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Kainic Acid as Conformationally Constrained Glutamic Acid Analog in Peptide Synthesis[1]

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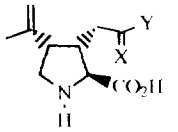
Abstract : A synthetic protocol is described which allows the incorporation of the γ-amide (Kan) and the γ-benzyl ester [Kai(Bzl)] and ether [Kol(Bzl)] of kainic acid (Kai) into peptide chains as exemplified with the synthesis of the substance P (SP) analogs [Kan^5]-SP_{5-11}, [Glp^5, Kan^6]-SP_{5-11}, [Kan^6]-SP_{6-11}, [Glp^5, $Kai(Bzl)^{11}$]-SP_{5-11} and [Glp^5, $Kol(Bzl)^{11}$]-SP_{5-11}.

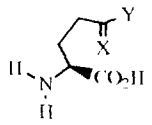
We have recently indicated that (-)-α-kainic acid (Kai, **1**), a natural product isolated from the marine alga *Digenea simplex* with powerful neuroexcitatory activity in the mammalian central nervous system, may be used as a conformationally restricted analog of glutamic acid (Glu) in biologically important compounds.[2] We now wish to report on the development of a methodology which allows the incorporation of derivatives of Kai modified at the γ-carboxy function, such as the γ-amide (Kan, **2**)[3] and the γ-benzyl ester [Kai(Bzl), **3**] and ether [Kol(Bzl), **4**], into peptide chains. The C-terminal hexa (**5**)- and heptapeptides (**6**) of the mammalian tachykinin substance P (SP, **7**) were used as models in the present work. Structure-activity relationship studies have shown that the biological activity of **7** mainly depends on the amino acid sequence of peptides **5** and **6**. Moreover, replacement of *N*-terminal glutamine (Gln) by pyroglutamic acid (Glp) and of C-terminal methionine (Met) by Glu(Bzl) in these peptides results in analogs with higher and equipotent respectively contractile activity to that of the parent peptide **7**.[4] Using the synthetic protocol described below, the SP analogs **8-12** were successfuly prepared. These SP analogs contain Kan and Glp in place of Gln at positions 6 or 5 and 5, respectively and Kai(Bzl) and Kol(Bzl) in place of Met at position 11. It should be pointed out that the Kai analog with a reduced γ-carboxy function, abbreviated here as Kol, represents a conformationally restricted analog of the hydroxyamino acid δ-hydroxynorvaline (Hnv).[5]

Incorporation of compounds **2** and **3** into peptide chains and the preparation of derivatives of ether **4** suitable for use in peptide synthesis required the use of Kai-intermediates selectively protected at one of the two available carboxy functions. Such an intermediate, namely lactone **13**, had been already described and successfully used for the preparation of a series of Kai γ-amides.[3] Lactone **13**, readily available through *N*-protection of Kai with the *tert*-butoxycarbonyl (Boc) group, followed by iodolactonization of the resulting Boc-Kai, served in our work as the key-intermediate for the

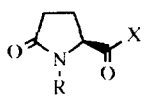
conversion of Kai to both Kai-NH_2, for use as the C-terminal amino acid, and Kan for use in the *N*-terminal region of the peptide chain. In both cases, the commercially available triphenylmethylamine ($TrtNH_2$) was employed as an anhydrous and convenient source of ammonia.[6]



Kai (1) : X=O, Y=OH
Kan (2) : X=O, Y=NH_2
Kai(Bzl) (3) : X=O, Y=OBzl
Kol(Bzl) (4) : X=H_2, Y=OBzl



Glu : X=O, Y=OH
Gln : X=O, Y=NH_2
Hnv: X=H_2, Y=OH



Glp : R=H, X=OH
Trt-Glp-OBt : R=Trt, X=OBt

Gln-Phe-Phe-Gly-Leu-Met-NH_2 **SP_{6-11} (5)**

Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **SP_{5-11} (6)**

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **SP (7)**

Kan-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **[Kan^5]-SP_{5-11} (8)**

