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Synthesis and NMR Studies of *N*-Substituted Derivatives of Kainic Acid Dimethyl Esters

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Dimethyl kainate derivatives incorporating the amino protecting groups, 9-fluorenylmethoxycarbonyl (Fmoc), *tert*-butoxycarbonyl (Boc) and triphenylmethyl (trityl, Trt), have been synthesized from (–)-α-kainic acid [(2*S*,3*S*,4*S*)-2-carboxy-4-(1-methylethenyl)-3-pyrrolidinylacetic acid] by means of *N*-protection followed by esterification with methanol in the presence of triphenylphosphine and diethyl azodicarboxylate. Coupling of *N*-Fmoc protected 4-methylaminobenzoyl chloride with dimethyl kainate followed by *N*-deprotection with piperidine provided dimethyl 1-(4-methylaminobenzoyl)kainate. The ^{1}H and ^{13}C NMR spectra of two of the compounds reveal the presence of two conformers due to restricted rotation around the amide bond. The ^{1}H and ^{13}C assignments of the four compounds were carried out by one- and two-dimensional NMR techniques.

(–)-α-Kainic acid [(2*S*,3*S*,4*S*)-2-carboxy-4-(1-methylethenyl)-3-pyrrolidinylacetic acid], (**1**) a natural product isolated from the marine alga *Digenea simplex*, exhibits powerful neuroexcitatory activity which is attributed to its interaction with specific populations of receptors of the major excitatory transmitters of the mammalian central nervous system, glutamic and aspartic acids.[1] In order to carry out structure–activity relationship studies and develop specific affinity-labelling substrates for the receptors of kainic acid, we have now synthesized a series of useful intermediates (Fig. 1) which allow easy access to biologically interesting analogues of kainic acid. The structures of these intermediates, which are actually *N*-substituted dimethyl kainate derivatives, were fully determined using one- and two-dimensional NMR techniques.

The synthetic pathways employed in the synthesis of the dimethyl kainate derivatives **2–5**, from the commercially available kainic acid, are outlined in Fig. 2. The fully protected derivatives **2** and **3**, bearing 9-fluorenylmethoxycarbonyl (Fmoc) and *tert*-butoxycarbonyl (Boc) groups which are useful for *N*-protection in peptide synthesis, were synthesized in order to allow incorporation of photoaffinity labels in the isopropenyl side chain of kainic acid.[2]

The *N*-tritylated dimethyl kainate (**4**) was synthesized in order to produce the α-monoester **6** (see the Experimental part) which provides an alternative to similar monoesters of kainic acid developed to allow the synthesis of γ-amides of kainic acid.[2,3] This strategy was based on the reported selective saponification of *N*-tritylglutamic acid (Glu) dimethyl ester.[4]

Compound **5**, a dimethyl kainate derivative bearing the 4-methylaminobenzoyl group at the *N*-position, was projected as a key intermediate for the synthesis of the Methotrexate analogue **9** with kainic acid, a conformationally restricted analogue of Glu, replacing Glu itself. Methotrexate, a powerful inhibitor of dihydrofolate reductase, is being successfully used as an anti-cancer drug.[5] Moreover, compound **9** was of interest to us from the point of view that the folates show kainate-like neurotoxicity.[6]

Experimental

General. Capillary melting points were taken on a Buchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl Zeiss precision polarimeter. IR spectra were recorded on a Perkin-Elmer 457 grating spectrophotometer. Mass spectra were obtained on a Fisons VG 7070E mass spectrometer at an electron bombardment energy of 70 eV, using the direct inlet probe

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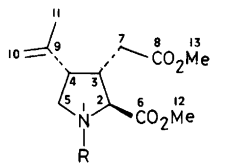
at a temperature of 250°C. The 400.13 and 100.62 MHz ^1H and ^{13}C NMR spectra were obtained at 298 K on a Bruker AM 400 WB instrument using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as an internal standard. The heteronuclear spin-echo experiment (SEFT) was performed using the gated decoupler method. The direct carbon–proton chemical correlations were established using the inverse heteronuclear experiment (HMQC). Both experiments were optimized for a one-bond ^1H–^{13}C coupling constant of 143 Hz. All starting materials and reagents used in the present work were purchased from Aldrich (Germany). Flash column chromatography (FCC) was performed on silica gel (Merck, Silica gel 60, 230–400 mesh) and TLC on precoated silica gel films (Merck, Silica gel $60F_{254}$, 0.2 mm on aluminium foil). The solvent systems used were: (A) BuOH–AcOH–H_2O (4:1:5, upper phase), (B) $CHCl_3$–MeOH (9:1), (C) PhMe–EtOAc (8:2), (D) PhMe–hexane–EtOAc (7:3:2), (E) PhMe–EtOAc (6:4). All compounds gave satisfactory spectral data.

