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THE SYNTHESIS AND PHARMACOLOGY OF SOME NOVEL EXCITATORY AMINO ACID ANALOGUES

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To My Family,

and Sue

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CONTENTS

(Contd.)

Statement of Advanced Studies Undertaken

Attendance of meetings of the Amino Acid Discussion Group and at the University of London Intercollegiate Neuropharmacology Course. Also, lectures at the Royal Society of Chemistry and colleges of the University of London. Additionally, participation in Research Seminars at the City of London Polytechnic, and presentation "Some Aspects of Kainic Acid Analogue Synthesis" at Wellcome Research, Eden Park.

Publications

Antiepileptic action of excitatory amino acid antagonists in the photosensitive baboon, Papio papio.
Meldrum, B.S., Croucher, M.J., Badman, G.T. and Collins, J.F.
Neuroscience Letters 39 (1983) 101-104.

Kainic acid derivatives with anticonvulsant activity.
Collins, J.F., Dixon, A.J., Badman, G.T., De Sarro, G., Chapman, A.G., Hart, G.P. and Meldrum, B.S.
Neuroscience Letters 51 (1984) 371-376.

Novel kainic acid analogues; effects on cyclic GMP content of adult rat cerebellar slices.
Anand, H., Roberts, P.J., Badman, G.T., Dixon, A.J. and Collins, J.F
Biochemical Pharmacology 35(3) (1986) 409-415.

Notes on Stereochemical Conventions

During the main text of this work, in order to facilitate comparison of data with published work in the biological and pharmacological press, the D- and L (Dextro- and Laevo-rotatory) stereochemical convention has been used in place of the IUPAC R,S (Rectus, Sinister) system. Thus, L-glutamate corresponds to (2S) glutamic acid, and D-glutamate to (2R) glutamic acid. The strictly more correct R,S nomenclature is followed in Chapter Five (Synthetic Details).

The Synthesis and Pharmacology of Some Novel Excitatory Amino Acid Analogues

G.T.Badman 1986

Certain amino acids, notably L-glutamate and L-aspartate, are believed to fulfil a role as excitatory neurotransmitters in the mammalian Central Nervous System (CNS). Investigation has revealed the presence of three, and possibly a fourth, type of receptor for such neurotransmitters. These receptors have been named after the most potent and selective agonist of each; thus they are usually referred to as N-methyl-D-aspartate (A1), quisqualate (A2), kainate (A3) and 2-amino, 4-phosphono butyric acid (A4); the designations in brackets being that of the nomenclature of Fagg and Foster[1].

Various further investigations have implicated these excitatory amino acid receptors in a number of serious pathological disorders, especially epilepsy, Huntington's Chorea, Alzheimer's disease (senile dementia) and the brain tissue damage occurring following Ischaemia. Potent and selective antagonists of such excitatory neurotransmitters are therefore much to be desired from a therapeutic standpoint, as well as the additional data that could be derived regarding the pharmacology and biochemistry of the receptors by using such antagonists in *in vitro* test systems[2].

The naturally-occurring material kainic acid, isolated from the Japanese seaweed *Digenea simplex* is a potent and selective agonist at one of the excitatory receptor sites (A3); the iontophoretic application of kainic acid to regions of mammalian brain tissue which possess high densities of this A3 receptor type causes tissue destruction in a pattern which corresponds very closely to that observed in the neuropathological and neurobiochemical onset of Huntington's Chorea. While some progress has been made towards a potent and selective A1 receptor antagonist, there are, as yet, no equivalent A3 antagonists. Therefore, the aim of this project has been to synthesise a selective and efficient A3 receptor antagonist, based on the parent kainic acid molecule, which could eventually provide much additional physiological and biochemical information regarding both the A3 receptors *per se* and the pathology of the neurodegenerative diseases.

Accordingly, a number of kainate-based molecules have been synthesised. Particular attention has been paid to analogues where the C-3 side chain has been modified by conversion of the carboxylic acid to a different functional group. These synthetic procedures were facilitated by the discovery and subsequent development of a selective and high-yielding mono-esterification process which converts *t*-BOC-kainate to *t*-BOC kainate C-2 monomethyl ester in around 70% yield, without concurrent formation of any of the C-3 monoester.

Additionally, considerable efforts have been devoted towards the derivatisation of the C-4 side chain, especially to increase the chain length while still retaining a degree of unsaturation. There is very considerable scope for further development of this particular avenue of research.

The compounds that have been synthesised have undergone testing for physiological and biochemical activity. Quantities of the potent and selective A1 receptor antagonists 2-APP and 2-APH have also been produced according to literature methods, for testing and comparative purposes.

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1. Foster, A.C. and Fagg, G.E. Brain Res. Rev. 7 (;984) 103-164.
2. Meldrum, B.S. Clinical Science 68 (1985) 113-122.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

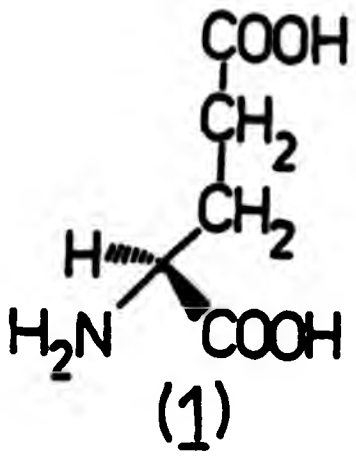
1.1.1 Excitatory Acidic Amino Acids: Neurotransmission

In 1959, Curtis *et al*[1,(also 2&3)] first demonstrated an excitatory effect on individual Central Nervous System (CNS) neurones by L-glutamic acid (L-glu), L-aspartic acid (L-asp) and various other similar, molecules. Since then, considerable numbers of other acidic amino acids have been shown to possess similar activity. Specifically, a neurotransmitter role was proposed for these compounds, and subsequent experimentation and investigation confirmed the initial hypothesis (for reviews, see[4,5,6,7,8]).

In order for a molecule to be classed as a neurotransmitter, it has to fulfil a number of basic criteria. These are generally defined as follows[9,10]:

1. *Synthesis and Storage*: The substance must be synthesised and/or stored pre-synaptically.
2. *Release*: The substance must be released from storage upon pre-synaptic stimulation, and must be observed in the extracellular fluid (ECF) in the vicinity of the synapse.
3. *Identity of Action:* The application of the putative neuro-transmitter post-synaptically must mimic the effect seen when standard pre-synaptic stimulation is applied.
4. *Disposal*: There must be a suitable mechanism for the removal of the substance from the post-synaptic region.
5. *Pharmacological Effect:* Specific antagonists must be able to block both the natural transmission and the effect of externally-applied transmitter.

The above criteria may best be illustrated by reference to a putative excitatory acidic amino acid transmitter, L-glutamate (1)[8,11], although it should be noted that the same requirements apply to all other neurotransmitters, excitatory or inhibitory.



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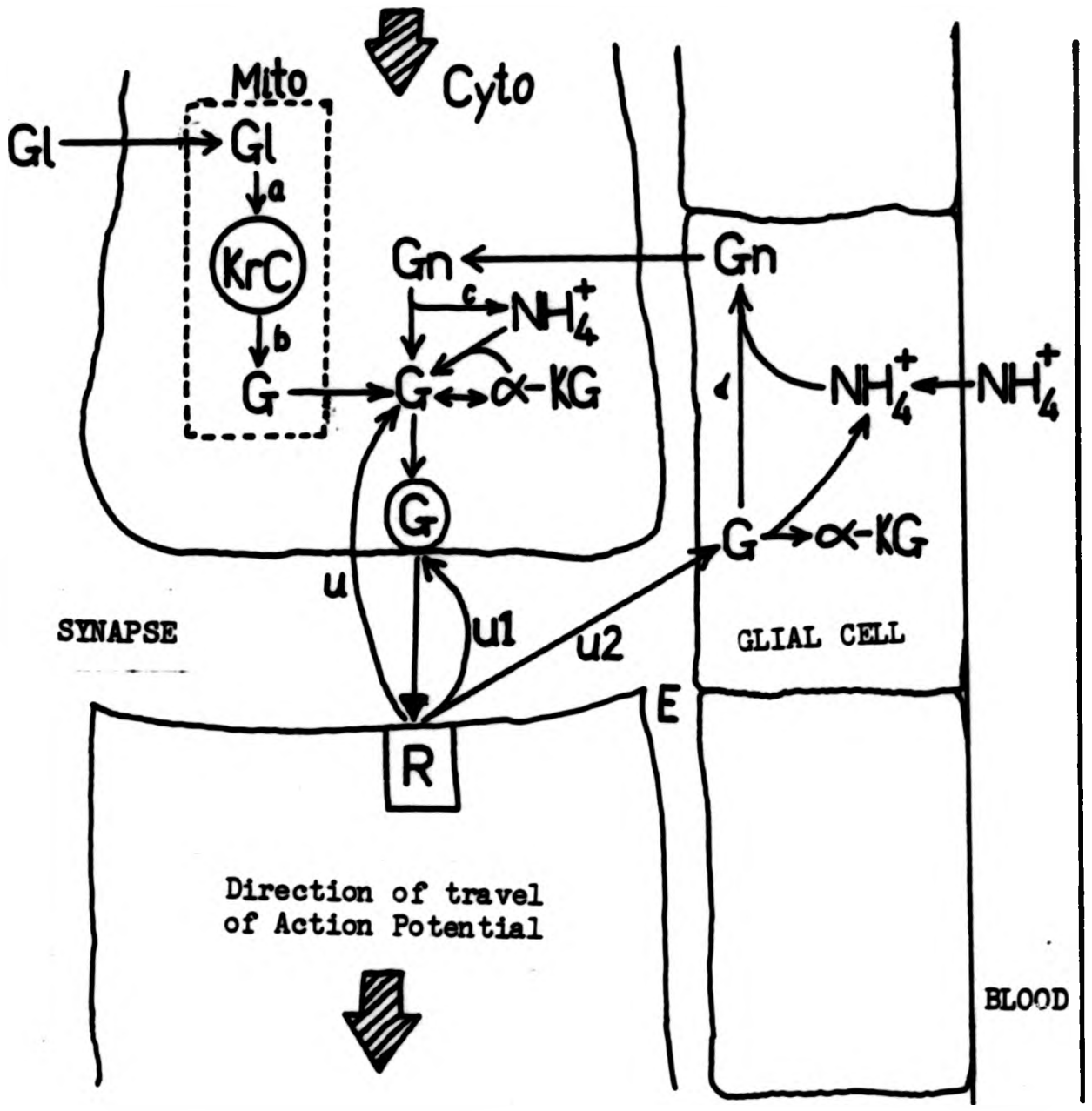
1.1.2 Glutamic Acid as a Neurotransmitter

Hayashi[12] first reported the excitatory effect of glutamic acid on the cerebral cortex in 1952. The work of Curtis *et al*[1] demonstrated the effect at the neuronal level, not just of L-glutamate, but of a number of other related compounds as well. This discovery prompted the postulation of a neurotransmitter rôle for glutamate; however, the investigation of the possible involvement of the molecule in neurotransmission is complicated by the diversity of other roles which this amino acid plays, being distributed throughout the body and incorporated into various metabolic and catabolic pathways.

Considerable work was therefore required to provide the necessary evidence of the proposed neurotransmitter activity, and the findings of a number of groups researching the problem have been combined to produce a generally-accepted model of the proposed biochemistry of glutamate as a neurotransmitter at CNS synapses (Fig. 1).

The model shows all the necessary biochemical requirements of neurotransmission.

The glutamate is synthesised either from glutamine (2) by



KEY:

G	Glutamate	G	Pre-synaptic Vesicle
Gn	Glutamine	R	Post-synaptic Receptor
Gl	Glucose	Mito	Mitochondrial Metabolism
α-KG	α-Keto Glutarate	Cyto	Cytoplasmic Metabolism
a	Glycolysis	KrC	Kreb's Cycle
b	GABA Shunt	u	(Re)uptake to Cytoplasm
c	Glutaminase	u1	(Re)uptake to Vesicle
d	Glutamine Synthetase	u2	Uptake to Glial Cell
E	Extra-Cellular Fluid		

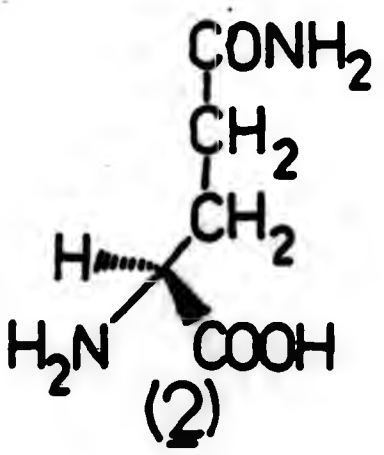
Fig.1 Some Metabolic Aspects of Glutamate as a Neurotransmitter.

cytoplasmic enzymes (70%), or by mitochondrial glycolosis of glucose (3) *via* the Krebs (TCA) Cycle (30%)[13].

When the afferent nerve impulse (Action Potential, AP) arrives at the pre-synaptic membrane a depolarisation results, leading to an influx of Ca^{2+} ions[14]. This influx of calcium ions causes the release of glutamate both from the synaptic vesicles and the cytoplasmic pool; the latter probably being the more important source[15,16].

The glutamate then extant in the ECF at the synapse proceeds to combine with the post-synaptic receptor and propagates the AP along the efferent[17].

The disposal processes then remove the glutamate either by re-uptake into the pre-synaptic vesicles or cytoplasmic pool, or, preferentially, by uptake into glial cells where the predominant process is the conversion of glutamate into glutamine[18,19,20]. Thus high local levels of the excitant are reduced. Indeed, the neuronal and glial cell pathways appear complementary; the neuronal nett production of glutamate from glutamine[21] is balanced by the glial cells' conversion of glutamate to glutamine[18].



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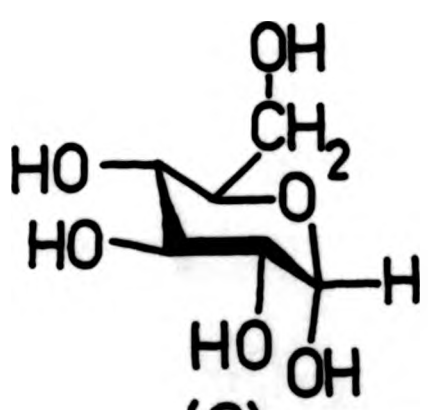

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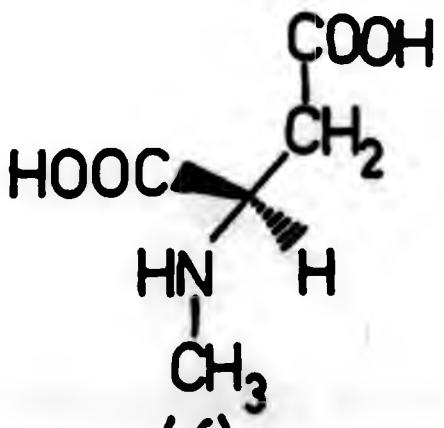
1.2 Excitatory Amino Acid Receptors

1.2.1 General Classification

All neurotransmitters must interact with a post-synaptic receptor, combination with which leads to the physiological effects which propagate the nerve impulse (Action Potential, AP) along the efferent neurone[9,10].

Investigation into the receptors which are activated by L-glutamate soon revealed a heterogeneous population of receptor sub-types. Electrophysiological experiments by McLennan[22] and by Watkins[8] and their colleagues, studying a large number of acidic amino acid analogues, led to the proposal that there were three distinct excitatory receptor types, initially known after the three most potent and selective receptor agonists, being N-methyl-D-aspartate (NMDA) (4), quisqualic acid (QA) (5), and kainic acid (KA) (6), respectively. More recently, Fagg and Foster[23] have proposed a receptor nomenclature for the three varieties noted above, and also for a fourth sub-type of L-glutamate receptors, the L-2-amino-4-phosphono-butanoic acid preferring site, (APB) (7), the four types being classified as A1 (NMDA), A2 (QA), A3 (KA) and A4 (APB) respectively.

These four putative excitatory amino acid receptor sub-types all differ in a number of respects, the characteristics of which are described hereafter.



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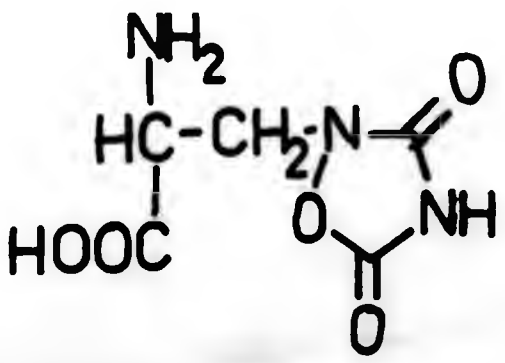

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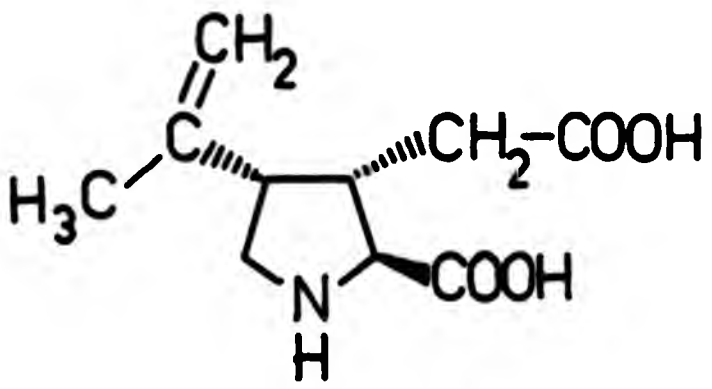

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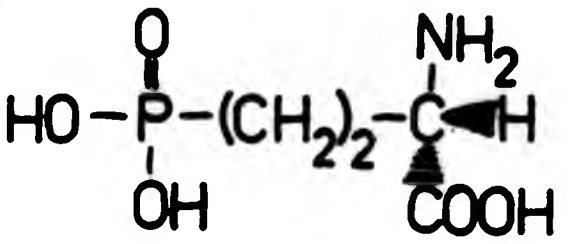

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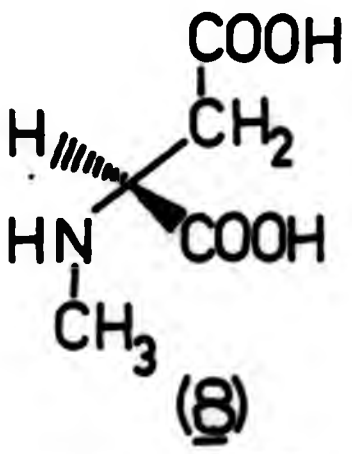
1.2.2 The A1 Receptor

The A1 receptor is currently the best characterised of all the excitatory amino acid receptor sites, largely because potent and selective antagonists as well as agonists for the site are known. Pharmacologically, these receptors are defined by their high selectivity for NMDA (4), with decreasing agonist potency also shown by NMLA (8), ibotenic acid (9), L-homocysteate (10), D-glutamate (11), *cis*-1-amino-1,3-dicarboxycyclopentane (*cis*-ADCP) (12) and *trans*-2,3-piperidine dicarboxylic acid (*trans*-2,3-PDA) (13)[8,24,25].



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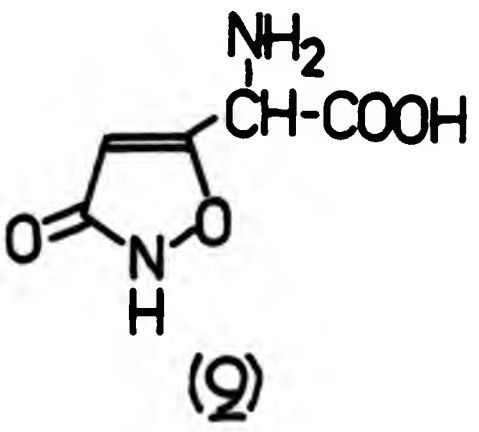

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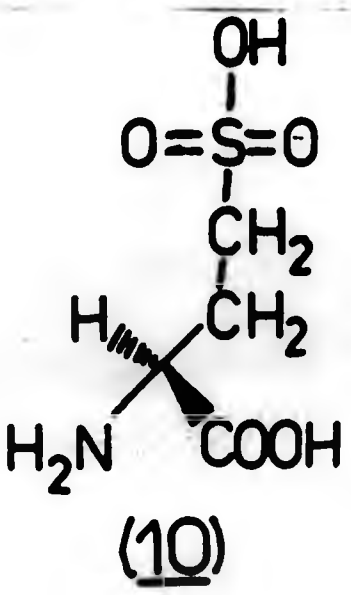

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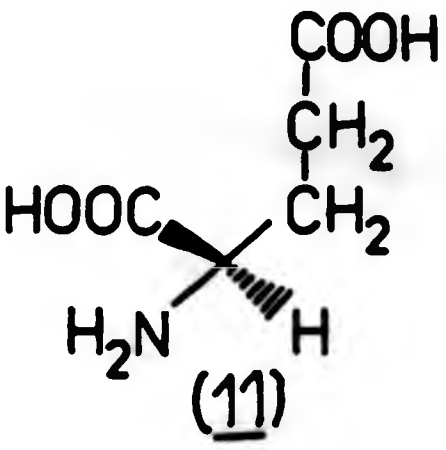

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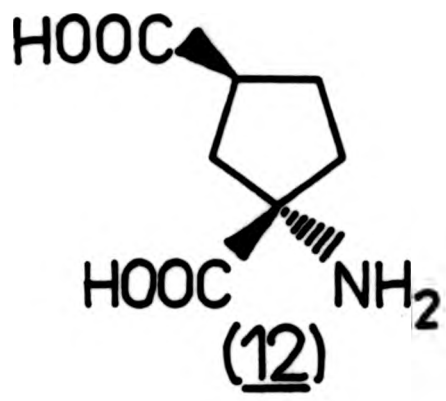

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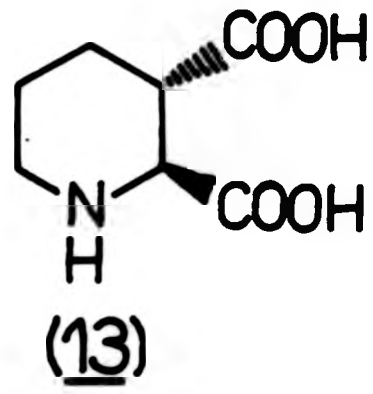
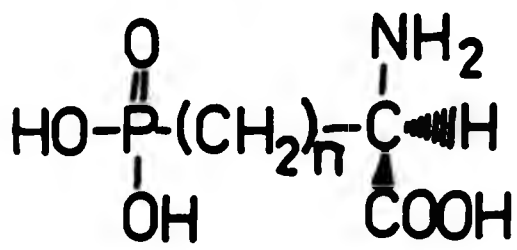


(13)

The most potent and selective antagonists at the A1 receptor site are the D-isomers of some of the α-amino-ω-phosphono-carboxylic acids, e.g. 2-amino-5-phosphono-pentanoic acid (APP) (14), 2-amino-7-phosphono-heptanoic acid (APH) (15); less potent antagonists include the D-isomers of long-chain (C>5) α-amino-dicarboxylic acids, e.g. D-α-amino-adipate (16), D-α-aminosuberate (17); long-chain diamino-dicarboxylic acids, e.g. D,D-α,ε-diaminopimelate (18); HA-966 (1-hydroxy-3-amino-2-pyrrolidone) (19); and Mg^{2+} ion[8,24,25].



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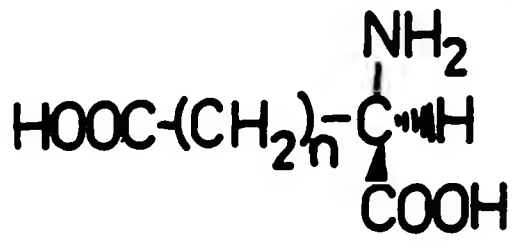
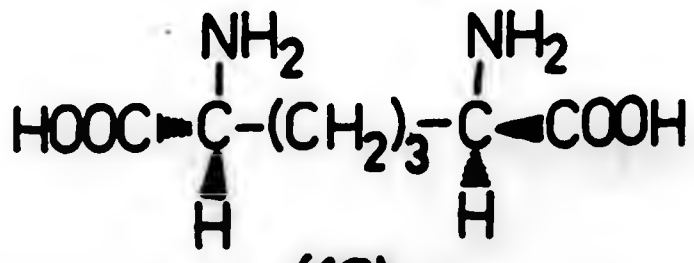
(15) n=5



(16) n=3
(17) n=5



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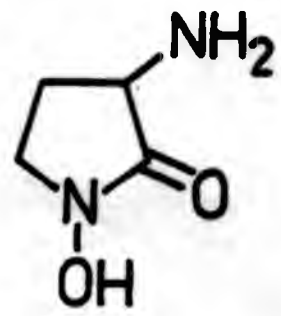


(19)

While Mg^{2+} antagonises L-glutamate binding at this site, binding appears to be independent of Cl^-, Na^+ and Ca^{2+} ions[26,27].

A complete survey of the anatomical distribution of A1 receptor sites is difficult to present, since most workers have concentrated their investigations on one or two areas of the brain, *e.g.* hippocampus, cerebellum. Nevertheless, data suggests populations of A1 receptors in ventral root synapses of the spinal cord[28,29], in cerebral cortex[30], cuneate nucleus[30], caudate nucleus[31], lateral geniculate body[32], cochlear nucleus[33,34], olfactory cortex[35], cerebellum[36] and hippocampus[37]. In this latter region, using thin slices of rat brain, Monaghan *et al* have demonstrated that [^{3}H]-L-glutamate binding sites where the radio-ligand is readily displaced by low concentrations of NMDA, exist predominantly in the stratum oriens and stratum radiatum of the CA1 field and the inner portions of the dentate gyrus molecular layer; lower levels were observed in the stratum oriens and stratum radiatum of CA3, the outer molecular layer of the dentate gyrus and the stratum lucidum and the dentate gyrus hilus[26].

The identity of these sites with the pharmacologically-defined A1 receptor is further suggested by the fact that the potent NMDA antagonist APP has an affinity constant (K_i) at these sites of 2.3 ± 0.2 μM (n=2), which corresponds well with its physiological potency (apparent K_d = 1.4 μM)[29]. Compounds which have been shown[8] to interact with the NMDA-sensitive receptor, e.g. ibotenate, D-α-aminoadipate and D- and L-aspartate, are all potent displacers of the NMDA-sensitive [^{3}H]-L-glutamate binding in stratum radiatum (50% inhibitory concentration, or IC_{50}, less than 100 μM (n=4))[26].

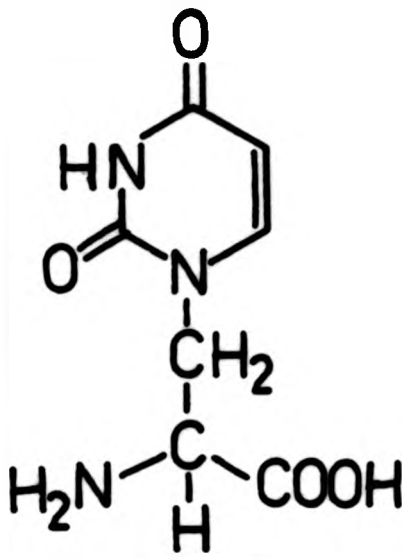
The physiological role of the A1 receptor at acidic amino acid-using synapses is thought to include participation in the polysynaptic dorsal root-evoked ventral root responses[28,29,38,39]. A post-synaptic

location for the receptor site is suggested by the evidence that homogenate sub-cellular fractions essentially free of pre- and extra-synaptic membranes, but enriched in post-synaptic densities, contain A1 (and A2) receptors[27]. Post-synaptic densities are prominent post-junctional features of excitatory (Type I) synapses[40]; they are thought to provide a matrix or structural framework, wherein the transmitter receptors and other molecules involved in the post-synaptic neuronal response are emplaced.

Having demonstrated an involvement of A1 receptors in spinal motoneurone activation, it has also been shown that A1 receptor antagonists do not affect many of the synaptically-evoked excitatory responses in other brain pathways, thus creating some doubt as to the possible participation of A1 receptors in these systems[41,42,43]. However, A1 receptors have been identified by autoradiography throughout the brain, and have been implicated in a number of pathological conditions specific to that organ, namely, Long Term Potentiation (LTP)[44], Epilepsy[45,46] and various Neurodegenerative disorders[47,48,49,50,51]. Nowak *et al* have found that activation of the A1 receptor by glutamate, aspartate or NMDA opens cation channels, which can be blocked by Mg^{2+} in a voltage-dependent manner; this voltage-dependent "switch" may be controlling the way in which NMDA-sensitive receptors participate (or not) in excitatory pathways[52].

1.2.3 The A2 Receptor

The A2, or quisqualate-preferring receptor, is activated selectively by quisqualic acid (5); other agonists include L-glutamate (1), Willardiine (1-(2-amino-2-carboxyethyl)-uracil (20), α-amino-3-hydroxy-5-methyl-4-isoxysolepropionate (AMPA) (21) and 4-bromo, homo-ibotenate (22)[24]



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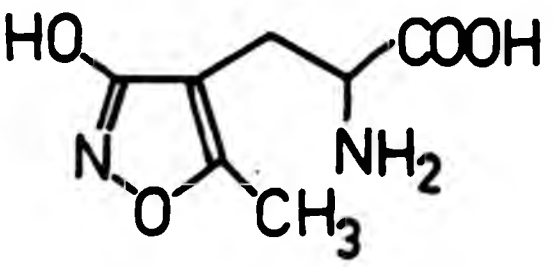

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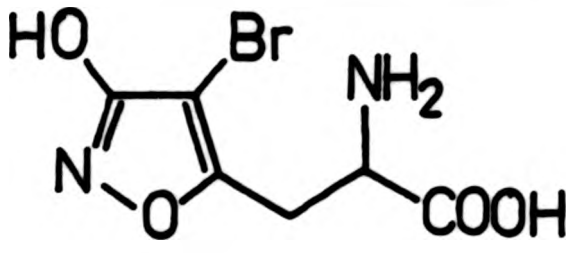
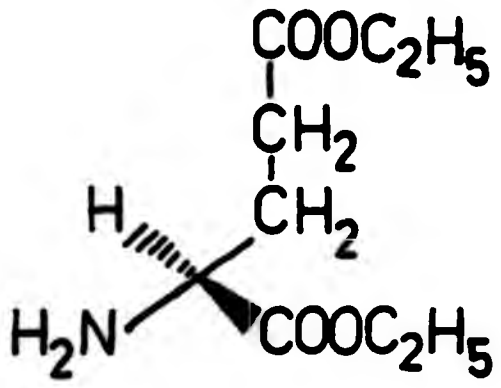


(22)

Glutamate diethylester (GDEE) (23) has some antagonist effect against this site[22], and both glutamyl-taurine (Glu-tau) (24) and glutamyl-aminomethansulphonate (GAMS) (25) show antagonist activity at both the A2 and A3 receptor[53].



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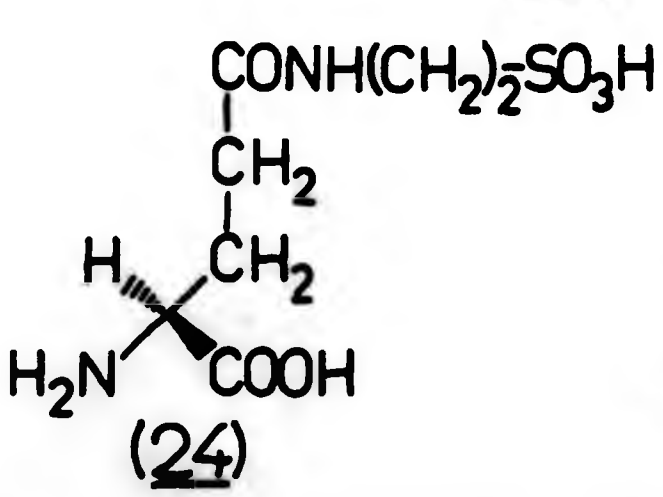

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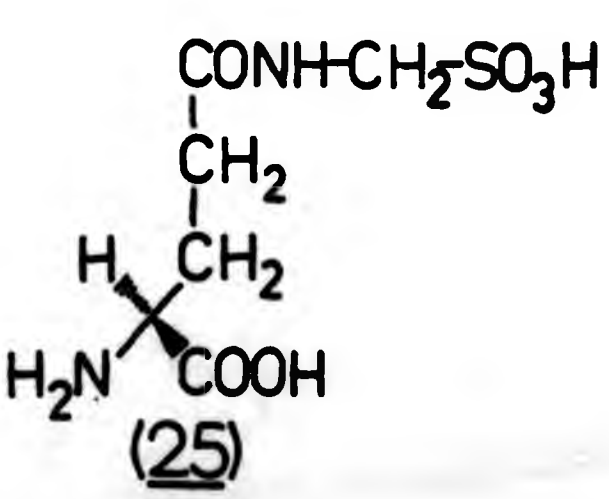

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The great problem with investigative work on the A2 (and indeed the A3) receptor is that really potent and selective antagonists have not yet been discovered; hence most of the pharmacological work on these receptors has been based on "subtractive" studies where A1 receptor activity has been blanked out by the use of the available (and potent) A1 antagonists, and the residual activity assigned as A2/A3 etc., on the basis of local distribution and agonist binding studies.

The A2 receptor seems to be independent of definite anion or cation requirements[23], but activity is enhanced by both Ca^{2+} and Cl^{-} ions[54]. This enhancement due to Ca^{2+} cations is in direct contrast to the observed inhibitory effect of same at the A3 receptor[55] and therefore this forms one way of differentiating between the two types.

Anatomical distribution of the A2 site has not been comprehensively studied, most investigations centring on specific regions within the brain; however, various groups have reported quisqualate-preferring receptors in the cerebral cortex[56], hippocampus[57,58] (mainly in the stratum radiatum C1 area)[26], cerebellum (molecular layer)[54] and the caudate nucleus[54]. Interestingly, it has been suggested that the relative proportions of A2 and A1 receptors are complementary[54]. That is, where one type exists as a high-density population of the total L-glutamate binding sites within a given area, the other is present in only small quantities, between them accounting for almost all the L-glutamate binding not occurring at A4 receptors; for example, in hippocampal slice preparations, the stratum radiatum (C1) area contains L-glutamate binding sites which are practically insensitive to both Kainate and APB; A1 receptors account for about 75-80% of the total, the remainder being A2. Conversely, in the molecular layer of the cerebellum, the A2 sites are in the majority, comprising somewhere in excess of 60% of the total L-glutamate binding sites; the remainder are A1. Other areas of the brain are believed to have approximately

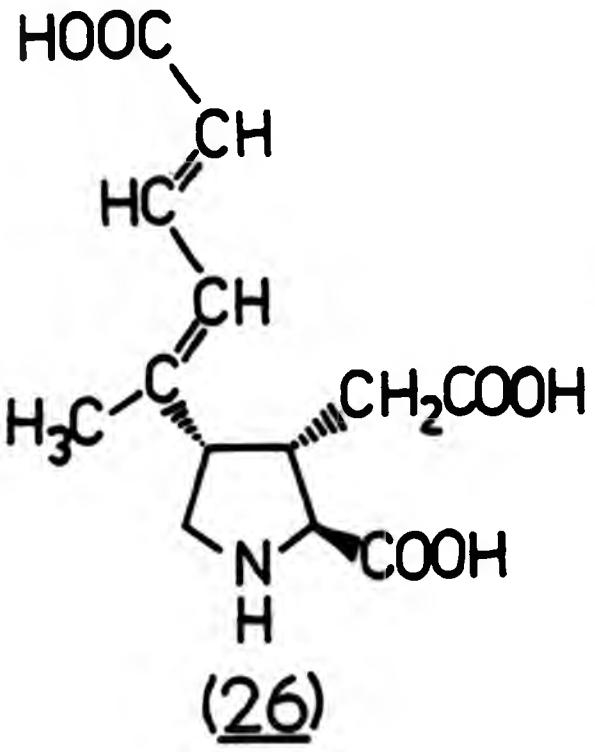
equal proportions of A1 and A2 receptors.

Little published speculation as to the pathological significance of this proposed complementary relationship has appeared, but it would be interesting to know whether the two receptor types represent parallel excitatory systems, perhaps operating at different times or under different conditions; the Mg^{2+}-dependent switch mechanism indicated for the A1 receptor might have some relevance to this.

Physiologically, little is known about the nature of the A2 receptor except that it is present in post-synaptic densities (along with the A1 receptor)[27] and therefore a post-synaptic role in amino acid excitatory transmission would seem probable. Autoradiographic data suggests that the synapses may be distinct from the A1 type synapses[54]. The quisqualate-preferring receptor seems to participate less in excito-toxic effects than either the A1 or the A3 types; other than that, there is little or no information available on possible involvement in physiological or pathological conditions.

1.2.4 The A3 Receptor

Kainate-preferring (A3) receptors are insensitive to A1 antagonists or to GDEE (23); therefore it was proposed that they constitute a third excitatory amino acid receptor sub-type[22]. The A3 receptor is potently activated by kainic acid (6), domoic acid (26) and 5-bromo-Willardiine (27), with moderate activity also displayed by quisqualate (5), L-glutamate (1) and AMPA (21). L-aspartate (28), Willardiine (20) and NMDA (4) are all virtually inactive[24]. There are no really potent and selective antagonists at this receptor and the aim of this project was to attempt to remedy this deficiency. GAMS (25) and Glu-tau (24) both show some antagonist activity here and at the A2 receptor[53].



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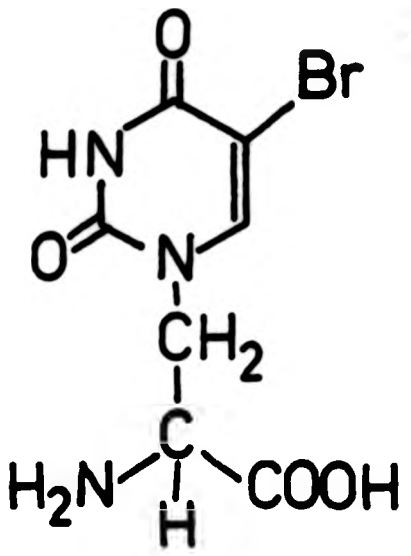

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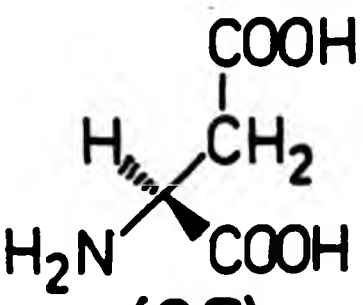

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Ca^{2+} also acts as an antagonist[55], millimolar concentrations in buffer solutions inhibiting L-glutamate binding to A3 receptors in stratum lucidum of hippocampal preparations; the sites previously sensitive to kainic acid in low nanomolar concentrations being no longer definable. Conversely, Cl^- ions caused an enhancement of binding to A3 receptors[54].

Research into the anatomical localisation of A3 receptor sites indicates that the kainate-preferring receptors are very widely distributed throughout the CNS. Investigators have made use of what appears to be a relatively pure population of A3 receptors in preparations of dorsal root fibres of immature rats[24], in addition, autoradiographic techniques indicate receptor populations in the cerebral cortex (a trilaminar pattern of distribution, with greater densities in the superficial and deep layers as opposed to the middle layers)[59], in the

striatum and nucleus accumbens of the basal ganglia (both having high densities, whereas the globus pallidus shows very few binding sites)[59]; the septum (lateral portion has more sites than the medial aspect)[59]; in the hippocampus, (where the kainate receptors seem to be localised almost entirely in the stratum lucidum of areas CA3 and CA4, corresponding to the Mossy fibre synaptic termination zone[60]. Lesser activity is also seen in the commissural/associational fibre zone of the dentate gyrus; other hippocampal areas have a low level of binding activity). Investigators differ as to the localisation of sites in rat brain cerebellum, with Henke *et al*[61] observing predominant labelling in the molecular layer, whereas other groups find a high density in the granular layer and less in the molecular layer[59,62]. Low levels of binding activity are also reported for the thalamus, hypothalamus, mid-brain and hind-brain areas[55,63,64,65].

Whereas A1 and A2 receptors have been demonstrated to be present in the post-synaptic densities of rat brain synaptic plasma membranes (SPM), thus indicating a post-synaptic role for the NMDA and QA-preferring receptors, the A3 receptor has not been identified in a similar position, although they have been shown to be localised in Synaptic Junction (SJ) preparations[60]; this may suggest that this sub-type of receptor is not post-synaptic, perhaps functioning pre- or extra-synaptically instead. Alternatively, the A3 receptor may be bound to the PSDs more loosely than the A1 and A2 sub-types and hence become detached during the preparation of enriched fractions thereof. Lesioning studies have also been unable to determine any further specific information as to a pre- or post-synaptic localisation[59,66].

The nature of the A3 receptor therefore requires much further investigation before a complete account of its physiological significance can be determined.

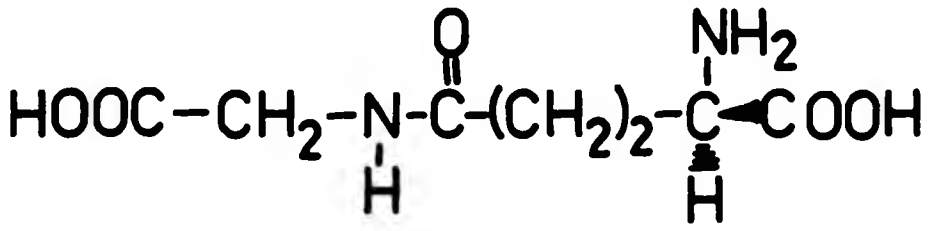
1.2.5 The A4 Receptor

Although tentatively described as the APB-preferring receptor, it might be more accurate to describe this receptor as APB-sensitive, since it is not known whether APB acts as an agonist or an antagonist at this site. It was discovered somewhat later[67] than the three other types because it is not stable to freeze-thaw cycles[68] which are commonly used in the formulation of brain preparations for study. The site is completely dependent on the presence of Cl^- and Ca^{2+} ions[69]. L-APB (7) is the most potent inhibitor of L-glutamate binding at the putative A4 receptor[29] while L-aspartate (28), L-glutamate (1), quisqualate (5) and L-homocysteate (10) are all fairly good displacers. The L-isomers of these compounds are all more effective than the corresponding D-forms. NMDA (4) and kainate (6) are almost inactive at this site[69,70]. D,L-α-aminoadipate (16) and D,L-α-aminosuberate (17) are of similar potency as displacers to D,L-APB. The phosphonate derivatives APP (14) and APH (15) are rather less active, while γ-DGG (29), *cis*-2,3-PDA (30) and HA-966 (19) are almost completely inactive.

Low concentrations of Na^+ ions inhibit binding[23].

Anatomical distribution data is incomplete, but suggests that there are populations of A4 receptors highest in brainstem and thalamus/hypothalamus, with decreasing levels in hippocampus and midbrain, and lower levels in the spinal cord, cortex, striatum and cerebellum[35,69,70,71].

The A4 receptor sites have been identified as being present in synaptic membranes, but are thought to be absent from post-synaptic densities and synaptic junction fractions[27]. It has been suggested that the A4 site has characteristics similar to those of the receptors which mediate the antagonistic effects of L-APB at some CNS excitatory synapses, perhaps being a modulatory receptor rather than a true excitatory type in the sense of the A1, A2 and possibly A3 sites[23].



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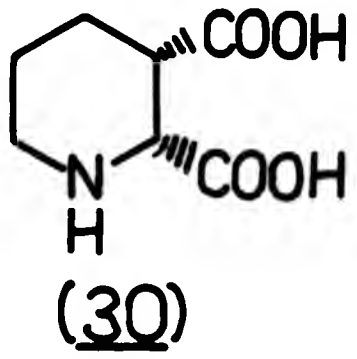

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Table 1 summarises the data on the different receptor types.

Table 1. Summary of Excitatory Acidic Amino Acid Receptor Sub-types.

Receptor Sub-type	A1	A2	A3	A4
Trivial Name	N-methyl-D-aspartate (NMDA)	Quisqualic acid (QA)	Kainic acid (KA)	L-4-phosphono-2-amino butanoic acid (L-APB)
Ionic Requirements	Independent of Cl^-, Ca^{++}, Na^+. Inhibited by Mg^{++}.	Enhanced by Ca^{++}, Cl^-.	Enhanced by Cl^-. Inhibited by Ca^{++}.	Requires Cl^-. Enhanced by Ca^{++}. Inhibited by Na^+.
Freeze/Thaw Labile?	No	No	No	Yes
Most Potent & Selective Agonists	NMDA, ADCP, Ibotenate	QA, AMPA	KA, Domoate	APB*, L-Glutamate*
Proposed Function	Mediate transmission at excitatory synapses LTP Induction			?Modulate synaptic excitation
Most Potent & Selective Antagonists	D-2-APH, D-2-APP, Asp-Amp	GDEE, Glu-Tau, GAMS	Glu-Tau, GAMS	APB*, L-Glutamate*
Possible Therapeutic Use of Antagonists	Convulsive and neuro-degenerative disorders e.g. epilepsy, ischaemia	Unknown at present	Unknown at present	Unknown at present
Radioligands	($[^3H]$-L-Glutamate) $[^3H]$-2-APP	($[^3H]$-L-Glutamate) $[^3H]$-AMPA	($[^3H]$-L-Glutamate) $[^3H]$-Kainate	($[^3H]$-L-Glutamate) $[^3H]$-APB
Notes	Excitotoxicity; best characterised sub-type	Lacks potent and selective antagonists	Excitotoxicity; needs selective antagonists	*Not known if agonist or antagonist at this site

1.3 Excitatory Amino Acid Receptors in Brain Physiology and Pathology

1.3.1 Long Term Potentiation (LTP)

Long Term Potentiation is a sustained increase in synaptic efficacy which follows brief tetanic stimulation of excitatory pathways[73,74]. It has been suggested that this phenomenon, although not precisely understood either mechanistically or physiologically, may provide the physiological basis for engram formation, that is, the formation of the memory trace pattern[75]. It is most commonly believed that the short-term memory consists of the storage of engrams as some continuously-reverberating pattern of electrical impulses within the cortex[76].

It has been shown that interference with A1 receptors (which are certainly present within the cortex) *via* APP prevents the induction of LTP, even though the antagonist does not itself directly affect the EPSP of the neuronal population. This suggests that the activation of the A1 receptor is required to initiate LTP[41].

It may be deemed conceivable that the brief bursts of tetanic stimulation required in the first instance to commence the LTP process may raise the membrane potential above the critical threshold level which "switches on" the A1 receptor via the Mg^{2+}-dependent gate[52] (see previous details). The A1 receptors which do not seem to participate generally in low-frequency synaptic transmission could thus be activated and allow the induction of LTP to proceed[77]. What happens next is a matter of some debate, but Baldry and Lynch have suggested that a receptor-mediated rise in Ca^{2+} levels intradendritically acts *via* a protease (Calpain I) to unmask latent transmitter (glutamate) receptors in sub-synaptic membranes[78]. Difficulties with this scheme have been noted[23]. However, evidence that A1 receptors are involved seems very strong and any use of A1 receptor antagonists *in vivo* for possible therapeutic reasons (qv) would require caution until the effects on learning and memory have been established[75].

1.3.2 Epilepsy

Epilepsy is a disease characterised by paroxysmal abnormal electrical activity in the brain, often accompanied by loss of consciousness and convulsions. An epileptic attack begins with sudden, excessive local discharges of grey matter at an abnormal focus; generalised epileptic convulsions develop when the electrical activity spreads into adjacent normal brain tissue[79]. Any therapeutic materials employed to combat epilepsy therefore theoretically have two potential modes of action: they could either inhibit or damp out the seizure focus itself, or prevent the spread of the abnormal activity to the normal brain tissue. Most currently used drugs are thought to act by the second mechanism[79].

There are four types of epilepsy: Grand Mal or major epilepsy; the patient suffering loss of consciousness and clonic/tonic convulsions, often being preceded by a premonitory aura of apprehension or fear/anxiety, sometimes accompanied by epigastric discomfort.

Petit Mal, or minor epilepsy, is characterised by brief periods of clouding, or loss, of consciousness during which the sufferer stops his or her activities for a few moments before resuming them without being aware of the interruption. Frequent attacks of this type may seriously handicap the patient.

Psychomotor epilepsy comprises bouts of abnormal sensations or behaviour; for example, a patient might have a fit of rage of which he or she cannot recall anything later on.

Focal, or Jacksonian epilepsy is normally associated with gross organic lesioning of the cerebral cortex. Characteristically the condition produces convulsive twitching of isolated muscle groups; the spasms may remain localised or progress to more generalised convulsions and loss of consciousness[79].

Epileptiform seizures can be artificially induced by a wide variety of methods (for review, see[80]); chemical stimulation by convulsants such as pentylenetetrazole or leptazol; electrically by stimulation of the brain with externally applied electric shocks; or in certain specialised animal models such as the baboon *Papio papio* which is sensitive to intermittent photic stimulation, or the DBA/2 strain of mice which display a well-defined range of seizure responses after audiogenic stimulation, *e.g.* by the ringing of a bell[81].

As has been noted previously, the acidic amino acids L-glutamate, L-aspartate, NMDA, quisqualate, kainate, *etc.*, all have excitatory effects when applied to CNS neurones; when perfused or injected intracerebrally, or administered by intravenous or intraperitoneal methods, these compounds all cause seizures and also produce the ensuing neuronal degeneration and death of a similar pattern to that observed in such pathological conditions as stroke, cerebral palsy, epilepsy, ageing and Alzheimer's disease, Huntington's Chorea and other chronic neurodegenerative disorders[75].

The implication of excitatory amino acids and their receptors in epilepsy has been further indicated by the discovery that A1 receptor antagonists can prevent seizures in certain animal models[45,82]; the work of Meldrum and colleagues using APH in the photosensitive baboon[83] and the audiosensitive DBA/2 mice being particularly noteworthy[81]. Corresponding results have also been achieved for the suppression of paroxysmal depolarisations and bursts with *in vitro* test systems[84]. Additionally, the A1 antagonists protect against certain chemical convulsants including NMDA, but not including kainate[85]. Seizures induced by convulsants which are believed to act mainly on GABAergic inhibition have also been prevented; although these compounds have also been shown to enhance the stimulated release of D-[^{3}H]-aspartate in *in vitro* studies employing brain "mini-slices"[86]. Thus it may be

possible that such seizures would evolve from the action of excitatory amino acids which could be prevented by the A1 antagonists such as APH, rather than by, (or in addition to) any GABAergic effects.

The observation that A1 receptor antagonists prevent many types of seizure (but not those induced by kainate) might be taken as an indication that most of the epilepsy models examined are mediated in some way by the A1 receptor[75,87]; data on the quisqualate-type A2 receptor is rather lacking, but the fact that kainate seems able to produce convulsions in the presence of A1 antagonists[85] suggests either that an alternate route of seizure propagation via the A3 receptor is in operation or that the activation of the A3 receptor in some way overcomes the antagonist action of substances such as APH at the A1 receptor site.

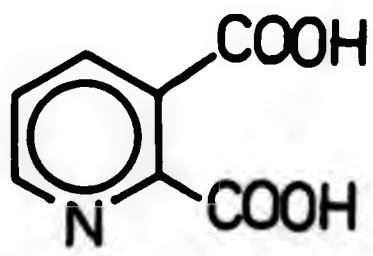
1.3.3 Neurodegenerative Disorders: General

Administration of excitatory amino acids can lead to convulsions in the first instance, but the pathological damage is not confined to a transient seizure condition. As long ago as 1957 it was realised that neuronal cells (in this case, retinal amacrine cells) degenerate after systemic administration of monosodium glutamate to infant mice[88]. Since at this time initial studies on glutamate as a neurotransmitter were being pursued, there was some doubt as to the veracity of the claim that it could also act as a toxin; however, further investigation showed that neuronal damage does indeed occur in infant rodents (the damage in adults being less severe) following subcutaneous administration of various endogenous neuroexcitatory amino acids. Oral routes of administration produced similar results[89,90]. Under these conditions, the damage is localised mainly in the arcuate nucleus of the hypothalamus, the area postrema and the circumventricular organs; that is, the territories outside, and unprotected by, the Blood-Brain

Barrier (BBB)[91]. When the damage is analysed at the cellular level, the lesion is found to be restricted to local neurones and does not affect non-neuronal elements such as axons[91]. The first degenerative changes appear in post-synaptic dendritic structures in a pattern consistent with mediation by the neuroexcitatory effects of acidic amino acids[92]. Compounds able to produce these destructive effects as a result of their excitatory action have been termed "excitotoxins"[93].

A similar pattern of damage is observed in the hippocampus (with focal swelling of dendrites in vulnerable neurones) after status epilepticus[94] or following sustained electrical stimulation of excitatory pathways to the hippocampus, *e.g.* the perforant path[95]. Such sustained-burst firing of vulnerable neurones, with attendant enhancement of calcium entry and subsequent cell damage due to high intracellular Ca^{2+} ion concentrations, has been claimed to be the common mechanism in status epilepticus, cerebral ischaemia and excitotoxic neuronal death[96].

The implied association of receptor involvement with the action of excitotoxins such as NMDA, quisqualate and kainate is supported by the discovery that A1 receptor antagonists, particularly APH, block the excitotoxic actions of an endogenous neurotoxin, quinolinic acid (31)[97]. This material has been demonstrated to increase in concentration within the rat brain during ageing[48], raising the possibility that excitotoxic processes may contribute towards the degenerative changes associated with the senile dementias[75,87].



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Kainic acid, when injected focally into the brain, causes selective local and distant neuronal damage[98]; for example, in the hippocampus, CA3 pyramidal cells are particularly vulnerable (which corresponds well with the observed distribution of kainate-preferring receptors within the stratum lucidum), whereas the dentate granule cells are relatively resistant[99]. Some sites can be protected by prior lesioning of the glutamatergic input (cortico-striatal); this reduces the vulnerability of striatal neurones to kainate[100]. Similar protective action has been observed after destruction of the excitatory afferents to the optic tectum[101] and the hippocampus[102].

Injection of A1 agonists such as NMDA, ibeotenate and quinolinic acid produce a different pattern of neuronal degeneration to kainate [102,103]; this damage can be prevented by co-administration of APH[104]. Unlike kainate toxicity, ibotenate toxicity is not affected by the destruction of the excitatory afferents[103].

It seems possible that there are two distinct types of direct neurotoxic action produced by the excitotoxic amino acids[75]. Firstly, there appears to be a straightforward action on the A1 receptors, independent of the excitatory afferents and blockable by the action of A1 receptor antagonists. This post-synaptic excitotoxicity is probably associated with patterns of burst firing and induction of dendritic calcium spikes[105].

Secondly, there is a less direct approach *via* the kainate (A3) receptor, which is not interfered with by the A1 antagonists but does seem prone to protection by the prior destruction of the glutamatergic excitatory inputs[100]. This would seem to suggest a complex interaction where synaptic release of excitatory and perhaps also inhibitory neurotransmitters is involved[75]. There is also an indirect neurotoxic action associated with the induction and spread of seizure activity that shows patterns of damage identical with those observed in

animals undergoing status epilepticus induced by drugs other than the neurotoxic amino acids[106].

1.3.3.1 Huntington's Chorea

In 1976 it was shown that intrastriatal injection of kainic acid in rats produced the type of neuropathological and neurochemical features that occur in Huntington's Disease; marked neuronal degradation in the striatum, with selective loss of GABAergic and cholinergic neurones[107]. Associated with these changes is a drop in levels of glutamate decarboxylase and choline acetyl-transferase activity, while tyrosine hydroxylase levels are normal. These changes in enzyme concentrations are seen both with the natural and the kainate-induced pathological disorders[107,108]. Various studies in laboratory animals during or after striatal nerve cell degeneration caused by kainate or similar compounds confirms that behavioural, functional and pharmacological aspects of the induced and the naturally occurring conditions are analagous[109].

The clinical symptoms of the disease (which is thought to be genetically inherited) usually appear in patients aged forty to fifty, with irregular, rapid spontaneous limb movements (choreic movements). The disease progresses to athetoid movements (writing, grimacing and characteristic sideways head movements) and usually dementia, mood disturbance and impaired judgement[110]. Interestingly, it has been noted that kainate and quinolinate are less toxic during ontogeny than later, possibly corresponding to an increased susceptibility of older animals to the excitotoxicity, analagous to the late pathological onset of Huntington's Chorea[47].

1.3.3.2 Cerebral Ischaemia

Exposure of hippocampal cells to anoxic conditions, either as a cell culture *in vitro*[111] or by occlusion of the carotid artery *in*

vivo[112] result in ischaemic neuronal degeneration. The damage resembles that seen in the aftermath of status epilepticus and preferentially involves pyramidal neurones in hippocampal CA3 and CA1 regions, small pyramidal neurones in cortical lamina III, small interneurones in the striatum and cerebellar Purkinje cells[75]. This pattern of selective vulnerability in the hippocampus develops during the first two hours of re-perfusion, the vulnerable neurones showing an accumulation of intracellular calcium with a distribution in the parikarya and dendrites suggesting the involvement of excitatory inputs and burst firing[112]. This would seem to indicate that a suppression of excitatory transmission and associated burst firing might be of value in protection against ischaemic damage[75].

The results of Meldrum *et al* using the A1 antagonist APH are therefore particularly significant; focal injection of small quantities of APH into the dorsal hippocampus provides good local protection against the acute neuronal pathological changes induced by forebrain ischaemia in the rat[49].

Previous therapeutic use of barbiturates against experimental focal ischaemia has led to a suggestion that the use of calcium entry blockers would prove efficacious[113]. This seems somewhat unselective in that calcium entry controls the responses of *all* excitable cells (*e.g.* those in the heart and vasculature as well as the targets in the CNS) whereas the success of APH suggests that a more specific targetting policy could be pursued instead[75].

1.3.3.3 Alzheimer's Disease

Senile dementia of this type is characterised by a diffuse loss of cortical neurones[114]. Ascending neuronal systems are also affected, particularly a loss of cholinergic neurones which connect the nucleus basilis to the frontal and temporal cortex[115], and of noradrenergic fibres ascending from the locus coeruleus[116].

Although there is no direct evidence that an excitotoxic mechanism determines the course of this disease, it has been noted that ageing rats show raised levels of the endogenous excitotoxin, quinolinic acid[48]; some individuals having concentrations comparable to those shown to induce pathological changes in neuronal cell cultures[117].

1.4 Project Objective: A Selective Kainate (A3) Receptor Antagonist

1.4.1 Summary of Data

α-Kainic acid, a sterically rigid, cyclic analogue of L-glutamate, possesses, in common with a variety of other compounds, a potent excitatory action on certain areas of the CNS. In addition to its excitatory properties, α-kainate is a very potent lesioning agent, producing neurodegenerative symptoms in both *in vivo* and *in vitro* test conditions which closely resemble, and may be identical to, those observed in a number of pathological conditions[75].

The areas of greatest kainate toxicity appear to correspond with, and are presumably modulated by, the areas of highest density of the A3 receptor, a sub-class of excitatory amino acid receptors of which α-kainic acid appears to be the most potent and selective agonist known[23]. Currently, no specific, potent A3 receptor antagonists are known.

With the recent advances made in A1 receptor antagonists, and the most promising potentially useful therapeutic value of same, it is clearly to be hoped that the discovery of an A3 receptor antagonist might lead to similarly good results against the neurodegenerative disorders in which the A3 receptor has been implicated.

