to be the most efficient route to these compounds, with the diethyl cyanomalonate anion providing the α-carboxyl and 2-(aminomethyl) groups and the halogenated ester providing the side chain containing the ω-carboxyl group (Scheme 4.1). Alkylation would be followed by ester hydrolysis, monodecarboxylation and nitrile reduction to give the 2-(aminomethyl)dicarboxylic acid (**4-1**).
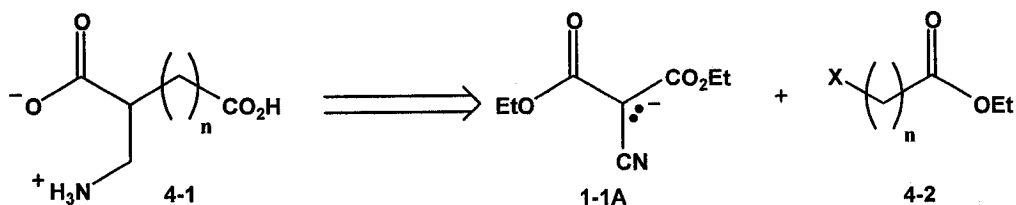
Scheme 4.1 Retrosynthetic analysis of 2-(aminomethyl)dicarboxylic acids.

4.1.1 Biochemical Roles of Acidic α-Amino Acids

Aspartic acid and glutamic acid each play an active role in cellular metabolism and both are directly linked to the citric acid cycle via oxaloacetate and 2-oxoglutarate (α-ketoglutarate). Aspartate aminotransferase is a pyridoxal phosphate enzyme that catalyzes the interconversion of aspartic acid and oxaloacetate.[1] Glutamate dehydrogenase is an NAD^+ or $NADP^+$ dependent enzyme that catalyses the oxidative deamination of glutamic acid to 2-oxoglutarate.[2]

Aspartic acid and glutamic acid are also involved in the biosynthesis of other amino acids. Several amino acids including lysine, methionine, threonine and isoleucine derive part of their carbon skeleton from aspartic acid in which the first step, transfer of a phosphate group from ATP to the side chain carboxyl group, is catalyzed by an aspartokinase.[3] Asparagine also derives its carbon skeleton from aspartic acid through the action of asparagine synthetase.[4] This enzyme catalyzes the reaction between ATP and aspartate to form β-aspartyl adenylate which reacts with ammonia to form asparagine.

Many amino acids including aspartate, alanine, cysteine, histidine, methionine and tryptophan[5] are synthesized by glutamate transaminases (aminotransferases), a group of pyridoxal phosphate dependent enzymes that catalyze the transfer of the amino group from glutamic acid to an α-keto acid.[6] Besides its amino group, glutamate also provides its carbon skeleton for the synthesis of several amino acids. Glutamine synthetase catalyzes the ATP-dependent formation of glutamine from glutamic acid and ammonia. Glutamine is involved in the synthesis of peptides, proteins, nucleotides and amino sugars.[7] The important neurotransmitter, γ-aminobutanoic acid (GABA) is derived from

the glutamate decarboxylase catalyzed decarboxylation of glutamic acid.[8] *N*-Acetylglutamate γ-semialdehyde, derived from glutamic acid is a precursor to both proline and ornithine.[9] Glutamic acid is also a component of glutathione (γ-glutamylcysteinylglycine), a tripeptide that reacts with peroxides and free radicals in the cell. γ-Glutamylcysteine synthetase catalyzes the reaction between glutamate and cysteine, while glutathione synthetase catalyzes the reaction between the γ-glutamylcysteine dipeptide and glycine.[10]

Pyrimidine nucleotides derive part of their structure from aspartate.[11] In the initial step, aspartate transcarbamylase catalyses the reaction between carbamoyl phosphate and the γ-carboxyl group of aspartic acid, giving *N*-carbamoyl aspartate, which cyclizes to give the six-membered ring precursor to pyrimidines.

Glutamate racemase catalyzes the interconversion of L-glutamic acid and D-glutamic acid, providing D-glutamic acid for the synthesis of bacterial cell wall peptidoglycan, a cross-linked polysaccharide-peptide complex that provides rigidity to the cell wall of bacteria.[12] Since peptidoglycan is not present in mammalian cells, its biosynthetic pathway is a very attractive target for antibiotics that act as enzyme inhibitors. Inhibition of glutamate racemase could lead to a defective peptidoglycan layer, a weak cell wall and a bacterial cell that is susceptible to lysis.

α-Aminoadipic acid (2-aminohexanedioic acid) is involved in the biosynthetic pathway that yields lysine.[13] In bacteria, α-aminoadipic acid is incorporated into penicillin and cephalosporin β-lactam antibiotics.[14] α-Aminopimelic acid, originally found in plants, (2-aminoheptanedioic acid) is an inhibitor of a succinylase in the

diaminopimelic acid pathway[15] and carbapenam synthetase in the carbapenam β-lactam antibiotic pathway.[16]

From the examples presented above, acidic α-amino acids, especially aspartic acid and glutamic acid, are involved in a wide variety of metabolic processes. The enzymes that catalyze these reactions are potential targets for inhibition by the 2-(aminomethyl)dicarboxylic acids prepared in this thesis. The structural relationships between acidic amino acids and 2-(aminomethyl)dicarboxylic acids are presented in the following section.

4.2 β²-Amino Acid Homologues of Acidic α-Amino Acids

4.2.1 Structural Relationships Between Acidic α-Amino Acids and 2-(Aminomethyl)dicarboxylic Acids

The structures of the acidic α-amino acids (4-3) aspartic acid, glutamic acid, α-aminoadipic acid and α-aminopimelic acid can be viewed as the 2-amino derivatives of a homologous series of dicarboxylic acids (Figure 4.1). The same structural relationship is also present in a series of 2-(aminomethyl)dicarboxylic acids (4-1), potentially available by the route outlined in Chapter 1 for the synthesis of β²-amino acids from diethyl cyanomalonate (1-1). Each 2-(aminomethyl)dicarboxylic acid differs from the α-amino acid sharing the same dicarboxylic acid backbone by the presence of a methylene group separating the amino group from the dicarboxylic acid backbone (Figure 4.1).

NH₃⁺		

4-3

4-1

n	α-Amino Acid	Dicarboxylic Acid
1	Aspartic Acid	Butanedioic Acid
2	Glutamic Acid	Pentanedioic Acid
3	α-Aminoadipic Acid	Hexanedioic Acid
4	α-Aminopimelic Acid	Heptanedioic Acid
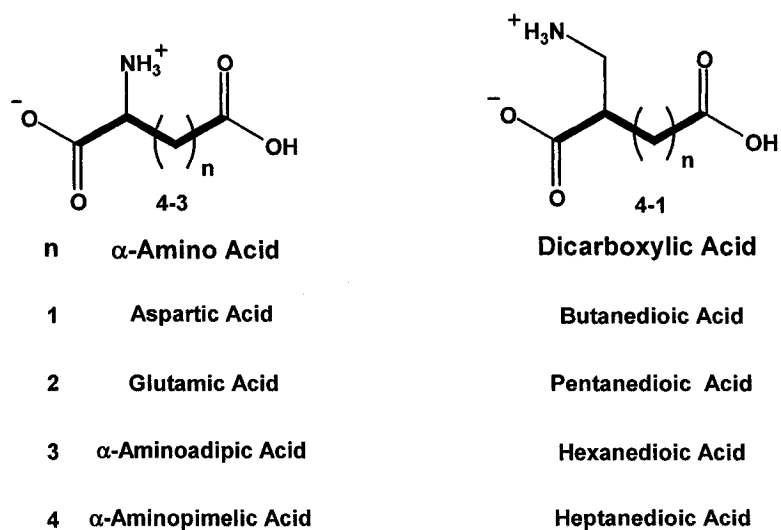
Figure 4.1 Common dicarboxylic acid backbone in acidic α-amino acids and their 2-(aminomethyl)dicarboxylic acid homologues.

Alternatively, the series of α- and β^2-amino acids can be viewed as sharing an ω-amino carboxylic acid backbone (Figure 4.2). From this perspective, the amino acids with equal numbers of carbon atoms differ in the position of the carboxylic acid group, either α or β to the amino group.

The structural similarities between the acidic α- and β^2-amino acids suggest that the latter may serve as substrate analogues or inhibitors in the enzyme-catalyzed reactions of the α- amino acids. Moreover, each β^2-amino acid is structurally related to two α-amino acids (Figure 4.1, Figure 4.2), expanding the number of enzymes to be assessed for potential interactions. Two members of the series of acidic β^2-amino acids are known. Their known biological interactions and their chemical syntheses are described in the following subsections.
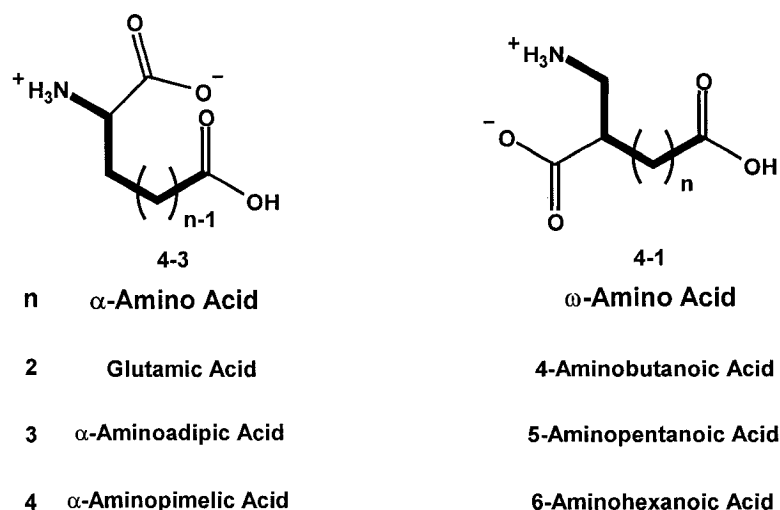
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	4-3	4-1
	α-Amino Acid	ω-Amino Acid

n	α-Amino Acid	ω-Amino Acid
2	Glutamic Acid	4-Aminobutanoic Acid
3	α-Aminoadipic Acid	5-Aminopentanoic Acid
4	α-Aminopimelic Acid	6-Aminohexanoic Acid

Figure 4.2 Common ω-aminocarboxylic acid backbone in acidic α-amino acids and 2-(aminomethyl)dicarboxylic acids.

4.2.2 Biological Activities of 2-(Aminomethyl)dicarboxylic Acid Homologues

Glutamate is an important excitatory neurotransmitter.[17] Its β^2-amino acid homologue, 2-(aminomethyl)butanedioic acid, also demonstrated neuroexcitatory effects upon the toad spinal cord.[18,19] The anticoagulant properties of copolymeric styrene with sulfonate and dicarboxylic acids, including 2-(aminomethyl)butanedioic acid and 2-(aminomethyl)pentanedioic acid were shown to have heparin-like properties.[20] In addition, the interaction of 2-(aminomethyl)pentanedioic acid with several enzymes that catabolize glutamate, including glutamine synthetase and enzymes that are involved in glutathione biosynthesis, has been investigated.[21]

Glutamine synthetase, an enzyme that catalyzes the conversion of glutamic acid to glutamine using ammonia and ATP, accepts 2-(aminomethyl)pentanedioic acid as a substrate.[21] About 50% of the amino acid was converted to its monoamide (2-(aminomethyl)glutaramate), indicating that one enantiomer was selected by the enzyme.

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A hydroxamate assay of glutamine synthetase (NH_3 replaced by NH_2OH), utilizing 2-(aminomethyl)pentanedioic acid as the substrate, resulted in about 50% of the starting amino acid forming the hydroxamate, which underwent non-enzymatic cyclization to 2-piperidone-5-carboxylic acid (6-oxo-3-piperidinecarboxylic acid, 5-carboxy-2-piperidone). Results of the hydroxamate assay were used to develop an enzymatic resolution of DL-2-(aminomethyl)pentanedioic acid.[21]

The racemic mixture of 2-(aminomethyl)pentanedioic acid was incubated with glutamine synthetase under the hydroxamate assay conditions until 50% of the original amino acid had been converted to 2-piperidone-5-carboxylic acid. The reaction mixture was applied to an ion exchange column which was eluted with water so the unreacted 2-(aminomethyl)pentanedioic acid, "isomer B" remained bound, while the 2-piperidone-5-carboxylic acid passed through. 2-Piperidone-5-carboxylic acid was hydrolyzed under acidic conditions to give "isomer A" of 2-(aminomethyl)pentanedioic acid. "Isomer B" of 2-(aminomethyl)pentanedioic acid was obtained by eluting the original ion exchange column with a 2.5 M ammonia solution. Although this method appeared to resolve the two enantiomers of 2-(aminomethyl)pentanedioic acid, the yields were quite low, 5% for "isomer A" and 48% for "isomer B" with an overall amino acid recovery of 53%.[21]

With resolved enantiomers of 2-(aminomethyl)pentanedioic acid available, each was tested separately against glutamine synthetase. The stereochemical preferences of the enzyme were determined by incubating the individual enantiomers separately with the enzyme. Incubation with "isomer A" resulted in 95% amino acid utilization. Incubation with "isomer B" only gave 7% utilization, which was attributed to contamination from "isomer A", possibly from amino acid not converted to the hydroxamate or cyclized

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during resolution. The K_M value for "isomer A" was determined to be 80 mM, while the

value for L-glutamate was determined to be 3.8 mM, indicating the natural substrate

binds more efficiently than its β^2-amino acid homologue. The relative V_{max} values show

that L-glutamate is catabolized 10 x faster than 2-(aminomethyl)pentanedioic acid.

γ-Glutamylcysteine synthetase, an enzyme involved in the synthesis of the

tripeptide glutathione (γ-glutamylcysteinylglycine) that catalyzes the reaction between

the γ-carboxyl group of glutamine and the α-amino group of cysteine, was also tested

with 2-(aminomethyl)pentanedioic acid as an inhibitor. When DL-2-

(aminomethyl)pentanedioic acid was incubated with γ-glutamylcysteine synthetase, L-α-

aminobutyrate and an ATP generating system, almost all of the amino acid substrate was

utilized by the enzyme. However, when the individual enantiomers were examined, it

was determined that "isomer A" was used preferentially (50% utilization after 15 min)

over "isomer B" (50% utilization after almost 5 hours). A large scale preparation of the

product, γ-2-(aminomethyl)glutaryl-α-aminobutyrate, was also carried out. The product

contained approximately 90% "isomer A" when the reaction was stopped at 41%

conversion of the DL-2-(aminomethyl)pentanedioic acid starting material.[21]

The K_m value for DL-2-(aminomethyl)pentanedioic acid was determined to be 4.1

mM. However, since "isomer A" is used preferentially, its K_m can be estimated to be

around 2 mM. The K_m measured for L-glutamate was 1.8 mM, indicating that the

homologue binds to this enzyme almost as well as the natural substrate. However, the

relative V_{max} value for L-glutamate is ten times that of 2-(aminomethyl)pentanedioate.[21]

γ-2-(Aminomethyl)glutaryl-α-aminobutyrate was a poor inhibitor of γ-glutamyl

cyclotransferase and γ-glutamyl transpeptidase, enyzmes of the γ-glutamyl cycle.[21]

However, these results show that a β^2-amino acid can effectively bind and undergo catalysis by an enzyme whose natural substrate is an α- amino acid.

These enzymes share a common feature as they catalyze reactions that occur at the γ-carboxyl group of glutamic acid. However, β^2-amino acid analogues of glutamate have not been tested against glutamate catabolizing enzymes where catalysis occurs at the α-carbon.

4.2.3   Literature Syntheses of 2-(Aminomethyl)dicarboxylic Acids

4.2.3.1   2-(Aminomethyl)butanedioic Acid

The initial synthesis[22] of 2-(aminomethyl)butanedioic acid (**4-1A**) in 1961 involved the addition of benzylamine to itaconic acid (**4-4**, 2-methylenebutanedioic acid) in aqueous solution (Scheme 4.2). Subsequent removal of the *N*-benzyl group by hydrogenolysis yielded 2-(aminomethyl)butanedioic acid (**4-1A**). The entire carbon skeleton of the final amino acid is provided by itaconic acid, while the amino group is derived from benzylamine. This synthesis used relatively cheap and widely available starting materials, and the racemic product was obtained in only two steps with an overall yield of 56%.
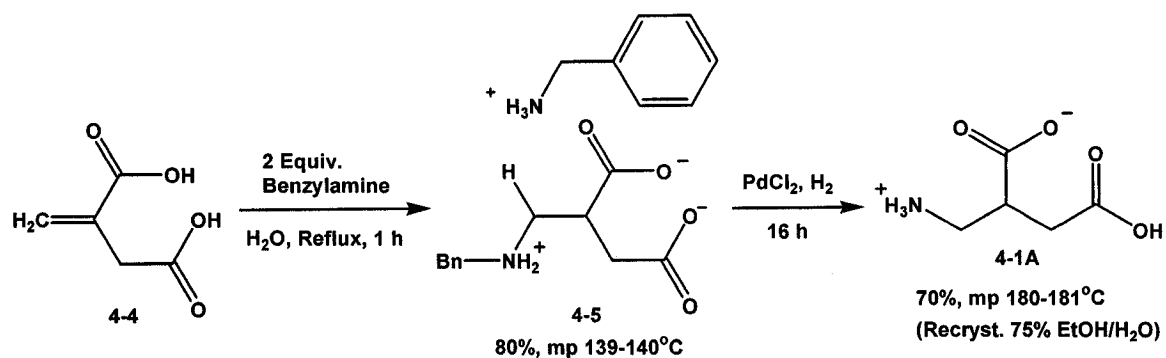
Scheme 4.2 Synthesis of 2-(aminomethyl)butanedioic acid from itaconic acid.[22]

2-(Aminomethyl)butanedioic acid (**4-1A**) has been synthesized[23] also by the addition of ammonia to the double bond of dimethyl itaconate (**4-4A**) followed by ester hydrolysis (Scheme 4.3). No details concerning reaction conditions or yields were reported. A similar addition of ammonia to the double bond of diethyl itaconate (**4-4B**), followed by ester hydrolysis, has been used to produce 2-(aminomethyl)butanedioic acid (Scheme 4.4).[24]
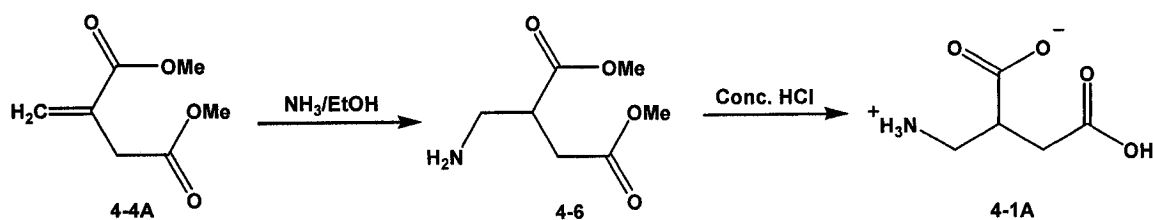
Scheme 4.3 Synthesis of 2-(aminomethyl)butanedioic acid from dimethyl itaconate.[23]

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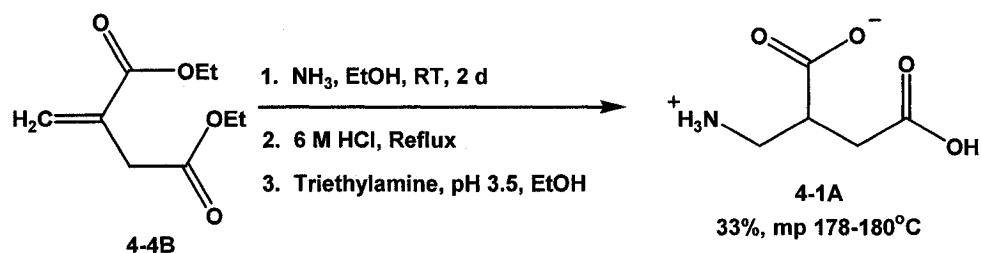
Scheme 4.4 Synthesis of 2-(aminomethyl)butanedioic acid from diethyl itaconate.[24]

In a fourth variation of this synthetic approach to 2-(aminomethyl)butanedioic acid (**4-1A**),[25] ammonia was added to the double bond of ammonium itaconamate (**4-4D**, Scheme 4.5). Ammonium itaconamate was obtained as a mixture from itaconic anhydride (**4-4C**) and ammonia. A low recovery from the recrystallization of the racemic product may have contributed to the very poor overall yield of 3%.
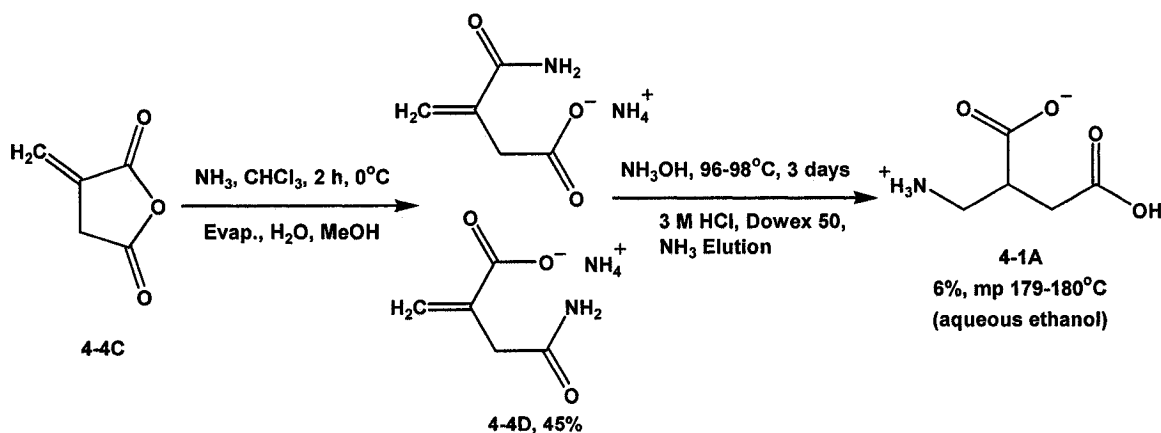
Scheme 4.5 Synthesis of 2-(aminomethyl)butanedioic acid from itaconic anhydride.[25]

A classical malonate ester approach was employed in the fifth synthesis[20] of 2-(aminomethyl)butanedioic acid (**4-1A**, Scheme 4.6). In the first step, diethyl malonate (**1-11**) was alkylated using only 0.5 equivalent of ethyl bromoacetate, possibly to avoid dialkylation. The intermediate product, diethyl 2-carboethoxybutanedioate (**4-7**, 1,1,2-tricarbethoxyethane), was converted to a 3° carbanion and alkylated with bromomethylphthalimide to give **4-8**. A stronger base is required for the second alkylation due to the decreased acidity of the remaining hydrogen. The racemic amino acid **4-1A** was obtained using vigorous acid hydrolysis to remove the protecting groups. In this reaction sequence, the carbon backbone of 2-(aminomethyl)butanedioic acid (**4-1A**) is constructed using two carbon-carbon bond forming reactions. For each, the length of the electrophile can be varied, and two series of amino acid homologues could be generated. However, the efficiency of this reaction sequence cannot be evaluated because yields were not reported.
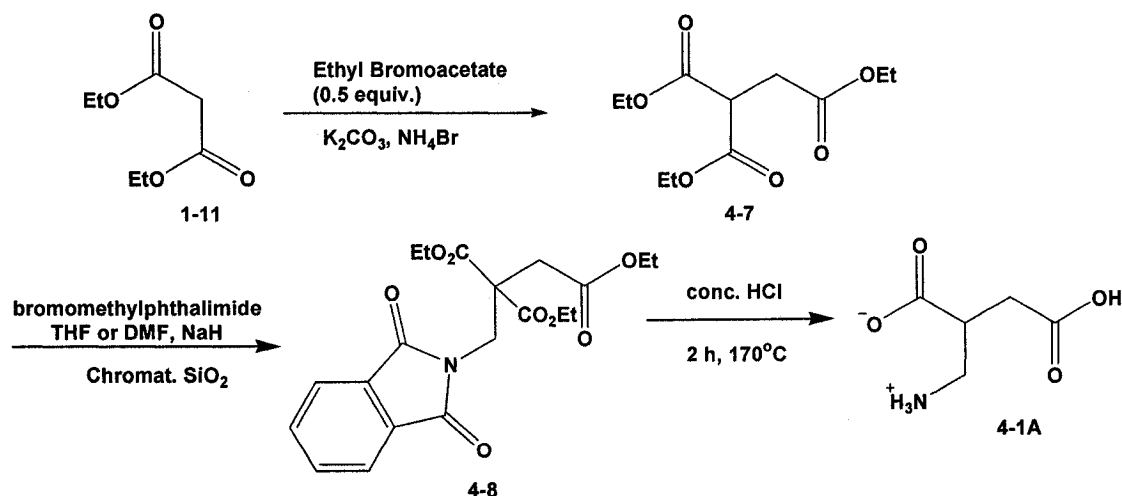
Scheme 4.6 Synthesis of 2-(aminomethyl)butanedioic acid from diethyl malonate.[20]

More recently, the Evans' chiral auxiliary[26] was used for the enantioselective

synthesis of (S)-2-(aminomethyl)butanedioic acid (**4-1A**, Scheme 4.7 and Scheme 4.8).[18]

The first step in the synthesis involved a stereoselective alkylation of an enolate (**4-10**)

derived from an acyloxazolidone containing either a vinyl (**4-9A**) or a 3,4-

dimethoxyphenyl group (**4-9B**, Scheme 4.7). Oxidation of the vinyl or 3,4-

dimethoxyphenyl group (Scheme 4.8) gave a carboxylic acid **4-13** which was converted

to a protected amine (**4-14**, Z, benzyloxycarbonyl). The initially formed acyl azide

underwent a Curtius rearrangement to give an isocyanate which was attacked by benzyl

alcohol. The Evans' chiral auxiliary and methyl ester group were both removed using

LiOH and H_2O_2, and the Z group was removed via catalytic hydrogenation. The overall

yields of (S)-2-(aminomethyl)butanedioic acid (**4-1A**) were 20% (R = vinyl) and 16% (R

= 3,4-dimethoxyphenyl).
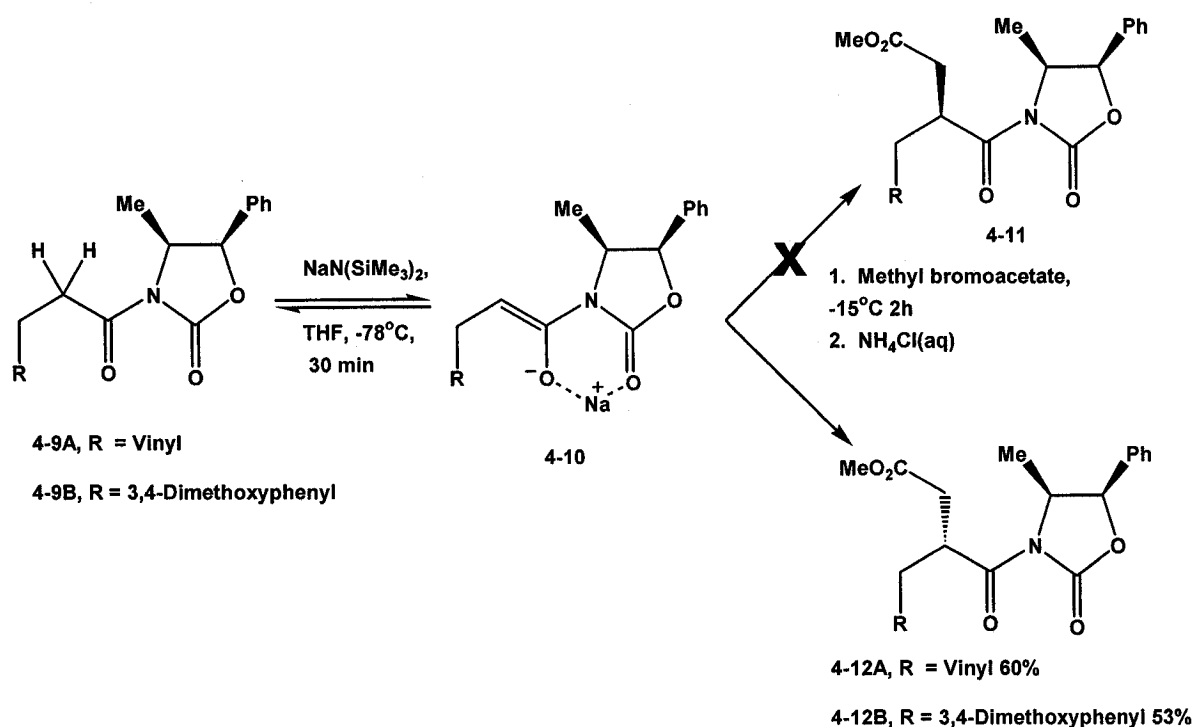
Scheme 4.7 Stereoselective alkylation of the Evans' chiral auxiliary.[26]
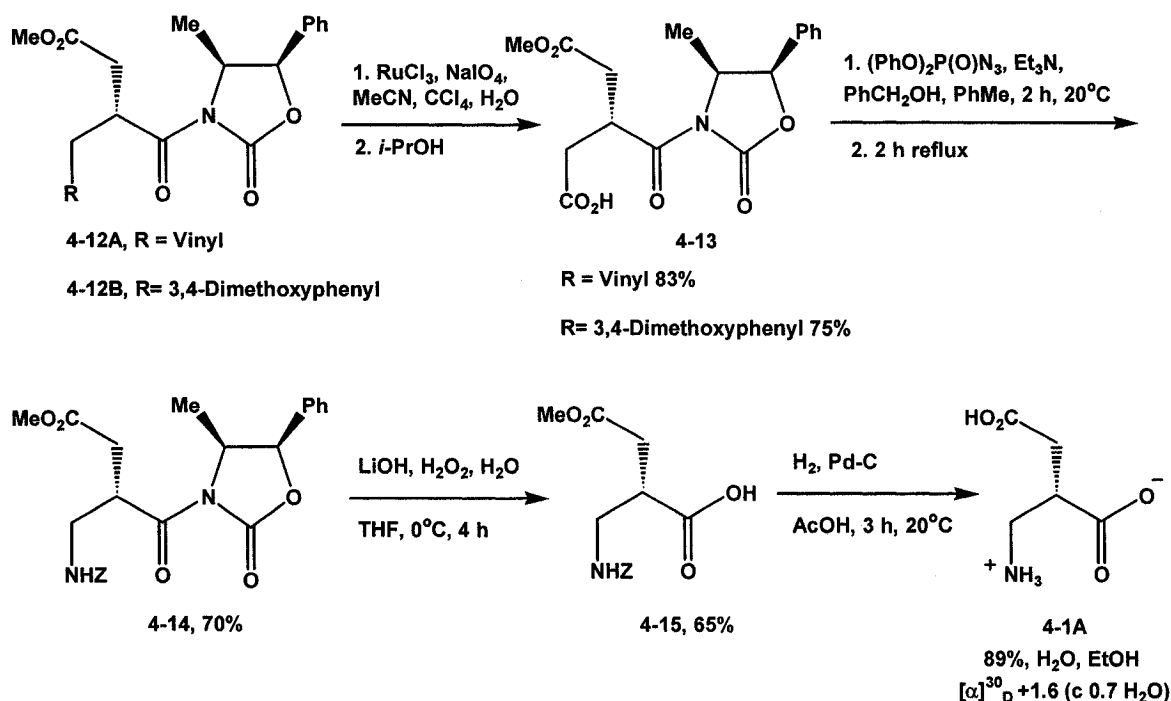
Scheme 4.8 Transformation of the alkylated Evans' chiral auxiliary (Scheme 4.7) into (S)-2-(aminomethyl)butanedioic acid.[26]

The stereochemistry of the amino acid product was confirmed by converting it to

a lactam ester of known configuration (**4-16**, Scheme 4.9). The necessary reference

sample was synthesized from itaconic acid (**4-4**) and (S)-1-phenylethylamine. The

pyrrolidonecarboxylic acid product (**4-17**) was obtained as a 1:1 mixture of diastereomers

which were separated by fractional recrystallization. An X-ray crystal structure obtained

on the less soluble diastereomer showed an S configuration at carbon four. Reductive

cleavage of the N-(α-methylbenzyl) group and esterification yielded an ester lactam (**4-

16**) of the same configuration as that obtained from the amino acid and verified that (S)-

2-(aminomethyl)butanedioic acid (**4-1A**) was produced by the initial stereoselective

alkylation reaction.

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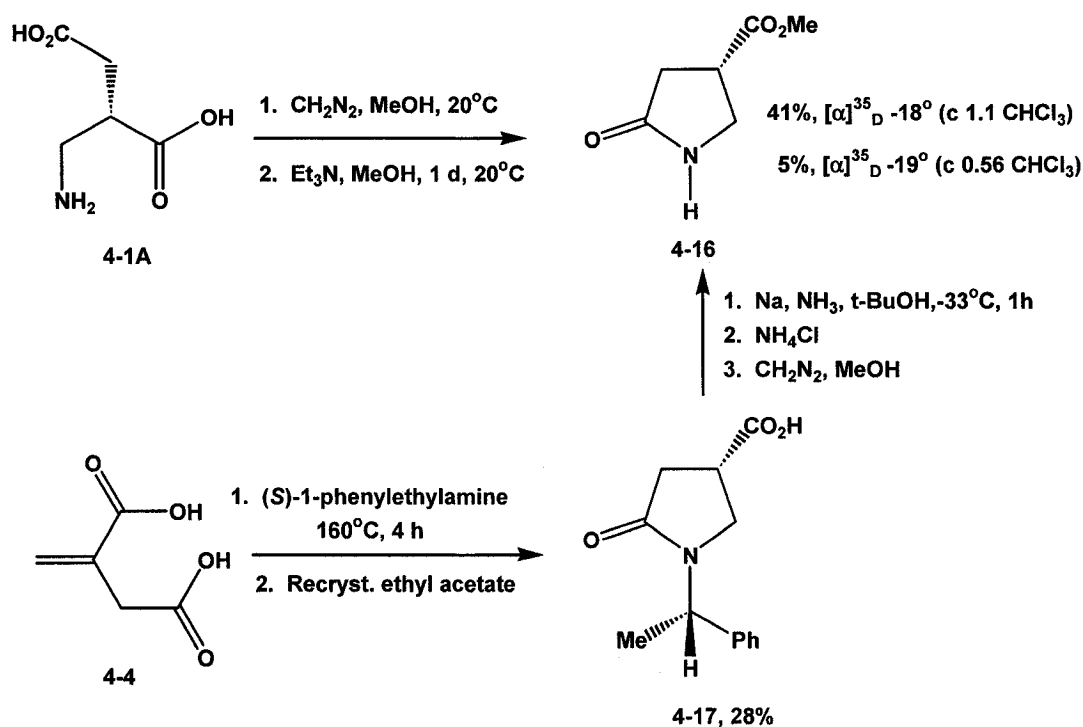
Scheme 4.9 Confirmation of stereochemistry of (S)-2-(aminomethyl)butanedioic acid.[26]

A 2-methyl derivative of 2-(aminomethyl)butanedioic acid was prepared as its

hydrochloride salt during the synthesis of aminomethyl substituted cyclic imides (Scheme

4.10).[27] Methyl cyanoacetate (**1-12B**) underwent reductive methylation followed by

alkylation with ethyl bromoacetate to give dimethyl 2-cyano-2-methylbutanedioate (**4-**

19). Hydrogenation and acid catalyzed hydrolysis yielded 2-(aminomethyl)-2-

methylbutanedioic hydrochloride (**4-21**) in a 24% overall yield.
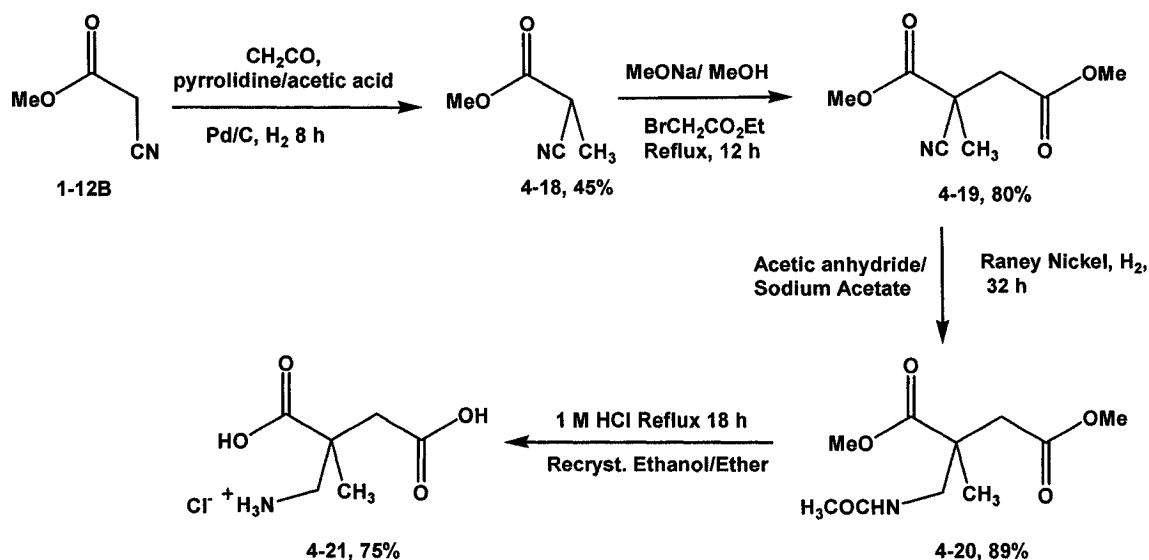
Scheme 4.10 Synthesis of 2-(aminomethyl)-2-methylbutanedioic acid.[27]