Glp-Kan-Phe-Phe-Gly-Leu-Met-NH_2 **[Glp^5, Kan^6]-SP_{5-11} (9)**

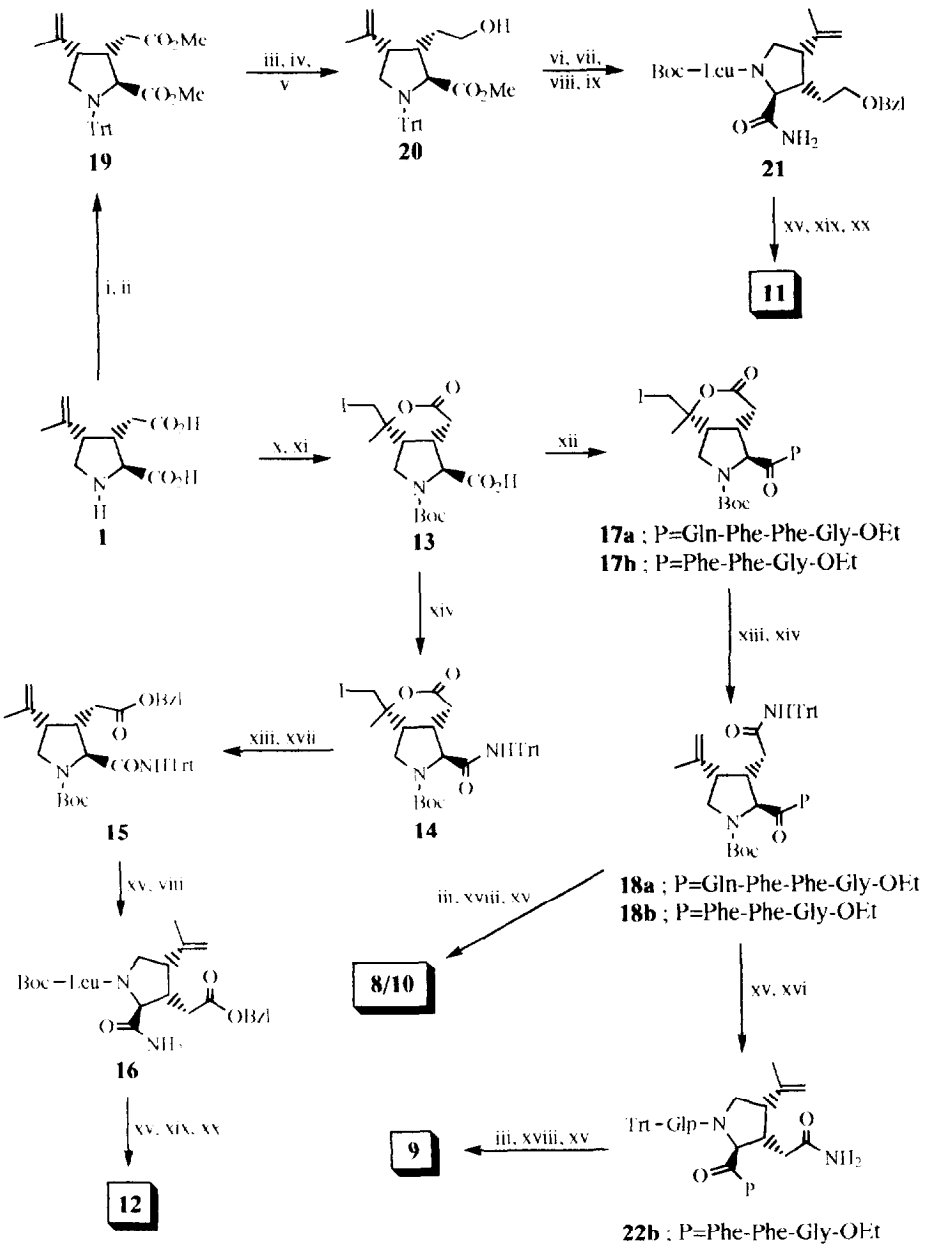
Kan-Phe-Phe-Gly-Leu-Met-NH_2 **[Kan^6]-SP_{6-11} (10)**

Glp-Gln-Phe-Phe-Gly-Leu-Kai(Bzl)-NH_2 **[Glp^5, $Kai(Bzl)^{11}$]-SP_{5-11} (11)**

Glp-Gln-Phe-Phe-Gly-Leu-Kol(Bzl)-NH_2 **[Glp^5, $Kol(Bzl)^{11}$]-SP_{5-11} (12)**