Quite apart from any postulated medicinal role for an A3 receptor antagonist, such a compound would be of great value to the pharmacologist and the biochemist simply in order to more fully elucidate

and characterise the structure, distribution, pharmacology and biochemistry of the A3 receptor.

The primary objective of this project, then, was to synthesise a specific, and hopefully potent, A3 receptor antagonist.

As a secondary objective, the chemistry of α-kainic acid and its analogues and stereoisomers would be investigated.

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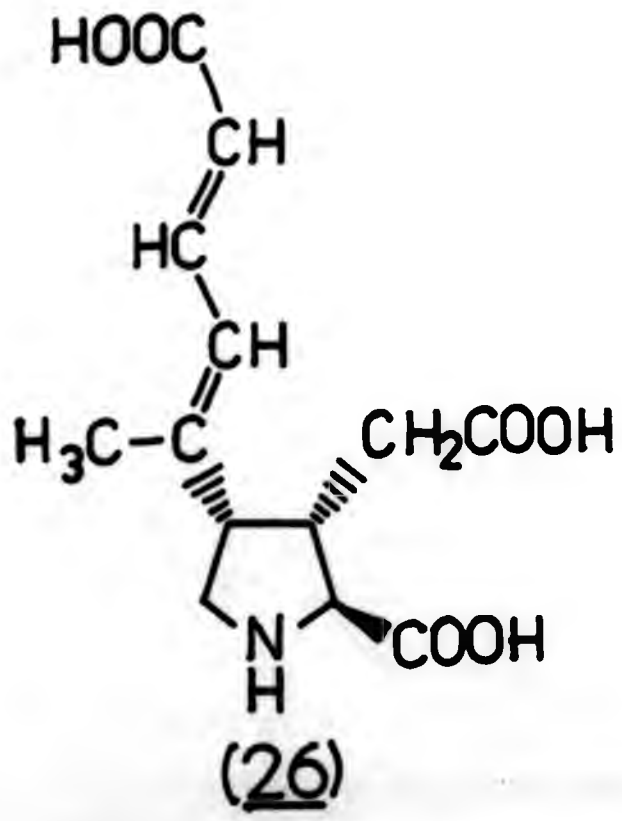
CHAPTER TWO

CHEMICAL STRATEGY

2.1 Kainate: Prior Investigations of Structure/Activity Relationships

In order to evaluate potential approaches to therapeutic analogues of α-kainic acid, it is convenient to consider the information available in terms of prior work by other groups whose investigations may furnish useful structure-activity information. Such previous studies have indicated that certain modifications to the structure of α-kainate adversely affected the membrane-binding capability, and hence presumably their affinity for the A3 receptor; most notably, the C-4 isopropylidene side chain is required for action at the receptor. Domoic acid (26), with a lengthened and doubly-unsaturated side chain at the 4-position is a potent displacer of kainate at the receptor; but α-ketokainate (32) retains only a moderate degree of activity, and dihydrokainate (33) even less (some 2-3 orders of magnitude less than α-kainic acid). A simple stereoisomer α-allokainate (34) also exhibits a very low ability to displace α-kainate[1,2].



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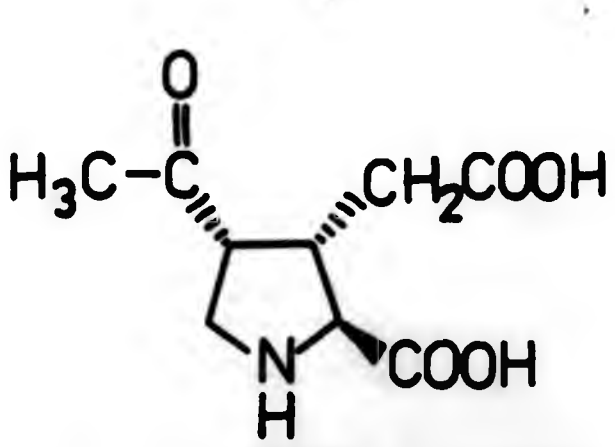

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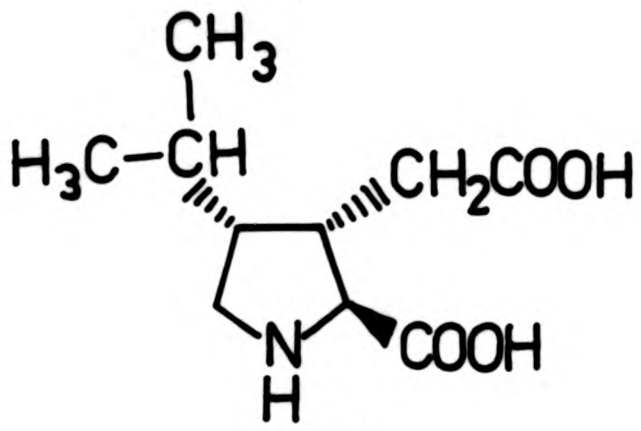

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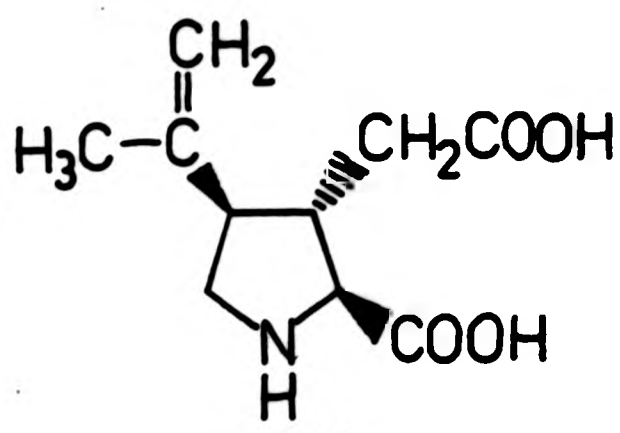


(34)

A number of lactone and hydroxy-acid derivatives tested (in which the unsaturated side-chain is replaced) are reported to have antagonised the specific $^{22}Na^{+}$ efflux produced by excitatory doses of NMDA and kainate, although membrane data is not available[3].

On the whole, however, very few kainate analogues have been synthesised and tested for biological activity, so the field is therefore open to a considerable degree of general investigation simply to provide basic structure-activity information.

2.2 Objectives: Target Molecules

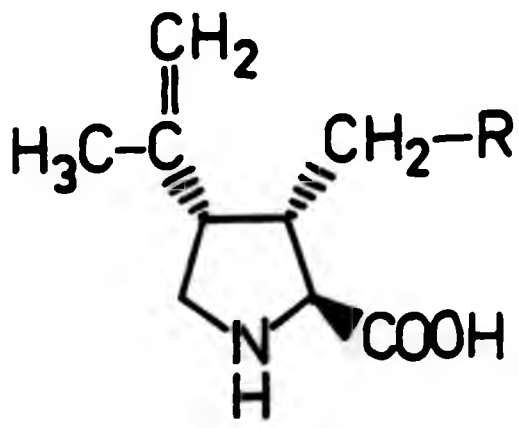
The lack of thorough prior investigation into the synthesis of kainate analogues allows considerable scope for the chemist wishing to produce novel kainate-based compounds. The limited data available regarding proposed structure-activity relationships of such compounds as had already been produced limited the possibilities only slightly.

Firstly, it appeared desirable to preserve the integrity of the C-4 iso-propenyl side chain, since the reduction of the alkenyl moiety or oxidation to the ketone had not produced particularly active compounds. Indeed, the most potent displacer of α-kainate binding, domoic acid (26), has identical structure and stereochemistry to α-kainate except that the C-4 side chain is longer by CH:CH.COOH.[2] While it might prove useful,

then, to elongate the C-4 side chain, while retaining a degree of unsaturation, it would be unlikely to produce viable compounds by reducing or replacing it.

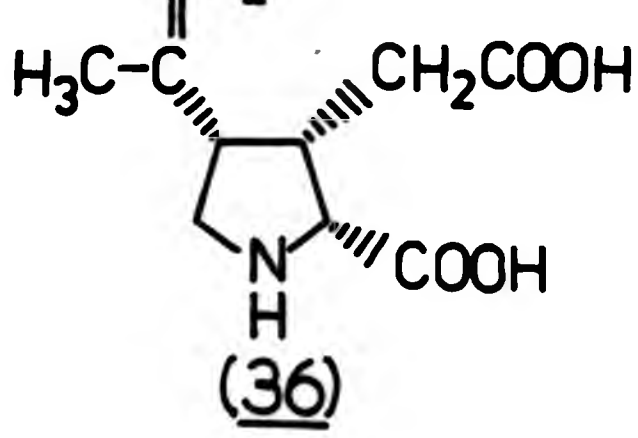
Secondly, a comparison with L-glutamate suggests that the α-amino acid part of the molecule may well be a common receptor recognition "handle" and that the secondary amino function and the C-2 carboxyl group should remain substantially unaltered, although certain N-protected derivatives might prove useful in order to facilitate transport across the Blood-Brain Barrier[4].

Initial efforts were therefore directed towards effecting various alterations to the C-3 carboxy-methyl side chain. Possible target molecules include the replacement of the carboxyl group with an alternative acid group, *e.g.* sulphonate (35a) or phosphonate (35b); a replacement of the carboxylic acid with some different functional group (35c); a chain-elongation process to homo-kainate or even to *bis*-homo-kainate (35e); or some form of coupling reaction incorporating part of the carboxyl group, such as peptide formation (35f).



R	No.
-COOH	(6)
$-SO_3H$	(35a)
$-PO_3H_2$	(35b)
-CN, -OH, etc.	(35c)
$-CH_2COOH$	(35d)
$-(CH_2)_2COOH$	(35e)
-CONHR'	(35f)

Compounds produced in the α-series (as depicted above) could also be duplicated in the β-series, based on β-kainic acid (36) where the stereochemistry at C-2 is reversed. The β-kainate analogues would therefore correspond to D-glutamate rather than the L- form.



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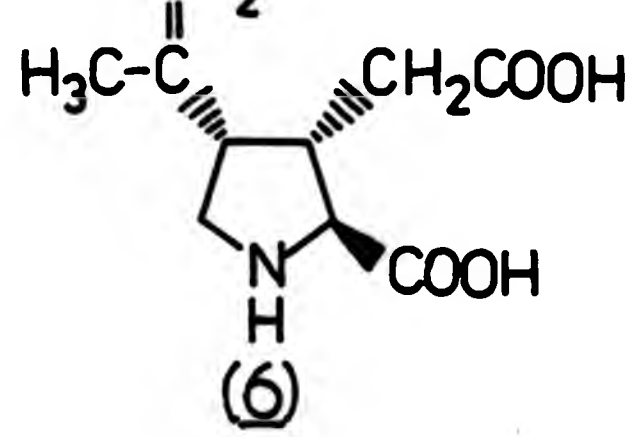


(6)

By no means all the chemistry was directed towards alternations at the C-3 carboxyl, however; investigation of compounds where the C-4 side chain underwent various reactions also proceeded, as well as various stereochemical manipulations.

2.3 Kainate: Some General Chemical Aspects

The chemistry of kainic acid presents the chemist with some challenging problems. The considerable degree of polarity evident in a dicarboxylic amino acid of this type makes it necessary to introduce, by means of appropriate protecting groups, a sufficient lipophilicity to enable various common reactions to be carried out under typically non-polar, non-protic solvent conditions.

Additionally, specific reagents and reaction sequences need to be carefully chosen to minimise undesirable reactions at sites on the molecule other than the intended reacting position. This potential problem stems from the diversity of functional groups on the kainate

molecule. For example, the alkene moiety will be prone to attack by a number of reagents which might otherwise be used to effect some synthetic manipulation elsewhere in the molecule; in such cases, if no alternate reagent was practicable or available, a protection and subsequent deprotection of the double bond would need to be considered.

Empirical experience has also shown that through-space intramolecular interactions are often of importance; examination of models or suitable molecular graphics facilities shows favourable conformations for steric or reactive interactions under certain conditions.

2.4 Kainate; Difficulties of Materials Analysis

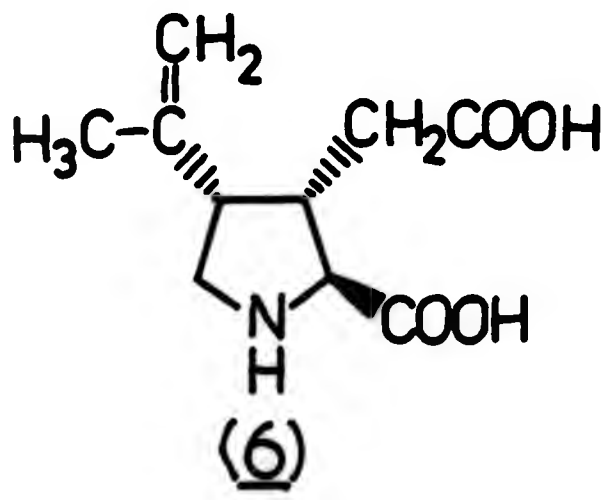
A major consideration at all times during these syntheses using kainic acid was the comparatively high price of the raw material, α-kainic acid (Ca. £40.00 per gramme at current prices), a fact which necessitates the use of comparatively small-scale reactions, which in turn leads to the preclusion of extensive use of certain analytical techniques; the complexity of the molecular structure and that of likely reaction intermediates renders other methods difficult. Experience at the bench demonstrated that kainate analogues, even material obtained commercially "pure" gives most misleading results when subjected to elemental analysis; examination of data obtained suggests some complex inclusion of water, anywhere from ½ mole water to 1 mole kainate and upwards[5]. Kainate and its analogues also appear somewhat refractory to standard mass spectrometric analysis; rapid decomposition and fragmentation occurring on heating, with the result that the observation of molecular ions is very difficult.

However, we were fortunate to be able to make extensive use of two important analytical techniques in order to elucidate structural information, and confirm intermediates and products: 22.5 MHz ^{13}C Nuclear

Magnetic Resonance Spectroscopy using advanced pulse sequence techniques (DEPT) and Fast Atom Bombardment (FAB) Mass Spectroscopy. In some cases, use was also made of a 100.62 MHz NMR service.

2.5 Kainate; Stereochemical Aspects:

The kainate molecule contains three chiral centres, at the C-2, C-3 and C-4 atoms, and there are thus theoretically eight possible stereoisomers. In fact, as far as is known there are no reports of kainate analogues with anything other than the R absolute configuration at C-3; since methods of inverting the stereochemical configuration at both C-2 and C-4 are known[6,7], the number of possible stereoisomers readily available is only four: α-kainate (1), β-kainate (36), α-allokainate (34) and β-allokainate (37).



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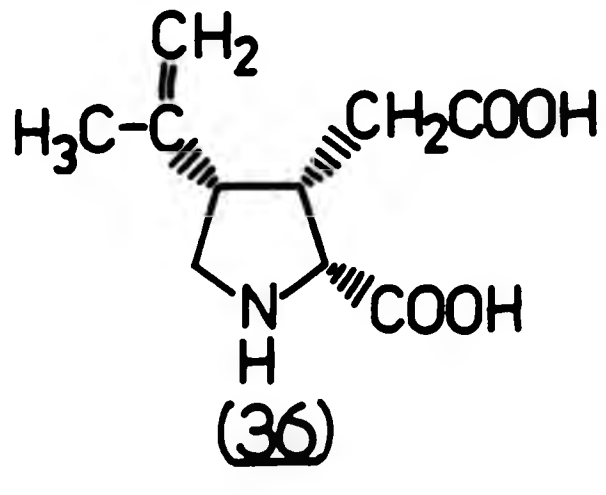

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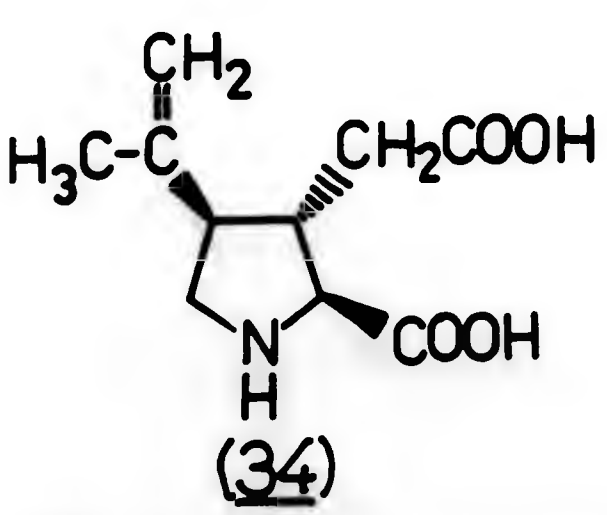

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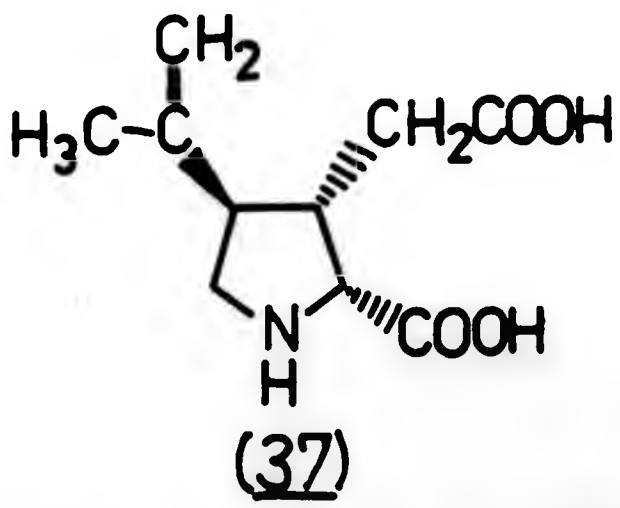
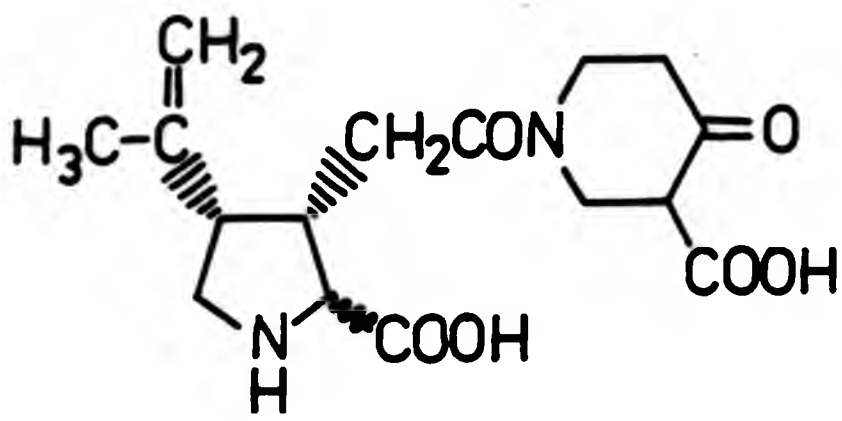


(37)

Since previous work had indicated that α-allokainate was considerably less active than standard α-kainate[2], attention was focused almost exclusively on the α- and occasionally β-derivatives, *i.e.* stereochemical conversions at the C-2 position.

Some recent pharmacological results have suggested that β-kainate may be an α-kainate antagonist under certain conditions[8]. Whether or not there is any general relationship between α- (agonist) and β- (antagonist) homologues is unclear, but results obtained with two of the compounds synthesised during this project do not indicate any such relationship; both were potent excitants: (38) and (39).



(38) C-2α
(39) C-2β

2.6 Synthesis

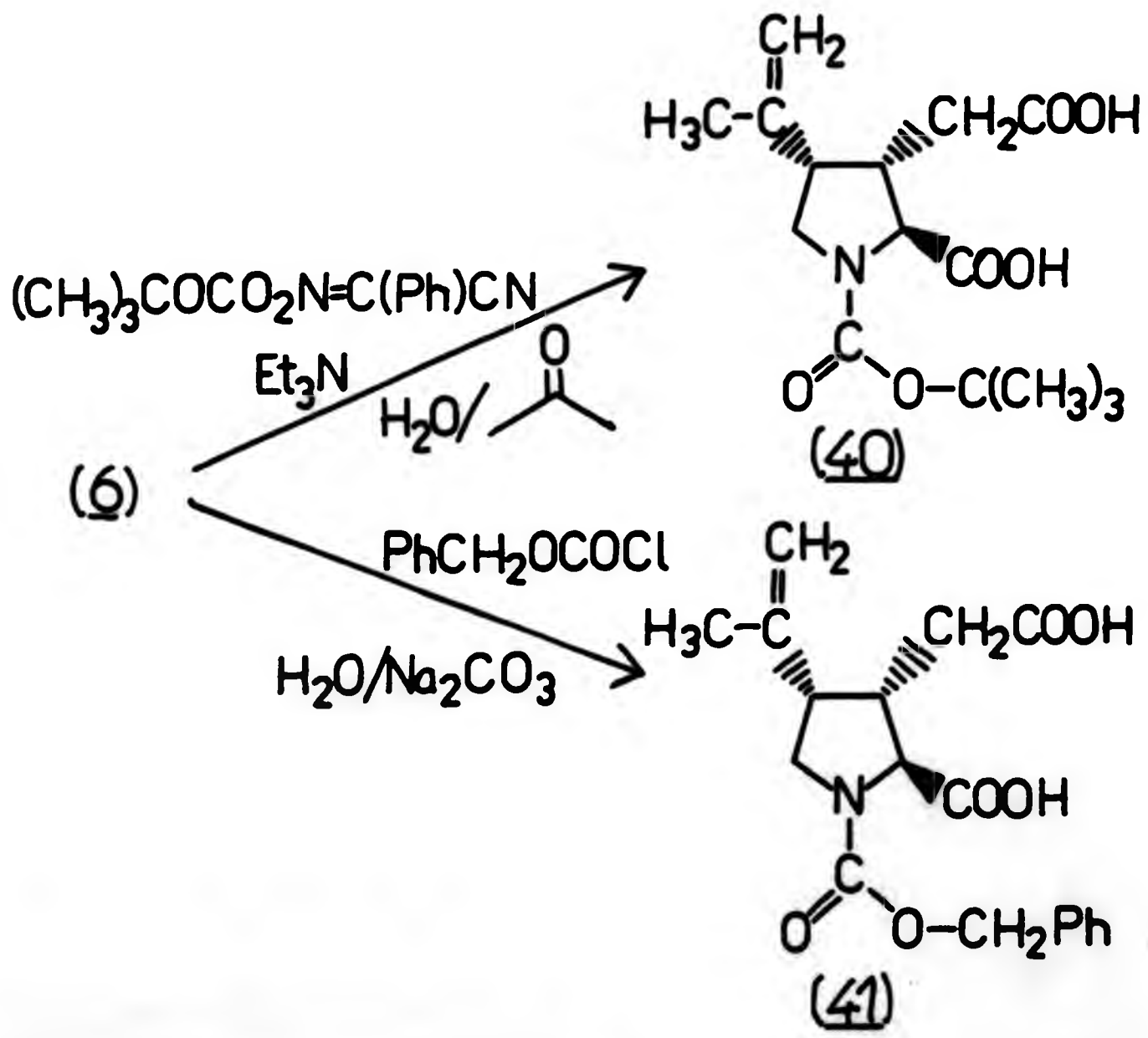
2.6.1 N-Protection

Almost all the reaction series employing kainic acid entailed, as a first step, N-protection by one or other of the commonly used N-protecting groups. The importance of such a technique is primarily two-fold; firstly and obviously, they protect and inactivate the amino-function from attack by, or reaction with, other reagents employed to effect a change elsewhere in the molecule. Secondly, the solvolysis in typical organic solvents of what is, in fact, a highly polar hydrophilic (lipophobic) molecule, is increased. As a purely incidental feature of protection with the carboxy-benzyl (CBZ) group, an additional

advantage becomes apparent; the intermediates thus produced show clearly increased visibility in TLC examination; the CBZ group develops and stains clearly in acidic ammonium molybdate solution. This can be a considerable advantage when the double bond on the C-4 side chain is reduced or replaced, thus eliminating the most obvious TLC recognition "handle" in the basic parent molecule.

N-protection was usually effected using the *tertiary*-butyl oxycarbonyl group (*t*BOC), which was added by the use of "BOC-ON" (2-(*tert.*-butoxycarbonyl-oxyimino)-2-phenyl acetonitrile in water-acetone solution made basic with triethylamine[9]; yields varied but were always in excess of 90%. (Scheme 1).

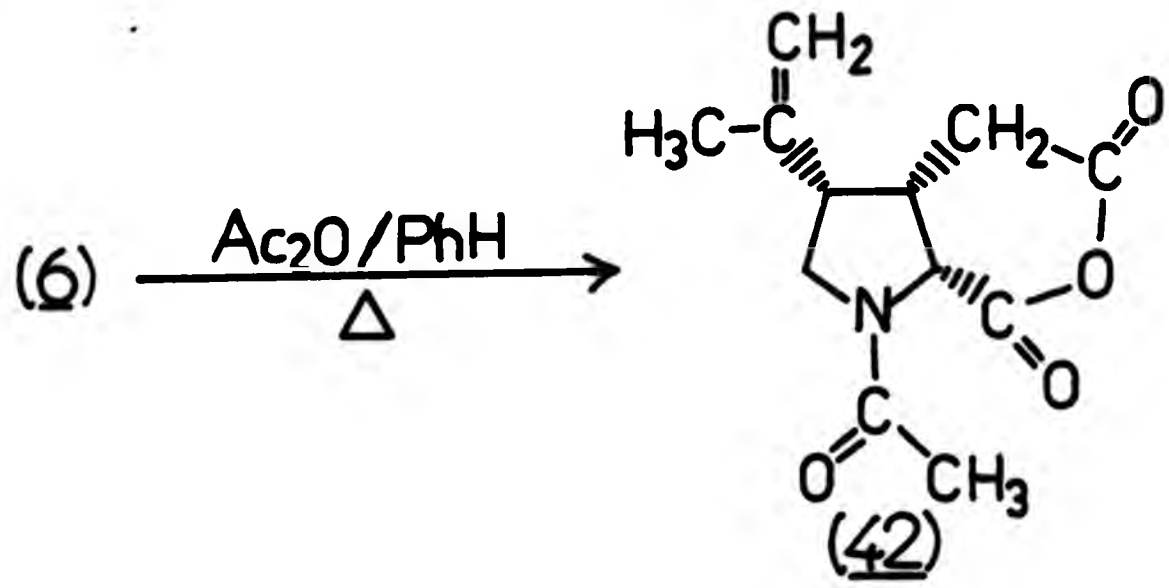
Scheme 1



Alternatively, the carboxybenzyl group was occasionally employed; addition was by action of benzyl chloroformate to a vigorously-stirred solution of α-kainic acid in water, in the presence of excess sodium carbonate[10]; the work-up was commonly rather more difficult than with the *t*BOC group but yields were still in the region of 85-90%. (Scheme 1).

During conversion of α- to β-kainate, the amino group was protected as the N-acetyl derivative[6], although this was more a by-product of the use of acetic anhydride to alter the stereochemistry than anything else. Isolated yields of the recrystallised anhydride intermediate (42) were in the 60-70% range (Scheme 2).

Scheme 2



(42)

The whole subject of kainate N-protection clearly invites much greater investigation from a pharmacologically-directed synthesis angle; clearly, if a therapeutically useful, centrally-acting kainate variant was found, some form of protecting group would be needed at nitrogen (and/or the carboxylic acid groups) to facilitate transport across the Blood-Brain Barrier, and then be easily removed by some endogenous enzyme system (s) to unmask the active molecule. Whether or not any of the three above groups used during this project could fulfil this requirement was not investigated; from a chemical point of view they performed adequately in a straightforward synthetic sense; their pharmacological efficacy remains untested.

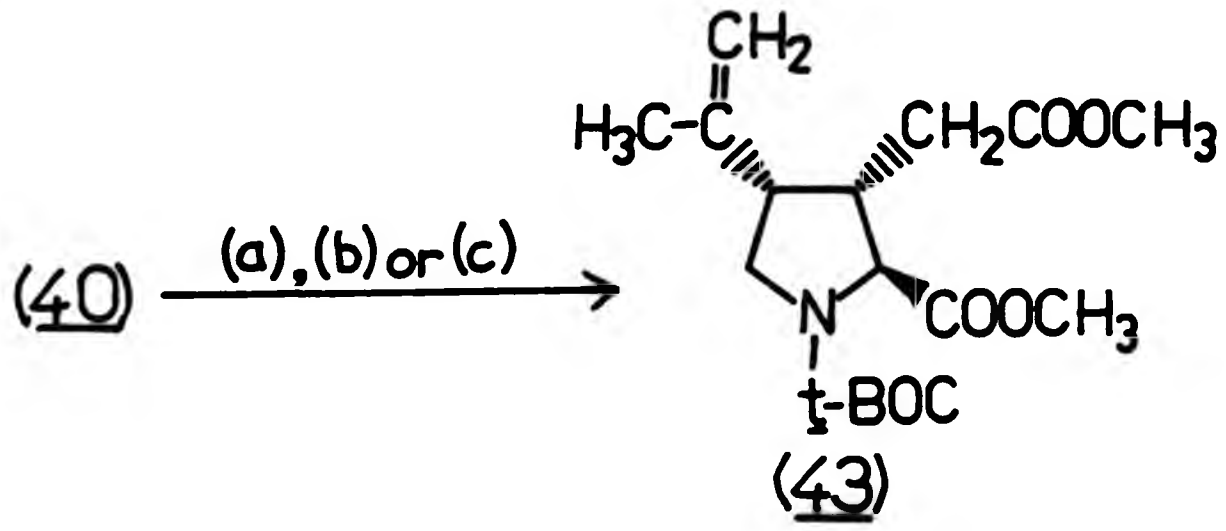
2.6.2 Esterification

In addition to protecting the amino function, it was also necessary to protect the carboxyl groups, either separately or together.

2.6.2.1 Diesterification; Identical Ester Groups

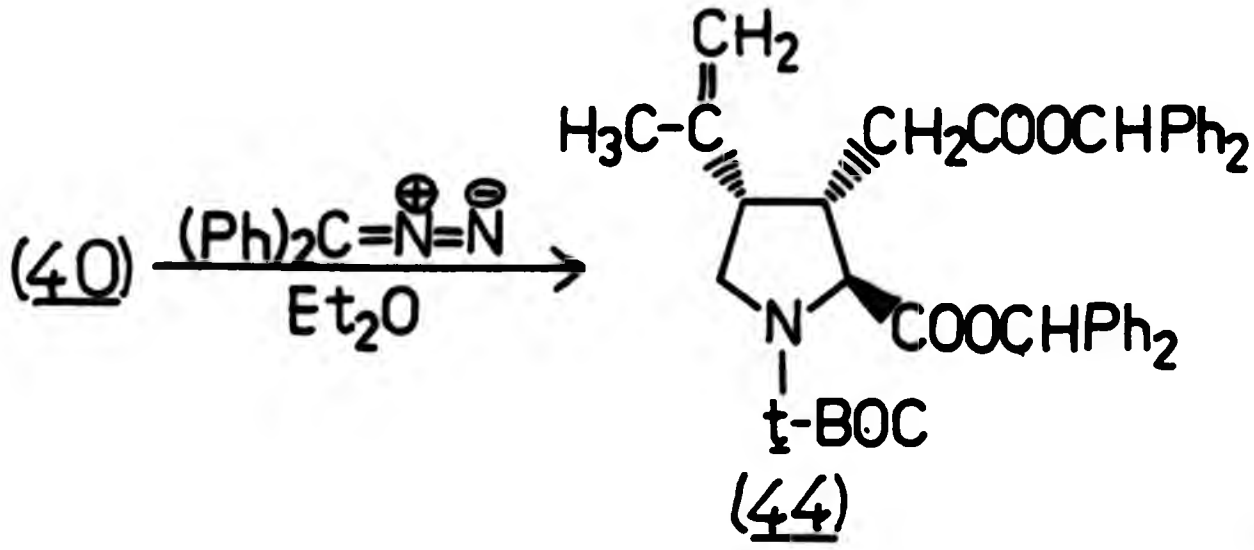
While other group members pursued a number of different types of ester and diester functionalisation, the major interest in this project centred on the *t*BOC-dimethyl diester variant (43). This was produced by any one of several methods, including (a) excess ethereal diazomethane[11], (b) Fischer esterification[12], or, (c) oxalyl chloride and methanol[13]. (Scheme 3).

Scheme 3



Such diesters were used mainly as intermediates in reaction sequences centring on the C-4 side chain, where the protection of the carboxyl functions was necessary. Some of the alternative diester types were assessed for their ability to selectively deprotect to give one mono-ester or the other (particularly the di-benzhydryl diester (44)[14], (Scheme 4), but micro-scale reactions indicated that such an approach was hardly feasible and certainly far less economic than the selective mono-esterification methods subsequently discovered.

Scheme 4

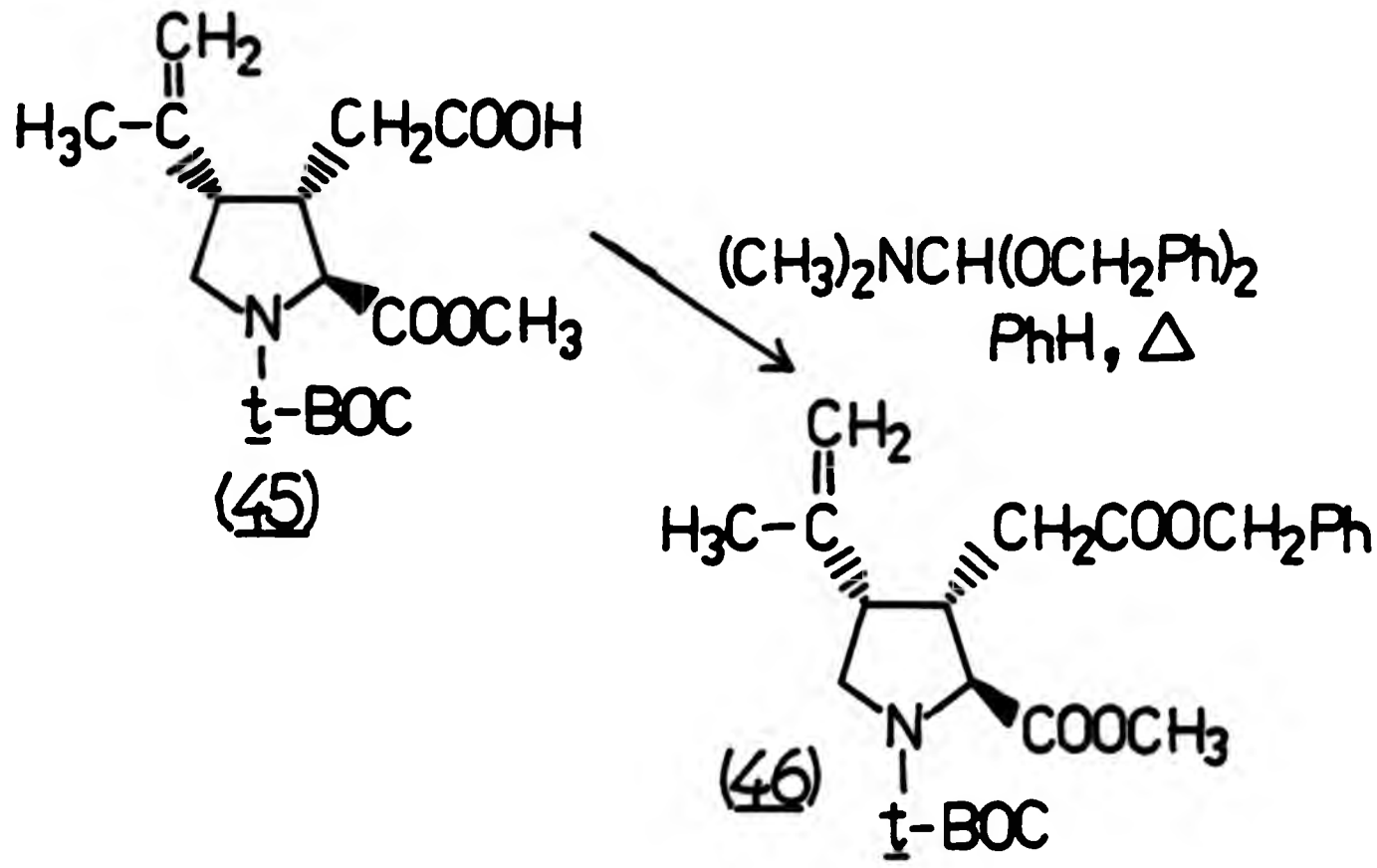


2.6.2.2 Diesterification; Mixed Diester Groups

Under certain circumstances it was necessary to protect the two carboxyl functions with non-identical protecting groups.. An example of this technique occurred during an attempted Hunsdiecker reaction pathway when the C-2 carboxyl was protected as the methyl ester (45) and the C-3 carboxylic acid then esterified using N,N dimethyl formamide dibenzylacetal[15] to give a benzyl ester on this acid group (46) (Scheme 5). This arrangement prevented a subsequent unwanted intramolecular reaction between the C-3 and C-4 side chains, and also allowed the later removal of the benzyl ester under mild conditions (hydrogenation), thus unblocking the C-3 acid whilst leaving the C-2 methyl ester *in situ*.

Some effort was also directed towards synthesising various mixed diesters (such as (46)) and then attempting to selectively deprotect the C-2 methyl ester, whilst leaving the C-3 carboxyl blocked. This did not prove very practicable, however, and a different system was eventually employed.

Scheme 5



2.6.2.3 Monoesterification; A Question of Selectivity

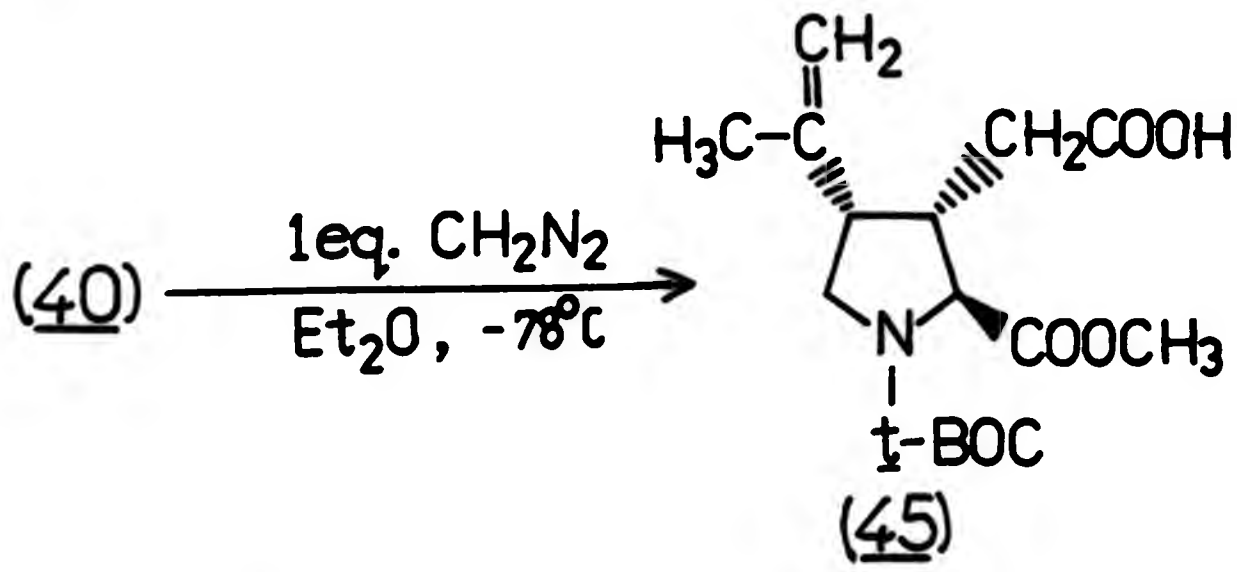
As a declared aim of this project was to produce kainate analogues where the C-3 side chain was to be the main site of alteration, there was clearly a requirement for a selective process to deactivate and/or protect the C-2 carboxylic acid and leave the C-3 acid group vulnerable to reaction and conversion.

With this in mind, therefore, a fortuitous observation during the routine production of some dimethyl diester (43) proved to be of outstanding significance. While using an ethereal solution of a two-fold excess of diazomethane[11], it was noted that, as the addition of approximately one equivalent of diazomethane proceeded, the yellow diazo-solution decolourised extremely rapidly; addition of more than one equivalent of ethereal diazomethane produced a much slower decolourisation, consistent with the hypothesis that one of the two carboxylic acid groups might be reacting much more rapidly than the other, leading to the fast loss of colour up to the addition of about one equivalent of

the diazo- reagent; subsequent reaction at the other acid site was then less rapid and gives rise to the slower decolourisation.

Investigation of this phenomenon by TLC showed that the addition of the diazomethane to the solution of the N-protected compound (40), (if the diazo- reagent was present only in equivalent concentration), did appear to favour the production of one ester over the other; in fact, when the reaction was carried out at -78°C, with slow addition of the ethereal diazomethane to the solution of the α-N-*t*BOC-kainate (40), separation of the products by silica chromatography yielded 70% of one monoester, and approximately 15% each of the diester and unreacted starting material. Later evaluation, which will be described in subsequent sections of this account, showed that the monoester selectively produced by this method corresponds to the C-2 monoesterified product (45). (Scheme 6).

Scheme 6



(45)

A convenient route has thus been discovered for the preparation of an intermediate that provides a good starting point for reactions on the C-3 side chain.

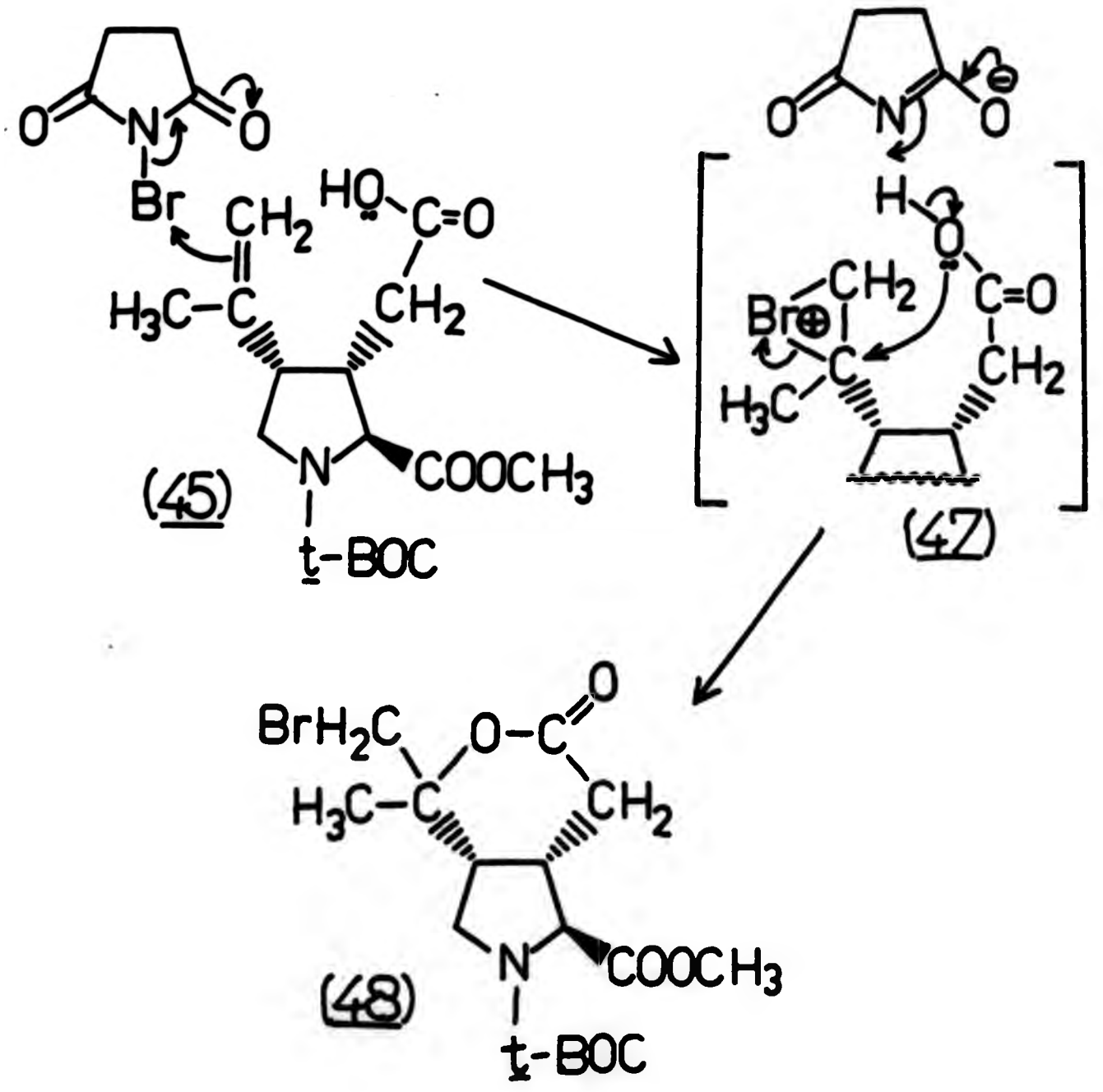
2.6.2.4 Selective C-2 Monoesterification; Confirmation of Regio-Selectivity by Bromohydrin Reaction

During the course of a reaction sequence intended to protect the C-4 side chain alkenyl moiety as the bromohydrin[16], confirmation of the

regioselectivity of the monoesterification was achieved. The monoester (45) was treated with N-bromosuccinimide in an attempt to form the bromohydrin at the alkene site. A rapid reaction ensued, but rather than stop at the bromohydrin the presumed intermediate (47) proceeded to react intra-molecularly to form the cyclic bromo-lactone (48), presumably via the mechanism shown (Scheme 7).

Such a reaction can only occur if the C-3 carboxyl is unblocked; the monoesterification must therefore have proceeded at the C-2 acid.

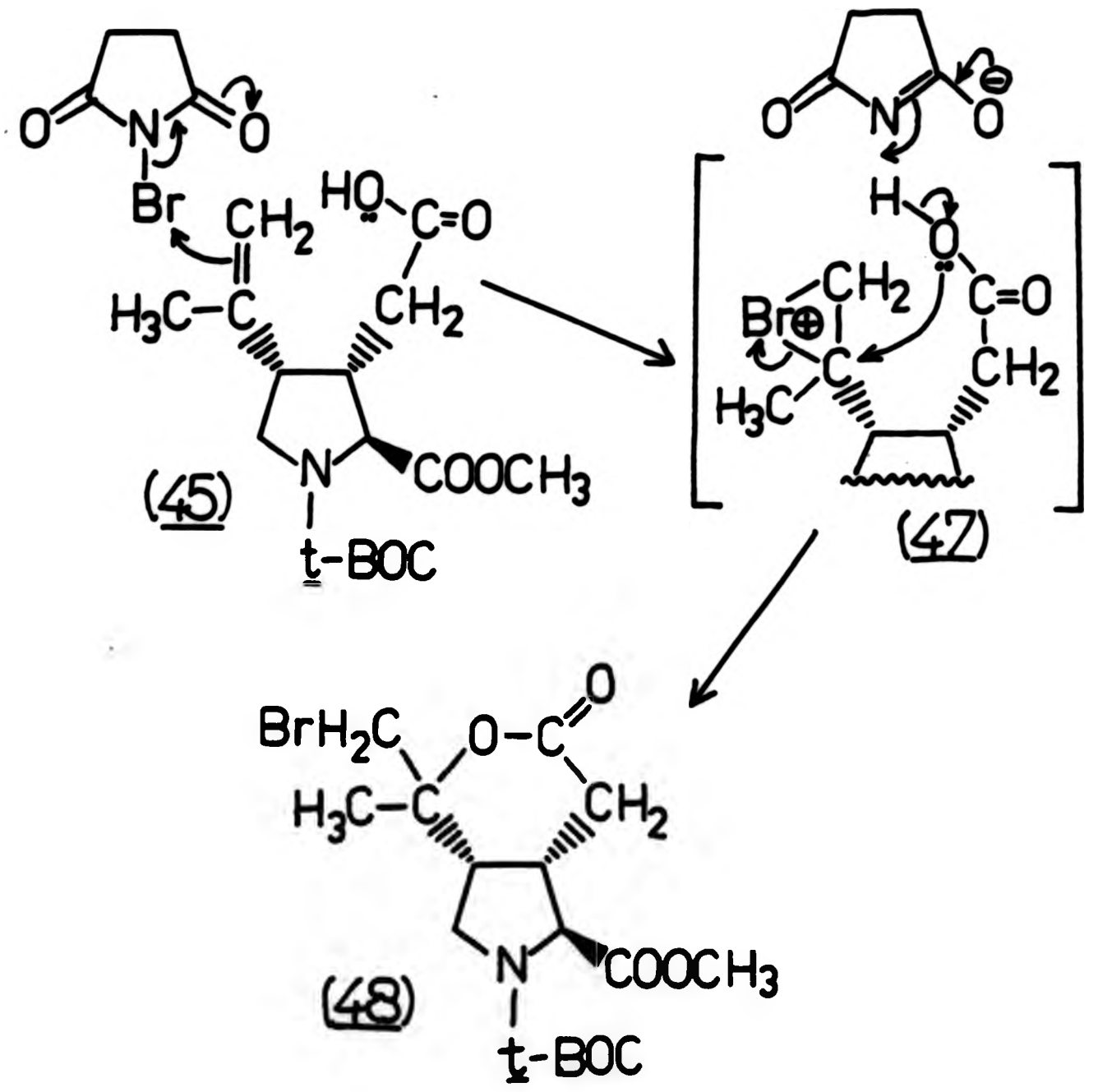
Scheme 7



regioselectivity of the monoesterification was achieved. The monoester (45) was treated with N-bromosuccinimide in an attempt to form the bromohydrin at the alkene site. A rapid reaction ensued, but rather than stop at the bromohydrin the presumed intermediate (47) proceeded to react intra-molecularly to form the cyclic bromo-lactone (48), presumably via the mechanism shown (Scheme 7).

Such a reaction can only occur if the C-3 carboxyl is unblocked; the monoesterification must therefore have proceeded at the C-2 acid.

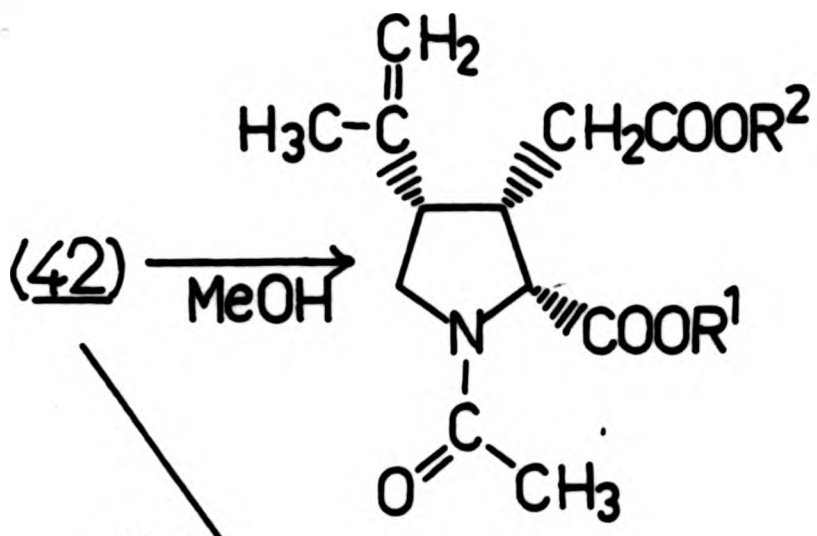
Scheme 7.



2.6.2.5 Monoesters; Undefined Anhydride Intermediates

Previous workers had observed that the β-N-acetyl kainate anhydride (42), formed by reaction of acetic anhydride on α-kainic acid (1), could be opened in methanol to yield a mixture of the two β-N-acetyl kainate monoesters (49), (50); or with water to give β-N-acetyl kainic acid (51)[6]. (Scheme 8)

Scheme 8

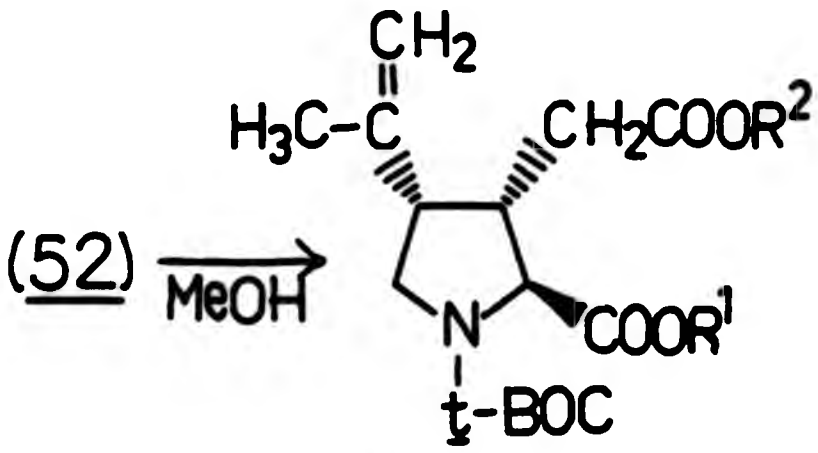


	R^1	R^2
(49)	$-CH_3$	-H
(50)	-H	$-CH_3$

(51)

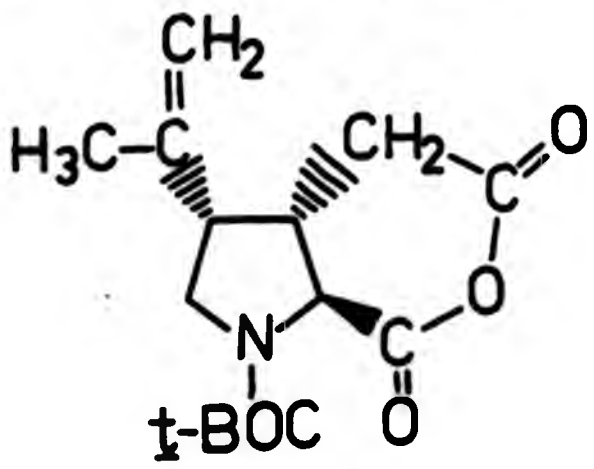
However, reaction of acetic anhydride and α-kainic acid already protected at nitrogen by a large group (*e.g.* *t*BOC or CBZ) gave rise to an anhydride intermediate (52) which, when opened in methanol, yielded a mixture of both the monoesters (45), (53) and some diester (43), all of which possess α-stereochemistry at the C-2 atom (Scheme 9).

Scheme 9

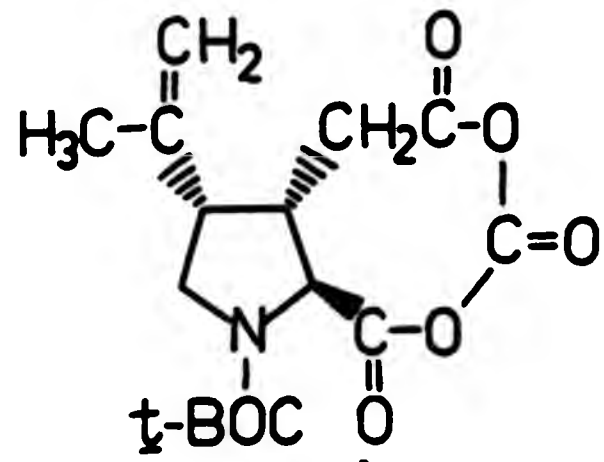


	R^1	R^2
(45)	$-CH_3$	-H
(53)	-H	$-CH_3$
(43)	$-CH_3$	$-CH_3$

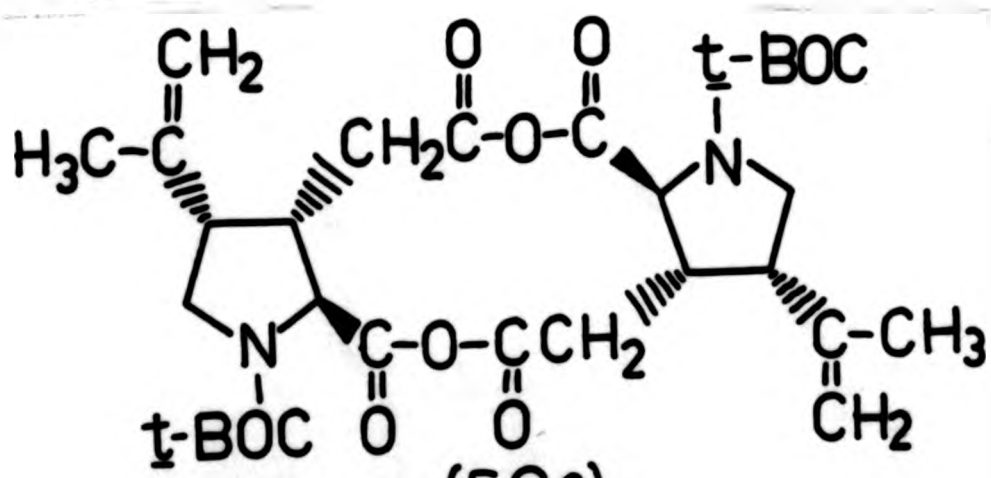
The structure of this anhydride intermediate (52) has eluded identification by us; evidence from ^{13}C NMR shows an absence of acetyl $-\underline{C}H_3$ carbons, indicating that this intermediate is not a simple di- mixed anhydride; presumably the putative structure (52a) would be so strained as to be too unstable to be considered seriously, leading to the possibility that either a unimolecular species such as (52b), or a dimolecular type like (52c) is involved.



(52a)



(52b)



(52c)

Mass spectrometric analysis under EI or CI conditions fails to provide any clues, and the FAB technique requires the solvolysis of the material in glycerol, which simply produces a molecular ion at M/E 313 (*t*BOC-kainic acid). ^{13}C NMR at 90 or 400 MHz did not shed any further light on the problem, so at present the precise structure of (52) remains unknown.

Opening this intermediate anhydride in methanol does provide the best current method for production of the C-3 carboxy-monoester (53), (see Scheme 9), although the other monoester (45) is also produced in equal quantities, and the chromatographic separation of the two is difficult and tedious. If large quantities of (53) were required, some more efficient synthesis would clearly be necessary.

