SYNTHESIS AND CD CHARACTERISTICS OF POSITION 3 ANALOGUES OF ANTIARRHYTHMIC PEPTIDE*

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Received July 13, 1988
Accepted September 2, 1988

Six analogues of the antiarrhythmic peptide (Gly-Pro-Hyp-Gly-Ala-Gly), in which Hyp at position 3 has been replaced with other amino acids such as Gly, Ala, D-Ala, Ser, Thr and Lys have been synthesized. The compounds were obtained by a stepwise peptide coupling strategy in solution. A relationship between antiarrhythmic activities and CD spectra has been discussed.

Antiarrhythmic peptide (AAP)**, originally isolated from bovine atria[1] and identified as Gly-Pro-Hyp-Gly-Ala-Gly (ref.[2]) showed a protective effect against experimental drug induced arrhythmia in cultured myocardial cells of rats[1] and whole hearts of dogs, rats and mice[3]. In order to get peptides with enhanced antiarrhythmic activity, structure-activity relationship studies were carried out. Since Hyp residue present at position 3 is the only amino acid with any side chain functionality, an attempt was made to establish its role in the expression of biological activity. Following six analogues: [Lys^3]AAP, [Thr^3]AAP, [Ser^3]AAP, [Gly^3]AAP, [Ala^3]AAP and [D-Ala^3]AAP in which Hyp was replaced with either hydrophillic or neutral amino acids were synthesised and screened for their antiarrhythmic activity in rats. As reported earlier[4] two analogues [Lys^3]AAP and [Thr^3]AAP showed potent antiarrhythmic activity (delay in the onset of early arrhythmia) at 10 mg/kg i.v. and its protective effect (121% and 126% prolongation respectively) was much higher than that of AAP (14% prolongation). Out of the rest, [D-Ala^3]AAP was found to be completely inactive[4] whereas [Ser^3], [Ala^3] and [Gly^3]AAP were slightly more active[4] than AAP (41%, 30% and 44% prolongation respectively).

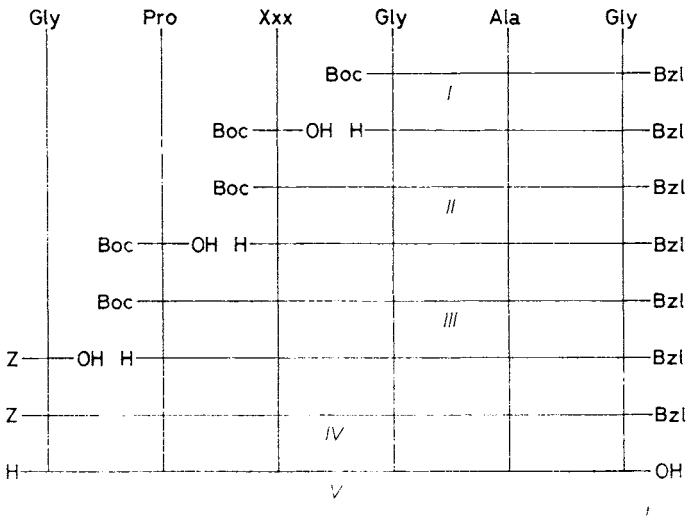
The synthesis of all peptides was achieved in solution by a stepwise manner (Scheme 1). For peptide bond formation mixed anhydride[5], DCC/HOBt[6] and symmetrical anhydride[7] procedures were used. The enantiomeric purity and amino acid composition of peptides were monitored by ^{13}C NMR at each step of the synthesis.

The CD spectrum of AAP in methanol (Fig. 1) is characterised by a maximum

* CDRI Communication No. 4297.

** Abbreviations follow the published recommendations (Eur. J. Biochem. *138*, 9 (1984)). In addition we used: NMM, N-methylmorpholine; DCC, N,N'-dicyclohexyl carbodiimide.

at 220 nm and a small minimum near 235 nm while a strong negative trend may be anticipated around 200 nm (Figs 1 and 2). When Hyp^3 residue is replaced with Lys, Ser, Thr, Ala and Gly, more or less similar CD patterns are observed although there are slight differences in both the intensities and positions of the 235 nm band.



