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Chemical Studies on Excitatory Amino Acids

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1993

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Introduction p1

References p5

Chapter 1

Synthesis of New Kainoids p7

Chemical Studies on Excitatory Amino Acids

References p30

Chapter 2

Structure-Activity Relationships of Kainoids p31

Experimental p39

References p40

Chapter 3

Stereochemical Discrimination of Kainoid

by Means of ¹H-NMR Spectrum p41

Experimental p75

References p82

Kimiko Hashimoto

Chapter 4

Synthesis of Conformationally Restricted Analogs

of Kainic Acid p83

Experimental p85

References p113

Hokkaido University

1993

Contents

Introduction

References

.....p1

.....p5

Chapter 1

Synthesis of New Kainoids

Experimental

References

.....p7

.....p13

.....p30

Chapter 2

Structure-Activity Relationships of Kainoids

Experimental

References

.....p31

.....p39

.....p40

Chapter 3

Stereochemical Discrimination of Kainoid

by Means of ^1H NMR Spectrum

Experimental

References

.....p41

.....p75

.....p82

Chapter 4

Synthesis of Conformationally Restricted Analogs

of Kainic Acid

Experimental

References

.....p83

.....p95

.....p113

Conclusion	p114
References	p115

¹ HNMR spectra	p116
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Publication List	p136
------------------	-----------

Acknowledgments	p141
-----------------	-----------

Introduction

About twenty years ago, studies concerning the constituents of the poisonous mushroom, *Clitocybe acromelalga*, were started in this laboratory. This mushroom has been known to cause unique symptoms of poisoning, that is, in a few days after erroneous ingestion one feels a sharp pain and a marked reddish edema on hands and feet, and it continues for about a month. The symptom is similar to acromelalgia and erythromelalgia. Since these fungi were distributed only in Japan and especially in Tohoku, Hokuriku and the surrounding country, the symptoms has been thought to be due to an endemic disease. The isolation of toxic principles was carried out by monitoring the lethal effect on mice, because the same symptoms in man were not reproduced in mice and frogs. Among the isolated toxic compounds, acromelic acids A (13) and B (14) (Fig. 1) caused the most potent lethal effect on mice.¹

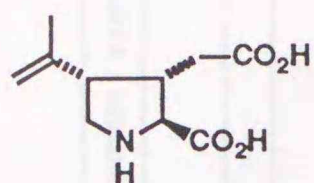
These non-proteinogenic amino acids have a common structure with kainic (1)² and domoic acids (4)³, the former being isolated from an alga and used as a medicine in exterminating roundworm in Japan and the latter also being isolated from an alga and also has an exterminating affect on roundworm and pinworm. Moreover, kainic and domoic acids have attracted attention in the field of neuroscience for their strong neuroexcitatory action on glutamatergic neurons. This exterminating activity is probably due to their excitotoxicity nature on worms. Acromelic acids were expected to have similar activity on neuro-cells like those of kainic and domoic acids.

The synthetic study of acromelic acids performed in 1986⁴ revealed the extremely potent activity over that of kainic and domoic acids. For example, these acids showed 100 times more potent depolarization than that of kainic acid in the crayfish neuro-muscular junction⁵ and in the prepa-

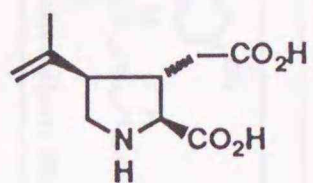
ration of new-born rat spinal motoneuron⁶, they exhibited the strongest activity among the related compounds known so far. These kainoid amino acids, kainic, domoic and acromelic acids, include the glutamic acid moiety in their structures that act on the glutamate receptors (or excitatory amino acids (EAA) receptors).⁷ These receptors are distributed in the central nervous system of vertebrate and in the peripheral system of invertebrate. EAA receptors are classified into two major groups, ionotropic receptors and metabotropic receptors, and the former is further classified into three subtypes according to the selective agonists, NMDA (N-methyl-D-aspartic acid), AMPA (α -amino-3-hydroxy-5-methyl-4-oxazolepropionic acid) and kainate (kainic acid) receptor subtypes (Fig. 2).⁸ But the AMPA and kainate subtypes are not clearly separated and sometimes they are shown as a single subtype. EAA receptors, especially the NMDA receptor, are known to be involved in memory and learning,⁹ and are also related to Parkinsonism and Huntington's diseases.¹⁰

Recently, a mussel poisoning that occurred in Canada was attributed to domoic acid and its analogs.¹¹ This accident revealed that these kainoids also cause brain damage and loss of memory. Seventeen naturally occurring kainoid members are known currently and some synthetic studies have been done,¹² but there is no effective route to supply a sufficient amount and variety of these amino acids. Because of a shortage of kainate-type reagents, the nature and role of kainate-type receptors remain unclear. Therefore, a new synthetic route is essential to supply these chemicals to the neuroscience field. These reagents will contribute to revealing the nature of the kainate type receptor and allow the study of the mechanisms of neurotransmission and neurodegenerative disorders. Thus, the study EAA and the receptors by means of organic chemistry was attempted.

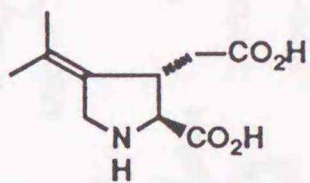
Fig 1 Naturally occurring kainoids



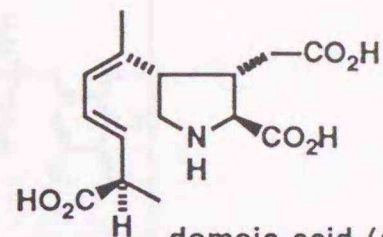
α -kainic acid (1)



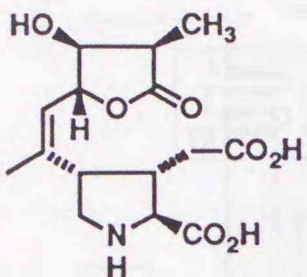
α -allokainic acid (2)



isokainic acid (3)



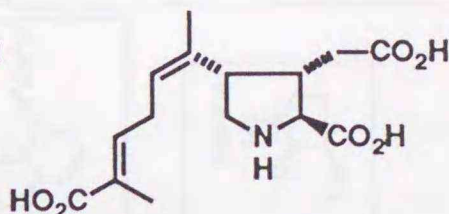
domoic acid (4)



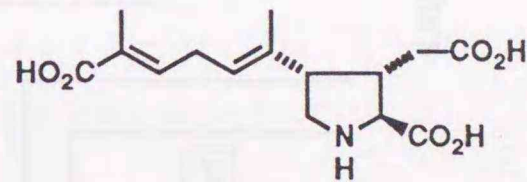
domoilactone A (5)



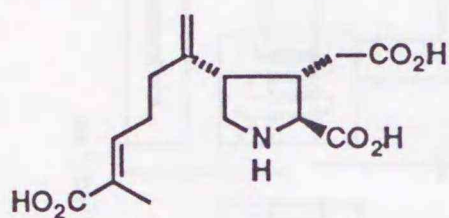
domoilactone B (6)



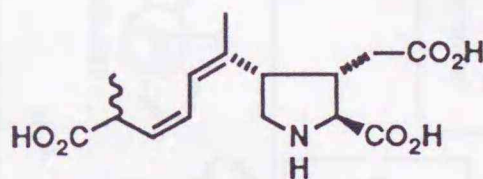
isodomoic acid A (7)



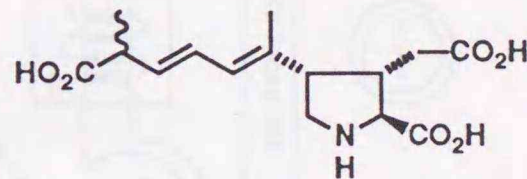
isodomoic acid B (8)



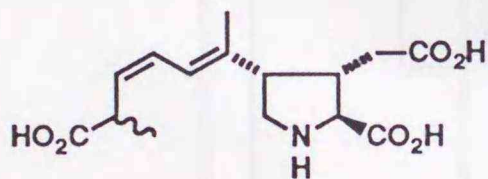
isodomoic acid C (9)



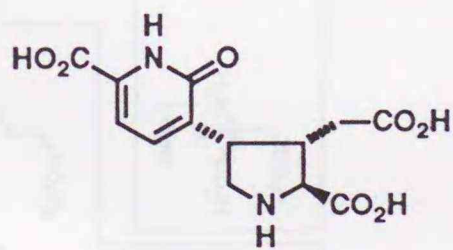
isodomoic acid D (10)



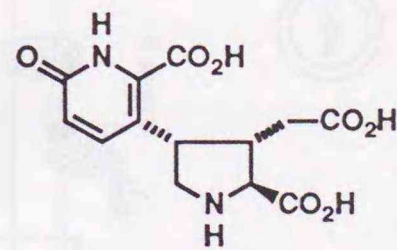
isodomoic acid E (11)



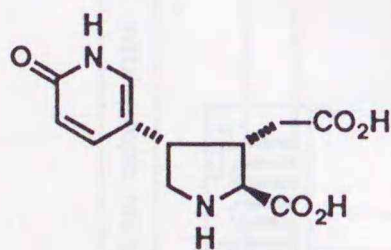
isodomoic acid F (12)



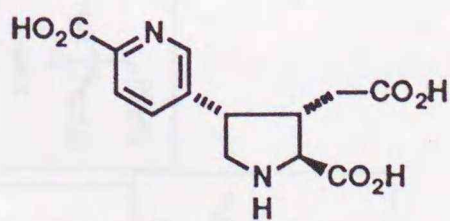
acromelic acid A (13)



acromelic acid B (14)



acromelic acid C (15)

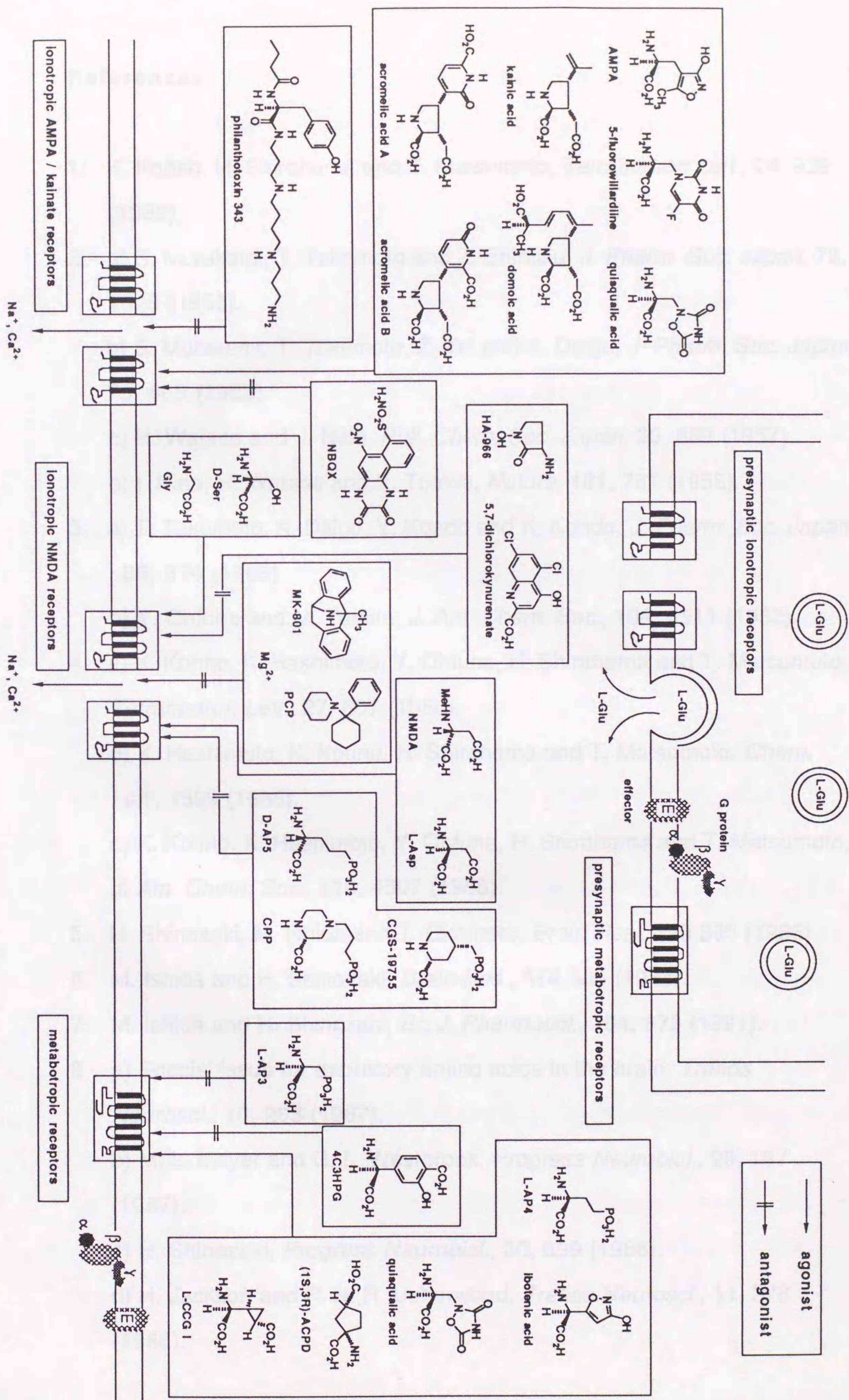


acromelic acid D (16)



acromelic acid E (17)

Fig 2 Excitatory amino acid receptors



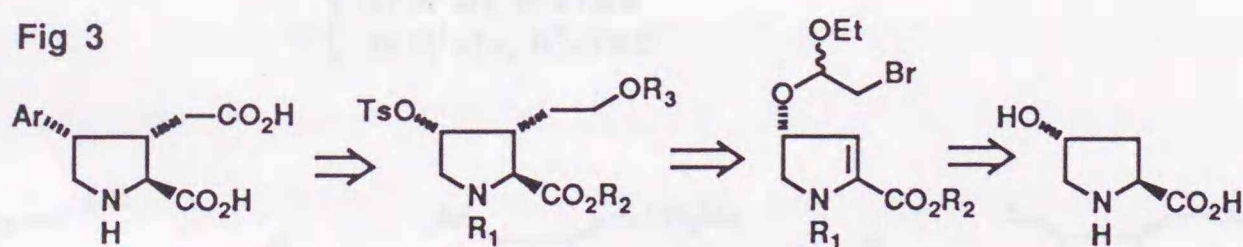
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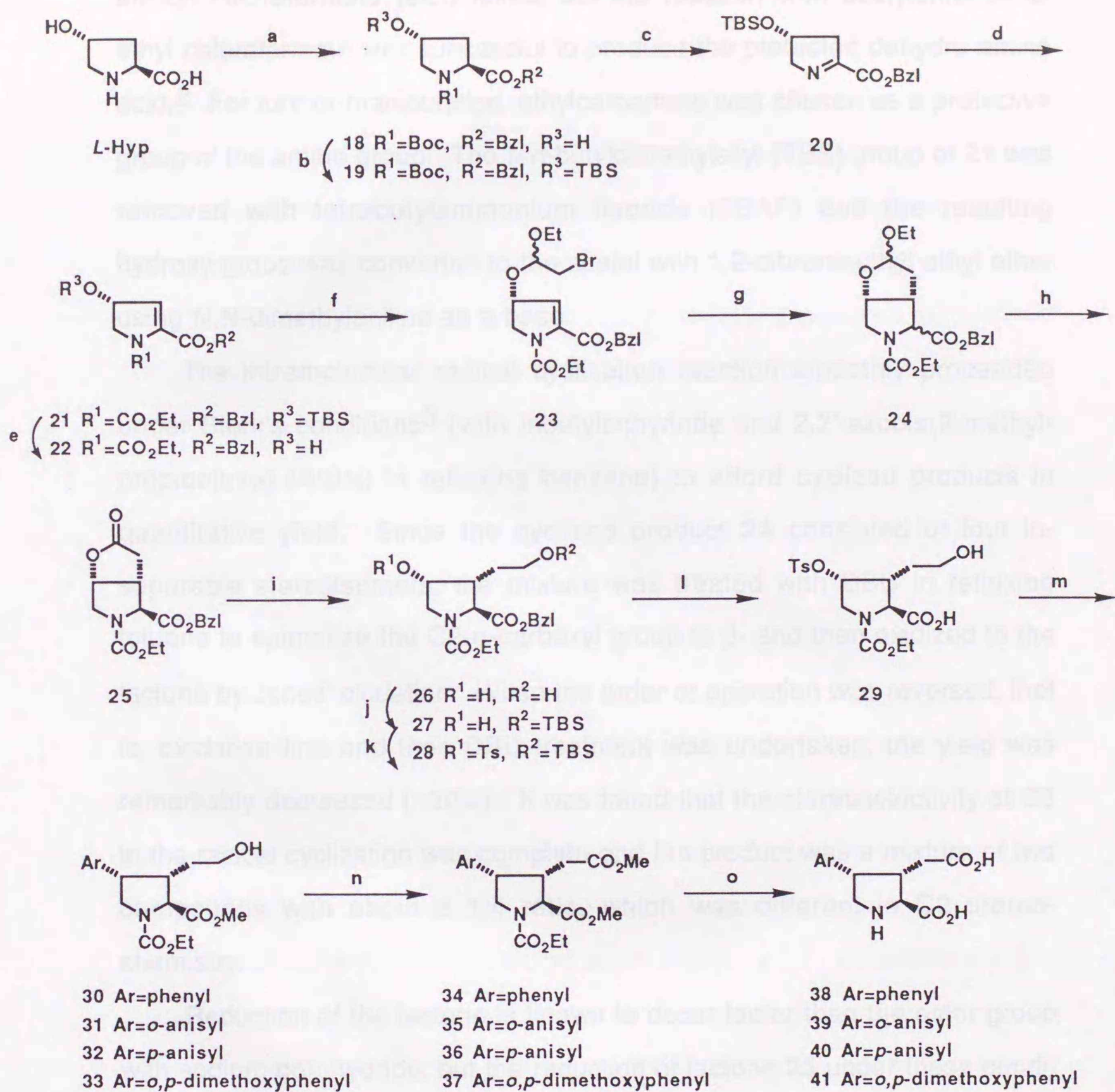
Chapter 1 Synthesis of New Kainoids

A structural feature of kainoids is the 4-substituted 3-carboxymethylproline whose stereochemistry is (2*S*, 3*S*, 4*S*)-, except for allokainic acid (2) and isokainic acid (3). This stereochemical arrangement is essential for their biological activity (depolarizing activity). For example, allokainic acid having a (4*R*)-configuration and the C4 of isokainic acid is a sp^2 carbon but they show almost no activity.¹ Next the strength of the depolarizing activity increases in the following order, kainic, domoic, acromelic acids A and B. They are structurally different only in the C4-substituent. Accordingly, the relationship between the structure of the C4-substituent and its biological activity was the first problem. To solve this problem, a new synthetic route, which should have flexibility for the introduction of various C4-substituents with proper stereochemistry, was needed. The newly devised synthetic plan is outlined in Fig. 3.



Starting from *L*-Hyp (trans-*L*-4-hydroxyproline), stereoselective introduction of the C3 acetic acid unit using an intramolecular radical cyclization reaction followed by the substitution reaction of C4-tosylate using Ar_2CuLi with retention of configuration would lead to kainoids with the proper stereochemistry. The amino, carboxyl and hydroxyl groups of *L*-Hyp were protected as the *tert*-butylcarbamate, benzyl ester and silyl ether, respectively (Fig. 4). Carbamate **19** was deprotected using TFA, then without further purification, chlorination of the resulting amine with *tert*-butylhypochlorite followed by dehydrochlorination with triethylamine in one pot gave the 1-pyrroline derivative **20**. Protection of the imine with di-*tert*-butyl dicarbonate

Fig 4



(a) i) Boc_2O , NaHCO_3 ii) BzI , Et_3N (b) TBSI , imidazole (c) i) TFA ii) $t\text{BuOCl}$ then Et_3N (d) ClCO_2Et , pyridine (e) TBAF (f) 1,2-dibromoethyl ethyl ether, N,N -dimethylaniline (g) $n\text{Bu}_3\text{SnH}$, AIBN (h) i) DBU / toluene ii) Jones' ox (i) $\text{BH}_3\text{-DMS}$ (j) TBSI , imidazole (k) TsCl , pyridine (l) i) H_2 / Pd-C ii) TBAF (m) Ar_2CuLi then CH_2N_2 (n) PDC / DMF then CH_2N_2 (o) Ba(OH)_2

(Boc₂O), 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BocON) and benzyl chloroformate (ZCl) failed, but the reaction with acetylchloride or ethyl chloroformate was successful to produce the protected dehydro amino acid.² For further manipulation, ethylcarbamate was chosen as a protective group of the amino group. The *tert*-butyldimethylsilyl (TBS) group of **21** was removed with tetrabutylammonium fluoride (TBAF) and the resulting hydroxy group was converted to the acetal with 1,2-dibromoethyl ethyl ether using N,N-dimethylaniline as a base.

The intramolecular radical cyclization reaction smoothly proceeded under Stork's conditions³ (with tributyltinhydride and 2,2'-azobis(2-methylpropionitrile) (AIBN) in refluxing benzene) to afford cyclized products in quantitative yield. Since the cyclized product **24** consisted of four inseparable stereoisomers, the mixture was treated with DBU in refluxing toluene to epimerize the C2- α -carboxyl group to β - and then oxidized to the lactone by Jones' oxidation. When the order of operation was reversed, that is, oxidation first and then DBU treatment was undertaken, the yield was remarkably decreased (~20%). It was found that the stereoselectivity at C3 in the radical cyclization was complete and the product was a mixture of two compounds with about a 1:4 ratio, which was different in C2 stereochemistry.

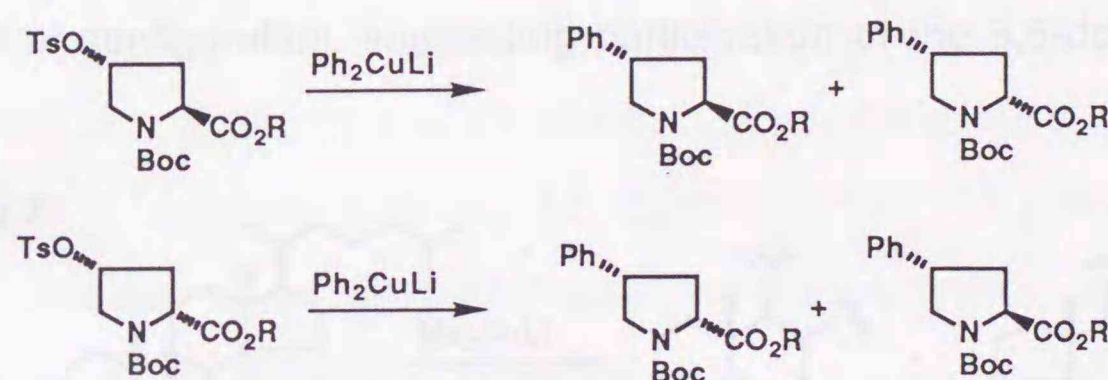
Reduction of the lactone is known to occur faster than the ester group with sodium borohydride, but the reduction of lactone **25** under these conditions resulted in almost intactness in ethanol or a low yield in the mixture of ethanol and methanol. Reduction with other reagents, such as the borane tetrahydrofuran complex, sodium trimethoxyborohydride, or sodium tri(*tert*-butoxy)borohydride resulted in intactness or reduction of both carbonyls. The α -amino ester is known to be easily reduced to an alcohol with weak reducing reagents through the coordination of the reagent with nitrogen. The only successful conditions were the use of borane dimethylsulfide

complex (three equivalents) in THF, dropwise addition of the reagent at 0°C and gradual elevation to room temperature.

The primary hydroxyl group of **26** was protected as a silyl ether and the secondary one was tosylated. Tosylation of **27** using DMAP as a catalyst produced a chlorinated product instead of the tosylate, but using excess tosyl chloride in concentrated pyridine afforded the desired product. The next substitution reaction is the crucial step in this synthesis.

Thottathil reported that the reaction of *cis*- and *trans*-hydroxyprolines with phenylcuprate proceeds with retention of configuration at C4 (Fig. 5).⁴

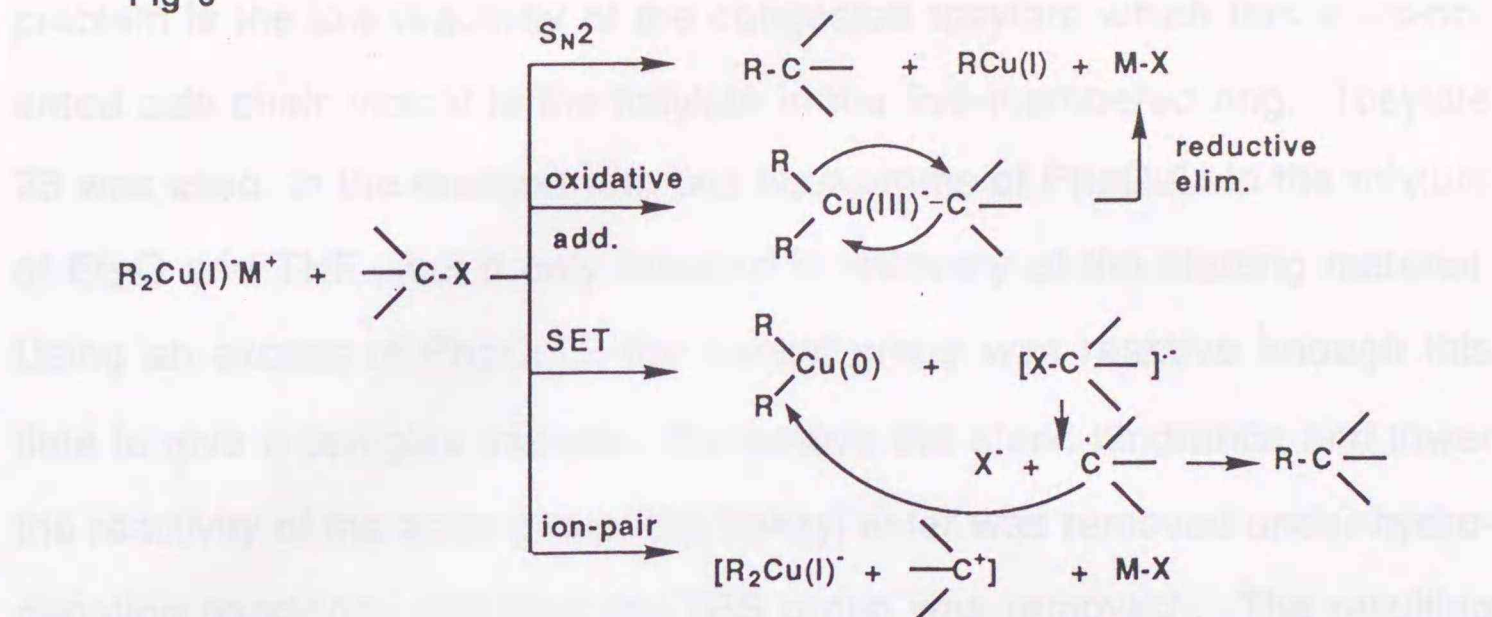
Fig 5



The mechanisms of the alkylhalide and tosylate substitution reaction with organocuprate is complicated and the reaction pathway is affected by the nature of the leaving group, the hybridization of the carbon and other functional groups in the substrate.⁵ In addition, the organocuprate varies as a function of solvent, mode of preparation, and presence of additives. The proposed mechanisms for the substitution reaction involves (1) direct substitution by R through an S_N2 process, (2) attack by the cuprate to form a transient Cu(III) intermediate, followed by reductive elimination, (3) a single electron transfer process, and (4) formation of ion-pair species (cuprate(I)-cation complex) (Fig. 6).

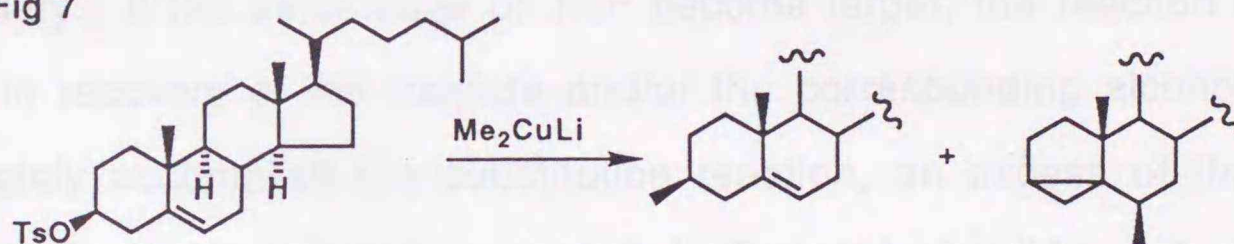
The former two processes proceed stereospecifically with inversion of configuration, the third gives the products with random stereochemistry and in the fourth process, the results vary by the function of the proximity. For

Fig 6



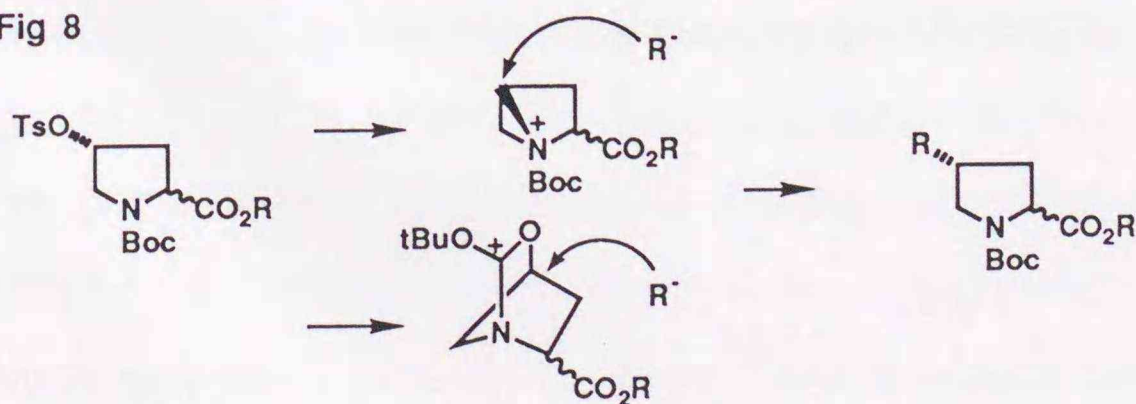
example, steroidal tosylate of cholesterol reacts with $(CH_3)_2CuLi$ with retention of configuration, suggesting participation of the 5,6-double bond (fig. 7).⁶

Fig 7



In the case of hydroxy proline, there are three possible candidates for the participating group: the carboxyl, amino and Boc groups. Among them, the carboxyl group was easily removed as the stereochemistry of C4 is always retained irrespective of the C2-carboxyl configuration. Two other possibilities have remained unsolved, that is, the aziridine route and carbamate participation (Fig. 8). Further investigations into this problem are now in progress.

Fig 8



For the synthesis of the kainoid using this substitution reaction, one problem is the low reactivity of the congested tosylate which has a *cis*-oriented side chain vicinal to the tosylate in the five-membered ring. Tosylate **28** was used in the reaction with two equivalents of Ph_2CuLi in the mixture of Et_2O and THF, but it only resulted in recovery of the starting material. Using an excess of Ph_2CuLi , the benzyl group was reactive enough this time to give a complex mixture. To remove the steric hindrance and lower the reactivity of the ester group, the benzyl ester was removed under hydrogenation conditions and then the TBS group was removed. The resulting tosylate **29** was exposed to the same reaction to afford the desired compound. Et_2O was a suitable solvent for this reaction, but, because of the low solubility of the tosylate **29**, addition of a small amount of THF was necessary. If the percentage of THF become larger, the reaction would result in recovery of the tosylate and/or the corresponding alcohol. To completely accomplish the substitution reaction, an excess of the aryl cuprate (12~16 equivalents) was needed. By employing this method, four aromatic groups, phenyl, *o*-anisyl, *p*-anisyl and *o,p*-dimethoxyphenyl, were introduced to the common skeleton of the kainoid. Oxidation of the primary alcohol to a carboxylic acid followed by hydrolysis of the esters and usual deprotection of the carbamate afforded new members of the kainoid, **38~41**.⁷

Experimental

General

Melting points are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO IR-S spectrometer on NaCl cell. ^1H NMR spectra were recorded on a Hitachi R-250H (250MHz), a JEOL Model JMN-FX-400 (400MHz), and a JEOL GX-500 (500MHz) spectrometers. Chemical shifts were reported as δ values in ppm relative to TMS (tetramethylsilane) in CDCl_3 as an internal standard or relative to HOD (4.8ppm) in D_2O . Low and high resolution mass spectra were obtained on a JEOL Model JMS-DX 300 and a JMS-DX303 spectrometers. Silica gel (Merck 60) was used for column chromatography. For the purification of amino acids, Dowex 50Wx8 and Amberlite IRC-50 were used for the column chromatography, paper electrophoresis (PEP) was performed on Whatman 3MM filterpaper using a model 20-TR apparatus (MS-kiki Co. Ltd.) and separations were effected at pH4.6, pyridine-acetic acid- H_2O (3:3:994 by volume), 600V, 1.5h, and recrystallizations were performed from hot H_2O .

Benzyl (2*S*,4*R*)-1-tert-butyloxycarbonyl-4-hydroxylpyrrolidine-2-carboxylate (18)

To a solution of *L*-Hyp (*L*-4-hydroxyproline, 20 g, 153 mmol) in 1N sodium hydrogen carbonate (180 ml, 180 mmol) was added di-tert-butylcarbonate (Boc_2O , 36.6 g, 168 mmol) dissolved in 1,4-dioxane (180 ml) and the mixture was stirred at room temperature overnight. The solution was acidified to pH2 with 1N hydrochloric acid and was extracted with ethyl acetate (200 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated to afford carbamate. Resulting carboxylic acid was used without further purification.

To a solution of carboxylic acid in N,N-dimethylformamide (350 ml) was added triethylamine (106 ml, 762mmol) and benzylchloride (25 ml, 217 mmol) under argon and the mixture was stirred at room temperature overnight. The solution was poured into water and then extracted with ethyl acetate (200 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (20-40% ethyl acetate-hexane) to give benzyl ester (35.8 g, 73%) as a colorless oil. $[\alpha]_D -57.7^\circ$ (c 1.00, CHCl₃) ; EI-HR-MS, m/z 322.1647 (M)⁺ calcd for C₁₇H₂₄O₅N, 322.1654 ; IR (neat) 3440, 2980, 1750, 1690, 1410, 1260, 1180, 1155, 1085, 1050, 980, 890, 860, 750 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.35 (ca5H, s), 1.46 (ca4H, s), 2.07 (1H, ddd, J=4.9, 7.9, 12.8Hz), 2.20~2.37 (1H, m), 3.45 (ca0.5H, brd, J=11.5Hz), 3.56 (ca0.5H, brd, J=11.5Hz), 3.59 (ca0.5H, dd, J=4.3, 11.6Hz), 3.65 (ca0.5H, dd, J=4.3, 11.6Hz), 4.40~4.55 (2H, m), 5.06~5.30 (2H, m), 7.36 (5H, s).

Benzyl (2*S*,4*R*)-4-(tert-butyldimethylsilyl)oxy-1-tert-butyloxy-carbonylpyrrolidine-2-carboxylate (19)

To a solution of alcohol **18** (17.8 g, 55 mmol) and imidazole (24.0 g, 353mmol) in N,N-dimethylformamide (DMF, 120 ml) was added tert-butyldimethylsilylchloride (TBSCl, 17.0 g, 113mmol) in DMF (60 ml) at 0°C under argon and the mixture was stirred for two hours. The mixture was poured into water and extracted with ether (150 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. Crude product was purified by column chromatography on silica gel (0-20% ether-hexane) to afford silyl ether (20.5 g, 85%) as an oil. $[\alpha]_D -37.1^\circ$ (c 0.89, CHCl₃) ; EI-HR-MS, m/z 436.2502 [(M+H)]⁺ calcd for C₂₃H₃₈O₅SiN, 436.2520 ; IR (neat) 2920, 1750, 1700, 1455, 1395, 1250, 1175, 1150, 1115, 1090, 1015, 895, 835, 775 cm⁻¹ ; ¹HNMR (250MHz,

CDCl₃) 0.04 (ca4.5H, s), 0.10 (ca1.5H, s), 0.85 (ca6.7H, s), 0.92 (ca2.3H, s), 1.35 (ca5.4H, s), 1.46 (ca3.6H, s), 1.93~2.06 (1H, m), 2.11~2.25 (1H, m), 3.31 (ca0.5H, dd, J=3.1, 11.6Hz), 3.40 (ca0.5H, dd, J=3.1, 11.6Hz), 3.57 (ca0.5H, dd, J=4.9, 11.6Hz), 3.62 (ca0.5H, dd, J=4.9, 11.6Hz), 4.33~4.42 (ca1.5H, m), 4.48 (ca0.5H, t, J=7.3Hz), 5.07~5.29 (2H, m), 7.3~7.4 (5H, m).

Benzyl (4*R*)-4-[(*tert*-butyldimethylsilyl)oxy]-1-pyrroline-2-carboxylate (20)

To a solution of carbamate **19** (20.5 g, 47 mmol) in dichloromethane (100 ml) was added trifluoroacetic acid (TFA, 100ml) under argon and the mixture was stirred for 30 min at room temperature. The mixture was evaporated at about 30°C and the residue was poured into aqueous sodium carbonate with ice and then extracted with ethyl acetate (150 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. Resulting amine was used to the next step without further purification.

To a stirred solution of the amine (16.0 g) in ether (320 ml) was added *tert*-butylhypochlorite (4.7 ml, 42 mmol) at -30~-40°C under argon and the mixture was stirred for 30 min. To this solution was added triethylamine (6.1 ml, 44 mmol) and additional ether (100 ml) and then the mixture was stirred at room temperature overnight. Resulting precipitate was removed by filtration and the filtrate was evaporated to yield 1-pyrroline (14.5 g, 92%). This compound is easily aromatized by the usual silica gel column chromatography and thus used to the next step without purification. But purification by silica gel 60 extra pure (Merck 7754) would give good results for preparation of analytical sample. $[\alpha]_D^{25}$ 34.5° (c 1.50, CHCl₃) ; EI-HR-MS, *m/z* 333.1757 (M)⁺, calcd for C₁₈H₂₇O₃SiN 333.1761 ; IR (neat) 2920, 1725, 1625, 1555, 1500, 1460, 1410, 1250, 1215, 1110, 1070, 1025, 995, 910, 835, 780, 750 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) -0.02 (ca2H, s), 0.06,

(ca4H, s), 0.84 (ca6H, s), 0.86 (ca3H, s), 1.85 (ca0.5H, dd, $J=6.6$, 12.8Hz), 2.45 (ca0.5H, dd, $J=6.1$, 12.8Hz), 2.61 (ca0.5H, dd, $J=4.0$, 10.7Hz), 2.80~3.04 (ca0.5H, m), 3.60 (ca0.5H, s), 3.90 (ca0.5H, d, $J=17.7$ Hz), 4.08~4.24 (ca1.5H, m), 4.52~4.58 (ca0.5H, m), 5.00 (ca0.5H, d, $J=11.5$ Hz), 5.10 (ca0.5H, d, $J=11.5$ Hz), 5.21 (ca0.5H, d, $J=12.0$ Hz), 5.30 (1H, d, $J=12.0$ Hz), 7.3~7.5 (5H, m).

Benzyl (4*R*)-4-(tert-butyldimethylsilyl)oxy-1-ethoxycarbonyl-2-pyrroline-2-carboxylate (21)

To a solution of 1-pyrroline **20** (14.5 g, 44 mmol) in dichloromethane (140 ml) was added pyridine (15.6 ml, 193 mmol) and ethyl chloroformate (10.6 ml, 111 mmol) at -15°C under argon and the mixture was stirred for an hour at room temperature. The reaction was quenched with aqueous ammonium chloride and the mixture was poured into water and extracted with ether (150 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography (10-20% ether-hexane) to afford 2-pyrroline (12.5 g, 71%) as an oil. $[\alpha]_D -44.7^\circ$ (c 1.10, CHCl₃); EI-HR-MS, m/z 405.1953 (M)⁺ calcd for C₂₁H₃₁O₅SiN, 405.1971.; IR (neat) 2920, 1720, 1625, 1460, 1410, 1375, 1375, 1330, 1300, 1250, 1175, 1075, 910, 835, 780 cm⁻¹; ¹HNMR (250MHz, CDCl₃) 0.08 (6H, s), 0.88 (9H, s), 1.21 (3H, t, $J=7.0$ Hz), 3.75 (1H, dd, $J=3.4$, 12.2Hz), 3.96 (1H, dd, $J=7.9$, 12.2Hz), 4.13 (2H, q, $J=7.0$ Hz), 4.94 (1H, ddd, $J=3.1$, 3.4, 7.9Hz), 5.27 (2H, s), 5.67 (1H, d, $J=3.1$ Hz), 7.39 (5H, m).

Benzyl (4*R*)-1-ethoxycarbonyl-4-hydroxy-2-pyrroline-2-carboxylate (22)

To a solution of silyl ether **21** (8.5 g, 21 mmol) in tetrahydrofuran (THF, 85 ml) was added 1M solution of tetrabutylammonium fluoride (TBAF) in

THF (26 ml, 26 mmol) at 0°C under argon and the mixture was stirred for 30 min. The reaction was quenched with aqueous ammonium chloride and the mixture was poured into water and extracted with ethyl acetate (80 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography (20-40% ethyl acetate-hexane) to give alcohol (5.0 g, 82%) as an oil. $[\alpha]_D -59.3^\circ$ (c 1.19, CHCl_3) ; EI-HR-MS, m/z 291.1104 (M)⁺ calcd for $\text{C}_{15}\text{H}_{17}\text{O}_5\text{N}$, 291.1107 ; IR (neat) 3420, 2960, 1710, 1625, 1415, 1380, 1335, 1300, 1230, 1175, 1135, 1040, 955, 910, 830, 755 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.21 (3H, t, $J=7.0\text{Hz}$), 2.09 (1H, d, $J=7.9\text{Hz}$), 3.85 (1H, dd, $J=2.8, 13.1\text{Hz}$), 3.97 (1H, dd, $J=7.6, 13.1\text{Hz}$), 4.13 (2H, q, $J=7.0\text{Hz}$), 4.79~4.89 (1H, m), 5.27 (2H, s), 5.76 (1H, d, $J=3.1\text{Hz}$), 7.30~7.50 (5H, m).

Benzyl (4*R*)-1-ethoxycarbonyl-4-(2-bromo-1-ethoxy)ethoxy-2-pyrroline-2-carboxylate (23)

To a solution of alcohol **22** (5.0 g, 17 mmol) in dichloromethane (100 ml) was added *N,N*-dimethylaniline (5.1 ml, 40 mmol) and 1.3M 1,2-dibromoethyl ethyl ether in ether (29ml, 38 mmol, prepared from ethyl vinyl ether with bromine) at room temperature under argon and the mixture was stirred overnight. The reaction was quenched with aqueous ammonium chloride and poured into water and extracted with ether (100 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel to afford acetal (7.5 g, 99%) as an oil. EI-HR-MS, m/z 442.0872 [$(M+H)$]⁺ calcd for $\text{C}_{19}\text{H}_{25}\text{O}_6\text{NBr}$, 442.0865 ; IR (neat) 2960, 1740, 1710, 1625, 1455, 1415, 1380, 1335, 1300, 1230, 1175, 1130, 1025, 910, 755 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.21 (ca4H, t, $J=7.0\text{Hz}$), 1.23 (ca2H, t, $J=7.0\text{Hz}$), 3.27~3.41 (2H, m), 3.50~3.70 (2H, m), 3.94 ~4.00 (2H, m), 4.13 (2H, q,

$J=7.0\text{Hz}$), 4.74 (ca0.5H, t, $J=5.5\text{Hz}$), 4.77 (ca0.5H, t, $J=5.5\text{Hz}$), 4.81~4.88 (1H, m), 5.27 (2H, s), 5.75 (ca0.5H, d, $J=3.1\text{Hz}$), 5.76 (ca0.5H, d, $J=3.1\text{Hz}$), 7.30~7.50 (5H, m).

Benzyl (1*R*,5*R*)-7-ethoxy-3-ethoxycarbonyl-6-oxa-3-aza-bicyclo[3.3.0]octane-2-carboxylate (24)

To a stirred solution of acetal **23** (5.0 g, 12 mmol) and 2,2'-azobis(isobutyronitrile) (AIBN, 104 mg, 0.63mmol) in refluxing benzene (270 ml) was added a solution of tributyltinhydride (3.6 ml, 13 mmol) in benzene (27 ml) and the mixture was stirred for an hour. The cooled mixture was concentrated to about 50 ml in vacuo and was added a saturated solution of potassium fluoride (20 ml) and ether (50 ml) and stirred for about 10 min. Resulting precipitate was filtered off and the filtrate was evaporated. The crude product was purified by silica gel column chromatography (20-40% ethyl acetate-hexane) to afford cyclized product as a mixture of four stereoisomers (4.5 g, 100%, concerning C2 and C7) as an oil. EI-HR-MS, m/z 363.1696 (M)⁺ calcd for $C_{19}H_{25}O_6N$ 363.1682 ; IR (neat) 2960, 2750, 1705, 1420, 1380, 1340, 1170, 1115, 1055, 995, 895, 750 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) (a mixture of four isomers) 0.89~2.34 (9H, m), 2.98~4.30 (6H, m), 4.56~4.97 (2H, m), 5.15~5.35 (3H, m), 7.30~7.50 (5H, m).

Benzyl (1*R*,2*S*,5*R*)-3-ethoxycarbonyl-7-oxo-6-oxa-3-aza-bicyclo[3.3.0]octane-2-carboxylate (25)

To a solution of acetal **24** (5.0 g, 14 mmol) in toluene (60 ml) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 6.0 ml, 40 mmol) and then the mixture was stirred at refluxing temperature under argon overnight. The cooled mixture was poured into water and extracted with ethyl acetate (60 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in

acetone (100 ml) and to the solution of 8N Jones' reagent (20 ml, 160 mmol) was added at 0°C. The mixture was stirred at room temperature for 1.5 hours and then quenched with iso-propanol. The solution was neutralized with aqueous sodium hydrogen carbonate and filtered through celite. The filtrate was extracted with ethyl acetate (100 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (20-30-40% ethyl acetate-benzene) to give lactone (3.9 g, 85%) $[\alpha]_D -47.5^\circ$ (c 1.12, CHCl₃) ; EI-HR-MS, m/z 333.1189 (M)⁺ calcd for C₁₇H₁₉O₆N, 333.1212 ; IR (neat) 2980, 1780, 1745, 1700, 1415, 1380, 1340, 1290, 1250, 1170, 1115, 1065, 1045, 1015, 925, 740 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.10 (ca1.5H, t, J=6.7Hz), 1.28 (ca1.5H, t, J=6.7Hz), 2.65 (1H, dd, J=2.1, 18.0Hz), 2.93 (1H, dd, J=9.2, 18.0Hz), 3.15 (1H, m), 3.67~3.78 (1H, m), 3.98~4.22 (3H, m), 4.26 (ca0.5H, d, J=2.7Hz), 4.39 (ca0.5H, d, J=2.7Hz), 5.02 (1H, t, J=5.5Hz), 5.17 (1H, d, J=12.2Hz), 5.24 (1H, d, J=12.2Hz), 5.30~5.50 (5H, m) along with C2-epimer (460 mg, 10%).

Benzyl (2*S*,3*R*,4*R*)-1-ethoxycarbonyl-4-hydroxy-3-(2-hydroxy-ethyl)pyrrolidine-2-carboxylate (26)

To a solution of lactone **25** (15.0 g, 45 mmol) in tetrahydrofuran (THF, 300 ml) at 0°C was added dropwise borane dimethylsulfide complex (13.2 ml, 139 mmol) and the mixture was stirred at room temperature overnight. The reaction was quenched with 1N hydrochloric acid and the mixture was poured into water and then extracted with ethyl acetate (300 ml x 3). The combined extracts were washed with brine dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography (1-5% methanol-chloroform) to afford diol (14.2 g, 94%) as a white crystal. mp. 162° ; $[\alpha]_D -33.3^\circ$ (c 1.05, CHCl₃) ; FAB-HR-MS, m/z 338.1607 [(M+H)]⁺ calcd for C₁₇H₂₄O₆N 338.1604 ; IR (nujol)

3360, 3200, 2900, 1740, 1680, 1450, 1375, 1205, 1165, 1010, 885 cm^{-1} ; ^1H NMR (250MHz, CD_3OD) 1.03 (ca1.5H, t, $J=7.0\text{Hz}$), 1.24 (ca1.5H, t, $J=7.0\text{Hz}$), 1.70~1.99 (2H, m), 2.24~2.42 (1H, m), 3.50~3.65 (4H, m), 3.87~4.15 (3H, m), 4.26 (1H, brs), 5.14 (1H, D, $J=12.5\text{Hz}$), 5.15 (1H, d, $J=12.5\text{Hz}$), 7.30~7.50 (5H, m).

Benzyl (2*S*,3*R*,4*R*)-3-[2-(tert-butyldimethylsilyl)oxyethyl]-1-ethoxycarbonyl-4-hydroxypyrrolidine-2-carboxylate (27)

To a solution of diol **26** (10.0 g, 30 mmol) and imidazole (5.7g, 84 mmol) in *N,N*-dimethylformamide (DMF, 100 ml) under argon was added a solution of tert-butyldimethylsilylchloride (TBSCl, 5.3 g, 35 mmol) in DMF (50 ml) at 0°C and the mixture was stirred at 0°C ~room temperature overnight. The mixture was poured into water and extracted with ethyl acetate (200 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was chromatographed on silica gel (20-40% ethyl acetate-hexane) to yield silyl ether (8.7 g, 65%) as an oil. $[\alpha]_{\text{D}} -41.9^\circ$ (c 1.14, CHCl_3) ; FAB-HR-MS, m/z 452.2443 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_{23}\text{H}_{38}\text{O}_6\text{NSi}$ 452.2469 ; IR (neat) 3380, 2920, 1730, 1670, 1425, 1380, 1345, 1260, 1205, 1165, 1140, 1105, 1020, 995, 895, 775, 740 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 0.08 (6H, s), 0.90 (9H, s), 1.05 (ca1.5H, t, $J=7.3\text{Hz}$), 1.23 (ca1.5H, t, $J=7.3\text{Hz}$), 1.80~1.97 (2H, m), 2.14~2.30 (1H, m), 3.50~3.68 (2H, m), 3.70~3.86 (2H, m), 3.93~4.20 (3H, m), 4.32~4.37 (1H, m), 5.10~5.29 (2H, m), 7.33 (5H, brs).