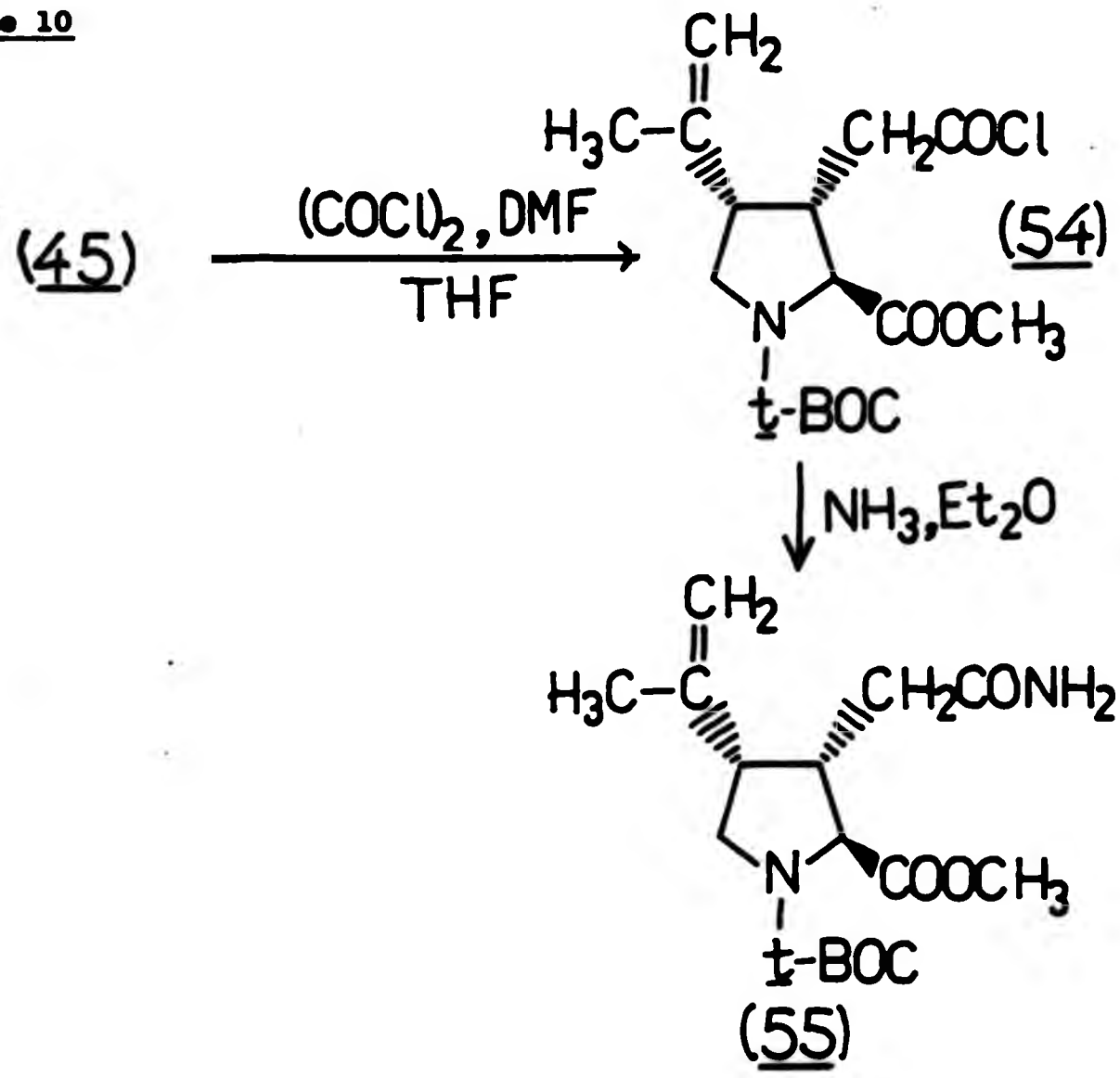
2.6.3 Functional Group Conversion: Acid to Nitrile

The C-3 carboxylic acid group was converted, via the amide[17], to a nitrile[18], thus furnishing a simple functional group conversion product.

2.6.3.1 Conversion of C-3 carboxylic acid to the Amide

The *t*-BOC monoester (45) was converted to the acid chloride (54) by the action of oxalyl chloride with a catalytic amount of DMF in THF; the intermediate acid chloride was not isolated, but converted directly to the amide (55) by evaporation of all solvent and excess reagent, the residues being dissolved in dry ether and ammonia bubbled through the solution. Column chromatography on silica gave the pure amide in around 90% yield; see Scheme 10.

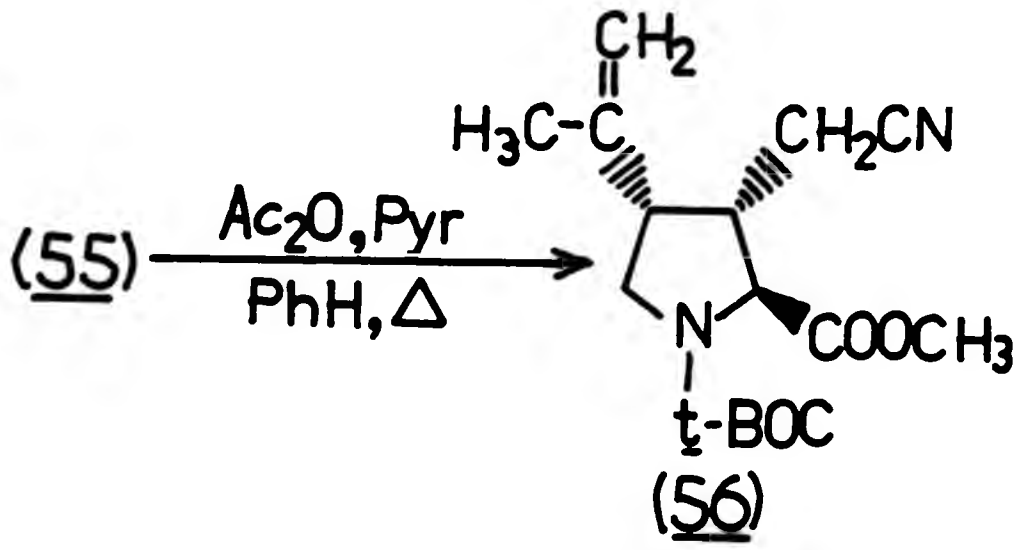
Scheme 10



2.6.3.2 Conversion of Amide to Nitrile

The amide was converted to the nitrile by refluxing in dry benzene with excess acetic anhydride and one equivalent of pyridine (the latter to consume any by-product acetic acid). Chromatography on silica afforded the required product (56) pure, in approximately 90% yield. Examination of ^{13}C NMR showed a characteristic nitrile peak at δ119 (with concurrent loss of one of the carboxy signals visible with (45) and (55)). The methylene carbon adjacent to the new nitrile group exhibited a considerable upfield movement on the spectrum from δ33.99 to δ17.42; see Sche ; 11.

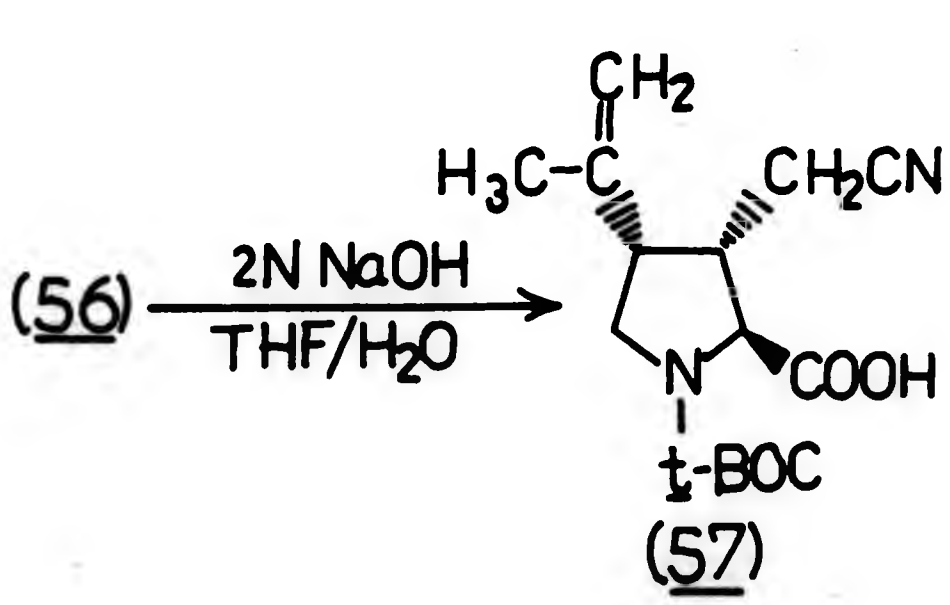
Scheme 11



2.6.3.3 Deprotection of nitrile; Removal of esters

The removal of methyl ester protecting groups applied not only to the nitrile (56), but also to all other kainate intermediates which required removal of the esters blocking the carboxylic acid functional group(s). The general method[19] employed was to dissolve the intermediate (in this case (56)) in THF, and to add an excess of 2N sodium hydroxide; the mixture was stirred vigorously overnight to ensure complete deprotection; yield after an extractive work-up was 90%. See Scheme 12.

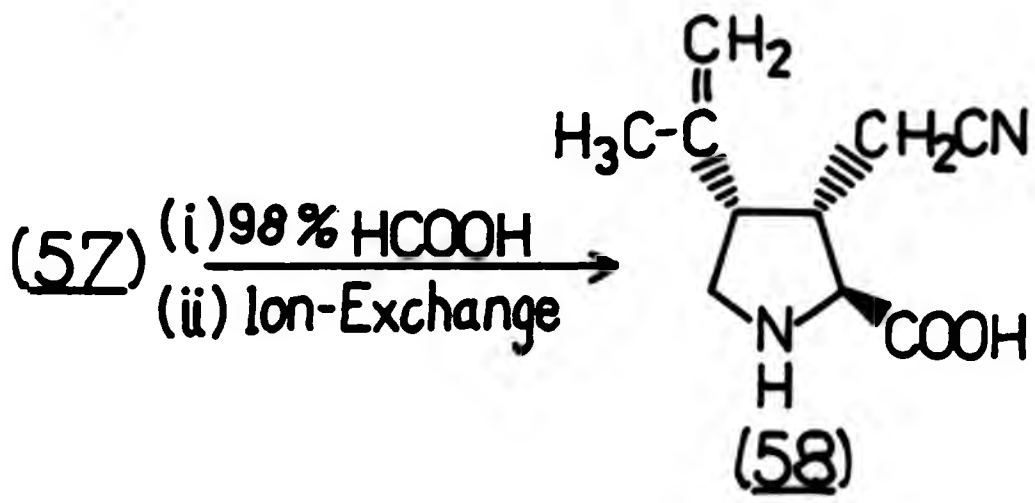
Scheme 12



2.6.3.4 Deprotection of nitrile; Removal of *t*-BOC

Two methods of removing the *t*-BOC group were employed at varying times; initially, trifluoroacetic acid at 0°C for 3 hours[20] was used; later a literature search suggested that 98% formic acid at room temperature overnight[21] was a rather milder reagent. Formate thus became the reagent of choice for the removal of the *t*-BOC group. Following the use of formic acid to effect the deprotection at nitrogen, the residues were ion-exchanged on Dowex W50X8 (H^+) resin to yield the completely deprotected derivative (58), in 90% yield from (57), as per scheme 13.

Scheme 13



Interestingly, removal of this product from the ion-exchange resin required elution with a more concentrated ammonia solution than normally necessary for non-nitrile kainate compounds; presumably the nitrile nitrogen must be able to assume sufficient quaternary character to contribute significantly towards the molecules' binding to the anion exchange resin. NMR and FAB mass spectroscopy confirmed the product structure.

2.6.4 Amide Couplings Through the C-3 Carboxyl

The availability of the selectively esterified C-2 monoester (45) was exploited to provide a range of compounds where the exposed C-3

carboxylic acid was incorporated into an amide (peptide) linkage[17], thus creating "Kainyl-R" dipeptides, where R is the new moiety introduced.

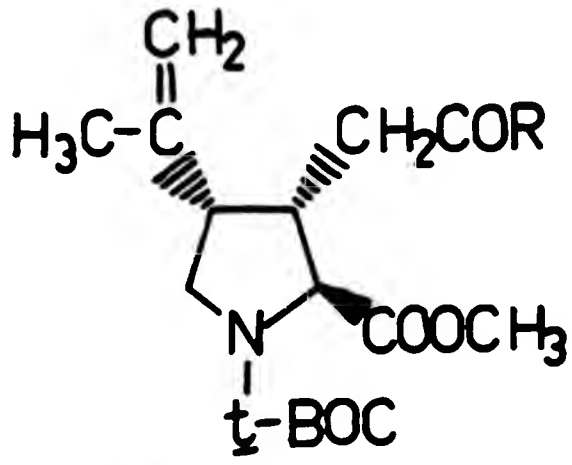
2.6.4.1 Cyclic amide adducts

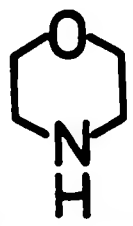
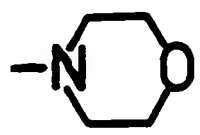
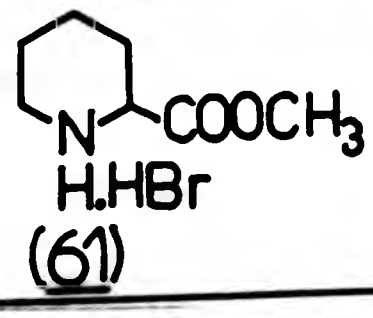
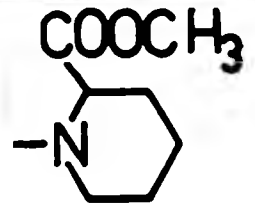
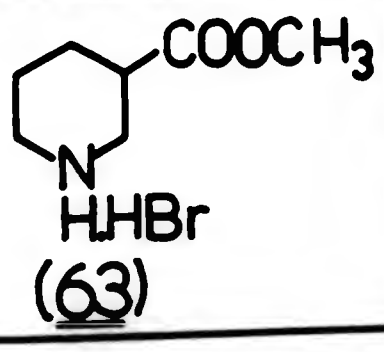
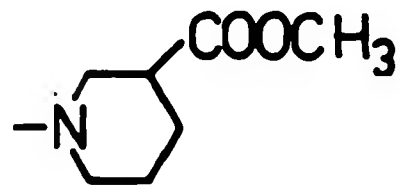
Initial amide production efforts were directed towards the coupling of monoesterified *a*-N-*t*-BOC kainate (45) with cyclic (secondary) amines corresponding to a range of ω-amino carboxylic acids; the cyclic compounds represent rigid conformers of the freely-rotating straight-chain types.

All individual analogues were synthesised according to the same general pattern; the acid chloride (54), (formed from (45) by the action of oxalyl chloride and DMF in THF) was dissolved in THF and an excess of pyridine added. The moiety to be added was then introduced in an appropriately-protected form and the resulting mixture refluxed for as long as required to complete the reaction (checking progress by consumption of starting material on TLC), and the reaction mixture given an extractive work-up followed by silica chromatography; yields of products varied from 33-75%. Table 2 summarises the various peptide coupling reactions carried out using the cyclic adducts.

Table 2.

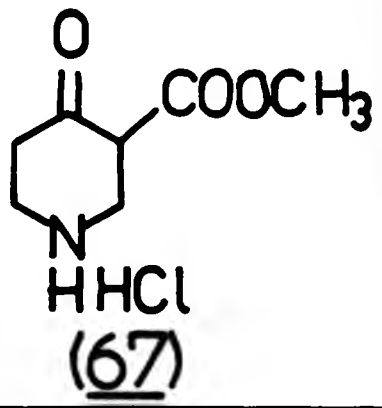
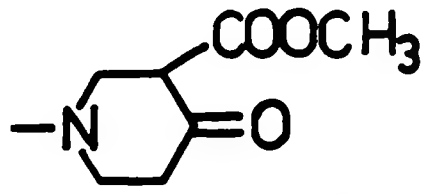
α-series



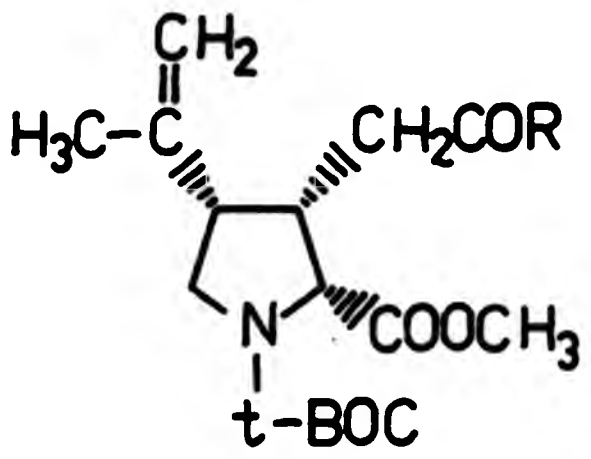
Adduct Reagent	Yield	R
 (59)	64%	 (60)
 (61)	74%	 (62)
 (63)	33-45%	 (64)
(65)	72%	 (66)

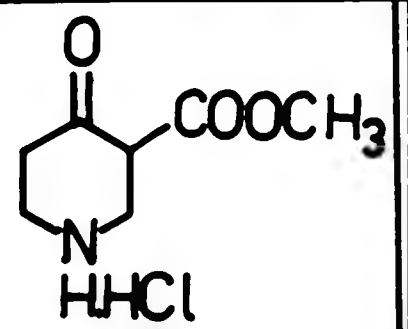
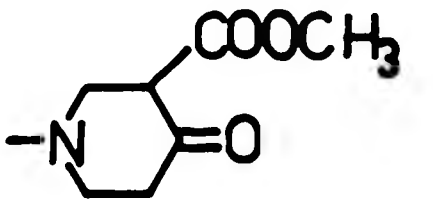
(Contd.)

Table 2 (Contd.)

Adduct Reagent	Yield	R
 (67)	41%	 (68)

β-series

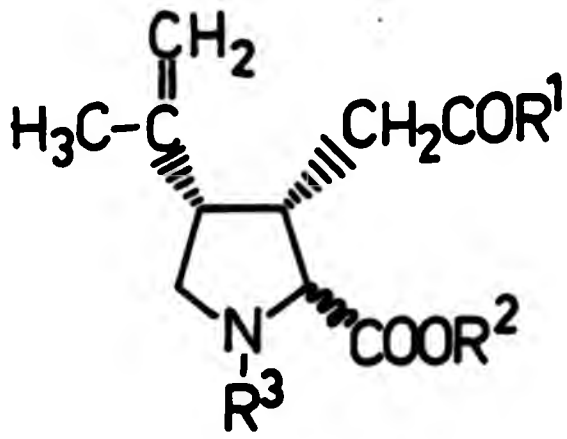


Adduct Reagent	Yield	R
 (67)	45%	 (69)

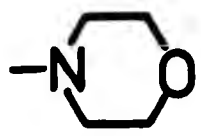
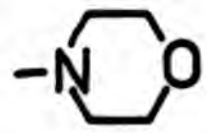
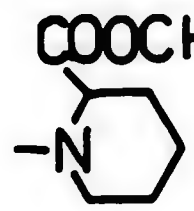
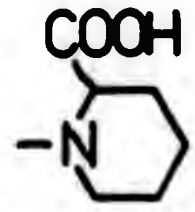
2.6.4.2 Deprotection of cyclic amide adducts

Having completed the purification of the amide coupling products, all were subsequently deprotected, firstly to remove all methyl ester groups present[19], and subsequently to remove the N-protecting *t*-BOC moiety[21]. De-esterification was performed by the action of a mixture of 2N sodium hydroxide and THF, stirred at room temperature overnight. After an extractive work-up, the de-esterified intermediates were treated with 98% formic acid overnight at room temperature to remove the *t*-BOC group. The products were then purified using ion-exchange. Table 3 summarises this deprotection process.

Table 3.

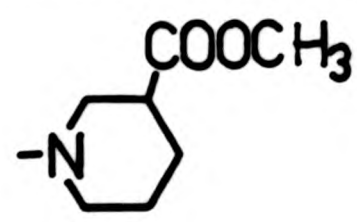
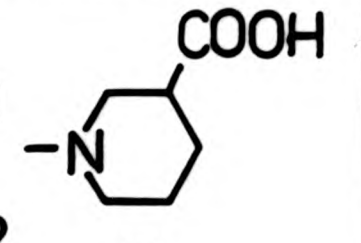
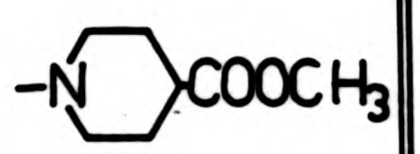
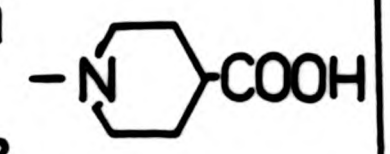
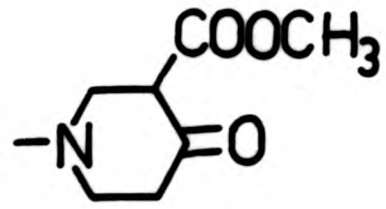
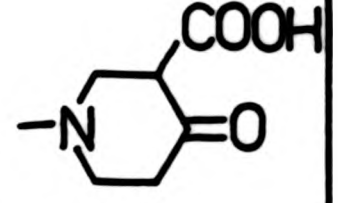


α-Series

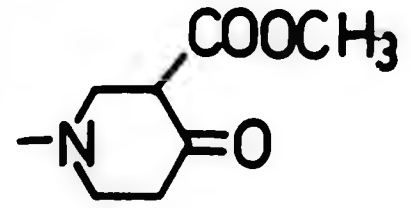
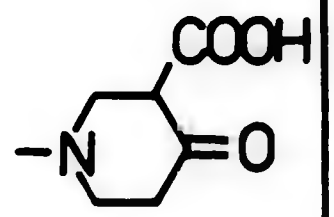
No.	Protected R^1, R^2, R^3	Yield	Deprotected R^1, R^2, R^3
(60)	R^1 =  R^2 = $-CH_3$ R^3 = -t-BOC	60%	R^1 =  R^2 = -H R^3 = -H (70)
(62)	R^1 =  R^2 = $-CH_3$ R^3 = -t-BOC	87%	R^1 =  R^2 = -H R^3 = -H (71)

(Contd.)

Table 3 (Contd.)

No.	Protected R^1, R^2, R^3	Yield	Deprotected R^1, R^2, R^3
(64)	R^1 =  R^2 = $-CH_3$ R^3 = $-t$-BOC	89%	R^1 =  R^2 = $-H$ R^3 = $-H$ (72)
(66)	R^1 =  R = $-CH_3$ R^3 = $-t$-BOC	91%	R^1 =  R^2 = $-H$ R^3 = $-H$ (73)
(68)	R^1 =  R^2 = $-CH_3$ R^3 = $-t$-BOC	77%	R^1 =  R^2 = $-H$ R^3 = $-H$ (38)

β-Series

No.	Protected R^1, R^2, R^3	Yield	Deprotected R^1, R^2, R^3
(69)	R^1 =  R^2 = $-CH_3$ R^3 = $-t$-BOC	83%	R^1 =  R^2 = $-H$ R^3 = $-H$ (39)

2.6.4.3 Assessment of Cyclic Amide Addition Products

With the exception of the kainyl-morpholine adduct (70), all the cyclic amide adducts represent the combination of kainic acid with conformationally rigid analogues of ω-amino carboxylic acids. Thus, (71) corresponds to glycine, (72), to β-alanine, (73) to γ-aminobutyric acid (GABA) and (68) and (69) to 2-methyl-keto-β-alanine. While such a series would naturally provide a certain amount of structure-activity relationship data, (not just between the analogues of this series, but also to straight-chain ω-amino carboxylic acid adducts (qv)), two of the peptides synthesised correspond to the introduction of kainate coupled to conformationally rigid versions of known inhibitory transmitters[22]. Thus, (71) could be looked upon as a form of kainyl-glycine, and (73), kainyl-GABA.

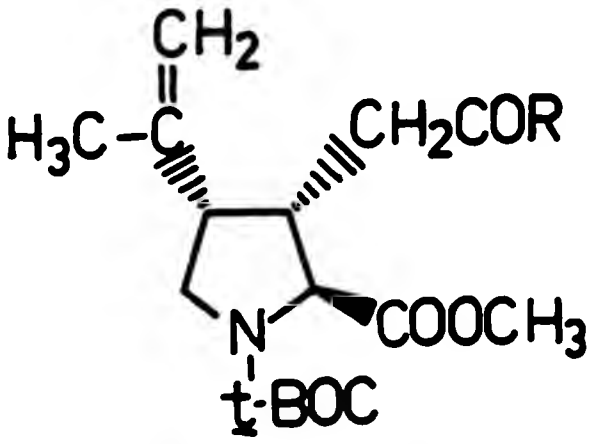
The pharmacological assessment of the validity of the notion that introducing compounds related to inhibitory transmitters might prove to be potentially interesting will be discussed in later sections.

2.6.4.4 Straight-Chain Amide Adducts

As part of an extensive series of peptide coupling syntheses by group members, three straight-chain amide adducts were prepared as part of this project. Two were simply ω-amino carboxylic acids, while the third, which involved coupling kainate with 2-bromoethylamine was intended to provide an intermediate with a leaving group replaceable by an alternative acid function (*e.g.* phosphonate, sulphonate); thus eventually obtaining the phosphonate or sulphonate analogue of kainyl-β-alanine. Although the intended replacement of the bromide leaving group was not successfully realised during the course of this project, it will still form a potentially interesting class of compounds for pharmacological investigation.

In common with the cyclic amide adducts described in 2.6.4.1, the straight-chain variants were synthesised by combination of the α-N-*t*BOC-kainyl acid chloride (54) with the appropriately-protected amines in THF and excess pyridine, refluxing as necessary to ensure completion of the reaction. The addition products were isolated by an extractive work-up followed by silica chromatography. Table 4 illustrates the materials used and yields obtained.

Table 4.

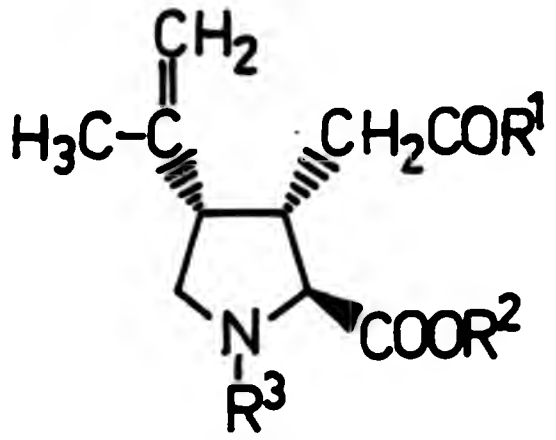


Adduct Reagent	Yield	R
$HCl.H_2N(CH_2)_3COOCH_3$ (74)	28-30%	$-HN(CH_2)_3COOCH_3$ (75)
$HCl.H_2N(CH_2)_4COOCH_3$ (76)	50%	$-HN(CH_2)_4COOCH_3$ (77)
$HBr.H_2N(CH_2)_2Br$ (78)	40%	$-HN(CH_2)_2Br$ (79)

2.6.4.5 Deprotection of Straight-Chain Amide Adducts

Deprotection of the straight-chain adducts was effected by first removing any methyl esters in the molecule by the action of dilute aqueous sodium hydroxide and THF[19], followed by removal of the N-protecting group using 98% formic acid[21], followed by ion-exchange chromatography. Although both the acid adducts were treated in this way, the bromide (79) was retained in a fully-protected state for possible conversion to a sulphonate or phosphonate. Table 5 gives comparative details of such deprotections as were carried out.

Table 5.

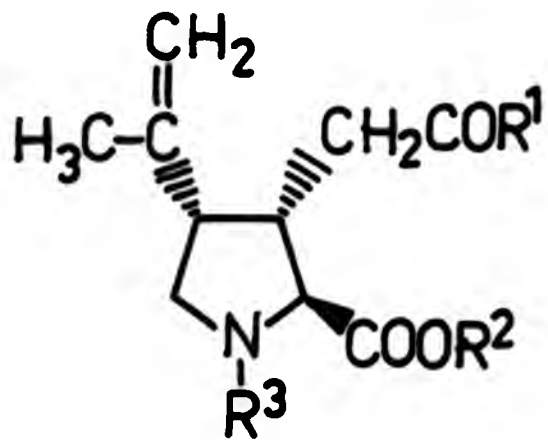


No.	Protected R^1, R^2, R^3	Yield	Deprotected R^1, R^2, R^3
(75)	R^1 -HN$(CH_2)_3$COOCH$_3$ R^2 -CH_3 R^3 -t-BOC	81%	R^1 -HN$(CH_2)_3$COOH R^2 -H R^3 -H (80)
(77)	R^1 -HN$(CH_2)_4$COOCH$_3$ R^2 -CH_3 R^3 -t-BOC	65%	R^1 -HN$(CH_2)_4$COOH R^2 -H R^3 -H (81)

2.6.4.6 Branched Chain Amide Adducts

α-kainyl-L-glutamate was prepared by the addition of L-glutamate dimethylester hydrobromide to a solution of the α-N-*t*-BOC kainyl acid chloride (54) in THF with an excess of pyridine. Subsequent deprotection of the methyl esters with dilute base in aqueous THF and removal of the *t*-BOC group by action of 98% formic acid, followed by ion-exchange chromatography afforded the product (84). Table 6 summarises details of the coupling and subsequent deprotection reactions.

Table 6.



Adduct Reagent	Yield	R^1, R^2, R^3
$HBr.H_2N-CH(CH_2)_2COOCH_3)COOCH_3$ (82)	61%	R^1-HN-CH($(CH_2)_2COOCH_3$)$COOCH_3$ R^2 $-CH_3$ R^3 -t-BOC (83)

(Contd.)

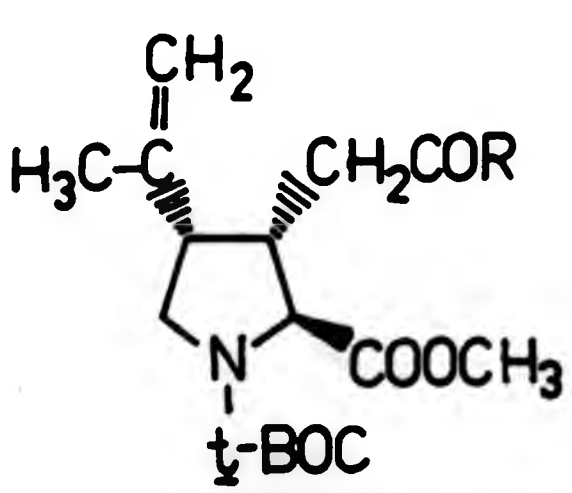
Table 6 (Contd.)

Protected R^1, R^2, R^3	Yield	Deprotected R^1, R^2, R^3
R^1 = $-HN-CH(CH_2)_2COOCH_3$ / $COOCH_3$	77%	R^1 = $-HN-CH(CH_2)_2COOH$ / $COOH$
R^2 = $-CH_3$		R^2 = $-H$
R^3 = $-t$-BOC (83)		R^3 = $-H$ (84)

2.6.4.7 Potential Exploitation of Amide Adducts

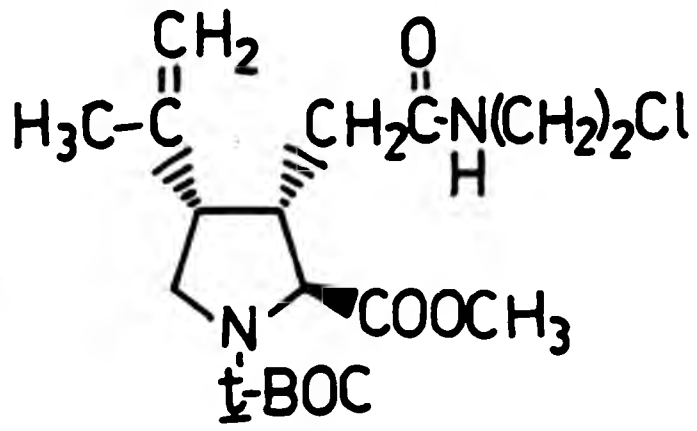
The adducts incorporating one or more new carboxylic acid groups attached at the kainate C-3 carboxyl were intended to be assessed pharmacologically in their own right, such investigation providing structure-activity data amongst these related compounds in addition to any potential therapeutic value discovered. The kainyl-aminoethyl-bromide adduct (79), however, was intended merely as an exploitable first step in producing kainate-aminoethanesulphonate (85) and kainate-aminoethanephosphonate (86); see Fig. 4.

Fig. 4



R= $-NH(CH_2)_2SO_3H$ (85)
$-NH(CH_2)_2PO_3H_2$ (86)

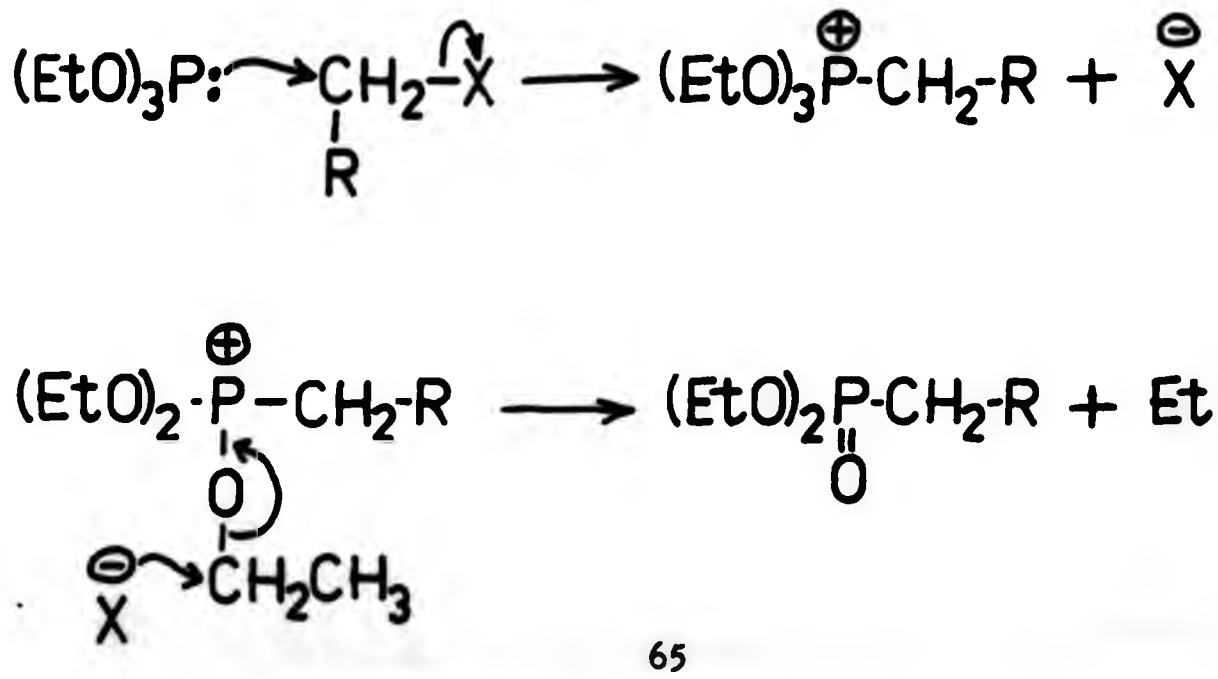
The kainyl-aminoethylbromide adduct (79) was refluxed with triethylphosphite (Arbuzov reaction)[23]; this was intended to yield the protected phosphonate derivative, but was not successful. It is possible that the choice of bromide as the leaving group was the basis of the problem; literature research indicated that the requirement for a successful Arbuzov reaction is a good nucleophile (rather than a good leaving group)[24] and hence the reactivity series runs Cl>Br>I; therefore the synthesis of kainyl-aminoethylchloride (87) would have been of more use in providing a suitable starting material.



(87)

Additionally, steric hindrance from the C-4 side chain could have prevented the approach of the triethylphosphite to the methylene group; see Scheme 14.

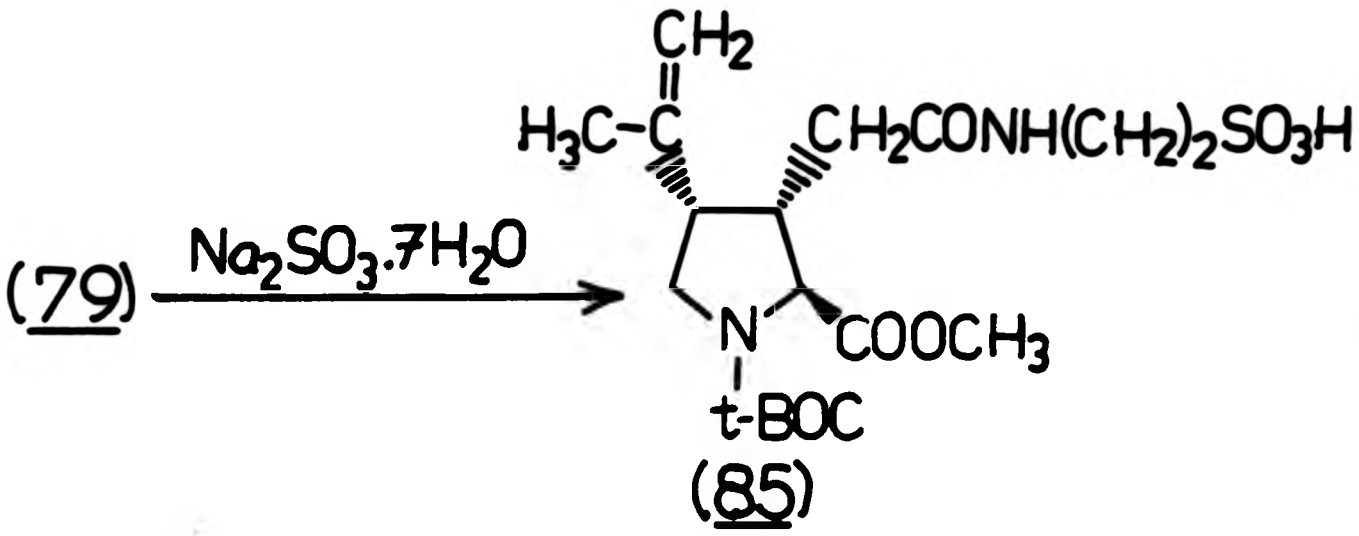
Scheme 14



Before any further investigation along these lines could be carried out, an alternative approach to the phosphonate proved successful; kainate was coupled directly to diethyl-aminoethylphosphonate and the phosphonate esters subsequently removed[25].

An attempt to synthesise the kainyl-aminoethane sulphonate (85) by reaction of the aminoethylbromide adduct (79) with sodium sulphite[26] (see Scheme 15) was also unsuccessful, as were attempts to preform a suitably protected sulphonate derivative and then couple that to the intermediate kainyl acid chloride (54).

Scheme 15

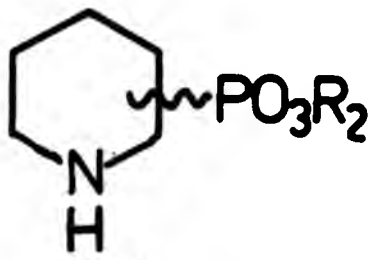


2.6.4.8 Attempted Synthesis of Cyclic Adducts Incorporating Phosphonate or Sulphonate Groups

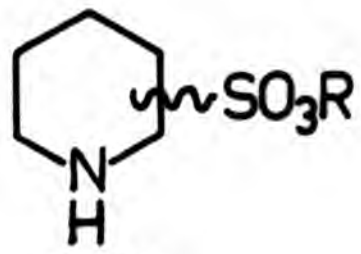
In addition to the ultimately unsuccessful series of reactions directed towards kainate adducts incorporating straight chain amino-alkyl sulphonates or phosphonates (analogous to the straight chain amino-alkyl carboxylic acid adducts that were synthesised successfully), some efforts were directed towards producing cyclic amines with phosphonate (88) or sulphonate (89) groups attached to the ring; see Fig. 5. The R group(s) correspond to some suitable protecting group(s) to enable these intermediates to be coupled to the kainyl acid chloride (54). Such cyclic amide adducts would then correspond to the series of cyclic

carboxylic acid adducts synthesised previously; (71), (72) and (73).

Fig. 5



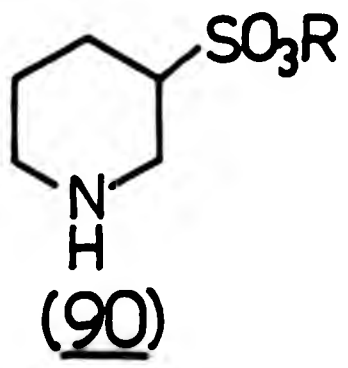
(88)



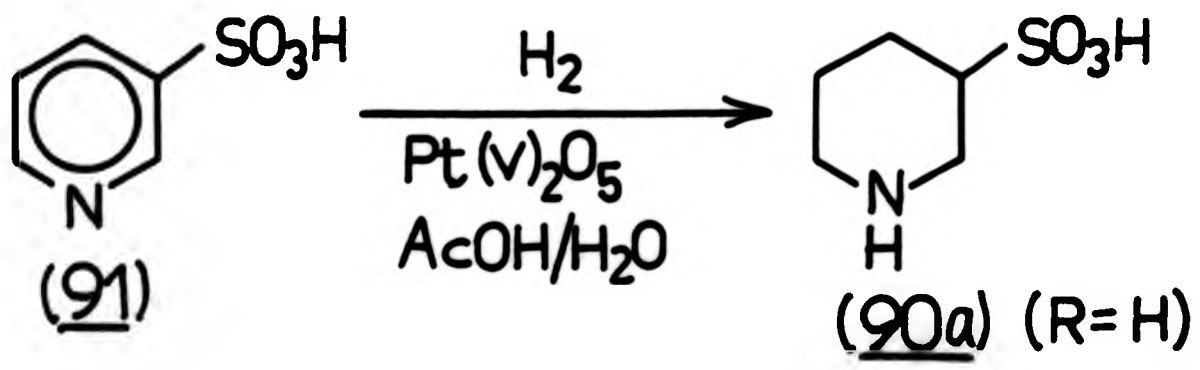
(89)

Limited attempts to produce a protected version of 3-sulphonyl-piperidine (90) were based on the use of 3-sulphonyl-pyridine (91). We were unable to esterify this material, although it was possible to reduce it, using hydrogenation on a platinum catalyst[27], to 3-sulphonyl piperidine (90a), as per Scheme 16. However, subsequent attempts to N-protect with a carboxy-benzyl group[10] preparatory to attempting an esterification of the sulphonate also proved unproductive.

Scheme 16



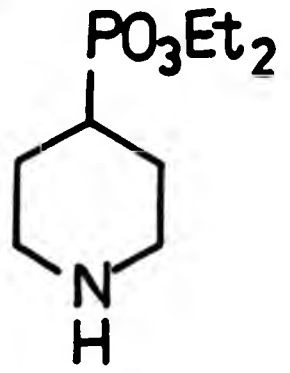
(90)



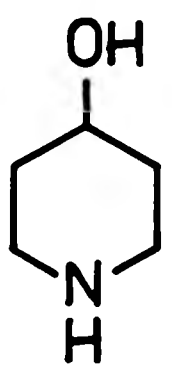
(91) (90a) (R=H)

Greater success attended the initial stages of an attempted synthesis

of diethyl 4-phosphonyl piperidine (92) from 4-hydroxy piperidine (93).



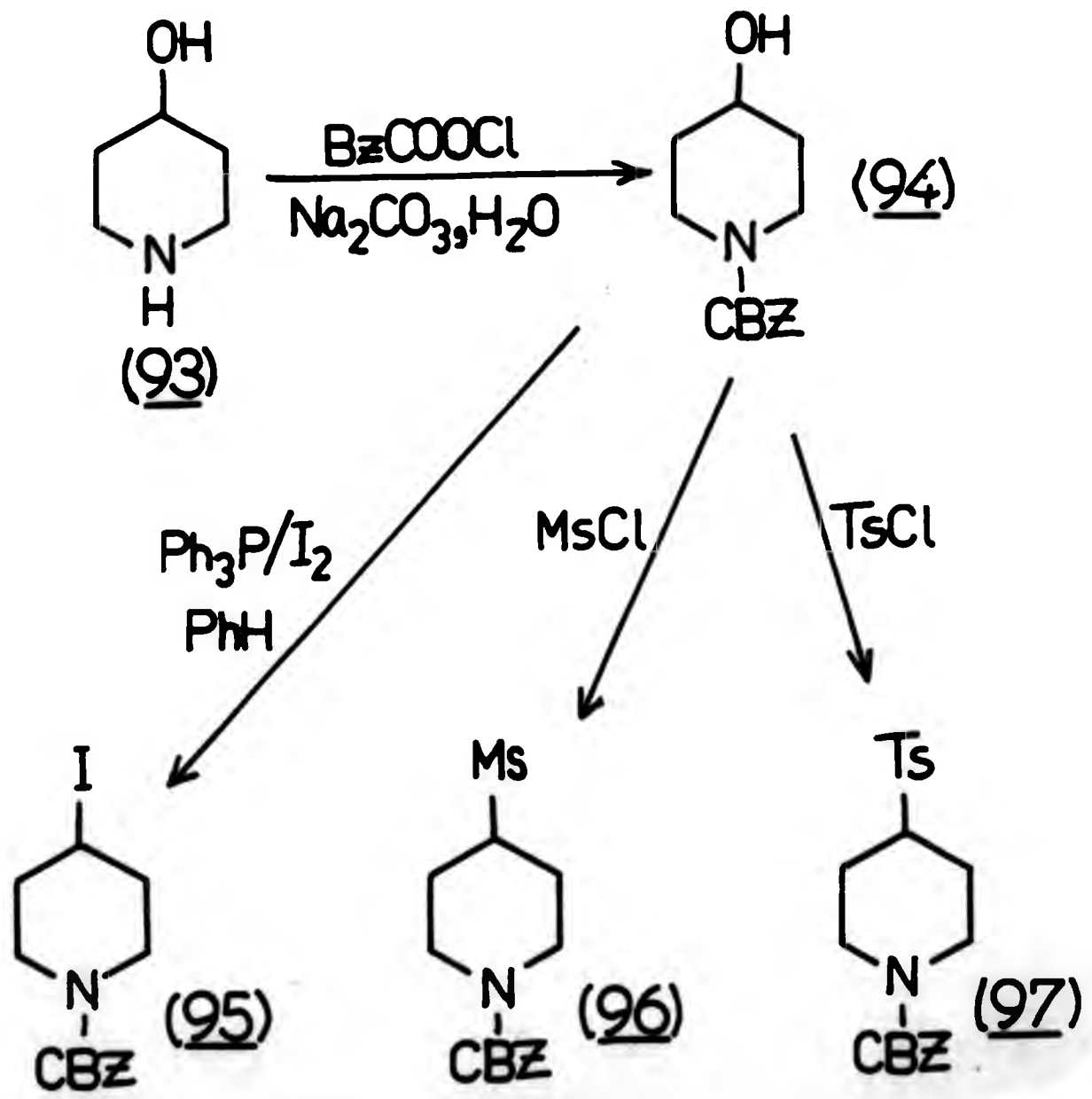
(92)



(93)

The hydroxyl compound (93) was first converted to the N-CBZ protected material (94); this could then be converted to the iodide (95)[28], mesylate (96)[29] or tosylate (97)[30]; see Scheme 17.

Scheme 17

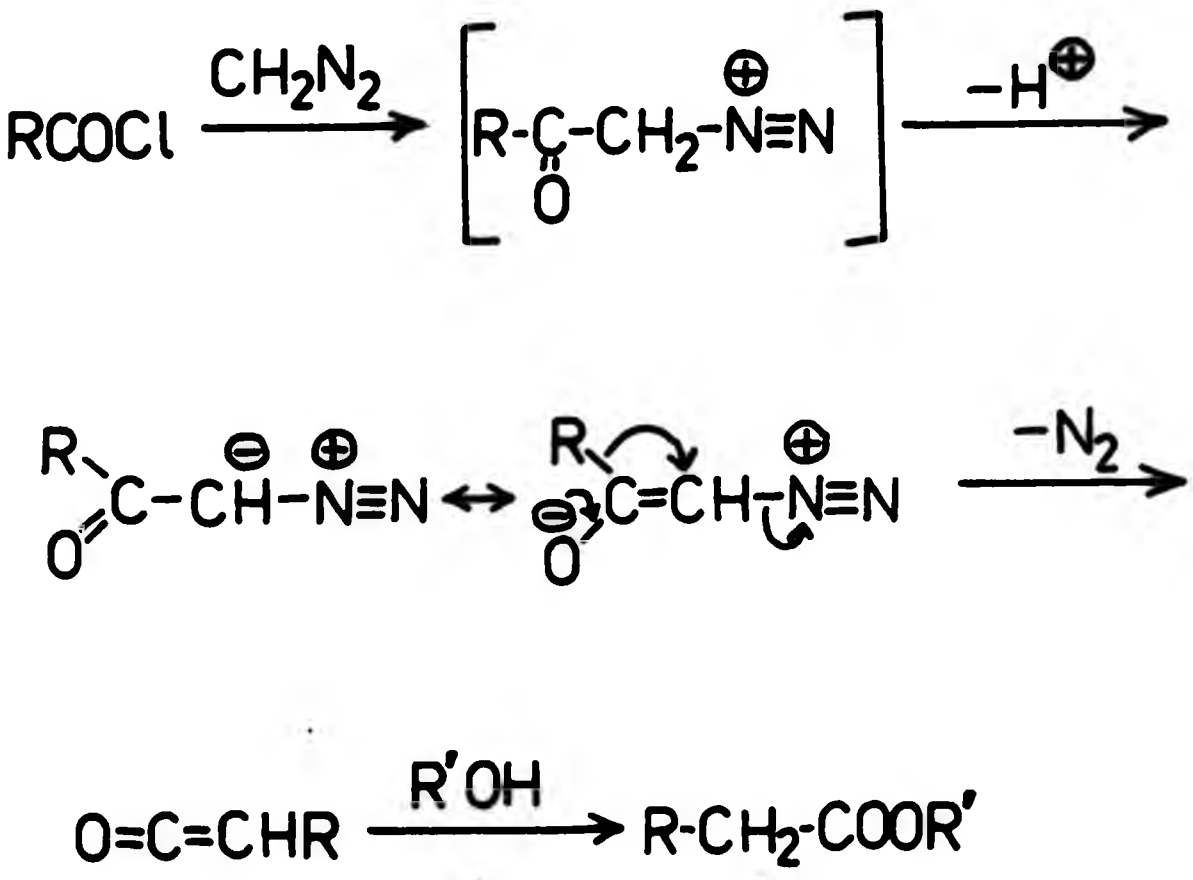


However, replacement of any of these groups with phosphonate, either by Arbuzov reaction (with the iodide) or use of sodium diethyl phosphite (with the mesylate or tosylate) could not be successfully achieved.

2.6.5 Chain Extension at C-3; Arndt-Eistert Reaction

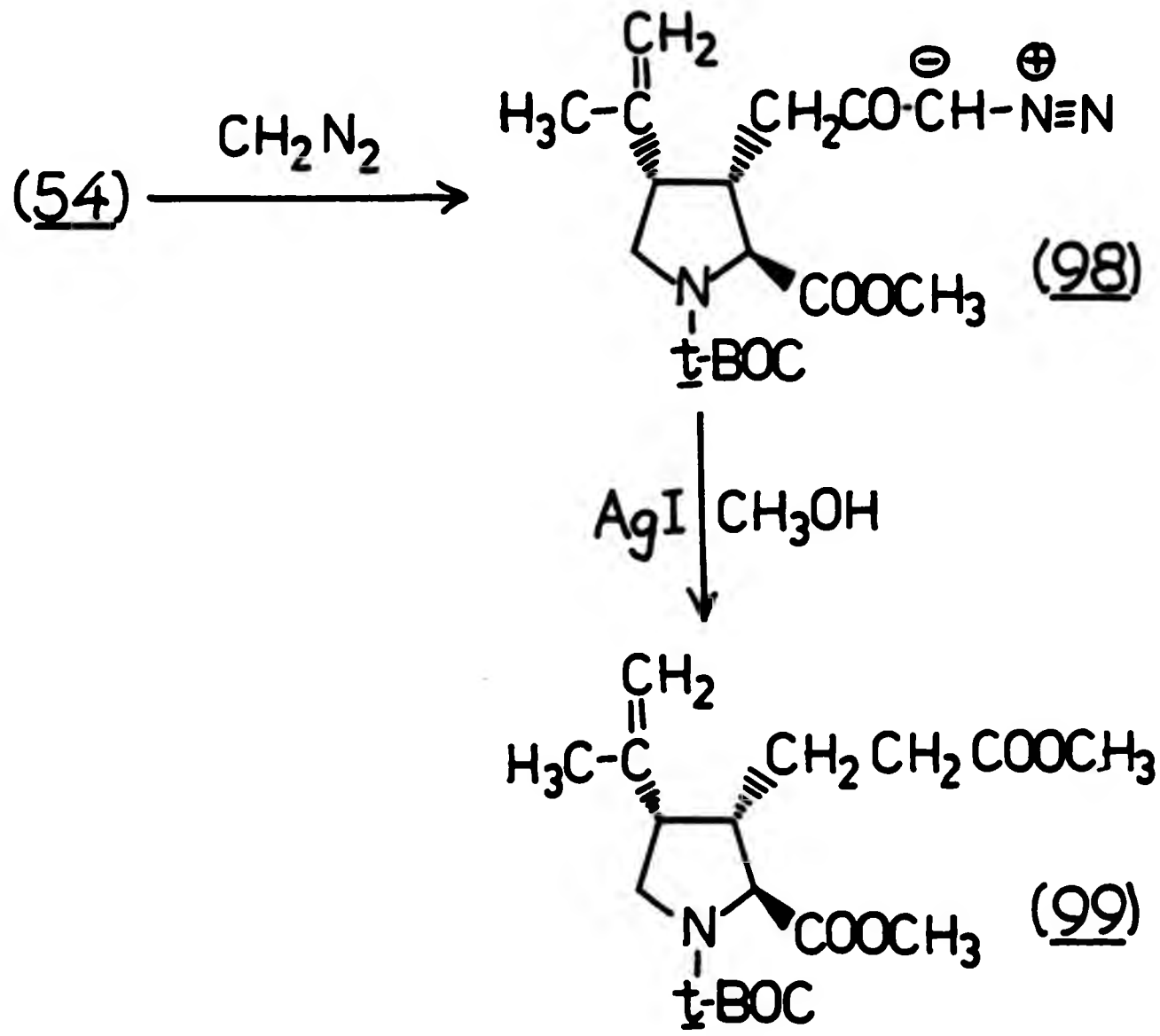
The success of the selective mono-esterification process at the C-2 carboxyl prompted attempts to homologate the C-3 side chain by an insertion of methylene group(s), using the Arndt-Eistert reaction, as outlined in Scheme 18.

Scheme 18



The acid chloride (54) was treated with a three-fold excess of ethereal diazomethane; the resulting diazoketone intermediate (98) was not isolated, but treated with silver (I) oxide in methanol in an attempt to furnish the rearranged, C-3 chain extended homo-kainate dimethyl ester (99); (Scheme 19).

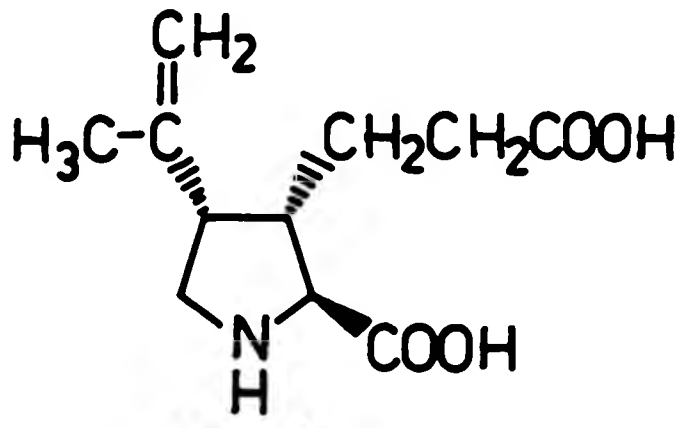
Scheme 19



However, examination of the ^{13}C NMR of the isolated product indicated the presence of small quantities of unreacted diazoketone (98), (quaternary carbon peak at δ = 200.72 ppm, corresponding to the C=O ketone carbonyl). Separation of the rearranged desired product and the unrearranged intermediate by chromatography was not practicable, and further reaction with methanol and silver (I) oxide failed to completely eliminate the diazoketone.

Complementary work by other group members[25] showed that a more suitable reagent to effect the desired rearrangement of the diazoketone to the chain-extended diester is silver benzoate; using this material, the diazoketone could be completely reacted[32], and the homo-kainate diester thus formed deprotected by the normal sequence of dilute base

to remove the esters, 98% formic acid to remove the N-protecting group and then ion-exchange chromatography to purify the product (35d).



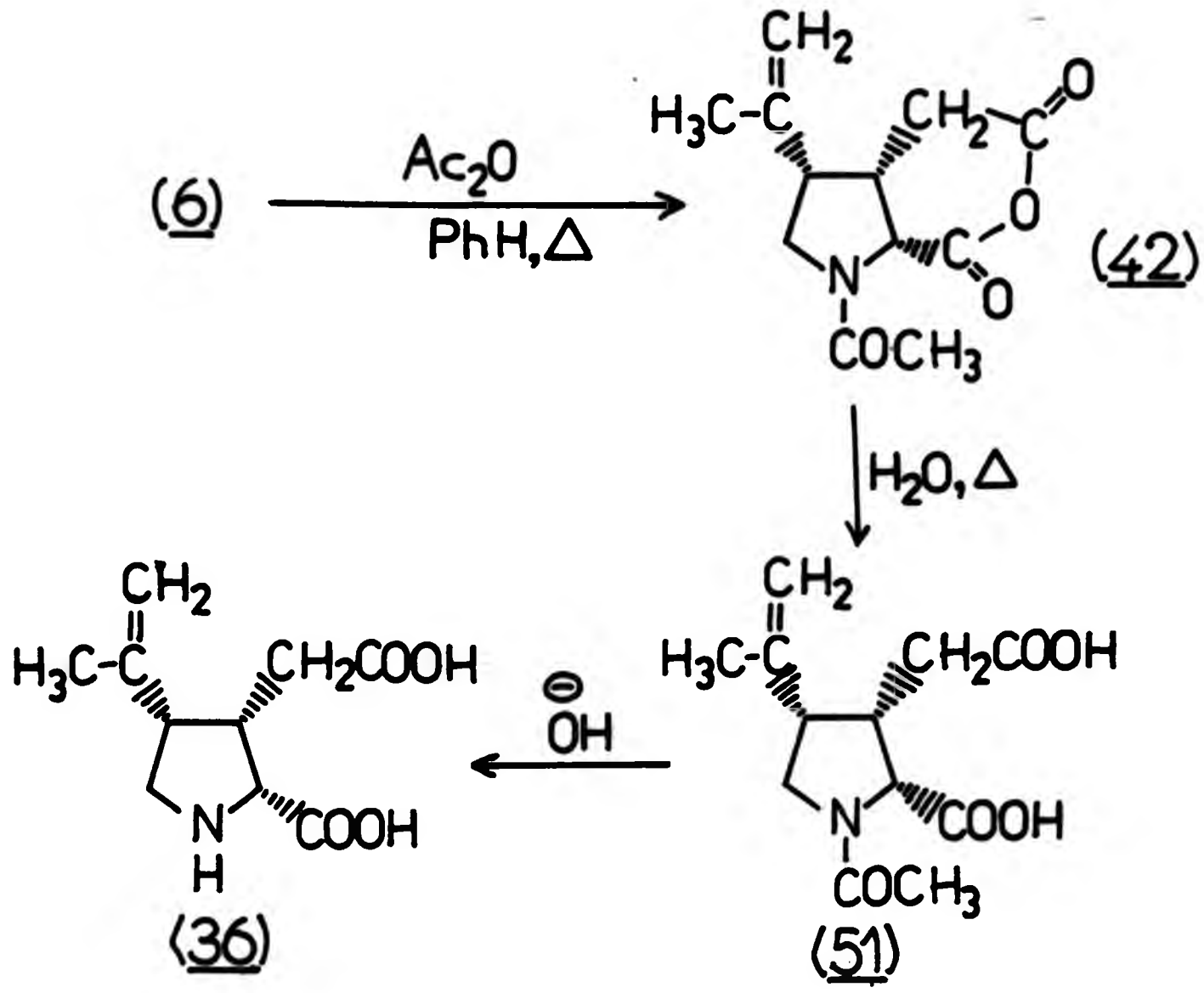
(35d)

2.6.6 Production of β-Kainic Acid

Japanese workers investigating kainic acid chemistry provided a simple and effective method for the conversion of α- into β-kainic acid[6]. *i.e.* inverting the stereochemical configuration at C-2; α-kainate (6) is suspended in dry benzene and refluxed with excess acetic anhydride; N-acetyl-β-kainate anhydride (42) is formed. Hydrolysis of (42) in water opens the anhydride to give N-acetyl-β-kainic acid (51), which is subsequently treated with aqueous potassium hydroxide to yield β-kainate (36) (Scheme 20). Significant differences are observed in the ^{13}C NMR and in the optical rotation of the α- and β-diastereoisomers.