In formulae *II–IV*: *a*, Xxx = Lys(Z) *b*, Xxx = Thr(Bzl) *c*, Xxx = Ser(Bzl)
d, Xxx = D-Ala *e*, Xxx = Ala *f*, Xxx = Gly

In formula *V*: *a*, Xxx = Lys *b*, Xxx = Thr *c*, Xxx = Ser *d*, Xxx = D-Ala
e, Xxx = Ala *f*, Xxx = Gly

SCHEME 1

The presence of a maximum at 220 nm and 215 nm in AAP and [Gly^3]AAP respectively may be atributed to Hyp^3 and Gly^3 residues (based on earlier CD studies on polypeptides related to collagen[8,9]). The CD curve for [D-Ala^3]AAP, however, is quite different from AAP as it exhibits a minimum near 230 nm and a large positive trend may be anticipated near 200 nm.

When the CD spectrum for position 3 analogues was measured in water (Figs 3 and 4) similar results were observed. The CD curves for AAP and its analogues *Va–Vc*, *Ve* and *Vf* are characterised by presence of a large negative band at 202 nm. [D-Ala^3]AAP, on the contrary, exhibits entirely different CD spectrum. Our results, thus, suggest that the analogues *Va–Vc*, *Ve* and *Vf* have similar distributions of φ, ψ angles in methanol and water and the insertion of D-Ala residue in position 3 perturbs the distribution resulting in the observed change in the spectral patterns.

Further it is interesting that the change in CD pattern for position 3 analogues of AAP is parallel to a certain extent with the changes in biological activity of these peptides. Specifically, analogues with a CD pattern similar to that of AAP have high biological activity and the one with perturbed CD spectrum has almost no activity. It appears from our CD data that the spatial structure of analogues *Va – Vc*, *Ve* and *Vf* are essentially similar to that of AAP and that the whole molecular shape of AAP might be contributing to its antiarrhythmic activity.

EXPERIMENTAL

The spectra were recorded on a Bruker WM 400 FT NMR spectrometer. Chemical shifts are expressed in δ-scale downfield (i) from TMS in DMSO, (ii) from $CDCl_3$ in $CDCl_3$ and (iii) from 1,4-dioxane in D_2O. Reverse phase HPLC analyses were performed on Waters HPLC system

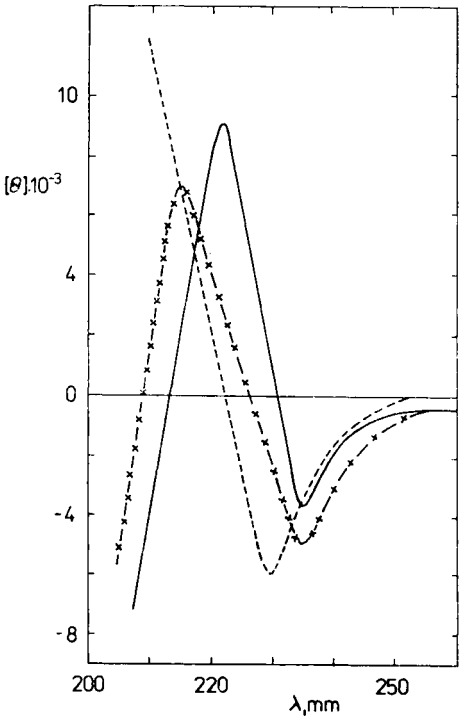


FIG. 1

CD spectra of AAP (———), [Gly^3]AAP (-x-x-) and [D-Ala^3]AAP (— — —) in MeOH

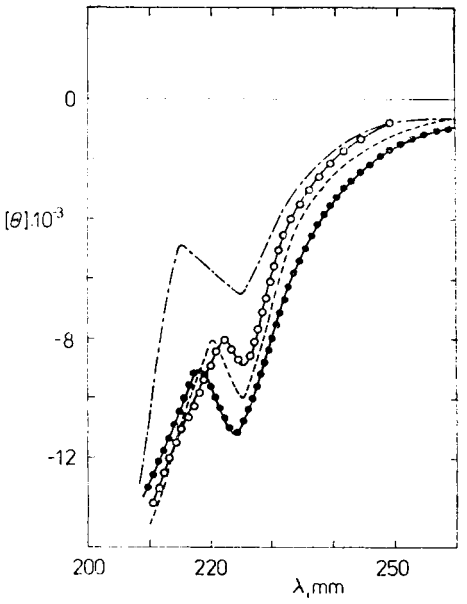


FIG. 2

CD spectra of [Ala^3]AAP (-·-·-·-), [Ser^3].AAP (-○-○-), [Lys^3]AAP (— — —) and [Thr^3]AAP (-●-●-) in MeOH

using C_{18} Novapak column and methanol — 0·025% trifluoroacetic acid as mobile phase. The detection was usually done at 212 nm. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. The circular dichroic spectra were recorded on a Jobin Yvon Mark III dichrograph. All spectra were recorded at room temperature in solutions of 0·25—1 mg/ml. The cell with path length of 0·05 cm was used.

