Pergamon

Tetrahedron, Vol. 52, No. 6, pp. 1931-1942, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)01058-0

Acyclic Analogs of Kainoids: Their Syntheses and Depolarizing Activities

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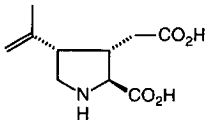
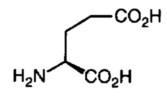
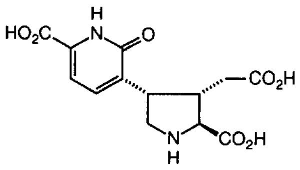
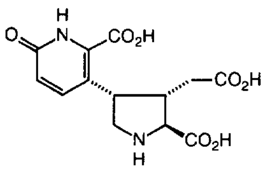
Abstract: Four stereoisomers of the β-benzylglutamic acids were synthesized by the Michael addition of benzylphenylsulfide to protected (4*S*)- and (4*R*)-4-amino-2-penten-5-olide and to protected (2*S*)- and (2*R*)-3-dehydro-2-hydroxymethyl-5-pyrrolidone. The β-methallyl- and β-isobutylglutamic acids were also synthesized in a similar manner. The depolarizing activities of these synthesized compounds were observed.

Kainic acid (**1**) has been used as a successful probe for the analysis of the physiological functions of excitatory amino acids since they have potent excitatory activity on mammalian central neurons.[1] In particular, it introduced the excitotoxic concept that glutamic acid (**2**) destroys neurons by excessive activation of the excitatory receptors. The neuronal degeneration has been shown to mimic the symptoms observed in patients suffering from neuronal diseases such as epilepsy and Huntington's chorea. The new kainoids, acromelic acids[2] A (**3**) and B (**4**), which are aromatic kainoids and isolated by us from poisonous mushroom, were found to exhibit extremely potent depolarizing activity, almost 3 to 5 times stronger than kainic acid in rat spinal motoneurons, and destroy the lower part of the spinal cord while kainic acid destroys the brain.[3] The aromatic kainoids, which have various aromatic substituents at C4 of the proline skeleton, have therefore attracted pharmacologists' interests and have been expected to be useful reagents to study the function of excitatory receptors. Recently, we synthesized several aromatic kainoids[4] and demonstrated the importance of the chemical properties and the stereochemistry of the C4-substituent on the biological activity.[5] Considering the interaction between the kainoid and the receptor, the three functional groups, namely the two carboxyl and one amino groups of the glutamic acid moiety, in addition to the C4-substituent should be adequately arranged to favorably fit into the receptor. The pyrrolidine ring should play a role to arrange the spatial position of these four functional groups in the kainoid molecule. In order to clear the part of the pyrrolidine ring, the syntheses of aliphatic analogs of the kainoid, which were formally derived by the elimination of the C5-carbon atom from the pyrrolidine ring, were attempted. (Fig. 1) We would like to describe here the syntheses and the relative


kainic acid (**1**)


L-glutamic acid (**2**)


acromelic acid A (**3**)


acromelic acid B (**4**)

strength with respect to biological activities of four stereo isomers of β-benzylglutamic acid, and the β-methallyl- and β-isobutylglutamic acids.[6]

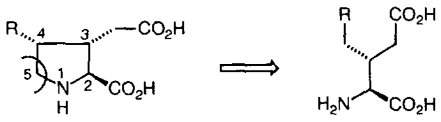
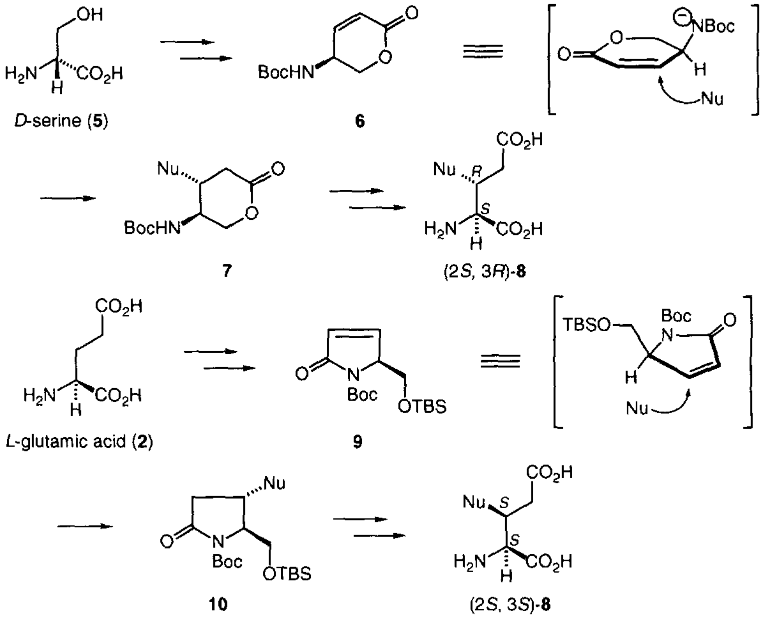


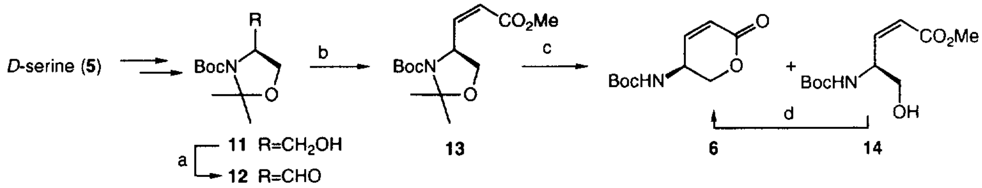
Fig. 1.

The samples used for the pharmacological test should be enantiomerically pure. The stereoselective introduction of the substituents into the glutamic acid framework at the C3 position was accomplished using Michael addition reactions employing two differently modified glutamic acids as the acceptors, **6** and **9** (Scheme 1). One of them, aminodihydropyron **6** was prepared from *D*-serine (**5**) (Scheme 2). According to



Scheme 1. Synthetic plan toward 3-substituted glutamic acids.

