Palladium(II)-Catalyzed Olefin-Coupling Reactions of Kainic Acid: Effects of Substitution on the Isopropenyl Group on Receptor Binding

Gregory A. Conway,† Joon Sup Park,† Linda Maggiora,† Mathias P. Mertes,*,† Noemi Galton,‡ and Elias K. Michaelis‡

Departments of Medicinal Chemistry and Human Development, University of Kansas, Lawrence, Kansas 66045.
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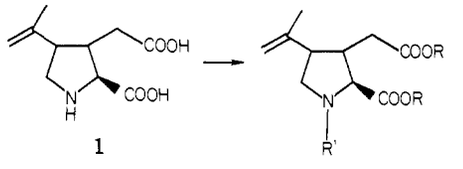
Two palladium-catalyzed carbon–carbon bond forming reactions were found to be useful for the modification of a protected amino acid derivative containing a sterically hindered isopropenyl group. Arylation of the terminal methylene group of the dimethyl ester of *N*-(ethoxycarbonyl)kainic acid (**3**) was accomplished by treatment with an aromatic amine, palladium(II) acetate, and *tert*-butyl nitrite. Substitution of the allylic methyl group of **3** was accomplished by conversion to the π-(allyl)palladium complex (**5**) which, on subsequent treatment with the carbanions of *tert*-butyl acetoacetate or phenylthioacetone, gave the alkylated products. Both the (*Z*)- and (*E*)-3-nitrophenyl derivatives (**8a,b**) of kainic acid were active in the standard binding assay. Unexpectedly, the cis compound in the nitrophenyl series (**8a**), which more closely resembles the extended conjugation found in domoic acid, was found to be 20 times less potent than the trans derivative **8b**. The latter had one-fifth the receptor-binding affinity of kainic acid.

Kainic acid, a unique amino acid isolated from the seaweed *Digenea simplex*, has proven to be an invaluable pharmacological probe for the study of neurochemical pathways in the brain that are mediated by L-glutamic acid.[1] In the design of some affinity-labeling reagents for receptor studies, we explored the application of the palladium(II)-catalyzed olefin-coupling reaction to give regiocontrolled synthesis of vinyl-substituted aromatic derivatives of kainic acid. This reaction was compared to a related olefin modification route employing the reaction of the π-(allyl)palladium derivative of kainic acid with carbanions to give substitution on the allylic methyl group of kainic acid.

Chemistry. Heck and co-workers[2] reported the palladium(II)-catalyzed synthesis of simple styrenes from the reaction of aryl halides and olefins. By using an amine catalyst and triphenylphosphine as the activating ligand, high yields of respective styrenes were obtained via the cis addition of the phenylpalladium complex to the olefin and cis elimination of the hydridopalladium complex from the intermediate. Application of this reaction under the described conditions using 4-bromo-1-nitrobenzene or 3-iodo-1-nitrobenzene and the olefin **3** failed to give any



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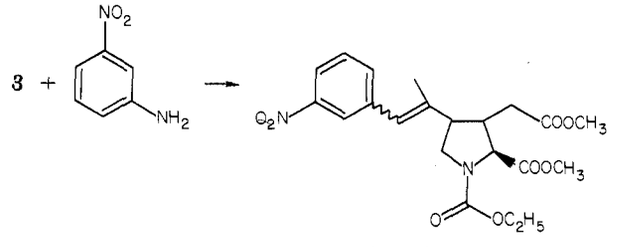
2a, R = CH_3; R' = H
b, R = H; R' = $CO_2CH_2C_6H_5$
3, R = CH_3; R' = $CO_2C_2H_5$



4a, *Z* isomer
b, *E* isomer

detectable styrene derivative or loss of the starting olefin **3**. In a modification of this reaction, the oxidative addition complex[3] formed from 3-iodo-1-nitrobenzene and tetrakis(triphenylphosphine)palladium also failed to react with **3** using the reported conditions.

Fujiwara and co-workers[4] described a unique reaction wherein arylamines in the presence of palladium(II) salts and acetic acid react with olefins to give low yields of styrene derivatives. Treatment of the protected kainic acid derivative **3** with 3-nitroaniline, palladium acetate, and *tert*-butyl nitrite afforded, after chromatography on silica, a 5% yield of the cis isomer **4a** and a 10% yield of the trans isomer **4b**. The principal criteria for assignment of the geometrical isomers are the ultraviolet spectral differences and nuclear magnetic resonance assignment for **4a** and **4b**. Compound **4b** has an intense ultraviolet absorption band at 246 nm; in contrast, compound **4a** showed an equally intense band at 232 nm, a blue shift of 14 nm which would suggest that the aromatic ring is somewhat out of plane with the olefin. Space-filling models show severe steric interactions when the phenyl group is substituted cis to the pyrrolidine ring. This interaction is not as evident in the model for the trans isomer **4b**. The chemical shifts in proton nuclear magnetic resonance for these compounds show that the vinylic proton in **4b** is a singlet at 6.22 ppm, whereas the vinylic proton in **4a** is a doublet integrating for one proton at 6.38 and 6.30 ppm. The chemical shifts in both cases approximate those expected for styryl protons. However, the doublet pattern for the vinylic proton of the **4a** isomer was unexpected. This doublet was not a result of long-range coupling with the methyl group or the carbon 4 ring proton, since decoupling experiments in both regions failed to collapse the doublet in **4a**. The only reasonable alternative is that the doublet represents the chemical shifts for the vinylic proton in two isomers where steric interactions restrict the rotation of the bond joining the β-styryl carbon and the pyrrolidine ring. Further observations that support the cis–trans assignments are the allylic methyl proton resonances in **4a** that appear as a doublet at 1.10 and 1.02 ppm, an upfield shift of 0.7 ppm from the assigned resonance for the allylic methyl protons in **4b**, and a significant upfield shift for the ring protons at carbon 5 (0.7 ppm).