Benzyl (2*R*,3*R*,4*R*)-3-[2-(tert-butyldimethylsilyl)oxyethyl]-1-ethoxycarbonyl-4-(4-toluenesulfonyloxy)pyrrolidine-2-carboxylate (28)

To a solution of alcohol **27** (7.5 g, 16.6 mmol) in pyridine (40 mmol) was added *p*-toluenesulfonyl chloride (TsCl, 15.7 g, 82.4 mmol, recrystallized

from benzene) and the mixture was stirred at room temperature overnight. The mixture was poured into water and extracted with ether (100 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica gel column chromatography (20-40% ethyl acetate-hexane) to give tosylate (8.6 g, 86%) as an oil. $[\alpha]_D^{25}$ 5.6° (c 1.01, CHCl₃) ; EI-HR-MS, m/z 605.2465 calcd for C₃₀H₄₃O₈NSiS 605.2479 ; IR (neat) 2920, 1700, 1595, 1415, 1340, 1250, 1205, 1175, 1090, 1000, 890, 835, 775, 745 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 0.00 (ca2H, s), 0.04 (ca4H, s), 0.87 (ca4.5H, s), 0.89 (ca4.5H, s), 1.06 (ca1.5H, t, J=7.0Hz), 1.24 (ca1.5H, t, J=7.3Hz), 1.70~1.86 (2H, m), 2.48 (3H, s), 2.54~2.75 (1H, m), 3.28~4.20 (7H, m), 5.02, (1H, brs), 5.12~5.30 (2H, m), 7.36 (5H, brs), 7.38 (2H, d, J=8.6Hz), 7.80 (2H, d, J=8.6Hz).

(2*R*,3*R*,4*R*)-1-ethyloxycarbonyl-3-(2-hydroxy)ethyl-4-(4-toluene-sulfonyloxy)pyrrolidine-2-carboxylic acid (29)

To a solution of benzyl ester **28** (9.0 g, 15 mmol) in methanol (180 ml) was added a suspension of 10 % Pd on charcoal (500 mg, 0.47 mmol) in water (10 ml). The degassed mixture was stirred under hydrogen atmosphere overnight. The suspension was filtered through celite and the filtrate was evaporated. The residue was dissolved in tetrahydrofuran (THF, 120 ml) and to this solution was added 1M tetrabutylammonium fluoride in THF (TBAF, 36 ml, 36 mmol) at 0°C under argon. The mixture was stirred for an hour at room temperature and then poured into aqueous sodium carbonate, washed with ether (100 ml x 2). The aqueous phase was acidified to pH2 with 1N hydrochloric acid and extracted with ethyl acetate (100 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated to give carboxylic acid as an amorphous solid (5.4 g, 82%). $[\alpha]_D^{25}$ 16.9° (c 1.10, CH₃OH) ; FAB-HR-MS, m/z 402.1241 [(M+H)]⁺ calcd for C₁₇H₂₄O₈NS 402.1223 ; IR (nujol) 3440,

2880, 1725, 1680, 1590, 1440, 1360, 1345, 1215, 1160, 1090, 1030, 975, 900, 825, 770, 750 cm^{-1} ; ^1H NMR (250MHz, CD_3OD) 1.19 (ca1.5H, t, $J=7.0\text{Hz}$), 1.20 (ca1.5H, t, $J=7.0\text{Hz}$), 1.68~1.90 (2H, m), 2.47 (3H, s), 2.44~2.62 (1H, m), 3.25~3.36 (1H, m), 3.43 (1H, t, $J=5.5\text{Hz}$), 3.51 (1H, dd, $J=2.8, 13.4\text{Hz}$), 3.68 (1H, d, $J=13.4\text{Hz}$), 3.90 (1H, d, $J=9.8\text{Hz}$), 4.00~4.16 (2H, m), 5.06 (1H, brs), 7.45 (2H, d, $J=7.9\text{Hz}$), 7.79 (2H, d, $J=7.9\text{Hz}$).

Preparation of aryl lithium

Phenyl lithium was purchased from Aldrich Co. and other aryl lithium reagents were prepared from corresponding aryl bromides with lithium metal.

o-Anisyl lithium

Dry ether (40 ml) and lithium metal (800 mg, 115 mmol, prewashed with ethanol) were placed in a three necked round-bottomed flask equipped with a stirrer, dropping funnel and reflux condenser. To this suspension was added a solution of 2-bromoanisole (6.2 ml, 50 mmol) in ether (10 ml) over an hour at 0°C under argon. After additional stirring for 30 min, the solution was transferred into a bottle which was tightly stoppered and kept in a refrigerator. The prepared solution was titrated by a solution of 1M sec-butanol in *m*-xylene to find that the concentration of aryl lithium was 0.85M.

p-Anisyl lithium

A solution of aryl lithium was prepared as the same manner as above, except for the amount of *p*-bromoanisole (6.3 ml, 50 mmol). Concentration of the prepared aryl lithium was 0.88M.

o,p-Dimethoxyphenyl lithium

A solution of aryl lithium was prepared as the same method as above, except for the amount of *o,p*-dimethoxyphenyl bromide (7.2 ml, 50 mmol). Concentration of the aryl lithium was 0.69M.

General procedure for substitution reaction of tosylate with aryl cuprate

To a suspension of copper(I) bromide dimethyl sulfide complex (4.31 g, 21 mmol, recrystallized from dimethylsulfide-hexane) in ether (33 ml) and tetrahydrofuran (THF, 11 ml) at 0°C under argon was added a solution of p-anisyl lithium (0.88M in ether, 48 ml, 42 mmol) and the mixture was stirred for 15 min. To the solution was added a solution of tosylate **29** (700 mg, 1.7 mmol) in THF (7 ml) and the mixture was stirred at room temperature overnight. To the cooled solution at 0°C was added an aqueous ammonium chloride and the solution was stirred till the color of the solution became blue. The mixture was poured into an aqueous solution of sodium carbonate and washed with ether (50 ml x 2). The aqueous layer was acidified to pH2 with 1N hydrochloric acid and extracted with ethyl acetate (100 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in methanol and to the solution ethereal diazomethane was added. After evaporation of the solvents the crude product was purified by silica gel column chromatography (20-40% ethyl acetate-benzene) to give ester (472 mg, 71%). Other aryl cuprates were also reacted with tosylate as the same manner as above except for the amount of aryl cuprates used in the reaction, diphenylcopper lithium (8 equivalent to tosylate), di(o-anisyl)copper lithium (18 equivalent) and di(o,p-dimethoxyphenyl)copper lithium (18 equivalent) to give corresponding substituted esters in 57, 58, 71% yield respectively.

Methyl (2S,3S,4S)-1-ethoxycarbonyl-3-(2-hydroxy)ethyl-4-phenylpyrrolidine-2-carboxylate (**30**)

$[\alpha]_D -41.2^\circ$ (c 0.95, CHCl₃) ; EI-HR-MS, m/z 321.1583 (M)⁺ calcd for C₁₇H₂₃O₅N 321.1576 ; IR (neat) 3460, 2960, 1755, 1705, 1435, 1390,

1355, 1270, 1210, 1180, 1135, 1060, 1025, 895, 760 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.22 (ca1.5H, t, $J=7.0\text{Hz}$), 1.28 (ca1.5H, t, $J=7.0\text{Hz}$), 1.37 (2H, q, $J=6.7\text{Hz}$), 2.70 (1H, ddd, $J=6.1, 12.2, 12.8\text{Hz}$), 3.59 (2H, t, $J=6.4\text{Hz}$), 3.76 (ca1.5H, s), 3.78 (ca1.5H, s), 3.50~4.10 (3H, m), 4.06~4.11 (3H, m), 7.10 (2H, d, $J=7.3\text{Hz}$), 7.21~7.36 (3H, m).

Methyl (2S,3S,4S)-1-ethoxycarbonyl-3-(2-hydroxy)ethyl-4-(2-methoxy)phenylpyrrolidine-2-carboxylate (31)

$[\alpha]_{\text{D}} -65.8^\circ$ (c 1.075, CHCl_3); EI-HR-MS, m/z 351.1689 (M^+) calcd for $\text{C}_{18}\text{H}_{25}\text{O}_6\text{N}$ 351.1682; IR (neat) 3460, 2960, 1750, 1695, 1500, 1470, 1430, 1385, 1350, 1245, 1205, 1175, 1125, 1055, 1025, 760 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.21 (ca1.5H, t, $J=7.3\text{Hz}$), 1.27 (ca1.5H, t, $J=7.3\text{Hz}$), 1.20~1.40 (2H, m), 2.74~2.88 (1H, m), 3.46~3.66 (2H, m), 3.76 (ca1.5H, s), 3.78 (ca1.5H, s), 3.79 (3H, s), 3.70~4.30 (6H, m), 6.87 (1H, t, $J=7.3\text{Hz}$), 6.91 (1H, d, $J=7.3\text{Hz}$), 7.03 (1H, d, $J=7.3\text{Hz}$), 7.22 (1H, t, $J=7.3\text{Hz}$).

Methyl (2S,3S,4S)-1-ethoxycarbonyl-3-(2-hydroxy)ethyl-4-(4-methoxy)phenylpyrrolidine-2-carboxylate (32)

$[\alpha]_{\text{D}} -43.9^\circ$ (c 1.60, CHCl_3); EI-HR-MS, m/z 351.1678 (M^+) calcd for $\text{C}_{18}\text{H}_{25}\text{O}_6\text{N}$ 351.1682; IR (neat) 3460, 2960, 1750, 1700, 1615, 1520, 1475, 1430, 1385, 1350, 1250, 1180, 1125, 1020, 825 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.21 (ca1.5H, t, $J=7.0\text{Hz}$), 1.27 (ca1.5H, t, $J=7.0\text{Hz}$), 1.37 (2H, q, $J=6.7\text{Hz}$), 2.58~2.73 (1H, m), 3.49~4.28 (8H, m), 3.75 (ca1.5H, s), 3.77 (ca4.5H, s), 6.83 (2H, d, $J=8.6\text{Hz}$), 7.01 (2H, d, $J=8.6\text{Hz}$).

Methyl (2S,3S,4S)-1-ethoxycarbonyl-3-(2-hydroxy)ethyl-4-(2,4-dimethoxy)phenylpyrrolidine-2-carboxylate (33)

$[\alpha]_{\text{D}} -66.6^\circ$ (c 1.575, CHCl_3); EI-HR-MS, m/z 381.1809 (M^+) calcd for $\text{C}_{19}\text{H}_{27}\text{O}_7\text{N}$ 381.1788; IR (neat) 3460, 2960, 1750, 1700, 1615, 1590,

1515, 1470, 1425, 1385, 1350, 1295, 1260, 1205, 1130, 1025, 920, 830, 750 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.21 (ca1.5H, t, $J=7.3\text{Hz}$), 1.28 (ca1.5Hz, t, $J=7.3\text{Hz}$), 1.32 (2H, q, $J=7.3\text{Hz}$), 2.68~2.83 (1H, m), 3.50~4.26 (8H, m), 3.76, 3.77, 3.78 (total 9H, s each), 6.42 (1H, d, $J=9.2\text{Hz}$), 6.44 (1H, s), 6.93 (1H, d, $J=9.2\text{Hz}$).

Oxidation of alcohols to carboxylic acids (preparation of diesters 34~37)

A solution of alcohol **31** (Ar = o-anisyl, 152 mg, 0.43 mmol) and pyridinium dichromate (488 mg, 1.3 mmol) in N,N-dimethyl formamide (3 ml) under argon was stirred at 45 °C overnight. The cooled mixture was acidified to pH2 with 1N hydrochloric acid and extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with water, brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in methanol and to this solution ethereal diazomethane was added. After evaporation, resulting product was chromatographed on silica gel (20-40% ethyl acetate-hexane) to afford diester (100 mg, 61%). Other derivatives (Ar = phenyl, p-anisyl, o,p-dimethoxyphenyl) were also reacted with PDC in the same manner as above to give corresponding diester in 60~70% yield.

Methyl (2S,3S,4S)-1-ethoxycarbonyl-2-methoxycarbonyl-4-phenylpyrrolidine-3-acetate (34)

$[\alpha]_D -8.1^\circ$ (c 1.25, CHCl_3); EI-HR-MS, m/z 349.1550 (M^+) calcd for $\text{C}_{18}\text{H}_{23}\text{O}_6\text{N}$ 349.1526; IR (neat) 2960, 1750, 1715, 1425, 1390, 1355, 1270, 1210, 1180, 1135, 1030, 785, 710 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.22 (ca1.5H, t, $J=7.0\text{Hz}$), 1.29 (ca1.5H, t, $J=7.0\text{Hz}$), 2.04 (ca0.5H, dd, $J=7.3, 16.5\text{Hz}$), 2.06 (ca0.5H, dd, $J=7.3, 16.5\text{Hz}$), 2.24 (ca0.5H, dd, $J=7.9, 16.5\text{Hz}$), 2.28 (ca0.5H, dd, $J=7.9, 16.5\text{Hz}$), 2.97~3.10 (1H, m), 3.60 (ca1.5H, s), 3.61

(ca1.5H, s), 3.77 (ca1.5H, s), 3.78 (ca1.5H, s), 3.6~4.30 (6H, m), 7.06 (2H, d, J=7.3Hz), 7.22~7.36 (3H, m).

Methyl (2S,3S,4S)-1-ethoxycarbonyl-2-methoxycarbonyl-4-(2-methoxy)phenylpyrrolidine-3-acetate (35)

$[\alpha]_D$ -66.8° (c 0.90, CHCl₃) ; EI-HR-MS, m/z 379.1613 (M)⁺ calcd for C₁₉H₂₅O₇N 379.1631 ; IR (neat) 2960, 1750, 1715, 1610, 1505, 1475, 1425, 1390, 1355, 1250, 1205, 1180, 1135, 1030, 765 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.21 (ca1.5H, t, J=7.3Hz), 1.29 (ca1.5H, t, J=7.3Hz), 2.01~2.25 (2H, m), 3.14~3.27 (1H, m), 3.58 (ca1.5H, s), 3.60 (ca1.5H, s), 3.76 (ca4.5H, s), 3.78 (ca1.5H, s), 3.75~4.26 (6H, m), 6.86 (1H, t, J=7.3Hz), 6.91 (1H, d, J=7.3Hz), 7.01 (1H, dd, J=1.8, 7.3Hz), 1.23 (1H, dt, J=1.8, 7.3Hz).

Methyl (2S,3S,4S)-1-ethyloxycarbonyl-2-methoxycarbonyl-4-(4-methoxy)phenylpyrrolidine-3-acetate (36)

$[\alpha]_D$ 4.0° (c 1.50, CHCl₃) ; EI-HR-MS, m/z 379.1632 (M)⁺ calcd for C₁₉H₂₅O₇N 379.1631 ; IR (neat) 2960, 1750, 1715, 1620, 1590, 1525, 1475, 1425, 1390, 1355, 1255, 1210, 1185, 1135, 1035, 895, 840, 780 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.21 (ca1.5H, t, J=7.3Hz), 1.28 (ca1.5H, t, J=7.3Hz), 2.03 (ca0.5H, dd, J=7.3, 16.5Hz), 2.04 (ca0.5H, dd, J=7.3, 16.5Hz), 2.24 (ca0.5H, dd, J=8.3, 16.5Hz), 2.27 (ca0.5H, dd, J=8.3, 16.5Hz), 2.91~3.05 (1H, m), 3.60 (ca1.5H, s), 3.62 (ca1.5H, s), 3.60~4.30 (6H, m), 3.76 (ca1.5H, s), 3.78 (ca4.5H, s), 6.83 (2H, d, J=8.9Hz), 6.97 (2H, d, J=8.9Hz).

Methyl (2S,3S,4S)-1-ethyloxycarbonyl-2-methoxycarbonyl-4-(2,4-dimethoxy)phenylpyrrolidine-3-acetate (37)

mp. 123~125° ; $[\alpha]_D$ -48.4° (c 1.10, CHCl₃) ; EI-HR-MS, m/z 409.1760 (M)⁺ calcd for C₂₀H₂₇O₈N, 409.1737 ; IR (nujol) 2940, 1750, 1715, 1620, 1590, 1520, 1460, 1390, 1350, 1270, 1215, 1165, 1140, 1035, 920, 850, 820, 780 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.21 (ca1.5H, t, J=7.3Hz), 1.29 (ca1.5H, t, J=7.3Hz), 2.00~2.24 (2H, m), 3.07~3.22 (1H, m), 3.58 (ca1.5H, s), 3.60 (ca1.5H, s), 3.73 (3H, s), 3.76 (ca1.5H, s), 3.77 (ca1.5H, s), 3.78 (3H, s), 3.70~4.30 (6H, m), 6.40~6.46 (2H, m), 6.90 (1H, dd, J=2.4, 9.2Hz).

Deprotection of diesters, 34~37

To a solution of diester **34** (Ar = phenyl, 42 mg, 0.12 mmol) in ethanol (1.0 ml) was added water (1.0 ml) and barium hydroxide octahydrate (226 mg, 0.72 mmol) and the mixture was stirred under refluxing overnight. The cooled solution was neutralized with 1N hydrochloric acid and passed through Dowex 50Wx8 (H⁺ form, water-5% ammonium hydroxide) to afford an amino acid as an ammonium salt. The salt was then passed through Amberlite IRC-50 (H⁺ form, water) to give salt-free amino acid. This was further purified by recrystallization from water to give amino acid as colorless crystals. (30 mg, 60%). Other esters were also deprotected as the same manner as above to give corresponding amino acids in 40~60% yield.

(2S,3S,4S)-2-carboxyl-4-phenylpyrrolidineacetic acid (**38**)

mp. >310° (decomp) ; $[\alpha]_D$ 18.7° (c 0.075, H₂O) ; FAB-HR-MS, m/z 250.1058 [(M+H)]⁺ calcd for C₁₃H₁₆O₄N, 250.1079 ; IR (nujol) 3400, 2920, 2860, 1720, 1640, 1470, 1460, 1385, 1325, 1275, 1195, 885, 760 cm⁻¹ ; ¹HNMR (500MHz, D₂O, pD=3.8, HOD=4.8ppm) 2.24 (1H, dd, J=9.0, 17.1Hz), 2.57 (1H, dd, J=5.9, 17.4Hz), 3.24 (1H, quintet, J=6.4Hz), 3.77 (1H, dd, J=8.5, 11.0Hz), 3.96 (1H, q, J=7.8Hz), 3.99 (1H, dd, J=7.6, 11.0Hz), 4.13 (1H, d, J=6.4Hz), 7.30~7.33 (2H, m), 7.42~7.51 (3H, m).

(2S,3S,4S)-2-carboxy-4-(2-methoxy)phenylpyrrolidine-3-acetic acid (39)

mp. >310° (decomp) ; $[\alpha]_D$ 28.0° (c 0.025, H₂O) ; FAB-HR-MS, m/z 280.1209 [(M+H)]⁺ calcd for C₁₄H₁₈O₅N 280.1185 ; IR (nujol) 2920, 2860, 1720, 1620, 1470, 1460, 1380, 1250 cm⁻¹ ; ¹HNMR (500MHz, D₂O, pD=4.0, HOD=4.8ppm) 2.20 (1H, dd, J=10.0, 16.0Hz), 2.64 (1H, dd, J=4.9, 16.6Hz), 3.28 (1H, dddd, J=4.9, 7.3, 8.3, 10.0Hz), 3.081 (1H, dd, J=8.3, 12.2Hz), 3.89 (1H, dd, J=8.3, 12.2Hz), 3.91 (3H, s), 4.06 (1H, q, J=8.3Hz), 4.13 (1H, d, J=7.3Hz), 7.06 (1H, dt, J=1.0, 7.3Hz), 7.13 (1H, d, J=7.3Hz), 7.18 (1H, dd, J=1.5, 7.3Hz), 7.45 (1H, dt, J=1.5, 7.3Hz).

(2S,3S,4S)-2-carboxy-4-(4-methoxy)phenylpyrrolidine-3-acetic acid (40)

mp. 283~284° ; $[\alpha]_D$ 29.1° (c 0.055, H₂O) ; FAB-HR-MS, m/z 280.1179 [(M+H)]⁺ calcd for C₁₄H₁₈O₅N, 280.1185 ; IR (nujol) 3120, 2920, 2860, 1720, 1635, 1520, 1460, 1380, 1325, 1300, 1270, 1255, 1175, 120, 1035, 890, 820 cm⁻¹ ; ¹HNMR (500MHz, D₂O, pD=4.5, HOD=4.8ppm) 2.22 (1H, dd, J=9.3, 16.9Hz), 2.55 (1H, dd, J=5.9, 16.9Hz), 3.19 (1H, dddd, J=5.9, 7.3, 8, 9.3Hz), 3.72 (1H, dd, J=8.8, 11.2Hz), 3.89 (1H, q, J=8Hz), 3.90 (3H, s), 3.96 (1H, dd, J=8.0, 11.2Hz), 4.09 (1H, d, J=7.3Hz), 7.07 (2H, dd, J=2.0, 6.8Hz), 7.25 (2H, dd, J=2.0, 6.8Hz).

(2S,3S,4S)-2-carboxy-4-(2,4-dimethoxy)phenylpyrrolidine-3-acetic acid (41)

mp. 283~284° ; $[\alpha]_D$ 24.0° (c 0.125, H₂O) ; FAB-HR-MS, m/z 310.1270 [(M+H)]⁺ calcd for C₁₅H₂₀O₆N 310.1290 ; IR (nujol) 3120, 2920, 2860, 1730, 1620, 1520, 1470, 1385, 1330, 1300, 1255, 1215, 1190, 1130, 1035, 945, 880, 840 cm⁻¹ ; ¹HNMR (500MHz, D₂O, pD=4.2, HOD=4.8ppm) 2.22 (1H, dd, J=10.0, 16.8Hz), 2.64 (1H, dd, J=4.6, 16.8Hz), 3.23 (1H, dddd,

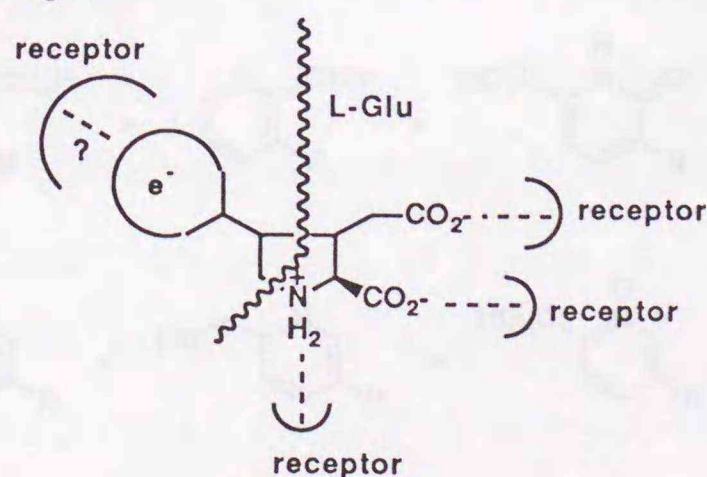
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Chapter 2. Structure-activity relationships of kainoids

In the mode of interaction between the transmitter and the receptors, the three-points-binding hypothesis¹ meets with general acceptance, that is, three polar groups, two carboxylate and an ammonium group, bind to receptors to cause depolarization. Glutamic acid freely changes its conformation in the solution state. There is an assumption. Each subtype of the glutamate receptor selectively takes a particular conformation of glutamic acid and its three polar groups interact with the appropriate receptor site. The aforementioned strong depolarization of the kainoid may be explained by this assumption, that is, the glutamic acid moiety of the kainoid is suitably constrained for the kainate type receptor to selectively bind. On the other hand, another interpretation would be possible. Kainoids are not endogenous compounds and have additional structures other than the glutamic acid moiety. This additional part may interact with the allosteric site of the receptor and produce abnormal excitation (Fig. 9). These two assumptions, due to a conformational change in the Glu-part and an additional substituent effect, have not been studied and a conclusive explanation still remains unclear. The solution of this problem could be to study the structure of the receptors and design a new agonist and antagonist.

Fig 9



In the case of the kainoid, the activity varies based on the structural change of the C4-substituents. Depolarizing activities of synthetic and natural kainoids were tested in the preparation of newborn rat spinal moto-

Chemical structures illustrating the retrosynthetic analysis of various substituted benzene and heterocyclic compounds, showing the conversion of complex molecules into simpler precursors like substituted benzenes, pyridines, and amino acids (L-Glu).

Chemical structures and their relative frequencies (from left to right):

- Structure 1: A substituted alkene with a carboxylic acid group (HO_2C) and a hydrogen atom (H) on the same carbon, and a substituent R on the other carbon.
- Structure 2: A substituted pyridine ring with a carboxylic acid group (CO_2H) and a substituent R .
- Structure 3: A substituted benzene ring with a methoxy group (OMe) and a substituent R .
- Structure 4: A substituted pyridine ring with a carboxylic acid group (HO_2C) and a substituent R .
- Structure 5: A substituted benzene ring with a hydroxyl group (OH) and a substituent R .
- Structure 6: A substituted alkene with a substituent R .
- Structure 7: A substituted pyridine ring with a carboxylic acid group (HO_2C) and a substituent R .
- Structure 8: A substituted pyridine ring with a hydroxymethyl group (HOCH_2) and a substituent R .
- Structure 9: A substituted pyridine ring with a carboxylic acid group (HO_2C) and a substituent R .
- Structure 10: A substituted benzene ring with a substituent R .
- Structure 11: $L\text{-Glu}$ (L-Glutamic acid).
- Structure 12: A substituted benzene ring with two methoxy groups (MeO) and a substituent R .
- Structure 13: A substituted alkene with a substituent R .
- Structure 14: A substituted pyridine ring with a substituent R .
- Structure 15: A substituted benzene ring with a methoxy group (MeO) and a substituent R .

neuron and dorsal-root fiber (See Figs. 10 and 11).⁴ These preparations show different sensitivities to agonists, which would be partly due to the different distribution of some receptor subtypes or a slightly different structural distinction of each subtype. The latter preparation shows a weak response to AMPA-type compounds, so it is useful to discriminate the kainate-type compound from the AMPA-type compound. In the following discussion, the activity in the former preparation was utilized.

The neuroexcitatory activity of the kainoid is considerably more potent than the real neurotransmitter, glutamic acid. For example, kainic acid is about 100 times more potent than glutamic acid. Among the newly synthesized compounds, MFPA (**39**) showed the strongest depolarization. It is about 10 times more potent than kainic acid.

To find the cause of the activity difference in the variety of C4-substituents, an ab initio calculation about the C4 substituents was performed using the model compounds whose kainoid skeleton was simplified to a methyl group. A Gaussian 80 program was employed using STO-3G as a basis set. Structure was not optimized and Pople's standard geometry⁵ was used. HOMO and LUMO energies and atomic orbital coefficient of HOMO of the nearest three carbons to C4 are shown in Tables 1 and 2, since kainic acid is the smallest molecule and has an isopropenyl group, which consists of three carbons calculation about the nearest three carbons are probably effective. Kainoid compounds are classified into two groups, a group of neutral compounds and that of ionized compounds in water solution and the data are arranged separately in two groups.

The order of neuroexcitatory potency is parallel to their HOMO energies except for *p*-anisyl compound **40**, *o,p*-dimethoxyphenyl compound **41**, and pyridine *N*-oxide (see Tables 1 and 2) in both groups. The former two compounds have low activities in spite of their higher HOMO energies.

Table 1 HOMO and LUMO orbital energy and atomic orbital coefficient of HOMO concerning several substituents at C-4 of kainoids-1 :undissociated fragments in aqueous solution

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basis set : STO-3G]

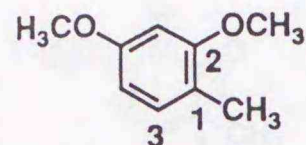
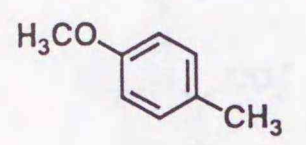
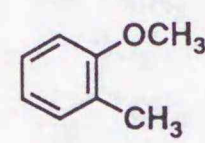
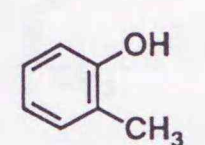
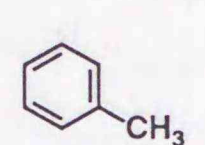
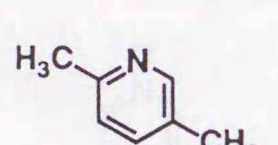
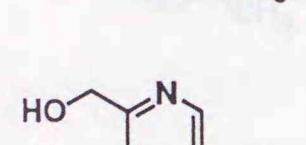
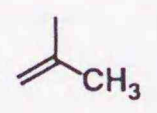
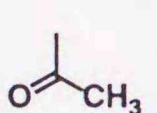
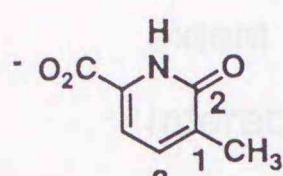
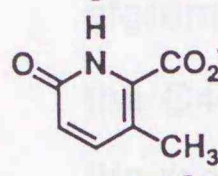
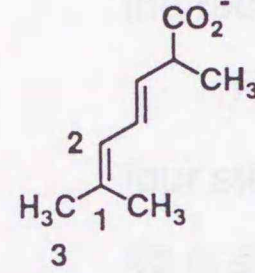
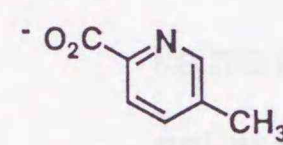
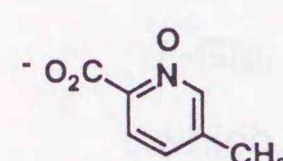
	orbital energy / hartree		atomic orbital coefficient of HOMO		
	HOMO	LUMO	C-1	C-2	C-3
	-0.22108	0.27256	0.50859	0.29100	0.10759
	-0.23337	0.26582	0.48565	0.18479	0.25725
	-0.23722	0.26682	0.40513	0.42123	-0.07631
	-0.23880	0.26421	0.39687	0.43465	-0.09934
	-0.26583	0.26870	0.51937	0.26596	0.30678
	-0.26957	0.25005	0.51924	0.38980	0.16232
	-0.27942	0.23745	0.50357	0.39334	0.14456
	-0.28992	0.32451	0.58069	0.65195	-0.10544
	-0.36423	0.27360	0.58289	-0.08472	0.67795

Table 2 HOMO and LUMO orbital energy and atomic orbital coefficient of HOMO concerning several substituents at C-4 of kalnoids-2 :dissociated fragments in aqueous solution

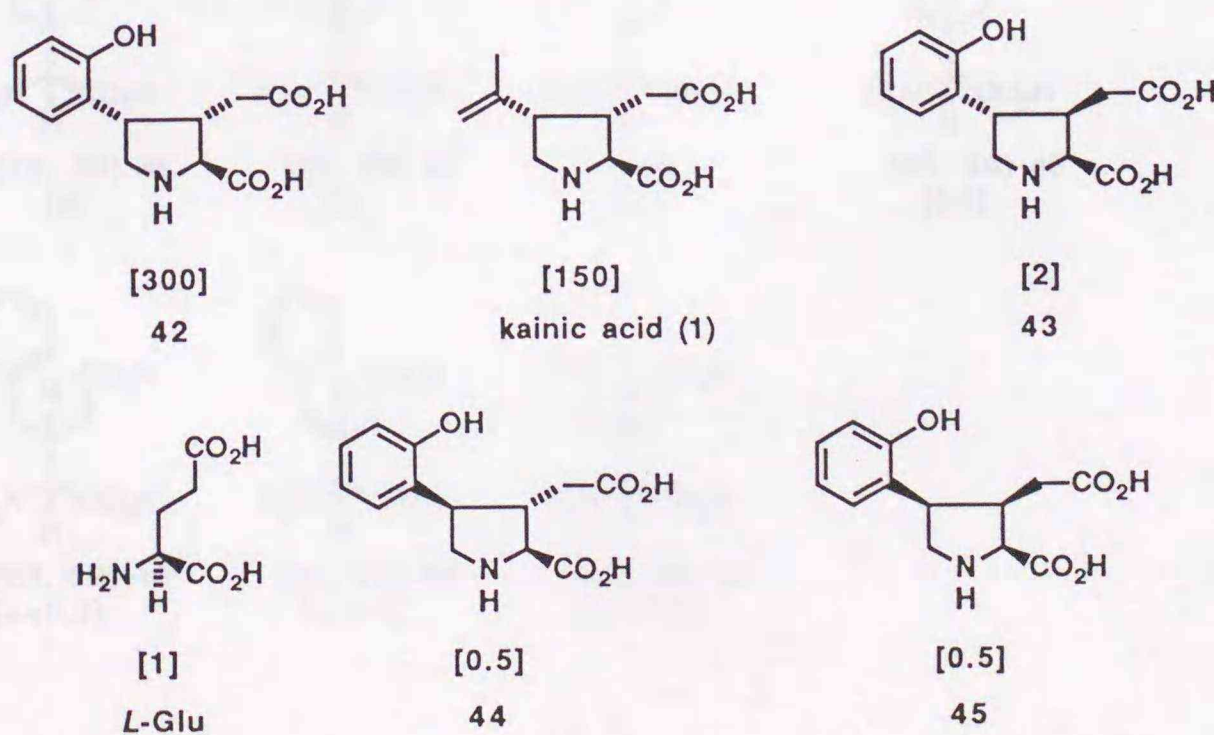
[Program : GAUSSIAN 80
basis set : STO-3G]

	orbital energy / hartree		atomic orbital coefficient of HOMO		
	HOMO	LUMO	C-1	C-2	C-3
	-0.03714	0.34879	0.53196	-0.00689	0.16898
	-0.04179	0.35837	0.49250	0.36046	-0.11447
	-0.10505	0.37862	0.49320	0.32832	-0.04927
	-0.10674	0.39200	0.49148	0.28339	0.11356
	-0.02625	0.37715	0.16116	-0.29464	0.36196

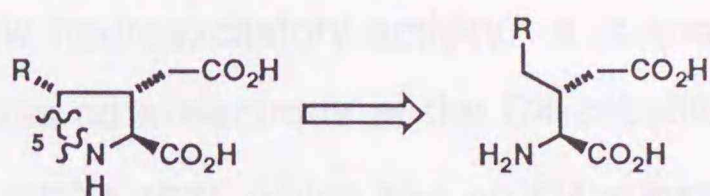
Probably the steric bulkiness around the *p*-position prevents the substrate from binding with receptors. Pyridine *N*-oxide also showed very low activity despite its higher HOMO energy. This may be explained by the small orbital coefficient of HOMO at C1 relative to those of the compounds which consist of the same number of atoms. The orbital coefficient roughly indicates the extent of orbital distribution and the small orbital expanse may limit the interaction. In this case, the next-HOMO energy complies with the aforementioned tendency. These considerations lead to the conclusion that the C4-substituent of the kainoid directly interacts with the allosteric site of the receptor to give electrons of the C4- π -group to the receptor.

The importance of the C4- π -group was also realized by synthesizing four stereoisomers of the phenol kainoid (Fig. 12).⁶ The excitatory activity of **42** is strongest among them, which has the same stereochemistry as that of kainic acid. One of the isomers, **43**, has the same stereochemistry at C4 but not at C3 with those of kainic acid and showed a slightly stronger effect than *L*-Glu. The other two isomers, **44** and **45**, exhibited very low activities which were weaker than *L*-Glu. These results indicated that the stereochemistry of the C4-group is more important than the conformation of the Glu part.

Fig 12 Relative depolarizing activity in the isolated newborn rat spinal motoneuron [L-Glu=1]

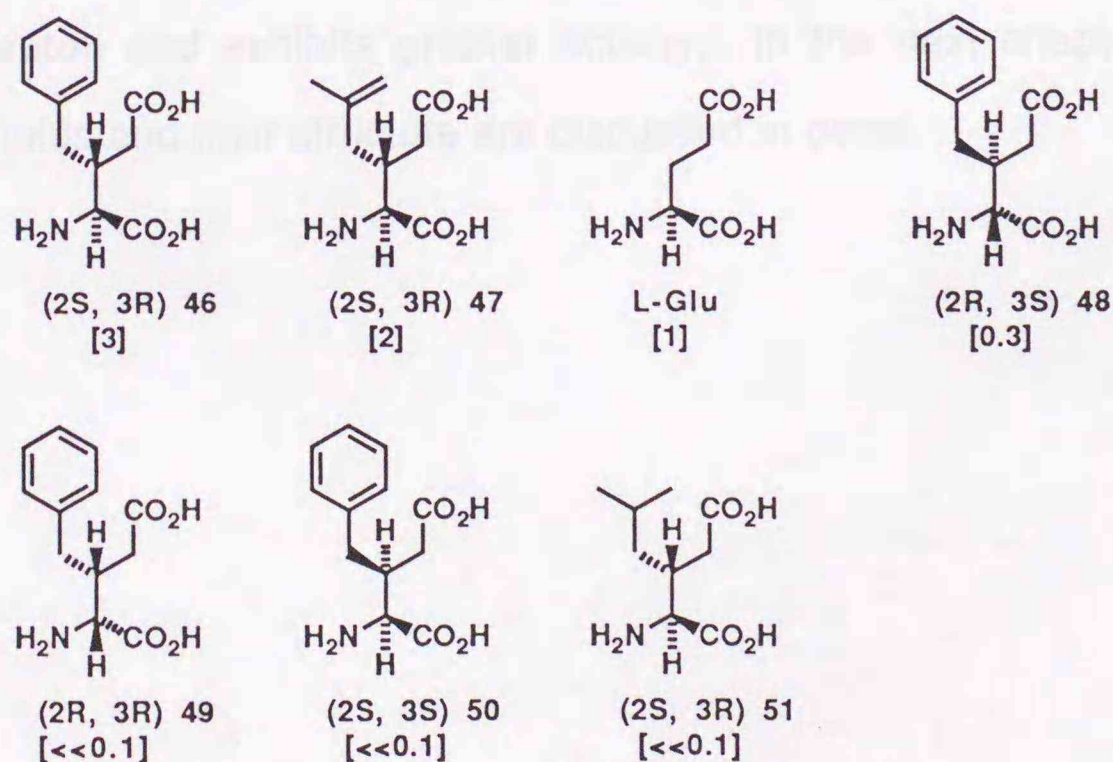


Another example is in the case of the acyclic kainoid.⁷ Four stereoisomers of β -benzylglutamic acid (**46**, **48~50**), (2*S*,3*R*)-3-isobutenyl- (**47**) and (2*S*,3*R*)-3-isobutylglutamic acids (**51**) were prepared. These compounds could be derived from the kainoid with removal of the C5-methylene group. Their depolarizing activities became much weaker than the kainoid,



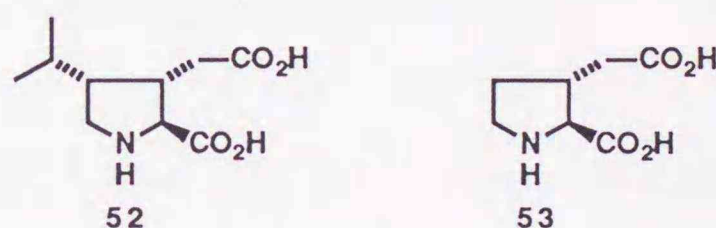
and (2*S*, 3*R*)-3-benzylglutamic acid (**46**) still kept its kainate-type selectivity. It increased depolarization in the preparation of the crayfish neuromuscular junction induced by glutamic acid and its activity was not affected by the NMDA antagonist, AP5. A decrease in the activity clearly indicates the importance of spatial arrangement of the phenyl group which corresponds to the π -group of the C4-substituent in the kainoid. The pyrrolidine ring is necessary to keep the proper conformation of the C4-substituent. The activity of the acyclic kainoid is shown in Fig. 13, and relative excitatory potencies are shown in parenthesis with L-Glu as 1. The isomers, **46** and **47**, which have

Fig 13 Relative potency ratio of depolarization in the isolated newborn rat spinal motoneuron [L-Glu=1]



the same stereochemistry with those of kainic acid, showed stronger activities than L-Glu. But **51**, which also has the same configuration as those of kainic acid, showed almost no activity.

These facts indicated that it was more important to have a π -group in the molecule than to keep the glutamic acid moiety in a suitable configuration so as to show neuroexcitatory activity. It is known that dihydrokainic acid (**52**), which has no π -electrons at the C4-substituent, exhibited almost no activity,⁸ and CPPA (**53**), which has no C4-substituent, does not show kainate-type selectivity any longer and binds to NMDA-type receptors.⁹ The existence, the nature and the conformation of the C4- π -group clearly plays a key role for subtype selectivity and potency of depolarization.



Concerning the conformations of the pyrrolidine ring in the kainoid, they are classified into two groups, kainic acid and domoic acid types based on ¹HNMR data. These differences in conformations seem to be due to the nature of the C4-substituent. The latter group shows a large *J*-value of the α -proton and exhibits greater activity. In the next chapter, ¹HNMR of kainoids and their structure are discussed in detail.

Experimental

Ab initio calculations were performed at Hokkaido University Computing Center.

The methods of the neurobiological test were described in the following references : refs. 4, 6, 7.

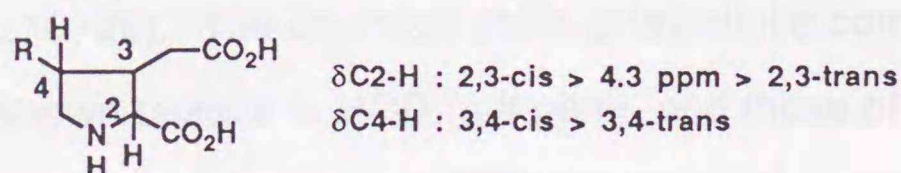
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Chapter 3. Stereochemical discrimination of kainoids by means of ^1H NMR spectrum

In the course of a structural study on acromelic acids, the relative stereochemistries were deduced by comparison with the ^1H NMR spectrum of other kainoids. Their stereochemistries were finally determined by total synthesis.¹ In these studies, our group reported an empirical rule for the structural determination of kainoids by ^1H NMR spectrum analysis, that is, the relative configuration between C2 and C3 would be determined by the chemical shift of C2-H. The C2-H appears upfield relative to 4.3 ppm when the compound has 2,3-trans stereochemistry; on the other hand, in the 2,3-cis compound, it appears downfield relative to 4.3 ppm (In the previous paper the border was set at 4.2 ppm, because the peak of HOD was read at 4.7 ppm, but it appears near 4.8 ppm relative to DSS).¹



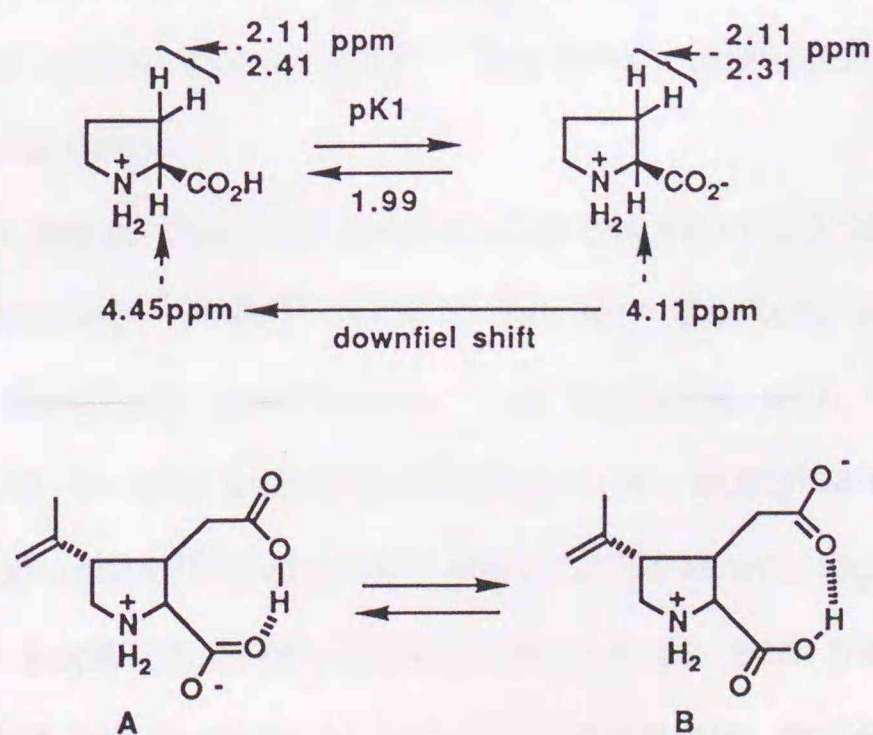
On the other hand, Goldberg *et al* reported a rule for the relation between configurations at C3 and C4 in ^1H NMR.² They pointed out that the chemical shift of C4-H of the 3,4-cis isomer appears downfield of that of the 3,4-trans isomer when comparing only the two pairs of synthetic isomers. No scope and limitation were provided for utilizing these rules. Because I have many kainoids, these rules were refined. Furthermore, by analyzing the ^1H NMR spectra of the kainoids, the conformation-activity relationship could be examined.

Acidic amino acids, e.g. glutamic acid, aspartic acid, and kainoids, form various types of ionized species in solution depending on the pH. The conformation of the acids varies with a change in the type of ionized species. Thus the chemical shifts and J-values vary as a function of pH.³ The avail-

able pH range for the rule was examined by measurement of the ^1H NMR of kainic acid and β -kainic acid at various pHs (Tables 3 and 4, Fig. 14). The chemical shift of each proton changes around the pKa; that is, the chemical shift of C2-H changes around the pK1 and pK3, C3-H and C4-H change around pK3, C5-H2 changes around the pK3, and C6-H2 changes around pK2. These changes are caused by the dissociation of the amino and carboxyl groups near each proton. On the other hand, the J-values of kainic acid changed little at various solution pHs. The pKa values of glutamic, kainic, β -kainic, and domoic acids are reported as in Fig. 15.⁴ Chemical shifts of the protons of interest, C2-H and C4-H, change around pH2 and pH10, and pH10 respectively. Thus the present rules can be applied in the range of pH2 to 10, but especially the 2,3-relationship should be determined with careful analysis at the borders of pH2 and 10. In this usable pH region, the ^1H NMR of various kainoids was investigated (Tables 5~17 and Figs. 16~26). The chemical shifts of synthetic compounds in this laboratory are shown relative to HOD (4.8 ppm), and those of other reported compounds are expressed relative to TSP (0.0 ppm; *) or not indicated (#). The pH values are indicated in parenthesis. For some of these data, the pH values are not indicated, but the purified compound is hardly soluble in water so that the pH of the resulting solution would be pH2~5, which is an appropriate pH region. First, the 2,3-relationship was checked in comparison with four possible stereoisomers in four groups, the kainic, ketokainic, dihydrokainic acids and phenol kainoid groups. Eleven derivatives in the former three groups were prepared from kainic acid according to the methods of Takemoto, and Nakamori *et al* (Fig. 27).^{4a,6} The former three groups all satisfy the rule, but the dihydrokainic acid group does not. Although the dihydrokainic acid group was ruled out, the C2-H of the 2,3-trans compounds always appear at a higher field than that of the 2,3-cis compounds. Thus the chemical shift of the sp^3 substituted

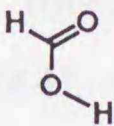
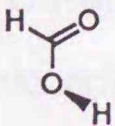
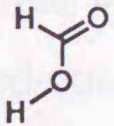
compounds at C4 should be compared with the two isomers. Other synthetic and reported compounds all satisfy this rule, except for isodomoic acids E and F, whose spectra were not measured under appropriate pH conditions. Regarding why the rule is valid for the 2,3-relationship, three explanations are offered. One is the anisotropy effect of single bonds, but this rule holds irrespectively of the stereochemistry of C4. Since the conformation of the pyrrolidine ring varies with the configurational change in the C4-substituent, the effective anisotropy of single bonds is also transformed. The validity of this rule irrespectively of the C4-stereochemistry does not suggest that the origin of the regularity of the C2-H chemical shift is the anisotropy of single bonds.

The next explanation involves the internal protonation of the C2-carboxylate. The chemical shift of the C2-H of proline moves downfield by protonation of the zwitter ion into the acid form. In the case of kainoid, the intramolecular γ -carboxylic acid protonates the α -carboxylate to produce an equilibrium of A and B. In the 2,3-cis compounds, the equilibrium is biased to B more than in the 2,3-trans compounds resulting in down field shift of C2-H. Thus, the C2-H of 2,3-cis compounds would always appear downfield of that of 2,3-trans compounds in the pH region between pK2 and pK3. The internal protonation may explain the origin of the rule.



The third explanation is the anisotropic effect of α -carboxylate. The zwitter ion would form a hydrogen bond between the ammonium and carboxylate groups. This hydrogen bond would be effectively formed when the proton on the nitrogen is in the plane of the carboxylate group. In the case of formic acid, the torsional energy is represented as in formula (1).

$$V = \frac{4.029}{2} (1 - \cos \alpha) + \frac{11.70}{2} (1 - \cos 2\alpha) \quad (1)$$

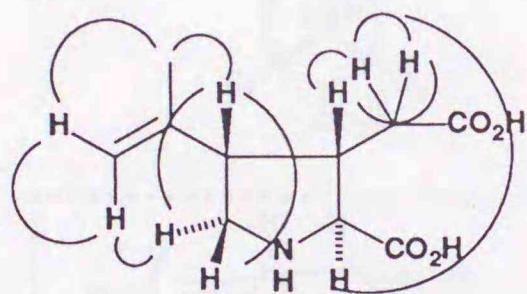
		
α (dihedral angle; O=C-O-H)		
0°	90°	180°
V (torsional energy; kcal/mol)		
0	13.7	4.0

This indicates that a stable hydrogen bond is effectively made when the proton is coplanar with the carboxylate group. In the 2,3-cis compounds, this hydrogen bond is disturbed to some extent by the steric hindrance of the vicinal carboxymethyl group resulting in a relatively small downfield shift of C2-H because of the anisotropic effect of the carbonyl group. On the other hand, the 2,3-trans compounds easily form this hydrogen bond without steric hindrance to restrict the dihedral angle, N-H-O-CO, to 0°, resulting in an upfield shift of C2-H. The third explanation is also usable for the origin of the rule.

