

Synthesis of a New Kainic Acid Based Selective Ligand as a Potential Photoaffinity Label of Non-NMDA Excitatory Amino Acid Receptors in Chicken Brain

Emmanuel Sivas,^a Georgia Voukelatou,^a Elias D. Kouvelas,^a George W. Francis,^b Dagfinn W. Aksnes^b and Dionissios Papaioannou*.^c

^aDepartment of Physiology, Medical School, University of Patras, Patras, Greece, ^bDepartment of Chemistry, University of Bergen, Allégaten 41, N-5007 Bergen, Norway and ^cDepartment of Chemistry, School of Natural Sciences, University of Patras, Patras, Greece

Sivas, E., Voukelatou, G., Kouvelas, E. D., Francis, G. W., Aksnes, D. W. and Papaioannou, D., 1994. Synthesis of a New Kainic Acid Based Selective Ligand as a Potential Photoaffinity Label of Non-NMDA Excitatory Amino Acid Receptors in Chicken Brain. – Acta Chem. Scand. 48: 76–79. © Acta Chemica Scandinavica 1994.

The synthesis of two analogues of kainic acid (KA) incorporating photo-activatable moieties attached either on the γ -carboxy function (γ -amide **1**) or the isopropenyl side-chain (amide **2**) is described. The synthesis of the former amide involves coupling of *N*-(*tert*-butoxycarbonyl)-protected α -diphenylmethyl kainate with 2-(4-azidobenzamido)ethylamine (**5**) followed by trifluoroacetic acid mediated complete deprotection. Amide **2** was synthesized by palladium-mediated allylic amination, with 4,4'-dimethoxybenzhydramine (DMBA), of *N*-(9-fluorenylmethoxycarbonyl)-protected dimethyl kainate, followed by splitting the DMB-group with formic acid, coupling with *N*-hydroxysuccinimidoyl 4-azidobenzoate and finally complete deprotection by saponification. Preliminary pharmacological studies in chicken brain membranes showed that amide **2** is a stronger inhibitor of [³H]KA binding on chicken cerebellar membranes than is amide **1** and that amide **2** has specificity only for the cerebellar, as opposed to the telencephalon, type of non-NMDA binding sites.

The actions of excitatory amino acids (EAAs) are mediated by at least four distinct receptor systems. *N*-Methyl-D-aspartic acid (NMDA), kainic acid (KA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are of the ionotropic type. The fourth, the metabotropic receptor, activated by quisqualic acid (Quis) and *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD) stimulates inositol phospholipid metabolism.¹ The non-NMDA, AMPA and KA receptors, despite regional differences, e.g., chicken brain,² and different ranks of relative potencies of agonists,³ do share several properties.⁴ To increase our understanding of non-NMDA EAA receptors we decided to use the advantages offered by photoaffinity cross-linking,⁵ by developing a photoaffinity label based on KA. The synthesis of analogues of KA incorporating a photolabile moiety, at either the γ -carboxy function or on the isopropenyl side-chain (γ -amide **1** and amide **2**, respectively, Fig. 1) is described. Results are given for binding studies of these analogues in the membrane fraction of chicken cerebellum and telencephalon. In cerebellum the

predominant class of non-NMDA binding sites are KA sensitive, while in telencephalon the AMPA-sensitive class of sites predominates.³

The synthesis of **1** and **2** is outlined in Fig. 1. The aryl azide⁶ photolabel was used because of its known propensity to form adducts with nucleophiles, e.g., amines. Incorporation of the *p*-azidobenzoyl moiety into KA required ethane-1,2-diamine as a spacer group. The key intermediate **3** for the synthesis of **1** may be prepared from KA (**4**) and its crystal structure has been described.⁷ Amine **5** was obtained, in 65% yield, using a sequence involving monotrylation of ethane-1,2-diamine, acylation of the other amino function with *N*-hydroxysuccinimidoyl 4-azidobenzoate (HSAB) in the presence of diisopropylethylamine (DIEA) and removal of the triphenylmethyl (Trt) group with trifluoroacetic acid (TFA). Coupling of **3** and **5** was performed with dicyclohexylcarbodiimide (DCC)–1-hydroxybenzotriazole (HOBt) in the presence of triethylamine (TEA). Removal of the amino and α -carboxy protecting groups with an ice-cold 55% solution of TFA in CHCl₃ for 2 h, in the presence of anisole, provided a 50% overall yield of the trifluoroacetate salt of amide **1**. Initial pharmacological studies (Table 1) showed that this amide was a weak inhibitor of [³H]KA binding

* To whom correspondence should be addressed.

