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THE SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF QUISQUALIC ACID AND SOME NOVEL EXCITATORY AMINO ACID MIMETICS.

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MEMORANDUM

Except where otherwise stated, the work reported in this Thesis is solely the work of the author.

This Thesis is dedicated to my mother, and to Benjamin, Susan, Anthony and David.

<u>"DON'T QUIT"</u>

"When things go wrong, as they sometimes will,
When the road you are trudging seems all uphill,
When the funds are low, and the debts are high,
And you want to smile, but you have to sigh,
When care is pressing you down a bit,
Rest, if you must - but don't quit.

Life is queer with its twists and turns,
As every one of us sometimes learns,
And many a failure turns about
When he might have won had he stuck it out;
Don't give up, though the pace seems slow -
You might succeed with another blow.

Often the goal is nearer than
It seems to a faint and faltering man,
Often the struggler has given up
When he might have captured the victor's cup,
And he learned too late, when the night slipped down
How close he was to the golden crown.

Success is failure turned inside out-
The silver tint of the clouds of doubt-
And you never can tell how close you are,
It may be near when it seems afar;
So stick to the fight when you're hardest hit-
It's when things seem worst that you mustn't quit."

Those who lived through this thesis with me will understand the moral of this poem, which was sent to me by my sister at a particularly difficult time.

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Finally, I hope that all my family and friends will forgive me for my preoccupation throughout the course of this work.

ABSTRACT.

The Synthesis and Pharmacological Evaluation of Quisqualic acid and some Excitatory Amino Acid Mimetics.

P.I. Okonkwo.

L-Glutamic acid and L-aspartic acid are believed to be the principal initiators of excitatory synaptic neurotransmission in the mammalian central nervous system. A number of molecules of either natural or synthetic origin have since been identified as possessing agonist or antagonist properties at glutamate binding sites. One of the most potent agonists is quisqualic acid, (69), and this Thesis features two aspects of the synthetic organic chemistry and the pharmacology of quisqualic acid and some of its analogues.

The first objective of the work reported here was to devise and carry out a practical synthesis of tritiated quisqualic acid, in order to facilitate pharmacological studies of the location of quisqualate receptors in the brain. The successful route was based on Bycroft's synthesis of DL-quisqualic acid (143), and the method has been dispatched to a commercial radiochemical company.

The second objective was to synthesize, and test pharmacologically, a range of structural analogues of quisqualic acid. In general, these analogues were either variants on the ring structure, or were acyclic analogues or their peptide derivatives. A number of these structures posed significant synthetic challenges, and new methodology has had to be applied in the synthesis of several. Radioligand binding studies of the analogues, using tritiated glutamic acid and tritiated AMPA, revealed that only one, the six-membered ring compound (S)-3-(2-tetrahydro-1,2,4-oxadiazin-3,6-dionyl)alanine (85), bound strongly to L-AMPA receptors, and only one, the aminopyrrolidinedione, (119), bound moderately strongly to glutamate receptors. A brief and somewhat speculative analysis of this data is presented in the Pharmacology Discussion section of this Thesis.

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LIST OF ABBREVIATIONS.

ACN	Acetonitrile.
AcOH	Acetic acid
ALS	Amyotropic lateral sclerosis.
AMPA	α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid.
AP5	2-Amino-5-phosphonopentanoic acid.
BMAA	β-N-methyl-amino-L-alanine.
BOAA	β-Oxalylamino alanine
$(Boc)_2O$	Di-tert-butyldicarbonate.
BuOH	n-Butanol.
Cbz	Benzyloxycarbonyl.
$CDCl_3$	Deuterated chloroform.
CNQX	6-Cyano-7-nitroquinoxalin-2,3-dione.
CNS	Central nervous system.
CPP	2-Carboxy-4-(3-phosphonopropyl)piperazine.
DABCO	1,4-Diazabicyclo[2.2.2]octane.
D-AP7	D-2-Amino-7-phosphonoheptanoic acid.
DAPA	2,3-Diaminopropionic acid.
DCC	Dicyclohexyl carbodiimide.
DCl	Deuterium chloride.
DCM	Dichloromethane.
D_2O	Deuterium oxide.
DEAD	Diethyl azodicarboxylate.
DGG	D-γ-Glutamyl-glycine.
DMF	Dimethylformamide.
DMSO	Dimethyl sulphoxide.
DNQX	6,7-Dinitroquinoxalin-2,3-dione.
EDC	1-Ethyl-3-[(3-dimethylamino)propyl]carbodiimide.
EPSPs	Excitatory post synaptic potentials.
EtOAc	Ethyl acetate.
Et_3N	Triethylamine.
GABA	γ–Amino-butyric acid.
GAD	Glutamic acid decarboxylase

GDEE	Glutamate diethyl ester.
HCl	Hydrochloric acid.
H_2O	Water.
HOBT	1-Hydroxybenzotriazole.
KSCN	Potassium thiocyanate.
L-AP4	L-2-Amino-4-phosphonobutanoic acid.
MeOH	Methanol.
MK801	(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine
mRNA	Messenger ribonucleic acid.
NAALADase	N-Acetylaspartylglutamate degrading enzyme
NaOD	Sodium deuteroxide.
NMDA	N-Methyl-D-aspartate.
NMM	N-Methylmorpholine.
NMR	Nuclear magnetic resonance.
PCP	Phencyclidine.
PCR	Polymerase chain reaction.
PDA	Piperidine dicarboxylic acid.
P_2O_5	Phosphorus pentoxide.
PTSA	*p*-Toluenesulphonic acid.
REMA	Repetitive excess mixed anhydride.
TCP	1-(2-Thienyl)-1-cyclohexylpiperidine
THF	Tetrahydrofuran.
TLC	Thin layer chromatography.
TPP	Triphenylphosphine.

CHAPTER ONE.

INTRODUCTION.

1.1 The central nervous system.

The central nervous system (CNS) consists of two parts, the brain and spinal cord. In man, the CNS consist of perhaps one hundred billion interconnecting neurones; these are formed prenatally, and, if damaged, cannot be replaced. During the life span of the average adult approximately 3% of neurones are lost as a result of wear and tear.[1]

1.2. The neurone.

The neurone (fig 1.1) is the basic functional unit of the brain. It consists of a cell body (which contains the nucleus and deoxyribonucleic acid (DNA)), dendrites, axon and synapse. There are two main types of neurone; sensory, which convey information from the body to the CNS, and motor, which convey information from the CNS to the rest of the body.

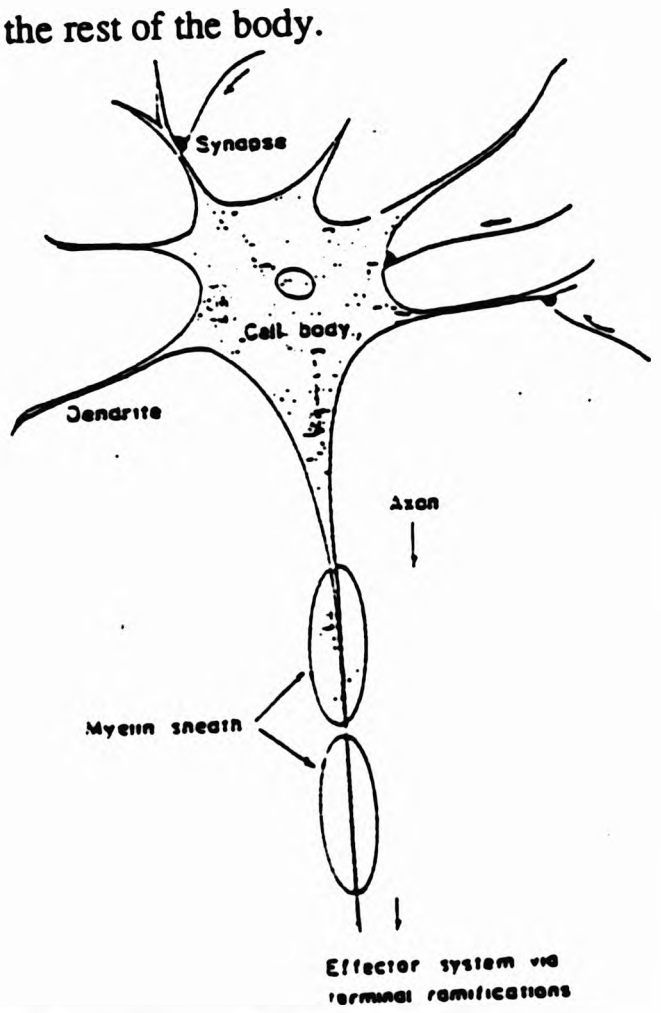


Fig 1.1 Schematic representation of neurone.

The synapse is the point of functional contact between the axon terminal and other cells. It is also responsible for the biosynthesis, delivery, reception and removal of synaptic neurotransmitters, and thus provides a flow of chemical information from presynaptic to postsynaptic loci. (Fig 1.2).

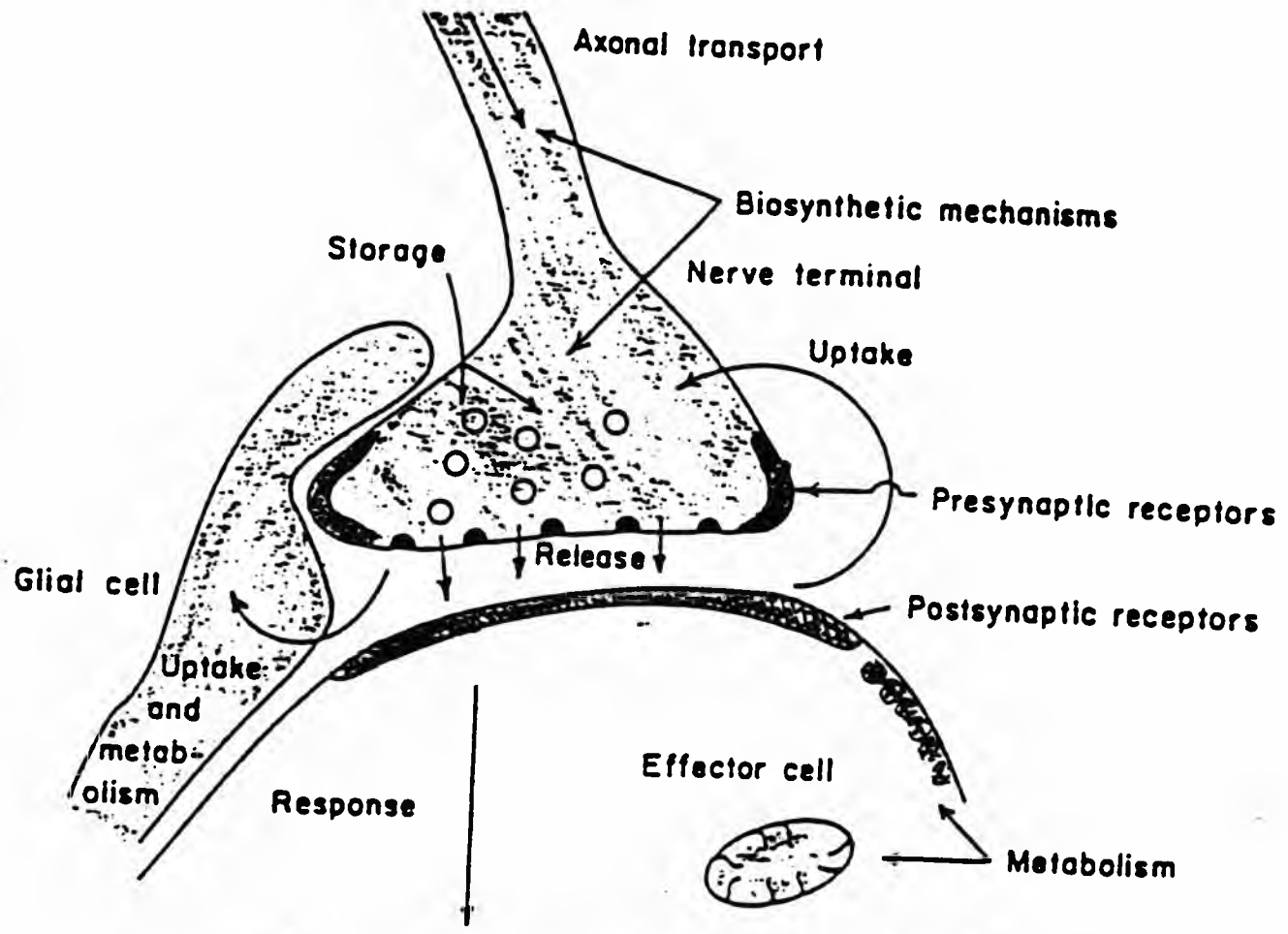
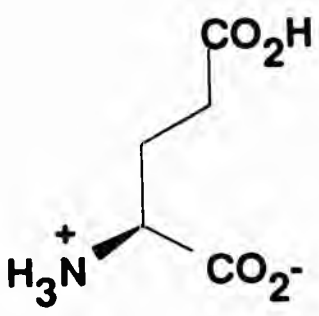


Fig. 1.2 Schematic representation of synaptic function.

1.3. Neurotransmission.

Transmission of a nerve impulse along the axon of a neurone in the form of depolarization causes a change in the ion permeability of the axonal membrane. This results in the transfer of sodium ions into the axon from the exterior and of potassium ions out of the axon. The arrival of the impulse at the presynaptic terminal triggers the release of calcium ions into the cell through the voltage-regulated calcium ion channels. This in turn triggers the release of neurotransmitters into the synaptic cleft, and their diffusion across

the synaptic space, to the postsynaptic receptor membranes. L-glutamic acid (1), the archetypal excitatory amino acid, binds to receptors that are linked to sodium ion channels. The influx of sodium ions causes the neurone to become depolarized; this process initiates the events of neuronal transmission. The sum of these depolarizations is known as the excitatory post synaptic potential (EPSP). The release of an inhibitory neurotransmitter such as GABA (2) results in the influx of chloride into the cell membranes. This causes hyperpolarization of the cell membranes, known as the inhibitory post synaptic potential (IPSP).



(1)

(2)

1.4. Criteria for neurotransmitters.

The criteria that should be satisfied for positive identification of a substance as a neurotransmitter in the CNS are:

1. Release The substance must be released from storage by stimulation of the nerve.

2. Identification of action. Postsynaptic application of the putative neurotransmitter should mimic the effect of presynaptic stimulation.

3. Effect. Specific antagonists which block or potentiate transmission should have corresponding effects on the response to the applied substance.

4. Storage The substance and its associated enzymes must be present in

presynaptic nerve endings.

5. Inactivation. One or more mechanisms for inactivation of the transmitter should be present.

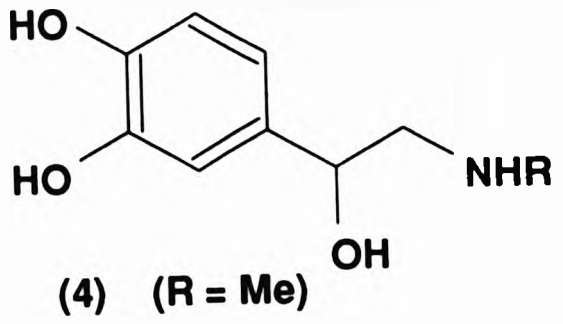
These are the criteria on which characterization of various reported neurotransmitters has been based. The real situation is usually a distortion of the ideal, and it is rarely possible to meet all the theoretical criteria. Often the first criterion is the most difficult to fulfil.

1.5. Neurotransmitters.

The primary observation that synaptic communication might utilize chemical agents was made by by Elliot.[2] This was extended by Loewi[3] who demonstrated that an agent liberated on stimulation of the vagus nerve of the frog heart could, when administered to the heart of a second frog, mimic the effect of a parasympathetic stimulation. This substance, which Loewi called "vagusstoff", was later isolated from the spleen by Dale *et al.*[4] and identified as the neurotransmitter acetylcholine (ACh) (3). These pioneering studies increased our understanding of the nature of chemical transmitters, and led to the accepted view that neurotransmitters are chemical components which are synthesized, stored and released from presynaptic vesicles.

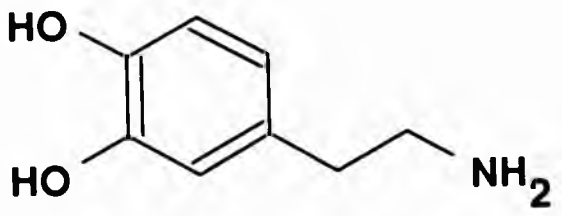
There is considerable diversity in the structure of molecules which have been identified as putative neurotransmitters,[5] these include the phenylethylamines, such as adrenaline (4), noradrenaline (5) and dopamine (6); the indole, 5-hydroxytryptamine (7); the imidazole, histamine (8); acetylcholine (3); the excitatory amino acids, L-glutamic acid (1) and L-aspartic acid (9); the inhibitory amino acids, glycine (10), taurine (11) and γ-aminobutyric acid (2), as well as peptides, such as substance P (12).

(3)



(4) (R = Me)

(5) (R = H)



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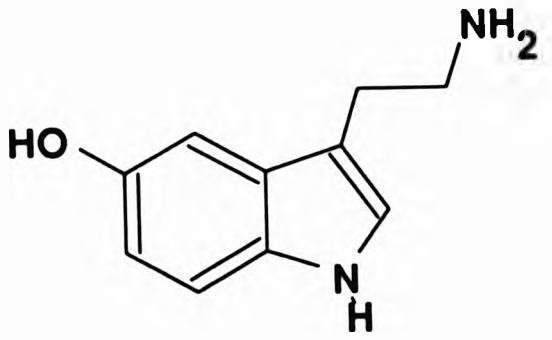

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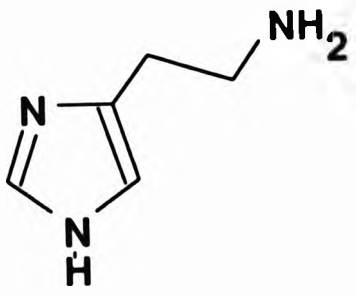

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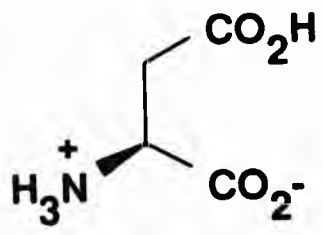

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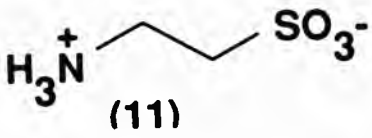


(11)

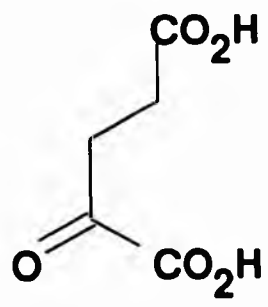
H.Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met.NH_2

(12)

1.6. Excitatory neurotransmitters.

Historically, identification of acidic amino acids as neuronal excitants was first reported by Hayashi,[6] who, in 1952, discovered that L-glutamic acid (1), and L-aspartic acid (9) caused convulsions when introduced into the central nervous systems (CNS) of monkeys and dogs. Eight years later, Curtis *et al.*[7] demonstrated the excitation of single neurones in the CNS by L-glutamate (1), L-aspartate (9), and structurally related acidic amino acids.[8] For the next ten years, iontophoretic studies provided evidence in support of transmitter roles for L-glutamic (1) and L-aspartic (9) acids.[9]

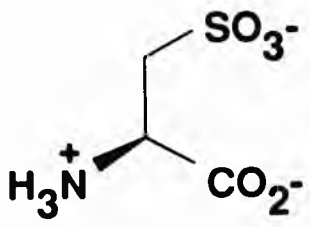
Since its isolation, L-glutamic acid has been the subject of numerous areas of research. Its involvement in biological processes is now well accepted. For example in mammals, it is involved in metabolic pathways, such as fatty acid synthesis, regulation of ammonia; (in the absence of a fully functional urea cycle, ammonia detoxification involves glutamic acid formation through amination of α-ketoglutaric acid (13) catalyzed by the enzyme glutamate dehydrogenase[10]); it is incorporated into proteins and peptides, and is involved in the control of osmotic and amniotic balance; it is also a precursor for certain co-factors such as folic acid and glutathione. Its action as a major excitatory neurotransmitter in the CNS is well documented.[11]



(13)

Additionally, it is the major precursor of γ-aminobutyric acid (GABA) (2), an inhibitory neurotransmitter in the CNS; the formation of GABA from L-glutamic acid is catalyzed by the enzyme glutamate decarboxylase.[12] The efficiency of the blood-brain barrier in the isolation of sensitive neurones from circulating glutamate is responsible for the low concentration of the amino acid in the central nervous system (CNS). Extraneuronal concentrations are maintained at low levels by avid uptake systems. Defects in these mechanisms may be involved in certain neuropathological states, whereby sustained elevations of extracellular glutamate may ultimately result in cell death.[13] Research into the role of L-glutamic acid as a neurotransmitter, particularly neurochemical aspects has, however, been fraught with difficulties because of its involvement in numerous biological processes. It is only recently that convincing evidence[7] has emerged about the specific role of the amino acid in behavioural and neuropathological manifestations. Encouraged by developments in the demonstration of GABA as a depressant of spinal neurones,[14] Curtis *et al.* used the then newly developed technique of microiontophoresis to apply the amino acids L-glutamic acid (1), L-aspartic acid (9) and L-cysteic acid (14), to single spinal neurones of the cat. These researchers observed that the amino acids had the ability to produce membrane depolarization.[11,15,16] These early observations, and others,[17] suggested that L-glutamic acid and L-aspartic acid represented a new class of neuronal excitants, distinguishable from calcium chelating agents and cholinomimetics. These findings were substantiated by Takeuchi,[17] who, in 1964, discovered that responses to L-glutamic acid, iontophoretically applied to crayfish muscle, were the same as those observed by Curtis.[15] Similar observations were made by Usherwood[18] when L-glutamic acid was applied to locust neuromuscular

junction.



(14)

1.7 Concept of the receptor.

The concept of the receptor site, with which drugs, hormones and neurotransmitters act to produce their biological effect, originated at the end of the nineteenth century through the studies of Langley[19] and Ehrlich.[20] Langley, working on atropine and pilocarpine, and Ehrlich, on antibodies, demonstrated that in order for drugs and antibodies to act specifically, they must first interact with specific "receptive substances" or receptors. The receptor concept has been substantiated in the last decade by the isolation and purification of macromolecular substances that fulfil all the criteria of being a receptor. It is now generally accepted that authentic receptors should have the following properties.[21]

1. Specificity. Receptors are specific cellular components with which specific ligands interact to induce a physiological response.

2. Saturability. The concept of saturability of receptors follows from ligand binding studies and is depicted as a dose-response curve, is usually plotted as log (dose) vs. response.

3. Selectivity. The selectivity of receptors towards agonists and antagonists allows deductions to be made about the complementary functional groups which are involved in interactions with ligands. Selectivity is thought to be greater with agonists than with antagonists.

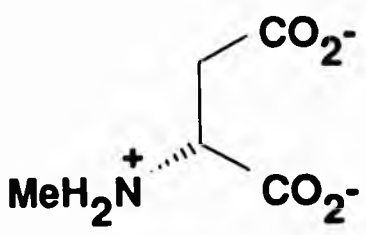
4. Reversibility. Since transmitters, hormones, and most drugs act in a reversible manner, it follows that the binding of these agents to receptors should be reversible.

Electrophysiological and binding studies have provided the now generally accepted view that a receptor is a form of transducer that, when activated by interaction with an agonist, effects the characteristic response.[22]

1.8. Excitatory amino acids.

Following the discovery of the likely neurotransmitter role of L-glutamic acid, [14] a large number of acidic amino acids and related compounds were tested and their pharmacological effects evaluated. Among these were N-methyl-D-aspartate (15), D-homocysteic acid (16), and ibotenic acid (17),[15] which were found to be more potent as agonists than glutamate in the mammalian CNS .

In 1970, Shinozaki[23] investigated certain anthelmintics for their excitant properties on rat neurones, in a programme of research that was to prove extremely fruitful. One of these compounds was kainic acid (18). This amino acid, which was first isolated in 1953 by Takemoto[24] from the seaweed *Digenea simplex*, was found to be a potent excitant in the mammalian CNS.



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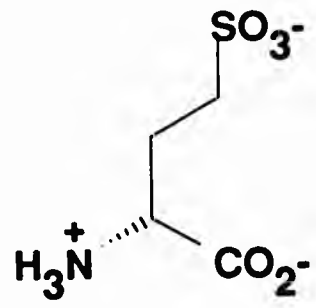

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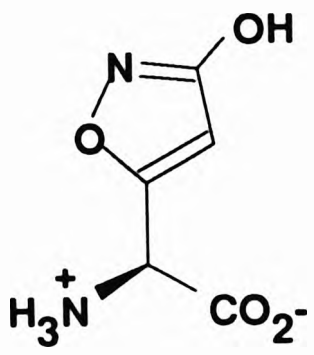

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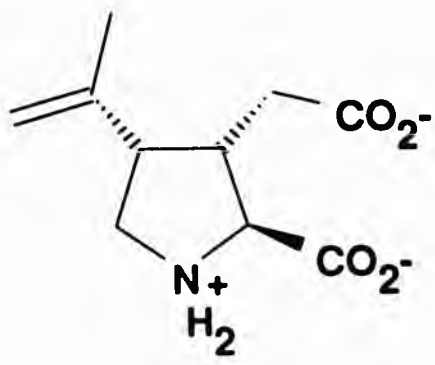
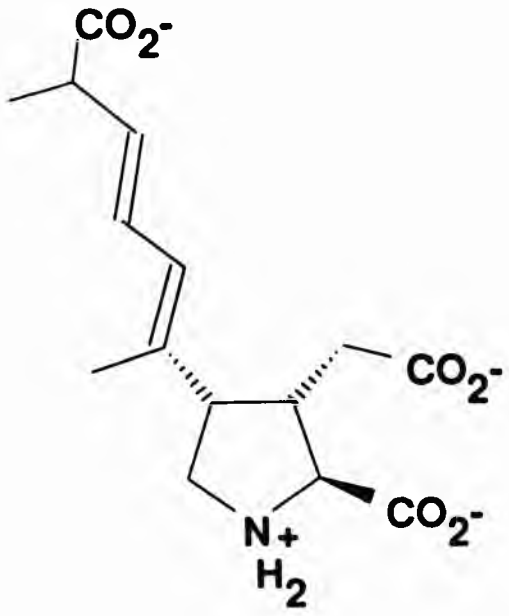


(18)

This finding led Watkins and co-workers to test the anthelmintic domoic acid, (19) a structural analogue of kainic acid isolated from the marine algae *Chondria armata*. When tested on frog and rat spinal neurones, it was found that the amino acid was a more potent agonist than L-glutamic acid, and was equipotent to kainic acid.[13] Continuing with his investigations into anthelmintics, Shinozaki[25] observed that the amino acid quisqualic acid (20) (isolated by Takemoto in the early seventies)[24] had potent excitant properties on the crayfish neuromuscular junction. This prompted Watkins and co-workers to investigate quisqualic acid for its excitant properties on the frog and rat spinal neurones. They observed that on both kinds of neurones quisqualic acid was at least two orders of magnitude more potent than L-glutamic acid, and equal to or stronger than kainic acid.[13]



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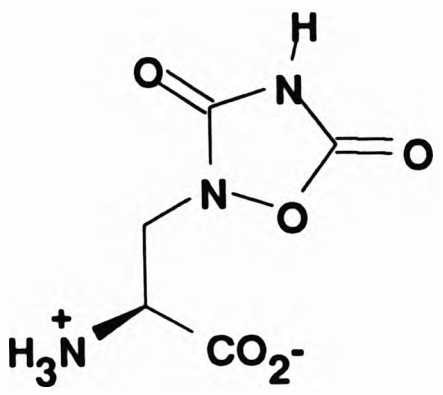
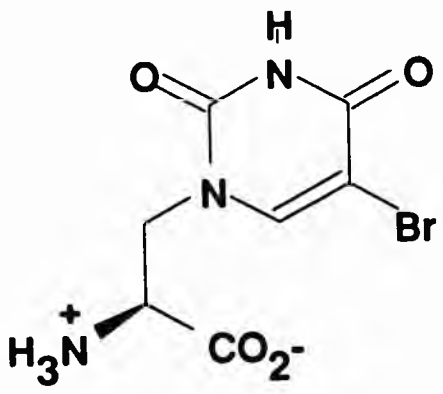


(20)

These observations extended the range of structures known at the time which could replace the terminal carboxymethyl group of glutamate with enhancement of activity. Among these compounds were bromowillardine (21), a potent agonist at the kainate receptor; and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (22), a structural analogue of quisqualic acid and a known neuroexcitant.[26]

Other exogenous agonists identified include β-N-oxalylamino-L-alanine (BOAA) (23), a quisqualate agonist, and trans-2,3-piperidine dicarboxylic acid (24), an NMDA agonist.[27,28]



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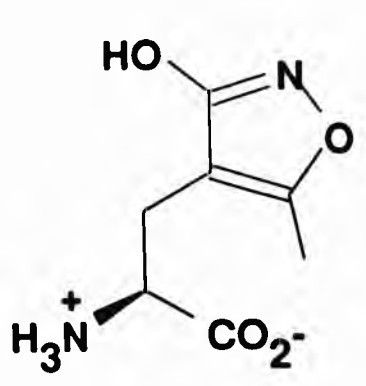

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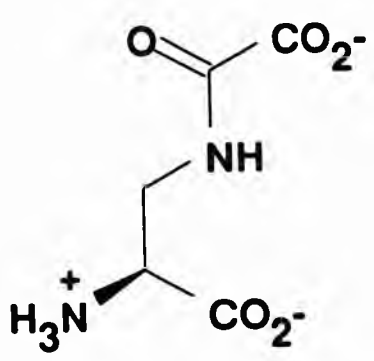

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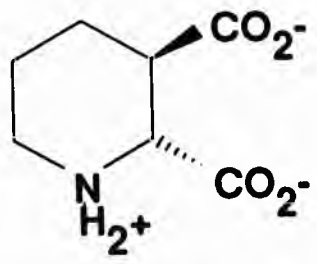

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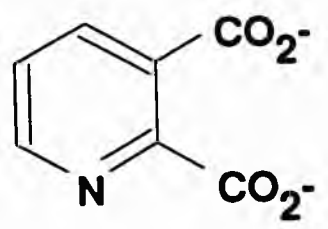
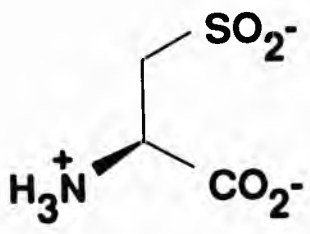


(25)

Endogenous agonists include L-glutamic acid, L-aspartic acid, quinolinic acid (25), and sulphur-containing excitatory amino acids such as cysteic acid (14), homocysteic acid (the L-epimer of (16)), cysteine sulphinic acid (26), homocysteine sulphinic acid (27), and S-sulphocysteine (28). These are all neuronal excitants, the effects of which are thought to be mediated primarily *via* the NMDA receptor.[29,30]



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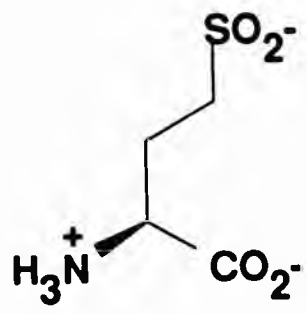

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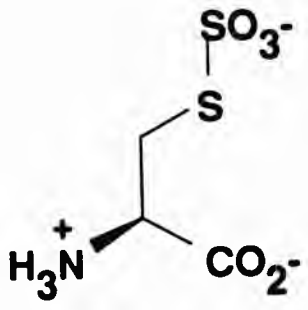
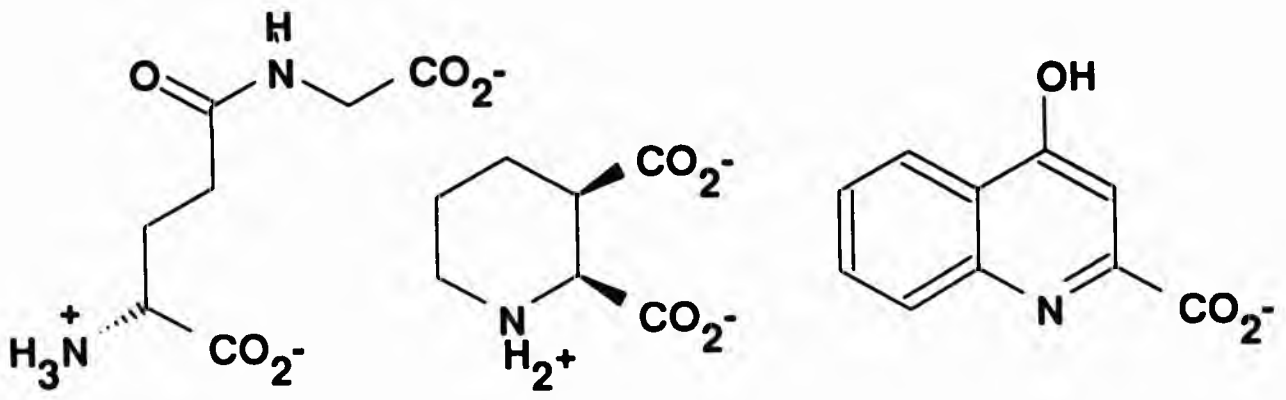


(28)

The side-chains of these compounds contain ionizable acidic groups which presumably interact with a basic region on the receptor.

A greater understanding of the physiological and pathological roles of excitatory amino acid receptors was provided by the identification of useful antagonists. Among the acidic amino acids identified were the non-selective antagonists; γ-D-glutamyl-glycine (29), cis-2,3-piperidine dicarboxylic acid (30), and kynurenic acid (31).[31]



(29) (30) (racemic) (31)

1.9. Excitatory amino acid receptors.

Excitatory amino acid receptors are now accepted as the major neurotransmitter receptors mediating synaptic excitation in many sites in the mammalian,[32] locust,[33,34] and cockroach[35] central nervous systems (CNS). As structure-activity relationships of agonists and antagonists at these excitatory amino acid receptors began to emerge, the possibility that perhaps these responses were mediated by more than one receptor subtype became a focal point of research. Early speculation focused on the possibility of there being only one type of excitatory amino acid receptor, and that this receptor

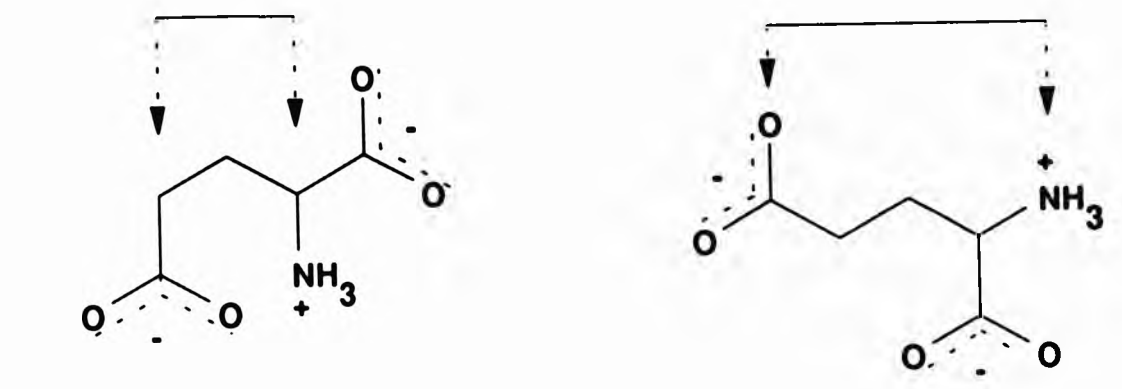
contained at least two or possibly three charged sites which interacted with oppositely charged groups in the amino acid molecule.

Two point receptor. Three point receptor.

Fig 1.3. Early receptor model.[7].

It was further hypothesized[36] that perhaps the glutamate receptor (in vertebrates) was part of a lipoprotein complex, or possibly was coupled to a divalent metal ion. While the principles on which such speculations were based remained tenable, the likelihood became increasingly doubtful as evidence began to emerge that the observed responses may have been mediated *via* two or more distinct receptor subtypes.[37] This hypothesis was substantiated by Piggott,[38,39] who investigated the population of glutamate sites on the common garden snail *Helix aspera*, and concluded that there were two types of glutamate receptors; an excitatory receptor with a preference for the folded conformation (resembling L-aspartate), and an inhibitory receptor with preference for the extended conformation (resembling ibotenic acid) (fig.1.4).

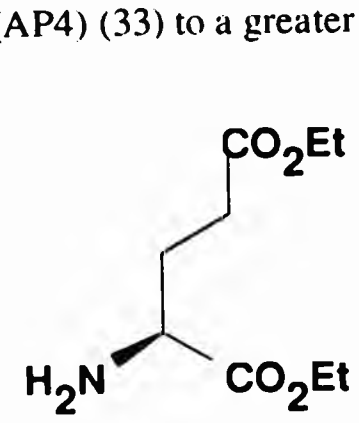
Fig. 1.4



This tentative hypothesis was further investigated by Duggan[40] who examined the relative potencies of N-methyl-D-aspartic acid (15), and kainic acid (18) on the same group of feline spinal cells. This researcher found that the relative potencies of N-methyl-D-aspartic acid and kainic acid parallelled those of L-glutamic acid and L-aspartic acid. Furthermore M^{c}Culloch[41] found regional differencies in sensitivity to the amino acids in different regions of the spinal cord, glutamate and kainate receptors being more populous in the dorsal horn cells than in the Ranshaw cells, which had an increased population of aspartate and NMDA receptors. The inference that kainic acid and N-methyl-D-aspartic acid acted on different amino-acid receptors led to comparative studies on the excitations produced by NMDA and kainate receptor agonists[42].

As soon as the idea of two distinct receptor subtypes became established, the possible existence of a third receptor subtype was proposed.[43] This was prompted by the observation that iontophoretically applied L-glutamate diethyl ester (GDEE) (32) blocked quisqualic acid (20) induced responses but not kainic acid-induced responses of the cat spinal neurones.[44] Although these observations were treated with caution, owing to the non-selectivity of GDEE as an antagonist, the findings were substantiated by the evidence[45] that kainic acid-induced responses were antagonized by 2-amino-4-phosphonobutyric acid

(AP4) (33) to a greater degree than were quisqualic acid-induced responses.



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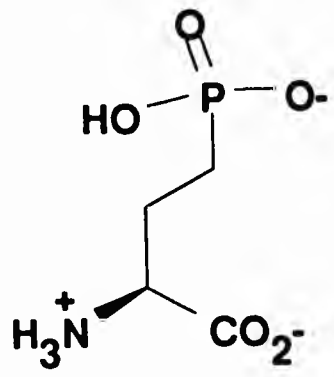

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Furthermore, in cat spinal neurones, kainic acid-induced responses were antagonized by γ-D-glutamylglycine, (DGG) (29), but those induced by quisqualic acid (20) were not.[42]

Additionally, Mayer[46,55] found that in cultured neurons, kainate and quisqualate activate channels with dissimilar conductance and desensitization properties. Since these pioneering discoveries by Curtis,[14] Watkins[9] and others[11,15] considerable evidence[47-51] has accumulated indicating that excitatory amino acid induced responses may be mediated by up to four subtypes of receptors; notably N-methyl-D-aspartate, kainate, quisqualate and L-2-amino-4-phosphonobutyrate (L-AP4). The first three of these receptor subtypes were defined on the basis of their high selectivity for the appropriate agonist; N-methyl-D-aspartic acid, kainic acid, and quisqualic acid respectively. The L-AP4 receptor was discovered because a subpopulation of synaptically induced excitatory responses were antagonized by L-AP4, but those mediated by NMDA, kainate, or quisqualate were not.

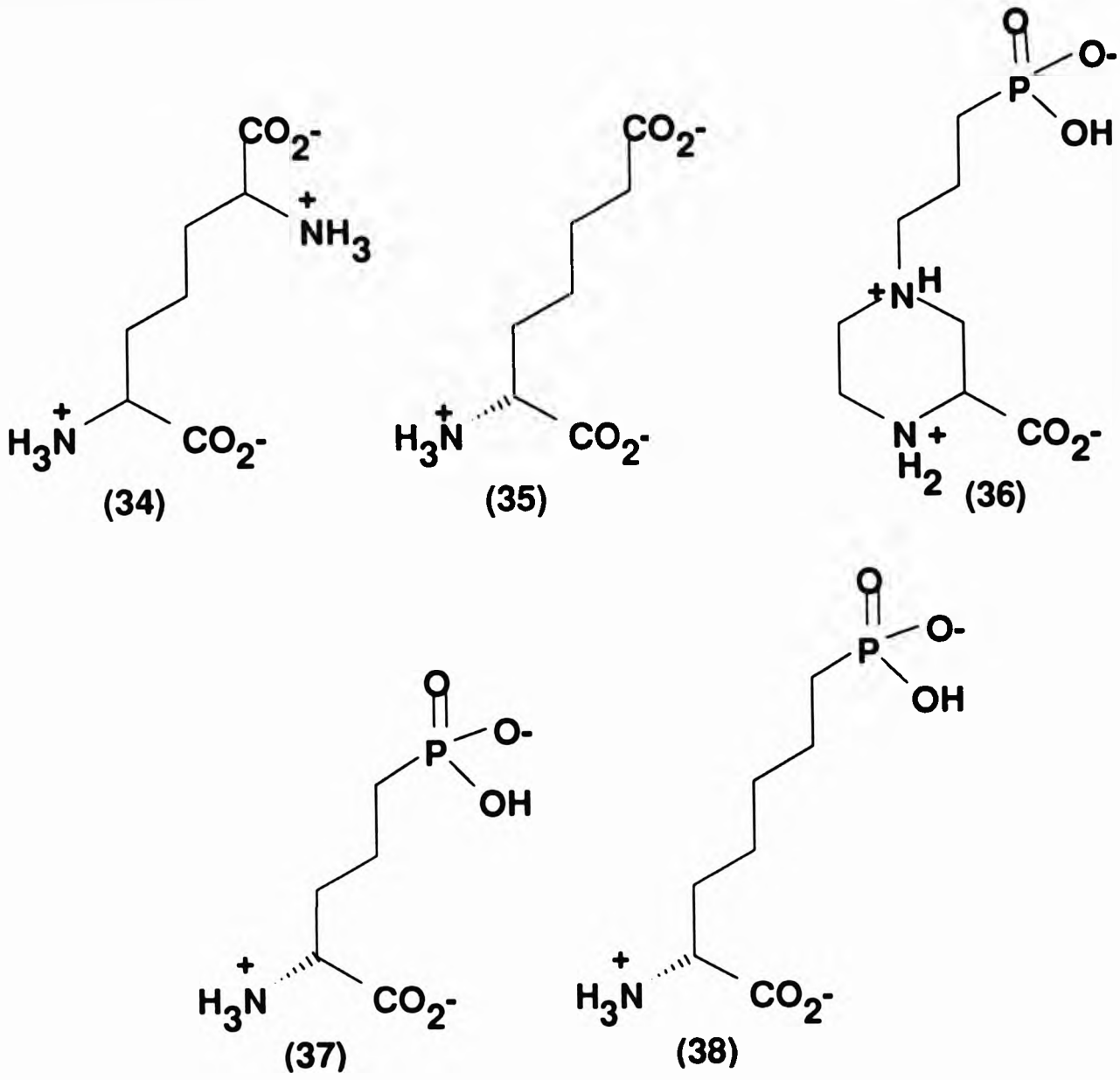
Recently however, "quisqualate receptors" have been found to be heterogeneous.[52,53] The term quisqualate receptor is no longer applied to the receptor responsible for the mediation of the fast excitatory postsynaptic

potential and has been termed the AMPA receptor, following the discovery of the more selective ligand α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (22). A second type of quisqualate-sensitive receptor is the metabotropic receptor. The latter receptor is coupled to the generation of inositol-1,4,5-triphosphate (IP_3), and the mobilization of calcium ions.

The story is further complicated by the likelihood that kainic acid acts on the classical quisqualate receptor.[54]

1.9.1. NMDA receptor.

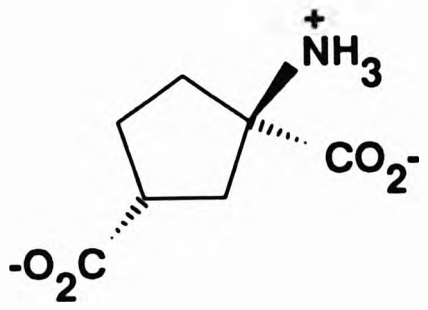
Since it was first recognized as a distinct receptor subtype,[54] the NMDA receptor has become undeniably the best characterized, and hence the best understood of all the excitatory amino acid receptors.[55] The availability of selective antagonists (which distinguish it from other types of excitatory amino acid receptors) has contributed to the understanding of this receptor subtype. Among these antagonists are α-diaminopimelic acid (34) and D-α-aminoadipic acid (35) 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (36),[56] the D-isomers of some of the α-amino-ω-phosphono-carboxylic acids, notably 2-amino-5-phosphonopentanoic acid (AP5) (37), 2-amino-5-7-phosphonoheptanoic acid (AP7) (38), and others.[57]



Based on structure-activity relationships, a model comprising three points of attachment was proposed for recognition at the NMDA receptor. Two of these points interact with the α-amino acid ammonium and carboxylate functions, and are common to both agonists and competitive antagonists.[58] Two further points accomodate the ω-acidic group, one for the shorter chain D- and L-forms (such as NMDA, L-glutamate), and a separate point for the longer chain antagonists which possess the D-stereochemistry, such as D-AP5 (37).

Electrophysiological data do not give conclusive structure-activity information for the responses to agonists of the NMDA receptor, because most of the NMDA agonists also activate more than one excitatory amino acid receptor.

However, relative receptor affinities available from combined radioligand binding and electrophysiological studies support the view that L-glutamic acid has the highest affinity as an agonist at the NMDA receptor. N-methyl-D-aspartic acid itself has moderately high affinity. Other agonists include L-homocysteic acid (16), ibotenic acid (17), *trans*-1-amino-1,3-dicarboxycyclopentane (39), *trans*-2,3-piperidinedicarboxylic acid (*trans*-2,3-PDA) (24), and N-methyl-L-aspartic acid (40). (a weaker agonist than NMDA).[58]



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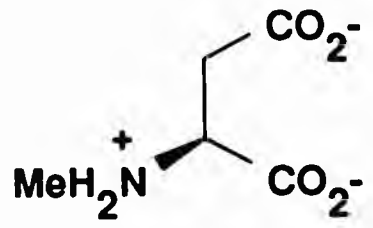

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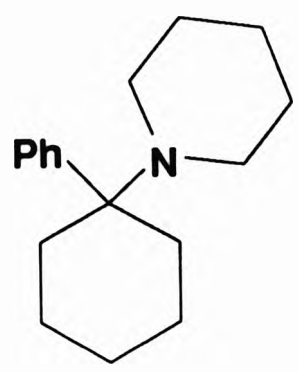
D- and L- forms of short chain, acidic, amino acids have variable preference, with the D-enantiomer generally possessing higher affinity than the L-enantiomer.[58] The ω-terminal acidic amino acid group preference for agonist activity decreases in the order CO_2H, > SO_2H > SO_3H, >>PO_3H_2.[59]

In addition to direct interactions at the NMDA ligand binding site, the NMDA receptor system is subject to allosteric control. Evidence[60,61] has accumulated which indicates that the dissociative anaesthetics phencyclidine (PCP) (41), ketamine (42) and MK801 (43), and σ-opiate compounds act as non-competitive antagonists at the NMDA receptor. The specific sites have been identified by labelling with the PCP analogue ^3H (1-(2-thienyl)cyclohexyl)piperidine (^3H-TCP) (44), or with ^3H-MK 801[62] and shown to lie within the ion channel.



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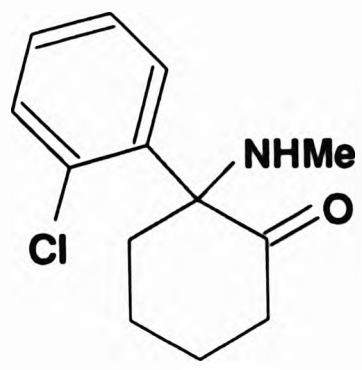

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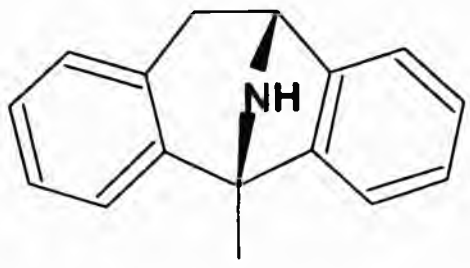

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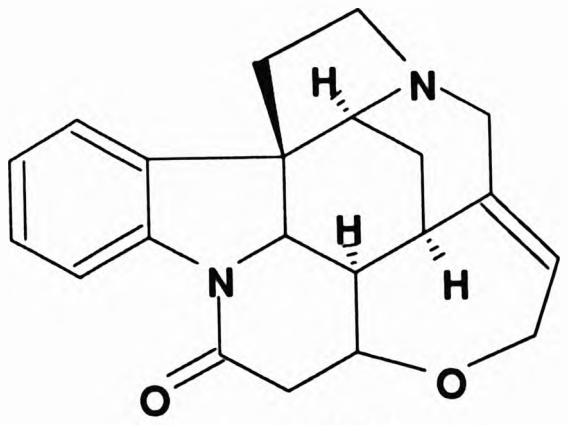
Autoradiographic experiments showed a high correlation between the anatomical distribution of NMDA-sensitive ^{3}H L-glutamate sites and ^{3}H-TCP binding sites in the brain. Furthermore, although dissociative anaesthetics had no effect on the binding of ^{3}H-glutamate or ^{3}H-CPP, NMDA receptor agonists increased ^{3}H-TCP or ^{3}H-MK801 binding, which suggests[21,22,62,63] that PCP and related substances bind to an activated site of the NMDA receptor complex .

Another allosteric site on the NMDA receptor is a binding site which recognizes the amino acid glycine (10). Using the patch-clamp technique, Johnson and Ascher[64] observed that glycine potentiates NMDA responses by increasing the frequency of channel opening. This observation is consistent with the finding that the distribution of ^{3}H-glycine binding sites in the CNS

was similar to that found for NMDA, but quite different from that of ^{3}H-strychnine (45) binding sites.[6,39,65,66]



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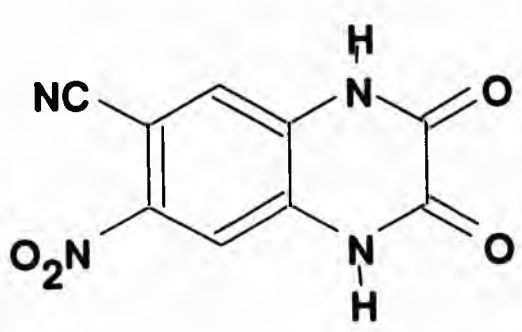
Anatomically, (as shown by autoradiographical and radioligand binding studies), NMDA receptors are localized subcellularly at the postsynaptic density (consistent with synaptic rather than extrasynaptic function),[67] and are present in high density in the cerebral cortex, hippocampus, striatum, septum and amygdala.[67] Using thin brain slices, Monaghan *et al* [67]. have demonstrated that populations of NMDA receptors exists predominantly in the stratum oriens and stratum radiatum of the CA1, as well as in the inner areas of the dentate gyrus. Lower levels were observed in the stratum oriens and the radiatum of CA3, and in the outer molecular layer of the dentate gyrus, and the stratum lucidum.[68] In support of this, compounds such as D-α-aminoadipic acid, ibotenic acid, and D- and L-aspartic acids, which have been shown to interact with the NMDA receptor, are all displacers of the NMDA-sensitive ^{3}H-L-glutamate binding in the stratum radiatum.[67]

The location and electrophysiological properties of the NMDA receptor suggest that it plays a crucial role in pathological conditions and synaptic plasticity. Its high concentration in the principal region affected in various

degenerative diseases has led to the speculation that the receptor may be involved in conditions such as Huntington's Chorea and Alzheimer's disease.[68-73] It is also associated with other neuronal injuries such as epilepsy and stroke.[73]

1.9.2. "Quisqualate receptor".

The quisqualate receptor was the least understood of the three excitatory amino acid receptors originally identified in the 1970's, possibly due in part to the unavailability of ^{3}H-quisqualic acid. Quisqualic acid (20) was first described as a potent excitant at the crayfish neuromuscular junction,[25] and in the frog and rat spinal cord.[74] The use of quisqualic acid in binding studies has led to the conclusion that the amino acid is not selective for what was initially known as the quisqualate receptor subtype. It also exhibits high affinity for other excitatory amino acid receptors; namely, the kainate, AMPA, and metabotropic receptors, some transport sites, such as the chloride-dependent L-AP4-sensitive sites, and certain dipeptide degrading enzymes, such as that responsible for cleavage of N-acetyl-aspartylglutamate (NAALADase).[52] A major breakthrough in the study of the quisqualate-sensitive ionotropic receptor was the isolation of a new class of excitatory amino acid antagonists, the quinoxalinediones[75] such as CNQX (46)and DNQX (47) .



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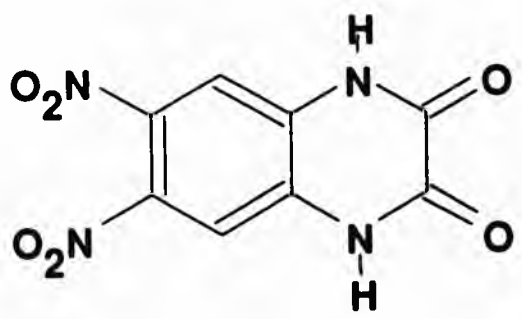

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Binding and electrophysiological studies of this novel group of compounds have led to an increased understanding of the function of the quisqualate-sensitive receptor subtype responsible for the fast excitatory postsynaptic potentials (EPSPs). This receptor is the AMPA receptor, following the identification of the more selective ligand α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (22). The other receptor which shows high sensitivity to quisqualate (CNQX/DNQX-insensitive) is the metabotropic receptor, the activation of which is coupled to the generation of inositol 1,4,5-trisphosphate (IP_3), and the mobilization of calcium ions.[76] For the purposes of this review the term "quisqualate receptor" will refer to the quis/AMPA receptor. The metabotropic receptor will be treated separately.

1.9.3. Quis/AMPA receptors.

Since its synthesis, isolation, and pharmacological evaluation, the structural analogue of quisqualic acid, AMPA (22), has proved a useful tool in advancing the understanding of excitatory amino acid receptors. The availability of ^{3}H-AMPA, and its specificity in binding and electrophysiological studies, has provided distinct evidence[77] of the differences between the NMDA and non-NMDA receptors, notably kainate, AMPA and the newly-defined metabotropic receptor. To date no specific antagonist for this receptor has been discovered. However, two analogues of kynurenic acid (31), the quinoxalinediones CNQX (46) and DNQX (47), show some selectivity and relatively high affinity for the ^{3}H-AMPA binding sites and block AMPA and quisqualate-evoked excitation.[77] Molecular studies of the AMPA receptor by Honore' *et al.* demonstrated that the use of thiocyanates potentiates ^{3}H-AMPA binding to its

receptors, possibly due to a shift of a high affinity binding site of the receptor.[78]

Receptor autoradiography using ^{3}H-AMPA in conjunction with kainate and NMDA (to prevent binding to other receptor types) suggests that, anatomically, the AMPA receptors are localized in the telencephalic regions, with high levels in the hippocampus, cortex, lateral septum, stratum, and the molecular layer of the cerebellum.[79] This distribution corresponds closely to that of NMDA receptors, and thus supports the speculation that the two receptor subtypes probably act in concert in their mediation of neuronal postsynaptic activation.

Physiologically, little is known about the AMPA receptor. There are suggestions that it may participate in neurotoxicity in the hippocampus[80] neuronal degeneration of the cerebral cortex,[7] and ischaemic brain injury. [7] However, the mechanism by which these actions may be mediated is not clearly understood.

1.9.4. Metabotropic receptor.

The discovery of this newly-defined receptor confirmed a long-held hypothesis[76,81-83] that the quisqualate receptor may be coupled to more than one site in the CNS. The receptor was identified by the study of phosphatidylinositol turnover in brain slices, following intracellular injection of rat brain .[83] In 1988, the first potent and selective non-NMDA receptor antagonists were developed by Honore.[75] Use of these quinoxalinediones, particularly (CNQX) (46), in ligand binding as well as in electrophysiological

studies, by virtue of their blockade of quisqualic acid-induced excitations of the AMPA receptor, but not those exhibited by the metabotropic receptor, provided evidence in support of the existence of more than one quisqualate receptor.

Pharmacologically, metabotropic receptors are preferentially activated by quisqualic acid (20), ibotenic acid (28), and L-glutamic acid (1), and not by NMDA (15), AMPA (22), or kainic acid (18). There are no specific antagonists known, as yet, for these receptor subtypes, although it has been suggested that AP4 (33) may be an indirect antagonist.[83]

Research into the anatomical localization of metabotropic receptors indicates that a subpopulation of ^{3}H-L-glutamate binding sites (quisqualate-sensitive, but AMPA-insensitive) may represent their recognition sites.[84] The development of selective and potent competitive antagonists would facilitate further characterization of these receptors.

Little is known of their physiological role in the CNS, although it has been suggested that they may be involved in neurodegenerative disorders and synaptic plasticity.[85]

1.9.5. Kainate receptor.

Alpha-kainic acid (18), a cyclic analogue of L-glutamic acid (1), was isolated from the seaweed *Digenea simplex*. by Takemoto *et al.*[24] Its neuroexcitatory properties were first described in 1970 by Shinozaki,[23] and it is the prototypic

agonist of the kainate receptor. Since its identification as an excitatory amino acid receptor, only limited progress has been made in kainate neurochemistry, possibly due to the lack of specific kainate antagonists. However, some non-selective antagonists have been identified including kynurenic acid (31), γ-D-glutamyl-glycine (29), CNQX (46) and DNQX (47).[75] Some progress has been made in the identification of potent and selective kainate agonists, notably domoic acid,[27,86] (19) and 5-bromowillardine (21).[87] It would appear that the presence of the bromine atom in the latter is significant, since willardine has little activity at the kainate site.[87]

Moreover, quisqualic acid has moderate activity at the kainate site, as do L-glutamic acid (1). Electrophysiological studies of kainate activity in the CNS have indicated that the activation of the receptor may be attributable to the C-4 unsaturated side chain of kainic acid.[25,27,31] Furthermore, the (S)-configuration at C-2, and the *cis*-relationship between the C-3 and C-4 substituents, are essential to its activity.[27] Understanding of the kainate receptor will be dependent upon the identification of specific and selective antagonists for the receptors.

Research into the anatomical distribution of kainate receptors indicated that they were predominant in the mammalian C fibres,[27,87,88] but appear to be quite diffuse in many regions of the CNS. Autoradiographic studies have indicated receptor populations in the cerebral cortex, and high densities in the striatum, and nucleus accumbens of the basal ganglia.[89] Localised concentrations of the receptor have also been reported in the hippocampus,[88] with low levels in the thalamus, hypothalamus, mid-brain, and hind-brain of the CNS.[90] Only limited investigations [87] have been made of what appears (in view of the lack of NMDA and AMPA selectivity in this region of the

nervous system to be a pure kainate population, i.e. the C-fibres. It is unclear, however, whether or not these kainate receptors are identical to kainate receptors in other regions of the CNS.[88] The kainate receptor has also been suggested to be involved in neuropathological states such as epilepsy and spasticity, neurodegenerative conditions which are associated with strokes, Huntingtons Chorea, Alzheimer's disease, and schizophrenia.[91]

The excitotoxic effect of kainic acid was first reported by Olney,[92] who observed that the amino acid had the same order of potency for its excitatory and its neurotoxic activities. Compounds which interact as agonists at the kainate receptor, such as domoic acid, also exhibit neurotoxic activity.[93]

1.9.6. L-AP4 receptor.

This subpopulation of glutamate receptors was discovered because L-AP4 (33) was a potent antagonist of evoked excitatory responses at a subpopulation of the glutamate receptor.[94,95] Although the L-AP4 receptor was discovered somewhat later than the other excitatory amino acid receptors, it is rather surprising that to date very little is known about the mechanism by which it mediates its responses.