The β-kainate thus formed provides the starting material for the synthesis of the β-analogues of any interesting α-compounds.

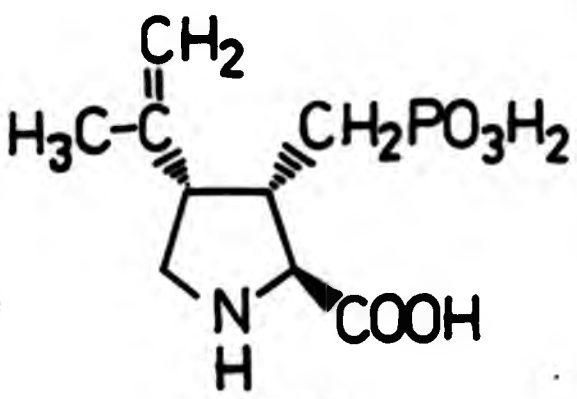
Scheme 20



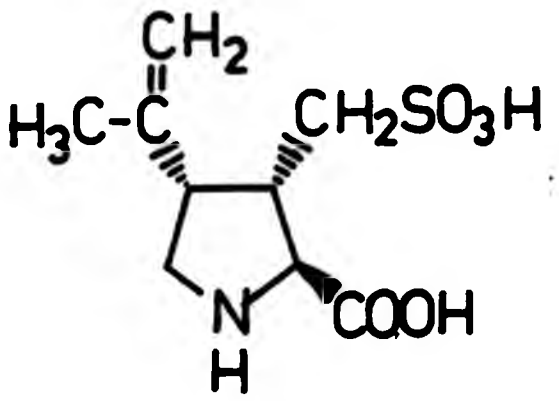
2.6.7 Carbon Chain-Shortening at C-3 Side Chain; Replacement of Carboxylic Acid Group with Other Acids

Pharmacologists pinpointed kainate analogues (35a) and (35b) as being of considerable potential interest; the replacement of the C-3 side-chain carboxylic acid with a phosphonic or sulphonic acid, whilst retaining the same overall chain length, would enable the effect of stronger acids at this position to be evaluated. It was noted that the potent A1 receptor antagonists 2-APP (15) and 2-APH (14) both contain the phosphonic acid grouping, and GAMS (25) contains the sulphonic acid group, indicating that such acids can possess considerable activity in biological systems[33].

The aim, then, was to attempt a synthesis of (35a) and (35b)



(35b)

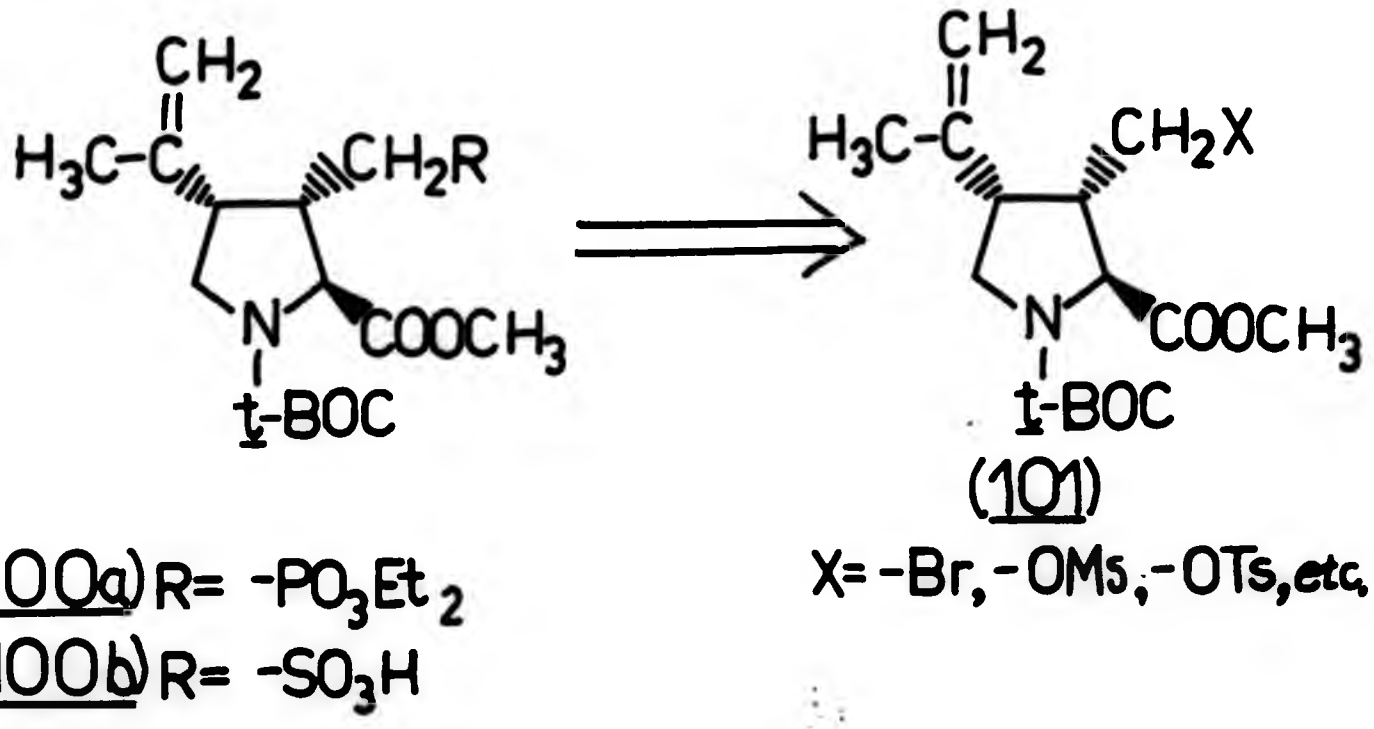


(35a)

2.6.7.1 Retrosynthetic Analysis; Possible Approaches to Chain-Shortening Reactions

A retro-synthetic analysis of the desired products (35a) and (35b) suggested that a suitably protected intermediate (101) was the logical precursor of the protected variant (100a, 100b) of the final products; (see Scheme 21).

Scheme 21

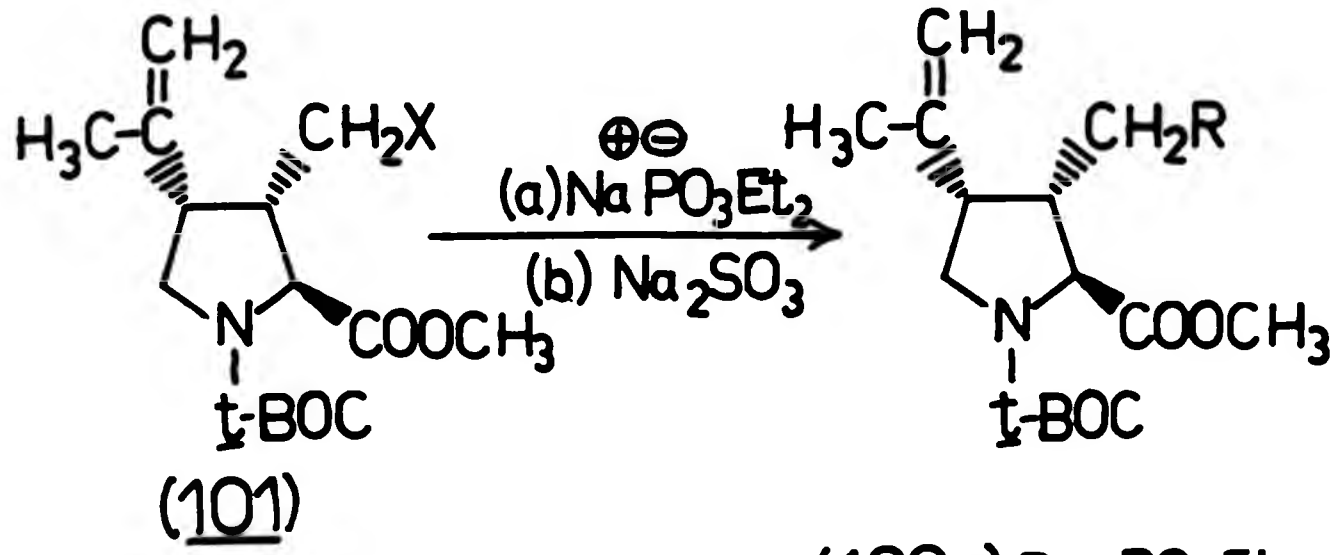


(100a) R= $-PO_3Et_2$
(100b) R= $-SO_3H$

(101)
X= -Br, -OMs, -OTs, etc.

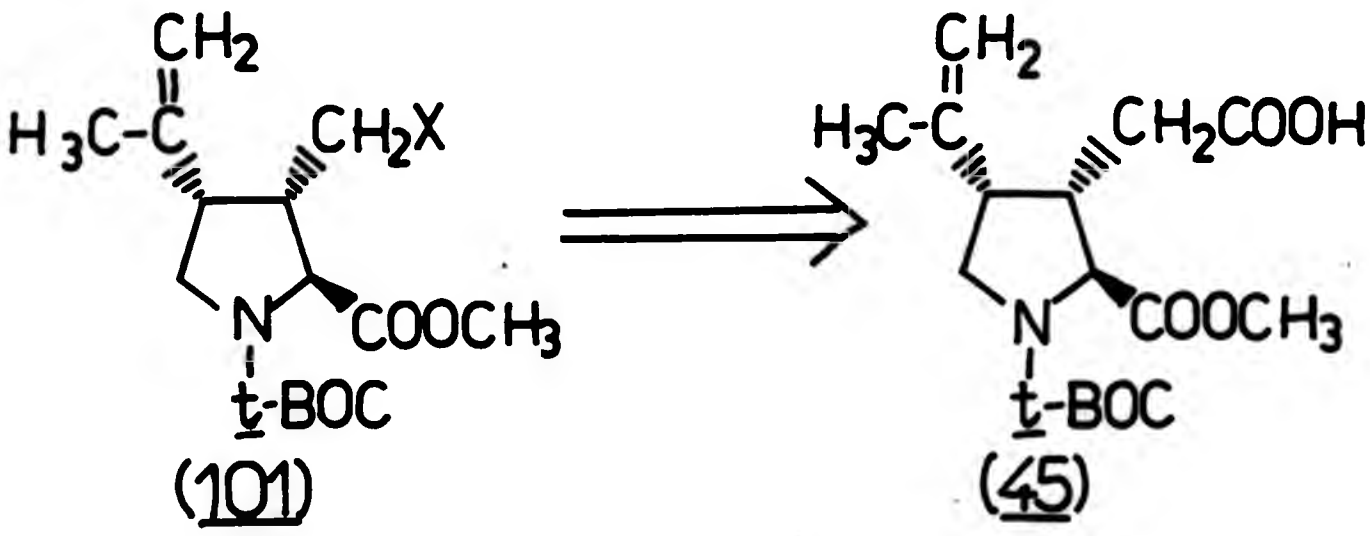
If such an intermediate as (101) could be synthesised, the replacement of the leaving group X by phosphonate or sulphonate might be effected by reaction with (a) sodium diethyl phosphonate or, (b), sodium sulphite, respectively; (Scheme 22).

Scheme 22



Retrosynthetically, the intermediate (101) should be obtainable from the carboxyl at C-3, either as the free acid or in some activated form. (Scheme 23).

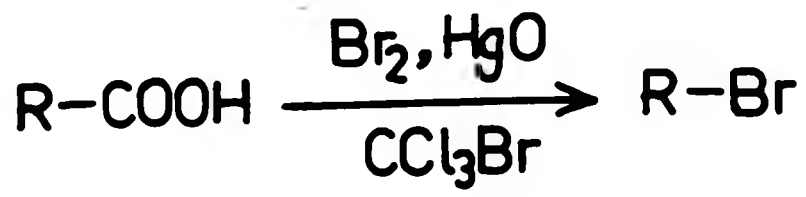
Scheme 23



2.6.7.2 Chain Shortening; Attempted Hunsdiecker Reaction

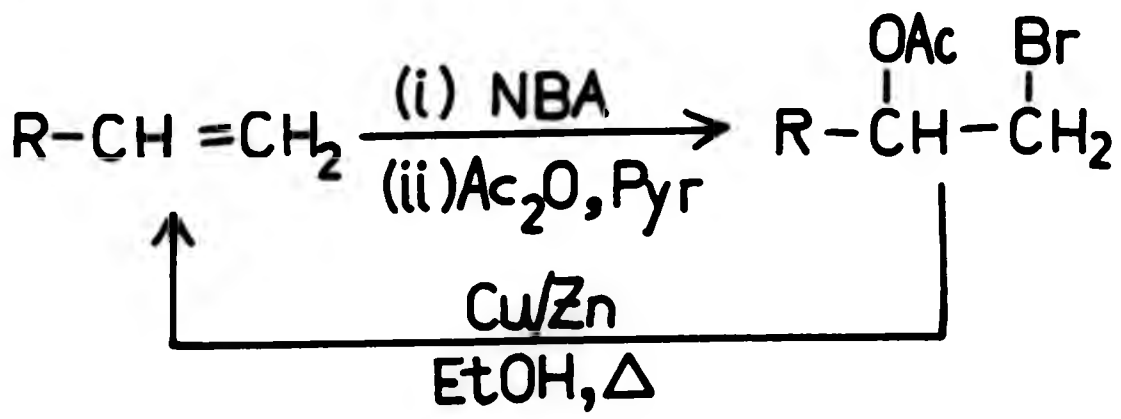
The conversion of the carboxyl intermediate (45) to the chain-shortened (101) is obviously the key step, involving, as it does, the removal of one carbon atom from the carbon skeleton. A literature search indicated that the transformation of a carboxylic acid to a bromide with one less carbon atom could be accomplished by the Hunsdiecker reaction[34]. (Scheme 24).

Scheme 24



Obviously, such a use of elemental bromine around the unprotected double bond would lead to an unwanted reaction at that site; therefore, the alkene moiety would require protection during the Hunsdiecker stage of the reaction sequence, and by preference the mode of protection should be such that it could be easily removed under mild conditions. Investigation of the appropriate literature suggested that one possible option for such a protecting group would be to form a bromohydrin acetate at the alkene site, which could be subsequently removed by re-fluxing in ethanol with a copper-zinc couple[35]; (Scheme 25 shows the general system).

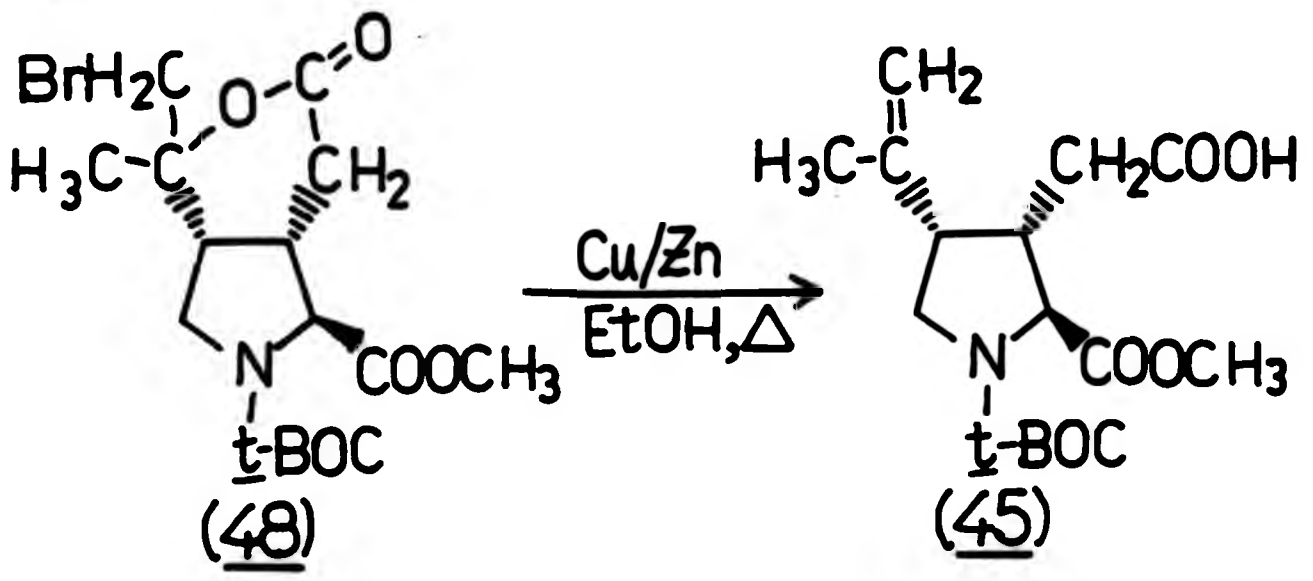
Scheme 25



As noted previously (see section 2.6.2.4), the reaction of the monoester (45) (unprotected at the C-3 carboxylic acid) with N-bromo-succinimide (NBS) led to an intra-molecular bromo-lactone ring formation, forming (48) in high yield. This clearly indicated that some form of temporary protection at the C-3 carboxylic acid group would be required whilst the bromo-hydrin formation was proceeding on the C-4 alkenyl side-chain. Additionally, however, the bromo-lactone (48) provides a

useful model for the copper-zinc couple method of re-forming the double bond; therefore, a preliminary experiment employing copper-zinc couple in refluxing ethanol to open the lactone (48) and re-form the double bond was made. (Scheme 26); a compound identical to the monoester (45) by TLC and ^{13}C NMR examination was formed in 74% yield.

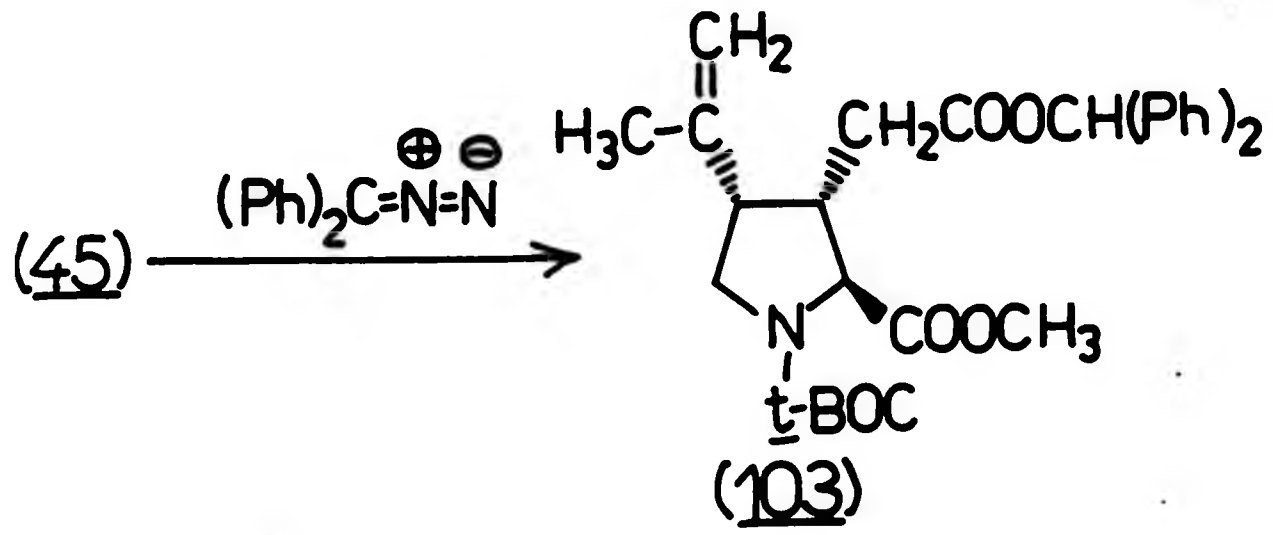
Scheme 26



With the utility of the copper-zinc couple method of re-forming the double bond thus proven, investigation proceeded into the selection of a protecting group for the C-3 carboxylic acid group.

The major requirement of such a protecting function was that the removal of same would not employ conditions that would simultaneously result in the deprotection of the methyl ester blocking the C-2 carboxyl. However, the fact that the alkenyl side chain would be protected before the deprotection of the C-3 carboxylic acid-blocking function meant that hydrogenation could be considered as a candidate method for deprotecting the C-3 carboxyl protecting group. Such a mild technique would not affect the C-2 methyl ester, the bromohydrin acetate planned for the C-4 side-chain, or indeed the *t*-BOC group protecting the amine function. Thus, attention was focused on potential protecting groups removable by hydrogenation; the first such to be tried was the benzhydryl (diphenyl methyl) type, formed by the reaction of the monoester (45) with diphenyldiazomethane[14] (Scheme 27).

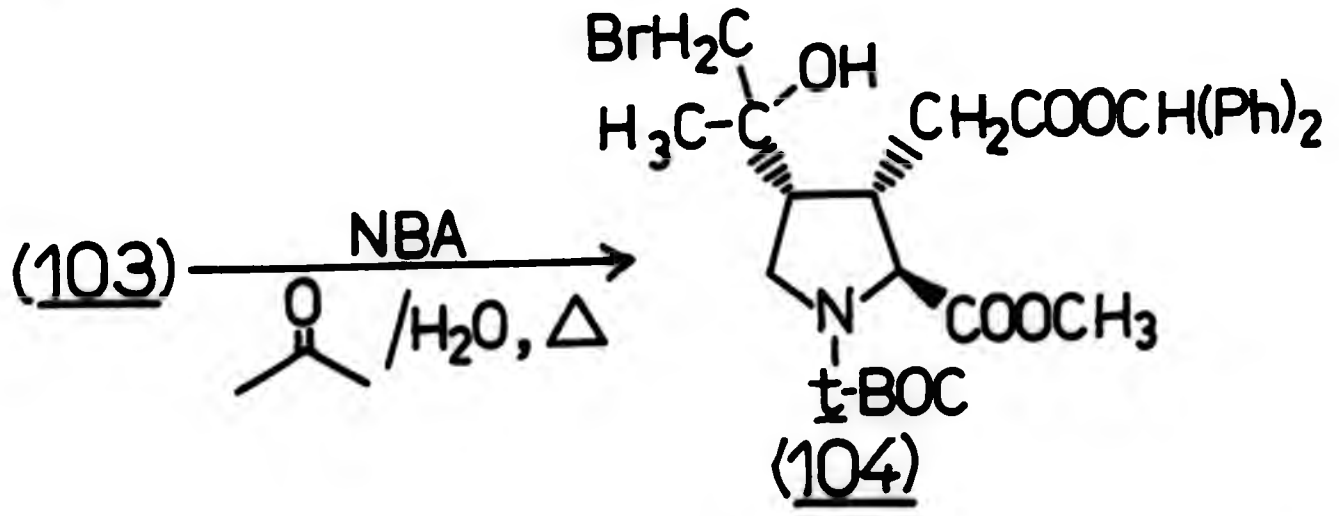
Scheme 27



The mixed diester (103) would not react with N-bromo succinimide to form the bromo-hydrin (presumably the steric hindrance by the phenyl groups obstructed the approach of the reagent to the alkene site).

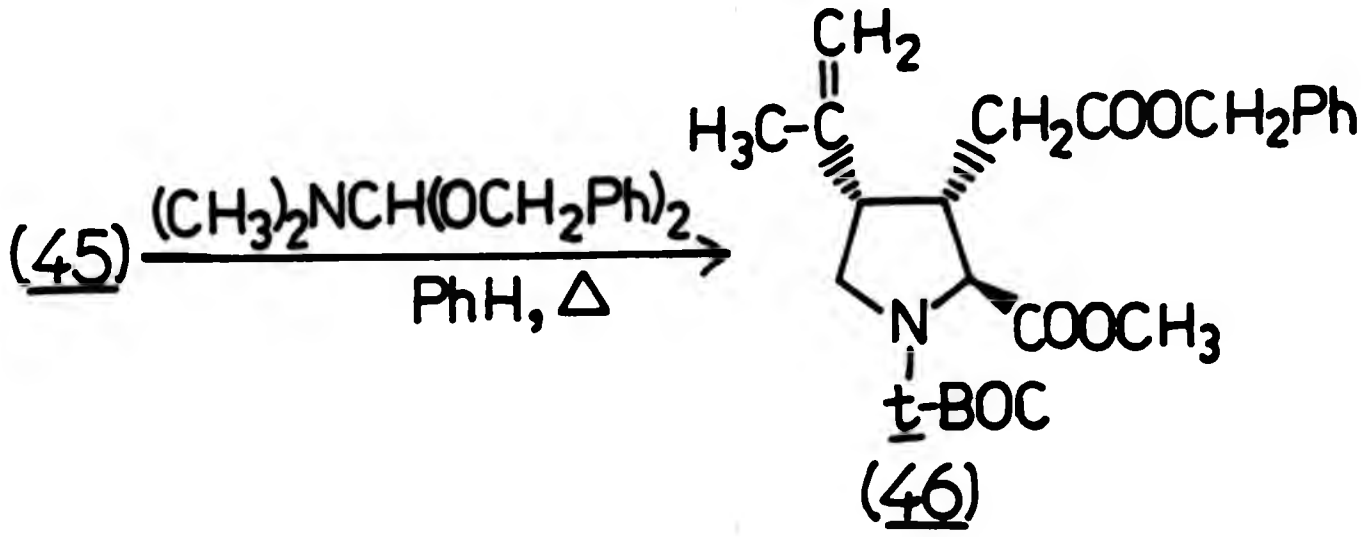
However, using a smaller brominating agent, N-bromo acetamide (NBA), the bromo-hydrin was formed successfully. (Scheme 28). Attempts to convert the hydroxy function of the product (104) to the corresponding acetate derivative using acetic anhydride and pyridine[36] failed, however.

Scheme 28



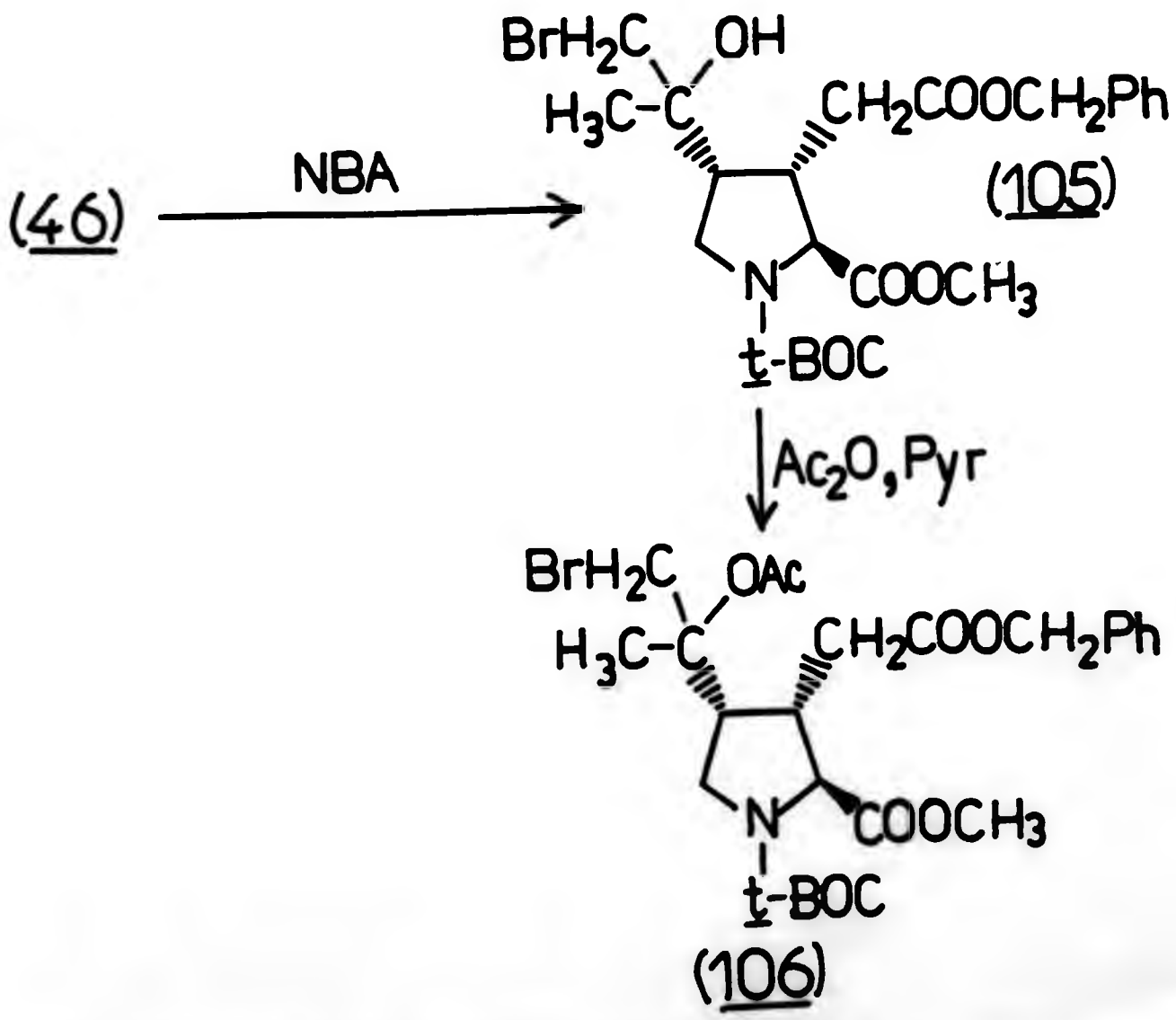
Since the difficulty encountered in these transformations appeared likely to be due to the steric effects of the benzhydryl group, it was decided to employ a smaller C-3 carboxyl-protecting group, (albeit one still labile to hydrogenation for eventual removal); hence the benzyl group was introduced by action of N,N-dimethylformamyl-dibenzyl acetal[15] on the monoester (45); (see Scheme 29).

Scheme 29



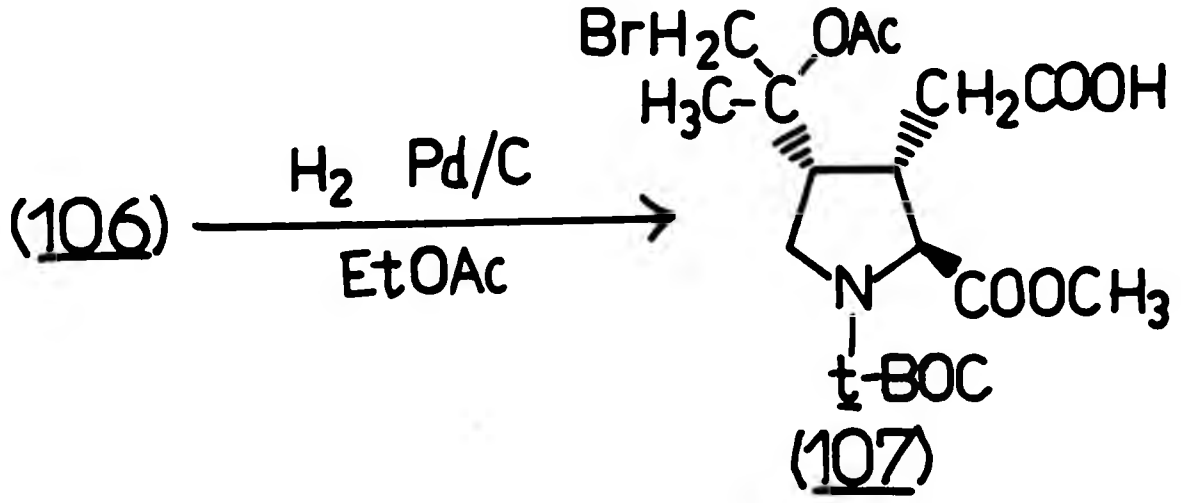
The mixed diester (46) reacted with NBA in refluxing acetone/water solution to form the bromohydrin (105) which was not isolated, but reacted immediately with acetic anhydride and pyridine to form the corresponding bromohydrin acetate (106) (Scheme 30).

Scheme 30



The fully-protected material (106) was then de-protected at the C-3 carboxyl group by hydrogenation over a palladium on charcoal catalyst[37]; (Scheme 31). This gave the desired intermediate (107) in approximately 50% yield overall from the monoester (45).

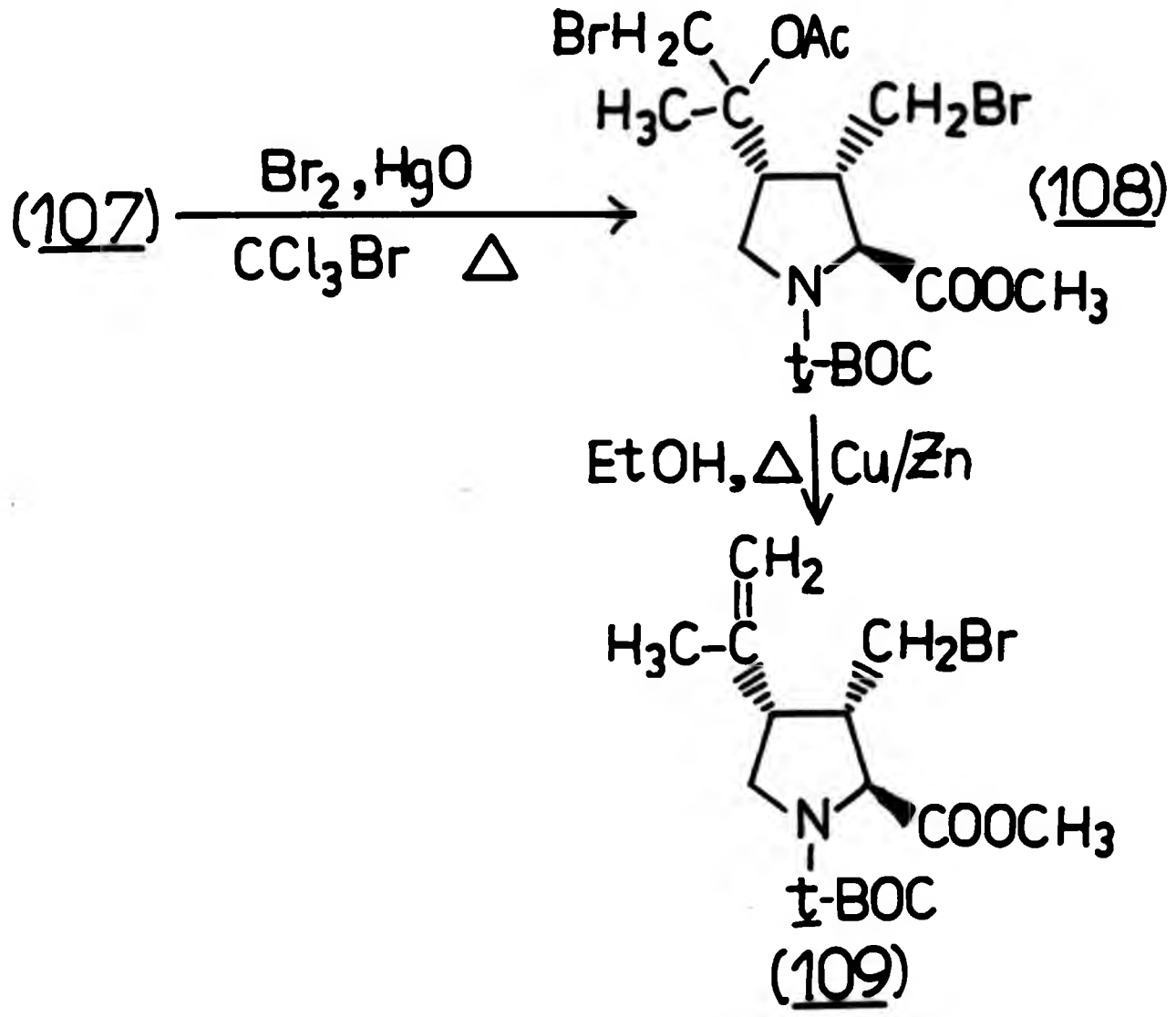
Scheme 31



Having thus synthesised the precursor molecule for the Hunsdiecker reaction (107), the reaction itself, in modified form[38], was attempted. A slurry of mercuric oxide and (107) in bromotrichloromethane, was added dropwise to a solution of bromine in the same solvent. The mixture was heated for 2 hours and then excess bromine was removed, the remaining material filtered several times through celite to remove the mercuric residues, and the residues after evaporation of solvent chromatographed on silica.

Such products as were isolated after this procedure did not, however, appear to be the desired compound (108). ^{13}C NMR did not indicate a single, clean conversion product, and there were significant traces of fractions where an alkenyl moiety had been (re)generated. Additionally, the material comprising the major fractional component was treated with copper-zinc couple in an attempt to regenerate the double bond completely; this reaction was not successful and no significant quantities of unsaturated products were recovered from a mixture of several components. (Scheme 32 illustrates the attempted reaction sequence).

Scheme 32



The reasons for the failure to obtain the desired product are not known; the reaction was repeated with similarly negative results.

The lack of success with this attempted Hunsdiecker reaction was disappointing, but other methods of chain-shortening were attempted.

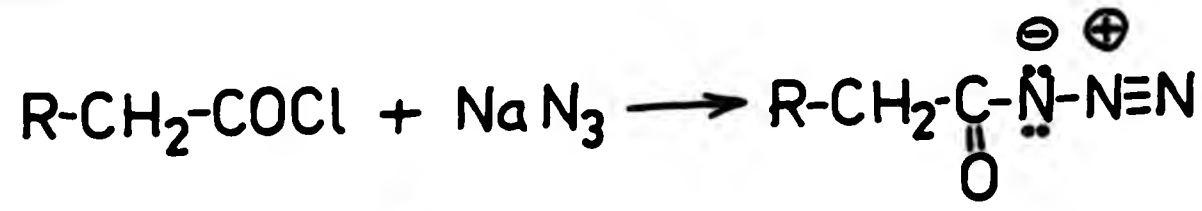
2.6.7.3 Chain Shortening; Attempted Curtius Reaction

Any reaction sequence leading to the chain-shortening of the C-3 side-chain and formation of some potentially convertible functional group on the new terminal carbon could be employed towards the eventual synthesis of some compound analogous to (101). To this end, an attempt was made to carry out the Curtius reaction[39] on the acid chloride (54).

The Curtius reaction converts an activated carboxylic acid (*e.g.* acid chloride) to an amine with one less carbon atom, (Scheme 33), via the highly unstable and reactive acyl nitrene, which decomposes with

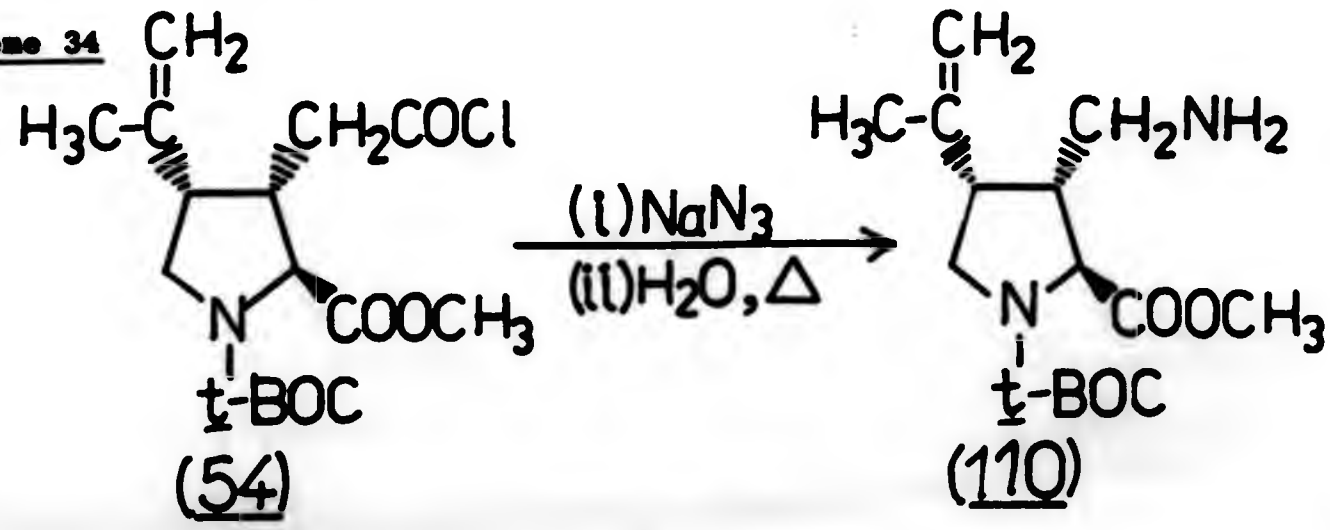
rearrangement to the isocyanate; this latter then reacts with water to form the corresponding, thermally-unstable carbamic acid, which itself decomposes to the amine with the loss of the terminal carbon atom as carbon dioxide.

Scheme 33



After the initial reaction of the acid chloride (54) with sodium azide, the intermediate formed, (which was assumed to be the isocyanate), was refluxed in benzene, toluene and water, in an attempt to decompose it to the amine; however, the result of the latter reflux was a large number of decomposition components in the reaction mixture, none of which could be identified as the required amine (110); the Curtius reaction, (as outlined in Scheme 34), was therefore unsatisfactory for the formation of the chain-shortened amine of our kainate compounds.

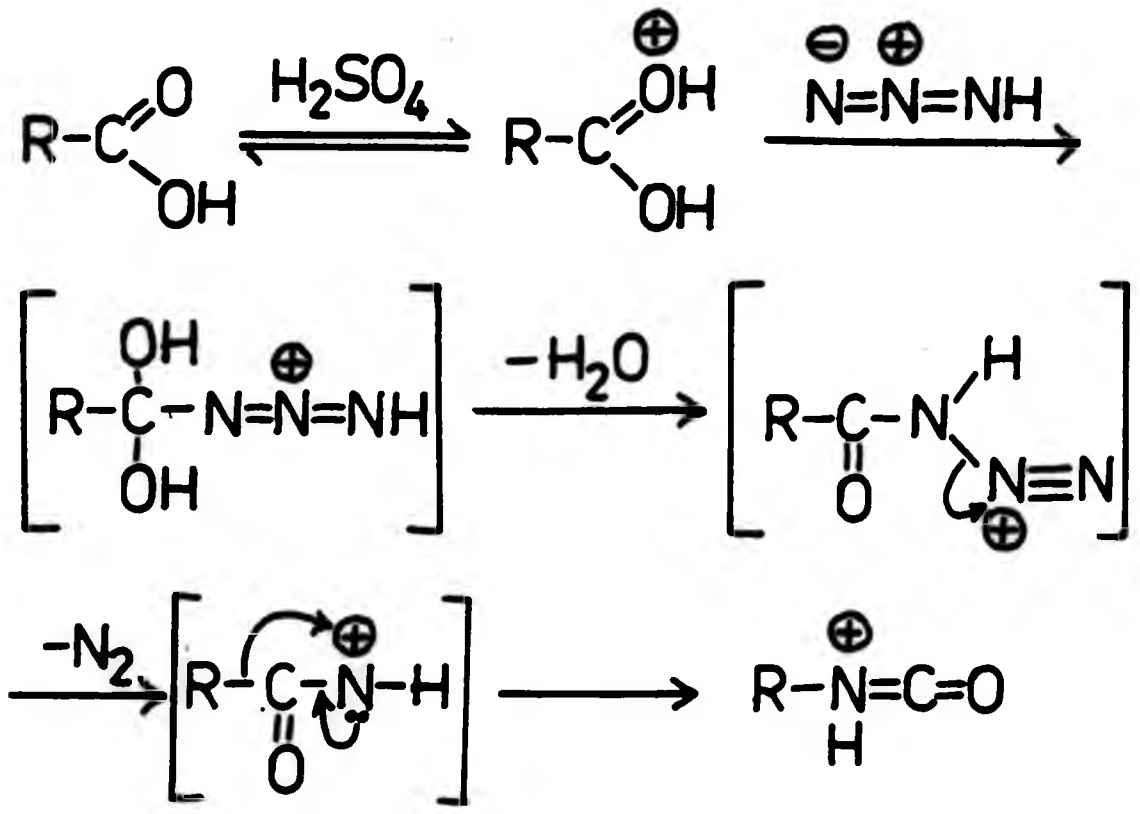
Scheme 34



2.6.7.4 Chain-Shortening; Attempted Schmidt Reaction

In common with the Curtius reaction[39] (see section 2.6.7.3), the Schmidt reaction[40] entails the use of an azide in a reaction which results in the replacement of a terminal carboxyl group with an amine. The reaction is commonly carried out by dissolving the free acid in chloroform and adding concentrated sulphuric acid; sodium azide is then added to the reaction mixture and is converted almost immediately into hydrazoic acid, which reacts with the protonated carboxylic acid; the unstable intermediates thus formed decomposing to the protonated isocyanate which reacts with water to give the amine; (see Scheme 35).

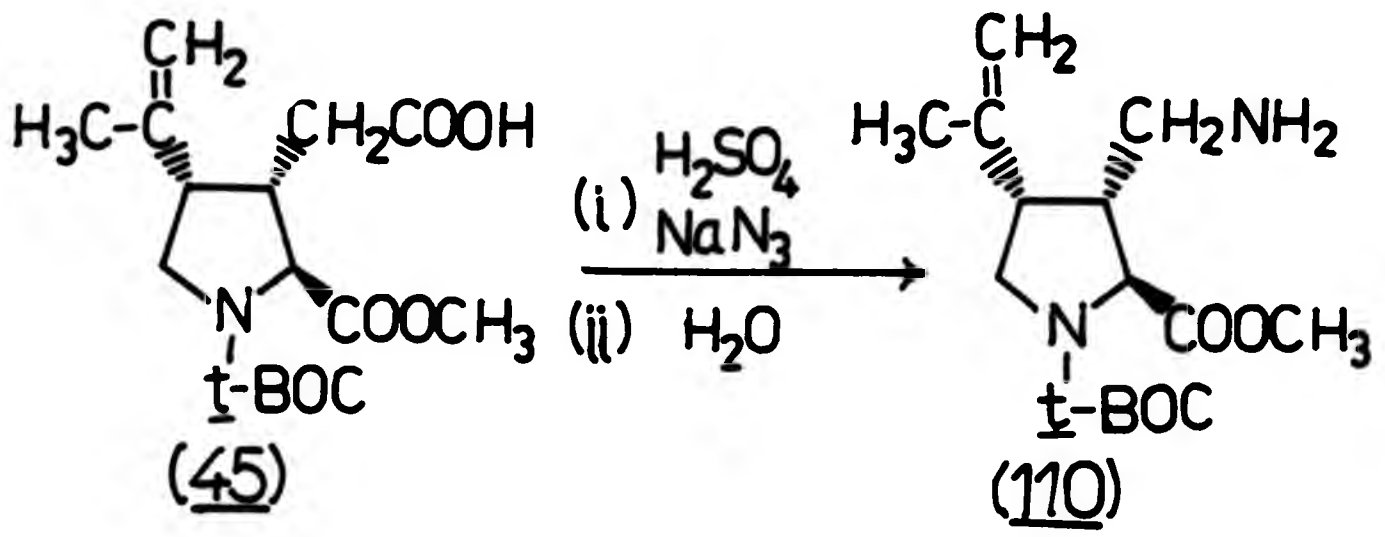
Scheme 35



However, the reaction did not appear to proceed satisfactorily in our hands; the concentrated sulphuric acid rapidly removed the N-protecting *tert*-butyloxycarbonyl group, thus creating problems as far

as easily following the reaction by TLC was concerned; additionally, a slower reaction, possibly intramolecular in character, and presumably catalysed by the acid, led to the loss of permanganate-reducing activity when material was examined by TLC in a partition system (*e.g.* n-butanol/acetic acid/water), thus indicating that the unsaturated alkenyl moiety was being converted to a saturated compound. Partition system TLC also showed that several different ninhydrin-active spots were present, suggesting a number of degradative reactions were occurring. The attempted Schmidt reaction outlined in Scheme 36 was therefore abandoned.

Scheme 36

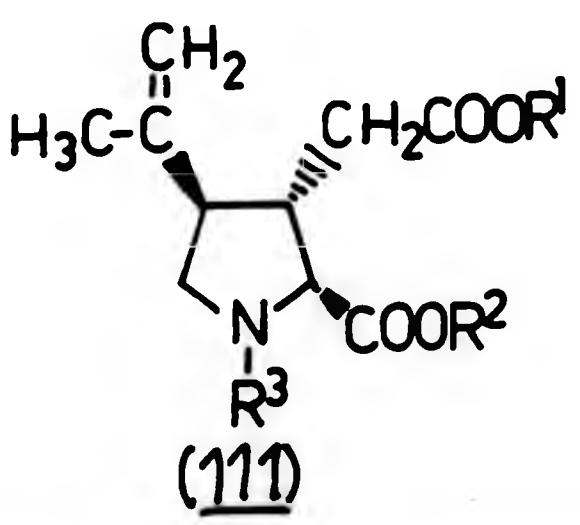


2.6.7.5 Chain-Shortening; Analysis

No outstanding and incontravertible successes were achieved in our attempts to degrade the carboxyl function on the C-3 side chain by one carbon unit, whilst replacing it with some alternate functional group, which could act either as a leaving group in subsequent nucleophilic displacement reactions involving phosphonate or sulphonate equivalents, or be easily convertible into one. While we employed only three of the several known methods to effect our desired degradative/conversion reaction, it was felt that persisting with this approach by means of other literature reactions could well prove equally unproductive, and thus would not usefully repay any effort invested in them.

It is clear from the results of the experiments that we carried out that there appears to be some intrinsic factor(s) involved which contribute substantially towards our lack of progress with this type of conversion. Certainly it is clear that some of the reagents used may have been reacting elsewhere in the molecule than where they were targeted, thus producing undesirable side-products. Additionally, the failure to attain the desired rearrangements may be due in part to steric hindrance caused by the proximity of the C-4 isopropenyl side chain (either in the protected or unprotected state) to the reaction/rearrangement side on the C-3 side chain. Additionally, if the alkenyl moiety is unprotected, intramolecular reactions could also occur fairly readily.

Conceivably, C-3 side chain manipulations of this type could prove easier if applied to allo-kainate derivatives (111), where the likely steric interactions between C-3 and C-4 chains are at a minimum and intra-molecular reactivity between the two is correspondingly diminished. In the absence of a suitable quantitative method of epimerising the allo-kainate derivatives back to the standard kainate types, however, the reaction sequences, even if successful, would only provide allo-kainate analogues, whereas the compounds of primary interest would remain the standard kainate series.

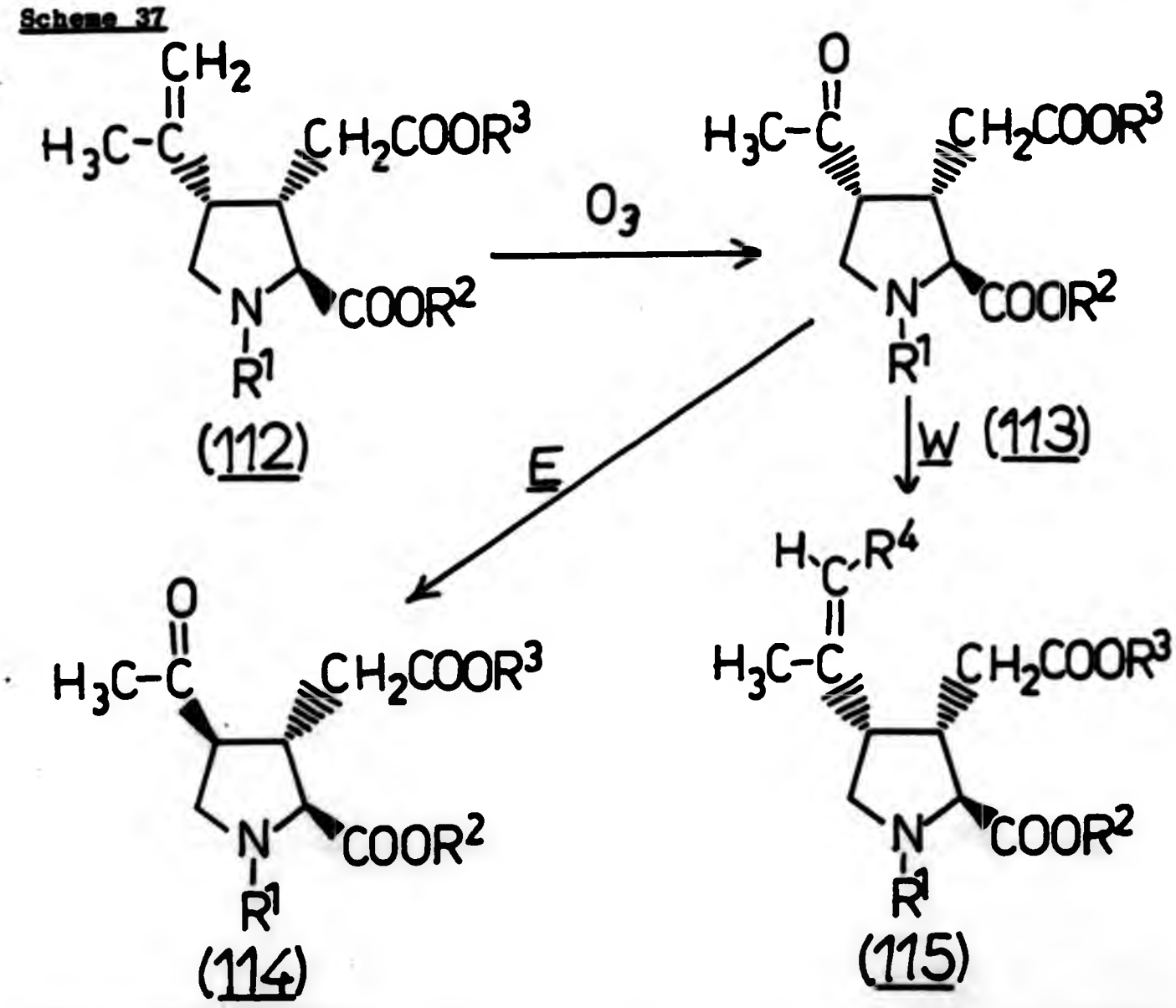


(111)

2.6.8 C-4 Side Chain Manipulations

The presence of an alkenyl function in kainic acid obviously provides a potentially reactive site towards a number of reagents, some of which have already been mentioned in this account. In addition to these, a further simple reaction, whereby the double bond is oxidised to a ketone[41] with the loss of $=CH_2$, could provide the precursor to two distinct series of compounds; one in which the C-4 acetyl side chain thus produced could be epimerised to give allo-keto-kainate derivatives by keto-enol tautomerism, and the other whereby the ketone formed the substrate for a series of Wittig[42] or Horner-Wittig[43] reactions directed towards extending the carbon skeleton with a view to producing chain-extended analogues at C-4, with or without the retention of a degree of unsaturation; Scheme 37 outlines the two divergent options:

Scheme 37



For legend, see over.

R^1 = t-BOC, -CBZ

$R^2 = R^3$ = $-CH_3$

R^4 = VARIOUS

E = EPIMERISATION

W = WITTIG

2.6.8.1 Ozonolysis; Production of Keto-Kainate

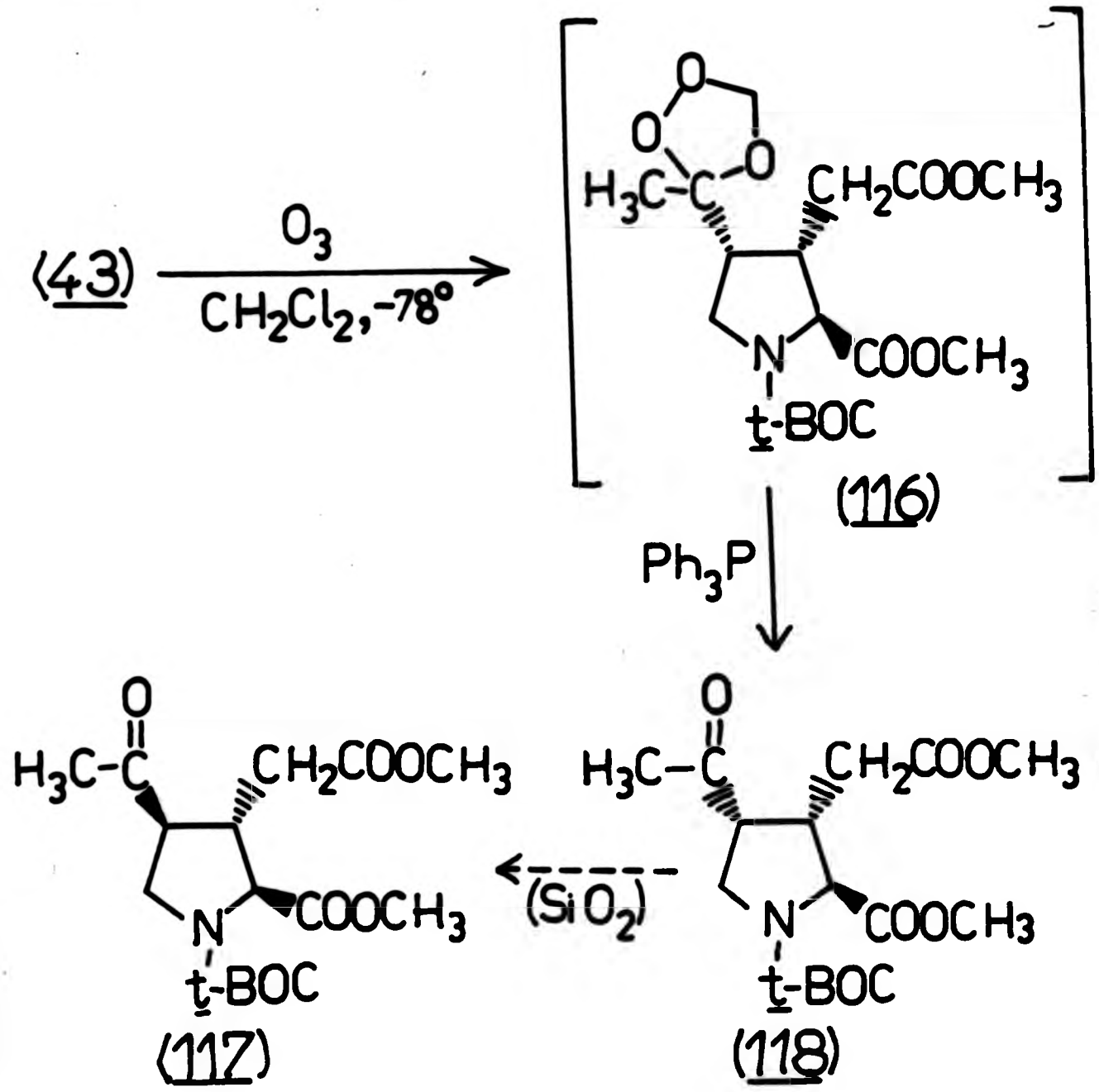
The alkenyl moiety was found to be reactive towards ozone[41], the gas being bubbled through a solution of suitably-protected kainate material in methylene chloride at -78°C. This moderately stable ozonide (presumed structure (116)) was not isolated but directly decomposed to the ketone by action of triphenyl phosphine, the pure ketone (117) being isolated by rapid column chromatography on silica. (Slower columning could epimerise the material at C-4 and the product would become contaminated with the corresponding allo-kainate derivative (118))[5]; see Scheme 38.

