^{1}H NMR Study of Protected and Unprotected Kainoid Amino Acids: Facile Assignment of C-4 Stereochemistry

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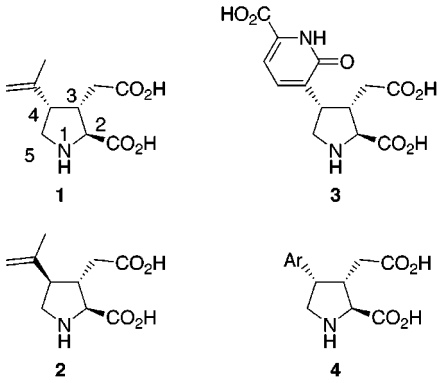
The kainoid amino acids exhibit potent neuroexcitatory activity in the mammalian central nervous system. Around their pyrrolidine ring, a trans disposition between the C-2 and C-3 substituents and a cis relationship between the C-3 and C-4 substituents are crucial for their potent biological activity. During synthetic studies into the kainoids, we have established a straightforward, empirical rule, which allows the facile assignment of C-4 stereochemistry to both protected and unprotected kainoids. When pairs of C-4 epimers are available, the rule indicates that, when their ^{1}H NMR spectra are compared, one of the methylene protons on the C-3 side chain appears at significantly lower chemical shift in the C-3, C-4 cis isomer than the corresponding signal for the proton in the spectrum for the C-3, C-4 trans isomer. In addition, the rule states that the difference in chemical shift between the two individual protons on the C-3 side chain of the C-3, C-4 cis isomer is significantly greater than the corresponding difference for the C-3, C-4 trans isomer. The rule is demonstrated for kainoids possessing an unsaturated substituent at C-4 and when comparing spectra in D_2O for pairs of unprotected C-4 epimers, the spectra were recorded at approximately the same pD.

Introduction

The kainoids are a class of naturally occurring, non-proteinogenic amino acids, which have prompted considerable synthetic and neuropharmacological research owing to their unique structure and interesting biological properties.[1] They are pyrrolidine dicarboxylic acids with three contiguous stereocenters about the pyrrolidine ring, and this structure is typified by the parent member (−)-α-kainic acid (**1**). Common to all the kainoids is a trans relative disposition between the C-2 and C-3 substituents, and with the exception of (+)-α-*allo*-kainic acid (**2**), they possess a cis disposition between the groups at C-3 and C-4.[2] The differing nature of the substituent at C-4 gives rise to the various members of the kainoid family and modifies their biological activity.[3] Kainoids display potent neuroexcitatory activity in the mammalian central nervous system and therefore have an important role in neuropharmacological research.[4] They behave as conformationally restricted analogues of the neurotransmitter L-glutamic acid and have been shown to act at the kainate class of ionotropic glutamate receptors.[5] Acromelic acid A (**3**) was isolated from a rare, toxic Japanese mushroom *Clitocybe acromelalga* in 1983 and was found to be the most potent naturally occurring kainoid neuroexcitant.[6] Since then, a range of "unnatural" C-4 aryl acromelic acid analogues **4** have been synthesized which



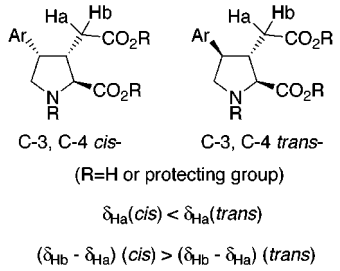


Figure 1. Rule for assigning C-4 stereochemistry.

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(1) For a comprehensive review see Parsons, A. F. *Tetrahedron* **1996**, *52*, 4149.

(2) Murakami, S.; Takemoto, T.; Shimizu, Z. *J. Pharm. Soc. Jpn.* **1953**, *73*, 1026.

(3) (a) Shirahama, H. In *Organic Synthesis in Japan Past, Present and Future*; Noyori, R., Ed.; Tokyo Kagaku Dozin Co. Ltd.: Tokyo, 1992; p 373. (b) Horikawa, M.; Shima, Y.; Hashimoto, K.; Shirahama, H. *Heterocycles* **1995**, *40*, 1009.