A number of studies suggest that the effect of L-AP4 is mediated presynaptically in the CA1 region of the hippocampus, lateral olfactory tract and in the spinal cord, where L-AP4 is thought to increase post-synaptic excitations induced by NMDA, kainate, quisqualate, and L-glutamate.[95,96] However, the precise membrane binding site through which L-AP4 exerts these effects has been difficult to elucidate. It has been suggested that its actions may be mediated *via* a chloride-dependent L-glutamate binding

site.[97,98]

The anatomical distribution of the the receptor remains equivocal, although populations of the L-AP4 receptor in the brainstem, hypothalamus, spinal cord, cortex and the cerebellum have been cited.[95,98,99] Its precise physiological profile has yet to be defined.

1.10. Pathological aspects of excitatory amino acids.

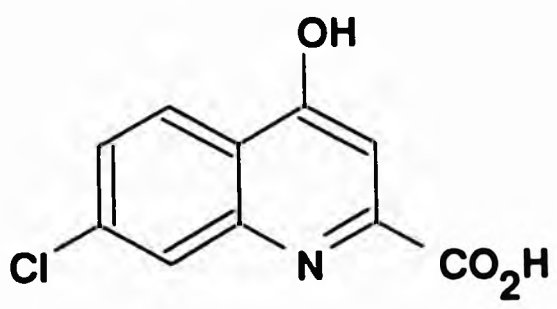
A major impetus to the study of excitatory amino acids has been the implication of their receptors, particularly the NMDA receptor, in neuropathological conditions. Since the discovery in 1957[100], that systemic administration of L-glutamic acid to mice caused degeneration of retinal neurones, research has centred on the role of excitatory amino acids in neurodegenerative diseases. These research programmes, coupled with the development of selective antagonists for the excitatory amino acid receptors, have provided clear evidence[101-103] of their involvement in neuropathological states. The involvement of excitatory amino acids in abnormal neuronal activities, such as epilepsy, is well documented.[104] They are also thought to be associated with chronic neurodegenerative disorders, such as Huntington's Chorea,[104] senile dementia of the Alzheimer type,[105] Parkinsonism,[105] amyotrophic lateral sclerosis (ALS).[104] They are also thought to be involved in the pathogenesis of stroke, traumatic brain injury, cerebral ischaemia and hypoxia.[106]

1.10.1. Epilepsy.

Epilepsy is a clinical paroxysmal abnormal electrical activity in the brain, due to sudden synchronous high-voltage discharges arising from a number of hyperexcitable neurones. Neurochemical and electrical changes occur with disturbances of the inhibitory mechanisms and spread of electrical discharges beyond the locus. Clinical manifestations include disturbances in sensation, alterations in perception and/or coordination, loss of conciousness, convulsive movements, and incontinence.[107]

Epileptiform foci may result from many different pathological conditions, such as trauma, vascular lesions, neoplasms, infections and atrophy of the brain. Metabolic and nutritional factors, such as hypoglycaemia, electrolyte imbalances, pyridoxine deficiency, and disturbance of fat metabolism are all contributary factors. Various toxic states, including lead and carbon monoxide poisoning, uraemia and certain allergic conditions are causative factors. Certain endocrine aspects, including changes which occur at puberty, premenstrually, and during pregnancy are important. Age is also very important, and in young children, febrile convulsions may lead to temporal lobe epilepsy later in life. Artificial induction of epileptiform activity in a variety of animal models has been reported.[108-110] These inductive methods include photic stimulation of the baboon *papio papio* and of the mouse (DBA/2 strain,[111-114]); audiogenic stimulation such as bell-ringing; electrical stimulation of the brain with externally applied electrodes; i.e a form of kindling; and chemical stimulation with known convulsants, such as leptazol, and/or excitatory amino acids such as NMDA, kainic acid, L-glutamic acid, L-aspartic acid, and quisqualic acid.[113]

The involvement of excitatory amino acid receptors in the production of seizure-like attacks was demonstrated by the use of excitatory amino acid antagonists. Often, burst-firing techniques were used for the induction of epileptiform seizures in conjunction with metal ions such as potassium. Meldrum observed that spontaneous bursts in the region of CA3 region of hippocampal slices superfused with 8.5mM potassium ions or with 200nM kainic acid were eliminated by 2-5mM CNQX, but were insensitive to competitive NMDA antagonists.[113] Furthermore, Aram *et al* showed that NMDA-evoked depolarizations were blocked by NMDA receptor antagonists, such as AP7.[112] This was substantiated by Meldrum and his colleagues, who observed that photo-induced convulsions in baboons were also blocked by AP7.[113] Corresponding results have been reported for the suppression of epileptiform bursts with 7-chlorokynurenic acid (48), a selective antagonist for the glycine co-agonist site of the NMDA receptor.[111] The work of Coutinho-Netto *et al.*, who identified anti-convulsant effects of locally-applied AP5 in cobalt-induced seizure in the rat neocortex, is noteworthy.[114] Antagonism of NMDA receptors was also observed by Croucher and his colleagues,[113] who demonstrated that intracerebroventricular administration of AP5 and AP7 caused potent anticonvulsant effects against audiogenic seizures in DBA/2 mice. Thus, the NMDA receptor has been seized upon as the ultimate mediator of epileptiform seizures. Data on the role of the AMPA receptor is rather scanty, although the selectivity of the quinoxalinedione NBQX (49) for the AMPA receptor[115,116] over the NMDA receptor should facilitate the study of AMPA receptors in epileptiform seizures. The availability of a specific non-NMDA receptor antagonist would permit analysis of the role of the receptor in the propagation of epileptic seizures.



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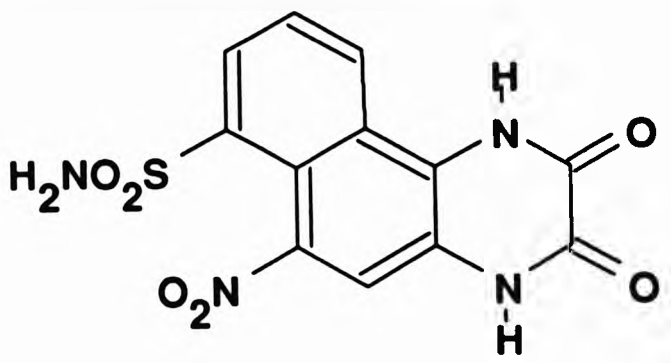

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1.10.2. Huntington's disease.

Huntington's disease is a severe neurological disorder, marked by degeneration of motor neurones. Usually the disorder is manifest during middle life, with the onset of involuntary choreic movements that become more severe with time, resulting in death some fifteen years after the onset. The aetiology of this genetically inherited disease remains unknown. It is of biochemical interest that epilepsy is fairly common when the disorder is first diagnosed. Anatomically the greatest atrophy is seen in the basal ganglia, a large group of nuclei which controls movement through the thalamus and motor cortex. Attempts to discover the biochemical basis for Huntington's disease have shown that levels of the inhibitory amino acid γ-aminobutyric acid (GABA) (2) are lowered.[117] Significantly, the levels of glutamic acid decarboxylase (GAD) (an enzyme which catalyzes the synthesis of GABA) were also significantly decreased.[118]

The changes in enzyme concentration were observed in both the postmortem tissues of choreic brain and experimental animals in which the disorder had been induced by administration of kainic acid.[119] Interestingly, there appears

to be no morphological evidence in support of specific excitotoxic neuronal damage, nor is there evidence in support of increased levels of quinolinic acid (25).

Clinical management of Huntington's disease has been fraught with difficulty. One choice of treatment would have been to increase the levels of GABA in the brain; however since GABA does not cross the blood-brain barrier, this mode of treatment is not feasible, unless suitable prodrug forms of GABA can be produced.

1.10.3. Cerebral ischaemia and hypoxia.

Ischaemic neuronal degeneration is evident after any stress, such as cardiac arrest, profound arterial hypotension, focal ischaemia, or hypoglycaemia,[120] that critically depletes cerebral energy metabolism. Neuropathologists have generally attributed epileptic brain damage to systemic hypoxia, to cerebral arterial spasm or to cerebral oedema, leading to compression of the arteries.[121-123] This is often associated with status epilepticus, involving pyramidal neurones in hippocampal CA1 and CA3 regions.[124] Microscopically, the brain damage takes the form of nerve cell loss and reactive gliosis.[125] Excitotoxic mechanisms are thought to contribute to this type of neuronal loss, which follows cerebral ischaemia. Postsynaptic neuronal changes which characterize excitotoxic damage have been identified in the various animal models of ischaemia.[126] Evidence in support of excitotoxic involvement in cerebral ischaemia was provided by the finding that competitive and non-competitive NMDA receptor antagonists (CPP and TCP respectively)[126] protected cerebral neuronal cells of adult mice, cats and rats

from damage, following cerebral artery occlusion.[125] The volume of neuronal damage in such cases was reduced by 40-65%.[125]

1.10.4. Alzheimer's disease.

Alzheimer's disease is caused by overproduction of fibrous protein in the neurones of the brain, and abnormally formed microtubules and microfilaments fill the cells, often to such an extent that other vital subcellular organelles, such as mitochondria, are excluded, resulting in cell death.[127] The cholinergic neurones, which connect the nucleus basilis to the frontal temporal cortex, are particularly affected,[128] as are glutamatergic and serotoninergic neurones.[129] The involvement of noradrenergic fibres has also been reported.[130]

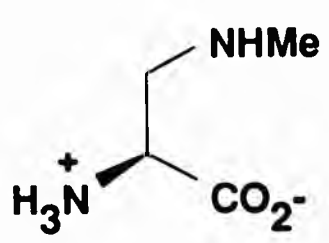
1.10.5. Amyotropic lateral sclerosis.

Amyotropic lateral sclerosis (ALS), also known as motor neurone disease, is a progressive degeneration of the corticobulbar, spinal, and lower motor neurones.[131] It leads, by encroachment, to a mixture of spastic and atropic changes in cranial and spinal musculature. Damage to the myelin sheath occurs secondary to damage in the lateral columns. This condition is likened to Guam disease, a neuropathological condition sometimes referred to as ALS, Parkinsonism or dementia.[131]

1.10.6. Guam disease.

Like ALS, Guam disease is a chronic neurological disease. It is linked to the

dietary consumption of β-N-methylamino-L-alanine (BMAA) (50), an amino acid found in the seeds of *cycas circinalis*.[132]



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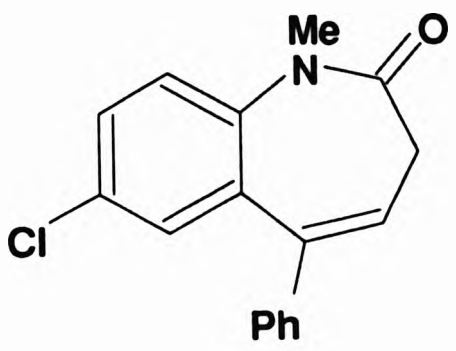
The amino acid BMAA is thought to be an exogenous excitotoxin.[132] Under normal conditions (*in vitro*), it is neither excitatory nor toxic. However, in the presence of bicarbonate ions it becomes excitotoxic, possibly due to the formation of an α-methyl carbamate.[132] Its excitotoxic activity is thought (by virtue of its blockade by the NMDA antagonists AP5 and AP7), to be mediated largely *via* the NMDA receptor.[133,134] However, the observation that the amino acid has a weak excitotoxic action on NADPH-diaphorase-positive neurones suggests that part of its action may be mediated *via* a non-NMDA receptor.[135]

1.10.7 Neurolathyrism and BOAA

Neurolathyrism is a spastic disorder occurring in East Africa and southern Asia. It is associated with the consumption of the chick pea *Lathyrus sativus*, which contains the excitotoxin β-N-oxalylamino-L-alanine (BOAA) (23). This behaves as a glutamate-like excitant on spinal neurones, acting principally as an agonist at AMPA receptors.[136,137]

1.11. Therapeutic potential of excitatory amino acids.

There are many mechanisms which control the synaptic release of excitatory neurotransmitters. Among these are autoreceptors, whose response is analogous to glutamate or its analogues, $GABA_A$ receptors linked to the benzodiazepine receptor site, adenosine receptors, and $GABA_B$ receptors. Thus, in brain slices or in other *in vitro* preparations, it is possible to demonstrate a decrease in the evoked release of excitatory amino acids in the presence of benzodiazepines such as (51), baclofen (52), and excitatory amino acid antagonists such as MK801 (43).



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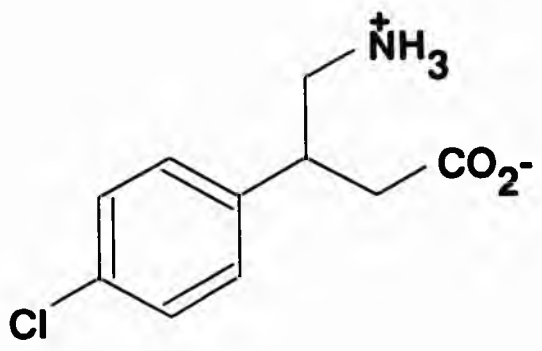


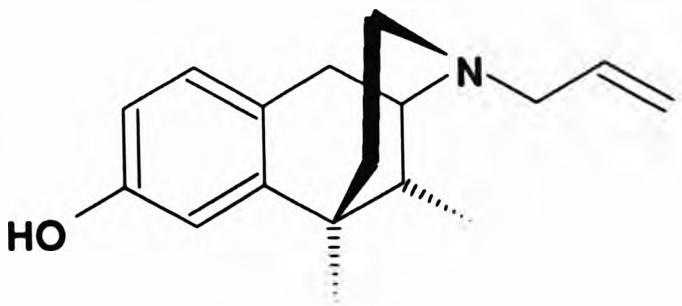
(52)

The postsynaptic action of excitatory amino acids can be blocked by analogues that compete for the particular receptor site. A wide range of glutamate analogues has been tested as potential antagonists at postsynaptic NMDA, quisqualate, kainate and AMPA receptor sites. The NMDA receptor antagonists were more efficacious than the quis/AMPA or kainate receptor antagonists. Compounds with a highly potent and selective action on the NMDA receptor, such as 2-amino-7-phosphonoheptanoic acid (AP7) (38) and

3-((+/-)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (36), are potent anticonvulsants when injected intracerebroventricularly in mice.

To be clinically useful as an anticonvulsant, an NMDA receptor antagonist must cross the blood-brain barrier, and should suppress seizures with acceptably low toxicity towards physiological systems. The early reports by Meldrum and Croucher *et al*,[113] that systemic administration of AP7 to mice, rats, and baboons eliminated seizures at doses devoid of unwanted side-effects, engendered considerable optimism for the clinical usefulness of this NMDA antagonist. Unfortunately, however, recent reports[138-142] suggest that the anticonvulsant effect of AP7 may overlap with unwanted behavioural effects. The recent identification of the more potent and selective NMDA antagonists such as CPP (36) and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine, (MK801) (43) has provided new insight into the therapeutic potential of NMDA antagonists. CPP was found to be 5-12 times more potent as an NMDA antagonist than either AP5 or AP7.[143,144]

Electrophysiological studies suggest that MK801 is a potent and noncompetitive blocker of NMDA induced responses. The only compounds shown to compete effectively at the MK801 binding site were the σ-opioid type drugs, PCP (41), SKF 10047 (53), and ketamine (42).



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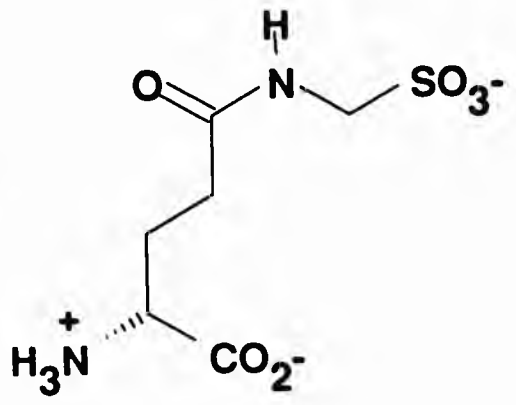


(54)

Furthermore, Foster[97] and his colleagues reported that systemic injection of MK801 protected against neurodegeneration of cerebral cells following intracerebral injection of NMDA. Some compounds with a preferential antagonist action at the kainate or quis/AMPA receptors are also anticonvulsants. Intracerebroventricular injection of γ-D-glutamyl-aminomethylsulphonate (54) and various piperazine derivatives (eg BBPP) exhibited anticonvulsant effects, although the exact receptor site at which these actions are mediated remains equivocal.[144]

It is clear from the above reports that excitatory amino acid antagonists have potential value in the search for clinically useful anticonvulsants. However, comprehensive biochemical characterization, identification, and isolation of the different excitatory amino acid receptors is urgently required for the attainment of the full therapeutic potential of such antagonists.

ADDENDUM

Recent advances in molecular biology have led to the cloning and expression of glutamate excitatory amino-acid receptors. Thus, the NMDA receptor is

now divided into two subunits, termed NMDAR-1 and NMDAR-2[145,146] The recent expression and cloning of one of the NMDA receptor subunits, NMDAR-1, by Nakanishi *et al.*[146] revealed prominent structural similarities with the classical glutamate NMDA receptor, including Ca^{2+} ion permeability, voltage-dependent blockade by Mg^{2+} ions and enhancement by glycine[145]. Its pharmacological profile was found to be similar to that of the classical NMDA receptor; for example, L-glutamate, quisqualic acid, ibotenic acid and homocysteic acid were found to be effective agonists, whilst AP5 and CPP were antagonists. In contrast, the NMDAR-2 subunit , when co-expressed with NMDAR-1, markedly potentiates responses to NMDA or glutamate, but shows no intrinsic electrophysiological response to agonist application,[147,148] which suggests that the subunit is distinctively different from NMDAR-1.

The story is further complicated by the findings of Seeburg,[1149] and Nakanishi [150], who reported the existence of at least four additional NMDA receptor subunits termed NMDAR-A to NMDAR-D. In addition, cDNA cloning of a polypeptide of a putative NMDA receptor has also been reported.[151]

The recent advances in molecular biology and the identification of a cDNA clone for the functional AMPA/kainate receptor has provided evidence of the existence of closely related subunits of the classical AMPA receptor.[152] Cloned cDNA encoding the receptors was obtained *via* the polymerase chain reaction (PCR) from which four molecular subunits termed GluR-A to GluR-D or GluR-1 to GluR-4 were fully characterized.[153,154,146]

The pharmacological profile of the recombined expressed receptors was studied[155] by ligand binding techniques, using low concentrations of [^{3}H] AMPA and [^{3}H] KA. The results demonstrated only specific [^{3}H] AMPA binding. Competitive binding to these receptors was most effective with

quisqualic acid and L-glutamic acid.

In addition, the molecular evidence for the existence of high-affinity kainate receptors was provided by the cloning of cDNA for kainate binding proteins from frog brain[156] and chick cerebellum.[157] That which was cloned from frog brain provided a high affinity binding site, but no ion channel.[145] Additional numbers of the same receptor subclass GluR-6 and GluR-7 have also been cloned.[155] GluR-6, which possesses 80% sequence identity with GluR-5, was found more predominantly in the CNS than was GluR-5. Interestingly, its mRNA distribution was found[158] to resemble that of high affinity KA-binding sites in CA3 of the hippocampus, the caudate putamens and cerebellar granule cells.

Furthermore, a high affinity KA receptor subunit termed KA-1 was isolated by Werner *et al.*[159] This clone was found to be abundant in CA3 and dentate gyrus of the hippocampal formation, consistent with the localization of certain classical high-affinity KA sites in the CNS. A new class of this subunit termed KA-2 has also been identified.[154]

Molecular cloning by cross hybridization and polymerase chain reaction (PCR)-mediated DNA amplification techniques has provided evidence of the existence of at least six subtypes of the metabotropic receptor. These were termed mGluR-1 to mGluR-6.[160-164] cDNA clones for the six different subtypes of the mGluR family were isolated by molecular screening of a rat brain cDNA library[165] The mGluR's showed distinct signal transduction *via* different G proteins and are expressed in specialized neuronal glial cells in the CNS.[166] These six mGluR's were further divided into three subgroups according to similarities in their sequences and by their agonist selectivities. Nakanishi[146] investigated the properties of these individual receptors in

Chinese hamster ovarian cells and found that mGluR-1 and mGluR-5 stimulated inositol triphosphate (IP3) formation and intracellular calcium mobilization as well as the induction of arachidonic acid release. Furthermore, these two receptors also showed the same agonist selectivity, being found to be most sensitive towards quisqualic acid.[146,167] However, these receptors differ slightly in their sensitivity towards pertussis toxin (PTX). In contrast, mGluR-2 is linked to the inhibitory cAMP cascade[159], and at mGluR-4 receptors, AP4 is the most potent agonist, being one order of magnitude more potent than L-glutamic acid.[167-169] Furthermore, mGluR's-2,3 and 4 were found to be more sensitive to PTX, which suggests that these receptors are coupled to PTX-sensitive G (Gi) proteins.

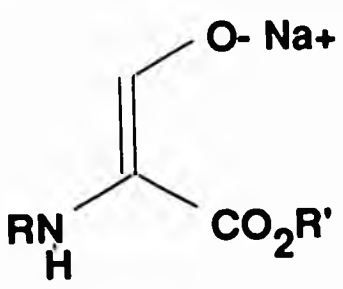
CHAPTER TWO.

2. CHEMISTRY DISCUSSION.

2.1. INTRODUCTION.

The search for new glutamate agonists and antagonists has prompted many chemists to synthesize various novel amino acids. The last ten years saw an explosion in the field of amino acid research, with particular interest being focused on the glutamate agonist, quisqualic acid. This area of research was initiated by the observation by Shinozaki *et al.*,[23] who discovered that quisqualic acid had powerful neuroexcitant properties.

In 1980 Bycroft and his colleagues commenced a synthetic strategy aimed at developing structure activity relationships at the quisqualate receptor.[170] These workers developed the methodology by which quisqualic acid was synthesized, in its racemic form. The key intermediates which were developed at this time include the enolate (2:1), and the tosylate (2:2) derivatives. While the tosylate undergoes displacement by nucleophiles, yielding compounds of the types (2:3), the enolate undergoes condensation reaction with hydroxylamine salts yielding compounds such as (2:4). Both intermediates have been employed in the synthesis of DL-quisqualic acid and related analogues.



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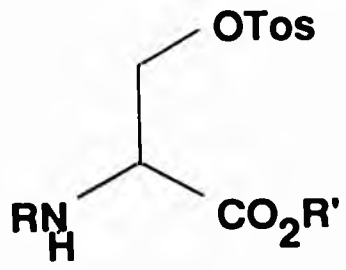

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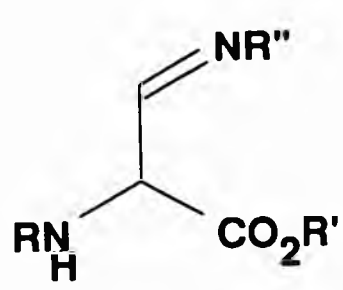

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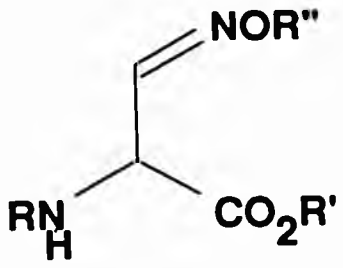


(2:4)

The principal question addressed in this research programme concerned the functional group requirements for the maintenance of agonist and/or antagonist activity and the development of specific mimetics which act only at one defined receptor (the quisqualate receptor being the receptor of interest).

Receptor models focused on the question of whether two or all three of the functional groups in the amino acid moiety were involved in the binding of the amino acid to its receptor sites. The alternative models proposed by Curtis *et al.*[7] for the glutamate receptor comprised a two point receptor (possessing a positive and a negative site of attachment) and a three point receptor in which there are two positively-charged and one negatively-charged binding sites. The question of whether a two or a three point receptor is responsible for the mediation of quisqualate responses remains

unclear, possibly due to the unavailability of ^{3}H agonists and/or antagonists. It was on this basis that the decision was made to search for synthetic methodology by which tritiated quisqualic acid may be synthesized.

In order to evaluate potential changes in the quisqualate moiety with respect to synthetic targets, it is convenient at this point to consider the information available in terms of structure activity relationships at the quisqualate receptor. To date however, except for preliminary reports by Honore,[75] who synthesized a selective antagonist (DNQX) (a non-amino acid), only a few analogues of quisqualic acid have been synthesized and tested for their pharmacological efficacies; notably Bycroft's substituted hydantoin (86), which showed no activity at the quisqualate receptor, and glutamate diethyl ester (32), a non-selective antagonist. The lack of prior investigation into the synthesis of quisqualic acid analogues and the paucity of data with regards to structure-activity relationships allows the chemist considerable scope in the search for novel quisqualic acid analogues

2.1.1. AIMS AND OBJECTIVES.

Although the identity of quisqualic acid has been known for a long time, very little progress has been made in the study of its receptor, possibly due to the lack of tritiated quisqualic acid and the unavailability of selective antagonists at the quisqualate receptor. From the foregoing introduction, it is clear that of prime importance in the study of the quisqualate receptor is the availability of labelled quisqualic acid. Initial efforts were therefore directed

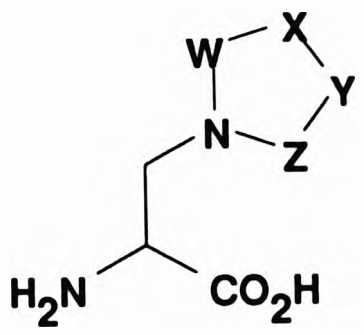
towards the development of the methodology by which labelled quisqualic acid may be synthesized.

Even though the Ferrosan compounds (46) and (47) exhibited selectivity in their action as antagonists, it was decided that quisqualic acid analogues (i.e compounds possessing the heterocyclic ring systems and the propionic side chain) should be synthesized. The reason for this was that whereas the Ferrosan compounds, non-amino acids, are selective only at the AMPA receptor, analogues which are structurally related to quisqualic acid may act not only at the AMPA site, but also at the newly defined metabotropic receptor site. Futhermore, since quisqualic acid is a structural analogue of glutamic acid, analogues of quisqualic acid may also show some activity at the NMDA receptor.

It was therefore decided that three types of quisqualic acid analogues would be synthesized and tested for their pharmacological effiects. Firstly, modification of the heterocyclic side-chain of the amino acid would provide a series of β-functionalized L-alanines, such as (2:5) and (2:6).

Secondly, protection of the α-amino or carboxylate function of quisqualic acid, or peptide coupling, would provide analogues wherein one or other of these groups was no longer charged, for example, dipeptides such as (2:7).

Thirdly, the acidic NH of the quisqualic acid heterocyclic side-chain would be alkylated to give compounds such as (2.8).



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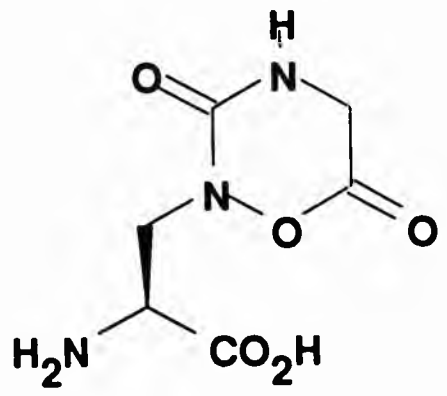

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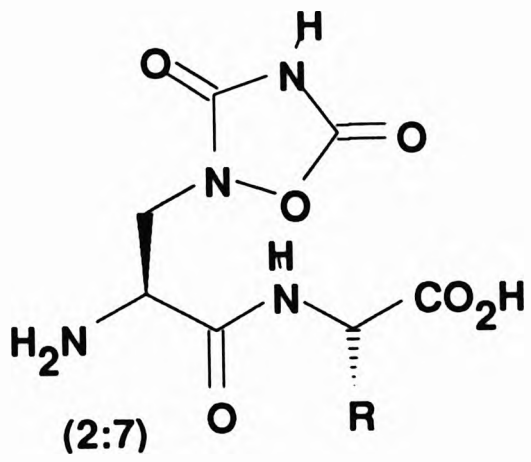

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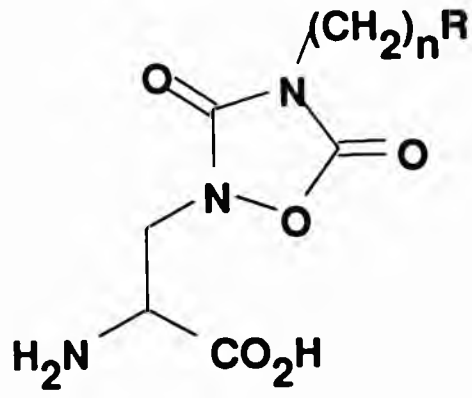


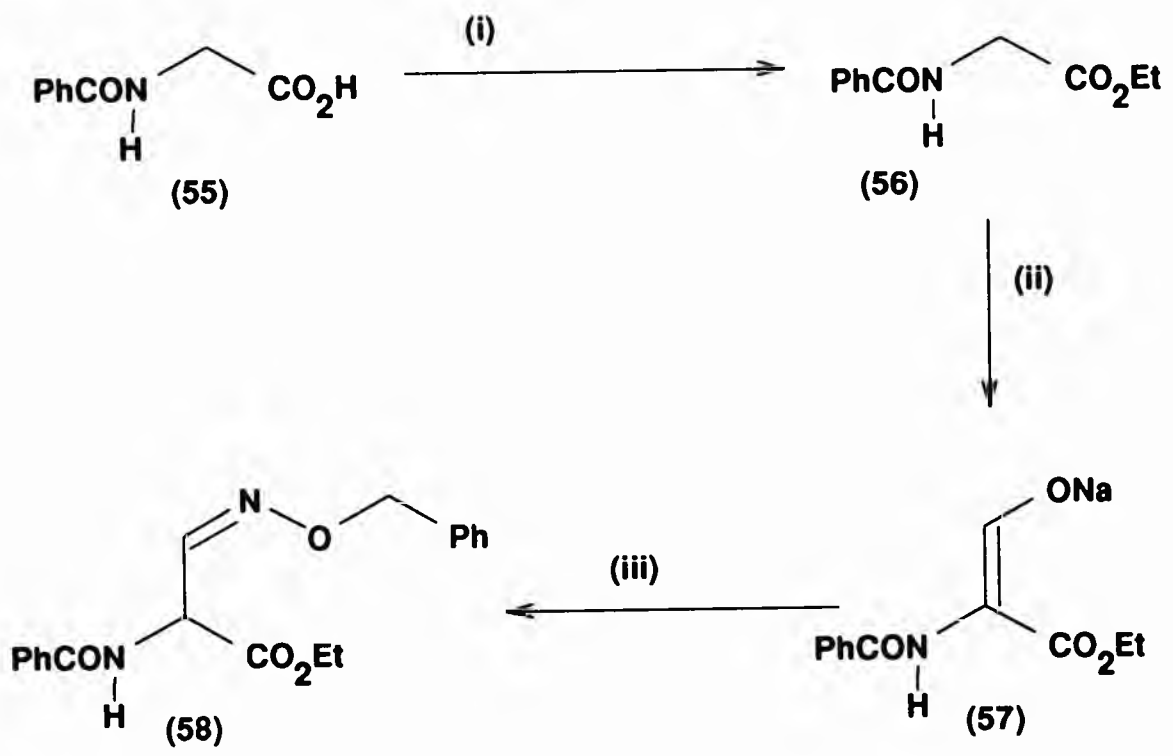
(2:8)

Pharmacological evaluation of these compounds should hopefully allow some conclusion to be reached regarding the functional group requirements of the quisqualate receptor.

2.2. SYNTHESIS OF DL-QUISQUALIC ACID.

The synthetic strategy adopted for the work reported here was based on the synthon approach developed by Bycroft[170] for the synthesis of DL-quisqualic acid. This approach utilizes benzoyl protected glycine (55) which was converted to the ethyl ester (56) (scheme 1), by acid-catalyzed esterification. The ester, a white solid, was obtained from extractive workup in good yield (98%). Its physical and spectroscopic properties were consistent with the proposed structure.

Scheme 1.



Reagents: (i) *para*-toluenesulphonic acid, EtOH, 20°C

(ii) HCO_2Et, NaOEt, EtOH

(iii) $PhCH_2ONH_2$.HCl, DCM

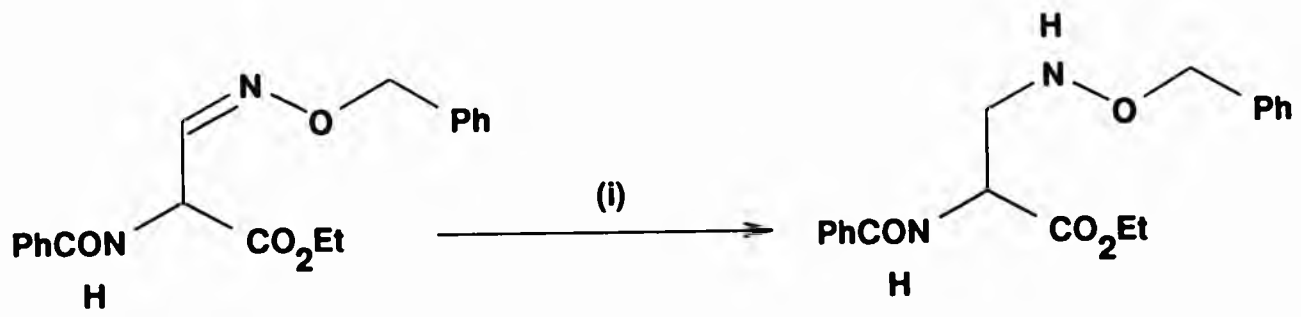
Condensation of the ester (56) with ethyl formate and sodium ethoxide afforded N-benzoyl-2,3-dehydroserine as its sodium enolate (57). Examination of the ^1H NMR spectrum showed the olefinic proton in the aromatic region at δ 8.6, with

concomitant loss of the methylene signals. Structural assignment was completed by the mass spectrum which exhibited an $[M+H]^+$ at $^m/_e$ 252.

Condensation of the sodium enolate (57) with O-benzylhydroxylamine hydrochloride in a two phase system (water/dichloromethane) gave the benzyl oxime (58) in 63% yield, following flash chromatography. The ^1H NMR spectrum of (58) exhibited resonances due to the benzyl group at δ 5.1 (CH_2Ph), and δ 7.3. The presence of the methine double doublet at δ 5.41, and the olefinic proton of the oxime at δ 7.65, suggested that the sodium salt (57) had in fact been converted to the protected oxime (58). The mass spectrum exhibited a molecular ion at $^m/_e$ 340 which was consistent with the structure.

Sodium cyanoborohydride reduction of the O-benzyloxime (58) in methanolic HCl at pH 3-4 afforded the protected hydroxylamine (59), The reaction was stirred overnight to ensure complete reduction. The yield after extractive workup and flash chromatography was 66%. The absence of any absorption assignable to the oxime in the infrared spectrum of the product, and the absence of the olefinic proton in the ^1H NMR, together with the presence of a methylene multiplet at δ 3.48, and an upfield shift of the methine signals from δ 5.14 in the oxime (58) to δ 4.87 in the benzyloxyamine (59), confirmed the structure.

Scheme 2.



(58) (59)

Reagents: (i) $NaBH_3CN$, MeOH, pH3-4

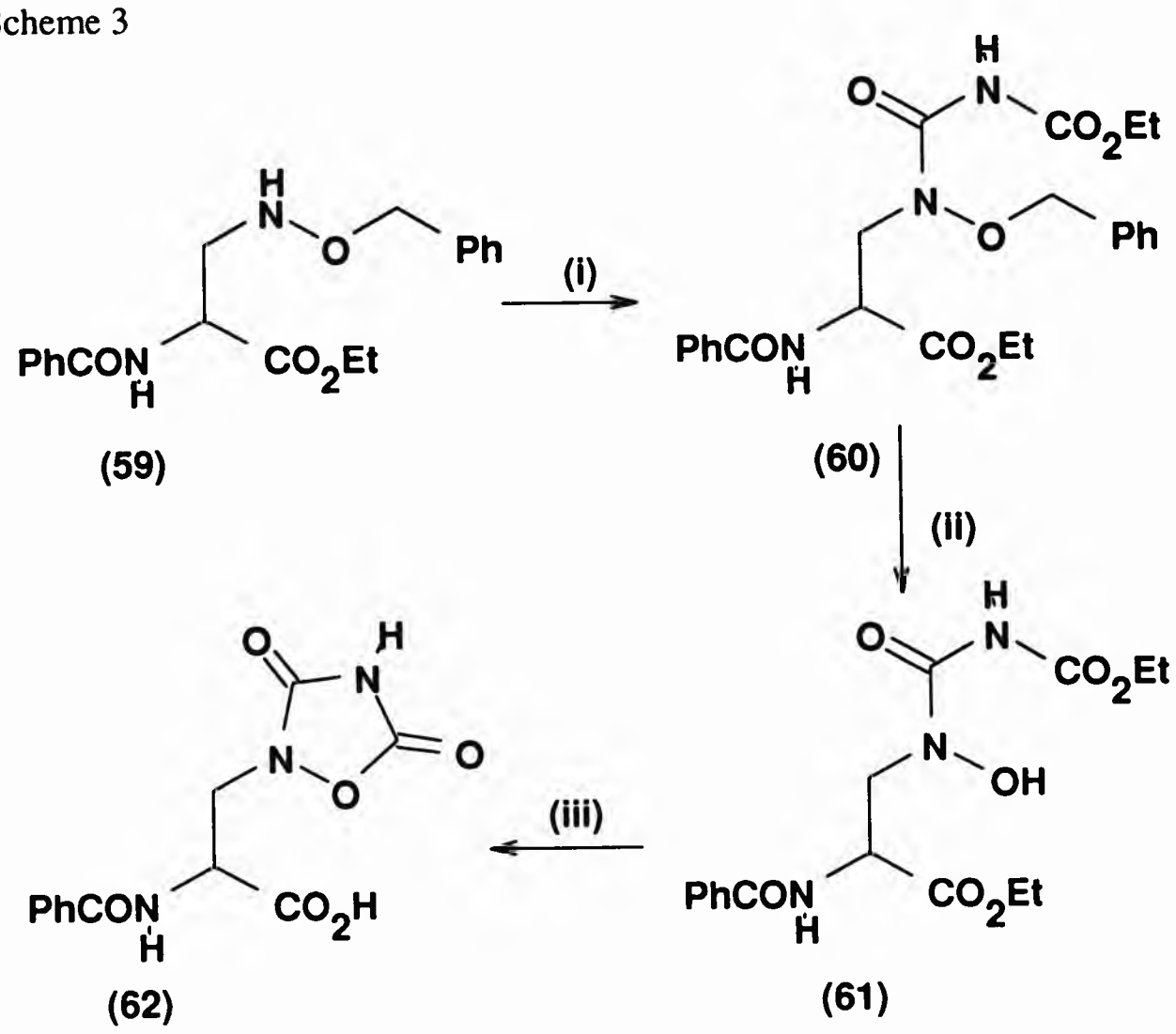
The importance of the pH of the reaction deserves some comment. The optimum pH for cyanoborohydride reduction of oximes is pH 3-4. This ensures that the oxime nitrogen remains protonated, and is therefore more susceptible to nucleophilic attack by the borohydride ion. At higher pH, reduction is often ineffective because the oxime nitrogen is unprotonated, whilst at lower pH the borohydride is solvolyzed.[171]

2.2.1. Construction of the oxadiazolidine ring.

This was initiated by the elaboration of the benzyloxylamine (59). The amine was condensed with ethoxycarbonyl isocyanate in dry ether to afford the N-benzyloxyureide (60) as an amorphous solid in good yield (68%) (scheme 3). The base peak in the mass spectrum was the molecular ion ($^{m}/_{e}$ 457).

The protected hydroxyurea (60) was debenzylated in ethanol, by facile catalytic hydrogenation over palladium on activated charcoal. Recrystallization from diethyl ether/ethanol afforded (61) in 74% yield. The loss of the benzylic protons in the ^{1}H NMR spectrum of the product showed that deprotection of the N-hydroxyl group had occurred.

Scheme 3



Reagents: (i) EtO_2CNCO, Et_2O

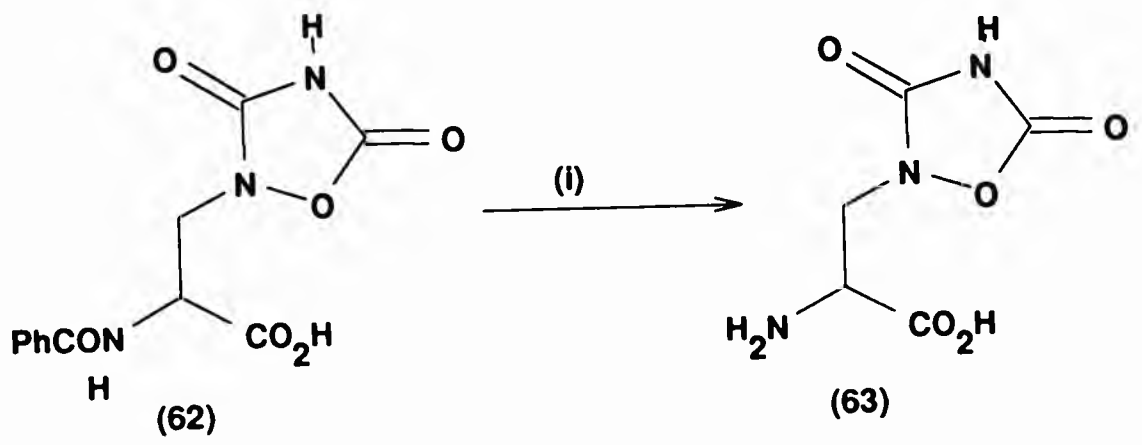
(ii) H_2, Pd-C, EtOH

(iii) 2eqv. NaOH, then HCl aq.

Treatment of the hydroxyurea (61) with two equivalents of sodium hydroxide effected both cyclization to the oxadiazolidinedione, and hydrolysis of the ethyl ester. The white solid, which precipitated on acidification with concentrated HCl, was recrystallized from water and ethanol to afford N-benzoylquisqualic acid (62) in 88% yield. The absence of ethyl signals in its ^1H NMR spectrum, and an $[M+H]^+$ at $^m/_e$ 294 in the mass spectrum, confirmed the structure (scheme 3).

With the isolation of the benzoyl protected cyclized material, all that remained was removal of the N-protecting group to give the racemic compound. To this end, treatment of (62) with 6M HCl followed by ion exchange chromatography afforded the amino acid (63) in 35% yield. The low yield of the final product may be attributable to the vigorous conditions required for the removal of the benzoyl protecting group. An interesting feature of the NMR spectrum of the product (in $NaOD/D_2O$) was an obvious upfield shift of the methine signal relative to that of the methylene protons, whereas in DCl/D_2O, the methine signal was downfield of the methylene signal.

Scheme 4.



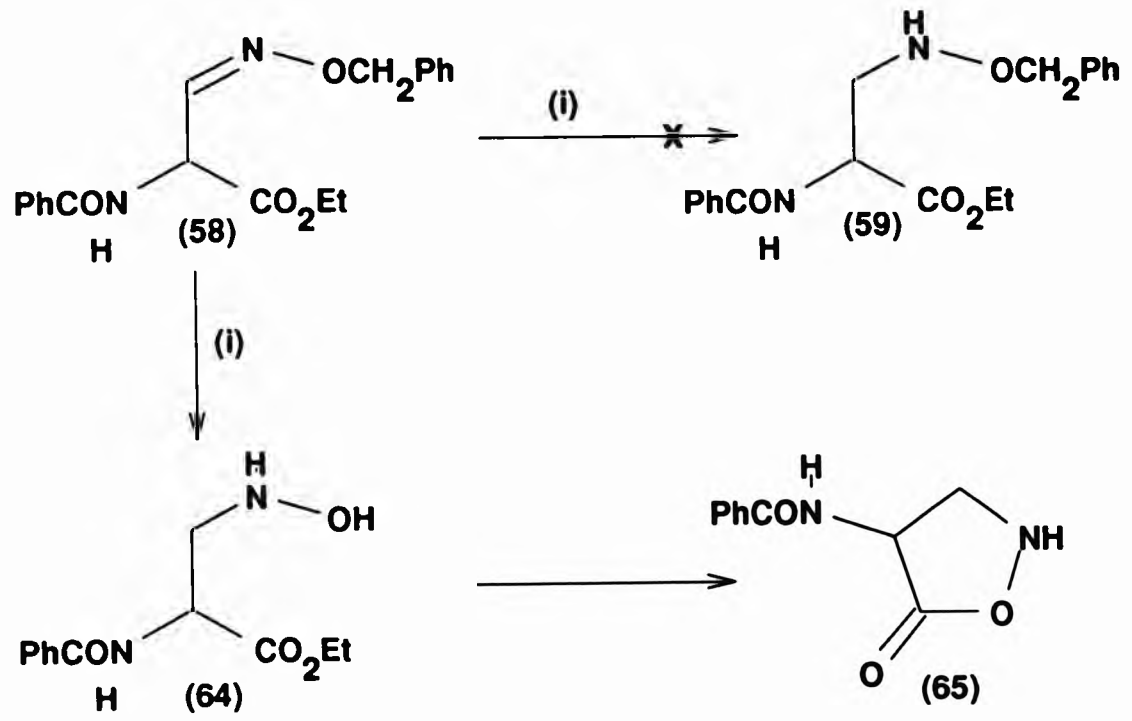
Reagents: (i) 6N HCl, reflux

2.2.2. Methodology for the synthesis of "tritiated" quisqualic acid.

Initial attempts at synthesis were focused on the methodology by which DL-quisqualic acid (63) was prepared, using the intermediate benzyloxime (58). The possibility of double bond reduction by facile catalytic hydrogenation to give the corresponding protected hydroxylamine derivative (59) was

considered (scheme 5). Substitution of tritium gas for hydrogen in such a reaction would provide a convenient method of incorporating a label into quisqualic acid moiety. However, the O-benzyl protecting group would be unlikely to survive under such conditions.

Scheme 5



Reagents: (i) H_2, Pd-C, EtOH

Thus, in a model reaction, catalytic hydrogenation of the oxime (58) did not produce the desired benzyloxylamine (59), but resulted in the loss of the O-benzyl group to give (64). Under basic conditions this would simply cyclize to a five membered ring system (65) (scheme 5).

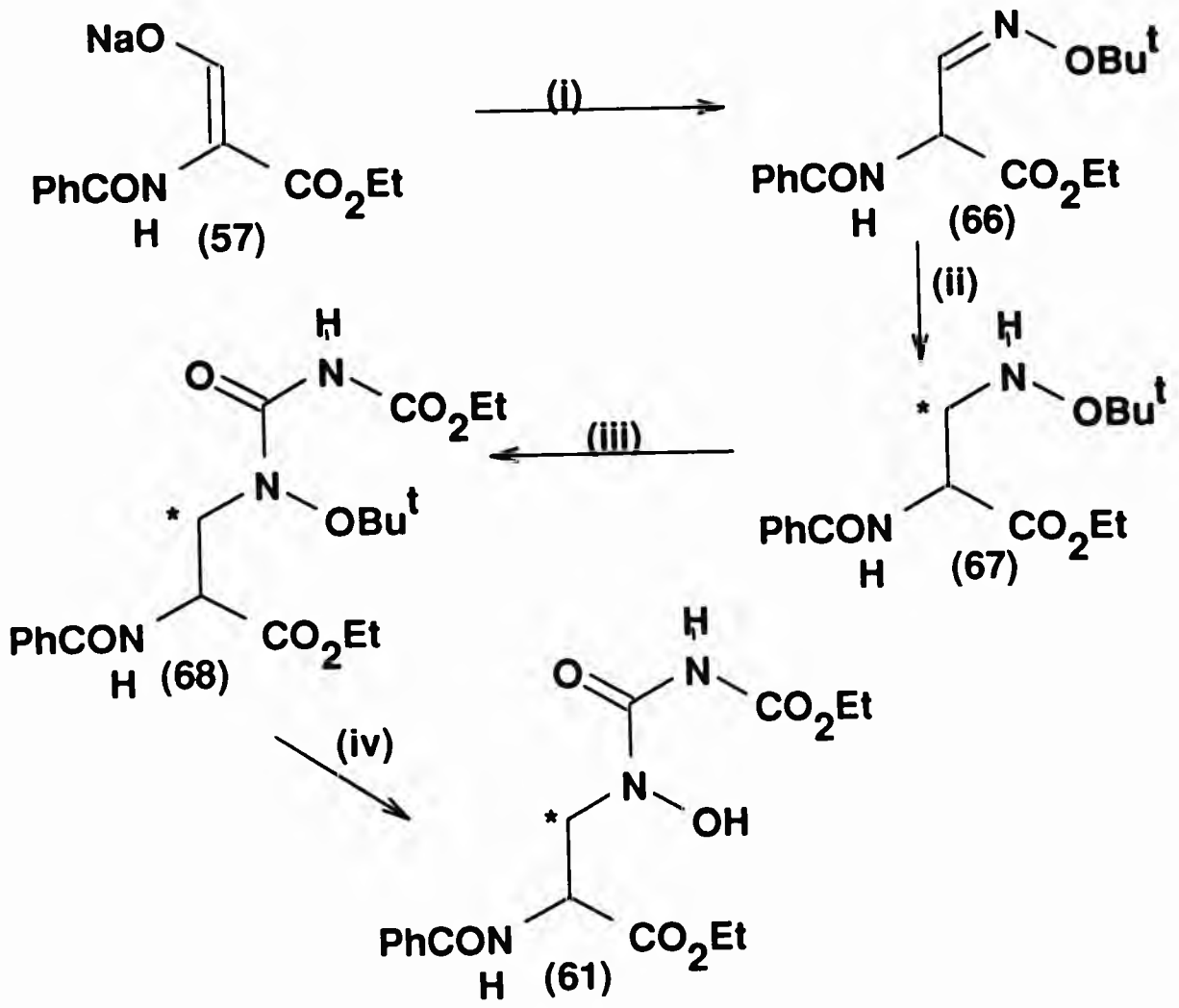
In order to circumvent this problem, a new starting material was sought which would incorporate a protecting group stable to catalytic hydrogenation over palladium. The starting material of choice was the O-t-butyloxime (66), prepared by condensation of the sodium enolate (57) with tert-butylhydroxylamine hydrochloride in a water/dichloromethane mixture. The protected oxime (66), a white solid, was obtained from extractive workup

and flash chromatography in good yield (62%). The base peak in the mass spectrum was the molecular ion ($^{m}/_{e}$ 306). The absence of the olefinic proton, and the presence of the a nine proton singlet in its ^{1}H NMR spectrum were consistent with the proposed structure.

The method chosen for the insertion of the "tritium" atom was *via* hydrogenation of the t-butyl protected oxime (66) Thus the oxime was dissolved in a mixture of acetic acid (95%) and concentrated sulphuric acid (5%). The reaction mixture was hydrogenated over palladium on activated charcoal for two hours. Extractive workup gave the t-butyloxylamine (67) in 40% yield. (scheme 6). The upfield shift of the methine proton in its ^{1}H NMR spectrum from δ 5.4 in (55) to δ 4.8 in the t-butyloxylamine, coupled with the appearance of a signal assignable to the methylene protons, suggested that the oxime had been reduced. In principle, tritium gas could replace hydrogen in this reaction.

The importance of the concentrated sulphuric acid concentration in this reaction is noteworthy. Any increase in the concentration of sulphuric acid to greater than 5% results in the acidolysis of the t-butyl group. The yield can be improved by the use of 2-3% concentrated sulphuric acid. In the absence of concentrated sulphuric acid, the reduction fails. (scheme 6)

Scheme 6.



Reagents: (i) $Bu^tONH_2.HCl$, H_2O-DCM

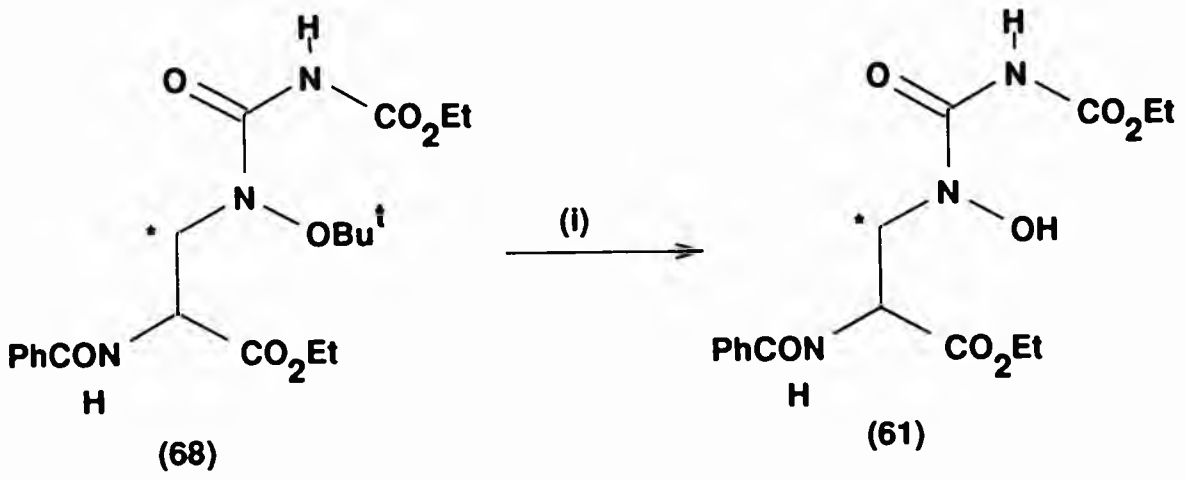
(ii) AcOH, H_2SO_4, Pd-C, H_2 (or $^3H_2$)

(iii) EtO_2CNCO, Et_2O

(iv) TFA

(* denotes tritium label)

Treatment of the reduced system (67) with ethoxycarbonyl isocyanate in dry ether gave the t-butyloxyurea (68), as an amorphous solid, the structure of which was confirmed by ^1H NMR and mass spectrometry.



Scheme 7.

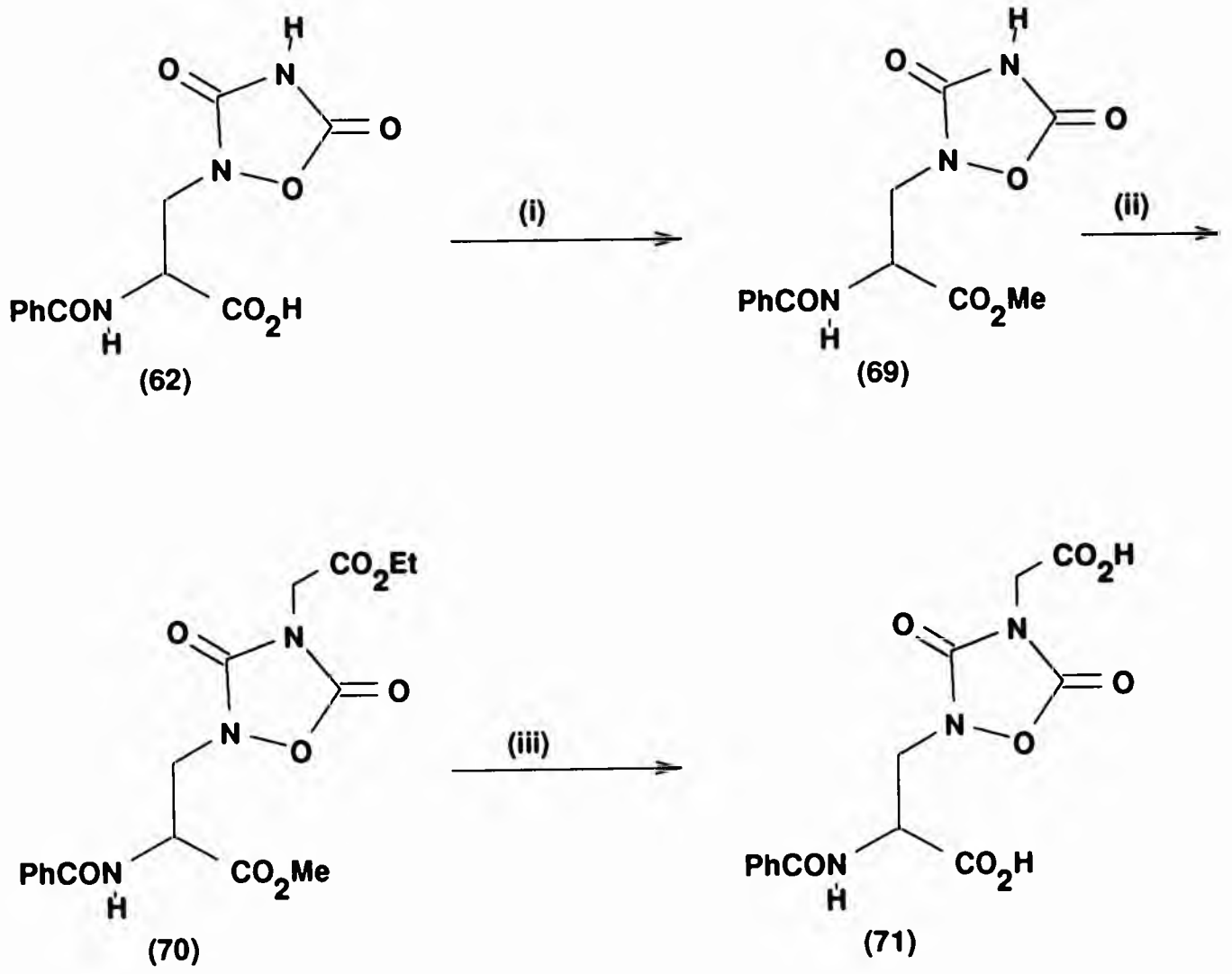
Reagents: (i) 1N HCl in AcOH

Acidolysis of the t-butyl group was effected by treatment of (68) with 1M hydrogen chloride in acetic acid. After one hour at room temperature, and extractive work up, the hydroxyurea (61) was isolated in 52% yield. The loss of the t-butyl group was evident from the disappearance of the signal at δ 1.28 in the ^{1}H NMR spectrum, and an M^{+}. at $^{m}/_{e}$ 367 in its mass spectrum confirmed the structure. Cyclization in sodium hydroxide, and hydrolysis of the ethyl ester groups, as outlined in scheme 5, gave the protected amino acid, which, upon hydrolysis in 1M hydrogen chloride in acetic acid, followed by ion exchange chromatography, afforded DL-quisqualic acid (63).

It should be added that, to date, this is still the only reported procedure by which a tritium label may be incoporated into the quisqualate moiety. The availability of labelled quisqualic acid should facilitate the study of the quisqualate receptor.

2.3. Attempted synthesis of N–ω-carboxymethyl DL-quisqualic acid (72).

The alkylation of quisqualic acid was initiated by the esterification of the benzoyl protected amino acid (62) which was converted to its methyl ester (69) by treatment with thionyl chloride in methanol (scheme 8). The structure of (69) was confirmed by spectroscopic characterization.



Scheme 8.

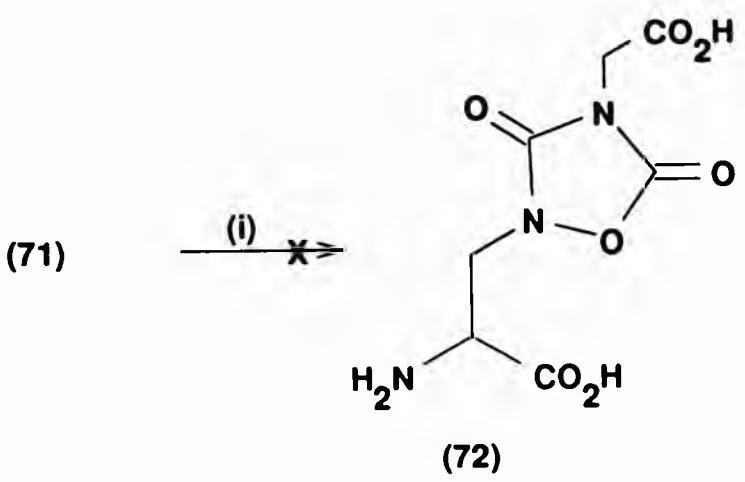
Reagents: (i) $SOCl_2$, MeOH

(ii) NaH, $BrCH_2CO_2Et$, DMF, 50°C

(iii) NaOH aq.

