Tetrahedron Letters, Vol.31, No.21, pp 2967-2970, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

PROBING THE TOPOGRAPHY OF KAINATE RECOGNITION SITES: SYNTHESIS OF A NOVEL OXETANE CONTAINING KAINIC ACID ANALOGUE.

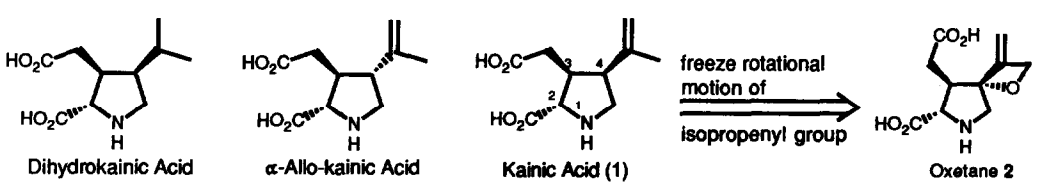
Alan P. Kozikowski* and Abdul H. Fauq
Departments of Chemistry and Behavioral Neuroscience, University of Pittsburgh, Chevron Science Center, Pittsburgh, PA 15260

Summary: A short synthesis of the conformationally rigidified spirocyclic-kainic acid analogue **2** is described starting from kainic acid. This relatively conservative change in the structure of kainic acid has been found to lead to a loss of affinity for glutamate recognition sites.

Kainic acid is a potent excitotoxic agent which is capable of inducing brain lesions closely resembling those seen in Huntington's disease and several other neurodegenerative disorders.[1] Kainic acid, like other neuroexcitotoxins, appears to act via postsynaptic receptors, causing the neuron to depolarize repeatedly or persistently. While excitotoxin induced neuronal cell death has been attributed to ATP depletion, such changes may occur downstream from initial cytosolic Ca^{++} increases which occur through voltage-independent ion channels. The dramatic changes in intracellular Ca^{++} levels may in turn overactivate certain kinases and proteases resulting in eventual cell death.[2]

In order to learn more about the kainic acid recognition site with the intention of discovering potent and selective kainic acid receptor antagonists, we have begun a program to prepare several derivatives of this molecule. Specifically, we have chosen to examine the preparation of analogues modified in the vicinity of its isopropenyl group.

Since α-allo-kainic acid (possessing a reversed orientation of the isopropenyl side chain) and dihydrokainic acid possess less than 2% of the excitatory potency of kainic acid at the crustacean neuromuscular junction, it can safely be assumed that the isopropenyl appendage plays a critical role in binding and functional activation at the kainic acid recognition site.[3]



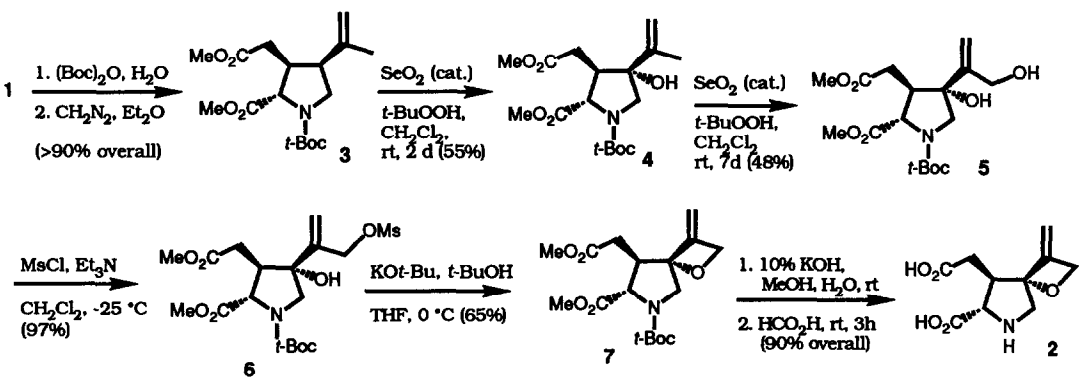
Dihydrokainic Acid | α-Allo-kainic Acid | Kainic Acid (1) | Oxetane 2

Consequently, we became interested in examining the effect of freezing the rotational motion of the isopropenyl group by constructing the spirocyclic oxetane **2**. *Selection of this conformationally rigidified analogue for synthesis was guided by the notion that the rotational motion of the isopropenyl group might play an essential role in the signal transducing event, and that accordingly the rigid structure* **2** *might be incapable of eliciting a biological response through a complementary change in ligand and receptor conformation.*[4]

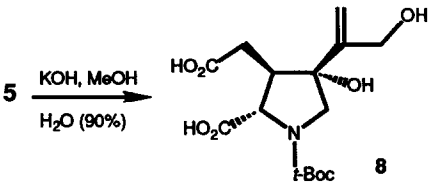
Herein we describe an efficient synthetic route to the optically pure **oxetane** analogue **2** starting from kainic acid. *This route is based on our discovery that the allylic hydroxylation of a fully protected kainic acid derivative proceeded with complete retention of stereochemistry at the C-4 stereocenter.*