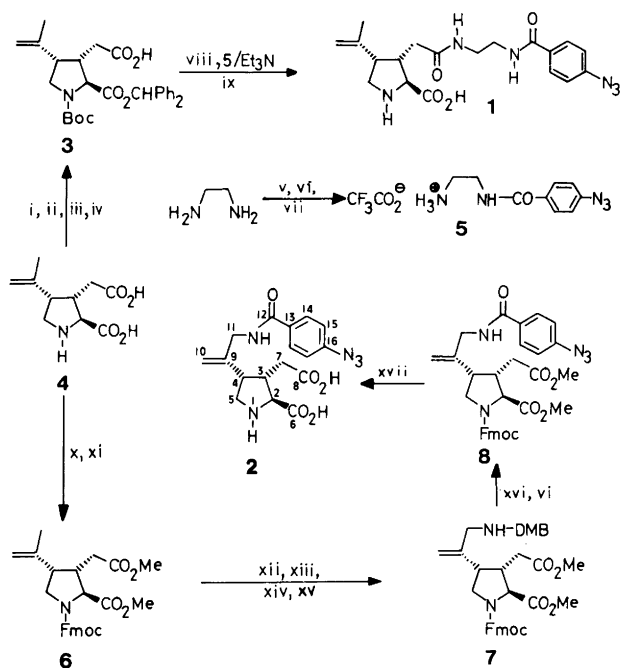


Fig. 1. Synthesis of KA analogues **1** and **2**. Reagents and conditions are indicated by roman numerals as follows: (i) *tert*-butyl S-(4,6-dimethylpyrimidin-2-yl)thiocarbonate-TEA in dioxane-H₂O; (ii) I₂-KI-NaHCO₃ in H₂O; (iii) Ph₂CHOH-TPP-DEAD in THF; (iv) Zn in AcOH-H₂O; (v) TrtCl in CHCl₃; (vi) HSAB-DIEA in DMF; (vii) 10% TFA in CH₂Cl₂; (viii) DCC-HOBT in DMF; (ix) 55% TFA in CHCl₃-anisole (10:1), 2 h at 0°C; (x) FmocCl-Na₂CO₃ in dioxane-H₂O; (xi) MeOH-TPP-DEAD in THF; (xii) Pd(CF₃CO₂)₂ in Me₂CO; (xiii) Bu₄NCl; (xiv) DIPHOS; (xv) DMBA; (xvi) 88% aq. HCO₂H; (xvii) NaOH in H₂O-MeOH.

in chicken brain cerebellar membranes. Since naturally occurring powerful KA agonists, e.g., domoic acid⁸ involve only changes in the isopropenyl side chain of KA, we decided to attach amine **5** at this position.

The photolabile moiety was introduced in the isopropenyl side chain of KA by Pd-mediated allylic functionalization.⁹ The required derivative **6** was obtained from KA, in 82% yield, by *N*-protection¹⁰ with FmocCl-Na₂CO₃ followed by esterification with MeOH in the presence of triphenylphosphine (TPP)-diethyl azodicarboxylate (DEAD).¹¹ Attempts to attach **5** to the

Table 1. Percentage inhibition of [³H]KA binding on chicken cerebellar membranes by KA analogues **1** and **2**, at various concentrations. Values are mean (standard error of the mean) from five experiments.

Concentration/mM	% Inhibition	
	γ-Amide 1	Amide 2
0.1	7(1)	32(2.5)
0.2	13(1.9)	52(3.4)
0.4	25(1.5)	73(5.3)
0.6	36(1.8)	79(5.5)
0.8	44(2.2)	82(5.4)

side chain of **6** failed. An alternative approach utilised the KA analogue **2**, obtained from the intermediate **7**, which bears an allylic amino function and allows attachment of the *p*-azidobenzoyl photolabile moiety by *N*-acylation. Sequential treatment of **6** with Pd(CF₃CO₂)₂, Bu₄NCl, 1,2-bis(diphenylphosphino)ethane (DIPHOS) and finally 4,4'-dimethoxybenzhydrylamine (DMBA) provided **7** (55% overall yield). Removal of the DMB-group with 88% aq. formic acid followed by acylation in the presence of DIEA, gave the intermediate **8** (42% yield), saponification of which effected simultaneous deprotection of the amino and the carboxy groups and yielded the KA analogue **2** (62% yield).