Melting points were determined in glass capillaries and are uncorrected. Homogeneity of all the amino acid derivatives and peptides was established by TLC on silical gel G plates using the solvent systems: (A) 1-BuOH–AcOH–H_2O (4 : 1 : 5, upper layer); (B) $CHCl_3$–MeOH (9 : 1); (C) $CHCl_3$–MeOH (4 : 1).

Boc-Lys(Z)-Gly-Ala-Gly-OBzl (*IIa*)

The protected tripeptide *I* (ref.[10]) (0·78 g, 2 mmol) was treated with a 1 : 1 mixture of CH_2Cl_2 and TFA (6 ml) for 30 min at room temperature. The solvent was removed under reduced pressure and the residue treated with HCl/THF to give HCl.Gly-Ala-Gly-OBzl. THF was removed under reduced pressure and the residue dried over NaOH pelletes in vacuo. The hydrochloride salt was dissolved in dry DMF (7 ml), neutralised with NMM (0·22 ml, 2 mmol) at 0°C and the

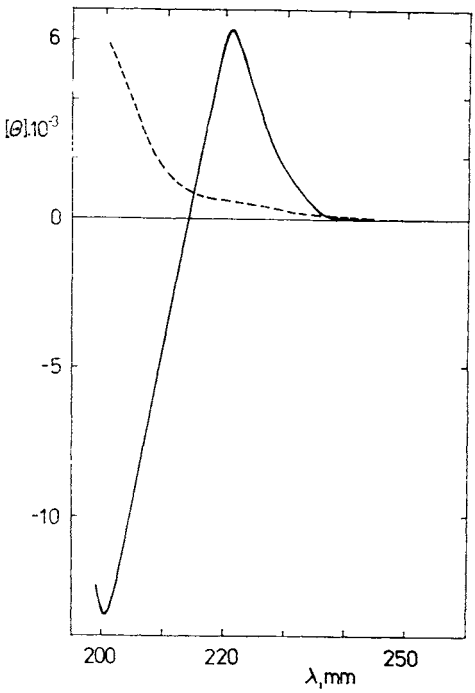


FIG. 3

CD spectra of AAP (———) and [D-Ala³]. .AAP (———) in water

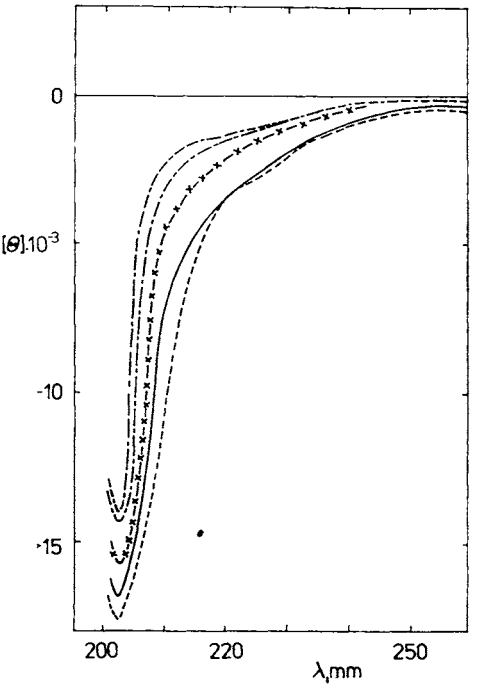


FIG. 4

CD spectra of [Ser³]AAP (—·—·—), [Gly³]. .AAP (–·–·–), [Ala³]AAP (–x–x–), [Lys³]. .AAP (———), [Thr³]AAP (— — —) in water