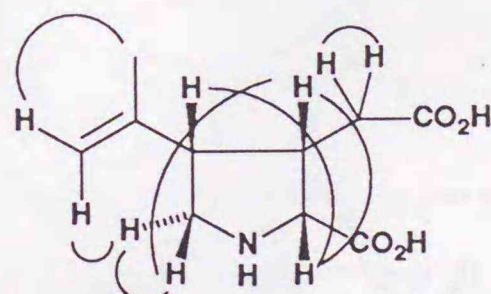
Next, the stereochemical relationship between C3 and C4 was examined by comparing ^1H NMR data of the four isomers of the four groups (Table 7). Goldberg synthesized four kainoids with the following C4-substituents in α - and β -configurations, i. e., acetyl and carboxylic acid groups. He compared the chemical shifts of C4-H with regard to two pairs of compounds, each of which contained 3,4-cis and trans isomers, and suggested that the protons of 3,4-cis compounds appeared downfield of

those of 3,4-trans compounds. This rule is satisfactory for synthetic kainic, ketokainic acids and phenol kainoids but not for the dihydrokainic acid group. This is due to the anisotropy of the C4-substituent with an sp^2 carbon. The kainic acid NOE experiments (Fig. 28) and X-ray crystallographic analysis⁷ show that the C4- π -group in the 3,4-cis compounds is almost perpendicular to the pyrrolidine ring. Thus the downfield shift of C4-H is probably induced. On the other hand in the 3,4-trans compounds, rotation of the C4-substituent has little effect on the chemical shift of C4-H. Dihydrokainic acids are ruled out since they do not have an sp^2 -group. Many examples in which the allylic strain controls the conformation of molecules are known. In the kainoids, the allylic strain and vicinal cis-substituted group must constrain the angle of the C4-substituent perpendicular to the pyrrolidine ring.

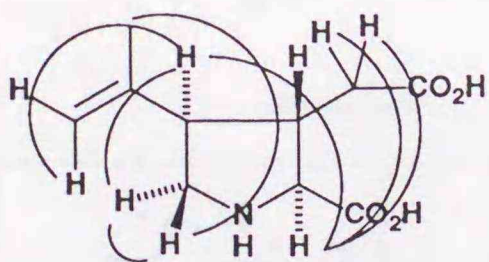
Fig 28



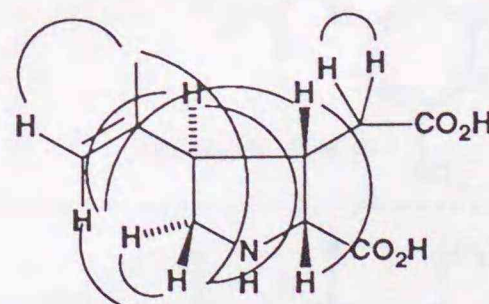
α -kainic acid (pD = 3.6)



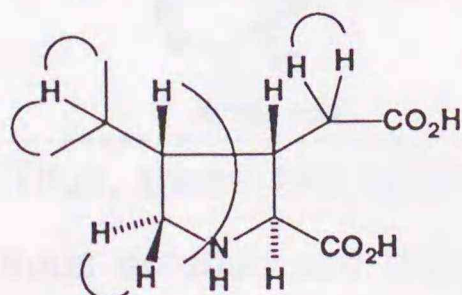
β -kainic acid (pD = 3.4)



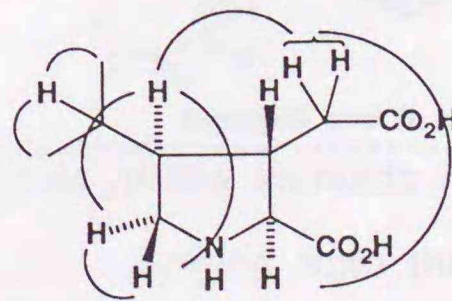
α -alkokainic acid (pD = 4.3)



β -alkokainic acid (pD = 4.5)

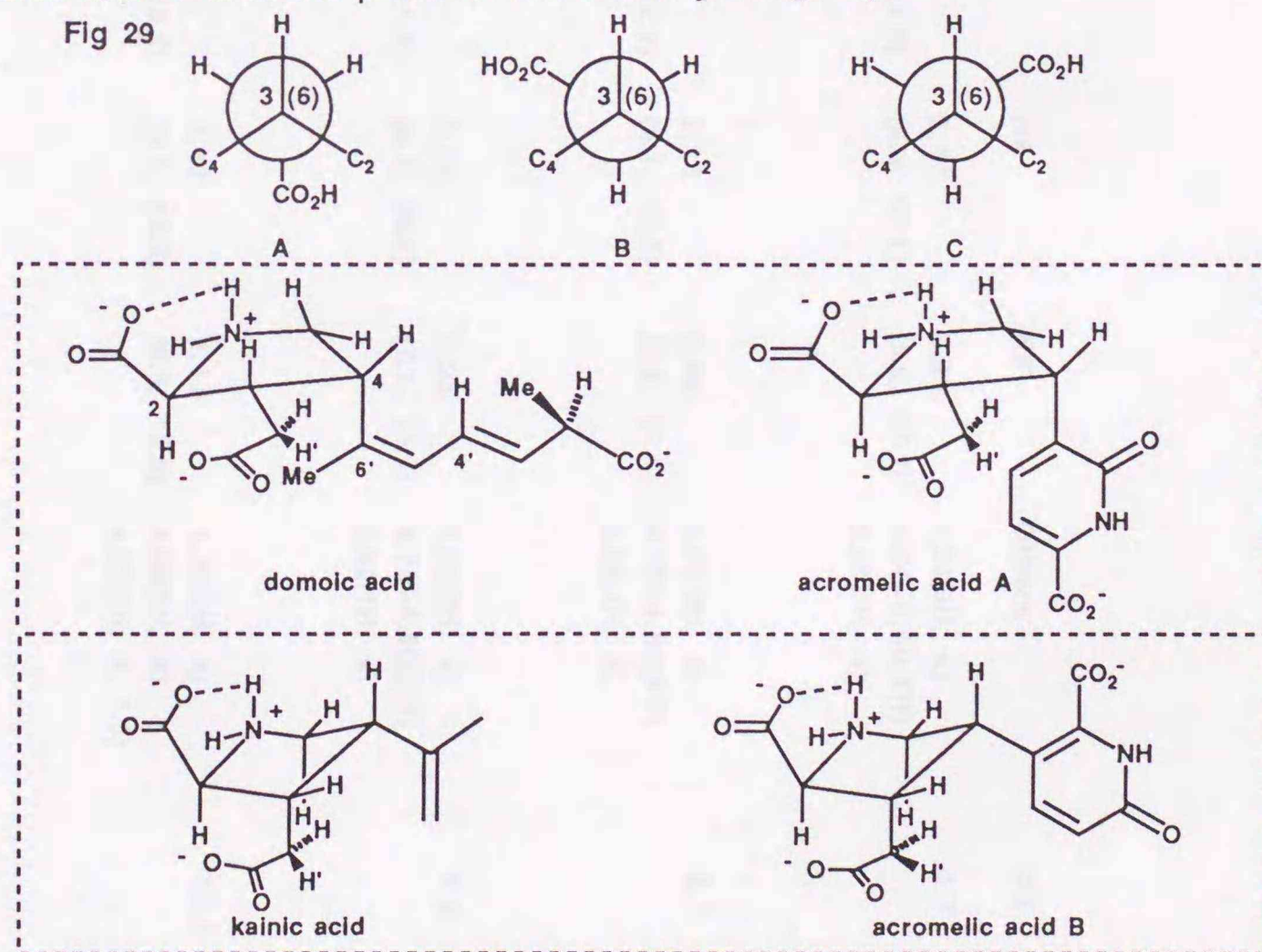


dihydro- α -kainic acid (pD = 4.2)



Dihydro- α -alkokainic acid (pD = 4.6)

^1H NMR analysis of kainoids indicated that their conformations were divided into two groups, the kainic acid and domoic acid groups. The latter group has a larger J-value of the C2-H, which means that C2-H and C3-H are nearly anti-coplanar, and a small $\Delta J = J_{4,5} - J_{4,5'}$ value which means that C4-H is located between the C5-methylene protons, that is, C4-H assumes an equatorial position. Further, the gauche rotamers around the C3-C6 bond are shown in Fig. 29. Considering the J-values between C3-H and C6-H₂, B and C would be satisfactory but in conformer B, the C4-substituent and acetic acid residue are so near as to cause serious steric hindrance and thus only conformer C is available. The angle of the carboxylate plane around the C6-CO₂H bond is suggested also from the chemical shifts of C6-H and H'. C6-H' always appears downfield with a small J-value, which indicates that H' is in the plane of the carboxylate group.



Thus, these two types of conformation of the kainoids in a neutral aqueous solution are depicted in Fig. 29. Judging from the biological activities of acromelic acids A and B which show comparative activities, these differences in their conformation little affect their activities.

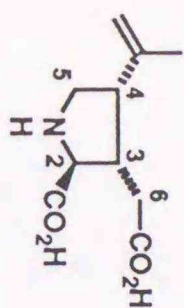


Table 3 ^1H NMR of kainic acid at various pHs

	H2	H3	H4	H5	H5'	H6	H6'	Others	pD
4.43 (3.9)	3.17 (3.9, 5.9, 7.3, 8.3)	3.11 (7.1, 7.3, 12.0)	3.47 (12.0, 12.0)	3.66 (7.1, 12.0)	2.47 (8.3, 17.1)	2.53 (5.9, 17.1)	1.74(3H, s) 4.70~4.80(1H) 5.06(1H, s)	0.7	
4.10 (3.4)	3.08 (3.4, 6.4, 7.3, 8.1)	3.02 (7.1, 7.3, 12.0)	3.44 (12.0, 12.0)	3.64 (7.1, 12.0)	2.36 (8.1, 16.6)	2.46 (6.4, 16.6)	1.77(3H, s) 4.70~4.80(1H) 5.05(1H, s)	3.7	
4.04 (3.4)	3.02 (3.4, 7.1, 7.3, 8.1)	2.97 (7.3, 7.3, 10.7)	3.42 (10.7, 11.7)	3.61 (7.3, 11.7)	2.13 (8.1, 15.4)	2.25 (7.1, 15.4)	1.77(3H, s) 4.70~4.80(1H) 5.02(1H, s)	7.5	
3.23 (5.4)	2.76 (5.4, 6.8, 7~8, 8.3)	2.59 (7.1, 7~8, 10.5)	2.80 (10.5, 10.5)	3.17 (7.1, 10.5)	2.12 (8.3, 15.6)	2.17 (6.8, 15.6)	1.71(3H, s) 4.68(1H, s) 4.87(1H, d, 1.5)	12.8	

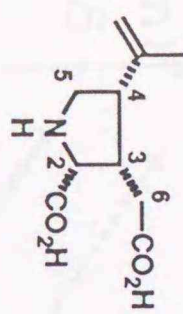


Table 4 ^1H NMR of β -kainic acid at various pHs

	H2	H3	H4	H5	H5'	H6	H6'	Others	pD
4.64 (6.1)	3.22 (4.9, 6, 6.1, 7.9)	3.40 (6, 8, 12)	3.50 (12, 12)	3.57 (8, 12)	2.22 (7.9, 17.1)	2.43 (4.9, 17.1)	1.77(3H, s) 5.08(2H, s)	0.9	
4.35 (6.7)	3.16 (5.8, 6, 6.7, 6.7)	3.30 (6, 8, 12)	3.45 (8, 12)	3.51 (12, 12)	2.22 (5.8, 17.1)	2.35 (6.7, 17.1)	1.78(3H, s) 5.08(2H, s)	3.3	
4.32 (6.1)	3.14 (3.7, 6, 6.1, 7.3)	3.24 (6, ?, ?)	3.42~3.54	3.42~3.54	2.04 (3.7, 16)	2.14 (7.3, 16)	1.80(3H, s) 5.01(2H, s)	6.2	
3.73 (5.5)	2.78~2.96 (3.1, 5.5, 9.8, ?)	2.78~2.96	3.12~3.24	3.12~3.24	1.89 (3.1, 15.9)	2.00 (9.8, 15.9)	1.77(3H, s) 4.7~5.0(2H)	12.1	

Fig 14 pD dependence of the chemical shifts of the protons of kainic and β -kainic acids

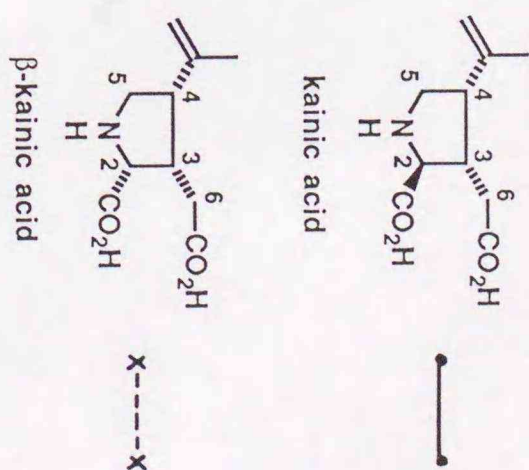
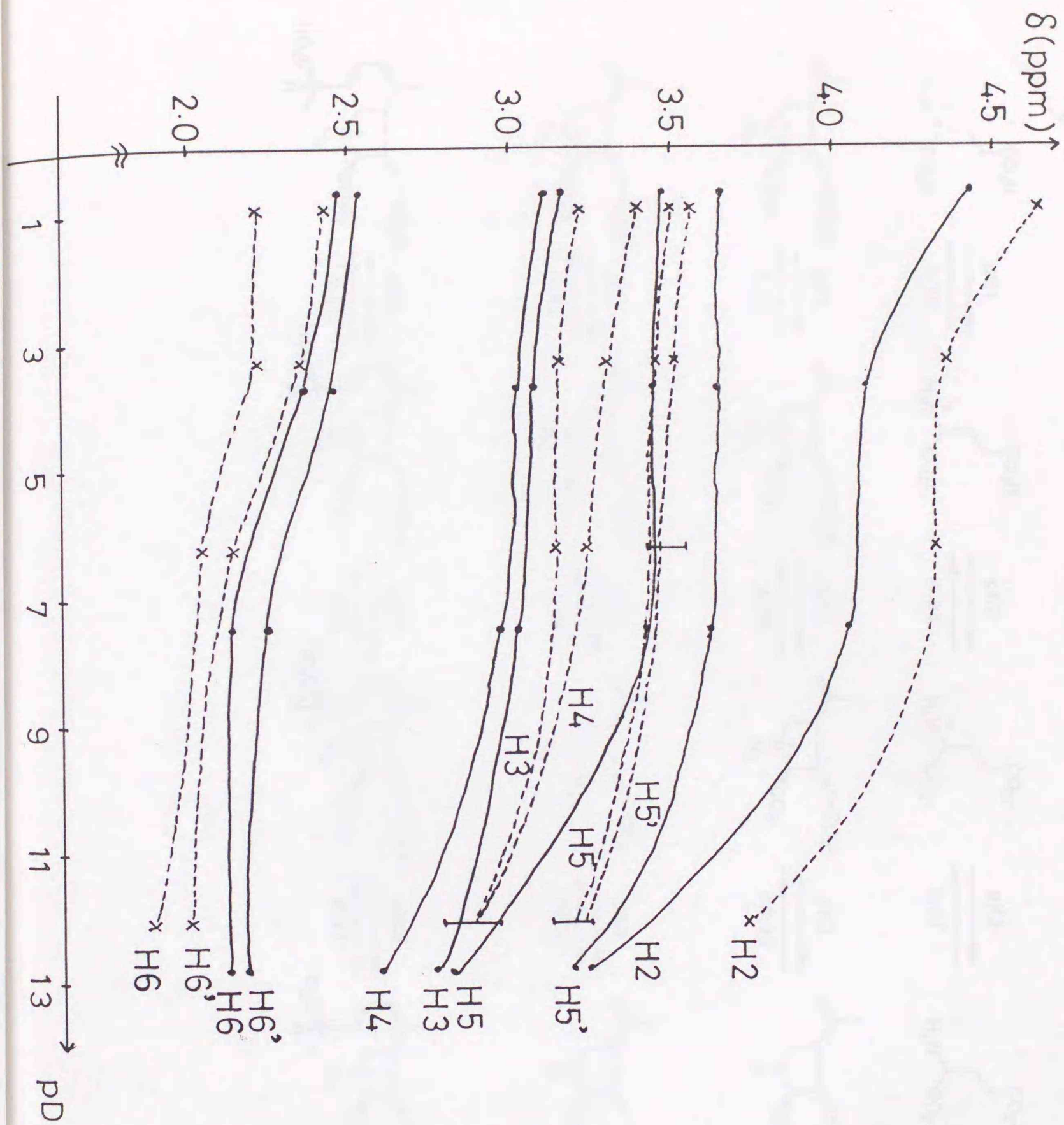


Fig 15

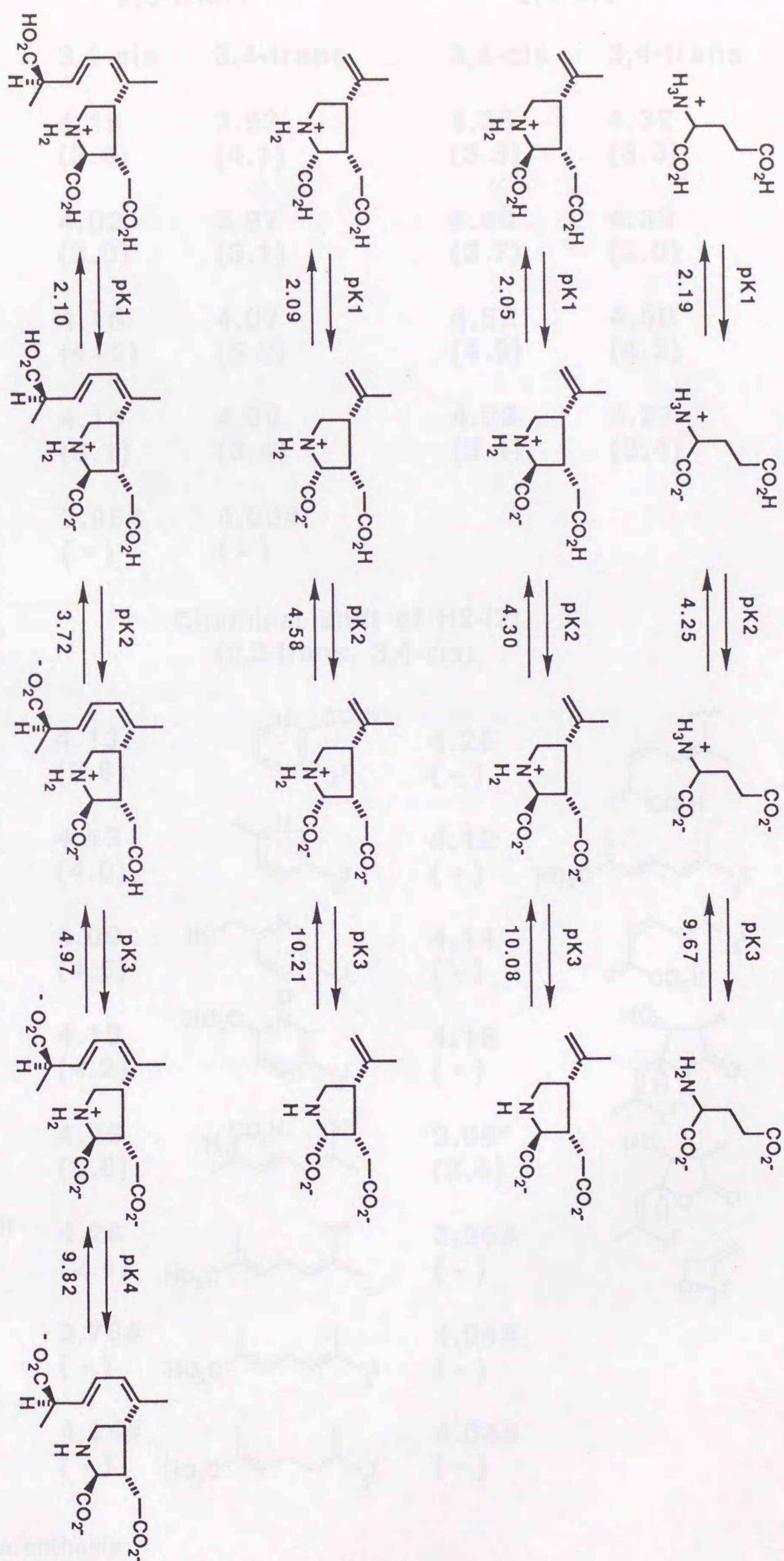


Table 5

Chemical shift of H2-(1)

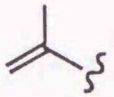
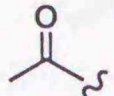
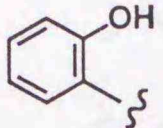
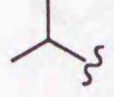
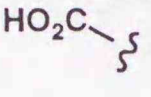
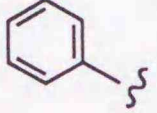
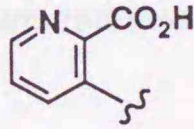
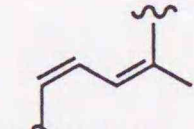
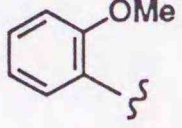
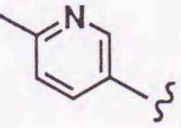
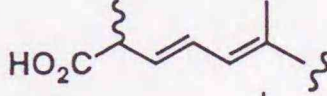
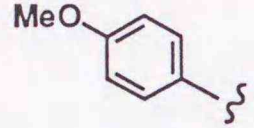
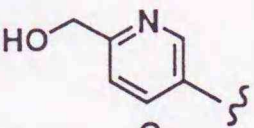
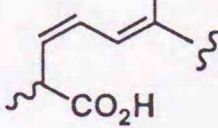
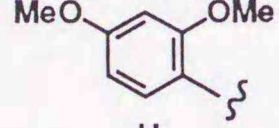
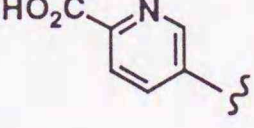
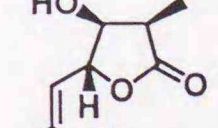
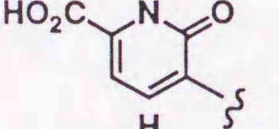
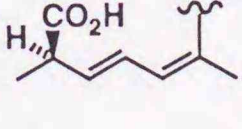
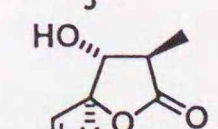
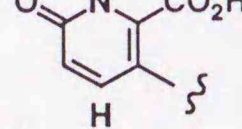
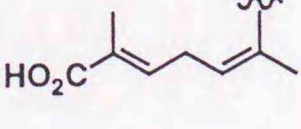
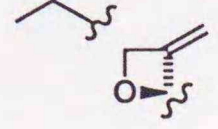
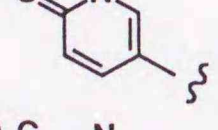
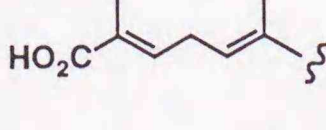
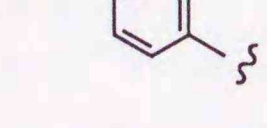
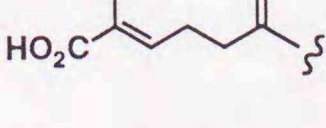
	2,3-trans		2,3-cis	
	3,4-cis	3,4-trans	3,4-cis	3,4-trans
	4.19 (3.4)	3.92 (4.1)	4.36 (3.3)	4.32 (3.3)
	4.02 (3.0)	3.97 (3.1)	4.40 (2.7)	4.33 (2.0)
	4.16 (4~5)	4.07 (5.2)	4.52 (4.9)	4.50 (4.3)
	4.14 (3.1)	4.00 (3.4)	4.23 (3.4)	4.27 (3.4)
	3.96# (-)	4.00# (-)		

Table 6

Chemical shift of H2-(2)
(2,3-trans, 3,4-cis)

	4.13 (3.8)		4.26 (-)		4.15* (1.9)
	4.13 (4.0)		4.12 (-)		4.36* (1.8)
	4.09 (4.5)		4.14 (-)		4.39* (1.6)
	4.10 (4.2)		4.18 (-)		3.97* (-)
	4.26 (3.0)		3.98* (3.4)		4.03* (-)
	4.26 (2.4)		3.96# (-)		3.96# (-)
	3.78# (-)		4.04# (-)		
	4.24# (-)		4.08# (-)		

pD is in parenthesis.

Chemical shift is shown relative to HOD (4.8 ppm) or
TSP (0.0 ppm ; *) or not indicated (#).

Table 7

Chemical shift of H4

	2,3-trans		2,3-cis	
	3,4-cis	3,4-trans	3,4-cis	3,4-trans
	3.52 (3.4)	2.88 (4.1)	3.31 (3.3)	2.74 (3.3)
	3.82 (3.0)	3.42 (3.1)	3.89 (2.7)	3.50 (2.0)
	3.93 (4~5)	3.6~3.8 (5.2)	4.13 (4.9)	3.52 (4.3)
	1.98 (3.1)	2.09 (3.4)	2.21 (3.4)	2.27 (3.4)
	3.48# (-)	3.17# (-)		

pD is in parenthesis.

Chemical shift is shown relative to HOD (4.8ppm)
or not indicated (#).

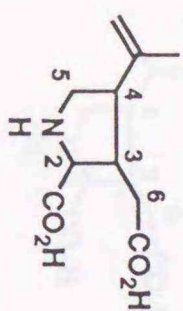
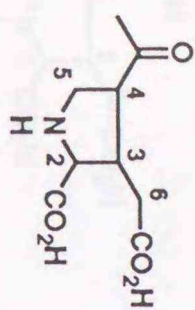


Table 8 Kainic acid derivatives

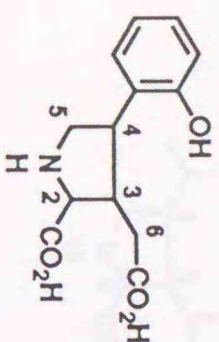
	H2	H3	H4	H5	H5'	H6	H6'	Others	pD
2,3-trans 3,4-trans (α-allo)	3.92 (9)	2.6 (4, 7, 9, 11)	2.88 (8, 11, 11)	3.32 (11, 11)	3.52 (8, 11)	2.59 (7, 15)	2.77 (4, 15)	1.72(3H, s) 4.98(2H, s)	4.1
2,3-trans 3,4-cis (α)	4.19 (3.4)	3.17 (3.4, 6.3, 7.0, 8.3)	3.11 (7.0, 7.3, 12.2)	3.52 (12.2, 12.2)	3.73 (7.3, 12.2)	2.47 (8.3, 16.6)	2.56 (6.3, 16.6)	1.85(3H, s) 4.85(1H, s) 5.14(1H, s)	3.4
2,3-cis 3,4-trans (β-allo)	4.32 (9)	2.87 (6, 9, 9, 10.5)	2.74 (8.5, 10.5, 10.5)	3.25 (10.5, 12)	3.65 (8.5, 12)	2.44 (9, 17)	2.53 (6, 17)	1.73(3H, s) 4.97(1H, s) 5.02(1H, s)	3.3
2,3-cis 3,4-cis (β)	4.36 (6)	3.17 (6, 6, 6, 7)	3.31 (6, 8, 12)	3.46 (12, 12)	3.52 (8, 12)	2.23 (6, 17)	2.35 (7, 17)	1.79(3H, s) 5.07(2H, s)	3.3

Table 9 Ketokainic acid derivatives



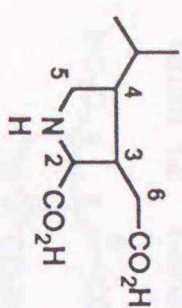
	H2	H3	H4	H5	H5'	H6	H6'	Others	PD
2,3-trans 3,4-trans (α-allo)	3.97 (7)	3.04 (5, 6, 7, 9)	3.42 (6, 6, 8)	3.62 (6, 13)	3.67 (8, 13)	2.73 (9, 17)	2.88 (5, 17)	2.30(3H, s)	3.1
2,3-trans 3,4-cis (α)	4.02 (6.5)	3.16 (6, 6.5, 6.5, 9.5)	3.82 (6.5, 6.5, 6.5)	3.57 (6.5, 11.5)	3.60 (6.5, 11.5)	2.54 (9.5, 17)	2.76 (6, 17)	2.31(3H, s)	3.0
2,3-cis 3,4-trans (β-allo)	4.33 (7.8)	3.20 (5.5, 6.9, 7.8, 8.1)	3.50 (5.5, 5.9, 8.3)	3.58 (5.9, 12.2)	3.72 (8.3, 12.2)	2.60 (8.1, 17.1)	2.65 (6.9, 17.1)	2.35(3H, s)	2.0
2,3-cis 3,4-cis (β)	4.40 (6.8)	3.48 (6.5, 6.8, 6.8, 6.8)	3.89 (6.5, 8.3, 9.3)	3.51 (8.3, 12.7)	3.68 (9.3, 12.7)	2.47 (6.8)	2.47 (6.8)	2.32(3H, s)	2.7

Table 10 Phenol derivatives



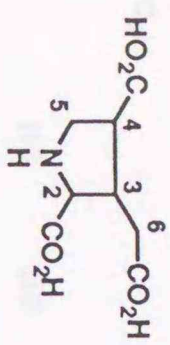
	H2	H3	H4	H5	H5'	H6	H6'	Others	PD
2,3-trans	4.07	3.00	3.6~3.8	3.6~3.8	3.6~3.8	2.52	2.69	6.95(1H, dd, 1.0, 7.8)	5.2
3,4-trans	(8.8)	(4.4, 7.8, 8.8, ?)	(?, ?, ?)	(?, ?)	(?, ?)	(7.8, 15.1)	(4.4, 15.1)	6.98(1H, ddd, 1.0, 7.0, 7.3)	
(α -allo)								7.24~7.28(2H, m)	
2,3-trans	4.16	3.16	3.93	3.81	3.86	2.01	2.47	6.94(1H, ddd, 1.5, 7.8, 7.8)	4~5
3,4-cis	(7.8)	(5.4, 7.5, 7.8, 9.8)	(7.3, 7.5, 7.8)	(7.3, 11.7)	(7.8, 11.7)	(9.8, 16.1)	(5.4, 16.1)	6.96(1H, dd, 1.5, 7.8)	
(α)								7.10(1H, dd, 1.5, 7.8)	
								7.27(1H, ddd, 1.5, 7.8, 7.8)	
2,3-cis	4.50	3.28	3.52	3.60	3.88	2.45	2.57	6.98(1H, dd, 1.0, 7.8)	4.3
3,4-trans	(8.8)	(6.3, 8.8, 8.8, 9.8)	(8.3, 9.8, 10.3)	(10.3, 11.2)	(8.3, 11.2)	(6.3, 16.6)	(8.8, 16.6)	7.01(1H, ddd, 1.0, 7.2, 7.3)	
(β -allo)								7.28(1H, dd, 1.0, 7.2)	
								7.30(1H, ddd, 1.0, 7.3, 7.8)	
2,3-cis	4.52	3.60	4.13	3.72	3.92	2.24	2.28	6.93(1H, dd, 1.0, 7.8)	4.9
3,4-cis	(7.3)	(4.9, 7.3, 7.5, 8.8)	(7.5, 8.3, 10.7)	(8.3, 11.7)	(10.7, 11.7)	(8.8, 16.5)	(4.9, 16.5)	6.95(1H, ddd, 1.0, 7.3, 7.3)	
(β)								7.22(1H, dd, 1.0, 7.3)	
								7.27(1H, ddd, 1.0, 7.3, 7.8)	

Table 11 Dihydrokainic acid derivatives



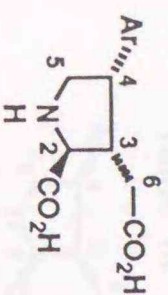
	H2	H3	H4	H5	H5'	H6	H6'	Others	PD
2,3-trans	4.00	2.63	2.09	3.23	3.54	2.75	2.75	0.91(3H, d, 6.8)	3.4
3,4-trans	(6.3)	(6.3, 6.3,	(6.3, 6.8,	(9.3, 12.0)	(7.8, 12.0)	(6.3)	(6.3)	0.98(3H, d, 6.8)	
(α-allo)		6.3, 6.3)	7.8, 9.3)					1.77(1H, octet, 6.8)	
2,3-trans	4.14	3.01	1.98	3.08	3.65	2.26	2.68	0.91(3H, d, 6.5)	3.1
3,4-cis	(1)	(1, 4.5,	(5.5, 8,	(12, 12)	(8, 12)	(12, 16)	(4.5, 16)	0.95(3H, d, 6.5)	
(α)		5.5, 12)	12, 12)					1.60(1H, d x septet 12, 6.5)	
2,3-cis	4.27	2.79	2.27	3.14	3.62	2.49	2.61	0.92(3H, d, 6.8)	3.4
3,4-trans	(8.8)	(6.4, 7.5,	(6.8, 7.5,	(9.0, 12.0)	(8.1, 12.0)	(7.6, 16.6)	(6.4, 16.6)	0.99(3H, d, 6.8)	
(β-allo)		7.6, 8.8)	8.1, 9.0)					1.82(1H, octet, 6.8)	
2,3-cis	4.23	3.15	2.21	3.10	3.53	2.23	2.51	0.92(3H, d, 6.4)	3.4
3,4-cis	(5.9)	(4.6, 5.5,	(5.5, 8.3,	(12, 12)	(8.3, 12)	(7.6, 16.8)	(4.6, 16.8)	0.96(3H, d, 6.4)	
(β)		5.9, 7.6)	11.0, 12.0)					1.65(1H, d x septet, 11.0, 6.4)	

Table 12 Carboxyl derivatives



	H2	H3	H4	H5	H5'	H6	H6'	Others	PD
2,3-trans	4.00	2.88~3.04	3.17	3.67	3.67	2.79	2.88~3.04	—	—
3,4-trans	(8.1)	(7.4, 8.1,	(8.1, 8.1,	(8.1)	(8.1)	(7.4, 16.5)	(?, 16.5)	—	—
(α-allo)		8.1, ?)	8.1)						
2,3-trans	3.96	2.9~3.2	3.48	3.58	3.70	2.58	2.9~3.2	—	—
3,4-cis	(9.9)	(6.2, 9.5,	(3.3, 6.2,	(6.2, 12.3)	(3.3, 12.3)	(9.5, 17.1)	(?, 17.1)	—	—
(α)		9.9, ?)	6.2)						

Table 13 Some kainoids-1



Ar	H2	H3	H4	H5	H5'	H6	H6'	Others	PD
	4.13 (6.4)	3.24 (5.9, 6.4, 7.8, 9.0)	3.96 (7.6, 7.8, 8.5)	3.77 (8.5, 11.0)	3.99 (7.6, 11.0)	2.24 (9.0, 17.1)	2.57 (5.9, 17.1)	7.30~7.33(2H, m) 7.42~7.51(3H, m)	3.8
	4.13 (7.3)	3.28 (4.9, 7.3, 8.3, 10.0)	4.06 (8.3, 8.3, 8.3)	3.81 (8.3, 12.2)	3.89 (8.3, 12.2)	2.20 (10.0, 16.0)	2.64 (4.9, 16.6)	3.91(3H, s) 7.06(1H, dt, 1.0, 7.3) 7.13(1H, dd, 1.0, 7.3) 7.18(1H, dd, 1.5, 7.3) 7.45(1H, dt, 1.5, 7.3)	4.0
	4.09 (7.3)	3.19 (5.9, 7.3, 8, 9.3)	3.89 (8, 8, 8.9)	3.72 (8.8, 11.2)	3.96 (8.0, 11.2)	2.22 (9.3, 16.9)	2.55 (5.9, 16.9)	3.90(3H, s) 7.07(2H, dd, 2.0, 6.8) 7.25(2H, dd, 2.0, 6.8)	4.5
	4.10 (7.8)	3.23 (4.6, 7.8, 8.3, 10.0)	3.99 (8.1, 8.3, 8.8)	3.76 (8.8, 12.2)	3.85 (8.1, 12.2)	2.22 (10.0, 16.8)	2.64 (4.6, 16.8)	3.90(6H, s) 6.65(1H, dd, 2.4, 8.3) 6.70(1H, d, 2.4) 7.10(1H, d, 8.3)	4.2
	4.16 (7.8)	3.16 (5.4, 7.8, 9.8, 7~8)	3.93 (7.3, 7.8, 7~8)	3.81 (7.3, 11.7)	3.86 (7.8, 11.7)	2.01 (9.8, 16.1)	2.47 (5.4, 16.1)	6.94(1H, ddd, 1.5, 7.8, 7.8) 6.96(1H, dd, 1.5, 7.8) 7.10(1H, dd, 1.5, 7.8) 7.27(1H, ddd, 1.5, 7.8, 7.8)	4~5

Table 14 Some kainoids-2

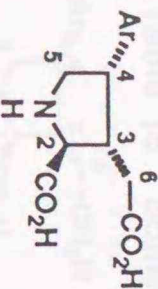
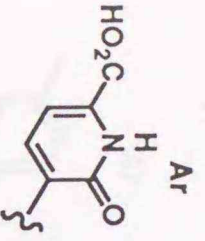
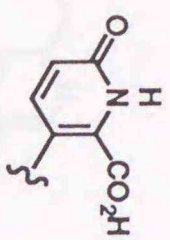
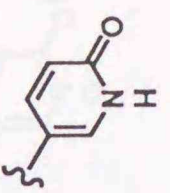
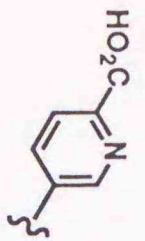
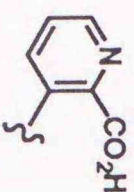
									
H2	H3	H4	H5	H5'	H6	H6'	Others	pD	
 4.26 (7.3)	3.31 (4.9, 7.3, 7.8, 10.3)	3.95 (7.3, 7.3, 7.8)	3.82 (7.3, 11.7)	3.86 (7.3, 11.7)	2.39 (10.3, 17.9)	2.80 (4.9, 17.9)	7.03(1H, d, 7.1) 7.65(1H, d, 7.1)	3.0	
 4.26 (5.9)	3.40 (5.9, 6.6, 8.5, 8.8)	4.7~4.8 (7.8, 8.5, 12.0)	3.76 (12.0, 12.0)	3.90 (7.8, 12.0)	2.48 (8.8, 16.6)	2.68 (6.6, 16.6)	6.83(1H, d, 9.5) 7.78(1H, d, 9.5)	2.4	
 3.78 (5.9)	2.89 (5.9, 5.9, 7.9, 9.5)	3.44 (7.9, 7.9, 10.4)	3.29 (10.4, 11.9)	3.59 (7.9, 11.9)	2.01 (9.5, 16.5)	2.28 (5.9, 16.5)	6.38(1H, d, 9.5) 7.15(1H, d, 2.6) 7.36(1H, dd, 2.6, 9.5)		
 4.24 (5.4)	3.39 (5.4, 6.4, 8.3, ?)	~4.20~ (9.3, ?, 13.8)	3.87 (13.8, 13.8)	4.17 (9.3, 13.8)	2.26 (8.3, 17.1)	2.60 (6.4, 17.1)	8.35(1H, brs) 8.45(1H, brs) 8.69(1H, brs)		
 4.26 (4.9)	3.56 (4.9, 6.3, 7.8, 8.8)	5.04 (7.8, 7.8, 11.7)	3.95 (11.7, 11.7)	4.01 (7.8, 11.7)	2.42 (8.8, 16.6)	2.62 (6.3, 16.6)	8.10(1H, dd, 5.9, 8.3) 8.58(1H, d, 8.3) 8.74(1H, d, 5.9)		

Table 15 Some kainoids-3

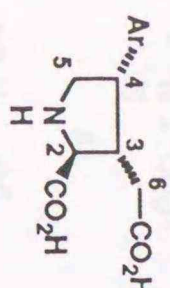
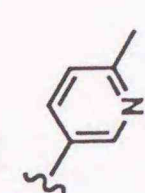
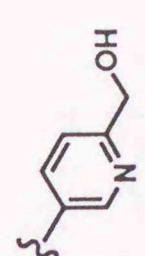
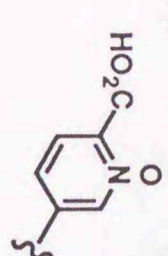
									
Ar	H2	H3	H4	H5	H5'	H6	H6'	Others	pD
	4.12 (5.9)	3.27 (5.6, 5.9, 6, 9.5)	~4.0~ (6, 7.8, 12.7)	3.78 (12.7, 15.1)	4.02 (7.8, 15.1)	2.07 (9.5, 16.4)	2.44 (5.6, 16.4)	2.73(3H, s) 7.73(1H, d, 8.3) 8.16(1H, dd, 2.0, 8.3) 8.47(1H, d, 2.0)	-
	4.14 (5.9)	3.29 (5.9, 5.9, 6, 9.3)	~4.0~ (6, 7.8, 12.7)	3.81 (12.7, 14.7)	3.93 (7.8, 14.7)	2.14 (9.3, 16.4)	2.50 (5.9, 16.4)	4.90(2H, s) 7.74(1H, d, 8.3) 8.10(1H, dd, 2.4, 8.3) 8.50(1H, d, 2.4)	-
	4.18 (5.9)	3.35 (5.9, 6.4, 8, 9.0)	3.80 (7, 8, 8)	4.01 (8, 12)	4.03 (7, 12)	2.36 (9.0, 17.0)	2.64 (6.4, 17.0)	7.75(1H, dd, 1.5, 8.3) 7.77(1H, d, 8.3) 8.34(1H, d, 1.5)	-

Table 16 Some kainoids-4

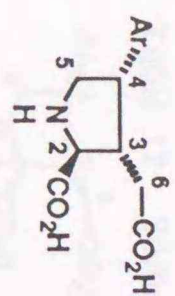
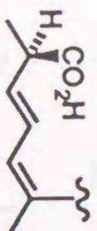
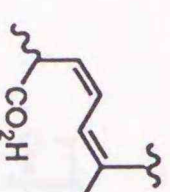
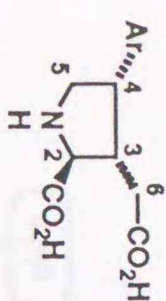
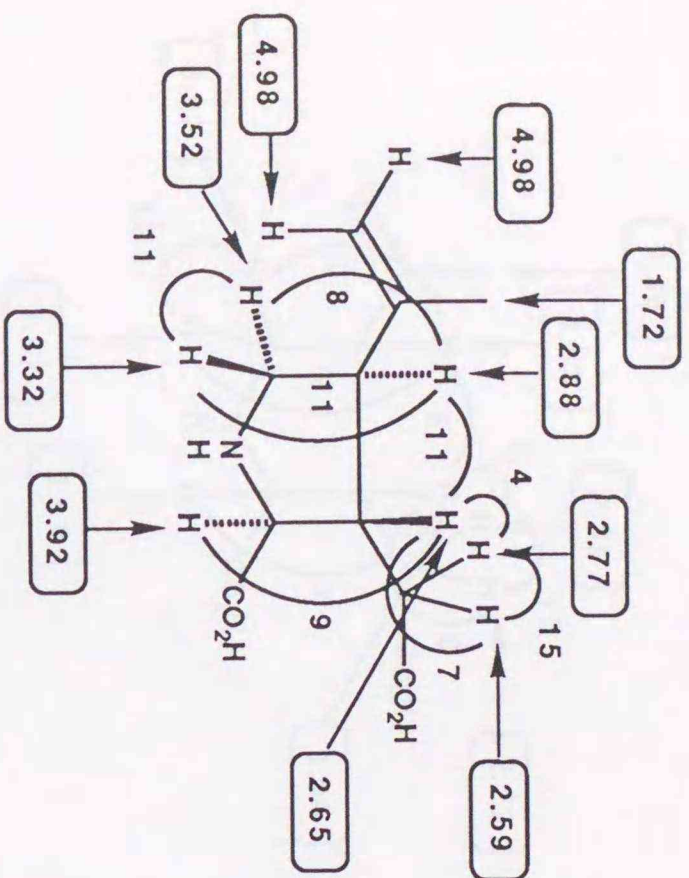
									
Ar	H2	H3	H4	H5	H5'	H6	H6'	Others	pD
	3.98 (8.1)	3.05 (5.8, 8.1, 8.4, 9.1)	3.84 (7.3, 7.9, 8.4)	3.49 (7.3, 12.3)	3.71 (7.9, 12.3)	2.50 (9.1, 16.8)	2.75 (5.8, 16.8)	1.27(3H, d, 7.1) 1.81(3H, s) 3.30(1H, dq, 7.8, 7.1) 5.78(1H, dd, 7.8, 14.9) 6.13(1H, d, 11.1) 6.35(1H, dd, 11.1, 14.9) 1.75(3H, s) 1.82(3H, s) 2.84(1H, ddd, 7.5, 7.5, 16.5) 2.96(1H, ddd, 7.0, 7.9, 16.5) 5.52(1H, dd, 7.5, 7.9) 6.61(1H, dd, 7.0, 7.5)	3.4
	3.96 (7.5)	2.98 (6.0, 7.5, 8.0, 9.0)	3.62 (7.0, 8.0, 10.0)	3.45 (10.0, 11.0)	3.67 (7.0, 11.0)	2.45 (9.0, 16.0)	2.61 (6.0, 16.0)	1.72(3H, s) 1.82(3H, s) 2.91(2H, d, 7.0) 5.25(1H, dd, 7.0, 7.0) 6.70(1H, dd, 7.0, 7.0)	-
	4.04 (3.5)	2.99 (3.5, 7.0, 7.0, 8.6)	2.91 (7.0, 8.6, 12.0)	3.46 (12.0, 12.0)	3.60 (7.0, 12.0)	2.38 (7.0)	2.38 (7.0)	1.86(3H, s) 2.21(2H, m) 2.39(2H, m) 4.93(1H, s) 5.15(1H, s) 6.72(1H, t, 7.0) 1.25(3H, d, 7.0) 1.85(3H, s) 3.69(1H, dq, 10.0, 7.0) 5.49(1H, t, 10.0) 6.28(1H, dd, 10.0, 11.6) 6.43(1H, d, 11.6)	-
	4.08 (3.0)	3.09 (3.0, 6.9, 8.6, 7.5)	2.99 (6.9, 7.2, 12.1)	3.43 (12.1, 12.1)	3.59 (7.2, 12.1)	2.27 (8.6, 16.8)	2.41 (7.5, 16.8)	1.86(3H, s) 2.21(2H, m) 2.39(2H, m) 4.93(1H, s) 5.15(1H, s) 6.72(1H, t, 7.0) 1.25(3H, d, 7.0) 1.85(3H, s) 3.69(1H, dq, 10.0, 7.0) 5.49(1H, t, 10.0) 6.28(1H, dd, 10.0, 11.6) 6.43(1H, d, 11.6)	-
	4.15 (8.1)	3.10 (6.3, 7.9, 8.1, 8.3)	3.87 (7.7, 7.9, 7.9)	3.52 (7.9, 12.4)	3.72 (7.7, 12.4)	2.53 (8.3, 17.1)	2.75 (6.3, 17.1)	1.86(3H, s) 2.21(2H, m) 2.39(2H, m) 4.93(1H, s) 5.15(1H, s) 6.72(1H, t, 7.0) 1.25(3H, d, 7.0) 1.85(3H, s) 3.69(1H, dq, 10.0, 7.0) 5.49(1H, t, 10.0) 6.28(1H, dd, 10.0, 11.6) 6.43(1H, d, 11.6)	1.9