4.2.3.2 2-(Aminomethyl)pentanedioic Acid

The initial synthesis of 2-(aminomethyl)pentanedioic acid (**4-1B**) was carried out in 1967 to confirm the structure of an intermediate obtained in the chemical degradation of nicotinic acid (Scheme 4.11).[28] Diethyl 2-cyanopentanedioate (**4-23**) was synthesized via Michael addition of the ethyl cyanoacetate carbanion (**1-12A**) to methyl acrylate (**4-22**).[29] Ester hydrolysis yielded disodium 2-cyanopentanedioate (**4-24B**), which was hydrogenated over PtO_2 to reduce the nitrile to the amine. The amino acid was initially isolated as a hygroscopic hydrochloride salt (**4-25**) in 64% yield. The free amino acid (**4-1B**) was obtained by boiling the hydrochloride salt in pyridine, but a yield for the free amino acid was not reported.
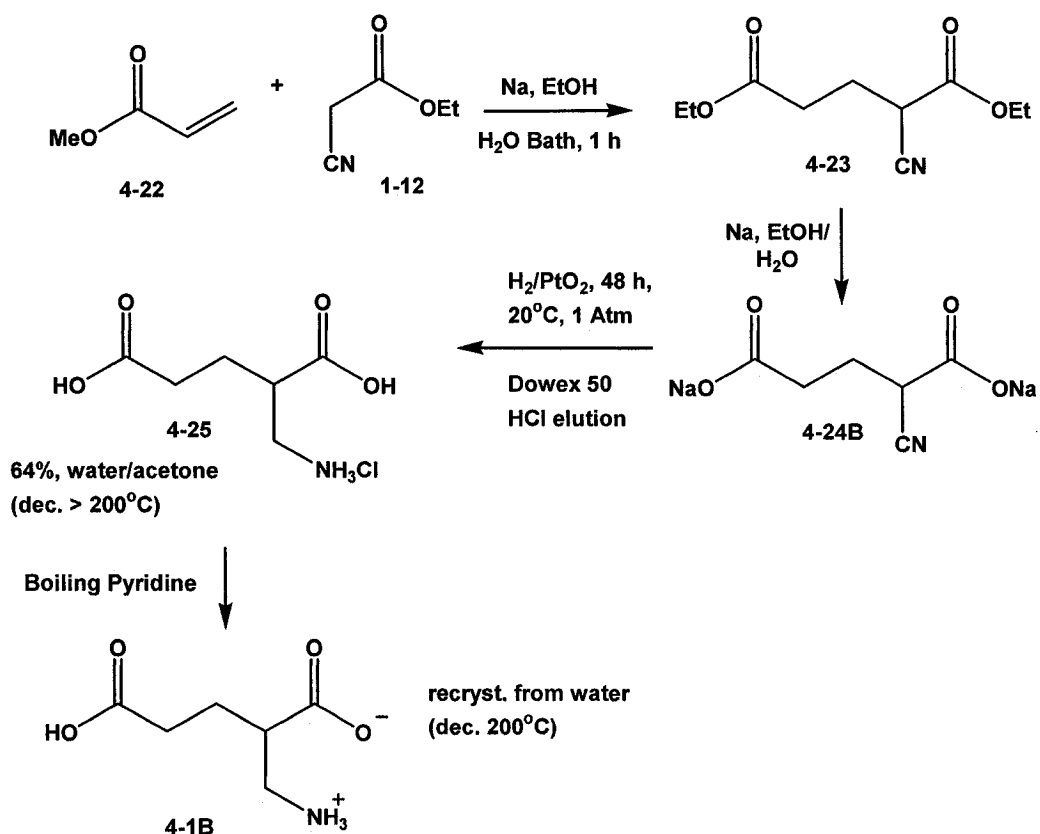
Scheme 4.11 Synthesis of 2-(aminomethyl)pentanedioic acid.[28]

A second synthesis[21] combined the addition approach used in the synthesis of 2-(aminomethyl)butanedioic acid (**4-1A**) with nitrile groups serving as precursors to the carboxylic acids (Scheme 4.12). The amino group was introduced by the addition of ammonia to the double bond in 2,4-dicyano-1-butene (**4-26**) and acid hydrolysis of the two nitriles gave racemic 2-(aminomethyl)pentanedioic acid (**4-1B**) in 32% overall yield.
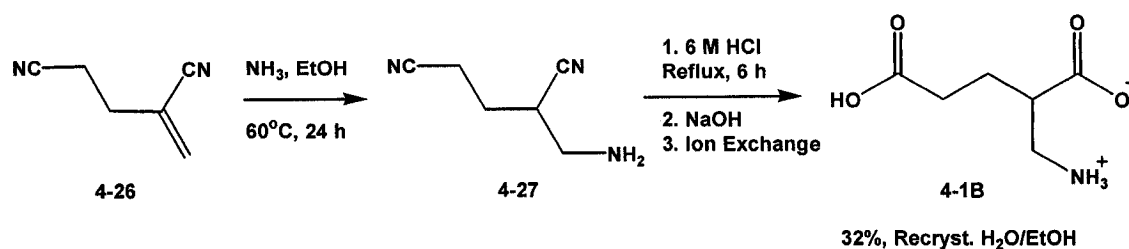
Scheme 4.12 Synthesis of 2-(aminomethyl)pentanedioic acid.[21]

The route used to synthesize 2-(aminomethyl)butanedioic acid from diethyl malonate (**1.11**, Scheme 4.6) was adapted to produce 2-(aminomethyl)pentanedioic acid (Scheme 4.13).[20] A Michael addition of the diethyl malonate carbanion (**1-11A**) to ethyl acrylate (**4-22A**) gave a triester **4-28**, which was deprotonated and alkylated with an equimolar amount of bromomethylphthalimide. After chromatography on a silica gel column, the phthalimido triester **4-29** was deprotected under vigorous conditions to give racemic 2-(aminomethyl)pentanedioic acid (**4-1B**). No yields were reported for this reaction sequence.
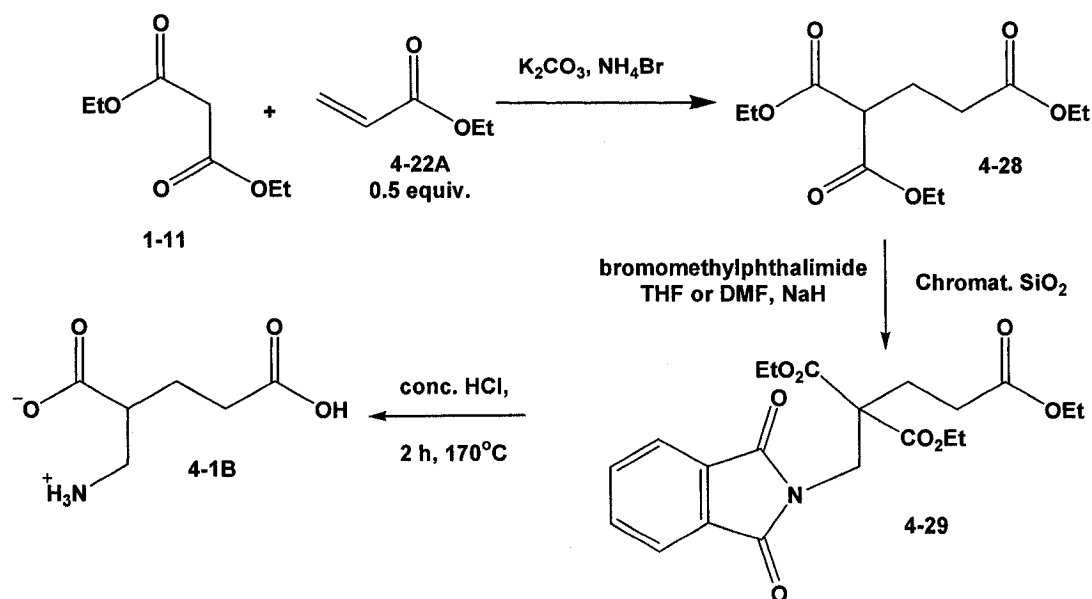
Scheme 4.13 Synthesis of 2-(aminomethyl)pentanedioic acid from diethyl malonate.[20]

4.2.4 Properties of 2-(Aminomethyl)butanedioic Acid and 2-(Aminomethyl)pentanedioic Acid

2-(Aminomethyl)butanedioic acid (**4-1A**) has been described as soluble in water

and insoluble in absolute ethanol, and has been crystallized from aqueous ethanol. The

racemic mixture melted at 180°C,[22,25] while (S)-2-(aminomethyl)butanedioic acid had a

melting point of 161°C and an optical rotation of $[\alpha]^{30}{}_D$ +1.6 (c 0.7 H$_2$O).[18] Elemental

analysis indicates that the amino acid crystallizes from water/ethanol as the

monohydrate.[18,25] As a β-amino acid, it did not react with copper carbonate, but gave a

positive ninhydrin test. When chromatographed on paper with phenol, 2-

(aminomethyl)butanedioic acid had an R$_f$ value of 0.4 (Asp 0.19, Glu 0.31)[22] and had a

retention time of 122 min in the amino acid analyzer (citrate buffer) with pH 3.25 and

4.25 and a buffer change at 60 min.[25]

Elemental analysis indicates 2-(aminomethyl)pentanedioic acid (**4-1B**) is not the hydrate.[21,28] Meister reports the melting point as 171-173°C recrystallized from water/ethanol,[21] but Scott reports the melting point as ≥ 200°C.[28] Titration of this compound yielded pK_a values of 2.7, 4.5 and 9.7.[21] 2-(Aminomethyl)pentanedioic acid also gives a positive test with ninhydrin.[28]

^{1}H NMR spectroscopy (D$_2$O) of **4-1B** gave the following results: δ_H 3.09 (2H), 2.61 (quint., 1H), 1.84 (q, 2H) and 2.39 (t, 2H). IR spectroscopy (KBr) gave absorption bands at 1505 cm^{-1} (-NH$_3$$^+$), 1380 and 1605 cm^{-1} (-COO$^-$) and at 1220, 1720, and 3110 cm^{-1} (-COOH). The mass spectrum of the diammonium salt showed peaks corresponding to 2-piperidone-5-carboxylic acid, its decarboxylation product as well as peaks corresponding to loss of CH$_2$CH$_2$COOH and CH(COOH)CH$_2$NH$_2$.[21] The ionization method was not reported.

The IR spectrum of the HCl salt of **4-1B** gave bands at 1220 cm^{-1} and 1720 cm^{-1} corresponding to the C-O stretch and C=O stretch respectively. The free amino acid was obtained by boiling the hydrochloride salt in pyridine. This gave a crystalline solid which also decomposed around 200°C. The IR spectrum of the free amino acid gave bands at 1230 cm^{-1} and 1710 cm^{-1} corresponding to the carbonyl group and bands at 1640-1650 cm^{-1} and 1510 cm^{-1} corresponding to the NH$_3$$^+$ group.[28]

4.3 Synthesis of 2-Cyanodicarboxylic Acids and 2-(Aminomethyl)dicarboxylic Acids

The synthetic approach based on the alkylation of diethyl cyanomalonate (**1-1**) was used to generate a series of homologous 2-(aminomethyl)dicarboxylic acids (**4-1**) as potential enzyme inhibitors and for ESI-MS studies.

4.3.1 Preparation of Alkylated Diethyl Cyanomalonates

Alkylation of diethyl cyanomalonate (**1-1**) was carried out under optimized

conditions (80°C, DMSO) developed from experiments described in Chapter 2 of this

thesis. Alkylation of tetrabutylammonium diethyl cyanomalonate (**1-1G**) with ethyl

bromoacetate (Scheme 4.14, n = 1), giving diethyl 2-carboxyethyl-2-cyanobutanedioate

(**3-2**), is described in Chapter 3 as an intermediate in GABA synthesis. However, initial

attempts to alkylate tetrabutylammonium diethyl cyanomalonate with commercially

available ethyl 3-bromopropanoate (80°C, DMSO, 2 h) led to a mixture of products. In

addition to the desired product (50%), ^{1}H and ^{13}C NMR signals from ethyl cyanoacetate

(monodecarboethoxylated diethyl cyanomalonate, 30%), diethyl 2-cyanopentanedioate

(monodecarboethoxylated product, 10%) and unreacted ethyl 3-bromopropionate (10%)

were observed. The presence of the monodecarboethoxylated side products was the

result of Krapcho decarboethoxylation.[30]

4-30A, n = 1, X = Br, R = Et, 0.75 h   3-2, n = 1, R = Et: 88%
4-30B, n = 2, X = I, R = Et, 1.5 h   4-31A, n = 2, R = Et: 81%
4-30C, n = 3, X = I, R =Et, 1.5 h   4-31B, n = 3, R = Et: 84%
4-30D, n = 4, X = I, R = H, 2 h   4-31D, n = 4, R = H: 48%
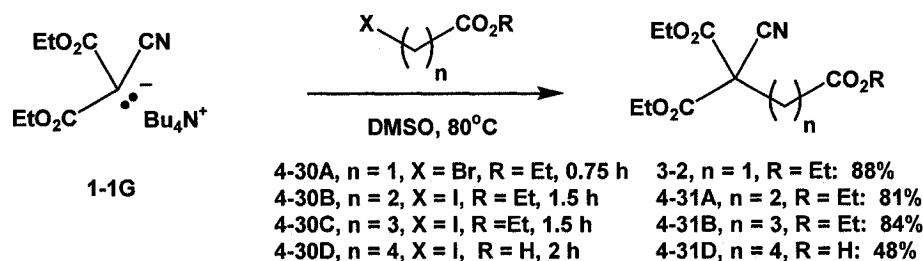
Scheme 4.14 Alkylation of diethyl cyanomalonate.

Although Krapcho decarboethoxylation is presented in this context as an

unwanted side reaction, syntheses utilizing monodecarboethoxylation (Chapter 5) and

didecarboethoxylation (Chapter 3) steps are discussed elsewhere in this thesis. The ^{1}H

NMR signals for diethyl 2-cyanopentanedioate increased in intensity as the reaction

length increased, indicating that prolonged heating of the reaction mixture resulted in

greater side product formation. As the alkylation reaction proceeds, the concentration of

halide ion increases, increasing the likelihood of Krapcho decarboethoxylation.

To overcome this problem, the more reactive alkyl iodides (4-30) were prepared

from the corresponding bromides (4-32) via the Finkelstein reaction (Scheme 4.15).[31]

The iodoesters or iodoacid were obtained in high yield and used as isolated from the

reaction mixture. When the more reactive electrophile ethyl 3-iodopropanoate was used,

prolonged heating of the reaction mixture was not necessary and side product formation

was avoided. Consequently, diethyl 2-carboxyethyl-2-cyanopentanedioate (4-31A) was

obtained as a clean product (Scheme 4.14).

$$Br\diagdown\diagup CO_2R \xrightarrow[\text{Reflux 4 h}]{\text{NaI, Acetone}} I\diagdown\diagup CO_2R$$

4-32A, n = 2, R = Et
4-32B, n = 3, R = Et
4-32C, n = 4, R = H

4-30A, n = 2, R = Et: 92%
4-30B, n = 3, R = Et: 87%
4-30C, n = 4, R = H: 88%
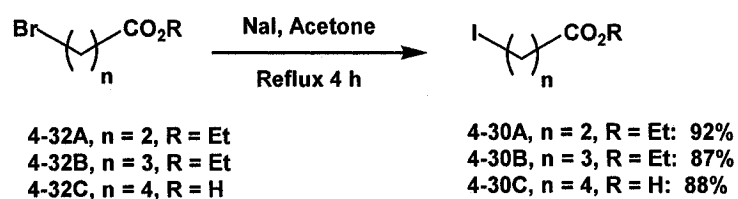
Scheme 4.15 Preparation of iodoesters and iodoacid from corresponding bromides.

When the reaction conditions developed for the alkylation of diethyl

cyanomalonate by ethyl 3-iodopropanoate (4-30A, 2 h, 80°C) were used for the ethyl 4-

iodobutanoate (4-30B) alkylation reaction. However, signals corresponding to unreacted

ethyl 4-iodobutanoate and the monodecarboethoxylated side-product (< 10%), in addition

to the desired product, diethyl 2-carboxyethyl-2-cyanohexanedioate (4-31B) were

observed in the ^{13}C NMR spectrum of the reaction mixture. Since prolonged heating of

the alkylation reaction mixture has been shown to be the predominant factor in monodecarboethoxylation, the reaction time was reduced from 2 h to 1.5 h. As a result, the NMR signals corresponding to the monodecarboethoxylated product were considerably reduced, but the NMR signals corresponding to unreacted ethyl 4-iodobutanoate remained. In an attempt to eliminate unreacted ethyl 4-iodobutanoate, the alkylation reaction was heated for 1.5 h using a 50% excess of tetrabutylammonium diethyl cyanomalonate. After work up, no signals corresponding to ethyl 4-iodobutanoate were detected by NMR spectroscopy. However, a small amount (< 10%) of ethyl cyanoacetate, most likely a side product from heating the excess diethyl cyanomalonate, was detected in the ^{1}H NMR spectrum of the isolated product. A simple extraction of the product mixture with 1 M NaOH eliminated the ethyl cyanoacetate signals and gave diethyl 2-carboxyethyl-2-cyanohexanedioate (4-31B) as a clean product by NMR spectroscopy.

Alkylation of tetrabutylammonium diethyl cyanomalonate (1-1G) by halide substituted carboxylic acids has been demonstrated in Chapter 2 of this thesis, and alkylation using 5-iodopentanoic acid (4-30D) yielded 1-ethyl 2-carboxyethyl-2-cyanoheptanedioic acid (4-31C, Scheme 4.14). Alkylation of diethyl cyanomalonate using a bromo or iodoester was preferred over the corresponding acid since it involved a simpler reaction workup and gave a cleaner product in greater yield (>80% for the esters vs. <50% for the corresponding acids). 5-Iodopentanoic acid was used in the alkylation of diethyl cyanomalonate because it was readily available.

4.3.2 Synthesis of 2-Cyanodicarboxylic Acids and Their Salts

A series of 2-cyanodicarboxylic acids (**4-33**) was prepared from the alkylated diethyl cyanomalonates by ester hydrolysis and monodecarboxylation under basic conditions (Scheme 4.16). A water-methanol mixture was chosen to assist the dissolution of the alkylated diethyl cyanomalonate. To minimize hydrolysis of the nitrile, the reaction was heated at reflux for only 1.5 h using 1.1 equivalents of base for each ester or acid functional group. As a result, the nitrile dicarboxylic acids were obtained in high yield, and no nitrile hydrolysis was detected by NMR spectroscopy.

Initially, the disodium salts (**4-24**) were prepared directly from 2-cyanobutanedioic acid (**4-33A**) and 2-cyanopentanedioic acid (**4-33B**) by ion exchange. However, purification of the disodium salts by recrystallization was not feasible; disodium 2-cyanobutanedioate was recovered from methanol in a yield of only 9% and disodium 2-cyanopentanedioate did not recrystallize from hot aqueous ethanol. As an alternative, the benzylammonium salts of the 2-cyanodicarboxylic acids were prepared by mixing one equivalent of benzylamine with the diacid. In general, the bis(benzylammonium) salts (**4-34**) proved easy to recrystallize from ethanol/ether, giving pure products with reasonable yields (60-70%). The disodium 2-cyanodicarboxylate salts (**4-23**) were prepared from the purified bis(benzylammonium) salts by ion-exchange chromatography and were used in enzyme inhibition studies without further purification.
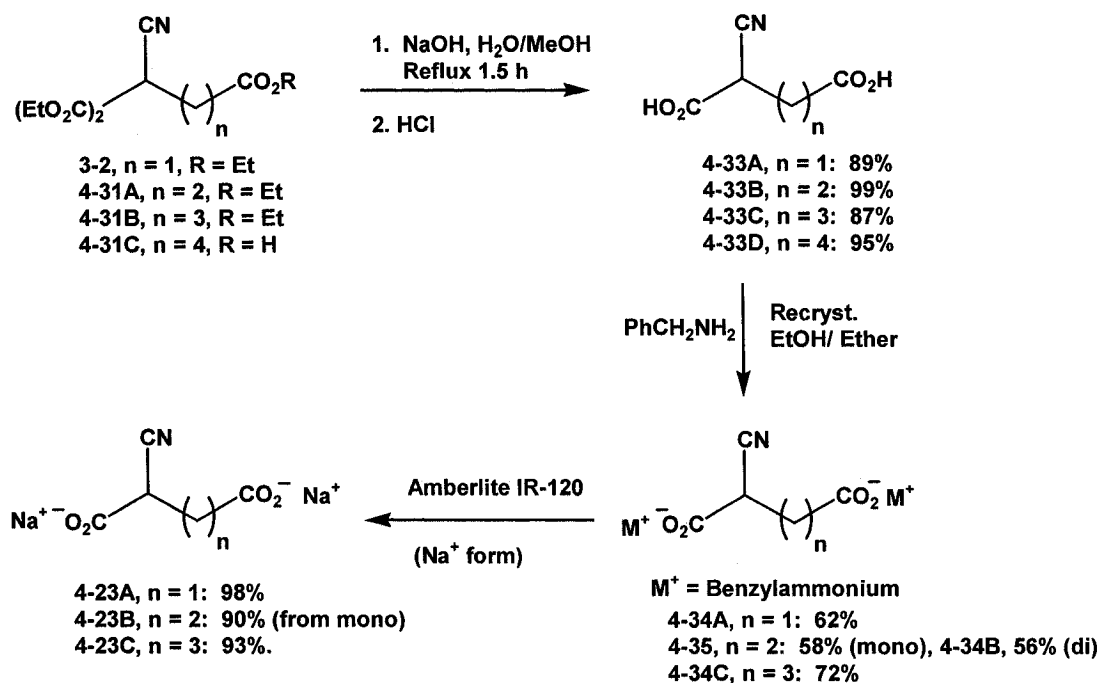
Scheme 4.16 Synthesis of 2-cyanodicarboxylic acids and their salts.

This synthetic route towards 2-(aminomethyl)dicarboxylic acids (**4-1**) also

produces a homologous series of 2-cyanodicarboxylic acids (**4-33**), which are nitrile

analogues of α-amino acids. Cyanoamino acid analogues of α-amino acids where the α-

carboxyl group has been replaced by a nitrile group have shown inhibitory effects on an

E. coli glutamate decarboxylase.[32] For the 2-cyanodicarboxylic acids prepared in this

chapter, the α-amino group was replaced by a nitrile group. Due to the acidity of the α-

carbon, these nitrile compounds may function as transition state analogues of enzymes

that act at the α-carbon of glutamic acid.

4.3.3 Synthesis of 2-(Aminomethyl)dicarboxylic Acids

Initial preparations of 2-(aminomethyl)dicarboxylic acids (**4-1**) involved ester hydrolysis and monodecarboxylation of the alkylated diethyl cyanomalonate followed directly by nitrile reduction and isolation by ion-exchange chromatography. A much cleaner amino acid product, however, was obtained by reduction of recrystallized bis(benzylammonium) 2-cyanodicarboxylate salts (**4-34**, Scheme 4.17). Since the bis(benzylammonium) salt of 2-cyanoheptanedioic acid (**4-33D**) could not be recrystallized, nitrile reduction was carried out directly on the diacid.

Reduction of the nitrile using $CoCl_2/NaBH_4$[33,34] is conveniently carried out in methanol or aqueous solution, solvents appropriate to dissolve the polyfunctional water soluble compounds prepared in this thesis. This nitrile reduction procedure utilizing $CoCl_2/NaBH_4$ in methanol was first reported in 1969.[33] The black precipitate observed during the reaction, later identified as Co_2B, catalyzes the heterogeneous reduction of the nitrile through coordination to the nitrile.[35]
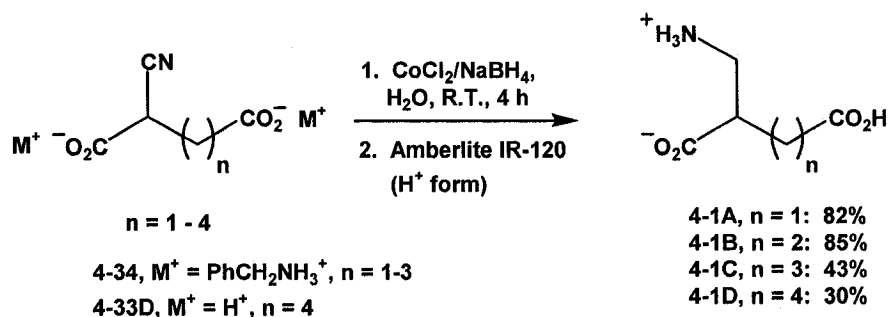
Scheme 4.17 Synthesis of 2-(aminomethyl)dicarboxylic acids.

The nitrile reduction was carried out in aqueous solution, and due to the exothermic nature of the reaction, sodium borohydride had to be added in small portions

to the amino acid/ $CoCl_2$ solution. During the addition of $NaBH_4$, generation of cobalt boride as a black solid and the evolution of hydrogen gas occurred. The reaction mixture was stirred at room temperature for four hours until bubbling had completely ceased. The pH was brought down to 4.5-5, to assist the binding of the amino acid to the ion-exchange column. This left a significant amount of undissolved cobalt boride in the reaction mixture, which was removed by gravity filtration before the sample was applied to the ion exchange column. It was found that this precipitate would otherwise react with the acidic ion exchange column, causing the release of hydrogen gas.

A significant factor in the yield of the amino acid was the volume of water used in the reduction reaction. Typically, if 4-5 mmol of nitrile were dissolved in 25-50 mL of water, a maximum yield of 50% was obtained. However, if only 10-15 mL of water were used, the yield increased considerably to >80%.

4.4 Cyclization of 2-(Aminomethyl)dicarboxylic Acids Upon Heating

A discrepancy between the melting points for the 2-(aminomethyl)butanedioic acid (**4-1A**) and 2-(aminomethyl)pentanedioic acid (**4-1B**) products and literature reports was noted. On heating in an open capillary tube using a Gallenkamp melting point apparatus, 2-(aminomethyl)butanedioic acid gave a melting point of 123-124°C. When a few crystals were placed on an open cover slip and heated using a Fisher-Johns apparatus, the appearance of the crystals changed from transparent to translucent at 124°C and the crystals melted at 180-184°C. The sample was heated to 190°C, allowed to cool to 110°C and heated again to 190°C. No change was observed during the second

179

heating. The melting point for racemic 2-(aminomethyl)butanedioic acid is reported twice in the literature as 180°C (Zilkha, *et. al.* used a Fisher-Johns m.p. apparatus).[22,25]

Similarly, 2-(aminomethyl)pentanedioic acid (**4-1B**) gave a melting point of 164-165°C (open capillary tube, Gallenkamp melting point apparatus) which was lower than the 171-173°C reported in the literature.[21] When a few crystals of 2-(aminomethyl)pentanedioic acid were placed on an open cover slip and heated slowly, melting occurred at 166-168°C. Heating was continued up to 190°C, and the sample was allowed to cool to 130°C. On heating a second time, the sample melted at 182-184°C.

Differential scanning calorimetry (DSC) was used to further investigate the thermal behaviour of these compounds. For 2-(aminomethyl)butanedioic acid (**4-1A**), an endothermic process was recorded at approximately 110°C. This was followed by melting at approximately 170°C. No change was observed during a second heating. Similarly, heating of 2-(aminomethyl)pentanedioic acid(**4-1B**) produced an endothermic event at approximately 155°C, followed by melting at approximately 185°C. A second heating was not performed. Decomposition of both samples was noted above 190°C, and each sample lost 20-30% of its initial mass during the DSC heating cycles.

Whether the mass loss was associated with the endothermic events at 110°C and 155°C or with the decomposition above 190°C was investigated by heating two carefully weighed solid samples of 2-(aminomethyl)pentanedioic acid (**4-1B**) at 135°C for 13 h. When the cooled solid material was weighed, a decrease of 11.4% in mass was measured for both samples. The new material had a melting point of 178-180°C, and the chemical shifts and the splitting patterns of the signals from the ^{1}H NMR spectrum differed from those of the starting material.

To determine the length of time for this decrease in mass to occur upon heating, weighed samples of 2-(aminomethyl)butanedioic acid (**4-1A**) and 2-(aminomethyl)pentanedioic acid (**4-1B**) were placed in an oven at 130°C for 1-h intervals, removed, allowed to cool, weighed and reheated until the mass change became negligible. A constant mass was achieved for 2-(aminomethyl)butanedioic acid after 8 h with a 21% decrease in mass. Heating of 2-(aminomethyl)pentanedioic acid for 5 h resulted in no further decrease in mass, with the total mass lost at 20%. The ^{1}H NMR spectra recorded from these samples after heating matched with those recorded from previously heated samples, and the ^{13}C NMR spectra showed the same number of signals as those of the initial amino acids, but at different chemical shifts.

The endothermic events recorded by DSC and the decrease in mass observed upon heating the amino acids indicate the loss of a volatile component. Elemental analysis data in the literature[18,25] suggests that 2-(aminomethyl)butanedioic acid (**4-1A**) exists as the monohydrate, but the 21% decrease in mass noted above, corresponds to the loss of two molecules of water (36.04 g/mol), from the monohydrated amino acid (165.15 g/mol).

In the same experiment, the 2-(aminomethyl)pentanedioic acid (**4-1B**) sample lost 20.1% mass after heating for 5 h at 130°C, also corresponding to the loss of two molecules of water from the amino acid monohydrate (179.18 g/mol). In the previous experiment, only an 11.4% decrease in mass was obtained on heating 2-(aminomethyl)pentanedioic acid. This smaller mass loss corresponds to the loss of a single water molecule (18.02 g/mol) from the amino acid (161.16 g/mol), and the non-hydrated form of 2-(aminomethyl)pentanedioic acid from the literature.[21] The products of

the two heating experiments have identical ^{1}H NMR spectra, suggesting that different

hydration states of the 2-(aminomethyl)pentanedioic acid samples led to the different

losses of mass.

In order to generate enough product for a full spectroscopic characterization and

comparison to the starting material, samples of both β-amino acids were heated at 130°C

for 16 h. 2-(Aminomethyl)pentanedioic acid (**4-1B**) gave a colourless powder (70%

yield) with a melting point of 169-171°C with a ^{1}H NMR spectrum distinct from the

starting material (Figure 4.3). 2-(Aminomethyl)butanedioic acid (**4-1A**), gave a

colourless, hygroscopic solid (62% yield). Unfortunately due to its hygroscopic nature, a

melting point could not be obtained.
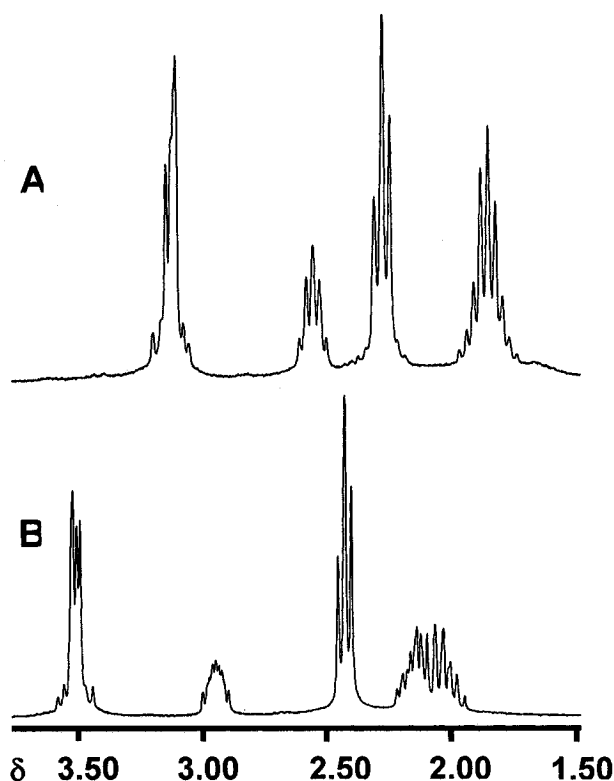
Figure 4.3 ^{1}H NMR spectra (D$_2$O) for 2-(aminomethyl)pentanedioic acid: (A) before
heating; (B) after heating at 140°C for 16 h.

The negative ion ESI-MS of the products from heating 2-(aminomethyl)butanedioic acid(**4-1A**) showed a strong signal at *m/z* 128. Similarly, the product from 2-(aminomethyl)pentanedioic acid (**4-1B**) gave a strong signal at *m/z* 142. In each case, the [M-H]⁻ ions of the product were 18 mass units lower than [M-H]⁻ ions expected for the amino acids, confirming the loss of water upon heating. Also, no signals corresponding to [M-H]⁻ of either β-amino acid were detected in the mass spectra of the products obtained from heating. For an in-depth discussion of the ESI-MS fragmentation behaviour of these compounds, refer to Chapter 5 of this thesis.

The IR spectrum of the product from 2-(aminomethyl)butanedioic acid showed an N-H stretch at 3201 cm⁻¹ and a carbonyl stretch at 1640 cm⁻¹, characteristic of a 5-membered lactam. These stretches were not observed in the IR spectrum of 2-(aminomethyl)butanedioic acid(**4-1A**). Similarly, the IR spectrum of the product from 2-(aminomethyl)pentanedioic acid showed an N-H stretch at 3200 cm⁻¹ and a carbonyl stretch at 1640 cm⁻¹, characteristic of a 6-membered lactam. These stretches were not observed in the IR spectrum of 2-(aminomethyl)pentanedioic acid (**4-1B**).

Given the IR and MS data, as well as the chemical change indicated by the ¹H and ¹³C NMR spectra (e.g. Figure 4.3 and subsections 4.4.2 and 4.4.3), it is most likely that heating converted the β-amino acids to the corresponding lactams (**4-36**, Scheme 4.18). Cyclization upon heating has been demonstrated for α-aminodicarboxylic acids. Heating glutamic acid[36] and α-aminoadipic acid[37] at their melting points have produced their corresponding five and six-membered lactams. Aspartic acid and α-aminopimelic acid do not cyclize as readily as the corresponding lactams would consist of four and seven-membered rings.[38]
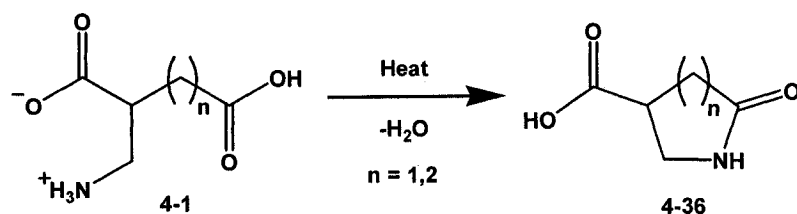
Scheme 4.18 Cyclization of 2-(aminomethyl)dicarboxylic acids to form lactams.

4.4.1 Structural Characterization of 2-(Aminomethyl)butanedioic Acid and 2-(Aminomethyl)pentanedioic Acid

Full structural characterizations of 2-(aminomethyl)butanedioic acid (**4-1A**) and

2-(aminomethyl)pentanedioic acid (**4-1B**) were carried out for comparison to the

proposed lactam products (**4-36**). This was also necessary to determine the location of

deuterium in several deuterium labelled 2-(aminomethyl)pentanedioic acid samples that

are discussed in Chapter 5.

2-(Aminomethyl)butanedioic acid (**4-1A**) melted at 123-124°C and gave IR bands

(Nujol, cm^{-1}) at 3122 (NH_3^+), 1717 (CO_2H) and 1670 (CO_2^-). Abundant ions at m/z 148

and 146, corresponding to [M+H]$^+$ and [M-H]$^-$, respectively, were detected by positive

and negative ion ESI-MS.

2-(Aminomethyl)pentanedioic acid (**4-1B**) melted at 164-165°C and the IR

spectrum (Nujol) showed characteristic bands (cm^{-1}) at 3133 (NH_3^+), 3000 br. (CO_2-H),

1717 (CO_2H), 1609 (CO_2^-) and 1224 (CO_2H) which correspond well with values reported

in the literature (KBr).[21,28] In the positive ion and negative ion modes of ESI-MS, 2-

(aminomethyl)pentanedioic acid produced expected ions at m/z 162 and 160

corresponding to [M+H]$^+$ and [M-H]$^-$, respectively.

4.4.1.1 2-(Aminomethyl)butanedioic Acid: Assignment of NMR Signals

Three aliphatic signals at δ_C 36-41 and two carboxyl signals near δ_C 178 were present in the ^{13}C NMR spectrum of 2-(aminomethyl)butanedioic acid (**4-1A**, Table 4.1). The ^1H NMR (500 MHz, D_2O) spectrum showed five groups of signals between δ_H 2.50-3.30. In the HSQC spectrum, the ^1H signals at δ_H 2.58 and 2.72 correlated with one ^{13}C signal at δ_C 36.5, indicating a pair of diastereotopic hydrogens bonded to the same carbon. Similarly, a second pair of diastereotopic hydrogens at δ_H 3.15 and 3.24 correlated with a single carbon atom at δ_C 40.8. By the process of elimination, the remaining 1-H multiplet signal at δ_H 2.92-2.97 must arise from H-2; it correlated with the ^{13}C signal at δ_C 41.1. In the COSY spectrum, the δ_H 2.92-2.97 multiplet correlated with each of the other four ^1H NMR signals, thereby confirming the assignment of H-2. The signals at δ_H 2.58 and 2.72 correlated with each other and with the signal at δ_H 2.92-2.97 and the signals at δ_H 3.15 and 3.24 correlated with each other and to the signal at δ_H 2.92-2.97.

The HSQC and COSY correlations are consistent with a five-spin system arranged as -CH_2-CH-CH_2-, but were unable to distinguish H-2' from H-3. On the other hand, four bonds separate H-2' from C-4, whereas H-3 and C-4 are only two bonds apart, suggesting that H-2' and H-3 would show different patterns of heteronuclear long-range coupling. In the HMBC spectrum, long-range coupling was observed between the signals at δ_H 2.58 and 2.72 (assigned to H-3) and the signals for both carboxyl carbons, whereas the signals at δ_H 3.12 and 3.19 (H-2') only correlated with one carboxyl carbon signal (δ_C 178.9 or C-1). The assignments for 2-(aminomethyl)butanedioic acid are summarized in Table 4.1. Note that the chemical shifts of H-2' and C-2' are very similar to those of H-2'

and C-2' in 2-(aminomethyl)pentanedioic acid (Table 4.2, next subsection) which were

assigned unambiguously using COSY correlations.

Table 4.1 Assignment of NMR signals for 2-(aminomethyl)butanedioic acid.

4-1A
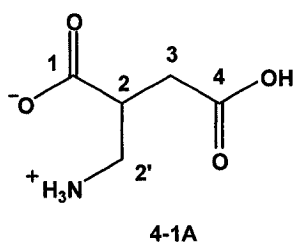
Position	^{13}C NMR	^{1}H NMR	COSY	HMBC
1	178.9	-	-	H-2', H-3
2	41.1	2.92-2.97 (m, 1H)	H-3, H-2'	H-2'
3	36.5	2.58 (dd, J = 16, 7, 1H) 2.72 (dd, J = 16, 6, 1H)	H-2	H-2'
4	177.8	-	-	H-3
2'	40.8	3.15 (dd, J = 13, 4, 1H) 3.24 (dd, J = 13, 9, 1H)	H-2	H-2, H-3

4.4.1.2 2-(Aminomethyl)pentanedioic Acid: Assignment of NMR signals

Four aliphatic carbon signals in the range δ_C 25-44 and two carboxylate carbon

signals near δ_C 179 were present in the ^{13}C NMR spectrum of 2-

(aminomethyl)pentanedioic acid (**4-1B**, Table 4.2). The ^{1}H NMR (500 MHz, D$_2$O)

spectrum showed five signals: two 2-H multiplets, one 1-H multiplet and two 1-H

doublet of doublets. Literature ^{1}H NMR (220 MHz, D_2O) data[21] gave the following results: δ_H 3.09 ("BC part of an ABC spectrum", 2H), 2.61 (quint., 1H), 1.84 (q, 2H) and 2.39 (t, 2H), which correspond well with the data collected in this investigation.