† Department of Medicinal Chemistry.
‡ Department of Human Development.

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(2) (a) Dieck, H. A.; Heck, R. F. *J. Am. Chem. Soc.* **1974**, *96*, 1133–1136. (b) Heck, R. F.; Nolley, J. P., Jr. *J. Org. Chem.* **1972**, *37*, 2320–2322. (c) Ziegler, C. B., Jr.; Heck, R. F. *Ibid.* **1978**, *43*, 2941–2946.
(3) Fitton, P.; Rick, E. D. *J. Organomet. Chem.* **1971**, *28*, 287–291.
(4) Akiyama, F.; Miyazaki, H.; Kaneda, K.; Teranishi, S.; Fujiwara, Y.; Abe, M.; Taniguchi, H. *J. Org. Chem.* **1980**, *45*, 2359–2361.

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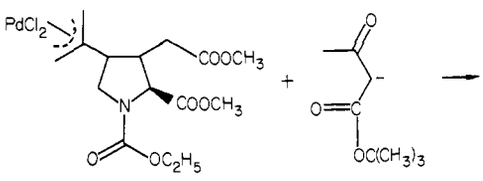
Compound **4a** in Me_2SO-d_6 showed a singlet for the vinyl proton at 6.30 ppm. However, heating up to 100 °C in this solvent failed to coalesce the doublet for the allylic methyl group at 1.10 and 1.02 ppm. Downfield shifts are observed in ring protons at carbon 5 (~0.7 ppm). Downfield shifts are observed in **4a** compared to **4b** for the assigned ring proton at carbon 2 (Δ = 0.38 ppm) and the methylene of the carboxymethyl chain at C3 ($\Delta \simeq$ 0.4 ppm). The ^{13}C nuclear magnetic resonance spectrum of **4a** shows a doublet at 46.5 and 45.2 ppm, which is assigned to carbon 5 of the pyrrolidine ring, and the ring carbon assignments for carbons 3–5 are about 3- to 5-ppm upfield than observed shifts for the corresponding carbons in structures **3**, **4b**, **5a,b**, and kainic acid (**1**). These field effects are possibly a result of the proximity of the aromatic ring to the pyrrolidine ring when they are cis as depicted in structure **4a**. The ^{13}C magnetic resonance spectrum for **4b** shows doubling of the assigned peaks for ring carbons 2, 3, and 5 and the fully substituted olefinic carbon, which suggests that **4b** also is a mixture of two rotational isomers.

Another versatile route for selective alkylation of isopropenyl groups employes palladium(II) activation of the allylic group to give the π-(allyl)palladium complex.[5] This organometallic species gives rise to regioselective carbon–carbon bond formation at the allylic methyl group of an olefin. The reaction of this electrophilic reagent with stabilized anions[6] should be compatible with the functional groups presented in the protected kainic acid derivative **3**. Treatment of **3** with palladium trifluoroacetate,[7] followed by conversion to the chloride complex, gave a 74% yield of the π-(allyl)palladium complex **5** of protected kainic acid.



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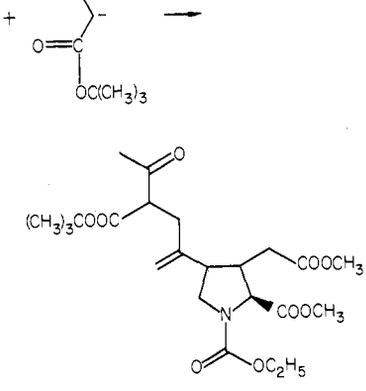

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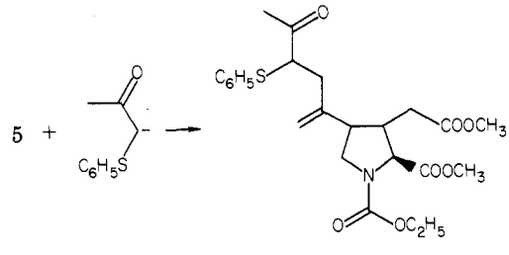

7

The anions of *tert*-butyl acetoacetate and phenylthioacetone reacted with **5** to afford the methyl alkylated products **6** (60%) and **7** (16%). The proton magnetic resonance assignments for both **6** and **7** were substantially the same as found in the starting material **3** with the exception of the vinylic protons. In compound **6**, one of the vinylic protons appears as a doublet at 4.95 and 4.85 ppm, and the other proton is a singlet at 4.77 ppm. The vinylic protons in compound **7** each appear as doublets at 5.00, 4.93, and 4.87, 4.85 ppm, respectively. Decoupling experiments using **7** failed to collapse these doublets, the conclusion being that compounds **6** and **7** are each a mixture of two isomers. Isomerization at the ring carbon-4 bond is excluded, since treatment of the π-(allyl)palladium complex **5** with cyanide ion and methanol reversed the reaction to give the protected kainic acid **3**. If isomerization at ring carbon-4 had occurred the product would be the protected derivative of allokainic acid[8] for which we found a singlet for the two vinyl protons at 4.76 ppm.