Boc-kainic acid and Fmoc-kainic acid were prepared from kainic acid monohydrate following general reported procedures for the synthesis of Boc[7] and Fmoc[8] protected amino acids, respectively. Boc-kainic acid, obtained in 89% yield, had m.p. 150–151°C (lit.[9] 149–151°C), $[\alpha]_D^{25} = -31.7°$ (*c* 1, MeOH) [lit.[9] $[\alpha]_D^{22} = -26.4°$ (*c* 1, MeOH)] R_f (A) 0.79. Fmoc-Kai,



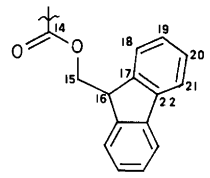
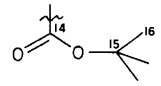
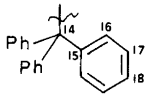
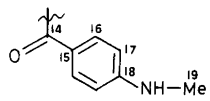
Compound	R-group
2	
3	
4	
5	

Fig. 1. Structures of the compounds Fmoc-Kai(Me)-OMe (**2**), Boc-Kai(Me)-OMe (**3**), Trt-Kai(Me)-OMe (**4**) and Mabz-Kai(Me)-OMe (**5**). C-atom identifications are indicated on the structures.

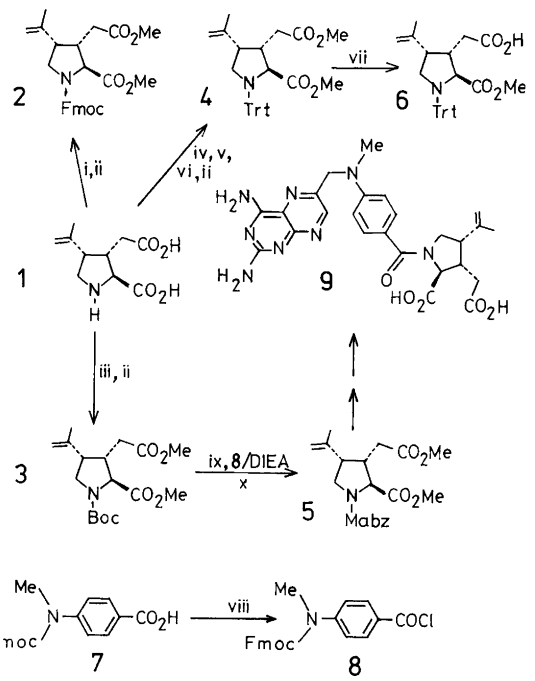


Fig. 2. Flow chart for the preparation of the kainate derivatives **2**–**6**. Reagents were as follows: (i) FmocCl–Na_2CO_3 in dioxane–H_2O, (ii) MeOH–TPP–DEAD, (iii) *tert*-butyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate–Et_3N in dioxane–H_2O, (iv) Me_3SiCl–Et_3N, (v) TrtCl, (vi) MeOH, (vii) 2 M NaOH in dioxane–MeOH, (viii) $SOCl_2$, (ix) TFA–DCM (1:1), (x) 20% Pip in DMF.

obtained in 92% yield, had m.p. 102–105°C, $[\alpha]_D^{25} = -37.2°$ (*c* 1, $CHCl_3$), R_f (A) 0.77.

Trt-kainic acid, isolated as the corresponding crystalline bisdiethylammonium salt (Trt-kainic acid·2DEA), was obtained in 93% yield using a reported procedure[10] which allows the preparation of Trt protected amino acids through one-pot pertrimethylsilylation of amino acids followed by N–Si bond scission with TrtCl. Trt-kainic acid·2DEA, recrystallised from acetone–hexane, had m.p. 146–148°C, $[\alpha]_D^{25} = -44.1°$ (*c* 2, MeOH), R_f (B) 0.09, IR (KBr): 2712, 2481, 1727, 1628, 1554, 1392, 748 and 717 cm^{-1}. Free Trt-kainic acid can be easily obtained from the corresponding bisDEA salt by partitioning the latter between diethyl ether and an ice-cold 5% aqueous citric acid solution. The aqueous layer is reextracted twice with diethyl ether and the combined ethereal layers washed twice with sat. NaCl solution, dried (Na_2SO_4) and evaporated to dryness under reduced pressure to provide an almost quantitative yield of Trt-kainic acid.