The COSY spectrum of 2-(aminomethyl)pentanedioic acid allowed for complete proton assignment. The multiplet at δ_H 2.62-2.68 integrated to 1-H and correlated with a 2-H multiplet at δ_H 1.84-1.96 and the two doublet of doublets at δ_H 3.12 and 3.19. The signals at δ_H 3.12 and 3.19 each integrated for one H and only correlated with each other and the signal at δ_H 2.62-2.65. In the HSQC spectrum, the δ_H 3.12 and 3.19 signals both correlated with the same carbon signal at δ_C 40.7, suggesting that they are two diastereotopic protons on the same carbon. Together, these results assign the signal at δ_H 2.62-2.65 to H-2 and the signals at δ_H 3.12 and 3.19 to H-2'.

Once the proton signals were assigned, the HSQC spectrum allowed for complete assignment of all the aliphatic carbon signals as presented in Table 4.2. Assignment of the two carboxyl groups was made from the HMBC spectrum. Long-range coupling between the proton signal for H-4 (δ_H 2.38-2.48) and the carbon signal at δ_C 178.8 assigned that carboxyl group to C-5. Long-range coupling with the H-2' protons (δ_H 3.12, 3.19) assigned the carbon signal at δ_C 179.1 to the remaining carboxyl group at position C-1. The assignments for 2-(aminomethyl)pentanedioic acid are summarized in Table 4.2.

Table 4.2 Assignment of NMR signals for 2-(aminomethyl)pentanedioic acid.

4-1B
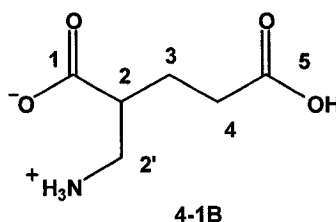
Position	^{13}C NMR	^{1}H NMR	COSY	HMBC
1	179.1	-	-	H-2'
2	44.0	2.62-2.68 (m, 1H)	H-3, H-2'	H-3, H-4, H-2'
3	25.3	1.84-1.96 (m, 2H)	H-2, H-4	H-2, H-4, H-2'
4	32.2	2.38-2.48 (m, 2H)	H-3	H-3
5	178.8	-	-	H-4
2'	40.7	3.12 (dd, J = 13, 5, 1H) 3.19 (dd, J = 13, 8, 1H)	H-2, H-2'α,β	H-2, H-3

4.4.2 Assignment of NMR Signals for 4-Carboxy-2-pyrrolidone

4-Carboxy-2-pyrrolidone (**4-36A**, 5-oxo-3-pyrrolidinecarboxylic acid) contains

the same structural fragment (–CH$_2$-CH-CH$_2$–) as 2-(aminomethyl)butanedioic acid (**4-**

1A). As was discussed previously for 2-(aminomethyl)butanedioic acid, the different

patterns of heteronuclear coupling observed in the HMBC spectrum were required for

complete assignment of NMR signals, as a COSY spectrum alone was insufficient.

Unambiguous assignments could not be made for 4-carboxy-2-pyrrolidone from NMR

spectra obtained in D$_2$O, because only one carbonyl ^{13}C NMR signal at δ_C 179.8 was

observed, indicating very similar chemical shifts for the carboxylic acid and the amide

carbons. Without two distinct ^{13}C NMR carbonyl signals, a full assignment could not be made from the HMBC spectrum in D_2O. Perhaps the carbonyl resonances would be resolved in another solvent. The ^{1}H NMR spectrum for 4-carboxy-2-pyrrolidone recorded in DMSO-d_6 was reported in the literature,[39] which corresponded well with data obtained in this investigation. Unfortunately, the ^{13}C NMR spectrum recorded in DMSO-d_6 did not give two distinct carbonyl peaks. However, two distinct carbonyl peaks were observed in the ^{13}C NMR spectrum recorded in methanol-d_4, so a complete signal assignment could be made for 4-carboxy-2-pyrrolidone in this solvent.

The ^{13}C NMR spectrum in methanol-d_4 gave three aliphatic signals between δ_C 35 and 49 and two distinct carbonyl signals at δ_C 179.8 and 180.1 (Table 4.3). The four ^{1}H NMR (500 MHz, methanol-d_4) signals between δ_H 2.40 and 3.55, consisted of two multiplets and two doublet of doublets.

The HSQC spectrum allowed for assignment of all of the aliphatic carbons. The two signals at δ_H 2.42 and 2.55 correlated with a single ^{13}C signal at δ_C 35.4, indicating that they are diastereotopic. The 1-H multiplet at δ_H 3.12-3.18 correlated to the ^{13}C signal at δ_C 42.2. The 2-H multiplet at δ_H 3.46-3.55 correlated with the signal at δ_C 48.5.

In the COSY spectrum, the 1-H signal at δ_H 3.12-3.18 correlated with the signal at δ_H 3.46-3.55 and the two doublet of doublet signals at δ_H 2.42 and 2.55. Therefore, the signal at δ_H 3.11-3.18 was assigned to H-2. The two doublet of doublet signals at δ_H 2.42 and 2.55 correlated with each other and with the H-2 signal at δ_H 3.12-3.18. The multiplet at δ_H 3.46-3.55 only correlated with the signal for H-2, giving the -CH$_2$-CH-CH$_2$- pattern mentioned earlier.

The protons corresponding to H-2' and H-3 were assigned from the HMBC spectrum by their differences in correlation to the two carbonyl groups. The proton signals at δ_H 3.11-3.18 (H-2) and 3.46-3.55 only correlated to the carbonyl signal at δ_C 179.8 with 2 and 3 bond correlations respectively. The proton signals at δ_H 2.42 and 2.55 showed strong 2-bond correlations to the carbonyl carbon signal at δ_C 180.1. Therefore, the ^{1}H signal at δ_H 3.44-3.55 was assigned to H-2' and the ^{13}C NMR signal at δ_C 179.8 was assigned as the carboxylic acid at C-1. Similarly, the two doublet of doublets at δ_H 2.43 and 2.55 were assigned to H-3 and the ^{13}C NMR signal at δ_C 180.1 was assigned to the amide carbon at C-4.

The HMBC spectrum also showed differing correlations between the individual diastereotopic H-3 protons (δ_H 2.42 and 2.55) and C-2'. Correlation was observed between the 1-H doublet of doublets at δ_H 2.42 and the carbon signal at δ_C 48.5 corresponding to C-2'. However, correlation was not observed between the second doublet of doublets (δ_H 2.55) assigned to H-3 and C-2'. This observed difference in correlation is the result of the Karplus relationship between the dihedral angle and the coupling constant.[40] Coupling constants are largest for dihedral angles that are 180° or 0° and smallest for angles that are 90°. The Karplus relationship applies to $^3J_{C-H}$ coupling as well as $^3J_{H-H}$ coupling.[41] The signal at δ_H 2.55 (H-3), showed no correlation to C-2', so its bond to C-3 must form an angle of approximately 90° with the bond between C-2 and C-2'. The bond between H-3 (δ_H 2.42) and C-3 probably forms an angle of approximately 180° with the bond between C-2 and C-2' which accounts for the strong correlation between δ_H 2.42 and δ_C 48.5. This observation is further evidence of ring formation, as

the restricted conformation in a five-membered ring would produce the different HMBC correlations that depend on a fixed dihedral angle.

The ^{1}H and ^{13}C NMR spectra of 4-carboxy-2-pyrrolidone recorded in methanol-d_4 or D$_2$O were similar in appearance. In the ^{1}H NMR spectrum, the signals corresponding to H-2' that appeared as two resolved doublet of doublets in D$_2$O appeared as a multiplet in methanol-d_4. Conversely, the signal corresponding to H-3, which appeared as a multiplet in D$_2$O appeared as two resolved doublet of doublets in methanol-d_4. Except for the HMBC carbonyl correlations the 2-D correlations in both solvents were the same for both solvents. The comparison of ^{1}H and ^{13}C NMR data for 2-(aminomethyl)butanedioic acid (D$_2$O) and 4-carboxy-2-pyrrolidone (D$_2$O, methanol-d_4) is presented in Table 4.3.

4.4.3 Assignment of NMR Signals for 2-Piperidone-5-carboxylic Acid

The three multiplets and two doublet of doublets present in the ^{1}H NMR (D$_2$O) spectrum of 2-piperidone-5-carboxylic acid (4-36B), correlated in the HSQC spectrum with the aliphatic carbons as indicated in Table 4.4. Separated signals for two pairs of diastereotopic hydrogens were indicated by the correlation of the two multiplet signals at δ_H 1.92-2.02 and 2.11-2.16 with the carbon at δ_C 23.0, and the correlation of the two doublet of doublet signals at δ_H 3.46 and 3.52 with the same carbon at δ_C 43.1.

The COSY spectrum allowed for complete assignment of the protons. The 1-H multiplet at δ_H 2.80-2.85 correlated with the two doublet of doublets signals at δ_H 3.46, 3.52 as well as the two multiplet signals at δ_H 1.98-2.02, 2.11-2.16 thereby assigning it as H-2. The two ^{1}H signals corresponding to the doublet of doublets at δ_H 3.46, 3.52 can be assigned to H-2' since they only correlate with each other and the signal at 2.80-2.85 ppm

(H-2). The two multiplet signals (δ_H 1.92-2.02 and 2.11-2.16) correlated to each other, the signal at H-2 (δ_H 2.80-2.85) and the 2-H triplet at δ_H 2.43. The signal at δ_H 2.43 only correlates with the two signals at δ_H 1.92-2.02 and 2.11-2.16 and therefore can be assigned to H-4, leaving the more highly correlated multiplet signals (δ_H 1.92-2.02, 2.11-2.16) to be assigned to H-3.

The assignment of the carboxyl carbon signals was made using HMBC. The signals corresponding to H-2' correlated with the carboxyl carbon signal at δ_C 179.1, thereby assigning it as C-1. The signals corresponding to H-4 correlated with the signal at δ_C 178.8, assigning the second carboxyl as C-5. The comparison of the structural assignments between 2-(aminomethyl)pentanedioic acid (**4-1B**) and 2-piperidone-5-carboxylic acid (**4-36B**) is summarized in Table 4.4.

Table 4.3 Comparison of 2-(aminomethyl)butanedioic acid and 4-carboxy-2-pyrrolidone chemical shifts.

4-1A

4-36A
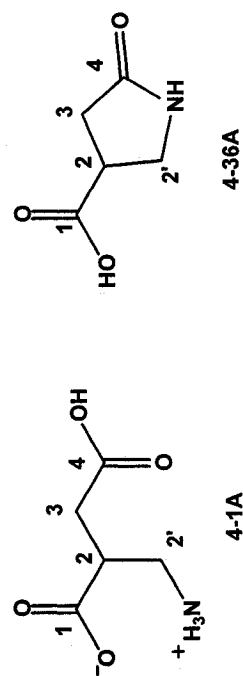
| | 2-(Aminomethyl)butanedioic acid | | 4-Carboxy-2-pyrrolidone | | | | | |
| | D$_2$O | | D$_2$O | | Methanol-d_4 | | | |
Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	COSY	HMBC
1	-	178.9	-	179.8	-	179.8	-	H2, H2'
2	2.92-2.97 (m, 1H)	41.1	3.36-3.43 (m,1H)	40.0	3.12-3.18 (m, 1H)	42.2	H3, H2'	H3, H2'
3	2.58 (dd, J = 16, 7, 1H) 2.72 (dd, J = 16, 6, 1H)	36.5	2.60-2.70 (m, 2H)	33.8	2.42 (dd, J = 17, 10,1H) 2.55 (dd, J = 17, 7,1H)	35.4	H2	H2, H2'
4	-	177.8	-	179.8	-	180.1	-	H3
2'	3.15 (dd, J = 13, 4, 1H) 3.24 (dd, J = 13, 9, 1H)	40.8	3.59 (dd, J = 10, 6, 1H) 3.70 (dd, J = 10, 9, 1H)	45.4	3.46-3.55 (m, 2H)	48.5	H2	H2, H3

Table 4.4 Comparison of 2-(aminomethyl)pentanedioic acid and 2-piperidone-5-carboxylic acid chemical shifts. See Figure 4.3 for a comparison of ^{1}H NMR spectra.

4-1B

4-36B
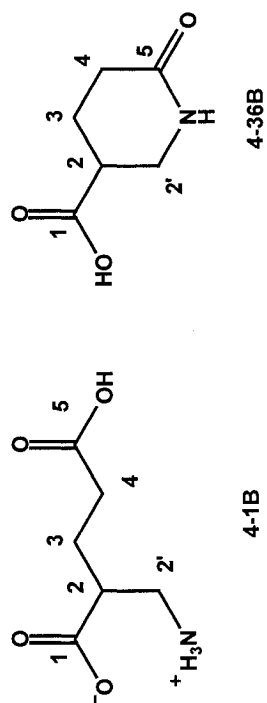
Position	2-(Aminomethyl)pentanedioic acid		2-Piperidone-5-carboxylic acid			
	^{1}H	^{13}C	^{1}H	^{13}C	COSY	HMBC
1	-	179.1	-	180.0	-	H3, H2'
2	2.63-2.68 (m, 1H)	44.0	2.80-2.85 (m, 1H)	39.0	H3, H2'	H3, H4, H2'
3	1.84-1.96 (m, 2H)	25.3	1.98-2.02 (m, 1H) 2.11-2.16 (m, 1H)	23.0	H2, H4	H2, H4, H2'
4	2.38-2.49 (m, 2H)	32.2	2.42-2.45 (m, 2H)	28.0	H3	H2, H3
5	-	178.8	-	175.1	-	H3, H4, H2'
2'	3.12 (dd, J = 13, 5, 1H) 3.19 (dd, J = 13, 8, 1H)	40.5	3.46 (dd, J = 13, 8, 1H) 3.53 (dd, J = 13, 6, 1H)	43.1	H2	H2, H3

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4.4.4 Evidence Supporting Cyclization of the 2-(Aminomethyl)dicarboxylic Acids

The melting points of the β-amino acids obtained experimentally did not correspond with those given in the literature. Upon heating a chemical change occurred, as was indicated by the differences observed in the ^{1}H NMR spectra, ESI-MS, the recorded loss of water and the different melting points of the products. The compound generated from a heated sample of 2-(aminomethyl)pentanedioic acid (**4-1B**) melted at 182-184°C, which is the reported[21] melting point (182-185°C) for 2-piperidone-5-carboxylic acid (**4-36B**). The cyclization of 2-(aminomethyl)pentanedioic acid by heating at 170-180°C has been reported in the literature.[21] The observed appearance change of 2-(aminomethyl)butanedioic acid (**4-1A**) at 123-124°C during the heating process is followed by melting at 180-184°C, corresponding to the values reported in the literature for 2-(aminomethyl)butanedioic acid.[22,25] The melting observed at 180-184°C was probably due to the lactam. The literature value for 4-carboxy-2-pyrrolidone (**4-36A**) was reported as 159-160°C.[39]

The cyclization of the 2-(aminomethyl)dicarboxylic acids (**4-1**) to form lactams (**4-36**) upon heating is clearly demonstrated by the spectroscopic results and is supported by the results of the various heating and DSC experiments. Cyclization of amino acids upon heating has been noted in EI mass spectrometry. Since amino acids are involatile, substantial heating is required for vapourization, which can result in unwanted reactions or decomposition. Amino acids such as glutamic acid[42] and creatine[43] appear to dehydrate and cyclize upon heating, forming pyroglutamic acid and creatinine, respectively. Cyclization of dipeptides upon heating has also been observed in EI mass spectrometry.[44] The terminal amino group of one amino acid reacts with the terminal

carboxylate group of the second amino acid, resulting in loss of water and cyclodipeptide formation. Therefore, the mass spectra collected are not solely of the amino acid, but contain significant peaks derived from the cyclization product.

4.5 Enzyme Inhibition

Due to their structural similarity to glutamic acid, several of the 2-(aminomethyl)dicarboxylic acids (**4-1**) and 2-cyanodicarboxylic acids (**4-33**) were tested as inhibitors against glutamate racemase and glutamate dehydrogenase, two glutamate catabolizing enzymes.

The 2-cyanodicarboxylic acids, 2-cyanobutanedioate ($K_i = 8$ mM) and 2-cyanopentanedioate ($K_i = 9$ mM) were found to be weak competitive inhibitors of glutamate racemase ($K_M = 1$ mM, L-Glu) isolated from *Fusobacterium nucleatum*.[45] However, 2-(aminomethyl)butanedioic acid ($K_i = 62$ mM) was found to be an extremely weak uncompetitive inhibitor.

2-(Aminomethyl)butanedioic acid ($K_M = 15.4$, $K_i = 7.3$) and 2-(aminomethyl)pentanedioic acid ($K_M = 15$, $K_i = 26$), as well as the sodium 2-cyanobutanedioate ($K_M = 4.8$, $K_i = 1.1$) and 2-cyanopentanedioate ($K_M = 6.7$, $K_i = 1.4$) were tested against glutamate dehydrogenase ($K_M = 29$ mM, L-Glu).[46] The 2-cyanodicarboxylic acids were better inhibitors of glutamate dehydrogenase than the 2-(aminomethyl)dicarboxylic acids.

4.6 Summary

Using reaction conditions developed in Chapter 2 of this thesis, the diethyl cyanomalonate anion was successfully alkylated by a series of halogenated esters and a carboxylic acid. The alkylated diethyl cyanomalonates were hydrolyzed and monodecarboxylated to give a homologous series of 2-cyanodicarboxylic acids, which were reduced to give a similar series of 2-(aminomethyl)dicarboxylic acids. An investigation of discrepancies between the literature melting point and the melting points obtained in this investigation revealed that the two shorter chain β-amino acids cyclize upon heating to form lactams. Both the nitriles and β-amino acids showed inhibitory behaviour towards two glutamate-catabolizing enzymes. ESI-MS studies of this collection of homologous 2-cyanodicarboxylic acids and 2-(aminomethyl)dicarboxylic acids for testing as potential enzyme inhibitors and for the ESI-MS studies is presented in the next chapter.

4.7 Experimental

4.7.1 General Procedures

Refer to Chapter 2 experimental section for general procedures (Section 2.5.1)

4.7.2 Preparation of Iodocarboxylic Acids and Iodoesters

A brominated carboxylic acid or ester was added to a solution of NaI (1.5 equiv.) in acetone (50 mL, dried over $CaSO_4$ for 16 h) and the mixture was heated at reflux with stirring for 4 h. The reaction mixture was allowed to cool to room temperature, filtered and the filtrate was evaporated *in vacuo*. The residual oil was suspended in saturated

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brine (50 mL) and extracted into ether (4 x 25 mL). The combined ether extracts were dried over MgSO$_4$ and evaporated *in vacuo* giving the iodocarboxylic acid or iodoester.

Ethyl 3-Iodopropanoate[31] (4-30A): A mixture of ethyl 3-bromopropanoate (41.0 g, 23 mmol) and sodium iodide (5.10 g, 34 mmol) yielded ethyl 3-iodopropanoate as an oil (4.75 g, 92%). ^{1}H NMR (CDCl$_3$) δ 1.28 (t, J = 7, 3H), 2.97 (t, J = 7, 2H), 3.33 (t, J = 7, 2H), 4.19 (q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ -3.4, 14.0, 38.2, 60.5, 170.5.

Ethyl 4-Iodobutanoate (4-30B): A mixture of ethyl 4-bromobutanoate (5.45 g, 28.0 mmol) and sodium iodide (6.29 g, 41.9 mmol) yielded ethyl 3-iodobutanoate as an oil (5.87 g, 87%). ^{1}H NMR (CDCl$_3$) δ 1.27 (t, J = 7, 3H), 2.13 (quin, J = 7, 2H) 2.44 (t, J = 7, 2H), 3.25 (t, J = 7, 2H), 4.14 (q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ 5.6, 14.3, 28.5, 34.9, 60.6, 172.4.

5-Iodopentanoic acid (4-30C): A mixture of 5-bromopentanoic acid (1.81 g, 10.0 mmol) and sodium iodide (2.25 g, 15.0 mmol) yielded 5-iodopentanoic acid as a solid (2.01 g, 88%). ^{1}H NMR (CDCl$_3$) δ 1.76 (m, 2H), 1.89 (m, 2H), 2.40 (t, J = 7, 2H), 3.20 (t, J = 7, 2H), 8.5 (br s, 2H). ^{13}C NMR (CDCl$_3$) δ 5.8, 25.6, 32.7, 32.9, 179.4.

4.7.3 Alkylation of Tetrabutylammonium Diethyl Cyanomalonate

Tetrabutylammonium diethyl cyanomalonate (1-1G, 2.00 mmol) and a halogenated carboxylic acid or ester (2.00 mmol) were dissolved in DMSO (1 mL/mmol tetrabutylammonium salt) and heated at 80°C. After cooling to room temperature, water

(50 mL) was added and the product was extracted (4 x 25 mL) into diethyl ether. The combined ether extracts were dried over $MgSO_4$ and evaporated *in vacuo*.

Diethyl 2-carboxyethyl-2-cyanopentanedioate (4-31A): After 1.5 h of heating, a mixture of tetrabutylammonium diethyl cyanomalonate (2.13 g, 5.00 mmol) and ethyl 3-iodopropanoate[31] (1.14 g, 5.00 mmol) yielded diethyl 2-carboxyethyl-2-cyanopentanedioate as an oil (1.16 g, 81%). ^{1}H NMR (CDCl$_3$) δ 1.24 (t, J = 8, 3H), 1.34 (t, J = 8, 6H), 2.54 (m, 4H), 4.19 (q, J = 8, 2H), 4.30 (q, J = 8, 4H). ^{13}C NMR (CDCl$_3$) δ 13.7, 14.0, 28.8, 29.8, 54.2, 60.8, 63.9, 114.3, 163.1, 171.0. EI-MS (70 eV) 258 (36), 240 (100), 167 (47), 139 (36), 111 (19), 101 (18). High Resolution MS (70 eV): calculated for $C_{13}H_{19}NO_6$ = 285.1212 amu, found = 285.1193 ± 0.0008 amu.

Diethyl 2-carboxyethyl-2-cyanohexanedioate (4-31B): After 1.5 h of heating, a mixture of tetrabutylammonium diethyl cyanomalonate (3.19 g, 7.50 mmol) and ethyl 4-iodobutanoate (1.21 g, 5.00 mmol) yielded, after an additional extraction with 1 M NaOH (2 x 10 mL), diethyl 2-carboxyethyl-2-cyanohexanedioate as an oil (1.26 g, 84%). ^{1}H NMR (CDCl$_3$) δ 1.26 (t, J = 7, 3H), 1.34 (t, J = 7, 6H), 1.75-1.87 (m, 2H), 2.19-2.26 (m, 4H), 2.40 (t, J = 7, 2H), 4.14 (q, J = 7, 2H), 4.33 (q, J = 7, 4H). ^{13}C NMR (CDCl$_3$) δ 13.6, 14.0, 20.4, 33.0, 33.1, 55.0, 60.3, 63.7, 114.6, 163.3, 172.0. EI-MS (70 eV) 299 (2), 254 (100), 226 (10), 154 (4). High Resolution MS (70 eV): calculated for $C_{14}H_{21}NO_6$ = 299.1369 amu, found = 299.1363 ± 0.0008 amu.

1-Ethyl 2-carboxyethyl-2-cyanoheptanedioic acid (4-31C): After 2 h of heating, a mixture of tetrabutylammonium diethyl cyanomalonate (2 x 2.13 g, 5 mmol) and 5-iodopentanoic acid (2.01 g, 8.80 mmol) yielded ethyl 2-carboxyethyl-2-cyanoheptanedioic acid as an oil (1.20 g, 48%). ^{1}H NMR (CDCl$_3$) δ 1.33 (t, J = 7, 6H), 1.51-1.61 (m, 2H), 1.67-1.76 (m, 2H), 2.15-2.23 (m, 2H), 2.40 (t, J = 7, 2H), 4.32 (q, J = 7, 4H). ^{13}C NMR (CDCl$_3$) δ 13.9, 24.1, 24.7, 33.5, 33.7, 55.4, 64.0, 115.0, 163.7, 178.9.

4.7.4   Ester Hydrolysis

A mixture of di- or triester and NaOH (3.3 equiv.) in methanol-water (5 mL + 45 mL) was heated at reflux for 1.5 h. Water (50 mL) was added to the cooled reaction mixture and the resulting solution was extracted with ether (2 x 25 mL). The aqueous phase was acidified using conc. HCl and extracted with ethyl acetate (4 x 50 mL). The combined ethyl acetate extracts were dried over MgSO$_4$ and evaporated *in vacuo*.

2-Cyanobutanedioic acid (4-33A): Hydrolysis of diethyl 2-carboxyethyl-2-cyanobutanedioate (2.71 g, 10.0 mmol) yielded 2-cyanobutanedioic acid as an oil (1.27 g, 89%). ^{1}H NMR (acetonitrile-d_3) δ 2.90 (d, J = 6, 2H), 3.95 (t, J = 6, 1H), 8.92 (br. s, 2H). ^{13}C NMR (acetonitrile-d_3) δ 20.8, 33.7, 117.6, 167.4, 172.2. ^{1}H NMR (D$_2$O) δ 2.98 (br. s, 2H) 4.01 (exch. t, 0.1H). ^{13}C NMR (D$_2$O) δ 33.1, 33.4 (exch.), 118.0, 168.9, 173.6. ESI$^-$-MS m/z 142 (100), 98 (19). CID (14%) of m/z 142: m/z 98 (100).

2-Cyanopentanedioic acid (4-33B): Hydrolysis of diethyl-2-carboxyethyl-2-cyanopentanedioate (0.74 g, 2.6 mmol) yielded 2-cyanopentanedioic acid as an oil (0.40

g, 99%); ^{1}H NMR (acetonitrile-d_3) δ 1.97-2.23 (m, 2H), 2.45 (t, J = 7, 2H), 3.77 (dd, J = 6, J = 8, 1H). ^{13}C NMR (CD$_3$CN) δ 25.2, 31.2, 37.2, 117.7, 168.3, 175.1. ^{1}H NMR (D$_2$O) δ 2.12-2.19 (m, 2H), 2.49 (t, J = 7, 2H), 3.86-3.91 (m, exch., 1H). ^{13}C NMR (D$_2$O) δ 24.2, 30.8, 37.1, 117.9, 169.6, 176.3. ESI$^-$-MS m/z 156 (100), 112 (16).

2-Cyanohexanedioic acid (4-33C): Hydrolysis of diethyl 2-carboxyethyl-2-cyanohexanedioate (0.95 g, 3.2 mmol) yielded 2-cyanohexanedioic acid as a colourless solid (0.47 g, 87%). m.p. 105-108°C. ^{1}H NMR (acetonitrile-d_3) δ 1.59-1.68 (m, 2H), 1.78-1.86 (m, 2H), 2.28 (t, J = 7, 2H), 3.64 (dd, J = 6, J = 8, 1H) 8.61 (exch., 2H). ^{13}C NMR (acetonitrile-d_3) δ 22.7, 29.4, 33.2, 37.9, 117.9, 168.3, 175.9.

2-Cyanoheptanedioic acid (4-33D): Hydrolysis of 1-ethyl-2-carboxyethyl-2-cyanoheptanedioic acid (1.20 g, 4.21 mmol) yielded 2-cyanoheptanedioic acid as a colourless solid (0.74 g, 95%). m.p. 100-102°C. ^{1}H NMR (acetonitrile-d_3) δ 1.32-1.44 (m, 2H), 1.46-1.58 (m, 2H), 1.75-1.83 (m, 2H), 2.22 (t, J = 7, 2H), 3.62 (dd, J = 7, J = 7, 1H), 8.94 (exch s, 2H); ^{13}C NMR (acetonitrile-d_3) δ 24.7, 26.8, 29.9, 33.7, 38.0, 118.0, 168.3, 175.9; ESI$^-$MS m/z 184 (30), 140 (100); CID (16%) of m/z 184: m/z 140 (100); CID (23%) of m/z 140: m/z 122 (100), 96 (9).

4.7.5 Formation of Benzylammonium Salts

Benzylamine (2 equiv.) was added to a solution of the 2-cyanodicarboxylic acid in water (50 mL). After 30 min, the solution was freeze dried and the resulting solid was recrystallized from ethanol/ether to yield a colourless, crystalline solid.

Bis(benzylammonium) 2-Cyanobutanedioate (4-34A): 2-Cyanobutanedioic acid (1.27 g, 8.88 mmol) yielded bis(benzylammonium) 2-cyanobutanedioate (1.97 g, 62%). mp 139-140°C (dec.). ^{1}H NMR (D$_2$O) δ 2.50-2.70 (m, 2H) (exch dd, J = 6, J = 9, 0.76H). ^{13}C NMR (D$_2$O) δ 37.8, 43.2, 121.1, 128.9, 129.3, 132.8, 172.2, 177.8. ESI-MS m/z 142 (17), 98 (100). CID (20%) of m/z 142: m/z 112 (5), 98 (100). CID (21%) of m/z 98: m/z 71 (45), 70 (32), 54 (6). CID (21%) of m/z 112: m/z 94 (25), 84 (55).

Benzylammonium 2-Cyanopentanedioate (4-35): 2-Cyanopentanedioic acid (0.62 g, 3.9 mmol) yielded benzylammonium 2-cyanopentanedioate (0.568 g, 58% yield). m.p. 95-97°C. IR (Nujol) cm^{-1}: 3230, 2250 (CN), 1764 (C=O). ^{1}H NMR (D$_2$O) δ 2.02-2.19 (m, 2H), 2.47 (t, J = 8, 2H), 4.15 (s, 2H), 7.44 (s, 5H). ^{13}C NMR (D$_2$O) δ 25.2, 31.6, 43.3, 120.1, 129.0, 129.4, 132.8, 172.0, 177.1. ESI-MS m/z 156 (100), 112 (22).

Bis(benzylammonium) 2-Cyanopentanedioate (4-34B): 2-Cyanopentanedioic acid (1.97 g, 12.6 mmol) yielded bis(benzylammonium) 2-cyanopentanedioate (2.59 g, 56%). m.p. 131-133°C. ^{1}H NMR (D$_2$O) δ 1.94-2.15 (m, 2H), 2.27 (t, J = 8, 2H), 3.44-3.49 (exch dd, J = 6, J = 8, 0.8H), 4.17 (s, 4H), 7.47 (s, 10H). ^{13}C NMR (D$_2$O) δ 27.0, 35.1, 40.3, 43.3, 120.1, 129.0, 129.4, 132.8, 172.6, 181.2. ESI-MS m/z 156 (30), 112 (100), 59 (5). CID (15%) of m/z 156: m/z 112 (67). CID (23%) of m/z 112: m/z 59 (100).

Bis(benzylammonium) 2-cyanohexanedioate (4-34C): 2-Cyanohexanedioic acid (0.27 g, 0.32 mmol) yielded bis(benzylammonium) 2-cyanohexanoate as a white crystalline solid (0.44 g, 72%). m.p. 118-119°C. ^{1}H NMR (D$_2$O) δ 1.60 (m, 2H), 1.75 (m, 2H), 2.12

(t, J = 8, 2H) 3.41 (t, J = 8, 1H), 4.13 (s, 4H), 7.43 (s, 10H). ^{13}C NMR (D$_2$O) δ 23.8, 29.9, 37.0, 40.5, 43.3, 120.9, 129.0, 129.4, 132.8, 172.9, 182.7. ESI'MS m/z 170 (35), 126 (100), 82 (15). CID (13%) of m/z 170: m/z 126 (100). CID (21%) of m/z 126: m/z 108 (100), 82 (24). CID (25%) of m/z 108: m/z 106 (100).

4.7.6 Formation of Sodium Salts

Regenerated Amberlite IR-120 resin (H$^+$ form, 30 cm x 2 cm column) was rinsed with 2 L of water, followed by 1 L of 1 M NaCl until the effluent was neutral. The column was then rinsed with 2 L of water until the effluent gave no precipitate with an AgNO$_3$ solution. The corresponding disodium salts were formed by applying an aqueous solution of the bis(benzylammonium) salt to an Amberlite IR-120 column (Na$^+$ form). The column was eluted with water. The first three 100 mL fractions were collected and concentrated *in vacuo*. The concentrate was treated with charcoal for 1 h and filtered through Celite. The filtrate was freeze dried to yield the disodium salts as colourless solids.

Disodium 2-Cyanobutanedioate (4-24A): Bis(benzylammonium) 2-cyanobutanedioate (0.402 g, 1.13 mmol) yielded disodium 2-cyanobutanedioate (0.207 g, 98%). m.p. 175-177°C (dec.). ^{1}H NMR (D$_2$O) δ 2.68-2.86 (m, 2H) 3.75-3.81 (exch dd, J = 6, J = 8, 1H). ^{13}C NMR (D$_2$O) δ 37.8, 121.3, 172.4, 178.1.

Disodium 2-Cyanopentanedioate (4-24B): Benzylammonium 2-cyanopentanedioate (1.06 g, 4.00 mmol) yielded disodium 2-cyanopentanedioate (0.72 g, 90% yield). m.p. 255-260°C (dec.). IR (Nujol) cm^{-1}: 2246 (CN), 1751 (C=O). ^{1}H NMR (D$_2$O) δ 2.06-2.20

(m, 2H), 2.35 (t, J = 8, 2H), 3.55-3.61 (m, 1H). ^{13}C NMR (D$_2$O) δ 26.3, 33.6, 40.3, 120.6, 172.6, 179.6. ESI$^-$-MS m/z 156(100), 112(26). CID (15%) of m/z 156: m/z 112 (100).

Disodium 2-Cyanohexanedioate (4-24C): Bis(benzylammonium) 2-cyanohexanedioate (0.290 g, 0.753 mmol) yielded disodium 2-cyanohexanedioate (0.15 g, 93% yield). ^{1}H NMR (D$_2$O) δ 1.73-1.82 (m, 2H), 1.91-2.00 (m, 2H), 2.32 (t, J = 7, 2H), 3.54-3.68 (exch., m, 0.8H). ^{13}C NMR (D$_2$O) δ 23.8, 30.0, 37.1, 40.7, 121.2, 173.2, 183.0.

4.7.7 Synthesis of 2-(Aminomethyl)dicarboxylic Acids

Solid NaBH$_4$ (10mmol/mmol of nitrile) was added in small portions to an aqueous solution (10-15 mL) of bis(benzylammonium) salt and CoCl$_2$ H$_2$O (2mmol/mmol of nitrile). After stirring at room temperature for 4 h, water (100 mL) was added and the pH was adjusted to 4.5 using conc. HCl. The mixture was filtered, and the filtrate was applied to an Amberlite IR-120 ion exchange column (H$^+$ form). The column was eluted with 0.5 M aqueous ammonia. The effluent was concentrated *in vacuo* and treated with activated charcoal for 1 h. The charcoal was removed by filtration through Celite and the filtrate was freeze dried.

2-(Aminomethyl)butanedioic Acid (4-1A): Reduction of bis(benzylammonium) 2-cyanobutanedioate (1.43 g, 4.00 mmol) yielded 2-(aminomethyl)butanedioic acid as a colourless solid (0.48 g, 82%). This was recrystallized from water/acetone to give a crystalline product (0.030 g). m.p. 123-124°C (open capillary), 180-184°C (open coverslip) (Lit. m.p. 180-181°C,[22] 179-180°C[25]). IR (Nujol) cm^{-1}: 3203, 1717, 1671,

1377. ^{1}H NMR (D$_2$O) δ 2.53-2.74 (m, 2H), 2.88-2.98 (m, 1H), 3.08-3.27 (m, 2H). ^{13}C

NMR (D$_2$O) δ 35.7, 40.5, 40.7, 177.0, 178.2. ^{1}H NMR (500 MHz D$_2$O) δ 2.58 (dd, J = 16,

7, 1H), 2.72 (dd, J = 16, 6, 1H), 2.92-2.97 (m, 1H), 3.15 (dd, J = 13, 4, 1H), 3.24 (dd, J =

13, 9, 1H). ^{13}C NMR (125 MHz D$_2$O) δ 35.5, 40.8, 41.1, 177.8, 178.9. ESI$^+$MS m/z 148

(100), 131 (7), 130 (20). CID (18%) of m/z 148: m/z 131 (11), 130 (100). CID (18%) of

m/z 131: m/z 113 (100). CID (22%) of m/z 130: m/z 112 (100), 84 (4). ESI⁻MS m/z 146

(100), 128 (7) 102 (27). CID (20%) of m/z 146: m/z 129 (6), 128 (34), 102 (100). CID

(20%) of m/z 128: m/z 111 (100), 99 (22), 98 (91), 84 (50), 55 (3). CID (20%) of m/z 102:

m/z 84 (33), 73 (100).

2-(Aminomethyl)pentanedioic acid (4-1B): Reduction of bis(benzylammonium) 2-

cyanopentanedioate (1.49 g, 4.00 mmol) yielded 2-(aminomethyl)pentanedioic acid as a

white solid (0.55 g, 85%). This was recrystallized from water to give a crystalline

product (0.037 g, 6%). m.p. 164-165°C (open capillary tube), 166-168°C (open coverslip)

(Lit.[21] m.p. 171-173°C). IR (Nujol) cm^{-1}: 3133, 1717, 1224, 1609, 1378. ^{1}H NMR (D$_2$O)

δ 1.79-1.89 (m, 2H), 2.34-2.40 (m, 2H), 2.52-2.63 (m, 1H), 3.02-3.18 (m, 2H). ^{1}H NMR

(500 MHz, D$_2$O) δ 1.85-1.96 (m, 2H), 2.38-2.48 (m, 2H), 2.62-2.68 (m, 1H), 3.12 (dd, J

= 13, J = 5, 1H), 3.19 (dd, J = 13, J = 9, 1H). ^{13}C NMR (125 MHz, D$_2$O) δ 25.3, 32.2,

40.8, 44.1, 178.8, 179.1. ESI$^+$MS m/z 162 (100), 145 (5), 144 (20), 98 (5). CID (18%) of

m/z 162: m/z 145 (10), 144 (100), 126 (5). CID (19%) of m/z 145: m/z 127 (100), 99 (3).

CID (22%) of m/z 144: m/z 126 (100), 98 (10). CID (18%) of m/z 126: m/z 98 (100). ESI⁻

MS m/z 160 (100), 143 (15), 142 (24), 116 (37), 99 (5), 98 (7). CID (20%) of m/z 160:

m/z 143 (55), 142 (29), 116 (60), 99(5). CID (20%) of *m/z* 143: *m/z* 99 (100). CID (23%) of *m/z* 142: *m/z* 112 (44), 98 (100). CID (20%) of *m/z* 116: *m/z* 98 (17), 87 (89).