The ^{13}C nuclear magnetic resonance spectra of **6** and **7** also show that they are isomeric mixtures; two peaks are observed in each compound for ring carbons 2–5 and in **6** for the methylene carbon of the carboxymethyl side chain. Compound **7** also shows two ^{13}C peaks for the allylic methylene and the unsubstituted vinyl carbon. We conclude from these observations and the obvious steric restrictions in space-filling models that, like compounds **4a** and **4b**, compounds **6** and **7** were isolated as mixtures of rotational isomers.

The results of these studies demonstrate that protected amino acids and similar derivatives containing an isopropenyl group can be modified by application of the palladium(II) aryl–olefin coupling reaction or through the π-(allyl)palladium complex. In the former case, substitution on the terminal olefin carbon occurred (**4a,b**). The reaction of the π-(allyl)palladium complex of **3** with carbanions gave the allylic methyl substituted products (**6** and **7**). The variable yields observed with these reactions (6–60%) are thought to be a result of steric hindrance; no serious attempts have been made to optimize the yields in either reaction.

Biological Results

For preparation of the products for receptor-binding affinity studies of these substituted kainic acid derivatives, it was found that the fully protected kainic acid **3** was hydrolyzed to kainic acid (**1**) by using pyridine and trimethylsilyl iodide in chloroform at 60 °C. A convenient method for following the reaction was to run the reaction in deuteriochloroform and follow the changes in the proton NMR spectra. It was found that the TLC and mass spectrum of this product were identical with kainic acid. Further confirmation that the reaction conditions did not epimerize the α-carbon was obtained from the biological results where the deprotection product of compound **3** had the same receptor affinity as kainic acid. Deprotection under these conditions converted the *cis*-nitrophenyl derivative **4a** to compound **8a** (Table I). The characteristic ^1H NMR chemical shifts for the vinyl hydrogen show much the same pattern of isomers in **8a** (δ 6.6, 6.9) as seen in the protected derivative **4a** (a doublet centered at δ 6.34), which again suggests **8a** is a mixture of rotational isomers. Deprotection of the trans compound **4b** to **8b** gave the vinyl proton signal as a singlet at δ 6.10; the same proton in the starting material **4b** had a chemical shift of δ 6.22. The concentration of aqueous solutions of compounds **8a,b** and kainic acid was determined by the colorimetric assay described by Troll.[9] The deprotection of compounds **6**

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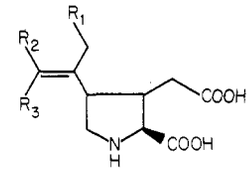
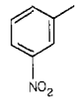
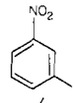
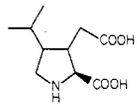
(8) Professor T. Takemoto of Tohoku University is acknowledged for his generous sample of α-allokainic acid.

Table I. Displacement by Kainate Analogues of Bound [^{3}H]Kainic Acid (20 nM)



compound	IC_{50},[a] nM
1 (kainic acid), $R_1 = R_2 = R_3 = H$	17.8
8a, $R_1 = R_2 = H$; R_3 = 	416.8
8b, $R_1 = R_3 = H$; R_2 = 	79.4
dihydrokainic acid 	645.6

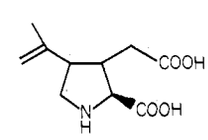
[a] All IC_{50} values represent the average of quadruplicate determinations from one to three experiments with two membrane preparations. The standard error of the mean was less than 10% of the value shown.

and **7** was attempted; however, the products were not tested because of stability problems under the conditions of the reaction.

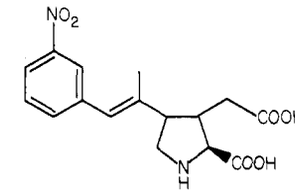
The interaction of compounds **8a** and **8b** with central nervous system receptors for kainic acid was determined. Binding of [^{3}H]kainic acid to nerve cell membrane receptor sites and the displacement of bound kainate by various analogues have been shown to correlate very well with the relative excitatory activity of these agents in the nervous system.[10-12] The concentration of each compound that produced 50% displacement (IC_{50} value) of [^{3}H]kainic acid is shown in Table I. The IC_{50} values of **8a,b** were compared to the [^{3}H]kainic acid displacement by unlabeled kainic acid and dihydrokanic acid (Table I). The excitatory activity of dihydrokainate is approximately 100 times less than that of kainic acid.[13] The IC_{50} for dihydrokainic acid displacement was approximately 40 times greater than that of kainate. Compound **8a** had an IC_{50} value that was close to the IC_{50} of dihydrokainic acid, which would indicate low biological activity. On the other hand, **8b** had an IC_{50} of 79.4 nM, which is very similar to the IC_{50} of L-glutamic acid (72 nM),[14] a known excitatory amino acid in the central nervous system.[1]