Preliminary experiments on [³H]KA binding in chicken cerebellar membranes showed (Table 1) that amide **2** showed much better inhibition of [³H]KA binding than did the γ-amide **1**. Thus, only the effects of **2** were further studied. The IC₅₀ values for inhibition of [³H]KA, [³H]AMPA and [³H]CNQX (tritiated 6-cyano-7-nitroquinoxaline-2,3-dione, an antagonist of non-NMDA receptors) binding to chicken cerebellar and telencephalon (values in parentheses) membranes at post hatching day 15 by amide **2** were 180 ± 15 (>600), >1000 (>1000) and 23 ± 2.1 (>1000) μM, respectively. These values suggest that **2** has specificity only for the cerebellar subclass of non-NMDA-type binding sites. This specificity is significantly lower than that of KA (IC₅₀ 1 ± 0.15 μM) although the C-4 unsaturated sidechain of KA, considered responsible for the specific activation of KA receptors,¹² is retained in amide **2**.

Detailed pharmacological studies of the non-NMDA receptors in chicken brain using amide **2** and other EAA agonists and antagonists as well as the results of applying radiolabelled **2** for photoaffinity labelling of cerebellar non-NMDA-type binding sites are now in progress.

Experimental

General. Melting points (uncorrected) were taken on a Büchi SMP-20 apparatus. Optical rotations were determined with a Carl-Zeiss precision polarimeter. IR spectra were recorded as Nujol mulls or neat on a Perkin-Elmer 457 grating spectrophotometer. ¹H and ¹³C NMR spectra were obtained at 400.13 and 100.63 MHz, respectively, on a Bruker AM400 spectrometer. The ¹³C-¹H chemical shift correlations were established using the heteronuclear shift correlation pulse sequence.¹³ The heteronuclear coupling-modulated spin-echo technique was used to distinguish methyl and methine from methylene and quaternary C-atoms.¹⁴ Flash column chromatography (FCC) was performed on Merck silica gel 60 (230–240 mesh) and TLC on Merck silica gel 60 F₂₅₄ films (0.2 mm) precoated on Al-foil. Spots were visualized with UV light at 254 nm, with ninhydrin, and Cl₂-KI-starch reagent. KA and HSAB were purchased from Sigma. [³H]AMPA (sp. act. 60 Ci mmol⁻¹), [³H]CNQX (sp. act. 18.6 Ci mmol⁻¹) and [³H]KA (sp. act. 58 Ci mmol⁻¹) were purchased from New England Nuclear. Membrane preparation

and displacement experiments of [^3H]AMPA and [^3H]CNQX and of [^3H]KA specific binding were performed as previously described.^{15,16}

Fmoc-KA was prepared from KA by a reported general procedure,¹⁰ {92% yield, m.p. 102–105°C, [α]_D²⁵ – 37.2° (*c* 1, CHCl₃), *R*_f 0.77 [BuOH–AcOH–H₂O (4:1:5; upper phase)]}. Dimethyl ester **6**, prepared by Mitsunobu esterification¹¹ of Fmoc-KA, was obtained as an oil {91% yield and had [α]_D²⁵ – 27.9° (*c* 4, CHCl₃), *R*_f 0.42 [PhMe–EtOAc (8:2)], IR (neat): 1740 and 1720 cm⁻¹, Anal. C₂₇H₂₉NO₆: C, H, N}. Full experimental details for the synthesis of γ -amide **1** may be obtained from the authors on request. Experimental details and physical data are given below for the pharmacologically more interesting KA analogue **2**. All new compounds gave satisfactory microanalytical (to within $\pm 0.4\%$ of the calculated values) and spectroscopic data.