Accordingly, kainic acid was first converted to the *t*-Boc protected diester **3** by reaction with *t*-Boc anhydride followed by diazomethane treatment.[5] Next, the diester was reacted with *tert*-butylhydroperoxide and a catalytic amount of selenium dioxide in CH_2Cl_2 at room temperature for 40 h. A 55% yield of the tertiary alcohol **4** was obtained as the major product (Scheme 1).[6]

Scheme 1. Synthesis of the Oxetane 2



Since the olefinic protons of the isopropenyl appendage appeared as two broad singlets in the ^1H NMR, we assumed that the hydroxylation reaction had taken place with α-stereochemistry. This assignment is in accord with a suggestion that the *cis*-relationship of the isopropenyl group and the acetic acid side chain results in steric compression which, in turn, is responsible for the different chemical shifts of the olefinic protons.[7] In the ^1H NMR of α-allo-kainic acid, these protons appear as a singlet. While we were unable to gather any substantive N.O.E. data in support of the stereochemical assignment, our speculation was fully confirmed by an X-ray analysis (Figure 1) of the diacid **8** prepared from **5** (*vide infra*) by aqueous KOH/MeOH



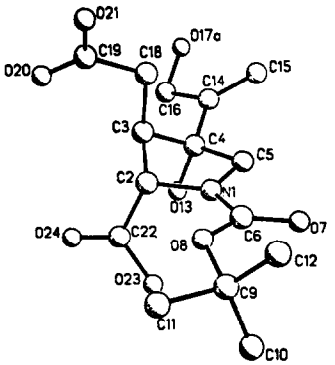
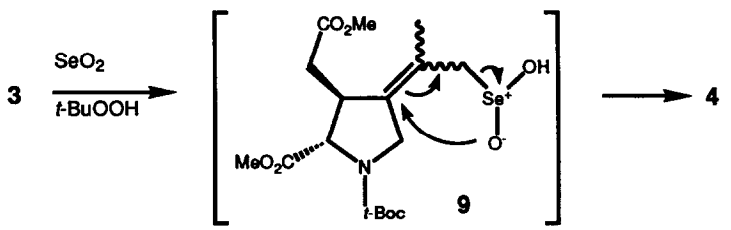


Figure 1. A Perspective Drawing of Alcohol **8** With Hydrogen Atoms Omitted for Clarity.

treatment. The stereochemical outcome of the allylic hydroxylation reaction can be rationalized by assuming that [2,3] sigmatropic rearrangement of the initially formed ene product, the seleninic acid **9**, occurs exclusively opposite the bulky β-oriented C-3 appendage.[6]



To procure the diol **5** required for preparation of the oxetane **2**, the tertiary alcohol **4** was resubjected to the selenium dioxide/*tert*-butylhydroperoxide reaction conditions for seven days. The desired diol **5** was isolated from this reaction in 48% yield (66% based on consumed starting material). From diol **5**, a facile route to the oxetane was now achieved. The diol was treated with methanesulfonyl chloride/triethylamine in methylene chloride at -25 °C. The resulting mono-mesylated product **6** was reacted in turn with potassium *tert*-butoxide in *t*-butanol/THF at 0 °C. The ring closed product **7** was isolated in 65% yield. The fully deprotected oxetane **2** was generated by sequential treatment with aqueous potassium hydroxide to effect ester hydrolysis and then with formic acid to cleave the *tert*-butyloxycarbonyl group.[8,9]

To our knowledge, the present work provides the first recorded attempt of the synthesis of a conformationally rigidified kainic acid analogue.[10] In binding experiments carried out to evaluate the affinity of oxetane **2** for glutamate receptors of the kainate, NMDA (N-methyl-D-aspartate), or quisqualate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) type, compound **2** exhibited an IC_{50} of > 100 μM at each of these three sites.[11] Accordingly, it is clear that rigidification of the isopropenyl group by introduction of a single oxygen atom impairs receptor recognition for reasons relating to conformational, steric, or electronic factors. Other analogues of kainic acid

are now being synthesized in order to gain a better understanding of the topographical properties of kainate recognition sites which contribute to the loss of binding affinity of **2**.

Acknowledgement. We are indebted to the Fidia Research Foundation for their support of this program. We thank Dr. Wroblewski of FGIN for carrying out the binding assays.

References

1 J. C. Watkins in *Kainic Acid as a Tool in Neurobiology*, E. G. McGeer, J. W. Olney, and P. L. McGeer, Eds., Raven Press, New York, 1978, pp. 37-69.

2. J. A. Dykens, A. Stern, and E. Trenkner, *J. Neurochem.*, 1987, **49**, 1222. For studies pertaining to the isolation of the kainic acid receptor, see D. R. Hampson, and R. J. Wenthold, *J. Biol. Chem.*, 1988, **263**, 2500.

3. J. T. Slevin, J. F. Collins, and J. T. Coyle, *Brain Res.*, 1983, **265**, 169.

4. See, *inter alia*, J. Saunders, G. A. Showell, R. J. Snow, R. Baker, E. A. Harley, and S. B. Freedman, *J. Med. Chem.*, 1988, **31**, 486.