Discussion

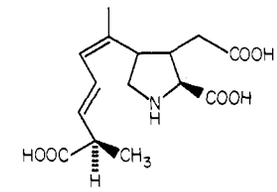
The neuroexcitatory activities recognized for kainic acid (**1**) and the related amino acid, domoic acid (**9**), are presumed to result from their interaction with specific populations of glutamate receptors in the mammalian CNS.[1,15]



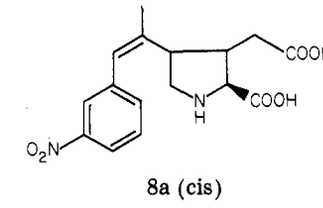
kainic acid (**1**)



8b (trans)



domoic acid (**9**)



8a (cis)

In an attempt to design specific affinity-labeling reagents for kainic acid "receptors" in the CNS, it was our intention to examine the effect of regio- and stereoselective substitutions on the isopropenyl group of kainic acid. Considering the high potency of domoic acid[16] (**9**), substitutions that mimic the extended conjugation and are in effect cis to the pyrrolidine ring (compound **8a**) should have a higher probability of activity than the corresponding trans isomer **8b**.

It was necessary before proceeding to the actual affinity-labeling reagents to obtain a preliminary evaluation of the biological activity of the intermediates that contain the major structural elements that would be found in the ultimate affinity labels. For this reason, the two nitrophenyl derivatives, **4a,b**, and two derivatives containing a propanone function on the allylic methyl group of kainic acid (**6** and **7**) were synthesized. Conversion of these protected derivatives to the corresponding amino acids without apparent side reactions was successful for the first two derivatives (**4a** → **8a**; **4b** → **8b**).

When this work was initiated it was predicted as a first approximation that the *trans*-nitrophenyl derivative **8b** would be more active than the *cis* derivative **8a**. This was based on the analogy of **8b** to the extended conjugation in domoic acid, which is at least twice as active as kainic acid.[1] This was found to be the case; **8b** has 20 times greater receptor affinity than **8a**. however, it was recently reported that the initial structural assignment for domoic acid was incorrect,[16] and the revised structure **9** is a cis substituted kainic acid, as is the less active nitrophenyl derivative.

The IC_{50} for kainic acid determined in the present study (17.8 nM) was nearly identical with those previously reported.[11,14] Of the two compounds tested, only **8b** exhibited an IC_{50} that approximated the values previously obtained for other excitatory amino acid analogues, such as quisqualic acid and L-glutamic acid.[11,12,14] On the basis of these results, it would be expected that the *trans*-nitrophenylkainic acid derivative would exhibit the highest degree of in vivo biological activity. Compound **8a** was only moderately more active than the weak kainic acid receptor agonist, dihydrokainic acid, and would be expected to have weak in vivo activity.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were measured with a Beckman IR-33, UV spectra were measured with a Cary Model 219 recording spectrophotometer, ^{1}H and ^{13}C NMR

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spectra were measured with Varian Models T-60 and FT-80 or a Bruker WP80 spectrometer; the reported assignments are relative to tetramethylsilane as an internal standard. Microanalyses were obtained from a Hewlett-Packard 185B, and mass spectra were obtained from a Varian CH5 or Ribermag R-10-10C mass spectrometer using either electron impact or chemical ionization. Unless indicated C, H, and N analyses were ±0.4% of the calculated values. High-performance LC was performed with a Waters Model M6000A using Partisil ODS preparative and analytical columns. Palladium acetate, purchased from Aldrich Chemical Co., and kainic acid, a product of Sigma Chemical Co., were used without further purification.

Methyl (2*S*,3*S*,4*S*)-3-(Carboxymethyl)-4-isopropenyl-2-pyrrolidinecarboxylate (2a). L-α-Kainic acid (200 mg, 0.94 mmol) was suspended in 20 mL of dry methanol and the solution was saturated with hydrogen chloride gas for 20 min at 5 °C. After stirring for 2 h at 0 °C, the solution was evaporated to give a viscous residue, which was dissolved in 5 mL of water and neutralized with sodium bicarbonate solution, and the product was extracted with chloroform. The washed organic layer was evaporated to dryness to give a quantitative yield of **2a**. An analytical sample was obtained by silica gel chromatography: ^{1}H NMR ($CDCl_3$) δ 4.68 and 4.52 (2 s, 2, $=CH_2$), 3.57 and 3.65 (2 s, 6, OCH_3), 3.6–2.9 (m, 3, C5 H_2, C2 H), 2.9–2.45 (m, 2, C3 H, C4 H), 2.6–2.1 (m, 2, CH_2COO), 1.55 (s, 3, CH_3); mass spectrum, *m/e* 241 (M^+). Anal. ($C_{12}H_{19}NO_4$, M_r 241.3) C, H, N.