(4) (a) Mayer, M. *Nature* **1997**, *389*, 542. (b) Clarke, V. R. J.; Ballyk, B. A.; Hoo, K. H.; Mandelzys, A.; Pellizzari, A.; Bath, C. P.; Thomas, J.; Sharpe, E. F.; Davies, C. H.; Ornstein, P. L.; Schoepp, D. D.; Kamboj, R. K.; Collingridge, G. L.; Lodge, D.; Bleakman, D. *Nature* **1997**, *389*, 599.

(5) (a) Cantrell, B. E.; Zimmerman, D. M.; Monn, J. A.; Kamboj, R. K.; Hoo, K. H.; Tizzano, J. P.; Pullar, I. A.; Farrell, L. N.; Bleakman, D. *J. Med. Chem.* **1996**, *39*, 3617 and references cited therein.

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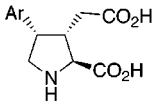
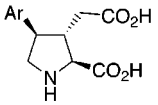
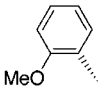
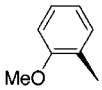
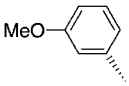
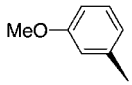
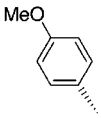
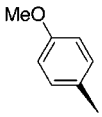
Table 1. C-3 Side-Chain Methylene Proton Chemical Shifts of Unprotected C-4 Arylkainoid Analogues (300 MHz, D_2O)

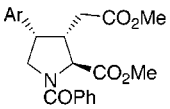


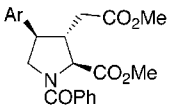


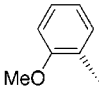
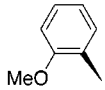
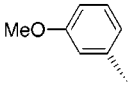
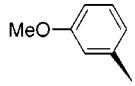
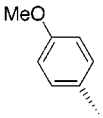
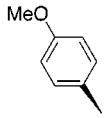
Ar (C-3, C-4 *cis* isomer)		δ_{Ha}	δ_{Hb}	Δ^a	Ar (C-3, C-4 *trans* isomer)		δ_{Ha}	δ_{Hb}	Δ^a
	**5**	1.78	2.15	0.37		**9**	2.29	2.47	0.18
	**6**	1.77	2.23	0.46		**10**	2.33	2.53	0.20
	**7**	1.81	2.18	0.37		**11**	2.25	2.42	0.17
	**8**	1.76	2.11	0.35		**12**	2.31	2.51	0.20

[a] $\Delta = \delta_{Hb} - \delta_{Ha}$.

Table 2. C-3 Side-Chain Methylene Proton Chemical Shifts of Protected C-4 Arylkainoid Analogues (300 MHz, $CDCl_3$)





Ar (C-3, C-4 *cis* isomer)		δ_{Ha}	δ_{Hb}	Δ^a	Ar (C-3, C-4 *trans* isomer)		δ_{Ha}	δ_{Hb}	Δ^a
	**13**	2.05	2.48	0.43		**17**	2.50	2.60	0.10
	**14**	2.13	2.35	0.22		**18**	2.48	2.59	0.11
	**15**	2.08	2.49	0.41		**19**	2.52	2.58	0.06
	**16**	2.07	2.48	0.41		**20**	2.49	2.57	0.08

[a] $\Delta = \delta_{Hb} - \delta_{Ha}$.

show similar or modified activity to the naturally occurring kainoids.[3]