Base induced alkylation of the heterocyclic imide with ethyl bromoacetate in DMF at 50°C produced the alkyl derivative (70) after chromatographic purification on silica. The spectroscopic characteristics of this material were consistent with its being the desired product.

Treatment of this material with aqueous sodium hydroxide and subsequent acidification with 2M HCl gave the diacid (71), which upon hydrolysis in refluxing 6M HCl gave what appears to be a decomposed material..



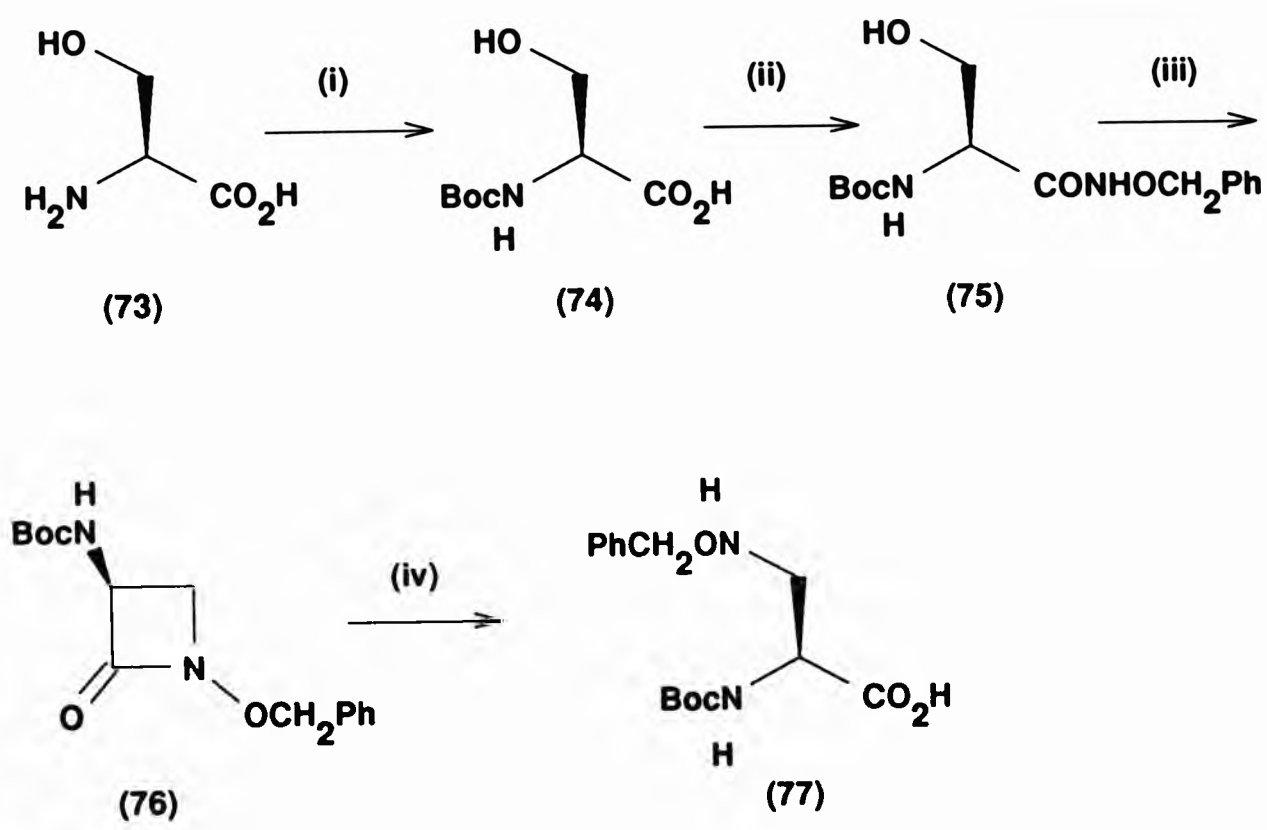
Scheme 9.

Reagents: (i) 6N HCl, reflux

2.4 SYNTHESIS OF L-QUISQUALIC ACID.

The synthetic approach to L-quisqualic acid was based on that developed by Mattingly and Miller[172] for the synthesis of 3-(tert-butoxycarbonyl)amino-1-benzyloxy-2-azetidinone (76). This synthon, which possesses a masked α-amino acid unit, was synthesized from L-serine (73). The synthesis of (76) based on this approach is illustrated in scheme 6. The initial step in the synthesis of quisqualic acid entails the conversion of L-serine to its N-t-butoxycarbonyl derivative (74) by known methodology.[173] The amino group of serine was protected using di-tert-butyldicarbonate ($(Boc)_2O$) in

isopropanol and aqueous sodium hydroxide at room temperature. The N-t-Boc derivative was obtained as a monohydrate, in yields often in excess of 85%. The ^1H NMR spectrum of (74) confirmed that the t-Boc group had been incorporated (singlet at δ 1.4). (Scheme 10).



Scheme 10.

Reagents: (i) $(Boc)_2O$, isopropanol, NaOH aq.
(ii) Bu^iOCOCl, NMM, THF,-25°C, 2 min., then $PhCH_2ONH_2.HCl$, NMM,THF,-15°C, 2hrs.
(iii) DEAD, TPP, THF,-78°C, then r.t., over night.
(iv) NaOH, aq. MeOH, r.t., 2hrs.

Acylation of O-benzylhydroxylamine hydrochloride with the activated N-t-Boc protected acid, which was carried out by the repetitive excess mixed anhydride (REMA) method,[174] proceeded smoothly in quantitative yield.

Thus t-Boc protected serine (74) was treated with one equivalent of isobutyl chloroformate and N-methylmorpholine in dry THF to give the mixed anhydride, which then reacted with O-benzylhydroxylamine to afford the hydroxamate (75), in an improved yield compared to that obtained by activation with EDC.[172] The reaction was carried out under anhydrous conditions, since the presence of water would have prevented the formation of the mixed anhydride, rendering the desired product unobtainable, or obtainable only in very low yield. To this end, the monohydrated t-Boc protected serine was dehydrated by azeotropic removal of water with toluene, prior to formation of the mixed anhydride. The reaction proceeded without racemization, presumably because the intermediate azlactone (formed after activation of the acid function) was configurationally stable under the reaction conditions, unlike the azlactones formed from amide-protected amino acids.[175] The benzyl protons in its ^{1}H NMR spectrum appeared at δ 5.1 and δ 7.3 respectively, and the characteristic amide signal occurred at δ 9.0. This, coupled with an $[M+H]^+$ (m/e 311) in the mass spectrum, confirmed the structure.

The optically pure hydroxamate was converted to the β-lactam (76) by the modified Mitsunobu[176] reaction (scheme 10). Thus, addition of the hydroxamate (75) to a preformed adduct of triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD) at -78°C afforded the desired product (76) in an isolated yield of 62%. The reaction was carried out under anhydrous conditions in dry THF under nitrogen to prevent hydrolysis of the preformed betaine. Thus TPP was dried over phosphorus pentoxide for seventy-two hours, and care was taken to ensure that the DEAD was not unnecessarily exposed to atmospheric moisture whilst being weighed.

Having stirred the reaction mixture at 25°C overnight, the crude product was preabsorbed on silica and purified by flash chromatography with ethyl acetate/hexane as the eluent. Isolation of the pure β-lactam (76) was complicated by the close similarity in polarity of the by-product diethyl hydrazinodicarboxylate. The FAB mass spectrum, with an $[M+H]^+$ at $^m/_e$ 293, and the characteristic carbonyl stretch (ν_{CO}=1720cm^{-1}) in the infrared, confirmed that the product was the desired β-lactam.

The β-lactam (76) was smoothly hydrolyzed to the hydroxylamino acid (77) by treatment with one equivalent of sodium hydroxide. The product was isolated after acidification with a saturated aqueous solution of citric acid. Care was taken to ensure that only one equivalent of base was used, since an excess of base would increase the risk of racemization, as suggested by Baldwin[177]. It was subsequently shown that racemization had not occurred during the base hydrolysis of the β-lactam (or at any at other stage), as the final product of the synthesis, L-quisqualic acid, had the same optical rotation as that of authentic L-quisqualic acid[177]. The mass spectrum of the product exhibited an $[M+H]^+$ at $^m/_e$ 311. Final confirmation of the product came from the infrared spectrum which demonstrated that the strained β-lactam had been converted to the hydroxylamino acid by the lowering of the carbonyl stretching frequency (ν_{CO} = 1650 cm^{-1}, compared with 1720cm^{-1} for the β-lactam).

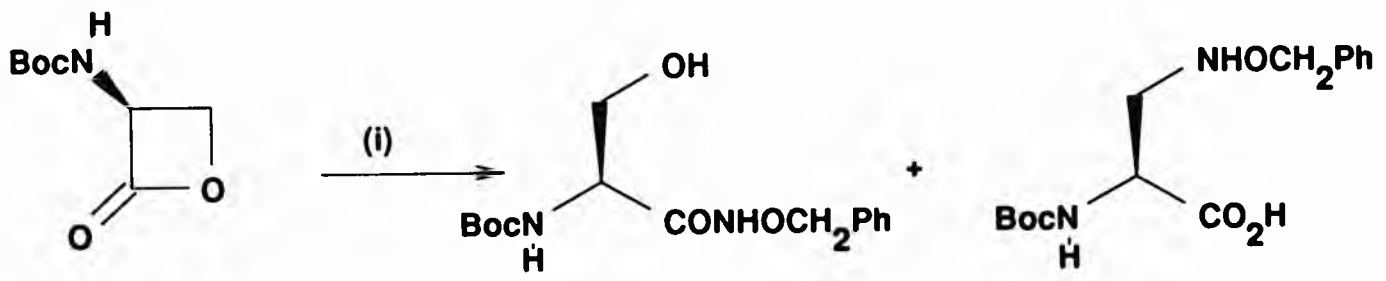
During the development of the L-quisqualic acid synthesis, the overriding problem which presented itself was the possibility that racemization might occur during the base induced hydrolytic opening of the β-lactam ring. Having taken the necessary precautions to circumvent this problem, by using a minimum amount of base for the ring-opening reaction, an alternative

approach to the hydroxylamino acid (77), which would minimize the risk of racemization, was considered.

A search of the literature led to a paper by Arnold *et al.*[176] which described a method whereby N-protected α-amino acid -β-lactones (such as (78)) may be opened with alkylation using appropriate nucleophiles to give the corresponding β-substituted alanine derivatives.

Thus a new synthetic methodology was devised by which enantiomerically pure L-quisqualic acid (18) may be obtained, by the alkylation of O-benzylhydroxylamine hydrochloride with the N-protected α-amino-β-lactone (78) (scheme 11).

Scheme 11



(78) (75) (77)

t = 0° or r.t.	100%	0%
t = 80°	40%	60%

Reagents: (i) $PhCH_2ONH_2$, MeCN

N-t-Boc serine (74) was converted to the β-lactone derivative (78), according to the literature precedent of Vederas *et al.*[178] by the modified

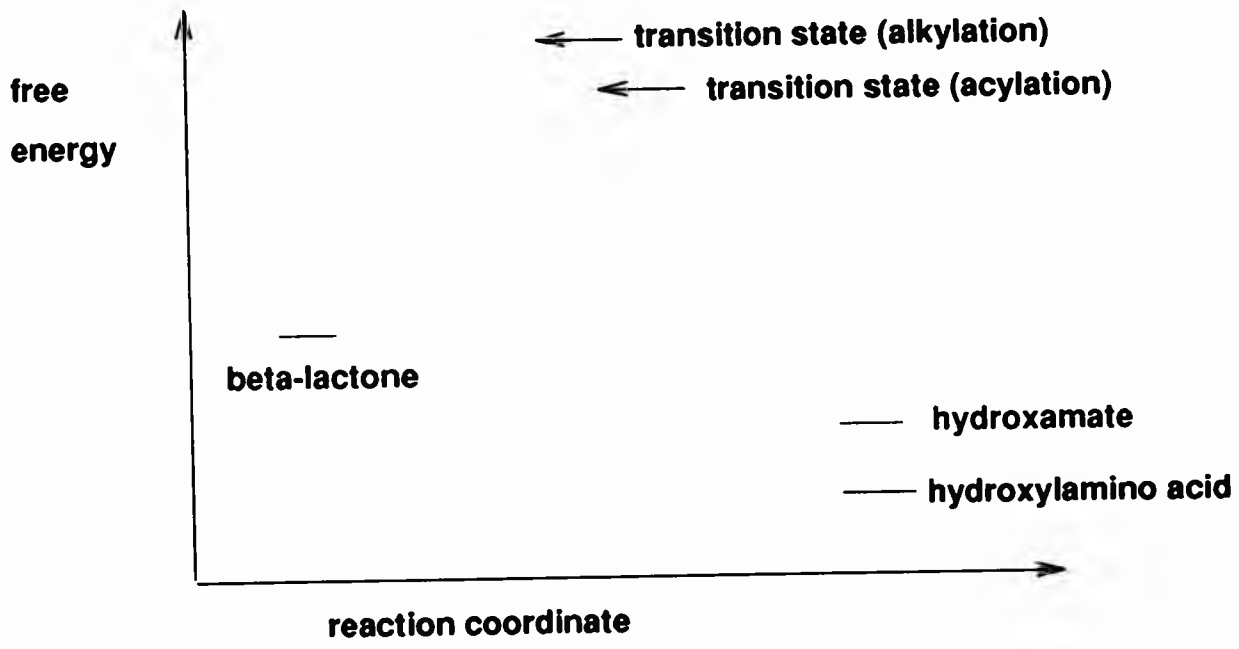
Mitsunobu reaction using triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD). The β–lactone was obtained in good yield (88%). An examination of the ^{1}H NMR spectrum revealed a downfield shift of the methylene signal from δ 3.7-4.1 in the t-Boc protected serine to δ 4.4 in the β-lactone. The methine resonance appeared at higher field δ 5.1. The FAB mass spectrum exhibited an $[M+H]^{+}$ at the required value of $^{m}/_{e}$ 188. The elemental analysis of the product was consistent with the desired structure.

Using O-benzylhydroxylamine as the nucleophile, several attempts were made to convert the β-lactone in THF to the corresponding hydroxylamino acid (77). These reactions invariably gave the hydroxamate (75), rather than the desired hydroxylamino acid derivative (77) (scheme 11). Temperature and solvent variation also afforded the acylation product (75). However, at 40°C in acetonitrile, thin layer chromatography (tlc) showed a mixture of two products, the more polar of which corresponded to the R_f of the hydroxylamino acid (77) obtained by the facile hydrolytic ring opening of the β-lactam (76).

Attempts to isolate this polar material by flash chromatography failed, nevertheless it was decided that the reaction should be repeated at a higher temperature. Thus the reaction mixture was heated under reflux (80°C) in acetonitrile. Tlc once again demonstrated the presence of two main products, which corresponded to the hydroxamate, (75) and the hydroxylamino acid (77). These were separated by extractive workup followed by chromatographic purification, and isolated in 40% and 60% yield respectively. The spectroscopic characteristics and melting point of the less polar product were found to be indistinguishable from those of the

hydroxamate (75) which was prepared directly from t-Boc-L-serine. Similarly, the more polar product exhibited identical physical and spectroscopic properties to those of the hydroxylamino acid (77). A possible explanation for this phenomenon may be attributed to the differences in the activation energies for formation of these two compounds. Thus, at lower temperatures, the acylation was favoured, whilst at higher temperatures the alkylation reaction was predominant. (fig 2.1)

Fig. 2.1

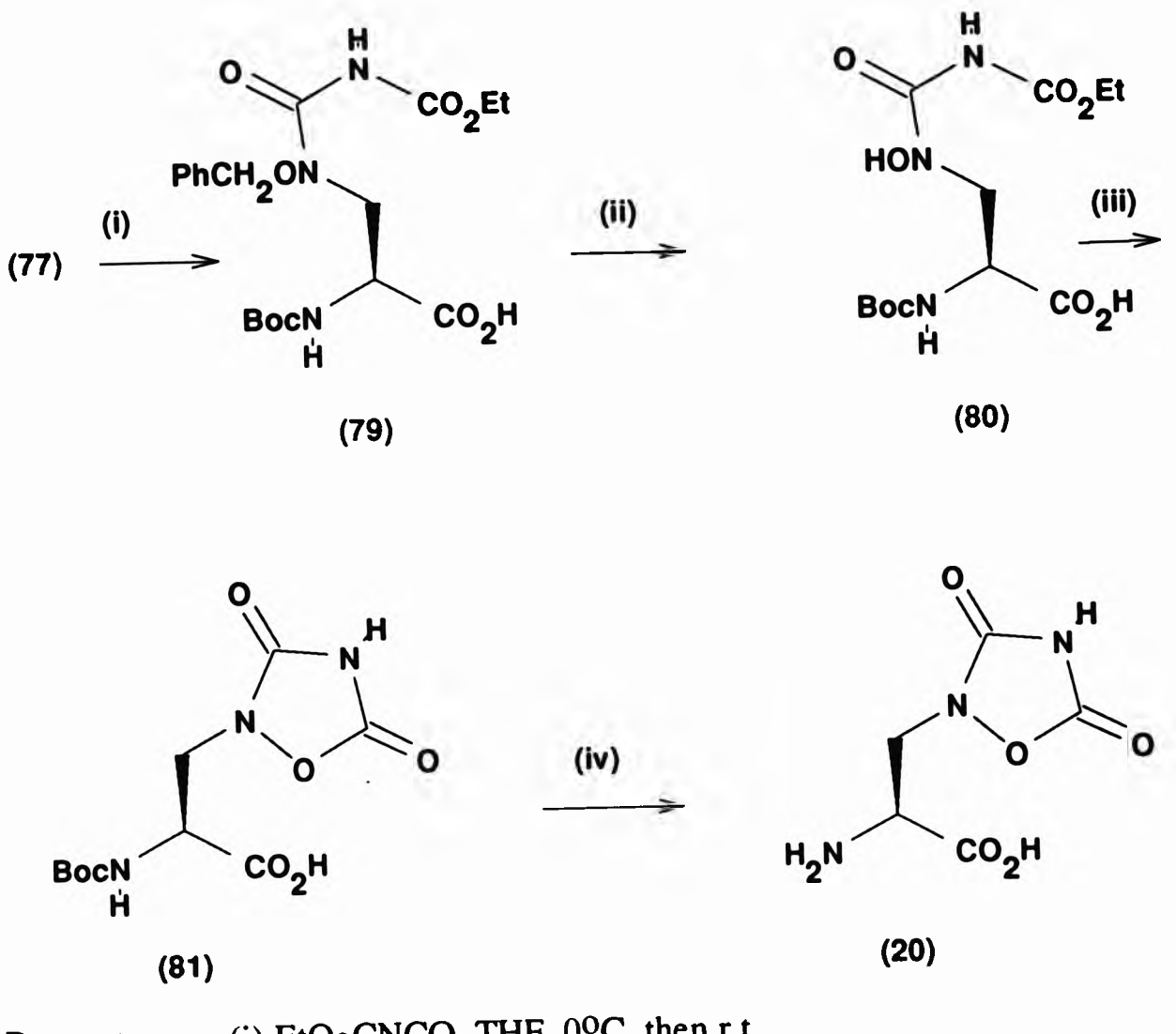


Alkylation of O-benzylhydroxylamine with the β-lactone (78) to give the benzyloxylamine derivative (77) has not been previously reported. Although the nucleophilic ring opening gave both the acyl and the alkyl derivative, this approach provides an alternative method by which L-quisqualic acid may be synthesized.

Condensation of the benzyloxylamine (77) with ethoxycarbonyl isocyanate in dry THF gave the substituted urea (79) (scheme 12). This was debenzylated to afford the corresponding N-hydroxy derivative (80) by

facile catalytic hydrogenolysis over palladium on activated charcoal in ethanol. The absence of the benzyl protons in the NMR spectrum, and an $[M+H]^+$ at $^m/_e$ 336 in the FAB mass spectrum, were consistent with the proposed structure.

Scheme 12



Reagents: (i) EtO_2CNCO, THF, 0^oC, then r.t.

(ii) H_2, Pd-C, EtOH, 1hr

(iii) NaOH aq. (2 eq.)

(iv) (a) 1N HCl in AcOH, (b) ion-exchange

Ring closure to form the oxadiazolidinedione (81), (t-Boc protected L-

quisqualic acid), was effected by cyclization with sodium hydroxide. (scheme 12).

The product (81) was isolated in good yield (58%) from extractive workup, after acidification. The absence of the ester signals in the ^1H NMR spectrum, and the upfield shift in the signals due to the side chain methylene protons, suggested that the cyclized derivative (81) had been isolated.

Acidolysis of the t-Boc protected L-quisqualic acid (81) in acetic acid containing 1M hydrogen chloride, followed by ion exchange chromatography, afforded L-quisqualic acid (20), in good yield (scheme 8). The physical and spectroscopic properties of the amino acid (20) were identical to those reported for L-quisqualic acid.[177]

2.5. SYNTHESIS OF SOME ANALOGUES OF QUISQUALIC ACID.

Quisqualic acid is a potent analogue of the excitatory neurotransmitter L-glutamic acid, exhibiting differing neuronal sensitivities in different regions of central nervous system. For example, it is excitatory in the cortex, and inactive in the spinal neurones The amino acid and its analogues are therefore of potential pharmacological significance. However, if the full potential of quisqualic acid activities is to be realized, especially with respect to receptor characterization and identification, extensive studies would have to be undertaken.

It was therefore considered that a worthy objective would be the synthesis of analogues of quisqualic acid, in order to assess the influence of structural modification on its neuropharmacological properties. In very simple terms, the following types of modifications seemed to present reasonable synthetic

targets, with a realistic possibility that their synthesis could be achieved:

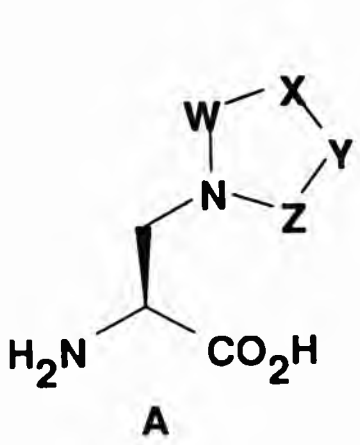
(a) modification of the nature of the atoms/groups in the ring.

(b) increase or decrease in ring size.

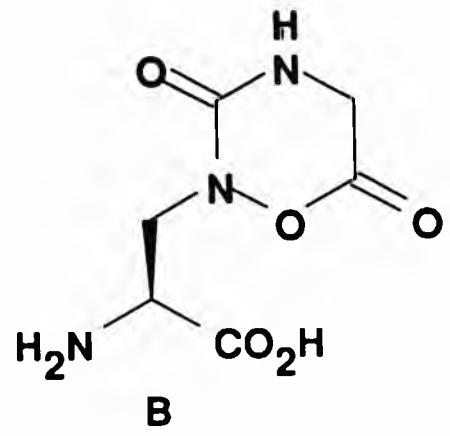
(c) preparation of conformationally less restricted analogues.

(d) modification of the α-amino acid functionalities.

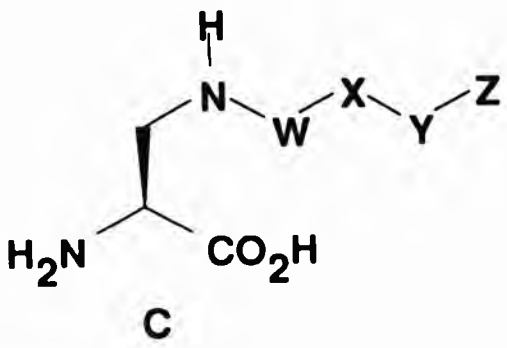
By way of illustration of each of these categories, structures such as (A-D) could be regarded as suitable synthetic targets.



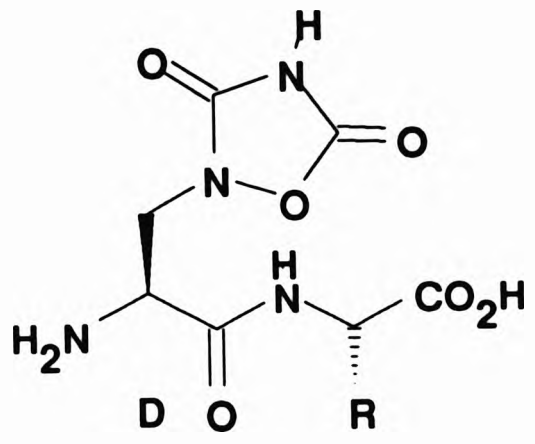
A



B



C



2.5.1. Synthesis of 3-[2-(tetrahydro-1,2,5-oxadiazin-3,6-dionyl)] alanine (85).

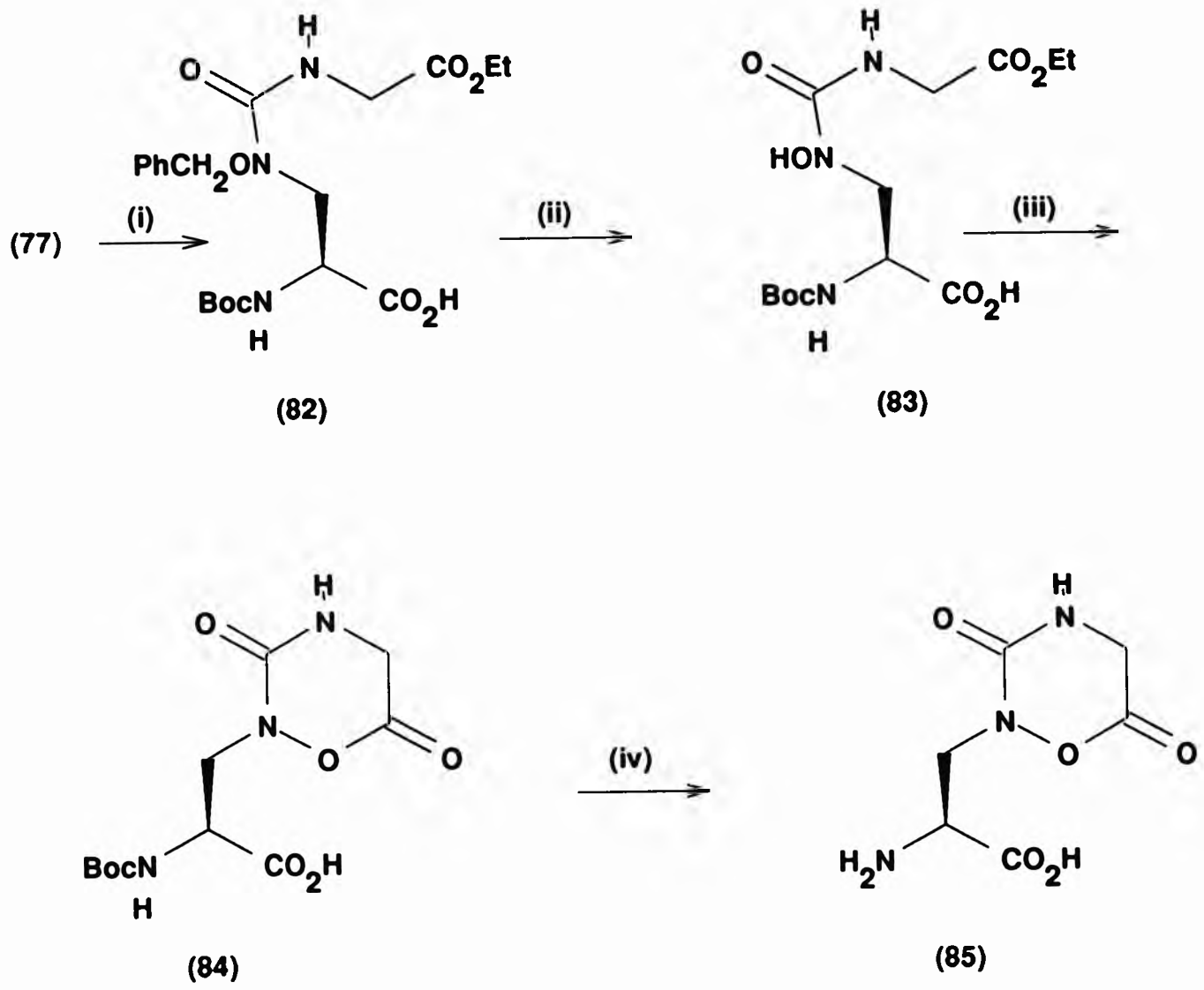
Preparation of a homologue of quisqualic acid (in this case, a six-membered ring analogue) would only require modification of the synthesis to allow the incorporation of a methylene group into the heterocyclic ring. One obvious

method would be to substitute ethyl isocyanatoacetate for ethoxycarbonyl isocyanate in the step wherein the protected hydroxylamino acid (77) is acylated.

In considering the best way to approach such a synthesis, it was decided that the intermediate (77), in the L-quisqualic acid synthesis already developed, would serve as a useful starting material, since it possesses a protected hydroxyamino group conveniently placed for acylation with ethyl isocyanatoacetate, and hence for construction of the tetrahydrooxadiazindione ring. (scheme 13). Reaction of the intermediate (77) with ethyl isocyanatoacetate in dry THF afforded the substituted urethane (82) in good yield (70%) after extractive work up. The evidence that isocyanatoacetate had been incorporated was provided by the presence of ethyl and methylene chemical shifts in the ^{1}H NMR spectrum, as well as an $[MH]^{+}$ at m/e 440 in its FAB mass spectrum.

The monoester was debenzylated by facile catalytic hydrogenolysis in ethanol. (scheme 13). After workup, and recrystallization, the hydroxyurea (83) was isolated in 68% yield. The absence of the phenyl and benzylic protons in the ^{1}H NMR spectrum, as well as the FAB mass spectrum, which exhibited an $[M+Na]^{+}$ at m/e 372, were taken as structural confirmation of the product.

Scheme 13



Reagents: (i) EtO_2CCH_2NCO, THF, 0°C, then r.t.

(ii) H_2, Pd-C, EtOH, 1hr.

(iii) NaOH aq. (2 eq.)

(iv) (a) 1N HCl in AcOH (b) ion-exchange.

Cyclization of the hydroxyurea (83) to the tetrahydrooxadiazinedione (84) was achieved by dissolution of the intermediate in a minimum amount of methanol, followed by the addition of two equivalents of sodium hydroxide. The mixture was stirred for four hours to ensure complete cyclization. The yield after extractive workup was 59%. Structural confirmation of the

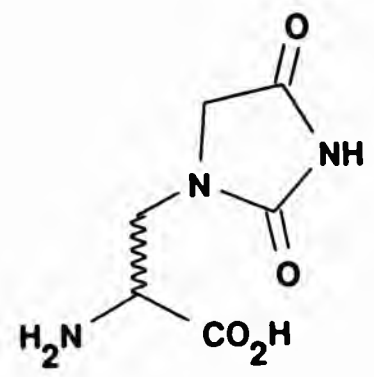
compound came from the ^1H NMR spectrum, which showed a considerable downfield shift of the tetrahydrooxadiazine dione methylene to δ 4.2 from δ 3.7 in the uncyclized material. The FAB mass spectrum of the t-Boc-protected amino acid (84) exhibited an $[M+H]^+$ at $^m/_e$ 304.

Removal of the t-butyloxycarbonyl N-protection was effected by acidolysis in acetic acid containing hydrogen chloride (1M). Thus, complete deprotection of the amino function was achieved by treatment of the urethane (84) with the aforementioned acid solution. The amino acid hydrochloride, which ensued on evaporation of solvent, was converted to the free amino acid (85) by ion exchange chromatography. Interestingly, elution of the amino acid from the anion exchange resin (acetate anion) required a slightly more concentrated acetic acid solution (5%) than did quisqualic acid. Presumably, the tetrahydrooxadiazinedione moiety contributed more towards binding to the anion exchange resin than did the oxadiazolidinedione of quisqualic acid. The ^1H NMR spectrum of the amino acid (85), coupled with the elemental analysis confirmed the structure.

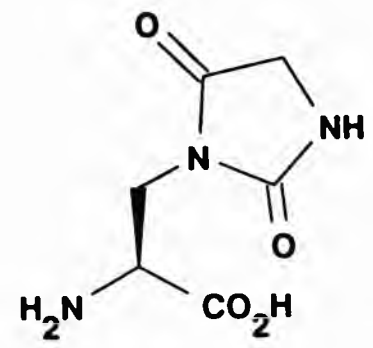
2.5.2. SYNTHESIS OF 3-[1-(1,3-IMIDAZOLIDIN-2,5-DIONYL)]ALANINE.

Having successfully synthesized the ring expanded analogue of quisqualic acid, attention was now directed towards the synthesis of analogues with variations in the functional groups of the heterocyclic ring system. The initial compound chosen was the hydantoin analogue (87) of quisqualic acid. The more obvious hydantoin analogue (86), wherein the ring oxygen of quisqualic acid has been replaced by a methylene group, has been prepared,

in racemic form, by Bycroft.[170]



(86)



(87)

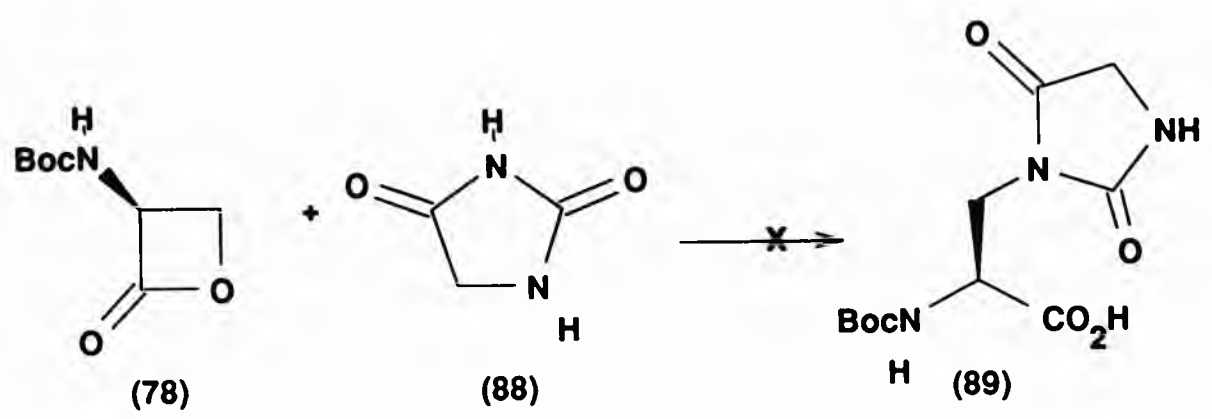
In view of the strong structural similarity of (86) to quisqualic acid it was somewhat suprising to find that the compound was completely inactive as an agonist or antagonist at the quisqualate receptor. The acidity of the substituted hydantoin ring system is notably less than that of the 1,2,4-oxadiazalidine-3,5-dione system of quisqualic acid; this may be sufficient to account for its total lack of activity.

This prompted the search for the methodology by which the hydantoin analogue (87) of quisqualic acid may be synthesized.

2.5.3. APPROACHES BASED ON THE ALKYLATION OF HYDANTOIN.

2.5.3.1. Approaches based on the alkylation of hydantoin with t-Boc propiolactone (78).

It has previously been established that, under suitable conditions, nucleophiles may be regiospecifically alkylated by the t-Boc-amino β-lactone (78) to yield substituted β-alanine derivatives. Thus it was hoped that, using hydantoin (88) as the nucleophile, the β-lactone (78) may be converted to the t-Boc protected hydantoin analogue (89) of quisqualic acid.

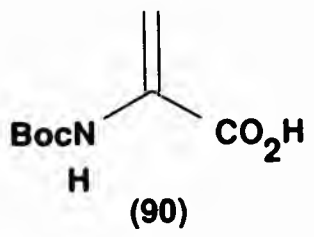


(78) (88) (89)

Reagents: bases : NaH, NaOH.

It was anticipitated that base abstraction of the proton from the more acidic nitrogen would render the hydantoin moiety sufficiently nucleophilic to attack and hence open up the β-lactone, affording the t-Boc protected analogue (89). Acidolysis, followed by ion exchange chromatography, would then yield the desired amino acid (87).

Initial attempts to synthesize (89) were initiated by alkylation of the hydantoin with t-Boc beta-lactone in the presence of sodium hydride in dry THF. The product, which was isolated from extractive work-up, was found upon characterization to be the elimination product t-Boc-dehydroalanine (90).



(90)

The product was identified by its ^1H NMR spectrum, which exhibited signals at δ 5.7 and 6.2 , assigned to the olefinic protons, and by an $[M+H]^+$

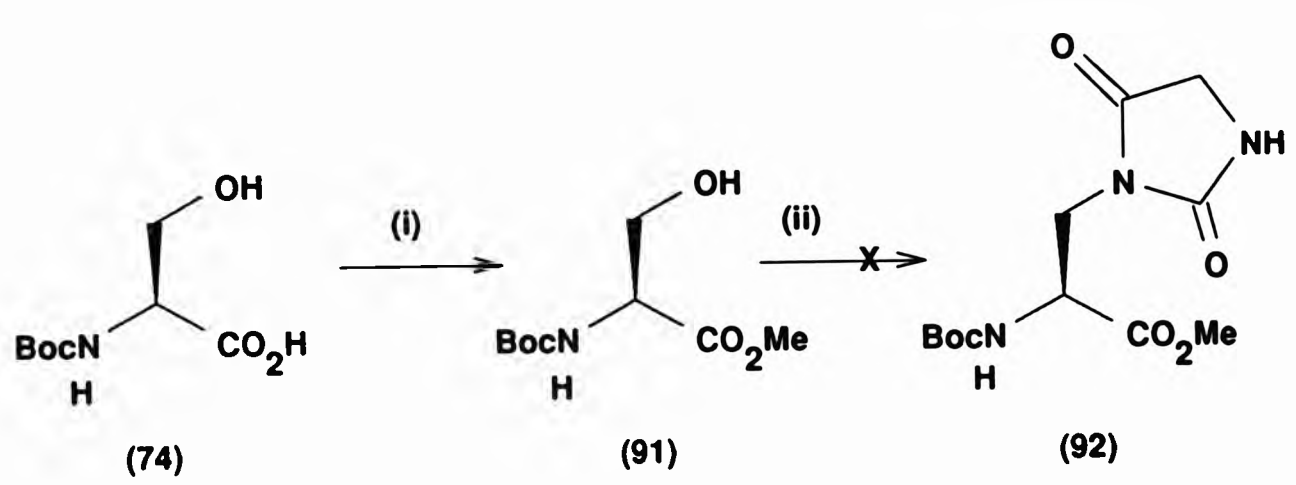
in its mass spectrum at $^{m}/e187$.

Repeated attempts to synthesize the analogue (89), by the alkylation of hydantoin with t-Boc β-lactone (78) under various conditions failed to yield the desired product. This approach was abandoned.

2.5.3.2. Attempted alkylation of hydantoin with t-Boc serine methyl ester (91).

Attempts to alkylate hydantoin (88) with t-Boc β-lactone (78) were not successful, possibly owing to the preferential reaction of the base at the C2 hydrogen; base abstraction of the proton simply resulting in an intramolecular rearrangment to give the protected dehydroalanine (90). An alternative approach was therefore sought for more suitable synthetic methodology. A literature search led to the possibility of synthesizing the hydantoin analogue by the alkylation of the heterocycle (88) with t-Boc serine methyl ester *via* the modified Mitsunobu reaction[179] (scheme 14).

Scheme 14

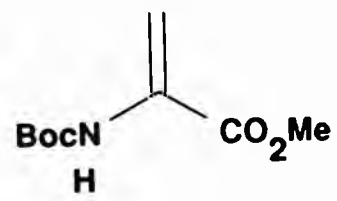


Reagents: (i) CH_2N_2, Et_2O

(ii) DEAD, TPP, -78°C, then (88)

Treatment of an ethereal solution of t-Boc serine (74) with diazomethane, followed by extractive workup, afforded the methyl ester (91) in good yield (87%). The presence of a three proton singlet at δ 3.7 in its ^1H NMR spectrum, and an $[M+H]^+$ in its mass spectrum at 219, were taken as structural confirmation of the methyl ester (91). (scheme 14).

N-t-Boc serine methyl ester (91) was added to a preformed adduct of diethyl azodicarboxylate and triphenylphosphine in dry THF at -78°C, followed by addition of hydantoin (scheme 14). A product was isolated which upon characterization (^1H NMR) appeared to be the methyl ester of dehydroalanine (93). The absence of any resonances assignable to the ring methylene of the hydantoin, or of the β-methylene, together with the presence of signals due to the olefinic protons, confirmed that the reaction had resulted in elimination from the activated serine derivative, rather than substitution. No further attempt was made to characterize this product.



(93)

2.5.3.3. Alternative approach to the synthesis of 3-[1-(1,3-imidazolidin-2,5-dionyl)] alanine (87).

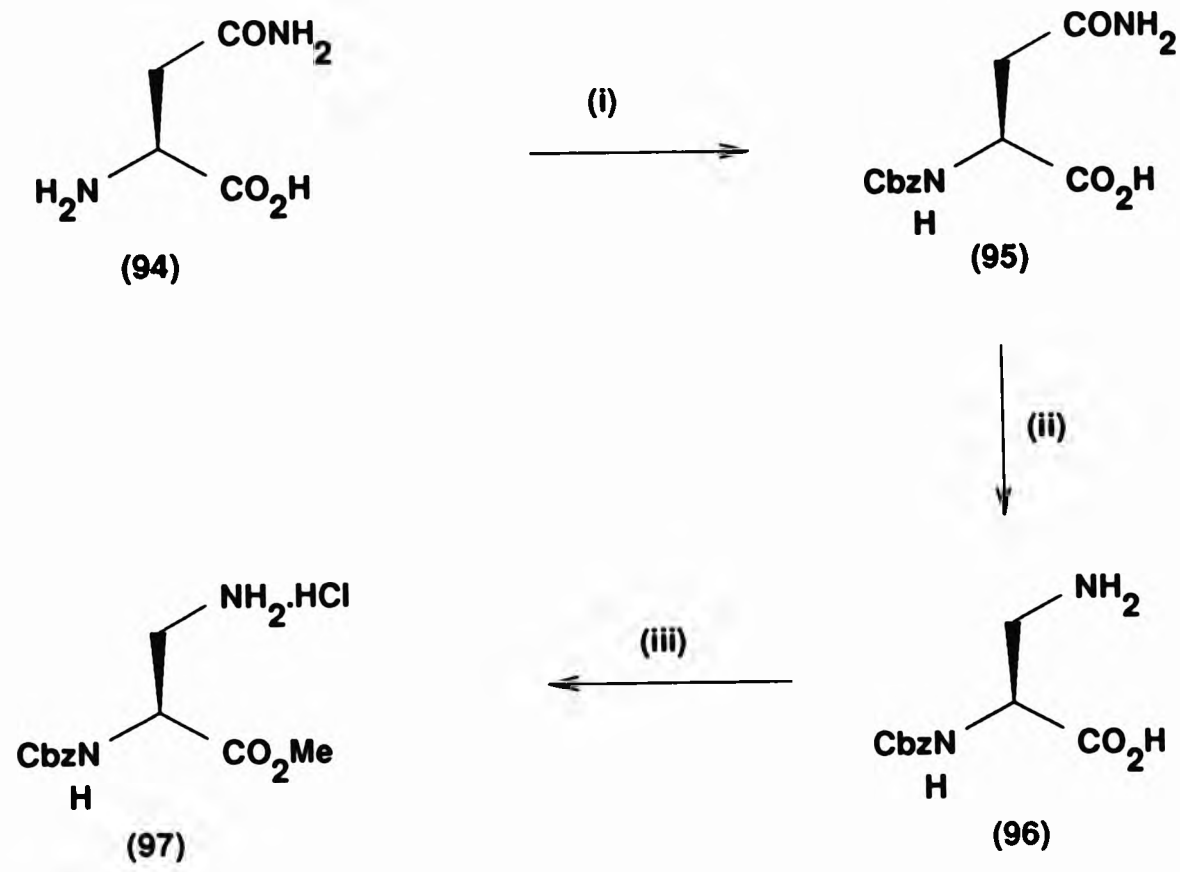
Evidently, attempts to synthesize (87), the hydantoin analogue of quisqualic acid, by the alkylation of the heterocycle had proved unsuccessful. It was therefore decided to explore the possibility of preparing the desired analogue

by a longer, but unambiguous, route (schemes 15-16).

None of the intermediates prepared thus far was suitable, therefore a new starting material had to be sought. L-Asparagine (94) was the starting material of choice. The amino acid was converted to its N-benzyloxycarbonyl derivative (95). A wealth of literature[173] exists for the synthesis of N-benzyloxycarbonyl protected amino acids. The method employed here is a particularly clean and convenient one using benzyl succinimidyl carbonate.[180] The use of this ester provides a smooth and efficient method for the conversion of L-asparagine (94) to its Cbz-protected derivative (95). The reaction, which was carried out in aqueous acetone, afforded the N-protected intermediate in an isolated yield of 86% (scheme 15). The ^{1}H NMR spectrum of the acid showed the phenyl resonances at δ7.4 and the benzyl methylene singlet at δ 5.1. Structural confirmation came from the mass spectrum which exhibited an $[M+H]^{+}$ at $^{m}/_{e}$ 267.

Cbz-protected asparagine (95) was converted to Cbz-protected diaminopropionic acid (Cbz-DAPA) (96) *via* the Hoffmann degradation[181] using bis(trifluoroacetoxy)iodobenzene in aqueous pyridine/ dimethylformamide (DMF) mixture. The amino acid was isolated after extractive workup, and recrystallization from water and ethanol, in an average yield of 90%. (scheme 15).

The ^{1}H NMR spectrum of (96) exhibited an downfield shift of the methylene from δ 2.5 in the amide to δ 3.5 in the amine. Methine resonances also appeared expectedly at a lower field δ 4.4, indicating that the amide (95) had been converted to the amine (96). The structural assignment was further confirmed by mass spectrometry $[M+H]^{+}$ ($^{m}/_{e}$ 239).



Scheme 15.

Reagents: (i) N-(benzyloxycarbonyloxy)succinimide, aq. acetone, $NaHCO_3$

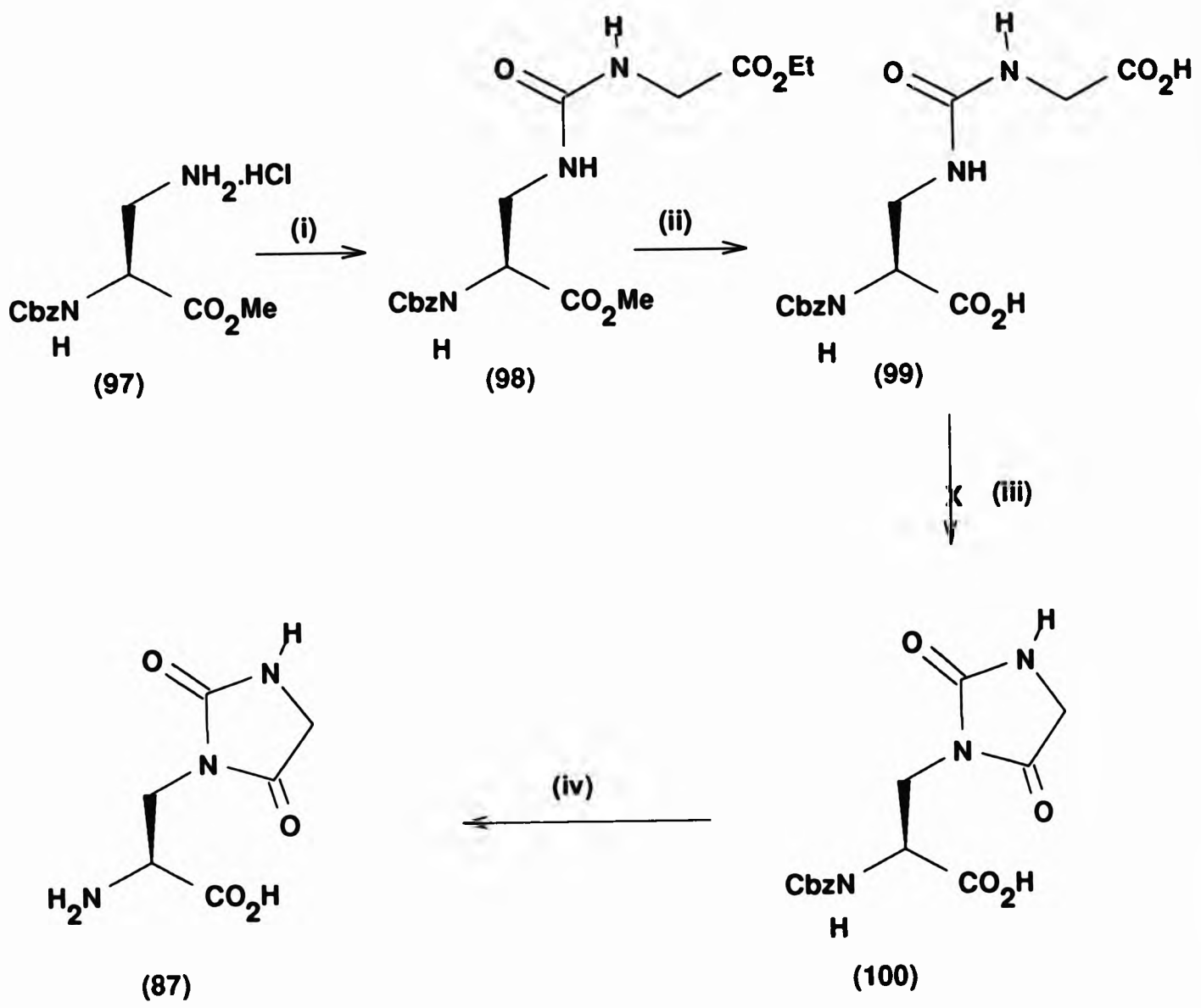
(ii) $PhI(OCOCF_3)_2$, aq. DMF, pyridine

(iii) $SOCl_2$, MeOH

Using thionyl chloride in dry methanol, the amino acid was smoothly esterified. The ester (97) was isolated in 78 % yield from trituration under ethereal methanol as a hydrochloride salt. (scheme 15). The spectroscopic characteristics of the product were consistent with the expected structure.

The most direct route to the construction of the hydantoin ring would be the condensation of the amino ester (97) with ethyl isocyanatoacetate, to give the substituted urea derivative (98), followed by cyclization on to the amide

nitrogen (scheme 16). Thus, Cbz-DAPA methyl ester hydrochloride was converted to the substituted urea (98). Its ^1H NMR spectrum indicated, by the presence of ethyl signals and an upfield methylene singlet, that the expected product had been isolated.



Scheme 16

Reagents: (i) EtO_2CCH_2NCO (1 eq.), THF, r.t.

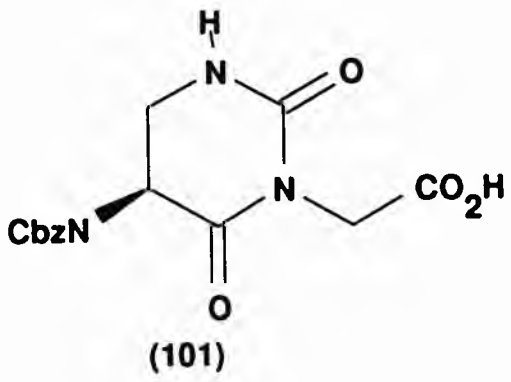
(ii) NaOH (2 eq.) MeOH aq.

(iii) $EtN{=}C{=}N(CH_2)_3NMe_2.HCl$, NMM, HOBT, DMF, r.t.

(iv) H_2, Pd-C

The diester (98) was smoothly hydrolyzed by treatment with two equivalents of 1N aqueous sodium hydroxide for two hours. The reaction mixture was acidified, and the diacid (99) was isolated as a foamy white solid (scheme 16). The absence of ethyl and methyl signals in its ^{1}H NMR spectrum, indicated that the hydrolysis had gone to completion. An $[M+H]^{+}$ at $^{m}/_{e}$ 340 in the FAB mass spectrum confirmed the structural assignment.

Structurally, it appeared feasible that cyclization of the intermediate (99) using a carbodiimide (a peptide type coupling reaction) in dry DMF would effect the desired cyclization. It was envisaged that the reaction would occur *via* amide bond formation between the activated carboxylic acid group adjacent to the methylene and the amide NH, (scheme 16). Although the intermediate (99) contained two carboxylic acid groups, formation of the five membered ring (100) should be kinetically favoured over that of the six membered ring (101); at worst, both compounds could be produced, and then separated by chromatographic methods.

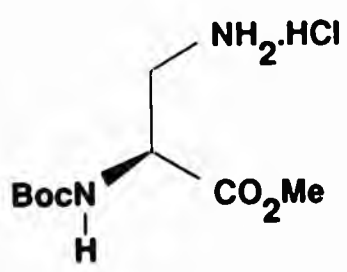


(101)

However, attempted cyclization of the adduct (99), using 1-ethyl-3-[3-(dimethylaminopropyl)]carbodiimide (EDC) in dry DMF, resulted in the formation of an intractable mixture, from which no single product could be

isolated. The mass spectrum of the crude reaction mixture did indicate the presence of an ion at m/e 322 corresponding to the desired product (100) or an isomer thereof. However, there were no fragments which could be assigned specifically to the hydantoin moiety. It was clear therefore that this method was not viable owing to the presence of two free carboxylic acid groups, either of which can become activated, and hence undergo chemical reaction. This method was therefore abandoned.

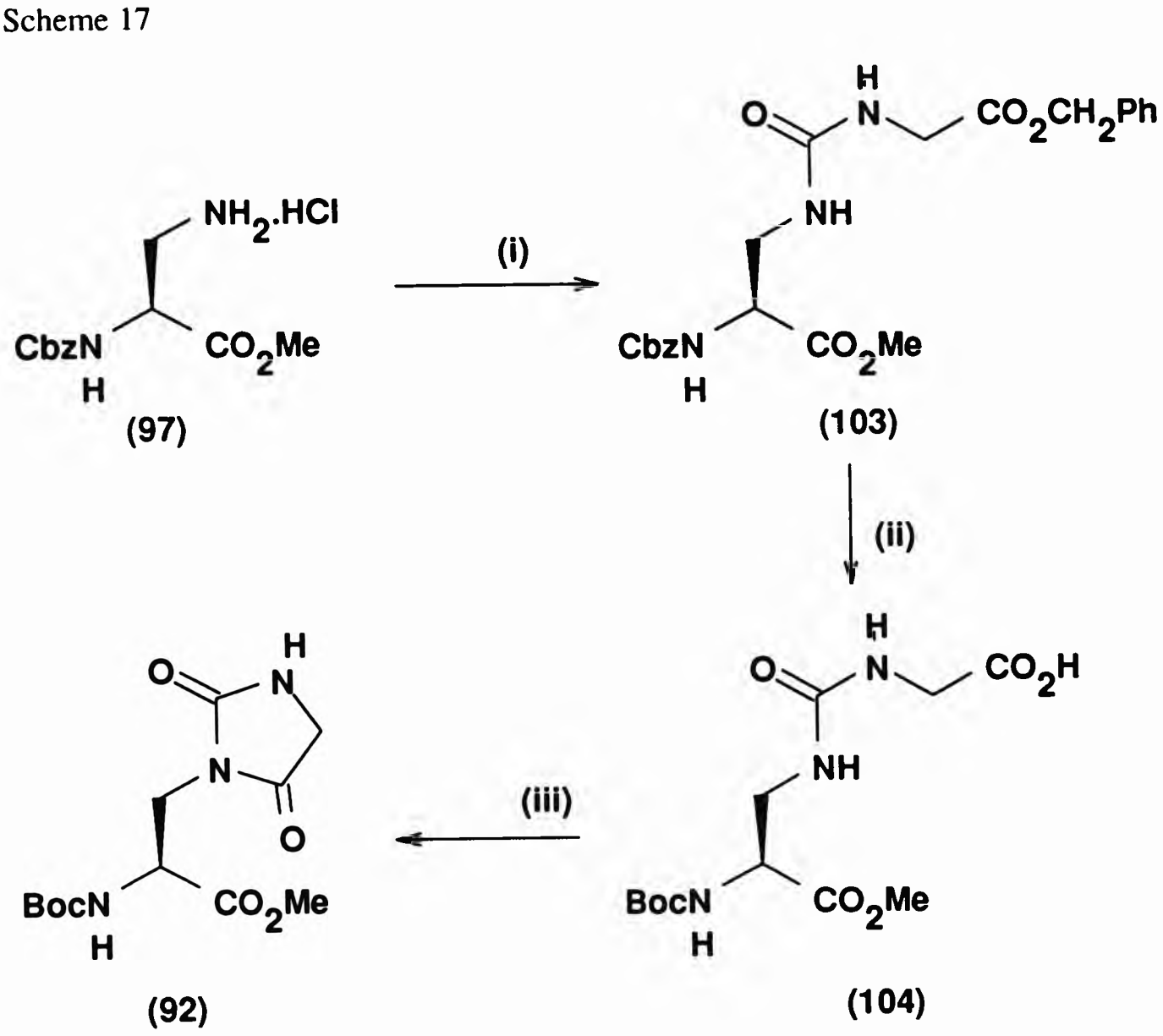
It appeared likely that a change in protecting group strategy could furnish the desired product. In considering the best way to approach such a synthesis, it was thought that the intermediate N-α-Cbz DAPA methyl ester (97), which had already been synthesized, would serve as a useful starting material. Condensation of this intermediate with benzyl isocyanatoacetate would afford a diester adduct. The benzyl ester could then be selectively removed by catalytic hydrogenation, yielding one carboxylic acid group which can undergo cyclization, after activation with carbodiimide. Initially, synthetic interest was focused on the corresponding t-Boc DAPA methyl ester hydrochloride (102) as the starting material. However, owing to the sensitivity of the t-Boc group to acid, synthesis of this intermediate was not undertaken.



(102)

The corresponding Cbz-protected DAPA methyl ester(97) was then chosen as the starting material (scheme 17).

Scheme 17



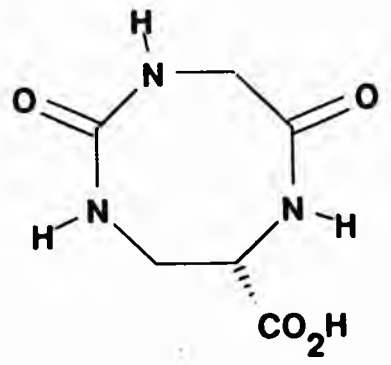
Reagents: (i) $PhCH_2O_2CCH_2NCO$, THF, 0^oc, then r.t.

(ii) $(Boc)_2O$, $NaHCO_3$, aq. MeOH, Pd-C, H_2, 6 hrs.

(iii) EDC, Et_3N, THF, r.t., o.n.

Clearly, from the above scheme, it is quite obvious that the removal of the benzyl ester by catalytic hydrogenation would result in the hydrogenolysis of

the benzyloxycarbonyl group. Attempted cyclization of the resulting amino acid could yield an eight membered ring system (105) or, more likely, intermolecular condensation products in preference to the desired five membered intermediate. As the amine is more nucleophilic than the amide, it would undergo reaction with the activated acid more readily.