2-(Aminomethyl)hexanedioic acid (4-1C): Reduction of bis(benzylammonium) 2-cyanohexanedioate (0.597 g, 1.55 mmol) yielded 2-(aminomethyl)hexanedioic acid as a white powder (0.116 g, 43%). m.p. 235-240°C (dec.). ^{1}H NMR (D$_2$O) δ 1.50-1.64 (m, 4H), 2.19-2.30 (m, 2H), 2.53-2.58 (m, 1H), 3.05-3.22 (m, 2H). ^{13}C NMR (D$_2$O) δ 23.6, 29.9, 37.56, 41.2, 45.4, 180.9. ESI$^+$MS *m/z* 176 (100), 158 (20), 140 (22). CID (18%) of *m/z* 176: *m/z* 158 (100), 140 (36). CID (19%) of *m/z* 158: *m/z* 140 (100). CID (19%) of *m/z* 140: *m/z* 123 (10), 112 (100), 111 (30), 97 (40). ESI⁻MS *m/z* 174 (100), 157 (26), 156 (10), 130 (10), 113 (7), 112 (15). CID (20%) of *m/z* 174: *m/z* 157 (100), 156 (28), 130 (5), 113 (5), 112 (25). CID (20%) of *m/z* 157: *m/z* 139 (11), 113 (100). CID (22%) of *m/z* 156: *m/z* 126 (7), 112 (88), 83 (16). CID (24%) of *m/z* 130: *m/z* 101 (89). CID (21%) of *m/z* 113: *m/z* 96 (55), 85 (40), 69 (73). CID (19%) of *m/z* 112: *m/z* 95 (100), 83 (5).

2-(Aminomethyl)heptanedioic acid (4-1D): Reduction of 2-cyanoheptanedioic acid (0.21 g, 1.1 mmol) yielded 2-(aminomethyl)heptanedioic acid as a white powder (0.064 g, 30%). m.p. 265-270°C (dec.). ^{1}H NMR (D$_2$O) δ 1.27-1.39 (m, 2H), 1.50-1.69 (m, 4H) 2.17-2.23 (m, 2H), 2.49-2.59 (m, 1H), 3.03-3.21 (m, 2H). ^{13}C NMR (D$_2$O) δ 25.9, 26.4, 29.8 37.5, 41.2, 45.4, 181.1, 183.9. ESI$^+$MS *m/z* 190 (100), 172 (18), 154 (28). CID (20%) of *m/z* 190: *m/z* 172 (100), 154 (72). CID (26%) of *m/z* 172: *m/z* 154 (100). CID (19%) of *m/z* 154: *m/z* 137 (90), 136 (100), 126 (77). ESI⁻MS *m/z* 188 (100), 171 (60), 170 (10). 127 (17). CID (20%) of *m/z* 188: *m/z* 171 (100), 170 (8), 127 (4). CID (20%) of

m/z 171: *m/z* 153 (13), 127 (77), 109 (13). CID (21%) of *m/z* 170: *m/z* 152 (7), 140 (81),

127 (9), 126 (100), 127 (5). CID (24%) of *m/z* 127: *m/z* 109 (100). CID (21%) of *m/z*

109: *m/z* 91 (40), 77 (100).

4.7.8 Lactam Synthesis

4-Carboxy-2-pyrrolidone (4-36A): 2-(Aminomethyl)butanedioic acid (0.205 g, 1.39

mmol) was placed in an oven at 130°C for 16 h. The product was dissolved in water (20

mL), treated with activated charcoal for 1 h and then filtered through Celite. Water was

removed by freeze drying, leaving 4-carboxy-2-pyrrolidone as a hygroscopic white solid

(0.112 g, 62%). (Lit.[39] m.p. 159-160°C); IR (Nujol) cm^{-1}: 3376, 1716, 3201, 1640. ^{1}H

NMR (250 MHz, D$_2$O) δ 2.60-2.73 (m, 2H), 3.30-3.43 (m,1H), 3.56-3.74 (m, 2H). ^{1}H

NMR (500 MHz, D$_2$O) δ 2.60-2.70 (m, 2H), 3.36-3.43 (m,1H), 3.59 (dd, *J* = 10, 6, 1H),

3.70 (dd, *J* = 10, 9, 1H). ^{13}C NMR (D$_2$O) δ 33.8, 40.0, 45.4, 179.8. ESI MS *m/z* 128

(100). CID (22%) of *m/z* 128: *m/z* 98 (100).

2-Piperidone-5-carboxylic acid (4-36B): 2-(Aminomethyl)pentanedioic acid (0.201 g,

1.25 mmol) was placed in an oven at 130°C for 16 h. The product was dissolved in water

(20 mL), treated with activated charcoal for 1 h and then filtered through Celite. Water

was removed by freeze drying, leaving 2-piperidone-5-carboxylic acid as a white powder

(0.126 g, 70%). m.p. 169-171°C (Lit.[21] m.p. 182-185°C); IR (Nujol) cm^{-1}: 3200, 1716

(CO$_2$H), 1640 (CONH). ^{1}H NMR (250 MHz, D$_2$O) δ 1.94-2.21 (m, 2H), 2.42 (t, *J* = 7,

2H), 2.89-2.99 (m, 1H), 3.44-3.58 (m, 2H). ^{13}C NMR (D$_2$O) δ 22.6, 28.8, 37.6, 42.6,

175.0, 177.25. ^{1}H NMR (500 MHz, D$_2$O) δ 1.98-2.02 (m, 1H), 2.11-2.16 (m, 1H), 2.42-

2.45 (m, 2H), 2.80-2.85 (m, 1H), 3.46 (dd, J = 13, J = 8, 1H), 3.53 (dd, J = 13, J = 5, 1H).

ESIMS m/z 142 (100). CID (23%) of m/z 142: m/z 112 (22), 98 (88).

Cyclization of 2-(Aminomethyl)pentanedioic acid: 2-(Aminomethyl)pentanedioic acid (A: 10.432 mg, B: 10.876 mg) was heated at 135°C for 13 h, allowed to cool to room temperature and then reweighed (A: 9.239 mg, B: 9.631 mg). The mass difference (A: 1.193 mg, 1.245 mg) amounted to loss of one water molecule (A: 11.44%, B: 11.45%, 161.16 g/mol x 11.44/100 = 18.44 g/mol (H_2O M.W. = 18.02 g/mol)). The samples were allowed to sit at room temperature for 6 days and reweighed (A: 9.252 mg, B: 9.637 mg) to see if any moisture was absorbed. The mass increase after 6 days was negligible (A: 0.013 mg and B: 0.006 mg). m.p. 178-180°C (Lit.[21] m.p. 182-185°C). ^{1}H NMR (D_2O) δ 1.96-2.15 (m, 2H), 2.42 (t, J = 7, 2H), 2.89-2.99 (m, 1H), 3.44-3.58 (m, 2H). ^{13}C NMR (D_2O) δ 22.6, 28.8, 37.6, 42.6, 175.0, 177.3.

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Chapter 5: Mass Spectrometric Studies of 2-Substituted Dicarboxylic Acids

5.1 Introduction

The development of electrospray ionization mass spectrometry (ESI-MS) has allowed mass spectrometric analysis to be carried out when non-volatile biological molecules, such as lipids,[1] nucleic acids[2] and proteins,[3] contain one or more ionizable functional groups. Use of low-energy collision induced dissociation (CID) combined with a soft ionization technique, such as ESI, has become prevalent in recent years to determine the amino acid sequence of peptides. Since peptides are composed of amino acids, an understanding of the fragmentation behaviour of the individual constituent amino acids is fundamental to determining the low energy fragmentation processes of peptides.[4,5]

The availability of a series of 2-(aminomethyl)dicarboxylic acids (β^2-amino acids) and 2-cyanodicarboxylic acids, prepared earlier in the thesis (Chapter 4) with chain lengths ranging from C_4 to C_7, permitted the effect of substituents on the fragmentation behaviour of dicarboxylic acid monoanions to be explored and to contrast the fragmentation modes of α- and β-amino acids in both positive and negative ion modes.

5.1.1 Positive Ion Fragmentation Processes of α-Aminodicarboxylic Acids

Positive ion fragmentation processes of amino acids have been studied much more extensively than negative ion fragmentation processes.[4,6,7] In general, the major fragmentation process observed upon CID of the [M+H]$^+$ ions derived from the majority of α-amino acids is the combined loss of water and carbon monoxide (46 u).[5,6,7]

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In α-aminodicarboxylic acids (**4-3**), the combined loss of water and carbon monoxide has been shown to occur from the α-carboxylate group.[4,5,8] Initially, water is lost from the α-carboxyl group generating an acylium ion (**5-2**), which fragments to give carbon monoxide and an immonium ion (**5-3**) as shown in Scheme 5.1. For the $[M+H]^+$ ions of $[4-^{13}C]$aspartic acid and $[5-^{13}C]$glutamic acid, a loss of 46 u consisting of water and ^{12}CO, was observed.[8] However, CID of protonated $[1-^{13}C]$glutamic acid resulted in a loss of 47 u, consisting of water and ^{13}CO, suggesting that the combined water and carbon monoxide loss occurs from the α-carboxyl group.[8]

5-1

n	m/z
1	134
2	148
3	162
4	176

5-2

5-3

n	m/z	(%)
1	88	(14)
2	102	(29)
3	116	(43)
4	130	(53)
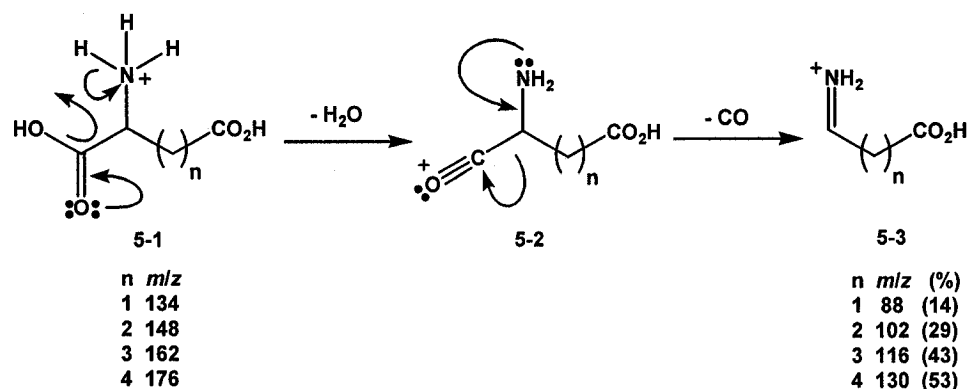
Scheme 5.1 Loss of water and subsequent loss of carbon monoxide from the α-carboxyl group of the $[M+H]^+$ ions derived from α-aminodicarboxylic acids (n = 1- 4) subjected to CID.

α-Amino acids with side chains containing hydroxyl groups, such as serine or threonine and α-aminodicarboxylic acids, such as aspartic acid and glutamic acid, show a strong loss of water (18 u) in addition to the combined loss of water and carbon monoxide from their $[M+H]^+$ ions.[5,7] The fragmentation that results in the loss of water alone from the $[M+H]^+$ ion (**5-1**) derived from α-aminodicarboxylic acids, however, is

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believed to primarily occur from the side chain carboxyl group.[5] Deuterium labelling has

shown that hydrogen atoms bonded to the carbon backbone are involved in the

fragmentation process leading to a proposed ketene intermediate (5-4A)[5] which can

cyclize to form a protonated lactam[4] (5-5, Scheme 5.2). In either case, further

fragmentation of the [M+H-H$_2$O]$^+$ ions leads to loss of an additional water molecule and

carbon monoxide giving an immonium ion as the product.[5]

n	m/z
1	134
2	148
3	162
4	176

n	m/z	(%)
1	116	(100)
2	130	(100)
3	144	(100)
4	158	(100)
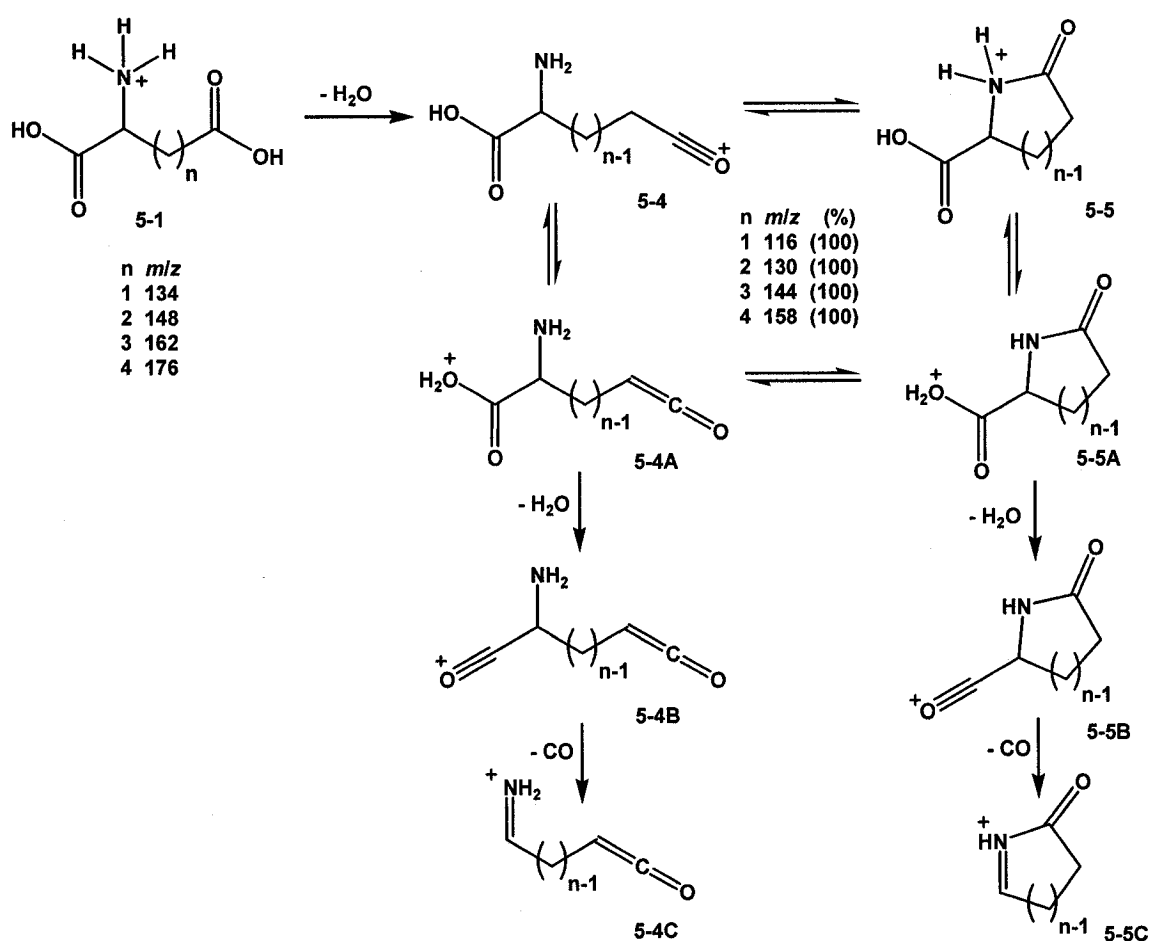
Scheme 5.2 Loss of water from the ω-carboxyl group and subsequent loss of water and carbon monoxide upon CID of the [M+H]$^+$ ion derived from α-aminodicarboxylic acids (n = 1- 4).

The $[M+H]^+$ ion of glutamic acid also showed a combined loss of two water

molecules and carbon monoxide (64 u) while the $[M+H]^+$ ion of aspartic acid showed a

combined loss of water and $CH_2=C=O$ (60 u) upon CID.[7] Under similar conditions, the

$[M+H]^+$ ions derived from the longer chain α-aminoadipic acid and α-aminopimelic acid

showed the same loss of water and combined loss of water and carbon monoxide.[8]

5.1.2   Negative Ion Fragmentation Processes of α-Aminodicarboxylic Acids

The negative ion ESI-MS/MS of a series of α-aminodicarboxylic acids (**4-3**, C_4-

C_7), showed the major fragmentation processes to be loss of water (18 u) and carbon

dioxide (44 u) upon CID of the $[M-H]^-$ ions (**5-6**).[9] Loss of ammonia also was observed

as a major fragmentation process upon CID of the $[M-H]^-$ ion derived from aspartic acid

(C_4). The combined loss of ammonia and carbon dioxide from the $[M-H]^-$ ion of aspartic

acid and the combined loss of water and carbon dioxide from the $[M-H]^-$ ions of α-

aminoadipic acid (C_6) and α-aminopimelic acid (C_7) were minor. Fragmentations of the

$[M-H]^-$ ions derived from a series of α-aminodicarboxylic acids are summarized in

Scheme 5.3.

Recently, negative ion ESI-MS studies on the monoanions of a series of acyclic,

aliphatic dicarboxylic acids ($HO_2C(CH_2)_nCO_2^-$, n = 1-10) showed the major

fragmentation pathways to be decarboxylation, loss of water or a combined loss of the

two.[10] These were the same fragmentation processes observed for α-aminodicarboxylic

acids.

5-7

- CO₂

n	m/z	(%)
1	88	(100)
2	102	(14)
3	116	(43)
4	130	(17)

5-6

n	m/z
1	132
2	146
3	160
4	174

- H₂O

5-8

n	m/z	(%)
1	114	(34)
2	128	(100)
3	142	(100)
4	156	(100)

- NH₃

5-9

n	m/z	(%)
1	115	(79)

- CO₂

5-10

n	m/z	(%)
3	98	(14)
4	112	(17)
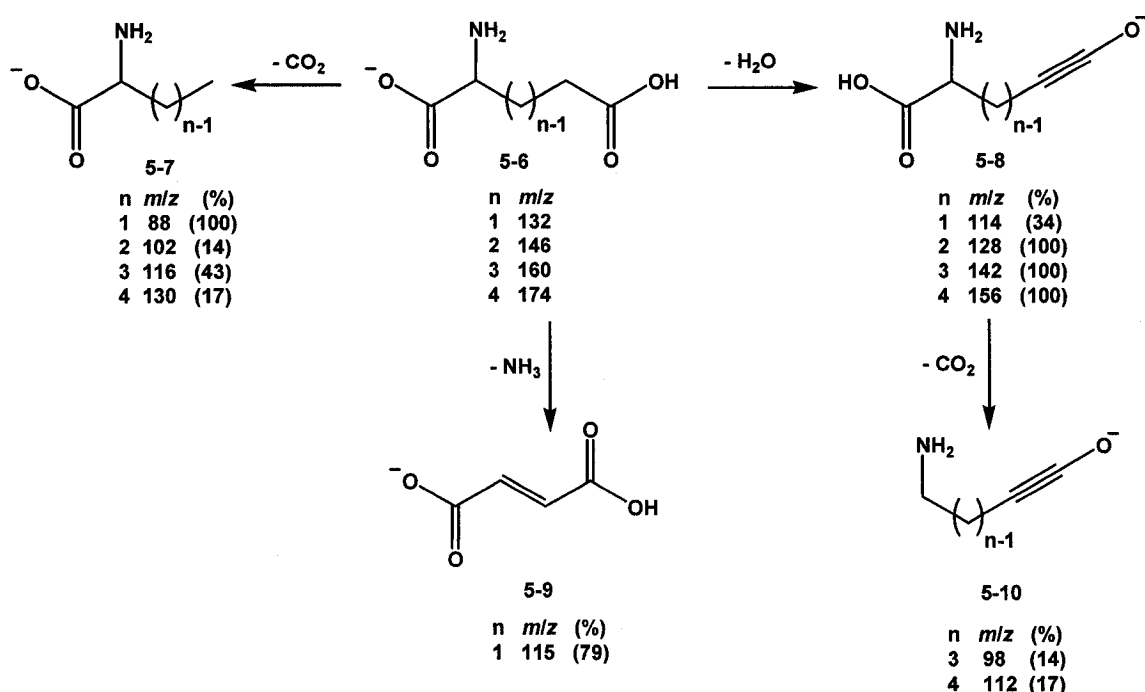
Scheme 5.3 Summary of fragmentation processes observed upon CID of the [M-H]⁻ ions derived from α-aminodicarboxylic acids.[10]

The fragmentation pattern obtained upon CID of acyclic dicarboxylic acids varied with the length of the carbon chain.[10] Decarboxylation was the major pathway observed for shorter dicarboxylic acids (n < 6), decreasing in importance as chain length increased, while loss of water was the major fragmentation observed for longer chain lengths (n =12). For intermediate length dicarboxylic acids ($6 \geq n \leq 10$), the combined loss of water and carbon dioxide was the major fragmentation process observed. The similar fragmentation behaviour displayed by the protonated α-aminodicarboxylic acids suggested that the amino substituent has little effect on the fragmentation behaviour of the monoanions of dicarboxylic acids.

Labelling studies using [4-¹³C]aspartic and [5-¹³C]glutamic acid showed that upon CID the side chain ω-carboxyl group was lost as carbon dioxide upon CID while the α-

carboxyl group was retained.[10] The α-carboxyl group is known to be more acidic than

the ω-carboxyl group and it could be that, in general, carbon dioxide is lost from the non-

ionized carboxyl group. The mechanism proposed for loss of carbon dioxide from a non-

ionized carboxyl group (5-11) involves a homolytic bond cleavage followed by

abstraction of a hydrogen atom (Scheme 5.4).[10]

5-11   **5-12**   **5-13**
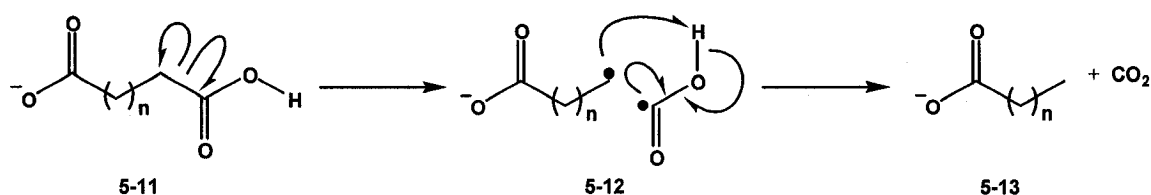
Scheme 5.4 Mechanism proposed for the loss of carbon dioxide from aliphatic
dicarboxylic acids.[10]

The loss of water occurs from the non-ionized carboxyl carbon as well.[10]

Dehydration is initiated by the ionized carboxyl group which results in the formation of a

ketene-hydroxide cluster ion **5-14**. A second proton transfer to the hydroxide ion results

in the formation of an alkyne oxide-water cluster ion **5-15** that loses water to give the [M-

H-H₂O]⁻ ion **5-16**.

CID of the [M-H]⁻ ion derived from aspartic acid (**5-16**) also resulted in loss of

ammonia, giving an ion **5-18** at *m/z* 115 [M-H-17]⁻.[9] The mechanism proposed in

Scheme 5.6 suggests that enolization of the side chain carboxyl group is assisted by the

α-carboxylate group which then transfers the proton to the departing amino group.
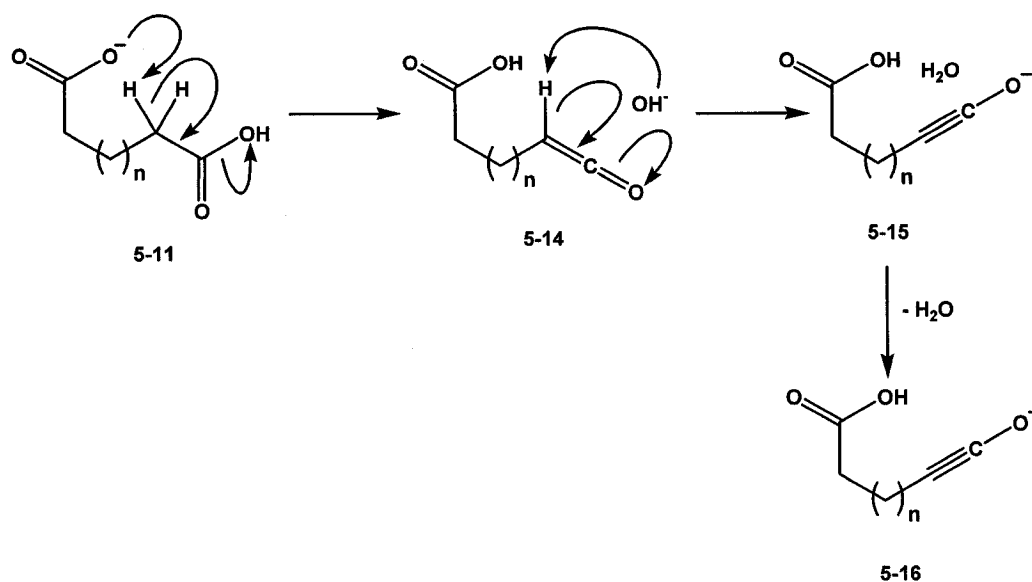
Scheme 5.5 Mechanism proposed for the loss of water from the monoanion of aliphatic dicarboxylic acids.[10]
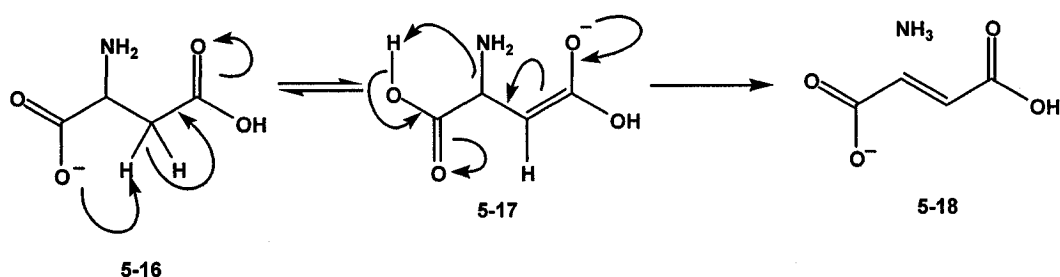
Scheme 5.6 Mechanism proposed for the loss of ammonia upon CID of the [M-H]⁻ anion derived from aspartic acid.

Overall, the fragmentation mechanisms involve interactions between functional groups in the gas-phase ions. The type of functional group and the relative positions of the functional groups in the compounds made available in this thesis are different, and lead to other fragmentation processes as described later in this chapter.

5.2 Preparation of Compounds for ESI-MS Study

Synthetic routes developed in this thesis were adapted to prepare several compounds designed specifically to help elucidate the mechanism of fragmentation processes observed by ESI mass spectrometry for the 2-cyanodicarboxylic acids and 2-(aminomethyl)dicarboxylic acids. In particular, the preparations of compounds in which the proton at C-2 was replaced with deuterium or a methyl group are described in the following subsections.

5.2.1 Deuterium Labelled 2-Cyano- and 2-(Aminomethyl)pentanedioic Acids

During the characterization of 2-cyanodicarboxylic acids prepared previously in Chapter 4, rapid exchange of the hydrogen on C-2 with D_2O was observed by ^1H NMR spectroscopy. For example, the ^1H NMR signal at δ_H 3.88, corresponding to the proton on C-2 of 2-cyanopentanedioic acid, appeared as a broad triplet and integrated to 0.65-H within 10 min of the sample being dissolved in D_2O. Also, in the ^{13}C NMR spectrum, a reduction in the intensity of the corresponding C-2 signal at δ_C 37.1 was observed. After 19 h in D_2O, the ^1H NMR signal at δ_H 3.88 had completely disappeared while the ^{13}C NMR signal at δ_C 37.1 appeared as three lines of equal intensity, indicating ^{13}C-^2H coupling. The other signals in the ^1H NMR spectrum each integrated to the same value. Negative ion ESI-MS of fully exchanged [2-^2H]-2-cyanopentanedioic acid, however, showed only 24% deuterium incorporation. The difference between the %^2H measured by ^1HNMR and ESI-MS was attributed to exchange with the carrier solvent (methanol) used in the ESI-MS experiment.

The facile exchange of the acidic proton located between the nitrile and the carboxyl group was utilized to introduce deuterium in the syntheses of [2-^{2}H]-2-cyanopentanedioic acid and two of the three isotopomers of 2-(aminomethyl)pentanedioic acid (Scheme 5.7). Purified samples of 2-cyanopentanedioic acid, available as its benzylammonium salt (**4-35**, Chapter 4), were chosen as the starting point for the preparation of deuterated compounds. Integration of the signal corresponding to C-2 (δ_H 3.59) in the ^{1}H NMR spectrum showed that approximately 90% exchange had occurred at C-2 within 5-10 min of mixing with D_2O. In general, deuterium was introduced at C-2 of benzylammonium 2-cyanopentanedioate by twice dissolving the nitrile in D_2O and then freeze-drying, after which there was no trace of the C-2 proton signal in the ^{1}H NMR spectrum.

Reduction of benzylammonium [2-^{2}H]-2-cyanopentanedioate (**5-20**) was carried out in methanolic NaBH$_4$/CoCl$_2$ (Scheme 5.7). The product, [2-^{2}H]-2-(aminomethyl)pentanedioic acid (**5-21**), retained 82% of the deuterium at C-2 by ^{1}H NMR integration; the other signals each integrated to their expected values, showing that deuterium was present only on C-2. Negative ion ESI-MS was used to confirm the presence of deuterium ($d_0 = 28\%$, $d_1 = 72\%$).
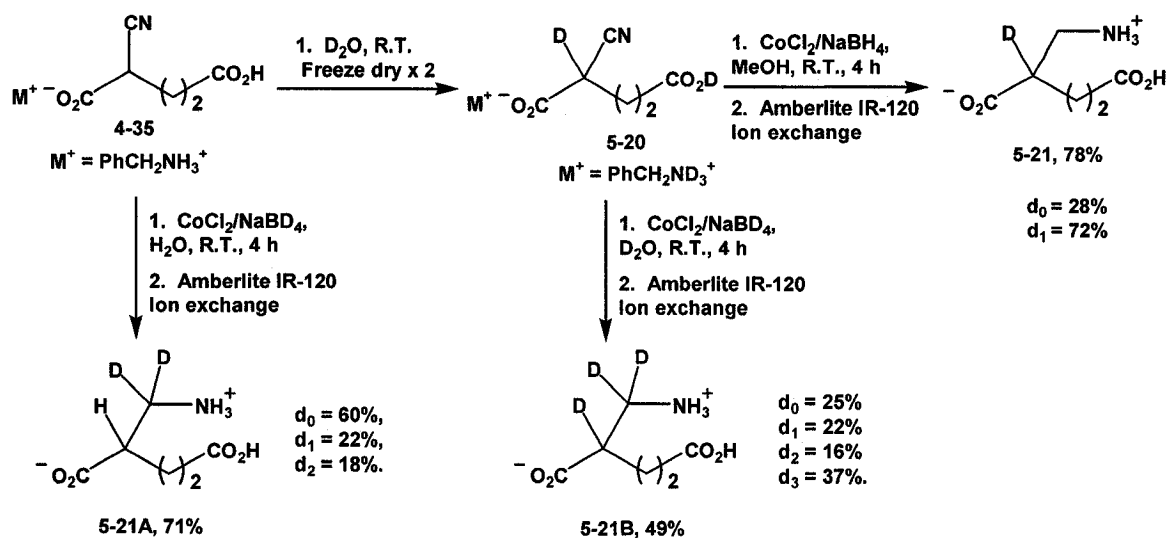
Scheme 5.7 Preparation of labelled 2-(aminomethyl)pentanedioic acid.

Deuterium was introduced at C-2' by reducing unlabelled benzylammonium 2-cyanopentanedioate with $NaBD_4$, giving **5-21A** (Scheme 5.7). Integration of the H-2' signal at δ_H 3.03-3.17 in the ^1H NMR spectrum, however, indicated only 25% deuterium incorporation. All other signals in the ^1H NMR spectrum integrated to the expected values and a total deuterium incorporation of 29% (d_0 = 60%, d_1 = 22%, d_2 = 18%) was determined by negative ion ESI-MS.

Similarly, a trideuterated sample (**5-21B**) was prepared by reducing benzylammonium $[2\text{-}^2H]$-2-cyanopentanedioate using $NaBD_4$ in D_2O (Scheme 5.7). Integration of the signals in the ^1H NMR spectrum of the product gave a deuterium incorporation of 74% in the C-2 position and 45% in the C-2' position. The signals corresponding to the protons at C-3 and C-4 each integrated to their expected values, indicating that deuterium exchange did not occur at these locations. A mixture of

deuterated species was indicated by negative ion ESI-MS (d_0 = 25%, d_1 = 22%, d_2 = 16%, d_3 = 37%).

While the deuterium labelling in these compounds was less than complete, the NMR analysis showed that the label was located at specific positions and by selecting the appropriate m/z values, the fragmentations of the desired labelled species could be studied by CID. The MS results are described later in this chapter.

5.2.2 2-Methyl Derivatives of 2-Cyanodicarboxylic Acids and 2-(Aminomethyl)dicarboxylic Acids

The 2-methyl derivatives of 2-cyanodicarboxylic acids and 2-(aminomethyl)dicarboxylic acids were prepared as summarized in Scheme 5.8. The syntheses of the triester starting materials are described earlier in this thesis (Chapter 4). The substrates for methylation were obtained by heating diethyl 2-carboxyethyl-2-cyanobutanedioate (3-2) and diethyl 2-carboxyethyl-2-cyanopentanedioate (4-31A) under Krapcho[11] conditions (Scheme 5.8). Each reaction was carefully monitored by ^{1}H NMR spectroscopy to determine the optimum time to ensure complete monodecarboethoxylation while minimizing didecarboethoxylation. Monodecarboethoxylation of diethyl 2-carboxyethyl-2-cyanobutanedioate was complete in 18 h at 90°C, giving 5-22A. However, ^{1}H and ^{13}C NMR signals corresponding to starting material were observed when diethyl 2-carboxyethyl-2-cyanopentanedioate (4-31A) was heated for 18 h under the same conditions. The reaction time was increased sequentially to 24, 30 and 36 h until all of the starting material was consumed. Beyond 39 h, however, signals corresponding to ethyl 4-cyanobutanoate appeared in the ^{1}H and ^{13}C NMR spectra, indicating that didecarboethoxylation had occurred.
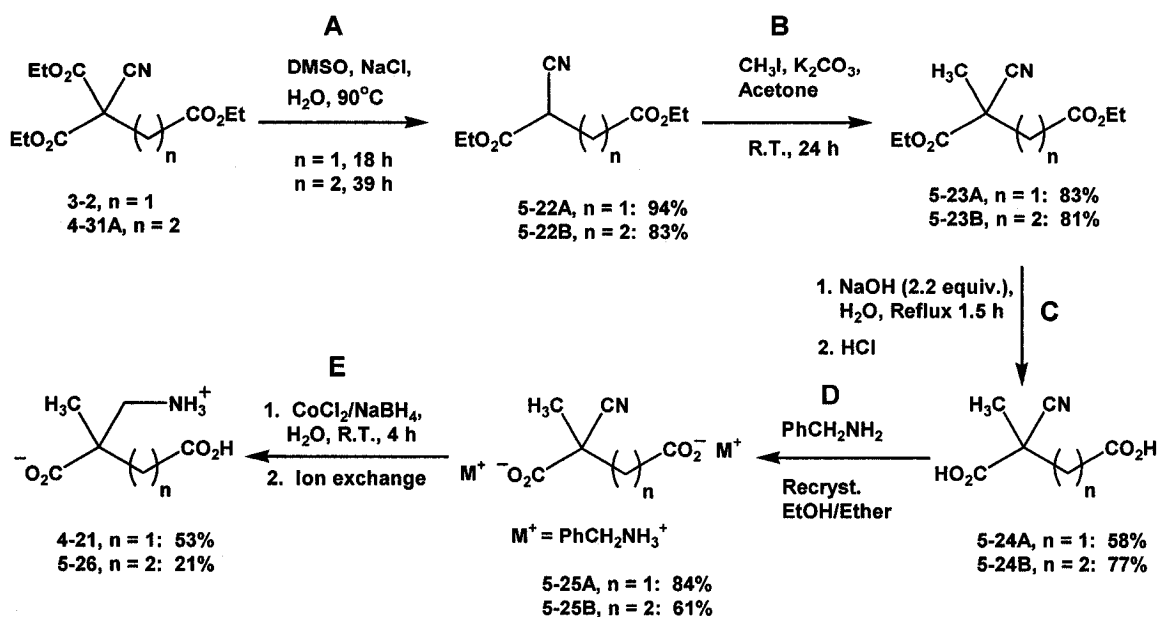
Scheme 5.8 Synthesis of 2-methylated derivatives of 2-cyanodicarboxylic acids and 2-(aminomethyl)dicarboxylic acids.

Methylation of the cyanodiesters was carried out by stirring the appropriate diethyl 2-cyanodicarboxylate (**5-22**) with methyl iodide and potassium carbonate in acetone at room temperature (Scheme 5.8-B). By using these relatively mild conditions, selective alkylation was achieved at C-2, avoiding methylation of the carbon adjacent to the ω-ester.

The 2-cyano-2-methyldicarboxylic acids **5-23A,B** were obtained by hydrolysis of the esters (Scheme 5.8-C) in base followed by acidification. NaBH$_4$ reduction[12] of the recrystallized bis(benzylammonium) salts (**5-25A,B**) gave the corresponding 2-(aminomethyl)-2-methyldicarboxylic acids **4-21** and **5-26** which were isolated by ion exchange (Scheme 5.8-E).

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5.2.3 Preparation of 4-Cyanobutanoic Acid and [4,4-^{2}H$_2$]-4-Cyanobutanoic Acid

Utilizing the same three-step reaction sequence developed for the synthesis of

sodium 3-cyanopropanoate (Chapter 3), ethyl 4-cyanobutanoate (5-28) was obtained

upon cyanide displacement[13] of bromide ion from commercially available ethyl 4-

bromobutanoate (5-27, Scheme 5.9). After ester hydrolysis in aqueous refluxing NaOH

(Scheme 5.9-C), the isolated nitrile acid 5-29 was applied to an Amberlite IR-120 ion-

exchange column (Na$^+$ form) to form its sodium salt (5-29A).
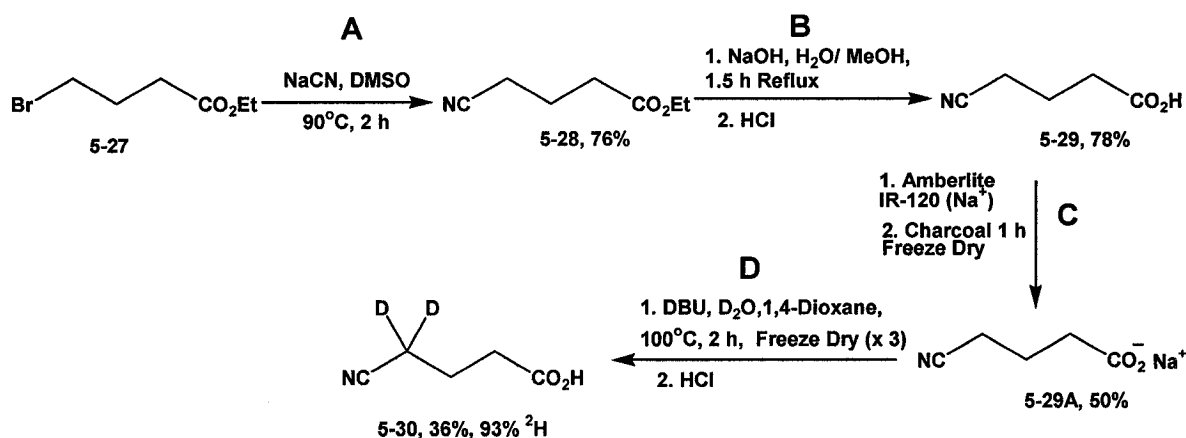
Scheme 5.9 Preparation of 4-cyanobutanoic acid and [4,4-^{2}H$_2$]-4-cyanobutanoic acid.