Table 17 Some kainoids-5

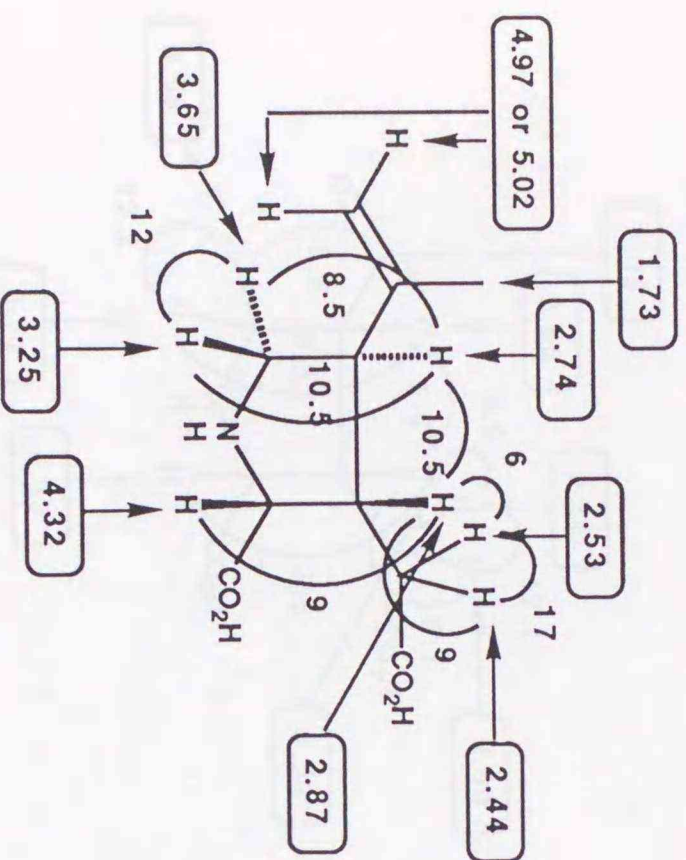


Ar	H2	H3	H4	H5	H5'	H6	H6'	Others	pD
	4.36 (4.0)	3.26 (4.0, 6.4, 6.4, 7.4)	3.23 (7.3, 7.4, 10.0)	3.58 (10.0, 11.9)	3.72 (7.3, 11.9)	2.53 (6.4, 16.9)	2.57 (6.4, 16.9)	1.33(3H, d, 7.0) 1.85(3H, s) 3.38(1H, dq, 7.9, 7.0) 5.87(1H, dd, 7.9, 15.2) 5.95(1H, d, 10.5) 6.55(1H, dd, 10.5, 15.2) 1.33(3H, d, 7.0) 1.87(3H, s) 3.73(1H, dq, 10.0, 7.0) 5.60(1H, dd, 10.0, 11.1) 6.19(1H, d, 11.0) 6.47(1H, dd, 11.0, 11.1) 1.18(3H, d, 7.0) 1.80(3H, s) 3.00(1H, dq, 5.5, 7.0) 4.23(1H, dd, 1.5, 5.5) 5.14(1H, dd, 1.5, 9.0) 5.53(1H, d, 9.0) 1.24(1H, 3H, d, 7.0) 1.83(3H, s) 2.78(1H, dq, 9.5, 7.0) 3.94(1H, dd, 7.5, 9.5) 4.92(1H, dd, 7.5, 9.5) 5.55(1H, d, 9.5) 5.01(2H, ABq, 11.2) 5.14(2H, brs)	1.8
	4.39 (4.6)	3.27 (4.6, 7.1, 7.2, 7.2)	3.32 (7.2, 7.3, 9.5)	3.63 (9.5, 11.9)	3.77 (7.3, 11.9)	2.53 (7.1, 17.1)	2.63 (7.2, 17.1)	1.33(3H, d, 7.0) 1.87(3H, s) 3.73(1H, dq, 10.0, 7.0) 5.60(1H, dd, 10.0, 11.1) 6.19(1H, d, 11.0) 6.47(1H, dd, 11.0, 11.1) 1.18(3H, d, 7.0) 1.80(3H, s) 3.00(1H, dq, 5.5, 7.0) 4.23(1H, dd, 1.5, 5.5) 5.14(1H, dd, 1.5, 9.0) 5.53(1H, d, 9.0) 1.24(1H, 3H, d, 7.0) 1.83(3H, s) 2.78(1H, dq, 9.5, 7.0) 3.94(1H, dd, 7.5, 9.5) 4.92(1H, dd, 7.5, 9.5) 5.55(1H, d, 9.5) 5.01(2H, ABq, 11.2) 5.14(2H, brs)	1.6
	3.97 (7.5)	3.00 (6.0, 7.5, 7.6, 9.0)	3.62 (7.6, 7.6, 7.6)	3.47 (7.6, 12.0)	3.72 (7.6, 12.0)	2.35 (9.0, 16.5)	2.50 (6.0, 16.5)	1.33(3H, d, 7.0) 1.80(3H, s) 3.00(1H, dq, 5.5, 7.0) 4.23(1H, dd, 1.5, 5.5) 5.14(1H, dd, 1.5, 9.0) 5.53(1H, d, 9.0) 1.24(1H, 3H, d, 7.0) 1.83(3H, s) 2.78(1H, dq, 9.5, 7.0) 3.94(1H, dd, 7.5, 9.5) 4.92(1H, dd, 7.5, 9.5) 5.55(1H, d, 9.5) 5.01(2H, ABq, 11.2) 5.14(2H, brs)	-
	4.03 (5.5)	3.05 (5.5, 7.0, 6.5, 8.5)	3.64 (7.0, 9.0, 9.0)	3.49 (9.0, 11.0)	3.69 (9.0, 11.0)	2.54 (8.5, 16.5)	2.59 (6.5, 16.5)	1.33(3H, d, 7.0) 1.80(3H, s) 3.00(1H, dq, 5.5, 7.0) 4.23(1H, dd, 1.5, 5.5) 5.14(1H, dd, 1.5, 9.0) 5.53(1H, d, 9.0) 1.24(1H, 3H, d, 7.0) 1.83(3H, s) 2.78(1H, dq, 9.5, 7.0) 3.94(1H, dd, 7.5, 9.5) 4.92(1H, dd, 7.5, 9.5) 5.55(1H, d, 9.5) 5.01(2H, ABq, 11.2) 5.14(2H, brs)	-
	3.96 (6.4)	3.17 (6.4, ?, ?)	—	3.63 (13.7)	3.87 (13.7)	2.67 (?, ?)	2.67 (?, ?)	1.33(3H, d, 7.0) 1.80(3H, s) 3.00(1H, dq, 5.5, 7.0) 4.23(1H, dd, 1.5, 5.5) 5.14(1H, dd, 1.5, 9.0) 5.53(1H, d, 9.0) 1.24(1H, 3H, d, 7.0) 1.83(3H, s) 2.78(1H, dq, 9.5, 7.0) 3.94(1H, dd, 7.5, 9.5) 4.92(1H, dd, 7.5, 9.5) 5.55(1H, d, 9.5) 5.01(2H, ABq, 11.2) 5.14(2H, brs)	-

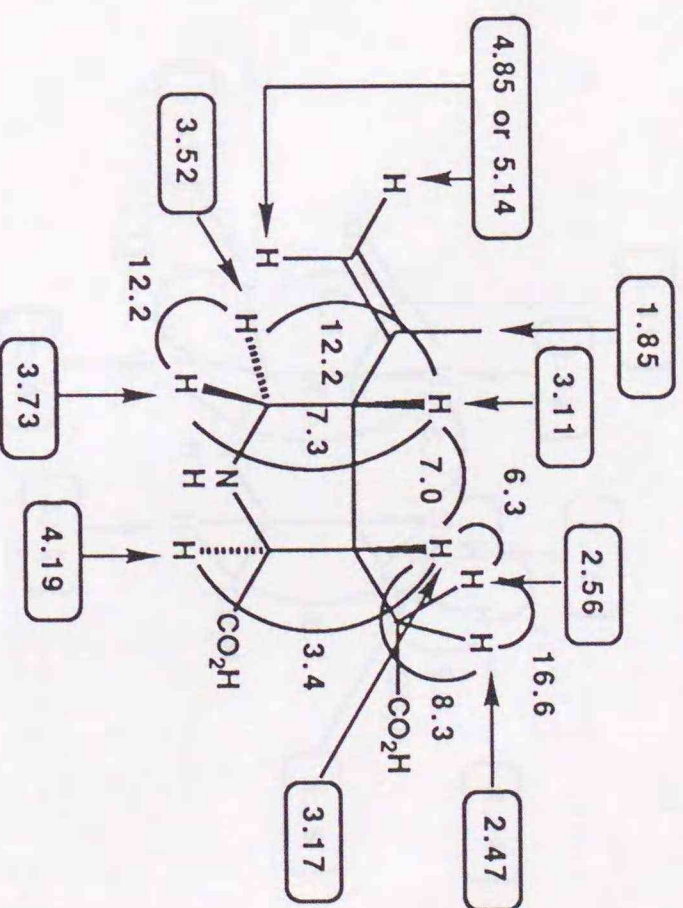
Fig 16 Kainic acid derivatives



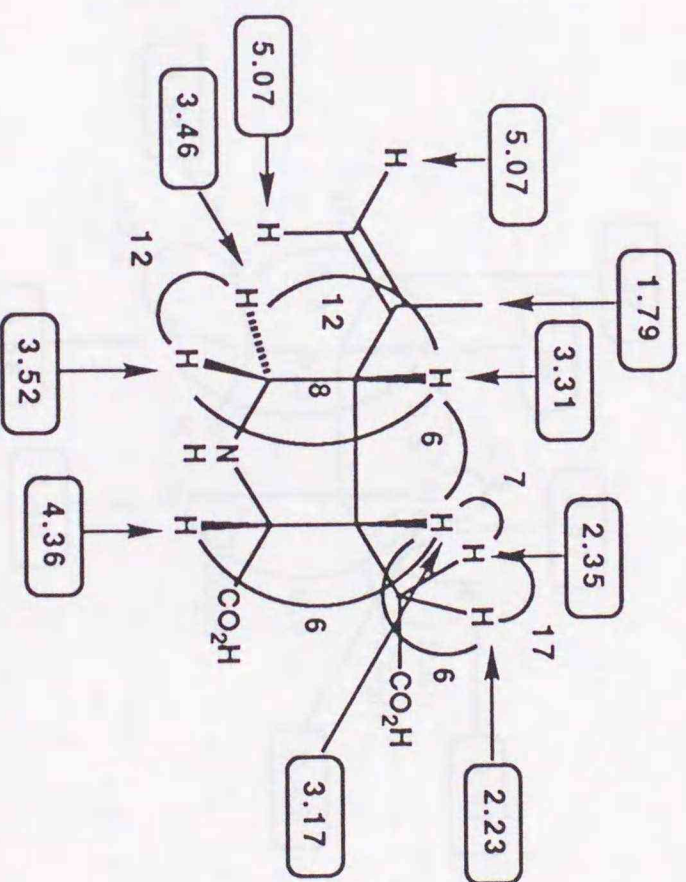
α -allokainic acid (pD=4.1)



β -allokainic acid (pD=3.3)

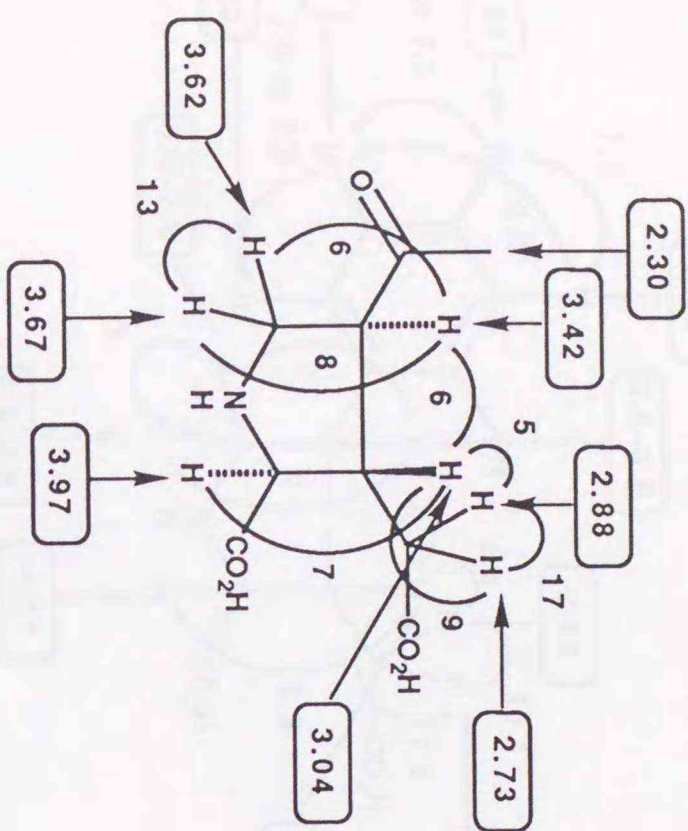


α -kainic acid (pD=3.4)

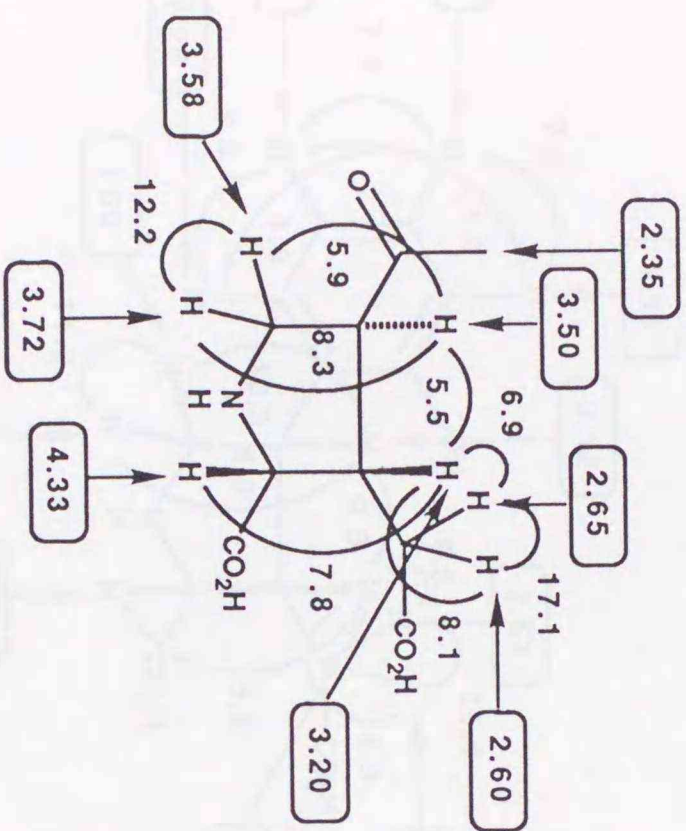


β -kainic acid (pD=3.3)

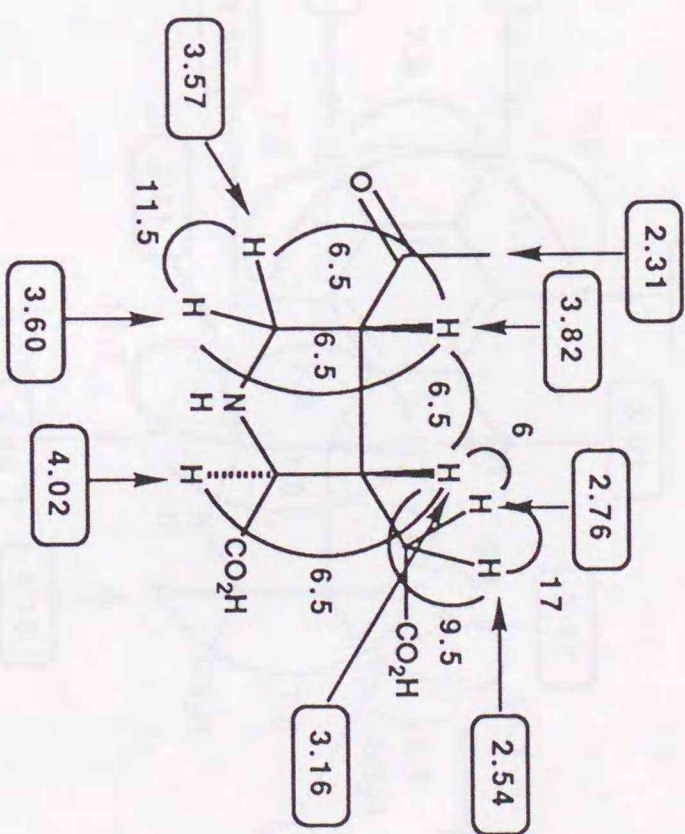
Fig 17 Ketokainic acid derivatives



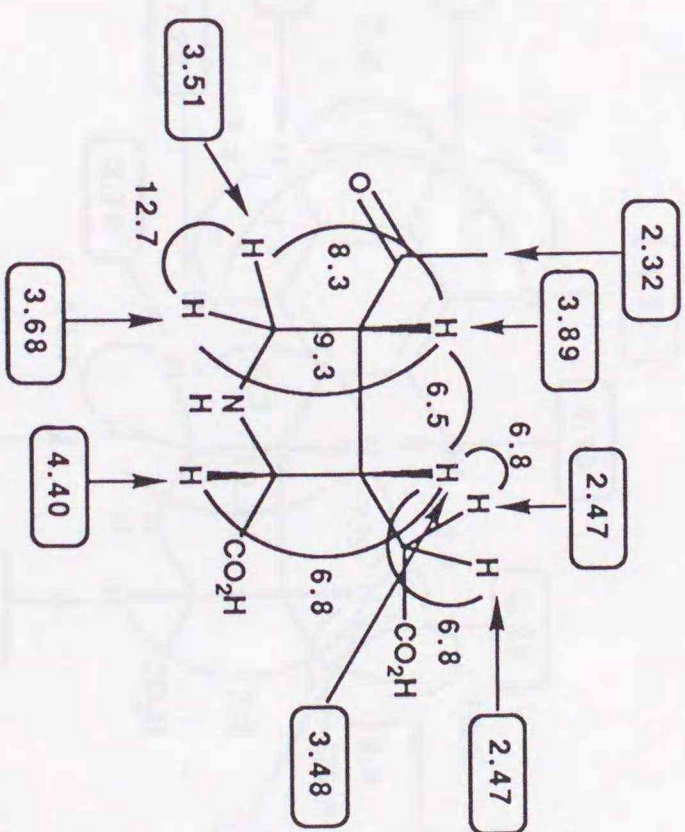
α -allotetokainic acid ($pD=3.1$)



β -allotetokainic acid ($pD=2.0$)

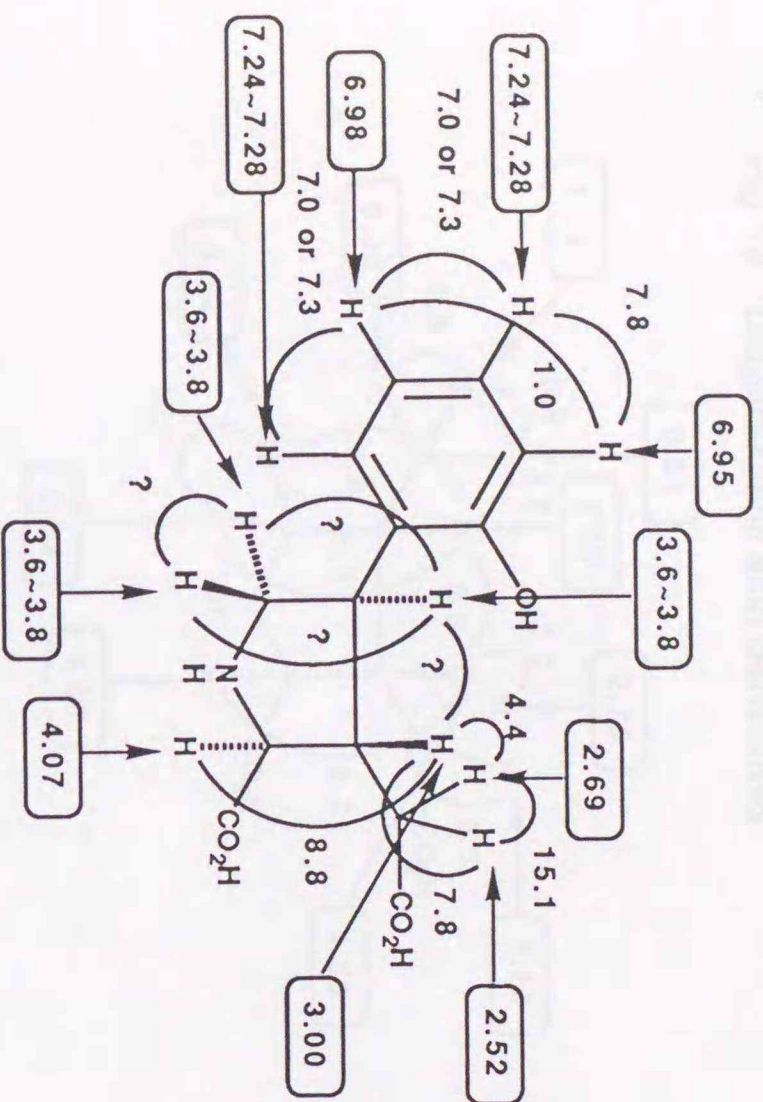


α -ketokainic acid ($pD=3.0$)

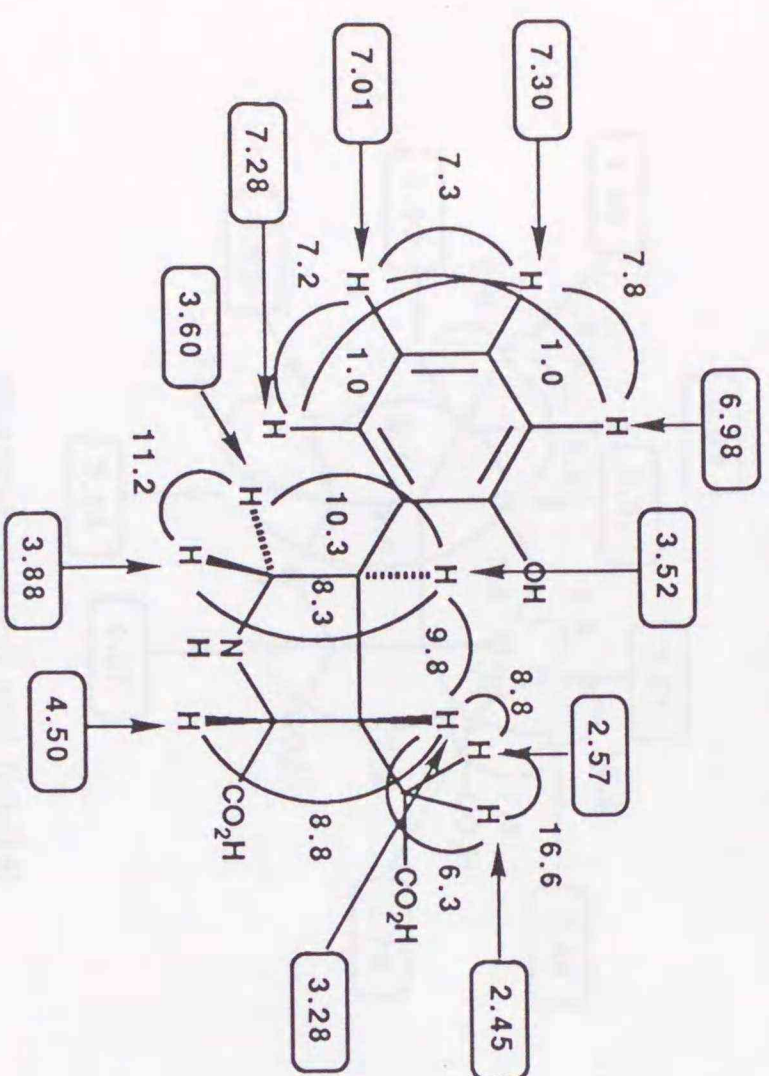


β -ketokainic acid ($pD=2.7$)

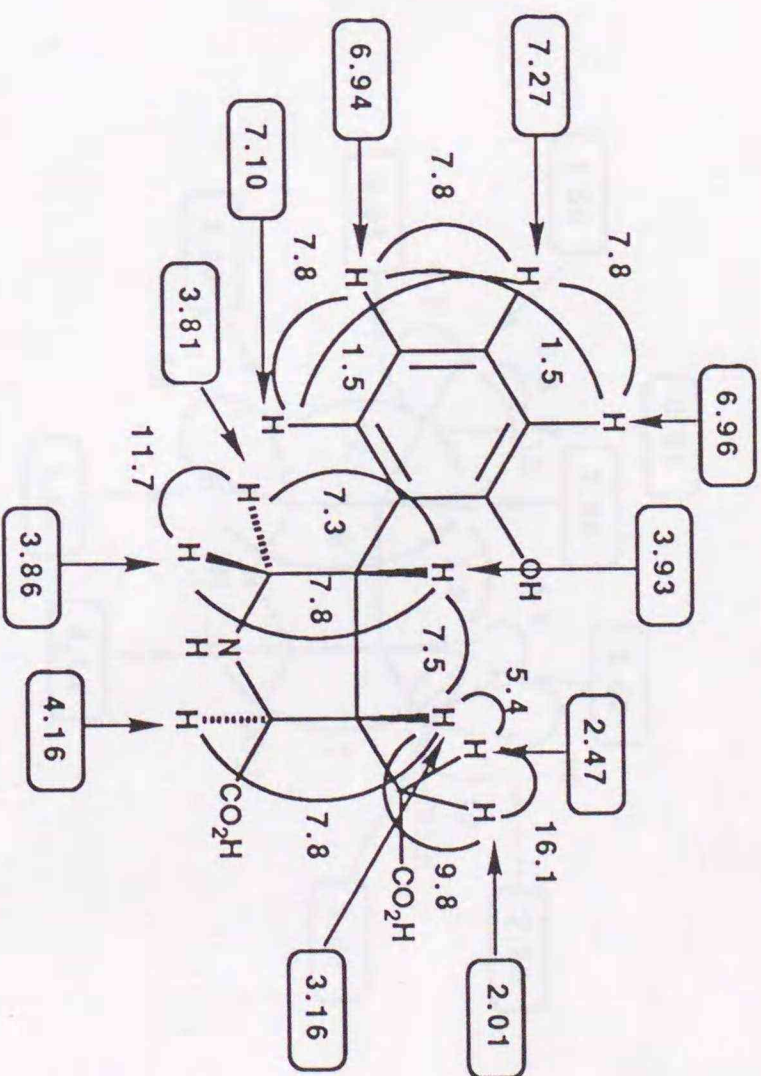
Fig 18 Phenol derivatives



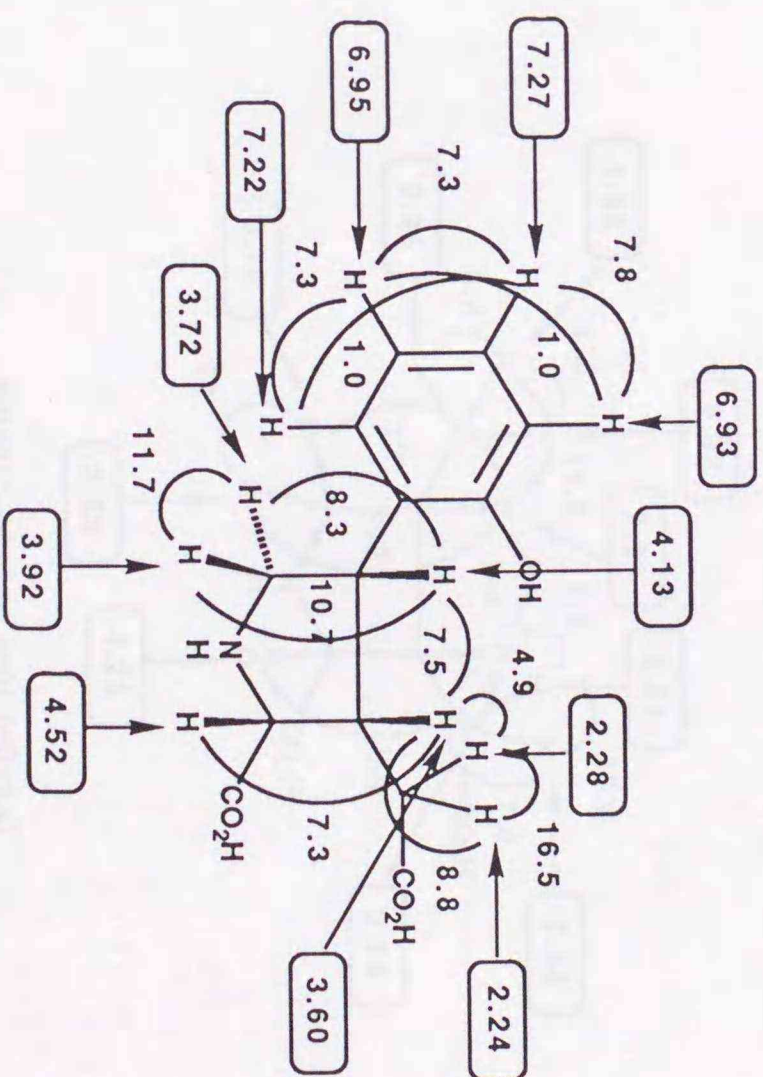
α -allo (pD=5.2)



β -allo (pD=4.3)

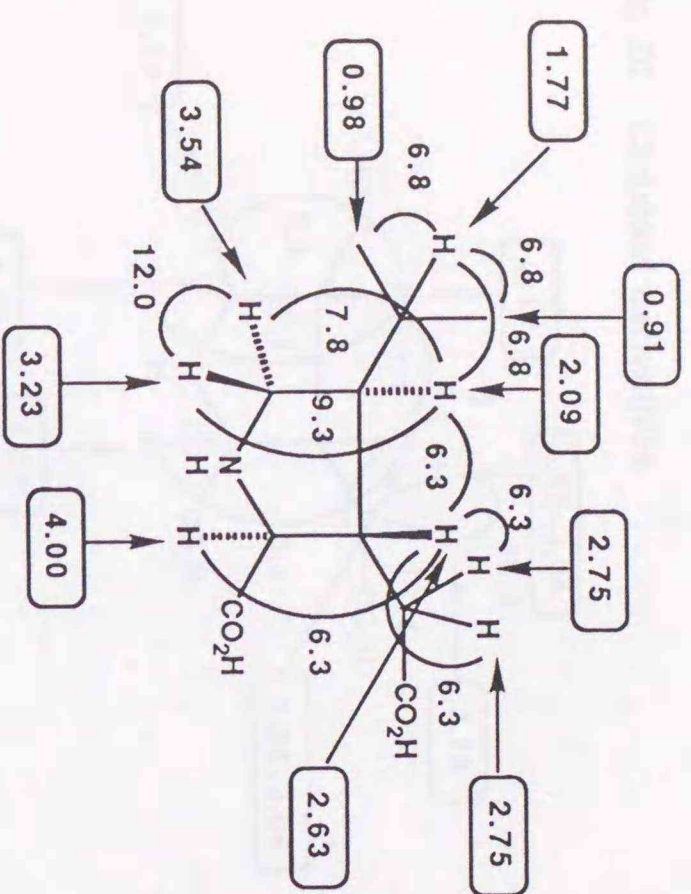


α (pD=4~5)

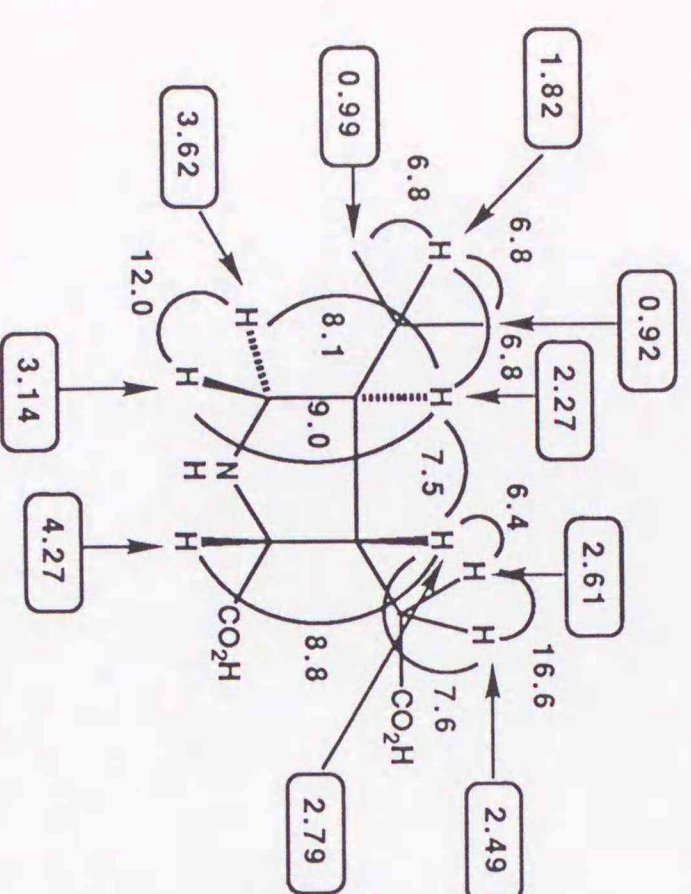


β (pD=4.9)

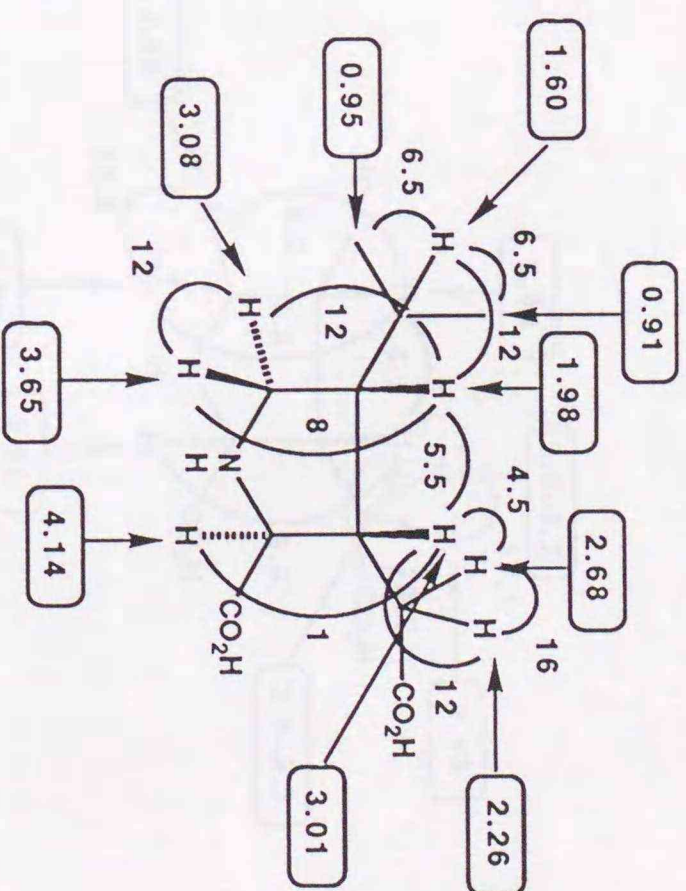
Fig 19 Dihydrokainic acid derivatives



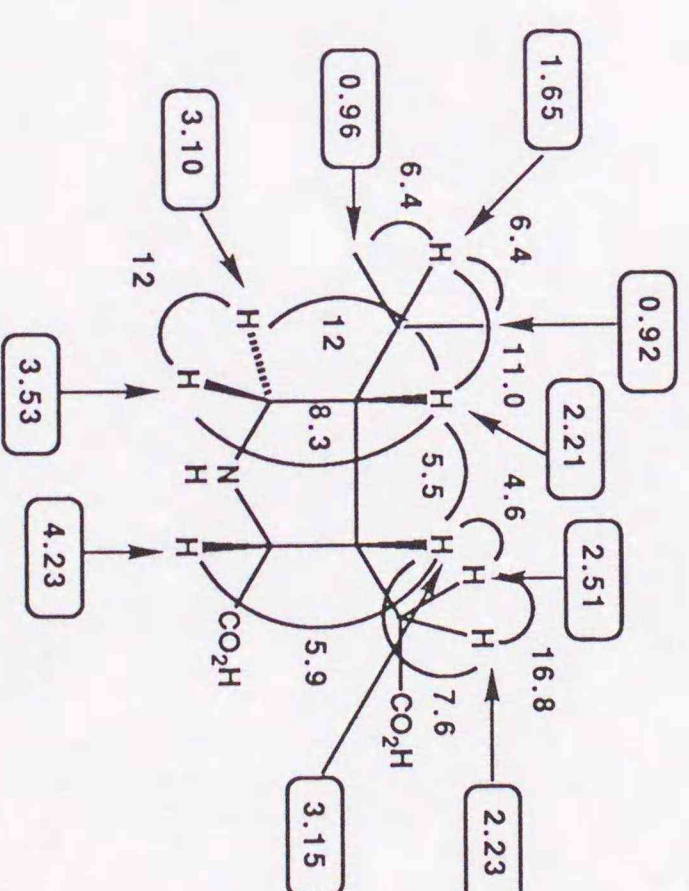
dihydro- α -alklokainic acid (pD=3.4)



dihydro- β -alklokainic acid (pD=3.4)



dihydro- α -kainic acid (pD=3.1)



dihydro- β -kainic acid (pD=3.4)

Fig 20 Carboxyl derivatives

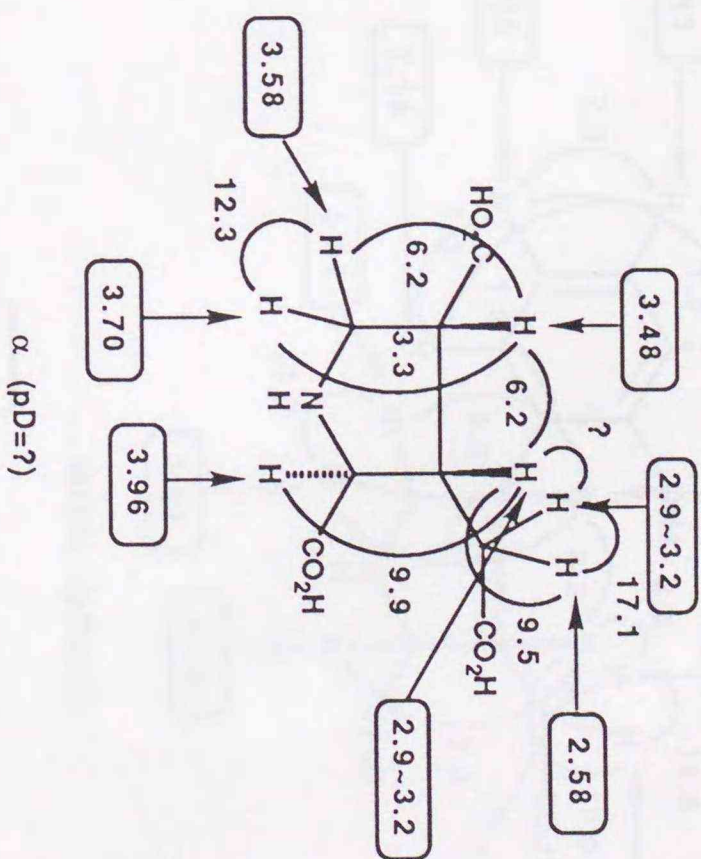
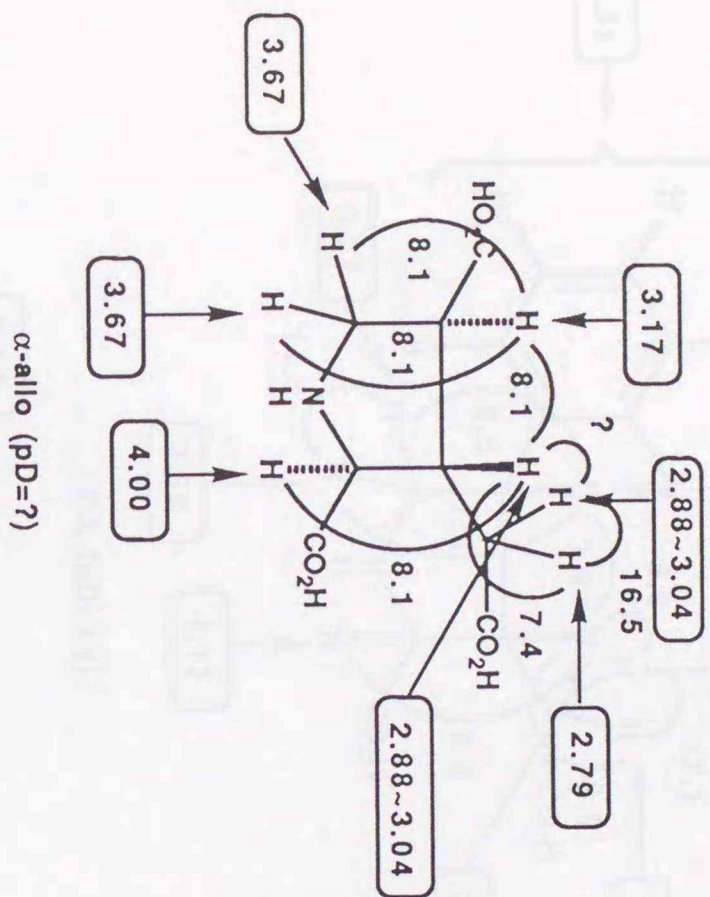
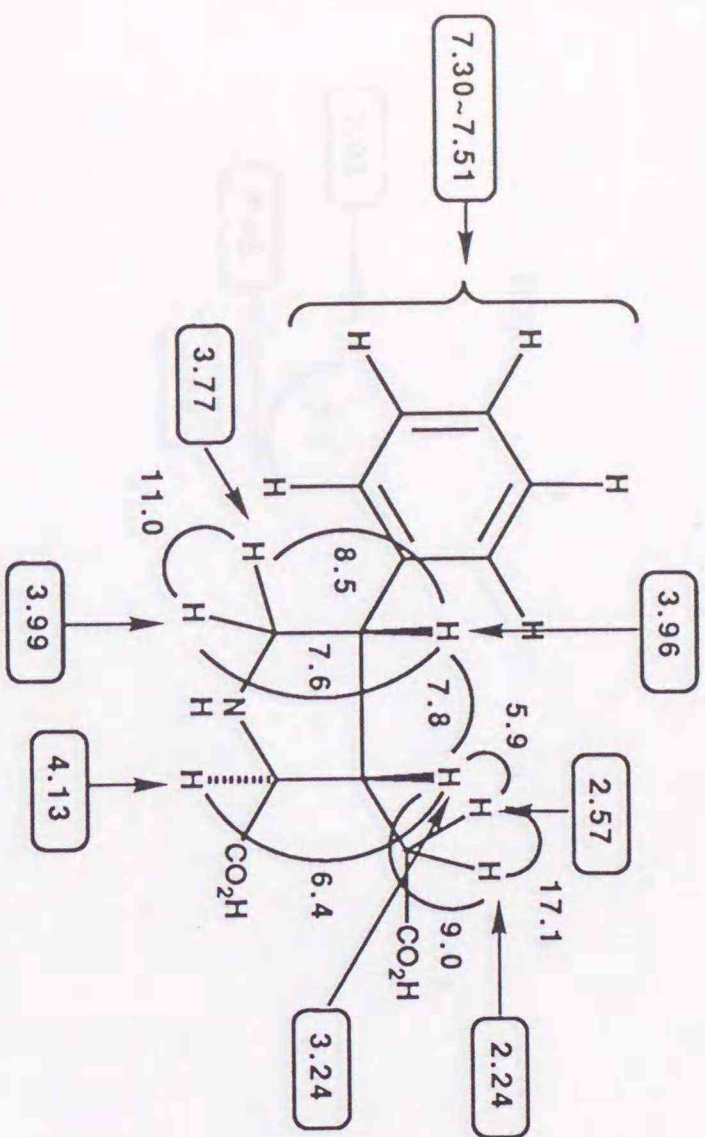
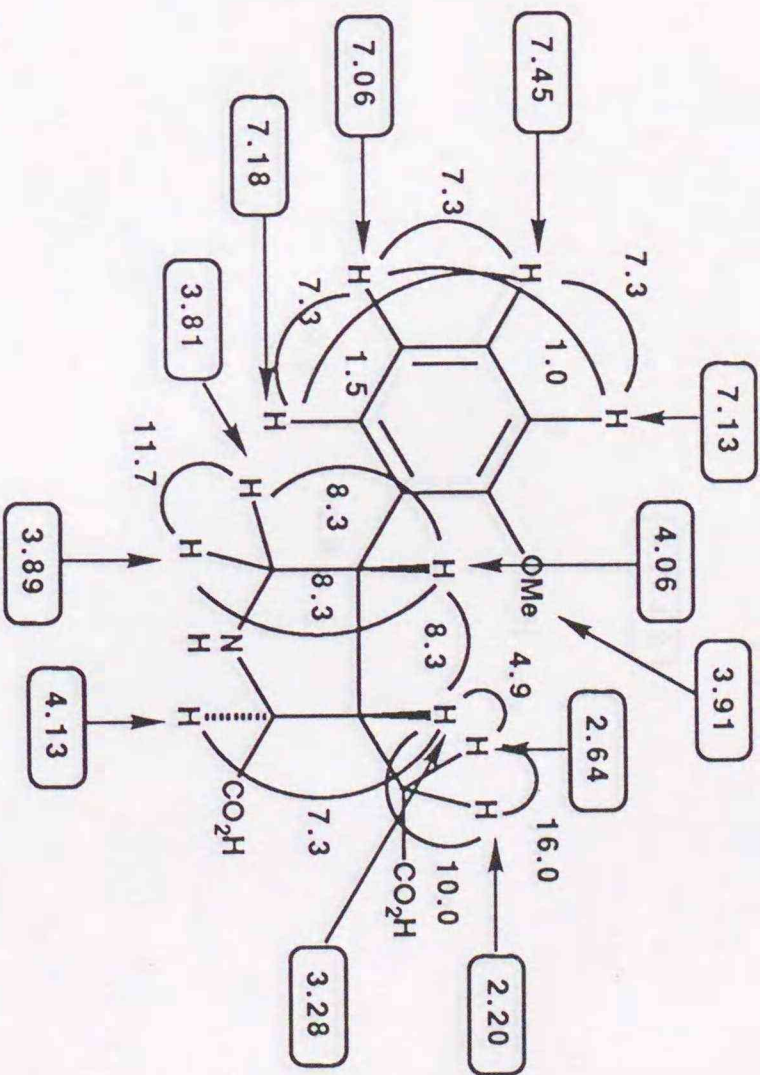


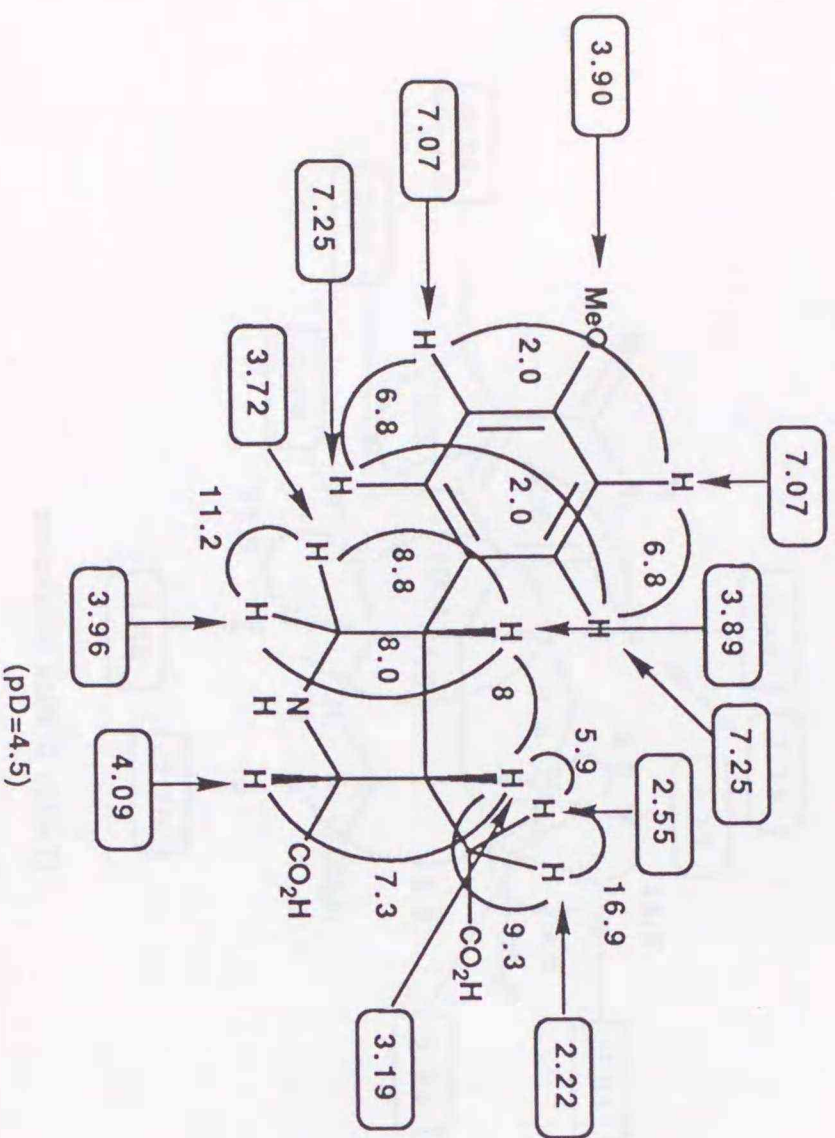
Fig 21 Some kainoids-1



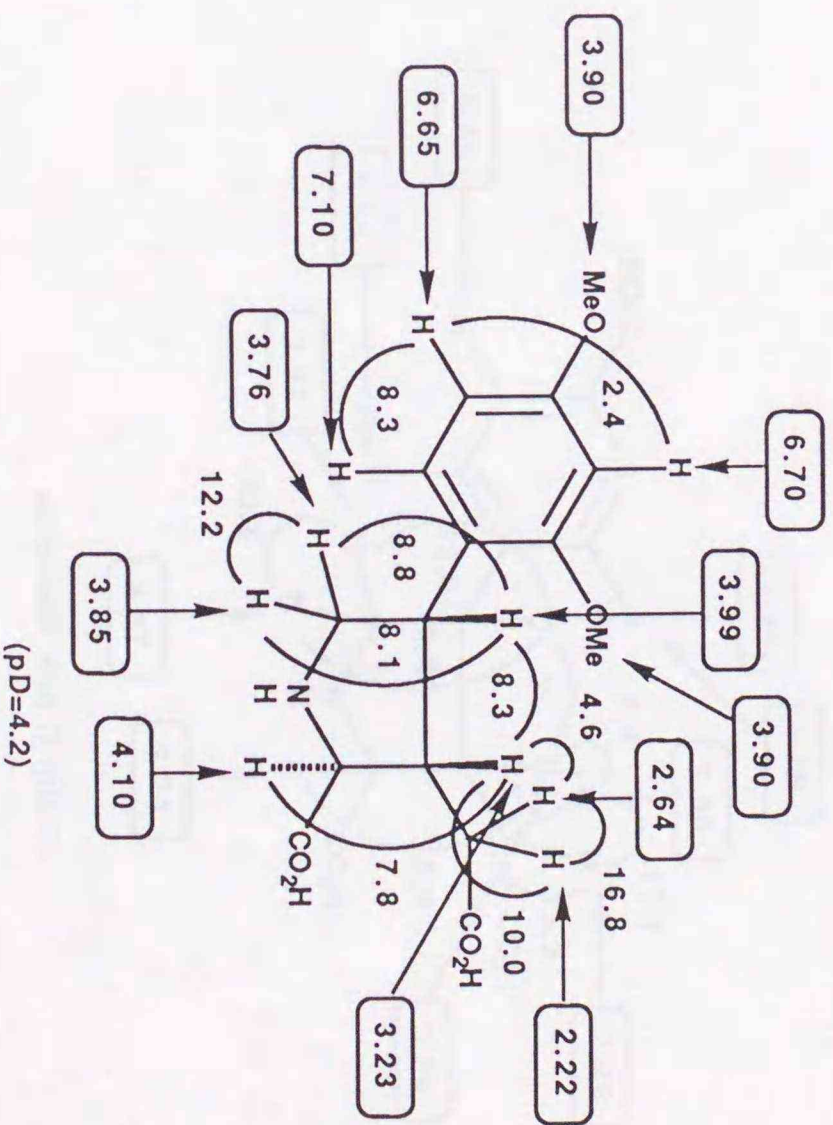
FPA (pD=3.8)