TABLE I

Properties of compounds *IIa*—*Vf*

Compound Yield, %	$[\alpha]_D^{25}$, ° (*c*)[a] M.p., deg	*k'* (system[b]) R_F (system)	Formula (M.w.)	Calculated/Found		
				% C	% H	% N
IIa	−17·1 (0·35)	9·3 (11 : 9)	$C_{33}H_{45}N_5O_9$	60·44	6·92	10·68
82	90	0·56 (A)	(655·8)	60·59	6·94	10·74
IIIa	−48·5 (0·35)	10 (3 : 2)	$C_{38}H_{52}N_6O_{10}$	60·62	6·96	11·16
70	134	0·42 (A)	(752·9)	60·72	6·90	11·25
IVa	−37·9 (0·3)	11·5 (13 : 7)	$C_{43}H_{53}N_7O_{11}$	61·20	6·33	11·62
85	167	0·41 (B)	(843·9)	61·31	6·20	11·56
Va	−65 (0·13)	14·1 (2 : 3)	$C_{20}H_{35}N_7O_7$	49·47	7·27	20·19
80	—[c]	0·34 (B)	(485·5)	49·60	7·31	20·32
IIb	−10·9 (0·55)	7·5 (11 : 9)	$C_{30}H_{40}N_4O_8$	61·63	6·90	9·58
87	112	0·50 (A)	(584·7)	61·61	6·89	9·61
IIIb	−39·6 (0·58)	8·3 (3 : 2)	$C_{35}H_{47}N_5O_9$	61·66	6·95	10·27
83	129	0·42 (A)	(681·8)	61·70	6·87	10·39
IVb	−41·2 (0·4)	10·2 (13 : 7)	$C_{40}H_{48}N_6O_{10}$	62·16	6·26	10·87
85	170	0·45 (B)	(722·9)	62·52	6·30	10·92
Vb	−54·5 (0·12)	12·1 (2 : 3)	$C_{18}H_{30}N_6O_8$	47·16	6·60	18·33
78	—[c]	0·39 (B)	(458·5)	47·29	6·47	18·47
IIc	−11·4 (0·35)	6·8 (11 : 9)	$C_{29}H_{38}N_4O_8$	61·04	6·71	9·82
80	126	0·54 (A)	(570·6)	61·17	6·76	9·97
IIIc	−37·1 (0·13)	7·8 (3 : 2)	$C_{35}H_{45}N_5O_9$	61·84	6·67	10·30
80	119	0·41 (A)	(679·8)	61·79	6·70	10·41
IVc	−89·8 (0·5)	9·8 (13 : 7)	$C_{39}H_{46}N_6O_{10}$	61·74	6·11	11·08
87	157	0·56 (B)	(758·8)	61·89	6·14	10·94
Vc	46 (0·12)	11·8 (5 : 6)	$C_{17}H_{28}N_6O_8$	45·94	6·35	18·91
78	—[c]	0·39 (B)	(444·4)	46·10	6·19	17·89
IId	−3·8 (0·26)	5·5 (11 : 9)	$C_{22}H_{32}N_4O_7$	56·89	6·94	12·06
90	145	0·58 (A)	(464·5)	56·72	6·71	12·15
IIId	−26·2 (0·35)	6·2 (3 : 2)	$C_{27}H_{39}N_5O_8$	57·75	7·00	12·47
93	129	0·43 (A)	(561·6)	57·64	6·81	12·30
IVd	−34·2 (0·35)	8·7 (13 : 7)	$C_{32}H_{40}N_6O_9$	58·89	6·18	12·88
87	112—114	0·48 (B)	(652·7)	58·71	6·00	12·72
Vd	−27·1 (0·3)	10·2 (2 : 3)	$C_{17}H_{28}N_6O_7$	47·66	6·59	19·62
82	—[c]	0·42 (B)	(428·4)	47·51	6·47	19·53
IIe	−14·1 (0·35)	5·2 (11 : 9)	$C_{22}H_{32}N_4O_7$	56·89	6·94	12·06
78·2	143	0·6 (A)	(464·5)	56·97	6·76	12·19

TABLE I
(*Continued*)