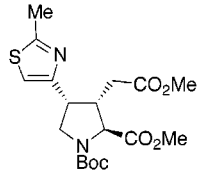
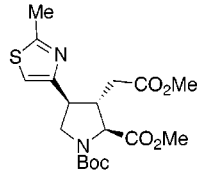
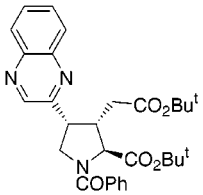
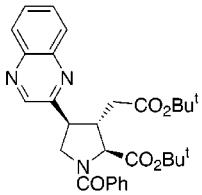
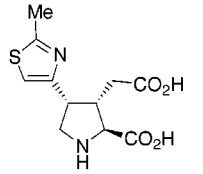
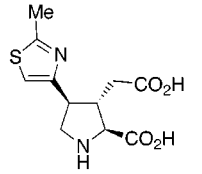
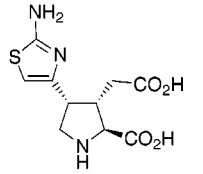
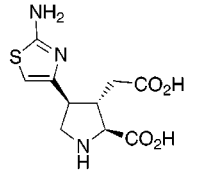
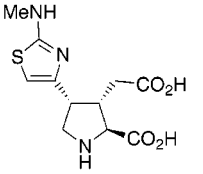
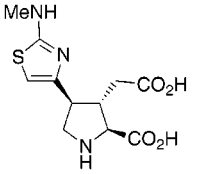
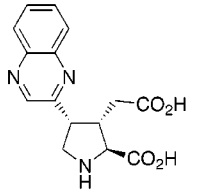
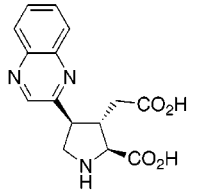
An unsaturated substituent at C-4 and a cis relative disposition between the C-3 and C-4 substituents are of paramount importance for strong kainoid neuroexcitatory activity.[7] Establishment of the correct stereochemistry at C-4 has been the most challenging task in kainoid synthesis. Simple and unambiguous methods to assign or confirm C-4 stereochemistry are therefore highly sought, particularly when synthesising new analogues. Structure elucidation by X-ray crystallography is frequently not possible and data obtained from ^{1}H NMR NOE experiments can often be misleading or inconclusive. Furthermore, signal overlap in the ^{1}H NMR spectra of these compounds occurs regularly, thus ruling out NOE experiments as a diagnostic tool. Shirahama and co-workers have recently reported methods for determining the relative stereochemistry of unprotected kainoid amino acids by ^{1}H NMR chemical shifts of the C-2 and C-4 protons in D_2O solution.[8] They found that the signals

(6) (a) Konno, K.; Shirahama, H.; Matsumoto, T. *Tetrahedron Lett.* **1983**, *24*, 939. (b) Konno, K.; Hashimoto, K.; Ohfune, Y.; Shirahama, H.; Matsumoto, T. *J. Am. Chem. Soc.* **1988**, *110*, 0, 4807.

(7) Hashimoto, K.; Horikawa, M.; Ishida, M.; Shinozaki, H.; Shirahama, H. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 743.

(8) Hashimoto, K.; Konno, K.; Shirahama, H. *J. Org. Chem.* **1996**, *61*, 4685.

Table 3. C-3 Side-Chain Methylene Proton Chemical Shifts of Protected and Unprotected C-4 Kainoid Analogues

Ar (C-3, C-4 *cis* isomer)	δ_{Ha}	δ_{Hb}	Δ^a	Ar (C-3, C-4 *trans* isomer)	δ_{Ha}	δ_{Hb}	Δ^a
 **21** 200MHz ($CDCl_3$)	2.13	2.33	0.20	 **27** 200MHz ($CDCl_3$)	2.52	2.66	0.14
 **22** 400MHz ($CDCl_3$)	2.13	2.35	0.22	 **28** 400MHz ($CDCl_3$)	2.48	2.59	0.11
 **23** 500MHz (D_2O)	1.89	2.48	0.59	 **29** 500MHz (D_2O)	2.44	2.68	0.24
 **24** 200MHz (D_2O)	1.75	2.35	0.60	 **30** 200MHz (D_2O)	2.29	2.52	0.23
 **25** 200MHz (D_2O)	2.05	2.49	0.44	 **31** 200MHz (D_2O)	2.26	2.48	0.22
 **26** 200MHz (D_2O)	1.82	2.60	0.78	 **32** 200MHz (D_2O)	2.62	2.84	0.22