Unambiguous assignment of all the protons of sodium 4-cyanobutanoate (5-29A)

was made using 2-D NMR spectroscopy. Sodium 4-cyanobutanoate gave three distinct

signals in its ^{1}H NMR spectrum (250 MHz, D$_2$O); a quintet at δ_H 1.96, and triplets at δ_H

2.38 and 2.56. The ^{13}C spectrum (126 MHz, D$_2$O) contained five signals at δ_C 16.2, 21.7,

36.2, 121.7 and 181.5. Obviously, the quintet at δ_H 1.96 reflected coupling of the H-3

protons to the four adjacent protons on C-2 and C-4. The HMBC spectrum allowed for

assignment of the two triplets. The ^{1}H NMR signal at δ_H 2.38 correlated with the

carbonyl signal at δ_C 181.5 and not with the nitrile signal at δ_C 121.7. Conversely, the ^{1}H

NMR signal at δ_H 2.56 correlated with the nitrile signal and not with the carbonyl signal.

Therefore, the ^{1}H NMR signal at δ_H 2.38 was assigned to H-2, adjacent to the carboxylate

and the ^{1}H NMR signal at δ_H 2.56 was assigned to H-4, adjacent to the nitrile.

To determine the optimum time for exchange and the extent of side product

formation, a series of ^{1}H NMR spectra were recorded over time on a sample of sodium 4-

cyanobutanoate heated in D_2O and NaOH (0.5 equiv.). The ^{1}H NMR signal at δ_H 2.56,

corresponding to the protons α to the nitrile decreased with time upon heating, while the

^{1}H NMR signal at δ_H 2.38 for the protons α to the carboxylate remained essentially

unchanged. The signals corresponding to the quintet at δ_H 1.96 became very complex as

time progressed, due to the exchange process and the formation of side products. Also,

^{1}H NMR signals found between δ_H 2.19-2.31 were attributed to the formation of side

products.

Exchange using DBU produced much cleaner results by ^{1}H NMR spectroscopy

(Figure 5.1). The ^{1}H NMR signals corresponding to H-4 disappeared after two

successive exchange reactions while the H-2 signal remained as a triplet. Also, the

quintet at δ_H 1.96 resolved as a triplet and the ^{13}C NMR signal for C-4 (δ_C 16.2)

disappeared following two exchanges with D_2O. As was seen with the sodium 3-

cyanopropanoate exchange reactions described in Chapter 3, the use of DBU as a base

resulted in relatively little nitrile decomposition during exchange.
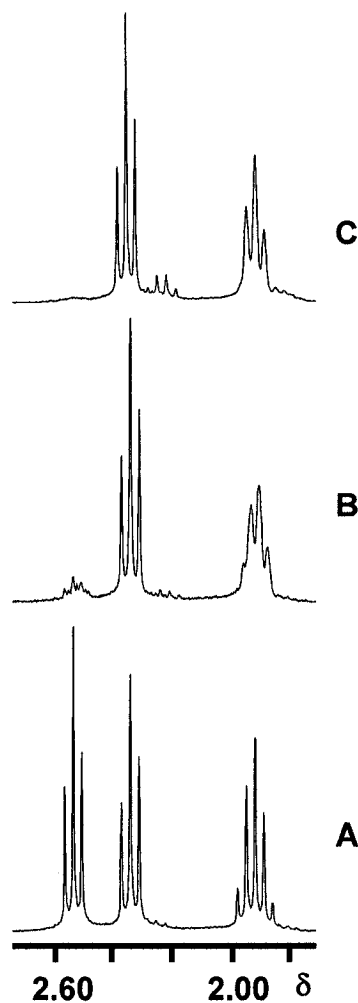
Figure 5.1 ^{1}H NMR spectra depicting the preparation of sodium [4,4-^{2}H$_2$]-4-cyanobutanoate (Each exchange: DBU, D$_2$O, 1,4-dioxane, 100°C, 2 h). (A) Unlabelled sodium 4-cyanobutanoate, (B) 1st exchange, (C) 2nd exchange.

Deuterium was selectively introduced onto C-4 of sodium 4-cyanopropanoate (5-29B) by using the modified literature[14] procedure developed in Chapter 3. This involved three consecutive exchanges at 100°C in D$_2$O and 1,4-dioxane with DBU followed by freeze-drying. Acidification of the reaction mixture after the final exchange and extraction into ethyl acetate gave [4,4-^{2}H$_2$]-4-cyanobutanoic acid (5-30) with 93% deuterium as measured by ^{1}H NMR spectroscopy and ESI-MS (Scheme 5.9-D). The ^{1}H

NMR spectrum of [4,4-^{2}H$_2$]-4-cyanobutanoic acid clearly showed the selective

incorporation of deuterium onto C-4 as the H-4 triplet had disappeared while the H-3

quintet had coalesced into a triplet and the H-2 signal remained a triplet (Figure 5.1).

5.2.4 Preparation of 5-Cyanopentanoic Acid

5-Cyanopentanoic acid (**5-32**) was prepared from diethyl 2-carboxyethyl-2-

cyanohexanedioate (**4-31C**), an intermediate in the synthesis of 2-

(aminomethyl)hexanedioic acid (**4-1C**), following a Krapcho[11] didecarboethoxylation and

ester hydrolysis (Scheme 5.10).

EtO$_2$C CO$_2$Et

NC   OEt

4-31C

NaCl, H$_2$O, DMSO
140°C, 18 h

NC   OEt

5-31, 61%

NaOH, H$_2$O/MeOH
Reflux, 1.5 h

NC   OH

5-32, 67%
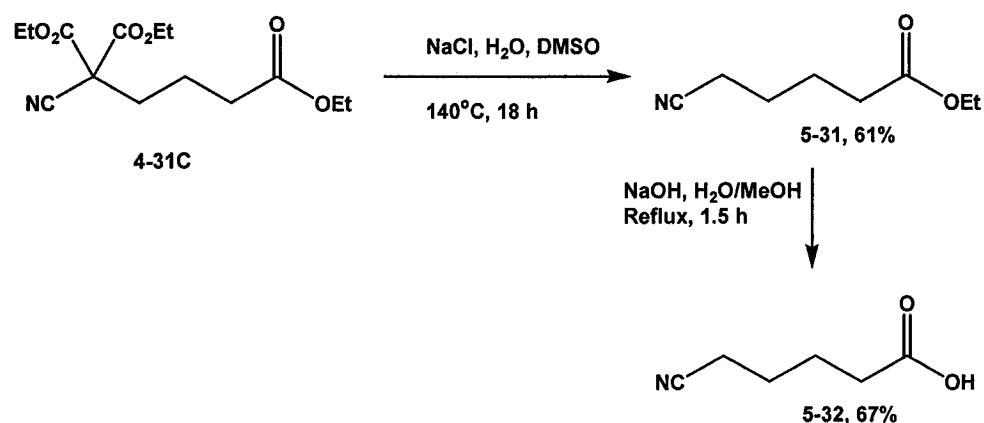
Scheme 5.10 Preparation of 5-cyanopentanedioic acid.

5.3 Positive Ion Mass Spectrometry of 2-(Aminomethyl)dicarboxylic Acids

The series of 2-(aminomethyl)dicarboxylic acids (**4-1**) prepared in this thesis

formed [M+H]$^+$ ions during the electrospray ionization process (Table 5.1). When

subjected to CID, the [M+H]$^+$ ions **5-33** showed only loss of water as a major

fragmentation process (Table 5.1 and Figure 5.2). A combined loss of two water

molecules, was more important at longer chain lengths (e.g., Figure 5.2-B), while a minor

loss of ammonia was observed for the two shorter chain 2-(aminomethyl)dicarboxylic

acids (e.g., Figure 5.2-A) and their 2-methyl derivatives. Unlike α-amino acids, the

combined loss of water and carbon monoxide was not observed upon CID of the $[M+H]^+$

ions derived from 2-(aminomethyl)dicarboxylic acids.

Table 5.1 Positive Ion MS/MS results for 2-(aminomethyl)dicarboxylic acids.

5-33
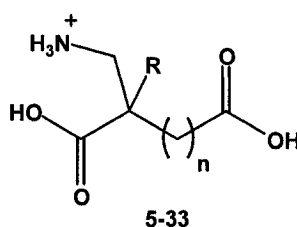
n	R	$[M+H]^+$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)		
				$[M+H-NH_3]^+$	$[M+H-H_2O]^+$	$[M+H-2H_2O]^+$
1	H	148	18	131 (11)	130 (100)	n.d.
1	CH$_3$	162	19	145 (4)	144 (100)	n.d.
2	H	162	18	145 (10)	144 (100)	126 (5)
2	CH$_3$	176	18	159 (3)	158 (100)	140 (12)
3	H	176	18	n.d.	158 (100)	140 (36)
4	H	190	20	n.d.	172 (100)	154 (72)

n.d. = not detected
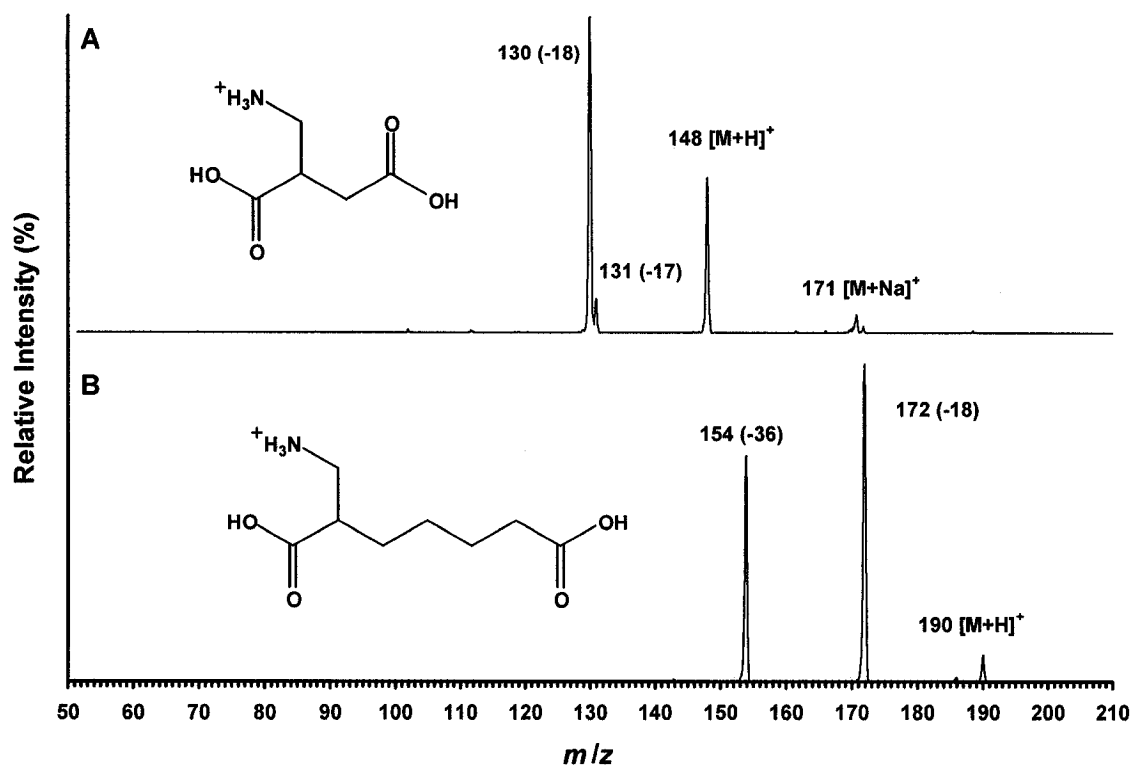
Figure 5.2 Comparison of the CID spectra of the [M+H]⁺ ions derived from: (A) 2-(aminomethyl)butanedioic acid; (B) 2-(aminomethyl)heptanedioic acid.

5.3.1 CID of [M+H]⁺ Ions Formed from Deuterium Labelled 2-(Aminomethyl)pentanedioic Acids

Several deuterated analogues of 2-(aminomethyl)pentanedioic acid were prepared (Section 5.2.1) to determine the role of the protons on C-2 and C-2' in mass spectrometric fragmentation processes. The results presented in Table 5.2 clearly show that deuterium atoms on C-2 and C-2' of 2-(aminomethyl)pentanedioic acid were retained for all observed fragmentation processes of the [M+H]⁺ ion.

Table 5.2 Positive Ion MS/MS results for labelled 2-(aminomethyl)pentanedioic acid.

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Entry	R^1	R^2	R^3	$[M+H]^+$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)		
						$[M+H-NH_3]^+$	$[M+H-H_2O]^+$	$[M+H-2H_2O]^+$
1	H	H	H	162	18	145 (10)	144 (100)	126 (4)
2	D	H	H	163	18	146 (10)	145 (100)	127 (6)
3	H	D	D	164	18	147 (13)	146 (100)	128 (6)
4	D	D	D	165	18	148 (10)	147 (100)	129 (6)

5.3.1.1 Loss of Water

The 2-(aminomethyl)dicarboxylic acids examined showed loss of water as the major fragmentation process observed upon CID of the $[M+H]^+$ ions. In principle, loss of water can potentially occur from either of the two carboxyl groups (Scheme 5.11-A,B). For α-aminodicarboxylic acids, however, it has been proposed that the loss of water occurs from the side chain carboxyl group (Scheme 5.2).[5] To investigate the regiochemistry of water formation, the $[M+H-H_2O]^+$ ions were generated in-source and subjected to CID.

CID of the $[M+H-H_2O]^+$ ions derived from the 2-(aminomethyl)dicarboxylic acids showed loss of water as the major fragmentation process with a combined loss of water and carbon monoxide as a minor process (Table 5.3 and e.g., Figure 5.3A). However, for the corresponding 2-(aminomethyl)-2-methyl-dicarboxylic acids, the

combined water-carbon monoxide loss was the major fragmentation process (Table 5.3

and e.g., Figure 5.3B). For the three longer chain 2-(aminomethyl)dicarboxylic acids, it

was possible to generate the $[M+H-2H_2O]^+$ ions in the source and to study their

fragmentation behaviour by CID. For each of the $[M+H-2H_2O]^+$ ions, loss of carbon

monoxide was a major process (Table 5.4), but the relative importance of other

fragmentations, including loss of ammonia, increased with increasing chain length.
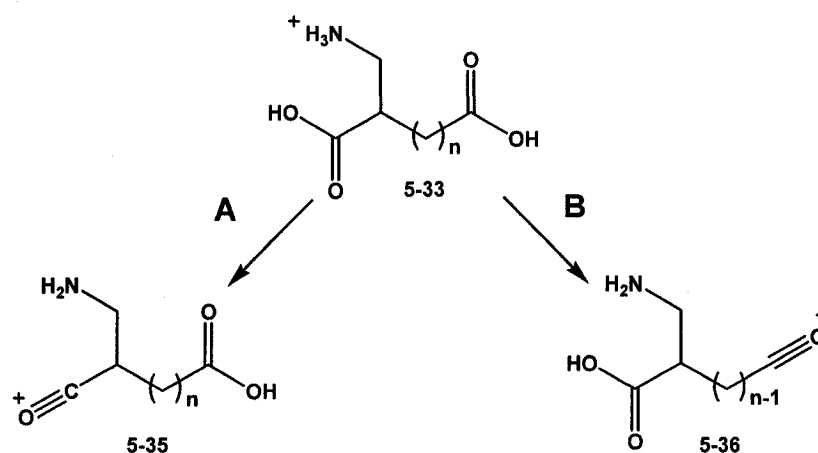
Scheme 5.11 Potential sites for loss of water from $[M+H]^+$ ion (**5-33**) derived from 2-(aminomethyl)dicarboxylic acids: (A) α-carboxyl group; (B) ω-carboxyl group.

Table 5.3 Product ions formed upon CID of the $[M+H-H_2O]^+$ derived from 2-(aminomethyl)dicarboxylic acids.

n	R	$[M+H-H_2O]^+$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)	
				$-H_2O$	$-(H_2O + CO)$
1	H	130	22	112 (100)	84 (4)
1	CH₃	144	19	126 (86)	98 (100)
2	H	144	22	126 (100)	98 (10)
2	CH₃	158	22	140 (30)	112 (100)
3	H	158	19	140 (100)	112 (3)
4	H	172	20	154 (100)	n.d.

n.d. = not detected

Table 5.4 Product ions formed upon CID of the $[M+H-2(H_2O)]^+$ ions derived from 2-(aminomethyl)dicarboxylic acids.

n	$[M+H-2(H_2O)]^+$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)			
			-17	-18	-28	Others
2	126	18	n.d.	n.d.	98 (100)	n.d.
3	140	19	123 (10)	122 (6)	112 (100)	111 (30), 97 (40)
4	154	19	137 (90)	136 (100)	126 (77)	124 (20), 112 (55)

n.d. = not detected
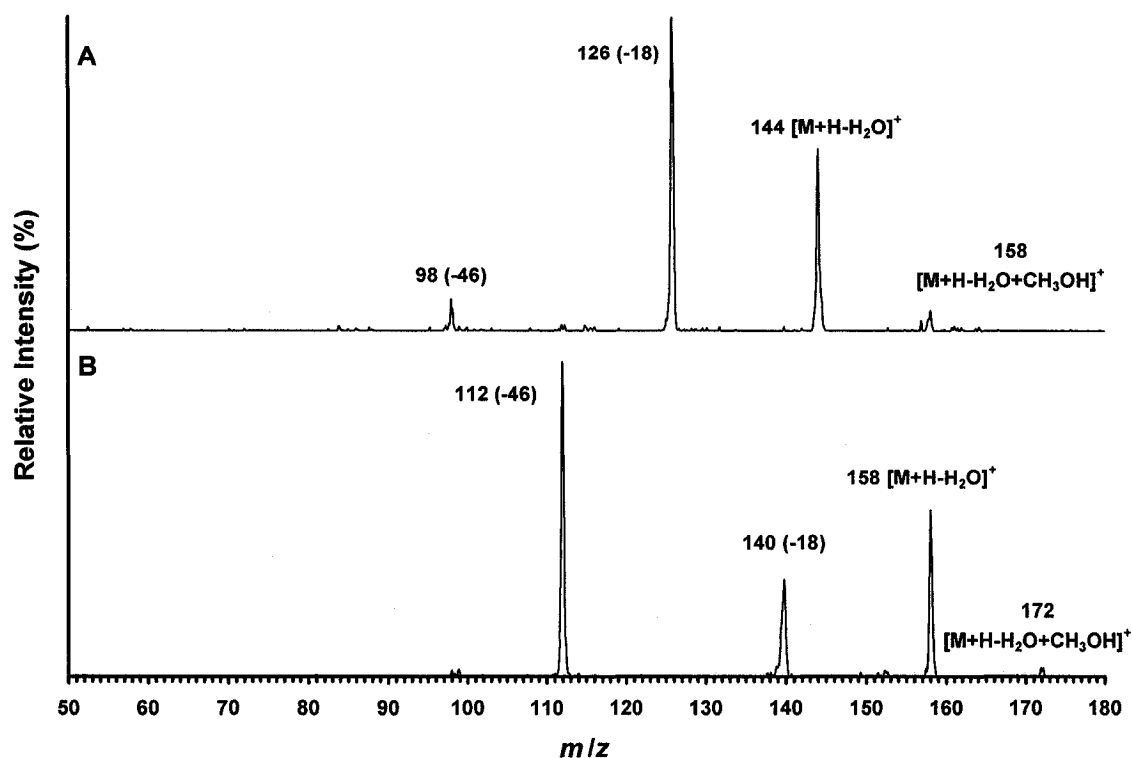
Figure 5.3 MS/MS of in-source generated $[M+H-H_2O]^+$ ions derived from: (A) 2-(aminomethyl)pentanedioic acid; (B) 2-(aminomethyl)-2-methylpentanedioic acid.

The results of the CID experiments on the in-source generated $[M+H]^+$, $[M+H-H_2O]^+$ and $[M+H-2H_2O]^+$ ions are consistent with successive losses of water, a second water and carbon monoxide from the $[M+H]^+$ ions of the 2-(aminomethyl)dicarboxylic acids. By analogy with the mechanism proposed for α-aminodicarboxylic acids, the initial loss of water from the $[M+H]^+$ ion could occur from the side chain carboxyl group, giving the $[M+H-H_2O]^+$ ion as an acylium ion or as a protonated lactam (Scheme 5.12). A second loss of water from the α-carboxyl group would yield another acylium ion (Scheme 5.12-C); evidence for the reaction of this ion with the solvent methanol is provided by a peak at m/z 158 in the MS/MS spectrum of the $[M+H-H_2O]^+$ ion derived from 2-(aminomethyl)pentanedioic acid (Figure 5.3A). The reaction of acylium ions with solvent in an ion trap has been documented.[15] The $[M+H-H_2O]^+$ ions derived from

the 2-(aminomethyl)dicarboxylic acids also showed a combined loss of water and carbon

monoxide. The peaks of the resulting $[M+H-(2H_2O+CO)]^+$ ions were much more intense

for ions derived from the initial 2-(aminomethyl)dicarboxylic acids substituted with a

methyl group at C-2 (Table 5.3, Figure 5.3B). Because of the α-methyl group, a more

stable 3° carbocation can be formed upon loss of carbon monoxide, as suggested in

Scheme 5.12. These results are analogous to what was observed upon CID of the $[M+H-H_2O]^+$ ion derived from glutamic acid where, following the loss of a second water

molecule, the fragmentation of the acylium ion to give carbon monoxide occurs readily

due to the formation of a stabilized immonium ion.[16]

n	R	m/z	(%)
1	H	112	(100)
1	CH₃	126	(86)
2	H	126	(100)
2	CH₃	140	(30)
3	H	140	(100)
4	H	154	(100)

n	R	m/z	(%)
1	H	84	(4)
1	CH₃	98	(100)
2	H	98	(10)
2	CH₃	112	(100)
3	H	112	(3)
4	H	n.d.	
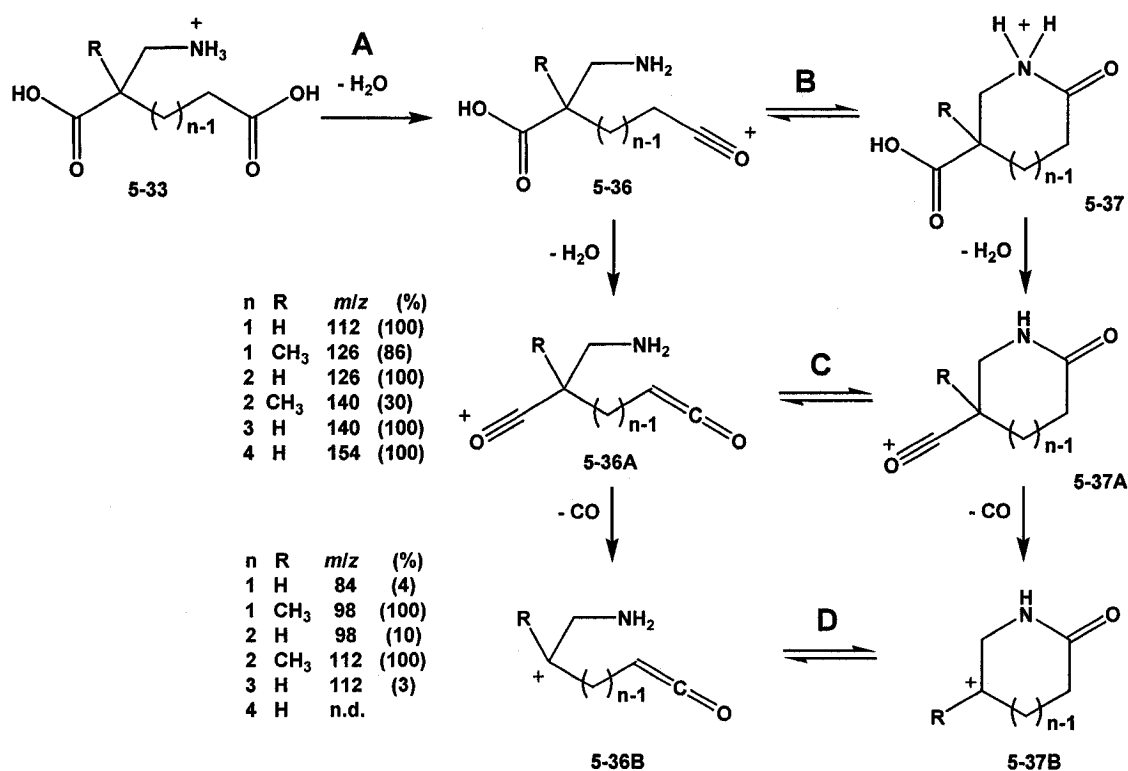
Scheme 5.12 Initial loss of water (A) and subsequent CID of the $[M+H-H_2O]^+$ ions derived from 2-(aminomethyl)dicarboxylic acids showing loss of water (C) and carbon monoxide (D).

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5.3.2 Summary for Positive Ion ESI-MS of 2-(Aminomethyl)dicarboxylic Acids

Upon CID, the $[M+H]^+$ ions (5-33) derived from 2-(aminomethyl)dicarboxylic

acids (4-1) showed loss of water as the major fragmentation process. Further

investigation involving CID of the $[M+H-H_2O]^+$ ion, revealed that this water molecule

originated from the ω-carboxyl group, correlating with the origin of the loss of water

observed in α-aminodicarboxylic acids. A distinguishing feature of the MS/MS spectra

of the 2-(aminomethyl)dicarboxylic acid $[M+H]^+$ ions is the lack of a peak corresponding

to the combined loss of water and carbon monoxide (- 46 u). This is in contrast to

MS/MS spectra obtained for $[M+H]^+$ ions derived from α-aminodicarboxylic acids

which, due to the presence of an α-amino group, can stabilize the carbocation formed

upon loss of water and carbon monoxide through formation of an immonium ion. For 2-

(aminomethyl)dicarboxylic acids, the β^2-amino structural feature consisting of the extra

methylene group between the α-carbon and the amino group, prevents the formation of

the immonium ion upon loss of carbon monoxide, so generation of a carbocation is a less

favourable process and is not observed upon CID of the $[M+H]^+$ ion.

5.4 Negative Ion Mass Spectrometry of 2-(Aminomethyl)dicarboxylic Acids

Each of the negative ion ESI mass spectra of the 2-(aminomethyl)dicarboxylic

acids (4-1) whose syntheses were described in Chapter 4 of this thesis, showed an intense

peak at the value expected for the $[M-H]^-$ ion (4-37).[10] Overall, CID of the $[M-H]^-$ ions

led to losses of ammonia (17 u), carbon dioxide (44 u) and water (18 u) as summarized in

Table 5.5. The relative importance of these fragmentation processes varied with chain

length; decarboxylation changed from the dominant process at short chain lengths (e.g.,

Figure 5.4A) to being insignificant at the longest chain length examined, whereas loss of

ammonia became the dominant process as the chain length increased (Figure 5.4B). If

the loss of ammonia is ignored, then the relative contributions of the water and carbon

dioxide losses are very similar to those measured for the equivalent series of

unsubstituted dicarboxylic acids,[10] suggesting that the aminomethyl group does not

participate in the fragmentation reactions leading to water and carbon dioxide.

Table 5.5 Product ions formed upon CID of 2-(aminomethyl)dicarboxylic acid monoanions.

4-37
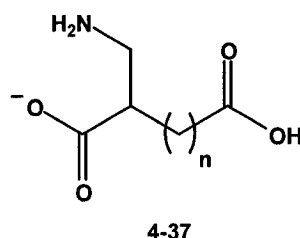
n	[M-H]⁻ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)			
			[M-H-NH₃]⁻	[M-H-H₂O]⁻	[M-H-CO₂]⁻	Combined Losses
1	146	20	129 (6)	128 (34)	102 (100)	n.d.
2	160	20	143 (92)	142 (48)	116 (100)	99 (8)
3	174	20	157 (100)	156 (28)	130 (5)	113 (5), 112 (25)
4	188	20	171 (100)	170 (8)	n.d.	127 (4)

n.d. = not detected

Similar results have been observed for the CID of the monoanions of the α-

aminodicarboxylic acids aspartic acid and glutamic acid.[10] Fragmentation processes

leading to loss of carbon dioxide and water were observed for both α-amino acid

monoanions, while aspartic acid also lost ammonia. The experiments carried out to

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examine each of these fragmentation processes for the 2-(aminomethyl)dicarboxylic acid

are described in the following subsections.

5.4.1 Loss of Ammonia

Elimination of ammonia (17 u) was observed to be a major fragmentation

pathway for 2-(aminomethyl)pentanedioic, 2-(aminomethyl)hexanedioic and 2-

(aminomethyl)heptanedioic acid and a minor pathway for 2-(aminomethyl)butanedioic

acid. Several deuterated and α-methyl analogues of 2-(aminomethyl)dicarboxylic acids

were prepared (Section 5.2) to investigate the mechanism of the fragmentation pathway

leading to the loss of ammonia.

Upon CID of the [M-H]$^-$ ion derived from 2-(aminomethyl)pentanedioic acid, the

relative intensities of the [M-H-17]$^-$ peak, corresponding to loss of ammonia and the [M-

H-18]$^-$ peak, corresponding to the loss of water, were 92% and 48%, respectively (Table

5.6, entry 1 and Figure 5.5A). Upon CID of the [M-H]$^-$ ion formed from [2-^{2}H]-2-

(aminomethyl)pentanedioic acid, the relative intensity of the [M-H-17]$^-$ peak was only

12% and the intensity of the [M-H-18]$^-$ peak increased to 100% (Table 5.6, entry 2),

indicating that a larger proportion of the neutral fragments formed had a mass of 18 u.

This supports the formation of NH_2D as a fragmentation product.

In contrast to the above results, the relative intensity of the [M-H-17]$^-$ (loss of

NH_3), and [M-H-18]$^-$ (loss of H_2O) peaks in the MS/MS spectrum of the [M-H]$^-$ ion

derived from the [2',2'-^{2}H$_2$]-2-(aminomethyl)pentanedioic acid (Table 5.6, entry 3) were

similar to those obtained for the unlabelled ion (Table 5.6, entry 1). Therefore, deuterium

on C-2' was retained in the [M-H-17]$^-$ product and did not play a role in the

fragmentation process generating ammonia. Similarly, the relative intensities of the [M-H-17]⁻ and [M-H-18]⁻ product ions (Table 5.6, entry 4) formed upon CID of the monoanion of [2,2',2'-²H₃]-2-(aminomethyl)pentanedioic acid can be attributed to the loss of deuterium on C-2 as NH_2D and the retention of deuterium at C-2'.
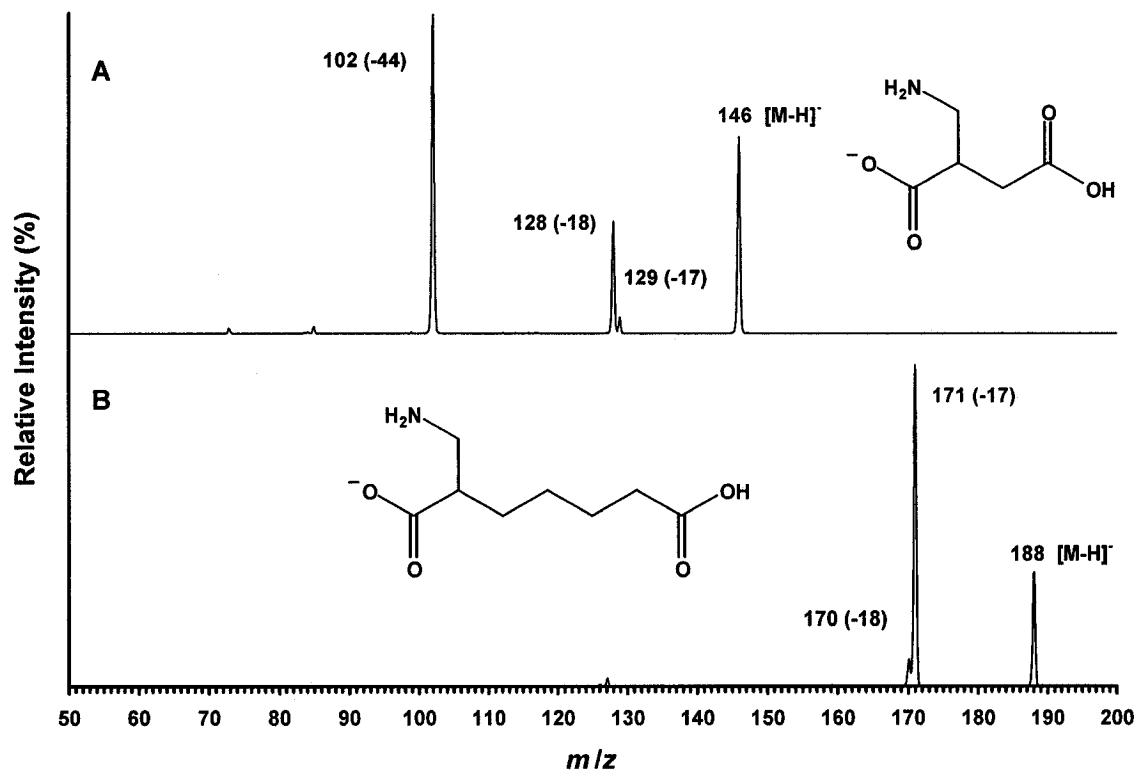
Figure 5.4 Effect of chain length on fragmentation processes initiated by CID: (A) [M-H]⁻ ion of 2-(aminomethyl)butanedioic acid; (B) [M-H]⁻ ion of 2-(aminomethyl)heptanedioic acid.

To investigate further the role of H-2 in the loss of NH_3, two amino acids in which H-2 was replaced by a methyl group were prepared. The syntheses of 2-(aminomethyl)-2-methylbutanedioic acid and 2-(aminomethyl)pentanedioic acid are described earlier in Section 5.2.2.

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While the MS/MS spectra of the [M-H]⁻ ions derived from 2-(aminomethyl)-2-methylbutanedioic acid and 2-(aminomethyl)-2-methylpentanedioic acid (Figure 5.5B) clearly showed neutral losses of water and carbon dioxide, no peak corresponding to a neutral loss of ammonia was detected. The absence of the fragmentation process leading to loss of ammonia in the 2-methyl analogues and the loss of NH_2D from $[2\text{-}^2H]$-2-(aminomethyl)pentanedioic acid demonstrate the involvement of H-2. A mechanism showing abstraction of H-2 by the side chain ω-carboxyl group as the initial step in the elimination of ammonia is presented in Scheme 5.13.

Table 5.6 Effect of ^2H labelling on product ions formed upon CID of the [M-H]⁻ ion derived from 2-(aminomethyl)pentanedioic acid.

4-37B

Entry	R 1	R 2	R 3	[M-H]⁻ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.) -17	-18	-19	-44	Combined Loss
1	H	H	H	160	20	143 (92)	142 (48)	-	116 (100)	99 (8)
2	D	H	H	161	21	144 (11)	143 (100)	142 (15)	117 (75)	99 (7)
3	H	D	D	162	22	145 (100)	144 (57)	-	118 (89)	101 (10)
4	D	D	D	163	21	146 (10)	145 (100)	144 (17)	119 (84)	101 (9)
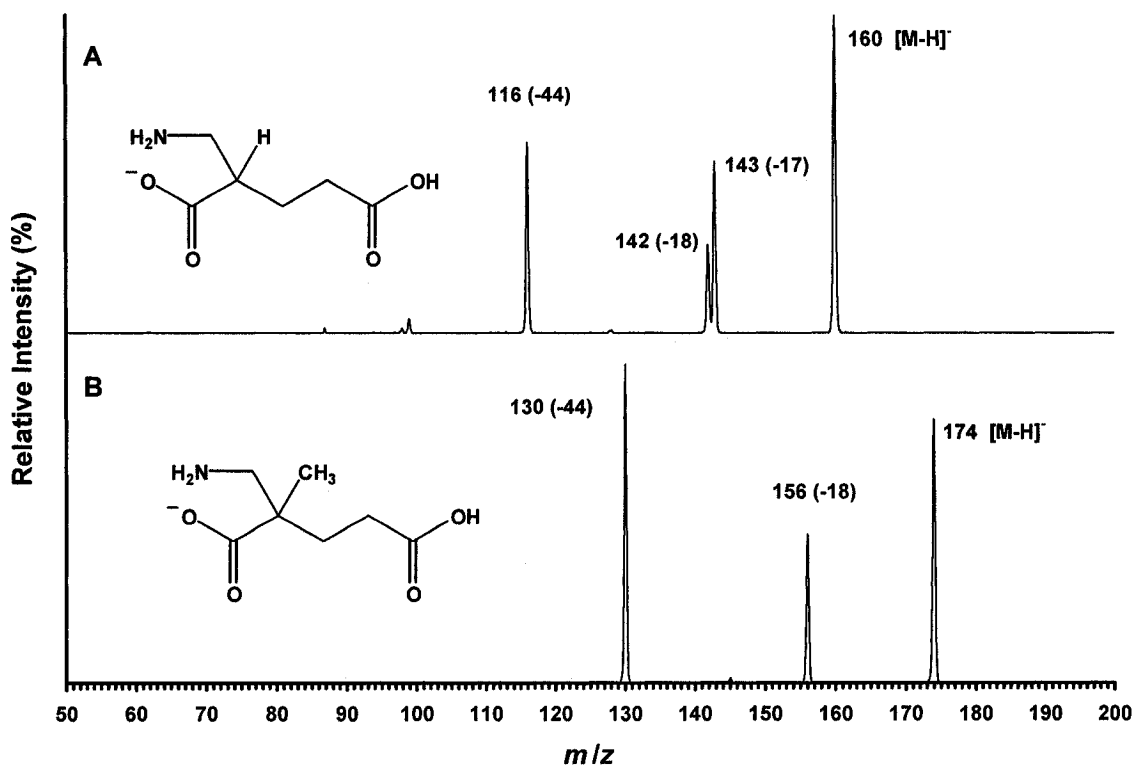
Figure 5.5 Effect of a methyl group on fragmentation: (A) MS/MS spectrum of the [M-H]⁻ ion derived from 2-(aminomethyl)pentanedioic acid; (B) MS/MS spectrum of the [M-H]⁻ ion derived from 2-(aminomethyl)-2-methylpentanedioic acid.
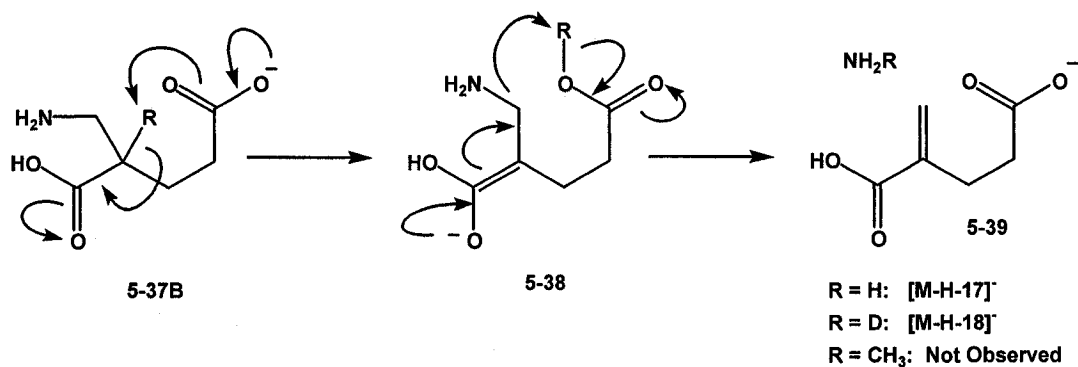
Scheme 5.13 Mechanism for the loss of ammonia from the monoanion of 2-(aminomethyl)pentanedioic acid.