Compound Yield, %	$[\alpha]_D^{25}$, ° (*c*)[a] M.p., deg	*k'* (system[b]) R_F (system)	Formula (M.w.)	Calculated/Found		
				% C	% H	% N
IIIe	−30·1 (0·35)	6 (3 : 2)	$C_{27}H_{39}N_5O_8$	57·75	7·00	12·47
87	156	0·42 (A)	(561·6)	57·89	6·78	12·32
IVe	−40 (0·35)	8·4 (13 : 7)	$C_{32}H_{40}N_6O_9$	58·89	6·18	12·88
88	181	0·54 (B)	(652·7)	58·72	6·27	12·96
Ve	−22·5 (0·15)	9·9 (2 : 3)	$C_{17}H_{28}N_6O_7$	47·66	6·59	19·62
80	—[c]	0·41 (B)	(428·4)	47·55	6·51	19·79
IIf	−10·1 (0·35)	4·8 (10 : 9)	$C_{21}H_{30}N_4O_7$	56·00	6·71	12·44
86	112	0·52 (A)	(450·5)	56·12	6·79	12·60
IIIf	−18·4 (0·35)	5·7 (3 : 2)	$C_{26}H_{37}N_5O_8$	57·03	6·81	12·79
83	127	0·45 (A)	(547·6)	57·11	6·81	12·65
IVf	−28·7 (0·35)	7·1 (13 : 7)	$C_{31}H_{38}N_6O_9$	58·30	6·00	13·16
80	148—50	0·43 (B)	(638·7)	59·42	5·67	13·21
Vf	−20·8 (0·15)	8·5 (2 : 3)	$C_{16}H_{24}N_6O_7$	46·60	5·87	20·38
89	—[c]	0·43 (A)	(412·4)	46·76	5·92	20·25

[a] In MeOH; [b] ratio of MeOH–0·05% TFA mixture; [c] amorphous.

solution mixed with Boc-Lys(Z) (ref.[11]) (0·72 g, 2 mmol) and HOBt (0·29 g, 2·2 mmol) in CH_2Cl_2 (10 ml). DCC (0·45 g, 2·2 mmol) in CH_2Cl_2 (5 ml) was added to the above mixture at 0°C under stirring, the reaction mixture was stirred for 2 h at 0°C and left overnight at room temperature. DCU was filtered off and washed with CH_2Cl_2 (8 ml). The filtrate was washed successively with 5% aq. citric acid, water, 5% Na_2CO_3 and finally with water untill neutral. The organic layer was dried (Na_2SO_4) and taken to dryness in vacuo. The residue was crystallised from EtOAc–ether to get *IIa*; yield 1 g (82%). ^{13}C NMR ($CDCl_3$): 41·70, 44·12 (2 C^{α}-Lys), 25·69, 25·73, 25·82 (C^{β}, C^{γ}, C^{δ}, Lys), 31·92 (C^{ε}-Lys). For analytical data see Table I.

Boc-Pro-Lys(Z)-Gly-Ala-Gly-OBzl (*IIIa*)

The protected tetrapeptide *IIa* (0·5 g, 0·76 mmol) was treated with formic acid (10 ml) for 5 h at room temperature. The solvent was removed under reduced pressure and the residue treated with HCl/THF at 0°C to give HCl. Lys(Z)-Gly-Ala-Gly-OBzl, THF was removed under reduced pressure and the residue dried over NaOH pellets in vacuo. The hydrochloride salt of the tetrapeptide thus obtained, was neutralised with NMM (0·885 ml, 0·76 mmol) in dry DMF (4 ml) and treated with Boc-Pro-ONp (ref.[12]) (0·28 g, 0·83 mmol) in the presence of HOBt (0·12 g, 0·73 mmol). The reaction mixture was stirred for 1 h at −10°C and for 10 h at room temperature. The solvent was evaporated to dryness, the residue taken up in ethyl acetate and washed with 5% citric acid, water, 5% $NaHCO_3$ and finally with water till neutral. The organic layer was dried over Na_2SO_4 and concentrated. The residue was crystallised from EtOAc–ether, yield 0·4 g.

^{13}C NMR (DMSO): 41·82, 43·99 (2 C^{α}-Gly), 48·79 (C^{α}-Ala), 18·01 (C^{β}-Ala), 51·81 (C^{α}-Lys), 25·51, 25·69, 28·91 (C^{β}, C^{γ}, C^{δ}-Lys), 31·86 (C^{ε}-Lys), 59·31 (C^{α}-Pro), 30·11 (C^{β}-Pro), 23·06 (C^{γ}-Pro), 45·91 (C^{δ}-Pro). The analytical data see Table I.