Benzyl (2*S*,3*S*,4*S*)-2-Carboxy-3-(carboxymethyl)-4-isopropenyl-1-pyrrolidinecarboxylate (2b). L-α-Kainic acid (1; 426 mg, 2 mmol) was dissolved in 5 mL of water containing 672 mg of sodium bicarbonate (8 mmol), and the solution was cooled to 3 °C. Benzyl chloroformate (510 mg, 3 mmol) was added slowly over a 2-h period, and the solution was stirred for an additional hour. After extraction with ether, the aqueous layer was acidified to pH 2 with hydrochloric acid. This solution was extracted with ether, and the ether layer was extracted with water, dried, and evaporated to give 450 mg of **2b** (65%) as an oil. An analytical sample was prepared by resolution on silica: ^{1}H NMR ($CDCl_3$) δ 10.5 (s, 2, COOH), 7.2 (d, 5, aromatic H), 5.0 (s, 2, phenyl CH_2), 4.83 and 4.7 (2 s, 2, $=CH_2$), 4.3–4.1 (m, 1, C2 H), 3.8–3.3 (m, 2, C5 H_2), 3.2–2.7 (m, 2, C3 H, C4 H), 2.5–2.7 (m, 2, CH_2COO), 1.63 (s, 3, CH_3); mass spectrum, *m/e* 347 (M^+). Anal. ($C_{18}H_{21}NO_6 \cdot H_2O$, M_r 365.4) H, N; C: calcd, 59.17; found, 59.91.

Ethyl Methyl (2*S*,3*S*,4*S*)-3-(Carboxymethyl)-4-isopropenyl-1,2-pyrrolidinedicarboxylate (3). L-α-Kainic acid (1 g, 4.7 mmol) was dissolved in a solution of 1.5 g of sodium bicarbonate in 47 mL of water at 2 °C. Ethyl chloroformate (0.8 g, 7.4 mmol) was added in 50-μL portions over a 20-h period; the solution was stirred an additional 2 h at 25 °C. The dry ether extract of the carbonate derivative of 1 obtained as described in the synthesis of **2b** was treated at 2 °C with ~0.5 M diazomethane in ether. The addition was stopped after the solution remained yellow; evaporation afforded a thick oil, which was purified on silica gel to give 1.27 g of **3** (86%): IR (neat) 1730, 1695, 1195 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.90 and 4.70 (2 s, 2, $=CH_2$), 4.33–3.90 (m, 3, CH_2O, C2 H), 3.75 and 3.67 (2 s, 6, OCH_3), 3.65–3.25 (m, 2, C5 H_2), 3.17–2.67 (m, 2, C3 H, C4 H), 2.26 (d, 2, CH_2COO), 1.69 (s, 3, $=CCH_3$), 1.21 (2 t, 3, CH_3); ^{13}C NMR ($CDCl_3$) 171.6 (COO), 154.0 (CO_2N), 140.7 ($C{=}CH_2$), 112.9 ($C{=}CH_2$), 63.3 (C2), 60.7 (CH_2O), 51.7, 51.0 (OCH_3), 47.3, 47.1 (C5), 45.4, 44.5 (C4), 41.3, 40.4 (C3), 32.1 (CH_2COO), 21.6 ($=CCH_3$), 14.9 (CH_2CH_3) ppm. Anal. ($C_{15}H_{23}NO_6$, M_r 313.4) C, H, N.

Dimethyl (*Z*)- and (*E*)-(2*S*,3*S*,4*S*)-3-[(Methoxycarbonyl)methyl]-4-(α-methyl-*m*-nitrostyryl)-1,2-pyrrolidinedicarboxylate (4a,b). A suspension of 2.66 g of palladium acetate (11.8 mmol) in 23 mL of acetonitrile was added to a 26-mL acetonitrile solution containing 2.04 g of 3-nitroaniline (14.8 mmol) and 2.48 g of the protected kainic acid derivative **3** (7.9 mmol). After the mixture was heated at 60 °C in a nitrogen atmosphere to give a red solution, an acetonitrile solution (3.8 mL) containing *tert*-butyl nitrite[17] (2.05 g, 17 mmol) was added in 0.1-mL portions over a 3-h period at 60 °C. The reaction mixture was stirred at 60 °C for an additional 4 h and overnight at 25 °C. Since some starting material (**3**) was evident on TLC, additional portions of palladium acetate (1 g), 3-nitroaniline (0.8 g), and *tert*-butylnitrite (0.8 g) were added to the reaction mixture at 60 °C, stirring was continued for 2 h, and the mixture was stirred at 25 °C for 2 days. The filtrate from the reaction mixture was mixed with 1 L of water, extracted several times with ether, and the ether was dried with magnesium sulfate. The residue on evaporation (3.7 g) was resolved on silica gel with increasing concentrations of ethyl acetate in hexane to give 160 mg of the cis compound **4a** (5%), which appeared from the spectral characteristics to be a mixture of rotational isomers: UV (MeOH) λ_{max} 232 nm (ε 19000); ^{1}H NMR ($CDCl_3$) δ 8.05–7.95 (m, 2, aromatic H), 7.45–7.35 (m, 2, aromatic H), 6.34 (d, 1, =CH), 4.48 (d, 1, C2 H), 4.15 (q, 2, J = 7 Hz, OCH_2), 3.72 and 3.67 (2 s, 6, OCH_3), 3.26–2.91 (m, 2, C3 H, C4 H), 3.0–2.6 (m, 2, C5 H_2), 2.6–2.4 (m, 2, CH_2COO), 1.23 (2 t (q), 3, J = 7 Hz, CH_2CH_3), 1.10 and 1.02 (2 s, 3, $=CCH_3$); ^{13}C NMR ($CDCl_3$) 171.4, 171.1 (CO_2), 152.3 (NCO_2), 148.4 (CNO_2), 141.9 (C2 C=), 135.6 (HC=), 129.2, 124.8, 123.9, 121.4 (aromatic CH) 126.8, 125.6 (isomers, aromatic CCH=), 64.1 (C5), 61.9 (OCH_2), 52.5 (OCH_3), 51.9 (OCH_3), 46.5, 45.2 (isomers C5), 42.3 (C3), 38.4 (C2), 32.3 (CH_2CO_2), 18.5 ($=CCH_3$), 14.6 (CH_2CH_3) ppm; mass spectrum, *m/e* 434 (M^+), 375 ($M - CO_2CH_3$), 361 ($M - CO_2CH_2CH_3$). Anal. ($C_{21}H_{26}N_2O_8$, M_r 434.4) C, H, N.