2.6.8.2 Epimerisation; Allo-Keto-Kainate

Other workers[5] demonstrated the complete conversion of 'standard' keto-kainate to allo-keto-kainate by an extension of the observation that the material would epimerise on a silica column; a slurry of silica, triethylamine and an ethereal solution of keto-kainate were stirred overnight at room temperature, at the end of which time conversion appeared to be 100% complete (by NMR).

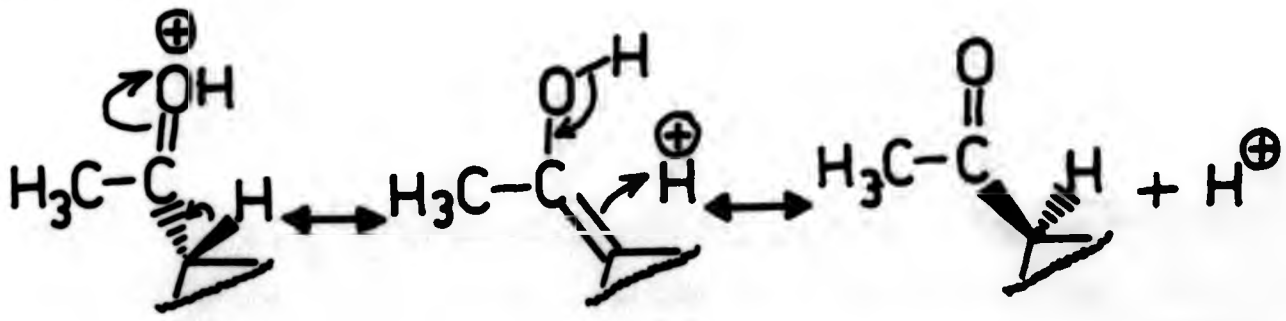
However, almost any reaction on keto-kainate employing acid or base conditions will tend to epimerise the C-4 centre to a greater or lesser extent; Scheme 39 shows the acid-catalysed keto-enol tautomerism which gives rise to the allo-keto form.

Scheme 38



Examination of models or suitable computer-based graphics demonstrated that the degree of steric interaction/hindrance between the C-3 and C-4 side chains is greatly reduced in the allo- form as opposed to the normal α-kainate epimer; one might reasonably conclude from this that the allo-epimer represents the favoured conformational arrangement, and

Scheme 39

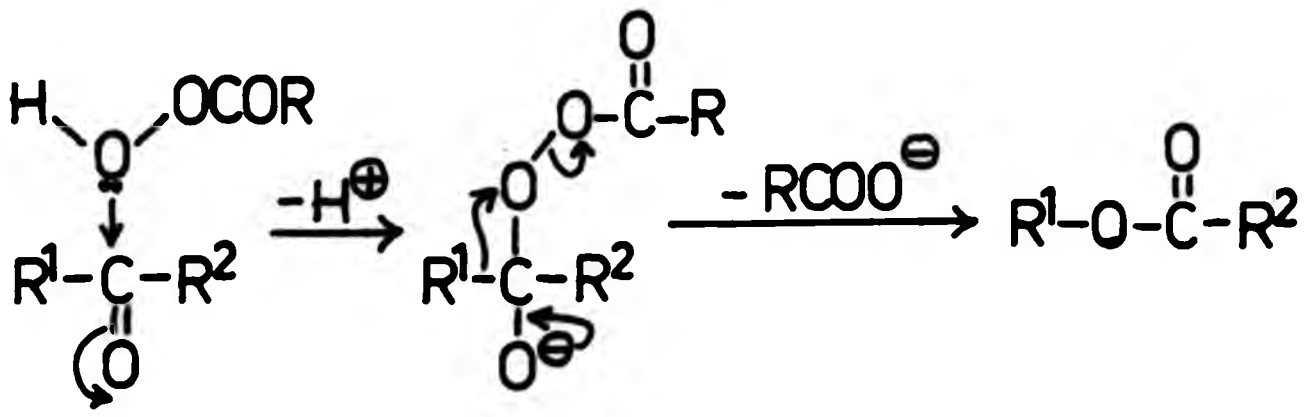


the ease of (accidental) epimerisation at C-4 would seem to support this theory.

2.6.8.3 Attempted Baeyer-Williger Reactions on Keto-Kainate

The Baeyer-Williger reaction[44] involves the use of peroxide-type oxidants, leading to a rearrangement to electron-deficient oxygen; as illustrated by Scheme 40.

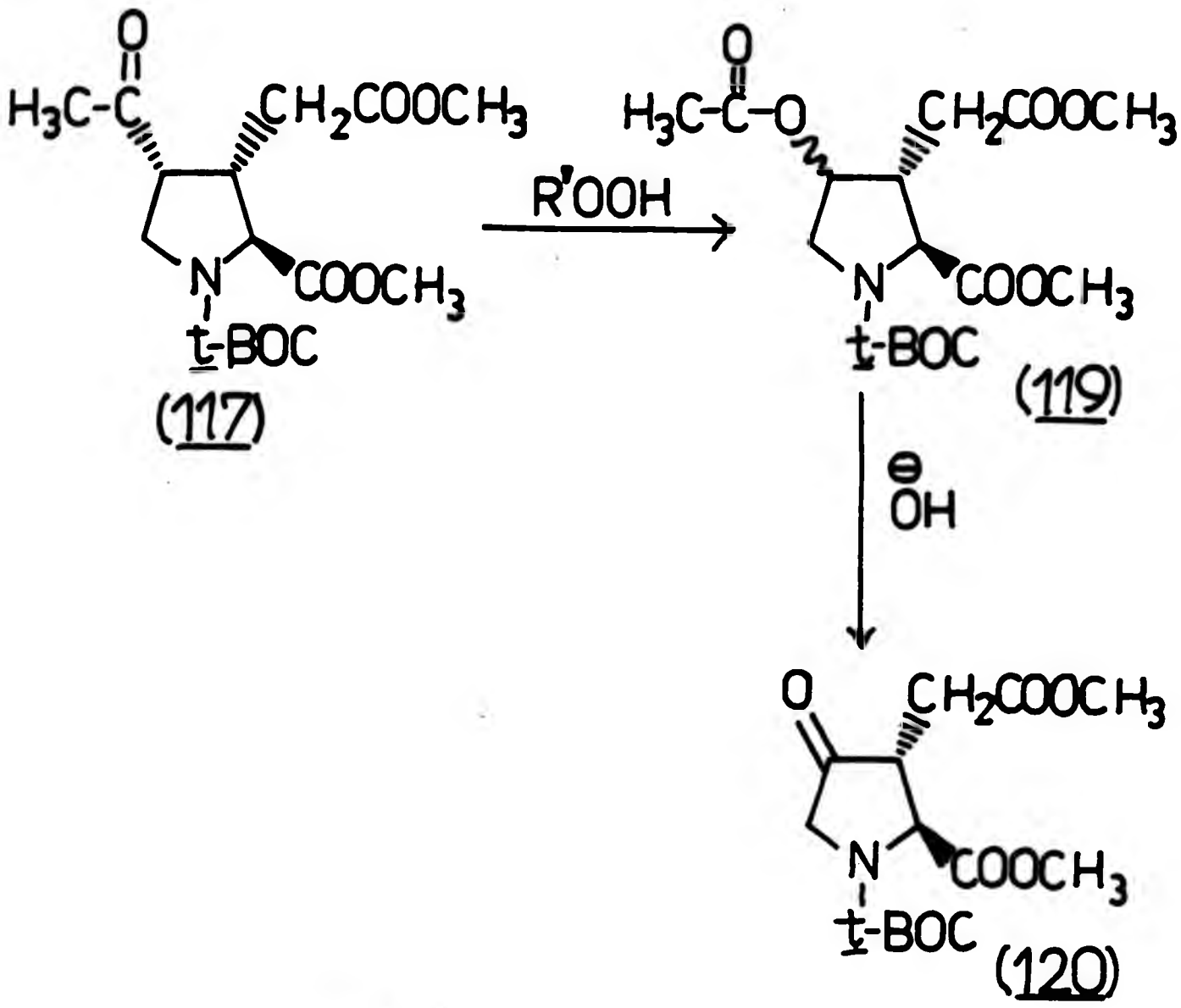
Scheme 40



The mechanism is thought to involve a nucleophilic attack by the per-acid on the carbonyl group to produce an intermediate which then rearranges, the corresponding anion of the acid of the original per-acid acting as a leaving group[45]. In an unsymmetrical ketone, (*i.e.* $R^1 \neq R^2$), such as the keto-kainate derivatives, the group which migrates during the rearrangement is that which is the better electron-donor; thus with alkyl groups, the ease of migration is in the order tertiary > secondary > primary > methyl.

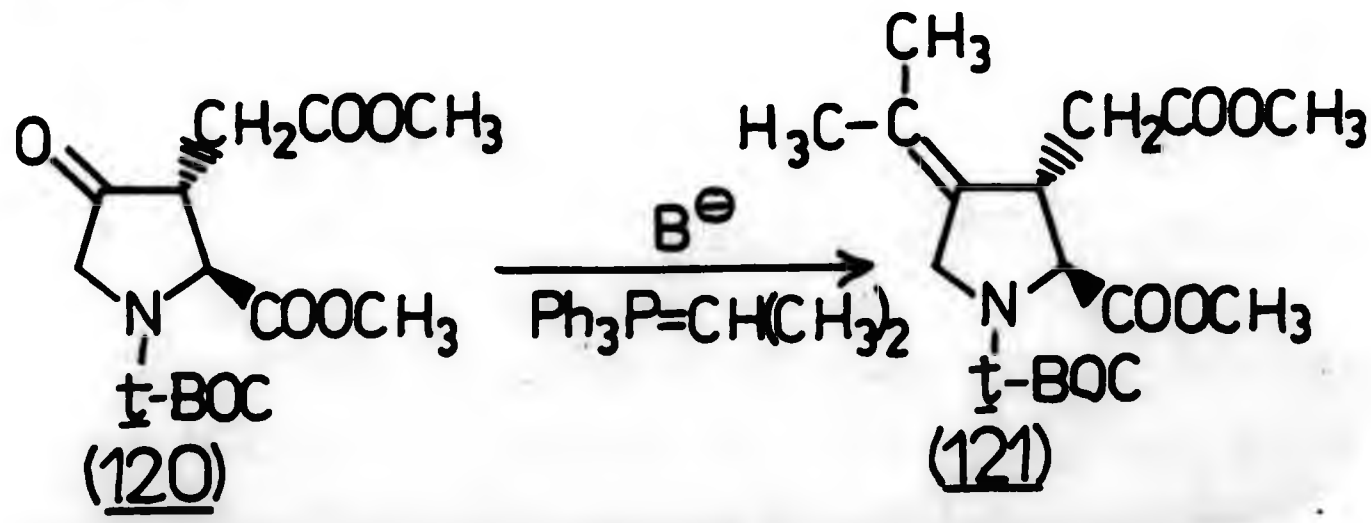
Within such priorities, it was felt that keto-kainate might be expected to undergo the rearrangement depicted in Scheme 41, to give the compound (119); it was envisaged that this material, when treated with aqueous base, would lose the acetyl moiety and form (120), where the keto-group is directly *exo*- to the pyrrolidine ring.

Scheme 41



The compound (120) was intended as an intermediate in producing iso-kainate derivatives, *e.g.* (121), where a Wittig-type reaction on the ketone group would provide the range of analogues (see Scheme 42) where the double bond of kainate is situated directly *exo*- to the ring as opposed to being in the terminal position on the iso-propenyl side chain.

Scheme 42



However, we were unsuccessful in our attempts to carry out the Baeyer-Williger reaction on either keto- or allo-keto-kainate derivatives with either sodium perborate[46], *meta*-chloroperoxybenzoic acid (mcpba) or 30% hydrogen peroxide as the oxidising agent[46,47]. Conceivably, 90% peroxide might have proved more "efficient" as an oxidant[48], but due to difficulties in obtaining such potentially hazardous material, we were unable to test this hypothesis.

2.6.8.4 Wittig-Type Reactions on Keto-Kainate; Attempted Chain Extensions at C-4

The Wittig reaction[42] involves the conversion of a carbonyl group into an alkene, with a corresponding increase in the carbon chain length; depending on the phosphorane reagent used, a wide range of alkenyl derivatives can be introduced. (See Scheme 43).

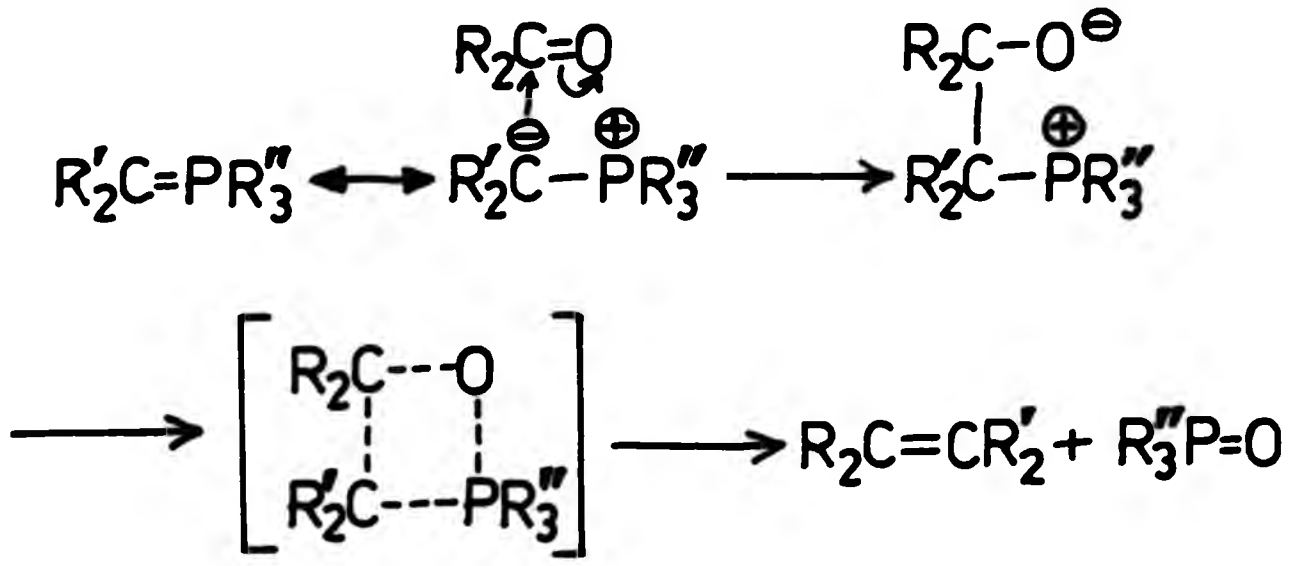
Scheme 43

$$R_2C{=}O + R'_2\overset{\ominus}{C}-\overset{\oplus}{P}R''_3 \longrightarrow R_2C{=}CR'_2 + R''_3P{=}O$$

The reaction is assumed to proceed via the mechanism shown in Scheme 44, with the phosphorane ylid making a nucleophilic attack on the carbonyl carbon, the intermediate forming a four-membered cyclic transition state which breaks down to form the alkenyl product and the phosphine oxide corresponding to the initial phosphorane[49].

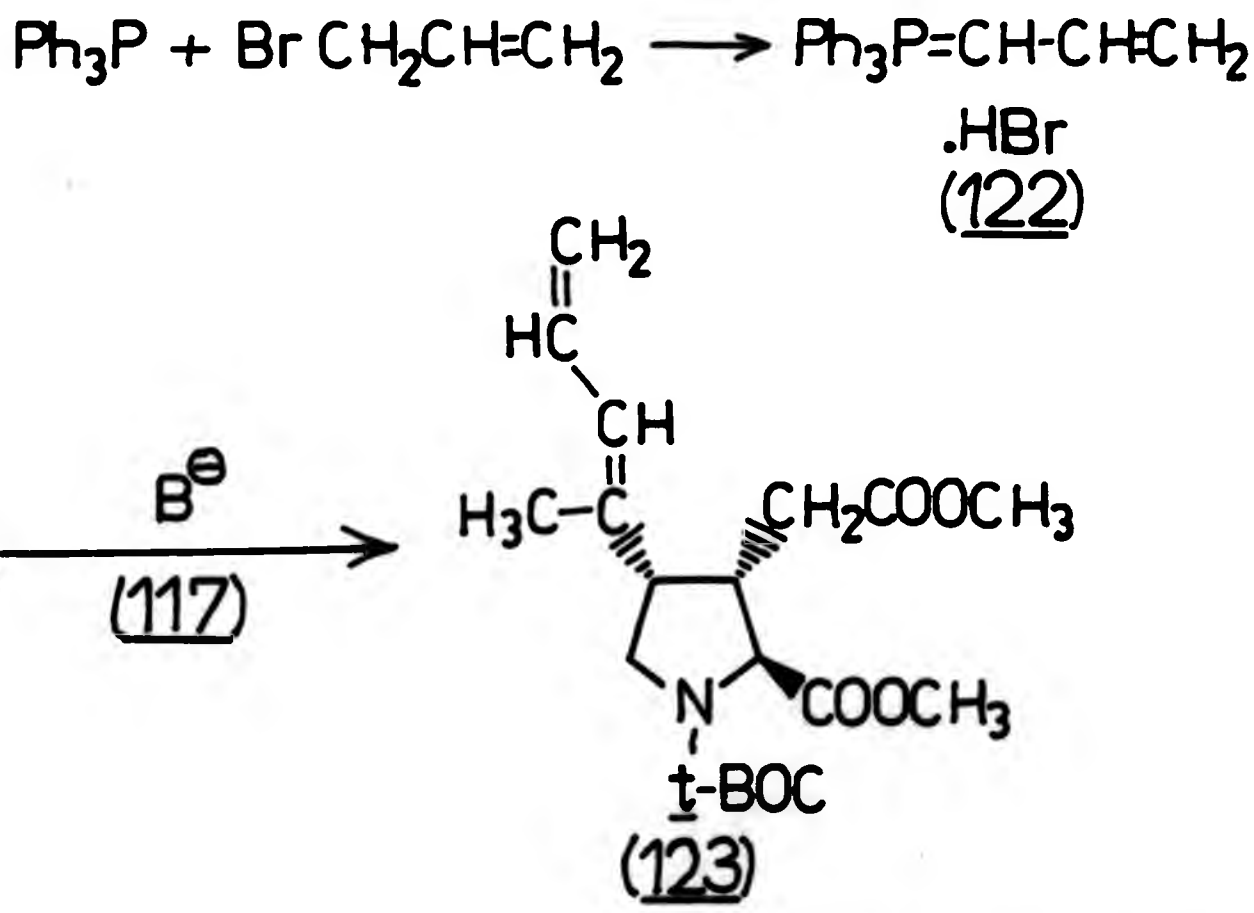
Although previous experimental work had demonstrated that the Wittig reaction could reform the standard α-N-*t*-BOC kainate dimethyl ester from the corresponding α-N-*t*-BOC keto-kainate dimethyl ester in moderate yield[26], we could achieve no success with an attempted Wittig reaction between α-N-*t*-BOC keto-kainate dimethyl ester and the phosphorane ylid formed from triphenyl phosphine and allyl bromide (Scheme 45)

Scheme 44



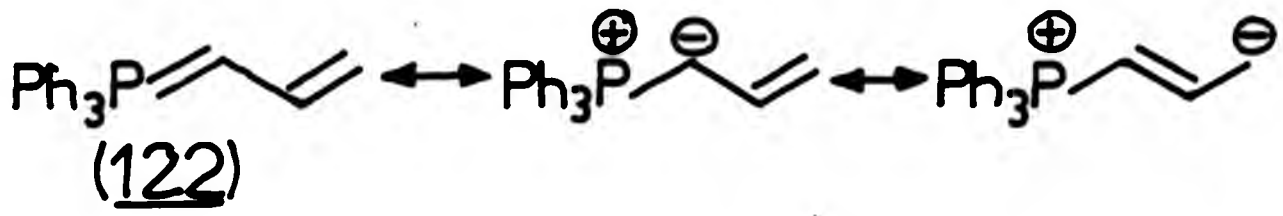
Such lack of success is perhaps not surprising if one considers the considerable likely resonance stability of the phosphorane (122) (see Scheme 46); such stability has been shown to make the ylid almost completely unreactive towards ketones, (although reaction with aldehydes might be possible)[50].

Scheme 45



Since the carbonyl group in keto-kainate is in the former category, a modification of the Wittig reaction is necessary in order to effect

Scheme 46



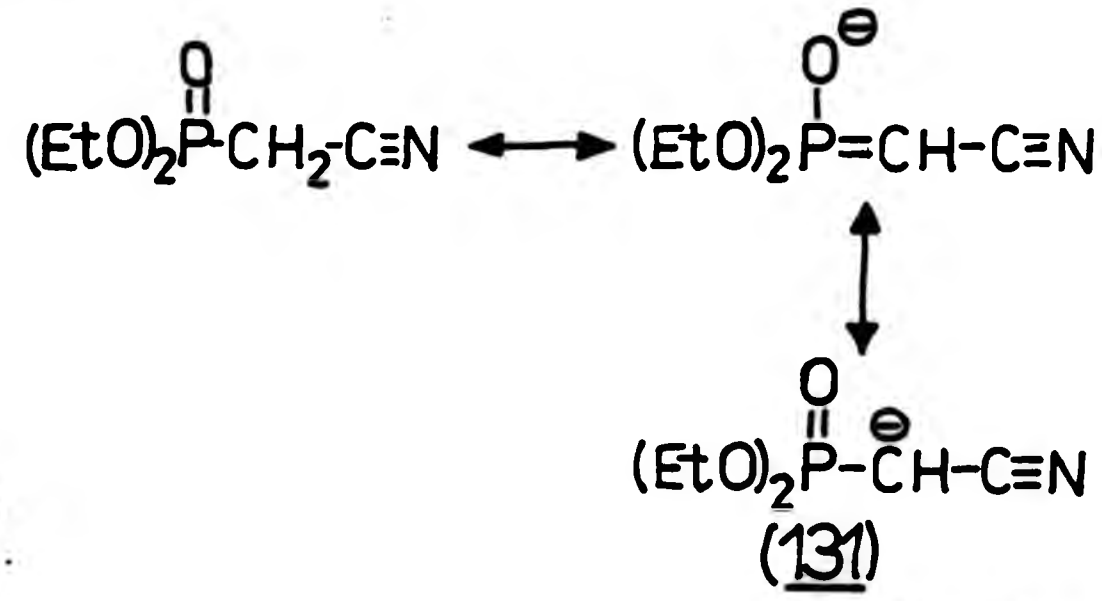
(122)

the required reaction between keto-kainate and phosphoranes of the resonance-stabilised type.

2.6.8.5 Horner-Wittig Type Reactions on Keto-Kainate; Chain Extensions at C-4

The Horner-Wittig modification[43] of the basic Wittig-type reaction incorporates the use of alkyl phosphonate esters in place of the alkyl phosphoranes of the standard Wittig reaction. As can be seen, the presence of an oxygen atom attached directly to phosphorous permits of an increase in the contribution of the resonance structure (131) in the resonance hybrid following proton removal by a strong base (*e.g.* sodium hydride) as used in the reaction; (see Scheme 47)[49].

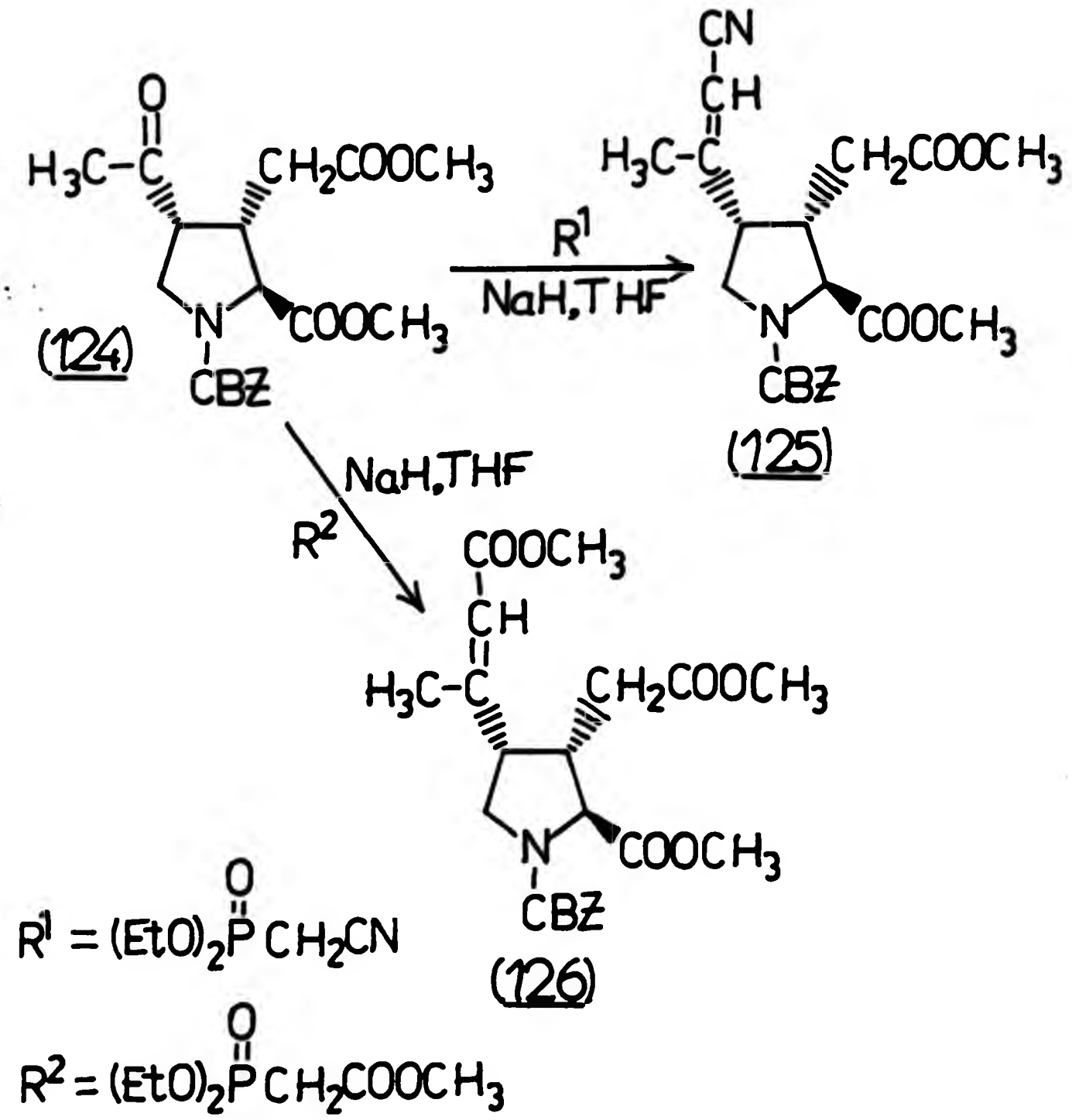
Scheme 47



(131)

Although lack of time prevented a full investigation of the potential of the Horner-Wittig reaction as applied to keto-kainate, both the acetonitrile and carbomethoxymethyl equivalents were added to N-CBZ protected keto-kainate dimethyl ester (Scheme 48), demonstrating the potential utility of this method.

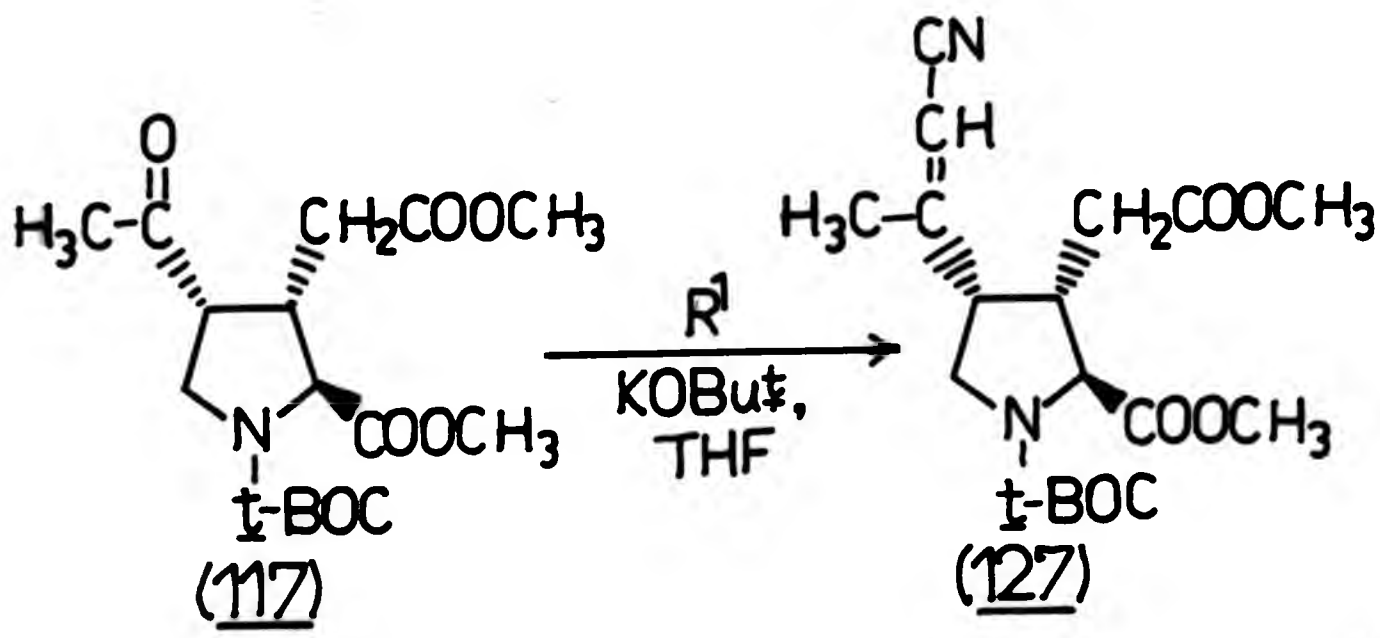
Scheme 48



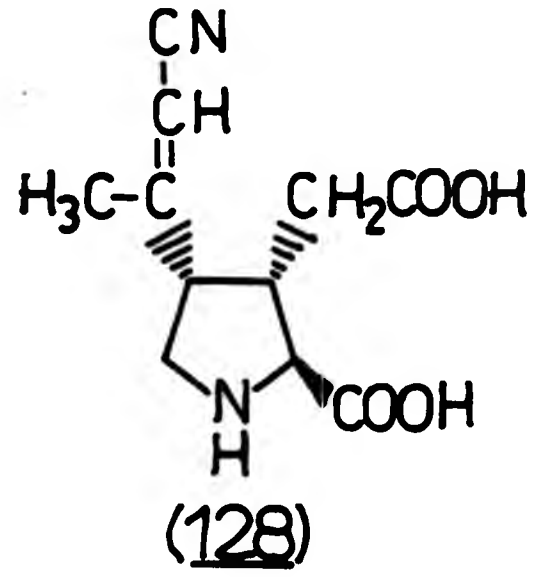
The use of the CBZ material meant that the entire reaction could be easily followed by TLC; thus a reasonably accurate idea of when all the starting material was consumed could be obtained. Although both (125) and (126) could be de-esterified by the action of dilute base in water/THF solution, the removal of the N-protecting group without recourse to hydrogenation would be almost impossible, since such a process would also reduce the newly re-formed alkenyl moiety in the C-4 side chain and hence should be avoided!

With some idea of the reaction conditions required, some preliminary work on the reaction between diethyl phosphonylacetonitrile and keto-kainate dimethyl ester protected at nitrogen with *t*-BOC (117) was carried out. Although visualisation of this starting material by TLC was extremely difficult, the formation of the unsaturated product (127) was possible and hence the reaction could be followed to some extent by observing the production of product visualised with alkaline permanganate. Scheme 49 shows this reaction.

Scheme 49



Chromatography gave the product (127) in some 60% yield; future work would have included the complete deprotection of (127) to give (128); small scale experiments (albeit without complete product characterisation) indicated the feasibility of such an approach.



(128)

2.6.8.6 C-4 Chain Elongation; Summary

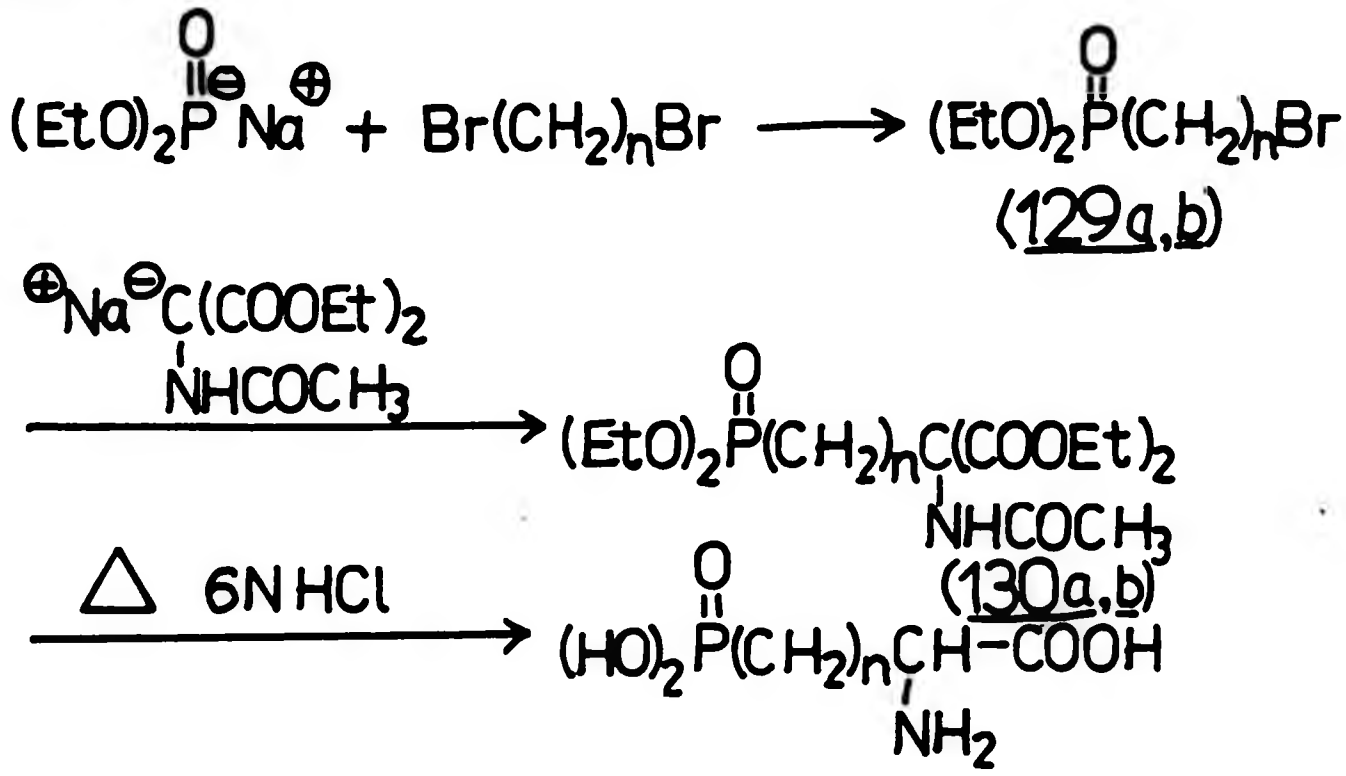
The obvious interest in kainate analogues chain-extended at the C-4 position could be investigated using the Horner-Wittig reaction on keto-kainate; the scope for the addition of various functional groups and increased carbon chain length being almost unlimited.

Although lack of time prevented us from a really thorough examination of such possibilities during the course of this project, it is to be hoped that subsequent investigations would develop this aspect of kainic acid chemistry to a very much greater degree than that achieved presently.

2.7 α-Amino, ω-phosphono-Carboxylic acids

Quantities of the anti-convulsants 2-APP (15) and 2-APH (14) were synthesised according to the manner of Curry[51] as modified by Joseph[52]. (Scheme 50).

Scheme 50



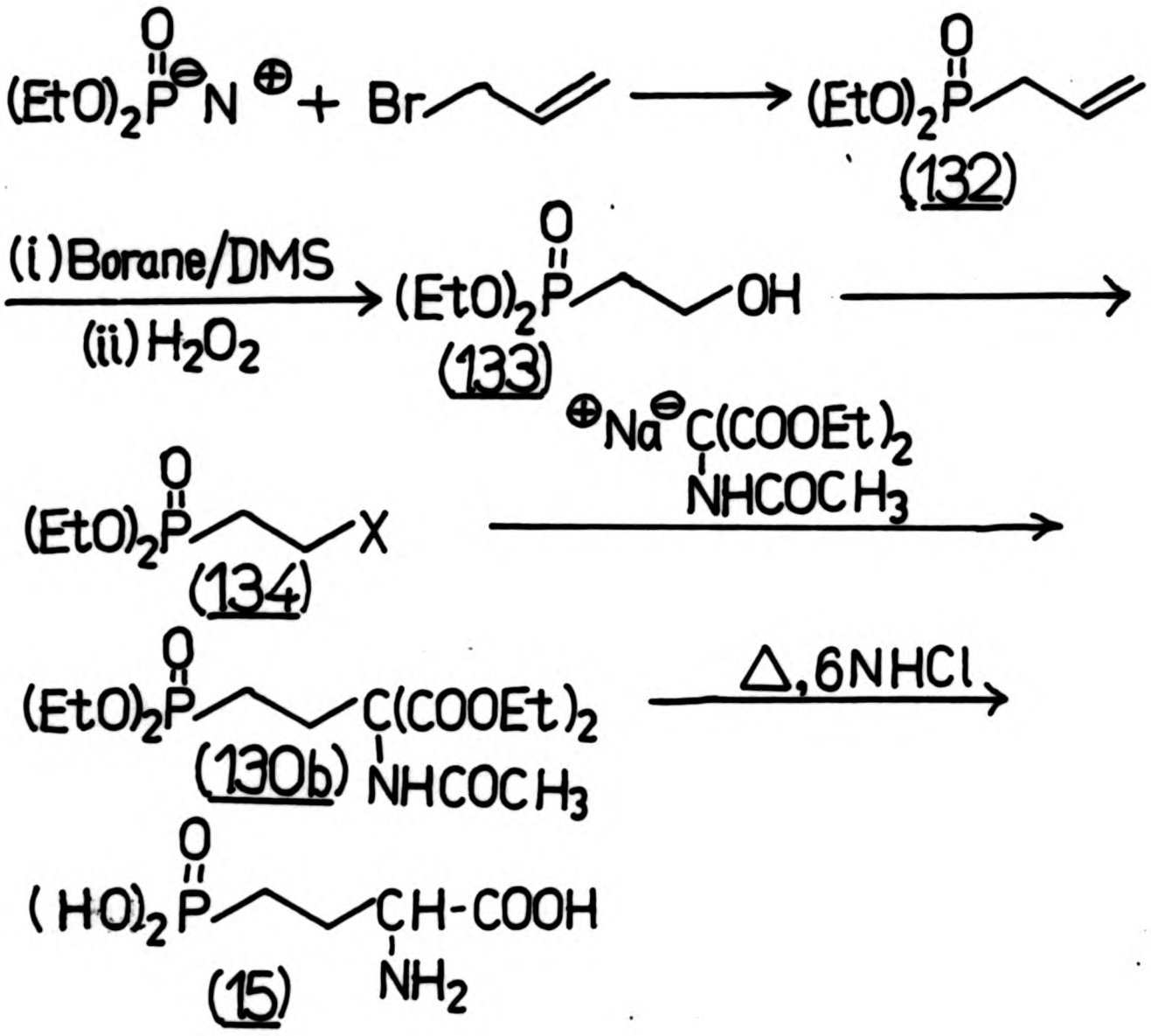
n = 3 = 2-APP (15) (b)
n = 5 = 2-APH (14) (a)

This method is neither particularly elegant, nor high-yielding. Although other commitments prevented the completion of the project, an alternative approach was briefly investigated. (Scheme 51).

While the method illustrated in Scheme 51 may appear rather more complicated than the previous system (as shown in Scheme 50), it would have the advantage of considerably reducing the amount of wastage and large-scale chromatography involved in the "standard" method.

The hydroxy-compound (133) represented the furthest extent of our progress in this reaction series; yields to this point were good. Further work in this area would be directed towards the synthesis of the intermediate (134) where X is a suitable leaving group (*e.g.* tosylate, mesylate, etc.).

Scheme 51


(EtO)2P(=O)⊖N⊕ + Br
(EtO)2P(=O)
(132)
(i) Borane/DMS
(ii) H2O2
(EtO)2P(=O)
OH
(133)
⊕Na⊖C(COOEt)2
NHCOCH3
(EtO)2P(=O)
X
(134)
(EtO)2P(=O)
C(COOEt)2
NHCOCH3
(130b)
Δ, 6NHCl
(HO)2P(=O)
CH-COOH
NH2
(15)

2.8 Kainate; Summary of Significant Chemistry

Certain aspects of the chemistry of kainic acid have been investigated.

Some attention has been given to protecting the secondary amine function, and it is obvious that much future work could be done at this site.

A simple and selective method for the esterification of the C-2 carboxylic acid has been discovered. This technique has enabled the synthesis of a variety of peptide adducts at the C-3 carboxyl, in addition to the conversion of the carboxyl group itself into alternative functional types, while retaining the C-2 carboxylic acid in unmodified form.

Attempts to reduce chain length of the C-3 side chain and subsequently replacing the terminal carboxylic acid with an alternative non-carbon acid group, *e.g.* phosphonate or sulphonate, have been unsuccessful.

The C-4 isopropenyl moiety has been converted to the corresponding keto-kainate compounds in both the normal and allo-stereochemical forms. The ketone thus produced has been used as the basis for homolgation reactions by the Horner-Wittig method, in order to reform the double bond and extend the carbon chain beyond it.

2.9 Kainate; Potential Future Development

Potential therapeutic agents will presumably require some form of N-protection which could provide solvolysis in lipophilic conditions, sufficient to enable transportation around the body and cross the blood-brain barrier, and then become de-protected *in vivo* by endogenous enzyme systems.

Although pharmacological testing of C-4 chain-extended kainate analogues has not yet been possible, it is to be hoped that future development of this work would focus upon producing an extensive array of kainate analogues with modified and extended C-4 side chains. The prospect of a kainate analogue where the C-3 carboxylic acid is replaced with a phosphonate or sulphonate function could also provide potentially interesting materials for testing; although this project failed to produce such a compound, it is possible that other methods, untried by us, could yield successful results.

2.10 α-amino, ω-phosphono carboxylic Acids; Summary

Quantities of the α-amino, ω-phosphono carboxylic acids 2-APP and 2-APH have also been produced by the method of Curry and Joseph; an alternative approach to their synthesis has also been briefly touched upon.

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CHAPTER THREE

KAINATE ANALOGUES AS POTENTIAL ANTI-CONVULSANTS

3.1 Anti-Convulsant Compounds: General

It is generally believed that the drugs currently employed as anti-convulsants are all centrally active by inhibiting the spread of the abnormal electrical activity associated with the various epileptic conditions[1]. Such inhibition is thought to operate either by enhancing and/or potentiating the effect of inhibitory transmitters, especially GABAergic processes[2], or by diminishing or curtailing excitatory transmission[3].

3.1.1 Anti-Convulsants by Interaction with Inhibitory Systems

A considerable body of literature exists describing the interaction of various compounds with the GABA inhibitory system: (e.g.[4,5,6]). Table 7 summarises the possible points of interaction (after Meldrum[7]) and indicates which compounds are believed to act at specific points.

TABLE 7

1. Mimic GABA action (GABA agonist) e.g. Muscimol, THIP (4,5,6,7 tetrahydroisoxazolo-[5,4-C]-pyridin-3-ol).
2. Act as GABA Pro-drug (Increases production of GABA) e.g. Benzoyl-GABA, Cetyl-GABA.
3. Facilitate Release of GABA from pre-synaptic neurone e.g. benzodiazepins.
4. Inhibit GABA-Transaminase (Prevents enzymic destruction of GABA at the synapse).
5. Enhancement of Affinity of GABA recognition site by allosteric interaction e.g. benzodiazepins, β-carbolines.
6. Action on Chloride Ionophore associated with GABA receptor site ($GABA_A$ type) e.g. Barbiturates, Etazolate.
7. Inhibition of GABA re-uptake e.g. Nipecotate.

It has been shown that interference of GABAergic inhibitory transmission by a variety of methods leads to focal or generalised seizures[8].

3.1.2 Anti-Convulsants by Interaction with Excitatory Systems

The excitatory effect of dicarboxylic amino acids when applied iontophoretically to the mammalian CNS has already been noted. When applied focally to the brain they cause convulsions[9,10]. No conclusive definition of their function in *in vivo* epileptogenesis has yet been determined[7], although anatomical pathways incorporating neurotransmission by excitatory amino acids are certainly implicated in the propagation of seizure activity away from the epileptic focus[3]; the extent of this involvement also requires elucidation. Even allowing for the fact that no complete description of the pathological significance of excitatory amino acid neurotransmission in epilepsy is yet available, some potentially therapeutic mechanisms for interefering with excitatory transmission can be indicated, in a similar manner to the mechanisms shown previously for enhancing GABAergic systems (albeit attempting to diminish transmission rather than to potentiate it as in the former case); see Table 8. (After Meldrum[7]).

TABLE 8

1. **Decrease the *in vivo* Rate of Synthesis** of putative excitatory transmitters, e.g. glutamate, aspartate, cysteine sulphinic acid, etc.
2. **Decrease Synaptic Release**, either by activity on pre-synaptic receptors or on a calcium-dependent protein kinase.
3. **Decrease Post-Synaptic Action** by selective receptor antagonists such as 2-APH, 2-APP, etc.

The work on A1 receptor antagonists noted earlier demonstrates the potential value of such compounds as anti-convulsants[11,12], and the

intention of this project was to produce some A3 receptor antagonists based on the kainate (agonist) molecule. Currently available compounds show little or no selective action against A3 receptor agonists. (See Table 9; after Meldrum[12]).

TABLE 9

Antagonist	NMDA (A1)	Quisqualate (A2)	Kainate (A3)
cis-2,3-PDA	+++	++	+++
γ-DGG	++++	+	+++
β-D-asp-β-alanine	++++	0	+++
D-α-aminoadipate	+++	0	+
2-APB	0	0	++
2-APP	++++	0	(+)
2-APH	++++	0	(+)
GDEE	0	-	-

0 = no activity; Relative potency + = Lowest, ++++ = Highest;
- = Not tested.

3.2 Anti-Convulsant Screening

Investigation of potential anticonvulsant compounds requires some method of screening to detect activity in a suitable model system. Because the epileptic condition would be somewhat difficult to simulate in *in vitro* experimental conditions, animal models are used to evaluate drug efficacy, potency and duration of action. Animal models can also provide additional information regarding putative mode, locus and mechanism of action; information on potential side-effects, undesirable or otherwise; information on the possible systemic toxicity of the compound being tested; while at a later stage in the drug development programme, information on any carcinogenic, mutagenic or teratogenic properties of the drug can be accumulated, along with data on absorption,

storage, metabolism, excretion and other kinetics of the potential drug. Finally, there may be the possibility of learning more of the pathoneurophysiology of seizures, or more generally of the basic mechanisms of the different types of epilepsy. (For Reviews, see[13,14]).

3.3 Animal Models of Epilepsy

A variety of models are available for inducing epileptic activity in animals[13,15]. It may be convenient to divide the animal models used into two major groups to start with: those that are genetically predisposed to epilepsy and those that are not. The former class would contain examples such as rodents that are prone, at certain ages, to sound-induced seizures[16], e.g. DBA/2 mice[17], or the photo-sensitive baboon *Papio papio*, which undergoes a series of seizure responses following stimulation with a flashing (stroboscopic) light source[18].

The latter class, on the other hand, contains all the animals which require some directly applied electrical, mechanical or chemical stimulus to produce the seizure response[13,15].

3.3.1 Non-Genetically Pre-Disposed Models

3.3.1.1 Electrically Induced Seizures (Maximal Electro-shock)[19]

Various types of animals, including mice, rats, rabbits, frogs, hamsters, sloths, monkeys and cats have been used in a wide variety of experiments assessing the induction of seizures by attachment of electrodes in different regions. Corneal electrodes have been used to generate partial or generalised seizure responses, while electrodes implanted in the cortex or hippocampus allow investigation of the spread of after-discharge activity through these areas from the artificial focus[13].

3.3.1.2 Chemically Induced Seizures

Chemical methods of inducing a seizure response have certain advantages over the electroshock method. They are usually easier to apply, and the convulsant thresholds can be determined more accurately. Sub-threshold effects such as pain, excitement and withdrawal are usually much less dramatic than with electroshock methods. Where some insight into the biochemical or pharmacological mode of action of the convulsant compound exists, information may be obtained about both the basic epileptic mechanisms and the mode of action of potential anti-convulsants. Chemical convulsants applied systemically are often very effective in activating a latent or dormant lesion.

Disadvantages include the fact that compounds administered systemically may make the discovery of the locus of action difficult, and they may also obscure biochemical events of significance by inducing changes in the animal's own biochemistry which are unrelated to their primary convulsogenic activity[13,20,21]. Notable chemical convulsants include pentylenetetrazole, picrotoxin, and bicuculline (the latter two interfering with GABAergic transmission), strychnine (possibly active by interfering with glycine (inhibitory) transmission), enkephalin, conjugated estrogens, tetanus toxin, acetylcholine (also cholinesterase inhibitors) and penicillin[13].

3.3.1.3 Epileptogenic Foci; Metals and Metal Salts

Aluminium oxide (alumina) paste, cobalt, tungstic acid and iron all, when applied or implanted to or in various areas of animal brains, produced epileptogenic foci which gave rise to seizure activity of varying severity over a varying time-scale[13,22].

3.3.1.4 Epileptogenic Foci; Local Freezing

Freezing a small area of the animals' cerebral cortex to cause the production of an epileptogenic focus has been demonstrated in rabbits, cats and dogs; the frozen sites were spontaneously active, the frequency

of discharge decreasing over a varying time-scale[13,23]. During (electrically) quiet periods, discharges could be induced by a repetitive, mild tactile sensory stimulation[24], or by systemic application of pentylenetetrazole[25].

3.3.1.5 Secondary Epilepsy and Kindling

It has been shown that production of an epileptogenic focus by application of metals or local freezing to/of exposed cortical areas can lead to the production of further epileptogenic foci "downstream" (in physiological terms), which can progress through semi-independent states to full epileptogenic activity after the surgical removal of the initially formed 'primary' focus. This phenomenon is known as secondary focus epilepsy, and illustrates the point that even "normal" neuronal tissue has a latent potential for becoming an epileptogenic focus without direct application of an external inducer[13,26].

The phenomenon termed "Kindling" is somewhat different; here, the incidence and severity of reaction to electroshock stimulus increased with more frequent and longer continued application[27]. Amygdaloid electrodes have been used to produce a response similar to the pathological limbic seizure[28], while thalamic stimulation gives rise to behavioural and electrographic signs characteristic of petit mal epilepsy[29], as does bilateral infraneocortical stimulation[30]. Both the latter two models can often continue into generalised grand mal epilepsy. For instance, Goddard *et al* demonstrated, in a series of experiments in cats, rats and monkeys, that brief stimulation via implanted electrodes at first produced no response; stimulation was continued, once per day, over a period of weeks. After seven days after-discharges were observed along with behavioural symptoms of epilepsy. The first, short, bilateral clonic convulsions set in after two weeks, initially only of short duration but increasing in duration and severity with continued daily stimulus[31].

3.3.2 Inborn (Genetically Pre-Disposed) Epilepsy Models

All the previously discussed models require, at least in the first instance, the direct physical application of some stimulus to the animal to induce a seizure condition. Animals with an inborn tendency towards epilepsy stemming from some genetic predisposition obviously possess certain advantages as model systems in that no physical contact with the stimulus is required, e.g. simply the sound of a ringing bell or the effect of stroboscopic illumination induces the epileptic condition. Direct physical interference with the animal, apart from the introduction of potential anticonvulsant compounds is therefore minimised[13].

During this project, the effectiveness of our products as potential therapeutic anticonvulsants was assessed using two such genetically predisposed models; the photosensitive baboon *Papio papio* from Senegal[18] and the DBA/2J audiogenic strain of mice[32,33].

3.3.2.1 Photosensitive Baboon

The Sengalese baboon species *Papio papio* shows a genetically-determined syndrome of photosensitive epilepsy that is manifest as a series of myoclonic responses, and sometimes tonic-clonic seizures, during stroboscopic stimulation. The myoclonic responses are graded thus: 1 = Myoclonus of eyelids; 2 = Myoclonus of neck and facial muscles; 3 = Myoclonus of trunk and all four limbs; 4 = Myoclonus sustained beyond the end of photic stimulation.

Anticonvulsant drugs effective against primary generalised seizures in man (such as barbiturates, benzodiazepins and valproate) block these responses, and the syndrome has proved to be a valuable model for testing novel anticonvulsants[34].

3.3.2.2 Audiogenic Mice

The DBA (= Dilute Brown Agouti coat colour) strain of the house mouse (*Mus musculus*) inbred since 1909[35], has been shown to be susceptible to sound-induced seizures[36]. The sensitivity of the mice appears

to vary with age, reaching a period of maximum susceptibility at some stage within the broad range of 16-39 days old (various groups report different results for the most sensitive period within this general envelope)[35,37,38]. Almost 100% of the mice of this strain undergo this age-dependent epileptiform response, progressing through a clearly-defined sequence of convulsions when initially exposed to a loud mixed-frequency sound (12-16 KHz, 90-120 db)[17]. Firstly, the mice undergo a wild running phase (stage 1), followed by clonic convulsions (stage 2); then a tonic extension (stage 3), ending in respiratory arrest (stage 4) or complete recovery. The four stages, numbered 1-4 in increasing order of severity, provide a convenient system for evaluating potential anticonvulsants; both as a basis whereby pharmacological data such as ED_{50} values against a specific phase (e.g. clonus) can be calculated, and also as providing a simple average "Seizure Response" value which can be used to compare various compounds[17].

Experiments using this model have shown that all the commonly used anticonvulsant drugs are active in suppressing the sound-induced seizures, and the ranking order of efficiency of the drugs is comparable in the two situations, thus establishing the value of using the seizure-susceptibe DBA/2 mice as a screening model for potential anticonvulsant drugs; see Table 10.

During this project, much use has been made of DBA/2 mice; the results of this screening are shown in the following section.

TABLE 10 Protection against sound-induced clonic seizures in DBA/2 mice by various anticonvulsant drugs (after Meldrum[17])

Anticonvulsant	ED_{50}(IP) ($mgkg^{-1}$)	Clinical Dose ($mgkg^{-1}day^{-1}$)	Plasma Level (gml^{-1})
Clonazepam	0.005	0.1	0.01-0.05
Diazepam	0.04-0.12	0.1-0.5	0.1-1.0
Phenobarbital	2.3-7.0	1-3	15-25
Diphenylhydantoin	2.3-13.9	4-5	10-20
Valproate	55-300	5-60	50-100
Ethosuximide	130-146	20	40-80
Trimethadione	135-142	10-60	15

3.4 Results of DBA/2 Anticonvulsant Screening

3.4.1 Methods and Materials; Intracerebroventricular (ICV) Administration

DBA/2 mice of mixed sexes, body weight 6-12 g, 21-28 days old, were exposed to auditory stimulation 45 minutes after intracerebroventricular (ICV) injection of 10 µl of a solution of 67 mM sodium phosphate buffer, (pH 7.4), alone or containing various amounts of the putative anti-convulsant compounds.

The injection co-ordinates were 1 mm posterior and 1 mm lateral to bregma; depth of injection 2.4 mm[42].

Table 11 shows the results of screening a range of kainate analogues synthesised during this project against the sound-induced seizures in DBA/2 mice; also included, for reference, are the results of some analogues synthesised by Dr. A.J. Dixon.

LEGEND: Control respresents the mice injected with the phosphate buffer only; n = number of mice used; WR = %Wild Running (SR = 1); Clonic = % clonic convulsions (SR = 2); Tonic = %tonic extension (SR = 3); RA = % Respiratory Arrest (SR = 4). SR = Average seizure response for dosage level. Temp. = Rectal temperature, measured immediately prior to testing. AJD = Compound synthesised by Dr. A.J. Dixon.

TABLE 11 Results of ICV testing of putative anticonvulsants on DBA/2 mice

COMPOUND	DOSE µmoles ICV	n	WR (SR=1)	Clonic (SR=2)	Tonic (SR=3)	RA (SR=4)	Mean SR	Temp mean±SEM	Toxicity Notes
FGG5/27	CONTROL	8	100	100	63	50	3.1	37.7±0.13	
(Z0)	0.03	8	100	100	75	63	3.1	37.5±0.19	Normal
	0.1	8	100	100	71	57	3.4	37.3±0.14	Normal
	0.3	8	100	100	75	25	3.0	37.6±0.15	Normal
FGG5/38	CONTROL	10	100	100	90	50	3.5	37.5±0.11	
(Z1)	0.03	11	100	91	82	64	3.5	37.5±0.33	Normal
	0.1	10	100	100	100	100	4.0	38.2±0.17	Slight Myoclonus
	0.3	9	100	89	78	55	3.2	37.7±0.24	Hyperactive + Myoclonus
FGG5/36	CONTROL	8	100	100	75	75	3.5	37.6±0.13	
(Z3)	0.003	8	100	100	75	63	3.4	37.8±0.14	Rearing, Myoclonus, Whole body tremor
	0.1	8	100	87	87	75	3.5	37.9±0.13	
	0.3	12	100	100	90	60	3.5	38.0±0.20	
FGG/836	CONTROL	8	72	72	72	57	2.4	37.3±0.22	
(144)	0.03	7	100	100	100	100	4.0	37.5±0.18	Ataxia, forelimb Myoclonus; at 0.3 moles, 4/4 died.
[β-73)]	0.1	7	100	100	86	86	3.7	37.1±0.22	
FGG5/39	CONTROL	9	100	100	100	25	3.3	37.5±0.20	
(Z2)	0.01	1	100	100	100	0	3.0	-	Clonic forelimbs

Contd.