(2*S*,3*S*,4*S*)-4-[1-(4-Azidobenzamidomethyl)ethenyl]-2-carboxypyrrolidin-3-ylacetic acid **2**. To a solution of the dimethyl ester **6** (0.33 g, 0.68 mmol) in dry Me₂CO (2 ml) were added activated molecular sieves (4 Å powder) (0.25 g) and Pd(CF₃CO₂)₂ (0.25 g, 0.72 mmol) under argon and the mixture was stirred at room temperature for 28 h. Bu₄NCl (0.25 g, 0.77 mmol) in dry Me₂CO (1 ml) was added and stirring was continued for a further 24 h. The resulting mixture was filtered through a Celite plug, the filter-cake washed with Me₂CO and the filtrate evaporated to dryness. The residue was subjected to FCC with EtOAc–PhMe (4:6), to give the corresponding oily π -(allyl)palladium complex in 65% yield as a mixture of isomers,¹⁷ *R*_f-values 0.47 and 0.53 [EtOAc–PhMe (4:6)]. The isomeric mixture (0.36 g, 0.6 mmol) was dissolved in dry THF (3 ml) and treated¹⁸ with DIPHOS (0.277 g, 0.7 mmol) at room temperature for 25 min under argon. DMBA¹⁹ (1.2 mmol, 0.29 g) was added and stirring was continued for a further 18 h. Dilution with EtOAc, followed by washing with ice-cold aq. NaHCO₃ solution (5%) and brine, drying (Na₂SO₄) and evaporation under reduced pressure, produced crude **7**. Purification by FCC with PhMe–EtOAc (1:1) gave pure compound **7** (55% yield) as an oil with *R*_f = 0.46 for this solvent system. A solution of **7** (0.15 g, 0.2 mmol) in 88% aq. formic acid (2 ml) was heated at 70°C for 1.5 h to remove the DMB-group. The reaction mixture was cooled to 0°C and brought to pH 7 by the dropwise addition of aq. NaHCO₃ solution (5%). Extraction with EtOAc (twice), drying (Na₂SO₄) of the organic layers and evaporation, gave the corresponding crude allylic amine [*R*_f = 0.4; CHCl₃–MeOH (8:2)] which was immediately dissolved in dry DMF (0.5 ml). HOBt (27 mg, 0.2 mmol) and pulverised HSAB (65 mg, 0.26 mmol) were sequentially added to the solution and the reaction mixture was stirred for 10 h; the pH was kept at ca. 8 by the dropwise addition of DIEA. Trituration with brine gave a precipitate of the crude product which was extracted into EtOAc. The organic layer was washed with aq. NaHCO₃ (5%) and then brine, dried, and evaporated to dryness. The residue

was subjected to FCC with PhMe–EtOAc (1:1) to give **8** (50 mg, 42% yield) as an oil.

Compound **8** (50 mg, 0.08 mmol) was dissolved in methanol (2 ml) and treated with 2 M NaOH (0.4 ml). After 2 h, the reaction mixture was cooled to 0°C and neutralized by the dropwise addition of 0.5 M aq. HCl. The resulting solution was evaporated at room temperature and crude **2** was purified by gel chromatography on Bio-Gel P-2 (Bio Rad), using a pyridinium acetate buffer (pH 7). The ninhydrin positive fractions were pooled and freeze-dried to give pure **2** (18 mg, 62% yield). Amino acid analysis (Beckman 120C) using resin W-1 (Beckman) and elution with 0.2 M and 0.4 M citrate buffers (pH 3.49 and 4.10) confirmed that **2** was free from KA.

The KA analogue **2** had m.p. 165–167°C (decomp.), [α]_D²⁵ – 6.7 (*c* 0.7, H₂O), *R*_f 0.29 [BuOH–AcOH–H₂O (4:1:5; upper phase)].

¹H NMR (D₂O, ref. Me₂CO at 2.17 ppm): δ 7.766 (2 H, m, *J* 7.73, 0.97 and 2.59 Hz, H-15), 7.152 (2 H, m, *J* 7.73, 0.97 and 2.59 Hz, H-14), 5.155 (1 H, s, H-10a), 4.945 (1 H, s, H-10b), 4.027 (1 H, d, *J* 2.33 Hz, H-2), 3.936 (2 H, s, H-11a/11b), 3.610 (1 H, dd, *J* 11.85 and 7.50 Hz, H-5a), 3.458 (1 H, t, *J* 11.74 Hz, H-5b), 3.065 (1 H, m, *J* 6.83, 2.33, 7.67 and 7.36 Hz, H-3), 2.951 (1 H, m, *J* 11.74, 6.83 and 7.50 Hz, H-4), 2.297 (1 H, dd, *J* 15.76 and 7.36 Hz, H-7a), 2.136 (1 H, dd, *J* 15.76 and 7.67 Hz, H-7b).