Z-Gly-Pro-Lys(Z)-Gly-Ala-Gly-OBzl (*IVa*)

The protected pentapeptide *IIIa* (0·3 g, 0·4 mmol) was dissolved in 5 ml of formic acid and the clear solution was left at room temperature for 6 h. The solvent was removed under reduced pressure and the residue treated with HCl/THF at 0°C to give HCl.Pro-Lys(Z)-Gly-Ala-Gly--OBzl. The residue after precipitation from methanol–ether was filtered and dried over NaOH pellets in vacuo. It was dissolved in dry DMF (3 ml), treated with NMM (0·04 ml, 0·4 mmol) and coupled with the symmetrical anhydride of Z-Gly[7] prepared from Z-Gly (0·5 g, 2·4 mmol) and DCC (0·25 g, 1·2 mmol) in CH_2Cl_2 (10 ml). The reaction mixture was stirred for 5 h at room temperature, the solvent was evaporated, the residue taken up in EtOAc and washed with 5% citric acid, water, 5% $NaHCO_3$ and finally with water till neutral. The organic layer was dried over Na_2SO_4 and concentrated. The residue was crystallised from EtOAc, yield 0·28 g. ^{13}C NMR (DMSO): 41·89, 43·51, 44·01 (3 C^{α}-Gly), 48·61 (C^{α}-Ala), 17·91 (C^{β}-Ala), 51·72 (C^{α}-Lys), 25·43, 25·64 28·83 (C^{β}, C^{γ}, C^{δ}-Lys), 31·91 (C^{ε}-Lys), 59·28 (C^{α}-Pro), 30·04 (C^{β}-Pro), 22·94 (C^{γ}-Pro), 45·82 (C^{δ}-Pro). For analytical data see Table I.

Gly-Pro-Lys-Gly-Ala-Gly (*Va*)

The protected hexapeptide *IVa* (0·2 g, 0·23 mmol) was dissolved in methanol (15 ml) and subjected to catalytic hydrogenation over 10% Pd/C in the presence of formic acid (0·3 ml) for 3 h. After removing the catalyst by filtration, methanol was evaporated under reduced pressure and the residue dried in a vacuum desiccator over P_2O_5 and NaOH. The residue was dissolved in methanol and passed through a column of IR 45 resin. The solvent was removed in vacuo and the residue triturated with dry ether to get a white solid. The product so obtained was filtered and precipitated twice from methanol–ether to give *Va*. Yield 0·088 g. ^{13}C NMR (D_2O): 42·49, 43·46, 44·21 (3 C^{α}-Gly), 58·96 (C^{α}-Pro), 29·01 (C^{β}-Pro), 24·61 (C^{γ}-Pro), 47·69 (C^{δ}-Pro), 49·44 (C^{α}-Ala), 16·72 (C^{β}-Ala), 52·13 (C^{α}-Lys), 26·01, 26·13, 28·98 (C^{β}, C^{γ}, C^{δ}-Lys), 32·78 (C^{ε}-Lys). For analytical data see Table I.

Boc-Thr(Bzl)-Gly-Ala-Gly-OBzl (*IIb*)

The protected tripeptide *I* (0·5 g, 1·2 mm) was treated with a 1 : 1 mixture of CH_2Cl_2 and TFA (5 ml) for 30 min at room temperature. The solvent was removed under reduced pressure and the residue treated with HCl/THF to give HCl.Gly-Ala-Gly-OBzl. THF was removed under reduced pressure and the residue dried over NaOH pellets in vacuo. The hydrochloride of the tripeptide was neutralized with NMM (0·14 ml, 1·2 mmol) in dry DMF (5 ml) and treated with the mixed anhydride prepared from Boc-Thr(Bzl) (0·43 g, 1·3 mmol) in the presence of NMM (0·15 ml, 1·3 mmol) and isobutylchloroformate (0·18 ml, 1·3 mmol) in dry THF (8 ml) at −15°C. The reaction mixture was stirred for 2 h at −10°C and then kept in the refrigerator overnight. The reaction was worked up in a similar manner as described for *IIa*. The residue was crystallised from EtOAc–ether, yield 0·64 g. ^{13}C NMR (DMSO): 40·66, 42·07 (2 C^{α}-Gly), 47·90 (C^{α}-Ala), 17·98 (C^{β}-Ala), 59·46 (C^{α}-Thr), 65·72 (C^{β}-Thr), 16·35 (C^{γ}-Thr). For analytical data see Table I.