5. O. Goldberg and V. I. Tiechberg, *J. Med. Chem.*, 1985, **28**, 1957; O. Goldberg, A. Luini, and V. I. Teichberg, *J. Med. Chem.*, 1983, **26**, 39.

6. K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, 1972, **94**, 7154. Small amounts of the primary allylic alcohol (5.6%) as well as the diol **6** (4.1%) were isolated from the reaction. The allylic oxidation of the silyl protected diol derived from kainic acid has been described previously, but no assignment of the tertiary alcohol stereochemistry was made: K. Konno, K. Hashimoto, Y. Ohfune, H. Shirahama, and T. Matsumoto, *J. Am. Chem. Soc.*, 1988, **110**, 4807.

7. P. D. Kennewell, S. S. Matharu, J. B. Tayler, and P. G. Sammes, *J. Chem. Soc. Perkin Trans. 1*, 1980, 2542; K. Kondo, Y. Kondo, T. Takemoto, and T. Ikenoue, *Bull. Chem. Soc. Japan*, 1962, **35**, 1899; For a discussion of the rotameric distributions of several kainoids, see: G. A. Conway, J. S. Park, L. Maggiora, M. P. Mertes, N. Galton, and E. K. Michaelis, *J. Med. Chem.*, 1984, **27**, 52.

8. Due to the presence of rotamers of the *t*-Boc derivatives, the ^{1}H and ^{13}C NMR's of compounds **3** to **8** are complicated.

9. Spectral data for the kainic acid analogue derived from the protected diester **4**, the trifluoroacetate salt of the fully deprotected diol derived from **5**, and oxetane **2** follow:

Kainic acid analogue derived from **4**: $[\alpha]_D^{23}$ - 51.4° (c 0.007 g/mL, H_2O); IR (nujol) 4350(d), 3500, 2000, 2925, 1722, 1699, 1601, 1574, 1415 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 5.08 (bs, 1 H), 4.90 (bs, 1 H), 4.20 (d, *J* = 2 Hz, 1 H), 3.76 (d, *J* = 12.4 Hz, 1 H), 3.46 (d, *J* = 12.4 Hz, 1 H), 3.01 (m, 1 H), 2.48 (dd, *J* = 16.6, 5.4 Hz, 1 H), 2.30 (dd, *J* = 16.6, 9.3 Hz, 1 H), 1.76 (s, 3 H); ^{13}C NMR (125 MHz, D_2O) δ 141.66, 114.97, 83.64, 65.83, 53.09, 47.32, 37.16, 18.70; MS (m/z) 229 (M^+), 211 (M^+ - H_2O), 198, 184, 166, 87; HRMS calcd for $C_{10}H_{15}NO_5$ 229.0950, found 229.0951.

Trifluoroacetate salt of deprotected diol derived from **5**: $[\alpha]_D^{23}$ - 23° (c 0.0435 g/mL, H_2O); IR (nujol) 3379, 1725, 1664, 1153 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 5.41 (bs, 1 H), 5.18 (bs, 1 H), 4.43 (d, *J* = 1.9 Hz, 1 H), 4.22 (d, *J* = 15 Hz, 1 H), 4.15 (d, *J* = 15 Hz, 1 H), 3.84 (d, *J* = 12.4 Hz, 1 H), 3.56 (d, *J* = 12.4 Hz, 1 H), 3.12 (m, 1 H), 2.56 (dd, *J* = 16.9, 5 Hz, 1 H), 2.35 (dd, *J* = 16.9, 9.7 Hz, 1 H); ^{13}C NMR (75 MHz, D_2O) δ 180.00, 176.30, 167.35, 146.5, 122.87, 118.50, 84.63, 68.97, 51.64, 46.17, 44.71, 37.6; MS (m/z, FAB) 246 (M^+), 207, 185, 115 (CF_3COOH + 1); HRMS calcd for $[C_{10}H_{16}O_6Na]^+$ 246.2421, found 246.2421.

2: $[\alpha]_D^{21}$ + 21.4° (c 0.0035 g/mL, MeOH/H_2O 1:2 v/v); IR (nujol) 3300, 3100, 2930, 1722, 1699, 1450, 1160 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 5.14 (bs, 2 H), 5.01 (ABq, *J* = 11.2 Hz, 2 H), 3.96 (d, *J* = 6.4 Hz, 1 H), 3.87 (d, *J* = 13.7 Hz, 1 H), 3.63 (d, *J* = 13.7 Hz, 1 H), 3.17 (m, 1 H), 2.67 (m, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 177.10, 173.73, 143.60, 110.63, 100.27, 77.47, 66.14, 55.34, 49.4, 36.20; MS (m/z) 209 (M^+ - H_2O), 182, 165, 136, 87, 69; HRMS calcd for $C_{10}H_{11}NO_4$ 209.0688, found 209.0687.

10. For the synthesis of some other novel kainic acid analogues, see: H. Anand, P. J. Roberts, G. Badman, A. J. Dixon and J. F. Collins, *Biochem. Pharmac.* 1986, **35**, 409.

11. J. C. Watkins, P. Krogsgaard-Larsen and T. Honoré, *Trends Pharmacol. Sci.*, 1990, **11**, 25.

(Received in USA 20 March 1990)