(105)

To circumvent this problem, a search of the literature led to methodology by which the Cbz-protected amino acid may be converted to the t-Boc protected amino acid in a one pot synthesis by catalytic hydrogenolysis.[182]

Thus, using Cbz-protected DAPA methyl ester as starting material, and benzyl isocyanatoacetate as the condensing agent, the intermediate (103) was isolated in 62% yield, after purification by flash chromatography. The ^1H NMR spectrum of the substituted urea (103) showed one methylene singlet at δ 3.9 ($NHCH_2CO_2Bzl$); resonances due to the benzyl ester and the Cbz protecting group appeared as two singlets at δ 5.1 (CH_2), and δ 7.4 (C_6H_5); indicating that acylation had taken place. The methyl ester appeared as a three proton singlet δ 3.7, and the β-methylene and methine protons resonated at δ 3.6, and δ 4.4 respectively. The mass spectrum, with an $[M+H]^+$ at m/e 434 confirmed the structure.

The adduct (103) was successfully debenzylated and reprotected with the t-Boc protecting group by catalytic heterogenous hydrogenation over palladium on charcoal in the presence of di-tert-butyl dicarbonate and sodium hydrogen carbonate in methanol. After six hours the t-Boc protected intermediate (104) was isolated in 52% yield. Confirmation of the structure came primarily from the ^{1}H NMR spectrum, which exhibited a nine proton singlet at δ 1.4 due to the t-Boc group. The absence of resonances in the aromatic region, as well as the loss of the benzylic methylene singlet at δ 5.1, showed that the desired exchange of protecting groups had occurred. The structural assignment was further confirmed by the mass spectrum of the product which exhibited an $[M+H]^{+}$ at $^{m}/e$ 320.

The ease of this protecting group exchange reaction renders the method a very useful synthetic tool for the amino acid chemist.

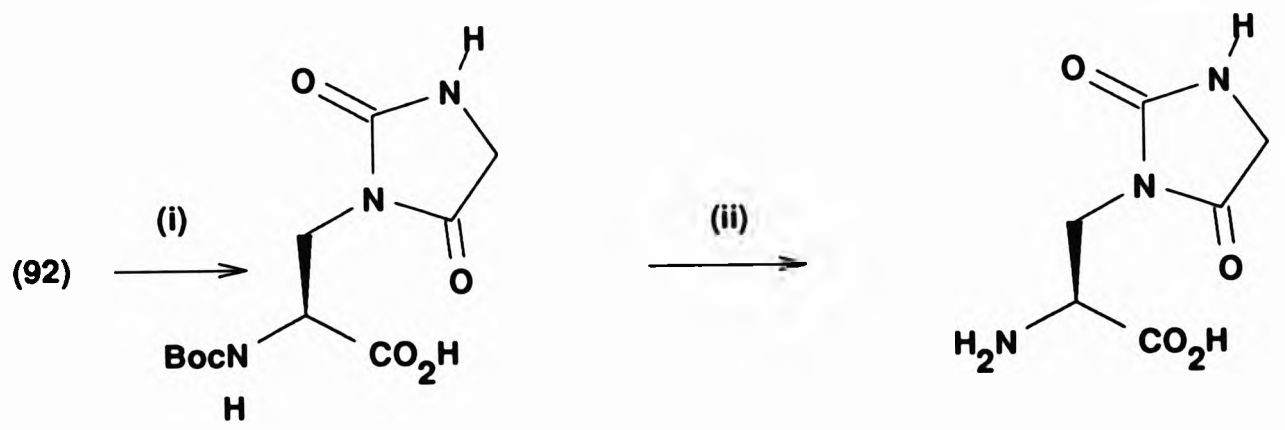
The monoester (104) was stirred in the presence of one equivalent of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) in dry THF. After workup and flash chromatography the cyclized adduct was obtained as a white solid. The reaction proceeded in respectable yield (68%), and the identity of the cyclized adduct (92) was confirmed by the proton NMR spectrum: the ring methylene protons resonated at slightly higher field (δ 3.9), resonances due to the side chain methylene and the methine occurred at δ 3.3 and δ 4.4. respectively. A three proton singlet at δ 3.7 was assigned to the methyl ester, and the t-Boc protons resonated at δ 1.4.

The cyclized intermediate (92) was smoothly hydrolyzed to the corresponding acid (89) using one equivalent of 1N aqueous sodium hydroxide. The protected amino acid was isolated in 71% yield after

extractive workup (scheme 14). Structural confirmation came from the 1H NMR spectrum which exhibited loss of the methyl ester signals at δ 3.7.

Acidolysis of the t-Boc protected compound using 1M hydrogen chloride in acetic acid, followed by ion exchange chromatography, afforded the amino acid (87) in an isolated yield of 69%. The loss of the t-Boc group was confirmed by ^{1}H NMR spectroscopy and mass spectrometry (scheme 18).

Scheme 18



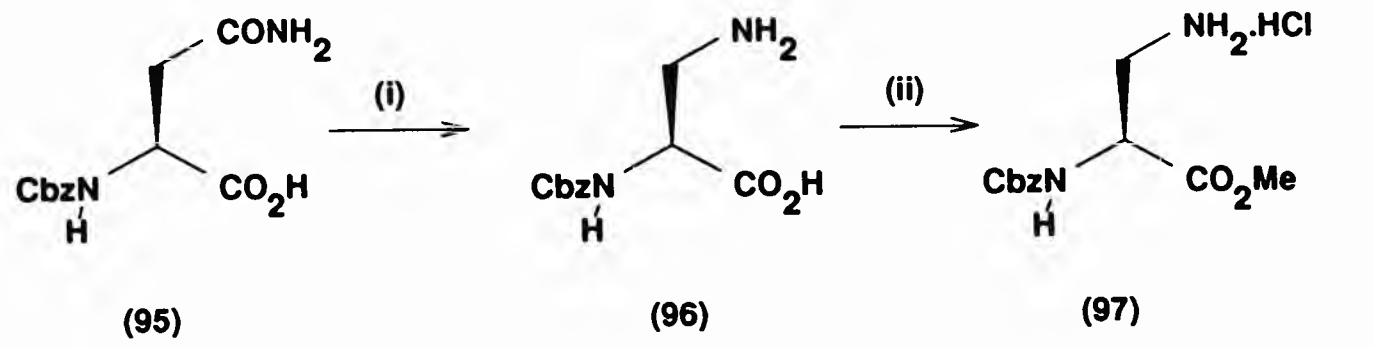
Reagents: (i) NaOH, aq. MeOH

(ii) (a) 1N HCl in AcOH, (b) ion-exchange

2.5.4. SYNTHESIS OF 3-(1-SUCCINIMIDYL) ALANINE (109).

Having succesfully synthesized the hydantoin analogue of quisqualic acid, in which the oxygen group in the heterocyclic ring was replaced with a carbonyl at position two, and the carbonyl with a methylene at position three, it was decided that methodology would be sought by which the succinimide analogue (109) of quisqualic acid may by synthesized.

Scheme 19



Reagents: (i) $PhI(OCOCF_3)_2$, DMF aq., pyridine, 20^oC, 3 hrs.

(ii) $SOCl_2$, MeOH

(iii) succinic anhydride, THF, reflux

(iv) EDC, Et_3N,THF

(v) KOH, MeOH aq.

The synthetic strategy adopted here was based on the synthon approach developed for the synthesis of the hydantoin analogue of quisqualic acid.

The synthon (97) possesses a substituted alanine side-chain with an amino group suitably positioned for acylation with succinic anhydride. Acylation of the synthon (97) with succinic anhydride proceeded as expected to give the acid (106), as a colourless crystalline solid, in 64% yield. (scheme 19).

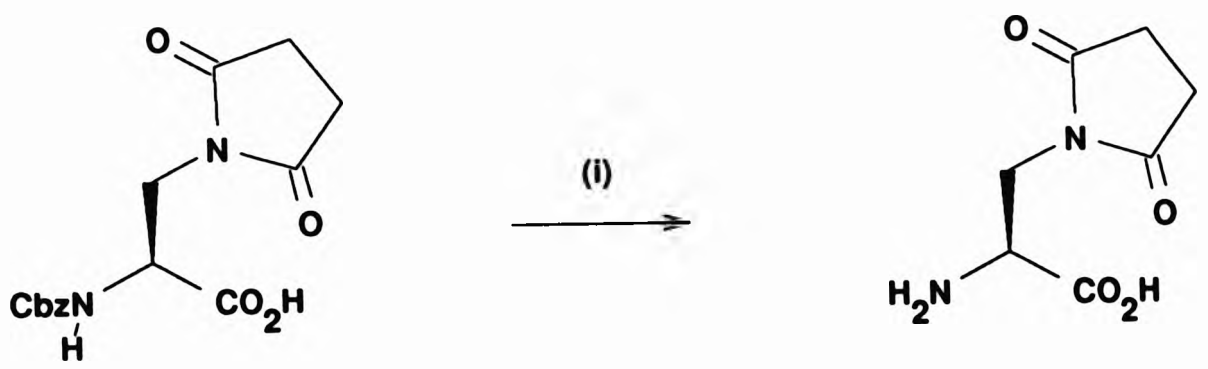
The ^{1}H NMR spectrum of the product exhibited a four proton double doublet (δ 2.3) assigned to the succinamide moiety, and the mass spectrum showed an $[M+H]^+$ at $^{m}/e$ 353.

Cyclization of the intermediate (106) was effected by carbodiimide activation of the carboxylic acid group. The cyclized product (107) was obtained as a colourless oil after extractive workup. The base peak in the mass spectrum was the molecular ion $[M+H]^+$ at $^{m}/e$ 335.

The ester function of (107) was smoothly hydrolyzed by treatment with one equivalent of potassium hydroxide in aqueous methanol for two hours. The reaction mixture was acidified and and the acid (108) isolated after extractive workup. Spectroscopic characterization of the acid was consistent with its being the expected product.

Cleavage of the benzyloxycarbonyl group of (108) was effected by heterogeneous catalytic hydrogenation over palladium on carbon in ethanol. The crude material, which was insoluble in ethanol, was dissolved in hot water, and the product (109), a white solid, was obtained on evaporation of solvent (scheme 20).

The absence of aromatic and benzylic methylene protons in the ^{1}H NMR spectrum of the product suggested that debenzylation had gone to completion. The mass spectrum exhibited an $[M+H]^+$ at $^{m}/e$ 187, confirming the structure.



(108) (109)

Scheme 20.

Reagents: (i) Pd-C, H_2, EtOH.

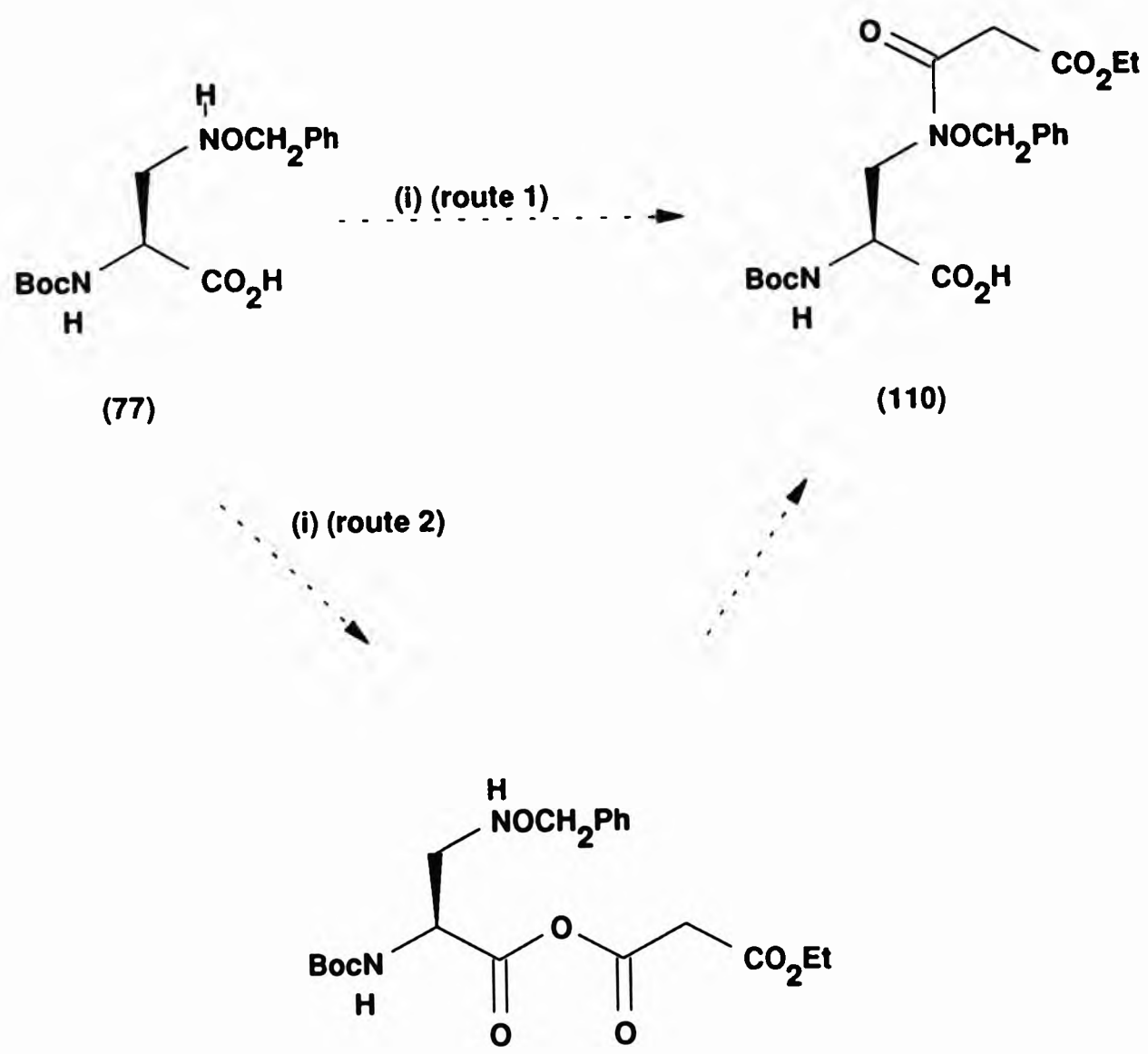
2.5.5. 3-[2-(1,2-OXAZOLIDINE-3,5-DIONYL)] ALANINE.

Continuing the series of analogues of quisqualic acid with changes to the heterocyclic ring system, it was decided that a synthetic methodology should be sought for the preparation of the carbon analogue (113), wherein the NH was replaced by a methylene group. It was considered that the synthesis of (113) would provide a useful analogue for comparative pharmacological investigation into the effect of the acidic NH at position 4 in the quisqualic acid molecule.

Functionally, the imide with its sp^2 hybridized nitrogen would be replaced with a tetrahedral carbon atom. Since the Van der Waals' radius of nitrogen is close to that of the carbon atom, the replacement of nitrogen by carbon is a suitable way to change the electron distribution pattern without substantially changing the shape or size of the molecule. The loss of the lone pair of electrons on the nitrogen may have pharmacological

significance. Furthermore, the loss of the very acidic N-H which ionizes at physiological pH could provide an interesting pharmacological outcome.

Scheme 21



Reagents: (i) Ethyl malonyl chloride, PhMe, reflux

The synthetic strategy adopted for the synthesis of the carbon analogue (113) utilizes the same synthon (77) developed for L-quisqualic acid synthesis (scheme 10). Thus the benzyloxyamine (77) was acylated with ethyl malonyl chloride in toluene under reflux to give the corresponding hydroxamate (110). The mechanism by which the reaction occurred is

unclear. The reaction may have proceeded *via* the formation of a mixed anhydride (route 2), or may have occurred by direct acylation of the hydroxylamine (route 1) (scheme 21)

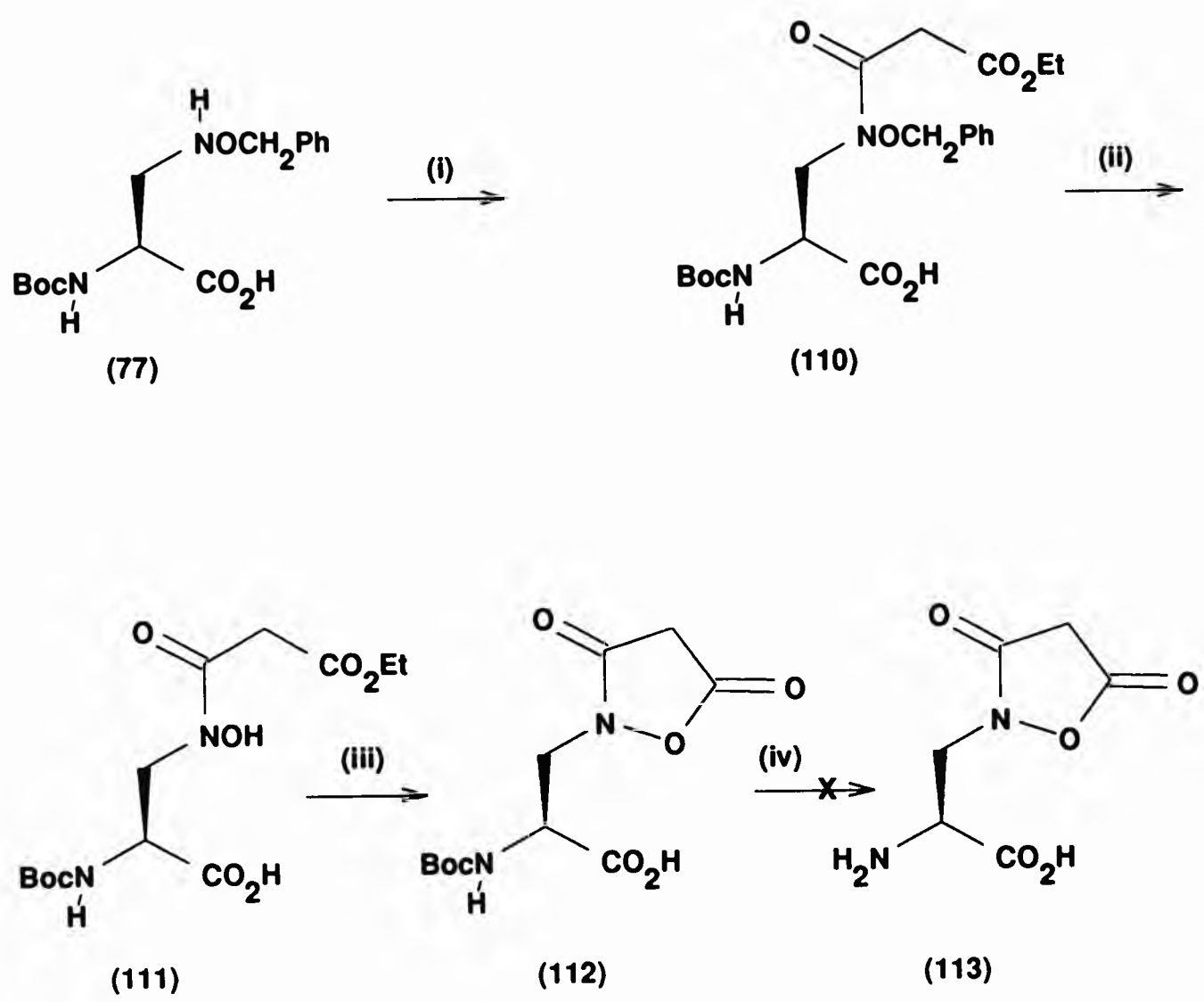
The product was collected as a colourless oil, after extractive workup and flash chromatography, in an isolated yield of 62%. The ^{1}H NMR spectrum of the product (110) exhibited signals due to the ethyl malonyl moiety, together with downfield shifts of the methine and β-methylene signals. This indicated that acylation of the hydroxylamino nitrogen had occurred. The mass spectrum, which gave an $[M+H]^{+}$ at $^{m}/_{e}$ 425, confirmed the structure.

The O-benzyl hydroxamate (110) was converted to the hydroxy derivative (111) by facile catalytic hydrogenolysis over palladium on charcoal in ethanol. (scheme 22). The ^{1}H NMR spectrum of the product showed the disappearance of the aromatic and benzyl methylene protons.
Interestingly, the loss of the benzyl group induced an upfield shift of the methylene from δ 4.2 to δ 3.6, due probably to shielding by the aromatic ring in the benzylated material. Structural confirmation came from the mass spectrum which exhibited an $[M+H]^{+}$ at $^{m}/_{e}$ 335.

Cyclization of the synthon (111) was effected by treatment with potassium hydroxide in methanol. The basic solution was acidified, and the isoxazolidinedione (112) was obtained from extractive workup in 64% yield. The loss of the ethyl protons in the ^{1}H NMR spectrum as well as a downfield shift of the side chain methylene signal from δ 3.4, to δ 3.9 suggested that cyclization had taken place. This, coupled with the an $[M+H]^{+}$ at $^{m}/_{e}$ 289 in its mass spectrum, was taken as structural

confirmation.

Scheme 22



Reagents: (i) Ethyl malonyl chloride, PhMe, reflux

(ii)H_2, Pd-C, EtOH

(iii) KOH, MeOH aq.

(iv) 1N HCl in AcOH, then ion-exchange chromatography

The t-Boc protected amino acid (112) was treated with 1M hydrogen chloride in acetic acid to effect deprotection. Ninhydrin development of the thin layer chromatogram gave the characteristic α–amino acid reaction,

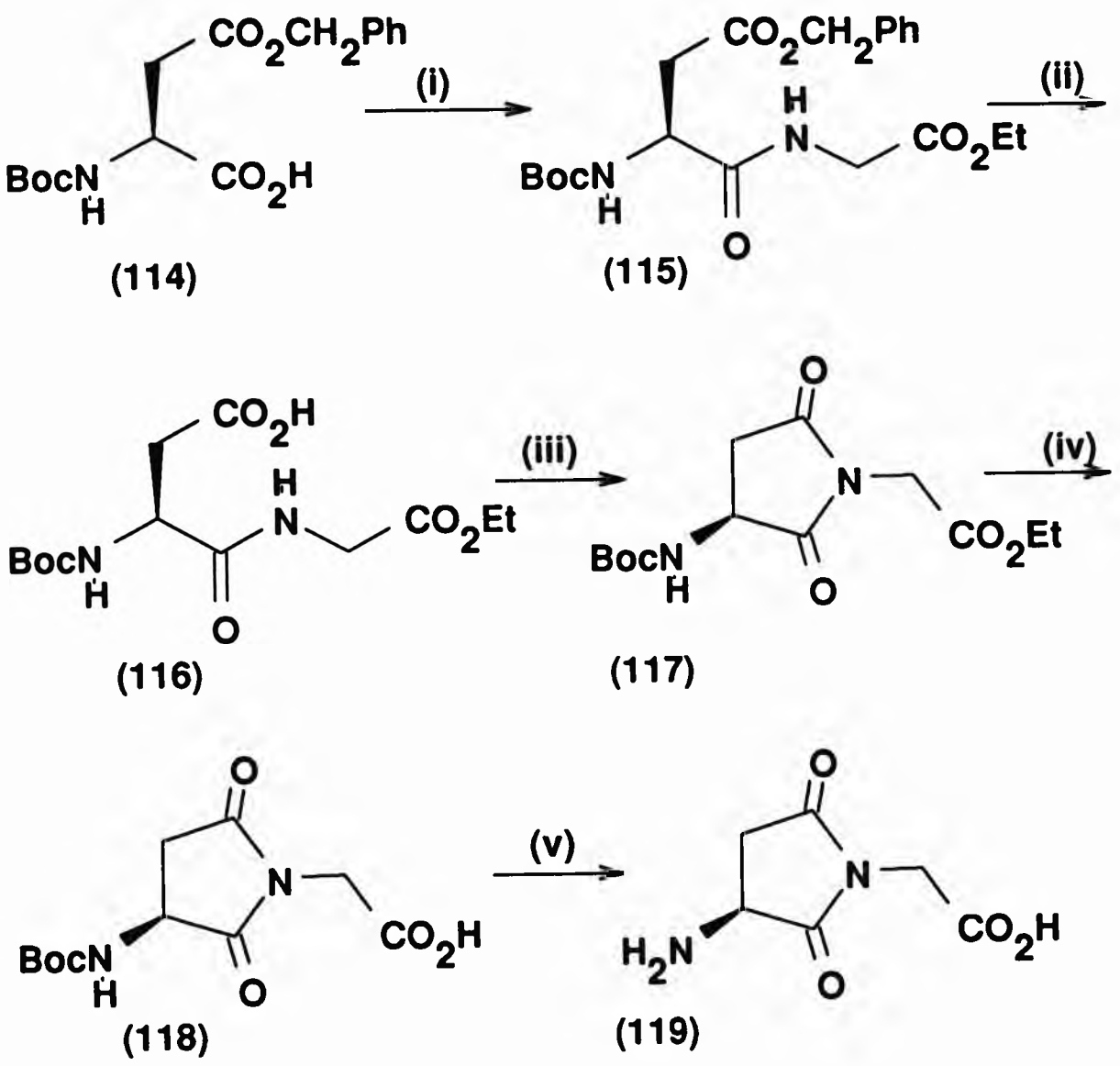
suggesting that the t-Boc group had been cleaved. However, evaporation of solvent and subsequent ion exchange chromatography gave an off-white solid, which appeared to have decomposed. The ^1H NMR and mass spectra of the product were inconclusive. No attempt was made to purify this product.

Having synthesized analogues of quisqualic acid which incorporated different functional groups into the heterocyclic ring, it was considered that an appropriate modification to the system would be to add a basic centre to the heterocyclic ring, by the introduction of an amino function at position 4 of the heterocyclic system. This is not unreasonable, as the potent NMDA antagonist CPP (cf. chapter 1) possesses a basic amino function (in addition the α-amino group). The methodology adopted for this synthesis (scheme 24) also introduces another chiral centre to the compound, but initial work was carried out on a simplified analogue based on glycine (scheme 23).

2.5.6. Synthesis of 1-[(3S)-3-Aminopyrrolidin-2,5-dionyl]-acetic acid (119).

L-aspartic acid β-benzyl ester was the starting material for this non-genetically coded amino acid. The amino ester was converted into its t-Boc derivative by treatment with $(Boc)_2O$ and sodium hydroxide (scheme 23). Spectroscopic characterization confirmed the structure.

Scheme 23



Reagents: (i) (a) mixed anhydride (IBCF), NMM, THF,-25^{o}C; then

(b) H.Gly.OEt.HCl, NMM, THF, -15^{o}C

(ii) H_2, Pd-C, EtOH

(iii) EDC, THF

(iv) NaOH aq.

(v) 1N HCl in AcOH; then ion-exchange chromatography

The adduct (114) was converted to the protected dipeptide by treatment with

glycine ethyl ester hydrochloride via a mixed anhydride reaction using isobutyl chloroformate and N-methylmorpholine. The presence of the methylene doublet at δ 4.0 and the chemical shifts assigned to the ethyl ester of the glycine moiety, as well as an $[M+H]^+$ at m/e 409 in its mass spectrum, confirmed the structure.

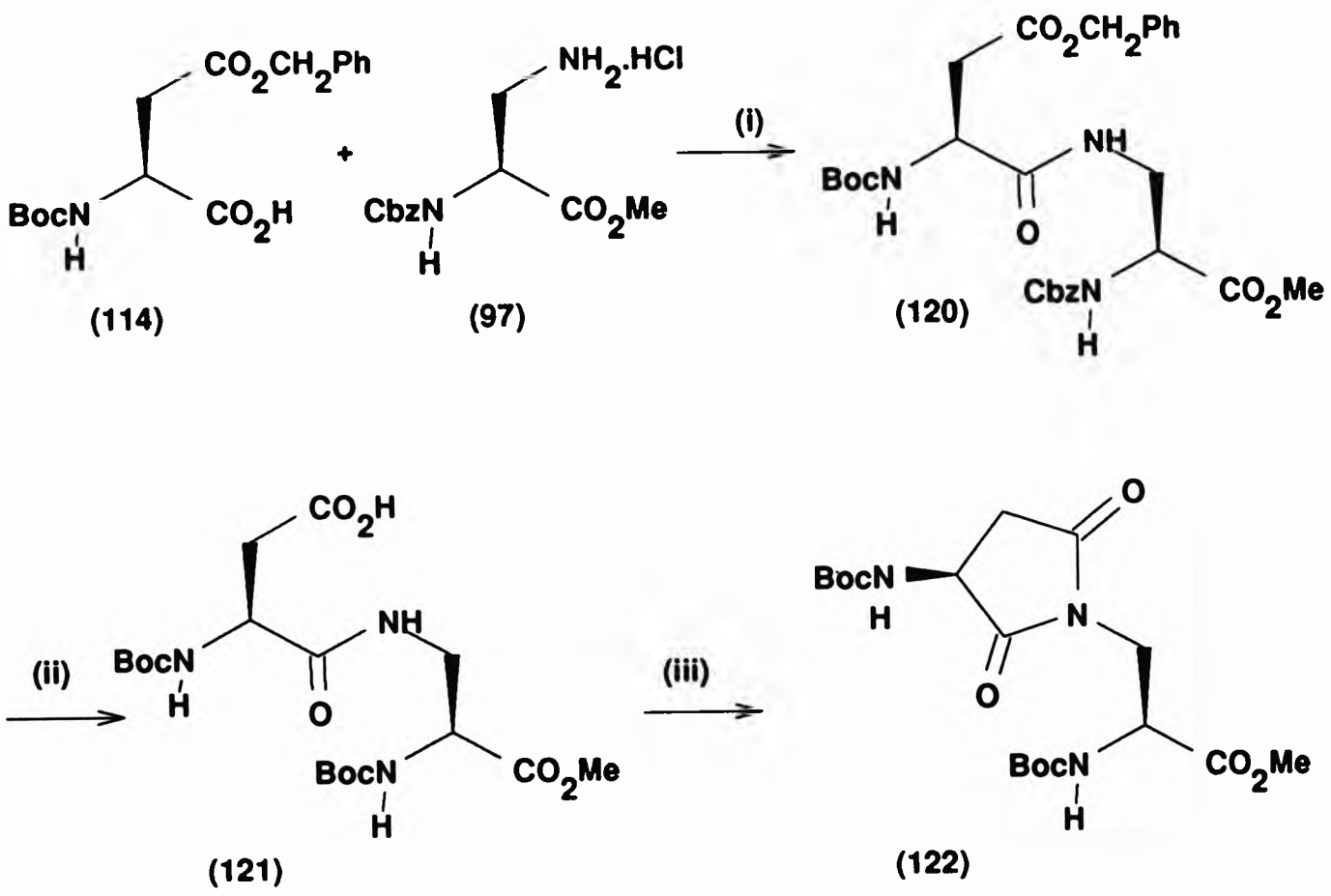
Deprotection of the benzyl group by catalytic hydrogenolysis gave the acid (116). Spectroscopic characterization confirmed the structure. Treatment of the adduct (116) with EDC in THF afforded the cyclized adduct (117) in isolated yield of 63%. Structural confirmation came primarily from ^1H nmr and mass spectrum which exhibited an $[M+H]^+$ at $^m/_e$ 301

Treatment of the adduct with aqueous sodium hydroxide gave the acid (118), which was deprotected using 1M hydrogen chloride in acetic acid, to give the amino acid hydrochloride, which was converted to the free amino acid (119), by ion exchange chromatography.

2.5.6.1. Synthesis of 3-[1-(3S)-3-Aminopyrrolidin-2,5-dionyl] alanine (124)

The starting material for this synthesis , as for the model chemistry, was L-aspartic acid β-benzyl ester. This was converted to its t-Boc derivative (114) by reaction with $(Boc)_2O$ and sodium hydroxide. The product was isolated after workup in 86% yield. The presence of a nine proton singlet in its ^1H NMR, and an $[M+H]^+$ at $^m/_e$ 324 in its mass spectrum confirmed that the t-Boc group had been incorporated.

Scheme 24



The synthon required for the construction of the heterocyclic nucleus was the intermediate α-N-Cbz-DAPA methyl ester hydrochloride (97) synthesized for the preparation of the hydantoin analogue (scheme 14). This adduct (97) and t-Boc β-benzyl aspartate (114) were coupled by standard peptide methodology using isobutylchloroformate and N-methylmorpholine, *via* a mixed anhydride intermediate (scheme 24). The amide (120) was

obtained from extractive workup in 66% yield. Structural confirmation came from its ^1H NMR spectrum, which exhibited resonances, in addition to those present in the aspartate moiety, which were assignable to the protected DAPA moiety. An $[M+H]^+$ at $^m/_e$ 558 in its mass spectrum confirmed the structure.

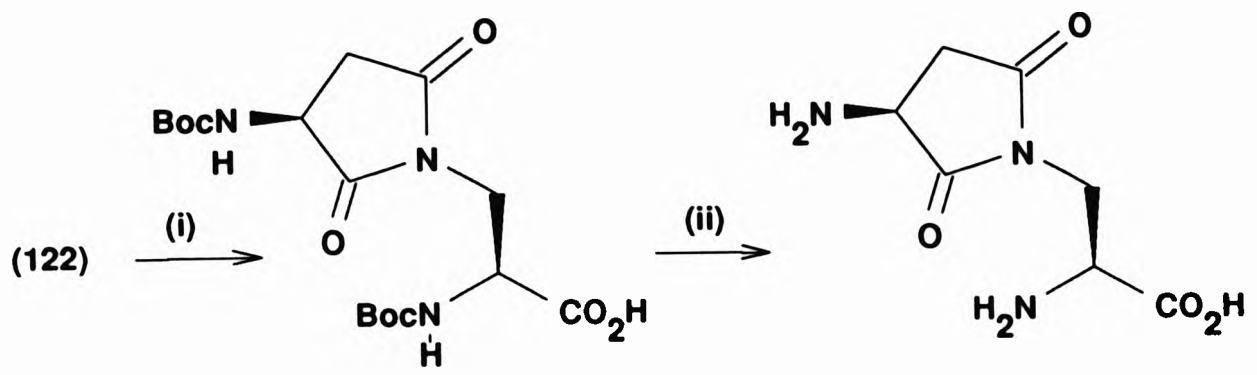
Catalytic hydrogenolysis of the Cbz group in the presence of $(Boc)_2O$ resulted in the reprotection of the amino function with a t-Boc group and also in cleavage of the benzyl ester. The reaction proceeded smoothly to afford the acid (121) in 59% yield. The ^1H NMR spectrum of this product confirmed the loss of the benzyl groups by the absence of the aromatic and benzylic methylene signals, and the presence of a second nine proton singlet at δ 1.4 demonstrated that reprotection by the t-Boc group had taken place.

The importance of the above Boc exchange reaction is that it ensures that there are no free amino groups available for reaction with the carbodiimide-activated carboxylic acid. Cyclization to give the five membered ring was effected by the reaction of the adduct (121) with EDC. The protected cyclized product (122) was obtained as a colourless oil after extractive workup. Structural confirmation came primarily from its infrared spectrum which exhibited absorptions assigned to the imide carbonyls at $1680cm^{-1}$. The absence of the carboxylic group was evident by the loss of a broad stretch at $2750cm^{-1}$. The mass spectrum, which gave an $[M+H]^+$ at $^m/_e$ 416, confirmed the structure.

Treatment of the cyclized adduct (122) with potassium hydroxide in methanol effected the ester hydrolysis (scheme 25). The product (123) was isolated after acidification followed by extractive workup. The loss of the

methyl ester group was confirmed by the absence of the a three proton singlet at about δ 3.7 in its ^1H NMR spectrum.

Scheme 25



(123) (124)

Reagents: (i) KOH, aq. MeOH.

(ii) 1N HCl in AcOH, then ion-exchange chromatography.

The t-Boc-protecting groups were smoothly acidolyzed by treatment of (123) with 1M hydrogen chloride in acetic acid solution at room temperature (scheme 25). The resulting amino acid dihydrochloride was converted to the monoacetate by ion exchange chromatography (acetate anion). A more concentrated acetic acid solution was required to elute the amino acid from the ion exchange resin. This may be attributable to the increased binding of the diamino acid to the resin bed. The loss of the t-Boc group was confirmed by ^1H NMR spectroscopy .

2.5.7. SYNTHESIS OF PYRAZOLE, TRIAZOLE, PYRROLIDINE AND PYRROLIDINONE ANALOGUES OF QUISQUALIC ACID.

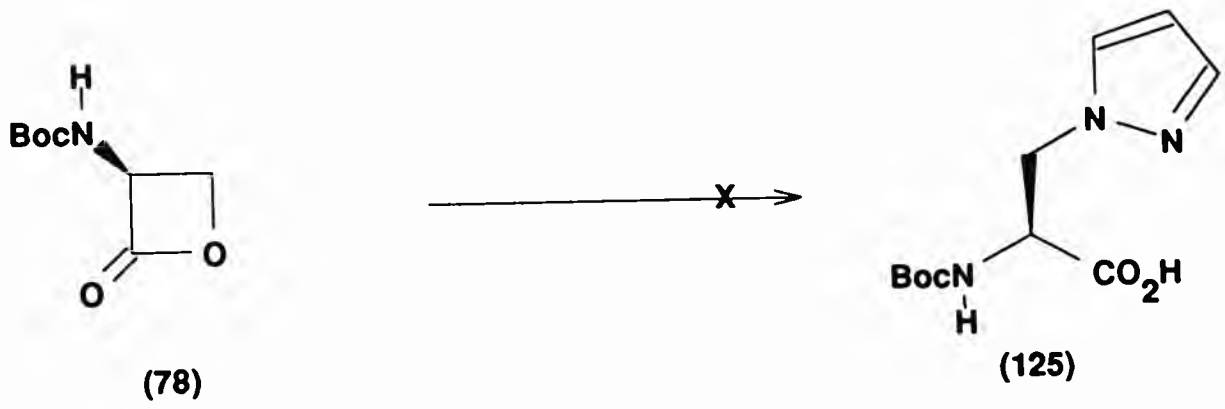
The objective was to develop synthetic methodology whereby various aromatic and non-aromatic heterocyclic analogues of quisqualic acid could be synthesized.

It was considered that alkylation of a variety of heterocycles with suitably activated derivatives of L-serine would provide a versatile approach to quisqualic acid analogues bearing a variety of functionalized chains. The synthesis of analogues which incorporate the pyrazole, pyrrolidine, triazole, and pyrrolidinone rings was investigated. The synthetic strategy adopted was based initially on alkylation of the heterocycle with the t-Boc α-amino β-lactone (78).

2.5.7.1. Attempted synthesis of α-N-t-butoxycarbonyl-3-[1-pyrazolyl] alanine (125).

Vederas *et al.*[178] reported several examples of synthetic routes whereby chiral β-lactones may be opened by nucleophiles to give pure enantiomers of N-protected β-substituted alanines. Using this synthetic methodology, attempts were made to alkylate pyrazole with (78). The reaction was carried out initially at room temperature, and then at 50°C, in dry acetonitrile. After workup, the colourless oil which ensued was found upon characterization to be a mixture of the starting material and t-Boc serine. The latter product presumably arose from hydrolysis of the β-lactone by adventitious water (scheme 26).

Scheme 26



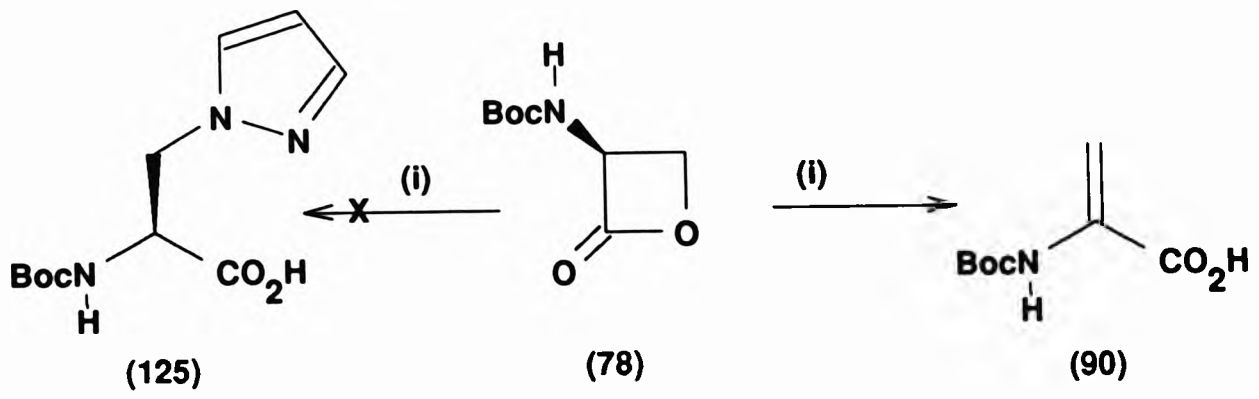
(78) (125)

Reagents: pyrazole, MeCN, r.t., then 50°C

Several attempts were made to alkylate pyrazole with the heterocycle (78). Reactions which were carried out in the absence of base led predominantly to the formation of t-Boc serine even at increased temperatures. It was decided therefore that the reaction should be repeated in the presence of base, in the hope that the preformed anion (by base abstraction of the acidic proton on the heterocycle) would undergo alkylation by the β-lactone more readily than neutral pyrazole (scheme 27). This hope was unfounded; reaction of pyrazole and the t-Boc β-lactone in the presence of sodium hydride at room temperature gave a colourless oil which upon characterization (^{1}H NMR) was found to be the elimination product (90). The presence of the olefinic protons at δ 5.7 and δ 6.2, and the absence of methylene and methine protons at δ 3.8, and δ 4.2, respectively, and the absence of signals in the aromatic region assignable to the pyrazole moiety, supported the case for elimination rather than alkylation. Confirmation came from the mass spectrum which exhibited an $[M+H]^+$ at $^m/_e$ 187. Repetition of the reaction at higher temperature gave an intractable mixture. The use of different solvents (THF, DMF, or EtOAc) under various

experimental conditions also failed to yield the desired product.

Scheme 27



(125) (78) (90)

Reagents: (i) pyrazole, NaH, MeCN, r.t.

Attempts to circumvent this problem by the use of a more hindered base, such as DABCO or potassium tert-butoxide, under different experimental conditions, also afforded the elimination product (90). An alternative approach was therefore sought for the synthesis of the pyrazole analogue of quisqualic acid.

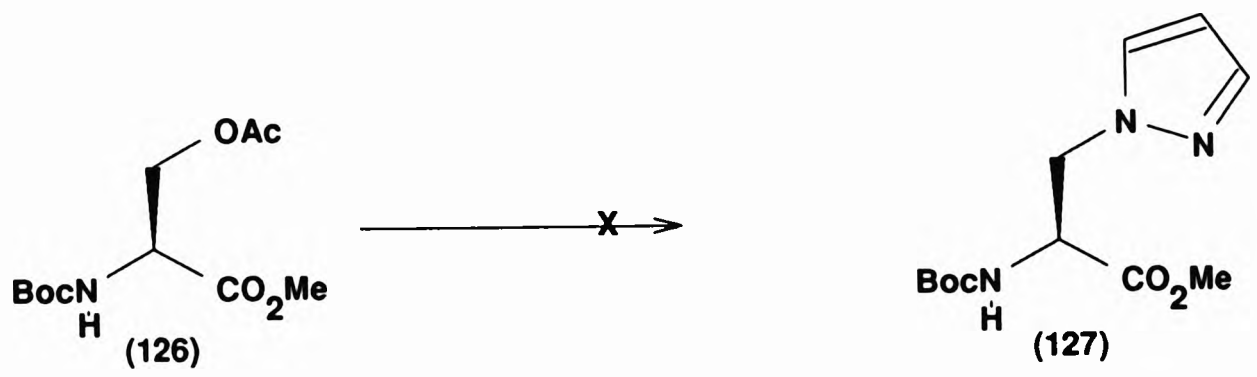
In view of the failure to alkylate pyrazole with the β-lactone (78), attention was directed towards the use of different starting materials with other leaving groups. N-protected β-substituted alanines became the starting materials of choice.

Initial interest was directed towards the alkylation of the heterocycle with N-t-butyloxycarbonyl-O-acetyl serine (126), which was prepared according to the procedure of Vederas *et al.*[178] The acetate was obtained in 78% yield after extractive workup.

Reaction of the acetate (126) and pyrazole in acetonitrile at room

temperature gave the starting materials only. The reaction mixture was heated at 50°C for two hours. Tlc showed the presence of mainly starting material, hence the reaction was heated under reflux for four hours. An intractable mixture was obtained after extractive workup; this method was abandoned (scheme 28).

Scheme 28

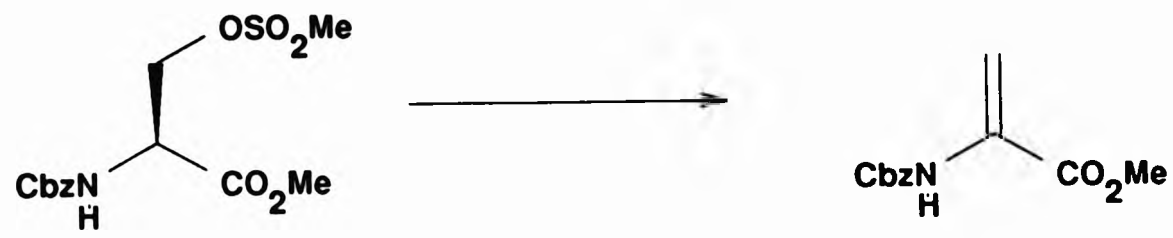


Reagents: pyrazole, MeCN, r.t., then 50°C

A new synthetic methodology was sought. It was considered that perhaps the mesylate(128), or the bromide (129), may be a better leaving group than the acetate in trying to effect an alkylation of this type.

Initially, attempted alkylation of pyrazole with the mesylate (128) (prepared as outlined on page 170) in acetonitrile at room temperature, then at 50°C, resulted in a colourless oil which upon characterization was found to be the elimination product, the protected dehydroalanine (130) (scheme 29).

Scheme 29

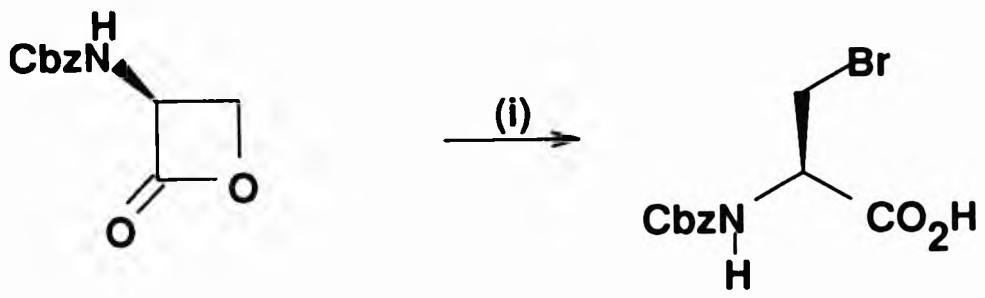


(128) (130)

Reagents: pyrazole, MeCN, r.t., then 50°C

The final attempt at pyrazole alkylation involved the use of N-benzyloxycarbonyl-β-bromo-L-alanine(129) The bromide was synthesized from the N-benzyloxycarbonylamino β-lactone (131) according to Vederas *et al.*[178] (scheme 30).

Scheme 30



(131) (129)

Reagents: (i) $MgBr_2.OEt_2$, dry Et_2O

The initial reaction of pyrazole with the halide (129) was carried out in toluene at room temperature in the presence of sodium bicarbonate. The apparent lack of reaction after two hours suggested that more vigorous conditions would be neccessary to facilitate the reaction. To this end, the reaction mixture was heated under reflux for four hours. The mass spectrum

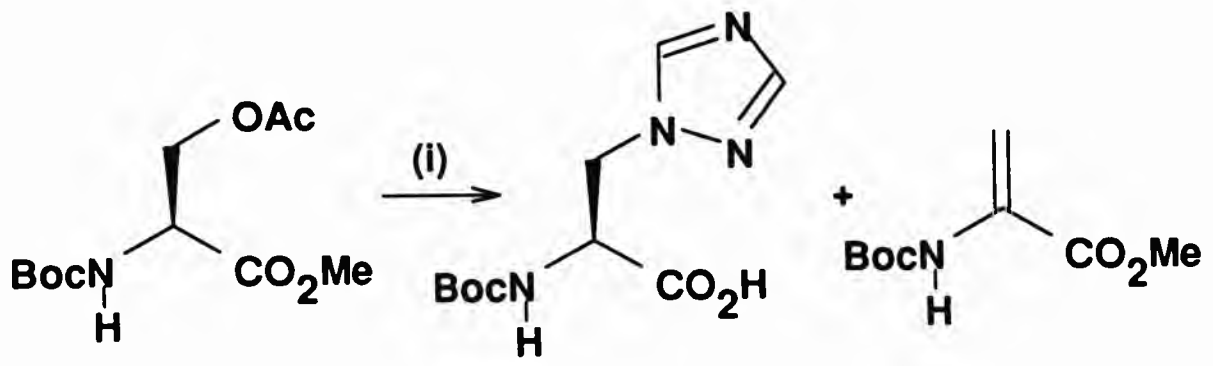
of the product (a colourless oil), and the ^1H NMR spectrum clearly indicated that some form of reaction had taken place, although evidence for the proposed structure was equivocal. Owing to the difficulties encountered in the synthesis of the pyrazole analogue of quisqualic acid, further approaches were not considered.

2.5.8. Synthesis of α-N-t-butoxycarbonyl-3-[1-triazolyl] alanine (132).

During the course of our studies in the attempted preparation of the pyrazole analogue, it was decided that the 1,2,4-triazole analogue of quisqualic acid should also be synthesized. As with the pyrazole, the aromatic nature of this amino acid (133) makes it an interesting synthetic target, from the pharmacological viewpoint. A synthetic approach to this system was therefore sought.

It was considered that the best approach to the synthesis of the triazole analogue would be to alkylate the heterocycle with the acetate (126), even though this method proved unsuccessful in the pyrazole synthesis. The triazole moiety, being more nucleophilic than pyrazole, might possibly undergo alkylation more readily (scheme 31).

Scheme 31



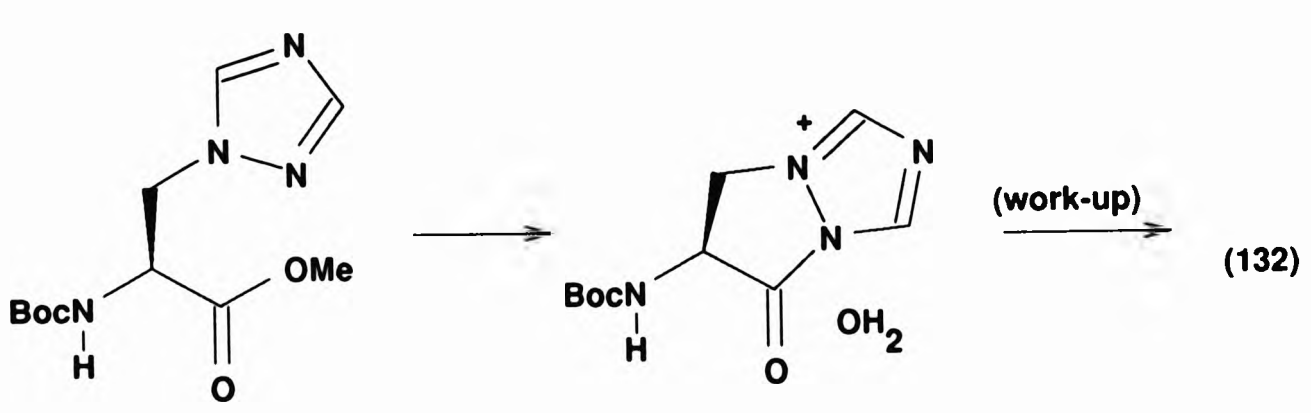
(126) (132) (93)

Reagents: (i) triazole, tetramethylguanidine, MeCN, reflux

The heterocycle and the acetate (126) were heated under reflux in acetonitrile, in the presence of tetramethylguanidine.

Chromatography gave the product as the acid (132) in 35% yield (scheme 31). The methyl ester had effectively been hydrolyzed *in situ* to the acid, possibly *via* a mechanism involving intramolecular attack of the triazole N-2 on the ester carbonyl (scheme 32).

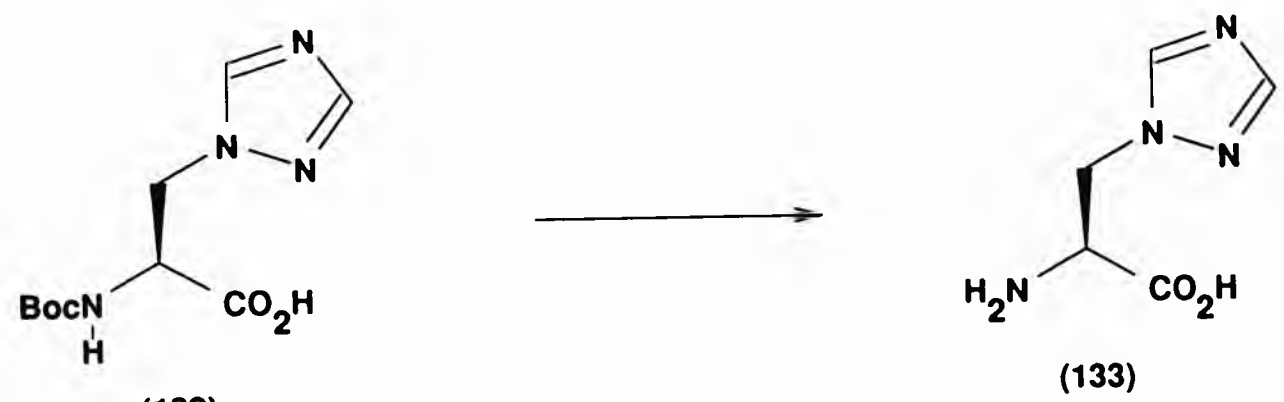
Scheme 32



The rather low yield may be attributable to the competing formation of the elimination product t-Boc-dehydroalanine methyl ester (93), which was removed by chromatographic separation. Structural confirmation of the

product came from its ^1H NMR spectrum, which exhibited two aromatic singlets at δ 8.0, and δ 8.4 respectively, due to the triazole moiety. The loss of the of the three proton singlet at δ 3.7 was a clear indication that the methyl ester had been hydrolyzed. The structural assignment was completed by the mass spectrum, which exhibited an $[M+H]^+$ at m/e 257. Future work would have included removal of the t-Boc group to give the amino acid (scheme 33)

Scheme 33

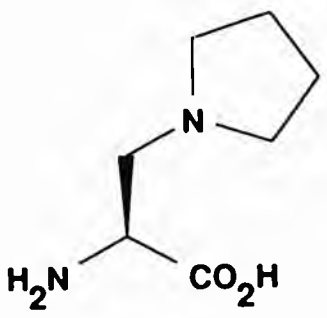


Reagents: 1N HCl in AcOH, then ion exchange chromatography.

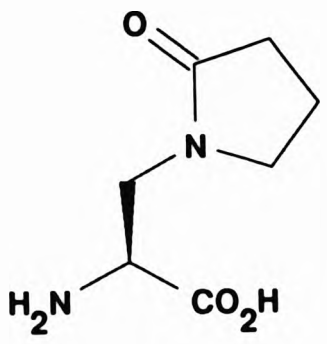
Deprotection was achieved on a small scale, albeit without spectroscopic characterization (positive ninhydrin test), which suggests that the approach may be feasible.

2.5.9. SYNTHESIS OF PROTECTED 3-[1-PYRROLIDINYL] ALANINE (136) AND 3-[1-(2-OXOPYRROLIDINYL)] ALANINE (137).

Having synthesized the triazole analogue of quisqualic acid (albeit t-Boc protected), by alkylation of the heterocycle, the decision was taken that compounds (134) and (135) should become synthetic targets.



(134)



(135)

The pyrrolidinone system (135) would be an analogue in which all but one of the functional groups on the quisqualic acid ring had been replaced by a methylene, while the pyrrolidine analogue (134) incorporates a side-chain devoid of any functional group other than the basic tertiary amine. Pharmacologically these two compounds, in conjunction with previously described analogues of quisqualic acid, represent an interesting research tool for the investigation of the ligand/receptor interactions of the quisqualate receptor.

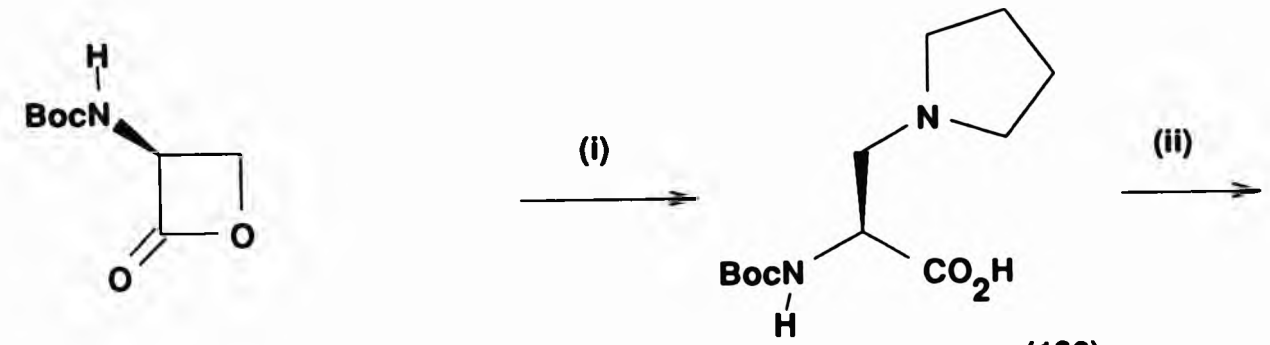
2.5.10. Synthesis of N-t-butoxycarbonyl-3-[1-pyrrolidinyl] alanine (136).

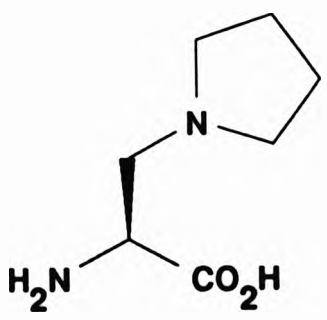
With some idea of the reaction conditions required, it was decided that preliminary work should be carried out on the reaction between pyrrolidine

and t-Boc β–lactone (78). The reaction, which was carried out at room temperature in acetonitrile in the presence of 1,4-diazabicyco[2.2.2]octane (DABCO), gave a mixture of the alkylated material (136), and the elimination product (90). Chromatographic purification afforded the t-Boc-protected amino acid (136) in 28% yield. Spectroscopic characterization of the product was consistent with the proposed structure.(scheme 34).

The adduct (136) was subsequently deprotected by treatment with a solution of 1M hydrogen chloride in acetic acid. The amino acid was collected as the hydrochloride salt in 76% yield (scheme 34). Structural confirmation came from spectroscopic characterization.

Scheme 34





(134)

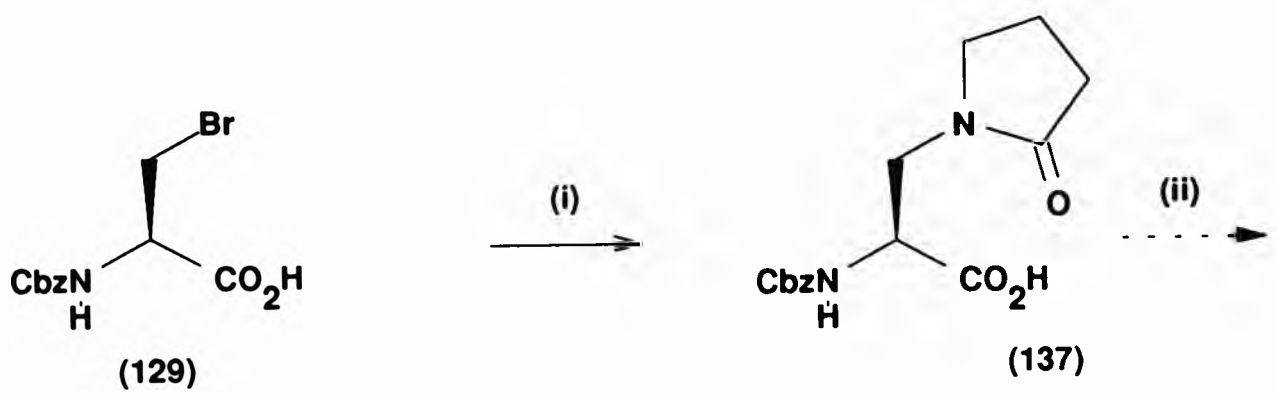
Reagents: (i) pyrrolidine, DABCO, MeCN

(ii) 1N HCl in AcOH, then ion-exchange chromatography

2.5.11. Synthesis of α-N-benzyloxycarbonyl-3-[1-pyrrolidin-2-onyl] alanine (137).

In parallel with the synthesis of (136) , preparation of the protected pyrrolidinone analogue (137) was in also progress. In this case, however, alkylation was effected by the use of the bromide (129) in toluene. (scheme 35).