Compounds *IIc*—*IIf*, *IIIb*—*IIIf*, *IVb*—*IVf*, *Vb*—*Vf*

These compounds were prepared in the same way as compounds *IIa*, *IIIa*, *IVa*, and *Va* and their analytical data are given in the Table I. Below only ^{13}C NMR spectral data are given.

Boc-Pro-Thr(Bzl)-Gly-Ala-Gly-OBzl (*IIIb*) (DMSO): 41·28, 44·21 (2 C^{α}-Gly), 48·51 (C^{α}-Ala), 18·09 (C^{β}-Ala), 59·62 (C^{α}-Thr), 67·01 (C^{β}-Thr), 59·10 (C^{α}-Pro), 30·01 (C^{β}-Pro), 23·12 (C^{γ}-Pro), 45·89 (C^{δ}-Pro).

Z-Gly-Pro-Thr(Bzl)-Gly-Ala-Gly-OBzl (*IVb*) (DMSO): 41·13, 43·01, 44·10 (3 C^{α}-Gly), 48·61 (C^{α}-Ala), 17·93 (C^{β}-Ala), 59·50 (C^{α}-Thr), 67·10 (C^{β}-Thr), 59·01 (C^{α}-Pro), 20·11 (C^{β}-Pro), 23·01 (C^{γ}-Pro), 45·45 (C^{δ}-Pro).

Gly-Pro-Thr-Gly-Ala-Gly (*Vb*) (D_2O): 42·61, 43·29, 44·04 (3 C^{α}-Gly), 59·01 (C^{α}-Pro), 28·56 (C^{β}-Pro), 24·21 (C^{γ}-Pro), 47·34 (C^{δ}-Pro), 49·32 (C^{α}-Ala), 16·91 (C^{β}-Ala), 60·10 (C^{α}-Thr), 67·85 (C^{β}-Thr).

Boc-Ser(Bzl)-Gly-Ala-Gly-OBzl (*IIc*) (DMSO): 41·21, 42·14 (2 C^{α}-Gly), 47·59 (C^{α}-Ala), 17·87 (C^{β}-Ala), 55·71 (C^{α}-Ser), 61·80 (C^{β}-Ser).

Boc-Pro-Ser(Bzl)-Gly-Ala-OBzl (*IIIc*) (DMSO): 46·67, 40·12 (2 C^{α}-Gly), 47·92 (C^{α}-Ala), 18·03 (C^{β}-Ala), 55·92 (C^{α}-Ser), 61·77 (C^{β}-Ser), 59·32 (C^{α}-Pro), 30·19 (C^{β}-Pro), 23·03 (C^{γ}-Pro), 45·99 (C^{δ}-Pro).

Z-Gly-Pro-Ser(Bzl)-Gly-Ala-Gly-OBzl (*IVc*) (DMSO): 40·91, 42·23, 43·86 (3 C^{α}-Gly), 47·81 (C^{α}-Ala), 17·91 (C^{β}-Ala), 55·98 (C^{α}-Ser), 61·65 (C^{β}-Ser), 59·51 (C^{α}-Pro), 30·23 (C^{β}-Pro), 23·13 (C^{γ}-Pro), 45·89 (C^{δ}-Pro).

Gly-Pro-Ser-Gly-Ala-Gly (*Vc*) (D_2O): 43·01, 43·81, 44·19 (3 C^{α}-Gly), 59·01 (C^{α}-Pro), 28·76 (C^{β}-Pro), 24·26 (C^{γ}-Pro), 47·80 (C^{δ}-Pro), 49·20 (C^{α}-Ala), 16·89 (C^{β}-Ala), 56·21 (C^{α}-Ser), 62·15 (C^{β}-Ser).

Boc-D-Ala-Gly-Ala-Gly-OBzl (*IId*) ($CDCl_3$): 41·46, 43·31 (2 C^{α}-Gly), 49·38, 49·49 (2 C^{α}-Ala), 17·21, 17·78 (2 C^{β}-Ala).

Boc-Pro-D-Ala-Gly-Ala-Gly-OBzl (*IIId*) (DMSO): 40·66, 42·00 (2 C^{α}-Gly), 47·46, 47·87 (2 C^{α}-Ala), 17·93, 18·04 (2 C^{β}-Ala), 59·67 (C^{α}-Pro), 33·21 (C^{β}-Pro), 24·31 (C^{γ}-Pro), 46·43 (C^{δ}-Pro).