By applying a source fragmentation voltage, it was possible to form the [M-H-NH$_3$]$^-$ ions from the 2-(aminomethyl)dicarboxylic acids and to investigate their fragmentations upon CID (Table 5.7). Note that loss of ammonia is a minor process for 2-(aminomethyl)butanedioic acid (Figure 5.4A) and this ion could not be generated in source. For the [M-H-NH$_3$]$^-$ ions examined, loss of water is a minor fragmentation process while decarboxylation occurs as the major process. These processes are analogous to the losses of water and carbon dioxide observed for the monoanions of aliphatic dicarboxylic acids[10] and are consistent with possible fragmentations of the monoanions of α-methylene dicarboxylic acids formed by elimination of ammonia (Scheme 5.13).

Table 5.7 Product ions formed by CID of [M-H-NH$_3$]$^-$ ions in source derived from 2-(aminomethyl)dicarboxylic acids.
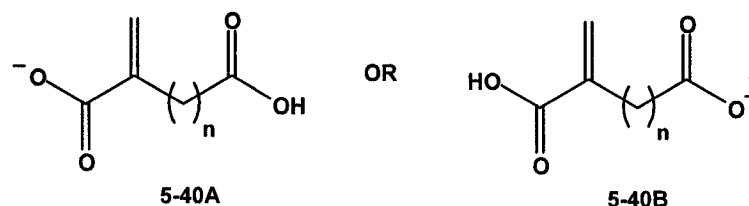
	[M-H-NH$_3$]$^-$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)		
n			-18	-44	-62
2	143	20	125 (2)	99 (100)	n.d.
3	157	20	139 (10)	113 (100)	n.d.
4	171	20	153 (16)	127 (100)	109 (17)

n.d. = not detected

5.4.2 Loss of Carbon Dioxide

As shown in Table 5.5, Figure 5.4 and Figure 5.5, the monoanions of the shorter chain β-amino acids, 2-(aminomethyl)butanedioic acid and 2-(aminomethyl)pentanedioic acid (including the 2-methyl derivatives) readily lost carbon dioxide upon CID. Decarboxylation, however, was a minor process for the monoanion of 2-(aminomethyl)hexanedioic acid and was not observed for the monoanion of 2-(aminomethyl)heptanedioic acid (Table 5.5). This dependence of carbon dioxide loss on chain-length is similar to that observed for a series of unsubstituted dicarboxylic acids.[10] Not unexpectedly, deuterium atoms at C-2 and C-2' were retained upon decarboxylation (Table 5.6).

Table 5.8 Product ions formed upon CID of the $[M-H-CO_2]^-$ ions derived from 2-(aminomethyl)dicarboxylic acid monoanions.

5-41A or 5-41B
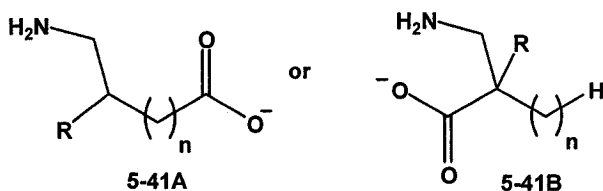
n	R	$[M-H-CO_2]^-$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)	
				-18	-29
1	H	102	21	84 (33)	73 (100)
1	CH₃	116	22	98 (8)	87 (100)
2	H	116	20	98 (19)	87 (100)
2	CH₃	130	23	112 (10)	101 (100)
3	H	130	24	112 (10)	101 (100)

As dicarboxylic acids, the monoanions of the 2-(aminomethyl)dicarboxylic acids

could lose carbon dioxide from either carboxyl group yielding a substituted β-amino acid

(5-43) or an ω-amino acid (5-42, Scheme 5.14). For aliphatic dicarboxylate anions,

carbon dioxide is lost from the non-ionized carboxyl group.

In this study, the fragmentation processes leading to the loss of the carboxyl group

from 2-(aminomethyl)dicarboxylate monoanions were investigated by ESI-MS/MS on

the in-source generated [M-H-CO$_2$]$^-$ ions. For each chain length, the in-source generated

[M-H-CO$_2$]$^-$ ion fragmented either by a minor loss of 18 u (H$_2$O) or a major loss of 29 u

(assigned below).
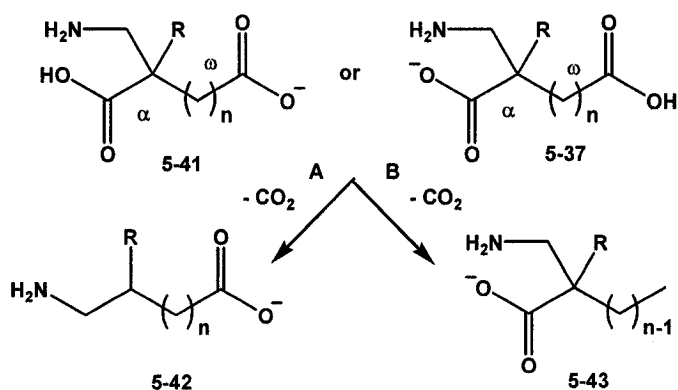
Scheme 5.14 Possible decarboxylation pathways for the monoanions of 2-
(aminomethyl)dicarboxylic acids (n = 1-4).

As shown in Scheme 5.14, loss of the α-carboxyl group by decarboxylation

would generate an ω-amino acid **5-42**. When the ESI mass spectra of a series of ω-amino

acids (H$_2$N(CH$_2$)$_n$CO$_2^-$, n = 3 - 7) were acquired, loss of water from the [M-H]$^-$ ion was

observed as the major fragmentation process (Figure 5.6B).[16] The loss of water from the

[M-H-CO$_2$]$^-$ ions of the 2-(aminomethyl)dicarboxylic acids, however, was a minor

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pathway (Table 5.8) implying that most of the [M-H-CO$_2$]$^-$ ions were formed by

decarboxylation of the ω-carboxyl group (Figure 5.6A).
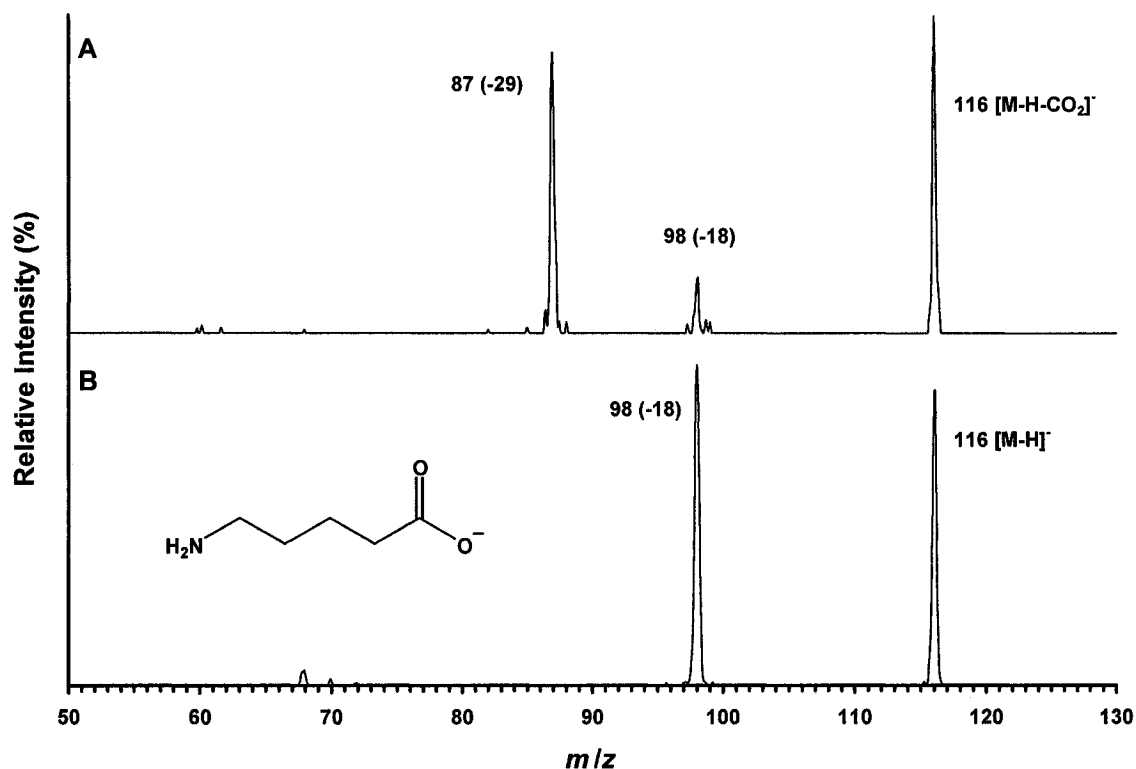
Figure 5.6 MS/MS spectra: (A) [M-H-CO$_2$]$^-$ ion derived from 2-(aminomethyl)pentanedioic acid; (B) [M-H]$^-$ ion derived from 5-aminopentanoic acid.

The major fragmentation of the in source [M-H-CO$_2$]$^-$ ions obtained by

decarboxylation of the [M-H]$^-$ ions was the loss of 29 u (Table 5.8, Figure 5.6A). The

odd value of this mass indicates that the neutral fragment contains nitrogen. A neutral

loss of 29 u also has been observed[17] as the predominant fragmentation process (Scheme

5.15) of the anions of simple β-amino acids such as β-alanine (n = 0) and β-alanine and

2-methyl-β-alanine (n = 1). A McLafferty-type rearrangement involving a 6-membered

cyclic transition state for the transfer of a proton from the β-amino group to the

carboxylate group yields $HNCH_2$ (**5-45**, 29 u) from β^2-amino acids[17] (Scheme 5.15). For

the [M-H-CO_2]⁻ ions derived from 2-(aminomethyl)dicarboxylic acids, the McLafferty-

type rearrangement would only be possible if the α-carboxyl group was retained and the

ω-carboxyl group was lost.
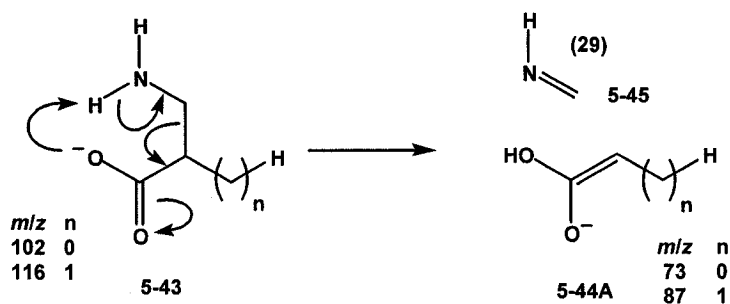
Scheme 5.15 McLafferty-type rearrangement resulting in loss of $HNCH_2$ (29) observed upon fragmentation of β-amino acids.[17]

Therefore, for 2-(aminomethyl)dicarboxylic acids, decarboxylation of the α-

carboxyl group (Scheme 5.14-A) occurs as a minor fragmentation process and loss of the

side chain ω-carboxyl group (Scheme 5.14-B) is the major fragmentation process.

5.4.3 Loss of Water

Loss of water is a fragmentation process that was observed upon CID of all of the

[M-H]⁻ ions derived from 2-(aminomethyl)dicarboxylic acids (Table 5.5). For the

monoanions of dicarboxylic acids, loss of water could occur from either carboxyl group

as shown in Scheme 5.16. In the deuterium labelling experiments (Table 5.6), a neutral

loss of 19 u was obtained from the [M-H]⁻ ion derived from [2-^{2}H]- (**5-21**) and [2,2',2'-

$^2H_3$]-2-(aminomethyl)pentanedioic acid (**5-22B**). Previous deuterium labelling studies

have shown that protons α to the carboxyl group are incorporated into the water lost from the monoanion of a dicarboxylic acid.[10] The loss of HOD from the monoanion of 2-(aminomethyl)pentanedioic acid (**4-1B**), therefore demonstrates that water is lost from the α-carboxyl group. Loss of water, however, was also observed when H-2 was replaced by a methyl group (Figure 5.5B). In this case two α substituents are adjacent to the α-carboxyl group, a structural arrangement that does not lead to loss of water.[10] Thus loss of water from the [M-H]$^-$ ions derived from the 2-methyl derivatives (**4-21, 5-26**) must occur at the ω-carboxyl group. Overall, reactions leading to loss of water from the [M-H]$^-$ monoanions from 2-(aminomethyl)dicarboxylic acids can occur from either carboxyl group (Scheme 5.16). When in-source generated [M-H-H$_2$O]$^-$ ions were subjected to CID, decarboxylation (-44 u) and a more minor loss of 30 u were observed (Table 5.9).

Additional structural information was obtained by subjecting in-source generated [M-H-H$_2$O]$^-$ ions to CID. At each chain length, neutral losses of 30 u and 44 u were observed. Note that replacement of H-2 with a methyl group had no significant effect on the fragmentation of the ion derived from 2-(aminomethyl)pentanedioic acid.
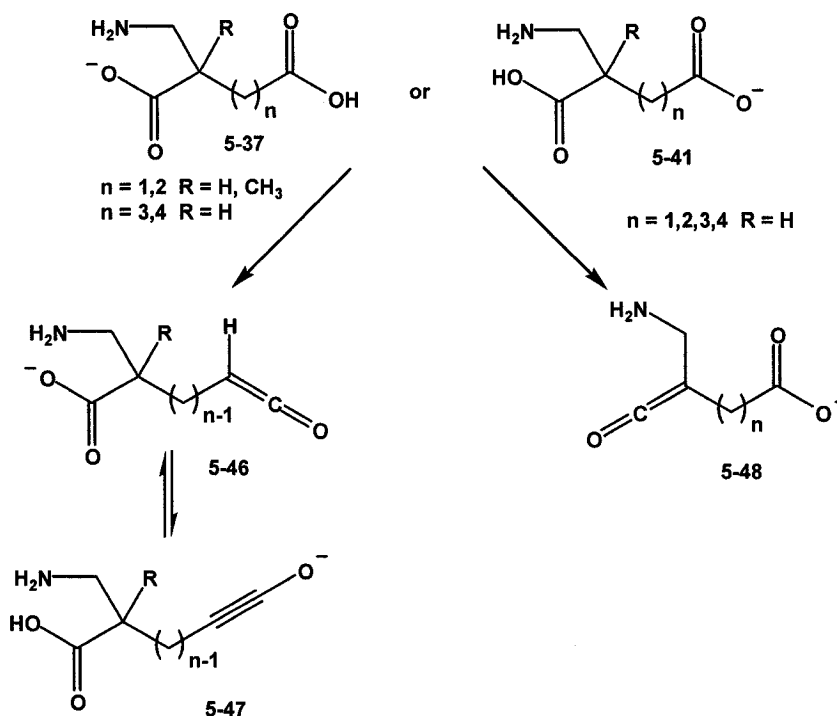
Scheme 5.16 Possible pathways for the loss of water for the monoanions of 2-(aminomethyl)dicarboxylic acids (n = 1-4).

Earlier in this thesis (Chapter 4), 2-(aminomethyl)pentanedioic acid (**4-1B**) and 2-(aminomethyl)butanedioic acid (**4-1A**) were shown to lose water and cyclize upon heating to form the corresponding lactams, 2-piperidone-5-carboxylic acid (**4-36B**) and 4-carboxy-2-pyrrolidone (**4-36A**), respectively. Negative ion ESI-MS of each lactam gave a [M-H]⁻ ion at the same m/z value as the [M-H-H₂O]⁻ ion derived from the corresponding 2-(aminomethyl)dicarboxylic acid.

Table 5.9 Product ions formed upon CID of $[M-H-H_2O]^-$ ions derived from 2-(aminomethyl)dicarboxylic acid monoanions.

5-48   5-46   5-47
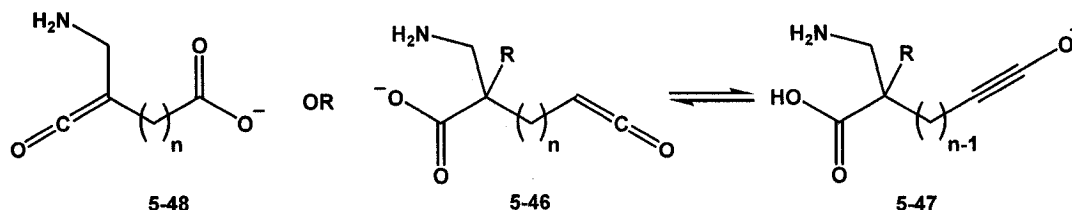
n	R	$[M-H-H_2O]^-$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)				
				-17	-29	-30	-44	-73 (44+29)
1	H	128	20	111 (100)	99 (22)	98 (21)	84 (50)	55 (4)
1	CH₃	142	20	n.d.	n.d.	112 (100)	n.d.	n.d.
2	H	142	23	n.d.	n.d.	112 (44)	98 (100)	n.d.
2	D	143	21	n.d.	n.d.	113 (8)	99 (100)	n.d.
2	CH₃	156	25	n.d.	n.d.	126 (50)	112 (100)	n.d.
3	H	156	22	n.d.	n.d.	126 (8)	112 (100)	83 (15)
4	H	170	21	152 (7)	127 (9)	140 (81)	126 (100)	97 (5)
5-Lactam[a]		128	22	n.d.	n.d.	98 (100)	84 (5)	n.d.
6-Lactam[b]		142	23	n.d.	n.d.	112 (25)	98 (100)	n.d.

[a] 4-carboxy-2-pyrrolidone, [b] 2-piperidone-5-carboxylic acid, n.d. = not detected

A comparison of the spectra obtained by CID of the 2-(aminomethyl)pentanedioic acid $[M-H-H_2O]^-$ ion (Figure 5.7A) and the 2-piperidone-5-carboxylic acid $[M-H]^-$ ion (Figure 5.7B) clearly shows that identical fragmentation processes occurred in each case, resulting in losses of 44 u and 30 u. Therefore, in-source deprotonation and dehydration of 2-(aminomethyl)pentanedioic acid (**4-1B**) must generate the same ion, or group of ions, as in-source deprotonation of 2-piperidone-5-carboxylic acid (Scheme 5.17). In other words, either the $[M-H-H_2O]^-$ ion derived from the amino acid must cyclize or the

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[M-H]⁻ ion derived from the lactam must ring-open or both processes could occur to

generate a mixture of ions shown in Scheme 5.17.
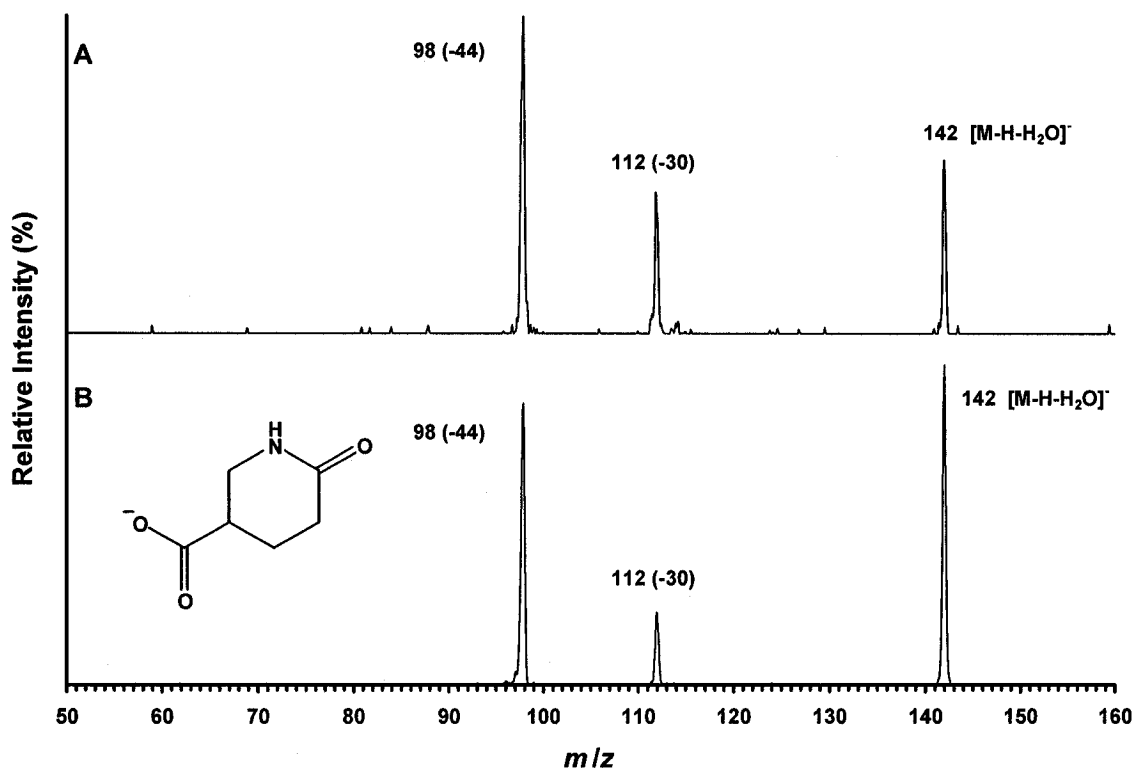
Figure 5.7 Identical MS/MS spectra of: (A) the [M-H-H₂O]⁻ ion derived from 2-(aminomethyl)pentanedioic acid; (B) the [M-H]⁻ ion derived from 2-piperidone-5-dicarboxylic acid.

The loss of carbon dioxide (44 u) as the major fragmentation pathway can be

rationalized as occurring from two of the three intermediates presented in Scheme 5.17.

Loss of carbon dioxide from the ionized lactam (**5-48**) would open the ring and give a

stabilized amide ion as the product (**5-49**). Alternatively, carbon dioxide could be lost

from the protonated carboxyl group of the alkyne oxide ion (**5-47**) as previously

suggested.[10]
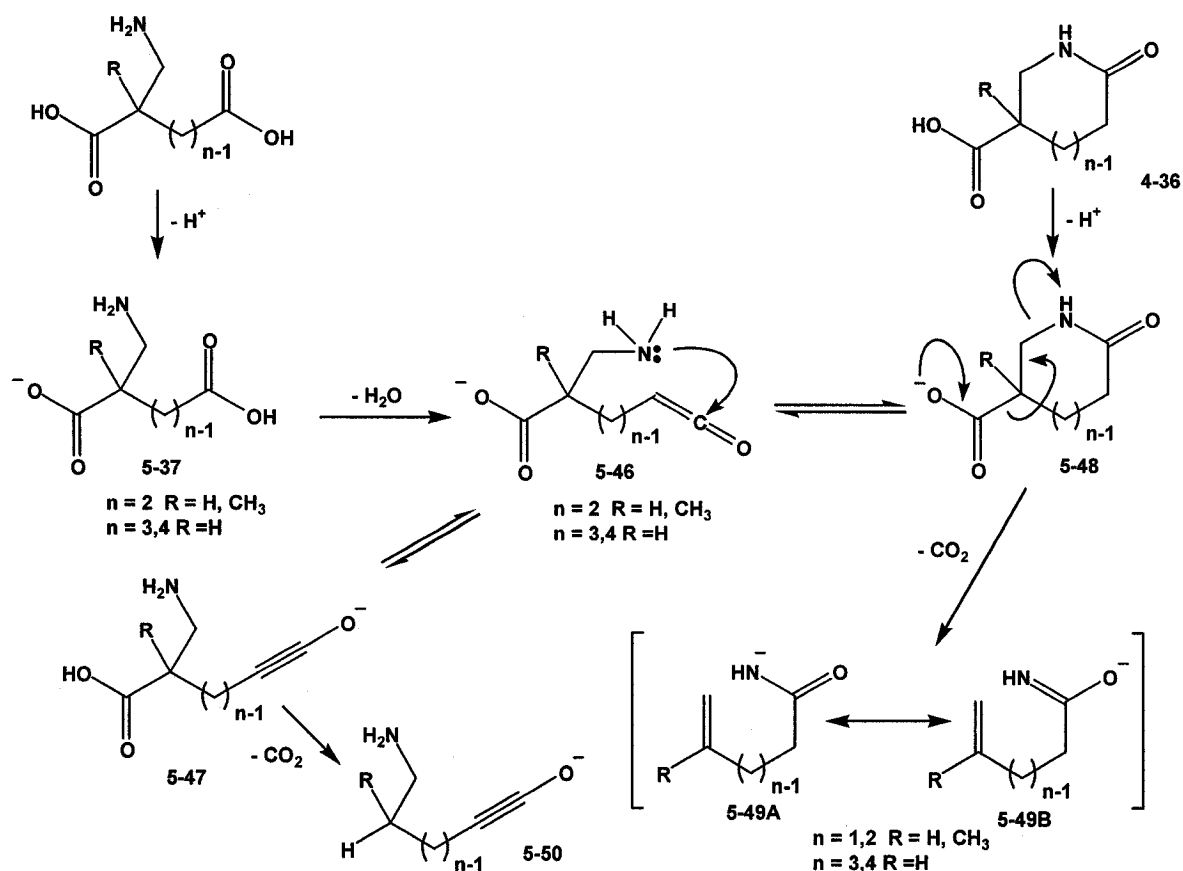
Scheme 5.17 Loss of water from the 2-(aminomethyl)dicarboxylic acid side chain carboxyl group, lactam formation and the subsequent loss of carbon dioxide.

The second fragmentation pathway observed upon CID of the [M-H-H$_2$O]$^-$ ions derived from 2-(aminomethyl)dicarboxylic acids (**4-1**) and the [M-H]$^-$ ions (**5-48**) derived from the corresponding lactams (**4-36**) was the loss of 30 u (Table 5.9). An indication of the composition of the 30 u neutral fragment was provided when CID was performed on the in-source generated [M-H-H$_2$O]$^-$ of [2',2'-^{2}H$_2$]-2-(aminomethyl)pentanedioic acid (m/z 144). The MS/MS spectrum showed the expected decarboxylation peak at m/z 100 (- 44 u) and a peak at m/z 112 indicating a loss of 32 u. The peak at m/z 112 was the same m/z as that for the ion produced by unlabelled amino acid (m/z 112), demonstrating that the deuterium on C-2' was lost with the neutral fragment upon CID. With a partial structure

of CH_2 (14 u), the remaining 16 u in the neutral fragment are most likely provided by the

adjacent NH_2. A neutral nitrogen-containing species of even mass would have an odd

number of electrons, suggesting the formation of •CH_2NH_2 (**5-54A**, 30 u) or •CD_2NH_2

(**5-54B**, 32 u) by homolytic cleavage of the C-2-C-2' bond (Scheme 5.18). Futhermore,

loss of •CH_2NH_2 demonstrates ring opening of the lactam anion (**4-51**, Scheme 5.18) and

the formation of an acylic ion (**4-52, 4-53**) that undergoes homolytic C-2-C-2' bond

cleavage.
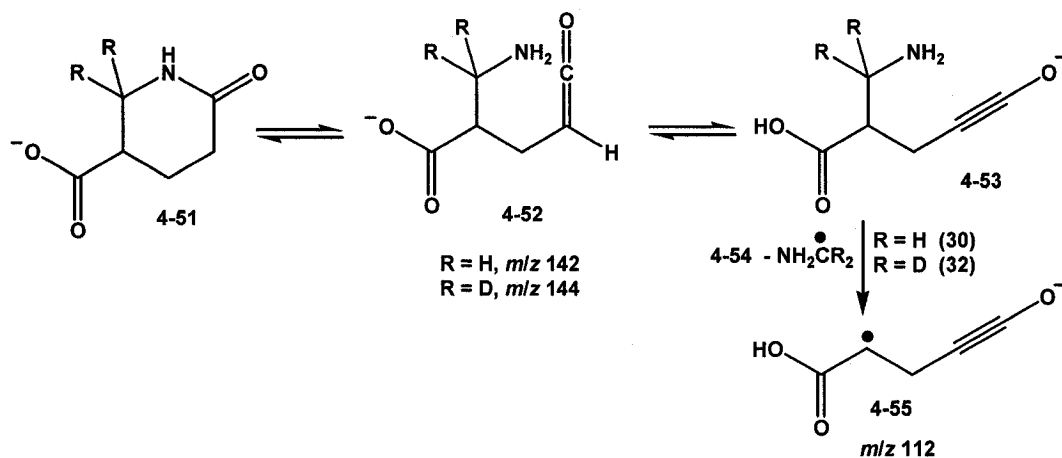
Scheme 5.18 Proposed loss of •CH_2NH_2 (30 u) or •CD_2NH_2 (32 u) upon CID of the [M-H-H_2O]⁻ ions derived from 2-(aminomethyl)pentanedioic acid.

The MS/MS spectrum of the in-source [M-H-H_2O]⁻ anion generated from 2-

(aminomethyl)butanedioic acid showed the losses of •CH_2NH_2 (30 u) and carbon dioxide

exhibited by the [M-H-H_2O]⁻ ions of the other 2-(aminomethyl)dicarboxylic acids

studied. The monoanion of the corresponding lactam, 4-carboxy-2-pyrrolidone (**4-36A**),

only showed these losses as well. However, the [M-H-H_2O]⁻ ion derived from 2-

(aminomethyl)butanedioic acid also showed neutral losses of 17 u (NH_3) and 29 u

(HNCH$_2$). Fragmentation of 2-(aminomethyl)-2-methylbutanedioic acid only showed a

loss of 30 u, suggesting that H-2 is important for the 17 u and 29 u neutral losses. Loss of

water from the α-carboxyl group would involve H-2 and lead to the different

fragmentation processes observed for the [M-H-H$_2$O]⁻ ion derived from 2-

(aminomethyl)butanedioic acid.

Loss of water from the non-ionized α-carboxyl group would involve abstraction

of the α proton, possibly assisted by the ω-carboxyl group to generate ion **5-56** (Scheme

5.19). Cyclization, by addition of the ω-carboxyl group to the ketene would place the

charge in a suitable position for the elimination of ammonia, generating a stabilized

enolate ion **5-58**. Alternatively, proton transfer from nitrogen to the ω-carboxylate group

in ion **5-56** would lead to loss of CH$_2$NH (29 u) and the formation of a stable alkyne

oxide ion **5-59**.
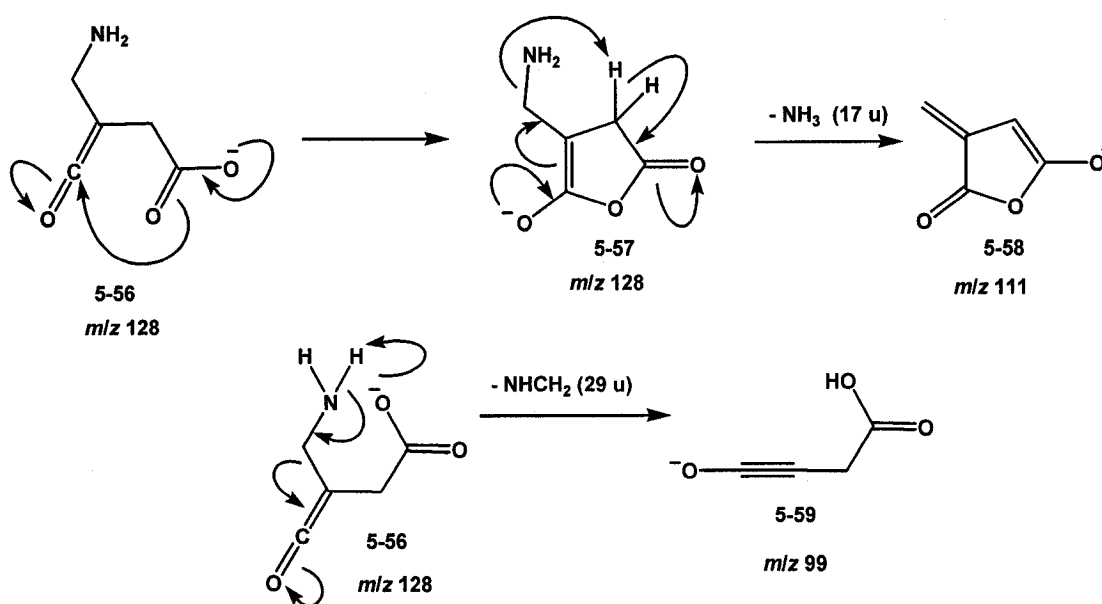
Scheme 5.19 Loss of water from the α-carboxyl group and subsequent fragmentations
from the monoanion of 2-(aminomethyl)butanedioic acid.

5.4.4 Summary for Negative Ion ESI-MS of 2-(Aminomethyl)dicarboxylic Acids

Upon CID, the [M-H]⁻ ions derived from 2-(aminomethyl)dicarboxylic acids (**4-1**)

fragmented to lose water and showed chain length dependent losses of carbon dioxide

and ammonia.

Lactams (**4-36**), prepared in Chapter 4, were essential for determining that the

initial water molecule lost upon CID of the [M-H]⁻ ions was from the ω-carboxyl group

of the 2-(aminomethyl)-2-methyldicarboxylic acids. The CID spectra of the [M-H-CO₂]⁻

ions derived from 2-(aminomethyl)dicarboxylic acids (**4-1**) differed from those obtained

from the [M-H]⁻ ions derived from the corresponding ω-amino acids, confirming that the

initial decarboxylation occurs from the ω-carboxylate group.

The major distinguishing feature between the 2-(aminomethyl)dicarboxylic acids

(**4-1**) and α-aminodicarboxylic acids (**4-3**) was the loss of ammonia. For α-

aminodicarboxylic acids, only glutamic acid showed a minor loss of ammonia while the

loss of ammonia was a major fragmentation process for 2-(aminomethyl)dicarboxylic

acids. Use of deuterated and α-methylated 2-(aminomethyl)dicarboxylic acids illustrated

the essential role of the H-2 proton and helped to elucidate the mechanism for loss of

ammonia.

5.5 Negative Ion Mass Spectrometry of 2-Cyanodicarboxylic Acids

Upon electrospray ionization in negative ion mode, each 2-cyanodicarboxylic acid

examined formed the expected monoanion (**5-61**) by deprotonation (Table 5.10).[10] In

each mass spectrum, however, the signal for the [M-H]⁻ ion was accompanied by another,

usually more intense, peak at an *m/z* value 44 mass units lower (Table 5.10, e.g. Figure

5.8A). The mass difference of 44 u between the two most intense signals suggested that each monoanion was undergoing in-source decarboxylation. Other minor in-source fragmentation processes were indicated by low intensity signals ($< 15\%$).

Since only monoanions (**5-61**) of the cyanodicarboxylic acids were observed by mass spectrometry, only one carboxyl group ionized under electrospray conditions. Due to the stabilizing effects of the electron withdrawing nitrile group, the carboxyl group α to the nitrile should be more acidic than the ω-carboxyl group. The fact that the gas phase acidity[18] of cyanoacetic acid (1354-1382 kJ/mol)[19] is considerably greater than that of acetic acid (1427^{19}-1459^{20} kJ/mol) supports this view and suggests that monoanions of 2-cyanodicarboxylic acids are ionized mostly at the α-carboxyl group.

Table 5.10 Negative ion ESI-MS and ESI-MS/MS data for 2-cyanodicarboxylic acids.