Scheme 35



Reagents: (i) pyrrolidinone, Et_3N, PhMe, reflux

(ii) H_2, Pd-C, EtOH.

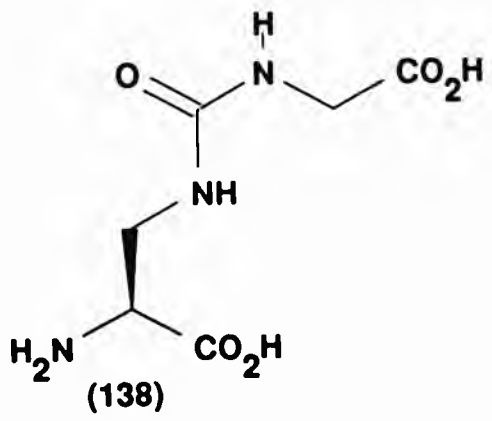
Heating pyrrolidinone and the bromide (129) in toluene effected the

alkylation. The product, a Cbz-protected amino ester (137), was obtained after extractive workup.

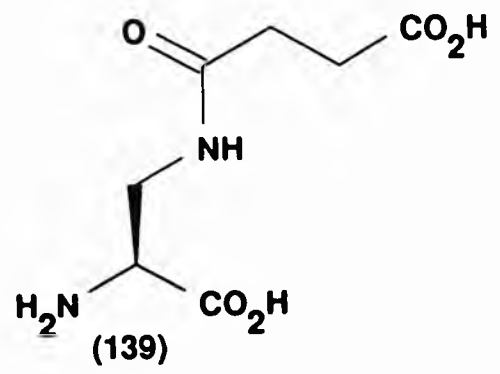
Unfortunately, however, deprotection of the pyrrolidinone analogue of quisqualic acid was not carried out, owing to lack of time.

2.5.12. NON-CYCLIC ANALOGUES OF QUISQUALIC ACID.

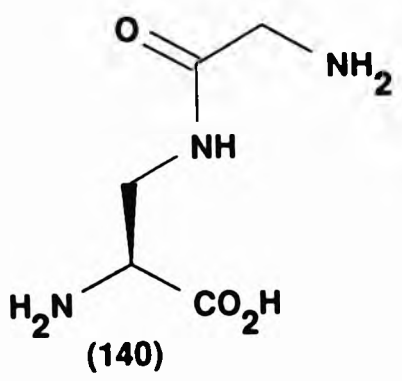
It was considered that less conformationally restricted analogues of some of the systems described above could be of interest as excitatory amino acid agonists and antagonists. Compounds (138)-(141)) were chosen as initial targets.



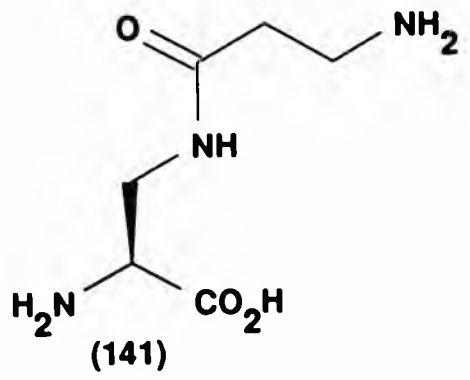
(138)



(139)



(140)



(141)

The synthetic routes chosen were directed towards the acylation of Cbz-protected diaminopropionic acid (96) with protected reagents derived from a

range of amino or dicarboxylic acids. Some of the compounds are non-cyclized analogues of systems already synthesized. Specifically, compound (138) is an acyclic analogue of the hydantoin (87), while (139) is a non-cyclized analogue of the succinimide (109). Compounds (140) and (141) were chosen as examples wherein the terminal side-chain functionality was complementary to that in (138) and (139), and hence would provide a comparative study of the two non-cyclized structural types.

The analogues were synthesized *via* the same general route (scheme 36). A solution of α-N-Cbz-DAPA (96) in dry THF was treated with the appropriate acylating agent. Extractive workup and flash chromatography gave the corresponding protected amide (142) in variable yields. See table 2 for a summary of the reactions undertaken.

The intermediates were subsequently deprotected under standard conditions. De-esterification was effected by treatment with sodium hydroxide to give the corresponding acids, followed by cleavage of the t-Boc group by treatment with hydrogen chloride in acetic acid. Ion-exchange chromatography (acetate resin), gave the amino acids. The individual amino acids were all satisfactorily characterized by ^{1}H NMR, mass spectrometry and elemental analysis.

Scheme 36

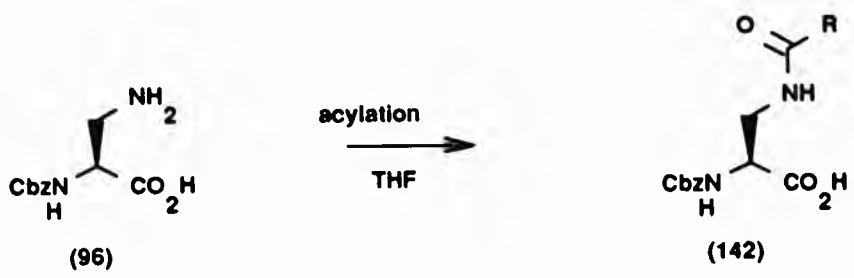
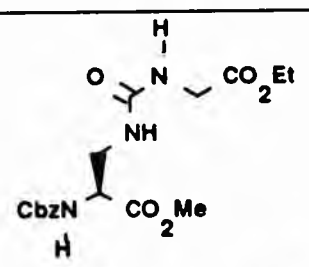
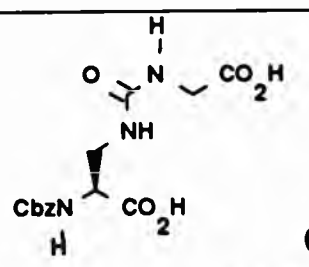
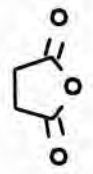
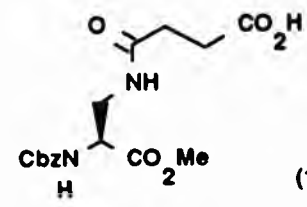
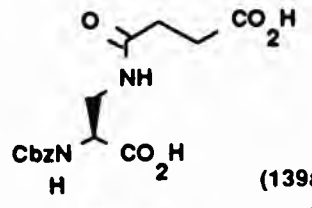
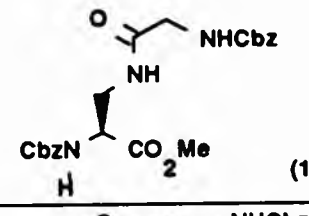
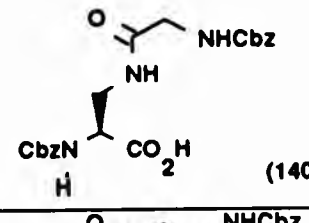
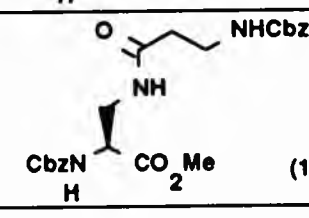
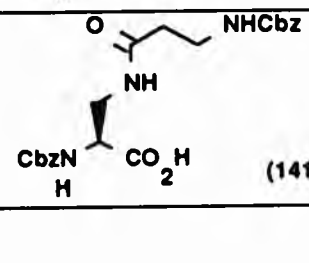


Table 2 (see scheme 36)

acylating agent	product	after hydrolysis	final prod.
EtO_2CCH_2NCO	 (98)	 (99)	(138)
	 (106)	 (139a)	(139)
Cbz.Gly.OH EDC	 (140a)	 (140b)	(140)
Cbz. -Ala.OH EDC	 (141a)	 (141b)	(141)

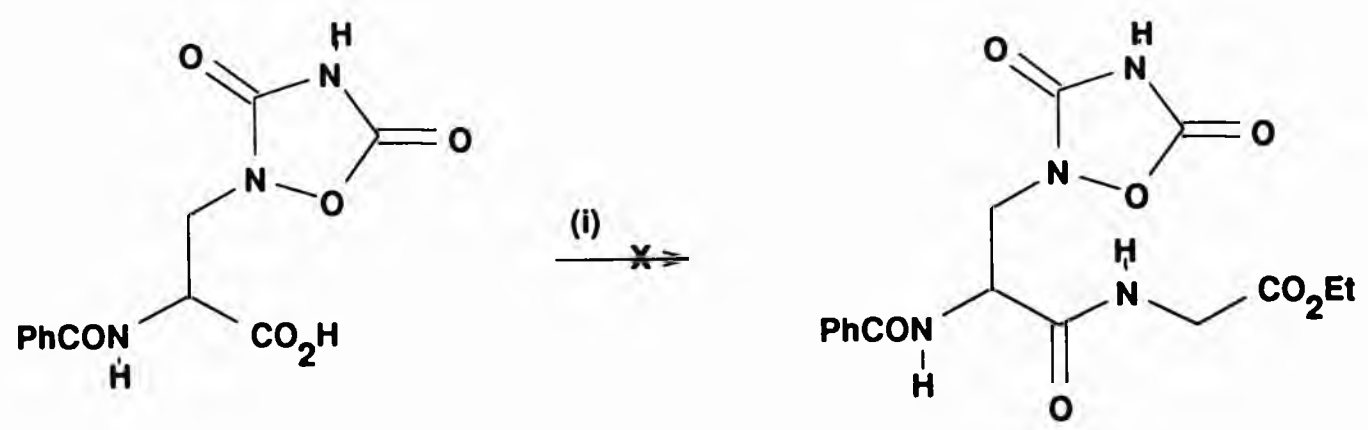
Having completed the synthesis of a series of quisqualic acid analogues in which various changes were introduced in the heterocyclic ring system, the emphasis now fell on the search for the methodology by which analogues with modification of the amino acid functions may be prepared.

2.5.13. SYNTHESIS OF SOME PEPTIDE ANALOGUES OF QUISQUALIC ACID.

2.5.13.1. Attempted synthesis of racemic quisqualyl-glycine ethyl ester (144).

An attempted synthesis of the peptide (144) employed N-benzoyl quisqualic acid, (62) which was converted to the the acid chloride by treatment with thionyl chloride. The acid chloride was not isolated, and hence not characterized, but was treated with glycine ethyl ester hydrochloride in dimethylformamide, in the presence of triethylamine. The reaction gave an intractable mixture, from which no single product could be isolated (scheme 37).

Scheme 37



(62) (144)

Reagents: (i) $SOCl_2$, then H.Gly.OEt.HCl, Et_3N, DMF

2.5.13.2. Synthesis of L-Quisqualyl-leucine (147).

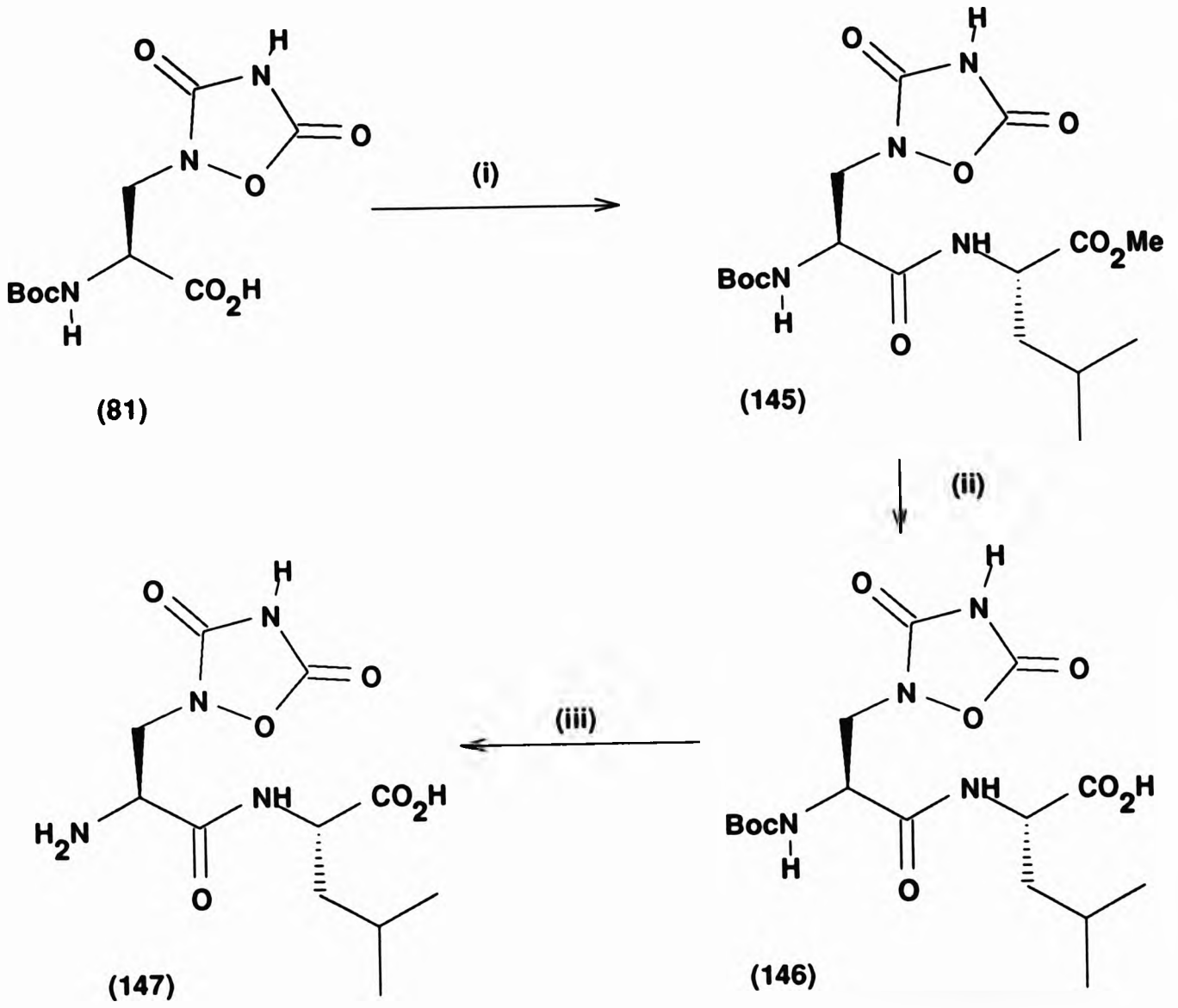
The amino acid leucine was chosen on the basis of its increased lipophilicity. A carboxyl-activated derivative of quisqualic acid was required, and therefore the carboxyl function of leucine was protected as its methyl ester. The reaction was carried out *via* the repetitive excess mixed anhydride (REMA) method. Thus, t-Boc protected quisqualic acid (81) was activated by conversion to the mixed anhydride with isobutyl chloroformate and N-methylmorpholine in DMF, then treated with leucine methyl ester hydrochloride and NMM in THF. After work-up and flash chromatography on silica, the protected peptide (145) was collected in 45% yield (scheme 38).

The ^1H NMR spectrum of the peptide exhibited resonances assignable to the leucine ester moiety, in addition to those due to the quisqualic acid portion of the molecule. The base peak in the mass spectrum was the $[M+H]^+$ at m/e 417.

Base hydrolysis of the ester was effected by treatment of (145) with sodium hydroxide at room temperature. The acid (146) was obtained in 68% yield. Its structure was confirmed by ^1H NMR, and mass spectrometry. Acidolysis of the t-Boc group with 1M hydrogen chloride in acetic acid solution, followed by ion exchange chromatography (acetate resin), afforded the dipeptide (147) in quantitative yield.

Ideally, comparative study of the peptides would have demanded the synthesis of several dipeptides incorporating different amino acid moieties coupled to quisqualic acid. Unfortunately, owing to lack of time, further peptide syntheses were not undertaken.

Scheme 38



Reagents: (i) mixed anhydride (IBCF), then

H. Leu.OMe.HCl,NMM, DMF

(ii) NaOH aq. (1 eq.), MeOH, 1 hr.

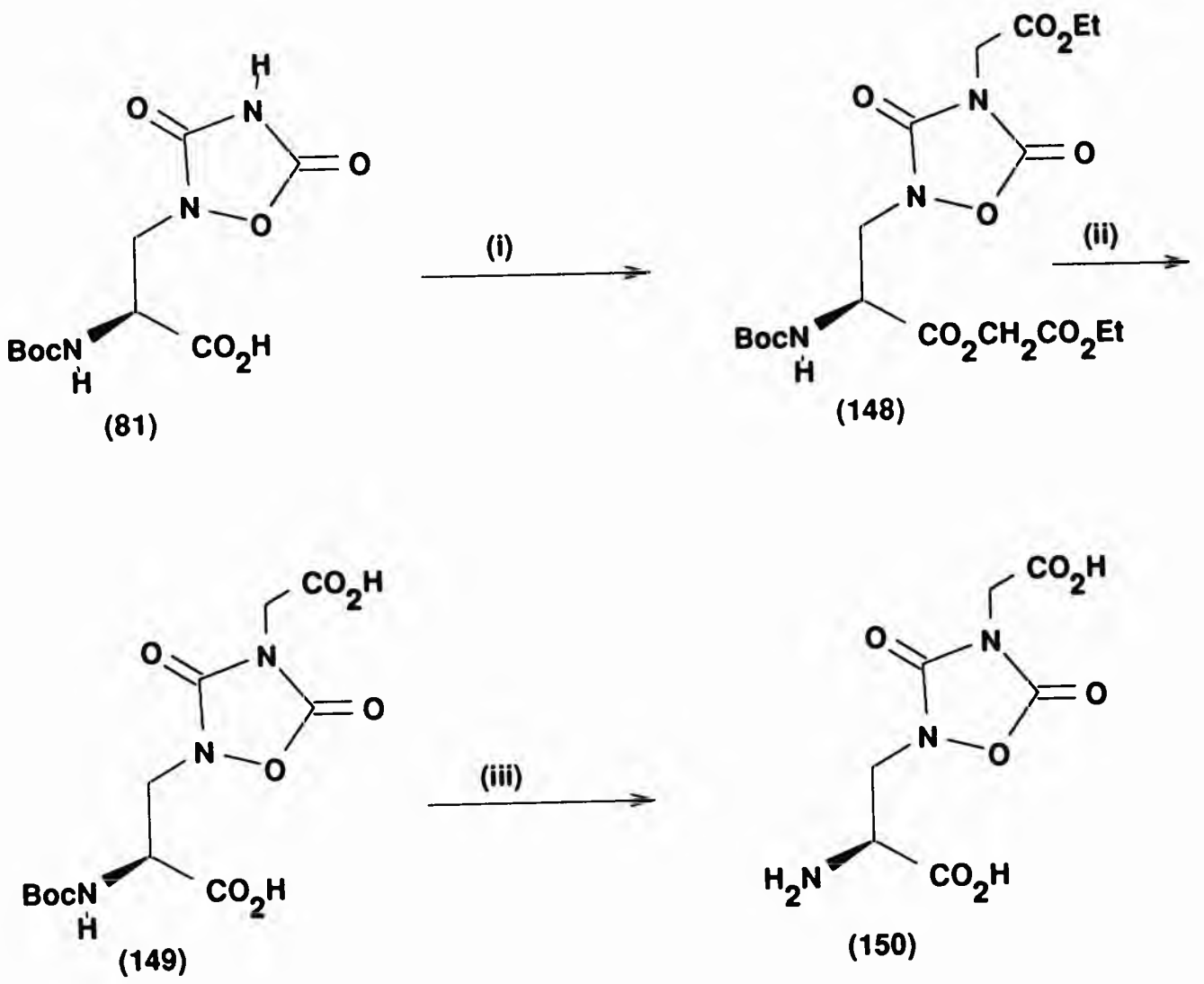
(iii) 1N HCl in AcOH, then ion-exchange chromatography

2.5.13.3. Synthesis of (S)-ε-N-carboxymethyl quisqualic acid(150).

The alkylation of quisqualic acid was initiated by the conversion of t-Boc quisqualic acid to the corresponding disodium salt by treatment with two

equivalents of sodium hydride in dry DMF. Alkylation was effected by treatment (*in situ*) of the sodium salt with ethyl bromoacetate. Chromatography gave the pure alkyl derivative (148) in 40% yield (scheme 39). Structural confirmation came primarily from its ^1H NMR spectrum.

Scheme 39



Reagents: (i) NaH, DMF, EtO_2CCH_2Br

(ii) KOH (3 eq.)

(iii) 1N HCl in AcOH, then ion-exchange chromatography

Hydrolysis of the ester (148) with three equivalents of potassium hydroxide to the corresponding diacid (149), and cleavage of the t-Boc group with 1M

hydrogen chloride in acetic acid solution gave, after ion-exchange chromatography, the alkylated amino acid (150). Interestingly, elution of the alkylated derivative from the ion exchange resin required a stronger concentration of the acetic acid (10%) than that (3%) used for the elution of quisqualic acid. This was presumably due to the carboxylic acid function in the N-ε side-chain.

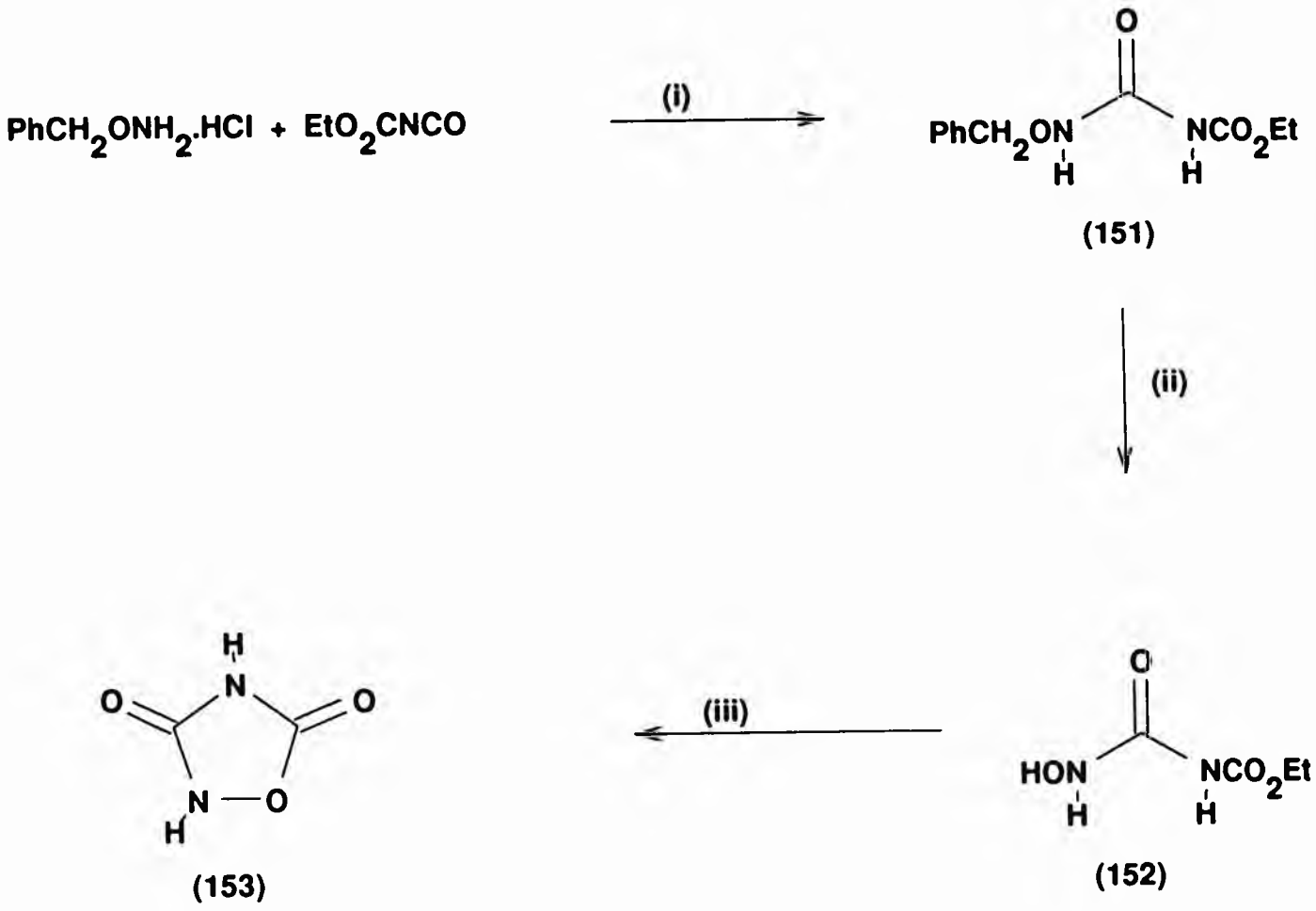
2.5.14. MISCELLANEOUS SYNTHESES.

2.5.14.1. 1,2,4-Oxadiazolidine-3,5-dione (153).

Prior to the successful synthesis of quisqualic acid, it was anticipated that a novel approach to the synthesis of quisqualic acid would be to alkylate the heterocycle (153) with the t-Boc β-lactone (78) to the corresponding t-Boc protected quisqualic acid. This would provide a convenient methodology by which the amino acid may be synthesized in its L-enantiomeric form.

Hence the heterocycle 1,2,4-oxadiazolidine-2,5-dione (153) was synthesized according to the method of Srivastava *et al.*,[183] by the condensation of O-benzylhydroxylamine hydrochloride with ethoxycarbonyl isocyanate. The reaction, which was carried out in dry THF, gave the hydroxamate (151) in good yield (92%). Spectroscopic characterization of the product confirmed the structure (scheme 40).

Scheme 40



Reagents: (i) dry THF, r.t.

(ii) H_2, Pd-C, EtOH

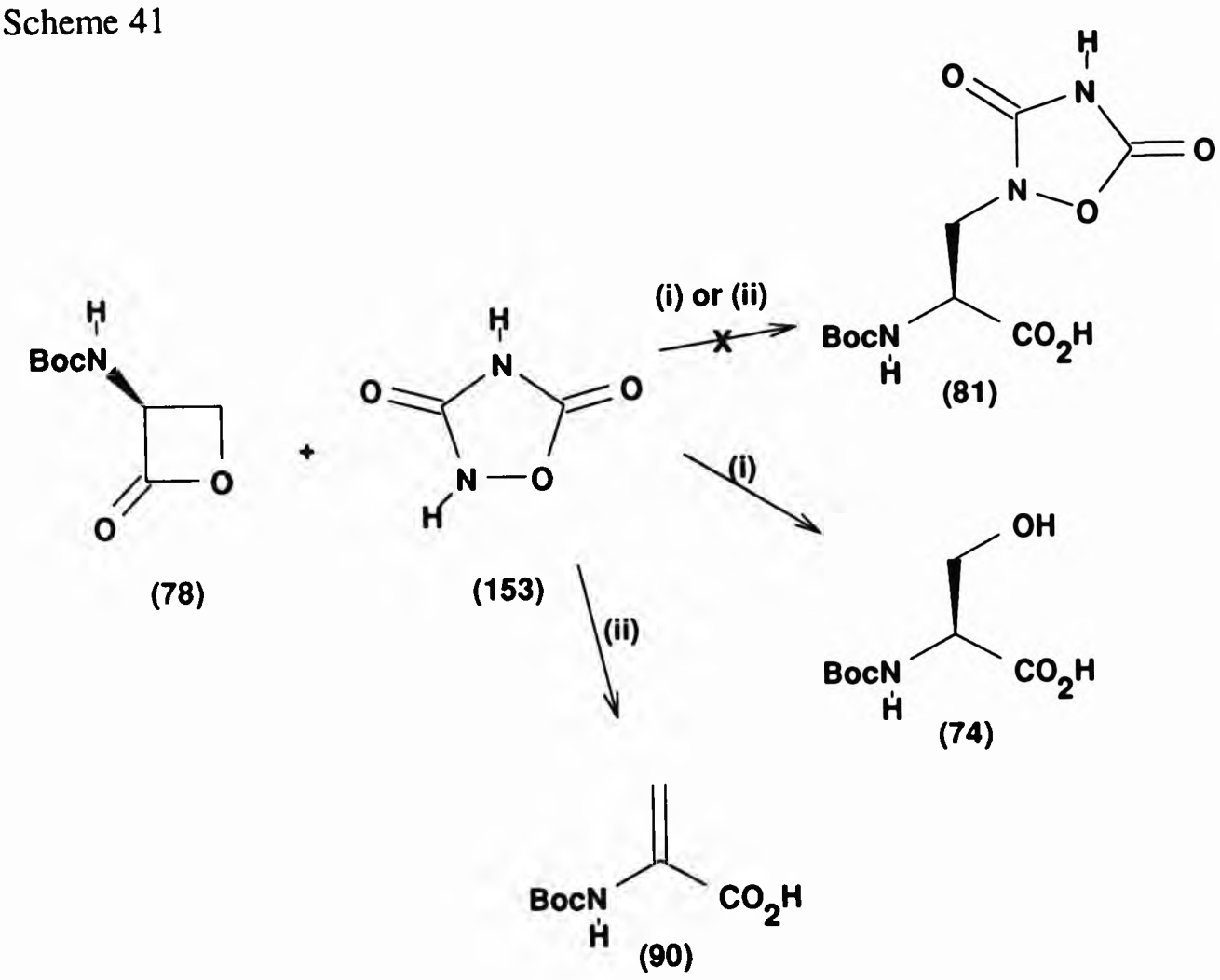
(iii) NaOH aq. MeOH, then ion-exchange chromatography

Debenzylation by catalytic hydrogenolysis in ethanol produced the hydroxyl derivative (152), which was cyclized to the heterocycle in aqueous sodium hydroxide. The structure of (153) was confirmed by mass spectrometry ($[M+H]^+$ at m/e 103) and elemental analysis which gave the empirical formula as $C_2H_2N_2O_3$.

2.5.14.2. Attempted alkylation of the heterocycle (153) with t-Boc β-lactone (78).

The reaction of the the heterocycle (153) with t-Boc β-lactone in dry ACN at room temperature, which would have been expected to give t-Boc protected quisqualic acid (81), resulted in the hydrolysis of the β-lactone to the protected serine derivative (74) (scheme 37). The presence of base led to the formation of the elimination product (90). Attempts to synthesize this compound under different conditions also failed to yield the desired product.

Scheme 41



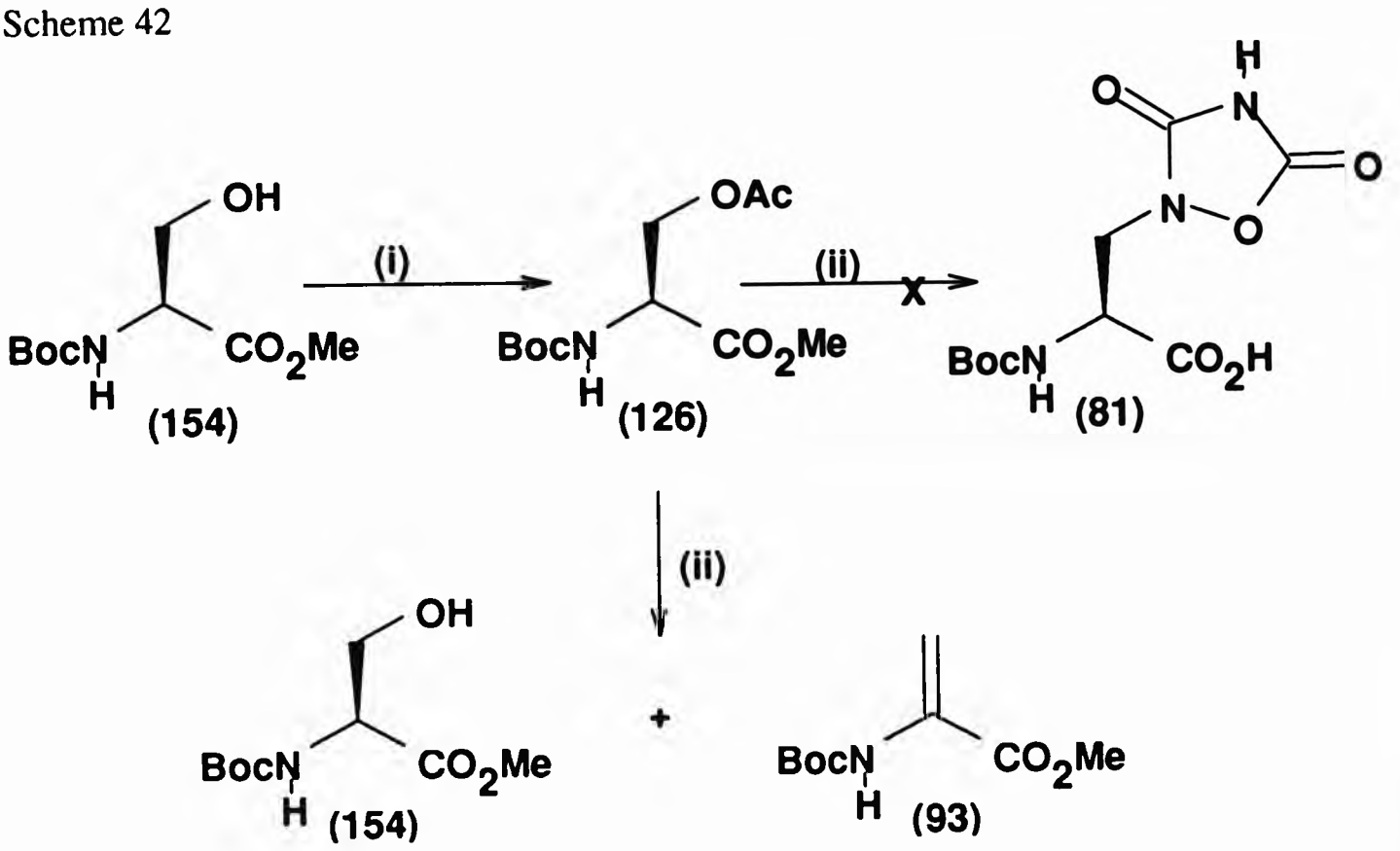
Reagents: (i) MeCN

(ii) MeCN, base

2.5.14.3. Attempts to alkylate the heterocycle (153) with t-Boc serinyl acetate methyl ester (126a)

In a different approach, attempts were made to alkylate the heterocycle by treatment with t-Boc serinyl-acetate methyl ester (126). At room temperature in dry ACN, the reaction gave protected serine and dehydroalanine, and at elevated temperatures, intractable mixtures were obtained. The use of different solvents, bases and experimental conditions also failed to yield the desired product. (scheme 42).

Scheme 42



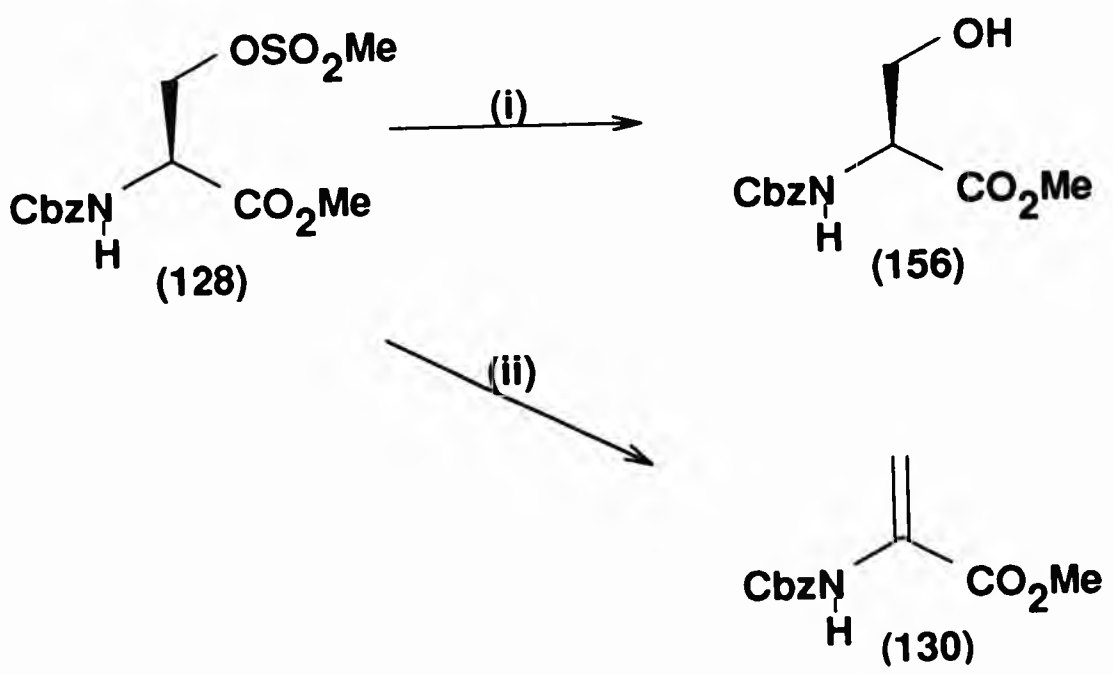
Reagents: (i) Ac_2O, pyridine, DCM

(ii) (153), dry MeCN, room temp.

2.5.14.4. Attempted synthesis of t-Boc quisqualic acid by the alkylation of the heterocycyle (153) with the mesylate (128).

It was hoped that the mesylate, being a better leaving group than the acetate, would furnish the alkylated material. Unfortunately, starting materials and protected serine (156) were obtained from the treatment of the heterocycle with the mesylate under neutral conditions in dry ACN. Base induced alkylation gave the elimination product .(130) (scheme 43).

Scheme 43



Reagents: (i) (153), dry ACN

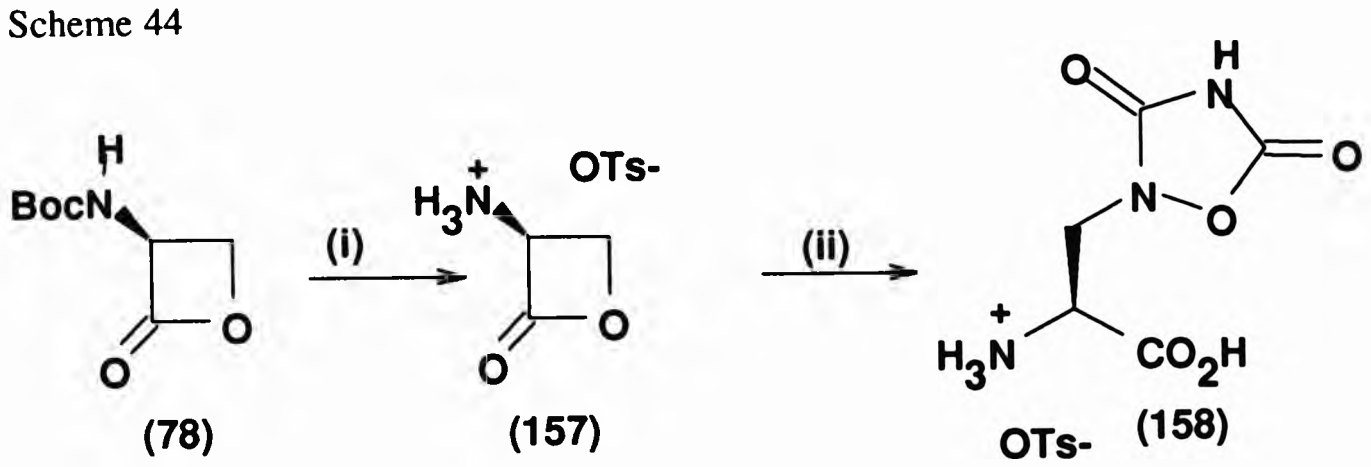
(ii) (153), base, dry ACN

2.5.14.5. Attempted alkylation of (153) with the PTSA salt of (3S)-3-aminopropiolactone (157).

In a different approach, a literature search[184] led to the possibility that the desired alkylation might be achieved by the use of the para-toluene sulphonic acid (PTSA) salt (157) of the β-lactone (78).

Thus the t-Boc β-lactone was converted to its PTSA salt (157) by the reaction with anhydrous p-toluenesulphonic acid in trifluoroacetic acid. Treatment of this with the heterocycle (153), in dry ACN at room temperature gave a white solid tentatively assigned as the protected quisqualate salt (158) (scheme 44). The chemical shift in its ^1H nmr spectrum suggested that alkylation may have taken place; the expected shift of the methylene from δ 4.95 in the lactone to δ 4.33 in the desired alkylated derivative was observed. Unfortunately, however, lack of time prevented further characterization of the product.

Scheme 44



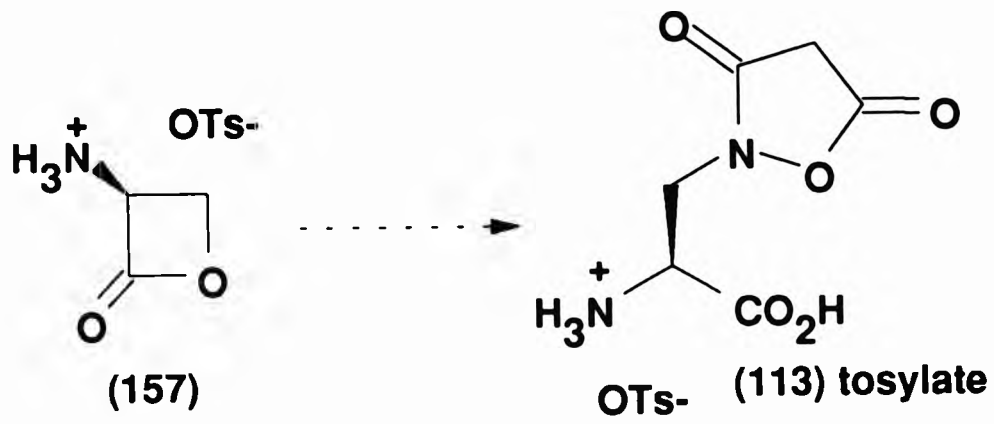
Reagents: (i) TFA, anhydrous para-toluenesulphonic acid

(ii) (153), dry ACN

2.5.15. Synthesis of 1,2-Oxazolidine-3,5-dione (159).

The unsuccessful attempt at deprotection of the carbon analogue (112) to the desired product (113) prompted the decision for the development of the methodology for an alternative method by which the analogue may be prepared. It was decided therefore that the heterocycle (159), should be synthesized. Alkylation of this with PTSA propiolactone (157) would then yield the tosylate salt of the carbon analogue (scheme 45).

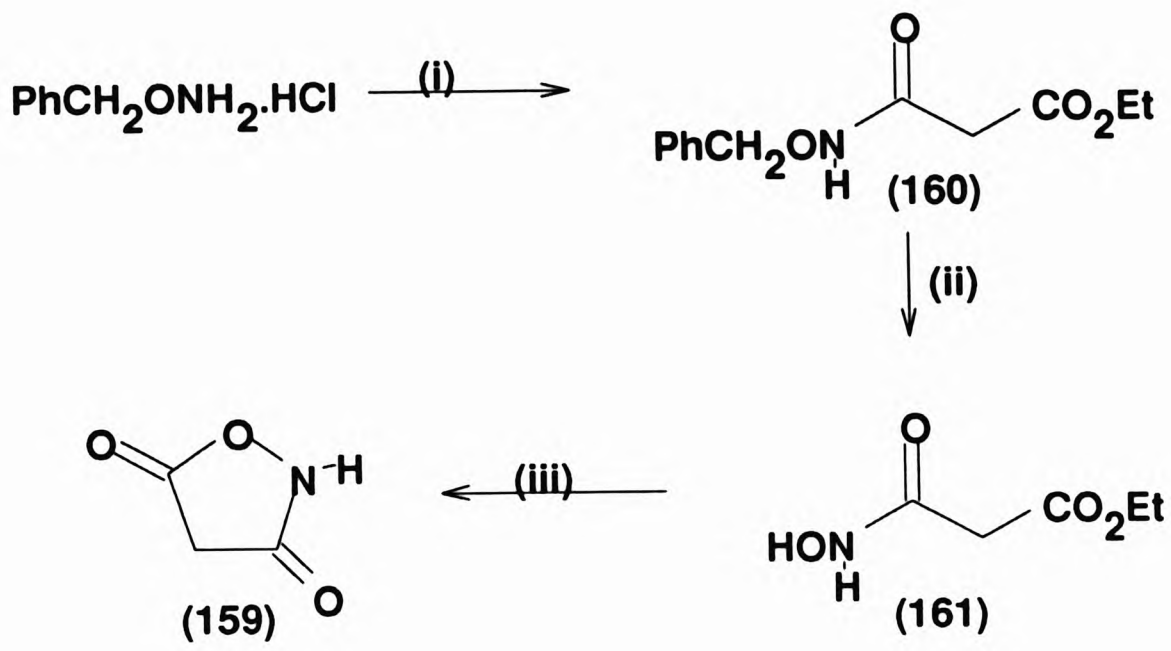
Scheme 45.



Reagents: (i) (159), base

To this end, O-benzylhydroxylamine hydrochloride was treated with ethyl malonyl chloride to afford the corresponding hydroxamate (160). Spectral characterization by ^1H nmr, mass spectrum and elemental analysis were consistent with the desired product. Catalytic hydrogenation in ethanol, using 10% palladium on activated charcoal gave the product as a foamy solid. An $[M+H]^+$ at m/e 149 in its FAB mass spectrum suggested the desired product (161) had been isolated. Cyclization of this intermediate by treatment with potassium hydroxide gave the heterocycle (159) as a white solid (scheme 46). Its FAB mass spectrum and elemental analysis confirmed the structure.

Scheme 46.



Reagents: (i) Ethyl malonyl chloride, K_2CO_3, PhMe

(ii) H_2, Pd-C, EtOH

(iii) KOH aq.

Unfortunately, lack of time prevented the alkylation of the heterocycle (159) with PTSA propiolactone (157).

CHAPTER THREE.

CHEMISTRY EXPERIMENTAL.

Materials and methods.

Nuclear magnetic resonance spectra were recorded on a Bruker AC200 Spectrometer.

The recorded chemical shifts are relative to tetramethylsilane (TMS) and the values are expressed as parts per million (ppm).

Mass spectra were recorded on a Kratos MS50TC.

Infrared spectra were recorded on a Bruker IFS 66

Elemental analyses were recorded on an Elemental Analyzer Model 1106.

Melting points were recorded on an Electrothermal Melting Point apparatus and are uncorrected.

Chemicals were obtained from the Aldrich Chemical Company.

Solvents were dried for reactions by the appropriate methods[153].

Flash chromatography was carried out on silica gel (Merck Kieselgel 60) (Art. 9385) (230-400 mesh ASTM).

Tlc was carried out on Merck Kieselgel 60 F252 (Art 5719).

Ion-exchange chromatography was performed on Dowex AG50-X8 cation exchange resin in the hydrogen form and Bio-Rad analytical grade anion exchange resin in the acetate form (200-400 mesh).

3.1 SYNTHESIS OF DL-QUISQUALIC ACID.

3.1.1. Preparation of ethyl hippurate.(56)

A catalytic amount of para-toluenesulphonic acid (0.31g, 1.62mmol) was added to a solution of hippuric acid (50g, 0.28mol) in ethanol (180ml). The solution was heated under reflux for twenty hours and excess ethanol was evaporated *in vacuo* to give a clear oil which was taken up in dichloromethane (60ml), washed with 2M sodium bicarbonate solution (3x20ml). The organic solvent was dried over sodium sulphate, filtered and the solvent was evaporated reduced pressure to afford a light oil. Crystallization from diethyl ether afforded ethyl hippurate as white crystals.

Yield: 49g. (84%).

m.p. 67^{o}C.(Lit.[170] 67.5^{o}C)

R_f: 0.76 (solvent hexane/ethyl acetate 2:1 v:v)

^{1}H nmr ($CDCl_3$): δ 1.29 (t, 3H, $\underline{CH_3}CH_2$); 4.2 (m, 4H, $CH_3\underline{CH_2}$ and$\underline{CH_2}$);

7.5 -7.9 (m, 5H, (PhCO).

^{13}C nmr ($CDCl_3$): 13.9 (s, 3H, $\underline{CH_3}CH_2$); 42.6 (s, 2H, HN$\underline{CH_2}$);

63.2 (s, 2H, $CH_3\underline{CH_2}$); 127.8-133.4 (s, 5H, $\underline{Ph}CO$);

171.9, and 172.4 (Ph$\underline{CO}$, and $\underline{CO_2}CH_2CH_3$).

Infrared spectrum: (nujol) ν_{max} 3340, 1760, 1640, 1580cm^{-1}.

3.1.2. Alternative method of preparation of ethyl hippurate (56).

A solution of hippuric acid (9.6g, 0.05mol), and triethyl orthoroformate (8.9g, 0.6mmol) in dimethylformamide (DMF) (6ml), was heated under reflux overnight at 125^{o}C. The oil bath temperature was increased to 176^{o}C, and the mixture was heated for another four hours. The brown solution was distilled,

and the fraction collected at b.p.140-142^{o}C was chromatographed on silica gel (40g) eluted with hexane /ethyl acetate (1:3 v:v). On evaporation of solvent ethyl hippurate was collected as white crystals. Recrystallization from diethyl ether gave the ester in 62% yield. (on a larger scale, yields were markedly decreased).

R_f: 0.76 (solvent EtOAc/hexane.

^{1}H nmr ($CDCl_3$): δ 1.3 (t, 3H, $CH_2\underline{CH_3}$); 4.2 (m, 4H, $\underline{CH_2}CH_3$ and $\underline{CH_2}$); 7.4-7.9 (m, 5H, $\underline{Ph}CO$).

3.1.3. N-Benzoyl-2,3-dehydroserine ethyl ester sodium salt (57).

Ethyl formate (14.8, 0.2mol), was added to a stirred and cooled solution of sodium ethoxide, (sodium 4.6g, 0.2mol) in dry ethanol (60ml). The resulting mixture was stirred for thirty minutes, and ethyl hippurate (43.7g, 0.21mol), was added over thirty minutes. The mixture was stirred at room temperature for forty hours. The yellow slurry was pour into a large volume of diethyl ether (600ml), and stired for a further hour, and filtered. The product, a cream solid, was air dried, powdered and dried in vacuo over P_2O_5.

Yield: 29.6g (55%).

m.p. 128^{o}C (decomposed).

^{1}H nmr: (D_2O): δ 1.24 (t, 3H, CH_3CH_2); 4.14 (q, 2H, CH_3,CH_2); 7.4- 7.6 (m, 5H, PhCO); 8.6. (s, 1H, CH=C).

Mass spectrum: molecular ion at $m/_e$ 257.

Anal. calcd. for $C_{12}H_{12}NO_4Na$ requires C 56.03; H 4.67; N 5.45.

Found: C 55.85; H 4.82; N 5.34.

3.1.4. Preparation of the enol from the sodium salt.

2M HCl (3ml) was added to a solution of the sodium salt (57) (150mg, 2mmol), in water (25ml) and the mixture was extracted with diethyl ether (3x25ml). The extracts were combined, dried over sodium sulphate, filtered and the filtrate was evaporated *in vacuo* to yield a light yellow oil which crystallized on standing.

Yield: 40mg, (87%).

^{1}H nmr ($CDCl_3$): δ 1.3 (t, 3H, $\underline{CH_3}CH_2$); 4.25 (q, 2H, $CH_3\underline{CH_2}$);
7.43-7.51 (m, 4H, PhCO, and CH=C); 7.78-7.87 (m, 2H, PhCO);
8.48 (broad s, 1H, NH); 12.2 (broad s,1H, HOC=C).

3.1.5. N-Benzoyl-3-oxo-alanine O-benzyl oxime ethyl ester (58).

A solution of the sodium salt (57), (13g, 0.05mol), in water and dichloromethane (70ml), was stirred at 0^{o}C, for fifteen minutes. O-Benzylhydroxylamine hydrochloride (7.34g, 0.046mol.) was added, and the mixture was warmed to room temperature and was left stirring overnight. The aqueous phase was separated and then extracted with dichloromethane (3x60ml). The combined extracts were dried over sodium sulphate, filtered and the filtrate was evaporated *in vacuo* to yield a light yellow oil which solidified on standing. Recrystallization from ether/hexane gave the oxime as a white solid.

Yield: 10.5g, (63%).

m.p. 85-87^{o}C, (Lit.[170] 88-89^{o}C).

R_f: 0.76 (solvent: hexane/ethyl acetate (2:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.28 (t, 3H, $\underline{CH_3}$,CH_2); 4.26, (q, 2H, $CH_3\underline{CH_2}$);
5.11 (s, 2H, $\underline{CH_2}$Ph); 5.41 (dd, 1H, $\underline{CH}CO_2C_2H_5$); 7.1-7.51 (m, 5H, $\underline{Ph}CH_2$); 7.65 (d, 1H, N=CH); 7.76-7.89,(m, 5H, PhCO)

Mass spectrum: molecular ion at $m/_e$ 340.

Anal. calcd. for $C_{19}H_{20}N_2O_4$ requires: C 67.04; H 5.92; N 8.28.

Found: C 67.09; H 6.12; N 8.41.

3.1.6. α-N-Benzoyl-3-benzyloxyamino alanine ethyl ester (59).

A trace of bromocresol green indicator was added to a solution of the oxime (58) (6.8g, 0.02mol), in methanol (50ml). The basic solution was acidified (yellow) with 2M methanolic HCl and sodium cyanoborohydride (1.26g, 0.02mol) was added in the one potion. The reaction mixture was maintained at pH2 by the gradual addition of methanolic HCl and was left stirring at room temperature overnight. The solvent was evaporated *in vacuo* and the residue was partitioned between chloroform and saturated sodium bicarbonate solution. The aqueous phase was extracted with chloroform (3x50ml) and the combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to yield a yellow oil (6.6g). After column chromatography on silica gel (30g) eluted with hexane/diethyl ether (2:1), the product was collected as a white solid on evaporation of solvent. Recrystallization from diethyl ether/hexane gave the pure hydroxylamine as a white crystalline solid.

Yield: 4.52g (66%).

m.p. 62-64°C.

^1H nmr ($CDCl_3$): δ 1.28 (t, 3H, $\underline{CH_3}CH_2$); 3.48 (m, 2H, $CH\underline{CH_2}$);

4.26 (q, 2H, $\underline{CH_2}CH_3$); 4.67 (s, 2H, $\underline{CH_2}Ph$); 4,87 (m, 1H, $\underline{CH}CO_2C_2H_5$);

6.77 (broad s, 1H, $\underline{NH}CH_2$); 7.26-7.28, (m, 10H, 2x$\underline{Ph}$).

Mass spectrum: $m/_e$ 342.

Anal. calcd. for $C_{19}H_{22}N_2O_4$ requires: C 66.50; H 6.48; N 8.18.

Found: C 66.87; H 6.90; N 8.43.

3.1.7. Diethyl-2-benzoylamino-4-benzyloxy-5-oxo-4,6-diazaheptane-1,7-dioate (60).

Ethoxycarbonyl isocyanate (0.3g, 2.5mmol) was added (dropwise) at 50°C, to a stirred solution of the hydroxylamine (59) in dry ether. After five to ten minutes, a colourless crystalline product began to separate out. Stirring was continued for another hour at room temperature and the product was collected by filtration, washed with cold ether and air dried. Recrystallization from diethyl ether gave a colourless amorphous solid.

Yield: (0.72g, 80%).

m.p. 98-100°C, (Lit.[170] 98-100°C).

R_f: 0.5 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.1-1.3 (m, 6H, 2x$CH_2\underline{CH_3}$);

3.5-4.4.3 (m, 6H, $CH\underline{CH_2}$+$(\underline{CH_2}CH_3)_2$ 4.5 (m, 1H, $\underline{CH}CH_2$);

5.1 (s,2H, $\underline{CH_2}Ph$); 5.8 (d, 1H, $\underline{NH}$); 6.7 (s, 1H, $\underline{NH}$);

7.3-7.8 (m, 10H, $CH_2\underline{Ph}$+$\underline{Ph}CO$);

Mass spectrum: molecular ion at m/e 457.

3.1.8. Diethyl-2-benzoylamino-4-hydroxy-5-oxo-4,6-diazaheptane-1,7-dioate (61).

A suspension of the benzyloxyureide (0.9g, 2mmol), in ethanol (20ml) and 5% palladium on activated charcoal (0.12g), was hydrogenated at room temperature and atmospheric pressure for two hours. The catalyst was removed by filtration through keiselguhr and the filtrate was evaporated to dryness, yielding a foamy solid (0.7g,96%). Recrystallisation from ethanol gave colourless plates.

Yield: (0.71g, 74%).

m.p. 146-148^{o}C.

R_f: 0.36 (solvent ethyl acetate/hexane 1:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.15 (t, 3H, $CH_2C\underline{H_3}$); 1.39 (t, 3H, $CH_2\underline{CH_3}$); 3.5 (m, 2H, $CH\underline{CH_2}$); 4.2 (m, 1H, $\underline{CH}CH_2$); 7.6 (m, 5H, $\underline{Ph}CO$); 9.02 (s, 1H, $\underline{OH}$-N).

Anal. calcd.: $C_{16}H_{21}N_3O_7$; requires C 52.32; H 5.72; N 11.44.

Found: C 52.01; H 5.96; N 11.12.

Mass spectrum: molecular ion at m/e 367.

3.1.9. N-Benzoylquisqualic acid (62).

The diester (61) (5.5g, 0.015mol), was dissolved in 4N sodium hydroxide solution (15ml), diluted with water (15ml). The mixture was left standing at room temperature for three hours and the solution was filtered, the filtrate was carefully acidified to pH3 with concentrated hydrochloric acid at 0^{o}C. The precipitated product was collected, washed with water and dried in a desiccator over P_2O_5.

Yield: 4.12g, (94%).

m.p. 191-193^{o}C.

^{1}H nmr (DMSO): δ 4.0 (m, 2H, CHCH2); 4.3 (m, 1H, CHCH2); 7.3 (d, 1H, NH); 7.5-7.8 (m, 5H, PhCO).

3.1.10 DL-Quisqualic acid (63).

N-Benzoylquisqualic acid (62) (5.68g, 0.02mol), was dissolved in 6N hydrochloric acid (240ml) and the solution was heated at 80^{o}C for twenty hours. On cooling, the benzoic acid which separated was removed by filtration and the aqueous phase was extracted with ethyl acetate (3x30ml) to remove residual benzoic acid, and the aqueous phase was concentrated *in*

vacuo. The resulting quisqualic acid hydrochloride was converted to the amino acid by ion exchange chromatography (Dowex 1X2-400, acetate resin). The column was first eluted with water (100ml), then with 3% acetic acid solution (200ml). The acidic eluates were combined and evaporated to dryness to afford DL quisqualic acid. Recrystallization from water and ethanol gave the amino acid as colourless plates.

Yield: 1.8g 48%, (Lit.[170] 30%).

m.p. 192°C (decomposed with effervescence) (Lit.[170] 190-191°C).

R_f: 0.17 (solvent: BuOH: AcOH: H_2O: 3:1:1 v:v).

^1H nmr (D_2O/NaOD): δ 3.4 (m, 1H, 1/2 CH$\underline{CH_2}$); 3.7 (m, 2H, $\underline{CH}$+1/2$\underline{CH_2}$).

Anal. calcd.: $C_5H_7N_3O_5$; C 31.75; H 3.70; N 22.22.

Found; C 31.03; H 3.78; N 21.67. (Calcd. for 0.25 H_2O: C 31.01, H 3.87, N 21.70)

*ν*max; (KBr), 3420, 3210, 2980, 2720. 1825, 1700, 1620.