COMPOUND	DOSE μmoles ICV	n	WR (SR=1)	Clonic (SR=2)	Tonic (SR=3)	RA (SR=4)	Mean SR	Temp mean±SEM	Toxicity Notes
FGG5/48	CONTROL	7	100	100	71	43	3.1	37.3±0.17	
(38)	0.03	6	100	83	83	67	3.3	37.4±0.19	5/6: Forelimb myoclonus
	0.3	1	100	100	100	0	3.0	-	3/4: Dead
FGG5/B48	CONTROL	9	100	100	100	25	3.3	37.5±0.20	
(39)		9	100	89	56	44	2.9	37.7±0.17	1/10: Clonic seizures; dead 4/10: Slow periodic twist
		4	100	100	75	50	3.3	38.8±0.06	1/4: Full limbic seizure 3/4: Slight myoclonus
FGG5/56	CONTROL	10	100	90	70	30	2.9	36.1±0.12	
(80)	0.01	8	100	87	87	87	3.5	36.9±0.27	Normal; slight myoclonus; 2/10 dead
FGG5/58	CONTROL	10	100	90	70	30	2.9	36.0±0.12	
(58)	0.01	8	100	100	100	50	3.5	37.1±0.23	Rearing; forepaw myoclonus. 2/10 dead
FGG5/72	CONTROL	10	100	90	70	30	2.9	36.1±0.12	
(84)	0.1	8	67	50	50	50	2.2	37.8±0.23	2/10 dead
FGG5/83	CONTROL	10	100	90	70	30	2.9	36.1±0.12	
(81)	0.1	10	100	72	72	29	2.7	37.8±0.16	Rearing; facial and forepaw myoclonus; 3/10 dead
FGG5/60	CONTROL	10	100	100	100	50	3.5	36.8±0.17	
(132)	0.1	9	88	88	77	44	3.0	36.9±0.17	} Some excitant effect
	0.3	8	88	75	63	25	2.5	37.8±0.15	
	0.6	9	100	44	44	19	2.0	37.5±0.20	

Contd.

COMPOUND	DOSE µmoles IC	n	WR (SR=1)	Clonic (SR=2)	Tonic (SR=3)	RA (SR=4)	Mean SR	Temp mean±SEM	Toxicity Notes
FGG5/β1	CONTROL	13	100	100	100	62	3.6	36.8±0.14	
(36)	0.033	10	90	90	80	50	3.1	37.7±0.15	See text 3.6.1
β-Kainate	0.1	10	90	50	40	20	2.0	37.5±0.15	
	0.33	10	40	10	10	0	0.6	38.0±0.15	
	1.0	10	30	0	0	0	0.3	38.5±0.33	
αKAGly	CONTROL	10	100	100	100	20	3.4	37.7±0.09	
(133)	0.1	10	100	80	80	30	2.9	38.3±0.08	
	0.33	10	80	50	30	10	1.7	37.8±0.21	See text 3.6.1
	0.66	10	30	20	20	10	0.8	38.9±0.23	
(AJD)	1.0	10	30	20	10	0	0.6	37.7±0.35	
αKAPhos	CONTROL	10	100	100	100	60	3.6	37.1±0.07	
(134)	0.033	8	100	100	88	53	3.5	37.7±0.20	
	0.1	10	100	100	70	60	3.3	38.2±0.21	See text 3.6.1
(AJD)	0.33	7	100	43	29	14	1.9	37.9±0.27	
βKAGly	CONTROL	10	100	90	80	50	3.2	37.2±0.17	
(135)	0.33	10	100	80	80	30	2.8	37.7±0.10	
	0.1	10	100	60	50	30	2.4	38.3±0.16	See text 3.6.1
	0.33	8	75	12.5	0	0	0.9	37.2±0.56	
(AJD)	0.66	8	62.5	0	0	0	0.6	38.3±0.29	

Contd.

COMPOUND	DOSE µmoles ICV	n	WR (SR=1)	Clonic SR=2)	Tonic (SR=3)	RA SR=4)	Mean SR	Temp mean±SEM	Toxicity Notes
βKA Phos	CONTROL	10	100	100	90	50	3.4	37.0±0.10	
(136)	0.1	10	100	100	100	40	3.4	37.4±0.11	
	0.33	10	100	100	80	50	3.3	37.1±0.08	See text 3.6.1
	0.66	10	90	90	90	40	3.1	37.1±0.18	
(AJD)	1.0	9	100	78	78	22	2.5	37.1±0.14	

3.4.2 Methods and Materials; Intraperitoneal (IP) Administration

Mice, as described for 3.4.1 were screened 45 minutes after intraperitoneal (IP) injection of either 0.9% saline (0.1 ml, pH 7) or the putative anticonvulsant, β-kainic acid[36].

Table 12 shows the results of screening β-kainic acid in DBA/2 mice by IP injection; legend as per ICV injections.

3.5 Results; DBA/2 Mice Screening; Kainate Behavioural Effects

All kainate analogues tested by ICV administration to DBA/2 mice proved to be excitant to a greater or lesser degree. From a potential therapeutic point of view, none of the compounds synthesised during this project showed any promise at all, although the results obtained may be a useful guide for future investigation in this field.

3.5.1 Behavioural Effects of Compounds Tested Possessing Some Anticonvulsant Effect

The compounds possessing the most significant anticonvulsant activity in the DBA/2 mouse screening system were β-kainic acid, α-kainate keto-nitrile, α- and β-kainyl glycine (AJD) and α-kainyl phosphonate (AJD). All the above analogues gave rise to similar post-injection behaviour, displaying one or more of the following features: (in decreasing order of occurrence) ataxia; brief, intermittent fore-paw myoclonus; tail rigidity; circling; muscle weakness (splayed hindlimbs); loss of righting reflex; tonic extension. All subjects were hyperactive during this time, which covered the period 5-20 minutes after ICV injection. Some of these compounds showed additional toxic or convulsant symptoms with a more delayed onset (a characteristic of 'typical' kainate seizures); for example, 45 minutes after injection, at the time of testing for anticonvulsant action, the higher doses of

TABLE 12 Results of IP testing of putative anticonvulsant (β-Kainic acid)

COMPOUND	DOSE ($mmol\ Kg^{-1}$ IP)	n	WR (SR=1)	Clonic (SR=2)	Tonic (SR=3)	RA (SR=4)	Mean SR	Temp mean+SEM	Toxicity Notes
	CONTROL	10	100	100	100	80	3.8	37.9+0.23	
(36)	0.33	10	100	100	70	70	3.4	38.1+0.17	
βKainate	0.66	10	100	100	50	30	2.8	37.8+0.15	
FGG5/β1	1.0	10	100	80	10	0	2.0	37.1+0.13	See text
	2.15	10	80	40	0	0	1.2	36.4+0.25	
	3.3	10	50	30	0	0	0.8	35.9+0.20	
	6.6	10	20	0	0	0	0.2	36.0+0.26	

α- and β-kainyl glycine produced forepaw myoclonus in about half of the animals, along with a loss of righting reflex. There was an 80% mortality rate at about 45 minutes after testing for the group injected with 1.0 mol kg^{-1} of β-kainyl glycine. α-kainyl aminomethylphosphonate causes continuous clonic seizures in half the mice tested 45 minutes after ICV injection of 0.33 mol kg^{-1}.

3.5.2 Behavioural Effects of Compounds Tested Without Noticeable Anticonvulsant Activity

All the other compounds listed in Table 11 and not included under 3.5.1 did not possess noticeable anticonvulsant activity. In most cases standard kainate-induced seizure activity was obvious after a delayed-onset period as described earlier; the more immediate effects were also obvious and in some cases included the fairly rapid death of between 20 and 100% of the sample population. The lesser effects such as myoclonus, ataxia, circling, rearing and hyperactivity were all much in evidence. The considerable motor interference and impairment produced by some of these compounds with their attendant excitatory and frequently fatal effects indicate that some of the analogues synthesised may be more toxic than the parent α-kainic acid molecule.

3.6 Kainate Pharmacology; Summary of Results

At an early stage of the project it was hoped that, by analogy to compounds known to antagonise activity at the A1 receptor, an A3 antagonist might prove to be based on the same stereochemical requirement - i.e. it would possess the D(+) configuration. In the kainate molecule, α-kainic acid possesses the L(-) configuration and β-kainate the D(+). By comparison, therefore, the hypothesis suggests that kainate analogues based on the β-stereochemistry would perhaps be more

likely to furnish the desired A3 antagonists than compounds based on the parent α-kainic acid molecule. The discovery that β-kainic acid itself possessed some anticonvulsant activity seemed to provide some measure of support for this theory.

However, results with other β-compounds were not encouraging, as in some cases they appeared to be more toxic than the corresponding α-analogue. In addition, Meldrum and co-workers have recently suggested that β-kainate may be exerting its anticonvulsant effect in the DBA/2 model by action at the A1 receptor[33], rather than the A3, which would suggest that an A3 antagonist based on the kainate molecule still awaits discovery.

From a structure-activity point of view, as far as the analogues tested go, one or two general trends are apparent. Although kainylglycine showed some slight activity in the DBA/2 test system, it and all the other peptides formed at the C-3 side chain proved to be more or less toxic. In particular, the peptides where a cyclic group had been added turned out to be extremely toxic. Replacement of the C-3 carboxy-acid group with a nitrile did not appear to reduce toxicity and neither did the C-3 chain extension by a methylene group (AJD). Both α- and β-compounds in this range were excitant. The kainyl aminomethyl phosphonate variants were also toxic and excitatory.

The class of kainate analogues which seemed to show the most general anticonvulsant activity in the DBA/2 screening system were those where the terminal $=CH_2$ of the isopropenyl side chain at C-4 was replaced by carbonyl oxygen to form the ketone. Presumably such a change would imply a different pattern of electron density with a general displacement towards the terminal oxygen atom rather than the more even distribution along the double bond in the alkene. Any alteration along these lines would almost certainly modify interactions at the receptor; changes in intra-molecular interactions leading to

further steric modifications in receptor "fit" might also occur.

Considering the known potency of domoic acid as a displacer of kainate at the receptor site[39], it might be speculated that production of kainate analogues with an extended C-4 side chain (whilst retaining an unsaturated character) could prove to be interesting. Investigations begun into synthetic approaches towards this end were unfortunately curtailed by lack of available time in this project; nevertheless, projected development of Wittig-type reactions to produce materials with differing C-4 side chains would provide potentially interesting analogues whose pharmacological evaluation should not be neglected.

3.7 Results of Photo-Sensitive Baboon Screening

In addition to the production of kainate analogues to investigate possible A3 receptor antagonists, quantities of the putative A1 receptor antagonists 2-APP and 2-APH were synthesised during this project, and their efficiency in preventing seizures in the photosensitive baboon *Papio papio*[34] assessed.

3.7.1 Methods and Materials

Adolescent baboons (*Papio papio*) were tested, whilst seated in a primate chair, by exposure to stroboscopic stimulation for up to five minutes, in a standardised fashion; compounds were administered intravenously and the animals were then observed for the following five hours with testing of myoclonic responses to stroboscopic stimulation at hourly intervals.

Fig. 6 shows the results of testing of 2-APH, and Fig. 7 the results of screening 2-APP in this model.

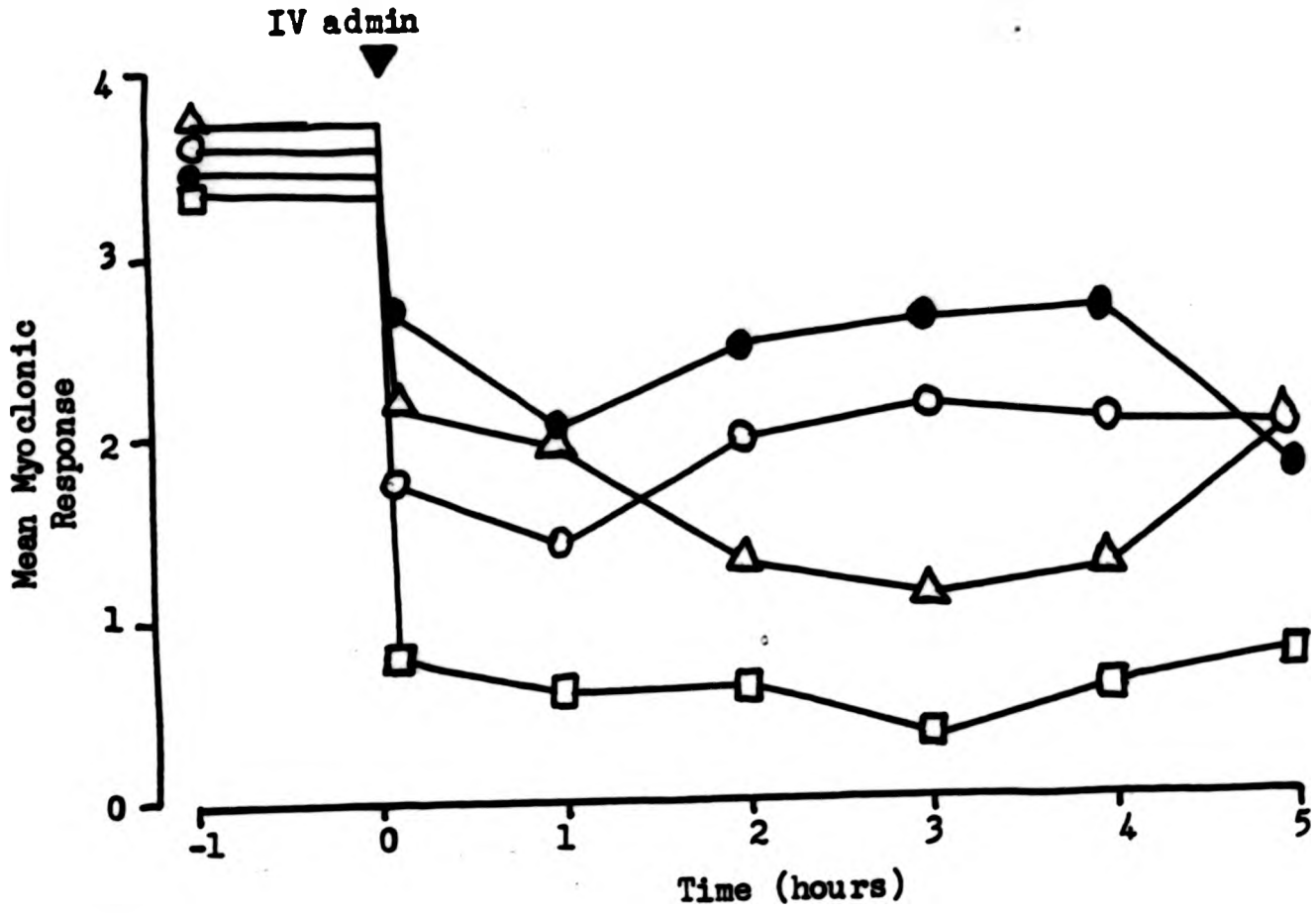


Fig.6 : Suppression of photically-induced myoclonic responses in the baboon Papio papio by 2-APH. (After Meldrum[18]).

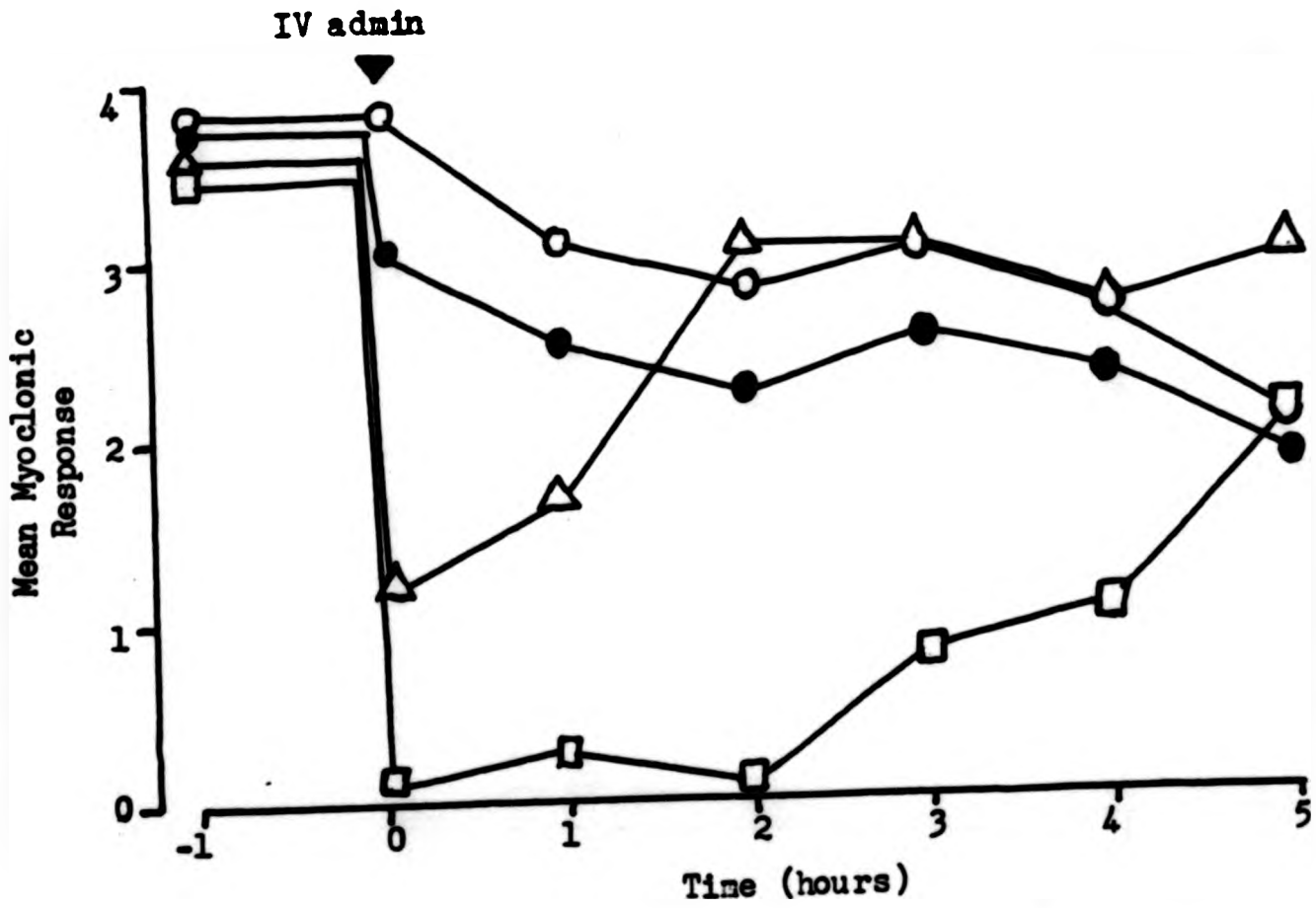


Fig.7 : Suppression of photically-induced myoclonic responses in the baboon Papio papio by 2-APP. (After Meldrum[18]).

LEGEND:

The mean myoclonic response to photic stimulation is plotted against time (hours) relative to drug administration at time zero. Groups of n animals were tested at each dose level; (n = 3 at all doses except 2-APP control group where n = 5). The following dose levels (mmol kg^{-1}) are shown:

Fig. 6: ● = saline; ○ = 0.1; △ = 0.33; □ = 1.0.

Fig. 7: ● = saline; ○ = 0.1; △ = 1.0; □ = 3.3

3.8 Photosensitive Baboon Screening; A1 Receptor Antagonism

The efficacy of 2-APH and 2-APP against photically-induced seizures in this test model indicates potential value worth exploring as an anti-convulsant, particularly in the case of 2-APH. There appeared to be no manifest neurological impairment, although the highest dose induced some retching and excess salivation in one animal immediately after administration of the drug. However, all animals appeared alert during photic stimulation. As shown in Fig. 6, 2-APH was effective in suppressing seizure response for five hours after intravenous (IV) injection. 2-APP showed a shorter time-course of action, the myoclonic responses being abolished about ten minutes after IV administration, but returning to normal after only 1-2 hours. Higher doses increased the protection time to 2-4 hours, after which myoclonic responses were again noted in response to stimulation. However, such dose levels caused some sedative effects, loss of muscle tone, and, in two out of three animals, excess salivation[18].

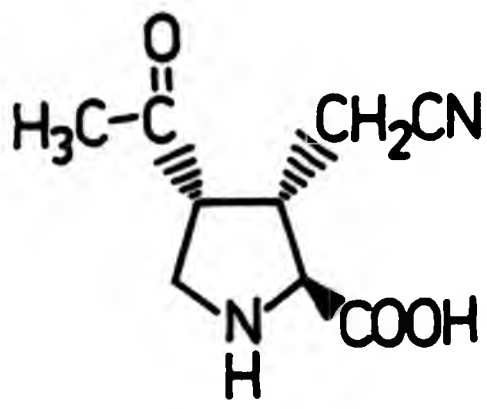
The greater efficacy of 2-APH as opposed to 2-APP corresponds to a similar pattern of results in testing using DBA/2 mice[3].

3.9 α-amino-ω-phosphonocarboxylic acids: Summary of Pharmacology

The results achieved during the investigation of the efficacy of 2-APH and 2-APP against photically-induced seizures in the baboon model confirm the potential value of these compounds as putative anticonvulsants. At typical effective dosages they appear to be generally lacking in systemic toxicity and gave few obvious side effects.

From a pharmacological standpoint, further research into possible side-effects such as impairment of processes required for transfer of information from short-term to long-term memory (although investigation of this phenomenon in animal models may prove rather difficult) will be required

Chemically speaking, the major challenge would be to produce some suitably protected form(s) of these compounds, particularly 2-APH, (which appears to be the most potent), such that lipid solubility is greatly increased and penetration of the blood-brain barrier is thereby enhanced; this would reduce the amount of material required for systemic administration as at present. Such protecting or masking groups should, of course, be removable by *in vivo* (enzymic) systems, preferably at or near the site of action, and possible candidates include various N-protecting groups or some form of Schiff Base formation at the amine[40]; esters on all or some of the acid functions, or perhaps the incorporation of said acid groups into lipid esters[41]. Indeed a combination of several such possibilities might prove necessary; certainly current results indicate a potential for rapid future development in this area.



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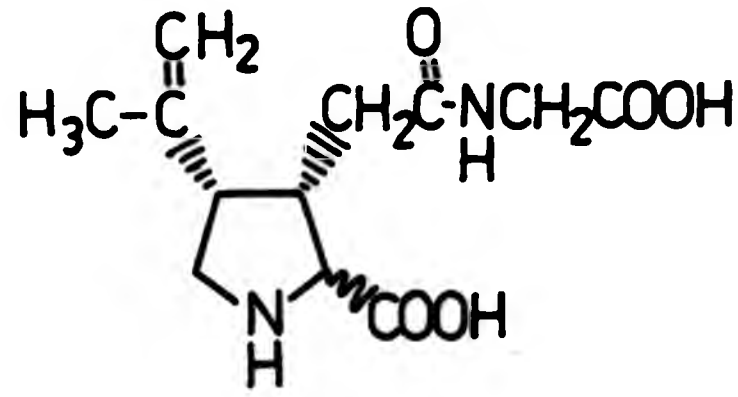
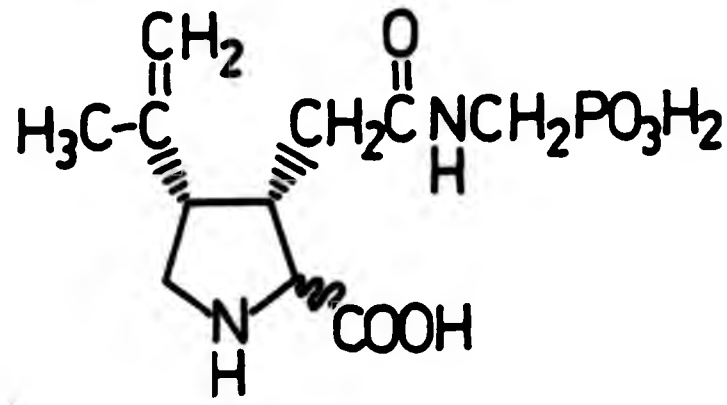


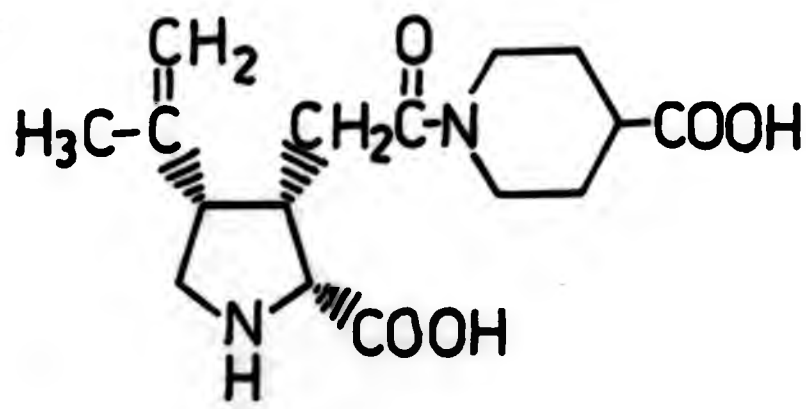
(133) C-2=α

(135) C-2=β



(134) C-2=α

(136) C-2=β



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CHAPTER FOUR

KAINATE NEUROCHEMISTRY

4.1 Aims of Kainate Neurochemistry

The neurochemical investigations of kainate analogues outlined hereafter were carried out by Dr. P.J. Roberts and his group at the University of Southampton, Department of Physiology and Pharmacology, to whom I am grateful for permission to quote from their results.

A range of kainate analogues were employed (including several which were not synthesised during this project) in order to determine possible structure-activity relationships to the effects of kainate-type compounds at the A3 receptor.

4.2 Neurochemical Aspects of the A3 Receptor

Various workers have confirmed an identity between the kainate-preferring receptor, as identified by electrophysiological experiments, [1,2,3] and the areas susceptible to binding of radio-labelled kainate[4,5,6]. This A3 receptor site (thus defined according to the nomenclature of Fagg and Foster[7]) has been shown to be further sub-divided into a high- and a low-affinity site, the former with a K_d of 5 nM and the latter in the range K_d = 27-72 nM[3,4,8]. An isolated report[8] detected a high-affinity binding site (K_d = 11 nM) only in intrasynaptosomal mitochondrial preparations which led to the suggestion that these organelles were the units responsible for the high-affinity binding observed in previous studies [3,9] which employed total particulate membrane preparations; however, the findings that high- and low-affinity sites are enriched in purified synaptic membranes[2,8] and synaptic junction fractions[4] tends to indicate that both populations of binding sites are present at the synapse[7].

4.3 Kainate Analogues: Potential Neurochemical Interactions at Endogenous Receptors

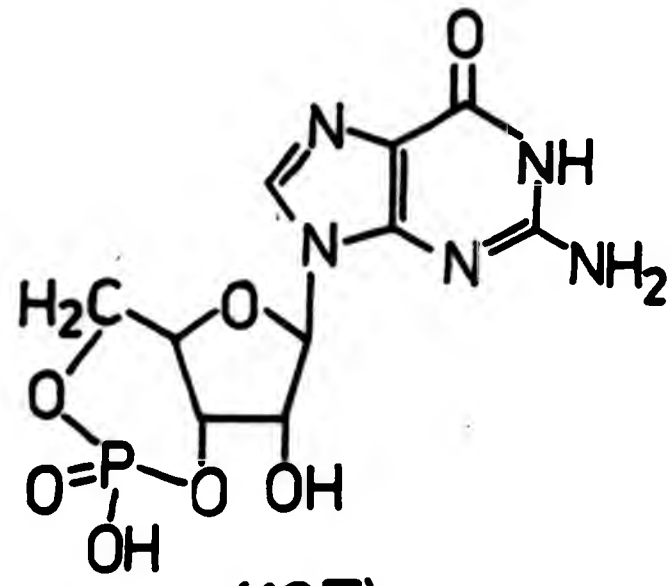
Although the endogenous neurotransmitter at the physiological A3 receptor has not been identified conclusively, a working hypothesis could implicate L-glutamate and/or L-aspartate in the fulfilment of this role[10,11,12]. As indicated in Chapter One (q.v.) a variety of conditions attach to the term "neurotransmitter", which may be briefly recapitulated here: (1) Synthesis and Storage, (2) Release, (3) Identity of Action, (4) Disposal, and, (5) Pharmacological Effect.

The introduction of any non-endogenous compounds to the receptor(s) and surrounding cellular environment could potentially influence one (or more) of the characteristics outlined above, thus leading to a change in the neurochemical environment of the synapse with probable concommittant alterations in specific transmission and possibly a general degradation in cellular function and condition.

The iontophoretic application of kainate and related compounds has been shown to have a neurotoxic and neurodegenerative effect[13]. Such activity must perforce be associated with, and/or accompanied by, changes in the neurochemical status of the tissue involved. For example, does the application of kainate analogues lead to an increased level of endogenous neurotransmitter at the synapse, perhaps by increased release from the pre-synaptic neurone, or by a reduction of (re-) uptake of endogenous transmitter by the cells surrounding the synapse?

Additional information regarding the activity of compounds as at the receptor site(s) can also be obtained by studying the efficacy of a given compound displacing a labelled ligand from the receptor site, thus indicating any specific preference for a receptor type.

Furthermore, the ability of a given excitatory amino acid to increase rat cerebellar cyclic-GMP (137) levels, via receptors whose pharmacological properties are similar to those found in electrophysio-



(137)

logical studies, provides another route of obtaining data regarding the interaction of analogues with excitatory amino acid receptors[14,15,16,17].

In the following sections, a brief evaluation of the potentially interactive aspects of the compounds synthesised during this project with endogenous neurotransmitter systems is given, followed by the results of some neurochemical investigations based on such biochemical aspects.

4.3.1 Endogenous Neurotransmitters: Release

The most reliable demonstration of the pre-synaptic release of a neurotransmitter is that in which the release of endogenous amino acid is monitored[18], or the release of labelled amino acid formed from some suitably labelled precursors[19] (*e.g.* [^{14}C]-Glucose) which have been shown to provide precursor material for amino acids in neurones[20,21,22]. An obvious difficulty incurred in studying the release of, say, L-glutamate is that release could occur from cellular compartments other than the nerve terminals, or of material bound to external cell surfaces[23,24,25].

The release of pre-synaptic L-glutamate can be effected by increased extracellular K^+ ion concentration or by electrical pulses[26]; the release of L-glutamate and L-aspartate in response to either electrical

or ionic stimulation is almost completely abolished in the absence of Ca^{++} ions[26]; this is similar to the situation at GABAergic synapses, where transmitter release is dependent on an inward current of calcium[27].

4.3.2 Endogenous Neurotransmitters: Uptake

Following release and subsequent activity at the receptor, some mechanism must be available to remove the transmitter substance from the synaptic cleft. An initial hypothesis relating to excitatory amino acid neurotransmitters was that excess neurotransmitter was inactivated enzymically; however, the failure of various microelectrophoretically applied enzyme inhibitors to affect (prolong) the *in vivo* excitation caused by the application of electrical or agonist stimulation suggested a lack of an effective enzymic degradation system situated externally on synaptic membranes or those of the immediately surrounding cells[28]. Conversely, the sensitivity of certain cells to electrophoretically applied L-glutamate and L-aspartate was enhanced by *para*-chloromercuriphenyl sulphonate, a compound which had previously been shown to block uptake of DL-aspartate and L-glutamate into cerebrocortical slices[29]. Additional evidence includes the fact that mono-N-alkylated aspartate derivatives, which, although possessing an excitatory effect, do not appear to be actively accumulated by neuronal tissue[30], were shown to prolong the excitation of neurones after termination of electrophoretic application for considerably longer than the unsubstituted D- or L-aspartate[31].

It has been noted that amino acids implicated in a neurotransmitter function (e.g. glycine, inhibitory transmitter in mammalian spinal cord[32]) can enter neural tissue by both high- (K_m = 1-50 μM) and low- (K_m = 0.1 - 1.0 mM) affinity transport systems; whereas other non-transmitter amino acids (e.g. glycine in the cerebral cortex, where it has not transmitter function[32]) have only low-affinity uptake systems[29,30,33].

No definite conclusion seems to have been reached as to the relative importance of the high- and low-affinity systems as regards the termination of excitatory amino acid neurotransmitter action, although Cox and Bradford[34] have suggested the possibility that the high-affinity site is most important in "scavenging" for small quantities of excitatory amino acids which could prevent a gradual build-up of the same in the extracellular spaces around the neurones involved in the excitatory amino acid transmission; such a theory would also provide an explanation of the presence of high-affinity transport systems in glial cells which are (comparatively) distant from the synaptic cleft itself.

Although kainic acid itself has been observed to act only as a weak inhibitor of low-affinity uptake[35] (with no apparent effect against the high-affinity system[36]) there is a potential for interaction between kainate analogues and the uptake systems which were investigated with our synthesised compounds.

4.3.3 Excitatory Amino Acids and Raised c-GMP Levels

Investigators studying neurotransmission in the granule cell parallel fibres, (where glutamate is thought to fulfil the transmitter role[37]) noted that stimulation (or depression) of these pathways *in vivo* increased (or reduced) cyclic-GMP (137) levels in the cerebellum[38,39].

Subsequent evaluation of this phenomenon indicated that the excitatory amino acids could provide the stimulus which would bring about an increase in cyclic-GMP concentrations, and that they did so *via* species of receptors whose pharmacological profile closely resembled that found in electrophysiological studies. Thus, the measurement of the nucleotide concentration changes has provided a means of assessing coupled excitatory amino acid-receptor function[14,15,16]. Although the precise physiological significance of the c-GMP accumulation in

response to excitatory stimuli does not seem to have been thoroughly defined, it has been suggested that the event is associated with neuronal depolarisation (and attendant calcium ion influx) which starts off the accumulative response[40].

4.3.4 Receptor Binding and Displacement

The measurement of changes in the cytoplasmic levels of cyclic-GMP provides an indirect assessment of the capability of a putative neurotransmitter agonist or antagonist to act at the synapse by combining with the receptor site in some fashion. Such measurements do not provide any direct perception of the interaction at the receptor site, however. While observation of the effects on c-GMP levels of potential inhibitors of excitatory amino acid-evoked responses in cerebellar levels of the nucleotide thus allow some insight into interaction at the receptor of the putative antagonist and the excitant, a rather more direct approach is to evaluate the ability of a given analogue to displace labelled agonist material from the receptor where it has bound. Various authors have examined the localisation of binding of [^{3}H]-α kainic acid to various anatomical areas within the brain, and have examined displacement by various excitatory agents; for examples see [1,2,3,5]. There is general agreement that the ranking order of displacer potency is domoic acid>kainic acid>quisqualic acid>α-keto-kainate >L-glutamate, (at both high- and low-affinity binding sites, although the absolute inhibitory potencies are greater at the former)[3,9].

A range of kainate analogues synthesised during this project were therefore tested to evaluate their ability to displace radiolabelled kainate from rat cerebellar tissue preparations.

4.4 Biochemical Testing; General

Four biochemical models were employed to evaluate the activity of kainate analogues at the receptor, these being:

(a) effect on release of D-[^{3}H]-aspartate from adult rat cerebellar minislices;

(b) effect on high-affinity D-[^{3}H]-aspartate uptake in adult rat cerebellar synaptosomes;

(c) Effect on adult rat cerebellar slice cyclic-GMP levels, and,

(d) effect on sodium-independent specific [^{3}H]-α-kainate binding to high-affinity sites.

4.4.1 Aspartate Release Model

4.4.1.1 Materials and Methods

Adult rat cerebellar minislices were perfused with normal Krebs bicarbonate buffer at 37°C. The slices were exposed to either 30 mM potassium alone in the first period of stimulation (S_1) or to 30 mM potassium plus the compound under test during the second period of stimulation (S_2). Each period was of four minutes duration. The ratio S_1/S_2 of D-[^{3}H]-aspartate release, as determined experimentally, was calculated.

4.4.1.2 Aspartate Release Model; Results

TABLE 13 Results of testing kainate analogues in aspartate release model

Compound	Concentration (μM)	Ratio S_2/S_1 (±SEM)
L-glutamate* (1)	300	1.96±0.13
	1000	2.84±0.28
α-kainate* (6)	14	1.12±0.17
	140	1.19±0.24
FGG5/60 (132)	56	0.89±0.11
	560	1.36±0.28
α-allokainate* (34)	24	0.91±0.14
	240	1.13±0.24
α-alloketokainate* (140)	65	1.16±0.19
	650	1.08±0.21
β-ketokainate* (32)	67	1.01±0.15
	670	0.97±0.20
β-alloketokainate* (141)	50	1.04±0.81
	500	1.75±0.35

Legend: Control S_2/S_1 = 1.00. Compounds marked * thusly not synthesised during this project and included only for comparison.

4.4.1.3 Aspartate Release Model; Discussion

Cox and Bradford[34] have indicated that in their test systems concentrations of kainic acid in the range 0.1 - 1.0 mM increased the median level of aspartate accumulation in the incubation medium of synaptosomal preparations of the rat cerebral cortex by about 60 - 200%, although they noted the difficulty in distinguishing between an effect on release and a possible inhibitory effect on uptake in their model.

The results for the range of our analogues tested in the cerebellar minislice model do not appear to offer any especially compelling evidence for a major effect on aspartate release in this model. While there is a general trend towards increased aspartate release following

administration of the kainate-type materials, the effect is nowhere near as pronounced as that elicited by L-glutamate. As a general conclusion, then, it would not seem unreasonable to state that, based on the above results in the given model system, the kainate analogues tested show only a moderate general ability to increase released concentrations of aspartate.

4.4.2 Aspartate Uptake Model

4.4.2.1 Materials and Methods

Adult rat cerebellar synaptosomes were incubated with the kainate compounds over a concentration range of 0.1 - 1.5 mM for three minutes in Krebs bicarbonate buffer at 37°C in the presence of 10 nM D-[^{3}H]-α-aspartate. The assays were initiated by the addition of P_2 synaptosomes and non-specific uptake determined in the presence of Na^+-free medium. IC_{50} concentrations were calculated from suitable inhibition versus concentration plots.

4.4.2.2 Aspartate Uptake Model; Results

TABLE 14 Results of testing kainate analogues in aspartate uptake model

Compound	IC_{50} (μM) ±SEM
α-dihydrokainate* (33)	110±16.5
α-kainic acid* (6)	350±52.5
α-allokainate* (34)	1050±158
β-kainate (36)	1325±200

Legend: Compounds marked * thusly not synthesised during this project and included for comparison.

Inactive compounds in this test system included: α-homokainate, β-homokainate, α-ketokainate, β-ketokainate, α-alloketokainate, β-alloketokainate, α-kainyl glycine, β-kainyl glycine, α-kainylaminomethylphosphonate, β-kainylaminomethylphosphonate.

4.4.2.3 Aspartate Uptake Model; Discussion

Previous authors have observed, while studying the effect on labelled glutamate uptake in varying model systems, differing results. Thus, while some workers[41] observed very considerable (60-75%) reductions in uptake, others[34,36] reported more modest (15-20%) decrements. In all cases, relatively high (0.3-1.0 mM) concentrations of kainate were required to elicit any significant inhibition of glutamate uptake.

While the model used to test our analogues is concerned with measuring the effect on aspartate rather than on glutamate uptake, the concentrations required to elicit a response are in the same range as the previous investigators noted.

α-dihydrokainate showed a markedly superior ability to the other analogues to inhibit uptake, which, generally, were almost completely inactive. It would seem that any modification to the C-3 side chain (by amide formation) or derivatisation of the alkenyl moiety at C-4 to the corresponding ketone removes any intrinsic uptake-blocking property of the parent molecule.

4.4.3 Cyclic-GMP Model

The investigation into cyclic-GMP levels in neuronal tissue following administration of kainate analogues was subdivided into two approaches: firstly, the elucidation of maximum response, i.e. determining the analogues with the greatest ability to increase cellular cyclic-GMP concentrations, and, secondly, the determination of the degree of inhibition of stimulation of rat cerebellar slice cyclic-GMP concentrations enhanced by excitatory amino acids.

4.4.4 Cyclic-GMP; Maximal Response

4.4.4.1 Materials and Methods

Adult rat cerebellar slices, 300 μm thick, were preincubated for 90 minutes in Krebs bicarbonate medium maintained at 37°C. After 1 minutes' administration of compound under test, the incubation was terminated, and, after suitable solubilisation and homogenation procedures, followed by centrifugation, the supernatant solution was assayed for cyclic-GMP by antibody binding techniques. (For more extensive detail, see[17]). Log-dose versus effect plots were used to calculate EC_{50} values for the compounds tested. The concentration required to produce the maximal effect in each case was also noted.

4.4.4.2 Cyclic-GMP, Maximal Response; Results

TABLE 15 Results of testing kainate analogues on cyclic-GMP maximal response model

Compound	Max. Response (μmol.cyclic-GMP/ mg protein)	Concentration (for Maximal Response)	EC_{50} (μM)
AD1/59* (138)	160	30	11
α-kainic acid* (6)	160	100	14
AD1/58* (139)	95	100	20
α-allokainate* (34)	130	100	24
β-alloketokainate* (141)	145	1000	50
FGG5/60 (132)	110	300	56
α-alloketokainate* (140)	80	1000	65
β-ketokainate* (32)	55	3000	67
quisqualic acid* (5)	80	300	100
N-methyl-D-aspartate* (4)	55	1000	100
α-homokainate* (35a)	200	3000	100
FGG5/58 (58)	100	1000	120
α-ketokainate* (142)	210	1000	141
α-dihydrokainate* (33)	100	300	150
β-kainic acid (36)	50	3000	158
α-kainyl glycine* (133)	50	1000	178
FGG5/56 (80)	60	3000	205
FGG5/83 (81)	35	3000	220
FGG5.72 (84)	40	3000	240
β-homokainate* (143)	30	3000	320

Legend: Compounds marked * thusly not synthesised during this project and included for comparison.

Inactive compounds in this test system included: AMPA, quinolinic acid, FGG5/27, FGG5/33, FGG5/36, FGG5/39, FGG5/β36 and FGG5/β48.

4.4.4.3 Cyclic-GMP, Enhancement Inhibition; Materials and Methods

Adult rat cerebellar slices, 300 µm thick, were preincubated for 90 minutes in Krebs bicarbonate medium maintained at 37°C. Putative antagonists were applied (in concentrations over the range 0.1 - 1.0 mM) to the incubation medium 1 minute prior to the addition of agonists, which was continued for 1 minute, at the end of which time the incubation was terminated and cyclic-GMP levels assayed as per 4.4.4.1. Plots of log-concentration versus percentage inhibition of enhancement provided the means of determining IC_{50} values. Agonists were added in concentrations corresponding to their EC_{50} values, as determined in previous experiments (cf 4.4.4.2).

4.4.4.4 Cyclic-GMP, Enhancement Inhibition; Results

TABLE 16 Results of testing kainate analogues in cyclic-GMP Enhancement Inhibition model

Compound	IC_{50} (μM) NMDA (100 μM)	QA (100 μM)	α-KA (14 μM)
α-kainyl glycine* (133)			265
FGG5/36 (73)			285
GAMS* (25)	NE	850	320
β-kainic acid (36)	>890		>320
FGG5/56 (80)			340
FGG5/83 (81)			355
FGG5/72 (84)			390
FGG5/27 (70)			>420
FGG5/48 (39)			510
FGG5/33 (71)			560
FGG5/39 (72)			620
FGG5/36 (144)			630
cis-2,3-PDA* (30)	820	NE	740
γ-DGG* (29)	780	1000	850
α-dihydrokainate* (33)			870
β-alloketokainate* (141)			920
α-ketokainate* (142)			920
α-homokainate* (35a)			>1000
GDEE* (23)	NE	680	>1000
AD1/59* (138)	>1000		NE
(-)-2-APP* (15)	210	NE	NE

Legend: NE = Not effective. Compounds marked * thusly not synthesised during this project and included for comparison.

Inactive compounds in this test system included: β-ketokainate, α-alloketokainate, α-allokainate, AD1/58, FGG5/58 and FGG5/60.

4.4.4.5 Cyclic-GMP Models; Discussion

The two subdivisions of this model show, when compared, a most interesting pattern of results, in that the compounds most potent at increasing cyclic-GMP levels were least effective at inhibiting α-kainate-induced enhancement of tissue c-GMP concentrations; conversely, the weakest stimulants of nucleotide accumulation (including the completely ineffective types) were the best inhibitors of kainate-induced rises in c-GMP levels. This would appear to indicate a degree of functional divergence in the analogues tested; those compounds which are, broadly speaking, of a similar general size and bulk to the parent α-kainate molecule appear to possess a kainate-like ability to increase c-GMP levels, e.g. AD1/58, AD1/59 (which differ from the parent only by one methyl ester group), α-allokainate (differs only in stereochemistry at C-4), etc. This trend holds reasonably firm as the least active compounds include those that are amongst the most different to the parent molecule (e.g. FGG5/27, FGG5/33, FGG5/36, FGG5/39, FGG5/β36 and FGG5/β48), by virtue of the large groups attached to the C-3 carboxyl *via* an amide coupling. Such analogues would not only present a much bulkier appearance to a potential site of interaction at the cellular level, (with possible attendant steric interaction and inhibition), but would be more likely also to possess different intrinsic steric conformations to the parent molecule as the addition of large and sterically hindering groups at C-3 might be expected to contribute to intra-molecular- in addition to any inter-molecular interactions at potential interactive sites on the membrane.

The inhibition of kainate-induced rises in cyclic-GMP concentration is thus unlikely to be affected by compounds which themselves induce such increases - if it is assumed that any inhibition is a result of competition for the interactive "receptor" site between

kainate and the analogues, then any compound able to raise the cyclic-GMP nucleotide level which successfully competes with kainate for the receptor site will then automatically contribute a certain measure of induction of increase in the c-GMP concentration rises. It would then follow that only those compounds which could compete for the binding sites, and, once emplaced, fail to produce any significant increase in cyclic nucleotide accumulation, would be the ones likely to inhibit the kainate-enhanced c-GMP level increases. In general, the results obtained from these experimental models are in agreement with this hypothesis.

4.4.5 [^{3}H]-α-Kainate Binding Displacement Model

4.4.5.1 Materials and Methods

The displacement of sodium-independent specific [^{3}H]-α-kainate binding from "high-affinity" sites by a range of kainate analogues was assayed thus; extensively washed cerebellar synaptic plasma membranes (cerebellar synaptosomes) were incubated at 2°C and 6 nM [^{3}H]-α-kainic acid administered simultaneously with a variety of analogues at differing (10 nM - 10 μM) concentrations. The incubation was continued for 90 minutes and the amount of displaced (non-specifically bound) [^{3}H]-α-kainate measured. Plots of percentage displacement of specific binding versus log-displacer concentration were obtained and IC_{50} values determined from them.

4.4.5.2 [^{3}H]-α-kainate Binding Displacement; Results

TABLE 17 Results of testing kainate analogues in the specific [^{3}H]-α-kainic acid binding displacement model.

Compound	IC_{50} (nM) ±SEM
α-kainate* (6)	25±4
domoic acid* (26)	40±6.5
FGG5/58 (58)	110±17
FGG5/60 (144)	115±17
α-ketokainate* (142)	130±20
α-homokainate* (35a)	251±40
α-allokainate* (34)	380±60
α-alloketokainate* (140)	610±90
L-glutamate* (2)	630±95
quisqualic acid* (5)	794±120
α-kainyl glycine* (133)	891±135
β-ketokainate (32)	1000±150

Legend: Compounds marked * thusly not synthesised during this project and included for comparison.

Inactive compounds in this test system included: NMDA, (-)-2-APP, *cis*-2,3-PDA, GDEE, γ-DGG, GAMS, Ibotenate, D-glutamate, D-aspartate, L-aspartate, AD1/58, AD1/59, FGG5/27, FGG5/33, FGG5/36, FGG5/β36, FGG5/39, FGG5/β48, β-kainic acid, FGG5/56, FGG5/72, FGG5/83, α-dihydrokainate, β-alloketokainate, β-homokainate, β-dihydrokainate, β-kainyl GABA.

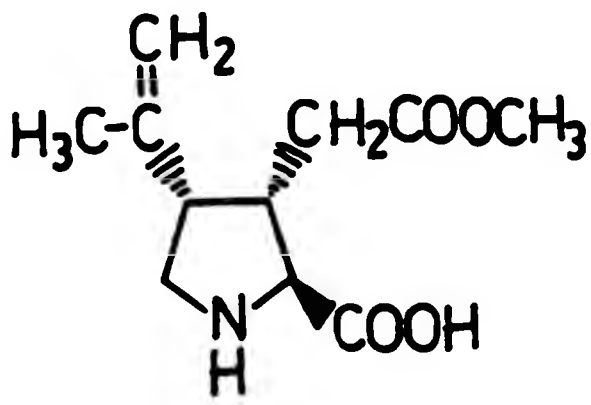
4.4.5.3 [^{3}H]-α-kainic acid Binding Displacement; Discussion

While it is difficult to generalise what the results obtained above mean in respect to activity at the receptor, it would be correct to state that the ability of a given compound to displace a compound attached to a binding (receptor) site is a function both of the affinity of the displacing agent for the interactive site, and its ability to

remain *in situ* after displacing the original molecule. Without the appropriate experimentation it would not be possible to state with absolute certainty the nature of the displacement recorded above, but it seems reasonable to infer that the figures shown for IC_{50} values could represent a measure of receptor affinity rather than an ability to bind irreversibly to the interactive site, as none of the compounds which appear to possess reasonable displacement ability contain the functional groups likely to interact much differently to the parent molecule at the binding site.

It would seem reasonable to suggest that those analogues which most closely resemble the parent α-kainate molecule would be the best displacers; in fact, this is not entirely the case. Both AD1/58 and AD1/59 were effective in raising cyclic-GMP levels and differ from the parent only in having one or the other carboxyl group esterified as the methyl function; however, neither shows a displacement capacity with an IC_{50} less than 1 μM. On the other hand, FGG5/58 and FGG5/60 both have the C-3 carboxyl converted to a nitrile function, and FGG5/60 also has the C-4 isopropenyl side chain changed to the ketone; both these compounds show c-GMP concentration-increasing ability in addition to displacing labelled kainate from the receptor at IC_{50} values of 110 - 115 nM.

One noticeable trend is that the C-4 stereochemistry does not appear to be critical (although it would appear that the normal (non-allo-) configuration is preferred) whereas the configuration at C-2 is more clearly relevant, the α-stereoisomers appear to be much more potent displacers than the corresponding β-analogues.



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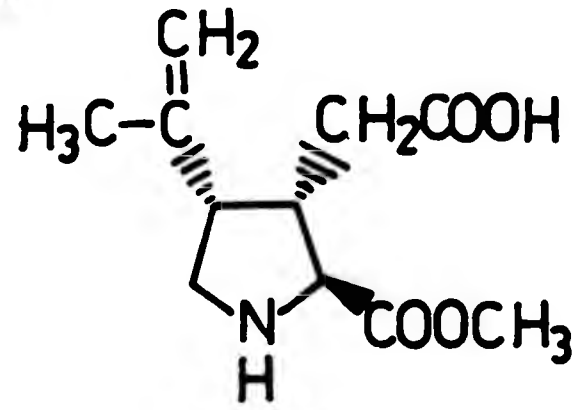

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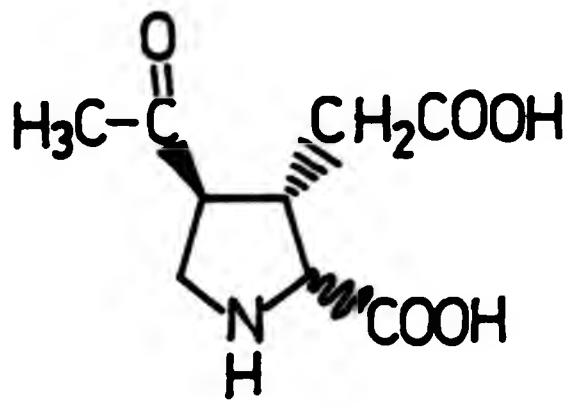

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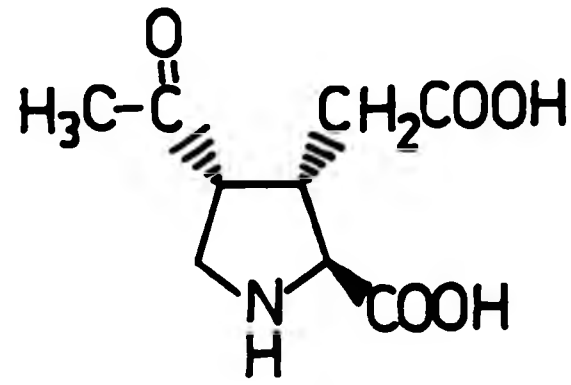
(141) C-2=β



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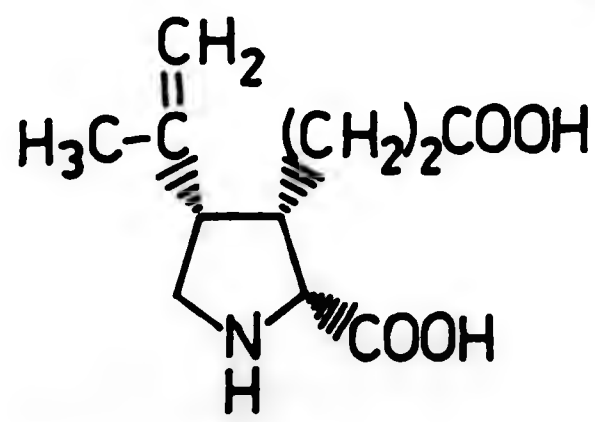

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CHAPTER FIVE

EXPERIMENTAL

5.1 Materials and Methods

90 MHz ^{1}H, 22.5 MHz ^{13}C and 36.2 MHz ^{31}P NMR spectra were recorded with a Jeol FX90Q Fourier Transform Spectrometer; the reported chemical shifts are relative to TMS (^{1}H and ^{13}C) and H_3PO_4 (^{31}P); values are expressed as parts per million (ppm).

100.62 MHz ^{13}C NMR spectra were recorded on the Warwick SERC High-Field NMR service.

FAB mass spectra were recorded on the Harwell PCMU service.

Solvents were dried for reactions by appropriate methods; see Vogel[1] for details.

IR spectra were recorded on a Perkin-Elmer 298 Spectrometer.

Column chromatography was performed on Kieselgel 60H (Merck); radial chromatography employed a TC Research Chromatotron with 1,2 and 4 mm plates coated with Kieselgel Silica 60G (PF254) (Merck).

Ion-exchange chromatography was performed using Dowex W50X8 resin (BDH) in the H^+ form.

5.2 Kainate Chemistry; Preparative Work

(2S,3R,4S) 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine. (6)

^{13}C NMR (D_2O); δ176.66 CH<u>C</u>OOH; δ173.74 CH_2<u>C</u>OOH; δ140.42 <u>C</u>:CH_2; δ114.04 C:<u>C</u>H_2; δ66.15 <u>C</u>-2; δ47.02 <u>C</u>-5; δ46.27 <u>C</u>-4; δ41.23 <u>C</u>-3; δ33.75 <u>C</u>H_2COOH; δ22.65 <u>C</u>H_3.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine (40)

α-Kainic acid (1 g, 4.7 mM) was dissolved in water (20 ml), slight warming being necessary to effect full solubilisation. Acetone (20 ml) and triethylamine (3 ml, 22 mM) were added. To this mixture, stirring at room temperature was added 2-(*tert*-butoxycarbonyloxyimino)-2-phenyl-acetonitrile (1.4 g, 5.69 mM, 1.2 equivalents); stirring was continued for 3 hours, at the end of which time the acetone was removed *in vacuo* and the aqueous residues made alkaline (pH 12) with 2N ammonium hydroxide solution. The basic aqueous solution was washed three times with ether (3 x 50 ml aliquots) and then acidified (pH 2) by cautious, dropwise addition of 12N hydrochloric acid. The acidified aqueous solution was then extracted with ether (3 x 50 ml aliquots); the combined ethereal extracts were dried over anhydrous sodium sulphate, filtered to remove the dessicant and then evaporated to dryness *in vacuo*; the residue (<u>40</u>) was a light, fluffy white solid; yield 1.402 g, 95%.

^{13}C NMR ($CDCL_3$); δ177.34, δ177.10 (d) CH<u>C</u>OOH; δ175.77 CH_2<u>C</u>OOH; δ155.51, δ154.21 N<u>C</u>OO-; δ141.15 <u>C</u>:CH_2; δ113.85, δ113.50 (d) C:<u>C</u>H_2; δ81.45, δ81.18 (d) <u>C</u>$(CH_3)_3$; δ64.01 <u>C</u>-2; δ47.89 <u>C</u>-5; δ46.02, δ45.40 (d) <u>C</u>-4; δ41.91, δ40.47 (d) <u>C</u>-3; δ32.91 <u>C</u>H_2COOH; δ28.50, δ28.39 (d) C($\underline{C}H_3)_3$; δ22.38 <u>C</u>H_3.

(2S.3R.4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carboxymethyl 4-isopropenyl azolidine. (45)

α-N-*t*-BOC kainate (40) (1 g, 3.2 mM) was dissolved in ether (200 ml) and the solution stirred and cooled to -78°C (195K). To the stirred, cold solution was added dropwise a solution of diazomethane (148 mg, 3.5 mM, 1.1 equivalents) in ether (50 ml) (prepared according to [2]) over a period of 1 hour. At the end of this time the mixture was allowed to warm to room temperature and glacial acetic acid was added dropwise to destroy any unreacted diazomethane. The solvent was then evaporated *in vacuo* and the oily residue taken up in the minimum volume of a mixture of ethyl acetate/hexane/acetic acid (33:65:2) and chromatographed on silica (20 g) to afford the monoesterified product (45) (780 mg, 75%), a clean, white solid.

^{13}C NMR ($CDCl_3$): δ176.55 $CH_2\underline{C}OOH$; δ172.65, δ172.49 (d) $\underline{C}OOCH_3$; δ154.51, δ153.86 (d) $N\underline{C}OO$; δ141.23 $\underline{C}:CH_2$; δ113.71, δ113.44 (d) $C:\underline{C}H_2$; δ80.50 $\underline{C}(CH_3)_3$; δ64.03, δ63.66 (d) C-2; δ53.22 $COO\underline{C}H_3$; δ47.73 C-5; δ45.99, δ45.21 (d) C-4; δ41.71, δ40.74 (d) C-3; δ32.94 $\underline{C}H_2COOH$; δ26.28 $C(\underline{C}H_3)_3$; δ22.21 $\underline{C}H_3$.

(2S,3R,4S) 1-carboxybenzyl, 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine (41)

α-kainic acid (5 g, 23.5 mM) was dissolved in water (50 ml) and sodium carbonate (10 g, 94 mM, 4 equivalents) added. To this mixture, vigorously stirred, was added an excess of benzyl chloroformate (10 ml, 10.16 g, 60 mM, 2.55 equivalents) and vigorous stirring continued overnight. At the end of this time the mixture was washed three times with ether (3 x 50 ml aliquots) and then cautiously acidified (pH 2) by dropwise addition of 12N hydrochloric acid; there was considerable effervescence and quantities of white solid, product (41), precipitated. The precipitate was filtered off; the filtrate was extracted with ethyl

acetate (3 x 50 ml aliquots), dried over magnesium sulphate, filtered to remove the dessicant and then concentrated to give a white solid; this was combined with the earlier precipitate and dried to afford (41) 7.7 g, 94%.

^{13}C NMR ($CDCl_3$): δ177.48, δ177.20 (d) CH<u>C</u>OOH; δ176.88, δ175.69 (d) CH_2<u>C</u>OOH; δ155.70, δ154.67 (d) N<u>C</u>OO; δ140.64 <u>C</u>:CH_2; δ136.09 Ar 4°<u>C</u>; δ128.56-δ127.69 (m) Ar <u>C</u>H (x5); δ113.60 C:<u>C</u>H_2; δ67.88, δ67.50 <u>C</u>H_2Ph; <u>C</u>H_2Ph; δ64.09, δ63.71 (d) <u>C</u>-2; δ47.90, δ47.57 (d) <u>C</u>-5; δ45.94, δ45.18 (d) <u>C</u>-4; δ41.77, δ40.52 (d) <u>C</u>-3; δ32.72 <u>C</u>H_2COOH; δ22.37 <u>C</u>H_3.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxy-methyl, 4-isopropenyl azolidine (43)

α-N-*t*BOC kainate (40) (1 g, 3.2 mM) was dissolved in ether (50 ml) and the solution cooled to 0°C (237K) with stirring. A solution of diazomethane (336 mg, 8 mM, 2.5 equivalents) (prepared according to [2]) in ether (50 ml) was added dropwise and the mixture stirred for 15 minutes, at the end of which time glacial acetic acid was added dropwise to destroy any unreacted diazomethane. The solvent was then removed *in vacuo* and the oily residue taken up in the minimum volume of ethyl acetate/hexane (2:1) and chromatographed on silica (20 g) to yield (40) 1.03 g, 95%, as a clear, viscous, pale yellow oil.