Z-Gly-Pro-D-Ala-Gly-Ala-Gly-OBzl (*IVd*) (DMSO): 40·91, 42·01, 43·56 (3 C^{α}-Gly), 47·51, 47·92 (2 C^{α}-Ala), 17·84, 18·11 (2 C^{β}-Ala), 59·82 (C^{α}-Pro), 33·12 (C^{β}-Pro), 24·11 (C^{γ}-Pro), 46·39 (C^{δ}-Pro).

Gly-Pro-D-Ala-Gly-Ala-Gly (*Vd*) (D_2O): 42·38, 43·20, 40·50 (3 C^{α}-Gly), 58·79 (C^{α}-Pro), 28·99 (C^{β}-Pro), 24·25 (C^{γ}-Pro), 47·35 (C^{δ}-Pro), 49·39, 49·53 (2 C^{α}-Ala), 16·91, 17·01 (2 C^{β}-Ala).

Boc-Ala-Gly-Ala-Gly-OBzl (*IIe*) ($CDCl_3$): 41·43, 43·29 (2 C^{α}-Gly), 49·28, 49·60 (2 C^{α}-Ala), 17·81, 18·01 (2 C^{β}-Ala).

Boc-Pro-Ala-Gly-Ala-Gly-OBzl (*IIIe*) (DMSO): 40·69, 42·09 (2 C^{α}-Gly), 47·51, 47·91 (2 C^{α}--Ala), 17·89, 18·10 (2 C^{β}-Ala), 59·61 (C^{α}-Pro), 33·18 (C^{β}-Pro), 24·29 (C^{γ}-Pro), 46·39 (C^{δ}-Pro).

Z-Gly-Pro-Ala-Gly-Ala-Gly-OBzl (*IVe*) (DMSO): 40·31, 42·11, 43·04 (3 C^{α}-Gly), 47·49, 47·87 (2 C^{α}-Ala), 17·93, 18·12 (2 C^{β}-Ala), 59·45 (C^{α}-Pro), 33·08 (C^{β}-Pro), 24·26 (C^{γ}-Pro), 46·37 (C^{δ}-Pro).

Gly-Pro-Ala-Gly-Ala-Gly (*Ve*) (D_2O): 42·41, 34·29, 44·00 (3 C^{α}-Gly), 58·81 (C^{α}-Pro), 28·15 (C^{β}-Pro), 24·19 (C^{γ}-Pro), 47·29 (C^{δ}-Pro), 49·47, 49·61 (2 C^{α}-Ala), 16·72, 16·91 (2 C^{β}-Ala).

Boc-Gly-Gly-Ala-Gly-OBzl (*IIf*) ($CDCl_3$): 41·23, 42·63, 43·12 (3 C^{α}-Gly), 49·39 (C^{α}-Ala), 17·11 (C^{β}-Ala).

Boc-Pro-Gly-Gly-Ala-Gly-OBzl (*IIIf*) (DMSO): 40·51, 42·09, 43·09 (3 C^{α}-Gly), 47·56 (C^{α}-Ala), 17·41 (C^{β}-Ala), 59·61 (C^{α}-Pro), 33·29 (C^{β}-Pro), 24·25 (C^{γ}-Pro), 46·39 (C^{δ}-Pro).

Z-Gly-Pro-Gly-Gly-Ala-Gly-OBzl (*IVf*) (DMSO): 40·48, 42·11, 43·14, 43·50 (4 C^{α}-Gly), 47·65 (C^{α}-Ala), 17·50 (C^{β}-Ala), 59·59 (C^{α}-Pro), 33·39 (C^{β}-Pro), 24·32 (C^{γ}-Pro), 46·31 (C^{δ}-Pro).

Gly-Pro-Gly-Gly-Ala-Gly (*Vf*) (D_2O): 42·38, 42·54, 43·11, 44·19 (4 C^{α}-Gly), 58·70 (C^{α}-Pro), 28·23 (C^{β}-Pro), 24·25 (C^{γ}-Pro), 47·34 (C^{δ}-Pro), 49·50 (C^{α}-Ala), 16·87 (C^{β}-Ala).

The author is grateful to Dr M. M. Dhar and Dr K. B. Mathur for their keen interest in this work. Technical assistance provided by Miss Deopali Tripathi is also gratefully acknowledged.

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