[a] $\Delta = \delta_{Hb} - \delta_{Ha}$.

for the C-2 proton appear at higher fields than 4.2 ppm when the compounds have C-2, C-3 trans stereochemistry, whereas in the C-2, C-3 cis compounds they appear lower than 4.2 ppm irrespective of the substituent at C-4. This rule held when the solutions were in the range pD 3−8. When a pair of C-2 epimers was available and the spectra were recorded at equal or nearly equal pD, the chemical shift of the C-2 proton of the C-2, C-3 trans isomer was higher than that of the corresponding C-2, C-3 cis isomer. For determination of relative stereochemistry between C-3 and C-4, they also showed that the signal for the C-4 proton of the C-3, C-4 cis isomers appears at lower fields than those of the corresponding C-3, C-4 trans isomers in each pair of C-4 epimers. This empirical rule was shown to hold for compounds possessing an unsaturated substituent at C-4 and when the spectra are recorded at the same or nearly equal pD. Goldberg has also noted a chemical shift difference of the H-4 protons between pairs of C-4 epimers.[9]

In the course of our synthetic studies toward both natural and unnatural kainoid amino acids, we have discovered an equally applicable but more general rule for assigning C-4 stereochemistry to pairs of C-4 epimers. Our empirical rule holds for both protected and unprotected kainoids and is reported here in detail.

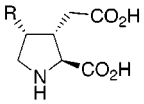
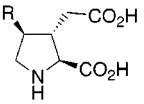
Results and Discussion

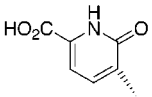
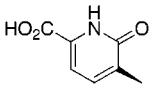
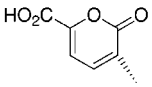
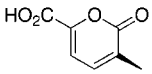
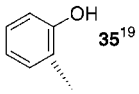
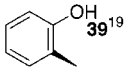
We have recently reported a concise and versatile synthesis of C-4 aryl kainoid analogues and their C-4 epimers starting from *trans*-4-hydroxy-L-proline.[10−14] Dur-

(9) Goldberg, O.; Luini, A.; Teichberg, V. I. *Tetrahedron Lett.* **1980**, *21*, 2355.

(10) Baldwin, J. E.; Bamford, S. J.; Fryer, A. M.; Wood, M. E. *Tetrahedron Lett.* **1995**, *36*, 4869.

Table 4. C-3 Side-Chain Methylene Proton Chemical Shifts of Unprotected Natural Kainoid (D_2O)

Ar (C-3, C-4 *cis* isomer)	δ_{Ha}	δ_{Hb}	Δ^a	Ar (C-3, C-4 *trans* isomer)	δ_{Ha}	δ_{Hb}	Δ^a
 **1**	2.38	2.47	0.09	 **2**	2.62	2.64	0.02
	400MHz				400MHz		
 **33**[8]	2.49	2.73	0.24	 **36**[8]	2.63	2.81	0.18
	400MHz				400MHz		
 **3**	1.98	2.45	0.47	 **37**	2.33	2.62	0.29
	300MHz				300MHz		
 **34**	2.17	2.58	0.41	 **38**	2.50	2.68	0.18
	300MHz				300MHz		
 **35**[19]	2.01	2.47	0.46	 **39**[19]	2.52	2.69	0.17
	500MHz				500MHz		

[a] $\Delta = \delta_{Hb} - \delta_{Ha}$.