^{13}C NMR ($CDCl_3$): δ172.68, δ172.41 (d) <u>C</u>O<u>C</u>H_3 (x2); δ154.40, δ153.72 (d) N<u>C</u>OO; δ141.45, δ141.31 (d) <u>C</u>:CH_2; δ113.47, δ113.22 (d) C:<u>C</u>H_2; δ80.26 <u>C</u>$(CH_3)_3$; δ64.03, δ63.71 (d) <u>C</u>-2; δ52.22, δ51.84 (d) COO<u>C</u>H_3 (x2); δ47.89, δ47.65 (d) <u>C</u>-5; δ46.05, δ45.29 (d) <u>C</u>-4; δ41.93, δ40.96 (d) <u>C</u>-3; δ32.99 <u>C</u>H_2COOCH_3; δ28.41, δ28.28 (d) C(<u>C</u>H_3)$_3$; δ22.29 <u>C</u>H_3.

(2S.3R.4S) 1-carboxybenzyl. 2-carbomethoxy, 3-carbomethoxymethyl, 4-isopropenyl azolidine. (171)

α-N-CBZ-kainate (41) (1 g, 2.87 mM) was dissolved in ether (100 ml) and the solution cooled to 0°C (273K) with stirring. A solution of diazomethane (336 mg, 8 mM, excess) in ether (50 ml) was added dropwise and the mixture stirred for 15 minutes, at the end of which time glacial acetic acid was added dropwise to destroy any unreacted diazomethane. The solvent was then removed *in vacuo* and the residue taken up in the minimum volume of ethyl acetate/hexane (1:1) and chromatographed on silica (25 g) to yield (171) (980 mg, 91%), as a clear, viscous oil.

(2S.3R.4S) 1-*tert*-butyloxycarbonyl, 2-carbodiphenylmethoxy, 3-carbodiphenylmethoxymethyl, 4-isopropenyl azolidine (44)

α-N-*t*BOC kainate (40) (500 mg, 1.6 mM) was dissolved in ether and diphenyldiazomethane (776 mg, 4 mM, 2.5 equivalents) (prepared according to [5]) added, and the mixture refluxed overnight. At the end of this time, the solvent was removed *in vacuo* and the residues taken up in the minimum volume of a mixture of ethyl acetate/hexane (1:5) and chromatographed on silica (15 g); thus affording the product (44), a clean white solid (856 mg, 83%).

^{13}C NMR ($CDCl_3$): δ170.81 COOR (x2); δ154.18, δ153.86 (d) NCOO; δ141.29, δ140.85 (d) C:CH_2; δ139.88 Ar 4°C (x4); δ130.02, δ127.09 (m) Ar CH (x20); δ113.33 C:CH_2; δ80.29, δ80.07 (d) $C(CH_3)_3$; δ77.79, δ77.31 (d) $CH(Ph)_2$ (x2); δ64.20, δ63.93 (d) C-2; δ47.89, δ47.24 (d) C-5; δ45.89, δ44.96 (d) C-4; δ41.88, δ40.79 (d) C-3; δ33.32 CH_2COOR; δ28.44, δ28.06 (d) $C(CH_3)_3$; δ22.27 CH_3.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3,4-([4',5']-2'-oxo, 6'-bromomethyl, 6'-methyl oxane) oxolidine (48)

α-N-*t*BOC kainate monomethyl ester (45) (300 mg, 0.92 mM) was dissolved in wet acetone (acetone/water 8:1) (9 ml) and N-bromosuccinimide (245 mg, 1.38 mM, 1.5 equivalents) added. The mixture was stirred for 2 hours at room temperature. TLC (chloroform/ethyl acetate 1:1) showed no starting material remaining. All solvent was then removed *in vacuo* and the residue partitioned between ether (50 ml) and 2N aqueous sodium hydroxide solution (30 ml). The ethereal layer was separated, dried and concentrated to yield (48), a white frothy solid; 340 mg, 92%.

^{13}C NMR ($CDCl_3$): δ171.50 (d) $\underline{C}OOCH_3$; δ168.40 (d) $CH_2\underline{C}OO-$; δ154,30, δ153.70 (d) $N\underline{C}OO$; δ81.64, δ81.48 (d) $\underline{C}OCH_2Br$; δ80.91 $\underline{C}(CH_3)_3$; δ66.15, δ65.79 (d) $\underline{C}$-2; δ52.52 $COO\underline{C}H_3$; δ44.99, δ43.88 (d) $\underline{C}$-5; δ39.95, δ38.82 (d), $\underline{C}$-4; δ38.33, δ36.62 (d) $\underline{C}H_2Br$; δ36.05, δ35.62 (d) $\underline{C}$-3; δ30.88, δ30.66 (d) $\underline{C}H_2COO-$; δ28.25 $C(\underline{C}H_3)_3$; δ25.52, δ24.11 (d) $\underline{C}H_3$.

Preparation of monoester (45) by action of copper-zinc couple on (48)

Copper-zinc couple (500 mg) (prepared according to [4]) was suspended in ethanol (30 ml); bromo-lactone (48) (300 mg, 0.74 mM) was added, and the mixture refluxed with stirring for 2½ hours. At the end of this period, examination of the reaction mixture by TLC showed only slight traces of (48), in addition to larger quantities of a material which possessed identical qualities on TLC to stock samples of monoester (45). The mixture was therefore concentrated *in vacuo* and the residues taken up in the minimum quantity of a mixture of ethyl acetate/hexane/acetic acid (33:65:2) and subjected to radial chromatography (chromatotron) on silica, thus affording a white solid (45) identical in all respects to material obtained by other methods. Yield 180 mg (84%).

^{13}C NMR ($CDCl_3$): as (45).

(2R.3R.4S) 1-acetyl, 2.3-([3.4]-1'-oxo, 6'-oxo oxetane) 4-isopropenyl azolidine (42) (After [3]).

α-Kainic acid (6) (1 g, 4.7 mM) was suspended in benzene (8 ml) and acetic anhydride (2 ml, 2.16 g, 20 mM, 4.5 equivalents) added. The mixture was refluxed for 2 hours, during which time the suspended material had all dissolved. After 2 hours, the solvent was removed *in vacuo* and the residues azeotroped with benzene. The white solid product was then recrystallised from the minimum volume of hot acetic anhydride, filtered and dried to yield (42) as fine white crystals; (780 mg, 70%).

^{13}C NMR ($CDCl_3$): δ178.40, δ176.50 (d) CH<u>C</u>OO- ; δ170.16, δ169.84 (d) CH_2<u>C</u>OO- ; δ166.26, δ165.12 (d) N<u>C</u>OCH_3; δ140.00, δ139.23 (d) <u>C</u>:CH_2; δ114.69, δ113.50 (d) C:<u>C</u>H_2; δ62.14, δ59.92 (d) <u>C</u>-2; δ48.54, δ48.22 (d) <u>C</u>-4; δ47.73, δ46.75 (d) <u>C</u>-5; δ34.73, δ32.94 (d) <u>C</u>-3; δ29.80, δ29.35 (d) <u>C</u>H_2COO- ; δ22.75, δ22.05 (d) <u>C</u>H_3, CO<u>C</u>H_3.

(2R,3R,4S) 1-acetyl, 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine (51). (After [3])

β-N-acetyl kainate anhydride (42) (750 mg, 3.17 mM) was dissolved in water (10 ml) and heated on a steam bath for 1 hour. The solvent was then evaporated *in vacuo* and the resulting white solid product dried, affording (51), yield 550 mg (95%).

^{13}C NMR (D_2O): δ176.66 CH<u>C</u>OOH; δ174.01 CH_2<u>C</u>OOH; δ173.68 <u>C</u>OCH_3; δ141.45 <u>C</u>:CH_2; δ113.66 C:<u>C</u>H_2; δ63.38 <u>C</u>-2; δ49.30 <u>C</u>-4; δ47.48 <u>C</u>-5; δ37.87 <u>C</u>-3; δ30.39 <u>C</u>H_2COOH; δ22.43, δ21.94 (d) <u>C</u>H_3, CO<u>C</u>H_3.

(2R,3R,4S) 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine (36) (After [3]).

β-N-acetyl kainate (51) (700 mg, 2.75 mM) was dissolved in 10% potassium hydroxide (aqueous) solution (14 ml), the mixture then being

refluxed for 6 hours, at the end of which time it was acidified (pH 2) by dropwise addition of 12 N hydrochloric acid and run onto an ion exchange column (Dowex W50X8, H^+ form), eluting firstly with water and then with 20% ammonia (aqueous) solution. The ninhydrin-active fractions were combined and concentrated, being evaporated *in vacuo* to yield (36) as a white solid; 500 mg (85%).

^{13}C NMR (D_2O): δ178.50 CH<u>C</u>OOH; δ172.44 $CH_2\underline{C}OOH$; δ140.85 <u>C</u>:CH_2; δ113.66 C:$\underline{C}H_2$; δ66.85 <u>C</u>-2; δ48.70 <u>C</u>-5; δ46.21 <u>C</u>-4; δ38.95 <u>C</u>-3; δ32.45 $\underline{C}H_2COOH$; δ23.57 $\underline{C}H_3$.

Preparation of anhydride intermediate (?52c) using acetic anhydride and α-N-*t*BOC kainic acid (40)

α-N-*t*BOC kainic acid (40) (1.33 g, 4.25 mM) was dissolved in benzene (25 ml) and a large excess of acetic anhydride (1.5 ml, 1.623 g, 16 mM) added. The mixture was refluxed for 3 hours, at the end of which time TLC showed only trace amounts of the starting material remaining. After standing overnight at room temperature to allow these traces to be consumed, the solvent was removed *in vacuo* and the residues azeotroped with benzene. The resulting gum was taken up in the minimum volume of ethyl acetate/hexane 1:1 and chromatographed on silica (25 g); the product (52?) was isolated as a white, foamy solid; yield 950 mg, (?%). 90 MHz and 400 MHz ^{13}C provided rather ambiguous results in that precise identification of the product proved impossible; a lack of acetyl-$\underline{C}H_3$ carbon atoms indicated that the material was not a mono- or di-mixed anhydride (see section 2.6.2.5 for fuller discussion). The most probable structure would appear to be that of an anhydride dimer (52c); di-[(2S,3R,4S)-1-*tert*-butyloxycarbonyl, 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine] anhydride.

^{13}C NMR ($CDCl_3$): δ167.72 - δ165.72 (m) <u>C</u>OOR; δ154.34, δ153.42 (d) N<u>C</u>OO; δ141.12, δ140.53 (d) <u>C</u>:CH_2; δ114.09, δ113.60 (d) C:$\underline{C}H_2$;

δ81.21, δ80.83 (d) C(CH$_3$)$_3$; δ64.36, δ64.14 (d) C-2; δ47.62, 47.40 C-5; δ45.45, δ44.96 (d) C-4; δ40.74, δ39.71 (d) C-3; δ34.02 CH$_2$COOR; δ29.33, δ28.17 (d) C(CH$_3$)$_3$; δ22.32, δ22.16 (d) CH$_3$.

(2S,3R,4S) -1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carboxychloromethyl, 4-isopropenyl azolidine (54)

α-N-*t*BOC kainyl monomethyl ester (45) (500 mg, 1.53 ml) was dissolved in THF (30 ml); oxalyl chloride (0.5 ml, 728 mg, 5.73 mM, excess) and DMF (catalyst; 1 drop) added. The well-stirred, effervescing mixture was kept at room temperature overnight under anhydrous conditions; at the end of that time, solvent and excess oxalyl chloride were removed *in vacuo*; the resulting yellowish amorphous solid (54) was not further isolated or characterised, but used immediately in further preparations.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbamylmethyl, 4-isopropenyl azolidine (55)

Kainyl acid chloride (54) (from (45), 500 mg, 1.53 mM) was dissolved in THF (50 ml) and gaseous ammonia bubbled steadily through the solution for 2 hours at room temperature; a white precipitate could be observed forming after a few minutes' bubbling. After 2 hours, the solvent was removed *in vacuo* and the white solid residue partitioned between water (50 ml) and ether (50 ml). The ethereal layer was evaporated and then taken up in the minimum volume of ethyl acetate and chromatographed on silica (20 g) to afford (55), a white solid; 450 mg (90% from (45)). ^{13}C NMR (CDCl$_3$): δ173.52, δ173.28 (d) CONH$_2$; δ172.79, δ172.52 (d) COOCH$_3$; δ154.45, δ153.86 (d) NCOO; δ141.7 , δ141.56 (d) C:CH$_2$; δ113.20 C:CH$_2$; δ80.48, δ80.29 (d) C(CH$_3$)$_3$; δ64.06, δ63.71 (d) C-2; δ52.22 COOCH$_3$; δ47.97, δ47.67 (d) C-5; δ46.10, δ45.24 (d) C-4; δ41.80, δ40.55 (d) C-3; δ34.21, δ33.99 (d) CH$_2$CONH$_2$; δ29.63, δ28.39 (d) C(CH$_3$)$_3$; δ22.35 CH$_3$.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-cyanomethyl, 4-isopropenyl azolidine (56)

Kainyl amide (55) (400 mg, 1.23 mM) was dissolved in ethyl acetate (25 ml) and acetic anhydride (0.5 ml, 541 mg, 5.3 mM, excess) added; the mixture was refluxed for 20 hours, cooled to room temperature and washed with water (25 ml). The organic phase was concentrated *in vacuo*, the residual material being taken up in the minimum volume of ethyl acetate/hexane 1:1 and chromatographed on silica (13 g), to afford the white, solid product (56), collapsing to a pale, clear oil on standing. Yield 300 mg, 80%.

^{13}C NMR ($CDCl_3$): δ171.79 $\underline{C}OOCH_3$; δ154.20, δ153.60 (d) $N\underline{C}OO$; δ139.72 $\underline{C}:CH_2$; δ119.10, δ118.07 (d) $\underline{C}N$; δ114.44, $C:\underline{C}H_2$; δ80.72 $\underline{C}(CH_3)_3$; δ63.52 $\underline{C}$-2; δ52.52 $COO\underline{C}H_3$; δ48.30, δ46.70 (d) $\underline{C}$-5; δ46.05, δ45.13 (d) $\underline{C}$-4; δ42.45, δ41.55 (d) $\underline{C}$-3; δ28.25 $C(\underline{C}H_3)_3$; δ22.54 $\underline{C}H_3$; δ17.93, δ17.42 (d) $\underline{C}H_2CN$.

IR (Thin film) 2220 cm^{-1} (C≡N)

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-cyanomethyl, 4-isopropenyl azolidine (57)

Fully protected kainyl nitrile (56) (300 mg, 0.97 mM) was dissolved in THF (20 ml) and 2N sodium hydroxide (aqueous) solution (20 ml), being stirred vigorously to ensure proper mixing. Stirring was continued, at room temperature, for 6 hours, at the end of which time the mixture was acidified (pH 2) by dropwise addition of 12N hydrochloric acid, concentrated to remove the lower-boiling THF and then extracted with ether (3 x 25 ml aliquots). The combined ethereal layers were dried (magnesium sulphate), filtered to remove the dessicant and concentrated *in vacuo* to yield (57), a white solid with an unpleasant smell; 260 mg (92%). This material was not characterised further, but proceeded straight into removal of the *tert*-butyl group as follows:

(2S,3R,4S)-2-carboxy, 3-cyanomethyl, 4-isopropenyl azolidine (58)

α-N-*t*BOC kainyl nitrile (57) (250 mg, 0.85 mM) was dissolved in 98% formic acid (5 ml) and stirred at room temperature overnight. At the end of this time the mixture was evaporated to dryness *in vacuo*, azeotroping with water. The residues were taken up in water and subjected to ion-exchange chromatography, eluting with 20% ammonia (aqueous) solution; the ninhydrin-staining fractions were combined, concentrated and dried to yield white solid (58); 150 mg, 92%.

^{13}C NMR (D_2O): δ172.92 C00H; δ139.17 C:CH_2; δ120.30 CN; δ114.41 C:CH_2; δ65.71 C-2; δ46.75 C-5; δ46.16 C-4; δ41.01 C-3; δ22.70 CH_3; δ17.82 CH_2CN.

Mass Spec. M/E = 195 (M+1)

(2R,3R,4S)-1-*tert*-butyloxycarbonyl, 2-carboxy, 3-carboxymethyl, 4-isopropenyl azolidine (145)

β-Kainic acid (36) (1 g, 4.69 mM) was dissolved in water (20 ml), slight warming being necessary to effect full solubilisation. Acetone (20 ml) and triethylamine (3 ml, 22 mM) were added. To this mixture, stirring at room temperature, was added 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (1.4 g, 5.69 mM, 1.2 equivalents), and stirring continued for 3 hours, at the end of which time the acetone was removed *in vacuo*, and the aqueous residues made alkaline (pH 12) with 2N ammonium hydroxide (aqueous) solution. The basic aqueous solution was washed three times with ether (3 x 50 ml aliquots) and then acidified (pH 2) by dropwise addition of 12N hydrochloric acid. The acidified aqueous solution was then extracted into ether (3 x 50 ml aliquots) and the combined ethereal extracts dried over anhydrous magnesium sulphate, filtered to remove the dessicant, and then evaporated to dryness *in vacuo*. The product, (145), a light, frothy white solid, was dried at the pump to yield 1.37 g (93%).

^{13}C NMR ($CDCl_3$): δ178.10 CH<u>C</u>OOH; δ176.80 CH_2<u>C</u>OOH; δ155.00, δ154.30 (d) N<u>C</u>OO; δ140.70 <u>C</u>:CH_2; δ113.20 C:<u>C</u>H_2; δ80.50 <u>C</u>$(CH_3)_3$; δ63.70 <u>C</u>-2; δ47.30 (d) <u>C</u>-5, <u>C</u>-4; δ39.40, δ38.40 (d) <u>C</u>-3; δ30.30, <u>C</u>H_2COOH; δ28.40, δ28.20 (d) C(<u>C</u>$H_3)_3$; δ22.40 <u>C</u>H_2.

(2R,3R,4S)-1-*tert*-butyloxycarbobyl, 2-carbomethoxy, 3-carboxymethyl, 4-isopropenyl azolidine (146)

β-N-*t*BOC kainate (145) (1 g, 3.2 mM) was dissolved in ether (200 ml) and the solution stirred and cooled to -78°C (195K). To the stirred, cold solution was added dropwise a solution of diazomethane (148 mg, 3.5 mM, 1.1 equivalents) in ether (50 ml) (prepared according to [2]) over a period of 1 hour. At the end of this time the solution was allowed to warm to room temperature and glacial acetic acid added dropwise to destroy any unreacted diazomethane. The solvent was then removed *in vacuo* and the residues taken up in the minimum volume of ethyl acetate/hexane/acetic acid (33:65:2) and chromatographed on silica (20 g), to afford the monoesterified product (146) (735 mg, 70%), a clean, white solid.

^{13}C NMR ($CDCl_3$): δ177.25 CH_2<u>C</u>OOH; δ171.35, δ170.92 (d) <u>C</u>OOH_3; δ154.67, δ154.13 (d) N<u>C</u>OO; δ140.75, δ140.47 (d) <u>C</u>:CH_2; δ113.71, δ113.50 (d) C:<u>C</u>H_2; δ88.72, δ88.45 (d) <u>C</u>$(CH_3)_3$; δ62.84, δ62.52 (d) <u>C</u>-2; δ52.06, δ51.84 (d) COO<u>C</u>H_3; δ47.78-δ47.13 (m) <u>C</u>-5, <u>C</u>-4; δ39.06, δ38.03 (d) <u>C</u>-3; δ29.63, δ29.42 (d) <u>C</u>H_2COOH; δ28.39, δ28.17 (d) C(<u>C</u>$H_3)_3$; δ22.59 <u>C</u>H_3.

(2R,3R,4S)-1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carboxychloromethyl, 4-isopropenyl azolidine (147)

β-N-*t*BOC kainyl monomethyl ester (146) (500 mg, 1.53 mM) was dissolved in THF (30 ml); oxalyl chloride (0.5 ml, 728 mg, 5.73 mM, excess) and DMF (catalyst, 1 drop) added. The well-stirred, effervescing mixture was kept at room temperature overnight under anhydrous conditions; at the

end of this time, solvent and excess oxalyl chloride were removed *in vacuo*. The resulting dirty yellowish amorphous solid (147) was not further isolated or characterised, but used immediately for further preparations.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(4'-morpholino)-amidomethyl, 4-isopropenyl azolidine (60)

α-N-*t*BOC kainyl monomethyl ester acid chloride (54) (from (45), 500 mg, 1.53 mM) was dissolved in THF (30 ml). Pyridine (1 ml, 980 mg, 12.4 mM, excess) and morpholine (1 ml, 1g, 11.48 mM, excess) added and the reaction stirred at room temperature for 8 hours. The solvent was then removed *in vacuo* and the residues partitioned between water (50 ml) and ether (50 ml). The aqueous layer was extracted twice more with ether (2 x 50 ml); the ethereal layers were combined and washed with 2N hydrochloric acid (50 ml), dried (magnesium sulphate) and concentrated to afford a white solid product (60); 410 mg (65% from (45)).
^{13}C NMR ($CDCl_3$): δ172.87, δ172.65 (d) $\underline{C}OOCH_3$; δ169.43 $\underline{C}ON$; δ153.90 $NC\underline{O}O$; δ142.39, δ141.96 (d) $\underline{C}:CH_2$; δ113.04 $C:\underline{C}H_2$; δ80.26 $\underline{C}(CH_3)_3$; δ66.85, δ66.50 (d) $\underline{C}$-2', $\underline{C}$-6'; δ64.25, δ63.82 (d) $\underline{C}$-2; δ52.28 $COO\underline{C}H_3$; δ48.46, δ47.81 (d) $\underline{C}$-5; δ45.70, δ45.07 (d) $\underline{C}$-4: δ45.70, δ42.07 (d) $\underline{C}$-3', $\underline{C}$-5'; δ41.77, δ40.41 (d) $\underline{C}$-3; δ31.18 $\underline{C}H_2CONH$; δ28.31 $C(\underline{C}H_3)_3$; δ22.43 $\underline{C}H_3$.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(4'-morpholino)-amido methyl, 4-isopropenyl azolidine (148)

The fully protected kainyl-morpholine adduct (60) (400 mg, 0.97 mM) was dissolved in a mixture of THF (15 ml) and 2N sodium hydroxide solution (15 ml) and stirred at room temperature for 2 hours. The THF was then removed *in vacuo*, and the aqueous residues washed with ether (25 ml) and then acidified (pH 2) by dropwise addition of 12N hydrochloric acid. The aqueous solution was extracted into ethyl acetate (3 x 30 ml); the

organic layers were combined, and dried (magnesium sulphate) and concentrated to yield a white solid (148), pure by TLC. This material was not characterised further, being deprotected at nitrogen at once, as follows: (yield, crude: 300 mg, 78%).

(2S,3R,4S) 2-carboxy, 3-(4'-morpholino)-amidomethyl, 4-isopropenyl azolidine (70)

The de-esterified kainyl-morpholine adduct (148) (300 mg, 0.76 mM) was dissolved in a large excess of 98% formic acid (5 ml) and stirred at room temperature for 2 hours. The solvent was then removed *in vacuo*, azeotroping with water. The residues were then taken up in the minimum volume of water and subjected to ion-exchange chromatography, eluting with 20% ammonia (aqueous) solution. The ninhydrin-active fractions were combined and concentrated to dryness *in vacuo*, yielding a white solid which was recrystallised from ethanol; (70); yield 160 mg, 71%. ^{13}C NMR (D_2O): δ174.27 COOH; δ172.48 CONH; δ141.44 C:CH_2; δ114.62 C:CH_2; δ67.22 C-2', C-6'; δ66.68 C-2; δ47.94 C-5; δ46.96 C-3', C-5'; δ43.28 C-4; δ42.19 C-3; δ32.17 CH_2CON; δ22.43 CH_3.

Preparation of carboxybenzyl (CBZ)-protected piperidine carboxylic acids (152), (153), (154)

The acid (a) piperidine-2-carboxylic acid, (144), (b) piperidine-3-carboxylic acid, (150), (c) piperidine-4-carboxylic acid (151)(1.29 g, 0.01 M) was dissolved in water (30 ml) and sodium carbonate (3.0 g, 0.029 M) added. The mixture was stirred until all solid material had dissolved. Benzyl chloroformate (2.0 ml, 2.39 g, 0.014 M) was added and the mixture stirred vigorously at room temperature overnight. At the end of this time the aqueous solution was washed with ether (3 x 50 ml aliquots), acidified (pH 2) by dropwise addition of 12N hydrochloric acid and extracted into ether (3 x 50 ml). The combined

ethereal layers were concentrated *in vacuo* to yield (a) 1-carboxybenzyl, 2-carboxy piperidine (152), 1.867 g, 71%; (b) 1-carboxybenzyl, 3-carboxy piperidine (153), 2.21 g, 84%; (c) 1-carboxybenzyl, 4-carboxy piperidine, 2.367 g, 90%; all as thick, clear yellow oils.

Esterification of N-protected piperidine carboxylic acids; preparation of (155), (156) and (157)

The N-protected piperidine carboxylic acids (a) (152) (1.867 g, 7.1 mM) (b) (153) (2.21 g, 8.4 mM), (c) (154) (2.367 g, 9 mM) were dissolved in methanol, to which was added dropwise a solution of dry hydrogen chloride in methanol [prepared by adding acetyl chloride (2ml) to methanol (20 ml)]. The mixture was refluxed overnight, the solvent then being removed *in vacuo* and the residues being partitioned between ether (50 ml) and 2N sodium hydroxide (aqueous) solution (50 ml). The aqueous phase was extracted twice into ether (2 x 30 ml). The ethereal layers were combined and evaporated *in vacuo* to afford the N-protected carbomethoxy piperidines (a) 1-carboxybenzyl, 2-carbomethoxy piperidine (155), (b) 1-carboxybenzyl, 3-carbomethoxy piperidine (156), (c) 1-carboxybenzyl, 4-carbomethoxy piperidine (157). These were not further isolated or characterised, but immediately deprotected at nitrogen as follows:

Deprotection at nitrogen of CBZ-piperidine carbomethoxy compounds; preparation of (61), (63) and (65)

The fully protected material (a) (155), (b) (156), (c) (157), was dissolved in a solution of hydrogen bromide in acetic acid (5 ml) and left standing at room temperature for 3 hours. The excess solvent was then removed *in vacuo*, caution being taken to minimise contact with lachrymatory benzyl bromide formed as a by-product, and the oily residues azeotroped with benzene. With continued azeotroping a

precipitate formed, which was filtered off and dried at the pump yielding (a) 2-carbomethoxy piperidine hydrobromide (61) 795 mg (50% from (152)), (b) 3-carbomethoxy piperidine hydrobromide (63) 1.39 g (74% from (153)), (c) 4-carbomethoxy piperidine hydrobromide (65), 1.13 g, (56% from (154)).

^{13}C NMR (D_2O):

(a) (61): δ170.17 $\underline{C}OOCH_3$; δ57.4 $\underline{C}$-2; δ54.4 $COO\underline{C}H_3$; δ44.9 $\underline{C}$-6; δ26.3 $\underline{C}$-3; δ22.0, δ21.9 (d) $\underline{C}$-4, $\underline{C}$-5.

(b) (63): δ174.90 $\underline{C}OOCH_3$; δ53.47 $COO\underline{C}H_3$; δ44.75, δ44.51 (d) $\underline{C}$-2, $\underline{C}$-6,; δ38.60 $\underline{C}$-3; δ24.98 $\underline{C}$-4; δ21.21 $\underline{C}$-5.

(c) (65): δ176.8 $\underline{C}OOCH_3$; δ53.5 $COO\underline{C}H_3$; δ43.8 $\underline{C}$-2, $\underline{C}$-6; δ38.7 $\underline{C}$-4; δ25.1 $\underline{C}$-3, $\underline{C}$-5.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(1'-(2'-carbomethoxy) perhydroazine)-amidomethyl, 4-isopropenyl azolidine (62)

Acid chloride (54) (from (45) (500 mg, 1.53 mM)) was dissolved in THF (30 ml); pyridine (5 ml) and (61) (0.51 g, 2.14 mM, 1.4 equivalents) were added. The stirred mixture was refluxed overnight, at the end of which time the solvent was removed *in vacuo* and the residues dissolved in ethyl acetate (50 ml). The organic solution was washed three times with 2N hydrochloric acid (aqueous) solution (3 x 30 ml aliquots), then with 2N sodium hydroxide (aqueous) solution (3 x 30 ml) and finally with 2N hydrochloric acid solution (30 ml) again. The organic layer was concentrated *in vacuo* and the yellow, oily residue dissolved in the minimum volume of ethyl acetate/hexane/acetic acid (65:33:2) and chromatographed on silica (20 g) to afford (62), a frothy white solid, collapsing to a pale, clear yellow oil on standing. Yield 510 mg; (74% from (45)).

^{13}C NMR ($CDCl_3$): δ172.95-δ170.81 (m) $\underline{C}OOCH_3$ (x2), $\underline{C}ON$; δ153.91 $N\underline{C}OO$; δ142.48, δ141.94 (d) $\underline{C}$:CH_2; δ113.04 C:$\underline{C}H_2$; δ80.12 $\underline{C}(CH_3)_3$; δ64.20-

δ63.57 (m) C-2, C-2'; δ52.20, δ52.09 (d) $COOCH_3$; δ48.59-δ47.81 (m) C-5; δ45.99-δ45.02 (m) C-4; δ43.29, δ43.10 C-6'; δ41.99, δ41.82 (d) C-3; δ31.50, δ31.39 (d) CH_2CON; δ28.41, δ28.31 (d) $C(CH_3)_3$; δ26.60 C-3'; δ25.25 C-5'; δ22.56, δ22.43 (d) CH_3; δ20.97, δ20.86 (d) C-4'.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(1'-(2'-carboxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (158)

The fully protected pipecolinate adduct (62) (510 mg, 1.13 mM) was dissolved in THF (20 ml) and 2N sodium hydroxide (aqueous) solution (20 ml). The mixture was stirred at room temperature overnight. At the end of this period the THF was removed *in vacuo*, and the aqueous residue washed with ether (3 x 30 ml), acidified (pH 2) by dropwise addition of 12N hydrochloric acid and extracted four times with ether (4 x 30 ml). The ethereal fractions were combined, dried (magnesium sulphate) and evaporated to yield a white, frothy solid (158) (450 mg, 94%), which was not isolated or further characterised, but immediately deprotected at nitrogen as follows:

(2S,3R,4S) 2-carboxy, 3-(1'-(2'-carboxy)perhydroazine)-amidomethyl 4-isopropenyl azolidine (71)

The de-esterified pipecolinate adduct (158) (450 mg, 1.06 mM) was dissolved in 98% formic acid (5 ml) and left standing at room temperature overnight, following which time all solvent was removed *in vacuo* and the residues dissolved in water and subjected to ion-exchange chromatography; the ninhydrin-active fractions were combined, and concentrated to yield a white solid product (71); 320 mg, 93%.

^{13}C NMR (D_2O): δ177.85 CON; δ174.09 NHCHCOOH; δ172.92 NCHCOOH; δ140.91 $C:CH_2$; δ114.09 $C:CH_2$; δ66.50 C-2; δ55.81 C-2'; δ47.40 C-5; δ46.48 C-4; δ44.59 C-6'; δ41.66, δ41.06 (d) C-3; δ32.13, δ31.85 (d) CH_2CON; δ28.01, δ27.47 (d) C-3'; δ25.41, δ24.92 (d) C-5'; δ22.86 CH_3; δ21.24 C-4'.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(1'-(3'-carbomethoxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (64)

Acid chloride (54) (from (45), 500 mg, 1.53 mM) was dissolved in THF (30 ml). Pyridine (5 ml) and (63) (0.51 g, 2.14 mM, 1.4 equivalents) were added. The stirred mixture was refluxed overnight, at the end of which time the solvent was removed *in vacuo* and the residues dissolved in ethyl acetate (50 ml). The organic solution was washed three times with 2N hydrochloric acid (aqueous) solution (3 x 30 ml), then with 2N sodium hydroxide (aqueous) solution (3 x 30 ml) and finally with 2N hydrochloric acid solution again. The organic layer was concentrated *in vacuo* and the residues dissolved in the minimum volume of ethyl acetate/hexane/acetic acid (65:33:2) and chromatographed on silica (25 g) to afford the product (64), an off-white solid; 310 mg, 45%. This material afforded a 90 MHz ^{13}C NMR spectrum which did not appear particularly clear; nevertheless, the material was deprotected and a subsequent NMR of the final product proved much more amenable to interpretation.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(1'-(3'-carboxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (159)

The fully protected nipecotate adduct (64) (300 mg, 0.66 mM) was dissolved in THF (20 ml) and 2N sodium hydroxide (aqueous) solution (20 ml). The mixture was stirred at room temperature overnight. At the end of this time the THF was removed *in vacuo* and the aqueous residues washed with ether (4 x 30 ml), acidified (pH 2) by dropwise addition of 12N hydrochloric acid and extracted four times into ether (4 x 30 ml). The ethereal fractions were combined, dried (magnesium sulphate) and evaporated to yield a white foamy solid (159); 365 mg, 94%. This material was not characterised further but immediately deprotected at nitrogen as follows:

(2S,3R,4S) 2-carboxy, 3-(1'-(3'-carboxy)perhydroazine)-amidomethyl 4-isopropenyl azolidine (72)

The de-esterified nipecotate adduct (159) (250 mg, 0.59 mM) was dissolved in 98% formic acid (5 ml) and stood at room temperature overnight. At the end of this time all solvent was removed *in vacuo* and the residues azeotroped with water. The residues were then dissolved in water and subjected to ion-exchange chromatography, the ninhydrin-active fractions being combined and concentrated to yield the product (72), a white frothy solid; 180 mg, 95%.

^{13}C NMR (D_2O): δ180.27 CON; δ173.90 NHCHCOOH; δ171.77 CHCOOH; δ140.93 C:CH_2; δ114.44, δ114.12 (d) C:CH_2; δ66.28 C-2; δ48.84 C-2'; δ47.38, δ47.02 (d) C5; δ46.51 C-4; δ44.99 C-6'; δ43.43; δ43.12 (d) C-3'; δ41.82 C-3; δ31.96 CH_2CON; δ27.63 C-4'; δ24.97, δ24.27 (d) C-5'; δ22.27 CH_3.

Mass. Spec. M/E = 325 (M + 1).

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(1'-(4'-carbomethoxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (66)

Acid chloride (54) (from (45) 500 mg, 1.53 mM) was dissolved in THF (30 ml). Pyridine (5 ml) and (65) (0.51 g, 2.14 mM, 1.4 equivalents) were added. The stirred mixture was refluxed overnight, at the end of which time the solvent was removed *in vacuo* and the residues dissolved in ethyl acetate (50 ml), the organic solution being washed three times with 2N hydrochloric acid (aqueous) solution (3 x 30 ml), and finally with 2N hydrochloric acid (30 ml) again. The organic layer was concentrated *in vacuo* and the residues dissolved in the minimum volume of ethyl acetate/hexane/acetic acid (65:33:2) and chromatographed on silica (20 g) to afford (66), the product, as a white frothy solid; 495 mg, 72%.

^{13}C NMR ($CDCl_3$): δ174.47 NHCHCOOCH_3; δ172.82, δ172.68 (d) CON;

δ168.92 $CH_2CHCOOCH_3$; δ154.32, δ153.83 (d) NCOO; δ142.37, δ141.96 (d) C:CH_2; δ113.14, δ112.95 (d) C:CH_2; δ80.10 $C(CH_3)_3$; δ64.12, δ63.74 (d) C-2; δ52.20, δ51.84 (d) $COOCH_3$ (x2); δ48.54, δ47.89 (d) C-5; δ45.99 C-4'; δ45.13, δ44.59 (d) C-4; δ41.93, δ41.20 (d) C-3; δ41.04, δ40.79 (d) C-2', C-6'; δ31.37 CH_2CON; δ28.28 $C(CH_3)_3$; δ27.87 C-3', C-5'; 22.37 CH_3

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(1'-(4'-carboxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (160)

The fully-protected isonipecotate adduct (66) (450 mg, 0.99 mM) was dissolved in THF (20 ml) and 2N sodium hydroxide (aqueous) solution (20 ml), the mixture being stirred at room temperature overnight. At the end of this time the THF was removed *in vacuo* and the aqueous residues washed with ether (3 x 30 ml), acidified (pH 2) by dropwise addition of 12N hydrochloric acid and extracted four times with ether (4 x 30 ml). The combined ethereal extracts were dried (magnesium sulphate) and evaporated to yield a white foamy solid (160); 394 mg, 94%. This material was not characterised further, but immediately deprotected at nitrogen as follows:

(2S,3R,4S) 2-carboxy, 3-(1'-(4'-carboxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (73)

The de-esterified isonipecotate adduct (160) (350 mg, 0.83 mM) was dissolved in 98% formic acid (5 ml) and stood at room temperature overnight. At the end of this time all solvent was removed *in vacuo* and the residues azeotroped with water. The residues were then dissolved in water and subjected to ion-exchange chromatography, the ninhydrin-active fractions being combined and concentrated to afford product (73), a white foamy solid: 260 mg, 97%.

^{13}C NMR (D_2O): δ182.03 CON; δ173.85 NHCHCOOH; δ171.57, δ171.35 (d)

COCOOH; δ141.02, δ140.85 (d) C:CH_2; δ114.58, δ113.98 (d) C:CH_2; δ66.25 C-2; δ47.57, δ47.29 (d) C-5; δ46.53, δ46.12 (d) C-2', C-6'; δ42.74-δ41.82 (m) C-3, C-4, C-4'; δ31.85 CH_2CON; δ29.09, δ28.60 (d) C-3', C-5'; δ22.81 CH_3.

(2R, 3R, 4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(1'-(4'-carbomethoxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (161)

The preparation was identical to that of (66) except that the corresponding β-diastereoisomer (147) acid chloride was used. Yield: 345 mg, 50%.

^{13}C NMR ($CDCl_3$): δ174.65 NHCHCOOCH$_3$; δ171.68 CHCOOCH$_3$; δ169.19, δ168.86 (d) CON; δ154.67, δ154.23 (d) NCOO; δ141,45 (d) C:CH_2; δ117.18, δ112.85 (d) C:CH_2; δ80.34 C(CH_3)$_3$; δ62.95 C-2; δ51.84 COOCH_3 (x2); δ47.46, δ47.19 (d) C-5; δ44.64, δ44.37 (d) C-2', C-6'; δ43.18 C-4; δ41.12, δ40.63 (d) CH_2CON; δ40.63 C-3; δ39.01 C-4'; δ28.17 C(CH_3)$_3$; δ27.73, δ27.41 (d) C-3', C-5'; δ22.64 CH_3.

(2R,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(1'-(4'-carboxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (162)

The preparation was identical to that of (160), except that the corresponding β-diastereoisomer (161) was involved. Yield: 298 mg, 92%.

(2R,3R,4S) 2-carboxy, 3-(1'-(4'-carboxy)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (144)

The preparation was identical to that of (73), except that the corresponding β-diasteroisomer (162) was involved. Yield: 205 mg, 90%. NMR data unavailable.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(1'-(3'-carbomethoxy, 4'-oxo)perhydrooxazine)-amidomethyl, 4-isopropenyl azolidine (68)

Acid chloride (54) (from (45) 500 mg, 1.53 mM) was dissolved in THF (30 ml). Pyridine (5 ml) and (67) (0.80 g, 1.98 mM, 1.3 equivalents) were added. The stirred mixture was refluxed overnight, at the end of which time the solvent was removed *in vacuo* and the residues dissolved in ethyl acetate (50 ml), the organic solution then being washed with 2N hydrochloric acid (aqueous) solution (3 x 30 ml), then with 2N sodium hydroxide (aqueous) solution (3 x 30 ml) and finally with 2N hydrochloric acid again (30 ml). The organic layer was concentrated *in vacuo* and the residues dissolved in the minimum volume of ethyl acetate/hexane/acetic acid (65:33:2) and chromatographed on silica (25 g) to afford (68), the product, a white frothy solid; 295 mg, 41%.

^{13}C NMR ($CDCl_3$): δ172.84-δ168.67 (m) $\underline{C}OOCH_3$ (x2) $\underline{C}ON$; δ153.86 (d) $N\underline{C}OO$; δ142.32, δ141.94 (d) $\underline{C}:CH_2$; δ113.12 C:$\underline{C}H_2$; δ80.26 $\underline{C}(CH_3)_3$; δ64.20, δ63.79 (d) $\underline{C}$-2; δ52.25, δ51.74 (d) $COO\underline{C}H_3$ (x2); δ48.51, δ47.86 (d) $\underline{C}$-5; δ45.94, δ45.10 (d) $\underline{C}$-4; δ41.69-δ41.25 (m) $\underline{C}$-3, $\underline{C}$-3'; δ38.82, δ37.87 (d) $\underline{C}$-2', $\underline{C}$-6'; δ31.91, δ31.29 (d) $\underline{C}H_2CON$; δ29.25 $\underline{C}$-5'; δ28.25 $C(\underline{C}H_3)_3$; δ22.37 $\underline{C}H_3$.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(1'-(3'-carboxy, 4'-oxo)perhydrooxazine)-amidomethyl, 4-isopropenyl azolidine (163)

The fully-protected oxo-nipecotate adduct (68) (250 mg, 0.54 mM) was dissolved in THF (20 ml) and 2N sodium hydroxide (aqueous) solution (20 ml) and stirred at room temperature overnight. At the end of this time the THF was removed *in vacuo* and the aqueous residue washed with ether (3 x 30 ml), acidified (pH 2) by dropwise addition of 12N hydrochloric acid and then extracted four times with ether (4 x 30 ml). The combined ethereal fractions were dried (magnesium sulphate) and evaporated to yield a white frothy solid (163); 206 mg, 88%. The material

was not further characterised, but immediately deprotected at nitrogen as follows:

(2S,3R,4S) 2-carboxy, 3-(1'-(3'-carboxy, 4'-oxo)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (38)

The de-esterified oxo-nipecotate adduct (163) (200 mg, 0.46 mg) was dissolved in 98% formic acid (5 ml) and stood at room temperature overnight. At the end of this time all solvent was removed *in vacuo* and the residues azeotroped with water. The residues were then dissolved in water and subjected to ion-exchange chromatography, the ninhydrin-active fractions being combined and concentrated to yield the product (38), a white, frothy solid; 135 mg, 87%.

^{13}C NMR (D_2O): δ178.72, δ178.34 (d) COCHCOOH; δ173.98, δ173.36 (d) NHCHCOOH, CON; δ140.96 C:CH_2; δ114.09 C:CH_2; δ66.55 C-2, δ47.24 C-5; δ46.37 C-4; δ45.64 C-6'; δ43.29 C-2'; δ41.77 C-3; δ35.62 C-3'; δ33.94 C-5'; δ31.83 CH_2CON; δ22.86 CH_3.

(2R,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-(1'-(3'-carbomethoxy, 4'-oxo)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (69)

This preparation was identical to that of (68), except that the corresponding β-diastereoisomer (147) acid chloride was used; yield: 320 mg, 45%.

(2R.3R.4S)1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(1'-(3'-carboxy, 4'-oxo)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (164)

This preparation was identical to that of (163), except that the corresponding β-diastereoisomer (69) was involved. Yield: 245 mg, 90%.

(2R,3R,4S) 2-carboxy, 3-(1'-(3'-carboxy, 4'-oxo)perhydroazine)-amidomethyl, 4-isopropenyl azolidine (39)

This preparation was identical to that of (38), except that the corresponding β-diastereoisomer (164) was involved. Yield: 190 mg, 92%. ^{13}C NMR (D_2O): δ178.88 CON; δ172.98, δ172.33 (d) COOH (x2); δ141.23 $C:CH_2$; δ114.04, δ113.50 (d) $C:CH_2$; δ67.07 C-2; δ48.70 C-5; δ46.32 C-4; δ44.15 C-6'; δ39.17 C-2'; δ35.92 C-3; δ34.24 C-3'; δ32.02 C-5'; δ28.39 CH_2CON; δ23.51, δ22.92 (d) CH_3.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-N-2'-bromoethyl-amidomethyl, 4-isopropenyl azolidine (79)

Acid chloride (54) (from (45) 500 mg, 1.53 ml) was dissolved in THF (30 ml). Pyridine (5 ml) and ethylamine bromide (436 mg, 1.5 equivalents) added. The mixture was refluxed overnight; at the end of this time the solvent was removed *in vacuo* and the residues dissolved in ether (50 ml) and washed with 2N hydrochloric acid (aqueous) solution (3 x 30 ml). The ethereal layer then being concentrated and the residues dissolved in the minimum volume of ether and chromatographed on silica (10 g), eluting with ethyl acetate/chloroform (1:1); product (79) isolated as a clear, pale yellow oil; 265 mg, 40%. ^{13}C NMR ($CDCl_3$): δ172.71 (d) $COOCH_3$; δ171.08 (d) CONH; δ153.50 NCOO; δ141.56 (d) $C:CH_2$; δ113.12 $C:CH_2$; δ80.23 $C(CH_3)_3$; δ64.03, δ63.76 (d) C-2; δ52.22 $COOCH_3$; δ48.11, δ47.57 (d) C-5; δ46.10, δ45.24 (d) CH_2Br; δ43.77 C-4; δ41.93-δ41.36 (m) CH_2CH_2Br, C-3; δ34.67 CH_2CONH; δ28.28 $C(CH_3)_3$; δ22.37 CH_3.

Methyl 4-aminobutanoate (74)

4-aminobutanoic acid (1.03 g, 0.01M) was dissolved in methanol (20 ml), to which was added dropwise a solution of acetyl chloride (1 ml) in methanol (20 ml); the mixture was refluxed overnight. At the end

of this time the solvent was removed *in vacuo* to give a clean, white solid; (74); 1.50 g, 97% (as the hydrochloride).

^{13}C NMR (D_2O): δ175.70 $\underline{C}OOCH_3$; δ53.10 $COO\underline{C}H_3$; δ39.90 $\underline{C}$-4; δ31.40 $\underline{C}$-2; δ23.00 $\underline{C}$-3.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-N-(n-carbomethoxy propyl)-amidomethyl, 4-isopropenyl azolidine (75)

Acid chloride (54) (from (45) 500 mg, 1.53 mM) was dissolved in THF (30 ml) and pyridine (5 ml) and (74) (0.31 g, 1.25 equivalents) added. The mixture was refluxed overnight, at the end of which time the solvent was removed *in vacuo* and the residues dissolved in ethyl acetate (50 ml). The organic solution was then washed with 2N hydrochloric acid (aqueous) solution (3 x 30 ml), then with 2N sodium hydroxide (aqueous) solution (3 x 30 ml) and finally with 2N hydrochloric acid again. The organic layer was then concentrated, dissolved in the minimum volume of ethyl acetate/chloroform (1:1) and chromatographed on silica (10 g) to afford the product (75), a white, frothy solid; 175 mg, 30%.

^{13}C NMR ($CDCl_3$): δ173.85-δ170.62 (m) $\underline{C}OOCH_3$ (x2), $\underline{C}ONH$; δ154.42, δ153.88 (d) $N\underline{C}OO$; δ141.99, δ141.69 (d) $\underline{C}:CH_2$; δ113.06 $C:\underline{C}H_2$; δ80.48, δ80.26 (d) $\underline{C}(CH_3)_3$; δ64.87, δ64.09 (d) $\underline{C}$-2; δ52.25, δ51.71 (d) $COO\underline{C}H_3$ (x2); δ47.94, δ47.59 (d) $\underline{C}$-5; δ45.26, δ45.05 (d) $\underline{C}$-4; δ41.93, δ41.23 (d) $\underline{C}$-3; δ39.06 $\underline{C}$-1'; δ35.08, δ34.75 (d) $\underline{C}H_2CON$; δ31.45 $\underline{C}$-3'; δ28.28 $C(\underline{C}H_3)_3$; δ24.60 $\underline{C}$-2'; δ22.37 $\underline{C}H_3$.

(2S,3R,4S) 1-*tert*--butyloxycarbonyl, 2-carboxy, 3-N-(n-carboxypropyl) amidomethyl, 4-isopropenyl azolidine (165)

The fully-protected GABA adduct (75) (170 mg, 0.4 mM) was dissolved in THF (10 ml) and 2N sodium hydroxide (aqueous) solution (10 ml) and stirred at room temperature overnight. At the end of this time the THF was removed *in vacuo* and the aqueous material washed with ether (3 x 30 ml).

The aqueous mixture was then acidified (pH 2) by dropwise addition of 12N hydrochloric acid and extracted three times with ethyl acetate (3 x 30 ml); the organic layers were combined, dried (magnesium sulphate) and concentrated to yield the product (165), a white frothy solid; 140 mg, 88%. This material was not characterised further, but was immediately deprotected at nitrogen, as follows:

(2S,3R,4S) 2-carboxy, 3-N-(*n*-carboxypropyl)-amidomethyl, 4-isopropenyl azolidine (80)

The de-esterified GABA adduct (165), (130 mg, 0.33 ml) was dissolved in 98% formic acid (5 ml) and stirred at room temperature overnight. At the end of this time all solvent was removed *in vacuo* and the residues azeotroped with water. The residue was then dissolved in water and subjected to ion-exchange chromatography, the ninhydrin-active fractions being combined and concentrated to afford a white solid material (80); yield 100 mg, 93%.

^{13}C NMR (D_2O): δ179.86 CON; δ174.01, δ173.85 (d) COOH (x2); δ140.42 C:CH_2; δ114.09 C:CH_2; δ66.47 C-2; δ47.19 C-5; δ46.48 C-4; δ41.55 C-3; δ39.55 CONHCH_2; δ35.48 CH_2CONH; δ32.78 CH_2COOH; δ24.81 $CH_2CH_2CH_2$; δ22.75 CH_3.

Mass Spec. M/E = 299 (M+1).

Methyl 5-aminopentanoate (76)

5-amino pentanoic acid (1.34 g, 0.01 M) was dissolved in methanol (25 ml) to which was added dropwise a solution of acetyl chloride (1 ml) in methanol (20 ml) and the mixture refluxed overnight, .t the end of which time the solvent was removed *in vacuo* to yield a white, solid material (76); 1.805 g, 95% (as the hydrochloride).

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-N-(*n*-carbomethoxy butyl)-amidomethyl, 4-isopropenyl azolidine (77)

Acid chloride (54) (from (45) 500 mg, 1.53 mM) was dissolved in THF (20 ml). Pyridine (5 ml) and (76) (320 mg, 1.25 equivalents) were added and the mixture refluxed for 30 minutes, at the end of which time the solvent was removed *in vacuo* and the residues partitioned between 2N hydrochloric acid (aqueous) solution (20 ml) and ethyl acetate (20 ml). The aqueous layer was extracted twice more into ethyl acetate (2 x 20 ml) and discarded, the organic layers being combined, dried (magnesium sulphate) and evaporated *in vacuo* to yield residues which were dissolved in the minimum volume of ethyl acetate/chloroform (1:1) and radially chromatographed on silica to afford the frothy white solid (77); 336 mg, 50%. The material was immediately de-protected as follows:

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-N-(*n*-carboxybutyl) amidomethyl, 4-isopropenyl azolidine (166)

The fully-protected valerate adduct (77) (300 mg, 0.68 mM) was dissolved in THF (10 ml) and 2N sodium hydroxide (aqueous) solution (10 ml) and the mixture stirred at room temperature overnight; at the end of this period the THF was removed *in vacuo* and the aqueous mixture washed three times with ether (3 x 25 ml), acidified (pH 2) by dropwise addition of 12N hydrochloric acid and extracted three times into ether (3 x 25 ml); the combined ethereal extracts were dried (magnesium sulphate) and concentrated to yield a white, frothy solid (166); 264 mg, 94%. The material was not characterised further, but immediately de-protected at nitrogen as follows:

(2S,3R,4S) 2-carboxy, 3-N-(*n*-carboxybutyl) amidomethyl, 4-isopropenyl azolidine (81)

The de-esterified valerate adduct (166) (250 mg, 0.61 mM) was

dissolved in 98% formic acid (5 ml) and stirred at room temperature overnight. At the end of this time the solvent was removed *in vacuo*, azeotroping with water, the residue then being dissolved in water and subjected to ion-exchange chromatography to afford the product (81), a white foamy solid; 131 mg, 69%.

^{13}C NMR (D_2O): δ183.40 CONH; δ173.85 COOH (x2); δ140.31 C:CH_2; δ114.09 C:CH_2; δ66.53 C2; δ47.13 C-5; δ46.43 C-4; δ41.55 CONHCH_2; δ39.98 C-3; δ37.33 CH_2COOH; δ35.48 CH_2CONH; δ29.66 CONHCH_2CH_2; δ23.73 CH_3; δ22.75 CH_2CH_2COOH.

(28) 2-N-carboxybenzyl amino-pentan-1,5-dioic acid (167)

L-glutamic acid (1) (3.00 g, 0.02 M) was dissolved in water (40 ml) to which was added sodium carbonate (8.48 g, 0.08 M, 4 equivalents) and benzyl chloroformate (4.25 ml; 50.8 g, 1.5 equivalents) and the mixture stirred vigorously at room temperature overnight. At the end of this time the aqueous mixture was washed three times with ether (3 x 50 ml), acidified (pH 2) (cautiously) by dropwise addition of 12N hydrochloric acid, and extracted into ether (3 x 50 ml). The ethereal layers were combined, dried (magnesium sulphate) and concentrated to yield the product (167) as a white amorphous solid, 4.80 g, 84%.

(28) 2-N-carboxybenzylamino, dimethyl pentan-1,5-dioate (168)

The N-protected glutamate (167) (4.5 g, 0.015 M) was dissolved in ether (80 ml) and treated with diazomethane (2.00 g, excess) in ether (60 ml), being stirred at room temperature for 3 hours. At the end of this time, residual diazomethane was destroyed by dropwise addition of glacial acetic acid; the solvent was then removed *in vacuo* to afford the di-methyl ester (168), which was neither isolated nor characterised further, but instead immediately deprotected at nitrogen as follows:

(28) 2-amino. dimethyl pentan-1,5-dioate (82)

The fully protected glutamate material (168) was dissolved in a solution of hydrogen bromide in acetic acid (10 ml) and stirred at room temperature for 4 hours. The solvent was then removed (care being exercised to minimise exposure to any lachrymatory benzyl bromide present), the off-white residues being repeatedly azeotroped with benzene, before being suspended in water (100 ml) and washed three times with ether (3 x 50 ml); the ethereal layers were discarded and the aqueous layer concentrated *in vacuo* and dried under vacuum to yield a thick, clear, yellow oil (82) (as the hydrobromide); 3.43 g (97% from (167)).

^{13}C NMR (H_2O): δ172.16 $\underline{C}HCOOCH_3$; δ169.95 $CH_2\underline{C}OOCH_3$; δ54.93, δ53.97 (d) $COO\underline{C}H_3$ (x2); δ50.17 $\underline{C}HCOOCH_3$; δ34.62 (?d) $\underline{C}H_2$ (x2).

(2S,3R,4S) 1-*tert*-butyloxycarbonyl,2-carbomethoxy, 3-(N-(2'S)2'-pentan-1',5' dicarbomethoxy)amidomethyl, 4-isopropenyl azolidine (83)

Acid chloride (54) (from (45), 500 mg, 1.53 mM) was dissolved in THF (20 ml) to which was added pyridine (5 ml) and (82) (490 mg, 1.25 equivalents), the mixture being refluxed for 20 minutes and then allowed to cool to room temperature and left standing at ambient overnight. The solvent was then removed *in vacuo* and the residues partitioned between 2N hydrochloric acid (aqueous) solution (30 ml) and ether (30 ml); the aqueous layer was extracted twice more with ether (2 x 30 ml) and the ethereal layers combined, dried (magnesium sulphate) and concentrated to afford (83), a clear, pale yellow oil. Yield; 453 mg, 61%.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carboxy, 3-(N-(2'S)2'-pentan-1',5'-dicarboxy)-amidomethyl, 4-isopropenyl azolidine (169)

The fully protected L-glutamate adduct (83) (440 mg, 0.91 mM) was dissolved in THF (10 ml) and 2N sodium hydroxide (aqueous) solution

(10 ml) and stirred at room temperature overnight. The THF was then removed *in vacuo* and the aqueous mixture washed with ether (3 x 25 ml), acidified (pH 2) by dropwise addition of 12N hydrochloric acid, and extracted three times into ether (3 x 25 ml). The ethereal extracts were combined, dried (magnesium sulphate) and concentrated to yield (169), a foamy white solid; 338 mg, 84%. This material was not characterised further, but immediately deprotected at nitrogen, as follows:

(2S,3R,4S) 2-carboxy, 3-(N-(2'S)2'-pentan-1',5'-dicarboxy) amidomethyl 4-isopropenyl azolidine (84)

The de-esterified L-glutamate adduct (169) (320 mg, 0.72 mM) was dissolved in 98% formic acid, (5 ml) and stirred at room temperature overnight, after which the solvent was removed *in vacuo*, azeotroping with water. The residues were then dissolved in water and subjected to ion-exchange chromatography, the ninhydrin-active fractions being combined and concentrated to afford the white, solid product (84); 227 mg, 92%.
^{13}C NMR (D_2O): δ179.53, δ178.23 (d) COOH (x3); δ173.74 CONH; δ140.37 C:CH_2; δ113.93 C:CH_2; δ66.42 C-2; δ55.10 CONHCH; δ47.08 C-5; δ46.37 C-4; δ41.50 C-3; δ35.00 CH_2CONH; δ32.18 CH_2COOH; δ27.90 CONHCHCH_2; δ22.75 CH_3.
Mass Spec: M/E = 343 (M+1).

Attempted Arbuzov reaction on (79)

The kainyl-aminoethyl bromide adduct (79) (250 mg, 0.56 mM) was dissolved in triethyl phosphite (10 ml) and refluxed for 24 hours. Examination of the reaction mixture by TLC indicated complete consumption of the starting material; however, no significant quantities of any material corresponding to any sort of kainate-based substance were isolable from the reaction mixture which was therefore discarded.

Attempted reaction of (79) and sodium sulphite

The kainyl-aminoethyl bromide adduct (79) (250 mg, 0.56 mM) was dissolved in THF (20 ml) and water (20 ml). To this solution was added sodium sulphite (90 mg, 1.25 equivalents). The mixture was refluxed, with stirring, for 3 hours, at the end of which time TLC showed that all the starting material had been consumed; the mixture was evaporated to dryness *in vacuo*, and the residues dissolved in 98% formic acid (5 ml) and stirred at room temperature overnight. The solvent was then removed *in vacuo* and the residues azeotroped with water before being subjected to ion-exchange chromatography; the ninhydrin-active fractions were collected, but TLC examination indicated a mixture of at least five components; separation being impracticable, the material was discarded.