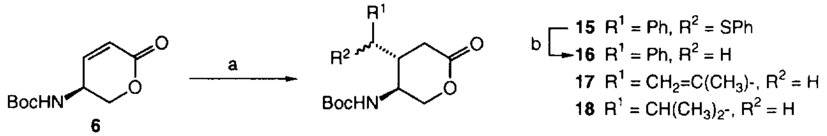
Garner's procedure,[7] **5** was converted to a protected hydroxymethyl glycinol **11** which was oxidized to an aldehyde **12** using Swern's reagent. The aldehyde **12** underwent a Wadsworth condensation employing bis(2,2,2-trifluoroethyl) (methoxycarbonyl)methylphosphonate[8] and potassium hexamethyldisilazide in the presence of 18-crown-6-ether in THF at -78°C to give the *cis*-α,β-unsaturated ester **13**[9] without any racemization in 87% yield through two steps. The treatment of **13** with *p*-TsOH in MeOH resulted in the deprotection of the acetonide followed by lactone formation to afford **6**[10] and the unlactonized ester **14** in 35% and 27% yield, respectively. The ester **14** was further treated with *p*-TsOH in CH_2Cl_2 to cyclize to **6** in 70% yield. On the other hand, lactam **9** was prepared from *L*-glutamic acid (**2**) without any racemization according to Ohfune's report.[10a]



a) $(COCl)_2$, DMSO, CH_2Cl_2, -78°C, then Et_3N b) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KH, $(TMS)_2NH$, 18-crown-6, THF, -78°C (87%, 2 steps) c) p-TsOH, MeOH, 25°C (35% (**6**), 27% (**14**)) d) p-TsOH, CH_2Cl_2, 25°C (70%)

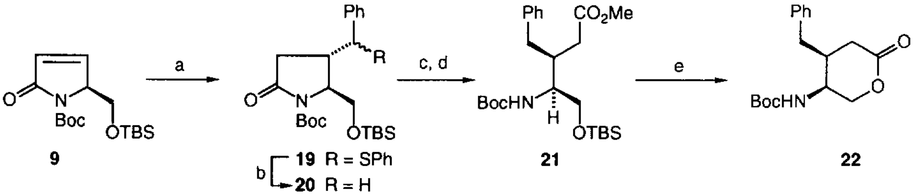
Scheme 2.

The Michael addition reactions to **6** and **9** were carried out as follows (Schemes 3 and 4). The anion produced from benzylphenylsulfide with butyl lithium added to **6** to give **15** as a diastereomeric mixture (1:1) in 47% yield. Desulfurization of the mixture **15** afforded a single product **16** in 88% yield. This clearly means that diastereoisomerism of **15** stems from two configurations of the benzyl carbon and the addition at the β-position of **6** stereoselectively occurred from one side. The determination of the stereochemistry of **16** is described later. The introduction of methallyl and isobutyl groups were also stereoselectively achieved with methallyl and isobutyl Grignard reagents in the presence of the cuprous bromide dimethylsulfide complex and adducts **17** and **18** were obtained in 24% and 60% yield accompanied by recovered **6** in 54% and 23%, respectively. The addition reaction of methallylphenylsulfide resulted in failure to produce a complex mixture. Methallyl-pyridylsulfide was added to give an adduct whose desulfurization was unsuccessful. Another Michael acceptor **9** also underwent the smooth addition of benzylphenylsulfide as well as the reaction of **6** to afford **19** as a diastereomeric mixture (1:1) in 73% yield. The perfect stereoselectivity of the addition reaction was again demonstrated by obtaining single product **20** through desulfurization of the addition product **19**. The basic hydrolysis of the lactam group and esterification of the resulting carboxylic acid converted **20** to **21** in 79% yield. The removal of the TBS group with aqueous hydrofluoric acid in acetonitrile furnished the pyranone **22** in 77% yield, which was diastereomeric to the previously obtained **16**.



a) $PhCH_2SPh$, TMEDA, n-BuLi, THF, -78°C (47% (**15**)), $CH_2{=}C(CH_3)CH_2MgCl$, CuBr-DMS, DMS, THF, -78°C (24% (**17**), 54% (**6**)) $(CH_3)_2CHCH_2MgCl$, CuBr-DMS, DMS, THF, Et_2O, -78°C (60% (**18**), 23% (**6**)) b) n-Bu_3SnH, AIBN, PhH, 80°C (88%)

Scheme 3.



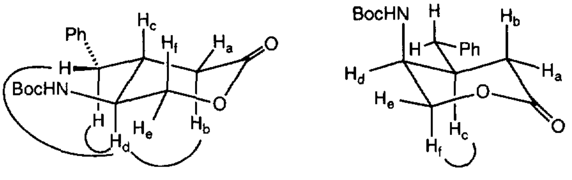
a) $PhCH_2SPh$, TMEDA, n-BuLi, THF, -78°C (73%) b) Raney Ni (W-2), acetone, H_2O, 56°C (98%) c) 1M $NaOH_{aq}$, MeOH, 25°C d) CH_2N_2, MeOH, 25°C (2 steps 79%) e) HF_{aq}, CH_3CN, 25°C (77%)

Scheme 4.