4-Methylaminobenzoic acid (Mabz) was converted into Fmoc-Mabz (**7**) using the same procedure as for the synthesis of Fmoc-kainic acid. Acid **7**, obtained in 78% yield had m.p. 184–185°C, R_f (B) 0.32, IR (Nujol): 1705 and 1680 cm^{-1}. *N*-Fmoc-4-methylaminobenzoyl chloride (Fmoc-Mabz-Cl, **8**) was prepared by treating acid **7** (1 g, 2.68 mmol) with $SOCl_2$ (0.87 ml, 12 mmol) in refluxing benzene (12 ml) for 2.5 h. Evaporation of the solvent,

trituration of the residue with hexane and recrystallisation from $CHCl_3$–petroleum ether (b.p. 40–60 °C) gave a 95% yield of **8**. Acid chloride **8** had m.p. 145–146 °C, IR (Nujol): 1780, 1740 and 1705 cm^{-1}.

General procedure for the synthesis of the N-*substituted kainic acid dimethyl esters* **2–4**. Dimethyl esters **2–4** were prepared using an adaptation of the Mitsunobu esterification procedure[11] as described below. Diethyl azodicarboxylate (DEAD) (0.8 ml, 5 mmol) was introduced dropwise into an ice-cooled solution of *N*-substituted kainic acid (2 mmol), MeOH (0.48 ml, 12 mmol) and triphenylphosphine (TPP) (1.30 g, 5 mmol) in anhydrous THF (10 ml). The resulting solution was kept at 0 °C for 15 min and then at room temperature for 30 min. The solution was again cooled to 0 °C and treated with additional portions of TPP (0.65 g, 2.5 mmol) and DEAD (0.4 ml, 2.5 mmol). The reaction mixture was kept at that temperature for 5 min and concentrated under reduced pressure. The resulting thick oils were subjected to FCC using as the eluant the solvent system C for **2** and **3**, and D for **4**, to give **2–4** as thick oils.

Dimethyl ester **2**, obtained in 91% yield, had $[\alpha]_D^{25}$ −27.9° (*c* 4, $CHCl_3$), R_f (C) 0.42. Anal. $C_{27}H_{29}NO_6$: C, H, N. IR (neat): 1740 and 1720 cm^{-1}. MS, *m/z* (% rel. int.): 463 (2, *M*), 432 (1.6, *M* − OMe), 404 (14, *M* − CO_2Me), 359 (9.4, *M* − OMe − CH_2CO_2Me), 240 (15, *M* − $CO_2CH_2C_{13}H_9$), 210 (13, 240 − CH_2O), 178 (100, $C_{13}H_8CH_2$), 165 (18, $C_{13}H_9$).

Dimethyl ester **3**, obtained in 90% yield had $[\alpha]_D^{25}$ −12.7° (*c* 2.1, $CHCl_3$), R_f (E) 0.52. Anal. $C_{17}H_{27}NO_6$: C, H, N. IR (neat): 1740 and 1700 cm^{-1}. MS, *m/z* (% rel. int.): 341 (0.2, *M*), 285 (25, *M* − C_4H_8), 282 (6, *M* − CO_2Me), 253 (21, *M* − C_4H_8 − CH_3OH), 240 (15, *M* − $CO_2C_4H_9$), 226 (32, *M* − C_4H_8 − CO_2Me), 210 (9, 240 − CH_2O), 182 (49, 240 − CO_2CH_2), 122 (21, 240 − CO_2CH_2 − HCO_2Me), 57 (100, C_4H_9).

Dimethyl ester **4**, obtained in 93% yield had $[\alpha]_D^{25}$ −13.5° (*c* 3.6, $CHCl_3$), R_f (D) 0.82. Anal. $C_{31}H_{33}NO_4$: C, H, N. IR (neat): 1740 and 1720 cm^{-1}. MS, *m/z* (% rel. int.): 483 (0.9, *M*), 424 (11, *M* − CO_2Me), 406 (5, *M* − Ph), 244 (100, TrtH), 240 (10, *M* − CPh_3), 165 (74, Ph_2C − H).