¹³C NMR (D₂O; ref. Me₂CO at 29.2 ppm): δ 180.21 and 178.33 (C-6 and C-8), 172.27 (C-12), 142.74 (C-16), 138.17 (C-13), 128.74 (C-9), 127.91 (C-15), 118.12 (C-14), 111.75 (C-10), 65.30 (C-2), 45.45 (C-11), 43.59 (C-5), 41.54 (C-4), 40.62 (C-3), 35.58 (C-7). Anal. C₁₇H₁₉N₅O₅: C, H, N.

IR (Nujol): 4400, 3200–2500, 2120, 1660, 1630 and 1580 cm⁻¹.

Acknowledgments. Financial support provided by the General Secretariat for Research and Technology of the Greek Ministry of Industry, Energy and Technology is gratefully acknowledged.

References

1. Monaghan, D. T., Bridges, R. J. and Cotman, C. W. *Ann. Rev. Pharmacol. Toxicol.* 29 (1989) 365.
2. Henley, J. M. and Barnard, E. A. *J. Neurochem.* 56 (1991) 702.
3. Krosgaard-Larsen, P., Honore, T., Hansen, J. J., Curtis, D. R. and Lodge, D. *Nature (London)* 284 (1980) 64.
4. Barnard, E. A. and Henley, J. M. *TIPS* 11 (1990) 500.
5. See for example: Voukelatou, G., Aletras, A. J. and Kouvelas, E. D. *Eur. J. Pharmacol.* 162 (1989) 179 and Pilch, P. F. and Czech, M. P. 'Affinity Cross-Linking of Peptide Hormones and their Receptors'. In: Venter, J. C. and Harrison, L. C., Eds., *Receptor Biochemistry and Methodology*, Liss, New York 1984, Vol. 1, p. 161.
6. Watt, D. S., Kawada, K., Leyva, E. and Platz, M. S. *Tetrahedron Lett.* 30 (1989) 899.

7. Papaioannou, D., Sivvas, E., Nastopoulos, V., Semertzidis, M. and Voliotis, S. *Acta Crystallogr. Sect. C45* (1989) 1649. For a similar synthesis see Goldberg, O. and Teichberg, V. I. *J. Med. Chem.* 28 (1985) 1957.
8. Takemoto, T., Daigo, K., Kondo, Y. and Kondo, K. *J. Pharm. Soc. Jpn.* 86 (1966) 874.
9. Trost, B. M. *Acc. Chem. Res.* 13 (1980) 385.
10. Chang, C.-D., Waki, M., Ahmad, M., Meinhofer, J., Lundell, E. O. and Hang, J. D. *Int. J. Peptide Protein Res.* 15 (1980) 59.
11. Mitsunobu, O. *Synthesis* (1981) 1.
12. Watkins, J. C., Krogsgaard-Larsen, P. and Honoré, T. *TIPS* 12 (1991) 4.
13. Bax, A. and Morris, G. A. *J. Magn. Reson.* 42 (1981) 501.
14. Brown, D. W., Nakashima, T. T. and Rabenstein, D. L. *J. Magn. Reson.* 45 (1981) 302.
15. Honoré, T., Drejer, J., Nielsen, E. O. and Nielsen, M. *Biochem. Pharmacol.* 38 (1989) 3207.
16. Vouklatou, G., Angelatou, F. and Kouvelas, E. D. *Int. J. Dev. Neurosci.* 4 (1986) 339.
17. Conway, G. A., Park, J. S., Maggiora, L., Mertes, M. P., Galton, N. and Michaelis, E. K. *J. Med. Chem.* 27 (1984) 52.
18. Åkermark, B. and Zetterberg, K. *Tetrahedron Lett.* (1975) 3733.
19. Trost, B. M. and Keinan, E. *J. Org. Chem.* 44 (1979) 3451.

Received May 7, 1993.