The stereochemistry of a diastereomeric pair of γ-lactones **16** and **22** was explored with ^{1}H NMR experiments. Since they were six-membered rings, the direct assignment of their stereochemistries by estimating the coupling constants among their protons could be basically possible. But the signals due to protons (H_d in Fig. 2) linked to the carbons bearing the amine groups of **16** and **22** were too broad to observe the *J*-values. Table 1 shows the readable coupling constants obtained from the spectra. For compound **16**, the constants, J_{ac}=6 and J_{bc}=10Hz, clearly assigned an equatorial configuration to the benzyl group. The configuration of the *N*-Boc group of **16** could not be assigned by ambiguous coupling constants, J_{de}=4 and J_{df}=6Hz. However, NOEs were observed between H_b and H_d and among H_d and the benzyl protons. Thus, both the benzyl group and *N*-Boc group should be assigned to an equatorial configuration as shown in Fig. 2. Two substituents of **16** were thus oriented *trans*. In the case of **22**, the constants, J_{ac}=6, J_{bc}=12, J_{de}=2 and J_{df}=2Hz suggested that the benzyl group and the *N*-Boc group are assigned to equatorial and axial positions, respectively. The observation of NOE between Hc and Hf also supported this assignment. The orientation of the two substituents was shown to be *cis*.

Compounds	H_a-H_b	H_a-H_c	H_b-H_c	H_d-H_e	H_d-H_f
16	16	6	10	4	6
22	18	6	12	2	2

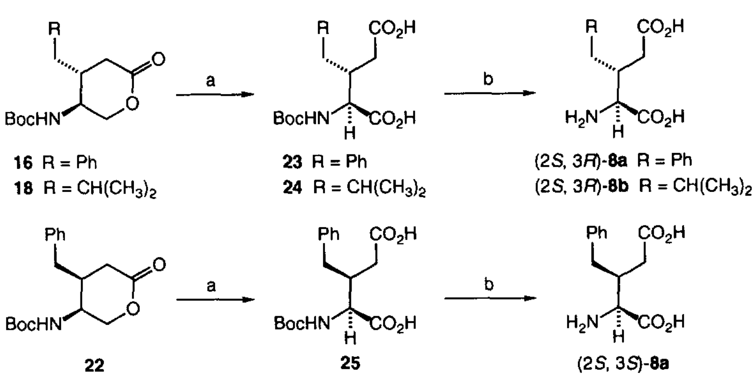
Table 1. Coupling constants (Hz) of pyranone derivatives **16** and **22**



16 **22**

Fig. 2.

Transformation of the lactones **16**, **18** and **22** to amino acids was next carried out (Scheme 5). Stirring with aqueous NaOH in the presence of $KMnO_4$, the lactones **16**, **18** and **22** underwent saponification and subsequent oxidation of the resultant hydroxyl group to afford dicarboxylic acids, **23**, **24** and **25** which were subjected to the next step without purification. Removal of their Boc group was performed by employing trifluoroacetic acid (TFA), and amino acid TFA salts (2*S*, 3*R*)-**8a**, (2*S*, 3*R*)-**8b** and (2*S*, 3*S*)-**8a** were



16 R = Ph
18 R = $CH(CH_3)_2$

23 R = Ph
24 R = $CH(CH_3)_2$

(2*S*, 3*R*)-**8a** R = Ph
(2*S*, 3*R*)-**8b** R = $CH(CH_3)_2$

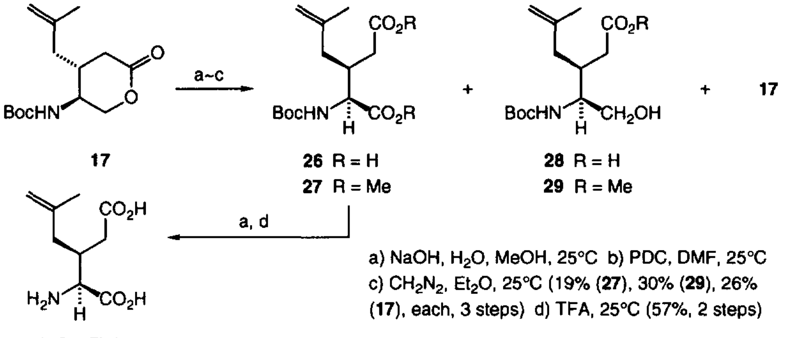
22

25

(2*S*, 3*S*)-**8a**

a) $KMnO_4$, NaOH, H_2O, 25°C b) TFA, 25°C (59% (2*S*, 3*R*)-**8a**, 57% (2*S*, 3*R*)-**8b**, 44% (2*S*, 3*S*)-**8a**, each, 2 steps)

Scheme 5.



a) NaOH, H_2O, MeOH, 25°C b) PDC, DMF, 25°C c) CH_2N_2, Et_2O, 25°C (19% (**27**), 30% (**29**), 26% (**17**), each, 3 steps) d) TFA, 25°C (57%, 2 steps)

Scheme 6.

obtained. The amino acids were freed from TFA and successively purified through columns of Amberlite IR-120B (H^+ form) and then IRC-50 (H^+ form). Then, free amino acids, (2*S*, 3*R*)-**8a**, (2*S*, 3*R*)-**8b** and (2*S*, 3*S*)-**8a** were obtained in 59%, 57% and 44% yield from the corresponding lactones, respectively. The oxidation of **17** which has an isopropenyl group susceptible to oxidation with permanganate was achieved with pyridinium dichromate after hydrolysis of the pyranone ring (Scheme 6). The produced mixture was treated with diazomethane to give the dimethylester **27**, monomethylester **29** and recovered **17** in 19%, 30% and 26% yield through 3 steps, respectively. The basic hydrolysis of **27** and removal of the Boc group afforded the (2*S*, 3*R*)-**8c**·TFA salt. The free amino acid (2*S*, 3*R*)-**8c** was obtained in a similar manner as above in 57% yield. *D*-Benzylglutamic acids, (2*R*, 3*S*)-**8a** and (2*R*, 3*R*)-**8a** were also synthesized through (4*R*)-**6** and (4*R*)-**9** starting from *L*-serine and *D*-glutamic acid, respectively.