5-61
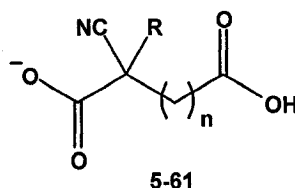
n	R	MS of [M-H]⁻, m/z, (Rel. Int.)			MS/MS of [M-H]⁻, m/z (Rel. Int.)		
		[M-H]⁻	[M-H-CO₂]⁻	Other	CID %	[M-H-CO₂]⁻	Other
1	H	142 (17)	98 (100)	n.d.	20	98 (100)	112 (5)
1	CH₃	156 (38)	112 (100)	n.d.	15	112 (100)	n.d.
2	H	156 (31)	112 (100)	59 (6)	15	112 (100)	n.d.
2	CH₃	170 (80)	126 (100)	n.d.	16	126 (100)	n.d.
3	H	170 (35)	126 (100)	88 (15)	13	126 (100)	n.d.
4	H	184 (30)	140 (100)	n.d.	16	140 (100)	n.d.

n.d. = none detected.
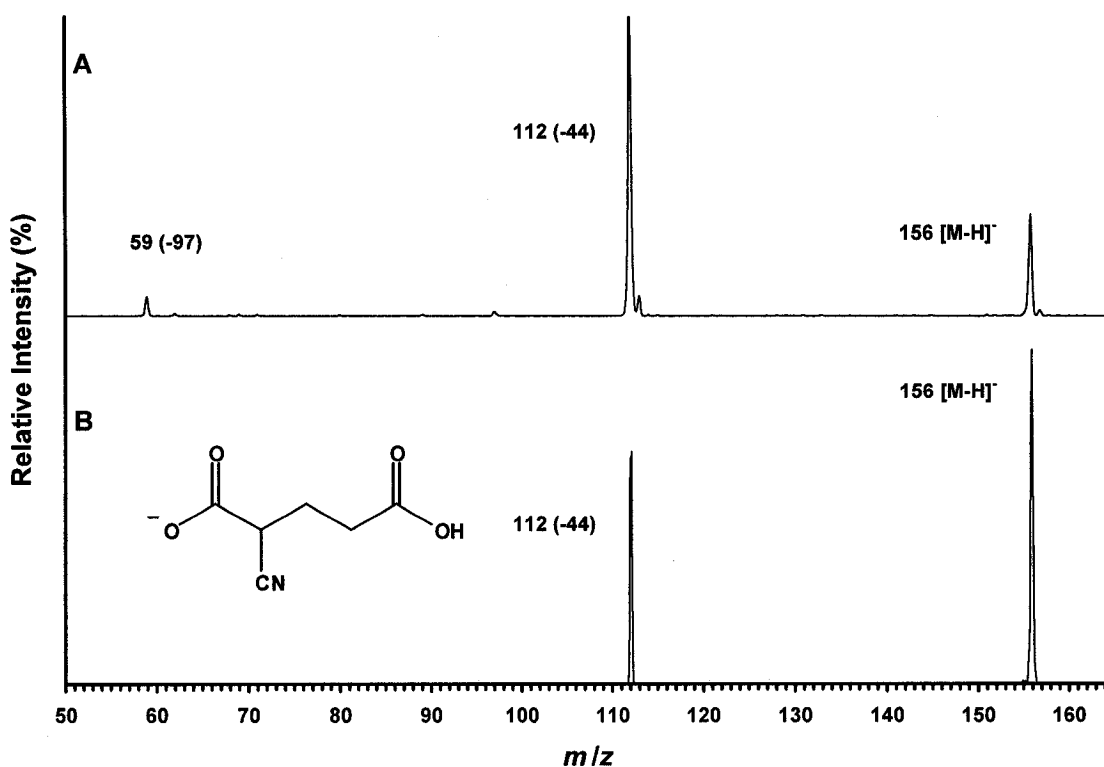
Figure 5.8 Negative ion mass spectra of deprotonated 2-cyanopentanedioic acid: (A) ESI-MS and (B) ESI-MS/MS.

5.5.1 Decarboxylation of 2-Cyanodicarboxylic Acid Monoanions

In principle, decarboxylation may occur from either the ionized α-carboxyl group (**5-62**) or the non-ionized ω-carboxyl group (**5-63**, Scheme 5.20). Previously, for a series of short-chain aliphatic dicarboxylic acids, decarboxylation of the non-ionized carboxyl group was observed.[10] Placement of a nitrile group on the α carbon of aliphatic dicarboxylic acids, however, opens up another potential decarboxylation pathway leading to a stabilized carbanion. While gas phase acidities indicate that the carbanion **5-62A** (1528-1569 kJ/mol for acetonitrile)[21,22] is less stable than the carboxylate ion **5-63** (1427-1459 kJ/mol for acetic acid),[19, 20] a facile intramolecular proton transfer would generate a carboxylate ion **5-62B** from **5-62A** (Scheme 5.20).

In the gas phase, monoanions of a series of aliphatic dinitriles have been generated using fast atom bombardment (FAB) and chemical ionization (CI).[23] Theoretical studies showed that the lowest energy conformation involved a hydrogen bond between the carbanion α to the nitrile and a hydrogen atom α to the ω-nitrile group. The fragmentation processes involved intramolecular proton transfers which generated anions β- or γ- to the second nitrile group that subsequently underwent fragmentations that varied with chain length. In this investigation, the stabilized carbanions formed upon decarboxylation of 2-cyanodicarboxylic acids may undergo fragmentation processes that initially involve a proton transfer.
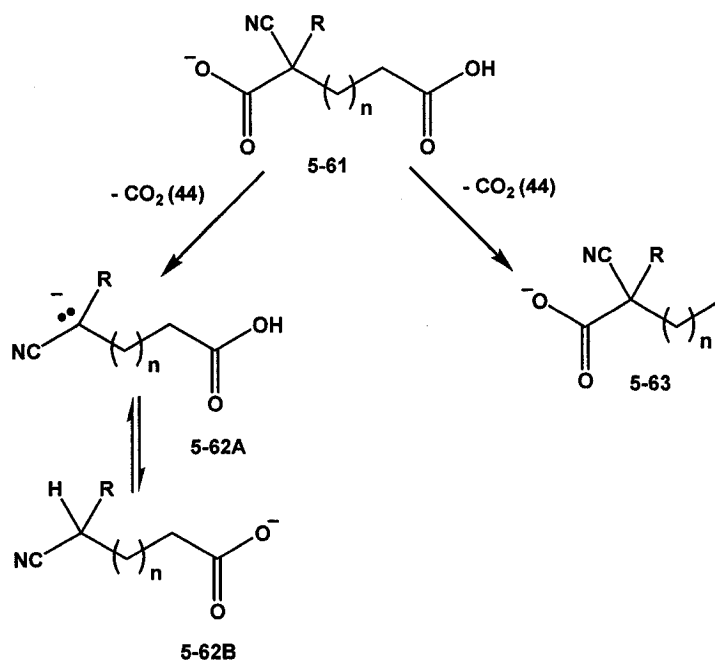
Scheme 5.20 Two possible decarboxylation pathways for 2-cyanodicarboxylic acids.

Upon CID at relatively low collision energy ($\leq$ 20% CID, Table 5.10 and Figure 5.8B), the monoanion of each 2-cyanocarboxylic acid (**5-61**) underwent decarboxylation, yielding only one product ion, 44 mass units lower than that of the deprotonated 2-cyanodicarboxylic acid. To investigate which carboxyl group was lost upon decarboxylation of the 2-cyanodicarboxylate anions upon CID, $[\text{M-H-CO}_2]^-$ ions were generated in-source and studied by CID. Note that the fragmentation of the $[\text{M-H-CO}_2]^-$ ions derived from 2-cyanodicarboxylic required CID energies approximately 50% higher than those needed to effect decarboxylation of the $[\text{M-H}]^-$ ion. The MS/MS spectra collected indicated that different fragmentation processes occurred for the $[\text{M-H-CO}_2]^-$ ions of different lengths. A corresponding series of ω-cyanocarboxylic acids were prepared as standards for comparison between the CID of their $[\text{M-H}]^-$ ions and the CID of the in-source generated $[\text{M-H-CO}_2]^-$ ions of the 2-cyanodicarboxylic acids.

There are several examples in this thesis where the electron withdrawing nature of a nitrile group was used to promote heterolytic bond cleavage (e.g., decarboxylation). The Krapcho decarboethoxylation reaction was utilized for the removal of esters adjacent to the nitrile, while leaving ester functional groups that were not adjacent to a stabilizing group intact. In the well established malonic acid and cyanoacetic acid syntheses, a carboxyl group adjacent to a second carboxyl group or nitrile group is formed by ester hydrolysis and then lost by thermal decarboxylation.[24] Earlier in this thesis, this procedure was used in the syntheses of β-amino acids to remove the carboxyl group adjacent to a nitrile and a second carboxyl group after base-catalyzed ester hydrolysis of the alkylated cyanomalonate esters. Therefore, the use of an electron-withdrawing nitrile group to stabilize the negative charge generated by a decarboxylation reaction is a

common synthetic strategy and it is expected that a comparable process occurs in the gas phase.

5.5.1.1 Decarboxylation of the Monoanion Derived from 2-Cyanobutanedioic Acid

Upon CID, the in-source generated $[M-H-CO_2]^-$ ion from 2-cyanobutanedioic acid (**4-33A**) yielded fragment ions formed by neutral losses of 27 u (HCN) and 44 u (CO_2) (Table 5.11, Figure 5.9A). A third ion was formed by a neutral loss of 28 u, a process more difficult to rationalize with the structure of the starting 2-cyanodicarboxylic acid.

The anion formed by deprotonation of 3-cyanopropanoic acid is a possible ion formed upon decarboxylation of the 2-cyanobutanedioate ion (**5-61A**, Scheme 5.21). The availability of a standard sample of 3-cyanopropanoic acid (**4-77**, Chapter 4) allowed the MS/MS spectrum to be acquired for the 3-cyanopropanoate ion (Figure 5.9B). Neutral losses of HCN and carbon dioxide from the $[M-H]^-$ ion were observed in the spectrum acquired using the ion-trap spectrometer.

Table 5.11 CID of the $[M-H-CO_2]^-$ of 2-cyanobutanedioic acid and the $[M-H]^-$ of 3-cyanopropanoic acid.

NC R² O
\|
R¹ OH
5-64
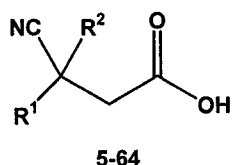
R¹	R²	$[M-H]^-$ (m/z)	$[M-H-CO_2]^-$ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)				
					-15	-18	-27	-28	-44
CO_2	H	142	98	22	-	-	71 (100)	70 (55)	54 (12)
H	H	98	-	23	-	-	71 (100)	-	-
CO_2	CH_3	156	112	23	97 (11)	94 (50)	-	84 (100)	-

Except for the loss of 28 u (m/z 70), CID of the [M-H-CO$_2$]$^-$ ion (m/z 98) derived

from the decarboxylation of 2-cyanobutanedioic acid (**5-61A**, Figure 5.9A) produced an

identical mass spectrum to that recorded upon CID of the [M-H]$^-$ ion derived from 3-

cyanopropanoic acid (**4-66**, Figure 5.9B). Also, the [M-H]$^-$ anion of [3,3-^{2}H$_2$]-3-

cyanopropanoate (**3-79**), prepared as an intermediate in the synthesis of [3,3-^{2}H$_2$]GABA

(**3-40**, Chapter 3), lost 27 u upon CID. Therefore, the proton lost in HCN most likely

derives from C-2, α to the carboxyl group and not from C-3, α to the nitrile. Loss of

HCN could involve the formation of an enolate anion (**5-67**, Scheme 5.22), followed by

elimination of HCN in which a proton is transferred from the carboxylic acid to cyanide

ion.
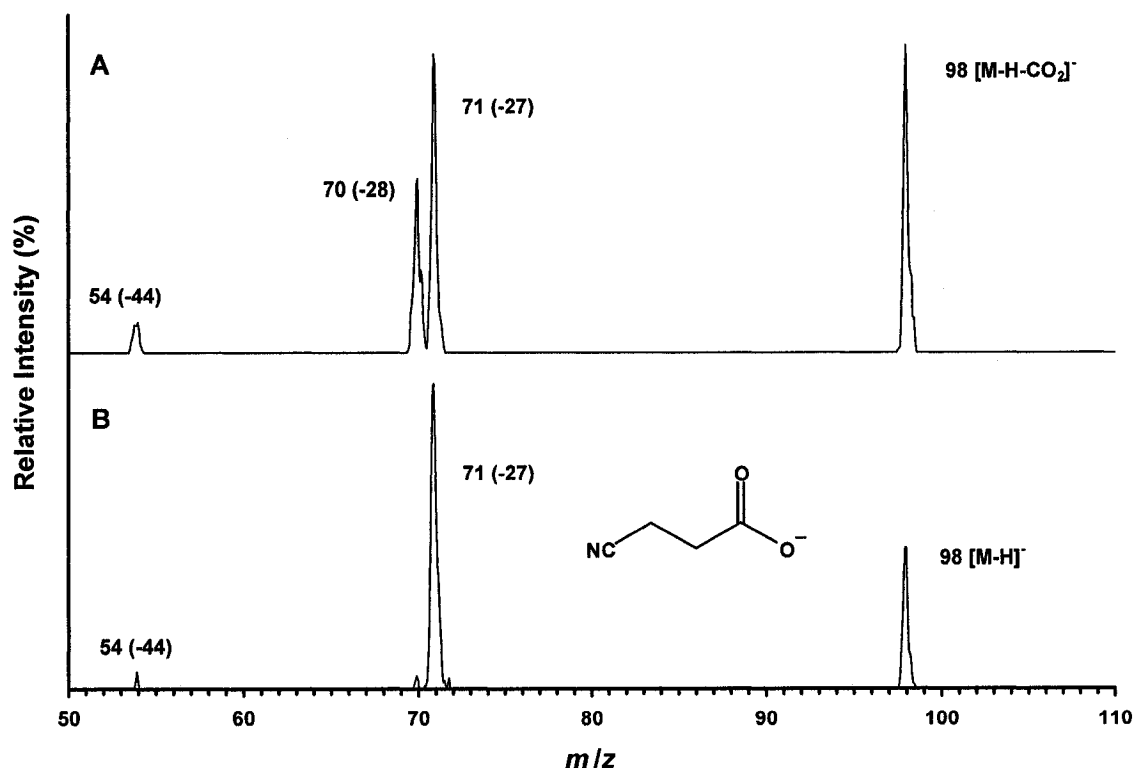
Figure 5.9 Ion trap MS/MS spectra: (A) CID of in-source generated [M-H-CO$_2$]$^-$ ion derived from 2-cyanobutanedioic acid; (B) CID of [M-H]$^-$ ion derived from 3-cyanopropanoic acid.
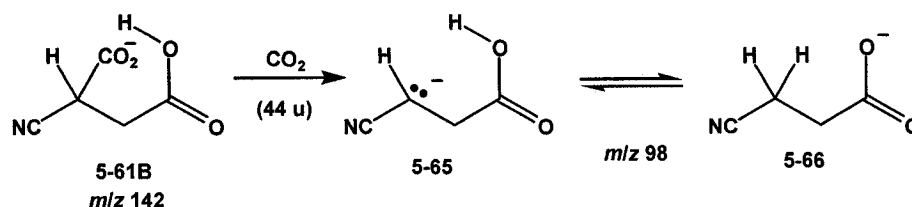
Scheme 5.21 Decarboxylation of 2-cyanobutanedioic acid (**5-61B**), formation of a nitrile-stabilized anion (**5-65**) and proton transfer to give 3-cyanopropanoate anion (**5-66**).
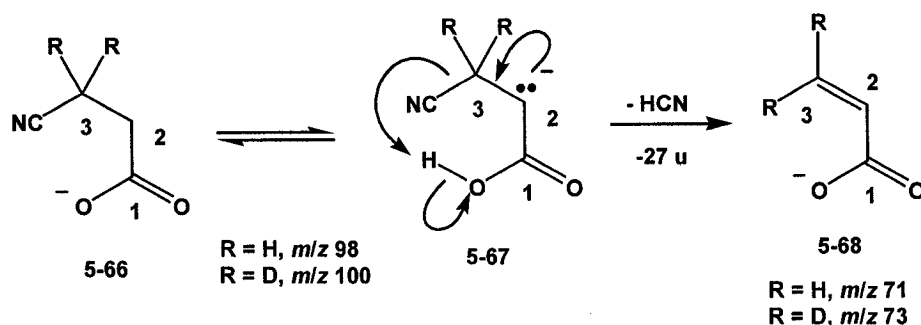
Scheme 5.22 Loss of HCN (27 u) upon CID of [M-H]⁻ derived from 3-cyanopropanoic acid showing retention of deuterium at C-3.

While the MS/MS spectra of the [M-H-CO₂]⁻ ions derived from 2-cyanobutanedioic acid (**4-33A**) and 2-cyano-2-methylbutanedioic acid (**5-24A**) each showed a strong peak corresponding to the loss of 28 u (Table 5.11), the loss of 28 u was not observed in the MS/MS spectrum of 3-cyanopropanoic acid (Figure 5.9B), suggesting the formation of two [M-H-CO₂]⁻ ions of different structure.

For the shorter chain 2-cyanodicarboxylic acid, the initial loss of the α-carboxyl group leads to placement of charge α to the nitrile. As shown in Scheme 5.23, proton transfer to nitrogen followed by attack of the ω-carboxyl group onto the carbon originally in the nitrile could lead to a cyclic [M-H-CO₂]⁻ ion, which upon ring opening would form carbon monoxide (28 u) and a resonance stabilized amide ion (**5-72, 5-73**).

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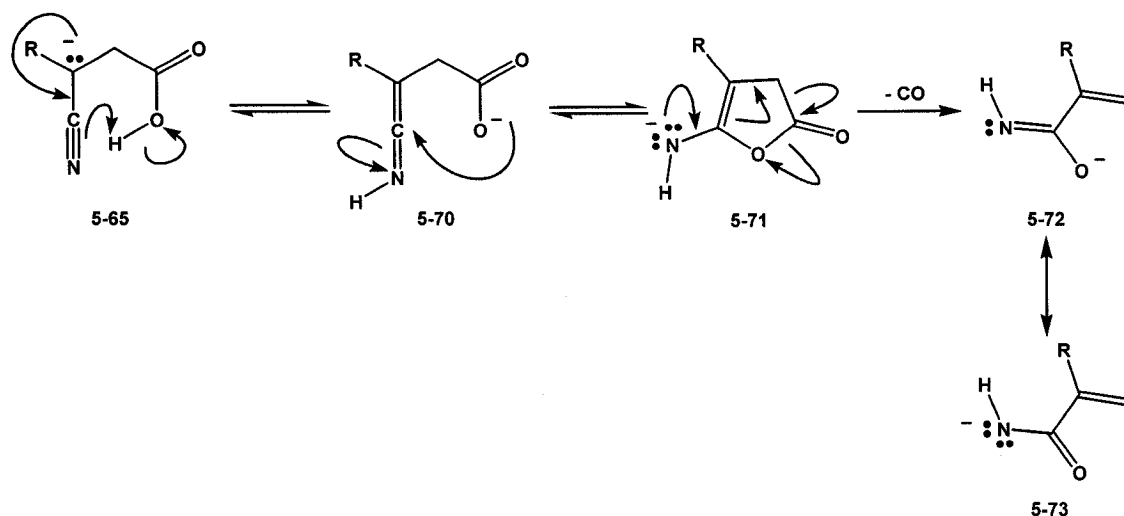
Scheme 5.23 Mechanism proposed for the loss of CO (28 u) upon CID of in-source generated [M-H-CO₂]⁻ ion derived from 2-cyanobutanedioic acid.

The MS/MS spectrum acquired for the in-source generated [M-H-CO₂]⁻ ion derived from 2-cyano-2-methylbutanedioic acid (**5-24A**) showed only one peak in common with the corresponding spectrum acquired for the [M-H-CO₂]⁻ ion of 2-cyanobutanedioic acid (Table 5.11). Instead of the neutral losses of 27 u (HCN) and 44 u (CO₂), neutral losses of 15 u (•CH₃) (Scheme 5.24) and 18 u (H₂O) were observed when a methyl group was positioned α to the nitrile. The methyl group also enhanced the relative intensity of the peak corresponding to the loss of 28 u.

Decarboxylation of the second carboxyl group during the CID of the [M-H-CO₂]⁻ ion derived from 2-cyanobutanedioic acid (Table 5.11) could occur via homolytic cleavage of a non-ionized carboxyl group giving a nitrile stabilized anion as shown in Scheme 5.25.

261
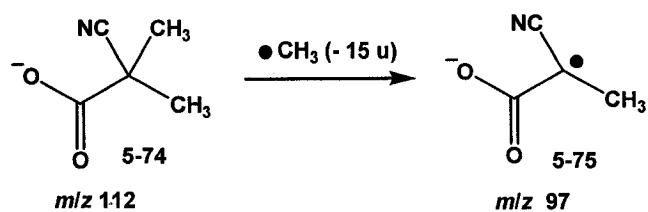
Scheme 5.24 Proposed loss of a methyl radical upon CID of the [M-H-CO₂]⁻ ion derived from 2-cyano-2-methylbutanedioic acid.
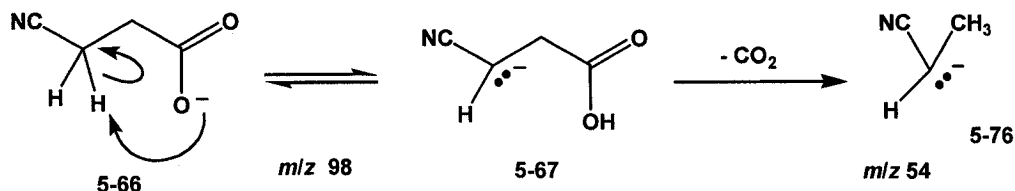
Scheme 5.25 Loss of carbon dioxide from the [M-H-CO₂]⁻ ion derived from 2-cyanobutanedioic acid upon CID.

In addition to the loss of carbon dioxide described above for the [M-H]⁻ ion of 2-cyanobutanedioic acid (**4-33A**), it may also lose cyanide, the m/z value of which is too low to be detected by the LCQ MS. Generation of cyanide ion (m/z 26), presumably accompanied by the formation of carbon dioxide and ethylene, was the major fragmentation process for 3-cyanopropanoic acid in the triple quadrupole mass spectrometer. This fragmentation process could also occur for the [M-H-CO₂]⁻ ion derived from 2-cyanobutanedioic acid.

5.5.1.2 Decarboxylation of the Monoanion Derived from 2-Cyanopentanedioic Acid

Upon CID, the [M-H-CO₂]⁻ ions derived in-source from 2-cyanopentanedioic acid (**4-33B**) and 2-cyano-2-methylpentanedioic acid (**5-24B**, Table 5.12 and Figure 5.10) each produced a predominant fragment ion at m/z 59 (100). 4-Cyanobutanoic acid (**5-**

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29A) was prepared as a standard compound for comparison of the CID of its [M-H]⁻ ion

with the CID of the [M-H-CO₂]⁻ ion derived from 2-cyanopentanedioic acid (Table 5.12).

Table 5.12 CID of the [M-H-CO₂]⁻ ions derived from 2-cyanopentanedioic acid and 2-cyano-2-methylpentanedioic acid.

5-77
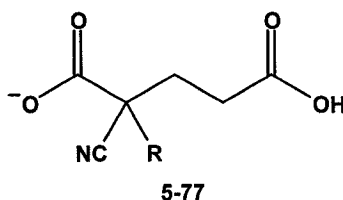
Entry	R	[M-H]⁻ (m/z)	[M-H-CO₂]⁻ (m/z)	CID Energy %	MS/MS Fragment Ions, m/z (Rel. Int.)	
						-72
1	H	156	112	23	59 (100)	n.d.
2	CH₃	170	126	22	59 (100)	54 (33)

n.d. = not detected

Upon electrospray ionization, 4-cyanobutanoic acid (**5-29A**) formed the expected

[M-H]⁻ ion at m/z 112. CID of the [M-H]⁻ ion of 4-cyanobutanoic acid (Figure 5.10C)

produced an ion at m/z 59, an ion in common with product ions formed upon CID of the

[M-H-CO₂]⁻ ions from 2-cyanopentanedioic acid (**4-33B**) and 2-cyano-2-methylpentanedioic acid (**5-24B**, Table 5.12, Figure 5.10A and Figure 5.10B).
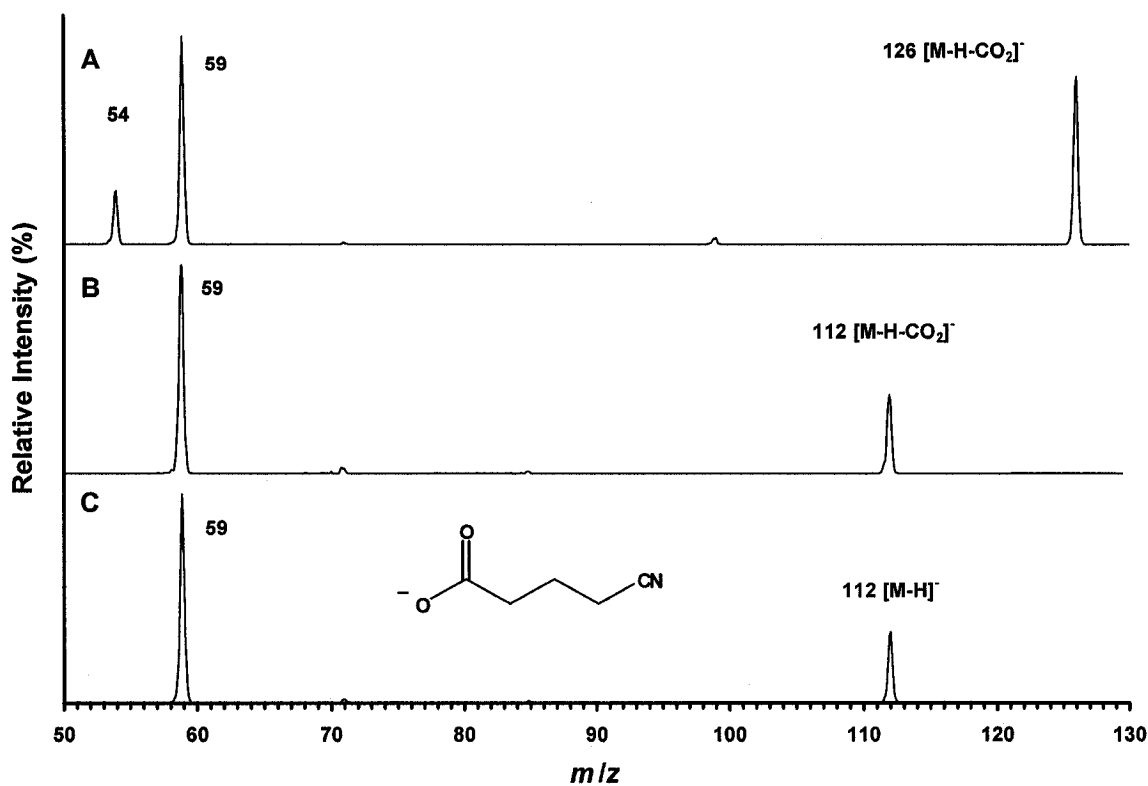
Figure 5.10 Formation of a common ion at m/z 59 upon CID: (A) $[M-H-CO_2]^-$ ion derived in-source from 2-cyano-2-methylpentanedioic acid; (B) $[M-H-CO_2]^-$ ion derived in-source from 2-cyanopentanedioic acid; (C) $[M-H]^-$ ion derived in-source from 4-cyanobutanoic acid.

The formation of an ion at m/z 59 from the 4-cyanobutanoate anion (**5-78**) is consistent with a McLafferty-type rearrangement (Scheme 5.26). This fragmentation process would be initiated by abstraction of the relatively acidic proton α to the nitrile by the ionized carboxyl group via a six-membered transition state. Subsequent formation of a carbon-carbon double bond in conjugation with the nitrile and cleavage of the C-2/C-3 bond would form the enolate ion of acetic acid (**5-81**, m/z 59) (Scheme 5.26).[17] Evidence for cleavage of the C-2/C-3 bond was provided by examining the fragmentation behaviour of a series of carboxylate ions with different α-substituents.[16] In addition, the proton transfer from C-4, adjacent to the nitrile, to the enolate ion was confirmed by CID

of the [M-H]⁻ derived from [4,4-²H₂]-4-cyanobutanoic acid (**5-30**, synthesis described in Section 5.2.3). In the QqQ spectrum, the resulting enolate ion (**5-81**) was one mass unit heavier (*m/z* 60) than that observed for the fragmentation of the unlabelled 4-cyanobutanoic acid (*m/z* 59) confirming that a deuterium atom was transferred during the rearrangement process.

R¹, R² = H, *m/z* 112
R¹, R² = D, *m/z* 114

R¹ = H, 52 u
R¹ = D, 53 u

R² = H, *m/z* 59
R² = D, *m/z* 60
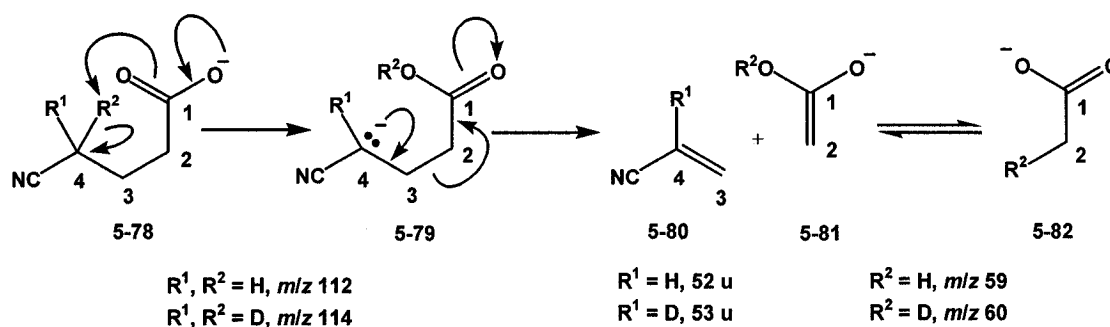
Scheme 5.26 McLafferty-type rearrangement upon CID of the [M-H]⁻ ion derived from 4-cyanobutanoic acid.

Decarboxylation of the α-carboxyl group of the 2-cyanopentanedioic acid anion would generate a carbanion stabilized by the adjacent nitrile (**5-79**, Scheme 5.27). The same carbanion is formed by intramolecular proton transfer from C-4 to the carboxylate group during the McLafferty-type rearrangement of the 4-cyanobutanoate anion (Scheme 5.26). The nitrile-stabilized carbanion may fragment directly to give the observed McLafferty-type rearrangement product **5-81** or it may have sufficient time to abstract a proton from the ω-carboxyl group, giving a 4-cyanobutanoate anion ionized on the carboxyl group (**5-83**). The observation of the ion at *m/z* 40 suggests that the carboxylate ion of 4-cyanobutanoate is formed.
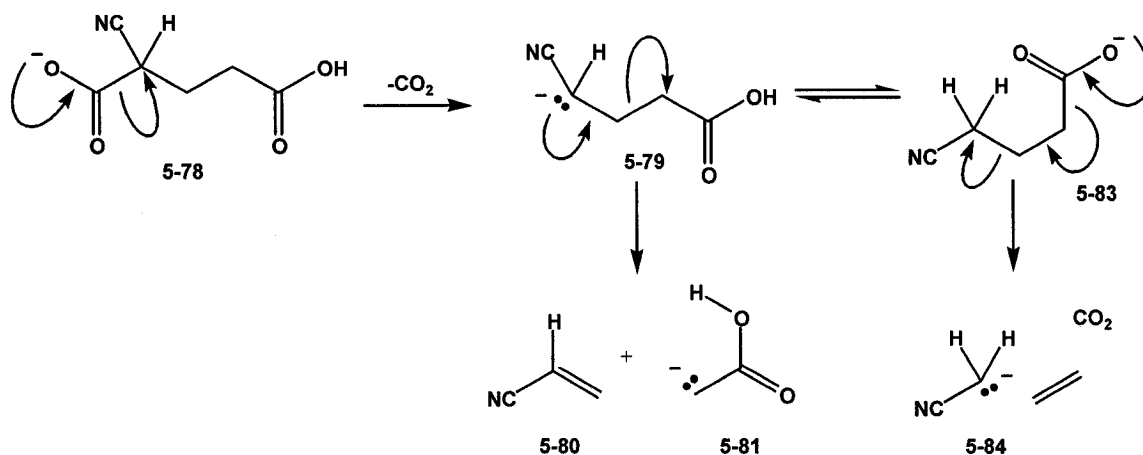
Scheme 5.27 Formation of two possible [M-H-CO$_2$]⁻ ions (**5-79** and **5-83**) by decarboxylation of 2-cyanopentanedioate (**5-78**), fragmentation of (**5-79**) to give an olefin (**5-80**) and an ion at m/z 59 (**5-81**) and fragmentation of (**5-83**) to give an ion at m/z 40 (**5-84**).

To investigate whether the proton transfer between the nitrile stabilized carbanion and the non-ionized carboxylate group occurred before the second step of the McLafferty-type rearrangement, a sample of 2-cyanopentanedioic acid was dissolved in MeOD/D$_2$O to introduce deuterium at C-2.

In the QqQ spectrometer, the [M-H]⁻ ion of the dideutero 2-cyanopentanedioic acid (Scheme 5.28, **5-85**, R=D) fragmented to yield ions at m/z 114 (- CO$_2$), m/z 60 (CH$_2$C(OD)O⁻) and m/z 42 (NCCD$_2$⁻). The formation of the dideutero acetonitrile anion **5-90** at m/z 42 demonstrates the intramolecular proton transfer from the carboxyl group to C-4 of the 4-cyanobutanoate ion (Scheme 5.28-C, **5-86**→**5-89**).
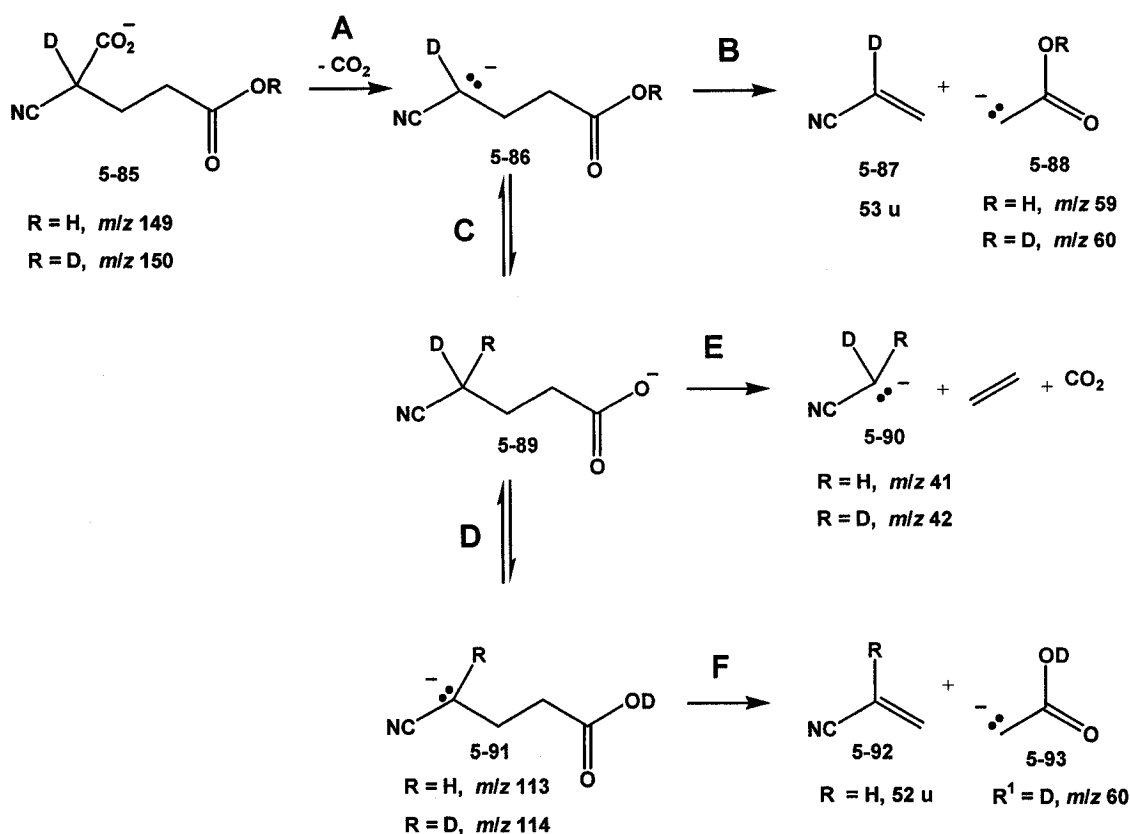
Scheme 5.28 Distribution of deuterium label in the product ions obtained by CID of the [M-H]⁻ ion derived from deuterated 2-cyanopentanedioic acids.

When dideutero 2-cyanopentanedioic acid was dissolved in MeOH/H₂O for injection into the QqQ spectrometer, only the monodeuterated [M-H]⁻ ion was detected. Faster exchange at oxygen would be expected, so deuterium would be located only at C-2 (**5-85**, R = H, Scheme 5.28). The spectrum (QqQ) of the [M-H]⁻ ion derived from [2-²H]-2-cyanopentanedioic acid showed peaks at m/z 113 and 41 as well as two peaks of equal intensity at m/z 59 and 60. The peaks at m/z 113 and 41 further support the formation of the carboxyl ionized 4-cyanobutanoate (**5-89**). The peaks of equal intensity at m/z 59 and 60 show that two different nitrile-stabilized ions (**5-86, 5-91**) are formed. This is only possible if the initial transfer of a proton from the ω-carboxyl group to C-4, giving a 4-

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cyanobutanoate anion with one hydrogen atom and one deuterium atom on C-4 (Scheme

5.28, **5-86**→**5-89**), is followed by a second proton transfer back to the carboxylate group

of either a proton, yielding ion **5-91**, or a deuteron, yielding ion **5-86**. The formation of

equal amounts of ions at m/z 59 and 60 suggests that the interconversion of ions **5-86**, **5-**

89 and **5-91** (Scheme 5.28) is fast, allowing equal amounts of ions **5-86** and **5-91** to form

before the second step of the McLafferty-type rearrangement occurs. The independent

generation of the intermediate carbanion and the labelling results also demonstrate that

the McLafferty-type rearrangement occurs in two distinct steps.

5.5.2 Decarboxylation Regiochemistry: Monoanions of 2-Cyanohexanedioic Acid and 2-Cyanoheptanedioic Acid

The [M-H-CO$_2$]⁻ ions formed by deprotonation and decarboxylation of 2-

cyanohexanedioic acid (**4-33C**) and 2-cyanoheptanedioic acid (**4-33D**) during

electrospray ionization exhibited similar fragmentation behaviour. For each, a major loss

of water was accompanied by a minor loss of carbon dioxide (Table 5.13, e.g., Figure

5.11A).

Decarboxylation of the α-carboxyl group from the [M-H]⁻ ion of 2-

cyanohexanedioic acid would generate deprotonated 5-cyanopentanoic acid, so it was

synthesized as a standard compound (**5-32**, Section 5.2.4). CID of the [M-H]⁻ ion (m/z

126) derived from 5-cyanopentanoic acid gave ions at m/z 108 and m/z 82 corresponding

to the loss of water (18 u) and carbon dioxide (44 u), respectively (Figure 5.11B). The

[M-H-CO$_2$]⁻ ions derived from 2-cyanohexanedioic acid (Figure 5.11A) and 2-

cyanoheptanedioic acid each fragmented in an identical fashion to the [M-H]⁻ ion derived

from 5-cyanopentanoic acid (Figure 5.11B), losing water and carbon dioxide. This is also

consistent with the loss of the ionized carboxyl group α to the nitrile during

decarboxylation of the [M-H]⁻ ions of 2-cyanodicarboxylic acids.

Table 5.13 CID of the [M-H-CO₂]⁻ ions of 2-cyanohexanedioic acid and 2-cyanoheptanedioic acid.