MFPA (pD=4.0)



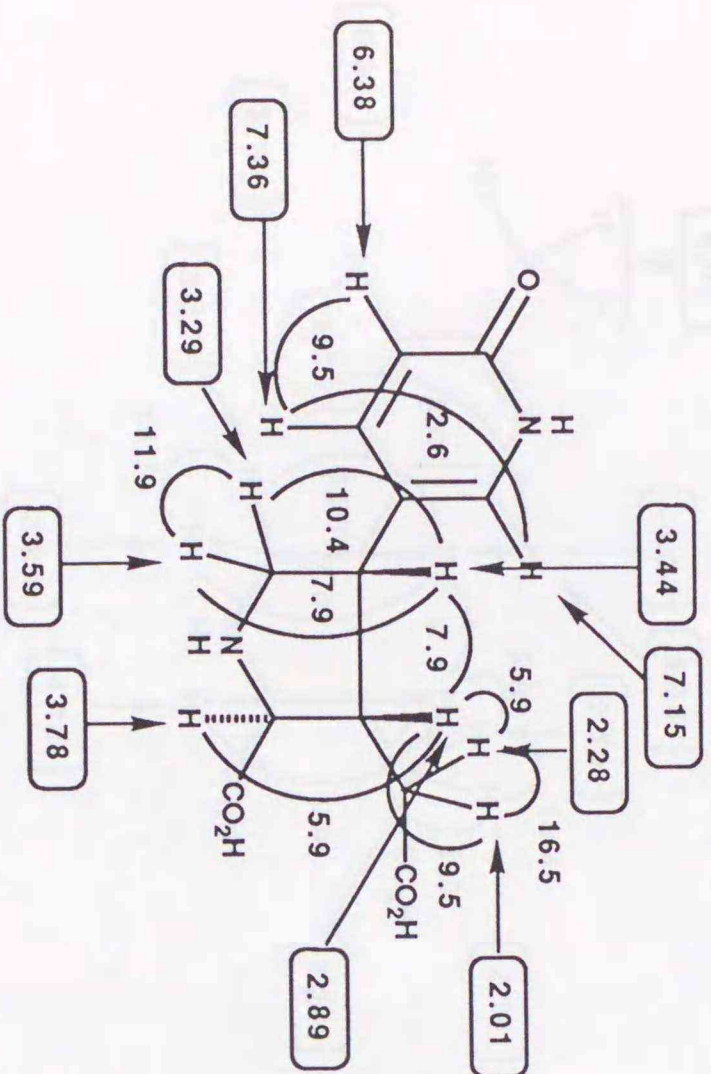
(pD=4.5)



(pD=4.2)

Chemical structure of a complex molecule with 13 labeled chemical shifts (ppm):

- 7.03
- 7.65
- 3.82
- 11.7
- 3.86
- 3.95
- 2.80
- 4.9
- 17.9
- 2.39
- 10.3
- 7.3
- 7.8
- 7.3
- 7.3
- 7.1
- 7.03
- 3.31
- 4.26



The chemical structure is a complex polycyclic molecule, likely a derivative of a nucleoside or a similar heterocyclic compound. It features a central pyrimidine-like ring system fused to a six-membered ring, which is further fused to a five-membered ring containing a nitrogen atom. The structure is substituted with several carboxylic acid groups (CO_2H) and various hydrogen atoms (H). The following table summarizes the proton assignments and coupling constants shown in the diagram:

Assignment / Coupling Constant	Location / Description
6.83	Proton assignment (top left)
7.78	Proton assignment (top left)
3.76	Proton assignment (top left)
9.5	Coupling constant between two protons in the top left region
12.0	Coupling constant between two protons in the top left region
7.8	Coupling constant between two protons in the top left region
3.90	Proton assignment (top left)
4.7~4.8	Proton assignment (top right)
2.68	Proton assignment (top right)
16.6	Coupling constant between two protons in the top right region
6.6	Coupling constant between two protons in the top right region
8.5	Coupling constant between two protons in the top right region
8.8	Coupling constant between two protons in the top right region
5.9	Coupling constant between two protons in the top right region
3.40	Proton assignment (top right)
2.48	Proton assignment (top right)
4.26	Proton assignment (bottom left)

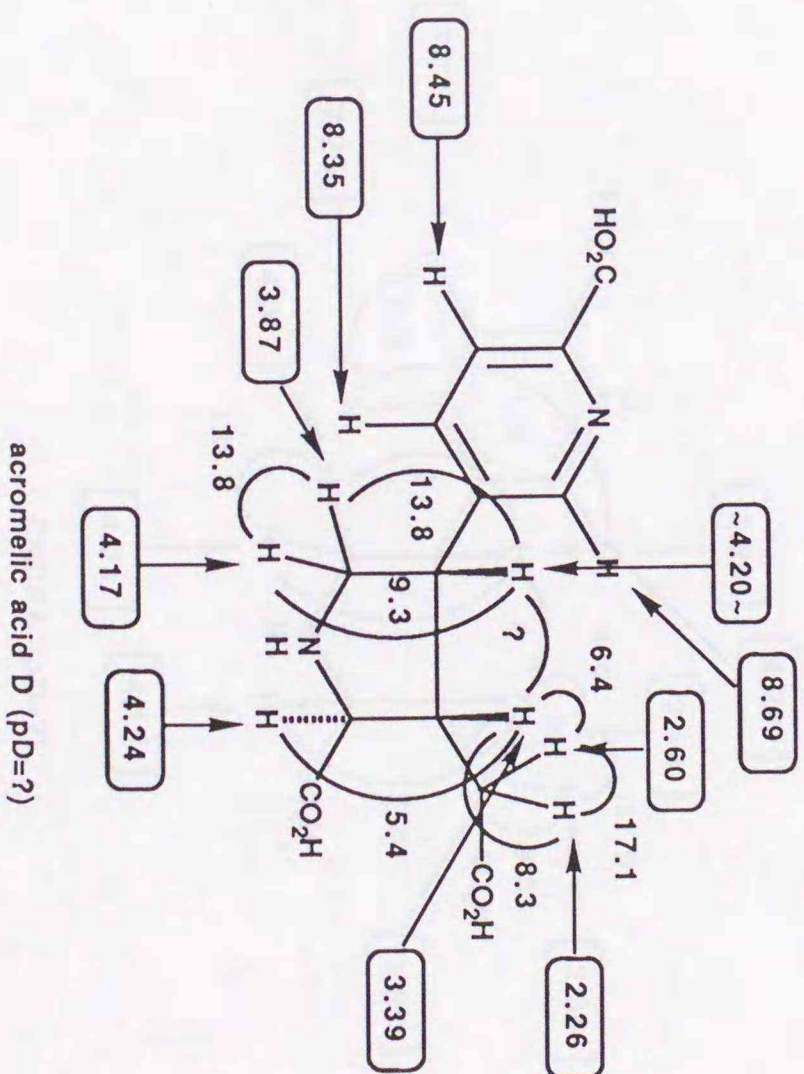
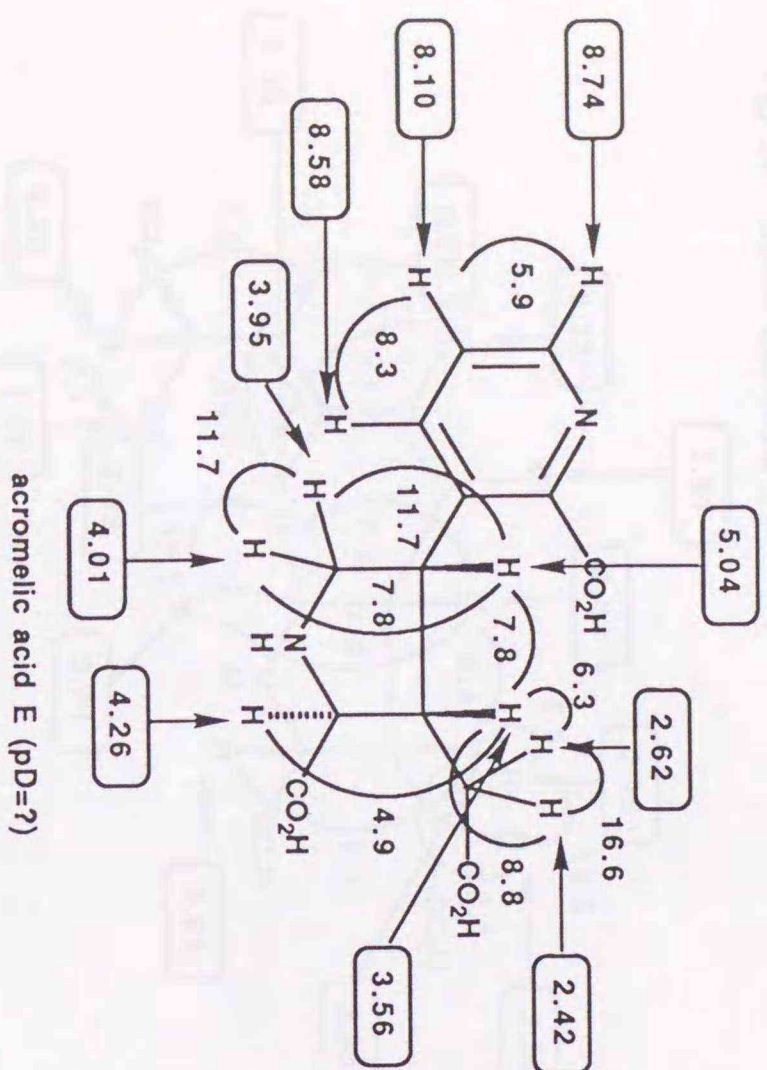
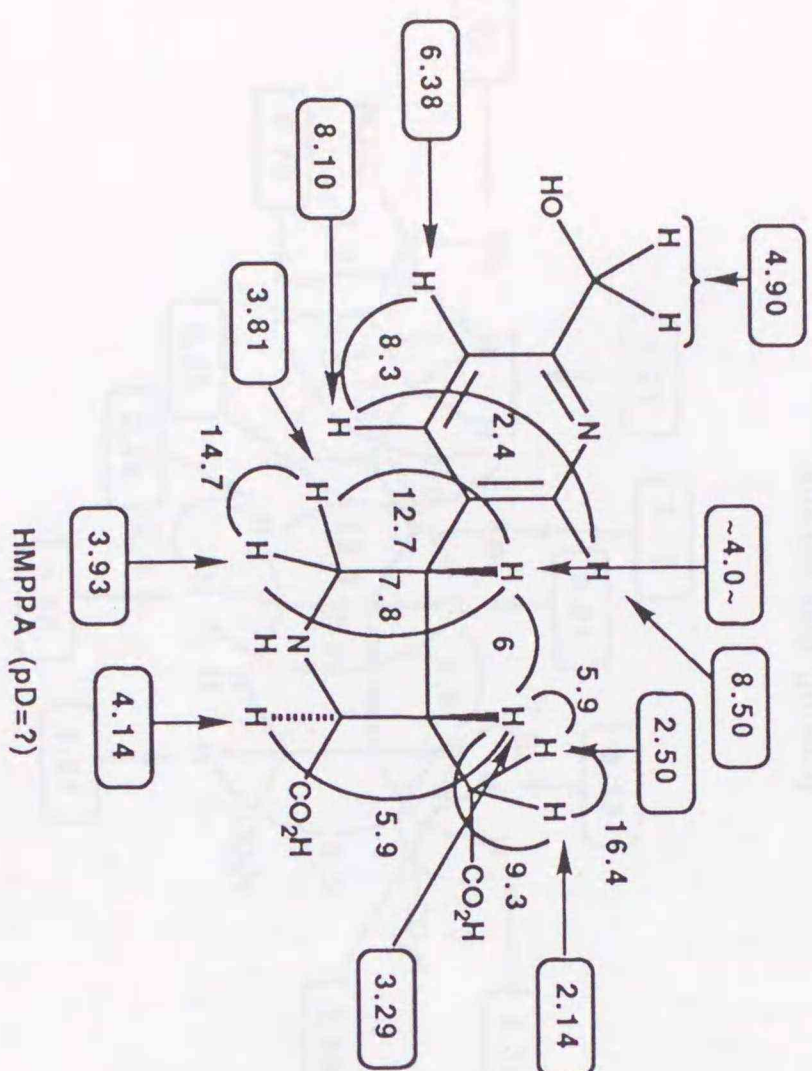


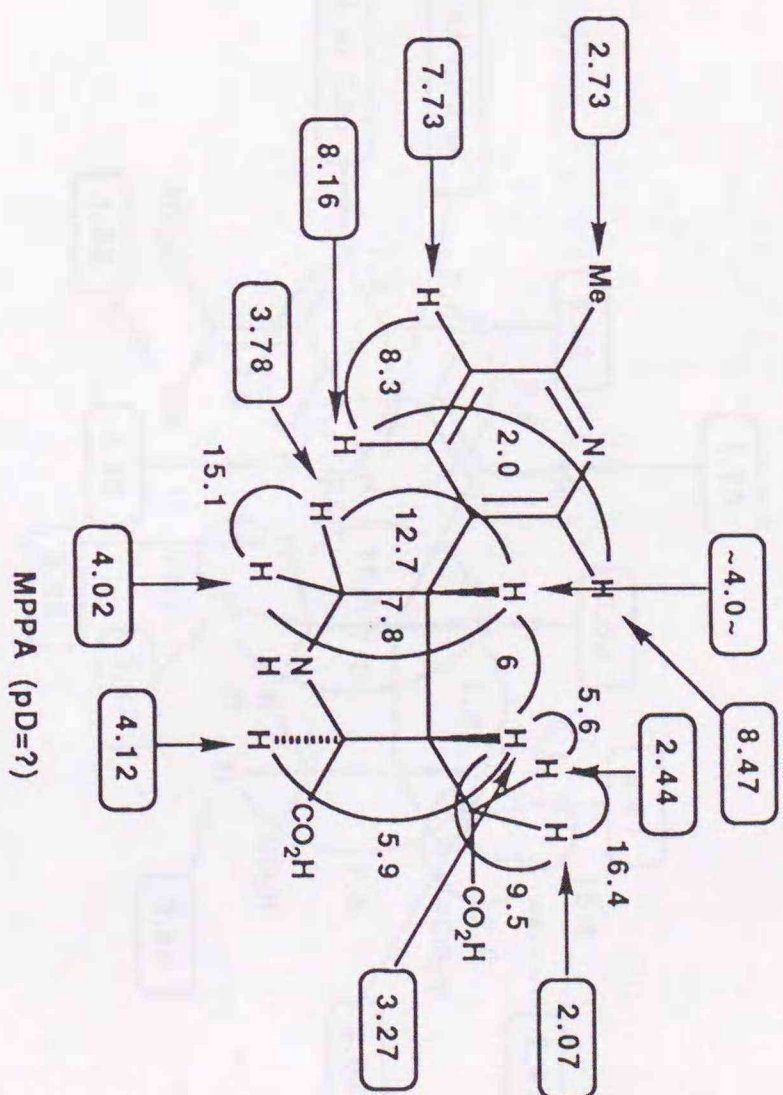
Fig 23 Some kainoids-3



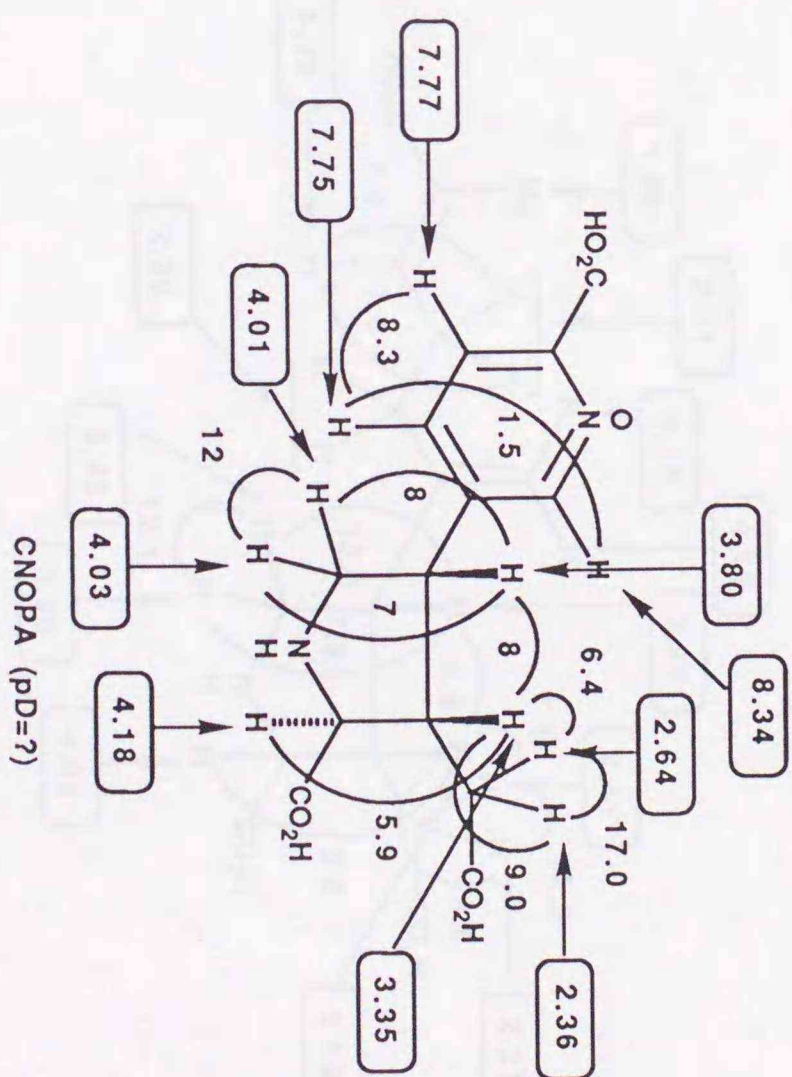
acromelic acid E (pD=?)



HMPPA (pD=?)

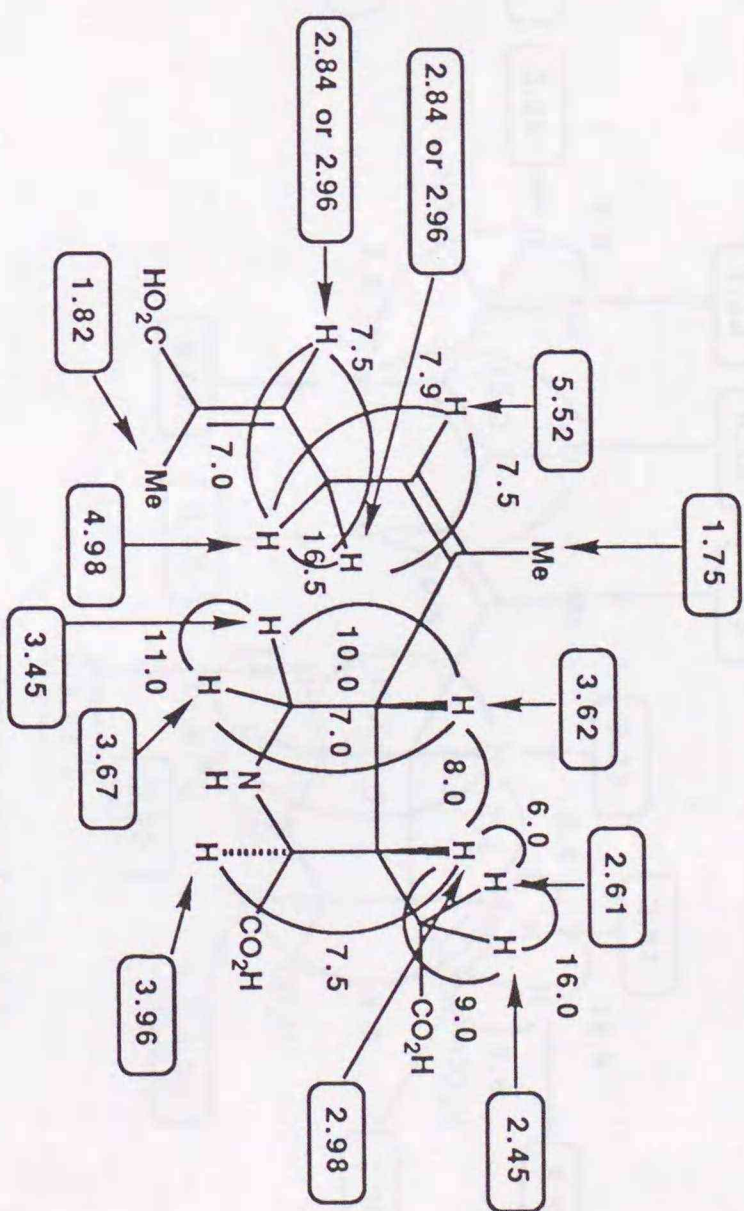
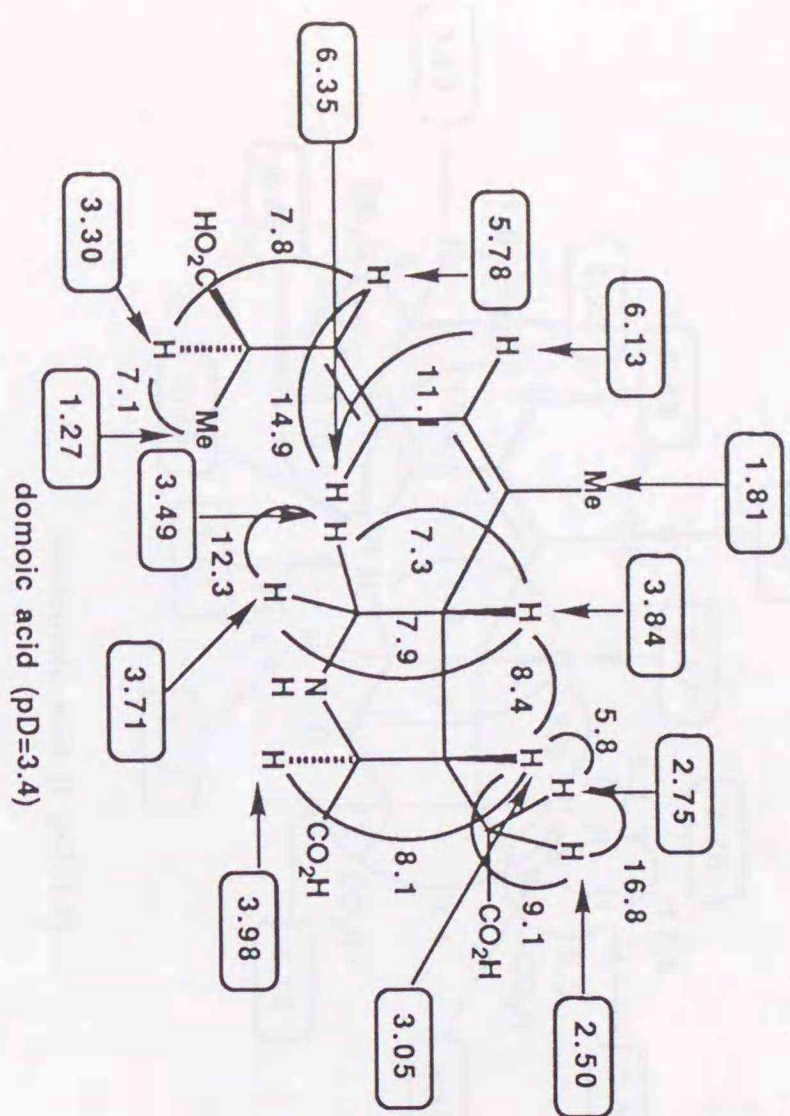


MPPA (pD=?)

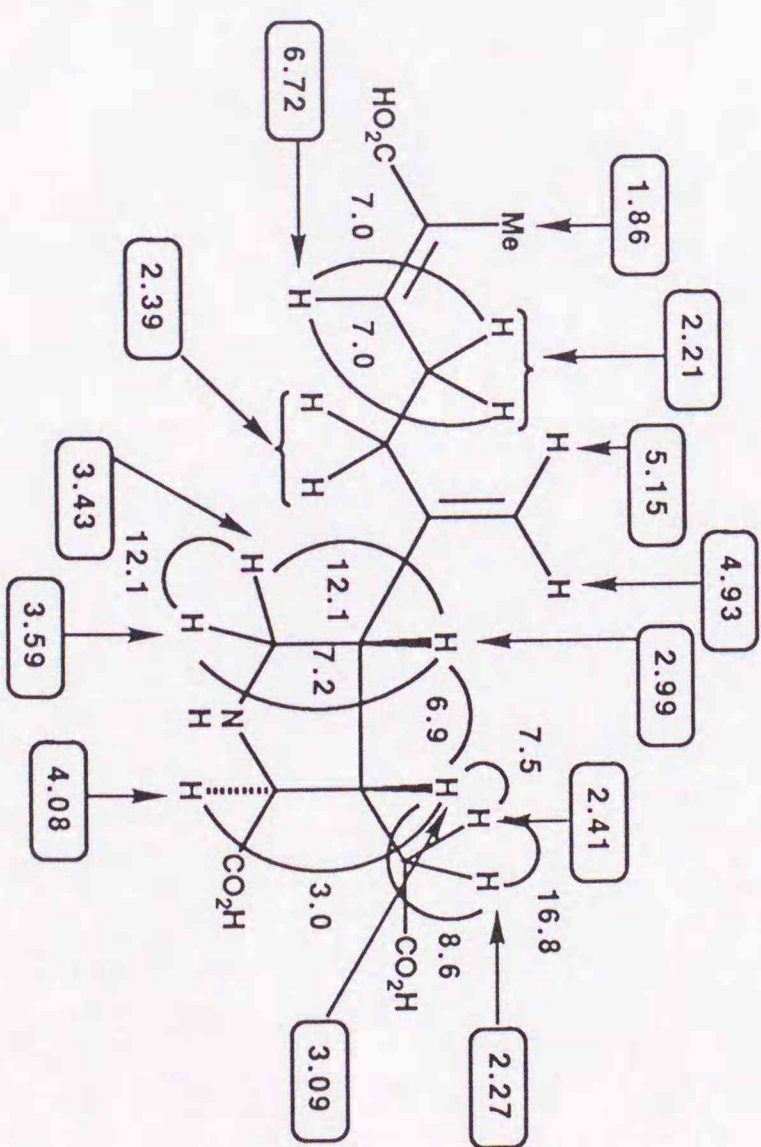
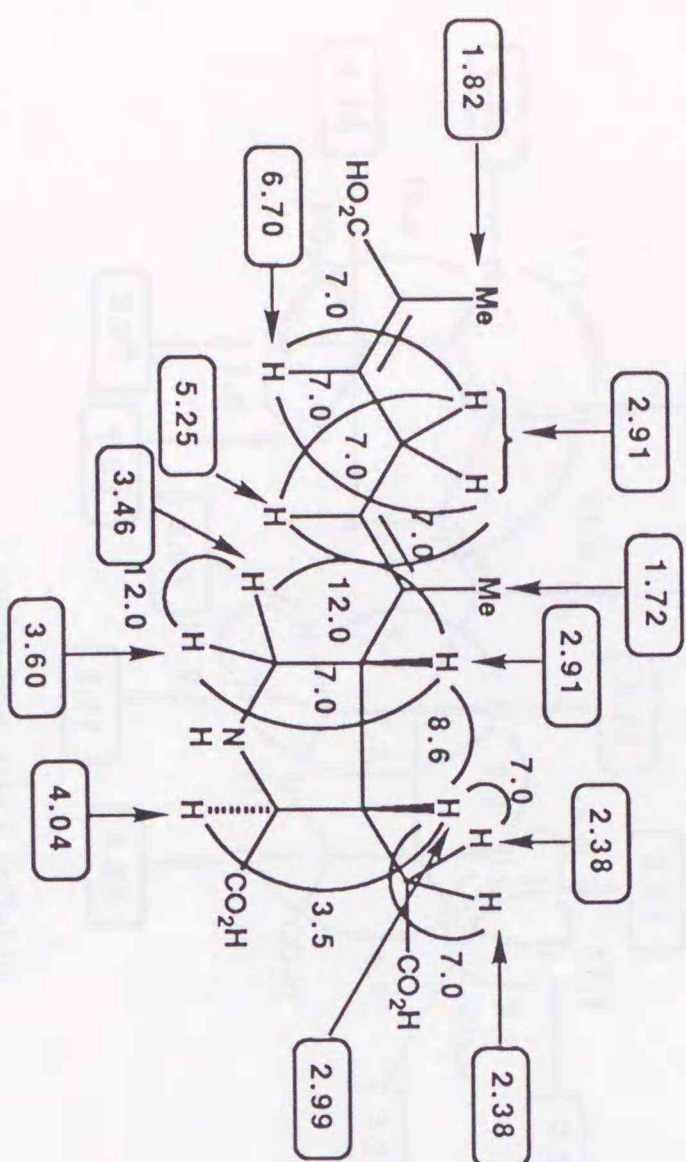


CNOPA (pD=?)

Fig 24 Some kainoids-4

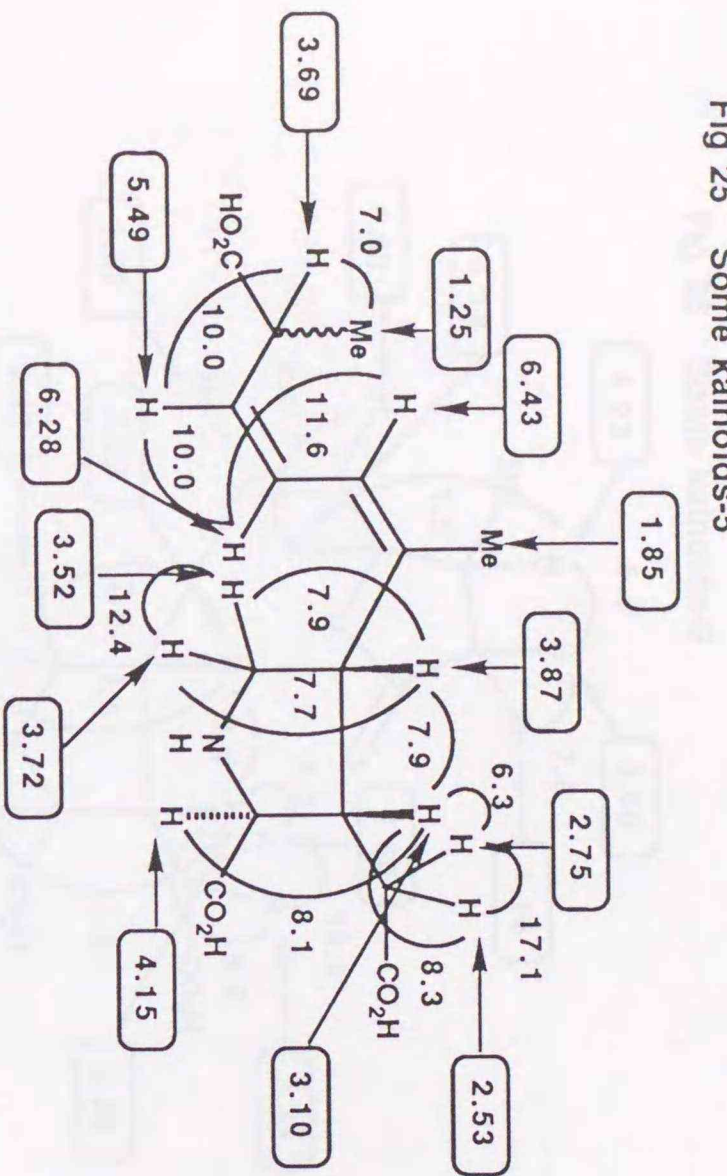


isodomoic acid A (pD=?)

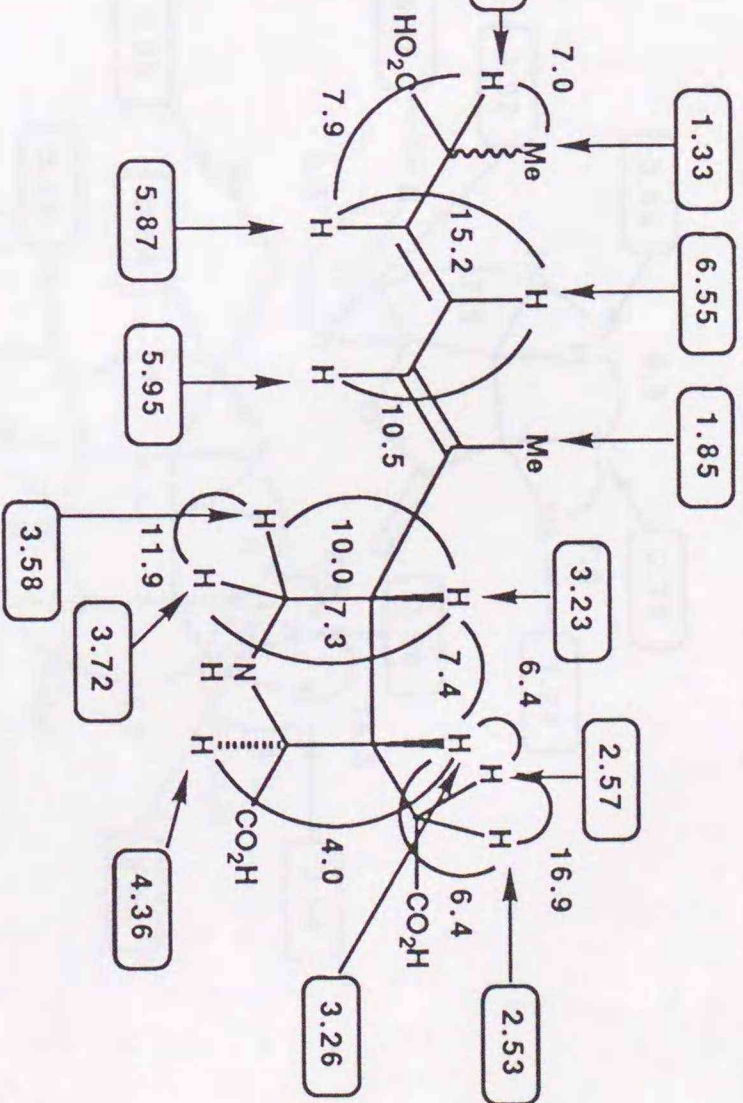


isodomoic acid C (pD=?)

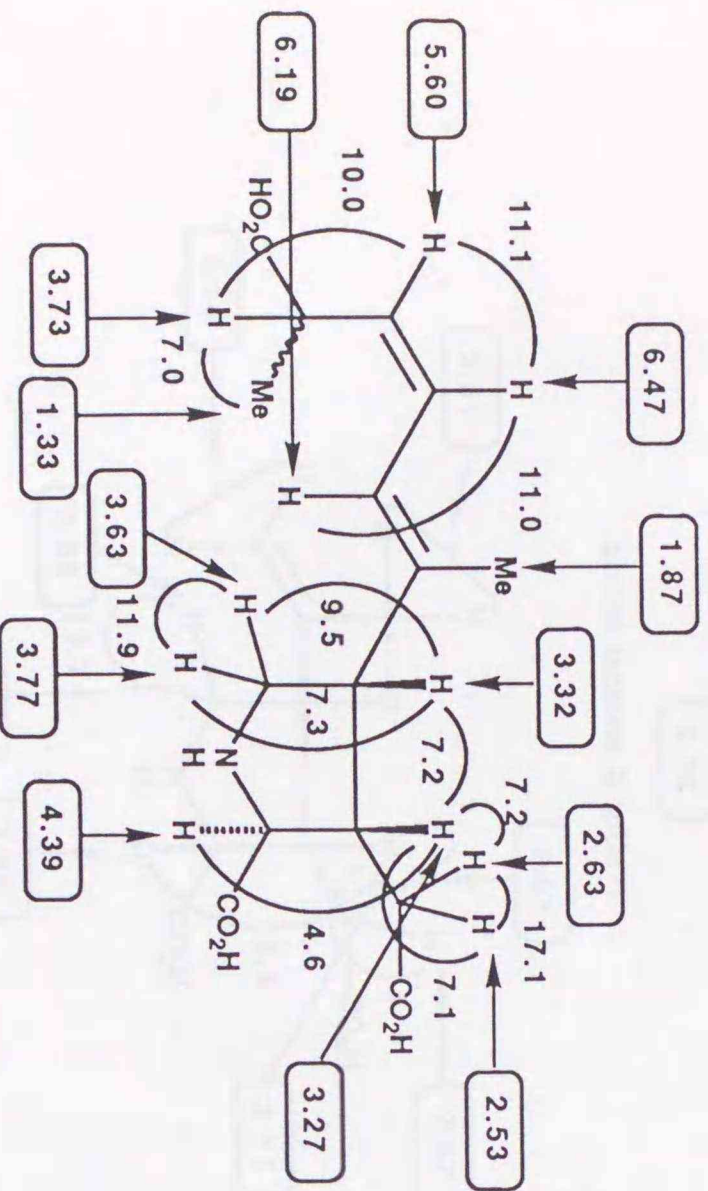
Fig 25 Some kainoids-5



isodomoic acid D (pD=1.9)



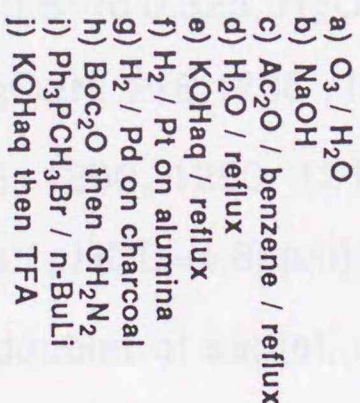
isodomoic acid E (pD=1.8)



isodomoic acid F (pD=1.6)



Fig 27



Experimental

Reduction of isopropenyl group

To a solution of kainic acid (100 mg, 0.43 mmol) in water (5 ml) at room temperature was added 10% Pd on charcoal (50 mg, 0.05 mmol) and the mixture was stirred under hydrogen overnight. The mixture was filtered and the filtrate was evaporated. The crude product was recrystallized from water to yield dihydrokainic acid (75mg, 74%).

β -kainic acid was reduced using Pt on alumina as a catalyst, otherwise this compound was epimerized at C4 to give an 1:1 mixture of isomers. Other kainic acid derivatives (allokainic and β -allokainic acids) were reduced in the same manner as the reduction of kainic acid.

Dihydrokainic acid

$[\alpha]_D -40.6^\circ$ (c 0.325, H₂O) ; FAB-HR-MS, m/z 216.1246 [(M+H)]⁺ calcd for C₁₀H₁₈O₄N, 216.1236 ; IR (nujol) 3100, 2920, 1730, 1625, 1470, 1460, 1385, 1335, 1290, 1250, 1210, 1180, 885, 870 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=3.1, HOD=4.8ppm) 0.91 (3H, d, J=6.5Hz), 0.95 (3H, d, J=6.5Hz), 1.60 (1H, doublet of septet, J=12, 6.5Hz), 1.98 (1H, ddt, J=5.5, 8, 12Hz), 2.26 (1H, dd, J=12, 16Hz), 2.68 (1H, dd, J=4.5, 12Hz), 3.01 (1H, dddd, 1, 4.5, 5.5, 12Hz), 3.08 (1H, t, J=12Hz), 3.65 (1H, dd, J=8, 12Hz), 4.14 (1H, d, J=1Hz).

Dihydroallokainic acid

mp. 251~254°C ; $[\alpha]_D 20.8^\circ$ (c 0.240, H₂O) ; FAB-HR-MS 216.1226 [(M+H)]⁺ calcd for C₁₀H₁₈O₄N, 216.1236 ; IR (nujol) 2900, 1715, 1630, 1595, 1460, 1380, 1315, 1270, 1220, 955, 885 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=3.4, HOD=4.8ppm) 0.91 (3H, d, J=6.8Hz), 0.98 (3H, d, J=6.8Hz), 1.77 (1H, octet, J=6.8Hz), 2.09 (1H, dddd, J=6.3, 6.8, 7.8, 9.3Hz), 2.63 (1H,

quintet, $J=6.3\text{Hz}$), 2.75 (2H, d, $J=6.3\text{Hz}$), 3.23 (1H, dd, $J=9.3, 12.0\text{Hz}$), 3.54 (1H, dd, $J=7.8, 12.0\text{Hz}$), 4.00 (1H, d, $J=6.3\text{Hz}$).

Dihydro- β -kainic acid

mp. $232\sim 234^{\circ}\text{C}$; $[\alpha]_{\text{D}} -25.7^{\circ}$ (c 0.140, H_2O) ; FAB-HR-MS 216.1223 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_{10}\text{H}_{18}\text{O}_4\text{N}$, 216.1236 ; IR (nujol) 3600, 3140, 2920, 1695, 1465, 1380, 1295, 1245, 1175, 1050, 935, 785, 735 cm^{-1} ; ^1H NMR (400MHz, D_2O , $\text{pD}=3.4$, $\text{HOD}=4.8\text{ppm}$) 0.92 (3H, d, $J=6.4\text{Hz}$), 0.96 (3H, d, $J=6.4\text{Hz}$), 1.65 (1H, doublet of septet, $J=11.0, 6.4\text{Hz}$), 2.21 (1H, dddd, $J=5.5, 8.3, 11.0, 12.0\text{Hz}$), 2.23 (1H, dd, $J=7.6, 16.8\text{Hz}$), 2.51 (1H, dd, $J=4.6, 16.8\text{Hz}$), 3.10 (1H, t, $J=12.0\text{Hz}$), 3.15 (1H, dddd, $J=4.6, 5.5, 5.9, 7.6\text{Hz}$), 3.53 (1H, dd, $J=8.3, 12.0\text{Hz}$), 4.23 (1H, d, $J=5.9\text{Hz}$).

Dihydro- β -allokainic acid

$[\alpha]_{\text{D}} 3.50^{\circ}$ (c 0.200, H_2O) ; FAB-HR-MS 216.1220 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_{10}\text{H}_{18}\text{O}_4\text{N}$, 216.1236 ; IR (nujol) 2910, 1715, 1615, 1465, 1380 cm^{-1} ; ^1H NMR (400MHz, D_2O , $\text{pD}=3.4$, $\text{HOD}=4.8\text{ppm}$) 0.92 (3H, d, $J=6.8\text{Hz}$), 0.99 (3H, d, $J=6.8\text{Hz}$), 1.82 (1H, octet, $J=6.8\text{Hz}$), 2.27 (1H, dddd, $J=6.8, 7.5, 8.1, 9.0\text{Hz}$), 2.49 (1H, dd, $J=7.6, 16.6\text{Hz}$), 2.61 (1H, dd, $J=6.4, 16.6\text{Hz}$), 2.79 (1H, dddd, $J=6.4, 7.5, 7.6, 8.8\text{Hz}$), 3.14 (1H, dd, $J=9.0, 12.0\text{Hz}$), 3.62 (1H, dd, $J=8.1, 12.0\text{Hz}$), 4.27 (1H, d, $J=8.8\text{Hz}$).

Ozonolysis of isopropenyl group

A solution of kainic acid (3 g, 13 mmol) in water (180 ml) was bubbled with ozone gas for an hour and then with oxygen gas for 10 min and the solution was evaporated at $20\sim 30^{\circ}\text{C}$. The residue was recrystallized from water to yield ketone (2.7 g, 89%). Other ketokainic acid derivatives were also prepared from corresponding isopropenyl compounds as well as kainic acid

Ketokainic acid

mp. $>300^{\circ}\text{C}$ (decomp) ; $[\alpha]_{\text{D}} 69.3^{\circ}$ (c 0.270, H_2O) ; FAB-HR-MS 216.0867 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_9\text{H}_{14}\text{O}_5\text{N}$, 216.0872 ; IR (nujol) 3080, 2940, 2860, 1720, 1705, 1645, 1470, 1415, 1380, 1310, 1255, 1200 cm^{-1} ; $^1\text{HNMR}$ (400MHz, D_2O , $\text{pD}=3.0$, $\text{HOD}=4.8\text{ppm}$) 2.31, (3H, s), 2.54 (1H, dd, $J=9.5$, 17Hz), 2.76 (1H, dd, $J=6$, 17Hz), 3.16 (1H, dq, $J=9$, 6.5Hz), 3.57 (1H, dd, $J=6.5$, 11.5Hz), 3.60 (1H, dd, $J=6.5$, 11.5Hz), 3.82 (1H, q, $J=6.5\text{Hz}$), 4.02 (1H, d, $J=6.5\text{Hz}$).

Alloketokainic acid

mp. $>300^{\circ}\text{C}$ (decomp) ; $[\alpha]_{\text{D}} -18.8^{\circ}$ (c 0.240, H_2O) ; FAB-HR-MS 216.0870 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_9\text{H}_{14}\text{O}_5\text{N}$, 216.0872 ; IR (nujol) 3200, 2930, 2860, 1740, 1715, 1615, 1460, 1420, 1395, 1360, 1315, 1240, 1225, 1195, 1170, 1085, 880 cm^{-1} ; $^1\text{HNMR}$ (400MHz, D_2O , $\text{pD}=3.1$, $\text{HOD}=4.8\text{ppm}$) 2.30 (3H, s), 2.73 (1H, dd, $J=9$, 17Hz), 2.88 (1H, dd, $J=5$, 17Hz), 3.04 (1H, quintet, $J=7\text{Hz}$), 3.42 (1H, q, $J=6\text{Hz}$), 3.62 (1H, dd, $J=6$, 13Hz), 3.67 (1H, dd, $J=8$, 13Hz), 3.97 (1H, D, $J=7\text{Hz}$).

β -Ketokainic acid

$[\alpha]_{\text{D}} 32.3^{\circ}$ (c 0.220, H_2O) ; FAB-HR-MS 216.0879 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_9\text{H}_{14}\text{O}_5\text{N}$, 216.0872 ; IR (nujol) 2900, 1715, 1615, 1460, 1380, 1170, 725 cm^{-1} ; $^1\text{HNMR}$ (400MHz, D_2O , $\text{pD}=2.7$, $\text{HOD}=4.8\text{ppm}$) 2.32 (3H, s), 2.47 (2H, d, $J=6.8\text{Hz}$), 3.48 (1H, dt, $J=6.5$, 6.8Hz), 3.51 (1H, dd, $J=8.3$, 12.7Hz), 3.68 (1H, dd, $J=9.3$, 12.7Hz), 3.89 (1H, ddd, $J=6.5$, 8.3, 9.3Hz), 4.40 (1H, d, $J=6.8\text{Hz}$).

β -Alloketokainic acid

$[\alpha]_{\text{D}} -16.6^{\circ}$ (c 0.350, H_2O) ; FAB-HR-MS 216.0888 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_9\text{H}_{14}\text{O}_5\text{N}$, 216.0872 ; IR (nujol) 2910, 1715, 1635, 1455, 1380, 1175 cm^{-1}

; ^1H NMR (400MHz, D_2O , $\text{pD}=2.0$, $\text{HOD}=4.8\text{ppm}$) 2.35 (3H, s), 2.60 (1H, dd, $J=8.1, 17.1\text{Hz}$), 2.65 (1H, dd, $J=6.9, 17.1\text{Hz}$), 3.20 (1H, dddd, $J=5.5, 6.9, 7.8, 8.1\text{Hz}$), 3.50 (1H, ddd, $J=5.5, 5.9, 8.3\text{Hz}$), 3.58 (1H, dd, $J=5.9, 12.2\text{Hz}$), 3.72 (1H, dd, $J=8.3, 12.2\text{Hz}$), 4.33 (1H, d, $J=7.8\text{Hz}$).

Kainic inversion (Inversion of configuration at C2-position of 2,3-trans kainoid to 2,3-cis compound through intramolecular acid anhydride)

A suspension of kainic acid (2.0 g, 8.7 mmol) in benzene (16 ml) and acetic anhydride (4.0 ml) was stirred at refluxing temperature under argon for 3 hours and the resulting solution was evaporated. The residue was recrystallized from acetic anhydride to yield anhydride with (R)-configuration at C2 (1.6g, 78%).

The anhydride (700 mg, 3.0 mmol) was dissolved in water (7.0 ml) and the solution was stirred at refluxing temperature for an hour. The solution was evaporated and the residue was recrystallized from water to give dicarboxylic acid (500 mg, 66%). The resulting acetamide (300 mg, 1.2 mmol) was dissolved in 1N potassium hydroxide (15 ml) and the solution was stirred at refluxing temperature overnight. The cooled solution was evaporated and passed through Dowex 50Wx8 (H^+ form, H_2O -5% NH_4OH) to give an ammonium salt. The solution of this salt was then passed through Amberlite IRC-50 (H^+ form, H_2O) to give salt-free amino acid. This was further purified by recrystallization from water to give β -kainic acid (130 mg, 52%). β -Allokainic acid was also prepared in the same manner as the preparation of β -kainic acid starting from allokainic acid.

β -Kainic acid

mp. $241\sim 243^\circ\text{C}$ (decomp) ; $[\alpha]_{\text{D}} 44.0^\circ$ (c 0.275, H_2O) ; FAB-HR-MS 214.1075 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}$, 214.1079 ; IR (nujol) ; 3540,

3140, 2920, 1695, 1620, 1570, 1425, 1380, 1335, 1275, 1235, 1205, 1185, 910, 790, 750 cm^{-1} ; ^1H NMR (400MHz, D_2O , $\text{pD}=3.3$, $\text{HOD}=4.8\text{ppm}$) 1.79 (3H, s), 2.23 (1H, dd, $J=6, 17\text{Hz}$), 2.35 (1H, dd, $J=7, 17\text{Hz}$), 3.17 (1H, quintet, $J=6\text{Hz}$), 3.31 (1H, dt, $J=12, 6\text{Hz}$), 3.46 (1H, t, $J=12\text{Hz}$), 3.52 (1H, dd, $J=8, 12\text{Hz}$), 4.36 (1H, d, $J=6\text{Hz}$), 5.07 (2H, s).