3.1.11 Methodology for the insertion of the tritium label.

3.1.11.1. N-benzoyl-3-oxoalanine-t-butyl oxime ethyl ester (66).

A solution of the sodium salt (57) (2g, 7.8mmol), in water (25ml) and dichloromethane (25ml), was treated at 0°C, with tert-butylhydroxylamine hydrochloride (1.07g, 8.6mmol). The mixture was warmed to room temperature and was left stirring overnight. The aqueous phase was separated, washed with dichlomethane (3x20ml) and the combined organic extracts were washed with brine, dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to afford a light yellow oil. Crystallization from ether/hexane gave the oxime as a white solid.

Yield: 1.5g, (60%).

m.p. 105-107°C.

R_f: 0.44 (solvent EtOAc/hexane 2:1 v:v).

^1H nmr ($CDCl_3$): δ 1.28 (s, 9H, t-butyl); 1.29 (t, 3H, $CH_2\underline{CH_3}$);

4.26 (q, 2H, $\underline{CH_2}CH_3$); 5.42 (d, 1H, CHCOOEt); 7.28 (s, 1H, NH);

7.41-7.88 (m, 5H, PhCO).

^{13}C nmr ($CDCl_3$): 14.02 (3H, $CH_2\underline{CH_3}$); 27.48 (9H, t-butyl);

53.26 (1H CHCOOEt); 62.26 (2H, $\underline{CH_2}CH_3$); 127-133.66 (5H, Ph);

142.16 (1H, -N-CH); 166.92 (COOEt); 168.55 (PhCO).

Mass spectrum: molecular ion at m/e 306.

Anal. calcd. for $C_{16}H_{22}N_2O_4$ requires: C 62.75; H 7.19; N 9.15.

Found: C 62.87; H 7.21; N 9.23.

3.1.11.2. N-Benzoyl-3-t-butylaminoalanine ethyl ester (67).

N-benzoyl-3-oxoalanine-t-butyl oxime ethyl ester (66) (2g, 6.5mmol) was dissolved in a solution of acetic acid (95ml), and concentrated sulphuric acid (5ml). The mixture was hydrogenated at room temperature and a pressure of 40psi in the presence of 5% palladium on charcoal (0.2g). The reaction was followed by thin layer chromatography, which showed complete disappearance of the starting material after two hours The catalyst was removed by filtration, and the acidic filtrate was neutralised with sodium hydroxide pellets. The product was extracted into chloroform (2x60ml) and the combined extracts were dried over sodium sulphate, filtered and the solvent evaporated *in vacuo* to yield a light yellow oil, which solidified on standing. Recrystallization from diethyl ether /hexane gave the hydroxylamine as colourless crystals.

Yield: 0.8g, 40%.

m.p. 64-66^{o}C.

R_f: 0.29 (solvent: hexane/ethyl acetate 1:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.17 (s, 9H, t-butyl); 1.33 (t, 3H, CH_3CH_2); 3.39-4.46 (m, 2H, CH_2-NOBu^{t}); 4.28 (q, 2H, CH_2CH_3); 7.27 (s, 1H, HN-CH) 7.49-7.90 (m, 5H, PhCO).

^{13}C nmr ($CDCl_3$) δ 14.98 (CH_3CH_2); 26 55 (t-butyl); 52.67 (CHCH2); 53.05 ($CHCH_2$); 61.49 (CH_2CH_3); 77.6 (HNCH); 127.1 (PhCO); 167.19 (PhCO); 171.42, (CO_2Et).

Anal cacld for $C_{16}H_{24}N_2O_4$ requires C 62.34; H 7.79; N 9.09.

Found: C 62.13; H 7.81; N 8.60.

ν_{max}: (nujol); 3330, 2950, 1780, 1760, 1740, 1720, 1520.

(The work reported above provides a method by which tritiated quisqualic acid can be synthesized).

3.1.11.3. Diethyl-2-benzoylamino-4-t-butyloxy-5-oxo-4,6-diazaheptane-1,7-dioate (68).

A solution of the hydroxylamine (67) (3.5g, 0.01mol) in dry ether (20ml), was treated dropwise with ethoxycarbonyl isocyanate (1.4g, 0.01mol), at 5°C. After stirring for an hour, the white solid which precipitated was filtered, washed with ice-cold ether, and air dried. Recrystallization from ether gave the diester as a white crystalline solid.

Yield: 4.1g, 94%.

m.p. 135-136°C.

R_f: 0.68 (solvent hexane/EtOAc 1:1 v:v).

^1H nmr ($CDCl_3$): δ 1.1 (s, 9H, t-butyl); 1.2 -1.4 (m, 6H, $(CH_2CH_3)_2$; 3.6-4.4 (m, 6H, $CHCH_2$+$(CH_2CH_3)_2$; 4.5 (m, 1H, $CHCH_2$).5.8 (d, 1H, NH); 6.8 (s, 1H, NH).

3.1.11.4. Diethyl-2-benzoylamino-4-hydroxy-5-oxo-4,6-diazaheptane 1,7-dioate (61)

The tert-butoxy amino derivative (68) (0.5g, 1.62mmol), was dissolved at 0°C, in a solution of trifluoroacetic acid (20ml). The solution was left standing at room temperature for two hours. On evaporation of solvent, the residue was taken up in ethyl acetate and washed with 2M sodium bicarbonate solution (3x40ml) and then with saturated brine, dried over sodium sulphate, filtered and the solvent evaporated *in vacuo* to give the product as a white solid.

Yield: 0.28g, 68%.

m.p. 148-150°C.

R_f: 0.36 (solvent:hexane/ethyl acetate)

^1H nmr ($CDCl_3$): δ 1.14 (t, 3H, CH_2CH_3); 1.4 (t, 3H, CH_2CH_3);

3.6, (m, 2H, CH$\underline{CH_2}$); 4.3 (m, 1H, $\underline{CH}CH_2$); 7.4-7.6 (m, 5H, $\underline{Ph}$);

9.00 (s, 1H, $\underline{NH}$).

Synthesis of" ^3H" DL quisqualic acid was carried out as outlined in (3.1.9.) and (3.1.10.) .

3.2. Alkylation of DL-quisqualic acid.

3.2.1. N-Benzoyl quisqualic acid methyl ester (69).

Thionyl chloride (5ml) was added (dropwise) under nitrogen, to dry methanol (40ml), at -40°C. The solution was stirred for fifteen minutes and N-benzoylquisqualic acid (3.46mmol) was added. The mixture was warmed to room temperature, then heated under reflux for three hours. On cooling, the solvent was evaporated and the residue was taken up in water and washed with ethyl acetate (3x30ml). The combined washings were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford a clear oil. Crystallization from ether and methanol gave the ester as a white solid.

Yield: 0.65g, 62%.

R_f: 0.28 (solvent DCM/MeOH 9:1 v:v).

m.p. 128-130°C.

^1H nmr ($CDCl_3$): δ 3.7 (s, 3H, O$\underline{CH_3}$);

4.1-4.3 (m, 4H, CH$\underline{CH_2}$+$\underline{CH2}$COOEt); 4.9 (m, 1H, $\underline{CH}$CH2); 6.9 (d, 1H, $\underline{NH}$); 7.3-7.6 (m, 5H, $\underline{Ph}$CO).

Mass spectrum: $[M+H]^+$ at m/e 308.

3.2.2. α-N-Benzoyl-ε-N-ethoxycarbonylmethyl quisqualic acid methyl ester (70).

A solution of the methyl ester (69) (0.5g, 1.6mmol) in DMF was treated with sodium hydride (0.04g, 1.7mmol). The mixture was stirred for thirty minutes at room temperature, followed by the addition of ethyl bromoacetate (0.28g, 1.7mmol). Stirring was continued for another fifteen minutes and the mixture was heated under at 50°C for four hours. On cooling, the solvent was evaporated and the residue was partitioned between dichloromethane and aqueous sodium bicarbonate solution. The crude product was extracted into the organic phase (3x20ml), washed with brine, dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to give a light yellow oil. Purification by flash chromatography on silica gel (15g) and elution methanol/dichlomethane 2:98 v:v gave the diester as a very light yellow oil.

Yield: 0.31g, 53%.

R_f: 0.40 (solvent EtOAc/Hexane 1:1 v:v).

^1H nmr ($CDCl_3$): δ 1.3 (t, 3H, $CH_2\underline{CH_3}$);

3.7 (s, 3H, $O\underline{CH_3}$); 4.2-4.5 (m, 4H, $CH\underline{CH_2}$+$\underline{CH2}COOEt$);

5.1 (m, 1H, $\underline{CH}CH2$); 7.1 (d, 1H, $\underline{NH}$); 7.4-7.8 (m, 5H, $\underline{Ph}CO$).

Mass spectrum: [molecular ion-CO_2] at m/e 349

3.2.3. α-N-Benzoyl-ε-N-carboxymethyl quisqualic acid (71).

The diester (70) (0.29g, 0.8mmol), was dissolved in methanol (5ml) followed by the addition of sodium hydroxide (0.07g, 1.7mmol) in water (15ml). The mixture was stirred at room temperature for two hours and alcohol was evaporated under reduced pressure. The aqueous phase was acidified to pH3 with 2M HCl and then extracted with ethyl acetate (3x20ml). The combined

extracts were dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to afford the acid as a foamy oil.

Yield: 0.13g, 50%.

R_f: 0.5 (solvent DCM/MeOH/AcOH 44:5:1 v:).

^{1}H nmr (DMSO): δ 3.6-3.7(m, 2H, CH<u>CH$_2$</u>); 3.8 (s, 2H, <u>CH$_2$</u>COOH);

4.3 (m, 1H, <u>CH</u>CH$_2$); 6.9. (d, 1H, NH);

7.3 (m, 5H, Ph).

3.2.4. Atempted synthesis of ε-N-carboxymethyl DL-quisqualic acid (72).

A solution of the adduct (71) (100mg, 0.28mmol) in 6M HCl was heated at 80^{o}C for twenty hours. Benzoic acid which precipitated was collected by filteration and the residue was evaporated to dryness to afford an oily residue. Ninhydrin developed tlc showed the presence of several products. These were neither isolated nor characterized.

3.3. SYNTHESIS OF L-QUISQUALIC ACID (20).

3.3.1. N-t-Butoxycarbonyl serine (74).

Di-tert-butyl dicarbonate (155.g, 0.71mol) in isopropanol (50ml) was added dropwise over an hour to a well stirrred solution of L-serine (73) (50g, 0.48mol) and sodium hydroxide (21g, 0.52mol), in water (50ml) and isopropanol (100ml). After a short period the temperature rose to approximately 40^{o}C, and a further 100ml of isopropanol was added. The turbid solution was stirred overnight, and the solvent was evaporated *in vacuo*. The residue was taken up in water, washed with petrol to remove residual (Boc)$_2$O, then acidified with 5% citric acid solution to pH3. The

aqueous phase was saturated with sodium chloride and the product was extracted into ethyl acetate (3x60ml), dried over sodium sulphate, filtered and the solvent evaporated *in vacuo*, to afford a colourless oil which solidified on standing to give the monohydrated t-Boc serine.

Yield: 86g 87%.

m.p.74-77^{o}C (lit. 75-78^{o}C [202])

R_f: 0.55 (solvent DCM/MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.8-4.1, (m, 2H, CH_2CH);

4.2-4.4 (m, 1H $CHCH_2$); 6.2 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 206.

3.3.2. (3S)-3-t-Butoxycarbonylamino-oxetan-2-one (78).

Triphenylphosphine (15.5g, 0.06mol) was dissolved in 150ml of dry THF. The solution was stirred under nitrogen at -78^{o}C, followed by the dropwise addition of diethyl azodicarboxylate (11.32g, 0.065mol). Stirring was continued and the off-white slurry which ensued was treated with a solution of t-Boc-L-serine (12.1g, 0.05mol) in 100ml of dry THF. The mixture was stirred overnight and concentrated *in vacuo*, to a give light brown/orange residue which was (pre-absorbed on silica gel), purified by flash chromatography using ethyl acetate/ hexane (40/60 v:v) as the eluent. The product, a white solid, was collected on evaporation of solvent.

Yield: 58%. (Lit [176] 71%)

m.p. 119-120^{o}C (Lit [176] 119.5-120^{o}C).

R_f: 0.4 (solvent DCM/MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.9 (m, 2H, $CHCH_2$);

4.3 (m, 1H, $CHCH_2$); 4.9 (s 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 188.

Anal. calcd. for $C_8H_{13}NO_4$ requires C 51.34 H 6.95 N 7.48

Found: C 51.04 H 6.93 N 7.24.

3.3.3. N-t-Butoxycarbonylserine-O-benzylhydroxamic acid (75).

N-tert-butoxycarbonyl-L-serine monohydrate (1.115g, 5mmol), was dehydrated by the azeotropic removal of water using toluene/ethyl acetate. The anhydrous oil which ensued was dissolved in dry tetrahydrofuran (THF) (20ml), cooled to -25°C, and N-methylmorpholine (505mg, 5mmol) was added The mixture was stirred at -25°C for five minutes and isobutyl chloroformate (682.5mg, 5mmol) was then added, and the mixture was stirred at -25°C for two minutes then treated with a cooled (-15°C) suspension of O-benzylhydroxylamine hydrochloride (1.6g, 10mmol), and N-methylmorpholine (1.01g, 10mmol), in dry THF (50ml). The reaction mixture was stirred for two hours at -15°C, then poured into 400ml of semi-saturated brine. The solution was acidified (pH3) by the gradual addition of 5% citric acid solution. The precipitated product was isolated by filtration and the filtrate was extracted into ethyl acetate, (3x20ml), dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo*. The white solid which ensued was added to that obtained by filtration, and recrystallized from hexane and ethyl acetate to afford the product as a white crystalline solid.

Yield: 1.2g, 62%.

m.p. 129-130°C.

R_f: 0.44 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.6-4.41 (m, 2H, CH_2CH); 4.9 (s, 2H, CH_2Ph); 5.5 (s, 1H, HN-t-Boc); 7.4 (m, 5H, Ph); 9.3 (s, 1H, HN-OCH2).

Mass spectrum $[M+H]^+$ at m/e 311

Anal. calcd. for $C_{15}H_{22}N_2O_5$ requires: C 58.06; H 7.10; N 9.03.

Found: C 57.57; H7.06; N 8.82.

3.3.4. (3S)-1-Benzyloxy-3-tert-butoxycarbonylamino-azetidin-2-one (76).

Diethyl azodicarboxylate (3.06g, 0.02mol) was slowly added at -78°C to a solution of triphenylphosphine (5.1g, 0.019mol), in dry THF (100ml). A light yellow slurry which ensued was treated by the slow addition of a solution of the hydroxamate (75), (5.92, 0.019mol) in dry THF (100ml). The mixture was stirred at -78°C for twenty minutes, then overnight at room temperature. The solvent was evaporated and the residue preabsorbed on silica gel (3g), then chromatographed on silica gel (30g), eluted with hexane/ethyl acetate 2:1. The product, a white crystalline solid, was collected on evaporation of solvent.

Yield: 2.3g 41% (Lit.[172] 66%).

R_f: 0.42 (solvent EtOAc/hexane 1:2 v:v).

m.p. 91-92°C (Lit.[172] m.p 91-92°C).

^1H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.2-3.5 (m, 2H, CH_2CH);

4.5 (m, 2H, CH_2CH); 4.9 (s, 3H, CH_2Ph +HN t-Boc); 7.4 (m, 5H, Ph)

Mass spectrum: $[M+H]^+$ at m/e 293.

Anal. calcd. for $C_{15}H_{20}N_2O_4$ requires: C 61.64, H 6.85, N 9.59.

Found: C 61.28, H 7.12, N 9.96.

Infrared spectrum: ν_{max} 3210, 2925,1720 cm^{-1}

3.3.5. (S)-α–N-tert-Butoxycarbonyl-3-benzyloxyamino alanine (77).

A solution of sodium hydroxide (0.15g, 3.75mmol) was added to a solution of N-α-tert-butoxycarbonyl-L-serine β lactam (76) (1g, 3.42mmol), in methanol (10ml). The mixture was stirred at room temperature for two hours and the basic solution was acidified (pH3), with saturated citric acid solution at O°C. The precipitated product was collected by filtration, and the aqueous phase

was extracted with ethyl acetate (3x20ml), dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford a colourless oil. This was combined with the precipitated solid, and then recrystallized from ethyl acetate/hexane to give the product as a white solid.

Yield: 0.64g, 64%.

m.p. 62-64^{o}C.

R_f: 0.79 (solvent:DCM/MeOH/AcOH 45:5:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4, (s, 9H, t-Boc); 3.1-3.4, (m, 2H, CH_2CH);

4.35-4.5 (m, 1H, $CH$$CH_2$); 4.7, (s, 2H, CH_2Ph); 4.8-5.0 (s, 1H, HN t-Boc)

7.3, (s, 5H, Ph),

Mass spectrum: $[M+H]^+$ at m/e 311.

Anal. calcd. for $C_{15}H_{22}N_2O_5$ requires: C 58.06 H 7.1 N 9.03.

Found: C 58.26 H 7.31 N 9.23.

Infrared spectrum: ν_{max} 1650 cm^{-1}

3.3.6. Synthesis of (77) by the alkylation of O-benzylhydroxylamine with t-Boc β–lactone (78).

A solution of t-Boc β-lactone (1g, 5.3mmol) and O-benzylhydroxylamine hydrochloride (0.95g, 5.9mmol) in dry acetonitrile (40), was heated at 80^{o}C for eight hours. The reaction which was followed by tlc showed the presence of two products. The solvent was evaporated *in vacuo*, and the residue was taken up in ethyl acetate, washed with water (3x25ml). The organic extract was dried over sodium sulphate, filtered and evaporated under reduced pressure to afford the acylated material (75) as a colourless oil. Crystallization from ethyl acetate/hexane gave the hydroxamate as a white solid.

Yield: 40%.

m.p. 129-130^{o}C.

R_f: 0.45 (DCM/MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.6-4.1 (m, 3H, CH and CH_2);

4.9 (s, 2H, CH_2Ph); 5.5 (d, 1H, NH); 7.4 (s, 5H, Ph);

9.3 (broad s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 311.

The aqueous phase was acidified to pH3 with 0.5M HCl at 0^{o}C and then extracted with ethyl acetate (3x20). The extracts were combined, dried over sodium sulphate, filtered and evaporated *in vacuo* to afford the aklylated product (77) as a colourless oil which solidified on standing.

Yield: 60%.

m.p. 62-64^{o}C.

R_f: 0.8 (DCM/MeOH/AcOH 45:4:1 v:v).

^{1}H nmr (DMSO): δ 1.4 (s, 9H, t-Boc); 3.1-3.4 (m, 2H, $CHCH_2$);

4.4 (m, 1H, $CHCH_2$); 5.5 (s, 2H, CH_2Ph); 5.8 (s, 1H, NH); 7.3 (s, 5H, Ph).

Mass spectrum: $[M+H]^+$ at m/e 311.

3.3.7. (2)-N-t-butoxycarbonyl-3-benzyloxyamino-alanine methyl ester.

Freshly prepared diazomethane[185] in ether was slowly added to an ethereal solution of the acid (74) (2.7g, 8.37mmol), in diethyl ether (100ml), until the evolution of nitrogen had ceased and the solution remained permanently yellow. A small amount of acetic acid solution was slowly added to destroy residual diazomethane. The ethereal solution was washed with cold water (3x30ml), dried over sodium sulphate, filtered and evaporated *in vacuo* to afford a colourless oil.

Yield: 2.8g, 92%.

R_f: 0.55 (solvent DCM/MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.2-3.5 (m, 2H, CH_2CH)

3.7 (s, 3H, OCH3); 4.45 (m, 1H, $CHCH_2$); 4.6 (s, 2H, CH_2Ph);

5.4 (broad s, 1H, HN-t-Boc); 5.8 (broad s, 1H, HN); 7.35 (s, 5H, Ph).

(Although the pilot synthesis of quisqualic acid utilized the methyl ester, it was found that on subsequent synthesis, esterification was not neccessary. Acylation of the acid (77) proceeded smoothly to yield the desired product).

3,3,8, (2S)-2-t-Butoxycarbonylamino-4-benzyloxy-5-oxo-4,6-diaza-heptan-1,7-dioic acid-7-ethyl ester (79).

A solution of the benzyloxyamine (77) (0.5g, 1.6mmol) in dry THF (30ml) was treated with a dropwise addition of with ethoxycarbonyl isocyanate (0.19g, 1.7mmol.) at 0^{o}C. The mixture was stirred at 0^{o}C for ten minutes, then at room temperature for one hour. The product which precipitated was collected by filtration and the aqueous filtrate was extracted with ethyl acetate (3x20ml), dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure, to afford a white solid which was combined with that obtained by filtration, and recrystallized from ether and hexane.

Yield: 0.5g, 74%.

m.p. 119-121^{o}C.

R_f: 0.64 (solvent DCM/MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.3 (t, 3H, CH_3CH_2); 1.4 (s, 9H, (t-Boc):

3.8-4.1 (m, 2H, CH_2CH); 4.2(q, 2H, CH_2CH_3); 4.6 (m, 1H, $CHCH_2$);

4.85 (s, 2H, CH_2Ph) 5.3 (s, 1H, NH-t-Boc); 7.4 (s, 5H, Ph); 7.8 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 426.

3.3.9. (2S)-2-t-Butoxycarbonylamino-4-hydroxy-5-oxo-4,6-diaza-heptan-1,7-dioic acid-7-ethyl ester (80).

A suspension of the acid (79) (200mg, 0.47mmol) in ethanol (25ml) and 5% palladium on charcoal catalyst (20mg) was hydrogenated at room temperature and atmospheric pressure for one hour. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The residue was taken up in ethyl acetate, washed with 0.1M HCl, dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo*, to afford the desired product as a colourless oil.

Yield: 0.12g, 76%.

R_f: 0.63 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.3 (t, 3H, $CH_2\underline{CH_3}$); 1.4 (s, 9H, t-Boc);

3.6 (s, 3H, $O\underline{CH_3}$); 3.6 and 4.2 (m, 2H, $\underline{CH_2}CH$); 4.2 (q, 2H, $\underline{CH_2}CH_3$);

4.8 (m, 1H, $CH_2\underline{CH}$); 5.7 (d, 1H, NH-t-Boc); 8.2) (s, 1H, NH);

9.0 (s, 1H, OH).

Mass spectrum: $[M+H]^+$ at m/e 336.

3.3.10. (2S)-α-N-t-Butoxycarbonyl quisqualic acid (81).

Sodium hydroxide (0.35g, 8.75mmol), in water (10ml), was added to the acid (80) (1.5g, 4.3mmol). The mixture was left standing at room temperature for three hours and filtered. The filtrate was acidified with saturated citric acid solution to pH3 at 0°C. The product was extracted into ethyl acetate (3x30ml) and the combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to yield a foamy oil. Crystallisation from dichloromethane and ether gave the cyclized acid as a white solid.

Yield: 1.2g, 97%.

m.p. 96-98°C

R_f: 0.11 (solvent DCM/MeOH 9:1 v:v).

^1H nmr (DMSO): δ 1.4 (s, 9H, t-Boc): 3.8 -4.1 (m, 2H, CH_2CH);

4.3 (m, 1H, $CHCH_2$); 7.2 (d, 1H, NH-t-Boc).

Mass spectrum: [M+Na] at m/e 312.

3.3.11. L-Quisqualic acid (20).

t-Boc L-quisqualic acid (500mg, 1.7mmol) was dissolved in 1M HCl in acetic acid solution (10ml). The solution was left standing at room temperature for one hour, during which time the amino acid hydrochloride began to precipitate out. On evaporation of solvent, the solid which ensued was taken up in water and washed, then with ether. The aqueous phase was concentrated *in vacuo* and converted to the free amino acid by ion exchange chromatography (acetate anion resin Dowex X8) eluted with water (100ml), then with 3% acetic acid solution (200ml). On evaporation of solvent, L-quisqualic acid was collected as a white solid.

Yield: 203mg, 62%

m.p. 192-193 decomposed with effervescence.

R_f: 0.22 (solvent: $BuOH/H_2O/AcOH$ 3:1:1 v:v).

^1H nmr (D_2O/NaOD): δ 4.0.(m, 1H, $1/2CH_2CH$);

4.1-4.2 (m, 2H, CH +$1/2$ CH_2)

Mass spectrum: $[M+H]^+$ at m/e 190

Anal. calcd for $C_5H_7N_3O_5$ requires: C 31.75: H 3.70; N 22.22.

Found: C 31.72: H 3.72: N 22.38.

$[\alpha]_D$ +17.2 (c= 1.0, 1M HCl).(Lit.[177] +17.0 (c=2.0, 6M HCl)).

3.4. SYNTHESIS OF SOME ANALOGUES OF QUISQUALIC ACID.

3.4.1.1. (S)-2-t-Butoxycarbonylamino-4,6-diaza-5-oxo-octan-1,8-dioic acid-8-ethyl ester (82)

A solution of the protected hydroxylamine (77) (3.4g, 0.011mol), in dry THF (50ml), was treated dropwise at 0°C, with ethyl isocyanatoacetate (1.5g, 0.02mol) in dry THF (20ml). The colourless solution was stirred for fifteen minutes at 0°C, then at room temperature overnight. The solvent was evaporated *in vacuo*, and the residue was partitioned between 0.5M HCl and ethyl acetate. The product was extracted into the organic phase, dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to give a foamy oil.

Yield: 3.43g, 70%.

R_f: 0.64 (solvent: DCM/MeOH/AcOH 45:5:1: v:v).

^1H nmr ($CDCl_3$): δ 1.1-1.25 (t, 3H, $\underline{CH_3}CH_2$); 1.4 (s, 9H, (t-Boc);

3.6-4.3 (m, 6H, $CH\underline{CH_2}$, and $\underline{CH_2CH_2}CH_3$); 4.7 (m, 1H, $\underline{CH}CH_2$);

6.0 (s, 1H, $\underline{HN}CH_2COOEt$); 6.4 (s, 1H, $\underline{HN}$ t-Boc.);

7.3-7.5 (s, 5H, $\underline{Ph}CH_2$).

Mass spectrum: $[M+H]^+$ at m/e 440.

ν_{max} (KBr): 3540 (broad), 1620, 1730cm^{-1}.

3.4.1.2. (2S)-2-t-Butoxycarbonylamino-4-hydroxy-4,6-diaza-5-oxo-octan-1,8-dioic acid-8-ethyl ester (83).

10% Palladium on activated charcoal (0.3g), was suspended in a solution of the O-benzylhydroxamate ester (82) (2.9g, 6.9mmol.) in ethanol (60ml). The suspension was hydrogenated at room temperature and atmospheric pressure for two hours. The catalyst was removed by filtration and the filtrate was

evaporated *in vacuo* to yield a foamy oil.

Yield: 2.36g, 96%.

R_f: 0.35 (solvent DCM/MeOH 3:1 v:v).

^1H nmr ($CDCl_3$): δ 1.2 (t, 3H, $\underline{CH_3}CH_2$); 1.4 (s, 9H, t-Boc);

3.8 (m, 2H, $CH\underline{CH_2}$); 4.2 (m ,4H, $\underline{CH_2}CH_3$ and $NH\underline{CH_2}$);

4.5 (m, 1H $\underline{CH}CH_2$); 6.1 (d, 1H, HN); 6.5 (m, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 350, [M+Na]+ at m/e 372

3.4.1.3. (2S)-2-t-Butoxycarbonyl-3-[2-(-tetrahydro-1,2,4-oxadiazin-3,6-dionyl)]alanine (84).

The hydroxyl derivative (83) (2.4g, 6.86mmol) was added to a solution of sodium hydroxide (0.6g, 15mmol) in water (20ml) and the solution was stirred at room temperature for two hours. (The reaction was followed by ferric chloride indicator). The solution was filtered and the filtrate was acidified by the gradual addition of 0.5M HCl to pH3. The product was extracted into ethyl acetate, dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to yield a foamy white solid.

Yield: 1.6g, 60%.

m.p. 72-74°C.

R_f: 0.57 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

^1H nmr (d-6 DMSO): δ 1.4 (s, 9H, t-Boc); 3.7 (m, 4H, $CH\underline{CH_2}$ and $\underline{CH_2}COOH$); 4.2 (m, 1H, $\underline{CH}CH_2$); 6.6 (d, 1H, NH); 7.1 (m, 1H, NH).

3.4.1.4. 3-(2-Tetrahydro-1,2,4-oxadiazin-3,6-dionyl)alanine (85).

The t-Boc protected system (84) (500mg, 1.7mmol), was dissolved in 1M HCl in acetic acid (20ml). The solution was left at room temperature for one hour, during which time amino acid hydrochloride began to precipitate out. On

evaporation of the solvent, the white solid which resulted was taken up in water and passed down an anion exchange column (acetate anion Dowex X8) eluted with 100ml of water, followed by 5% acetic solution, (200ml).The solvent was evaporated under reduced pressure and the product was collected as a white solid.

Yield: 280mg, 84%.

m.p. 191-193^{o}C (decomposed 185^{o}C then melted).

R_f: 0.17 (solvent BuOH/AcOH/H2O 3:1:1 :v:v)

^{1}H nmr (D_2O): δ 3.5 (s, 2H, $\underline{CH_2}CH$); 3.6-3.7 (m, 2H, $\underline{CH_2}NO$);

4.0 (m, 1H, $\underline{CH}CH_2$).

Mass spectrum: $[M+H]^+$ at m/e 204.

Anal. calcd. for $C_6H_9N_3O_5$ requires: C 41.37; H 4.43; N 20.69.

Found: C 41. 85; H 4.71; N 20.90.

$[\alpha]_D$ - 10.0 (c 0.1 , 1M HCl).

3.4.2. SYNTHESIS OF THE HYDANTOIN ANALOGUE OF QUISQUALIC ACID.

3.4.2.1. Attempted synthesis of 3-[1-(1,3-imidazolidin-2,5-dionyl)] alanine (87).

A solution of hydantoin (0.1g, 1.18mmol) in dry THF was added under nitrogen to a solution of the t-Boc β-lactone (78) (0.2g,1.07mmol) in dry THF. The mixture was stirred at room temperature for four hours. Tlc showed the presence of starting materials only, and the reaction was heated at 50^{o}C for another two hours. On cooling, solvent was evaporated and the residue was taken up in water, acidified to pHethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was

evaporated under reduced pressure to afford a colourless oil, which was identified as t-Boc serine.

Yield: 0.12g, 55%.

^{1}H nmr ($CDCl_3$): δ 1.5 (s, 9H, t-Boc); 3.8 (s, 3H, OCH_3);

5.7 (d, 1H, CH); 6.2. (d, 1H, CH); 7.0 (broad s, 1H, NH).

3.4.2.2. Synthesis of N-t-Butoxycarbonyl serine methyl ester (91).

A solution of t-Boc L-serine (2g, 9.76mmol) in ether (30ml) was treated with the slow addition of freshly distilled diazomethane in ether. Excess diazomethane was destroyed by the addition of a small amount of acetic acid solution. The ethereal solution was washed with cold water (3x40ml), dried over sodium sulphate, filtered and evaporated *in vacuo* to afford the ester as a colourless oil.

Yield:1.8g, 84%.

R_f: 0.42 (solvent DCM/MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.5-3.6 (m, 2H, CHCH_2);

3.7 (s, 3H, OCH_3); 4.2 (m, 1H, $CHCH_2$); 6.0 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 220

3.4.2.2. Attempted synthesis of 3-[1-(1,3-imidazolidin-2,5-dionyl)] alanine (87) by the Mitsunobu method.

A solution of triphenylphosphine (0.26g, 1mmol) in dry THF was stirred under nitrogen at -78^{o}C, for 10 minutes, followed by the dropwise addition of diethyl azodicarboxylate (0.17g, 1mmol). The off-white slurry which ensued was treated with t-Boc serine methyl ester (0.2g, 0.94mmol) and hydantoin (0.1g, 1mmol). The mixture was stirred at -78^{o}C for another fifteen minutes, warmed to room temperature, and stirred for two hours. The crude product

was purified by preabsorbed flash chromatography on silica eluted with hexane/ethyl acetate (2:1 v:v). The product, a colourless oil, was obtained on evaporation of solvent. (The product was identified by its ^1H nmr spectrum as the elimination product (90).

3.4.2.3. (2S)-2-Benzyloxycarbonylamino-3-amino-propionic acid (L-Cbz-DAPA.OH (96).

N-Benzyloxycarbonyl-L-asparagine (2.66g, 0.01mol) was added to a stirred solution of bis[trifluoroacetoxy]iodobenzene (6.5g, 0.015mol) in dimethylformamide (DMF) (40ml) and water (40ml). The mixture was stirred for fifteen minutes at room temperature, followed by the addition of pyridine (1.6ml, 0.02mol). After stirring at room temperature for three hours, the solvent was evaporated *in vacuo*. The residue was dissolved in water (100ml) and washed extensively with ether to remove residual bis[trifluoroacetoxy]iodobenzene and iodobenzene. The aqueous phase was concentrated *in vacuo* to afford the crude L-Cbz-DAPA, which was crystallized from ethanol/ether to give the compound as a white solid.

Yield: 1.89g , 79%. (Lit.[181] 84%)

m.p 225-226 ^oC (Lit.[181] m.p 228-230^oC)

R_f: 0.18 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

^1H nmr (DMSO): δ 2.5 (m, 2H, CH$\underline{CH_2}$); 4.4 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 2H, $\underline{CH_2}$Ph); 6.9 (s, 1H, $\underline{NH}$); 7.4 (s, 5H, $\underline{Ph}$); 7.5 (s, 2H, $\underline{2xNH}$).

Mass spectrum: $[M+H]^+$ at m/e 239

3.4.2.4. Methyl-(2S)-2-Benzyloxycarbonylamino-3-amino propionate (97).

Thionyl chloride (10ml) was slowly added to dry methanol (70ml), under nitrogen, at -40°C. The solution was stirred for a further fifteen minutes, followed by the addition of the acid (5g, 0.02mol). The mixture was stirred at -40°C for fifteen minutes, then warmed to room temperature. After ten minutes at room temperature, the reaction mixture was heated under reflux for three hours. The solvent was evaporated *in vacuo* and the residue was was triturated under ether, to give a colourless solid. Recrystallization from methanol/ether gave the methyl ester hydrochloride as a white solid.

Yield: 4.5g, 85%.

m.p. 152-154°C (decomposed, then melted).

R_f: 0.24 (solvent DCM/MeOH 9:1 v:v).

^1H nmr (DMSO): δ 3.0-3.3 (m, 2H, $\underline{CHCH_2}$); 3.7 (s, 3H, $\underline{OCH_3}$); 4.4 (m, 1H, $\underline{CHCH_2}$); 5.1 (s, 2H, $\underline{CH_2Ph}$); 7.4 (s, 5H, $\underline{Ph}$); 7.9 (d, 1H, $\underline{NH}$); 8.4 (s, 2H, $\underline{2xNH}$).

Mass spectrum: $[M+H]^+$ at m/e 253.

3.4.2.5. (2S)-1-Methyl-8-ethyl-2-benzyoxycarbonylamino-4,6-diaza-5-oxo-octan-1,8-dioate (98).

The adduct (97) (4g, 0.016mol) in dry THF was treated at 0°C, under nitrogen, with ethyl isocyanatoacetate (2.24g, 0.017mol) in dry THF. The reaction mixture was warmed to room temperature, and was left stirring for two hours. The solvent was evaporated and the residue was taken up in ethyl acetate (35ml), washed with saturated sodium bicarbonate and then with brine, dried over sodium sulphate, filtered and the filtrate was evaporated *in vacuo*, to afford the amide as a colourless oil.

Yield: 5.1g, 84.%.

R_f: 0.48 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.2 (t, 3H, $CH_2\underline{CH_3}$); 3.6 (m, 2H, $CH\underline{CH_2}$);

3.7 (s, 3H, OCH3) 4.2 (m, 2H, CH2COOEt); 4.4 (m, 1H, CHCH2);

5.1 (s, 2H, $\underline{CH_2}Ph$); 5.3 (s, 1H, NH); 6.2 (d, 1H, NH); 7.3 (s, 5H, Ph).

Mass spectrum: $[M+H]^+$ at m/e 382.

3.4.2.6. (2S)-2-Benzyloxycarbonylamino-4,6-diaza-5-oxo-octan-1,8-dioc acid (99).

The amide (98) (4.8g, 0.013mol) was treated with a solution of sodium hydroxide (1.06g, 0.026mol) in water (60ml). The mixture was stirred at room temperature for one hour, acidified to pH3 with 0.5M HCl and extracted with ethyl acetate (3x25ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford the diacid as a colourless oil.

Yield: 3.7g, 84%.

R_f: 0.63 (solvent DCM/MeOH AcOH 44:5:1 v:v).

^1H nmr (DMSO): δ 3.1–3.6 (m, 2H, $CH\underline{CH_2}$); 3.7 (d, 2H, $\underline{CH_2}COOH$);

4.0 (m, 1H, $\underline{CH}CH_2$); 5.0 (s, 2H, $\underline{CH_2}Ph$); 6.3 (m, 2H, 2x$\underline{NH}$);

7.4 (s, 5H, Ph); 7.5 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 340.

3.4.2.7. Attempted cyclization to (100).

EDC (0.29g, 0.15mmol) was dissolved in a solution of dry THF (30ml) containing the diacid (99) (0.5g, 1.45mmol) and triethylamine (0.15g, 1.53mmol). The mixture was stirred at room temperature for two hours and

the solvent was evaporated *in vacuo*. The resulting light yellow residue was taken up in water, acidified to pH3 with 2M HCl and extracted with ethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered and the solvent evaporated under reduced pressure to afford a light yellow oil which was found by tlc to contain multiple products. Attempts were not made to separate these products. The method was abandoned.

3.4.2.8. (2S)-1-Methyl-8-benzyl-2-benzyloxycarbonylamino-4,6-diaza-5-oxo-octan-1,8-dioate (103).

Benzyl isocyanatoacetate (0.3g, 1.99mmol) in dry THF (10ml), was added dropwise at 0°C, to a solution of (2S) 1-methyl-2-benzyloxycarbonylamino-3-aminopropionic acid hydrochloride (97) (0.5g, 1.98mmol) and triethylamine (0.8g, 1.12ml) in dry THF (50ml). The mixture was stirred at 0°C for fifteen minutes, then at room temperature overnight. The precipitated triethylamine hydrochloride was removed by filtration, and the filtrate was evaporated *in vacuo* to give a yellow oily residue which was partitioned between dichloromethane and aqueous sodium bicarbonate solution. The product was extracted into the organic phase (3x40ml), dried over sodium sulphate, filtered to remove the desiccant, and then evaporated *in vacuo*. The resulting residue was taken up in a minimum amount of ethyl acetate/hexane 2:1 and purified by flash chromatography on silica gel (30g) The product was isolated as a colourless oil following gradient elution with hexane/ethyl acetate (1:2 v:v).

Yield: 0.52g, 62%.

R_f: 0.55 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 3.6 (m, 2H, $CH\underline{CH_2}$); 3.7 (s,3H,$O\underline{CH_3}$);

3.9 (d, 2H ,$\underline{CH_2}COOCH_2Ph$); 4.4 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 4H, $2x\underline{CH_2}Ph$);

5.2 (m, 2H, 2xNH); 6.1 (d, 1H, NH); 7.4 (s, 10H, 2xPh).

Mass spectrum: $[M+H]^+$ at m/e 444.

3.4.2.9. (2S)-1-Methyl-2-t-butoxycarbonyl-4,6-diaza-5-oxo-octan-1,8-dioic acid (104).

Palladium on activated charcoal 10% (0.2g) was suspended in methanol containing the diester (103) (0.5g, 1.13mmol.), followed by the addition of a solution of sodium bicarbonate (0.5g, 5.95mmol) in water (10ml), and di-tert-butyldicarbonate $(Boc)_2O$) (1.3g, 5.96mmol) in methanol (70ml). The suspension was hydrogenated at room temperature and atmospheric pressure for six hours. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*, the resulting residue was taken up in water (30ml), washed with petrol to remove residual $(Boc)_2O$ and then acidified by the slow addition of 0.5M HCl at 0^oC, to pH3. The product was extracted into ethyl acetate (3x40ml), and the combined extracts were dried over sodium sulphate, filtered to removed the dessicant and the solvent was evaporated *in vacuo* to afford the product as a foamy solid.

Yield: 0.33g, 68%.

^1H nmr (DMSO): δ 1.4 (s, 9H, t-Boc); 3.3 (m, 2H, $CHCH_2$);

3.7 (s, 3H, OCH_3); 3.8 (d, 2H, CH_2COOH); 4.0 (m, 1H, $CHCH_2$);

6.3 (m, 2H, 2xNH); 7.1 (m, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 320.

3.4.2.10. α-N-t-Butoxycarbonyl-3-[1-(imidazolidine-2-dionyl)]alanine (92).

The t-Boc protected adduct (104), (0.3g, 0.94mmol) was dissolved in dry THF (60ml), followed by the addition of 1-ethyl-[3-

(dimethylamino)propyl]carbodiimide (EDC) (0.7g, 1.02mmol), and triethylamine (0.1g, 1.02mmol). The suspension was stirred at room temperature overnight. and the precipitated triethylamine hydrochloride was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was taken up in 60ml of ethyl acetate, washed with three 20ml portions of sodium bicarbonate and then with brine, dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to afford the crude (92). Purification by chromatography on silica gel (25g), eluted with ethyl acetate/hexane 2:1, gave the cyclized product as foamy oil.

Yield: 0.18g, 65%.

R_f: 0.39 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.5 (m, 2H, $CHCH_2$);

3.7 (s, 3H, OCH_3); 3.9 (s, 2H, CH_2COOH); 4.5 (m, 1H, $CHCH_2$);

5.5 (d, 1H, NH); 6.0 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 302.

3.4.2.11. α-N-t-Butoxycarbonyl-3-[1-(imidazolidin-2,5-dionyl]alanine (89).

Sodium hydroxide (0.02g, 0.50mmol) in water (20ml), was added to a solution of the cyclized hydantoin (92) (0.15g, 0.50mmol), in methanol (5ml). The solution was stirred at room temperature for two hours. Alcohol was evaporated *in vacuo* and the aqueous phase was carefully acidified with 0.5M HCl to pH3 at 0°C and extracted with ethyl acetate (3x30ml). The organic extracts were combined, dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to gave the t-Boc protected acid as a foamy solid.

Yield: 100mg, (71%).

R_f: 0.56 (solvent DCM/MeOH/AcOH).

^1H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.8 (m, 2H, $CHCH_2$);

4.2 (d, 2H, $\underline{CH_2}NH$); 4.4 -4.6 (m, 1H, $\underline{CH}CH_2$); 5.5 (s, 1H, $\underline{NH}$);

5.9-6.0 (s, 1H, $\underline{NH}$).

Mass spectrum: $[M+H]^+$ at m/e 288.

3.4.2.12. 3-[1-(1,3-Imidazolidin-2,5-dionyl)]alanine (87).

The-t-Boc protected acid (89) (72mg, 2.5mmol) was dissolved in a solution of 1M HCl/acetic acid solution (10ml). The solution was left standing at room temperature for one hour, during which time amino acid hydrochloride began to separate out. The solvent was evaporated and the residue was taken up in a minimum volume of water and converted to the free amino acid by ion exchange chromatography (acetate anion resin) eluted primarily with water 50ml, followed by 10% acetic acid solution. The amino acid was collected as a white solid on evaporation of solvent.

Yield: 0.31g, 66%.

m.p. 224-226^oC.

R_f: 0.19 (solvent $BuOH/H_2O/AcOH$ 3:1:1 v:v).

^1H nmr (D_2O): δ 3.6-3.8 (m, 2H, $CH\underline{CH_2}$); 3.9 (s, 2H, $\underline{CH_2}CO$);

4.2 (m, 1H, $\underline{CH}CH_2$).

Mass spectrum: $[M+H]^+$ at m/e 188.

Anal. calcd. for $C_6H_9N_3O_4$ requires C 38.50; H 4.81; N 22.43.

Found: C 38.72; H 5.00; N 22.61.

$[\alpha]_D$ - 11.76 (c 0.2, 1M HCl).

3.4.3. PREPARATION OF 3-(1-SUCCINIMIDYL)-ALANINE (109).

3.4.3.1. (2S)-2-Benzyloxycarbonylamino-4-aza-5-oxo-octan-1,8-dioic acid-1-methyl ester (106).

Succinic anhydride (1.10g, 11mmol) was added at 0°C, under nitrogen, to a stirred solution of Cbz-L-DAPA.OMe.HCl (97) (2.4g, 9.5mmol), and triethylamine (1.1g, 10.9mmol) in dry THF (50ml). The mixture was stirred at room temperature overnight and the solvent was evaporated *in vacuo*, to give a light yellow oil, which was partitioned between ethyl acetate and water. Excess succinic anhydride was extracted into ethyl acetate, and the aqueous phase acidified with 1M HCl, to pH3, and the product was extracted into ethyl acetate (3x40ml), the combined extracts were dried, over sodium sulphate, filtered and the filtrate was evaporated *in vacuo* to afford the amide as a colourless oil, which crystallized on standing.

Yield: 2.6g, 64%.

m.p. 92-94°C.

R_f: 0.18 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 2.4 (d, 2H, $\underline{CH_2}CH_2$); 2.6 (d, 2H, $CH_2\underline{CH_2}$);

3.5-3.7 (m, 5H, $CH\underline{CH_2}$ and $O\underline{CH_3}$); 4.4 (m, 1H, $\underline{CH}CH_2$);

5.1 (s, 2H, $\underline{CH_2}Ph$);

6.0 (d, 1H, NH); 6.6 (d, 1H, NH); 7.3 (s, 5H, Ph)

Mass spectrum: $[M+H]^+$ at m/e 353.

3.4.3.2. (2S)-N-Benzyloxycarbonylamino-3-(1-succinimidyl)-alanine methyl ester (107).

N,N-Diisopropylethylamine (0.57ml, 4.7mmol) and 1-hydroxybenzotriazole (HOBT) (0.7g, 5.18mmol), were added, under nitrogen, to a stirred solution

of the ester (1.67g, 4.7mmol) in dry THF (40ml). The solution was stirred at room temperature for fifteen minutes, followed by the addition of EDC, (1g, 5.22mmol). The reaction, which was followed by thin layer chromatography (tlc), was stirred for a further four hours. On evaporation of solvent the resulting residue was partitioned between aqueous sodium bicarbonate and ethyl acetate. HOBT was removed in the aqueous phase and the organic layer was washed with 0.1M HCl (to remove the urea by-product), then with brine. The solvent was dried over sodium sulphate, filtered and the filtrate was evaporated *in vacuo*, to give the cyclized product as a white solid.

Yield: 1.2g, 76%

m.p. 88-90°C.

R_f: 0.6 (solvent DCM/MeOH 9:1 v:v).

^1H nmr (DMSO): δ 2.3 (dd, 4H, 2x$\underline{CH_2}$); 3.3 (m, 2H, CH$\underline{CH_2}$);

3.7 (s, 3H, O$\underline{CH_3}$);

4.1 (m, 1H, $\underline{CH}CH_2$); 5.0 (s, 2H, $\underline{CH_2}$Ph); 7.3 (s, 5H, $\underline{Ph}$); 7.9 (d, 1H, $\underline{NH}$).

Mass spectrum: [MH+] at m/e 335.

3.4.3.3. (2S)-N-Benzyloxycarbonylamino-3-(1-succinimidyl) alanine (108).

Potassium hydroxide (0.2g, 3.56mmol), in water (10ml), was added to a solution of the ester, (1.1g, 3.6mmol) in isopropanol (30ml). The solution was stirred at room temperature for two hours, and alcohol was evaporated *in vacuo*. The resulting residue was partitioned between ethyl acetate and 1M hydrochloric acid. The product was extracted into ethyl acetate (3x40ml), and the combined organic extracts were dried over sodium sulphate and the solvent was evaporated *in vacuo*. The product was collected as a white solid after crystallization from ethyl acetate/hexane.

Yield: 900mg, 79%.

m.p. 142-144°C.

R_f: 0.26 (solvent DCM/MeOH 9:1 v:v).

^1H nmr (DMSO): δ 2.3 (dd, 4H, 2x$\underline{CH_2}$); 3.3 (m, 2H, CH$\underline{CH_2}$);

4.1 (m, 1H, $\underline{CH}CH_2$); 5.0 (s, 2H, $\underline{CH_2}$Ph); 7.3 (s, 5H, $\underline{Ph}$); 7.9 (d, 1H, $\underline{NH}$).

Mass spectrum: $[M+H]^+$ at m/e 321.

3.4.3.4. 3-(1-Succinimidyl)-alanine (109).

A suspension of (2S)-benzyloxycarbonylamino-3-succinimidopropanoic acid (108) (700mg, 2.18mmol) and 10% palladium on charcoal (70mg), in ethanol (25ml), was hydrogenated at room temperature and pressure for one hour. The catalyst was largely removed by filtration, and the product, which precipitated from the alcohol, was dissolved in hot water, and refiltered to remove residual catalyst. The product, a white solid, was collected on evaporation of solvent in 55% yield.

m.p. 240-242°C.

R_f: 0.25 (solvent BuOH/AcOH/H2O 3:1:1 v:v).

^1H nmr (D_2O/DCl): δ 2.4 (m, 2H, 2x$\underline{CH_2}$); 3.6 (m, 2H, CH$\underline{CH_2}$);

4.1 (m, 1H, $\underline{CH}$CH2).

Mass spectrum: $[M+H]^+$ 187.

Anal. calcd. for $C_7H_{10}N_2O_4$ requires: C 45.16 H 5.38 N 15 05.

Found: C 45.35 H 5.58 N 14.97.

$[\alpha]_D$ -29.0 (c 1.0 1M HCl).

3.4.4. SYNTHESIS OF 3-[2-(1,2-OXAZOLIDIN-3,5-DIONYL)] ALANINE (113).

3.4.4.1 (2S)-7-ethyl-2-t-butoxycarbonylamino-4-benzyloxy-4-aza-5-oxo-heptandioic acid (110).

Ethyl malonyl chloride (1.5g, 9.7mmol) was added (dropwise) under nitrogen, to a solution of the benzyloxyamine (77) (2g, 6.5mmol) and potassium hydroxide (1.8g, 0.013m), in toluene (60ml). The mixture was stirred at room temperature for ten minutes, then heated under reflux for two hours. On cooling, solvent was evaporated *in vacuo*, and the residue was taken up in water, acidified to pH3 with saturated citric acid solution and then extracted with three 30ml portions of dichloromethane. The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford the crude (110) as a light yellow oil. Purification by chromatography on silica gel (25g) eluted with DCM/MeOH (9:1 v:v) gave the amide as a foamy oil.

Yield: 1.4g: 51%.

R_f: 0.75 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

^1H nmr (DMSO): δ 1.1 (t, 3H, $CH_2\underline{CH_3}$); 1.4 (s, 9H, t-Boc);

3.5 (s, 2H, $\underline{CH_2}COOEt$); 3.8-4.2 (m, 4H, $CH\underline{CH_2}$ and $\underline{CH_2}CH_3$);

4.3 (m, 1H, $\underline{CH}CH_2$); 4.9 (s, 2H, $\underline{CH_2}Ph$); 6.9 (d, 1H, NH); 7.4 (s, 5H, Ph).

Mass spectrum: $[M+H]^+$ at m/e 425.

3.4.4.2. (2S)-7-ethyl-2-N-tert-butoxycarbonyl-3,6.tetrahydro-4.-N-hydroxyl-5-oxo-heptan-1,7-dioic acid (111).

A solution of the amide (110) (1.2g, 2.8mmol) in ethanol (50ml), was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladium on activated charcoal. After one hour, the catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to afford the hydroxyl derivative as a clear oil.

Yield: 0.8g, 86%.

R_f: 0.24 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

^1H nmr (DMSO): δ 1.0 (t, 3H, $CH_2\underline{CH_3}$); 1.4 (s, 9H ,t-Boc);

3.4 (s, 2H, $\underline{CH_2}COOEt$): 3.6-4.0 (m, 2H, $CH\underline{CH_2}$);

4.2 (m, 3H, $\underline{CH_2}CH_3$ and $\underline{CH}CH_2$); 6.6 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 335.

3.4.4.3. N-t-Butoxycarbonyl-3-(1,2-oxazolidin-3,5-dion-2-yl)-alanine (112).

Potassium hydroxide (0.3g, 5.42mmol), in water (20ml) was added to a solution of the hydroxamic acid (111), (0.9g, 2.70mmol) in methanol (2ml). The reaction, which was followed by thin layer chromatography, (developed with ferric chloride solution) was stirred at room temperature for one hour. Alcohol was evaporated, and the aqueous phase was acidified with 0.5M HCl to pH3 and extracted with ethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to afford the cyclized acid as an off-white solid.

Yield: 0.5g, 64%.

m.p. 147-150°C.

R_f: 0.59 (solvent DCM/MeOH/AcOH 44:5:1: v:v).

^1H nmr (DMSO): δ 1.4 (s, 9H, t-Boc); 3.2 (s, 2H, CH_2CO);

3.8-4.1 (m, 2H, $CHCH_2$); 4.3 (m, 1H, $CHCH_2$); 7.2 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 289.

3.4.4.4. 3-(1,2-oxazolidin-3,5-dion-2-yl) alanine.

N-tert-butoxycarbonyl-oxazolidine adduct (112) (0.3g, 1.04 mmol) was disolved in a solution of 1M HCl in acetic acid (15ml). The solution was left standing at room temperature for one hour and the solvent was evaporated under reduced pressure. The off-white residue which ensued was taken up in water and converted to the free base by ion exchange chromatography (acetate anion resin) eluted primarily with water followed by 3% acetic acid solution (120ml). The solvent was evaporated under reduced pressure to give the product as an off-white solid. (The product appeared to have decomposed, as shown by T.L.C. using ninhydrin development.)

3.4.5. SYNTHESIS OF [1-{ (3S)-3-AMINOPYRROLIDIN-2,5-DIONYL }] ACETIC ACID (119).

3.4.5.1. (5S)-1-Ethyl-7-benzyl-5-t-butoxycarbonylamino-3-aza-4-oxo-heptan-1,7-dioate (115).

N-tert-butoxycarbonyl-L-aspartic acid -4-benzyl ester (114) (3g, 9.32mmol) in dry THF was treated at -15°C, with N-methylmorpholine (0.04g, 9.32mmol and isobutylchloroformate (1.27g, 10mmol). The solution was stirred under nitrogen for two minutes, followed by the addition of a suspension of glycine ethyl ester hydrochloride (1.4g, 10mmol) and N-methylmorpholine (1g, 10mmol), in 50ml of dry THF. After being stirred for

two hours at -15°C the solution was poured into 500ml of semi saturated brine, and extracted with three 30ml portions of ethyl acetate. The combined extracts were dried over sodium sulphate, filtered and evaporated. The light yellow oil which ensued was purified by chromatography on silica gel (30g), eluted with hexane/ethyl acetate (2:1 v.v) to afford the product as a colourless oil.

Yield: 3g, 72%.

R_f: 0.2 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.3 (t ,3H, $CH_2\underline{CH_3}$); 1.4 (s, 9H, <u>t-Boc</u>);

2.7-3.1 (m, 2H, $CH\underline{CH_2}$); 4.0 (d, 2H, $\underline{CH_2}COOEt$); 4.2 (q, 2H, $\underline{CH_2}CH_3$);

4.6 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 2H, $\underline{CH_2}Ph$); 5.6 (s, 1H, <u>NH</u>); 7.0 (s, 1H, <u>NH</u>);

7.4 (s, 5H, <u>Ph</u>).

Mass spectrum: $[M+H]^+$ at m/e 409.

<u>3.4.5.2. (5S)-5-t-Butoxycarbonylamino-3-aza-4-oxo-heptan-1,7-dioc acid 1-ethyl ester (116)</u>

The diester (115) (2.8g, 6.68mmol) in ethanol (60ml), was hydrogenated in the presence of 10% palladium on activated charcoal (0.28g), at room temperature and atmospheric pressure. After one hour, the catalyst was removed by filtration, and the alcohol was evaporated *in vacuo*. The resulting residue was partitioned between ethyl acetate and 0.5M HCl. The product was extracted into the organic phase (3x30ml), dried over sodium sulphate, filtered and evaporated to afford the product as a colourless oil.

Yield: 1.89g 89%.

R_f: 0.35 (solvent Hex/EtOAc 2:1 v:v).

^1H nmr ($CDCl_3$): δ 1.2 (t, 3H, $CH_2\underline{CH_3}$); 1.4 (s, 9H, <u>t-Boc</u>);

2.42.7 (m, 2H, $\underline{CH_2}COOEt$); 3.8 (m, 2H, $CH\underline{CH_2}$); 4.2 (q, 2H, $\underline{CH_2}CH_3$);

4.4 (m, 1H, CHCH$_2$);7.0 (s,1H, NH); 8.1 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 319.

3.4.5.3. (3S)-1-Ethoxycarboxylmethyl-3-t-butoxycarbonyl-amino-pyrrolidine-2,5-dione (117).

The adduct (116) 2.0g, 6.3mmol), and triethylamine (0.70g, 6.93mmol), was dissolved in 50ml of dry THF. The solution was stirred under nitrogen for ten minutes followed by the addition of (EDC) (1.33g, 6.94mmol), and the mixture was left stirring overnight. The precipitated triethylamine hydrochloride was removed by filtration, and the filtrate was evaporated under reduced pressure. The oily residue which ensued was taken up in dichloromethane (60ml), washed with three 30ml portions of saturated sodium bicarbonate, dried over sodium sulphate, filtered and evaporated *in vacuo* to give the crude (117) as a yellow oil. Purification by chromatography on silica gel, with DCM/MeOH (9:1 v:v) as the eluent provided the cyclized product as a colourless crystalline solid.

Yield: 1.2g, 63%.

m.p. 89-91^{o}C.

R_f: 0.35 (solvent EtOAc/Hex 1:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.3 (t, 3H, CH2CH$_3$); 1.4 (s, 9H, t-Boc);

2.8-3.2 (m, 2H, CHCH$_2$); 4.2 (q, 2H, CH$_2$CH$_3$); 4.3 (s, 2H, CH$_2$COOEt);

4.4 (m, 1H, CHCH$_2$); 5.2)s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 301.

3.4.5.4. (3S)-1-Carboxymethyl-3-t-butoxycarbonylamino-pyrrolidine-2,5-dione (118).

A solution of potassium hydroxide (0.2g, 3.5mmol) in water (25ml) was

added to the ester 117) 0.9g, 3.02mmol), in methanol (10ml). The mixture was stirred at room temperature for one hour. The alcohol was evaporated, and the aqueous phase was acidified with 0.5M HCl to pH3, and extracted with ethyl acetate (3x30ml). The extracts were combined, dried over sodium sulphate, filtered and evaporated under reduced pressure to give the acid as a colourless oil.

Yield: 0.62g, 68%.

R_f: 0.74 (solvent DCM/MeOH) 9:1 v:v).

^1H nmr (DMSO): δ 1.4 (s, 9, t-Boc); 2.8 (m, 2H, $CHCH_2$);

3.7 (m, 2H, CH_2COOH); 4.1 (m, 1H, $CHCH_2$); 7.2 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 273.

3.4.5.5. SYNTHESIS OF [(3S)-3-AMINOPYRROLIDIN-2,5-DION-1-YL] ACETIC ACID (119).

The t-Boc protected acid (118) (0.52g, 1.88mmol), was dissolved in 15ml of 1M HCl in acetic acid solution. After one hour at room temperature, the solvent was evaporated *in vacuo*, and the resulting amino acid hydrochloride was converted to the free base by ion exchange chromatography (acetate anion resin), eluted primarily with water (50ml), followed by 10% acetic acid solution. The amino acid was collected as a white solid on evaporation of solvent.

Yield: 0.29, 89%.

m.p. 205°C (decomposed).

R_f: 0.14 (solvent BuOH/AcOH/H_2O 3:1:1 v:v).

^1H nmr (D_2O): δ 2.9 (m, 2H, $CHCH_2$); 3.8 (m, 2H, CH_2COOH);

4.2 (m, 1H, $CHCH_2$).

Mass spectrum: $[M+H]^+$ at m/e 173.

Anal. calcd. for $C_6H_8N_2O_4$ requires: C 41.86 H 4.65 N 16.28.

Found: 41.68 H 4.32 N 16.12.