Treatment of derivative **4** with 2 M NaOH in dioxane–methanol (2:1) at room temperature for 8 h produced exclusively the monoester **6**. α-Monoester **6**, obtained as the corresponding crystalline DEA salt (from acetone–hexane) in 82% yield had m.p. 143–145 °C, $[\alpha]_D^{25}$ = +11.8° (*c* 2, MeOH), R_f (B) 0.51, IR (KBr): 2712, 2481, 1727, 1628, 1554, 1392, 748 and 711 cm^{-1}.

*(2S,3S,4S)-Methyl 2-methoxycarbonyl-1-[4-(*N*-methylamino)benzoyl]-4-(1-methylethenyl)-3-pyrrolidinylacetate (***5***).* Dimethyl ester **3** (0.62 g, 1.8 mmol) was dissolved in an ice-cooled solution consisting of 5 ml each of trifluoroacetic acid (TFA) and dichloromethane (DCM). The resulting solution was kept for 1 h at 0 °C and then taken to dryness under reduced pressure. The residue, containing the TFA salt of dimethyl kainate, was dissolved in dry $CHCl_3$ and the resulting reaction mixture was brought to neutrality, by the addition of diisopropylethylamine (DIEA). The acid chloride **8** was then added to the solution, the resulting reaction mixture was brought to neutrality by adding more DIEA and subsequently allowed to attain room temperature. The solvent was then evaporated off, and the residue was taken up in EtOAc, washed sequentially with 5% aqueous citric acid solution, H_2O, 5% aqueous $NaHCO_3$ solution and brine, dried and finally taken to dryness to provide 1.15 g of crude Fmoc-protected **5** [R_f (E) 0.23)]. This without any further purification was treated with a 20% solution (10 ml) of piperidine (Pip) in DCM at room temperature for 0.5 h. The resulting solution was concentrated to dryness, the residue leached with hexane and the supernatant liquid decanted. This was repeated and the solid residue then recrystallised from EtOAc–hexane to give 0.51 g (80%) of pure **5**. Derivative **5** had m.p. 137 °C, $[\alpha]_D^{25}$ = −23.5° (*c* 1, $CHCl_3$), R_f (B) 0.54. Anal. $C_{20}H_{26}N_2O_5$: C, H, N. IR (KBr): 3338, 1741, 1735, 1626 and 1611 cm^{-1}. MS, *m/z* (% rel. int.): 374 (14, *M*), 342 (1, *M* − MeOH), 274 (3), 240 (3, *M* − COC_6H_4NHMe), 134 (100, COC_6H_4NHMe), 106 (17, MeNHPh).

Results and discussion

Comparison of the NMR spectra of the compounds **2–5** shows immediately that the compounds **2** and **3** provide, in general, pairs of resonances corresponding to two possible conformers about the amide bond. The ratio of conformers was 52:48 for **2** and 57:43 for **3**. Such conformational differentiation is not possible in the case of the tritylated kainate **4**. While similar conformers might exist in the case of **5**, they are not in fact detectable. We ascribe this to the efficient conjugation between the aryl and carbonyl groups leading to reduced bond order in the amide bond as compared with that found in **2** and **3**.

The assignment of the proton spectra is based on analysis of signal intensities, coupling patterns and consideration of information from 2D experiments. The ^{13}C spectra were then completely assigned using spin-echo spectra together with the data from ^1H–^{13}C chemical shift correlations. The ^1H and ^{13}C NMR resonances observed for compounds **2–5** are given in Tables 1 and 2, respectively. Resonances associated with the major conformers of **2** and **3** are given in italics when identifiable. In the case of compound **2**, however, it was not always possible to distinguish between resonances originating from the pair of conformers.

In the case of the compound **4**, the ring current shielding effect of the trityl moiety is clearly seen on H-2, H-3, H-5a, H-5b, H-7a, H-7b, H-10a and H-10b of the kainate. In particular the effect on H-7b is extreme, this resonance being observed at 0.217 ppm. A similar trend is not observed for the corresponding ^{13}C resonances. This is reasonable since the ring current effect, being independent of the observed nuclide, contributes only a few

Table 1. ^{1}H chemical shifts (δ) for the compounds Fmoc-Kai(Me)-OMe (**2**), Boc-Kai(Me)-OMe (**3**), Trt-Kai(Me)-OMe (**4**) and Mabz-Kai(Me)-OMe (**5**). Resonances associated with the major conformer are italicised when identifiable.