The depolarizing activity of the synthesized compounds was observed on the preparation of the newborn rat spinal motoneuron.[11] The results are shown in Fig. 3. Among the four stereoisomers of the benzylglutamic acid, the (2*S*, 3*R*)-isomer **8a** alone exhibited stronger activity than glutamic acid (**2**) itself. The (2*S*, 3*R*)-configuration was the same as that of the kainoid and this configuration was shown to be very important for exhibiting activity. (2*S*, 3*R*)-Methallylglutamic acid (**8c**) exhibited stronger activity than glutamic acid (**2**), while (2*S*, 3*R*)-isobutylglutamic acid (**8b**) exhibited almost no activity. This means that π-electrons on the β-substituent should enhance the depolarizing activity.[12] Though (2*S*, 3*R*)-**8a** and (2*S*, 3*R*)-**8c** are more

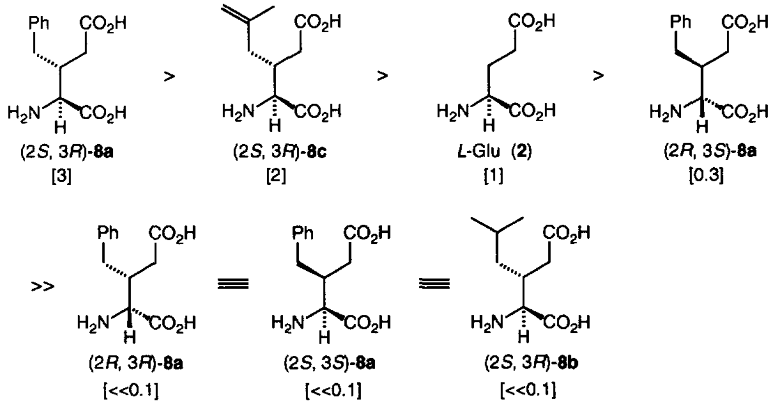


Fig. 3. Depolarizing activity in the isolated rat spinal cord.
Approximate relative activities to *L*-Glu are shown in parentheses.

potent in activity than glutamic acid, their potencies are much less when compared with that of kainic acid. Therefore, the pyrrolidine ring of the kainoid probably arranges its conformation adequately to fit the excitatory receptor. Furthermore, the depolarizing activities of (2*S*, 3*R*)-compounds **8a** and **8c** were not affected by AP5 (*D*-2-amino-5-phosphopentanoic acid) which is an NMDA (*N*-methyl-*D*-aspartic acid) antagonist. Also, they potentiated the depolarization caused by glutamic acid on the preparation of the crayfish neuro-muscular junction[13]. Therefore, both of the (2*S*, 3*R*)-β-benzyl and β-isobutenylglutamic acids (**8a**, **8c**) would be kainate type agonists. It is noteworthy that acyclic glutamic acid derivatives such as (2*S*, 3*R*)-**8a** and (2*S*, 3*R*)-**8c** work as kainate type agonists.

Experimental

General

Melting points are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a JASCO IR-S spectrometer on NaCl cell. ^{1}H NMR spectra were obtained by a Hitachi R-90H (90MHz), a JEOL FX-270 (270MHz) and a FX-500 (500MHz) spectrometers. Chemical shifts are reported in ppm down field from the peak of tetramethylsilane as an internal standard otherwise noted. When D_2O was employed as a solvent, the peak of HDO was determined as δ 4.70 ppm. Low and high resolution mass spectra were obtained on a JEOL Model JMS-DX 300, a JMS-DX 303 and a 01SG-2 spectrometers. Unless otherwise noted, nonaqueous reactions were carried out under an argon atmosphere. Ether (Et_2O) and tetrahydrofuran (THF) were distilled from sodium/benzophenone ketyl. Benzene, dichloromethane (CH_2Cl_2), dimethylsulfoxide (DMSO), diisopropylethylamine ($^i Pr_2NEt$), 1,1,1,3,3,3,-hexamethyldisilazane (HMDS), *N,N*-dimethylformamide (DMF), hexamethylphosphoramide (HMPA), hexane, pyridine, *N,N,N',N'*-tetramethylethylenediamine (TMEDA) and triethylamine (Et_3N) were distilled from calcium hydride. Methanol (MeOH) was distilled from magnesium methoxide. All other commercially obtained reagents were used without further purification. Analytical and preparative thin layer chromatographies were carried out by precoated silica gel plates (Macherey-Nagel DC-Fertigplatten SIL G25 UV_{254}). Silica gel used for column chromatographies were Merck Kieselgel 60 Art 7734.

(4*S*)-1,1-Dimethylethyl 4-[(Z)-3'-Methoxy-3'-oxo-1'-propenyl]-2,2-dimethyl-3-oxazolidinecarboxylate (13)

To a solution of oxalyl chloride (2.07 ml, 1.10 eq) dissolved in CH_2Cl_2 (50 ml) was added DMSO (3.37 ml, 2.20 eq) at -78°C. A solution of alcohol **11** (5.0 g, 21.6 mmol) dissolved in 50 ml of CH_2Cl_2 was added to the above prepared mixture at -78°C and stirred for 15 min. After addition of Et_3N (15.0 ml, 5.00 eq), the mixture was further stirred for 30 min and then the temperature was raised to 25°C. The mixture was poured into water (300 ml) and extracted with Et_2O (200 ml x 3) three times and the combined extracts were washed with brine, dried over Na_2SO_4 and evaporated to leave aldehyde **12** which was used to the next reaction without purification.