3.4.6. SYNTHESIS OF 3-[1-{(3S)-3-AMINO}PYRROLIDIN-2,5-DIONYL] ALANINE (124).

3.4.6.1. N-t-Butoxycarbonyl aspartic acid 4-benzyl ester (114).

Di-tert-butyl dicarbonate $(Boc)_2O$ (14.7g, 0.067mol) was added to a solution of L-aspartic acid 4-benzyl ester (10g, 0.045mol) and sodium hydroxide (1.8g, 0.045mol), in isopropanol (200ml). The mixture was stirred at room temperature overnight. Alcohol was evaporated in vacuo and the aqueous phase was carefully acidified with 0.5M HCl solution and extracted with ethyl acetate (3x40). The organic extracts were combined, dried over sodium sulphate, filtered and the filtrate was evaporated i*n vacuo* to afford the t-Boc protected ester as a white solid.

Yield: 13g 89%.

m.p. 94-96^oC.

R_f: 0.48 (DCM/MeOH 9:1 v:v)

^1H nmr (DMSO): δ 1.4 (s, 9H, t-Boc); 2.6-2.9 (m, 2H, $CHCH_2$); 4.3 (m, 1H, $CHCH_2$); 5.1 (s, 2H, CH_2Ph); 7.1 (s, 1H, NH); 7.4 (s, 5H, Ph).

Mass spectrum: $[M+H]^+$ at m/e 324.

3.4.6.2. (2S,6S)-1-Methyl-8-benzyl-2-benzyloxycarbonylamino-6-t-butoxycarbonylamino-4-aza-5-oxo-octan-1,8-dioate (120).

A solution of the t-Boc L-aspartic acid-β-benzyl ester (114) (4.04g, 0.013mol) in 70ml of dry THF was cooled to -25^oC, and N-methylmorpholine (1.2ml,

1.05eqv), and isobutyl chloroformate (1.8g, 0.013mol) was added with stirring. The mixture was stirred at -25°C for two minutes, then treated with a cooled (-15°C) suspension of Cbz-L-DAPA-methyl ester hydrochloride (97) (3g, 0.012mol), and N-methylmorpholine (1.2ml, 1.05eqv) in 50ml of dry THF. The mixture was stirred at -15°C for two hours, and then poured into 600ml of semi-saturated brine, stirred for fifteen minutes at room temperature, and extracted with dichloromethane (3x30ml). The combine organic extracts were dried over sodium sulphate, filtered, and evaporated *in vacuo* to give the crude (120). Purification by chromatography on silica gel, (30g) eluted with dichloromethane/methanol (9:1 v:v) gave the product as a foamy oil.

Yield: 4.8g 66%.

R_f: 0.73 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 2.6 3.0 (m, 2H, CH_2CO);

3.6-3.8 (m, 5H, OCH3 andCHCH2); 4.4 (m, 1H, $CHCH_2$);

5.1 (2s, 4H, 2xCH_2Ph); 5.6 (d, 1H, NH); 5.8 (d, 1H, NH); 6.8 (m, 1H, NH);

7.3 (2s, 10H, 2xPh).

Mass spectrum: $[M+H]^+$ at m/e 558.

3.4.6.3. (2S,6S)-1-methyl-2,6-di-(t-butoxycarbonylamino)-4-aza-5-oxo-octan-1,8-dioic acid (121).

10% Palladium on activated charcoal (0.4g) was suspended in ethanol (150ml) containing the diester (120) (4.00g, 7.2mmol), $(Boc)_2O$ (2.49g, 1.1mmol), and sodium carbonate (0.94g, 7.56mmol). The mixture was hydrogenated at room temperature and atmospheric pressure for six hours. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure, to afford an oily residue which was taken up in water,

washed with three 50ml portions of petroleum ether to remove residual $(Boc)_2O$. The aqueous phase was acidified to pH3 with 0.5M HCl at 0^oC, and the product was extracted into ethyl acetate (3x50ml), dried over sodium sulphate, filtered and evaporated *in vacuo* to afford the t-Boc protected acid as a foamy oil.

Yield: 1.8g 59%.

R_f: 0.49 (solvent ethyl acetate/hexane 2:1 v:v).

^1H nmr (DMSO): δ 1.4 (s, 18H, 2xt-Boc); 3.3 (m, 2H, CH_2COOH); 3.7 (s, 3H, OCH_3); 3.8 (m, 2H, $CHCH_2$); 4.0-4.2 (m, 2H, 2x$CHCH_2$); 6.9 and 7.1 (s, 2H, 2xNH); 8.1 (m, 1H, NHCO).

Mass spectrum: $[M+H]^+$ at m/e 434

3.4.6.4. α-N-t-Butoxylcarbonylamino-3-[1-{(3S)-3-t-butoxycarbonyl-amino}pyrrolidin-2,5-dionyl]-alanine methyl ester (122).

A stirred solution of the adduct (121) (0.9g, 2.14mmol), in 30ml of dry THF, was treated under nitrogen with 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (0.45g, 2.4mmol), and N-methylmorpholine (0.24g, 2.4mmol). Stirring was continued overnight at room temperature, and the solvent was evaporated *in vacuo*, to yield an oily residue which was taken up dichloromethane, washed with saturated sodium bicarbonate, then with brine, dried over sodium sulphate, filtered and evaporated *in vacuo* to afford the cyclized adduct as a foamy oil.

Yield: 0.45g, 56%.

R_f: 0.54 (solvent Hex/EtOAc 2:1 v:v).

^1H nmr ($CDCl_3$): δ 1.4 (s, 18H, 2xt-Boc); 2.6-3.0 (m, 2H, $COCH_2$); 3.5 (m, 2H, $CHCH_2$); 3.7 (s, 3H, OCH_3); 4.4 (m, 2H, 2x $CHCH_2$); 5.7 (s, 2H, 2xNH).

Mass spectrum: $[M+H]^+$ at m/e 416.

3.4.6.5. α-N-t-Butoxycarbonyl-3-[1-{(3S)-3-t-butoxycarbonylamino}-pyrrolidine-2,5-dionyl] alanine (123).

The methyl ester (122) (0.32g, 0.77mmol) was dissolved in 15ml of methanol containing potassium hydroxide (0.034g, 0.613mmol). The solution was stirred at room temperature for one hour. The solvent was evaporated *in vacuo*, and the residue was taken up in 20ml of water, acidified to pH3 with 0.5M HCl at 0°C and extracted with ethyl acetate (3x20ml), dried over sodium sulphate, filtered and evaporated under reduced pressure to give the product as a foamy oil.

Yield: 0.23g 74%.

R_f: 0.49 (solvent DCM/MeOH).

^1H nmr (DMSO): δ 1.4 (2s, 18H, 2xt-Boc); 2.6 (m, 2H, CH$\underline{CH_2}$); 3.3 (m, 2H, CH$\underline{CH_2}$); 3.9 (m, 1H, $\underline{CH}CH_2$); 4.2 (m, 1H, $\underline{CH}CH_2$); 6.9 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 402

3.4.6.6. 3-[1-(3S)-3-amino-pyrrolidine-2,5-dionyl] alanine (124).

The t-Boc protected acid (123)(0.23g, 0.55mmol), was dissolved in a solution of 1M HCl in acetic acid (15ml). The solution was left standing at room temperature for one hour. On evaporation of the solvent, the amino acid hydrochloride which ensued was taken up in a minimum amount of water, and converted to the free base by ion exchange chromatography, (acetate anion resin) eluted initially with 60ml of water, followed by 10% acetic acid solution. The diamino acid was collected as the diacetate salt as a white solid on evaporation of solvent.

Yield: 73mg, 61%.

m.p. 210°C (decomposed).

R_f: 0.12 (solvent BuOH/AcOH/H_2O 3:1:1 v:v).

^1H nmr (D_2O): δ 3.5-3.7 (m, 4H, 2xCH<u>CH_2</u>); 4.1-4.3 (m, 2H, 2x<u>CH</u>CH_2).

Mass spectrum: $[M+H]^+$ at m/e 262.

Anal. calcd. for $C_9H_{15}N_3O_6$: requires C 41.38 H 5.75 N 16.09.

Found: C 41.91: H 5.88; N 16. 40.

$[\alpha]_D$ +11.01 (c 0.25, 1M HCl).

3.4.7. Attempted synthesis of α-N-t-butoxycarbonyl-[1,2-pyrazol-1-yl]-3-alanine (125).

3.4.7.1. Reaction of the t-Butoxycarbonylamino-β-lactone (78) with pyrazole.

A solution of pyrazole (0.04g, 0.59mmol), in ACN (15ml) was treated under nitrogen with the t-Boc-amino-β-lactone (78) (0.1g, 0.54mmol). The mixture was stirred at room temperature for two hours, followed by the addition of sodium hydride (0.014g, 0.59mmol). The resulting mixture was then stirred for another two hours and solvent was evaporated under reduced pressure. The residue was taken up in water, acidified to pH3 with 0.5M HCl, extracted with ethyl acetate (3x15ml), dried over sodium sulphate, filtered and the filtrate was evaporated *in vacuo* to a clear oil, which was identified by its ^1H nmr spectrum as the elimination product (90).

The above reaction was repeated in different solvent (THF, DMF, EtOAc) and at different temperatures (25°C-100°C), but these conditions gave

intractable mixtures.

3.4.7.2. Reaction of the α-N-t-butoxycarbonylamino-β-lactone (78) with pyrazole in the presence of a tertiary amine.

DABCO (0.066g, 0.59mmol) was added to a stirred solution of pyrazole (0.04g, 0.59mmol).in dry THF (15ml). After stirring at room temperature for thirty minutes, the t-Boc-amino β-lactone (78) (0.1g, 0.53mmol) was added and the mixture was stirred for another two hours. The solvent was evaporated, the residue was acidified to pH3 with 0.5M HCl and extracted with ethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to give a colourless oil, which was found upon characterization to be the elimination product (90).

3.4.7.3. N-Benzyloxycarbonyl-L-serine

L-serine (5.25g, 0.05m) was dissolved in a mixture of water (70ml) and acetone (70ml), containing sodium bicarbonate (4.2g, 0.05m). The mixture was stirred at room temperature, and benzyl succinimidyl carbonate (12.4g , 0.05m) was added. Stirring was continued overnight. Acetone was evaporated *in vacuo*, and the aqueous phase was washed twice with dichloromethane (30ml), then acidified to pH3 with concentrated HCl. The product was extracted into ethyl acetate (3x50ml), washed with water, dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford the product as a white solid. Recrystallization from ether gave the protected amino acid as a white crystalline solid.

Yield: 9.8g 82%.

R_f: 0.23 (solvent DCM/MeOH 9:1 v:v).

m.p. 118-120°C.(Lit.[180] 119°C).

^{1}H nmr ($CDCl_3$): δ 3.7 (m ,2H, CH$\underline{CH_2}$); 4.1 (m, 1H, $\underline{CH}CH_2$);

5.1 (s, 2H, $\underline{CH_2}$Ph); 7.3-7.4 (m, 6H, $\underline{Ph}$ + $\underline{NH}$).

3.4.7.4. N-Benzyloxycarbonyl serine methyl ester.

Thionyl chloride (10ml) was slowly added at 0°C to 70ml of methanol. The solution was stirred for twenty minutes, followed by the addition of N-benzyloxycarbonyl serine (5g, 0.02mol). The reaction mixture was warmed to room temperature, then heated under reflux for three hours. The solvent was evaporated, the residue, a colourless oil, was taken up in ethyl acetate and washed with water and sodium bicarbonate solution, dried over sodium sulphate, filtered and the filttrate was evporated under reduced pressure to afford the product as a colourless oil.

Yield: 4.3g, 85%

R_f: 0.58 (solvent DCM/MeOH 9:1 v:v).

(Lit.[186] m.p.33-35°C)

^{1}H nmr ($CDCl_3$): δ 3.7 (s, 3H, O$\underline{CH3}$); 3.8 (m ,2H, CH$\underline{CH_2}$);

4.2 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 2H, $\underline{CH_2}$Ph); 7.3 (s, 5H, $\underline{Ph}$); 7.4 (d, 1H, $\underline{NH}$).

3.4.7.5. α-N-Benzyloxycarbonyl-O-methylsulphonyl serine methyl ester (128).

A solution of Cbz-L-serine methyl ester (1g, 4.57 mmol), in dichloromethane (30ml), was treated with methanesulphonyl chloride (0.58g, 5.07mmol) and triethylamine (0.51g, 5.07mmol. The mixture was stirred overnight, at room temperature, washed with saturated sodium bicarbonate and then with brine. The organic solvent was dried over sodium sulphate, filtered and evaporated under reduced presssure to afford an oily residue. Crystallization from

dichloromethane and ether gave the product as a white solid.

Yield: 1.1g, 73%%.

R_f: 0.47 (solvent hex/EtOAc 2:1 v:v).

m.p. 99-101°C.

^{1}H nmr ($CDCl_3$): δ 2.8 (s, 3H, $\underline{CH_3}S$); 3.7 (s, 3H, $O\underline{CH_3}$);

4.4-4.6 (m, 3H, $\underline{CHCH_2}$); 5.2 (s, 2H, $\underline{CH_2}Ph$); 5.7 (d, 1H, $\underline{NH}$);

7.4 (s, 5H, $\underline{Ph}$).

Mass spectrum: $[M+H]^+$ at m/e 332.

3.4.7.6. α-N-t-Butoxycarbonyl serine acetate methyl ester (126).

Acetic anhydride (3.09g, 0.03mol, 10eq), was added to a solution of t-Boc L-serine methyl ester (91) (0.65g, 3.02mmol), and pyridine (2ml) in dichloromethane (30ml). The mixture was stirred overnight at room temperature, washed with aqueous sodium bicarbonate, followed by 0.1M HCl to remove residual pyridine, and finally with brine. The organic solvent was dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to afford the acetate as a colourless oil.

Yield: 0.67g, 86%.

R_f: 0.52 (solvent ethyl acetate/hexane 2:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, $\underline{t\text{-}Boc}$); 2.0 (s, 3H, $\underline{CH_3}$); 3.7 (s, 3H, $O\underline{CH_3}$);

4.4. (m, 2H, $CH\underline{CH_2}$); 4.6 (m, 1H, $\underline{CH}CH_2$); 5.3 (s, 1H, $\underline{NH}$).

Mass spectrum: $[M+H]^+$ at m/e 262.

3.4.7.7. Reaction of the acetate (126) with pyrazole.

t-Boc-serine O-acetate methyl ester (0.51g, 2mmol), was added to a stirred solution of pyrazole (0.06g, 2.3mmol) in dry ACN (15ml) and the mixture

was stirred at room temperature for two hours and then at 50°C for two hours. Tlc showed presence of starting materials mainly, hence the mixture was heated under reflux for another two hours. The reaction gave an intractable mixture.

Attempted synthesis of the analogue(127) under the above conditions with Cbz serinyl-mesylate (128) also gave an intractable mixture.

3.4.7.8. (3S)-3-Benzyloxycarbonylamino oxetan-2-one (131).

Cbz–β-lactone was prepared as described for the synthesis of t-Boc β-lactone (78) (page 136). The crude product was purified by flash chromatography, with AcOH/ hexane (40/60 v:v) as the eluent. The white solid was obtained in 65% yield following recrystallization from ethyl acetate/hexane.

m.p. 130-133°C. (Lit.[176] 133-134°C)

^{1}H nmr ($CDCl_3$): δ 3.8 (m, 2H, CH$\underline{CH_2}$); 4.4 (m, 1H, $\underline{CH}CH_2$);

5.2 (s, 2H, $\underline{CH_2}$Ph); 6.2 (d, 1H, $\underline{NH}$); 7.3 (s, 5H, $\underline{Ph}$).

Mass spectrum: $[M+H]^+$ at m/e 222.

3.4.7.9. N-(Benzyloxycarbonyl)-β-bromo-L-alanine (129).

Magnesium bromide etherate (1.2g, 4.6mmol) (Aldrich) was added to a solution of Cbz-L-serine β-lactone (131) (0.3g, 1.3mmol.) in ether (70ml) and dry THF (7ml). The mixture was stirred at room temperature for fifteen minutes. The solvent was evaporated *in vacuo* and the residue was taken up in water, acidified with 2M HCl to pH3, and extracted with ether (3x40ml). After drying over sodium sulphate and filtration, the solvent was evaporated under reduced pressure to afford the crude bromide which was recrystallized from dichloromethane/hexane to give the pure bromide as a white solid.

Yield: 0.24g, 59%.

m.p. 71-73°C (Lit.[176] 70-71°C).

R_f: 0.49 (solvent (DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 3.5-4.0 (m, 2H, CH$\underline{CH_2}$); 4.8 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 2H, $\underline{CH_2}$Ph); 5.6-5.8 (d, 1H, $\underline{NH}$); 7.3 (s, 5H, $\underline{Ph}$).

Mass spectrum: $[M+H]^+$ at m/e 302,304.

3.4.7.10. Reaction of the bromide with pyrazole.

A stirred solution of pyrazole (0.04g, 0.59mmol) and sodium bicarbonate (0.5g, 0.59mmol) in ACN (15ml) was treated with the Cbz-protected bromide (129) (0.18g, 0.59mmol). The mixture was stirred at room temperature for two hours, then heated under reflux for four hours. The solvent was evaporated under reduced pressure and the residue was taken up in ethyl acetate and partitioned between the organic solvent and aqueous sodium bicarbonate. The product was extracted into ethyl acetate (3x20ml), the combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated in vacuo to give a light yellow oil, which was purified by flash chromatography on silica gel eluted with ethyl acetate/hexane 60:40 v:v. The product, a colourless oil, which was identified as the elimination product (130b) on the basis of its ^1H nmr spectrum, was collected on evaporation of solvent.

3.4.8. PREPARATION OF (S)-α–N-[1,2,4-TRIAZOL-1-YL] ALANINE (133).

3.4.8.1. (S)-α-N-t-Butoxycarbonyl-3-[1-(1,2,4-triazol-1-yl)] alanine (132).

Triazole (0.32g, 0.46mmol) and tetramethylguanidine (0.53g, 0.46mmol) were disssolved in 20ml of dry acetonitrile. The solution was stirred under

nitrogen, at room temperature, followed by the addition of t-Boc L-serine acetate methyl ester (126) (0.11g 0.42mmol). The mixture was stirred for another fifteen minutes at room temperature, then heated under reflux (80^{o}C) for three hours. On cooling solvent was evaporated at reduced pressure, and the residue was taken up in water and washed with two 25ml portions of ethyl acetate to remove residual triazole. The aqueous phase was acidified with saturated citric acid solution to pH3 and extracted with ethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford the adduct as a colourless oil which solidified on standing. Recrystallization from ethyl acetate/hexane gave the compound as a white solid.

Yield: 41mg, 35%.

R_f: 0.12 (solvent DCM/MeOH/AcOH 33:10:5 v:v).

m.p. 176-170^{o}C.

^{1}H nmr (DMSO): δ 1.4 (s, 9H, t-Boc); 4.4-4.6 (m, 3H, $CHCH_2$);

7.2 (s, 1H, NH); 8.0 (s, 1H, CH); 8.4 (s, 1H, CH).

Mass spectrum: $[M+H]^+$ at m/e 257.

3.4.8.2. (S)-3-(1,2,4-triazol-1-yl) alanine (133).

The t-Boc protected adduct (132) (50mg,0.2mmol) was dissolved in a solution of 1M HCl in acetic acid. This was left standing at room temperature for one hour and the solvent was evaporated under reduced pressure. The amino acid hydrochloride gave a ninhydrin positive spot on tlc. Owing to lack of time, this product was not characterized.

3.4.9. SYNTHESIS OF (S)-3-[1-PYRROLIDINYL] ALANINE (134).

3.4.9.1. (S)-N-t-butoxycarbonyl-3-[1-pyrrolidinyl] alanine (136).

The N-t-Butoxycarbonylamino β-lactone (78) (0.1g, 0.54mmol) was added under nitrogen to a solution of pyrrolidine (0.04g, 0.513mmol), and DABCO (0.06g, 0.56mmol) in 25ml of dry THF. The mixture was stirred overnight at room temperature and the solvent was evaporated under reduced pressure. The residue was taken up in water and acidified with 0.5M HCl to pH3 and extracted with ethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered, and the solvent was evaporated under reduced pressure to afford as a colourless oil. Crystallization from hexane and ether gave the product as a white solid.

yield: 90mg, 33%.

R_f: 0.63 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

m.p. 142-143^oC.

^1H nmr (DMSO): δ 1.4 (s, 9H, t-Boc); 1.6-2.0 (m, 4H, CH_2CH_2);

2.9-3.6 (m, 6H, CH_2CH_2 and $CHCH_2$); 4.3 (m, 1H, $CHCH_2$);

6.7 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 259.

3.4.9.2. 3-[1-Pyrrolidinyl] alanine (134).

The t-Boc protected adduct (136) (50mg, 0.2mmol) was dissolved in 1M solution of HCl acetic acid (10ml). After one hour at room temperature, the solvent was evaporated and the resultant amino acid hydrochloride was taken up in water and then evaporated off to remove residual acetic acid. (this was repeated three times). Excess acetic acid was then removed by high vacuum. The product was taken up in a small amount of water and freeze-dried to yield

the product as a sticky solid.

Yield: 200mg, 77%.

m.p. The compound is hygroscopic, therefore the melting point was not obtained.

R_f: 0.4 (solvent BuOH/ AcOH/ H_2O 3:1:1 v:v).

^1H nmr (D_2O): δ 1.9-2.1 (m, 4H, $\underline{CH_2}\underline{CH_2}$); 3.4-3.7 (m, 4H, 2x$\underline{CH_2}$N); 3.9-4.0 (m, 2H, CH$\underline{CH_2}$); 4.4 (m, 1H, $\underline{CH}CH_2$).

3.4.10. Synthesis of (S)-3-[1-pyrrolidin-2-onyl] alanine (135).

3.4.10.1. N-benzyloxycarbonyl-3-[1-pyrrolidin-2-onyl]-alanine (137).

A solution of N-Cbz-β-bromo-alanine (129) (0.5g, 1.7mmol) and triethylamine (0.18g, 1.8mmol) in toluene (20ml) was treated with pyrrolidin-2-one (0.15g, 1.7mmol). The mixture was heated under reflux for four hours, cooled and the solvent was evaporated under reduced pressure to afford an oily residue which was taken up water, acidified to pH3 with 2N HCl and extracted with ethyl acetate (3x25ml). The combined extracts were dried over sodium sulphate, filtered and evaporated under reduced pressure to give the Cbz-protected amino acid as a white solid.

Yield: 0.12g, 24%.

R_f: 0.53 (DCM/MeOH/AcOH 44:5:1 v:v).

^1H nmr ($CDCl_3$): δ 2.2 (m, 2H ,$\underline{CH_2}CH_2$); 2.4 (m, 2H, $\underline{CH_2}CO$); 3.6 (t, 2H, $\underline{CH_2}$N); 3.7-4.0 (m, 2H, CH$\underline{CH_2}$); 4.8 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 2H, $\underline{CH_2}$Ph); 5.7 (d, 1H, $\underline{NH}$); 7.4 (s ,5H, $\underline{Ph}$).

Mass spectrum: $[M+H]^+$ at m/e 307.

3.4.11. NON-CYCLIC AMINO ACIDS.

3.4.11.1. (2S)-2-Amino-4,6-diaza-5-oxo-octan-1,8-dioic acid (138).

The adduct (99) (0.8g, 2.6mmol) was dissolved in ethanol (50ml), followed by the addition of 10% palladium on activated charcoal (0.08g). The suspension was hydrogenated at room temperature and atmospheric pressure for one hour. The catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure, to afford the crude (138). Recrystallization from water and ethanol gave the amino acid as a white solid.

Yield: 0.34g, 64%.

R_f: 0.27 (solvent BuOH/AcOH/H_2O 3:1:1 v:v).

m.p. 236-238^oC.

^1H nmr (D_2O): δ 3.4-3.7 (m, 2H, CH$\underline{CH_2}$); 3.8 (s, 2H, $\underline{CH_2}$COOH);

4.1 (m, 1H, $\underline{CH}CH_2$).

Mass spectrum: $[M+H]^+$ at m/e 206.

Anal. calcd. for $C_6H_{11}N_3O_5$ requires C 35.12; H 5.36 N 20.49.

Found: C 34.05; H 5.43; H 19.63. (The product was found to contain 0.28 mol of water). (Calcd. for 0.28 H_2O: C 34.28, H 5.50, N 19.99)

$[\alpha]_D$ -20.03 (c 1.02, 1M HCl).

3.4.11.2. (2S)-2-Benzyloxycarbonylamino-4-aza-5-oxo-octan-1,8-dioic acid (139a).

The adduct (106) (0.7g, 2mmol) was dissolved in methanol (5ml), to which potassium hydroxide (0.12g, 2.1mmol) was added. The mixture was stirred at room temperature for one hour and solvent was evaporated. The residue was taken up in water (20ml), acidified to pH3 with 2M HCl and then extracted

into ethyl acetate (3x15ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to give the product as a colourless oil.

Yield: 0.5g, 74%.

R_f: 0.53 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

^1H nmr (DMSO): 2.2-2.5 (m, 4H, $\underline{CH_2CH_2}$); 3.3-3.5 (m, 2H, $CH\underline{CH_2}$); 4.0 (m, 1H, $\underline{CH}CH_2$); 5.1 (s, 2H, CH_2Ph); 6.9 (d, 1H, $\underline{NH}$); 7.3 (s, 5H, Ph); 7.9 (1H, $\underline{NH}$).

Mass spectrum: $[M+H]^+$ at m/e 339.

3.4.11.3. (2S)-2-Amino-4-aza-5-oxo-octan-1,8-dioic acid (139).

Palladium on activated charcoal (0.04g) was suspended in a solution of the diacid (139a) (0.4g, 1.18mmol). in ethanol (30ml). The suspension was hydrogenated at room temperature and atmospheric pressure for one hour. The catalyst was removed by filtration and the filtrate was evaporated to dryness to give the crude product as a white solid. Recrystallization from water and ethanol afforded the amino acid as a white solid.

Yield: 0.18g, 66%.

m.p. 223-225^oC.

R_f: 0.24 (solvent $BuOH/AcOH/H_2O$ 3:1:1 v:v).

^1H nmr (D_2O): δ 2.2 (m, 4H, $\underline{CH_2CH_2}$); 3.3-3.5 (m, 2H, $CH\underline{CH_2}$); 3.9 m, 1H, $\underline{CH}CH_2$).

Mass spectrum: $[M+H]^+$ at m/e 205.

Anal. calcd. for $C_7H_{12}N_2O_5$ requires: C 41.18; H 5.88; N13.73.

Found: C 40.36; H 5.98; N 13.27. (The compound was found to contain 0.4mol of water).(Calcd. for 0.4 H_2O: C 39.77, H 6.06, N 13.28)

$[\alpha]_D$. -28 5 (c 0.41, 1M HCl).

3.4.11.4. SYNTHESIS OF (S)-2,7-DIAMINO)-4-AZA-5-OXO-HEPTANOIC ACID (141).

3.4.11.4.1 (2S)-2,7-Bis(benzyloxycarbonylamino)-4-aza-5-oxo-heptanoic acid methyl ester (141a).

Cbz protected β-alanine (0.59g, 6.62mmol) was added (under nitrogen), to a solution of α-N-Cbz-L-DAPA methyl ester hydrochloride (97) (1.5g, 6.0mmol), EDC (1.21g, 6.0mmol) and triethylamine (0.64g, 6.0mmol) in dry THF. The mixture was stirred overnight and the solvent was evaporated *in vacuo* to afford an oily residue which was taken up in dichloromethane (40ml), washed with three 20ml portions of saturated sodium bicarbonate, dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to give the crude amide, which was purified by chromatography on silica gel (20g) eluted with DCM/MeOH 9:1 v:v). The amide was obtained as a white solid following crystallization from ethyl acetate/hexane.

Yield: 1.35g 51%.

m.p. 128-131^{o}C.

R_f: 0.3 (solvent ethyl acetate/hexane 2:1 v:v).

^{1}H nmr ($CDCl_3$): δ 2.4 (t, 2H, $\underline{CH_2}CH_2$); 3.4 (m, 2H, $CH\underline{CH_2}$); 3,6 (t, 2H, $CH_2\underline{CH_2}$); 3.7 (s, 3H, $O\underline{CH_3}$); 4.4 (m, 1H, $\underline{CH}CH_2$); 5.1 (2s, 4H, 2x$\underline{CH_2}$Ph); 5.4 (m, 1H, NH); 5.8 6.1 (d, 1H, NH); 6.1 (d, 1H, NH); 7.4 (2s, 10H, Ph).

3.4.11.4.2. (2S)-2,7-Bis(benzyloxycarbonylamino)-4-aza-5-oxo-heptanoic acid (141b).

Sodium hydroxide (0.09g, 2.2mmol) in water, (10ml), was added to a solution of the ester (0.98g, 2.2mmol), in methanol (10ml). The solution was stirred at room temperature for one hour acidified to pH3 with 2M HCl and then extracted with ethyl acetate (3x20ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to give the product as a colourless oil.

Yield: 0.74g, 78%.

R_f: 0.13 (solvent DCM/MeOH 9:1 v:v).

^1H nmr (DMSO): δ 2.2-2.4 (m, 2H, $\underline{CH_2}CH_2$); 2.5 (t, 2H, $CH_2\underline{CH_2}$);

3.2-3.5 (m, 2H, $CH\underline{CH_2}$); 3.7-3.9 (m, 1H, $\underline{CH}CH_2$);

5.0 (2s, 4H, 2x$\underline{CH_2}$Ph); 6.6 (d, 1H, NH); 7.3 (d, 1H, NH);

7.4 (2s, 10H, 2xPh). 8.2 (d, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 444.

3.4.11.4.3. (S)-2,7-Diamino-4-aza-5-oxo-heptanoic acid (141).

Palladium on activated charcoal (10%) (0.071g), was suspended in 25ml of ethanol containing the acid (141b) (0.71g, 1.7mmol). The mixture was hydrogenated at room temperature and atmospheric pressure for one hour. The catalyst was removed by filtration through celite and the filtrate was evaporated to dryness to give the crude diamino acid. Recrystallization from ethanol/water gave the diamino acid as a white solid.

Yield: 0.38g, 64%.

R_f: 0.21 (solvent $BuOH/H_2O/AcOH$ 3:1:1 v:v).

m.p. 231-232°C (decomposed, then melted with effervesence).

^1H nmr (D_2O/DCl): δ 3.0 (m, 2H, $\underline{CH_2}CH_2$); 3.4 (m, 2H, $CH_2\underline{CH_2}$);

3.7-3.8 (m, 2H, CH$\underline{CH_2}$); 4.3 (m, 1H, $\underline{CH}$CH2).

Mass spectrum: $[M+H]^+$ at m/e 176.

Anal. calcd. for $C_6H_{13}N_3O_3$ requires: C 41.14 H 7.43 N24.0.

Found: C 41.44 H 7.39 N 23.88.

3.4.11.5. Synthesis of (S)-2,6-diamino-4-aza-5-oxo-hexanoic acid (140).

3.4.11.5.1. (S)-2,6-Bis(benzyloxycarbonylamino)-4-aza-5-oxo-hexanoic acid methyl ester (140a).

A solution of Cbz-L-DAPA methyl ester (97) (2g, 8mmol), in dry THF was treated (under nitrogen) with triethylamine (0.85g, 8.4mmol), and EDC (1.6g, 8.4mmol). The solution was stirred at room temperature for fifteen minutes, followed by the addition of Cbz-glycine (0.63g, 8.4mmol). The mixture was stirred at room temperature overnight and the solvent was evaporated *in vacuo*, the resulting light yellow residue was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The aqueous phase was extracted with dichloromethane (3x25ml), dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to give an oily residue, which was purified by flash chromatography on silica gel (25g) with DCM/MeOH 9:1 v:v as the eluent.

Yield: 2.1g 61%.

R_f: 0.69 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 3.3-3.8 (m, 7H, $\underline{CH_2}NH_2$ and CH$\underline{CH_2}$ and O$\underline{CH_3}$); 4.4-4.5 (m, 1H, $\underline{CH}CH_2$); 5.0 (2xs, 4H, 2x$\underline{CH_2}$Ph); 6.3 (m, 2H, 2x$\underline{NH}$); 7.4 (s, 10H, 2x$\underline{Ph}$); 7.5 (d, 1H, $\underline{NH}$).

Mass spectrum: $[M+H]^+$ at m/e 444.

3.4.11.5.2. (2S)-2,6-Bis(benzyloxycarbonylamino)-4-aza-5-oxo-hexanoic acid (140b).

The ester (140a) (1.8g, 4.18mmol) was dissolved in a minimum amount of methanol (10ml) and treated with solution of sodium hydroxide (0.18g, 4.6mmol) in water (10ml). The mixture was stirred at room temperature for one hour followed by the evaporation of methanol. The aqueous phase was acidified to pH3 with 2M HCl, and patitioned between ethyl acetate. The crude product was extracted into ethyl acetate (3x20ml), dried over sodium sulphate, filtered and evaporated *in vacuo* to give an oily residue which was chromatographed on silica gel (15g), eluted with DCM/MeOH 9:1 v:v. The product was obtained as a colourless oil on evaporation of solvent.

Yield: 0.9g, 71%.

R_f: 0.77 (solvent DCM/AcOH/MeOH 44:1:5v:v).

^1H nmr (DMSO): δ 3.1-3.5 (m, 2H, CH$\underline{CH_2}$); 3.6 (d, 2H, $\underline{CH_2}$NH);

4.5 (s, 2H, $\underline{CH_2}$Ph); 5.0 (s, 2H, $\underline{CH_2}$Ph); 6.3 (d, 1H, $\underline{NH}$);

7.4 (2xs, 10H, 2x$\underline{Ph}$); 7.5 (d, 1H, $\underline{NH}$).

Mass spectrum: $[M+H]^+$ at m/e 430.

3.4.11.5.3. (2S)-2,6-Diamino-4-aza-5-oxo-hexanoic acid (140).

A suspension of 10% palladium on activated charcoal (60mg) and the acid (140b) (0.6g, 1.4mmol) in ethanol (50ml), was hydrogenated at room temperature and atmospheric pressure for one hour. The catalyst was removed by filtration, and the filtrate was evaporated under reduced pressure to afford the amino acid which was crystallized from ethanol/water.

Yield: 200mg (58%).

R_f: 0.2 (solvent BuOH/H_2O/AcOH 3:1:1 v:v).

m.p. 220-222°C.

^{1}H nmr (D_2O): δ 3.6-3.8 (m, 2H, CH<u>CH_2</u>); 3.9 (s, 2H, <u>CH_2</u>NH);

4.2-4.3 (m, 1H, <u>CH</u>CH_2).

Mass spectrum: $[M+H]^+$ at m/e 162.

Anal. calcd. for $C_5H_{11}N_3O_3$ requires: C 37.28 H 6.83 N 26.09.

Found: C 37.49 H 6.76 N 25.91.

$[\alpha]_D$ -29.03 (c 0.5, 1M HCl).

3.4.12. Synthesis of dipeptide analogues of quisqualic acid.

3.4.12.1. Attempted synthesis of quisqualyl-glycine (144).

A stirred solution of benzoyl-protected quisqualic acid (62) (0.5g, 2.1mmol) in dry THF (30ml), was treated with DCC (0.47g, 2.3mmol) and HOBT (0.31g 2.3mmol). The mixture was stirred at room temperature for thirty minutes, followed by the addition of glycine ethyl ester hydrochloride (0.32g 2.3mmol). Stirring was continued at room temperature overnight and the solvent was evaporated under reduced pressure. The resulting residue was partitioned between ethyl acetate and aqueous sodium bicarbonate and the crude product was extracted into the organic phase (3x30ml), and then washed with 2M HCl, then with water, followed by brine, dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to afford an intractable mixture, which was not characterized. This method was abandoned.

3.4.12.2. SYNTHESIS OF QUISQUALYL-LEUCINE (147)

3.4.12.2.1. α-N-t-Butoxycarbonyl quisqualyl-leucine methyl ester (145).

N-t-Boc protected quisqualic acid (81) (1g, 3.46mmol) was dissolved under nitrogen, in dry THF, cooled to -25°C, and treated with N-methylmorpholine (0.35g, 3.46mmol), then with isobutyl chloroformate (0.47g, 3.46mmol). The mixture was stirred at -25°C for two minutes, then treated with a cooled (-15°C) suspension of L-leucine methyl ester hydrochloride (0.63g, 3.46mmol) and NMM (0.37g, 3.6mmol). The reaction mixture was stirred for another two hours at -15°C and then poured into 500ml of semi-saturated brine, extracted with dichloromethane (3x35ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford the protected dipeptide as a colourless oil. Purification by flash chromatography on silica gel (20g), eluted with hexane/EtOAc (2:1 v:v) gave the product as a foamy solid on evaporation of solvent.

Yield: 1.1g (76%).

R_f: 0.36 (solvent hexane/ethyl acetate 1:1 v:v).

^1H nmr ($CDCl_3$): δ 0.9 (d, 6H, $(\underline{CH_3})_2$ 1.4 (s, 9H, t-Boc);

1.6 (m, 2H, $CH\underline{CH_2}$); 3.7 (s, 3H, $O\underline{CH_3}$); 4.0 (m, 2H, $CH\underline{CH_2}$);

4.5 (m, 2H, $2xCH\underline{CH_2}$); 5.4 (m, 1H, NH); 5.9 (d, 1H, NH); 7.4 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 417.

3.4.12.2.2. α-N-t-Butoxycarbonyl quisqualyl leucine (146).

A solution of the adduct (145) (0.9g, 216mmol) was dissolved in a solution of sodium hydroxide (90mg, 2.3mmol) in water (30ml). The mixture was stirred at room temperature for one hour. Alcohol was evaporated *in vacuo*, and the aqueous phase was acidified at 0°C to pH3, with 0.5M HCl and then extracted

with ethyl acetate (3x30ml). The combined extracts were dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to give the acid as a foamy solid.

Yield: 0.67g, 77%.

R_f: 0.57 (solvent DCM/MeOH 9:1 v:v).

^1H nmr (DMSO): δ 1.0 (dd, 6H, $(\underline{CH_3})_2$); 1.4 (s, 9H, t-Boc);

1.7 (m, 2H, $CH\underline{CH_2}$) 3.7 (s, 3H, $O\underline{CH_3}$); 4.1 (m, 2H, $CH\underline{CH_2}$);

4.5-4.7 (m, 2H, 2x$\underline{CH}CH_2$); 5.4 (s, 1H, NH); 6.0 (s, 1H, NH);

7.4 (s, 1H, NH).

Mass spectrum: $[M+H]^+$ at m/e 403.

3.4.12.2.3. L-Quisqualyl-L-leucine (147).

A solution of the t-Boc protected dipeptide (146) (0.5g, 1.2mmol) in 1M HCl/AcOH solution was left standing at room temperature for one hour. The solvent was evaporated *in vacuo* and the resulting peptide hydrochloride was converted to the free base by ion exchange chromatography (acetate anion resin) eluted primarily with water 50ml and then with 5% acetic acid solution. Acidic fractions were combined and concentated under reduced pressure, then freeze-dried to afford the peptide as an off-white crystalline solid.

Yield: 0.23g, 63%.

m.p. 138-140^oC (decomposition observed from 128^oC).

R_f: 0.53 (solvent $BuOH/H_2O/AcOH$ 3:1:1 v:v).

^1H nmr (D_2O): δ 2.8 (m, 6H, $(\underline{CH_3})_2$); 3.6-4.0 (m, 4H, $CH\underline{CH2}$ and $\underline{CH}CH_2$ and $1/2CH\underline{CH_2}$): 4.3 (m, 3H, $\underline{CH}CH_2$, and $\underline{CH}CH_2$, and $1/2CH\underline{CH_2}$).

Mass spectrum: $[M+H]^+$ at m/e 303.

Anal. calcd. for $C_{11}H_{18}N_4O_6$ requires: C 43.71; H 5.96; N 18.54.

Found:. C 41.43; H 6.12; N 16.86, (the product was found from spectroscopic and elemental analysis to contain 0.84mol of H_2O and 0.2mol of AcOH).

$[\alpha]_D$ -20.4 (c 0.6, 1MHCl).

3.4.13 Alkylation of L-quisqualic acid.

3.4.13.1. α–N–t-Butoxycarbonyl-ε-N–ethoxycarbonylmethyl quisqualic acid ethoxycarbonylmethyl ester (148).

t-Boc Quisqualic acid (0.5g, 1.7mmol), in dry DMF, was treated under nitrogen with sodium hydride (0.09g, 3.7mmol). The mixture was stirred at room temperature for thirty minutes, followed by the addition of ethyl bromoacetate (1.3g, 7.5mmol). The reaction mixture was stirred overnight and solvent was evaporated. The residue was taken up in water and acidified at 0°C, with 1M HCl to pH3, followed by extraction with ethyl acetate (3x30). The extracts were combined, washed with brine, dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressure to afford the crude product as a light yellow oil. Purification by flash chromatography on silica gel, with 3% MeOH in DCM as the eluent gave the product as a light yellow oil.

Yield: 0.48g, (55%).

R_f: 0.33 (solvent DCM/MeOH 9:1 v:v).

^1H nmr ($CDCl_3$): δ 1 2-1.6 (m, 15H, (2x$CH_2\underline{CH_3}$ and t-Boc);

4.0-4.4 (m, 8H, CH$\underline{CH_2}$ + $\underline{CH_2}$COOEt + 2x$CO_2\underline{CH_2}$Me);

4.6-4.8 (m,, 3H, O$\underline{CH_2}CO_2$Et +, $\underline{CH}$CH2); 5.4 (d, 1H, $\underline{NH}$);

3.4.13.2. α–N–t-Butoxycarbonyl-ε-N–carboxymethyl quisqualic acid (149).

A solution of the adduct (148) (0.45g, 0.97mmol) in methanol was treated with 3.3 equivalents of potassium hydroxide (0.18g, 3.2mmol). The mixture was stirred at room temperature for one hour, and then acidified at 0°C to pH 3 with 1M HCl, followed by extraction with ethyl acetate (3x25ml). The combined extracts were washed with brine, dried over sodium sulphate, filtered and the filtrate was evaporated under reduced pressue to afford the acid as a foamy oil.

Yield: 0.28g, (83%).

R_f: 0.32 (solvent DCM/MeOH/AcOH 44:5:1 v:v).

^{1}Hnmr (DMSO): δ 1.4 (s, 9H, t-Boc); 3.8-3.9 (m, 2H, CH$\underline{CH_2}$);

4.1 (s, 2H, $\underline{CH_2}$COOH); 4.3 (m, 1H, $\underline{CH}$CH2); 5.9 (d, 1H, $\underline{NH}$).

3.4.13.3. -ε-N–carboxymethyl quisqualic acid (150).

The intermediate (149) (0.25g, 0.72mmol) was dissolved in 1M hydrogen chloride in acetic acid (15). The solution was left standing at room temperature for one hour. The solvent was removed *in vacuo* and the resultant amino acid hydrochloride was converted to the free base by ion exchange chromatography, eluted with 10% acetic acid solution. The solvent was concentrated in vacuo, and then freeze dried, to afford the product as a white solid.

Yield: 25mg, (14%).

R_f: 0.25 (solvent BuOH/AcOH/H_2O 3:1:1 v:v).

^{1}H nmr (D_2O/DCl): δ 3.8 (s, 2H, CH_2COOH); 3.9-4.2 (m, 2H, $CHCH_2$);

4.3 (m, 1H, $CHCH_2$).

3.5. MISCELLANEOUS SYNTHESES.

3.5.1. N-Ethoxycarbonyl-N-benzyloxyurea (151).

Ethoxycarbonyl isocyanate (2.4g, 0.02m) was added, under nitrogen, to a solution of O-benzylhydroxylamine hydrochloride (3g, 0.019m) in 80ml of dry THF. The mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the resultant white solid was recrystallised from ether. The amide, a white crystalline solid, was collected by filtration.

yield: 4.1g, 92%.

m.p 123-125^{o}C.

R_f: 0.83 (solvent DCM./MeOH 9:1 v:v).

^{1}H nmr ($CDCl_3$): δ 1.3 (t, 3H,CH_2 $\underline{CH_3}$); 4.2 (q, 2H, $\underline{CH_2}CH_3$):

5.0 (s, 2H, $\underline{CH_2}$Ph); 7.4 (s, 5H, $\underline{Ph}$).

Mass spectrum: $[M+H]^+$ at m/e 239.

Anal. calcd. for $C_{11}H_{14}N_2O_4$ requires: C 55.46 H 5.88 N 11.76.

Found: C 55.41 H 5.91 N 11.51.

3.5.2. N-Ethoxycarbonyl-N-hydroxyurea (152).

The amide (4g, 0.017mol), in ethanol (150ml) was hydrogenated at room temperature and atmospheric pressure over palladium charcaol for ninety minutes. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to afford the crude hydroxylamine. Purification by recrystallization gave the pure ester as a white crystalline solid.

yield: 2.3g , 93%.

m.p. 143-145^{o}C.

R_f: 0.34 (solvent DCM/MeOH 9:1 v:v).

^{1}H nmr (DMSO): δ 1.2 (t ,3H, $CH_2\underline{CH_3}$); 4.1 (q, 2H, $\underline{CH_2}CH_3$);

8.9 (s, 1H, NH); 9.1 (s, 1H, NH); 9.3 (broad s, 1H, OH).

Mass spectrum: $[M+H]^+$ at m/e 149.

Anal. calcd. for $C_4H_8N_2O_4$ requires C 32.43 H 5.40 N 18.92.

Found: C 32.18 H 5.33 N 18.60.

3.5.3. 1,2,4-oxadiazolidine-2,5-dione (153).

2.5ml of 2M potassium hydroxide was added to the ester (150) (0.9g, 6.1mmol). The solution was stirred at room temperature for ten minutes, then warmed to 50^{o}C with stirring for fifteen minutes. On cooling, the resulting potassium salt was converted to the free base by ion exchange chromatography (Dowex 50X2-100, H^+ resin), eluted with 100ml of water. The heterocycle was collected as a white solid on evaporation of solvent.

Yield: 0.74g 94%.

m.p. 108-110^{o}C.

Anal. calcd. for $C_2H_2N_2O_3$ requires C 23.53; H 1.96 ; N 27.45.

Found: C 23.13; H 1.97; N 27.45.

Mass spectrum: $[M+H]^+$ at m/e 103.

3.5.4. Attempted synthesis of N-tert-butoxycarbonyl quisqualic acid by the alkylation of the heterocycle (153) with (3S)-(3-t-butoxycarbonylamino)-oxetan-2-one (78).

The heterocycle (153) (0.55g, 5.3mmol) in 10ml of dry acetonitrile (ACN) was stirred under nitrogen for ten minutes, and the β-lactone (78) (0.2g, 1.07mmol) was added. The mixture was stirred at room temperature overnight. The solvent was evaporated *in vacuo* to give an oily residue which was taken up in water and acidified to pH3 with 0.5M HCl and then extracted with ethyl acetate (3x25ml). The combined extracts were dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure to afford the product as an oil, which was found upon characterization to be t-Boc serine (74).

Yield: 0.12g, 67%.

^{1}H nmr ($CDCl_3$): δ 1.4 (s, 9H, t-Boc); 3.8 (m, 2H, $CHCH_2$);

4.3 (m, 1H, $CHCH_2$); 6.0 (s, 1H, NH).

Attempted alkylation of the heterocycle with the β-lactone using different solvents such as THF, DMF, EtOAc, and temperature variation ranging from room temperature to 100^{o}C either gave t-Boc serine or the the elimination product. At higher temperatures intractable mixtures were obtained.

Attempts were also made to alkylate the heterocycle with the mesylate (128), the acetate (126) and the bromide (129), in diferent solvents. At lower temperatures these reactions gave either protected serine (Cbz or t-Boc), or the elimination product. At higher temperatures intractable mixtures were obtained.

3.5.5. Alkylation of the heterocycle (153) with ptsa-β-lactone (157).

t-Boc β-lactone (78) (0.6g, 3.2mmol), was converted to the *p*-toluenesulphonic acid salt according to Vederas *et al.*[184] The protected propiolactone was treated with trifluoroacetic acid (10ml); after fifteen minutes, anhydrous ptsa (0.58g, 3.36mmol) was added and the solvent was evaporated under reduced pressure to afford the ptsa salt of the β-lactone, which was triturated with dry ether, to afford the salt as a white solid.

Yield: 0.80g, 96.5%

m.p. 175°C (decomposed) (Lit [184] 173°C decomposed).

^{1}H nmr (D_2O) δ 2.52 (s. 3H, ArC$\underline{H}_3$); 4.95 (m, 2H, C$\underline{H}_2$); 5.60 (m, 1H, C$\underline{H}$CH$_2$); 7.45 and 7.89 (ABq, 4H, Ar$\underline{H}$ x 4). 11.49 (s, 3H, N$\underline{H}_3^+$).

The heterocycle (153) (0.1g, 0.98mmol) in ACN was treated under nitrogen with ptsa β-lactone (0.28g, 1mmol). The reaction mixture was stirred at room temperature for four hours and solvent was evaporated. The residue was triturated under ether and the product was collected as a white solid after filtration.

Yield: 0.11g, 31%.

^{1}H nmr (D_2O) δ 2.40 (s, 3H, ArC$\underline{H}_3$), 4.33 (m, 2H, C$\underline{H}_2$), 4.39 (m, 1H, C$\underline{H}$CH$_2$), 7.35 and 7.67 (ABq, 4H, Ar$\underline{H}$x4)

3.5.6. Synthesis of 1,2-Oxazolidine-3,5-dione (159).

3.5.6.1. Ethyl malonyl O-benzylhydroxamate (160).

O-Benzylhydroxylamine hydrochloride (5.1g, 0.03mol) and potassium carbonate (4.4g, 0.032mol) was dissolved in 100ml of toluene. Whilst being

stirred under nitrogen at room tempterature, ethyl malonyl chloride (5g, 0.03m) was slowly added. The mixture was heated under reflux for two hours. On cooling, toluene was evaporated under reduced pressure and the residue was partitioned between aqueous sodium bicarbonate and dichloromethane. The product was extracted in to the organic phase (3x30ml), dried over sodium sulphate, filtered and the solvent was evaporated *in vacuo* to afford the crude hydroxamate (160). Purification by flash chromatography on silica gel with hexane/ethyl acetate (60:40;v:v) as the eluent gave the pure hydroxamate as a clear oil.

Yield: 4.8g, 68%.

Mass spectrum: $[M+H]^+$ at m/e 238.

3.5.6.2. Ethyl malonyl hydroxamate (161).

The O-benzyl hydroxamate (160) (3g, 0.01mol) was dissolved in ethanol (50ml), to which was added 10% palladium on activated charcoal (0.3g). The suspension was hydrogenated at room temperature and atmospheric pressure for one hour. The catalyst was removed by filtration and the filtrate was evaporated to dryness to afford the hydroxyl derivative as a foamy oil.

Yield: 1.4, 95%.

Mass spectrum: $[M+H]^+$ at m/e 148.

3.5.6.3. 1,2-Oxazolidine-3,5-dione (159).

A solution of the hydroxamate (161) (1.3g, 8.8mmol) and potassium hydroxide (0.54g, 9.7mmol) in water (50ml) was stirred at room temperature for fifteen minutes. The reaction which was followed by thin layer chromatography (developed by ferric chloride), was warmed to 50°C for ten minutes (to aid complete cyclization). On cooling the mixture was passed

down an ion exchange column, (Dowex 50 H+) and eluted with water. All acidic fractions were combined and evaporated to dryness to give the product as an off-white solid.

Yield: 0.8g, 89%.

m.p.115-116^{o}C.

Mass spectrum: $[M+H]^{+}$ at m/e 102.

Anal. calcd. for $C_3H_3NO_3$ requires: C 35.64; H 2.97; N 13. 86.

Found: C 35.68; H 3.12; N 13.95.

CHAPTER FOUR.

PHARMACOLOGY.

4. PHARMACOLOGICAL EVALUATION OF THE EFFECTS OF SYNTHESIZED L-QUISQUALIC ACID AND ANALOGUES ON THE BINDING OF L-GLUTAMATE AND AMPA TO WHOLE RAT BRAIN MEMBRANES.

4.1 INTRODUCTION.

Early electrophysiological studies opened up the field into the experimental potential of excitatory amino acid research. These early studies, which were initiated by Hayashi[6] and later by Curtis *et al.*,[14] in their search for transmitter roles for L-glutamic acid and L-aspartic acid in mammalian brain tissue, provided the now accepted view that L-glutamic acid and L-aspartic acid are initiators of synaptic transmission in the mammalian central nervous system.[7,14,15] These studies, which were performed in the hope of identifying potential neurotransmitter substances, led to the isolation and identification of other excitatory amino acids, notably: N-methyl-D-aspartic acid, kainic acid, and quisqualic acid. The observed responses exhibited by neurones when they were exposed to these compounds led to the theory that specific receptors exist through which these responses were mediated.[7,11,14,16]

4.1.2. Pharmacology of binding.

Pharmacologists coined the term "receptor" almost a century ago in order to describe the mode of action and the specificity of drugs. It is a fundamental assumption of any receptor theory, as stated explicitly by Ehrlich,[20] that for an agent to act it must be bound. Thus the most important criterion for identifying a binding site as a neurotransmitter receptor is the detailed pharmacology of the binding. Thus, compounds which effectively block or mimic the effects of the neurotransmitter should compete for receptor sites at low concentrations. Conversely, ineffective drugs should compete only at very high concentrations,

if at all. Electrophysiological and binding studies support the view that, when activated by an agonist,the receptor transduces its signal ultimately to provide the characteristic physiological response.

4.1.3. Radioligand receptor binding.

The intervention of neurotransmitter or drug interaction with biological receptors can be studied *in vitro* by following the binding of suitable radioactive ligands.[187-190] The first receptors studied by radioligand binding techniques were nicotinic cholinergic receptors.[191] These early experiments provided a major impetus to the design of practical procedures for radioligand binding studies.

Using these procedures, receptor sites for almost all the known neurotransmitters in the peripheral and central nervous systems have been studied. The measurement of radioligand binding to receptors provides a means of obtaining valuable information on the physiological interactions between ligand and receptor, which would not have been obtained solely by electrophysiological means. Furthermore, it allows a direct measurement of ligand-receptor interaction from which a quantification of the numbers of receptors present in membrane preparations may be elucidated.

4.1.4. Radioligand binding of excitatory amino acid.

The study of excitatory amino acid receptors has been an explosive area of investigation in recent years. Radioligand binding to excitatory amino acid receptors was first introduced (simultaneously) by Roberts[192] and Michaelis.[193] These researchers provided the first biochemical evidence for the existence of specific receptor binding sites for excitatory amino acids on nerve

membranes provided the initial methodology through which radioligand binding to central nervous system receptors might be investigated.

These early studies involved the binding of ^{3}H-L-glutamic acid to nerve cell membranes in the presence of non-radioactive agonists and antagonists as displacers . At this point, interpretation of results was based on results from electrophysiological characterization of the ligands. Results from these early pharmacological studies, and the availability of other tritiated compounds, notably ^{3}H-AMPA,[75] ^{3}H NMDA,[194] ^{3}H kainic acid,[195] ^{3}H-D-aspartate,[196] ^{3}H-DL-AP4[197] and ^{3}H-AP5[198] provided the necessary tools for probing a number of questions regarding the structure, function, and regulation of excitatory amino acid receptors.

4.1.5. Analysis of binding data.

4.1.5.1. Specific binding refers to the component of the radioligand binding which is actually bound to the receptor under investigation. Due to the low concentration of receptors in synaptic membranes (often in femtomoles per milligram of wet tissue) these can be readily saturated by the addition of an excess of "cold ligand", a compound that is a potent agonist or antagonist of the *in vivo* pharmacological action. For example, in an appropriate ^{3}H-L-glutamate binding assay, the addition 1mM of unlabelled NMDA to the ligand binding assay would displace the specifically-bound radioactive ligand from specific NMDA recptors.

4.1.5.2. Non-specific binding is that component of the binding that cannot be saturated with an excess of the appropriate displacer. This is often the result of

non-receptor phenomena such as the partitioning of the radioligand into the lipid environment of the membrane preparation.

4.1.5.3. Total binding is the sum of the specific and non-specific binding.

In practice, specific binding is usually derived from the difference between the total binding (measured when no displacer is present), and non-specific binding (measured in the presence of excess "cold ligand").

CHAPTER FIVE.

5. PHARMACOLOGY EXPERIMENTAL.

5.1. Materials and methods.

5.1.1. sources of material.

Unless stated to the contrary, the chemicals used were from the following sources:

Radiochemicals : Amersham International, P.O. Box 16, Amersham, or New England Nuclear, DuPont, Wedgewood Way, Stevenage.

Animals Adult male Wistar rats of approximately 250g in weight were used.

Measurement of radioactivity

Radioactivity was measured by an LKB Rack beta using a liquid scintillation spectrometer.

Amino acids and derivatives were from Tocris Neuramin, Bristol.

5.2. Radiolabelled ligands.

The vast majority of receptor labelling studies involve the binding of a radioactive ligand (agonist or antagonist) to a suitable membrane preparation derived from target tissues. Since many receptor sites have an equilibrium dissociation constant for ligand in the nanomolar range, the most crucial step in radioligand binding studies is the acquisition of radiolabelled ligand of high radiochemical specific activity.

In view of the low concentration of receptors in the membranes, the radioactive ligand should ideally possess the following properties:

1. High selectivity: This ensures that the ligand binds maximally to only the relevant receptor population, and to a minimal extent to non-receptor components of the membrane.

2.High affinity: This ensures that the ligand- receptor dissociation constant is in the range of the receptor concentration.

3 High specific activity: This ensures that appropriate low concentrations can be used and the specific binding may be readily and accurately determined.

4. Reversibility: Binding should be readily reversible with a time course consistent with the rate of termination of the physiological action, and that subcellular distribution of binding sites are compatible with those for neurotransmitter receptors.

For the work reported here ^{3}H-L-glutamate and ^{3}H-AMPA were used in the binding experiments. These ligands were chosen in order to study receptor binding to:

(i) all glutamate-sensitive receptor sites.

(ii) one class of non-NMDA receptors.

5.3. Tissue preparation.

The procedures used to prepare membranes to study the binding of ^{3}H-L-glutamate and ^{3}H-AMPA binding are shown schematically in fig. 5.3.

Brain tissue from adult male Wistar rats of approximately 250g was used for binding studies. The rats were killed, decapitated and their brains were rapidly removed and placed in ice-cold 0.32M sucrose in 5mM tris-acetate solution (pH 7.4). The tissue (excluding the cerebellum) was homogenized in a tight fitting Jencons glass homogenizer with ten strokes of a teflon pestle. The homogenate was centrifuged at 1000g for ten minutes. The pellet (P1) was discarded, and the supernatant was centrifuged at 17,000g for twenty minutes. The supernatant was discarded, and the pellet (P2) was suspended in 20ml of ice-cold 5mM tris-acetate buffer and rehomogenised.

The homogenate was lyzed by incubation at 0^{o}C for twenty minutes, followed by centrifugation at 17,000g for twenty minutes. Supernatant and the bilayer (a soft "buffycoat") were collected, (blood cells and the mitochondrial pellet were discarded) and homogenized, diluted to 50ml volume with 5mM tris-acetate buffer, and centrifuged at 17,000g for twenty minutes. The pellet (P4) was washed six to eight times by resuspension in 5mM tris-acetate, (pH 7.4), homogenized and centrifuged at 50,000g for ten minutes. The membranes were then finally resuspended in 50mM tris-acetate buffer and stored at -78^{o}C prior to the binding assay.

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5.4. [^{3}H]L-Glutamate binding assay.

The frozen pellet was allowed to thaw at room temperature, resuspended in 50ml of 50mM tris-acetate buffer (pH 7.4) and centrifuged at 50,000 rpm for 10 minutes. The pellet was finally resuspended in 20ml of tris-acetate buffer and a sample was removed for protein determination. An aliquot (600 µl) of the membrane suspension was transferred into 1ml Eppendorf tubes containing 50 µl (of varying concentration) of [^{3}H] L-glutamate, and 50 µl of 50mM tris-acetate buffer. Non-specific binding was determined by the addition of 50 µl of 1mM " cold" NMDA in 50mM tris-acetate buffer. The tubes were stoppered, shaken and then incubated for thirty minutes at room temperature.