The other isomer **4b** was isolated (333 mg, 10%) as a mixture (95% pure) as evidenced by liquid chromatography. This material was purified by silica gel chromatography, followed by preparative high-performance LC on a reverse-phase column with methanol/water (75:25) to give the trans isomer **4b**, again as an apparent mixture of rotational isomers: UV (MeOH) λ_{max} 246 nm (ε 18000); ^{1}H NMR (CD_3COCD_3) 8.07–7.92, 7.50–7.40 (m, 4, aromatic H), 6.22 (s, 1, =CH), 4.15–4.10 (m, 3, OCH_2, C2 H), 3.75 (s, 3, OCH_3), 3.65 (s, 3, OCH_3), 3.70–3.40 (m, 2, C5 H_2), 3.4–2.75 (m, 2, C3 H, C4 H), 2.36 (d, 2, CH_2COO), 1.81 (s, 3, $=CCH_3$), 1.25 (2 t (q), 3, J = 7 Hz, CH_2CH_3); ^{13}C NMR (CD_3COCD_3) 175.6 (CO_2), 171.0 (CO_2), 151.1 (NCO_2), 147.5, 145.7 (isomers, aromatic CNO_2), 140.1, 138.6 (isomers, $=CCH_3$), 135.8 (aromatic C=), 130.1, 126.1, 124.0, 121.8 (aromatic CH), 64.8, 64.5 (isomers, C2), 61.6 (OCH_2), 52.3 (OCH_3), 51.7 (OCH_3), 48.8, 48.3 (isomers C5), 47.9 (C4), 42.3, 42.2 (isomers C2), 33.6 (CH_2CO_2), 17.5 ($=CCH_3$), 14.7 (CH_2CH_3); mass spectrum, *m/e* 435 ($M^+ + 1$), 375 ($M - CO_2CH_3$), 361 ($M - CH_2COOCH_3$). Anal. ($C_{21}H_{26}N_2O_8$, M_r 434.4) C, H, N.

Ethyl Methyl (2*S*,3*S*,4*S*)-3-[(Methoxycarbonyl)methyl]-4-[1-[2-(*tert*-butoxycarbonyl)-3-oxobutyl]vinyl]-1,2-pyrrolidinedicarboxylate (6). A suspension of 97 mg of the protected kainic acid derivative **3** (0.31 mmol) and 103 mg of palladium trifloroacetate[7,18] (0.31 mmol) in 2.5 mL of dry ethyl acetate was stirred overnight in a nitrogen atmosphere at room temperature. A solution of tetra-*n*-butylammonium chloride (112 mg, 0.4 mmol) in 2.2 mL of dry ethyl acetate was added, and stirring was continued for an additional 24 h. The mixture was filtered with Celite, and the filtrate was evaporated to give 0.3 g of a brown oil. Thick-layer silica gel chromatography (ethyl acetate/hexane, 8:2) afforded 77 mg of the π-(allyl)palladium complex **5** (55%) as a yellow oil. The use of dry acetone as the solvent in this reaction gave a 74% yield of **5**: ^{1}H NMR ($CDCl_3$) δ 4.40–3.9 (m, 3, CH_2O, C2 H), 3.90–3.50 (m, 10, OCH_3, syn CH=CPd, C5 H_2), 3.4–3.05 (m, 2, C3 H, C4 H), 2.90 (s, 2, anti CH=CPd), 2.65 (s, 1, $CHCOOCH_3$), 2.51 (d, 1, $CHCOOCH_3$), 1.20 (m, 3, CH_2CH_3).