H-atom	Compound	Resonances (coupling)
H-2	**2**	4.248, 4.188 (1 H, two d, *J* 3.31, 2.75 Hz)
	3	*4.071* (0.6 H, d, *J* 3.60 Hz), 4.163 (0.4 H, d, *J* 3.85 Hz)
	4	3.698 (1 H, s)
	5	4.438 (1 H, d, *J* 5.50 Hz)
H-3	**2**	3.11–2.97 (1 H, m)
	3	2.877–2.807 (1 H, m)
	4	2.322 (1 H, m, *J* 11.36, 6.04, 3.42 Hz)
	5	2.912 (1 H, deceptive quintet, *J* 6.65 Hz)
H-4	**2**	2.93–2.84 (1 H, m)
	3	3.084–2.981 (1 H, m)
	4	3.345 (1 H, m, *J* 11.47, 6.24, 6.04 Hz)
	5	3.123 (1 H, unresolved m)
H-5a	**2**	3.76–3.70 (1 H, m)
	3	*3.720* (0.6 H, dd, *J* 10.76, 7.25 Hz), 3.651 (0.4 H, dd, *J* 10.65, 7.33 Hz)
	4	3.411 (1 H, dd, *J* 8.45, 6.24 Hz)
	5	3.936 (1 H, unresolved m)
H-5b	**2**	3.544, 3.455 (1 H, two dd, *J* 10.57, 8.95 Hz)
	3	*3.475* (0.6 H, dd, *J* 10.76, 8.19 Hz), 3.423 (0.4 H, dd, *J* 10.65, 8.51 Hz)
	4	2.569 (1 H, dd, *J* 11.47, 8.45 Hz)
	5	3.936 (1 H, unresolved m)
H-7a	**2**	2.338 (1 H, dd, *J* 16.77, 5.13 Hz)
	3	*2.355* (0.6 H, dd, *J* 16.83, 5.54 Hz), 2.335 (0.4 H, dd, *J* 16.93, 5.92 Hz)
	4	1.431 (1 H, dd, *J* 17.53, 3.42 Hz)
	5	2.507 (1 H, dd, *J* 16.78, 6.52 Hz)
H-7b	**2**	2.232 (1 H, dd, *J* 16.77, 9.52 Hz)
	3	*2.272* (0.6 H, dd, *J* 16.83, 9.02 Hz), 2.310 (0.4 H, dd, *J* 16.93, 9.02 Hz)
	4	0.217 (1 H, dd, *J* 17.53, 11.36 Hz)
	5	2.395 (1 H, dd, *J* 16.78, 7.02 Hz)
H-10a	**2**	4.955, 4.944 (1 H, two d, *J* 0.80 Hz)
	3	4.923, 4.921 (1 H, two s)
	4	4.711 (1 H, d, *J* 1.30 Hz)
	5	4.864 (1 H, s)
H-10b	**2**	4.713, 4.687 (1 H, two s)
	3	4.70 (1 H, s)
	4	4.381 (1 H, d, *J* 1.30 Hz)
	5	4.622 (1 H, s)
H-11	**2**	1.709, 1.695 (3 H, two s)
	3	1.694 (3 H, s)
	4	1.585 (3 H, s)
	5	1.633 (3 H, s)
H-12	**2**[a]	3.705, 3.698 (3 H, two s)
	3	*3.706*, 3.691 (3 H, two s)
	4[a]	3.556 (3 H, s)
	5	3.695 (3 H, s)
H-13	**2**[a]	3.776, 3.722 (3 H, two s)
	3	3.769, *3.760* (3 H, two s)
	4[a]	3.659 (3 H, s)
	5	3.780 (3 H, s)
H-15ab	**2**	4.471, 4.447, 4.409, 4.378 (2 H, four dd, *J* 10.27, 6.86 Hz)
H-16	**2**	4.278, 4.181 (1 H, two t, *J* 6.86 Hz)