Thus, coupling lactone **13** with $TrtNH_2$ provided the corresponding tritylamide **14** which upon treatment with Zn dust in glacial (gl.) AcOH, followed by esterification with benzyl alcohol under Mitsunobu type reaction conditions, gave the γ-benzyl ester derivative **15** in 65% overall yield. It should be noted that, due to its bulk, the coupling of $TrtNH_2$ with **13** was most efficiently performed, in 83% yield, using the coupling agent PyBrOP.[7] Treatment of **15** with trifluoroacetic acid (TFA)/CH_2Cl_2 (3:1) resulted in simultaneous deprotection of both amine and amide functions. The resulting trifluoroacetate salt was routinely coupled with Boc-Leu.H_2O, using *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt), to give the dipeptide amide **16** in 70% yield. On the other hand, coupling iodolactone **13** with the tetrapeptide ester H-Gln-Phe-Phe-Gly-OEt or the tripeptide ester H-Phe-Phe-Gly-OEt, in presence of the coupling agent BOP,[8] produced the corresponding iodolactones **17a** and **b** in 84-95% yields. These amino components were obtained from TFA-mediated *N*-deprotection of the corresponding peptides Boc-Gln-Phe-Phe-Gly-OEt and Boc-Phe-Phe-Gly-OEt, available in our laboratory from previous studies. Treatment of lactones **17a** and **b** with Zn/gl. AcOH, followed by coupling with $TrtNH_2$ in the presence of PyBrOP gave good yields (65-75%) of fully protected Kan peptides **18a** and **b**, respectively.

N-tritylation of Kai, followed by Mitsunobu type esterification gave the diester **19**, which was regiospecifically saponified at the γ-carboxy function.[2] The resulting Kai derivative was reduced at the γ-carboxy function in an one-pot experiment, involving activation with DCC/HOBt followed by $NaBH_4$ reduction,[5] to give the Kol derivative **20** in 78% yield. Alcohol **20** was sequentially benzylated at the hydroxy function with BzlBr/NaH, *N*-detritylated with $TsOH.H_2O$ in refluxing isopropanol, coupled with Boc-Leu.H_2O using the system DCC/HOBt and finally ammonolysed to give the dipeptide amide **21** in



Reagents : i, (a) Me_3SiCl/Et_3N, (b) TrtCl, (c) MeOH; ii, $MeOH/Ph_3P/EtO_2CN{=}NCO_2Et$; iii, NaOH; iv, DCC/HOBt; v, $NaBH_4$; vi, NaH/BzlBr; vii, $TsOH.H_2O$; viii, Boc-Leu/DCC/HOBt; ix, NH_3; x, Boc_2O; xi, $I_2/KI/NaHCO_3$; xii, BOP/H-Gln-Phe-Phe-Gly-OEt or BOP/H-Phe-Phe-Gly-OEt; xiii, Zn/gl. AcOH; xiv, $TrtNH_2$/PyBrOP; xv, TFA; xvi, Trt-Glp-OBt; xvii, BzlOH/ Ph_3P/ $EtO_2CN{=}NCO_2Et$; xviii, BOP / H-Leu-Met-NH_2; xix, BOP / Trt-Glp-Gln-Phe-Phe-Gly-OH; xx, 20% TFA in CH_2Cl_2.

76% overall yield.

The dipeptides **16** and **21** and the penta- and tetrapeptides **18a** and **b**, were subsequently used for the preparation of the projected SP analogs **8-12** as follows. Saponification of **18a** and BOP-mediated coupling of the resulting pentapeptide acid with H-Leu-Met-NH_2 gave the corresponding heptapeptide amide in 90% yield, which upon treatment with TFA afforded the SP analog **8**. Similarly, saponification of tetrapeptide ester **18b**, followed by BOP-mediated coupling of the resulting tetrapeptide acid with H-Leu-Met-NH_2 gave the corresponding hexapeptide amide, in 84% yield, which was also deprotected with TFA to give the SP analog **10**. On the other hand, *N*-deprotection of **18b** with TFA and coupling of the resulting tetrapeptide ester with Trt-Glp-OBt, a useful compound for the introduction of Glp in peptide chains,[9] gave the pentapeptide **22b** in 82% yield. Saponification of **22b**, followed by BOP-mediated coupling with H-Leu-Met-NH_2 gave the corresponding heptapeptide in 84% yield which upon TFA-mediated deprotection provided the SP analog **9**. For the preparation of analogs **11** and **12**, the tetrapeptide Boc-Gln-Phe-Phe-Gly-OEt was *N*-deprotected with TFA and subsequently coupled with Trt-Glp-OBt to give Trt-Glp-Gln-Phe-Phe-Gly-OEt in 82% yield, which upon saponification provided the corresponding pentapeptide acid. BOP-mediated coupling of this acid with the trifluoroacetate salts of the dipeptides H-Leu-Kai(Bzl)-NH_2 and H-Leu-Kol(Bzl)-NH_2 gave the heptapeptides Trt-Glp-Gln-Phe-Phe-Gly-Leu-Kai(Bzl)- and Kol(Bzl)-NH_2, in 88 and 85 % yield, respectively. These amides, upon treatment with 20% TFA in CH_2Cl_2 provided the desired SP analogs **11** and **12**. Biological evaluation of the thus obtained SP analogs **8-12** is now in progress.

REFERENCES AND NOTES

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