β -Allokainic acid

mp. $226\sim 228^\circ\text{C}$; $[\alpha]_{\text{D}} -21.7^\circ$ (c 0.175, H_2O) ; FAB-HR-MS 214.1066 $[(\text{M}+\text{H})]^+$ calcd for $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}$, 214.1079 ; IR (nujol) ; 3300, 3200, 2920, 1735, 1610, 1570, 1455, 1420, 1330, 1260, 1210, 1195, 1170, 1140, 935, 910, 860, 775 cm^{-1} ; ^1H NMR (400MHz, D_2O , $\text{pD}=3.3$, $\text{HOD}=4.8\text{ppm}$) 1.73 (3H, s), 2.44 (1H, dd, $J=9, 17\text{Hz}$), 2.53 (1H, dd, $J=6, 17\text{Hz}$), 2.74 (1H, dt, $J=8.5, 10.5\text{Hz}$), 2.87 (1H, ddt, $J=6, 10.5, 9\text{Hz}$), 3.25 (1H, dd, $J=10.5, 12\text{Hz}$), 3.65 (1H, dd, $J=8.5, 12\text{Hz}$), 4.32 (1H, d, $J=9\text{Hz}$), 4.97 (1H, brs), 5.02 (1H, t, $J=1.5\text{Hz}$).

Methyl (2S, 3S, 4S)-4-acetyl-1-tert-butyloxycarbonyl-2-methoxycarbonyl-3-pyrrolidine acetate

To a solution of alloketo-kainic acid (344 mg, 1.6 mmol) in 1N sodium hydrogen carbonate (4.0 ml) at room temperature was added a solution of di-tert-butyl carbonate (419 mg, 1.9 mmol) in 1,4-dioxane (4.0 ml) and the mixture was stirred overnight. The solution was acidified to pH2 and extracted with ethyl acetate (30 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in methanol and to this solution an ethereal diazomethane was added. The solvents were evaporated and the residue was chromatographed on silica gel (20-40% ethyl acetate-hexane) to give dimethyl ester (465 mg, 85%) as an oil. $[\alpha]_{\text{D}} -14.2^\circ$ (c 1.067, CHCl_3) ; EI-HR-MS, m/z 343.1649 $(\text{M})^+$ calcd for $\text{C}_{16}\text{H}_{25}\text{O}_7\text{N}$, 343.1632 ; IR (neat)

3000, 1750, 1710, 1485, 1445, 1410, 1370, 1260, 1210, 1165, 1135, 1005, 905, 865, 775 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.39 (ca6H, s), 1.45 (ca3H, s), 2.19 (3H, s), 2.45~2.72 (2H, m), 2.96~3.18 (2H, m), 3.47 (ca0.5H, t, $J=9.8\text{Hz}$), 3.51 (ca0.5H, t, $J=9.8\text{Hz}$), 3.66 (3H, s), 3.73 (3H, s), 3.8~4.0 (1H, m), 3.96~4.11 (1H, m).

Methyl (2S, 3S, 4R)-1-tert-butyloxycarbonyl-2-methoxycarbonyl-4-(2-propenyl)pyrrolidine-3-acetate (Dimethyl N-t-Boc-allocainate)

To a suspension of methyltriphenylphosphonium bromide (624 mg, 1.75 mmol) in toluene at room temperature under argon was added n-butyl lithium (1.60 M in hexane, 1.1 ml, 1.76 mmol) and the mixture was stirred for 10 min. The resulting yellow solution was cooled to -15°C and to this solution was added a solution of ketone (300 mg, 0.87 mmol) in toluene (5 ml) and stirred for 30 min. The reaction was quenched with aqueous ammonium chloride and the mixture was extracted with ethyl acetate 930 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was purified by silicagel chromatography to give olefin (370 mg, 60%) as an oil. $[\alpha]_D -27.9^\circ$ (c 0.95, CHCl_3) ; EI-HR-MS, m/z 341.1871 (M^+) calcd for $\text{C}_{17}\text{H}_{27}\text{O}_6\text{N}$, 341.1838 ; IR (neat) 2960, 1750, 1700, 1485, 1410, 1370, 1335, 1160, 1130, 905, 865, 765 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.39 (ca6H, s), 1.44 (ca3H, s), 1.71 (3H, s), 2.38~2.74 (4H, m), 3.32 (1H, t, $J=10.4\text{Hz}$), 3.63 (3H, s), 3.72 (ca2H, s), 3.73 (ca1H, s), 3.90 (ca0.5H, d, $J=8.5\text{Hz}$), 3.96 (ca0.5H, d, $J=8.5\text{Hz}$), 4.84 (1H, s), 4.88 (1H, s).

Allokainic acid

To a solution of diester (170 mg, 0.50 mmol) in ethanol (3.5 ml) at room temperature was added 1N potassium hydroxide (3.5 ml) and the mixture

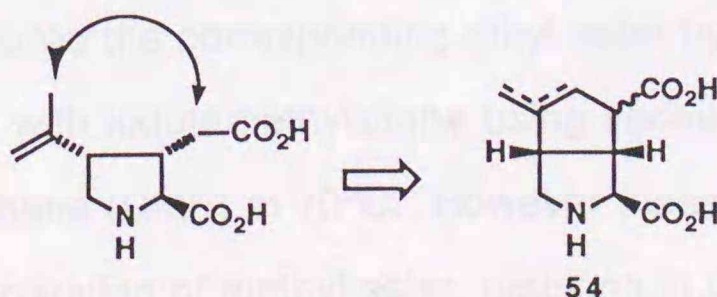
was stirred overnight. The solution was acidified to pH2 with 1N hydrochloric acid and extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in trifluoroacetic acid (TFA, 3.2 ml) at room temperature under argon and the mixture was stirred for 30 min. The solution was evaporated and the resulting TFA salt was passed through Dowex 50Wx8 (H⁺ form, H₂O-NH₄OH) to give ammonium salt. This salt was then passed through Amberlite IRC-50 (H⁺ form, H₂O) to give salt-free amino acid. The amino acid was further purified by recrystallization from water to give allokainic acid (70 mg, 65%). mp. 235~237°C ; [α]_D 11.0° (c 0.100, H₂O) ; FAB-HR-MS 214.1075 [(M+H)]⁺ calcd for C₁₀H₁₆O₄N, 214.1079 ; IR (nujol) 3580, 3160, 2940, 1730, 1700, 1630, 1580, 1460, 1400, 1325, 1280, 1200, 1170, 1100, 1055, 925, 845 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=4.1, HOD=4.8ppm) 1.72 (3H, s), 2.59 (1H, dd, J=7, 15Hz), 2.65 (1H, dddd, J=4, 7, 9, 11Hz), 2.77 (1H, dd, J=4, 15Hz), 2.88 (1H, dt, J=7.5, 11Hz), 3.32 (1H, t, J=11Hz), 3.52 (1H, dd, J=8, 11Hz), 3.92 (1H, d, J=9Hz), 4.98 (2H, s).

References

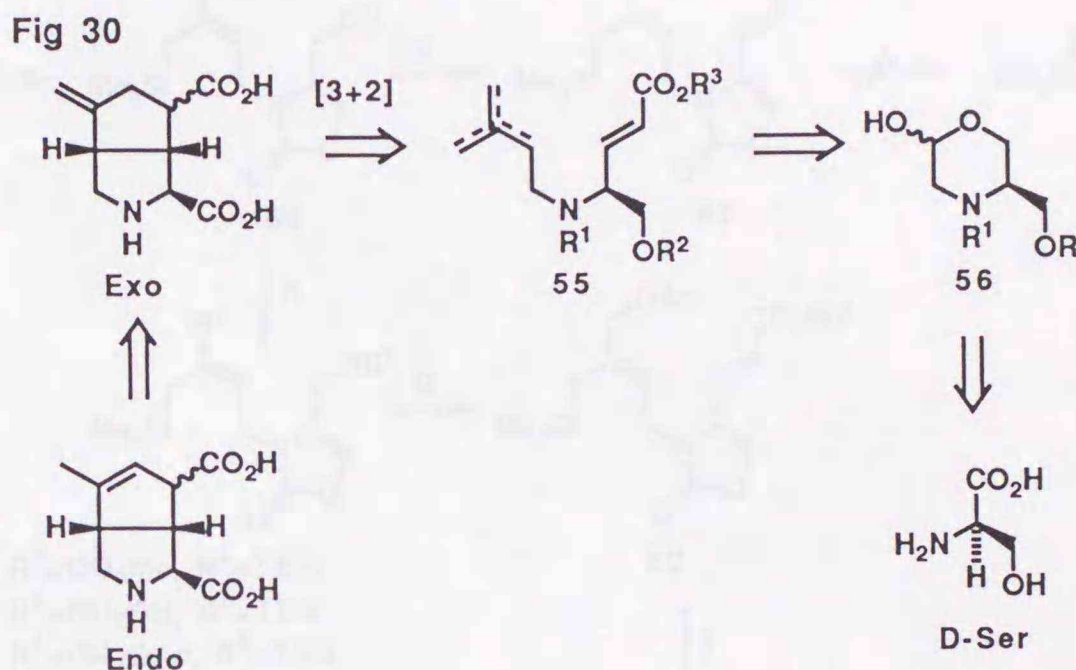
1. references 1 and 4 in the section of Introduction.
2. O. Goldberg, A. Luini and V. Teichberg, *Tetrahedron Lett.*, **21**, 2355 (1980).
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4. a) Y. Ueno, H. Nawa, J. Ueyanagi, H. Morimoto, R. Nakamori and T. Matsuoka, *J. Pharm. Soc. Japan*, **75**, 807 (1955).
b) H. Morimoto, *J. Pharm. Soc. Japan*, **75**, 901 (1955).
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5. a) reference 12 in the section of Introduction.
b) references 6 and 4a in Chapter 2.
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b) Y. Ueno, H. Nawa, J. Ueyenagi, H. Morimoto, R. Nakamori, T. Matsuoka, *J. Pharm. Soc. Japan*, **75**, 821 (1955).
c) R. Nakamori, *Proc. Jpn. Acad.*, **32**, 35 (1956).
7. references 2c and 2d in the section of Introduction

Chapter 4 Synthesis of conformationally restricted analogs of kainic acid

In Chapters 2 and 3, the mode of interactions of kainoid with its receptors were suggested. In these interactions, the C4 substituents of kainoid would play a role in neuroexcitation, that is, a direct interaction of the C4-substituent with its receptors and/or intramolecular regulation of the conformation of the glutamate moiety. The former mode of action was described in Chapter 2 as the donation of electrons (The strength of neuroexcitation depends on the HOMO energy of C4-substituents.) to its receptors, which would result in stronger depolarization. In the latter case, the polarity, bulkiness, and/or spatial arrangement of C4-substituents would affect the conformation of the internal glutamic acid moiety by restricting the conformation to a suitable one so that it would easily bind to kainate-type receptors. In order to clarify the mode of interaction between substrate and receptor, a new group of substrates was developed. The synthetic targets were bicyclo[3.3.0]octane derivatives **54**. These amino acids could be derived from kainic acid by linking the methyl of the isopropenyl group and the γ -methylene (glutamic acid numbering) of the glutamic acid part. Five-membered ring formation would restrict the spatial arrangement of both the γ -carboxylic acid and the C4-substituent. Syntheses of four isomers which have endo- or exo-type olefins with (R)-or (S)- γ -carboxyl groups, and testing of their biological activity would provide new information about the conformational requirement of the Glu-part and the effective position of the C4- π -electrons.



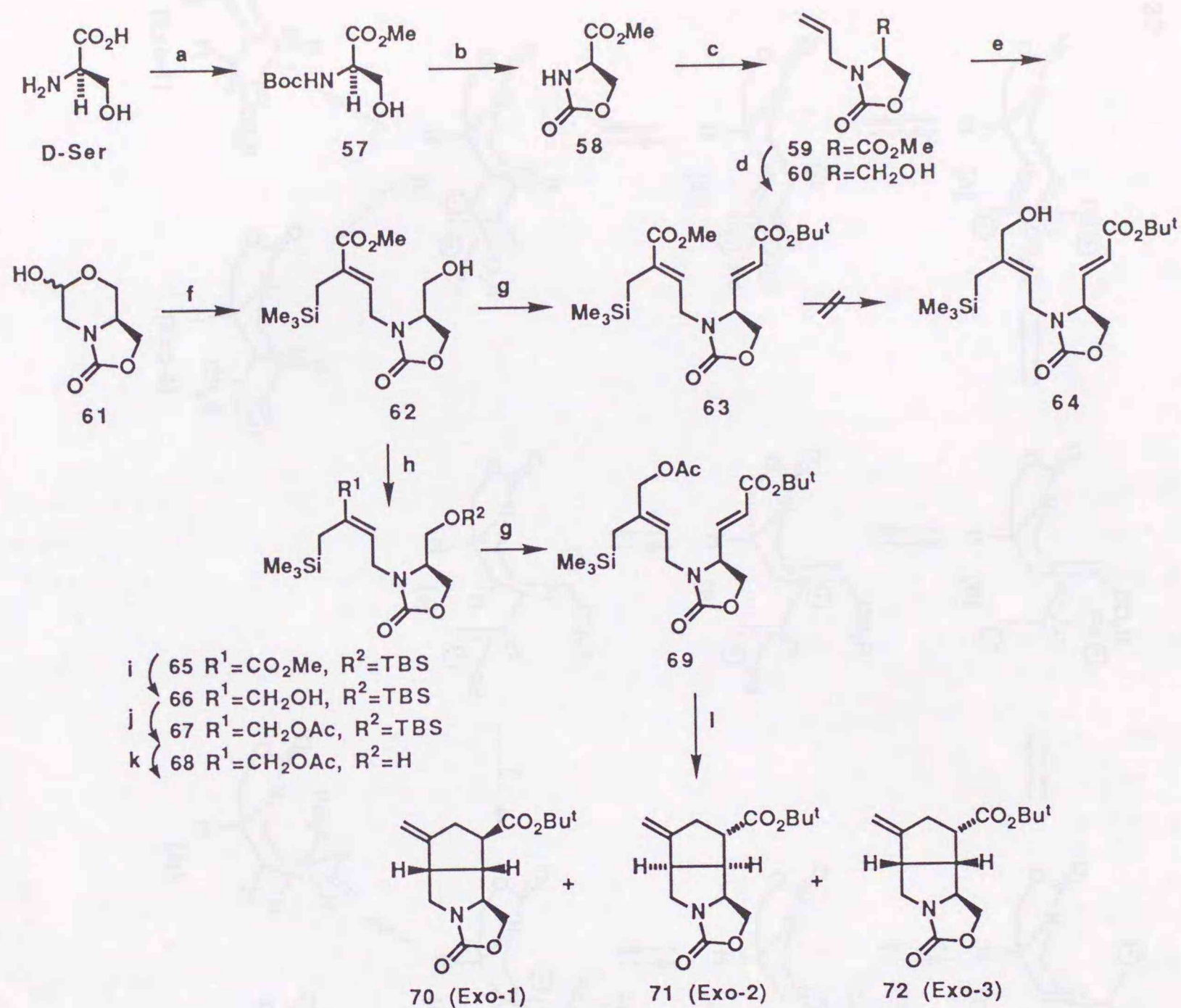
A retrosynthetic scheme is outlined in Fig. 30. Endo-isomers would be synthesized from Exo-isomers, and Exo-isomers would be constructed from **55** through [3+2] cyclization reaction. Further, **55** would be derived from lactol **56** by chain elongation, and the optically active lactol **56** would be synthesized from *D*-serine.



The amino group of *D*-serine was protected as a tert-butylcarbamate and the carboxylic acid was esterified with diazomethane (Fig. 31). The resulting alcohol **57** was reacted with thionyl chloride to afford cyclic carbamate **58**. Cyclic carbamate **58** was alkylated with allyl bromide and then reduced with sodium borohydride to give alcohol **60**. At this stage, no racemization occurred, which was confirmed by ^1H NMR measurement after conversion of the alcohol to the (R)-MTPA ester. The alcohol **60** was converted to lactol **61** by ozonolysis.

For the next Horner-Emmons reaction, trimethyl (2-trimethylsilylmethyl)phosphonoacetate **73** was prepared by a modified Hoffman method.¹ He prepared the corresponding ethyl ester by alkylation of triethyl phosphonoacetate with iodotrimethylsilane using sodium hydride as a base in 1,2-dimethoxyethane (DME) at 70°C. However these conditions were not suitable for the preparation of methyl ester, resulting in a low yield. Use of

Fig 31



(a) Boc₂O, NaHCO₃ then CH₂N₂ (b) SOCl₂ / THF (c) allyl bromide / NaH (d) NaBH₄ / MeOH
 (e) O₃ then DMS (f) (MeO)₂P(O)CH(CH₂TMS)CO₂Me / NaH (g) i) Swern ox
 ii) (MeO)₂P(O)CH₂CO₂Bu^t (h) TBSCl, imidazole (i) DIBAL, BF₃-Et₂O (j) Ac₂O, pyridine
 (k) CSA / ¹PrOH (l) Pd(OAc)₂, DIPHOS,BSA / m-xylene

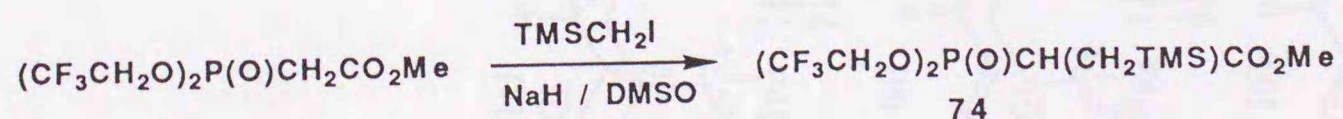
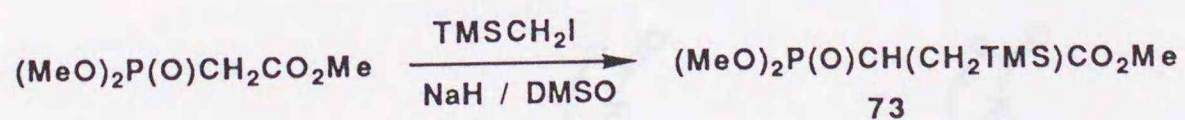


Fig 32

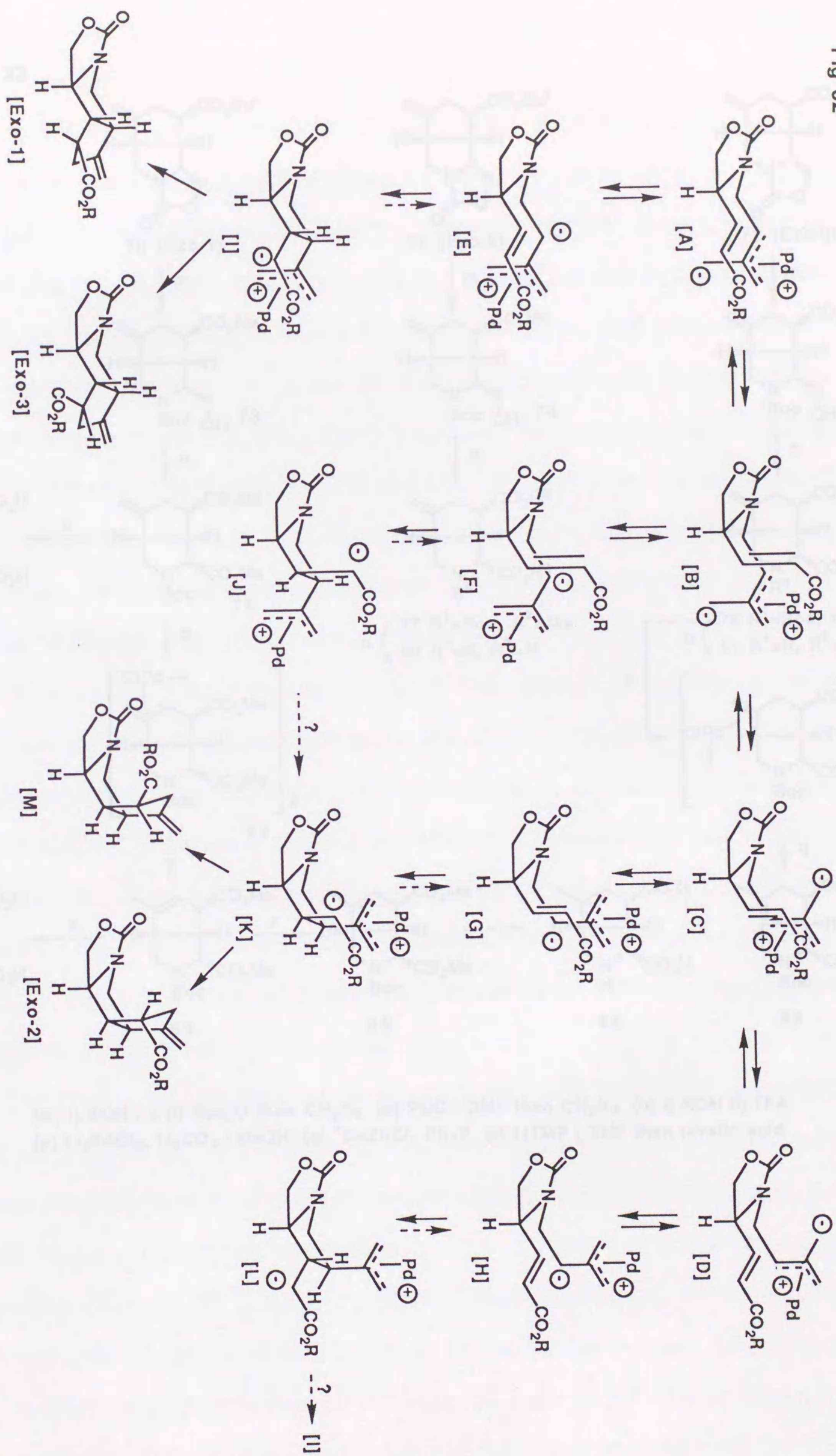
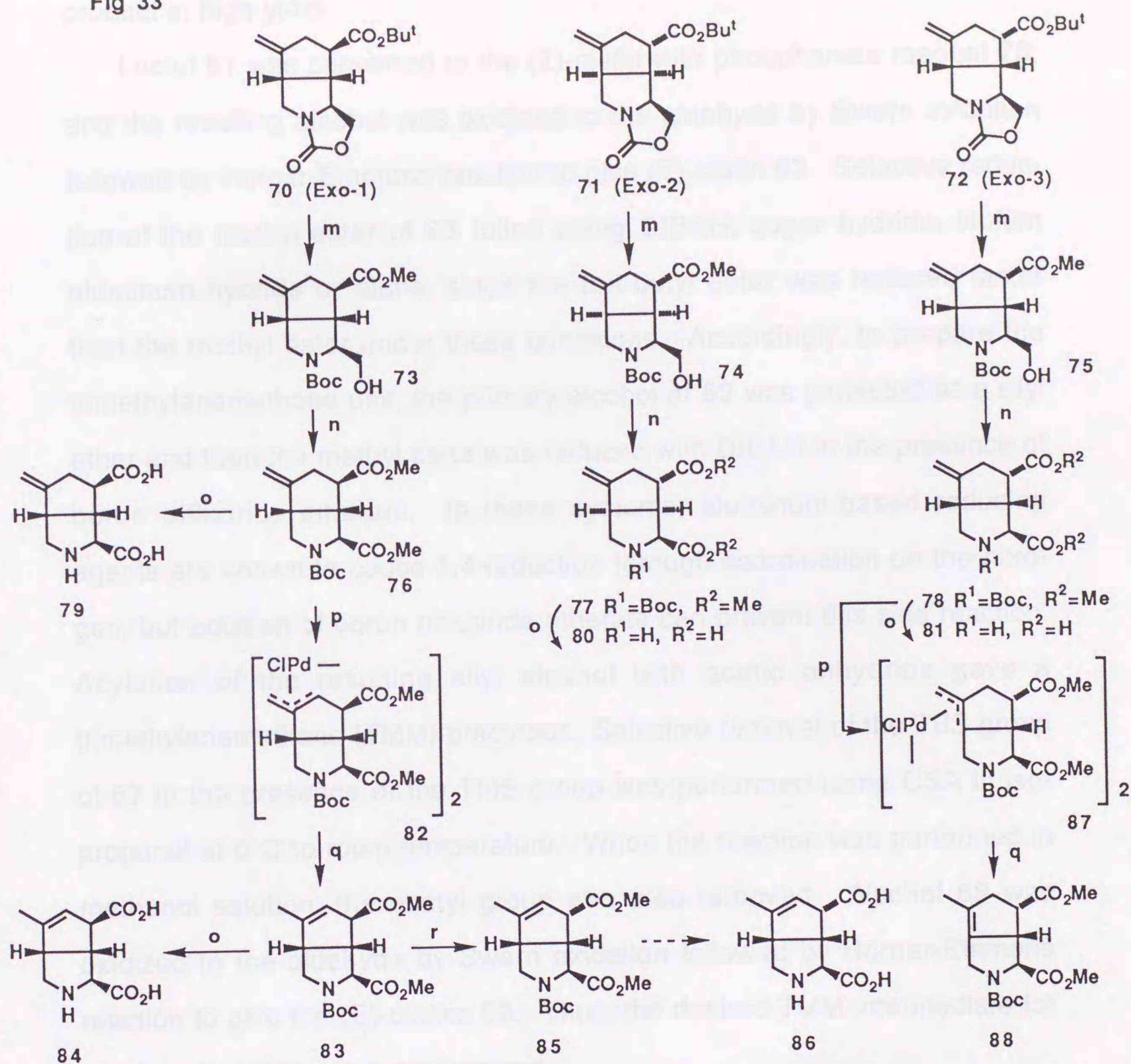


Fig 33



(m) i) KOH / Δ ii) Boc_2O then CH_2N_2 (n) PDC / DMF then CH_2N_2 (o) i) KOH ii) TFA
(p) $\text{Li}_2\text{PdCl}_4, \text{Li}_2\text{CO}_3 / \text{MeOH}$ (q) $n\text{BuZnCl}, \text{Ph}_3\text{P}$ (r) LiTMP / THF then pivalic acid

DMSO as a solvent instead of DME at room temperature affords the desired product in high yield.

Lactol **61** was converted to the (Z)-olefin with phosphonate reagent **73**, and the resulting alcohol was oxidized to the aldehyde by Swern oxidation followed by Horner-Emmons reaction to give (E)-olefin **63**. Selective reduction of the methyl ester of **63** failed using DIBAH, super hydride, lithium aluminum hydride or alane, since the tert-butyl ester was reduced faster than the methyl ester under these conditions. Accordingly, to prepare the trimethylenemethane unit, the primary alcohol of **62** was protected as a silyl ether and then the methyl ester was reduced with DIBAH in the presence of boron trifluoride etherate. In these systems, aluminum-based reducing agents are known to cause 1,4-reduction through coordination on the nitrogen, but addition of boron trifluoride etherate can prevent this side reaction. Acylation of the resulting allyl alcohol with acetic anhydride gave a trimethylenemethane (TMM) precursor. Selective removal of the TBS group of **67** in the presence of the TMS group was performed using CSA in isopropanol at 0°C to room temperature. When the reaction was performed in methanol solution, the acetyl group was also removed. Alcohol **68** was oxidized to the aldehyde by Swern oxidation followed by Horner-Emmons reaction to give the (E)-olefins **69**. Thus, the desired TMM intermediate for [3+2] cycloaddition was synthesized.

The [3+2] cycloaddition reaction, using the TMM precursor with olefin catalyzed by Pd(0) to produce a five-membered ring, was extensively investigated by Trost.² This reaction was applied to **69** in an intramolecular mode. Usually the intramolecular reaction occurs more easily than the intermolecular one, but **69** resisted cyclization even in refluxing toluene. This was probably due to the strain caused by the cyclic carbamate. The yield of this reaction has been known to increase as the temperature is elevated. m-xylene (bp. 138~139°C) was found to be a suitable solvent for this

reaction after all, and the use of mesitylene (bp. 162~164°C) resulted in decomposition of the substrate. Because this reaction was also known to be influenced by palladium reagent and its ligand, some reagents were tested. Tetrakis(triphenylphosphine)palladium(0) was not effective, but preparation of palladium(0) *in situ* using palladium(II) acetate with phosphine gave good results. The [3+2] cycloaddition reaction smoothly proceeded using 0.1 equivalent of palladium(II) acetate, 0.8 equivalent of diphenylphosphinoethane (DIPHOS) and 1 equivalent of N,O-bis(trimethylsilyl)-acetamide (BSA) to afford the cyclized products as a mixture of three stereoisomers, Exo-1 **70**, Exo-2 **71** and Exo-3 **72** in the ratio of 10:1:2 in 70% yield. Use of triisopropylphosphite as a ligand resulted in an increase in the yield of Exo-2 and Exo-3. To examine the effect of the geometry of the two olefins on this reaction, four isomers (E,E), (E,Z), (Z,E), (Z,Z) were exposed to these conditions but the product ratio was not changed. Incidentally, the (E)-isomer of the TMM unit was prepared by the corresponding Still's phosphonate reagent **74**³ (alkylation was performed in the same manner as those with trimethyl phosphonoacetate), and the (Z)-olefin acceptor was also synthesized with Still's reagent.

Trost stated "The characterization of the palladium-catalyzed trimethylenemethane cyclization as a stepwise but concerted-like reaction also provided a framework for rationalizing stereochemical results." and depicted "transition states for the cycloaddition of hydrindan substrates". Referring to this scheme, the following reaction course was suggested (Fig. 32). Four transition states for the cyclization to bicyclo[3.3.0]octanes are assumed and each of the transition states are surmised to have equilibrium-like A→E (E→A), B→F (F→B), C→G (G→C) and D→H (H→D). E and G will give cis bicyclo[3.3.0]octane systems through I and K, and F and H may give trans ones through J and L. In the present experiments, only cis compounds are obtained and Exo-1, Exo-2, and Exo-3 stem from I, K, and I,

respectively. Because the trans-bicyclo[3.3.0]octane system is fairly strained, the transition states of "concerted-like addition" to give trans-products may have a higher barrier. Further, the parallel arrangement (cis) of the positively charged trimethylenemethane moiety coordinated by palladium and the negatively charged acetic ester moiety may be stabilized. Thus only the cis system is obtained. The species E will give a cis-anti-cis tandem three five-membered ring system and G will give a cis-syn-cis system. The latter is more strained, and the former is easier to produce. The yield ratio, $(\text{Exo-1} + \text{Exo-3}) / \text{Exo-2} = (10 + 2) / 1$, clearly agrees with this explanation. Minute quantities of carboxylic ester group may rotate in the stage of I to give Exo-3. This rotation is probably effective for rationalizing that both of the substrates with (E)- and (Z)- α,β -unsaturated ester groups afford the same mixture of bicyclic compounds. Isomerization of J to K and L to I may be possible, because deprotonation may occur more easily at the bare portion of TMM because of the acidity of the appropriate proton.

Exo-1 **70**, Exo-2 **71** and Exo-3 **72** were hydrolyzed to the alcohol and individually reprotected with a Boc group (Fig. 33). Oxidation to the carboxylic acid, the usual deprotection [(i) 1N KOH, (ii) TFA], gave amino acids Exo-1 **79**, Exo-2 **80**, Exo-3 **81**. On the other hand, Endo-1 was prepared from Exo-1. The usual olefin isomerization reaction, such as with rhodium trichloride,⁴ tris(triphenylphosphine)ruthenium(II) chloride,⁵ bis(benzonitrile)palladium(II) chloride,⁶ CSA, on **76** was not successful in recovering the starting material. Accordingly, a two-step reaction, formation of the regioselective π -allyl complex followed by regioselective reduction of the complex, was applied to **76**. The desirable π -allyl complex was synthesized using lithium tetrachloropalladate with lithium carbonate in methanol,⁷ which was then regioselectively reduced with n-butylzinc chloride⁸ to afford Endo-1 **83**. Endo-1 **83** was deprotected by the same method as that for the Exo-series to give an amino acid **84**. This olefin

isomerization reaction was applied to Exo-3 **78**, but these reactions led to the undesirable Endo-product **88**. therefore, Endo-3 was synthesized from Endo-1 **83** by epimerization of the γ -ester (glutamic acid numbering). the corresponding enolate was formed with two equivalents of lithium 2,2,6,6-tetramethylpiperidide (LTMP) as a base at -78°C followed by kinetic protonation with pivalic acid at -100°C to afford the epimerized product **85** (Endo-3) in 60% yield along with recovery of Endo-1 **83** (6%). Endo-3 **85** was so easily epimerized at the γ -carbon by a weak base to reproduce Endo-1 that deprotection was not successful. The structures of Exo-1, Exo-2 and Exo-3 were determined by NOE experiments after conversion to appropriate compounds (Figs. 34 and 35).

Fig 34

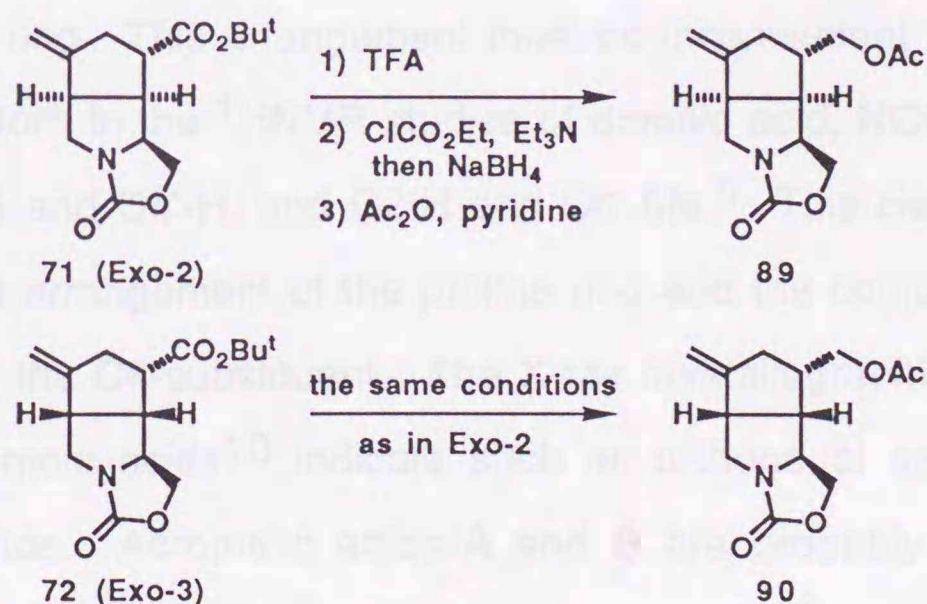
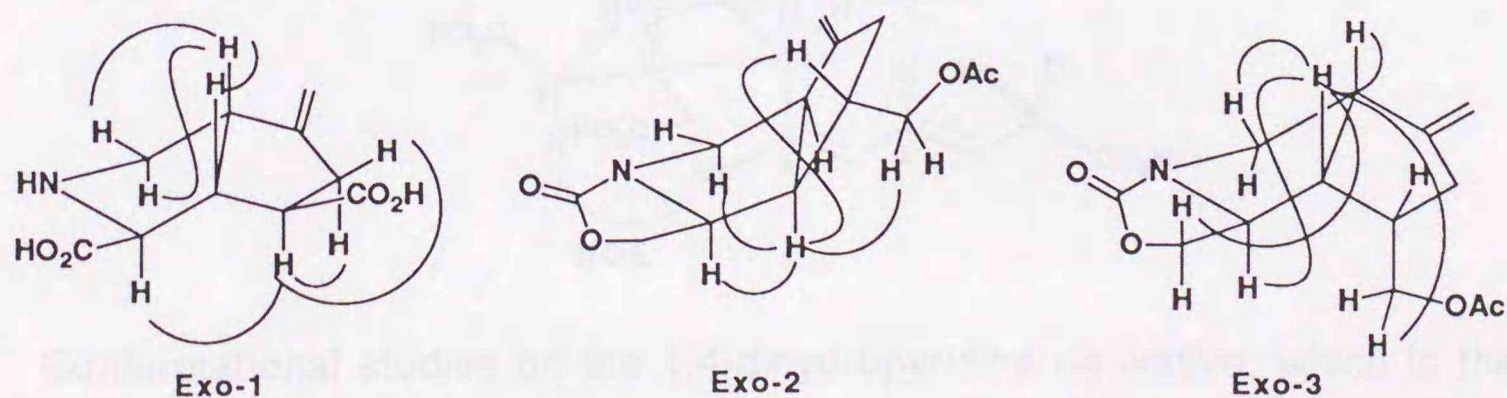


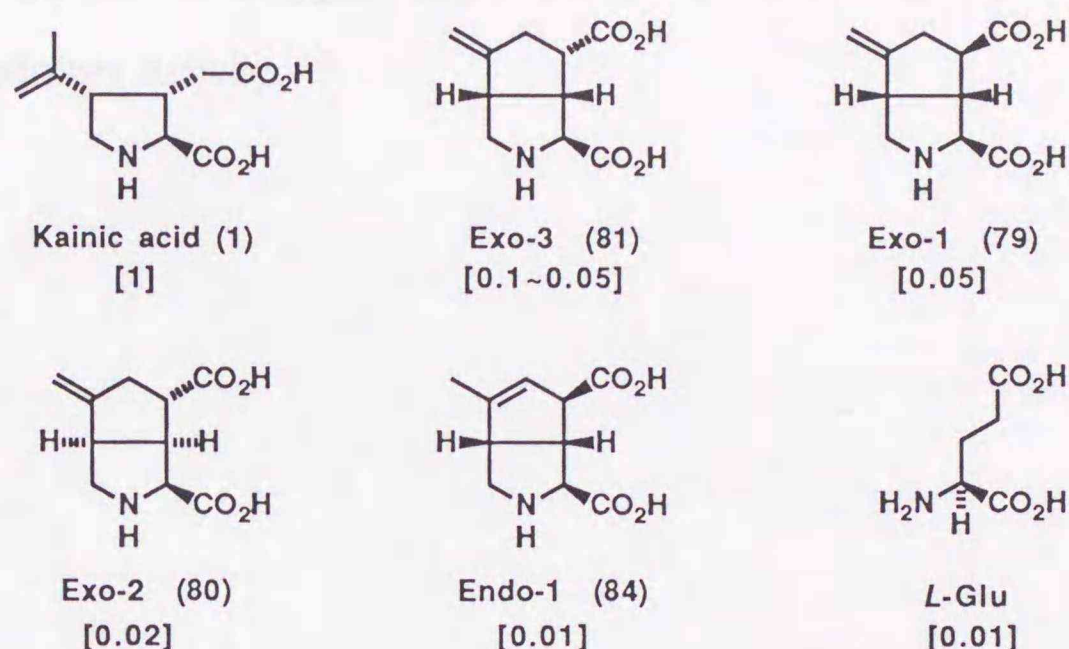
Fig 35 NOE experiments



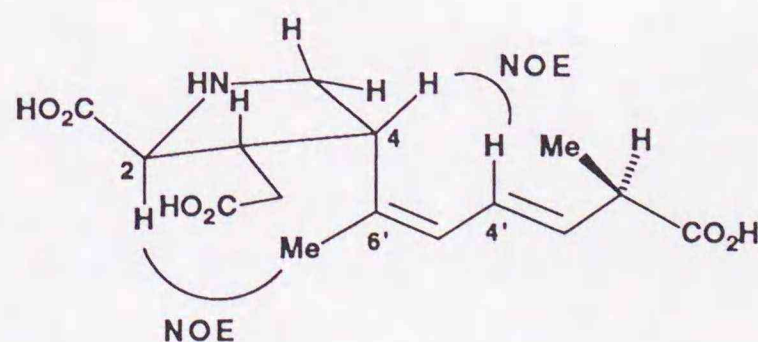
The depolarizing activity of these synthetic amino acids in new born rat spinal cord is shown in Fig 36. The number in parenthesis shows the activity relative to that of kainic acid as 1. All amino acids showed very weak

activity. These weak activities are probably mainly due to the fixed double bond plane of the isopropenyl group, because it is nearly in the same plane

Fig 36 Relative depolarizing activity in the isolated newborn rat spinal motoneuron [kainic acid =1]



as the proline ring. This arrangement must be inconvenient for interaction with the receptor. In the ^1H NMR studies of domoic acid, NOE is observed between C4-H and C4'-H, and C2-H and C6'-Me.⁹ This clearly indicates the orthogonal arrangement of the proline ring and the conjugated double bond plane of the C4-substituent. The X-ray crystallographic analyses of kainic and domoic acids¹⁰ indicate such an orthogonal arrangement in both compounds. Acromelic acids A and B are probably in the same situation.



Conformational studies on the 1,4-dihydropyridine derivative, which is the most effective calcium channel blocker,¹¹ shows that the orthogonal arrangement of two rings, the phenyl and dihydropyridine rings, is essential for pharmacological effect. The orthogonal arrangement is attributable to

allylic 1,3-strain. Such a situation should also be considered in the case of kainoid. To show pharmacological activity, orthogonal arrangement of the isopropenyl group relative to the proline ring is presumably essential. These experiments suggest the importance of C4- π -group orientation in neuroexcitatory activity.

Fig 37

* : 2~4Hz

Chemical structure of Exo-1 (pD=3.2) showing ^{13}C NMR chemical shifts (ppm) and coupling constants (Hz).

Chemical shifts (ppm):

- 2.73
- 2.89
- 3.02
- 3.27
- 3.36
- 3.51
- 3.71
- 4.00
- 4.5
- 4.5
- 4.9
- 5.06 and 5.17
- 5.5
- 5.9
- 8.3
- 8.5
- 12.0

Coupling constants (Hz):

- 2
- 2
- 6
- 8.3
- 12.0

Chemical structure labels: H , N , CO_2H .

Exo-1 (pD=3.2)



Experimental

Methyl (2*R*)-2-(*tert*-butyloxycarbonyl)amino-3-hydroxypropionate (57)

To a solution of *D*-serine (25 g, 238 mmol, >99%, $[\alpha]_D -13.5 \sim -15.5^\circ$, Wako) in 1N sodium hydrogen carbonate (250 ml, 250 mmol) was added a solution of *di-tert*-butylcarbonate (Boc_2O , 57 g, 261 mmol) in 1,4-dioxane (500 ml) and the mixture was stirred at room temperature overnight. The solution was acidified to pH 2 with 1N hydrochloric acid and then extracted with ethyl acetate (400 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in methanol and to this solution an ethereal diazomethane was added. Evaporation of the solvents gave ester (47 g, 90%) as an oil. $[\alpha]_D -10.2^\circ$ (c 1.00, CHCl_3); FAB-HR-MS, m/z 220.1193 $[(M+H)]^+$ calcd for $\text{C}_9\text{H}_{18}\text{O}_5\text{N}$, 220.1185; IR (neat) 3360, 2960, 1700, 1500, 1365, 1160, 1060, 855, 780 cm^{-1} ; ^1H NMR (250 MHz, CDCl_3) 1.44 (9H, s), 3.77 (3H, s), 3.88 (1H, dd, $J=3.7, 11.0\text{Hz}$), 3.96 (1H, dd, $J=3.7, 11.0\text{Hz}$), 4.38 (1H, brs), 5.46 (1H, brs).

Methyl (4*R*)-1,3-oxazolid-2-one-4-carboxylate (58)

To a solution of alcohol 57 (47 g, 215 mmol) in tetrahydrofuran (THF, 470 ml) under argon was added dropwise thionyl chloride (47 ml, 644 mmol) and the mixture was stirred at room temperature overnight. After evaporation of the solvent and excess thionyl chloride, the residue was chromatographed on silica gel to give cyclic carbamate (24.6 g, 79%) as an oil. $[\alpha]_D 17.5^\circ$ (c 1.00, CHCl_3); EI-HR-MS, m/z 145.0400 $(M)^+$ calcd for $\text{C}_5\text{H}_7\text{O}_4\text{N}$, 145.0375; IR (neat) 3300, 2960, 1750, 1480, 1440, 1405, 1365, 1220, 1115, 1055, 1005, 955, 920, 770 cm^{-1} ; ^1H NMR (250 MHz, CDCl_3) 3.81 (3H, s), 4.40~4.65 (3H, m), 3.81 (1H, s).

Methyl (4*R*)-3-allyl-1,3-oxazolid-2-one-4-carboxylate (59)

To a solution of cyclic carbamate **58** (31.4 g, 216 mmol) and allyl bromide (37.4 ml, 432 mmol) in *N,N*-dimethylformamide (DMF, 300ml) at 0°C under argon was added dropwise a suspension of sodium hydride (5.21 g, 217 mmol) in DMF (100 ml) and the mixture was stirred for an hour. The reaction was quenched with aqueous ammonium chloride and then the mixture was extracted with ethyl acetate (300 ml x 3). The combined extracts were washed with water and brine, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography (20-40% ethyl acetate-hexane) to afford allyl amine (33.5 g, 84%) as an oil. $[\alpha]_D^{25}$ 16.4° (c 1.07, CHCl₃) ; EI-HR-MS, *m/z* 185.0684 (M)⁺ calcd for C₈H₁₁O₄N, 185.0688 ; IR (neat) 2940, 1750, 1640, 1435, 1410, 1360, 1210, 1135, 1055, 990, 935, 765 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 3.72 (1H, dd, *J*=7.3, 15.3Hz), 3.79 (3H, s), 4.20~4.47 (4H, m), 5.23 (1H, d, *J*=17.7Hz), 5.24 (1H, d, *J*=9.8Hz), 5.68~5.87 (1H, m).

(4*S*)-3-Allyl-1,3-oxazolid-2-one-4-methanol (60)

To a solution of ester **59** (500 mg, 2.70 mmol) in methanol (5.0 ml) at 0°C was added sodium borohydride in small portions and the mixture was stirred for 30 min. The reaction was quenched with 1N hydrochloric acid and the mixture was extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (2-4% methanol-chloroform) to afford alcohol (386 mg, 91%) as an oil. $[\alpha]_D^{25}$ -40.0° (c 0.95, CHCl₃) ; EI-HR-MS, *m/z* 157.0748 (M)⁺ calcd for C₇H₁₁O₃N, 157.0739 ; IR (neat) 3360, 2900, 1720, 1425, 1245, 1075, 1030, 965, 925, 765 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 2.92 (1H, s), 3.61 (1H, dd, *J*=3.1, 12.2Hz), 3.67~3.92 (3H, m), 4.09 (1H, dd, *J*=4.9, 15.9Hz), 4.24~4.42 (2H, m), 5.24 (1H, d, *J*=9.8Hz), 5.27 (1H, d, *J*=18.1Hz), 5.72~5.89 (1H, m).

(6S)-3-Hydroxy-4,8-dioxo-1-azabicyclo[4.3.0]nonan-9-one (61)

A solution of olefin **60** (14.0 g, 89 mmol) in methanol (140 ml) at -78°C was bubbled with ozone gas for an hour and then with oxygen gas for 15 min. After addition of dimethylsulfide (14 ml) to this solution the temperature was warmed to room temperature. The solvent was evaporated and the resulting mixture was purified by silica gel chromatography (2-4% methanol-chloroform) to give hemiacetal (12.8 g, 90%) as an amorphous solid. EI-HR-MS, m/z 159.0542 (M)⁺ calcd for C₆H₉O₄N, 159.0531; IR (nujol) 3300, 2900, 1715, 1440, 1375, 1325, 1240, 1200, 1150, 1115, 1060, 1035, 1000, 975, 885, 840, 765 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) 2.87 (0.5H, dd, J=9.2, 12.8 Hz), 3.24 (0.5H, dd, J=2.1, 13.7 Hz), 3.50 (0.5H, T, J=11.2 Hz), 3.61 (0.5H, dd, J=3.1, 10.4 Hz), 3.78~4.16 (4H, m), 4.36~4.48 (1H, m), 4.77 (0.5H, dd, J=3.1, 8.8 Hz), 5.21 (0.5H, d, J=2.4 Hz).

preparation of trimethyl phosphono(trimethylsilylmethyl)acetate (73)

To a suspension of sodium hydride (2.76 g, 115 mmol) in dimethylsulfoxide (DMSO, 400 ml) under argon was added trimethylphosphonoacetate (18 ml, 111 mmol) and (iodomethyl)trimethylsilane (18 ml, 121 mmol) and the mixture was stirred at room temperature for two days. The reaction was quenched with water and the mixture was extracted with ethyl acetate (300 ml x 3). The combined extracts were washed with water and brine, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography to give phosphonate (27 g, 92%) as an oil.

Methyl (4S)-4-hydroxymethyl-1,3-oxazolid-2-one-3-[2-(trimethylsilyl)methyl]but-(2E)-enoate (62)

To a suspension of sodium hydride (2.23 g, 93 mmol) in tetrahydrofuran (THF, 80 ml) at 0°C under argon was added a solution of the phosphonate **73** (25.9 g, 97 mmol) in THF and the mixture was stirred for 15 min at room temperature. To a suspension of hemiacetal (12.8 g, 81 mmol) in THF (120 ml) at 0°C under argon was added this cooled phosphonate reagent, and the resulting mixture was stirred at room temperature overnight. The reaction was quenched with aqueous ammonium chloride and the mixture was extracted with ethyl acetate (300 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was chromatographed on silica gel (2-4% methanol-chloroform) to afford unsaturated ester (23 g, 95%) as crystals. mp. 66~68°C ; $[\alpha]_D$ -1.4° (c 2.725, CHCl₃) ; EI-HR-MS, m/z 301.1319 (M)⁺ calcd for C₁₃H₂₃O₅NSi, 301.1346 ; IR (nujol) 3300, 2880, 1705, 1430, 1315, 1240, 1150, 1090, 1025, 840 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 0.00 (3H, s), 1.83 (1H, d, J=13.4Hz), 1.92 (1H, d, J=13.4Hz), 3.60~3.96 (4H, m), 3.72 (3H, s), 4.16 (1H, dd, J=6.7, 17.0Hz), 4.23 (1H, t, J=8.9Hz), 4.36 (1H, t, J=8.9Hz), 6.50 (1H, t, J=6.7Hz).