To a suspension of KH (2.7 g, 35% in oil, 1.10 eq) in THF (10 ml) was added HMDS (5.0 ml, 1.10 eq) at 0°C. The temperature of the mixture was lowered to -78°C and a solution of 18-crown-6 (28.5 g, 5.00 eq) in THF (50 ml) and a solution of bis(2,2,2-trifluoroethyl)[(methoxycarbonyl)methyl]phosphonate (6.18 g, 1.10 eq) in THF (20 ml) were successively added and the reaction mixture was stirred for 10 min at -78°C. A solution of the above prepared aldehyde in THF (30 ml) was added at the same temperature and the mixture was stirred for 30 min. The reaction was quenched by the addition of NH_4Cl solution (300 ml) and the mixture was extracted with Et_2O (200 ml x 3) three times. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated. The residual mass was purified by column chromatography on silica gel (AcOEt/benzene, 4-8%) to give ester **13** (5.37 g, 87% from **11**). mp 51-52°C; $[\alpha]_D^{27}$ -24.4° (*c* 1.20, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.48 (s, 3H), 1.53 (s, 3H), 3.71 (s, 3H), 3.78 (dd, 1H, *J*=3, 9 Hz), 4.25 (1H, t, *J*=9 Hz), 5.39 (1H, dt, *J*=3, 9 Hz), 5.83 (d, 1H, *J*=12 Hz), 6.26 (dd, 1H, *J*=9, 12 Hz); IR (neat) 850, 1165, 1380, 1690, 1710 cm^{-1}; Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.96; H, 8.29; N, 4.73.

(2*Z*, 4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-penten-5-olide (6)

To a mixture of methyl ester **13** (2.00 g, 7.00 mmol) in MeOH (20 ml) was added *p*-TsOH (270 mg, 0.20 eq) and the solution was stirred for 12 h at 25°C. The reaction was quenched by Et_3N (0.20 ml, 0.20 eq) and the solvent was evaporated under reduced pressure. Products and starting material were separated by silica gel column chromatography (20 g) successively eluted with 8, 16 and 32% AcOEt/benzene to afford methyl ester **13** (300 mg, 15%), ester **14** (460 mg, 27%) and lactone **6** (520 mg, 35%).

The obtained alcohol was converted to the lactone as follows: To a solution of ester **14** (460 mg, 1.88 mmol) in CH_2Cl_2 (5 ml) was added *p*-TsOH (36.0 mg, 0.10 eq) and the mixture was stirred for 1 h at 25°C. After the solvent was removed, residue was purified by silica gel (15 g) column chromatography with 16% AcOEt/benzene to give the lactone **6** (280 mg, 70%): mp 128-129°C; $[\alpha]_D^{20}$ 105.0° (*c* 1.06, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.46 (9H, s), 4.35-4.52 (3H), 4.78 (1H, br s), 6.70 (1H, d, *J* =10 Hz), 6.88 (1H, dd, *J* = 5, 10 Hz); IR (nujol) 840, 1465, 1675, 1725, 3320 cm^{-1}; Anal. Calcd for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.19; H, 7.16; N, 6.49.

(3*R*, 4*S*)-3-Benzyl-4-[[(1,1-dimethylethoxy)carbonyl]amino]-5-pentanolide (16)

To a mixture of TMEDA (0.35 ml, 2.50 eq), HMPA (0.16 ml, 1.00 eq), benzylphenylsulfide (470 mg, 2.50 eq) and THF (5 ml) was added BuLi (1.50 ml, 1.57 M in hexane, 2.50 eq) at -78°C, and the solution was stirred for 3 min. To the mixture was added the lactone **6** (200 mg, 940 μmol) in THF (2 ml) with additional stirring for 5 min at -78°C. The reaction was quenched by aqueous NH_4Cl solution (10 ml). After warmed to room temperature, the mixture was extracted with Et_2O (10 ml x 3) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel (6 g) column chromatography provided diastereomeric mixture of Michael adducts (**15**) (183 mg, 47%).

Desulfurization was performed as follows: To a solution of **15** (170 mg, 410 μmol) and AIBN (14.0 mg, 0.02 eq) in benzene at 80°C was added Bu_3SnH (0.13 ml, 1.20 eq) dropwise. The mixture was heated at 80°C for 1 h and cooled to room temperature. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (5 g) with 8% AcOEt/benzene to afford desulfurized compound **16** (110 mg, 88%). Colorless crystals were obtained by recrystallization from Et_2O/hexane: mp 92-95°C; $[\alpha]_D^{25}$ -17.0° (*c* 1.00 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.45 (9H, s), 2.16 (1H, m), 2.27 (1H, dd, *J* = 10, 16 Hz), 2.56 (1H, dd, *J* = 6, 16 Hz), 2.59 (1h, dd, *J*=5, 14 Hz), 3.01(1H, dd, *J*=9, 14 Hz), 3.84 (1H, br s), 4.11 (1H, br dd, *J* = 6, 12 Hz), 4.34 (1H, dd, *J* = 4, 12 Hz), 4.63 (1H, m), 7.14-7.32 (5H, m); IR (neat) 710, 765, 880, 1710, 1740, 3300 cm^{-1}; Anal Calcd for $C_{17}H_{23}NO_4$: C, 66.87; H, 7.59; N, 4.59. Found: C, 66.51; H, 7.61; N, 4.43.

(3*R*, 4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(2-methyl-2-propenyl)-5-pentanolide (17)

CuBr•Me$_2$S (578 mg, 2.00 eq), Me$_2$S (1.5 ml) and THF (3 ml) were mixed at 25°C. After cooled to -78°C, to the mixture was added isopropenyl magnesium chloride (7.10 ml, 0.40 M in THF, 2.00 eq, prepared from isopropenyl chloride and magnesium in THF at -15°C) and the mixture was stirred for 10 min. To this solution was added lactone **6** (300 mg, 1.41 mmol) in THF (5 ml) with stirring for another 30 min. The reaction was quenched by addition of aqueous NH$_4$Cl solution (10 ml). The solution was warmed up to room temperature and extracted with AcOEt (10 ml x 3). Combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated *in vacuo*. Residual oil was purified by column chromatography on silica gel (10 g) using 15% AcOEt/hexane as a solvent to give an unreacted starting material (161 mg, 54%) and 2-methylpropen-2-yl adduct **17** (91.0 mg, 24%): ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.45 (9H, s), 1.72 (3H, s), 2.05 (2H, m), 2.23 (1H, dd, *J* = 9, 17 Hz), 2.38 (1H, dd, *J* = 4, 12 Hz), 2.70 (1H, dd, *J* = 6, 17 Hz), 3.72 (1H, br s), 4.15 (1H, dd, *J* = 6, 12 Hz), 4.37 (1H, dd, *J* = 4, 12 Hz), 4.70 (1H, m), 4.75 (1H, s), 4.86 (1H, s); IR (neat) 895, 1370, 1650, 1700, 1715, 1750, 3350 cm^{-1}.