ing these studies, four C-4 aryl acromelic acid analogues **5**−**8** and their C-4 epimers **9**−**12** were prepared. An obvious similarity between the ^{1}H NMR spectra in D_2O of **5**−**8** was immediately noted, and these spectra differed significantly from the spectra of their C-4 epimers **9**−**12**. The signals corresponding to the two methylene protons on the C-3 side chain showed a similar pattern within each of the two sets of C-4 epimers, but there were striking differences between the two sets of signals. From these initial observations, an empirical rule was established that readily and unambiguously distinguishes between a pair of C-4 epimers. Simple comparison of the ^{1}H NMR spectra can determine C-4 stereochemistry provided that an unsaturated substituent is located at C-4. Our rule is as follows: For a pair of C-4 epimers whose ^{1}H NMR spectra in D_2O are recorded at the same or approximately equal pD, one of the methylene protons on the C-3 side chain for the C-3, C-4 cis isomer appears at significantly lower chemical shift than the corresponding proton for the C-3, C-4 trans isomer (Figure 1). In addition, the difference in chemical shift between the two individual protons on the C-3 side chain for the C-3, C-4 cis isomer is significantly greater than the corresponding difference for the C-3, C-4 trans isomer. The data given in Table 1 for the chemical shifts of the C-3 side chain methylene protons for the pairs of C-4 epimers **5** and **9**, **6** and **10**, **7** and **11**, **8** and **12** clearly adheres to these rules. Final confirmation of the structure of kainoid **6** was gained by X-ray crystallography.[14]

After looking back at the protected derivatives of the pairs of epimers **5**−**8** and **9**−**12**, an analogous trend was observed in their ^{1}H NMR spectra. The same splitting patterns for the methylene protons on the C-3 side chain were displayed for each set of C-4 epimers **13**−**16** and **17**−**20** (Table 2).

Our rule for assigning the C-4 stereochemistry to unprotected kainoid amino acids could now be extended to include their protected derivatives. This result significantly improves the versatility of the rule since it is often useful to be able to identify a particular C-4 epimer before deprotection is performed.

When synthesizing our C-4 heteroaromatic kainoid analogues, this rule became particularly useful in identifying C-4 epimers of both protected and unprotected kainoids since ^{1}H NMR NOE experiments or X-ray crystallography were not possible in most cases.[15,16] The ^{1}H NMR data for the pairs of protected **21**−**26** and unprotected heteroaromatic kainoids **27**−**32** are given in Table 3.

Finally, we wanted to ensure that some of the known, naturally occurring kainoid amino acids and their C-4 epimers would satisfy our empirical rule. The ^{1}H NMR data for (−)-α-kainic acid (**1**) and (+)-α-*allo*-kainic acid (**2**) did indeed fit although the effect was not as pronounced as for the C-4 aryl compounds (Table 4).[8] The methyl ketones **33** and **36** synthesized by Shirahama were also in agreement but again the effect was not as significant as for the C-4 aryl compounds.[8] ^{1}H NMR data for acromelic acid A (**3**) and its C-4 epimer **37** were available to us from our synthesis of **3** as were the pyrone derivative **34** and its C-4 epimer **38**.[17,18] Again, both pairs of epimers followed the rule. The two C-4 epimers of the

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2-hydroxyphenylkainoid analogues **35** and **39** have been reported by Shirahama and, as with the C-4 aryl acromelic acid analogues shown in Table 1, conform to the observed trend.[19]

A plausible explanation for these observations is that for the protected and unprotected kainoids possessing the cis relative disposition between the C-3 and C-4 substituents one of the methylene protons on the C-3 side chain is situated in a shielding region of the unsaturated substituent at C-4. Its chemical shift will therefore be decreased relative to that of the corresponding proton in the C-3, C-4 trans- isomer. This anisotropic shielding of one of the methylene protons in the cis- isomers results in a greater difference in chemical shift between itself and the other methylene proton.

Summary and Conclusions

An empirical rule for assigning or confirming C-4 stereochemistry to both protected and unprotected kainoid amino acids has been suggested. The rule has been shown to hold for all pairs of C-4 epimers synthesized by us and by others which possess an aryl, heteroaromatic or other unsaturated substituent at the C-4 position. If both C-4 epimers of a particular compound are available then the rule can be quickly and easily applied to identify the correct epimer. When only one C-4 epimer is available, the rule provides a good indication of its identity. The rule will therefore be a valuable tool for determining, with confidence, the C-4 stereochemistry in future synthetic work in the kainoid field, particularly when X-ray crystallography is not possible and when ^{1}H NMR NOE experiments are unreliable or inconclusive. Characterization data for all compounds have been reported previously.[8,12−14,16,18,19]

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