5-61
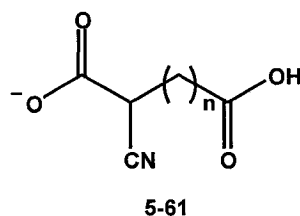
n	[M-H]⁻ (m/z)	[M-H-CO₂]⁻ (m/z)	CID	MS/MS Fragment Ions, m/z (Rel. Int.)	
				-H₂O	-CO₂
2	170	126	21	108 (100)	82 (24)
3	184	140	23	122 (100)	96 (9)

The loss of carbon dioxide from the [M-H-CO₂]⁻ ion (Figure 5.11A) is consistent

with formation of a nitrile-stabilized anion (**5-94**) and loss of carbon dioxide via

heterolytic cleavage of the carbon-carbon bond to the non-ionized carboxyl group

(Scheme 5.29). The loss of water appears to be similar to the loss of water observed

upon CID of the [M-H]⁻ ions of monocarboxylic acids where deprotonation α to the non-

ionized carboxyl group is assisted by the ionized carboxyl group. Formation of a nitrile-

stabilized anion (**5-94**), either by decarboxylation or by deprotonation of the ionized

carboxyl group, provides a base to assist the formation of the enolate ion (**5-96**) of the

longer-chain ω-cyanocarboxylic acids. Loss of water from the enolate ion would yield

the stabilized alkyne oxide anion (**5-97**, Scheme 5.29).

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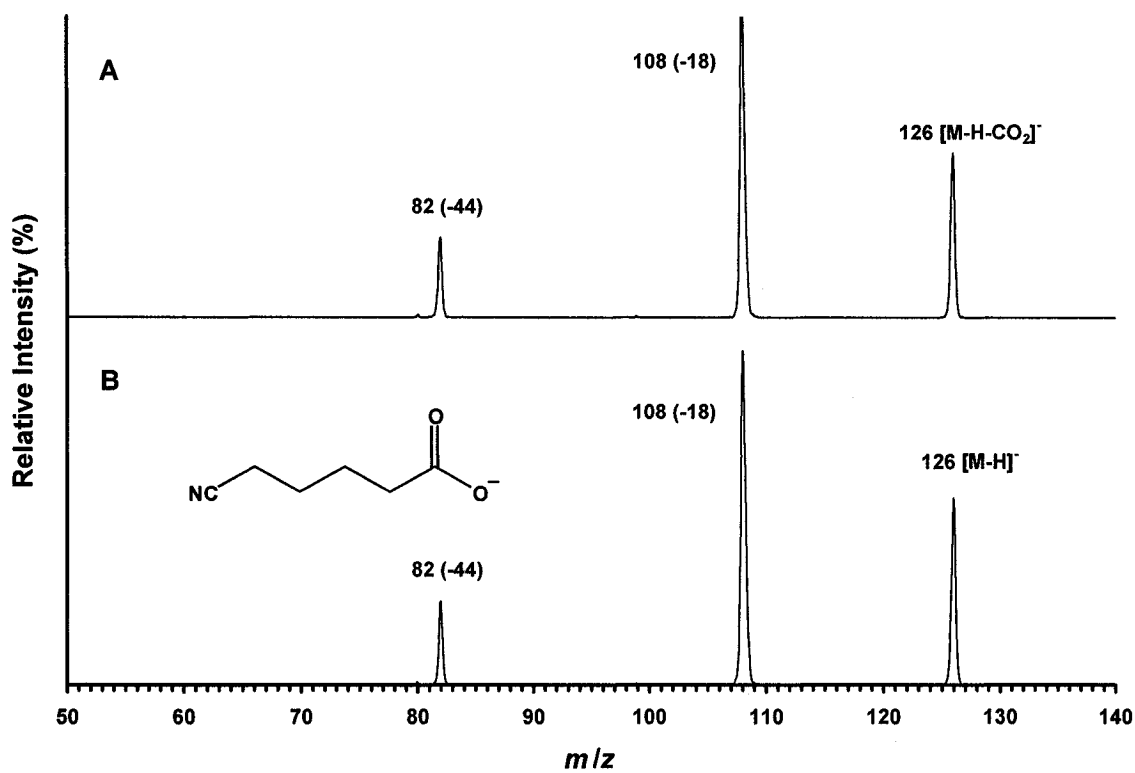
Figure 5.11 Common fragment ions at m/z 108 and m/z 82 indicating losses of water and carbon dioxide, respectively: (A) CID of in-source [M-H-CO₂]⁻ ion derived from 2-cyanohexanedioic acid; (B) CID of the [M-H]⁻ ion derived from 2-cyanopentanoic acid.

270
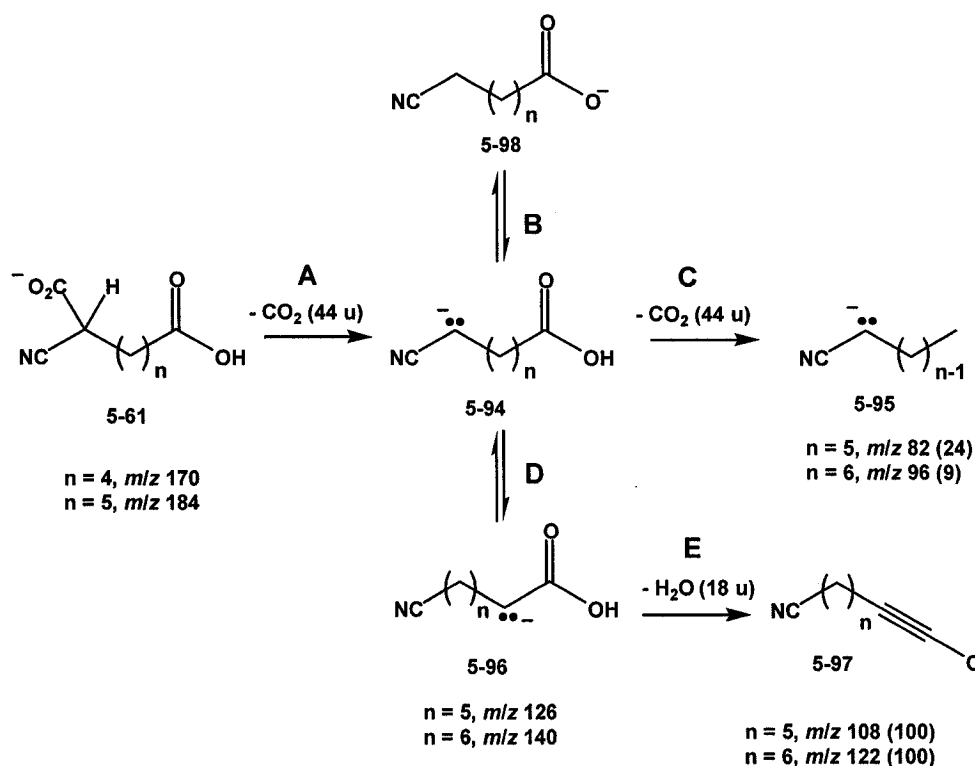
Scheme 5.29 Two fragmentation pathways observed for CID of the [M-H-CO₂]⁻ ions derived from 2-cyanohexanedioic acid (n = 4) and 2-cyanoheptanedioic acid (n = 5).

5.5.3 Summary for Negative Ion ESI-MS of 2-Cyanodicarboxylic Acids

The fragmentation processes of the series of cyanodicarboxylic acids examined by negative ion ESI MS are summarized in Scheme 5.30. All of the nitriles studied underwent facile in-source decarboxylation to generate [M-H-CO₂]⁻ ions. CID of the [M-H-CO₂]⁻ ions derived from the 2-cyanodicarboxylic acids and the [M-H]⁻ ions derived from the corresponding ω-cyanocarboxylic acids of the same chain length produced similar fragmentations, which showed that the initial decarboxylation occurred from the α-carboxyl group.

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Scheme 5.30 Summary of the fragmentation pathways of 2-cyanodicarboxylic acid monoanions.

5.6   Summary

A series of dicarboxylic acids, substituted on C-2 with either an aminomethyl or a nitrile group, were studied in detail by CID and ESI-MS. The mass spectrometric studies carried out in negative and positive ion mode allowed 2-(aminomethyl)dicarboxylic acids to be distinguished from α-aminodicarboxylic acids based on their fragmentation behaviour. In both positive and negative ion ESI-MS, fragmentation processes involving the ω-carboxyl group give similar results to those observed for α-amino acids. However, due to the 2-aminomethyl group, fragmentation processes involving the α-carboxyl group produced unique results, allowing for the identification of the β^2-amino acid structure.

The presence of an electron withdrawing nitrile group resulted in the loss of the ionized α-carboxylate group upon CID of [M-H]⁻ ions derived from 2-cyanodicarboxylic acids. This is in contrast to the other dicarboxylic acids examined, where decarboxylation occurred primarily from the non-ionized ω-carboxylate group. In general, fragmentation processes can be substantially altered by the addition of charge-stabilizing substituents.

5.7 Experimental

5.7.1 General Methods

Refer to Chapter 2 for general methods.

5.7.2 Preparation of Deuterium Labelled Dicarboxylic Acids

Benzylammonium [2-^{2}H]-2-cyanopentanedioate (**5-20**) was prepared by twice dissolving the unlabelled salt in D_2O and freeze-drying. Solid NaBH$_4$ or NaBD$_4$ (10 mmol/mmol nitrile) was added in small portions to an aqueous (D_2O or H_2O, 1 mL) or methanolic (MeOH, 5-10 mL) solution of benzylammonium 2-cyanopentanedioate and CoCl$_2$·6H$_2$O (2 mmol/mmol nitrile). After stirring at room temperature for 4 h, water (100 mL) was added and the pH was adjusted to 4.5 using conc. HCl. The mixture was filtered, and the filtrate was applied to an Amberlite IR-120 ion exchange column (H$^+$ form). The column was eluted with 0.5 M aqueous ammonia. The effluent was concentrated *in vacuo* and treated with activated charcoal for 1 h. The charcoal was removed by filtration through Celite and the filtrate was freeze dried yielding the labelled amino acid.

[2-²H]-2-(Aminomethyl)pentanedioic acid (5-21): A methanolic solution (MeOH, 5 mL) of benzylammonium [2-²H]-2-cyanopentanedioate (0.045 g, 0.17 mmol) was reduced using NaBH₄ (0.065 g, 1.7 mmol) and CoCl₂·6H₂O (0.081 g, 0.342 mmol) giving labelled 2-(aminomethyl)pentanedioic acid as a colourless powder (0.04 g, 78%). ¹H NMR (D₂O) δ 1.74-1.84 (m, 2H), 2.11-2.22 (m, 2H), 2.43-2.48 (m, 0.18H), 2.80-3.10 (m, 2H). ESI⁺MS m/z 161 (100), 160 (38). CID (22%) of m/z 161: m/z 144 (11), 143(100), 142 (15), 117 (75), 99 (7).

[2',2'-²H₂]-2-(Aminomethyl)pentanedioic acid (5-21A): An aqueous solution (H₂O, 1 mL) of benzylammonium 2-cyanopentanedioate (0.100 g, 0.379 mmol) was reduced using CoCl₂ 6H₂O (0.178 g, 0.746 mmol) and NaBD₄ (0.156 g, 3.73 mmol) giving labelled 2-(aminomethyl)pentanedioic acid as a colourless powder (0.043 g, 71%). ¹H NMR (D₂O) δ 1.74-1.94 (m, 2H), 2.25 (t, 2H), 2.48-2.58 (m, 0.9H), 3.03-3.17 (m, 1.5H). ESI⁺MS m/z 164 (28), 163 (37), 162 (100). CID (18%) of m/z 164: m/z 147 (13), 146 (100), 128 (6). ESI⁺MS m/z 163 [M+H]⁺. CID (18%) of m/z 163: m/z 146 (10), 145 (100), 127 (5). CID (18%) of m/z 162: m/z 145 (10), 144 (100), 126 (5). ESI⁺MS m/z 162 (30), 161 (37), 160 (100), 144 (12), 143 (19), 142 (39), 116 (10). CID (22%) of m/z 162: m/z 145 (100), 144 (57), 142 (14), 118 (89), 101 (10). CID (21%) of m/z 144: m/z 126 (55), 112 (40), 100 (100).

[2,2',2'-²H₂]-2-(Aminomethyl)pentanedioic acid (5-21B): An aqueous solution (D₂O, 1 mL) of benzylammonium [2-²H]-2-cyanopentanedioate (0.100 g, 0.379 mmol) was reduced, using CoCl₂·6H₂O (0.177 g, 0.743 mmol) and NaBD₄ (0.156 g, 3.71 mmol),

giving labelled 2-(aminomethyl)pentanedioic acid as a white powder (0.030 g, 49%). ^{1}H

NMR (D$_2$O) δ 1.72-1.89 (m, 2H), 2.16-2.30 (m, 2H), 2.44-2.55 (m, 0.74H), 3.00-3.14 (m,

1.11H); ESI$^+$MS m/z 165 (100), 164 (46), 163 (57), 162 (69). CID (18%) of m/z 165: m/z

148 (10), 147 (100), 129 (6). CID (18%) of m/z 164: m/z 147 (9), 146 (100), 128 (6). CID

(18%) of m/z 163: m/z 146 (10), 145 (100), 127 (8). CID (18%) of m/z 162: m/z 145 (16),

144 (100), 126 (11); ESI$^-$MS m/z 163 (100), 162 (44), 161 (60), 160 (66), 145 (50) 144

(27), 143 (19), 142 (42), 119 (10). CID (21%) of m/z 163: m/z 146 (10), 145 (100), 144

(17), 119 (84), 101 (9). CID (22%) of m/z 145: m/z 127 (17), 113 (6), 101 (90).

5.7.3 Preparation of 2-(Aminomethyl)-2-methyldicarboxylic Acids

5.7.3.1 Monodecarboethoxylation

A mixture of the alkylated diethyl cyanomalonate triester, NaCl (1 mmol/mmol

triester) and water (10 mmol/mmol triester) in DMSO (1 mL/mmol triester) was heated at

90°C. After cooling to room temperature, the reaction mixture was poured into saturated

brine (100 mL) and extracted with ether (4 x 50 mL). The combined ether extracts were

washed with brine (1 x 25 mL), which was back-extracted with ether (1 x 25 mL). The

combined ether layers were dried over MgSO$_4$ and evaporated in $vacuo$.

Diethyl 2-Cyanobutanedioate (5-22A): Heating diethyl-2-carboxyethyl-2-

cyanobutaneodioate (2.72 g, 10.0 mmol) for 18 h yielded diethyl 2-cyanobutanedioate as

an oil (1.88 g, 94%). ^{1}H NMR (CDCl$_3$) δ 1.29 (t, J = 7, 3H), 1.34 (t, J = 7, 3H), 2.66-

3.08 (m, 2H), 3.76 (t, exch., J = 7, 1H), 4.19 (q, J = 7, 2H), 4.33 (q, J = 7, 2H). ^{13}C NMR

(CDCl$_3$) δ 14.0, 14.2, 33.0, 33.8, 61.9, 63.4, 115.8, 165.1, 169.8.

Diethyl 2-Cyanopentanedioate (5-22B): Heating diethyl-2-carboxyethyl-2-

cyanopentanedioate (2.85 g, 10.0 mmol) for 39 h yielded diethyl 2-cyanopentanedioate as

an oil (1.77 g, 83%). ^{1}H NMR (CDCl$_3$) δ 1.28 (t, J = 7, 3H), 1.34 (t, J = 7, 3H), 2.16-

2.34 (m, 2H), 2.54-2.60 (m, 2H), 3.76 (dd, exch., J = 6, 0.5H), 4.16 (q, J = 7, 2H), 4.28

(q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ 14.0, 14.1, 24.8, 30.6, 36.4, 60.9, 63.0, 116.1, 165.6,

171.6. EI-MS (70 eV) 212.9 (8), 167.9 (100), 166.9 (24), 139.9 (47), 138.9 (36), 121.9

(43), 113.0 (37), 112.0 (25), 95 (20), 81.9 (48). High Resolution MS (70 eV): calculated

for C$_{10}$H$_{15}$NO$_4$ = 213.1001 amu, found = 213.1003 ± 0.0008 amu.

5.7.3.2 Methylation

Potassium carbonate (3.5 mmol/mmol diester) was added to a stirred solution of

2-cyanoester in acetone (20 mL, dried for 16 h over K$_2$CO$_3$). Methyl iodide (2 equiv.)

was added, and the reaction mixture was stirred at room temperature for 24 h. Acetone

was removed *in vacuo*. Water (100 mL) was added to the residue and the product was

extracted into ether (6 x 25 mL). The combined ether layers were washed with 1 M

NaOH (1 x 25 mL) and dried over MgSO$_4$. Solvent was removed *in vacuo*.

Diethyl 2-Cyano-2-methylbutanedioate (5-23A): Diethyl 2-cyanobutanedioate (1.27 g,

6.38 mmol) yielded diethyl 2-cyano-2-methylbutanedioate as an oil (1.13 g, 83%). ^{1}H

NMR (CDCl$_3$) δ 1.28 (t, J = 7, 3H), 1.35 (t, J = 7, 3H), 1.66 (s, 3H), 2.82 (AB system, J =

17, 1H), 3.04 (AB system, J = 17, 1H), 4.20 (q, J = 7, 2H), 4.30 (q, J = 7, 2H). ^{13}C NMR

(CDCl$_3$) δ 14.0, 14.1, 23.8, 40.6, 41.7, 61.6, 63.2, 119.4, 168.7, 168.8.

Diethyl 2-Cyano 2-methylpentanedioate (5-23B): Diethyl 2-cyanopentanedioate (1.77

g, 8.30 mmol) yielded diethyl 2-cyano-2-methylpentanedioate as an oil (1.52 g, 81%). ^{1}H

NMR (CDCl$_3$) δ 1.27 (t, J = 7, 3H), 1.34 (t, J = 7, 3H) 1.62 (s, 3H), 2.05-2.4 (m, 2H),

2.44-2.57 (m, 2H), 4.15 (q, J = 7, 2H), 4.28 (q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ 14.0,

14.1, 23.4, 30.3, 32.8, 43.2, 60.9, 63.0, 119.3, 168.7, 171.5. EI-MS (70 eV) 226.9 (9),

181.9 (100), 153.9 (40), 109.1 (21), 108.0 (26), 96.0 (54), 79.9 (22). High Resolution MS

(70 eV): calculated for C$_{11}$H$_{17}$NO$_4$ = 227.1157 amu, found = 227.1158 ± 0.0008 amu.

5.7.3.3 Ester Hydrolysis

A mixture of diester and NaOH (2.2 mmol/mmol diester) in methanol-water (5 mL + 45

mL) was heated at reflux for 1.5 h. Water (50 mL) was added to the cooled reaction

mixture and the resulting solution was extracted with ether (2 x 25 mL). The aqueous

phase was acidified using conc. HCl and extracted with ethyl acetate (4 x 50 mL). The

combined ethyl acetate extracts were dried over MgSO$_4$ and evaporated *in vacuo*.

2-Cyano-2-methylbutanedioic acid (5-24A): Hydrolysis of diethyl-2-cyano-2-

methylbutanedioate (1.49 g, 7.00 mmol) yielded 2-cyano-2-methylbutanedioic acid as a

colourless solid (0.64 g, 58%). ^{1}H NMR (acetonitrile-d_3) δ 1.56 (s, 3H), 2.82 (AB system,

J = 18, 1H), 2.95 (AB system, J = 18, 1H), 9.33 (exch. s, 2H). ^{13}C NMR (acetonitrile-d_3)

δ 24.0, 30.0, 41.3, 120.7, 170.7, 171.5.

2-Cyano-2-methylpentanedioic acid (5-24B): Hydrolysis of diethyl-2-cyano-2-

methylpentanedioate (1.52 g, 6.70 mmol) yielded 2-cyano-2-methylpentanedioic acid as

an oil (0.89 g, 77%). ^{1}H NMR (acetonitrile-d_3) δ 1.52 (s, 3H) 1.98-2.22 (m, 2H), 2.30-
2.54 (m, 2H), 9.42 (br.s, 2H). ^{13}C NMR (acetonitrile-d_3) δ 23.5, 30.5, 33.1, 44.1, 120.5,
170.9, 174.8.

5.7.3.4 Formation of Benzylammonium Salts

Benzylamine (2 mmol/mmol diacid) was added to a solution of the 2-
cyanodicarboxylic acid in water (50 mL). After 30 min, the solution was freeze dried and
the resulting solid was recrystallized from ethanol/ether to yield a colourless, crystalline
solid.

Bis(benzylammonium) 2-cyano-2-methylbutanedioate (5-25A): 2-Cyano-2-
methylbutanedioate yielded bis(benzylammonium) 2-cyano-2-methylbutanedioate (0.36
g, 84%). m.p. 132-133°C. ^{1}H NMR (D$_2$O) δ 1.48 (s, 3H), 2.49 (AB system, J = 16, 1H),
2.74 (AB system, J = 16, 1H), 4.17 (s, 4H), 7.46 (s, 10H). ^{13}C NMR (D$_2$O) δ 23.4, 43.3,
44.6, 45.1, 124.8, 129.0, 129.4, 132.9, 175.9, 177.6. ESI$^-$MS m/z 156 (39), 112 (100).
CID (15%) of m/z 156: m/z 112 (100). CID (24%) of m/z 112: m/z 97 (10), 94 (51), 84
(100).

Bis(benzylammonium) 2-Cyano-2-methylpentanedioate (5-25B): 2-Cyano-2-
methylpentanedioic acid in water (25 mL) yielded bis(benzylammonium) 2-cyano-2-
methylpentanedioic acid (1.07 g, 61%). m.p. 130-131°C. ^{1}H NMR (D$_2$O) δ 1.44 (s, 3H),
1.80-1.99 (m, 2H), 2.02-2.34 (m, 2H), 4.14 (s, 4H), 7.44 (s, 10H). ^{13}C NMR (D$_2$O) δ
23.3, 34.1, 34.5, 43.3, 46.9, 123.8, 129.0, 129.4, 132.8, 175.5, 181.2. ESI$^-$MS m/z 170

(81), 126 (100). CID (16%) of *m/z* 170: *m/z* 126 (100). CID (22%) of *m/z* 126: *m/z* 99 (3), 59 (100), 54 (33).

5.7.3.5 Nitrile Reduction

Nitrile reduction was carried out by adding solid $NaBH_4$ (10 mmol/mmol nitrile) in small portions to an aqueous solution (10-15 mL) of bis(benzylammonium) salt and $CoCl_2$ H_2O (2 mmol/mmol nitrile). After stirring at room temperature for 4 h, water (100 mL) was added and the pH was adjusted to 4.5 using conc. HCl. The mixture was filtered, and the filtrate was applied to an Amberlite IR-120 ion exchange column (H^+ form). The column was eluted with 0.5 M aqueous ammonia. The effluent was concentrated *in vacuo* and treated with activated charcoal for 1 h. The charcoal was removed by filtration through Celite and the filtrate was freeze dried.

2-(Aminomethyl)-2-methylbutanedioic acid (4-21): Reduction of bis(benzylammonium) 2-cyano-2-methylbutanedioate (1.04 g, 2.80 mmol) yielded 2-(aminomethyl)-2-methylbutanedioic acid as a white powder (0.24 g, 53%). m.p. 130-132°C. ^{1}H NMR (D_2O) δ 1.22 (s, 3H), 2.49 (AB, *J* = 16, 1H), 2.73 (AB, *J* = 16, 1H), 3.16 (s, 2H). ^{13}C NMR (D_2O) δ 23.2, 43.7, 45.7, 46.4, 179.3, 182.2. ESI^+MS *m/z* 162 (100), 144 (24), 98 (15). CID (19%) of *m/z* 162: *m/z* 145 (4), 144 (100). CID (19%) of *m/z* 144: *m/z* 126 (57), 98 (66). ESI⁻MS *m/z* 160 (100), 116 (21). CID (20%) of *m/z* 160: *m/z* 142 (17), 116 (100). CID (23%) of *m/z* 142: *m/z* 112 (100). CID (22%) of *m/z* 116: *m/z* 98 (8), 87 (86).

2-(Aminomethyl)-2-methylpentanedioic acid (5-26): Reduction of

bis(benzylammonium) 2-cyano-2-methylpentanedioate (0.73 g, 1.9 mmol) yielded 2-

(aminomethyl)-2-methylpentanedioic acid as a white solid (0.07 g, 20%). m.p. 145-

146°C. ^{1}H NMR (D$_2$O) δ 1.18 (s, 3H), 1.79-1.85 (m, 2H), 2.18-2.24 (m, 2H), 2.95 (d, J =

13, 1H), 3.11(d, J = 13, 1H). ^{13}C NMR (D$_2$O) δ 20.9, 32.3, 33.0, 44.8, 45.6, 182.0.

ESI$^+$MS m/z 176 (100), 158 (22). CID (19%) of m/z 176: m/z 159 (3), 158 (100), 140

(12). CID (22%) of m/z 158: m/z 138 (30), 112 (100). ESI$^-$MS m/z 174 (100), 156 (93),

130 (12). CID (20%) of m/z 174: m/z 156 (46), 130 (100); CID (25%) of m/z 156: m/z

126 (50), 112 (100). CID (23%) of m/z 130: m/z 112 (10), 101 (95).

5.7.4   Syntheses of 4-Cyanobutanoic Acid and [4,4-^{2}H$_2$]-4-Cyanobutanoic Acid

Ethyl 4-Cyanobutanoate (5-28):   Six separate reactions, each consisting of a solution of

ethyl 4-bromobutanoate (0.975 g, 5.00 mmol) in DMSO (4 mL) added to NaCN (0.490 g,

10.0 mmol) suspended in DMSO (4 mL), were heated at 90°C for 2 h. The reaction

mixtures were allowed to cool to room temperature and combined. Water was added (100

mL) and the mixture was extracted with diethyl ether (6 x 50 mL). The combined ether

layers were washed with water (2 x 25 mL) and this water layer was washed with ether (2

x 25 mL). The ether layers were combined, dried over MgSO$_4$ and evaporated *in vacuo*

yielding ethyl 4-cyanobutanoate as an oil (3.23 g, 76%). ^{1}H NMR (CDCl$_3$) δ 1.27 (t, J =

7, 3H), 1.93-2.04 (m, 2H), 2.45-2.52 (m, 4H), 4.16 (q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ

14.2, 16.5, 20.7, 32.4, 60.7, 119.1, 172.0.

4-Cyanobutanoic Acid (5-29): A mixture of ethyl 4-cyanobutanoate (1.41 g, 10.0

mmol) and NaOH (0.44 g, 11.00 mmol) in water (45 mL) and methanol (5 mL) was

heated at reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature,

and water (50 mL) was added followed by extraction with diethyl ether (2 x 25 mL). The

aqueous layer was acidified with concentrated HCl (pH paper red) and extracted with

ethyl acetate (4 x 50 mL). The ethyl acetate layers were combined, dried over $MgSO_4$

and evaporated *in vacuo* yielding 4-cyanobutanoic acid as an oil (0.88 g, 78 %). (lit.[25]

m.p. 39-40°C). ^{1}H NMR $(CDCl_3)$[25] δ 1.94-2.10 (m, 2H), 2.46-2.58 (m, 4H), 10.51 (br. s,

1H). ^{13}C NMR $(CDCl_3)$[25] δ 16.4, 20.4, 32.2, 118.9, 177.8. ESI MS (Quattro, 25 V Cone)

m/z 112 (100), 59 (28), 40 (24).

Sodium 4-Cyanobutanoate (5-29A): 4-Cyanobutanoic acid (0.88 g, 7.8 mmol) was

dissolved in water (50 mL) and applied to an Amberlite IR-120 column (Na^+ form, 30 cm

x 2 cm). The column was eluted with water and the first 8 fractions (100 mL each) were

collected and concentrated *in vacuo*. The concentrate (100 mL) was treated with charcoal

for 1 h and filtered through Celite. The filtrate was freeze dried to yield sodium 4-

cyanobutanoate as a colourless solid (0.53 g, 50%). m.p. 175-180°C (dec.). ^{1}H NMR

(D_2O) δ 1.89-2.02 (m, 2H), 2.38 (t, J = 7, 2H), 2.56 (t, J = 7, 2H). ^{1}H NMR (500 MHz,

D_2O) δ 1.95 (quin., J = 7, 2H, H-3), 2.37 (t, J = 7, 2H, H-2), 2.56 (t, J = 7, 2H, H-4). ^{13}C

NMR (125 MHz, D_2O) δ 16.2 (C-4), 21.7 (C-3), 36.2 (C-2), 121.7 (CN), 181.5 (C-1).

[4,4-^{2}H$_2$]-4-Cyanobutanoic Acid (5-30): A solution of sodium 4-cyanobutanoate

(0.0676 g, 0.500 mmol) and diazabicyclo[5.4.0]undec-7-ene (0.0374 mL, 0.250 mmol) in

1,4-dioxane (0.22 mL)/ D$_2$O (0.78 mL) was heated at 100°C for 2 h. After 2 h, the

reaction was allowed to cool to room temperature and then freeze dried. Two subsequent

exchange reactions were performed, as described above, each involving the addition of

fresh D$_2$O (0.78 mL), 1,4-dioxane (0.22 mL) and DBU (0.0374 mL). After the third

exchange, water (5 mL) was added to the freeze-dried reaction mixture. The solution was

acidified with conc. HCl (pH paper red) and extracted with ethyl acetate (4 x 5 mL). The

ethyl acetate layers were combined, dried over MgSO$_4$ and evaporated *in vacuo* yielding

[4,4-^{2}H$_2$]-4-cyanobutanoic acid as an oil (0.021 g, 36%). ^{1}H NMR (D$_2$O, K$_2$CO$_3$) δ 1.78-

1.84 (m, 0.07H), 1.91 (t, J = 7, 2H), 2.17-2.27 (m, 0.29H), 2.34 (t, J = 7, 2H). ^{13}C NMR

(D$_2$O) δ 20.4, 32.7, 126.2, 177.4. ESI⁻MS (Quattro, 25 V Cone) *m/z* 115 (6), 114 (100),

113 (6), 112 (2), 60 (15), 42 (18).

5.7.5 Synthesis of 5-Cyanopentanoic Acid

Ethyl 5-Cyanopentanoate (5-31): A mixture of diethyl 2-carboxyethyl-2-

cyanohexanedioate (0.586 g, 2.00 mmol), water (1.08 mL, 60.0 mmol) and NaCl (0.0293

g, 0.500 mmol) in DMSO (2 mL) were heated at 140°C for 18 h. The reaction mixture

was allowed to cool to room temperature; Water (25 mL) was added and the mixture was

extracted with diethyl ether (4 x 25 mL). The ether layers were combined and dried over

MgSO$_4$. The solvent was removed *in vacuo* giving ethyl 5-cyanopentanoate as an oil

(0.19 g, 61%). ^{1}H NMR (CDCl$_3$) δ$_H$ 1.19 (t, J = 7, 3H), 1.58-1.78 (m, 4H), 2.17-2.33 (m,

4H), 4.07 (q, J = 7, 2H). ^{13}C NMR (CDCl$_3$) δ_C 14.2, 16.9, 23.9, 24.9, 33.3, 60.5, 119.3, 172.8.

5-Cyanopentanoic Acid (5-32): A mixture of ethyl 5-cyanopropanoate (0.19 g, 1.5 mmol) and NaOH (0.066 g, 1.64 mmol) in water (13.5 mL) and methanol (1.5 mL) was heated at reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature. Water (25 mL) was added and the mixture was extracted with diethyl ether (2 x 25 mL). The aqueous layer was acidified with concentrated HCl (pH paper red) and extracted with ethyl acetate (4 x 25 mL). The ethyl acetate layers were combined, dried over MgSO$_4$ and evaporated *in vacuo* yielding 5-cyanopentanoic acid as an oil (0.14 g, 67%). ^{1}H NMR (D$_2$O) δ_H 1.90-2.00 (m, 2H), 2.13-2.19 (m, 2H), 2.58-2.2.65 (m, 4H); ESI'MS CID (21%) of m/z 126: m/z 108 (100), 143(100), 82 (25).

5.7.6 Mass Spectrometer Operating Conditions

Nitrile and amino acid samples were dissolved in methanol (HPLC grade) or aqueous methanol (1:1) at concentrations ranging from 0.5 to 1 mg mL^{-1}. Positive ion spectra (amino acids) and negative ion spectra (amino acids and nitriles) were obtained by flow injection analysis (20 μL min^{-1}). Regular MS and MS/MS were obtained at low collision energies on a Finnigan LCQ Duo ion trap or a Micromass Quattro triple quadrupole mass spectrometer. Spectra from the LCQ were run with the ESI needle at 4000 V and the capillary at 200°C. Nitrogen was used as the nebulizing gas, with helium as the damping and collision gas in the trap. CID experiments are reported in arbitrary percentage units from the Xcalibur software in which energies of 12-25% produced

fragmentation of most ions studied. For the Quattro mass spectrometer, the bath and nebulizer gases were nitrogen at optimized flow rates, the source temperature was set between 90°C and 100°C, the electrospray needle was at 3000 V, and the source cone voltage varied between 10 and 40 V. CID experiments were carried out using argon collision gas at energies reported in eV (laboratory frame). The argon was at a pressure to reduce the main beam by 50-80%. Spectra from the LCQ ion trap were averaged and processed using the supplied Xcaliber software. For the Quattro mass spectrometer, 10-30 spectra were combined and the background was subtracted and smoothed using MassLynx software.[17]

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Chapter 6: Conclusions

6.1 Thesis Accomplishments

Diethyl cyanomalonate (**1-1**) was synthesized in high yield. It formed stable, nonhygroscopic alkali metal and tetraalkylammonium salts easily which were very weakly basic, soluble in a variety of organic solvents and reactive towards alkylation by electrophiles. Under optimized reaction conditions (Bu_4N^+ salt, DMSO, 80°C), the diethyl cyanomalonate anion was alkylated by a variety of alkyl bromides and iodides, generating highly functionalized, useful molecules in high yield using simple work-up procedures.
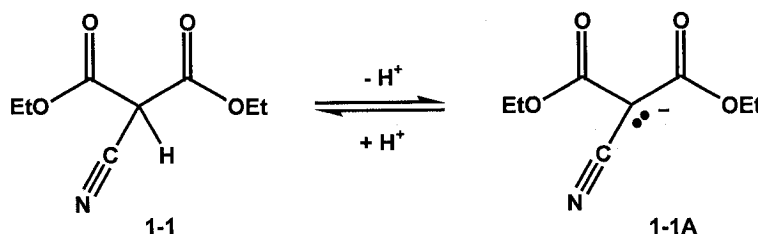
Scheme 6.1 Diethyl cyanomalonate (**1-1**) and its highly stabilized anion

The presence of three functional groups on the tertiary carbanion carbon also prevented unwanted dialkylation side reactions commonly encountered with active methylene compounds, especially ethyl cyanoacetate. Also, reaction of diethyl cyanomalonate with alkyl halides resulted in exclusive monoalkylation of the carbanionic carbon, there was no evidence of O-alkylation.

The nitrile and ester functional groups in diethyl cyanomalonate also provided synthetic flexibility. During amino acid synthesis, the ester functional group was

hydrolyzed to a carboxylic acid and the nitrile was reduced to an amine. The selective

removal of one or both esters by decarboethoxylation following alkylation of diethyl

cyanomalonate was also achieved. Following an initial alkylation of diethyl

cyanomalonate, selective monodecarboethoxylation gave a 2-cyanoester which was

readily alkylated to yield a dialkylated ethyl cyanoacetate derivative with two different

alkyl groups. The dialkylated compounds were converted to 2-(aminomethyl)-2-

methyldicarboxylic acids and 2-cyano-2-methyldicarboxylic acids, compounds which

provided valuable mechanistic information during ESI-MS fragmentation studies.

The synthetic flexibility of the alkylated diethyl cyanomalonates also led to the

preparation of deuterium labelled amino acids including [3,3-^{2}H$_2$]GABA (3-40), a

previously unavailable isotopomer of GABA and several isotopomers of 2-

(aminomethyl)pentanedioic acid (4-1B) which helped elucidate ESI-MS fragmention

pathways. An alkylated diethyl cyanomalonate, diethyl 2-carboxyethyl-2-

cyanobutanedioate (3-2), was used as an intermediate in a short synthesis of the important

ω-amino acid GABA (3-1), a synthetic route that could easily be adapted to generate

longer chain homologues.

Deuterium exchange adjacent to the nitrile observed during ester hydrolysis of

ethyl [2,2,3,3-^{2}H$_4$]-3-cyanopropanoate (3-53), led to the development of a general method

for introducing deuterium α to nitriles. After optimization of the exchange reaction

conditions (DBU, D$_2$O, 1,4-dioxane, 100°C), which included identification and

minimization of hydrolysis side products, DBU-catalyzed exchange selectively

introduced deuterium onto C-3 of sodium 3-cyanopropanoate (3-78), which, upon

reduction, gave the previously unavailable [3,3-^{2}H$_2$]GABA (3-40) labelled specifically at

C-3 with a high level of deuterium (98%). The exchange procedure was also applied towards the synthesis of $[4,4-^2H_2]$-4-cyanobutanoic acid, a compound crucial for the demonstration of the McLafferty-type rearrangment reaction of carboxylate anions observed in ESI-MS.

Diethyl cyanomalonate esters alkylated by halogenated esters and acids provided a homologous series of 2-(aminomethyl)dicarboxylic acids and 2-cyanodicarboxylic acids. The two shorter chain 2-cyanodicarboxylic acids and 2-(aminomethyl)dicarboxylic acids were shown to have inhibitory effects against the glutamate catabolizing enzymes, glutamate racemase and glutamate dehydrogenase. The 2-cyanodicarboxylic acids and 2-(aminomethyl)dicarboxylic acids prepared from diethyl cyanomalonate, were also invaluable additions to ESI-MS studies of dicarboxylic acids. The ESI-MS studies provided fundamental information on the behaviour of dicarboxylic acids substituted on C-2 with nitriles or aminomethyl functional groups.

6.2 Future Work

The investigation of the synthetic applications of diethyl cyanomalonate continues to be an important focus in this laboratory. Investigations of tandem dealkoxycarbonylation-intramolecular alkylation reactions, halogenating reagents and amino acid inhibitor synthesis, will potentially demonstrate broader synthetic applications of diethyl cyanomalonate.

The potential of 2-(aminomethyl)dicarboxylic acids as enzyme inhibitors remains largely unexplored. There are many other glutamate-catabolizing enzymes against which these compounds could be tested. Alkylation of diethyl cyanomalonate by a diverse

range of electrophiles could also be used to generate a wide variety of β^2-amino acids with different side chains, including those of the naturally occurring α-amino acids. Each β^2-amino acid is a potential inhibitor of the metabolism of α-amino acids and would be used to synthesize novel peptides. Ultimately a method to introduce chirality into the synthesis of β^2-amino acids from diethyl cyanomalonate would be desirable.

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