Incubation was terminated by centrifugation using a bench microfuge centrifuge at 13000g for four minutes. The supernatant was rapidly removed by careful suction and the pellet was washed superficially with aliquots of 50mM tris-acetate buffer to remove adhering/trapped unbound ligand. The tip of the tube containing the pellet was removed and placed in a glass scintillation vial (F.G.B.Trident Ltd) containing 500 µl of 2% sodium dodecyl sulphate. This was left at room temperature to solubilise overnight. To each sample was added 4ml of optiphase Hisafe scintillant. (Phamacia). The vials were thoroughly shaken to ensure that all the solubilized tissue had been dissolved in the scintillant and the bound radioactivity was measured by liquid scintillation counter using a medium quench counting efficiency of 60%. The experiments were carried out in quadruplicate.

Binding was then investigated using a single concentration of labelled L-glutamate (45Ci $mmol^{-1}$) and varying concentrations (10^{-3} to 10^{-11}M) of the

prepared L-quisqualic acid and analogues, and 600 µl of the prepared membranes. Each analogue was assayed three times in quadruplicate.

5.5. ^{3}H AMPA binding assay.

The experiment was carried out as outlined for L-glutamate binding assay with the following modifications:

1. 20nmol of tritiated AMPA (60 Ci mmol^{-1}) (50µl) was used in each assay.
2. The assays were carried out in the presence of 100mM potassium thiocyanate (chaotropic agent to enhance binding)
3. Synaptic membranes in 50mM tris-acetate buffer were incubated for one hour in quadruplicate, with varying concentrations of radiolabelled ligand alone, or the presence of a range of concentrations of L-quisqualic acid and analogues in a fixed concentration 20nM of [^{3}H]-AMPA.
4. Incubation was carried out at 4^{o}C
5. Incubation was terminated by centrifugation, and assayed as outlined for [^{3}H] L-glutamate above, for determination of their IC_{50} values.

6.RESULTS

The pharmacological profiles of L-quisqualic acid and a range of analogues were investigated by ligand binding studies. The objective of the experiments was to determine which, if any, of the prepared compounds were capable of displacing [^{3}H] L-glutamic acid and [^{3}H]-AMPA from cerebral tissue preparations.[199]

The percentage of inhibition was determined in a routine binding assay in the presence of 1mM NMDA (in [^{3}H] L-glutamate binding) and 1mM "cold" AMPA (in AMPA binding), to give the non-specific binding.

The data were analysed by the linear regression with IC_{50} values determined by computer analysis for the single phase sites and biphasic sites.

Plots of percent displacements of specific binding vs. log concentration of the displacer were obtained, and their $1C_{50}$ values determined from them. These are depicted in figures 6.1-6.4.

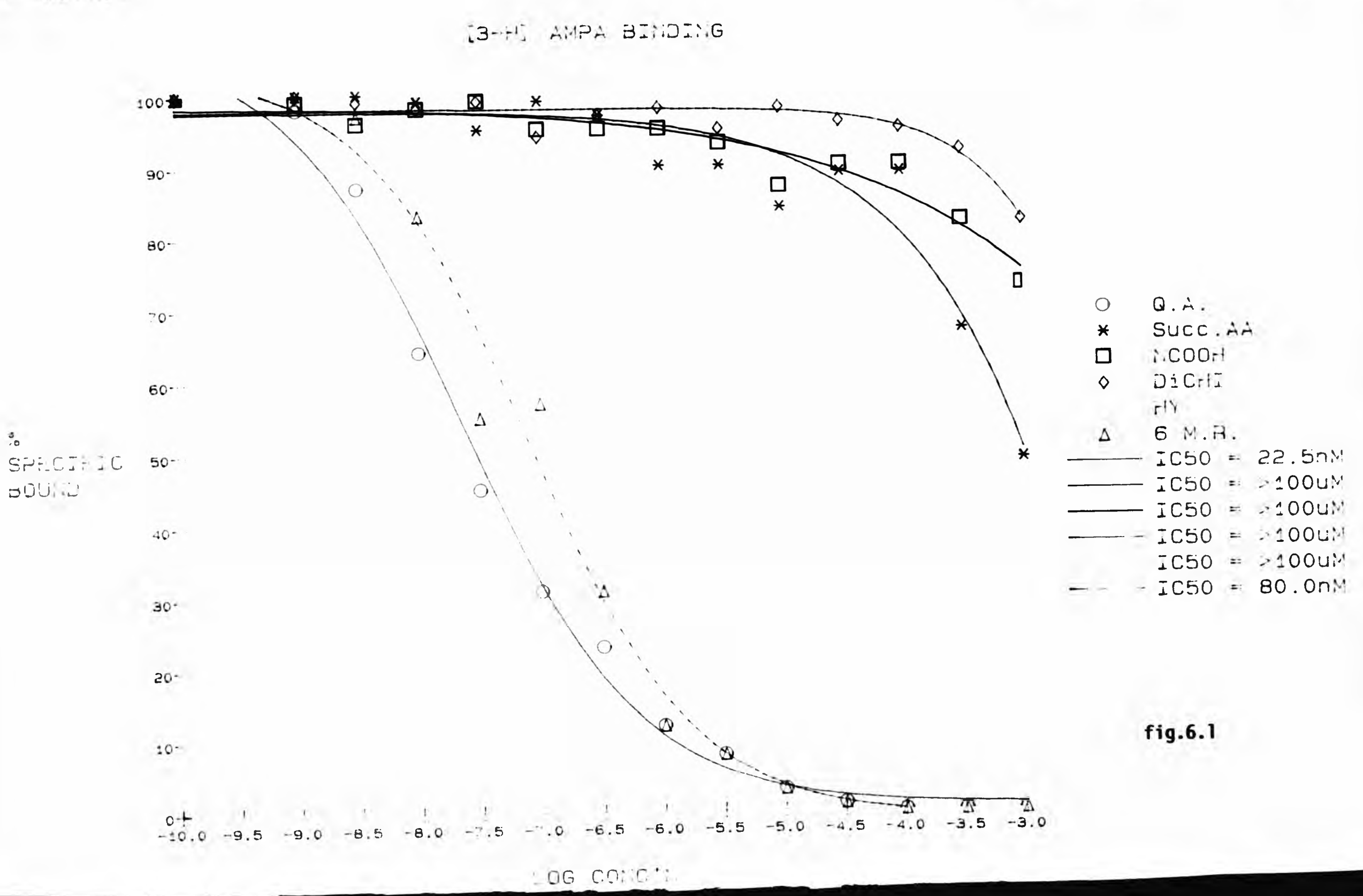
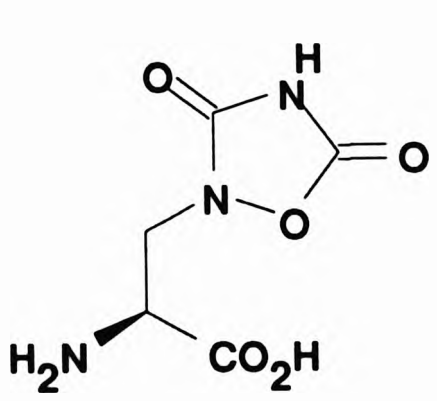

[3-H] AMPA BINDING
%
SPECIFIC
BOUND
100
90
80
70
60
50
40
30
20
10
0
-10.0 -9.5 -9.0 -8.5 -8.0 -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 -3.5 -3.0
LOG CONC
Q.A.
Succ.AA
NMDA
DiCHI
rIY
6 M.R.
IC50 = 22.5nM
IC50 = >100uM
IC50 = >100uM
IC50 = >100uM
IC50 = >100uM
IC50 = 80.0nM

fig.6.1

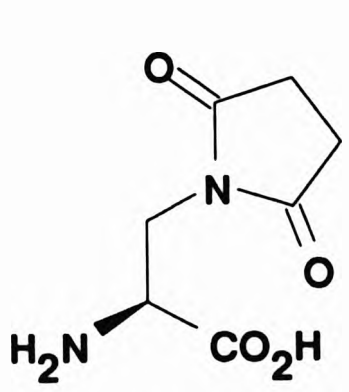
Fig. 6.1. Pharmacological profile of L-[^{3}H] AMPA binding to rat synaptic membrane.

Routine binding experiments were performed in the presence of increasing concentrations of synthesized L-quisqualic acid and cyclized analogues. Each analogue was assayed in quadruplicate and the data represents the mean of three experiments. For ease of interpretation the displacement curves and IC_{50} values for the cyclized analogues are presented in the composite figure 6.1.

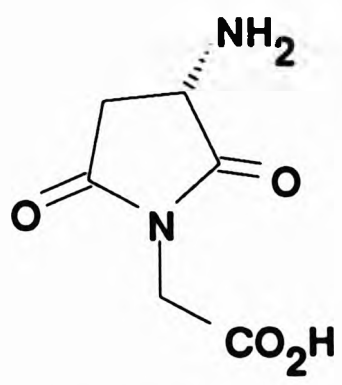
[O] L-quisqualic acid; [*] Succ. A.A [□] NCOOH; [✧] Dichi; [V] HY; [Δ] 6 M.R.



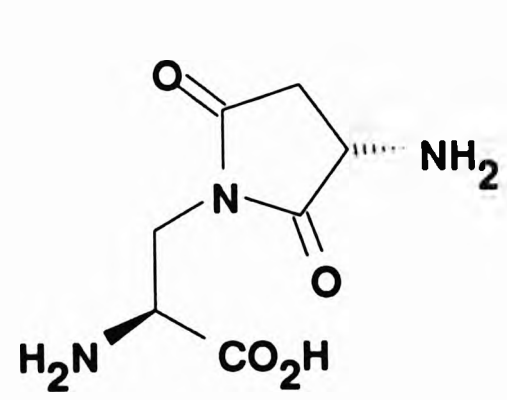
[O] L-Quisqualic acid



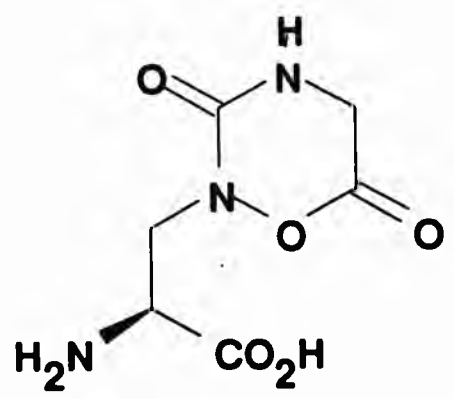
[ ] Succ. AA



[ ] NCOOH



[ ] Dichi



[ ] 6MR

Displacement curves and IC50 values for the uncyclized analogues are shown in fig.6.2 overleaf.

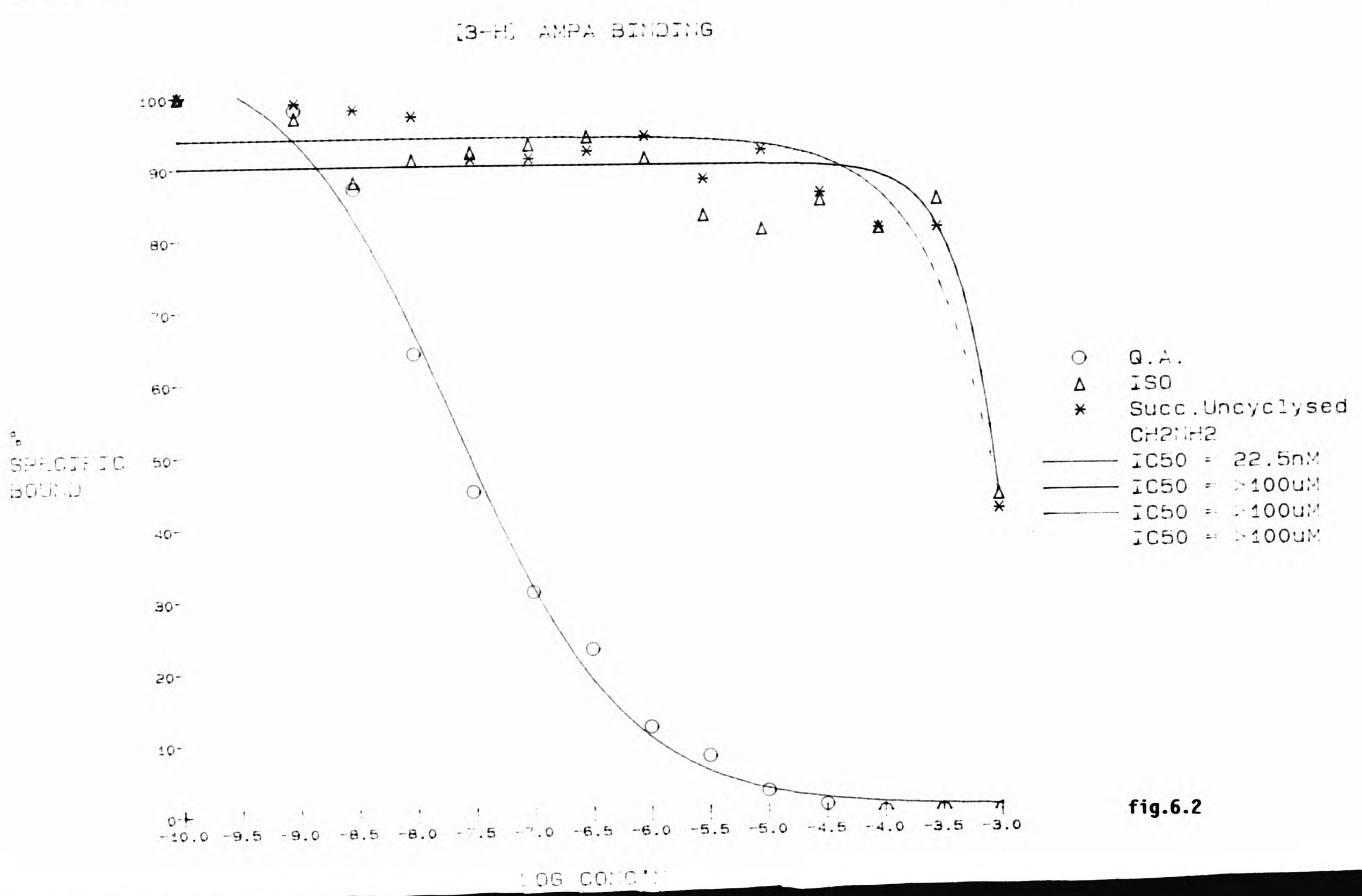
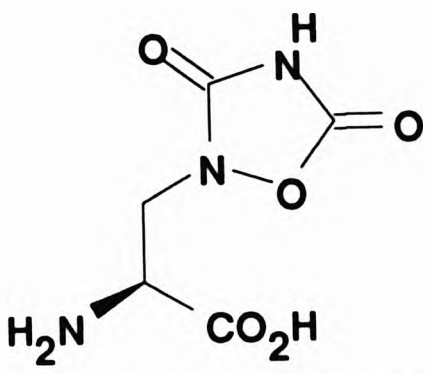

[3-H] AMPA BINDING
100
90
80
70
60
50
40
30
20
10
0
% SPECIFIC BOUND
-10.0 -9.5 -9.0 -8.5 -8.0 -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 -3.5 -3.0
LOG CONC'N
Q.A.
ISO
Succ.Uncyclysed CH2NH2
IC50 = 22.5nM
IC50 = >100uM
IC50 = >100uM
IC50 = >100uM

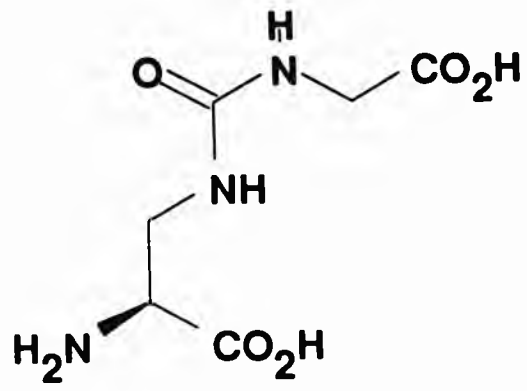
fig.6.2

Fig. 6.2.

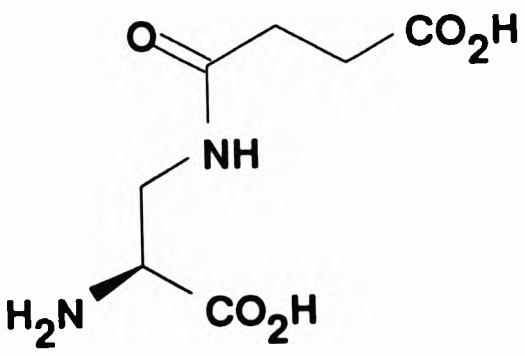
[O] Quisqualic acid; [A] ISO; [*] Succ uncyclized; [v] CH_2NH_2.



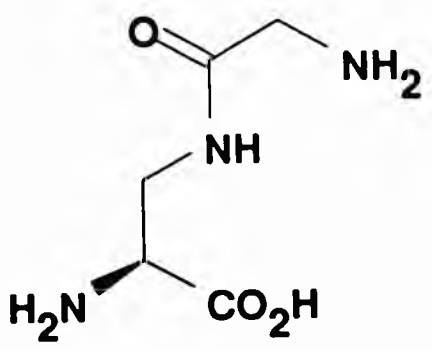
[O] L-Quisqualic acid



[ ] ISO



[ ] Succ uncyclized



[ ] CH_2NH_2

#GTABLE2_GLU

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[3-H] GLUTAMATE BINDING

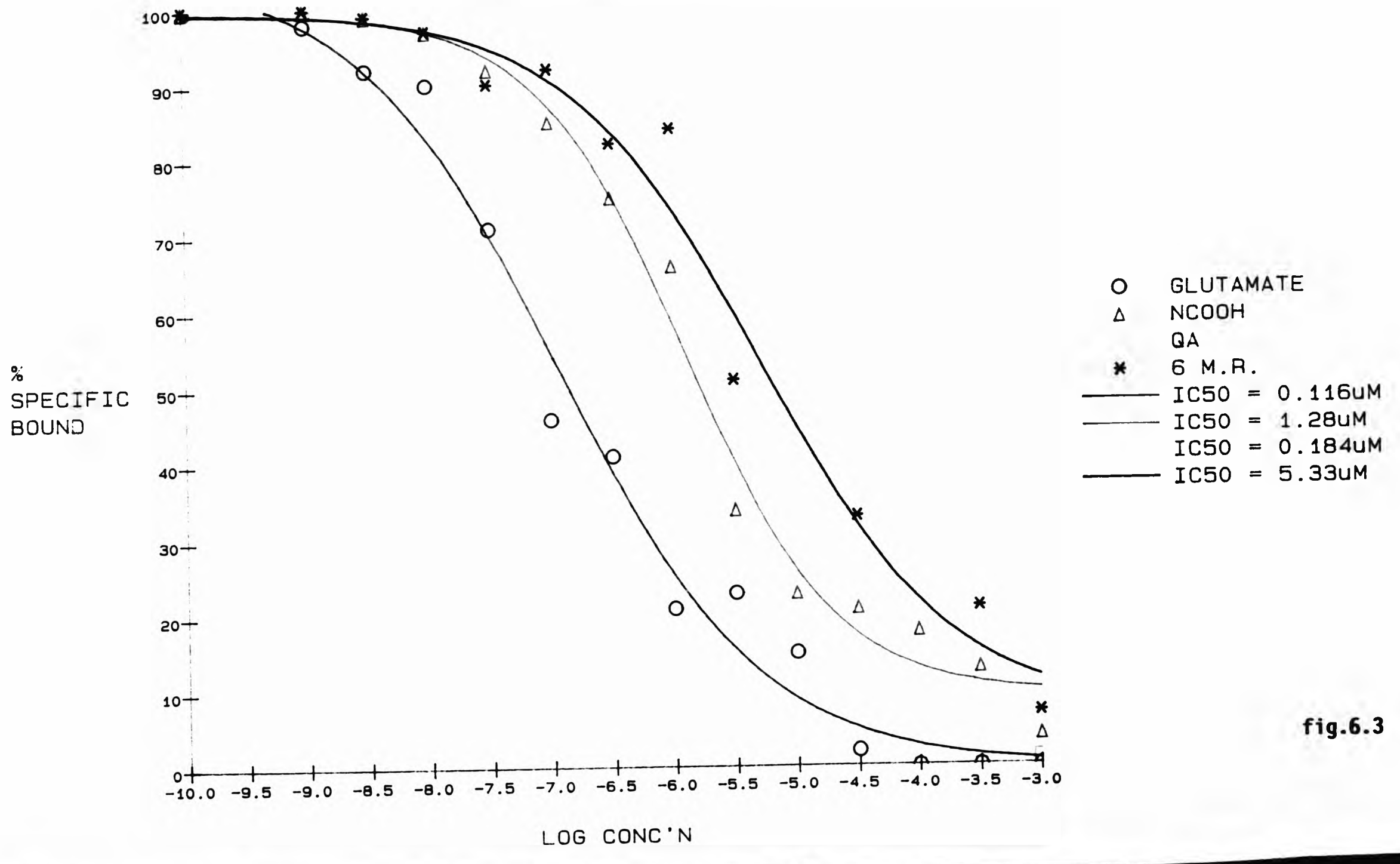
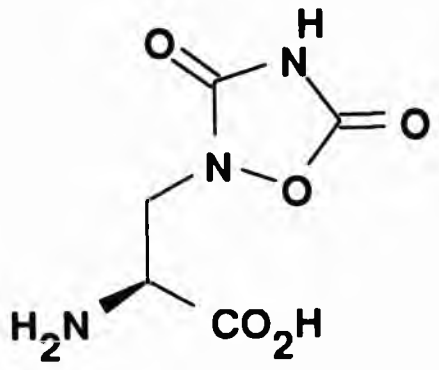


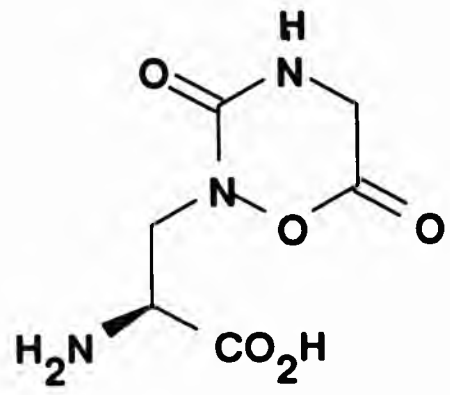
fig.6.3

Fig 6.3

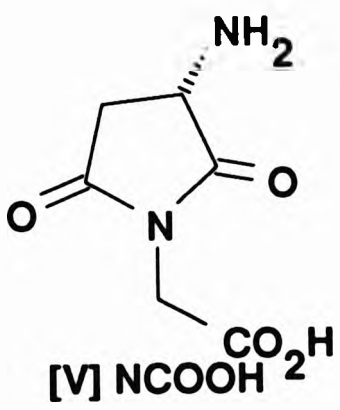
Pharmacological profile of L-[^{3}H] glutamate binding to the rat brain synaptic membranes. The composite figure shows displacement curves and the IC_{50} values for L-quisqualic acid and some of the prepared analogues. The experiment were performed in quadruplicate and the data represent the mean value of three independent experiments. [O] L-glutamic acid. [▯] Quisqualic acid; [v] 6MR; [*] NCOOH;



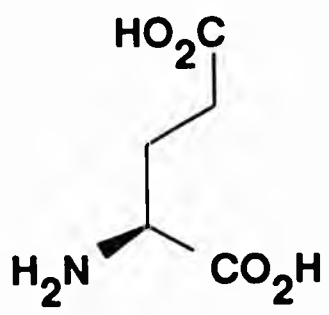
[▯] L-Quisqualic acid



[+] 6MR



[V] NCOOH



[O] L-Glu

#GTABLE2_GLU2

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[3-H] GLUTAMATE BINDING

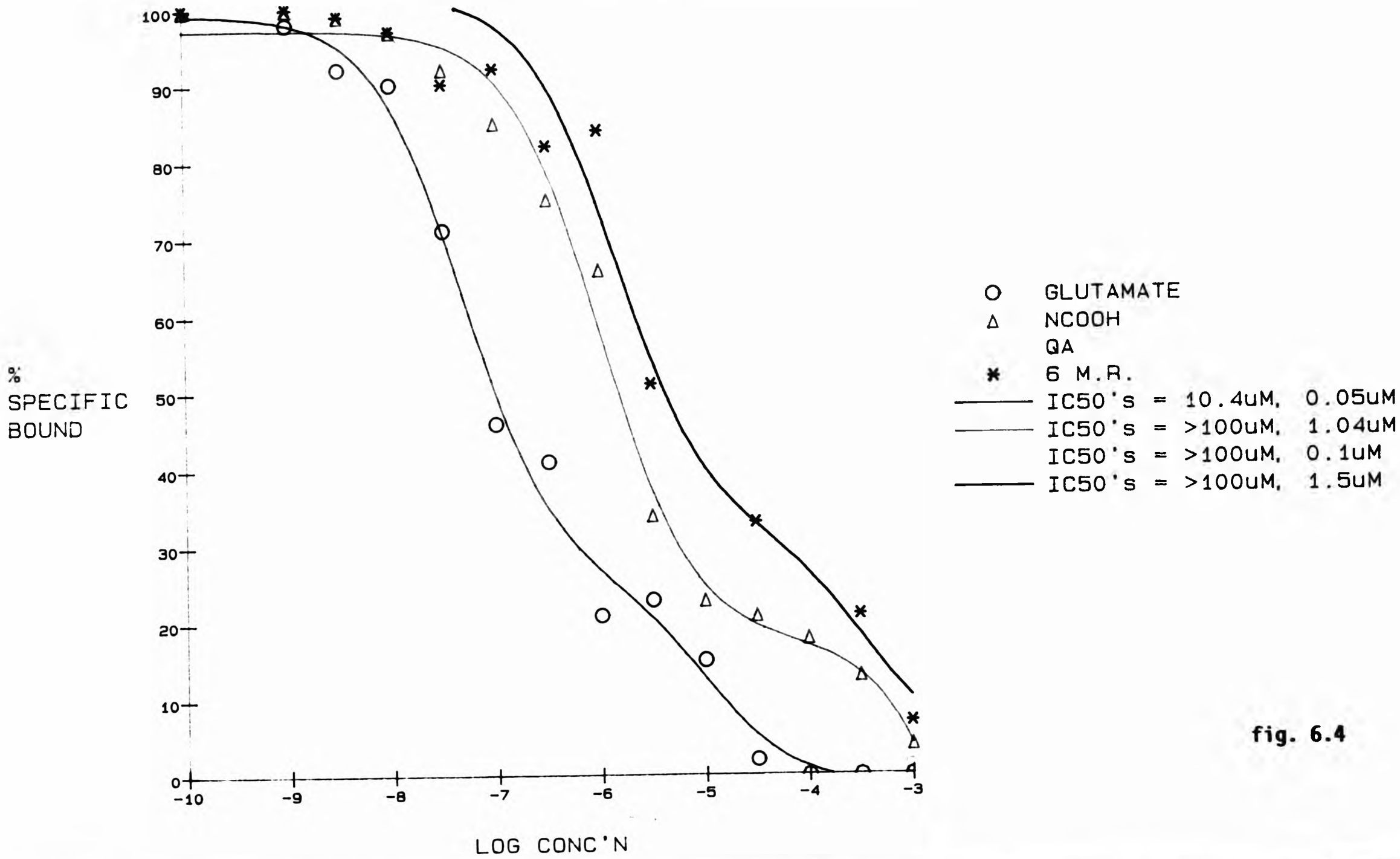
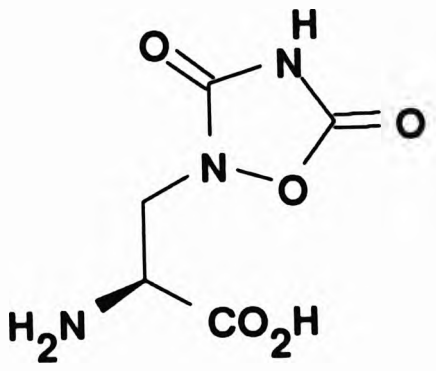


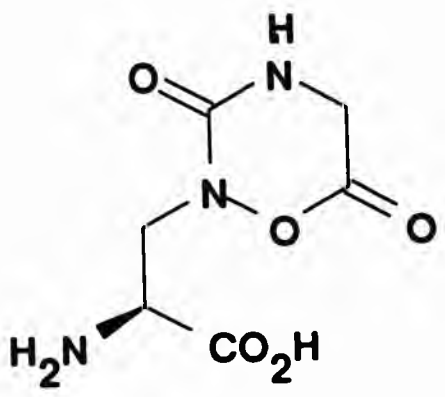
fig. 6.4

Fig. 6.4

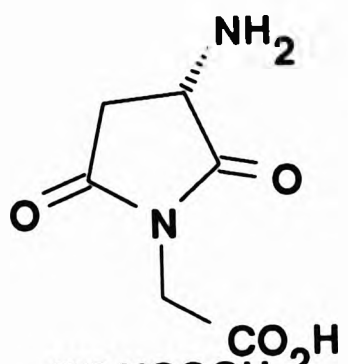
The composite figure gives the graph and the IC_{50}s value of the same data as those used in figure 6.3.



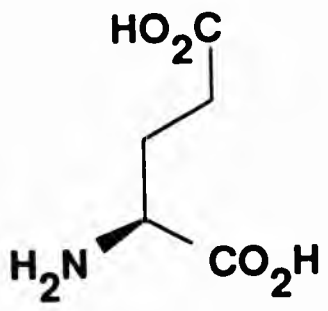
[□] L-Quisqualic acid



[+] 6MR



[V] NCOOH



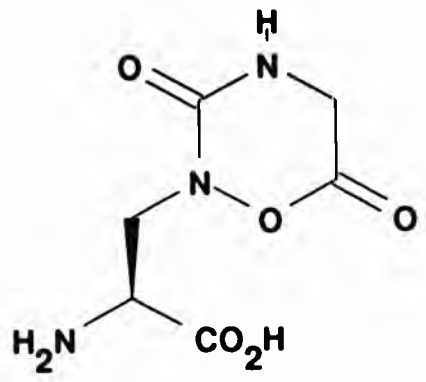
[O] L-Glu

6.1. Discussion.

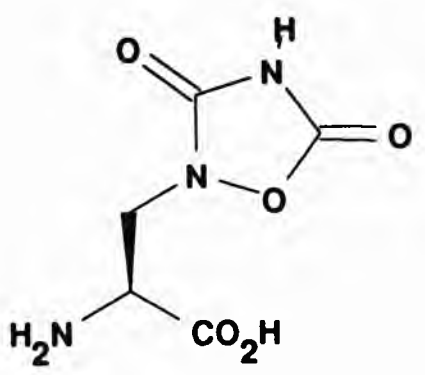
The results obtained for the binding of L-quisqualic acid and its analogues synthesized in this work are represented graphically in figures 6.1-6.4.

The IC_{50} value for the displacement of [^{3}H]AMPA by quisqualic acid (20) (in the presence of millimolar concentration of KSCN) was found to be 22.5 nM, this is in reasonable agreement with data obtained by other workers[52,80]. The variation between these results and those in the literature may be due to the type of buffer and detergent used in the binding experiments.

Of the synthesized quisqualate analogues, the six membered ring (85) (6.M.R. in figs 6.1-6.4) showed the highest activity in displacement of [^{3}H]AMPA binding, with an IC_{50} value of 80 nM. This finding may be attributable to the close structural relationship of the compound (85) to quisqualic acid (20).



(85)

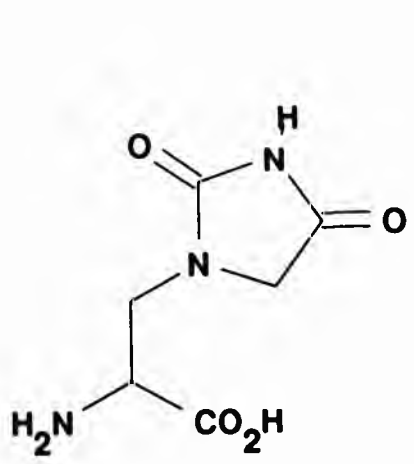


(20)

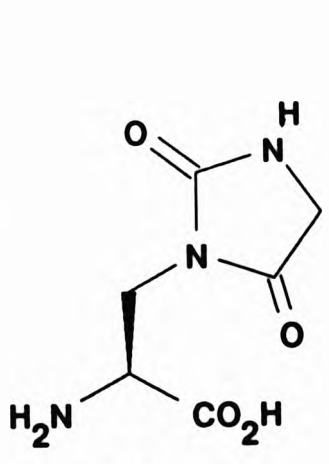
The reduction in activity of the analogue (85) in comparison to quisqualic acid (20) may be due to the effective decrease in acidity of the amidic ring NH of (85) compared with the corresponding group in quisqualic acid, which is imidic

rather than amidic. Alternatively, the alteration in ring size may adversely affect the orientation of the functional groups in the side chain which, presumably, contribute to binding to the receptor.

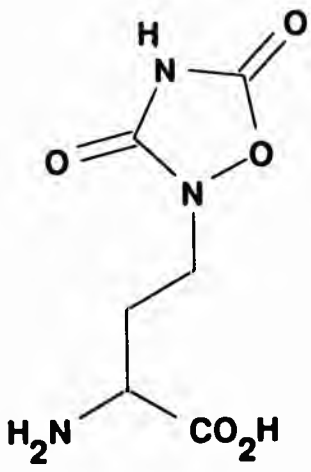
As regards the activities of the other QA analogues, it is particularly surprising, at first glance, that the hydantoin analogue (87), in which the ring oxygen in quisqualic acid is replaced with a carbonyl was essentially inactive in binding to the AMPA receptor. An explanation for this may be found in the X-ray crystal structure of (86), a hydantoin analogue prepared by Bycroft,[200] in which the ring oxygen in quisqualic acid is replaced by a methylene group. This compound, which was also inactive in binding to AMPA receptor, was found on X-ray crystallography[200] to possess a planar geometry, i.e. that the ring N bearing the aminopropionic acid substituent was planar, as would be expected of an amidic nitrogen. The corresponding N in quisqualic acid (20) was found to be pyramidal (with a low barrier to inversion), and therefore closer to the geometry of the sp^3 hybridised carbon in the corresponding position in L-glutamic acid.



(86)



(87)

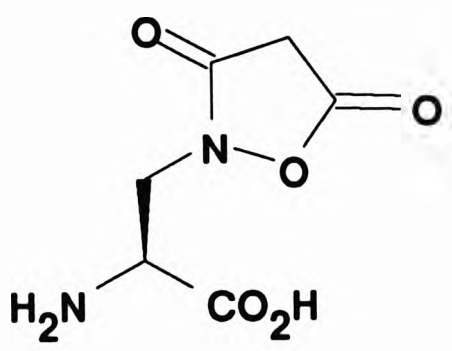


(159)

This finding was also in keeping with semi-empirical molecular orbital calculations which showed that a heteroatom α to the ring N induces pyramidalization at N, possibly as a way of reducing unfavourable interactions between lone pairs on adjacent heteroatoms.

It is of interest to note that, in experiments carried out in the locust (Schistocerca gregaria) muscle, the quisqualic acid analogue (159) (homoquisqualic acid)[201] in which the side-chain was elongated by one methylene group, showed activity at the quisqualate-sensitive glutamate receptor site. This supports the view that the pyramidal geometry of the ring N may be a requisite for quisqualate binding activity.

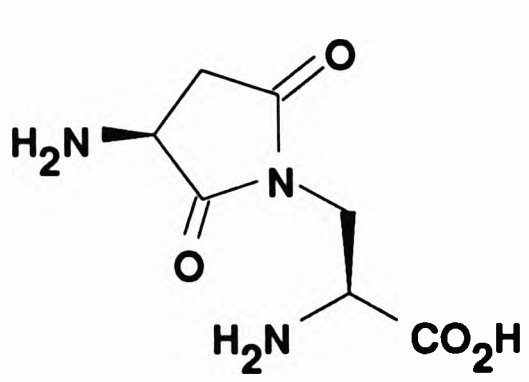
The importance of the ring NH in the quisqualic acid analogues would have been tested by studying the receptor binding of the methylene analogue (113), in which the heterocyclic ring NH is replaced with a methylene group. Precursors to (113) were designed and synthesized in this project, but unfortunately, the final amino acid was not isolated.



(113)

The diamino analogue (124), which introduced another amino function (and a second chiral centre) into the hydantoin ring, and effectively increased the

electron density of the compound, showed no activity at the receptor site. This may be due, in part, to the replacement of an acidic centre in the heterocycle with a basic group.



(124)

The change in ring conformation due to the presence of a planar nitrogen in (124) may be a contributory factor. There may also be unfavourable electrostatic or hydrogen bonding interactions with basic residues in the receptor, or simply steric congestion.

The other cyclized and the non-cyclized analogues, had IC_{50} values greater than 100μM, and were effectively inactive at displacing [^{3}H]-AMPA binding from the post-synaptic membrane.

The pharmacological evaluation of L-quisqualic acid showed a biphasic displacement of [^{3}H] L-glutamate binding from rat synaptic membranes, with IC_{50} values of 0.1μM for the high affinity site and >100μM for the low affinity binding site (figure 6.4). The observation of biphasic binding is in agreement with those of previous workers[51]. Interestingly, the aminopyrrolidinedione

(119) (NCOOH figs. 6.3 and 6.4), with the carboxymethyl side chain, showed some activity, exhibiting biphasic displacement of [^{3}H] L-glutamate binding to rat synaptic membrane with IC_{50} values of 1.04 μM for the high affinity site and >100μM for the low affinity site. It should be noted, however, that this compound showed no activity at the AMPA receptor. The other synthesized analogues were virtually inactive at displacing [^{3}H] L-glutamate binding including, quite suprisingly, the 6.M.R (85) .

Ideally, the best line of approach would have been to evaluate the pharmacological efficacies of a range of compounds as they were synthesized. The results obtained would therefore have provided a direction for future synthetic work. However, due to the inherent problems encountered during the course of this thesis, this approach was not possible.

7. CONCLUSION.

Much of the original work described in this thesis is synthetic organic chemistry. In the early part of the thesis, known methodology is modified and applied to the first synthesis of tritiated quisqualic acid, and the details have been supplied to a commercial radiochemical company.

In the main part of the Thesis, a series of cyclic and peptidic analogues of quisqualic acid is prepared and characterized. A feature of some of these syntheses has been that new methodology was required to answer the synthetic challenges; for example, preparation of L-quisqualic acid from serine *via* the propiolactone, and the synthesis of various analogues from L-asparagine.

Finally, a selection of the new analogues was studied pharmacologically, using

radioligand binding techniques with tritiated L-glutamic acid and tritiated AMPA as ligands. Two compounds, (119) and (85), showed significant binding to glutamate and AMPA receptors repectively. Aspects of both the synthetic and pharmacological studies will be published.

7.1 Suggestions for future work.

It may be envisaged that the work of this Thesis could be of value in future investigations by providing:

(a) ^{3}H quisqualic acid for pharmacological studies.

(b) quisqualate analogues which could be useful in future studies involving neuroreceptor classification.

(c) synthetic methodology of potential value if applied to other target structures.

The selectivity of the quinoxalinediones as antagonists of the AMPA receptor suggests that future synthetic ideas for quis/AMPA and/or metabotropic receptor agonists and antagonists should not be centered solely on amino acid type compounds, but also on structural analogues of quinoxalinediones.

Lastly, syntheses and pharmacological evaluation of synthesized compounds should be carried out in an iterative manner, and the pharmacological results would therefore provide a direction for future synthetic targets.

REFERENCES.

1. Bowman. W. C. and Rand, M. J. "Textbook of Pharmacology" (2^{nd} edn.) Blackwell Scientific Publications (Chapter 6).
2. Elliot, T. R., J. Physiol. (Lond.), XX, 31 (1904).
3. Loewi, O., Pflugers Arch. ges.Physiol., 193, 201 (1922).
4. Dale, H. H. and Dudley, H. W., J. Physiol. (Lond)., 68, 97 (1906).
5. Tringle, C. R. and Tringle, D. J., in "Chemical Pharmacology of the Synapse" Academic Press 1 (1976).
6. Hayashi, T., Jap. J. Physiol., 3, 46 (1952).
7. Curtis, D. R. and Watkins J. C., J. Neurochem., 6, 117 (1960).
8. Fagg, G.E. and Foster, A.C., Neuroscience, 9, 701 (1983).
9. Watkins, J. C., and Curtis, D. R., J. Physiol., 150, 656 (1960).
10. Benjamin, A. M. and Questel J. A., J. Neurochem., 23, 457-464 (1974).
11. Curtis D. R. *et al.*, Nature, 183, 611 (1959)
12. Stryer, L. in "Biochemistry", 2^{nd} edn.· p896 (W. H. Freeman).
13. Olney, J. W. in "Kainic acid as a tool in Neurobiology" (McGeer, E., Olney, J. W. and McGeer, P., eds.), 95 (1978) Raven Press.
14. Curtis, D. R. and Watkins, J. C., J. Physiol., 146, 185 (1959).
15. Curtis, D. R. and Watkins, J. C., J. Physiol., 158, 296 (1961).
16. Curtis, D. R. *et al.*, J. Neurochem., 6, 1 (1960).
17. Takeuchi. A. *et al.*, J. Physiol., 170, 296 (1964).
18. Usherwood, P. N. R. *et al.*, Nature, 28, 497 (1965).
19. Langley, J. N., Proc. Roy. Soc. Lond. B, 78, 170 (1906).
20. Ehrlich, P. in "Chemotherapeutics: Scientific principles, methods and results." Lancet, ii, 445 (1913).

21. Phillis, J. W., Ann. Rev. Physiol., 23, 501 (1961).

22. Florey, E., Ann. Rev. Physiol. Toxicol., 21, 156 (1981).

23. Shinozaki, H. and Komshi, S., Brain Res., 24, 368 (1970).

24. Takemoto, T. in "Kainic acid as a tool in Neurobiology", (Mc Geer, E.G., Olney, J. W. and McGeer, P. L., eds.) Raven Press,1 (1978).

25. Shinozaki, H. in "Kainic Acid as a tool in Neurobiology", (McGeer, E. G.,Olney, J. W. and McGeer, P. L., eds.), pp 17-35 Raven Press (1978).

26. Krogsgaard-Larsen, P. Honore', T., Trends in Pharmacol. Sci., 4, 31 (1983).

27. Watkins, J. C. in "Kainic Acid as a tool in Neurobiology", (McGeer, E.G., Olney, J. W. and McGeer, P. L., eds.), pp 37-69 Raven Press (1978).

28. Monaghan D. T. *et al.*, Annual. Rev. Pharmacol. Toxicol., 29, 365 (1989).

29. Mayer, M. L. *et al.*, Drug Dev. Res., 17, 263 (1989).

30. Evans, R. H. *et al.*, Br. J. Pharmacol., 67, 591 (1979).

31. Watkins, J. C. and Olverman, H. J., in "Excitatory Amino Acids in Health and Disease", (Lodge, D., ed.), pp13-45 (1988).

32. Curtis, D. R. *et al.*, J. Neurophysiol., 28, 497 (1965).

33. Usherwood, P. N. R. and Machilli, J., Exp. Biol., 17, 435 (1968).

34. Usherwood, P. N. R. and Grundfest, P., J. Neurophysiol., 28, 497 (1965).

35. Kerkut, G. A. and Walker, R.J., Comp. Biochem. Physiol., 17, 435 (1966).

36. Watkins, J. C., J. Theoret. Biol., 9, 37 (1965)

37. Cull-Candy, S.G., J. Physiol., 255, 449 (1976).

38. Johnstone, G. A. R. *et al.*, Nature, 248, 804 (1974).

39. Piggott, S. M. *et al.*, Comp. Biochem. Physiol., 51c, 91 (1975).

40. McCulloch, R. M. *et al.*, Exp.Brain Res., 19, 522 (1974).

41. Duggan, A. W., Exp. Brain Res., 19, 522 (1974).

42. Watkins, J. C., in "Glutamate: Transmitter in the CNS", (Roberts, P. J., Storm-Mathisen, J. and Johnstone, G. A. R., eds.) John Wiley and Sons, (1981).

43. Evans, R. H. and Watkins, J. C., Brain Res., 148, 536 (1978).

44. McLennan, H. and Lodge, D., Brain Res., 169, 83 (1979).

45. Davies, J. and Watkins, J. C. *et al.*, J. Physiol., 75, 461 (1979).

46. Mayer, M. L. et al., Proc Natl. Acad. Sci. USA, 86, 1411 (1989)

47. Evans, R. H. *et al.*, Br. J. Pharmacol., 75, 65 (1982).

48. Miller, R. F. and Slaughter, M. M., Science, 211, 182 (1981).

49. Koerner, J. F. and Cotman, C. W., Brain Res., 216, 192, (1981).

50. Davies, J. and Watkins, J.C. *et al.*, Comp. Biochem. Physiol., 72, 211 (1982).

51. Watkins, J.C.; Honore', T. and Krogsgaard-Larsen, P., Trends in Pharmacol. Sci., 11, 25 (1990).

52. Cha, J. J. and Greenamyre, J. T. *et al.*, J. Neurochem., 51, 469 (1988).

53. Honore', T. *et al.*, Science, 241, 701 (1988).

54. Watkins, J. C., Trends in Neurosci., 3, 61 (1980).

55. Mayer, M. L. and Westbrook, G. L., Prog. Neurobiol., 28, 197 (1986).

56. Davies, J. *et al.*, Brain Res., 382, 169 (1986).

57. Watkins, J. C. and Evans. R. H., Ann. Rev. Pharmacol. Toxicol., 21,

165 (1981).

58. Watkins, J. C. *et al.*, Trends in Pharmacol. Sci., Special Report, pp 4-12 (1991)

59. Watkins, J. C. and Olverman,H. J., Trends in Neurosci., 10, 265 (1987).

60. Anis, N. A. *et al.*, Br. J. Pharmacol., 79, 565 (1983).

61. Berry, S. C. *et al.*, Br. J. Pharmacol., 83, 179 (1984).

62. Wong. E. H. F. *et al.*, Proc. Natl. Acad. Sci. U.S.A., 83, 7104 (1986).

63. Largent, B. L. *et al.*, J. Pharmacol. Exp. Ther., 238, 739 (1986).

64. Johnson, J. W. and Ascher, P., Nature, 325, 529 (1987).

65. Monaghan, D. T. *et al.*, J. Neurosci., 5, 2909 (1986).

66. Bristow, D. R. *et al.*, J. Pharmacol., 126, 303 (1986).

67. Monaghan, D. T. *et al.*, Nature, 306, 176 (1983).

68. Schwarz, R. *et al.*, Life Sci., 35, 19 (1984).

69. Moroni, F. *et al.*, Neurosci. Letts., 47, 51 (1984).

70. Simon, R. P. *et al.*, Science, 226, 850 (1984).

71. Rothmans, S., J. Neurosci., 4, 1884 (1984).

72. Meldrum, B. S. Trends in Neurosci., 8, 47 (1985).

73. Meldrum, B. S. Epilepsia, 25, S149 (1984).

74. Biscoe, T. J. and Watkins, J. C. *et al.*, Nature, 255, 166 (1975).

75. Honore', T. *et al.*, Science, 241, 701, (1988).

76. Sladeczek, F. *et al.*, Nature, 317, 717 (1985).

77. Honore', T. Nielsen, M., Neuroscience Letts., 54, 27 (1985).

78. Honore', T. and Drejer, J., J. Neurochem., 51, 475 (1988).

79. Monaghan, D. T. *et al.*, Annual Rev. of Pharmacol. Toxicol., 29. 365 (1989).

80. Neilson, E. O. *et al.*, Eur. J. Pharmacol., 195, 197, (1988).

81. Sladeczek, F. *et al.*, Trends in Neurosci., 11, 546 (1988).

82. Barnard, E., A. *et al.*, in "Excitatory amino acids", Fidia Research Foundation Symposia Vol 6, (Meldrum B. S. *et al.*, eds), Raven Press (in press).

83 Schoepp, D. E. and Johnson B. G., J. Neurochem., 53, 273 (1989).

84. Cha, J.J. *et al.*, Neuroscience Lett., 113, 78 (1990)

85. Schoepp, D. D. et al., Trends in Pharmacol. Sci. Special Report, 74 (1991)

86. Watkins J. C. *et al.*, in "Excitatory Amino Acids and Neuronal Plasticity," (Ben-Ari, Y., ed.) Plenum Press (in press).

87. Agrawal, S. G. and Evans R. H., Br. J. Pharmacol., 87, 345 (1986).

88. Evans, R. H. *et al.*, Br. J. Pharmacol., 91, 531 (1987).

89. Monaghan, D. T. and Cotman, C. W., Brain Res., 252, 91 (1982).

90. Foster, A. C. *et al.*, Nature, 289, 73 (1981).

91. Campochiaro, P. and Coyle, J. T., Proc. Nat. Acad. Sci. U.S.A., 75, 2025 (1978).

92. Olney, L. W., in "Kainic acid as a tool in Neurobiology", (McGeer, E. G.,Olney, J. W. and McGeer, P. L., eds.), Raven Press, 95 (1978).

93. Teitelbaum, J. S. *et al.*, N. Engl. J. Med., 322, 1781 (1990).

94. Foster, A. C. and Fagg, G.E., Brain Res Rev., 7, 103 (1984).

95. Cotman, C. W. and Iversen, L. L.(eds.) "Excitatory Amino Acids in the Brain: Focus on NMDA Receptors", (Special Issue). Trends in Neurosciences, 10, 363 (1987).

96. Forsythe I. D. and Clements, J. D., J. Physiol., 429, 1 (1990).

97. Ascher, P. and Nowak, L., J. Physiol. Lond., 399, 247 (1988).

98. McDermott, A. G. *et al.*, Nature 321, 519 (1986).

99. Akaike, N. et al., Neurosci Letts., 87, 75 (1988)

100. Lucas, D. R. and Newhouse, J. P., Arch. Ophthalmol., 58, 193 (1957).

101. McCaslin, P. P. *et al.*, Brain Res., 417, 380 (1987).

102. Crunelli, V. *et al.*, J. Physiol. (London), 341, 627 (1983).

103. Lehmann, A., Agressologie, 5, 311 (1964).

104. Sloviter, R. S., Brain Res. Bull., 10, 675 (1983).

105. Moersbaecher, J. M., Pharmacol. Biochem. Behav., 32, 1080 (1990). (abstr).

106. Jones, D. A. *et al.*, in "Medical-Surgical Nursing: A Conceptual Approach" , McGraw-Hill book company, 1158 (1978).

107. Nicholas, A. V. in "Convulsive Disorders", in Grinkers Neurology, 7th ed., 111, 714 (1976).

108. Pfeiffer, C. C. *et al.*, Electroencephalogr. Clin. Neurophysiol., 8, 307 (1956).

109. Balzer, H. *et al.*, Naunyn-Schmiedebergs. Arch. Exp. Pathol. Pharmakol., 239, 520 (1960).

110. Meldrum, B.S. *et al.*, Electroencephalogra. Clin. Neurophysiol., 31, 563 (1971).

111. Dingledine R. *et al.*, "Excitatory Amino Acid Receptors in Epilepsy" in Trends in Pharmacological Sciences: A Special Report (edited by Lodge, D. and Collingridge, C.), 49 (1991).

112. Aram, J. A. *et al.*, Br. J. Pharmacol.,248, 320 (1989).

113. Meldrum, B. S. and Croucher *et al.* Science, 216, 899 (1982).

114. Coutinho-Netto, J. *et al.*, Epilepsia, 22, 289 (1981).

115. Kleckner, N. W. and Dingledine, R., Mol. Pharmacol., 36, 430 (1989).

116. Birch, P. J. *et al.*, Eur. J. Pharmacol., 156, 177 (1988).

117. Shouldson, I. *et al.*, Neurology, 26, 61 (1976).

118. McGeer, P. L. *et al.*, Brain Res., 32, 425 (1971).

119. Beal, M. F. *et al.* Nature, 321, 168 (1986).

120. Brierley, J. B. *et al.*, Arch. Neurol. Psychiat. (Chicago), 29, 367 (1973).

121. Spielmeyer, W., Z. ges. Neurol. Psych., 109, 501 (1927).

122. Scholz, W., Monogr. ges. Neurol. Psych., 75, (Springer-Verlag) (1951).

123. Scholz, W., Epilepsia, 1, 36 (1959).

124. Corsellis, J. A. N. *et al.*, in "Greenfields Neuropathology" (Blackwood, W. , ed.) Arnold, London.

125. Park, C. K. *et al.*, J. Cereb. Blood Flow Metab., 8, 757 (1988).

126. Bullock, R. *et al.*, J. Cereb. Blood Flow Metab., 10, 668 (1990).

127. Bowman, W.C. and Rand, M.J., in "Textbook of Pharmacology" (2nd. edn.) Blackwell Scientific Publications (Chapter 5)

128. Moroni, F. *et al.*, Neurosci. Lett., 47, 51 (1984).

129. Greenamyre, J.T. and Young, A.B., Neurobiol. Ag., 10, 593 (1989)

130. Felmus, M. *et al.*, Neurology, 26, 167 (1976).

131. Spencer, P. S. *et al.*, Lancet, ii, 1066 (1986).

132. Spencer, P. S. *et al.*, Science, 237, 517 (1987).

133. Weiss, J. H., and Choi, D. W., Science, 241, 973 (1988).

134. Ross, S. M. *et al.*, Brain Res., 425, 120 (1987).

135. Weiss. J. H. *et al.*, Brain Res., 497, 64 (1989).

136. Rao, S. L. N. *et al.*, Biochemistry, 14, 5218 (1964)

137. Bridges, R.J. *et al.*, J. Neurosci., 9, 2073 (1989)

138. McNamara, J. O. *et al.*, Neuropharmacology, 27, 563 (1988).

139. Sato, K. *et al.*, Brain Res., 63, 12 (1988).

140. Tricklebank, M. D. *et al.*, Eur. J. Pharmacol., 167, 127 (1989).

141. Peeters, B. W. *et al.*,Epilepsy Res.,3, 178 (1989).

142. Chapman, A. G. and Meldrum, B. S., Eur. J. Pharmamacol., 166, 201 (1989).

143. Slater, N. T. *et al.*, Neurosci. Lett., 60, 25 (1985).

144. Meldrum, B. S., "Pharmacological Approaches to the Treatment of Epilepsy "in " Current problems in epilepsy; New Anticonvulsant Drugs" (Meldrum, B. S. and Porter, R. J., eds.), 4, 22 (1986).

145. Moriyoshi, K. *et al.*, Nature, 354, 31 (1991)

146. Nakanishi, S., Science, 258, 597 (1992)

147. Meguro, H. *et al.*, Nature, 357, 36 (1992)

148. Monyer, H. *et al.*, Science, 256, 1217 (1992)

149. Mishina, M. *et al.*, Nature, 358, 673 (1992)

150. Seeberg, P.H. *et al.*, Trends in Pharmacological Sciences, 131, 291 (1992)

151. Hollman, M., O'Shea-Greenfield, A., Rogers, S.W., Heinemann, S., Nature, 342, 643 (1989)

152. Sakimura, K. *et al.*, Neuron, 8, 267 (1992)

153. Keinanen, K. *et al.*, Science, 249, 556 (1990)

154. Herb, A. *et al.*, Neuron, 8, 775 (1992)

155. Egebjerg, J., *et al.*, Nature, 351, 745 (1991)

156. Wada, K. *et al.*, Nature, 342, 684 (1989)

157. Gregor, P. *et al.*, Nature, 342, 689 (1989)

158. Bettler, B. *et al.*, Neuron, 8, 257 (1992)

159. Werner, P. *et al.*, Nature, 351, 742 (1991)

160. Masu, M. *et al.*, Nature, 349, 760 (1991)

161. Tanabe, Y. *et al.*, Neuron, 8, 169 (1992)

162. Abe, T. *et al.*, J. Biol. Chem., 267, 13361 (1992)

163. Houamed, K.M. *et al.*, Science, 252, 1318 (1991)

164. Bettler, B. *et al.*, Neuron, 5, 583 (1990)

165. Shigemoto, R. *et al.*, J. Comp.Neurol.,322, 121 (1992)

166. Recasens, M. *et al.* in "Current Aspects of the Neurosciences" (Osborne, N.N., ed.) Vol 3, pp 103-175, Macmillan, New York 1991

167. Nicoletti, F. *et al.*, J. Neurochem., 54, 771 (1990)

168. Nakajima, Y. *et al.*, J. Biol. Chem., 267, 2437 (1992)

169. Nakanishi, S. *et al.*, Recent Prog. Hormone Res., 46, 59 (1990)

170. Bycroft, B. W. *et al.*, J. Chem. Soc. Chem Commun., 1156 (1984).

171. Beyerman, H. C. *et al.*, Recueil, 92, 481 (1973).

172. Mattingly, P.G. and Miller, M.J., J. Org. Chem., 45, 410 (1980).

173. Greene, T.W. and Wuts, P.G.M., "Protective Groups in Organic Synthesis" (2nd. edn.) (Wiley-Interscience) Chapter 7 and references therein.

174. Beyerman, H.C., *et al.*, Recueil, 92, 481 (1973)

175. Jones, J. H. and Witty, M. J., J. Chem. Soc. Chem. Comm., 281 (1977).

176. Arnold L. D. *et al.*, J. Am. Chem. Soc., 107, 7105 (1985).

177. Baldwin, J.E. *et al.*, J. Chem. Soc. Chem. Comm., 256 (1985)

178. Vederas, J. C. *et al.*, J. Am. Chem. Soc., 107, 7105 (1985).

179. Robertson, A.D., personal communication.

180. Paquet, A., Can. J. Chem., 60, 976-980 1982.

181. Michinori, W, *et al.*, Synthesis, 266 (1980).

182. Ohfune, Y. *et al.*, Tet. Letts., 29, 2983 (1988)

183. Srivastava, P. C. *et al.*, J. Antibiot., 25, 151 (1972).

184. Vederas, J. C. *et al* J. Am. Chem. Soc., 110. 2237 (1988).

185. Vogel's Textbook of Practical Organic Chemistry (fourth edition), pages 291-292 (1981).

186. Hassall, C.H. *et al.*, J. Chem. Soc. C.,1495 (1968)

187. Cuatrecasas, P. and Hollenberg, M. D., Adv. Protein Chem., 30, 251 (1976).

188. Kahn, C. R., J. Cell Biol., 70, 261 (1976).

189. Snyder, S. H., Biochem. Pharmacol., 24, 1371 (1978).

190. Snyder, S. H. and Bennett, J. P. Jr., Annal. Rev. Physiol., 38, 153 (1976).

191. Changeux, J-P. *et al.*, C.R. Acad. Sci. Paris, 270, 2864 (1970).

192. Roberts, P. J. Nature (London), 252, 399 (1974).

193. Michaelis, E. K., Biochem. Biophys. Res. Commun., 65, 1004 (1975).

194. Foster, A. C. and Fagg, G. E., Brain Res. Rev., 7, 103 (1984).

195. London E. and Coyle, J. T., Mol. Pharmacol., 15, 492 (1979).

196. Roberts, P. J. and Sharif, A. N., in "Glutamate as a Neurotransmitter." (Di Chiara, G. and Gersa, G., eds), Raven Press, New York (1980).

197. Butcher, S. P., *et al.*, Brit. J. Pharmacol., 80, 355 (1983).

198. Jones *et al.*, J. Physiol., 340, 45 (1983).

199. Krogsgaard-Larsen *et al.*, Nature, 284, 64 (1980).

200. Bycroft, B. W. *et al.*, J.Comput.-Aided Mol. Des. ,2, 321 (1989).

201. Bycroft, B.W. *et al.*, Pesticide Science, 33, 244 (1991).

202. Schnabel, E., Justus Liebig's Ann. Chem., 702, 188 (1967)

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