(3*R*, 4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(2-methylpropyl)-5-pentanolide (18)

CuBr•Me$_2$S (723 mg, 2.50 eq), Me$_2$S (1 ml) and THF (2.5 ml) were mixed at 25°C. After cooled to -78°C, to the mixture was added isopropyl magnesium bromide (9.50 ml, 0.37 M in Et$_2$O, 2.50 eq, prepared from isopropyl bromide and magnesium in ether at -15°C) and the mixture was stirred for 10 min. To the solution was added lactone **6** (300 mg, 1.41 mmol) in THF (6 ml) with stirring for another 30 min. The reaction was quenched by addition of aqueous NH$_4$Cl solution (10 ml). The solution was warmed up to room temperature and extracted with AcOEt (10 ml x 3). Combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated *in vacuo*. Residual oil was purified by column chromatography on silica gel (10 g) using 10 and 20% AcOEt/hexane as a solvent to afford an unreacted starting material **6** (68.0 mg, 23%) and isobutyl adduct **18** (230 mg, 60%): $[\alpha]_D^{19}$ -60.8° (*c* 1.20, CHCl$_3$); ^{1}H NMR (270 MHz, CDCl$_3$) δ 0.89 (3H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 7 Hz), 1.26 (1H, ddd, *J* = 6, 9, 14 Hz), 1.45 (9H, s), 1.61 (1H, br s), 1.68 (1H, m), 1.92 (1H, tdd, *J* = 6, 9, 14 Hz), 2.21 (1H, dd, *J* = 9, 17 Hz), 2.71 (1H, dd, *J* = 6, 17 Hz), 3.70 (1H, br s), 4.15 (1H, br dd, *J* = 6, 12 Hz), 4.34 (1H, dd, *J* = 4, 12 Hz), 4.78 (1H, m); IR (neat) 1170, 1380, 1685, 1715, 1750, 3350 cm^{-1}; EI-MS *m/z* 271 (M$^+$); High Resolution EI-MS *m/z* 271.1843 (M$^+$, calcd for $C_{14}H_{25}NO_4$ 271.1785).

(2*S*, 3*S*)-3-Benzyl-1-[(1,1-dimethylethoxy)carbonyl]-2-[[(1,1-dimethylethyl)dimethylsilyloxy]methyl]-5-pyrrolidone (20)

To a mixture of BuLi (1.30 ml, 1.56 M solution in hexane, 1.17 eq) and THF (2 ml) were added TMEDA (300 μl, 1.20 eq) and benzylphenylsulfide (444 mg, 1.30 eq) successively, at -78°C with stirring. The colorless solution was turned to pale yellow one. After 15 min, the pyrrolidone **9** (559 mg, 1.70 mmol) was added dropwise at the same temperature. After stirring for 1 h at -78°C, the reaction was quenched by addition of saturated aqueous NH$_4$Cl solution (5 ml) and the mixture was warmed to room temperature, poured into water (20 ml) and extracted with Et_2O (25 ml x 3). Combined Et_2O solution was washed with brine (50 ml), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (34 g) with 20% Et_2O/hexane to provide a diastereomeric mixture (ca 1:1) of Michael adduct **19** (654 mg, 73% yield).

After a suspension of Raney Ni (W-2, ca. 2.00 g) in acetone (4 ml) was refluxed for 1 h, a solution of the sulfide **19** (200 mg, 380 μmol) in acetone (4 ml) and water (4 ml) were added successively. The resulting suspension was refluxed for 12 h, and filtered. After concentration *in vacuo*, the residue was purified by silica gel chromatography (6 g) using 2% acetone/$CHCl_3$ as a solvent to give the product **20** (156 mg, 98%): mp 44-46°C; $[\alpha]_D^{25}$ = -39.3° (*c* 1.00, $CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.85 (9H, s), 1.53 (9H, s), 2.17 (1H, d, *J* =18 Hz), 2.55 (1H, q, *J* = 8Hz), 2.67 (1H, dd, *J* = 8, 14Hz), 2.74 (1H, dd, *J* = 8, 14Hz), 2.81 (1H, dd, *J* = 8, 18 Hz), 3.60 (1H, dd, *J* = 2, 10 Hz), 3.85 (1H, dd, *J* = 4, 10Hz), 3.88 (1H, br s), 7.14-7.36 (5H, m); IR (neat) 700, 755, 840, 1715, 1760, 1790 cm^{-1}. Anal. Found: C, 65.50; H, 8.86; N, 3.30. Calcd for $C_{23}H_{37}NO_4Si$: C, 65.83; H, 8.89; N, 3.34.

Methyl (3*S*, 4*S*)-3-Benzyl-5-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4-[[(1,1-dimethylethoxy)carbonyl]amino]pentanoate (21)