Methyl (4*R*)-4-(*tert*-butyldimethylsilyl)oxymethyl-1,3-oxazolid-2-one-3-(2-trimethylsilylmethyl)but-(2*E*)-enoate (65)

To a solution of alcohol **62** (23 g, 76 mmol) and imidazole (15.2 g, 224 mmol) in N,N-dimethylformamide (DMF, 120 ml) at 0°C under argon was added a solution of *tert*-butyldimethylsilylchloride (TBSCl, 15.8 g, 105 mmol) in DMF (60 ml) and the mixture was stirred for an hour. The mixture was poured into water and extracted with ethyl acetate (300 ml x 3). The combined extracts were washed with water and brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel to give silyl ether (28.5 g, 90%) as crystals. mp. 56°C ; $[\alpha]_D$ -0.5° (c 0.95, CHCl₃) ; EI-HR-MS, m/z 415.2195 (M)⁺ calcd for C₁₉H₃₇O₅NSi₂,

415.2210 ; IR (nujol) 2920, 1725, 1440, 1250, 1185, 1155, 1130, 1080, 1045, 845, 785 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 0.00 (9H, s), 0.05 (6H, s), 0.87 (9H, s), 1.81 (1H, d, $J=13.4\text{Hz}$), 1.92 (1H, d, $J=13.4\text{Hz}$), 3.60 (1H, dd, $J=4.0, 10.7\text{Hz}$), 3.67 (1H, dd, $J=3.3, 10.7\text{Hz}$), 3.72 (3H, s), 3.82 (1H, dd, $J=6.7, 16.5\text{Hz}$), 3.75~3.87 (1H, m), 4.12 (1H, dd, $J=4.6, 8.5\text{Hz}$), 4.13 (1H, dd, $J=6.7, 16.5\text{Hz}$), 4.32 (1H, t, $J=8.5\text{Hz}$), 6.52 (1H, t, $J=6.7\text{Hz}$).

(4*R*)-4-(*tert*-butyldimethylsilyl)oxymethyl-1,3-oxazolid-2-one-3-(2-trimethylsilylmethyl)but-(2*E*)-en-1-ol (66)

To a solution of ester **65** (31.5 g, 76 mmol) in toluene (310 ml) at -78°C under argon was added boron trifluoride etherate 911.2 ml, 91 mmol) and a solution of diisobutylaluminum hydride (0.93M in hexane, 490 ml, 456 mmol) and the mixture was stirred for 30 min. The reaction was quenched with 1N hydrochloric acid and then the solution was acidified to pH4 . The resulting mixture was filtered through celite and the filtrate was washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (40-60% ethyl acetate-hexane) to give alcohol (23 g, 78%) as an oil. $[\alpha]_D -2.0^\circ$ (c 1.025, CHCl_3) ; EI-HR-MS, m/z 387.2269 (M)⁺ calcd for $\text{C}_{18}\text{H}_{37}\text{O}_4\text{NSi}_2$, 387.2261 ; IR (neat) 3400, 2940, 1730, 1420, 1245, 1120, 1050, 1005, 840, 780 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 0.05 (9H, s), 0.07 (6H, s), 0.89 (9H, s), 1.62 (2H, s), 3.58~3.71 (3H, m), 3.76~3.86 (1H, m), 3.98 (2H, s), 4.05~4.21 (2H, m), 4.29 (1H, t, $J=8.5\text{Hz}$), 5.35 (1H, t, $J=6.7\text{Hz}$).

(4*R*)-4-(*tert*-Butyldimethylsilyl)oxymethyl-1,3-oxazolid-2-one-3-[2-(trimethylsilyl)methyl]but-(2*E*)-enyl acetate (67)

To a solution of alcohol **66** (2.03 g, 5.2 mmol) in pyridine (10 ml) was added acetic anhydride (10 ml) and the mixture was stirred at room temperature overnight. The resulting solution was poured into aqueous

sodium carbonate with ice and extracted with ethyl acetate 950 ml x 3). The combined extracts were washed with aqueous cupric sulfate and brine, dried over anhydrous sodium sulfate and evaporated to give acetate (2.2 g, 98%) as an oil. $[\alpha]_D -11.0^\circ$ (c 1.075, CHCl_3); EI-HR-MS, m/z 429.2378 (M)⁺ calcd for $\text{C}_{20}\text{H}_{39}\text{O}_5\text{NSi}_2$, 429.2367; IR (neat) 2940, 1745, 1420, 1250, 1125, 1095, 1050, 845, 785 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 0.06 (9H, s), 0.07 (6H, s), 0.89 (9H, s), 1.56~1.62 (2H, m), 2.07 (3H, s), 3.63 (2H, d, $J=3.7\text{Hz}$), 3.65~3.81 (2H, m), 4.02~4.14 (2H, m), 4.30 (1H, t, $J=8.5\text{Hz}$), 4.41 (2H, s), 5.32 (1H, t, $J=6.7\text{Hz}$).

(4S)-4-Hydroxymethyl-1,3-oxazolid-2-one-3-[(2-trimethylsilyl)-methyl]but-(2E)-enyl acetate (68)

To a solution of silyl ether **67** (165 mg, 0.38 mmol) in isopropanol (3.3 ml) was added 10-camphorsulfonic acid (CSA, 247 mg, 1.1 mmol) and the mixture was stirred for three hours at room temperature. The mixture was poured into water and extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (0-1% methanol chloroform) to give alcohol (109 mg, 90%) as an oil. $[\alpha]_D -7.0^\circ$ (c 1.24, CHCl_3); EI-HR-MS, m/z 315.1512 (M)⁺ calcd for $\text{C}_{14}\text{H}_{25}\text{O}_5\text{NSi}$, 315.1503; IR (neat) 3400, 2940, 1735, 1430, 1375, 1250, 1085, 1035, 970, 850, 765 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 0.07 (9H, s), 1.62 (1H, d, $J=11.0\text{Hz}$), 1.64 (1H, d, $J=11.0\text{Hz}$), 2.08 (3H, s), 3.53~3.90 (3H, m), 3.89 (2H, d, $J=6.7\text{Hz}$), 4.20 (1H, dd, $J=6.4, 8.6\text{Hz}$), 4.33 (1H, t, $J=8.6\text{Hz}$), 4.42 (2H, s), 5.36 (1H, t, $J=6.7\text{Hz}$).

***tert*-Butyl (4S)-3-[[4-acetoxy-3-(trimethylsilyl)methyl]but-(2E)-enyl]-1,3-oxazolid-2-one-4-prop-(2E)-enoate (69)**

To a solution of oxalyl chloride (0.8 ml, 9.4 mmol) in dichloromethane (15 ml) at -78°C under argon was added dropwise dimethylsulfoxide (1.3 ml, 18.3 mmol). After stirring 2 min, to the solution was added a solution of alcohol **68** (1.45 g, 4.60 mmol) in dichloromethane (7 ml) and the mixture was stirred for 15 min. To this solution triethylamine (3.2 ml, 23.0 mmol) was added and the mixture was gradually warmed to room temperature. The reaction was quenched with aqueous ammonium chloride and the solution was poured into water and then extracted with ethyl acetate (30 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was used for the next step without further purification.

To a suspension of sodium hydride (122 mg, 5.08 mmol) in tetrahydrofuran (THF, 30 ml) at 0°C under argon was added trimethylphosphonoacetate (90.89 ml, 5.50 mmol) and the mixture was stirred at room temperature for 15 min. To the cooled solution at 0°C was added a solution of the aldehyde in THF (15 ml) and stirred for an hour. The reaction was quenched with aqueous ammonium chloride and the mixture was extracted with ethyl acetate (50 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography (20-40% ethyl acetate-hexane) to give unsaturated ester (1.8 g, 95%) as an oil. $[\alpha]_{\text{D}} -3.2^{\circ}$ (c 0.77, CHCl_3) ; EI-HR-MS, m/z 411.2061 (M^+) calcd for $\text{C}_{20}\text{H}_{33}\text{O}_6\text{NSi}$, 411.2077 ; IR (neat) 2940, 1750, 1410, 1365, 1300, 1245, 1225, 1150, 1055, 975, 850, 765 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 0.04 (6H, s), 1.48 (9H, s), 1.49 (1H, d, $J=9.8\text{Hz}$), 1.55 (1H, d, $J=9.8\text{Hz}$), 2.09 (3H, s), 3.53 (1H, dd, $J=8.5, 15.3\text{Hz}$), 3.99 (1H, dd, $J=6.4, 8.5\text{Hz}$), 6.04 (1H, dd, $J=5.5, 15.3\text{Hz}$), 4.27~4.46 (4H, m), 5.26 (1H, dd, $J=5.5, 8.5\text{Hz}$), 5.91 (1H, d, $J=15.8\text{Hz}$), 6.61 (1H, dd, $J=8.3, 15.8\text{Hz}$).

[3+2] cycloaddition

To a two necked flask equipped with stirrer, condenser and stoppered with septum was placed palladium(II) acetate (95 mg, 0.42 mmol) and 1,2-bis(diphenylphosphino)ethane (DIPHOS, recrystallized from ethanol, 1.5 g, 3.8 mmol) and the flask was filled with argon. To this flask was added m-xylene (40 ml), N,O-bis(trimethylsilyl)acetamide (BSA, 1.2 ml, 4.9 mmol) and a solution of **69** (1.9 g, 4.6 mmol) in m-xylene (20 ml) and then the temperature was gradually warmed to refluxing temperature. After stirring overnight, the cooled mixture was passed through a short silica gel column and evaporated. The residue was chromatographed on silica gel (5-10-15-20% ethyl acetate-hexane) to yield Exo-1 (**70**; 2.2 g, 55%) and a mixture of Exo-2 (**71**) and Exo-3 (**72**) (616 mg, 15%, a mixture of 1:2). Exo-2 and Exo-3 were further purified by silica gel column chromatography (15-20% ethyl acetate-benzene).

***tert*-Butyl (1*S*,2*R*,3*R*,6*S*)-5-methylene-9-oxo-10-oxa-8-azatri-cyclo[6.3.0.0^{2,6}]undecane-3-carboxylate (Exo-1; **70**)**

mp. 93°C ; $[\alpha]_D$ 17.7° (c 1.46, CHCl₃) ; EI-HR-MS, *m/z* 279.1469 (M)⁺ calcd for C₁₅H₂₁O₄N, 279.1470 ; IR (nujol) 2940, 1760, 1720, 1470, 1405, 1375, 1355, 1320, 1305, 1255, 1215, 1205, 1170, 1150, 1090, 1020, 990, 910, 795, 780 cm⁻¹ ; ¹HNMR (400MHz, CDCl₃) 1.44 (9H, s), 2.63 (1H, ddd, *J*=5, 7.5, 8Hz), 2.70~2.76 (3H, m), 2.95 (1H, dd, *J*=6.5, 12Hz), 3.34 (1H, brq), 3.73 (1H, ddd, *J*=3, 6.5, 7Hz), 4.09 (1H, dd, *J*=9, 12Hz), 4.54 (1H, dd, *J*=8, 9Hz), 4.85 (1H, q, *J*=2Hz), 5.03 (1H, q, *J*=2Hz).

***tert*-Butyl (1*S*,2*S*,3*S*,6*R*)-5-methylene-9-oxo-10-oxa-8-azatri-cyclo[6.3.0.0^{2,6}]undecane-3-carboxylate (Exo-2; **71**)**

$[\alpha]_D$ -29.7° (c 0.35, CHCl₃) ; EI-HR-MS, *m/z* 279.1483 (M)⁺ calcd for C₁₅H₂₁O₄N, 279.1470 ; IR (neat) 2980, 1765, 1735, 1405, 1375, 1280,

1260, 1215, 1155, 1075, 1010, 855, 780 cm^{-1} ; ^1H NMR (400MHz, CDCl_3) 1.46 (9H, s), 2.47~2.59 (2H, m), 2.68~2.78 (1H, m), 2.94~3.02 (1H, m), 3.34~3.40 (1H, m) 3.42 (1H, t, $J=9\text{Hz}$), 3.64 (1H, dd, $J=3.5, 9\text{Hz}$), 4.12 (1H, ddd, $J=3, 6, 8\text{Hz}$) 4.39 (1H, dd, $J=3, 9.5\text{Hz}$), 4.47 (1H, dd, $J=8.5, 9.5\text{Hz}$), 4.93 (1H, q, $J=2\text{Hz}$), 4.99 (1H, q, $J=2\text{Hz}$).

***tert*-Butyl (1*S*,2*R*,3*S*,6*S*)-5-methylene-9-oxo-10-oxa-8-azatri-cyclo[6.3.0.0^{2,6}]undecane-3-carboxylate (Exo-3; 72)**

$[\alpha]_D -40.0^\circ$ (c 0.30, CHCl_3); EI-HR-MS, m/z 279.1476 (M)⁺ calcd for $\text{C}_{15}\text{H}_{21}\text{O}_4\text{N}$, 279.1470; IR (neat) 2960, 1760, 1730, 1480, 1395, 1365, 1325, 1155, 1060, 1005, 890, 845 cm^{-1} ; ^1H NMR (400MHz, CDCl_3) 1.45 (9H, s), 2.04 (1H, ddd, $J=10, 12, 13\text{Hz}$), 2.69 (1H, ddd, $J=9, 10, 11\text{Hz}$), 2.90 (1H, dddd, $J=2, 4, 9, 18\text{Hz}$), 2.99 (1H, m), 3.08 (1H, dddd, $J=2.5, 4, 10, 18\text{Hz}$), 3.25 (1H, t, $J=9.5\text{Hz}$), 3.49 (1H, dd, $J=7.3, 9.5\text{Hz}$), 3.88 (1H, ddd, $J=5, 8.5, 10\text{Hz}$), 4.40 (1H, dd, $J=5.1, 8.8\text{Hz}$), 4.52 (1H, dd, $J=8.5, 8.8\text{Hz}$), 4.76 (1H, q, $J=2\text{Hz}$), 4.85 (1H, q, $J=2\text{Hz}$).

Hydrolysis of cyclic carbamate and reprotection with Boc group

To a solution of cyclic carbamate (Exo-1; **70**, 897 mg, 3.2 mmol) in ethanol (9 ml) was added 1N potassium hydroxide (9.0 ml, 9.0 mmol) and the mixture was stirred at refluxing temperature overnight. To the cooled solution was added a solution of *di-tert*-butyl carbonate (Boc_2O , 1.1 g, 5.0 mmol) in 1,4-dioxane and the resulting mixture was stirred at room temperature overnight. The solution was acidified to pH 2 with 1N hydrochloric acid and extracted with ethyl acetate (50 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in methanol and to this solution an ethereal diazomethane was added. After evaporation of the solvents, the crude

product was purified by silica gel chromatography (20-40% ethyl acetate-hexane) to give alcohol (850 mg, 85%) as an oil.

Exo-2 and Exo-3 were also reacted as well as the hydrolysis of Exo-1.

Methyl (1*R*,2*S*,5*S*,8*R*)-3-*tert*-butyloxycarbonyl-2-hydroxymethyl-6-methylene-3-azabicyclo[3.3.0]octane-8-carboxylate (Exo-1; 73)

$[\alpha]_D -5.7^\circ$ (c 1.59, CHCl₃) ; EI-HR-MS, m/z 311.1715 (M)⁺ calcd for C₁₆H₂₅O₅N, 311.1733 ; IR (neat) 3460, 2980, 1745, 1700, 1410, 1370, 1255, 1170, 1130, 1100, 1035, 890, 780 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.45 (9H, s), 2.60~2.96 (5H, m), 3.22 (1H, dd, J=7.3, 14.7Hz), 3.34 (1H, dd, J=6.4, 11.0Hz), 3.61 (1H, dd, J=7.3, 11.0Hz), 3.65~3.75 (1H, m), 3.70 (3H, s), 3.90 (1H, m), 4.91 (1H, d, J=1.5Hz), 4.98 (1H, d, J=1.5Hz).

Methyl (1*S*,2*S*,5*R*,8*S*)-3-*tert*-butyloxycarbonyl-2-hydroxymethyl-6-methylene-3-azabicyclo[3.3.0]octane-8-carboxylate (Exo-2; 74)

$[\alpha]_D -19.9^\circ$ (c 0.70, CHCl₃) ; FAB-HR-MS, m/z 312.1821 [(M+H)]⁺ calcd for C₁₆H₂₆O₅N, 312.1811 ; IR (neat) 3440, 2980, 1740, 1700, 1410, 1375, 1255, 1170, 1045, 890 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.45 (9H, s), 2.55~2.68 (1H, m), 2.79~2.94 (1H, m), 3.02~3.24 (3H, m), 3.65~3.84 (2H, m), 3.69 (3H, s), 4.00 (1H, dt, J=3.7, 6.1Hz), 4.89 (1H, s), 4.93 (1H, s).

Methyl (1*R*,2*S*,5*S*,8*S*)-3-*tert*-butyloxycarbonyl-2-hydroxymethyl-6-methylene-3-azabicyclo[3.3.0]octane-8-carboxylate (Exo-3; 75)

$[\alpha]_D -64.7^\circ$ (c 1.125, CHCl₃) ; EI-HR-MS, m/z 311.1707 (M)⁺ calcd for C₁₆H₂₅O₅N, 311.1733 ; IR (neat) 3420, 2980, 1740, 1695, 1485, 1440, 1410, 1370, 1295, 1255, 1165, 1130, 1075, 1050, 1035, 970, 850, 815, 765

cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.46 (9H, s), 2.53~2.68 (1H, m), 2.78~2.96 (2H, m), 2.98~3.20 (2H, m), 3.58~3.83 (5H, m), 3.70 (3H, s), 4.74 (1H, dd, J=2.4, 4.3Hz), 4.80 (1H, d, J=1.8Hz).

Oxidation of alcohol to carboxylic acid followed by esterification

A solution of alcohol (Exo-1; **73**, 448 mg, 1.44 mmol) and pyridinium dichromate (PDC, 1.6 g, 4.3 mmol) in N, N-dimethylformamide (DMF, 5 ml) was stirred at 45°C under argon overnight. To the cooled solution an ethereal solution of diazomethane was slowly added and the mixture was extracted with ethyl acetate (30 ml x 3). The combined extracts were washed with water and brine, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by silica gel chromatography (20-30% ethyl acetate-hexane) to yield diester (293 mg, 60 %). Exo-2 and Exo-3 were also oxidized in the same manner as one employed in the synthesis of Exo-1.

Dimethyl (1*R*,2*S*,5*S*,8*R*)-3-*tert*-butyloxycarbonyl-6-methylene-3-azabicyclo[3.3.0]octane-2,8-dicarboxylate (Exo-1; **76)**

[α]_D 2.5° (c 1.10, CHCl₃) ; EI-HR-MS, m/z 339.1660 (M)⁺ calcd for C₁₇H₂₅O₆N, 339.1682 ; IR (neat) 2960, 1745, 1705, 1480, 1440, 1395, 1365, 1280, 1250, 1205, 1170, 1125, 1080, 1020, 890, 780 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.39 (ca 4.5H, s), 1.45 (ca 4.5H, s), 2.64~3.00 (4H, m), 3.24~3.44 (2H, m), 3.73 (6H, s), 3.77~3.91 (1H, m), 4.18 (ca 0.5H, d, J=2.4Hz), 4.37 (ca 0.5H, d, J=1.8Hz), 4.93 (1H, d, J=4.3Hz), 4.99 (1H, s).

Dimethyl (1*S*,2*S*,5*R*,8*S*)-3-*tert*-butyloxycarbonyl-6-methylene-3-azabicyclo[3.3.0]octane-2,8-dicarboxylate (Exo-2; **77)**

mp. 78°C ; [α]_D -32.8° (c 0.665, CHCl₃) ; EI-HR-MS, m/z 339.1687 (M)⁺ calcd for C₁₇H₂₅O₆N, 339.1682 ; IR (nujol) 2920, 1750, 1735, 1705, 1465,

1400, 1380, 1365, 1320, 1260, 1205, 1120, 1025, 915, 890 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.38 (ca6H, s), 1.43 (ca3H, s), 2.59 (1H, ddt, $J=10.5, 16, 2.5\text{Hz}$), 2.84 (1H, dd, $J=8, 15\text{Hz}$), 3.02 (1H, q, $J=9\text{Hz}$), 3.38 (1H, q, $J=9.5\text{Hz}$), 3.22~3.42 (2H, m), 3.65 (3H, s), 3.66 (3H, s), 3.50~3.75 (1H, m), 4.41~4.53 (1H, m), 4.90 (1H, s), 4.95 (1H, s).

Dimethyl (1*R*,2*S*,5*S*,8*S*)-3-*tert*-butyloxycarbonyl-6-methylene-3-azabicyclo[3.3.0]octane-2,8-dicarboxylate (Exo-3; 78)

mp. 88°C ; $[\alpha]_D -119.6^\circ$ (c 1.05, CHCl_3); EI-HR-MS, m/z 339.1701 (M^+) calcd for $\text{C}_{17}\text{H}_{25}\text{O}_6\text{N}$, 339.1682; IR (nujol) 2920, 1765, 1745, 1695, 1465, 1405, 1380, 1370, 1340, 1330, 1305, 1260, 1220, 1170, 1160, 1120, 1105, 1085, 1010, 990, 940, 910, 875, 795 cm^{-1} ; ^1H NMR (250MHz, CDCl_3) 1.39 (ca4.5H, s), 1.45 (ca4.5H, s), 2.35~2.51 (1H, m), 2.58~2.74 (1H, m), 2.77~2.94 (2H, m), 3.04~3.27 (2H, m), 3.65 (3H, s), 3.71 (ca2H, s), 3.73 (ca1H, s), 3.74~3.84 (1H, m), 3.89 (ca0.7H, d, $J=10.4\text{Hz}$), 3.93 (ca0.3H, d, $J=9.8\text{Hz}$), 4.80 (1H, dd, $J=2.4, 4.3\text{Hz}$), 4.83 (1H, brs).

Hydrolysis of esters and removal of Boc group

To a solution of diester (Exo-1; **76**, 140 mg, 0.41 mol) in ethanol was added 1N potassium hydroxide 93 ml) and the mixture was stirred at room temperature overnight. The solution was acidified to pH2 with 1N hydrochloric acid and extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The crude product was dissolved in trifluoroacetic acid (TFA, 3 ml) at room temperature under argon and the solution was stirred for 30 min. After evaporation of TFA, the resulting TFA salt was passed through Dowex 50Wx8 (H^+ form, water-5% ammonium hydroxide) to afford ammonium salt. This was then passed through Amberlite IRC-50 (H^+ form, water) to give salt-free amino acid. The amino acid was further purified by

recrystallization from water to give colorless crystals (44 mg, 50%). Exo-2 and Exo-3 were also deprotected as well as deprotection of Exo-1 except for the temperature on hydrolysis (80°C).

(1*R*,2*R*,5*S*,8*S*)-4-methylene-7-azabicyclo[3.3.0]octane-2,8-dicarboxylic acid (Exo-1; 79)

mp. >300°C (decomp) ; $[\alpha]_D$ 64.2° (c 0.12, H₂O) ; FAB-HR-MS, *m/z* 212.0940 [(*M*+*H*)]⁺ calcd for C₁₀H₁₄O₄N, 212.0923 ; IR (nujol) 3060, 2900, 2840, 1715, 1620, 1590, 1455, 1405, 1385, 1335, 1320, 1300, 1275, 1255, 1195, 1165, 900, 880 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=3.2, HOD=4.8ppm) 2.73 (1H, ddt, *J*=4.5, 17, 2Hz), 2.89 (1H, dddd, *J*=2.5, 4, 6.0, 17.0Hz), 3.02 (1H, ddd, *J*=4.5, 6.0, 8.5Hz), 3.27 (1H, ddd, *J*=4.5, 5.9, 8.5Hz), 3.51 (1H, dddt, 4.5, 4.9, 8.3, 2Hz), 3.36 (1H, dd, *J*=4.9, 12.0Hz), 3.71 (1H, dd, *J*=8.3, 12.0Hz), 4.00 (1H, d, *J*=5.9Hz), 5.06 (1H, dt, *J*=4, 2Hz), 5.17 (1H, dt, *J*=2.5, 2Hz).

(1*S*,2*S*,5*R*,8*S*)-4-methylene-7-azabicyclo[3.3.0]octane-2,8-dicarboxylic acid (Exo-2; 80)

mp, 213~216°C, $[\alpha]_D$ -53.2° (c 0.25, H₂O) ; FAB-HR-MS, *m/z* 212.0924 [(*M*+*H*)]⁺ calcd for C₁₀H₁₄O₄N, 212.0923 ; IR (nujol) 2900, 2860, 1720, 1630, 1470, 1385, 1255, 1205, 910 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=4.0, HOD=4.8ppm) 2.57 (1H, ddt, *J*=9, 14.5, 2Hz), 2.73 (1H, dt, *J*=9, 7.5Hz), 2.80 (1H, dd, *J*=7.5, 14.5Hz), 3.42 (1H, dd, *J*=3.5, 12Hz), 3.49 (1H, dd, *J*=8.5, 12Hz), 3.53~3.63 (2H, m), 4.25 (1H, d, *J*=8Hz), 5.01 (1H, brs), 5.13 (1H, d, *J*=2Hz).

(1*R*,2*S*,5*S*,8*S*)-4-methylene-7-azabicyclo[3.3.0]octane-2,8-dicarboxylic acid (Exo-3; 81)

mp. >295°C (decomp) ; $[\alpha]_D$ -113.1° (c 0.16, H₂O) ; FAB-HR-MS, m/z 212.0927 [(M+H)]⁺ calcd for C₁₀H₁₄O₄N, 212.0923 ; IR (nujol) 3120, 2920, 2860, 1720, 1700, 1615, 1465, 1380, 1335, 1240, 1165, 1105, 1055, 925, 845 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=3.7, HOD=4.8ppm) 2.53 (1H, dt, J=43, 11Hz), 2.91 (1H, dddd, J=2, 4, 8, 17Hz), 3.08 (1H, ddd, J=8, 10, 13Hz), 3.06~3.18 (1H, m), 3.28 (1H, t, J=11Hz), 3.25~3.35 (1H, m), 3.61 (1H, dd, J=7, 11Hz), 3.94 (1h, d, J=11Hz), 4.88 (1H, dt, J=4, 2Hz), 4.95 (1H, dt, J=4, 2Hz).

Dimethyl (1*S*,4*R*,5*R*,6*S*)-7-*tert*-butyloxycarbonyl-2-methyl-7-azabicyclo[3.3.0]oct-2-ene4,6-dicarboxylate (Endo-1; 83)

A solution of diester **76** (200 mg, 0.59 mmol), lithium tetrachloropalladate(II) hydrate (185 mg, 0.71 mmol) and lithium carbonate (88 mg, 1.2 mmol) in methanol (4 ml) under argon was stirred at room temperature for an hour. The reaction was quenched with aqueous ammonium chloride and the mixture was extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The resulting π -allyl complex was used to the next step without further purification. To a suspension of zinc chloride (dried at 60°C in *vacuo*, 403 mg, 3.0 mmol) in tetrahydrofuran (THF, 8 ml) at 0°C under argon was added a solution of *n*-butyl lithium (1.65M, in hexane, 1.8 ml, 3.0 mmol). To this solution was added a solution of π -allyl complex and triphenylphosphine (recrystallized from hexane, 465 mg, 1.8 mmol) in THF (8 ml) and the mixture was stirred at room temperature overnight. The reaction was quenched with aqueous ammonium chloride and the mixture was extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel (20-30% ethyl acetate-hexane) to give Endo-1 (130 mg, 65 %) as an oil. $[\alpha]_D$ -111.6° (c 0.525, CHCl₃) ; FAB-HR-MS,

m/z 340.1751 [(M+H)]⁺ calcd for C₁₇H₂₆O₆N, 340.1760 ; IR (neat) 2960, 1750, 1715, 1485, 1440, 1400, 1370, 1210, 1175, 1130, 1080, 1015, 895, 860, 845, 780 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.38 (ca6H, s), 1.44 (ca3H, s), 1.73 (3H, s), 3.21~3.37 (2H, m), 3.51~3.66 (3H, m), 3.68 (3H, s), 3.74 (3H, s), 4.00 (ca0.7H, d, J=4.3Hz), 4.18 (ca0.3H, d, J=2.4Hz), 5.36 (1H, s).

(1*S*,4*R*,5*R*,6*S*)-2-methyl-7-azabicyclo[3.3.0]oct-2-ene-4,6-dicarboxylic acid (Endo-1; 84)

To a solution of Endo-1 **83** (203 mg, 0.60 mmol) in ethanol (4 ml) was added 1N potassium hydroxide (4 ml) and the mixture was stirred at room temperature overnight. The solution was acidified to pH2 with 1N hydrochloric acid and then extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in trifluoroacetic acid (4 ml) under argon and the mixture was stirred for 30 min at room temperature. The resulting TFA salt was passed through Dowex 50Wx8 (H⁺ form, water-5% ammonium hydroxide) to give ammonium salt. This salt was then passed through Amberlite IRC-50 (H⁺ form, water) to give salt-free amino acid. This was further purified by recrystallization from water to give Endo-1 (101 mg, 50%) as crystals. mp. >295°C (decomp) ; [α]_D -168.9° (c 0.35, H₂O) ; FAB-HR-MS, m/z 212.0924 [(M+H)]⁺ calcd for C₁₀H₁₄O₄N, 212.0923 ; IR (nujol) 3140, 2920, 2860, 1720, 1630, 1605, 1450, 1400, 1370, 1335, 1310, 1300, 1260, 1200, 980, 900, 890, 860 cm⁻¹ ; ¹HNMR (400MHz, D₂O, pD=3.3, HOD=4.8ppm) 1.76 (3H, d, J=1.0Hz), 3.38 (1H, ddd, J=1.5, 6, 8Hz), 3.44 (1H, dd, J=3.5, 12Hz), 3.49 (1H, dd, J=7, 12Hz), 3.60 (1H, m), 3.67 (1H, m), 3.94 (1H, d, J=6Hz), 5.51 (1H, brd, J=1Hz).

Dimethyl (1*S*,4*S*,5*R*,6*S*)-7-*tert*-butyloxycarbonyl-2-methyl-7-azabicyclo[3.3.0]oct-2-ene-4,6-dicarboxylate (Endo-3; 85)

To a solution of 2,2,6,6-tetramethylpiperidine (0.07 ml, 0.42 mmol) in tetrahydrofuran (THF, 1.4 ml) at 0°C under argon was added a solution of *n*-butyl lithium (1.65 M in hexane, 0.24 ml, 0.40 mmol) and the mixture was stirred for 15 min. To the cooled solution at -78°C was added a solution of Endo-1 **83** (69 mg, 0.20 mmol) in THF (1.4 ml) and the solution was stirred for 15 min. This was further cooled to -100°C and to this solution was added a cooled (-78°C) solution of pivalic acid (1M in THF, 0.6 ml, 0.6 mmol). The mixture was poured into water and extracted with ethyl acetate (20 ml x 3). The combined extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel to give Endo-3 (41 mg, 60%) as an oil along with recovery of Endo-1 (4 mg, 6%). $[\alpha]_D^{25}$ 3.2° (c 0.19, CHCl₃) ; FAB-HR-MS, *m/z* 340.1765 [(M+H)]⁺ calcd for C₁₇H₂₆O₆N, 340.1760 ; IR (neat) 2980, 1750, 1730, 1485, 1440, 1400, 1375, 1270, 1205, 1165, 1135, 1105, 1085, 1005, 895, 860, 765 cm⁻¹ ; ¹HNMR (250MHz, CDCl₃) 1.36 (ca6H, s), 1.42 (ca3H, s), 1.73 (3H, s), 3.14~3.25 (2H, m), 3.51 (1H, dd, J=6.7, 11.0Hz), 3.70 (3H, s), 3.72 (3H, s), 3.68~3.85 (2H, m), 4.00 (ca0.7H, d, J=5.5Hz), 4.12 (ca0.3H, s), 5.44 (1H, d, J=1.2Hz).

Reduction of *tert*-butyl ester and acetylation (Exo-2 (**89**) and Exo-3 (**90**))

tert-Butyl ester (Exo-2; **71**) was dissolved in trifluoroacetic acid (TFA, 0.5 ml) under argon and the solution was stirred for 30 min at room temperature. After evaporation of excess TFA, the resulting carboxylic acid was dissolved in tetrahydrofuran (THF, 0.4 ml) at 0°C under argon and to this solution was added triethylamine (0.03 ml, 0.19 mmol) and ethyl chloroformate (0.01 ml, 0.11 mmol). The mixture was stirred for 15 min and to this was added 90% ethanol (0.4 ml) and sodium borohydride (5.0 mg, 0.13 mmol). After stirring for 30 min, the reaction was quenched with

aqueous ammonium chloride and the mixture was extracted with ethyl acetate (5 ml x 3). The combined extracts were dried over anhydrous sodium sulfate and evaporated. The residue was purified by TLC. The resulting alcohol was dissolved in pyridine (0.2 ml) and to this solution was added acetic anhydride (0.2 ml). The mixture was stirred at room temperature overnight. This was poured into aqueous sodium carbonate with ice and extracted with ethyl acetate (5 ml x 3). The combined extracts were washed with aqueous cupric sulfate and brine, dried over anhydrous sodium sulfate and evaporated. The residue was purified by TLC to give acetate (7 mg, 50%). Exo-3 was also reacted in the same manner as the preparation of Exo-2.

**(1*S*,2*S*,3*S*,6*R*)-5-methylene-9-oxo-10-oxa-8-azabicyclo-
[6.3.0.0^{2,6}]undecane-3-methyl acetate (Exo-2; 89)**

$[\alpha]_D$ -61.1° (c 0.475, CHCl₃) ; EI-HR-MS, *m/z* 251.1143 (M)⁺ calcd for C₁₃H₁₇O₄N, 251.1157 ; IR (neat) 2960, 1760, 1745, 1400, 1370, 1330, 1245, 1080, 1040, 1005, 900, 780 cm⁻¹ ; ¹HNMR (400MHz, CDCl₃) 2.07 (3H, s), 2.10~2.20 (2H, m), 2.44 (1H, q, *J*=7Hz), 2.58 (1H, ddd, *J*=1, 10, 18Hz), 3.30 (1H, m), 3.37 (1H, dd, *J*=9, 12Hz), 3.70 (1H, dd, *J*=3, 12Hz), 3.96 (1H, dd, *J*=6.5, 11Hz), 4.04 (1H, dd, *J*=6, 11Hz), 4.10 (1H, dt, *J*=6.5, 5.5Hz), 4.49 (2H, d, *J*=5.5Hz), 4.95 (1H, q, *J*=2Hz).

**(1*S*,2*R*,3*S*,6*S*)-5-methylene-9-oxo-10-oxa-8-azabicyclo-
[6.3.0.0^{2,6}]undecane-3-methyl acetate (Exo-3; 90)**

mp. 108°C ; $[\alpha]_D$ -14.2° (c 0.225, CHCl₃) ; EI-HR-MS, *m/z* 251.1161 (M)⁺ calcd for C₁₃H₁₇O₄N, 251.1157 ; IR (nujol) 2920, 1750, 1470, 1400, 1380, 1315, 1245, 1070, 1055, 1010, 915 cm⁻¹ ; ¹HNMR (400MHz, CDCl₃) 1.66 (1H, dt, *J*=13, 10.5Hz), 2.05 (3H, s), 2.22 (1H, ddq, *J*=5, 10.5, 9Hz), 2.38 (1H, dddd, *J*=2, 4, 9, 17Hz), 2.96 (1H, dddd, *J*=2.5, 3.5, 9, 17Hz), 3.02

References

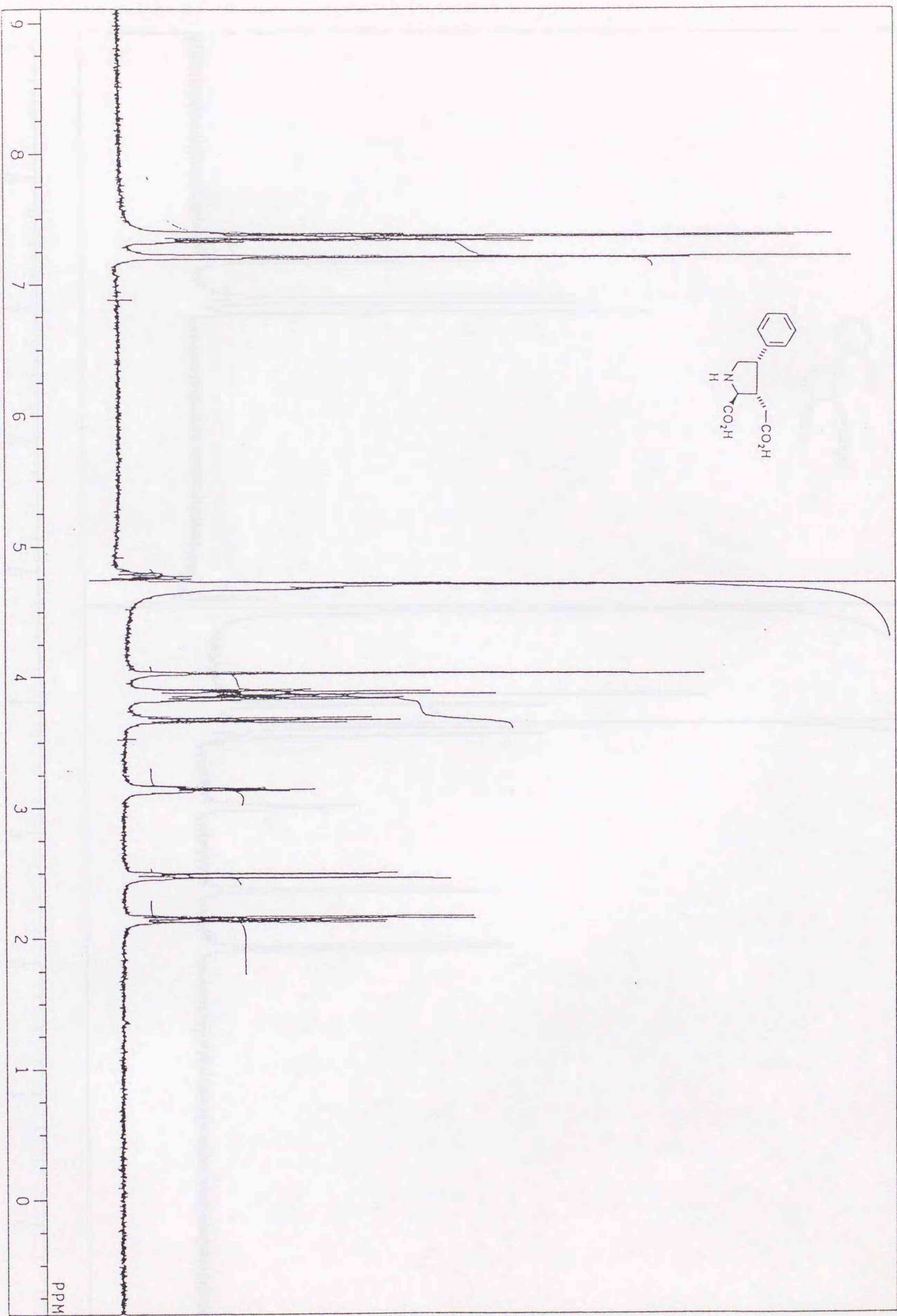
1. H. M. R. Hoffmann and R. Hennig, *Helv. Chim. Acta.*, **66**, 828 (1983).
2. a) B. M. Trost and T. A. Grese, *J. Org. Chem.*, **57**, 686 (1992).
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c) B. M. Trost, *Angew. Chem. Int. Ed. Engl.*, **25**, 1 (1986).
3. W. C. Still and C. Gennari, *Tetrahedron Lett.*, **24**, 4405 (1983).
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9. Private communication from Dr. Y. Ohfuné (SUNBOR).
10. kainic acid: references 2c and 2d in the section of Introduction.
domoic acid: K. Nomoto, T. Takemoto, M. Maeda, Y. In, M. Doi, M. Inoue and T. Ishida, *Biochem. Biophys. Res. commun.*, **187**, 325 (1992).
11. R. W. Hoffmann, *Angew. Chem. Int. Ed. Engl.*, **31**, 1124 (1992).

Conclusion

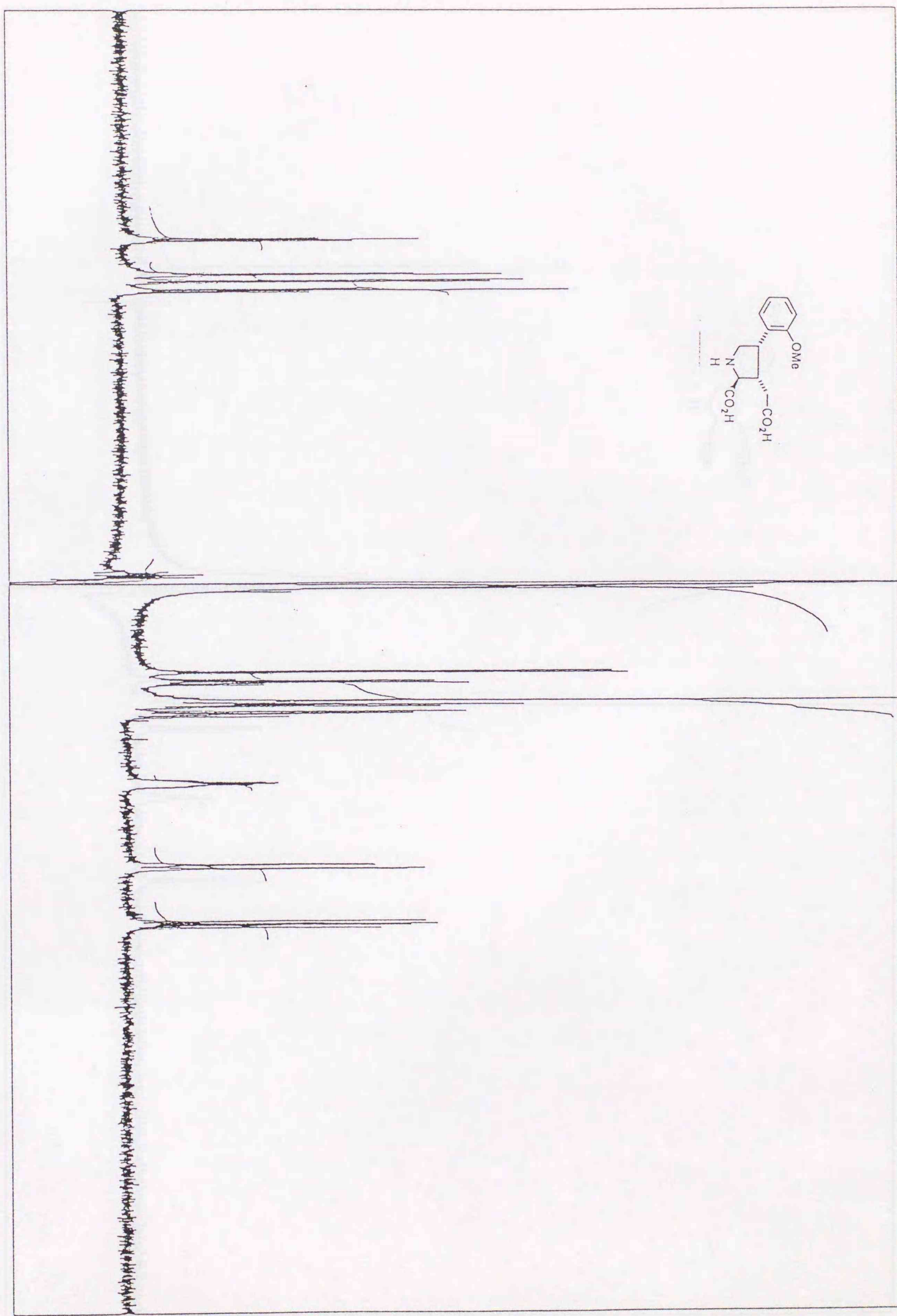
Many kainoids were synthesized by the newly developed synthetic methods and their depolarizing activities were estimated. The ab initio calculations for the π -systems of various C4-substituents and the detailed analysis of ^1H NMR spectra for both excitatory and non-excitatory kainoids were performed. These data suggest that the C4- π -group directly interacts with the receptors and the suitable spatial arrangement of both the L-Glu part and the C4- π -group of the substrate are necessary to bind to the receptors. Recently, the cloning of kainate-type receptors was reported¹ and some isolation studies² were also reported. However, the higher structures and the biological natures of these membrane proteins remain unclear. Further synthetic studies based on the above results would contribute to the elucidation of the structures and biological properties of kainate-type receptors.