Catalytic hydrogenation of (91); preparation of (90a)

3-sulphonyl pyridine (91) (500 mg, 3.15 mM) was dissolved in a mixture of water (80 ml) and glacial acetic acid (20 ml). A catalytic amount of platinum (V) pentoxide (50 mg) was suspended in the mixture and the whole subjected to hydrogenation at 3 atmospheres pressure for 16 hours. The solution was then filtered through celite to remove residual catalyst, and the filtrate concentrated to afford the hydrogenated product (90a), a white solid; 470 mg, 95%.

^{13}C NMR (H_2O) δ54.17 C-3; δ44.78, δ44.56 (d) C-2, C-6; δ24.24 C-4; δ21.32 C-5.

Attempted Esterification of (91)

3-sulphonyl pyridine (91) (500 mg, 3.15 mM) was dissolved in methanol (30 ml), to which was added dropwise a solution of acetyl chloride (1 ml) in methanol (20 ml); the mixture was then refluxed overnight. The solvent was removed *in vacuo*; however, the only substance recovered was unaltered starting material, in approximately 100% yield.

Preparation of N-carboxybenzyl 4-hydroxy piperidine (94)

4-hydroxy piperidine (93) (2.0 g, 0.24 M) was dissolved in water (50 ml) and sodium carbonate (8.48 g, 0.08 M, 4 equivalents) and benzyl chloroformate (4.25 ml, 5.08 g, 1.5 equivalents) were added; the mixture was stirred vigorously at room temperature overnight. The solution was then washed with ether (3 x 50 ml) and the aqueous layer cautiously acidified (pH 2) by dropwise addition of 12N hydrochloric acid. The solution was then extracted three times with ethyl acetate (3 x 50 ml); the organic phases were combined, dried (magnesium sulphate) and concentrated to yield the product (94), a white solid; 4.43 g, 95%.

^{13}C NMR ($CDCl_3$): δ155.26 N$\underline{C}$OO; δ136.57 Ar 4$^{o}\underline{C}$; δ128.39, δ127.91, δ127.64 (t) Ar $\underline{C}$H; δ67.07 $\underline{C}H_2$Ph; δ66.63 $\underline{C}$HOH; δ41.36 $\underline{C}$-2, $\underline{C}$-6; δ33.70 $\underline{C}$-3, $\underline{C}$-5.

Preparation of N-carboxybenzyl, 4-iodo piperidine (85)

N-carboxybenzyl, 4-hydroxy piperidine (94) (530 mg, 2.28 mM) was dissolved in benzene (40 ml); triphenyl phosphine (2.37 g, 9.05 ml, 4 equivalents), imidazole (0.62 g, 9.12 mM, 4 equivalents) and iodine (1.72 g, 6.84 mM, 3 equivalents) were added and the mixture refluxed for 5 hours, then cooled to room temperature. Saturated sodium bicarbonate (aqueous) solution (40 ml) was added and the mixture stirred vigorously for 5 minutes. Small portions of iodine (ca. 20 mg each) were added, with further vigorous stirring after each addition, until the upper (organic) layer contained excess iodine (brown colouration). The excess iodine was then destroyed by dropwise addition of 1M sodium thiosulphate (aqueous) solution (organic phase clears and becomes colourless). The organic layer was separated from the aqueous phase, washed with water (50 ml) and dried over magnesium sulphate, before being concentrated and chromatographed on silica (40 g), thus affording the product (95); yield 540 mg, 70%.

^{13}C NMR ($CDCl_3$): δ155.00 N<u>C</u>OO; δ136.50 Ar 4°<u>C</u>; δ128.40, δ127.90, δ127.80 (t) Ar <u>C</u>H; δ67.10 <u>C</u>H_2Ph; δ43.80 <u>C</u>-2, <u>C</u>-6; δ37.00 <u>C</u>-3, <u>C</u>-5; δ27.20 <u>C</u>-4.

Attempted Arbuzov reaction on (95)

Iodopiperidine (95) (450 mg, 1.32 mM) was dissolved in triethyl phosphite (10 ml) and refluxed for 24 hours. At the end of this time, TLC examination of the mixture indicated no apparent change in the starting material, longer reflux periods being similarly ineffective. The reaction was thus discarded.

Preparation of 1-carboxybenzyl, 4-O-tosyl piperidine (97)

1-carboxybenzyl, 4-hydroxy piperidine (94) (920 mg, 4 mM) was dissolved in pyridine (15 ml) and tosyl chloride (1.14 g, 6 mM, 1.5 equivalents) added. The mixture was stirred at room temperature for 24 hours; the solvent was then removed *in vacuo* and the residues partitioned between 2N hydrochloric acid (aqueous) solution (40 ml) and ethyl acetate (40 ml); the aqueous layer was extracted twice more with ethyl acetate (2 x 40 ml) and the three organic layers combined, concentrated and chromatographed on silica (25 g) eluting with ethyl acetate/hexane/acetic acid (49:49:2), affording product (97), a heavy, clear oil; 1.23 g, 80%.

Preparation of 1-carboxybenzyl, 4-O-mesyl piperidine (96)

N-protected 4-hydroxy piperidine (94) (1.18 g, 5.04 mM) was dissolved in pyridine (10 ml) and mesyl chloride (3.26 g, 25.2 mM, 5 equivalents) added. The mixture was stirred at room temperature for 2 hours, after which all solvent was removed *in vacuo* and the residues partitioned between 2N hydrochloric acid (aqueous) solution (40 ml) and ethyl acetate (40 ml); the organic layer was dried and concentrated to give (96); 1.53 g, 98%. R_f (Ethyl acetate/hexane/acetic acid (49:49:2) = 0.31; (R_f (94) = 0.22).

Attempted preparation of (92) from (97)

Sodium (0.1 g, 4.35 mM) was added to a solution of diethyl phosphite (0.66 g, 4.78 mM) in ether (40 ml) with stirring, until all the metal had dissolved. The solution was then added to a solution of tosylate (97) (1.23 g, 3.16 mM) in ether (40 ml), the mixture then being stirred at room temperature overnight. TLC examination the following day showed a complex mixture of components in the reaction mixture, which was therefore discarded.

Attempted preparation of (92) from (96)

Sodium (168 mg, 7.3 mM) was added to a solution of diethyl phosphite (1.01 g, 7.34 mM) in ether (40 ml), with stirring until all the metal had dissolved. This solution was then added to a solution of mesylate (96) (1.53 g, 4.89 mM) in ether (40 ml) and stirred at room temperature for 4 hours and then refluxed for 16 hours; TLC showed no apparent reaction; the mixture was thus discarded.

Attempted preparation of (92) from (94)

Hydroxy piperidine (94) (570 mg, 2.3 mM) was dissolved in benzene (40 ml); imidazole (625 mg, 9.2 mM, 4 equivalents), triphenyl phosphine (2.41 g, 9.2 mM, 4 equivalents) and diethyl phosphite (952 mg, 6.9 mM, 3 equivalents) were added. The mixture was refluxed for 48 hours, without any apparent reaction. The reaction was therefore abandoned.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxyethyl, 4-isopropenyl azolidine (99)

Acid chloride (54) (from (45) 1.0 g, 3.06 mM) was dissolved in ether (50 ml) and the solution added dropwise to a stirred solution of diazomethane (390 mg, 9.18 mM, 3 equivalents) in ether (50 ml), stirring being continued at room temperature overnight. The excess diazomethane was

then destroyed by dropwise addition of glacial acetic acid, and all solvent removed *in vacuo*, azeotroping with benzene. The white solid product (assumed to be the diazoketone (98)) was examined by infra-red spectroscopy; characteristic peaks at 2100 cm^{-1} (diazoketone) and at 1650 cm^{-1} (ketone) indicated that the assumption was correct. This material was not characterised further, but was dissolved in methanol (50 ml), to which solution was added silver (I) oxide (715 mg, 6.12 mM, 2 equivalents); the mixture was stirred at room temperature for 72 hours. The solution was then filtered through celite to remove residual silver oxide, and the filtrate concentrated to dryness *in vacuo*. The residues were then dissolved in ether (50 ml) and washed with water (2 x 30 ml) before being dried, (magnesium sulphate), concentrated and chromatographed on silica (30 g) eluting with ethyl acetate/hexane 1:3. This afforded white solid material, mainly (99), the required diester product, but contaminated with small quantities of the corresponding precursor diazoketone (98); yield (crude) 470 mg, 45%.

^{13}C NMR ($CDCl_3$): δ200.80 residual diazoketone; δ172.25-δ170.36 (m) $\underline{C}OOCH_3$ (x2); δ154.32, δ153.69 (d) N$\underline{C}$OO; δ141.91, δ141.37 (d) $\underline{C}$:CH_2; δ113.74, δ113.47 (d) C:$\underline{CH}_2$; δ88.21 $\underline{C}(CH_3)_3$; δ64.03, δ63.07 (d) $\underline{C}$-2; δ52.22, δ51.82 (d) COO$\underline{CH}_3$ (x2); δ47.65, δ46.73 (d) $\underline{C}$-5; δ46.05, δ45.29 (d) $\underline{C}$-4; δ41.93, δ40.79 (d) $\underline{C}$-3; δ32.99 $\underline{CH}_2COOCH_3$; δ28.39, δ28.28 (d) $C(\underline{CH}_3)_3$; δ22.27 (d) $\underline{CH}_2CH_2COOCH_3$; $\underline{CH}_3$.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbodiphenyl-methoxymethyl, 4-isopropenyl azolidine (103)

Monoester (45) (500 mg, 1.53 mM) was dissolved in ether and diphenyl-diazomethane (370 mg, 1.91 mM, 1.25 equivalents) added. The mixture was refluxed for 72 hours, at the end of which time all solvent was removed *in vacuo* and the pinky-white residues chromatographed on silica (20 g) eluting with ethyl acetate/hexane 1:5, thus affording the product

(103), a white solid; 664. mg, 88%. R_f (ethyl acetate/hexane 1:2) = 0.50.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbodiphenylmethoxymethyl, 4-(2'-hydroxy, 3'-bromo)isopropyl azolidine (104)

The benzhydryl ester (103) (500 mg, 1.01 mM) was dissolved in a solution of acetone/water 9:1 (10 ml) and N-bromoacetamide (152 mg, 1.11 mM, 1.1 equivalents) added. The mixture was refluxed for 90 minutes. The solvent was then removed *in vacuo* and the residues chromatographed on silica (20 g) to afford the white frothy solid (104); 405 mg, 68%. R_f (ethyl acetate/hexane 1:2) = 0.30.

Attempted preparation of (2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbodiphenylmethoxymethyl, 4-(2'-O-acetyl, 3'-bromo)isopropyl azolidine (170)

The benzhydryl bromohydrin (104) (350 mg, 0.6 mM) was dissolved in pyridine (4 ml) and acetic anhydride (1 ml, excess) added. The mixture was stirred at room temperature for 24 hours; examination by TLC showed no change in the starting material. Longer reaction times produced similarly negative results. The solvents were removed and the unreacted starting material recovered.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbobenzoxymethyl, 4-isopropenyl azolidine (46)

Monoester (45) (500 mg, 1.53 mM) was dissolved in dry benzene and N,N-DMF dibenzyl acetal (573 mg, 2.23 mM, 1.5 equivalents) added. The mixture was refluxed for 1 hour; all solvent was then removed *in vacuo* and the oily residues chromatographed on silica (20 g) to afford the product (46), a clear, heavy yellow oil; 611 mg, 95%.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbobenzoxy-methyl, 4-(2'-O-acetyl, 3'bromo) isopropyl azolidine (106)

The mixed benzyl/methyl di-esterified material (46) (611 mg, 1.45 mM) was dissolved in a mixture of acetone/water 9:1 (10 ml) and N-bromo-acetamide (300 mg, 2.18 mM, 1.5 equivalents) added. The mixture was refluxed for 2 hours. All solvent was then removed *in vacuo* and the residues dissolved in pyridine (5 ml) to which was added acetic anhydride, (2 ml, excess). The mixture was stirred at room temperature overnight, at the end of which time all solvent was removed *in vacuo* and the residues radially chromatographed on silica, eluting with ethyl acetate/hexane 1:2, to afford the frothy, white solid product (106); 450 mg, 56%. R_f (ethyl acetate/hexane 1:2) = 0.25.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carboxymethyl, 4-(2'-O-acetyl, 3'-bromo)isopropyl azolidine (107)

The bromohydrin acetate (106) (450 mg, 0.81 mM) was dissolved in ethyl acetate (150 ml) and 5% palladium on charcoal catalyst (100 mg) was added. The mixture was subjected to hydrogenation at three atmospheres pressure overnight. The mixture was then filtered through celite to remove residual catalyst, and the filtrate concentrated *in vacuo* to afford a frothy, white solid (107); 375 mg, 99%.

^{13}C NMR ($CDCl_3$): δ172.55-δ168.37 (m) $\underline{C}OOCH_3$, $\underline{C}OOH$, $OO\underline{C}CH_3$; δ153.80 (d) $N\underline{C}OO$; δ81.59, δ80.56 (d) $\underline{C}(CH_3)_3$, $\underline{C}OCOCH_3$; δ66.15, δ65.83 (d) $\underline{C}$-2; δ52.55, $COO\underline{C}H_3$; δ45.02, δ43.94 (d) $\underline{C}$-5; δ40.63-δ38.57 (m) $\underline{C}$-4; δ37.98-δ36.68 (m) $\underline{C}H_2Br$; δ36.03, δ35.65 (d) $\underline{C}$-3; δ30.33 $\underline{C}H_2COOH$; δ28.22 $C(\underline{C}H_3)_3$; δ25.52, δ24.10 (d) $COCO\underline{C}H_3$; δ14.14 $C\underline{C}H_3$.

Attempted preparation of (2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-bromoethyl, 4-((2'RS)2'-O-acetyl, 3'-bromo)isopropyl azolidine (108) Hunsdiecker Reaction

A slurry of the bromohydrin acetate (107) (370 mg, 0.79 mM) and mercury (II) oxide (500 mg, excess) in trichlorobromomethane (50 ml) was added dropwise to a stirred solution of bromine (320 mg, 2.02 mM) in trichlorobromomethane (50 ml) maintained at a temperature of 80°C. The mixture was stirred at this temperature for 2 hours, then all solvent was removed *in vacuo* (care being taken to minimise contact with any excess bromine) and the residues dissolved in ethyl acetate (70 ml) and filtered three times through celite to remove residual mercuric material. The solvent was removed *in vacuo* and the residues radially chromatographed on silica, eluting with ethyl acetate/chloroform 1:1, which afforded a white-brown solid material (600 mg; >100% of desired product) which appeared (TLC) to be composed of two almost co-incident components. ^{13}C NMR on this material failed to conclusively identify it as the required product; it was therefore decided to attempt the reformation of the isopropenyl side chain by action of a copper-zinc couple on this impure material.

Attempted preparation of (2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-bromomethyl, 4-isopropenyl azolidine (109)

The impure material (?108) (600 mg) was dissolved in ethanol (30 ml) and copper-zinc couple (500 mg, excess) added. The mixture was refluxed for 6 hours. Examination by TLC failed to visualise the formation of any unsaturated material (developing in alkaline potassium permanganate); reflux was continued for a further 16 hours, at the end of which time only a small quantity of visibly permanganate-active material had been formed, which proved impossible to isolate chromatographically, although TLC indicated that little or no starting material remained. Neither could any non-permanganate-active products be isolated.

Attempted preparation of (2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-aminomethyl, 4-isopropenyl azolidine (110) from (54); Curtius Reaction

Acid chloride (54) (from (45) 300 mg, 0.02 mM) in THF (30 ml) was added dropwise to a solution of sodium azide (90 mg, 1.38 mM) in acetone/water 1:1 (30 ml), and the mixture stirred at room temperature for 1 hour. At the end of this time, examination by TLC showed complete conversion of the starting material to a higher R_f spot; this was assumed to be the corresponding intermediate iso-cyanate, and all solvent was removed *in vacuo*; attempts to decompose the intermediate to the product amine by refluxing in benzene (30 ml), toluene (30 ml) or water (30 ml) all failed; a 16 hour reflux in water produced at least six minor components (TLC); the reaction mixture was therefore discarded.

Attempted preparation of (2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-aminomethyl, 4-isopropenyl azolidine (110) from (45); Schmidt Reaction

Monoester (45) (250 mg, 0.76 mM) was dissolved in chloroform (20 ml) to which was added firstly, concentrated sulphuric acid (20 drops) and then sodium azide (102 mg, 1.53 mM, 2 equivalents), and the mixture stirred at room temperature for 2 hours. Sodium nitrite (264 mg, 3.83 mM, 5 equivalents) was added, (causing considerable effervescence and evolution of nitrogen dioxide). Analysis of the reaction mixture following the addition of sodium nitrite showed a complex mixture of components indicating a considerable degree of degradative reaction of the starting material was occurring; the reaction mixture was therefore discarded.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-acetyl azolidine (117)

α-N-*t*BOC-kainate dimethyl ester (43) (1.16 g, 3.39 mM) was dissolved in methylene chloride (50 ml) and the solution cooled to -78°C (195K); ozone was bubbled through the solution until it assumed a blue colour; oxygen was then bubbled through the solution until the blue colouration was discharged. Triphenyl phosphine (2.3 g, 8.78 mM, 2.5 equivalents) was added, and the mixture stirred at room temperature overnight. All solvent was then removed *in vacuo* and the residues taken up in the minimum volume of ethyl acetate/hexane 1:2, tested with starch iodide paper (negative result) to ensure no ozone remained, and then chromatographed on silica (20 g) to afford (117), a heavy, clear oil. Yield: 650 mg, 58%.

^{13}C NMR ($CDCl_3$): δ206.79, δ206.31 (d) $\underline{C}OCH_3$; δ172.45-δ171.91 (m) $\underline{C}OOCH_3$ (x2); δ154.03, δ153.32 (d) N$\underline{C}$OO; δ80.35 $\underline{C}(CH_3)_3$; δ63.77 $\underline{C}$-2; δ52.34-δ51.75 (m) COO$\underline{C}H_3$ (x2); δ51.37, δ50.34 (d) $\underline{C}$-4; δ46.93, δ46.33 (d) $\underline{C}$-5; δ41.94, δ40.70 (d) $\underline{C}$-3; δ33.06, δ32.62 (d) $\underline{C}H_2COOCH_3$; δ30.29 $\underline{C}H_3$; 28.07 $C(\underline{C}H_3)_3$.

(2S,3R,4R) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-acetyl azolidine (118)

The α-keto kainate (117) (500 mg, 1.45 mM) was dissolved in ether (20 ml) and added to a stirred suspension of triethylamine (0.6 g) on silica [75-230 mesh] (6 g) in ether (30 ml); stirring was continued for 48 hours at room temperature, the solution filtered through celite to remove the silica, and the filtrate then being concentrated to yield (118), a clear, heavy oil; 400 mg, 80%.

^{13}C NMR ($CDCl_3$): δ205.29 $\underline{C}OCH_3$; δ172.14-δ171.41 (m) $\underline{C}OOCH_3$ (x2); δ153.26 N$\underline{C}$OO; δ80.48 $\underline{C}(CH_3)_3$; δ63.82, δ63.55 (d) $\underline{C}$-2; δ55.15, δ54.17

(d) C-4; δ52.20, δ51.79 (d) $COO\underline{C}H_3$ (x2); δ48.11 C-5; δ41.72, δ40.93 (d) C-3; δ36.65, δ36.22 (d) $\underline{C}H_2COOCH_3$; δ29.66, δ29.23 (d) $\underline{C}H_3$; δ28.20 $C(\underline{C}H_3)_3$.

Attempted preparation of (2S,3R,4RS) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-O-acetyl azolidine from (117); Baeyer-Williger reaction (*m*-chloroperbenzoic acid).

Fully protected keto-kainate (117) (410 mg, 1.31 mM) was dissolved in chloroform (25 ml) and *m*-chloroperbenzoic acid (295 mg, 1.70 mM, 1.3 equivalents) added. The mixture was stirred until all the solid material had dissolved, and the reaction vessel was then stood at room temperature in a dark place for 48 hours. At the end of this time, analysis by TLC indicated no reaction had occurred, so the mixture was refluxed for 24 hours, without apparent effect. The lack of reaction was confirmed by subsequent complete deprotection of the material; α-allokainic acid (34) was recovered in 78% overall yield.

Attempted preparation of (2S,3R,4RS) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-O-acetyl azolidine from (118); Baeyer-Williger Reaction (*m*-chloroperbenzoic acid)

Fully protected allo-keto kainate (118) (410 mg, 1.31 mM) was dissolved in chloroform (25 ml) and *m*-chloroperbenzoic acid (295 mg, 1.71 mM, 1.3 equivalents) added. The mixture was stirred until all solid material had dissolved, and the reaction vessel stood at room temperature in a dark place overnight. Examination by TLC showed no reaction had occurred, so a further portion of *m*cpba (295 mg, 1.71 mM, 1.3 equivalents) was added, and the mixture refluxed for 6 hours, at the end of which time TLC showed that the starting material appeared to have been used up. 2N sodium hydroxide (aqueous) solution (25 ml) was added and

the mixture stirred vigorously at room temperature overnight. The aqueous layer was then separated from the organic layer, and the former acidified (pH 2) by dropwise addition of 12N hydrochloric acid, and extracted three times into ethyl acetate (3 x 30 ml). The three organic layers were combined, dried (magnesium sulphate) concentrated and dissolved in 98% formic acid (5 ml). The formate solution was stirred at room temperature overnight, then evaporated to dryness *in vacuo*, azeotroping with water. The residues were then subjected to ion-exchange chromatography, the ninhydrin-active fractions being combined and concentrated to yield a white solid (34); 220 mg, (85% from (118)). ^{13}C NMR (H_2O) identified this material as (2S,3R,4R) 2-carboxy, 3-carboxymethyl, 4-acetyl azolidine, thus indicating that the desired Baeyer-Williger reaction had not occurred.
^{13}C NMR (H_2O): δ211.16 $\underline{C}OCH_3$; δ177.14 CH$\underline{C}$OOH; δ173.05 $CH_2\underline{C}OOH$; δ65.68 $\underline{C}$-2; δ54.82 $\underline{C}$-4; δ46.63 $\underline{C}$-5; δ42.27 $\underline{C}$-3; δ39.06 $\underline{C}H_2COOH$; δ29.20 $CO\underline{C}H_3$.

(2S,3R,4S) 1-carboxybenzyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-acetyl azolidine (124)

Fully-protected N-carboxybenzyl kainate (171) (1.18 g, 3.14 mM) was dissolved in methylene chloride (50 ml) and the solution cooled to -78°C (195K). Ozone was bubbled through the solution until it attained a transparent blue colouration; oxygen was then blown through the solution until the blue colouration was discharged. The reaction was then left stirring at room temperature overnight. Triphenyl phosphine (1.23 g, 4.71 mM, 1.5 equivalents) was added, and the mixture stirred at room temperature for 2 hours. The solvent was then removed *in vacuo*, and the residues chromatographed on silica (77 g), eluting with ethyl acetate hexane 1:1, affording the product (124), a white, frothy solid,

collapsing to a clear, pale yellow oil on standing; 930 mg (79%). ^{13}C NMR ($CDCl_3$): δ206.62, δ206.19 (d) $\underline{C}OCH_3$; δ172.03, δ171.81 (m) $\underline{C}OOCH_3$ (x2); δ154.61, δ154.05 (d) $N\underline{C}OO$; δ136.30 Ar 4°$\underline{C}$; δ128.47-δ127.72 (m) Ar $\underline{C}H$ (x5); δ67.26, δ67.12 (d) $\underline{C}H_2Ph$; δ64.33, δ63.76 (d) $\underline{C}$-2; δ52.52, δ52.28, δ51.87 (t) $COO\underline{C}H_3$ (x2); δ51.44, δ50.46 (d) $\underline{C}$-4; δ47.46, δ46.23 (d) $\underline{C}$-5; δ42.09, δ40.87 (d) $\underline{C}$-3; δ33.05, δ32.80 (d) $\underline{C}H_2COOCH_3$; δ30.39 $\underline{C}H_3$.

Attempted Baeyer-Williger reaction on (124): Hydrogen Peroxide

The CBZ-keto-kainate (124) (400 mg, 1.06 mM) was dissolved in a mixture of ethyl acetate/ethanol 4:1 (40 ml); trifluoroacetic acid (1 ml, excess) and 30% hydrogen peroxide (4 ml, excess) were added, and the mixture refluxed for 100 hours. At the end of this time, the TLC showed a mixture of components present, including unreacted starting material (124), the epimerised allo-starting material, and other minor components. The reaction mixture was therefore discarded.

Attempted Baeyer-Williger reaction on (124): Sodium perborate

The CBZ-kainate (124) (230 mg, 0.61 mM) was dissolved in a mixture of ethyl acetate/acetic acid 4:1 (40 ml) and sodium perborate tetra-hydrate (105 mg, 0.67 mM, 1.1 equivalents) added; the reaction mixture was stirred at room temperature for 72 hours without any apparent change; the solvent was then removed *in vacuo* and replaced with a mixture of ethyl acetate/trifluoroacetic acid 4:1 (40 ml) and stirred at room temperature for 48 hours; the only reaction apparent was a slow de-protection at nitrogen presumably caused by the tri-fluoro-acetic acid. The reaction was abandoned.

Allyl triphenyl phosphonium bromide (122)

Triphenyl phosphine (2.62 g, 0.01 M) and allyl bromide (1.32 g, 0.011 M, 1.1 equivalents) were dissolved in benzene (50 ml) and refluxed for 2 hours, the reaction mixture then being allowed to cool to room temperature and stand overnight. After approximately half an hour, a white precipitate could be seen forming. After standing overnight, the precipitate was filtered off, washed on the filter with benzene and dried *in vacuo* to afford (122), a shiny white powder; 2.0 g, 53%. ^{31}P NMR (H_2O): 20.3 rel. H_3PO_4.

Attempted preparation of (2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-(1'-methyl)buta-1',3'-dienyl azolidine (123) from (117) and (122). Wittig Reaction

The Wittig reagent (122) (800 mg, 2.09 mM) was suspended in THF (25 ml) under nitrogen, to which was added a solution of phenyl lithium in cyclohexane/ether (2 ml, 168 mg, 1 equivalent); the mixture assumed a yellow colouration, and, after stirring at room temperature for 3 hours the solution had darkened to a clear red-brown colour. To this solution was added (117) (500 mg, 1.33 mM); the red colouration of the solution was rapidly discharged. The mixture was then refluxed for 2 hours, at the end of which time the solvent was removed *in vacuo* and the residues partitioned between ethyl acetate (40 ml) and 2N sodium hydroxide (aqueous) solution (40 ml) and the layers separated; the aqueous layer was washed twice with ether (2 x 30 ml) and then acidified (pH 2) with 12N hydrochloric acid and extracted three times with ethyl acetate (3 x 40 ml). The combined organic layers were concentrated; TLC analysis indicated at least four components in the mixture; apart from possible product there appeared to have been epimerisation at C-4 and some de-esterification. NMR analysis of the materials isolated after

chromatography proved impossible, and, in view of the minimal yields of recovered material (>20%) the reaction was abandoned.

(2S,3R,4S) 1-carboxybenzyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-(1'-methyl, 2'-cyano) ethenyl azolidine (125)

To a solution of cyanomethyl diethyl phosphonate (1.0 g, 5.65 mM) in THF (20 ml) was added sodium hydride (136 mg, 5.65 mM, 1 equivalent); after the mixture had stopped effervescing a solution of (124) (600 mg, 1.6 mM) in THF (20 ml) was added dropwise and the mixture stirred at room temperature for 15 minutes, after which time the solvent was removed *in vacuo* and the reddish tarry residues partitioned between ethyl acetate (40 ml) and water (20 ml); the organic layer was separated and concentrated *in vacuo*, the residues being chromatographed on silica (22 g), eluting with ethyl acetate/chloroform 1:1, to afford the product (125), a white, frothy solid, collapsing to a clear, pale yellow oil on standing; yield 450 mg, 71%.

^{13}C NMR ($CDCl_3$): δ171.65, δ171.38 (d) $\underline{C}OOCH_3$ (x2); δ160.08 $\underline{C}N$; δ154.40, δ153.88 (d) $N\underline{C}OO$; δ136.09 Ar 4°$\underline{C}$; δ128.53-δ127.82 (m) Ar $\underline{C}H$ (x5); δ115.98 $\underline{C}$:CH; δ98.46 (d) C:$\underline{C}$HCN; δ67.45 (d) $\underline{C}H_2Ph$; δ63.74, δ63.41 (d) $\underline{C}$-2; δ52.68-δ52.09 (m) $COO\underline{C}H_3$; δ47.62, δ47.19 (d) $\underline{C}$-5; δ46.92, δ46.10 (d) $\underline{C}$-4; δ42.39, δ41.39 (d) $\underline{C}$-3; δ32.91 $\underline{C}H_2COOCH_3$; δ20.64 $\underline{C}H_3$.

(2S,3R,4S) 1-carboxybenzyl, 2-carboxy, 3-carboxymethyl, 4-(1'-methyl, 2'-cyano) ethenyl azolidine (172)

The fully-protected Horner-Wittig adduct (125) (400 mg, 1.0 mM) was dissolved in THF (20 ml) and 2N sodium hydroxide (aqueous) solution (20 ml), and stirred at room temperature overnight. The THF was then removed *in vacuo* and the aqueous residue washed three times with ether (3 x 30 ml). The aqueous layer was then acidified (pH 2) by dropwise

addition of 12N hydrochloric acid (a white precipitate formed) and extracted three times into ethyl acetate (3 x 40 ml); the organic layers were combined, dried (magnesium sulphate), and concentrated to yield a white, frothy solid (172); 260 mg, 70%.

^{13}C NMR ($CDCl_3$): δ175.20-δ174.52 (m) COOH (x2); δ160.06 C-N; δ155.21, δ154.56 (d) NCOO; δ135.76 Ar 4°C; δ128.61-δ127.66 (m) Ar CH (x5); δ116.01 C:CH; δ96.52 C:CHCN; δ67.85 (d) CH_2Ph; δ63.60 (d) C-2; δ46.83, δ46.10 (d) C-5; δ42.34 (d) C-4; δ41.17 (d) C-3; δ32.86 (d) CH_2COOH; δ20.64 CH_3.

(2S.3R.4S) 1-carboxybenzyl, 2-carbomethoxy. 3-carbomethoxymethyl, 4-(1'-methyl, 2'-carbomethoxy) ethenyl azolidine (126)

Diethyl phosphonyl methyl ethanoate (1.0 g, 4.76 mM) was dissolved in THF (30 ml) and sodium hydride (114 mg, 4.76 mM, 1 equivalent) added. After evolution of gas had ceased, a solution of (124) (550 mg, 1.46 mM) in THF (20 ml) was added, the mixture stirred at room temperature overnight, and then refluxed for 6 hours to complete the reaction. The solvent was then removed *in vacuo* and the tarry residues partitioned between water (30 ml) and ethyl acetate (30 ml), the aqueous layer was extracted twice into ethyl acetate (2 x 30 ml) and the three organic layers combined, dried (magnesium sulphate) and concentrated *in vacuo*, the residues being chromatographed on silica (20 g), eluting in ethyl acetate/chloroform 1:2, thus affording the product (126), a white, frothy solid, collapsing to a clear, yellow oil on standing; yield 300 mg, 48%.

^{13}C NMR ($CDCl_3$): δ171.90-δ171.06 (m) $COOCH_3$ (KA, x2); δ166.15 $COOCH_3$; δ154.10, δ153.72 (d) NCOO; δ136.22 Ar 4°C; δ128.45-δ128.01 (m) Ar CH (x5); δ119.16 C:CH; δ117.69 C:CH; δ67.36 CH_2Ph; δ64.12, δ63.84 (d) C-2; δ53.64-δ51.13 (m) $COOCH_3$ (x3); δ50.14, δ49.79 (d) C-5; δ47.57

(d) C-4; δ43.48, δ42.53 (d) C-3; δ33.54 $\underline{C}H_2COOCH_3$; δ15.28 $\underline{C}H_3$.

(2S,3R,4S) 1-*tert*-butyloxycarbonyl, 2-carbomethoxy, 3-carbomethoxymethyl, 4-(1'-methyl, 2'-cyano)ethenyl azolidine (127)

Diethylphosphoryl methylcyanide (1.01 g, 4.83 mM) was dissolved in THF (30 ml) and potassium *tert*-butoxide (542 mg, 4.83 mM, 1 equivalent) added. When evolution of gas had ceased, a solution of (117) (1.51 g, 4.39 mM) in THF (30 ml) was added and the mixture stirred at room temperature overnight. The solvent was then removed *in vacuo* and the residues chromatographed on silica (30 g), eluting with ethyl acetate/chloroform/acetic acid (49:49:2), to afford the product (127), a heavy, clear yellow oil; 1.0 g, 62%.

^{13}C NMR ($CDCl_3$): δ172.19, δ171.03 (d) $\underline{C}OOCH_3$ (x2); δ160.14 $\underline{C}N$; δ153.00 (d) $N\underline{C}OO$; δ116.07 $\underline{C}$:CHCN; δ99.14 C:$\underline{C}$HCN; δ80.75 $\underline{C}(CH_3)_3$; δ64.03, δ63.82 (d) $\underline{C}$-2; δ52.31, δ51.81 (d) $COO\underline{C}H_3$ (x2); δ51.14, δ49.54 (d) $\underline{C}$-5; δ48.76, δ48.11 (d) $\underline{C}$-4; δ43.53 (d) $\underline{C}$-3; δ35.40 $\underline{C}H_2COOCH_3$; δ28.20 $C(\underline{C}H_3)_3$; δ17.58 $\underline{C}H_3$.

5.3 α-amino, ω-phosphono-carboxylic acids; Preparative work.

1-bromo, 5-diethylphosphonyl pentane (129b).

Sodium (10.8 g, 0.51 M), finely divided, was added to a solution of diethylphosphite (70.75 g, 0.51 M) in ether (600 ml). The reaction proceeded smoothly and rapidly with the evolution of hydrogen; a reflux condenser was fitted to ensure that the reaction did not become too vigorous. When all the metal had dissolved, the solution of the sodium salt of diethyl phosphite was added to an excess of 1,5-dibromopentane (301.2 g, 1.31 mM) in ether (1000 ml) in a 2 litre round-bottomed flask, fitted with a magnetic stirrer, maintained at 0°C (273K). The reaction mixture was stirred at room temperature for 72 hours, then the flask was fitted with a reflux condenser and refluxed for 5 hours. The resulting solution, containing a fine, white suspended solid, was filtered through celite, evaporated *in vacuo* and chromatographed on silica (200 g) to afford the product (129b); a clear, yellow oil; 33.0 g, 23%.

^{13}C NMR ($CDCl_3$): δ61.53, δ61.24 (d, J = 6.41 Hz) O$\underline{C}H_2$; δ33.32 $\underline{C}$-5; δ32.27, δ33.22 (d, J = 1.22 Hz) $\underline{C}$-4; δ29.37, δ22.39 (d, J = 147.37 Hz) $\underline{C}$-1; δ28.64 $\underline{C}$-2; δ21.85, δ21.63 (d, J = 4.92 Hz) $\underline{C}$-3; δ16.63, δ16.36 (d, J = 6.10 Hz) $OCH_2\underline{C}H_3$:

^{31}P NMR ($CDCl_3$): δ31.8 (rel. H_3PO_4).

ethyl-2-N-acetylamino, 2-carboethoxy, 7-diethylphosphonyl heptanoate (130b)

Sodium (2.43 g, 0.12 M) was dissolved in ethanol (55 ml) and stirred until all the metal had dissolved. To this solution was added diethyl acetamidomalonate (24.92 g, 0.12 M), and the resulting yellowish solution evaporated to dryness *in vacuo*, azeotroping with toluene. To the resulting white crystalline material was added a solution of (129b)

(32.97 g, 0.12 M) dissolved in a mixture of toluene (240 ml) and diethyl carbonate (80 ml). The mixture was refluxed for 48 hours. The resulting solution was filted to remove a brown, solid precipitate, which was washed on the filter with toluene; the combined filtrates were concentrated, and the residues chromatographed on silica (100 g), to afford the product (130b); 27.09 g, 58%.

(2RS) 2-amino, 7-phosphono-heptanoic acid (15) (2-APH).

A solution of (130b) (27.09 g, 5.95 mM) in 6N hydrochloric acid (350 ml) was refluxed for 72 hours. The solution was then filtered and evaporated to dryness *in vacuo*, the residues being taken up in the minimum volume of methylated spirits; propylene oxide was added dropwise until a white solid precipitate began to form. The mixture was left to stand at room temperature with occasional stirring, until the accumulated solid product became non-sticky. The mixture was then filtered, the white solid collected on the filter being the product (15); recrystallised from water/ethanol to yield 7.60 g, 52%.
^{13}C NMR (D_2O): δ174.30 COOH; δ54.47 C-2; δ30.47 (s) C-6; δ30.47, δ29.55 (d, J = 20.75 Hz) C-5; δ30.28, δ24.27 (d, J = 135.50 Hz) C-7; δ24.46, δ24.27 (d, J = 5.27 Hz) C-4; δ22.86, δ22.67 (d, J = 4.99 Hz) C-3.
^{31}P NMR (D_2O): δ28.60 (rel. H_3PO_4). M.Pt.: 221°C (Lit. 228°C).

1-bromo, 3-diethylphosphonyl propane (129a)

Sodium (8.4 g, 0.37 M) was added to a solution of diethyl phosphite (55.20 g, 0.4 M) in ether (300 ml) and the solution stirred until all the metal had dissolved; this solution was then added to a solution of 1,3 dibromopropane (182 g, 0.9 M) in ether (500 ml) at 0°C (273K); when addition was complete, the mixture was allowed to warm to room temperature and the mixture stirred at ambient for 24 hours. The

solvent was then removed *in vacuo* and the residues chromatographed on silica (200 g) to afford the product (129a), a clear, yellow oil; 15.70 g, 15%.

^{13}C NMR ($CDCl_3$): δ62.3, δ61.9 (d, J = 6.1 Hz) O<u>C</u>H_2; δ34.4, δ33.1 (d, J = 18.9 Hz) <u>C</u>-1; δ29.2, δ19.7 (d, J = 142.8 Hz) <u>C</u>-3; δ26.1, δ25.9 (d, J = 4.3 Hz) <u>C</u>-2; δ16.8, δ16.4 (d, J = 6.1 Hz) $OCH_2\underline{C}H_3$.

^{31}P NMR ($CDCl_3$): δ30.70 (rel. H_3PO_4).

ethyl, 2-N-acetylamino, 2-carboethoxy, 5-diethylphosphonyl pentanoate (130a)

Sodium (1.28 g, 55.6 mM) was dissolved in ethanol (30 ml), to which solution was added diethylacetamidomalonate (13.84 g, 55.6 mM); to the resulting yellowish solution was added (129a) (15.7 g, 55.6 mM) and the mixture was refluxed for 48 hours, after which all solvent was removed *in vacuo* and the residues chromatographed on silica (50 g), yielding the product (130a), a heavy, pale yellow oil. 11.41 g, 47%.

(2RS) 2-amino, 5-phosphono pentanoic acid (14) (2-APP)

A solution of (130a) (11.41 g, 28.9 mM) in 6N hydrochloric acid (100 ml), was refluxed for 24 hours, at the end of which time the solution was filtered, and the filtrate evaporated to dryness *in vacuo*; the residues were taken up in the minimum volume of methylated spirits and propylene oxide added dropwise until a white solid precipitate began forming. The mixture was then stirred at room temperature until the precipitate became non-sticky. The white precipitate was then filtered off and recrystallised from water/ethanol to afford (14); 3.84 g, 67%.

^{13}C NMR (D_2O): δ173.68 <u>C</u>OOH; δ53.96 <u>C</u>-2; δ32.02, δ30.18 (d, J = 41.5 Hz) <u>C</u>-4; δ31.20, δ24.22 (d, J = 157.47 Hz) <u>C</u>-5; δ19.23 <u>C</u>-3.

3-diethylphosphonyl prop-1-ene (132)

Sodium (2.3 g, 0.1 M) was added to a solution of diethyl phosphite (13.9 g, 0.1 M) in ether (200 ml) and stirring continued until all the metal had dissolved. The solution was then cooled in an ice bath and dropwise addition of allyl bromide (140 g, 0.12 M) commenced, with stirring. The flask was fitted with a reflux condenser as the reaction became rather vigorous. (Caution!) When the vigorous reaction had ceased, the mixture was allowed to warm to room temperature, filtered through celite to remove the suspended sodium bromide, and concentrated to yield a clear, pale yellow oil with an unpleasant odour (132); 15.13 g, 85%.

^{13}C NMR ($CDCl_3$): δ127.80, δ127.31 (d, J = 10.98 Hz) C-2; δ120.21, δ119.62 (d, J = 13.41 Hz) C-1; δ62.06, δ61.81 (d, J = 6.10 Hz) $O\underline{C}H_2$; δ34.94, δ28.71 (d, J = 140.38 Hz) C-3; δ16.58, δ16.31 (d, J = 6.10 Hz) $OCH_2\underline{C}H_3$.

^{31}P NMR ($CDCl_3$): δ26.7 (rel. H_3PO_4).

3-diethylphosphonyl propan-1-ol (133)

To a stirred solution of allyl phosphonate (132) (8.6 g, 0.048 M) in hexane (50 ml) at 0°C (273K) under an atmosphere of argon, was added borane-methyl sulphide (1.21 g, 0.014 M); there was some effervescence and the solution became slightly cloudy. The solution was allowed to warm to room temperature and stirring was continued for 1 hour. Ethanol (50 ml) and 2N sodium hydroxide (aqueous) solution (10 ml) were added, stirring being continued; the mixture was cooled to 0°C (273K) and 30% hydrogen peroxide solution (30 ml, large excess) added. A white suspension formed. The mixture was allowed to warm to room temperature and stirring continued at ambient for 1 hour. At the end of this time, all solvent was removed *in vacuo* and the residues partitioned between

ice-cold water (50 ml) and ether (100 ml). The ethereal layer was separated and the aqueous extracted twice more with ether (2 x 100 ml), the combined ethereal layers being concentrated to yield a pale yellow oil, which was chromatographed on silica (30 g) to afford (133), a pale yellow oil; 1.79 g, 19%.

^{13}C NMR ($CDCl_3$): δ62.95, δ62.73 (d, J = 4.88 Hz) C-1; δ61.81, δ61.70 (d, J = 2.44 Hz) $O\underline{C}H_2$; δ38.25, δ32.18 (d, J = 136.72 Hz) C-3; δ24.70, δ24.00 (d, J = 15.87 Hz) C-2; δ16.58, δ16.31 (d, J = 6.10 Hz) $OCH_2\underline{C}H_3$.

^{31}P NMR ($CDCl_3$): δ29.6 (rel. H_3PO_4).

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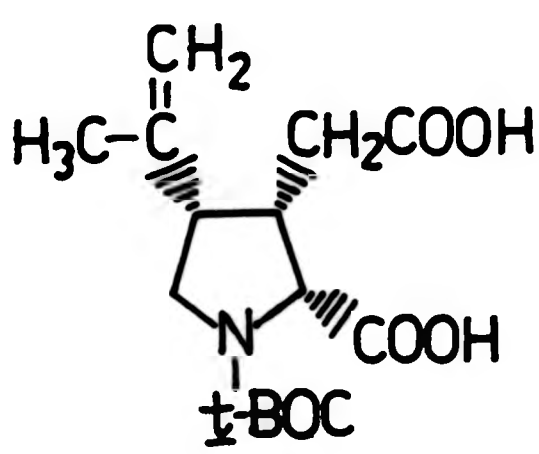

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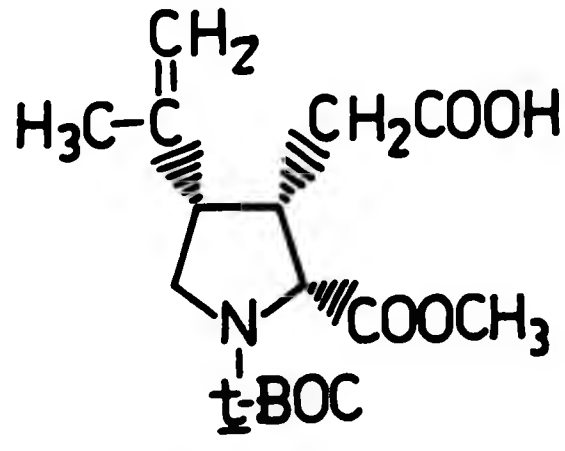

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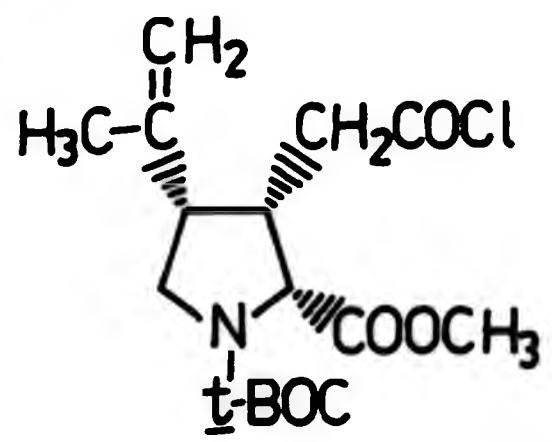
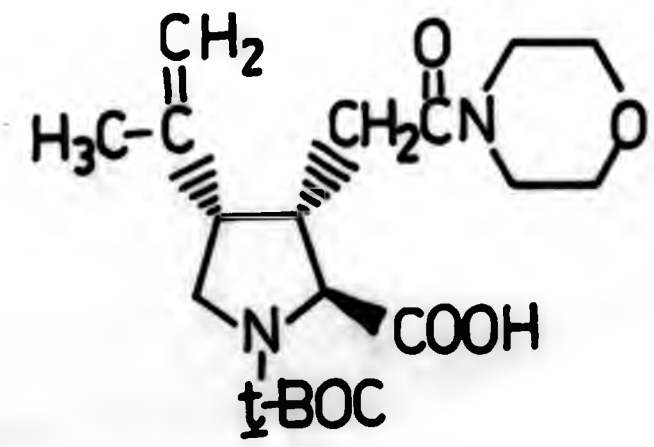
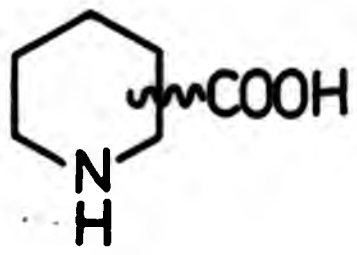


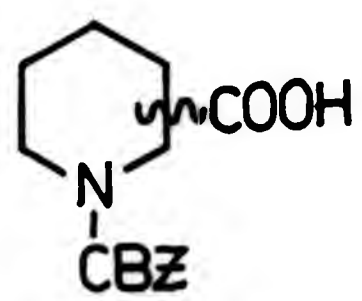
(147)



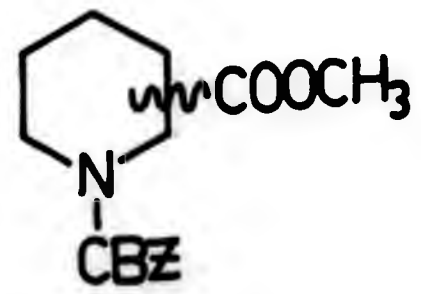
(148)



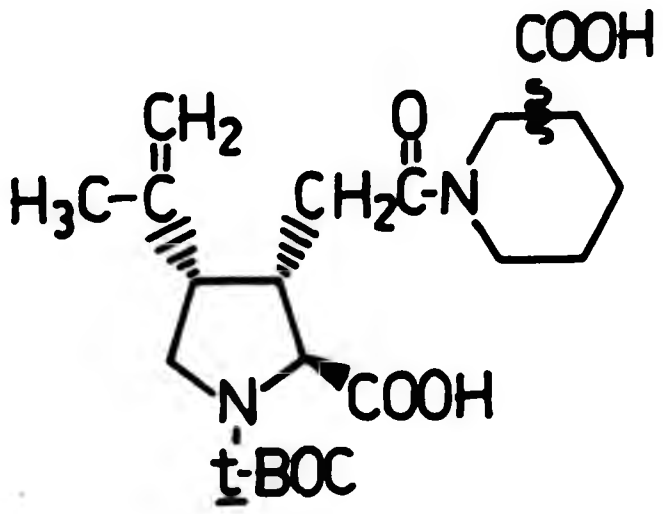
-COOH = C-2 (149)
-COOH = C-3 (150)
-COOH = C-4 (151)



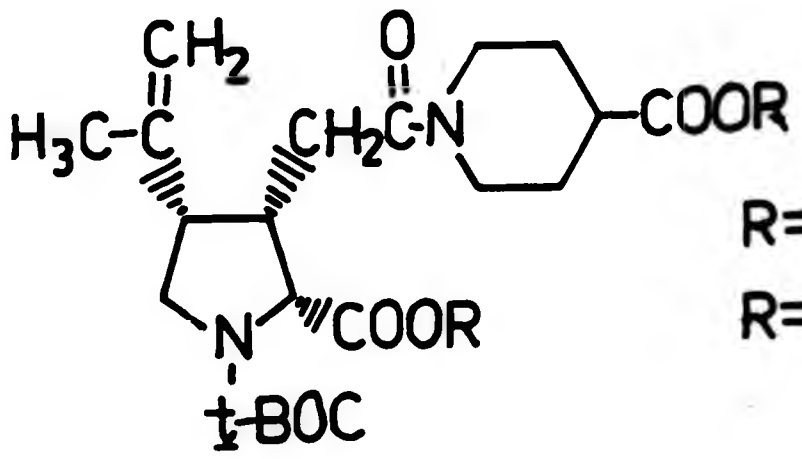
-COOH = C-2 (152)
-COOH = C-3 (153)
-COOH = C-4 (154)



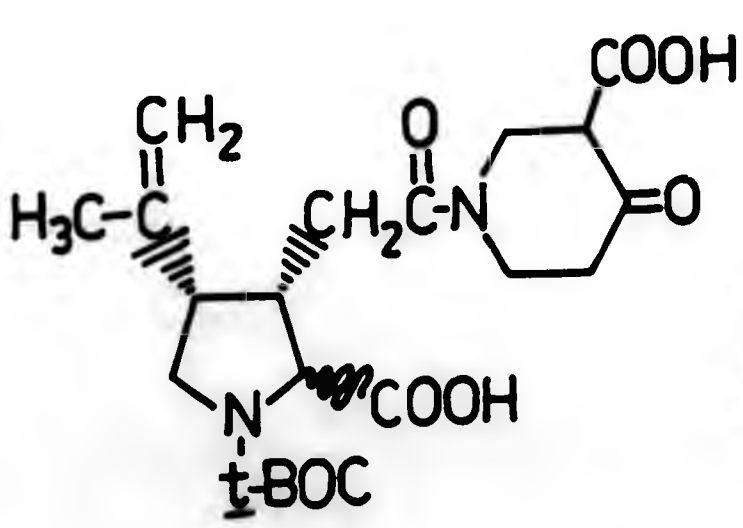
-COOR = C-2 (155)
-COOR = C-3 (156)
-COOR = C-4 (157)



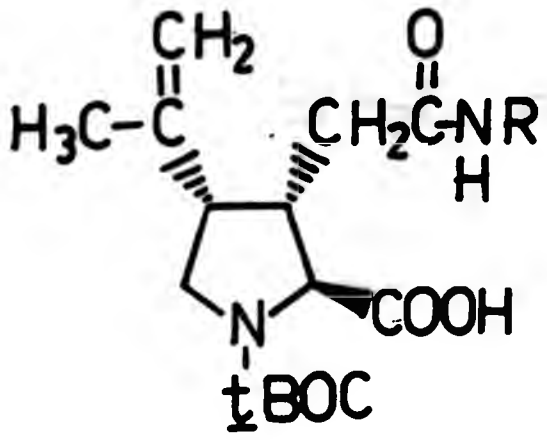
-COOH = 2′ (158)
-COOH = 3′ (159)
-COOH = 4′ (160)



R=CH3 (161)
R= H (162)

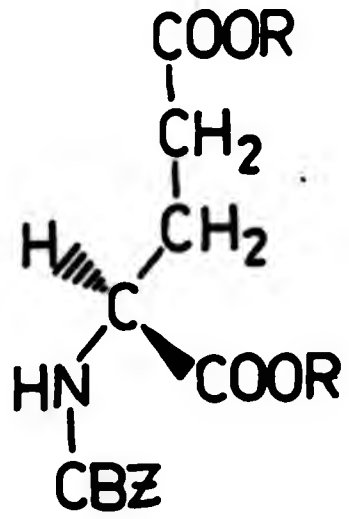


C-2=α (163)
C-2=β (164)



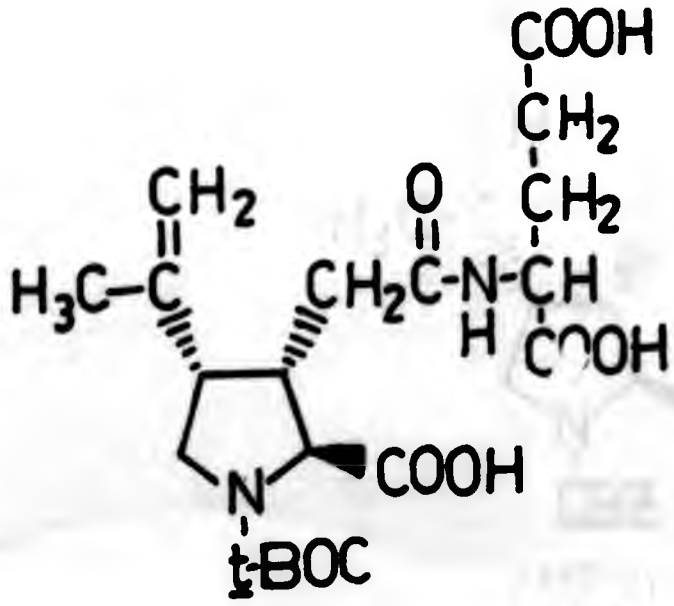
R= $(CH_2)_3COOH$ (165)

R= $(CH_2)_4COOH$ (166)

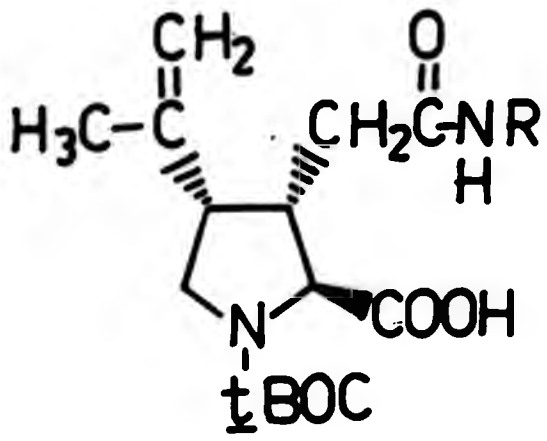


R = H (167)

R = CH_3 (168)

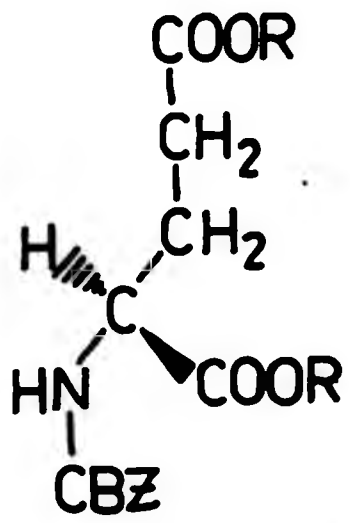


(169)



R=$(CH_2)_3COOH$ (165)

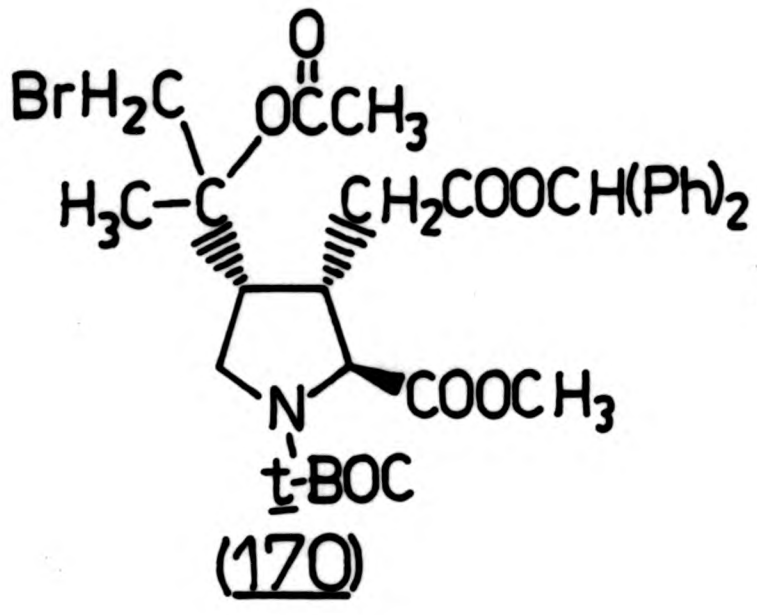
R=$(CH_2)_4COOH$ (166)



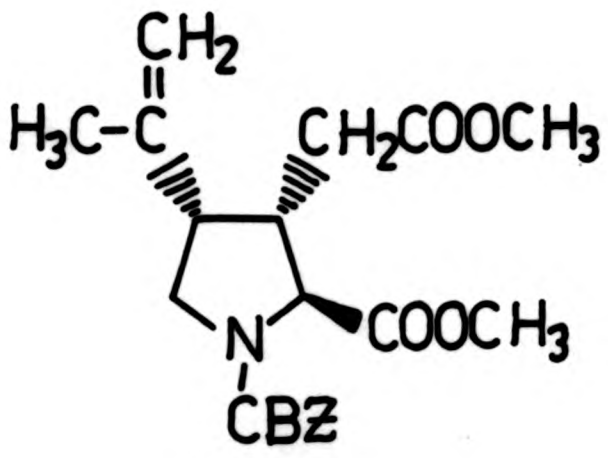
R = H (167)

$R=CH_3$ (168)

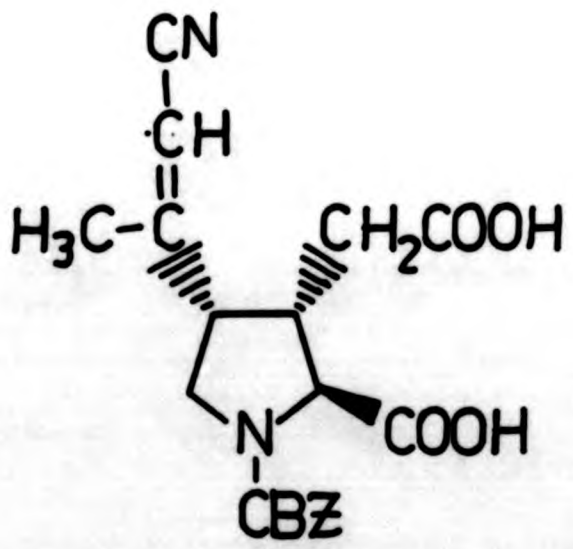
(169)


O
BrH2C
OCCH3
H3C–C
CH2COOCH(Ph)2
N
COOCH3
t-BOC

(170)


CH2
H3C–C
CH2COOCH3
N
COOCH3
CBZ

(171)


CN
CH
H3C–C
CH2COOH
N
COOH
CBZ

(172)

2

D7167'87

END