To a solution of **20** (260 mg, 620 μmol) in THF (1.3 ml), was added 1 M NaOH aqueous solution (1.30 ml), and stirred for 12 h at 25°C. After 1 M HCl aqueous solution (1.3 ml) was added to neutralize, the mixture was poured in water (10 ml) and extracted with AcOEt (15 ml x 3). The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Solution of CH_2N_2 in Et_2O was added dropwise to the solution of crude product in MeOH until the yellow color of CH_2N_2 remained. The solvent was evaporated under reduced pressure, and the residual oil was purified by column chromatography on silica gel (8 g). Elution with 20% Et_2O/hexane provided methyl ester **21** (220 mg, 79%): $[\alpha]_D^{25}$ -5.2° (*c* 1.00, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ 0.05 (6H, s), 0.90 (9H, s), 1.44 (9H, s), 2.26 (1H, dd, *J* = 6, 16 Hz), 2.39 (1H, dd, *J* = 6, 16 Hz), 2.48 (1H, t, *J* = 13Hz), 2.53 (1H, sept, *J* = 6Hz), 2.85 (1H, br dd, *J* = 5, 13 Hz), 3.58 (3H, s), 3.60 (2H, m), 3.74 (1H, br d, *J* = 8 Hz), 4.86 (1H, br d, *J* = 8 Hz), 7.17-7.29 (5H, m); IR (neat) 700, 755, 780, 840, 1705, 1720, 1735, 3280, 3880, 3450 cm^{-1}; EI-MS *m/z* 451(M$^+$); High-Resolution EI-MS *m/z* 451.2792 (M$^+$, calcd for $C_{24}H_{41}NO_5Si$ 451.2755).

(3*S*, 4*S*)-3-Benzyl-4-[[(1,1-dimethylethoxy)carbonyl]amino]-5-pentanolide (22)

To a solution of methyl ester **21** (130 mg, 290 μmol) in acetonitrile (1.5 ml) was added 10% aqueous HF solution (1 ml, excess) at 25°C and the mixture was stirred for 30 min. The reaction mixture was poured into water (10 ml) and extracted with AcOEt (10 ml x 3). The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5 g) with 4% acetone/$CHCl_3$ to give lactone **22**. The product was recrystallized by Et_2O/hexane (68 mg, 77%) ; mp 150-151°C; $[\alpha]_D^{25}$ -24.4° (*c* 1.10, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$) δ 1.48 (9H, s), 2.32 (1H, br dd, *J* = 12, 18Hz), 2.37 (1H, m), 2.47 (1H, m), 2.62 (1H, dd, *J* = 6, 18 Hz), 2.84 (1H, dd, *J* = 6, 14 Hz), 4.05 (1H, br s), 4.32 (1H, dd, *J* = 2, 12 Hz), 4.41 (1H, dd, *J* = 2, 12 Hz), 4.85 (1H, br s), 7.14-7.36 (5H, m); IR (neat) 700, 760, 1690, 1700, 1720, 1730, 1750, 3320 cm^{-1}; EI-MS *m/z* 249 (MH$^+$-^{t}Bu); High-Resolution EI-MS *m/z* 249.0988 (MH$^+$-^{t}Bu, calcd for $C_{13}H_{15}NO_4$ 249.1002).

(2*S*, 3*R*)-3-Benzylglutamic Acid (8a)

A mixture of lactone **16** (110 mg, 360 μmol), aqueous 1M NaOH solution (1.5 ml), and $KMnO_4$ (85.0 mg, 1.50 eq) was stirred for 12 h at 25°C. Then EtOH (3 ml) was added. The mixture was filtered through Celite and washed with EtOH. Filtrate was concentrated *in vacuo*. To the residual oil, was added TFA (2 ml, excess) and the mixture was stirred for 5 min at 25°C. After addition of water (5 ml), the mixture was concentrated under reduced pressure. Crude TFA salt was purified with a column of Amberlite IR-120B eluted with water and 3% aqueous NH_3 solution successively to afford ammonium salt of **8a**. After the solvent was evaporated *in vacuo*, the product was purified by an Amberlite IRC-50 column eluted with water to give salt free **8a** (50.0 mg, 59%, 2 steps from **16**). Recrystallization was performed from water: mp 155-157°C; $[\alpha]_D^{25}$ 15.7° (*c* 0.20, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 2.41 (1H, dd, J = 6, 17 Hz), 2.47 (1H, dd, J = 7, 17 Hz) 2.65 (1H, dd, J = 10, 15 Hz) 2.72 (1H, m), 2.86 (1H, dd, J = 5, 15 Hz), 3.84 (1H, d, J = 3 Hz), 7.28-7.38 (5H, m); IR (nujol) 710, 760, 1575, 1640, 1695, 2200-3600 cm^{-1}; EI-MS *m/z* 219 (M^+-H_2O); High Resolution EI-MS *m/z* 219.0886 (M^+-H_2O, calcd for $C_{12}H_{13}NO_3$ 219.0896).

(2*R*, 3*S*)-3-Benzylglutamic Acid (8a)

$[\alpha]_D^{21}$ -16.2° (*c* 0.20, H_2O)

(2*S*, 3*R*)-3-(2-Methylpropyl)glutamic Acid (8b)

A mixture of lactone **18** (100 mg, 369 μmol), CH_2Cl_2 (1 ml), 1 M aqueous NaOH solution (1 ml) and $KMnO_4$ (87.0 mg, 1.50 eq) was stirred for 12 h at 25°C. Then EtOH (3 ml) was added. The solution was filtered through Celite and the filtrate was concentrated under reduced pressure. After addition of water (10 ml), the solution was acidified to pH 2 with dilute aqueous HCl solution and extracted with AcOEt (10 ml x 3). Combined organic layer were dried over Na_2SO_4 and concentrated under reduced pressure. To the residual oil was added TFA (2 ml, excess) and the mixture was stirred for 30 min at 25°C. Then water (5 ml) was added and the mixture was concentrated *in vacuo*. Crude TFA salt was purified in the same procedure as **8a** to afford salt free **8b** (43.0 mg, 2 steps from **18** 57%). Recrystallizaion was performed from water: mp 130-132°C; ^{1}H NMR (500 MHz, D_2O) δ 0.79 (3H, d, J = 7 Hz), 0.83 (3H, d, J = 7 Hz), 1.19 (2H, m), 1.56 (1H, tt, J = 7, 13Hz), 2.46 (3H, m), 3.79 (1H, d, J = 2 Hz); IR (nujol) 1210, 1380, 1550-1660, 1720, 2300-3300 cm^{-1}; EI-MS *m/z* 204 (MH^+); High Resolution EI-MS *m/z* 204.1263 (MH^+, calcd for $C_9H_{18}NO_4$ 204.1236).