Table 1. Continued

H-atom	Compound	Resonances (coupling)
H-18	**2**	7.381–7.360 (2 H, br m)
H-19	**2**	7.347–7.268 (2 H, m)
H-20	**2**	7.605 (1 H, dd, *J* 11.06, 7.54 Hz), 7.538 (1 H, deceptive t, *J* 8.47 Hz)
H-21	**2**	7.767, 7.749 (2 H, two overlapping d, *J* 7.00 Hz)
H-16	**3**	1.472, 1.409 (9 H, two s)
H-16	**4**	7.584 (6 H, dd, *J* 7.32, 1.27 Hz)
H-17	**4**	7.248 (6 H, t, *J* 7.25 Hz)
H-18	**4**	7.151 (3 H, tt, *J* 7.30, 1.16 Hz)
H-16	**5**	6.555 (2 H, d, *J* 8.14 Hz)
H-17	**5**	7.509 (2 H, *J* 8.14 Hz)
H-18	**5**	2.854 (3 H, s)

[a] The chemical shifts of H-12 and H-13, the methoxy methyl hydrogens, may be interchanged.

percent to the total shielding of the ^{13}C nucleus. However, from the ^{1}H chemical shift data it is possible to conclude that the trityl group is under the kainate ring as normally drawn, i.e., the trityl group is on the same side of the ring as the isopropylidene and γ-methoxycarbonyl groups. In this compound too, the large geminal coupling for H-7 (17.53 Hz) and the small geminal coupling (8.45 Hz) for H-5 are noteworthy. H-3 and H-7b must have a *trans* relationship (11.36 Hz). The same applies to H-4 and H-5b (11.47 Hz) whereas the dihedral angle between the (C-2)–(H-2) and (C-3)–(H-3) bonds is roughly 90° [*J* (H-2,H-3) = 0]. The other three compounds do not show the same large variation in the vicinal coupling constants. This indicates that the conformation of the kainate ring of compound **4** deviates considerably from that of the other compounds

Table 2. ^{13}C chemical shifts (δ) for the compounds Fmoc-Kai(Me)-OMe (**2**), Boc-Kai(Me)-OMe (**3**), Trt-Kai(Me)-OMe (**4**) and Mabz-Kai(Me)-OMe (**5**). Resonances associated with the major conformer are italicised when identifiable.

	Compound number			
C-atom	**2**	**3**	**4**	**5**
C-2	*64.10*, 63.77	*64.07*, 63.72	65.10	64.00
C-3	*40.94*, 42.01	40.96, *41.93*	42.08	40.68
C-4	45.11, *46.06*	*45.30*, 46.05	44.96	47.02
C-5	*47.71*, 47.83	47.91, *47.64*	48.84	52.50
C-6(8)	172.33, 172.22, 172.13, 172.10	*172.71*, 172.52, *172.46*, 172.34	177.35 172.92	172.43, 172.28
C-7	32.90	33.01	30.78	33.08
C-9[a]	*141.31*, 141.17	141.48, *141.31*	141.72	123.16
C-10	113.52, *113.37*	*113.49*, 113.22	111.66	113.87
C-11	*22.46*, 22.44	22.40, *22.29*	22.96	22.12
C-12	51.91, 52.57	*51.86*, 52.23,	51.31,	51.85,
(C-13)		52.41	51.71	52.43
C-14	*154.83*, 154.45	154.38, *153.74*	76.61	169.83
C-15	*67.57*, 67.52	*80.30*, 80.26	143.15	141.93
C-16	47.32	28.45, *28.28*	129.62	111.13
C-17	144.12–143.72	–	127.56	129.71
C-18	125.31–124.95	–	126.35	151.24
C-19	128.24–127.02	–	–	30.27
C-20	128.24–127.02	–	–	–
C-21	*119.98*, 119.94	–	–	–
C-22[a]	141.40, *141.35*	–	–	–

[a] The chemical shifts of C-9 and C-22 may be interchanged for compound **2**.

presumably due to the closeness of the bulky trityl group and to changes in the geometry of the *N*-atom, pyramidal in **4**, but planar in **2**, **3** and **5**.

The chemical shifts of the kainate ring protons and carbons are markedly different for the two conformers of **2** and **3**, especially for the H-2 and H-5 protons which are in the vicinity of the amide bond. However, judging from the coupling constants, the change in conformation of the amide C–N partial double bond has only a small effect on the ring conformation.

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