Compound **5** (73 mg, 0.08 mmol) in 1.5 mL of dry tetrahydrofuran was added to a 10-mL solution of 194 mg of triphenylphosphine (0.74 mmol) in the same solvent. To this clear yellow solution under a nitrogen atmosphere was added a tetrahydrofuran solution (1.5 mL) of the sodium salt of *tert*-butyl acetoacetate prepared from 0.52 mmol of sodium hydride and 0.10 g of *tert*-butyl acetoacetate (0.67 mmol). Five minutes later, a yellow solid formed in the reaction mixture; this yellow suspension was stirred at 25 °C overnight. Ether (50 mL) was added to the reaction mixture, the resulting mixture was filtered, and the filtrate

(17) *tert*-Butyl alcohol was used in the procedure reported by Noyes, W. A. In "Organic Syntheses", Wiley: New York, 1943; Collect. Vol. II, pp 108–109.

(18) Stephenson, T. A.; Movehouse, S. M.; Powell, A. R.; Heffer, J. P.; Wilkinson, G. *J. Chem. Soc.* **1965**, 3632–3640.

was extracted with water. The ether layer was dried (magnesium sulfate) and evaporated to give 240 mg of a yellow solid, which was purified to give 27 mg of **6** (60%): ^{1}H NMR ($CDCl_3$) δ 4.90 (d, 1, =CH), 4.77 (s, 1, =CH), 4.32–3.95 (m, 3, OCH_2, C2 H), 3.76 (s, 3, OCH_3), 3.68 (s, 3, OCH_3), 3.70–3.42 (m, 2, C5 H_2), 3.20–2.65 (m, 2, C3 H, C4 H), 2.52–2.10 [m, 3, CH_2CO_2, $CH(CO)_2$], 1.6–1.2 (m, 2, $=CCH_2$), 1.54 [s, 9, $C(CH_3)_3$], 1.20 (q, 3, CH_2CH_3); ^{13}C NMR (CD_3COCD_3) 193.4 (CH_3CO), 179.5, 172.4 (CO_2CH_3), 169.0, 168.7 [isomers, $CO_2C(CH_3)_3$], 156.1 (OCON), 143.7 (C=), 113 (=CH_2), 82.0 [$OC(CH_3)_3$], 64.6, 64.3 (isomers C2), 61.3 (OCH_2), 59.3, 57.9 [isomers, $C(CO)_2$], 52.2, 51.6 (OCH_3), 48.4, 48.1 (isomers, C5), 45.1, 44.2 (isomers C4), 42.5, 41.6 (isomers, C3), 34.1 (CH_2C=), 33.7, 33.2 (isomers, CH_2CO_2), 28.0 ($COCH_3$), 27.7 [$C(CH_3)_3$], 14.7 (CH_2CH_3) ppm; mass spectrum *m/e* 469 (M^+), 413 [M – CH_2= $C(CH_3)_2$], 396 (M – $CO_2CH_2CH_3$). Anal. ($C_{23}H_{35}NO_9$, M_r 469.5) C, H, N.

Ethyl Methyl (2*S*,3*S*,4*S*)-3-[(Methoxycarbonyl)-methyl]-4-[1-[3-oxo-2-(phenylthio)butyl]vinyl]-1,2-pyrrolidinedicarboxylate (7). The sodium salt of phenylthioacetone was prepared in 1.5 mL of tetrahydrofuran under a nitrogen atmosphere by using 45 mg of phenylthioacetone (0.27 mmol) and 0.27 mmol of sodium hydride. This solution was added to 1.5 mL of a tetrahydrofuran solution containing 82 mg of the π-(allyl)palladium complex **5** (0.09 mmol) and 102 mg of triphenylphosphine (0.38 mmol) prepared as described in the synthesis of **6**. After the addition the reaction mixture turned red; stirring was continued at 25 °C for 38 h. Ether (50 mL) was added, the organic layer was washed several times with water, dried (magnesium sulfate), and evaporated to give 175 mg of a red oil. The portion of this residue that was soluble in 40% ethyl acetate in hexane was chromatographed on silica gel to give 14 mg (16%) of pure **7** as a yellow oil: IR ($CDCl_3$) 1740, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 5, aromatic), 4.99 and 4.94 (2 s, 1, =CH), 4.88 and 4.85 (2 s, 1, =CH) 4.20 (s, 1, C2 H), 4.3–3.95 (m, 2, OCH_2), 3.77 (s, 3, OCH_3), 3.68 (s, 3, OCH_3), 3.7–3.2 (m, 1, C5 H_2), 3.2–2.7 (m, 2, C3 H, C4 H), 2.6–2.1 (m, 3, CH_2CO_2, SCH), 2.26 (s, 3, $COCH_3$), 1.6–1.7 [m, 1, =CC*H*(H)CS], 1.27 [m, 1, =CCH(*H*)CS], 1.19 (q, 3, CH_2CH_3, J = 7 Hz); ^{13}C NMR (CD_3COCD_3) 203.5 ($COCH_3$), 172.8, 172.4 (CO_2CH_3), 154.9 (OCON), 143.1 (C=), 133.3, 133.1, 129.7 (2), 129.1, 128.6 (aromatic C), 114.4, 114.1 (isomers, =CH_2), 64.5, 64.2 (isomers, C2), 61.3 (OCH_2), 55.3 (SCCO), 52.2, 51.6 (OCH_3), 48.3, 48.0 (isomers C5), 45.2, 44.4 (isomers, C4), 42.3, 41.4 (isomers, C3), 37.0, 36.8 (isomers, $SCCH_2C$=), 33.2 (CH_2CO_2), 27.3 (CH_3CO), 14.6 (CH_3CH_2) ppm; mass spectrum, *m/e* 477 (M^+), 418 (M – CO_2CH_3), 404 (M – $CO_2CH_2CH_3$), 368 (M – SC_6H_5). Anal. ($C_{24}H_{31}NO_7S$, M_r 477.6) C, H, N.