(2*R*, 3*S*)-3-(2-Methylpropyl)glutamic Acid (8b)

$[\alpha]_D^{25}$ 1.7° (*c* 0.18, H_2O)

(2*S*, 3*S*)-3-Benzylglutamic Acid (8a)

To a mixture of lactone **22** (50.0 mg, 160 μmol) and 1 M aqueous NaOH solution (1 ml) was added $KMnO_4$ (39.0 mg, 1.50 eq), and the mixture was stirred for 12 h at 25°C. Then EtOH (3 ml) was added. The solution was filtered through Celite and the filtrate was concentrated under reduced pressure. To the residual oil was added TFA (1 ml, excess) and the mixture was stirred for 15 min at 25°C. After adition of water (5 ml) the mixture was concentrated *in vacuo*. Desalting of the TFA salt of **8a** by the same procedure as **8a** described above gave salt free **8a** (17 mg, 44%, 2 steps from **20**). Recrystallization was performed by water or 50% EtOH-water: mp 156-157°C; $[\alpha]_D^{25}$ 30.8° (*c* 0.20, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 2.23 (2H, d, J = 5

Hz), 2.58 (1H, m), 2.77 (2H, d, $J = 8$ Hz), 3.69 (1H, d, $J = 4$ Hz), 7.22-7.35 (5H, m); IR (nujol) 700, 750, 1470, 1695, 1710, 2400-3200 cm^{-1}; EI-MS *m/z* 219 (M$^+$-H_2O); High Resolution EI-MS *m/z* 219.0886 (M$^+$-H_2O, calcd for $C_{12}H_{13}NO_3$ 219.0896).

(2*R*, 3*R*)-3-Benzylglutamic Acid (8a)

$[\alpha]_D^{25}$ -34.6° (*c* 0.20, H_2O)

Dimethyl (2*S*, 3*R*)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-(2-methyl-2-propenyl)-pentanedioate (27)

A mixture of lactone **17** (123 mg, 457 μmol), MeOH (1 ml) and 1 M aqueous NaOH solution (0.5 ml) was stirred for 1.5 h at 25°C. The mixture was made acidic with addition of 1 M HCl to adjust to pH2, and then diluted with water (10 ml) and extracted with AcOEt (10 ml x 3). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Residual crude product was applied to the next reaction without further purification.

A mixture of the crude product (ca. 120 mg), DMF (2.5 ml) and PDC (314 mg, 2.00 eq) was stirred for 48 h at 25°C. The mixture was poured into 0.01 M aqueous NaOH solution (10 ml) and it was acidified with 1 M aqueous HCl solution to adjust to pH 2 followed by extraction with AcOEt (10 ml x 3). Combined extracts were dried over Na_2SO_4 and concentrated *in vacuo*. To a solution of residual oil in AcOEt was added a solution of CH_2N_2 in Et_2O until the yellow color of CH_2N_2 remained. The solvent was removed under reduced pressure and the residue was purified by silica gel (5 g) column chromatography eluted with 10% AcOEt/hexane to give methyl ester **27** (28.0 mg, 19%), lactone **17** (32.0 mg, 26%) and alcohol **29** (41.0 mg, 30%). **27**; $[\alpha]_D^{21}$ 21.9° (*c* 1.00, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (9H, s), 1.70 (3H, s), 1.91 (1H, dd, $J = 9$, 14 Hz), 2.01 (1H, dd, $J = 6$, 14 Hz), 2.32 (2H, m), 2.69 (1H, m), 3.68 (3H, s), 3.75 (3H, s), 4.56 (1H, br dd, $J = 3$, 8 Hz), 4.72 (1H, s), 4.81 (1H, s), 5.15 (1H, br d, $J = 8$ Hz); IR (neat) 895, 1060, 1370, 1650, 1720, 1745, 3360 cm^{-1}; EI-MS *m/z* 329 (M$^+$); High Resolution EI-MS *m/z* 329.1834 (M$^+$, calcd for $C_{16}H_{27}NO_4$ 329.2839).

(2*S*, 3*R*)-3-(2-Methyl-2-propenyl)glutamic Acid (8c)

A mixture of methyl ester **27** (63.0 mg, 191 mmol), MeOH (0.6 ml) and 1 M aqueous NaOH solution (0.6 ml) was stirred for 6 h at 25°C. The mixture was acidified by 1 M aqueous HCl solution and then it was poured into water (10 ml) and extracted with AcOEt (10 ml x 3). Combined extracts were dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. To the residual oil was added TFA (1.5 ml, excess) and the mixture was stirred for 15 min at 25°C. After addition of water (5 ml), the mixture was concentrated *in vacuo*. Residue was purified by ion exchange resin Amberlite IRC-50 by the same procedure as **8a**. Elution with water gave salt free amino acid **8c** (22.0 mg, 2steps from **27**, 57%). The product was recrystallized from water: $[\alpha]_D^{25}$ 14.5° (*c* 0.11, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 1.70 (3H, s), 2.11 (1H, dd, $J = 10$, 14 Hz), 2.18 (1H, dd, $J = 6$, 14 Hz), 2.48 (2H, m), 2.64 (1H, m), 3.85 (1H, t, $J = 2$ Hz), 4.80 (1H, s), 4.87 (1H, s); IR (nujol) 900, 915, 1670-1750, 2400-3200 cm^{-1}.

(2*R*, 3*S*)-3-(2-Methyl-2-propenyl)glutamic Acid (8c)

$[\alpha]_D^{25}$ -15.8° (*c* 0.12, H_2O)

ACKNOWLEDGMENT: The biological activities were measured by Drs. Shinozaki and Ishida (Tokyo Metropolitan Institute of Medical Science) whom we gratefully acknowledge.

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(*Received in Japan* 6 *November* 1995; *accepted* 1 *December* 1995)