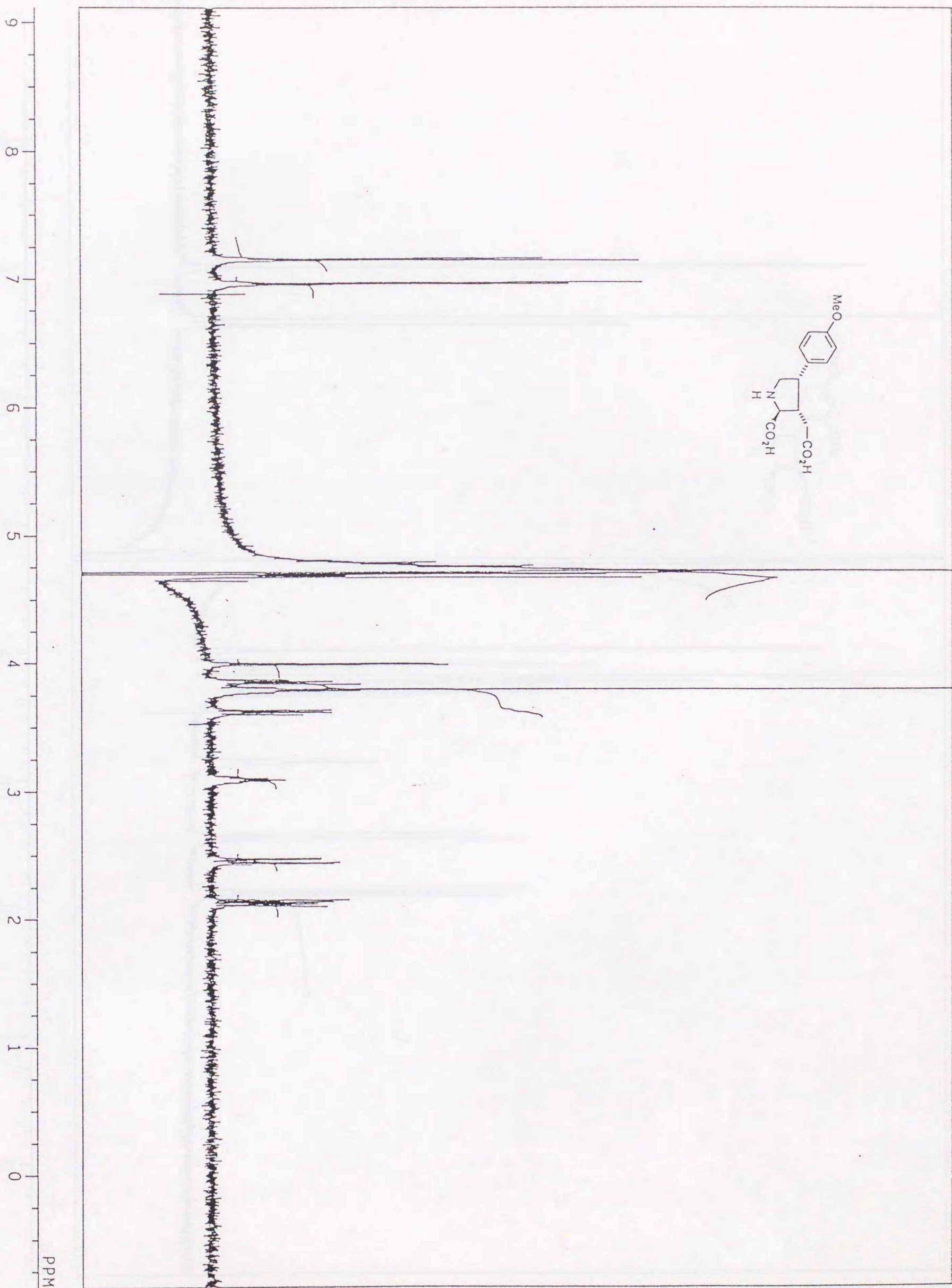
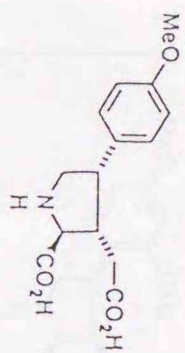
References

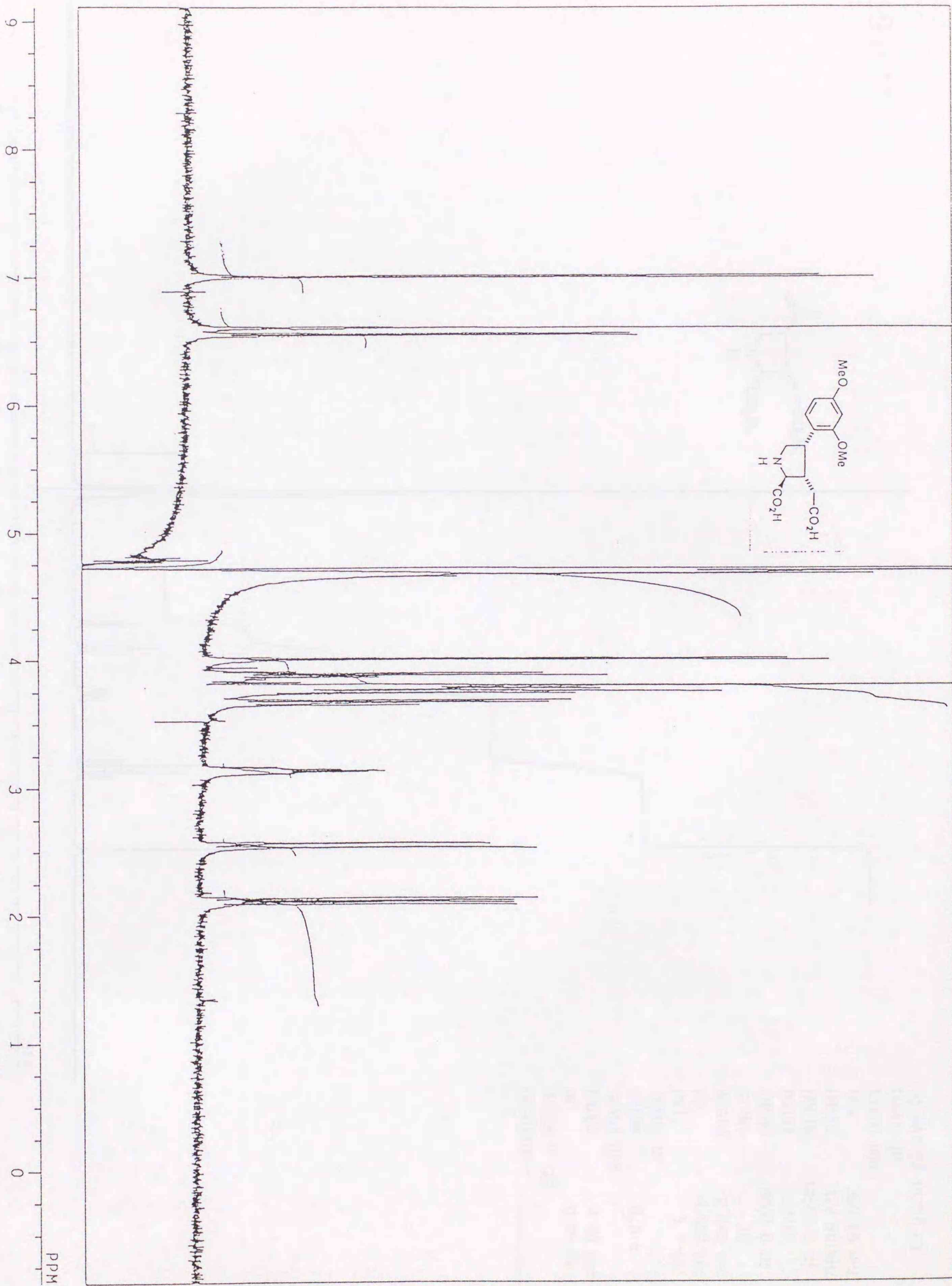
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c) D. R. Hampson, K. D. Wheaton, C. J. Dechesne and R. J. Wenthold, *J. Biol. Chem.*, **264**, 13329 (1989).
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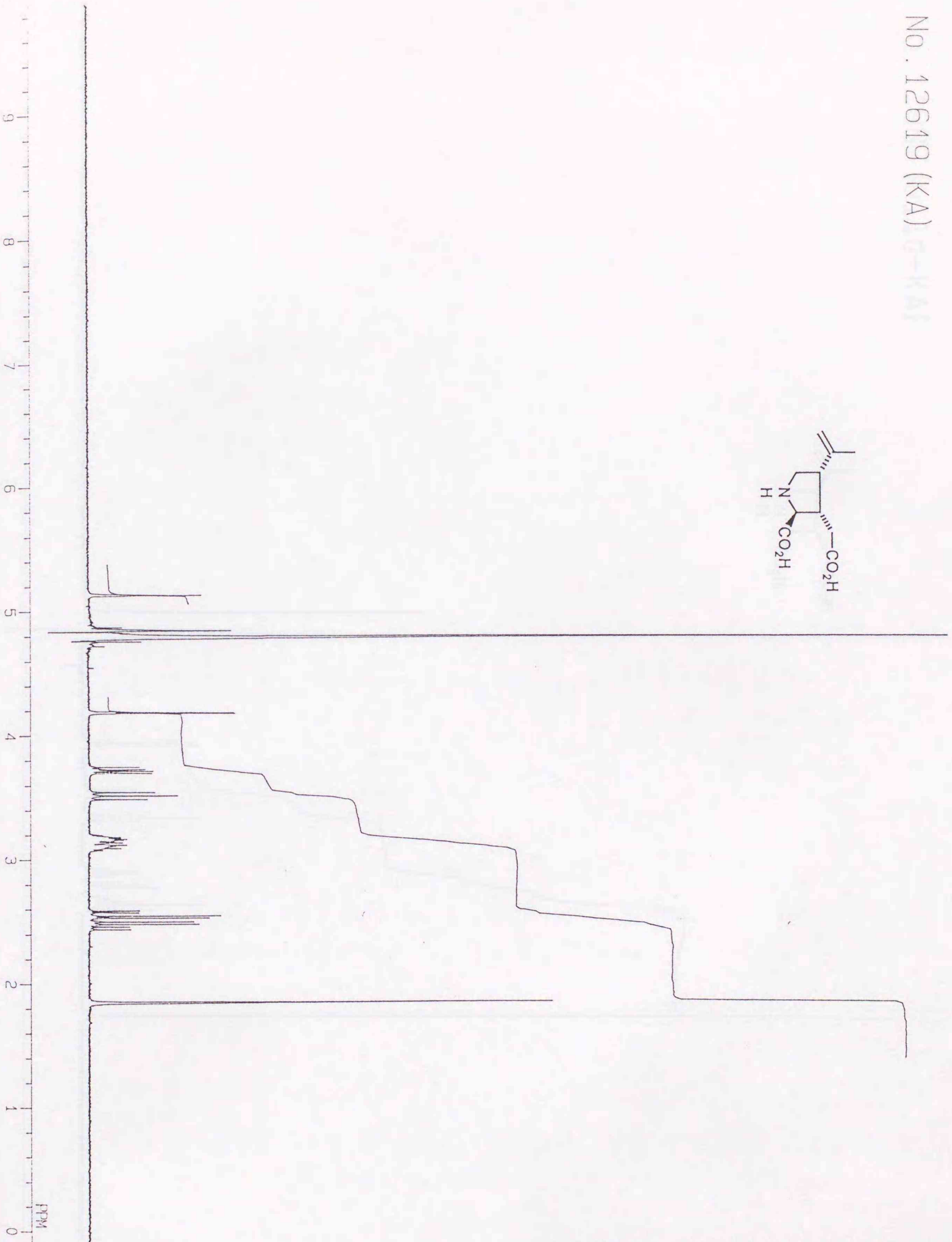
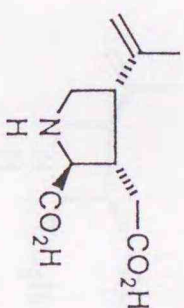
9
8
7
6
5
4
3
2
1
0
ppm





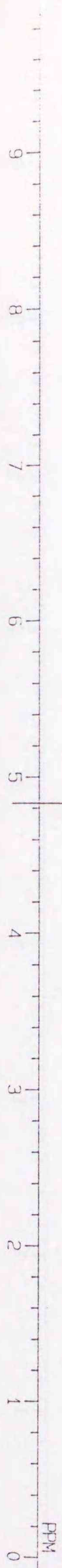
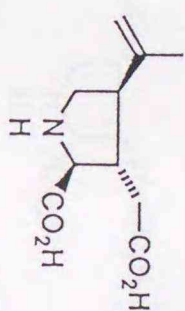


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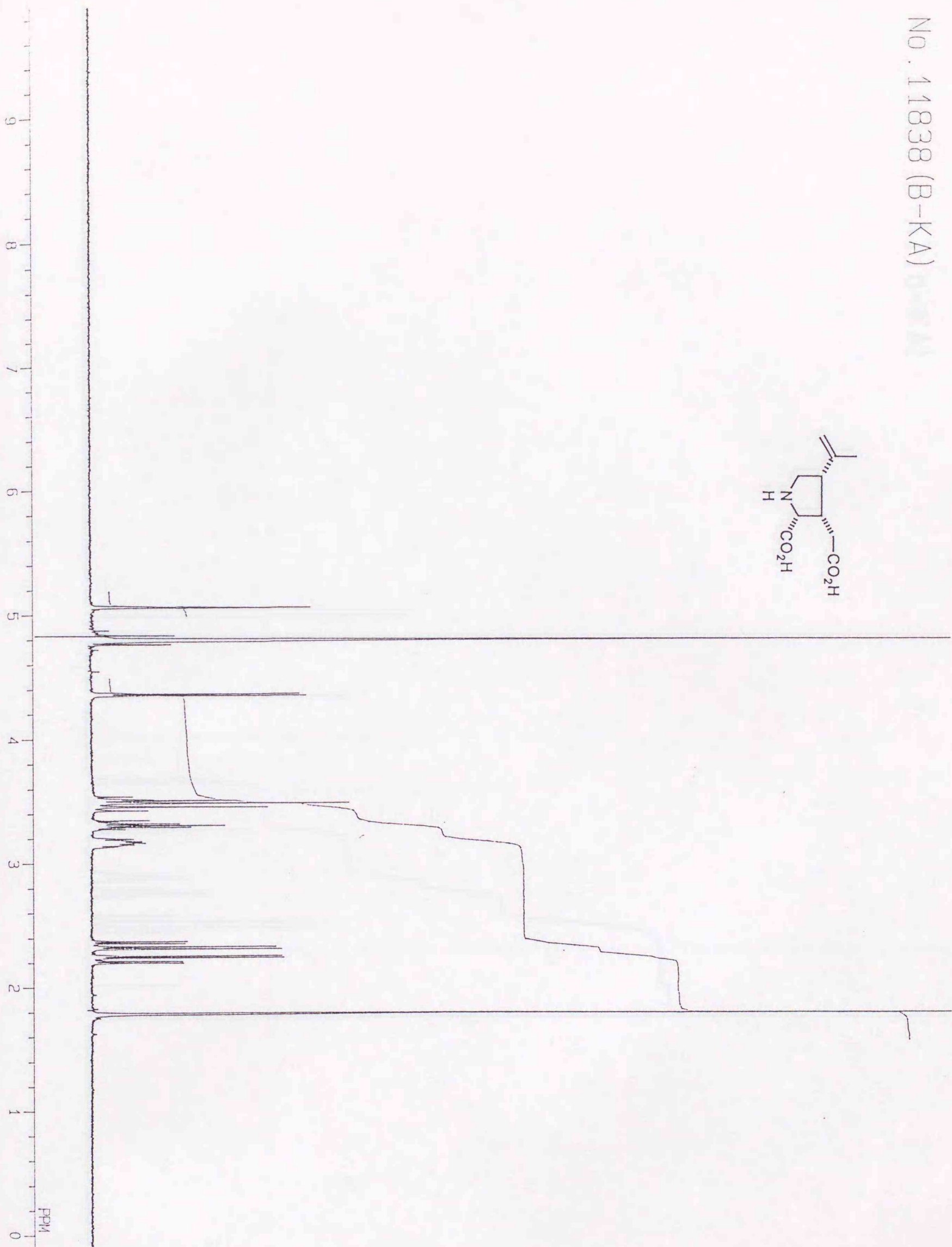
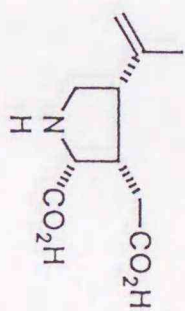


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 OBFIN 10500.0 Hz
 POINT 32768
 FREQ 8000.0 Hz
 SCANS 16
 ACQTM 2.048 sec
 PD 4.952 sec
 PM1 5.7 us
 IRNUC 1H
 CTEMP 0.0 c
 SLVNT D2O
 EXREF 4.80 ppm
 BF 0.25 Hz
 RGAIN 22
 OPERATOR :

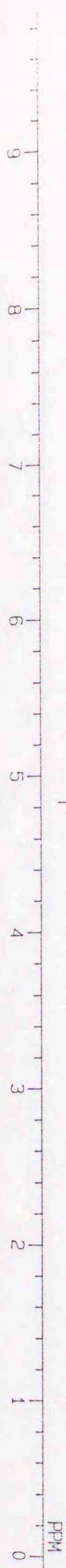
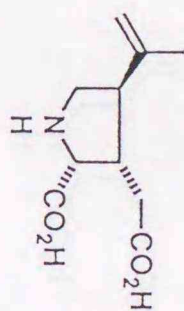
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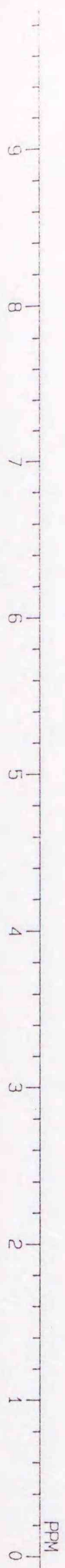
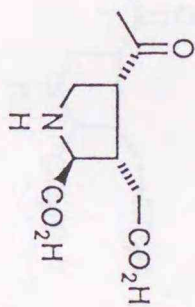
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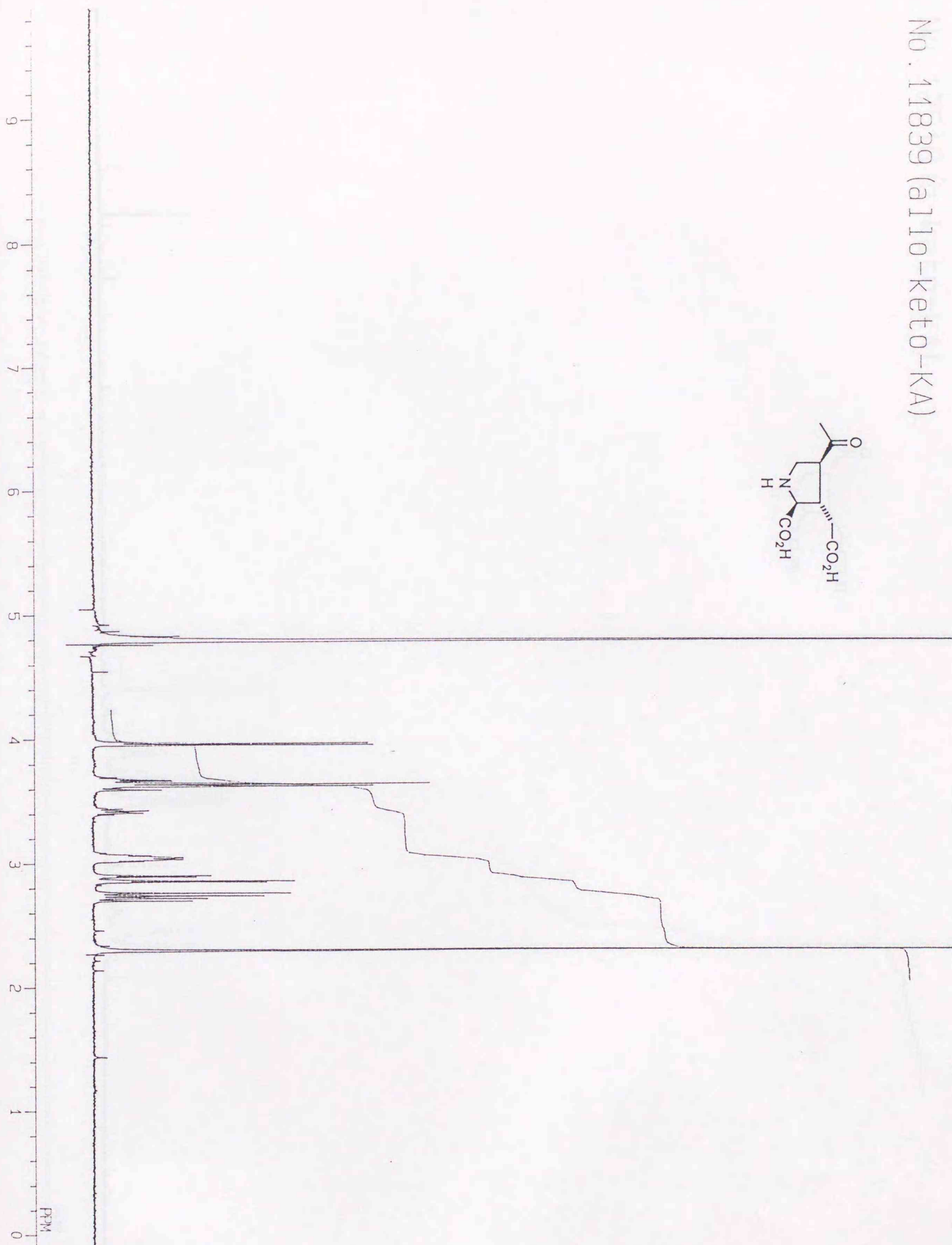
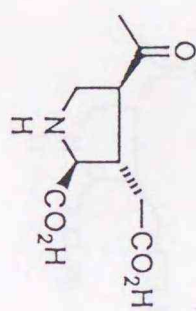
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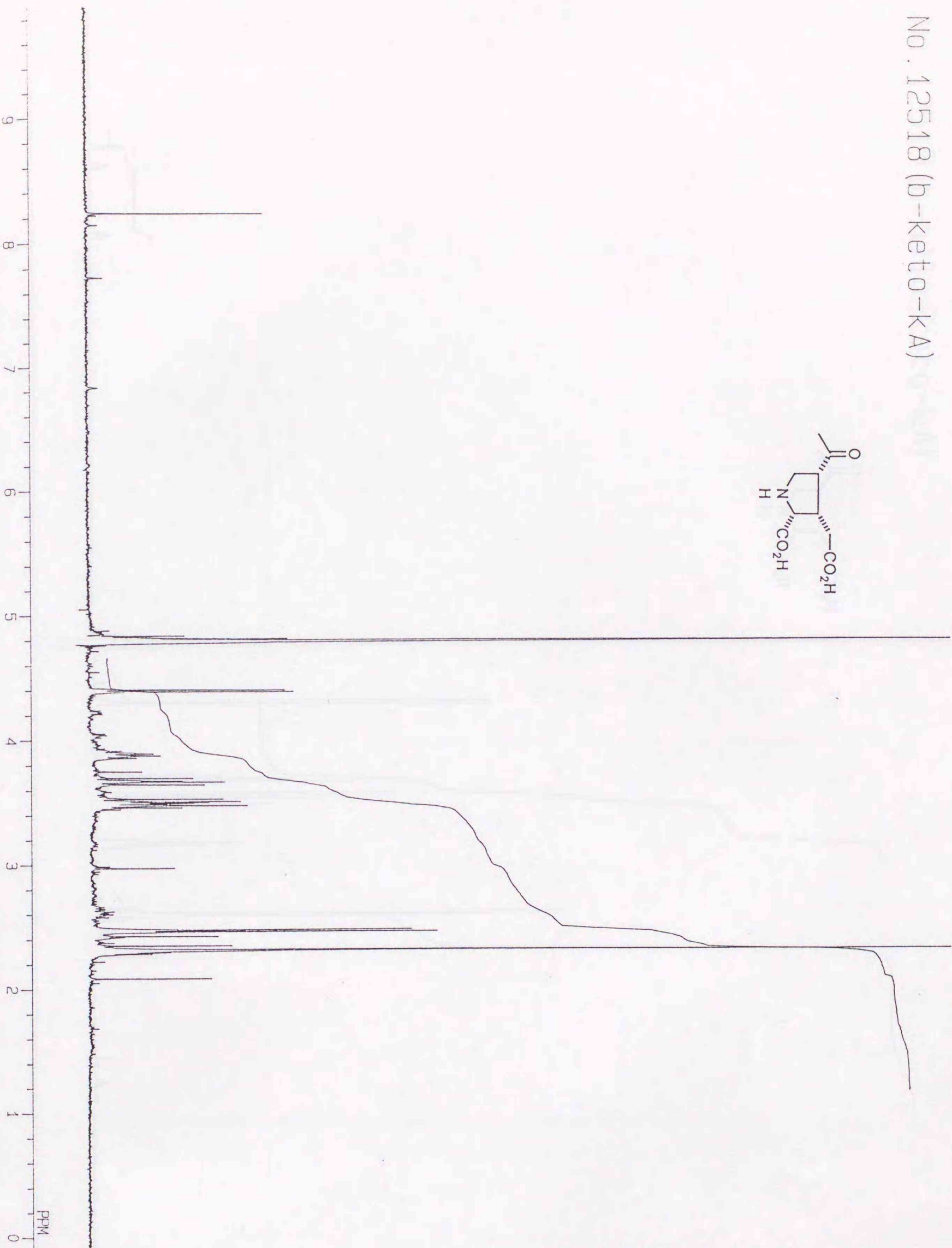
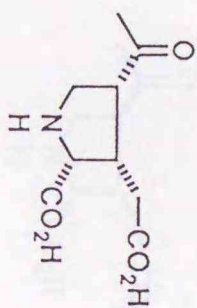
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No. 11839 (a110-keto-KA)

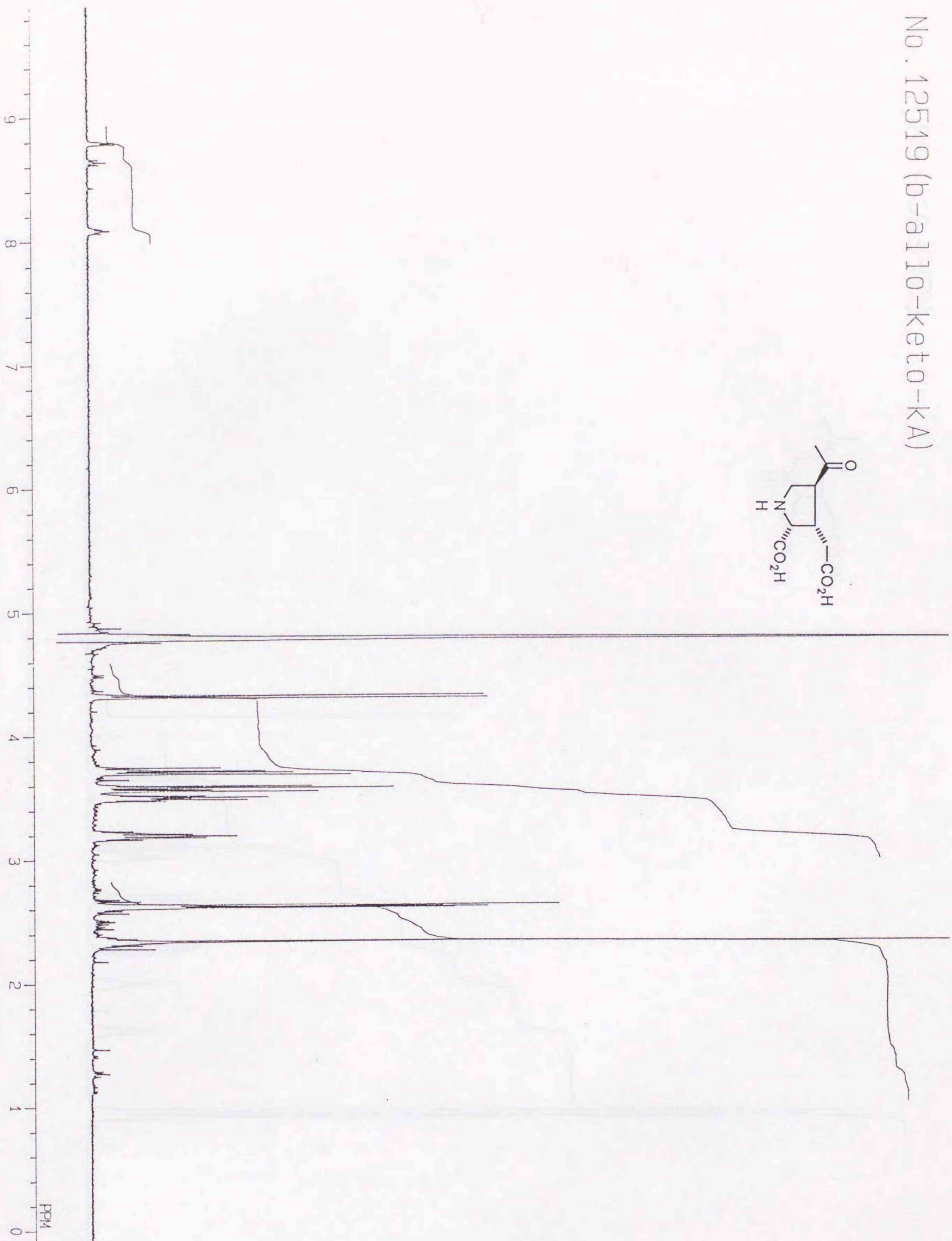
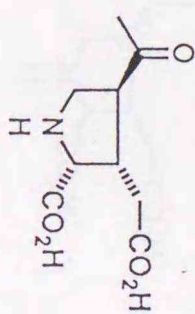


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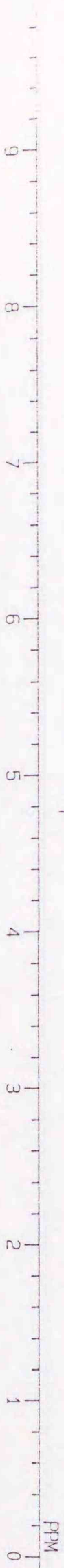
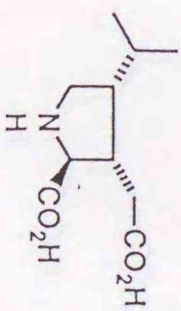


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 OBSET 124.00 KHz
 OBFIN 10500.0 Hz
 POINT 32768
 FREQU 8000.0 Hz
 SCANS 12
 ACQTM 2.048 sec
 PD 4.952 sec
 PM1 5.7 us
 IRNUC 1H
 CTEMP 0.0 C
 SLVNT D2O
 EXREF 4.80 ppm
 BF 0.25 Hz
 RGAIN 22
 OPERATOR : _____

No. 12519 (b-a110-keto-kA)

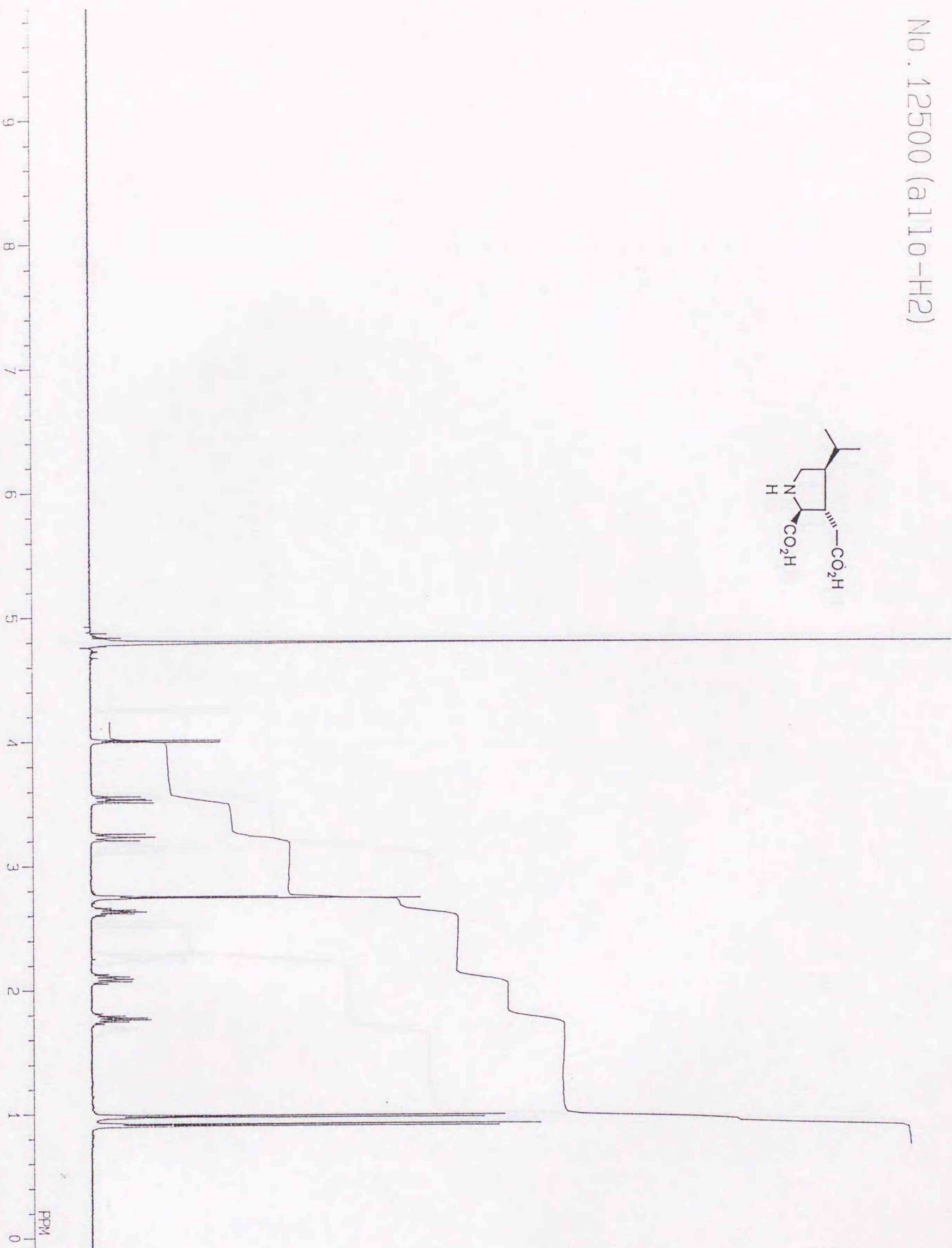
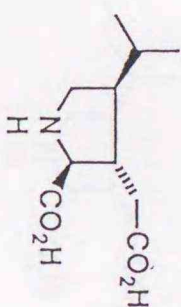


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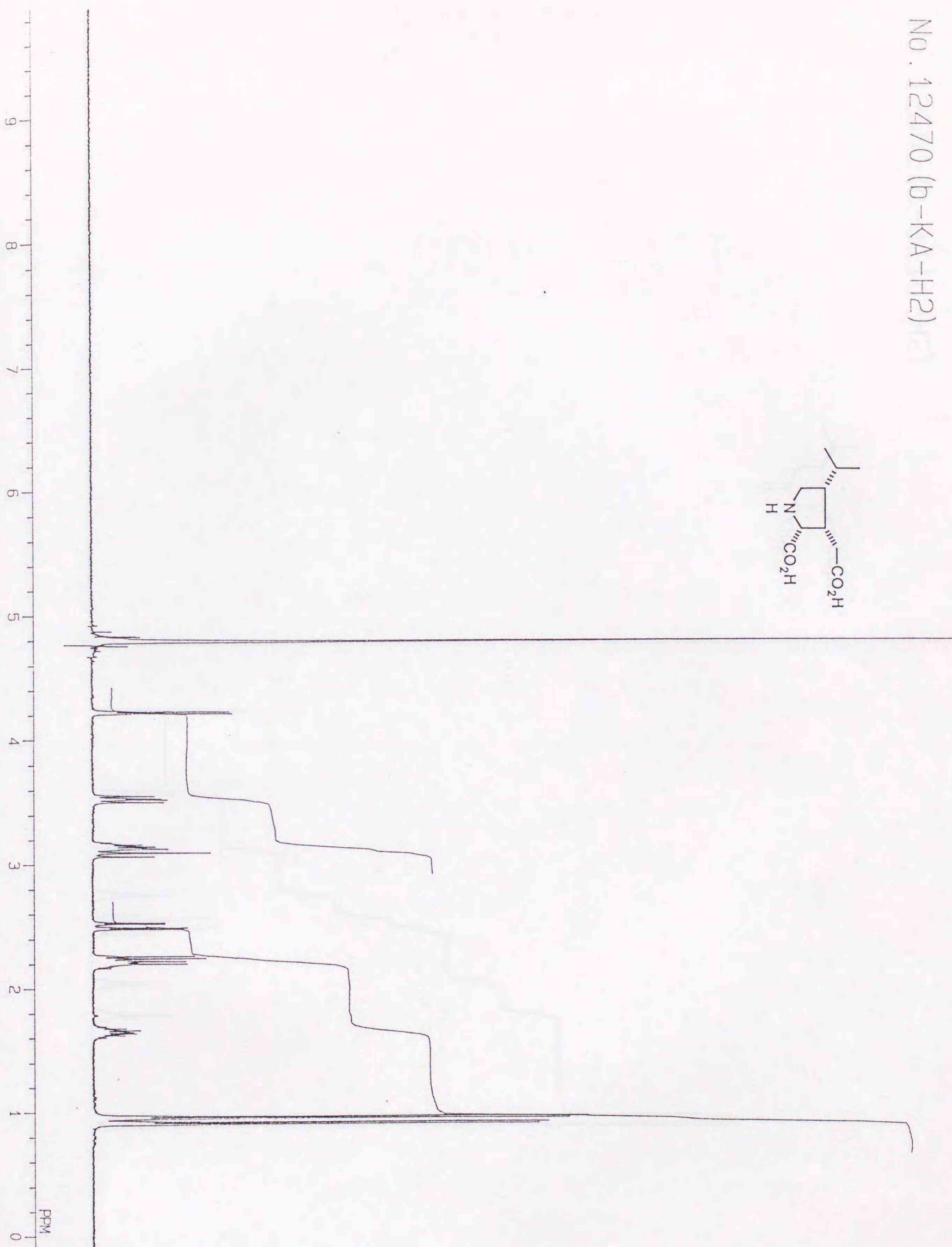
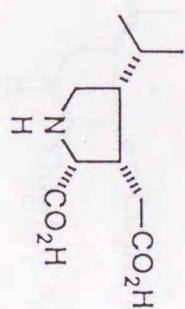
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 OFIN 10500.0 Hz
 POINT 32768
 FREQ 8000.0 Hz
 SCANS 4
 ACQTM 2.048 sec
 PD 4.952 sec
 PW1 5.7 us
 IPNUC 1H
 CTEMP 22.8 C
 SLVNT C6D6-
 EXREF 4.80 ppm
 BF 0.25 Hz
 RGAIN 21
 OPERATOR :

No. 12500 (a110-H2)

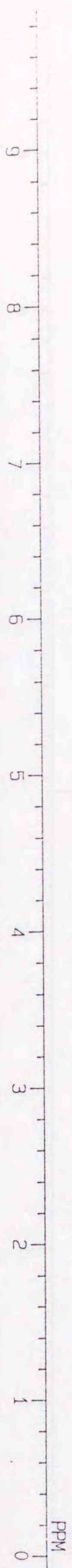
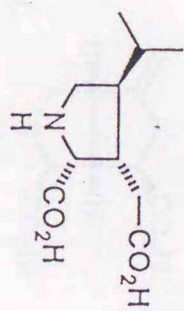


18-MAY-93 11:21:34
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 OBSET 124.00 KHz
 OBFIN 10500.0 Hz
 POINT 32768
 FREQ 8000.0 Hz
 SCANS 20
 ACQTM 2.048 sec
 PD 4.952 sec
 PM1 5.7 us
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 CTEMP 0.0 C
 SLVNT D2O
 EXREF 4.80 ppm
 BF 0.25 Hz
 RGAIN 23
 OPERATOR : _____

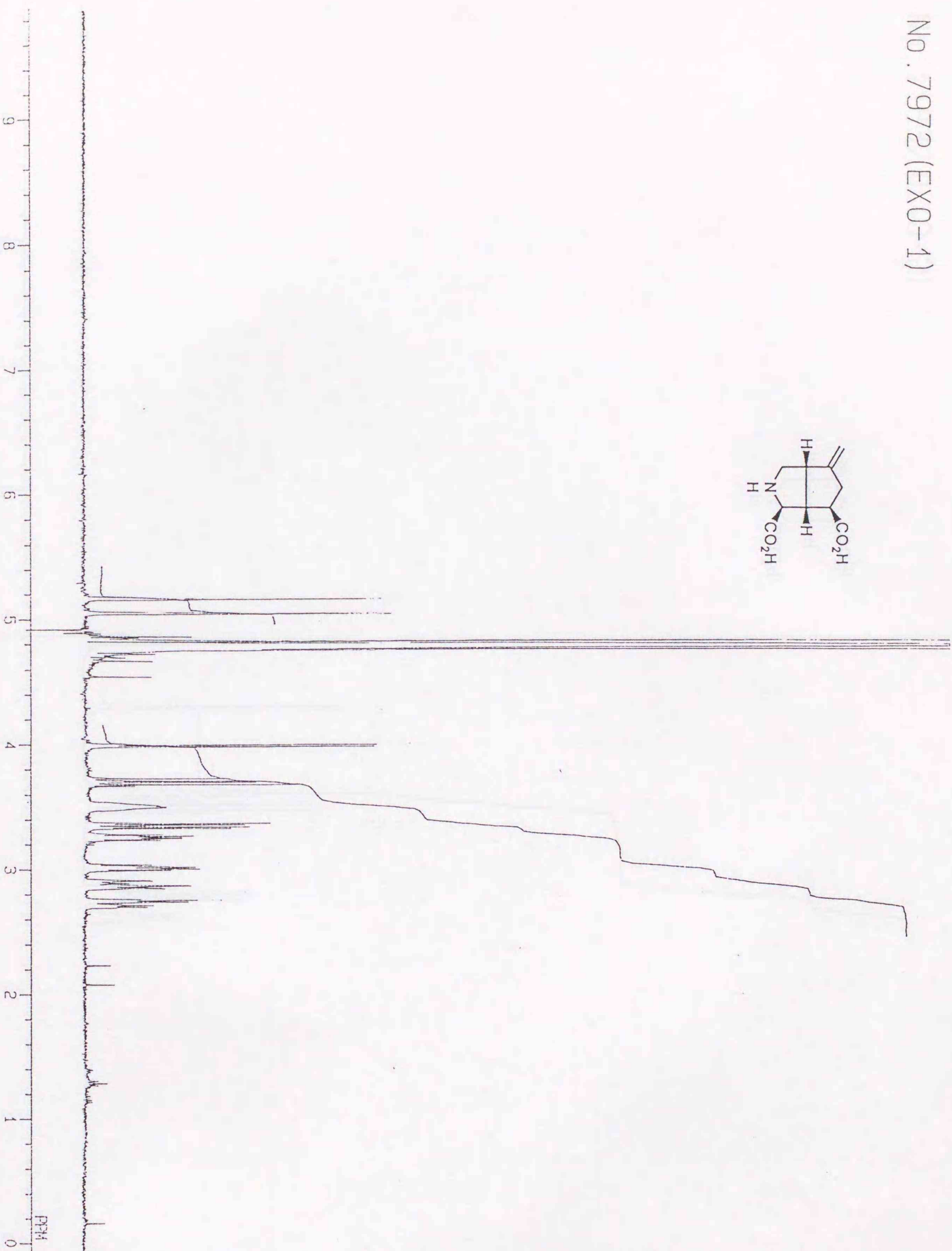
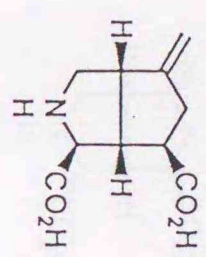
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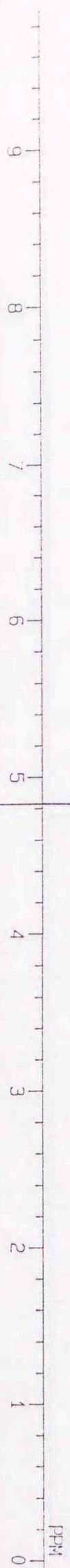
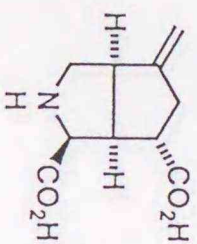


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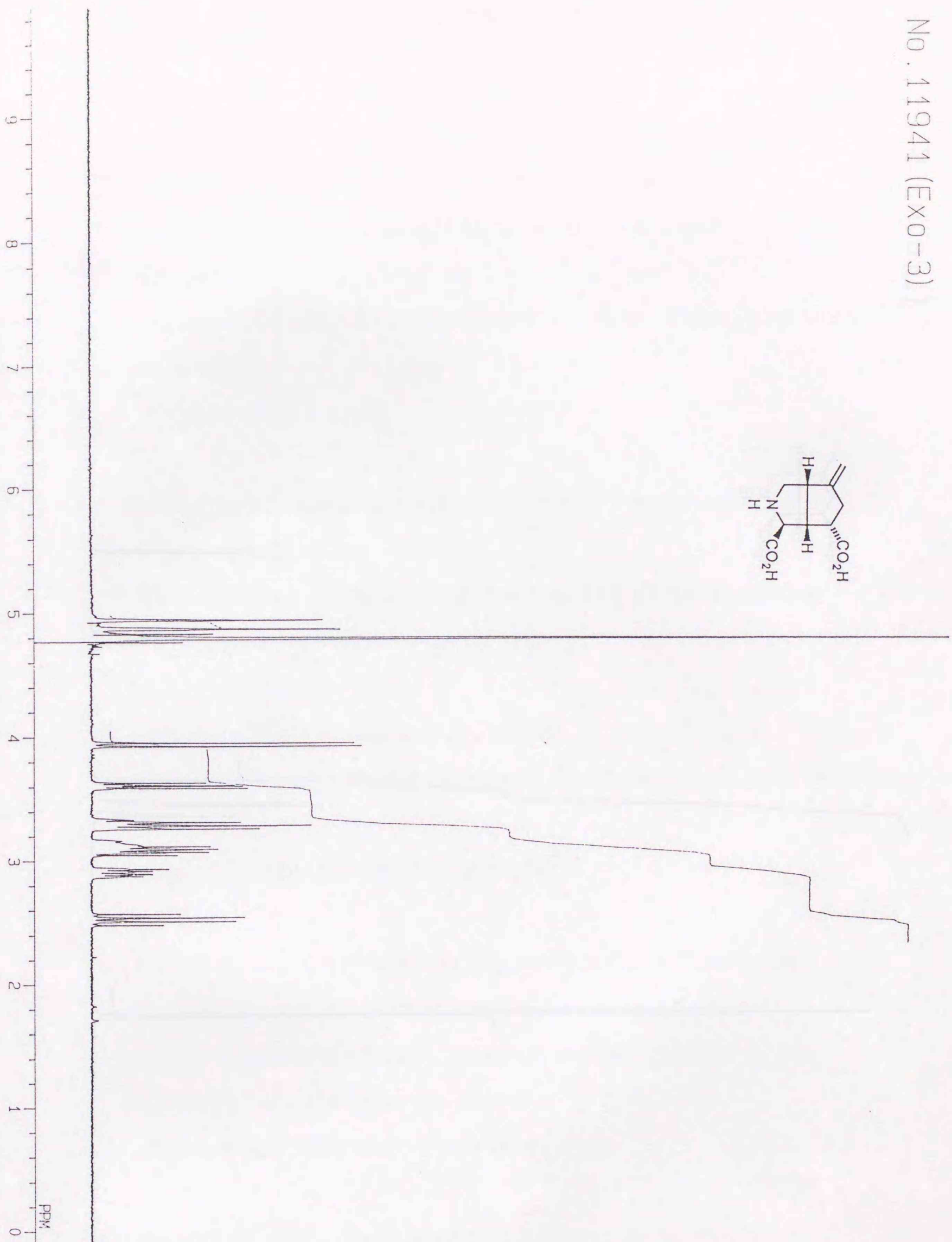
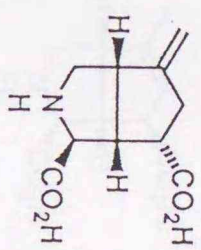
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 CLPNT 256
 TODAT 1
 CLFRQ 100.0 HZ
 SCANS 8
 ACQTM 2.048 sec
 PD 4.952 sec
 PW1 5.0 us
 PW2 10.0 us
 PW3 10.0 us
 P11 1.000 ms
 P12 1.000 ms
 P13 1.000 ms
 IRATN 511
 OBATN 511
 CTEMP 24.1 c
 CSPED 14 HZ
 SLVNT D2O
 LOOP1 1
 XS 96.2526 HZ
 CXS 0.0000 HZ
 XE 3997.8240 HZ
 CXE 100.0000 HZ
 TH 0.00000
 DET 72.5 us
 DELAY 50.0 us
 OPERATOR :

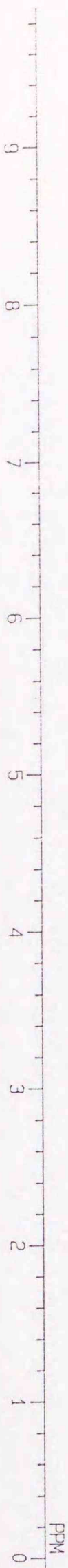
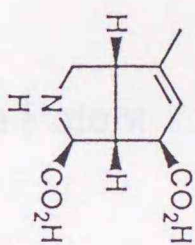
NO. 11837 (EX0-2)



02-FEB-93 10:01:52
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 OBSET 124.00 KHz
 OBF IN 10500.0 Hz
 POINT 32768
 FREQU 8000.0 Hz
 SCANS 12
 ACQTM 2.048 sec
 PD 4.952 sec
 PW1 5.7 us
 IRNUC 1H
 CTEMP 30.0 C
 SLVNT D2O
 EXREF 4.80 ppm
 BF 0.25 Hz
 RGAIN 20
 OPERATOR : _____

No. 11941 (Exo-3)





Publication List

[Original papers]

1. Neurotoxic Ciguatera-like Syndrome of *Halimeda* Mushrooms *Gymnophyes*

Speciality

Mitsuyasu Tanaka, Kinuko Hashimoto, Toshiaki Ohtsuka, and Haruhisa

Shirahama

Phytochemistry in press

2. Efficient Synthesis of Aspartic Acid B and C, Which Are

Neurotoxic Amino Acids

Mitsuyasu Tanaka, Kinuko Hashimoto, and Haruhisa Shirahama

Journal of Organic Chemistry 52, 1234-1238 (1987)

3. Isolation of the Acid Group of Gymnophyes

Mitsuyasu Tanaka, Kinuko Hashimoto, Toshiaki Ohtsuka, and Haruhisa

Shirahama

Phytochemistry 21, 1001-1002 (1982)

4. Configuration of Various Hydroxy Methyl Groups That Potent

Neurotoxic Activity in the Sea Cucumber *Halimeda* System

Kinuko Hashimoto, Masahito Horikawa, Michiko Ichida, Haruhisa

Shirahama, and Haruhisa Shirahama

Biological Chemistry Letters 2, 742-744 (1987)

5. Novel Neurotoxic Amino Acid (p-Cl)-Glycine Aspartic Acid

Kinuko Hashimoto, Kinuko Hashimoto, and Haruhisa Shirahama

Neuroscience 34, 141-144 (1987)

Publication List

[Original papers]

- 1 Neurotoxic Oligoisoprenoids of Hallucinogenic Mushroom *Gymnopilus Spectabilis*
Masayasu Tanaka, Kimiko Hashimoto, Toshikatsu Okuno, and Haruhisa Shirahama
Phytochemistry in press.
- 2 Efficient Syntheses of Acromelic Acids B and E, Which Are Potent Neuroexcitatory Amino Acids
Manabu Horikawa, Kimiko Hashimoto, and Haruhisa Shirahama
Tetrahedron Lett., **34**, 331~334 (1993).
- 3 Chirality of The Acyl Group of Gymnopilin
Masayasu Tanaka, Kimiko Hashimoto, Toshikatsu Okuno, and Haruhisa Shirahama
Phytochemistry, **31**, 4355~4356 (1992).
- 4 Configurational Variants of Hydroxyphenylkainoids: Their Potent Depolarizing Activities in The Rat Central Nervous System
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- 5 Novel Neuroexcitatory Amino Acid from *Clitocybe Acromelalga*
Kimiaki Yamano, Kimiko Hashimoto, and Haruhisa Shirahama
Heterocycles, **34**, 445~448 (1992).

- 6 Synthesis and Neuroexcitatory Activity of Some Acromelic Acid Analogs
Katsuhiro Konno, Kimiko Hashimoto, and Haruhisa Shirahama
Heterocycles, **33**, 303~311 (1992).
- 7 Synthesis of New Acromelic Acid Congeners: Novel Neuroexcitatory
Amino Acids Acting on Glutamate Receptor
Kimiko Hashimoto, Haruhisa Shirahama
Tetrahedron Lett., **32**, 2625~2628 (1991).
- 8 Simple Analogs of Acromelic Acid, Which Are Highly Active Agonists of
Kainate Type Neuroexcitant
Kimiko Hashimoto, Manabu Horikawa, and Haruhisa Shirahama
Tetrahedron Lett., **31**, 7047~7050 (1990).
- 9 Syntheses of Acyclic Analogues of Kainoids And Neuroexcitatory Activity
Miwa Yanagida, Kimiko Hashimoto, Michiko Ishida, Haruhiko
Shinozaki, and Haruhisa Shirahama
Tetrahedron Lett., **30**, 3799~3802 (1989).
- 10 Acromelic Acids A and B. Potent Neuroexcitatory Amino Acids Isolated
from *Clitocybe Acromelalga*
Katsuhiro Konno, Kimiko Hashimoto, Yasufumi Ohfune, Haruhisa
Shirahama, and Takeshi Matsumoto
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- 11 Improved Procedures for Preparation of 2-Pyridones and 2-
Hydroxymethylpyridones from Pyridine N-Oxides
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Matsumoto

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12 Synthesis of Acromelic Acid B, A Toxic Principle of *Clitocybe*

Acromelalga

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13 A New Pyridine Synthesis Starting from α,β -Unsaturated Carbonyl Compounds

Katsuhiro Konno, Kimiko Hashimoto, Haruhisa Shirahama, and Takeshi Matsumoto

Tetrahedron Lett., **27**, 3865~3868 (1986).

14 Synthesis of Acromelic Acid A, A Toxic Principle of *Clitocybe*

Acromelalga

Katsuhiro Konno, Kimiko Hashimoto, Haruhisa Shirahama, and Takeshi Matsumoto

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[Reviews]

1 きのこ毒

白濱晴久、橋本貴美子

一億人の化学シリーズ、13巻、「生物毒の世界」日本化学会編
(大日本図書) p93~116 (1992).

2 神経毒、悪酔毒

橋本貴美子、白濱晴久

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3 Syntheses of Neuroexcitatory Kainoids

Kimiko Hashimoto and Haruhisa Shirahama

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4 グルタミン酸レセプターに作用する物質

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5 Chemistry of Glutamate Analogues

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Horikawa

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6 Synthesis and Neuroexcitatory Activity of New Kainoids

Kimiko Hashimoto and Haruhisa Shirahama

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有機合成化学協会誌、47, p212~223, p267 (1989).

8 Acromelic Acids A and B, Very Potent Excitatory Amino Acids from A
Poisonous Mushroom

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Neurotox '88: Molecular Basis of Drug and Pesticide Action, G. G. Lunt Ed, Elsevier Science Publishers BV, Amsterdam, p105~122 (1988).

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