Biological Testing. The protected kainic acid derivatives were hydrolyzed according to the general procedure. Compound **3** (40 mg, 0.128 mmol) in 0.4 mL of deuteriochloroform in a screw-cap NMR tube was deoxygenated by a stream of nitrogen gas. In an inert atmosphere were added pyridine (0.017 mL, 0.21 mmol) and trimethylsilyl iodide (0.15 mL, 1.02 mmol), and the tube was sealed. The reaction mixture was heated to 60 °C, and the progress of the reaction was monitored by ^{1}H NMR analysis. A decrease in the intensity of the proton signals for the ethyl group of the carbonate function and the methyl group of the esters concurrent with the increase in the proton signals for methyl iodide and ethyl iodide were easily discerned in the NMR spectra. After 36 h, the deprotection of **3** was complete. The solution in the NMR tube was transferred, leaving a dark solid residue, and rapidly neutralized to a pH of ~6 by the addition of 0.1 N potassium hydroxide. This solution was evaporated to give a solid residue, which was dissolved in 0.5 mL of water and placed on 10.5-mL bed volume of Dowex 50 W (H form, 200–400 mesh). Elution with water removed the neutral materials; elution with 1 N ammonium hydroxide, followed by evaporation, gave kainic acid (**1**; 18.1 mg, 66%), as a discrete peak. Silica gel chromatography (1-propanol/water, 4:1) and the mass spectrum were identical with those of kainic acid.

By the above method, the (*Z*)-nitrophenyl derivative **4a** (18.5 mg, 0.43 mmol) was hydrolyzed at 60 °C over 42 h with 7 μL of pyridine (0.085 mmol) and 50 μL of trimethylsilyl iodide (0.34 mmol) to give 3.4 mg of the (*Z*)-nitrophenyl-substituted amino acid **8a**, which was a single spot on silica gel TLC.

The (*E*)-nitrophenyl derivative **4b** (5.6 mg, 0.013 mmol) was treated at 60 °C for 16 days with a total of 4.1 μL of pyridine (0.05 mmol) and 40 μL of trimethylsilyl iodide (0.28 mmol) to give 4.7 mg of the (*E*)-nitrophenyl-substituted kainic acid **8b** as a single spot on TLC.

A quantitative spectrophotometric assay described by Troll[9] was used to determine the concentration of the amino acid derivatives for biological testing. Aliquots (5 μL) of approximately 0.01 M aqueous solutions of the amino acid derivatives in 1-mL volumetric tubes were treated with 20 μL of 1 M sodium bicarbonate, 40 μL of 0.02 M 1,2-naphthoquinone-4-sulfonate, and 150 μL of water. After the solution was mixed and then left standing for 10 min, 20 μL of 1 M sodium acetate buffer (pH 5) and 20 μL of 0.1 M ascorbic acid in 0.001 M hydrochloric acid were added. The resulting solution was diluted to 1.0 mL and mixed, and the absorbance was recorded at 480 nm. These readings were compared to standards using kainic acid, which under the conditions described followed Beer's law for the range of 0.05 to 1.0 absorbance unit (ϵ = 4600 at 480 nm).

Assay of [^{3}H]Kainic Acid Binding. A modification of the centrifugation assay of London and Coyle[11] was used. All assays were conducted in plastic minivials with 100 μg of rat brain synaptic membrane protein and 20 nM [^{3}H]kainic acid per assay. The samples were incubated at 2 °C for 45 min. The assay was terminated by centrifugation of the samples at 19 000 for 10 min. The pellets were rinsed with ice-cold buffer (50 mM Tris/Cl) and dissolved in 0.1 N sodium hydroxide prior to scintillation counting. Nonspecific binding of [^{3}H]kainic acid was determined by measuring the amount of [^{3}H]kainic acid bound in the presence of excess unlabeled kainic acid (10^{-4} M) as was described previously.[14]

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Registry No. 1, 487-79-6; 1 (ethoxycarbonyl derivative), 87682-49-3; **2a**, 4071-37-8; **2b**, 73903-33-0; **3**, 87682-50-6; **4a**, 87682-51-7; **4b**, 87682-52-8; **5**, 87682-57-3; **6**, 87682-53-9; **7**, 87696-37-5; **8a**, 87682-54-0; **8b**, 87682-55-1; 3-nitroaniline, 99-09-2; palladium trifloroacetate, 42196-31-6; *tert*-butyl acetoacetate sodium salt, 64770-14-5; phenylthioacetone sodium salt, 87682-56-2; dihydrokainic acid, 52497-36-6.