58. Synthesis of Angiotensins, Bradykinins and Substance P Octapeptides in which the Residues Phe and Tyr have been Replaced with Car and of [Car[1], Leu[5]]-Enkephalin[1])

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Summary

Thirteen peptides, analogues of bradykinin (BK), enkephalin, Substance P (SP) and [Sar[1]]-angiotensin II ([Sar[1]]-AT_{II}) have been synthesized by the solid-phase method. In all these peptides the residue Phe and Tyr were substituted with the boron-containing amino-acid L-*o*-carboranylalanine (Car). The purity and identity of the peptides were established by TLC., cellulose-electrophoresis and amino acid analysis.

Introduction. – The synthesis of the new amino-acid L-*o*-carboranylalanine (Car) was primarily intended to develop an analogue of phenylalanine (Phe), containing in its side-chain a maximal concentration of the boron isotope ^{10}B [1–3]. This amino acid (Car) could perhaps be inserted in the sequence of peptide hormones or of antibodies in order to enable ^{10}B to be carried and accumulated specifically in tumoral tissues. Capture of a slow neutron by the ^{10}B isotope will disintegrate with 2.3 MeV

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Abbreviations: AT_{II}, angiotensin II; BK, bradykinin; Enk, enkephalin; SP, substance P; BAW, 1-butanol/acetic acid/water 10:2:3; BAWP, 1-butanol/acetic acid/water/pyridine 30:6:20:12; BIWCl, 1-butanol/2-propanol/water/monochloroacetic acid 65:15:20:3; BHA, benzhydrylamine; Boc, butyloxycarbonyl; Bzl, benzyl ether; Car, L-*o*-carboranylalanine; DCC, dicyclohexylcarbodiimide; DMF, *N,N*-dimethyl formamide; Pra, L-properglyglycine; TEA, triethyl amine; TLC., thin-layer chromatography; Tos, *p*-toluenesulfonyl; DEA: diisopropylethylamine; TFA: trifluoroacetic acid; HF: hydrogen fluoride.

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this nucleus into a ^{4}He and a ^{7}Li nucleus which are able to kill an adjacent tumor cell. Boron compounds have already been introduced in certain organs and tissues of animals [4], antibodies [5] and viruses [6] for basic studies of this technique and for autoradiographic purposes [7]. Treatment of neoplastic diseases with boron compounds and slow neutrons have also been attempted on humans [8]. Our early studies to demonstrate the structural similarity between Phe and Car were carried out on the enzyme α-chymotrypsin using the tripeptide inhibitor Z-Ala-Ala-P*, where P* is the amino acid containing an aromatic nucleus in the side-chain and Z the benzyloxycarbonyl group. Z-Ala-Ala-Car-OH was 3 times more potent than Z-Ala-Ala-Phe-OH in inhibiting α-chymotrypsin [9]. Such high affinity of the Car-containing tripeptide was taken as an indication that the globular side-chain of Car fits and binds strongly to the hydrophobic pocket of α-chymotrypsin [10]. Extension of this approach to a small peptide hormone was achieved by the synthesis of a Car-containing enkephalin analogue [11]. The binding of this [Car^4, Leu^5]-enkephalin to opiate receptors in rat brain extracts was 3 times higher than that of the natural peptide [Leu^5]-enkephalin [11].

Based on these experimental findings suggesting fairly good similarity between Phe and Car, we decided to synthesize several peptide hormones to find out if Car could be of use in structure-activity studies of other peptides hormones. For this purpose, peptides were chosen which contain aromatic amino-acids such as Phe or Tyr in their natural sequences.

We mainly describe here the methods and the procedures used for synthesis, purification and analyses of 12 analogues of bradykinin (BK), Substance P (SP), angiotensin (AT) and of [Car^4, Leu^5]-enkephalin *(Table 2)*. The chemical characteristics of all these peptides will be given in detail herein, the biological activities in forthcoming publications [12] [13].

Results and discussion. – The insertion of Car in peptide chains to replace the natural residues Phe or Tyr brings about certain changes of the physical-chemical properties of the resulting peptide analogues, and the synthesis of such analogues therefore requires modification of the solid-phase method described by *Merrifield* [14]. We have coupled all amino acids but Car by the symmetrical anhydride method [15] since the formation of the symmetrical anhydride of butyloxycarbonyl (Boc) protected Car did not run to completion. This is probably due to steric hindrance between the large carborane side-chains of Car. For this reason the coupling of Boc-Car to the peptide chain was performed by the classic DCC[1]) method, without hydroxy-benzotriazole [16]. The higher lipophilicity of the carborane side-chain of Car with respect to those of Phe and Tyr [17] brought about a decrease of the hydrophilic properties of the whole peptide and therefore a decrease of solubility in water. This was a disadvantage when the compound had to be tested in biological assays, because it had to be dissolved in aqueous physiological mediums. However the addition of a basic amino acid, such as Lys or Arg improved the water solubility of our peptides. In some instances, after hydrogen fluoride cleavage of the peptide-resin ester, the crude peptide was almost insoluble in 0.2 M acetic acid and could not be applied to a Sephadex-G-15 column for a first purification. In these cases we used a Sephadex-LH-20 column and methanol because all Car-containing peptides **1–13**

were soluble in methanol. Thereafter the peptides were better soluble in aqueous mediums and further purified on Sephadex G-15 and, if necessary, with partition chromatography [18].

The higher lipophilicity of the Car-peptides was also reflected by the increased Rf values on TLC. with respect on the corresponding natural peptides. The 2 octapeptides analogues of SP (compounds **10** and **11** of *Table 2*) contain Met-amide at the C-terminal; these peptides, susceptible to oxidation of their sulfur atom in Met, were tested to exclude this possibility. The peptides were first treated with thioglycol to reduce the possibly oxidized peptides but no new products appeared on TLC. Then small amounts of the pure peptides were oxidized with hydrogenperoxide and new products with lower Rf values on TLC. were detected, owing to the formation of Met-sulfoxides. Therefore no oxidation occurred during synthesis and purification of the SP analogues.

All the analogues synthesized gave the characteristic green flame for organic boron compounds when burnt in the non-luminous *Bunsen* flame.

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Experimental Part

t-Butyloxycarbonyl-amido acids, peptide reagents, chloromethylated resin (copolystyrene – 1% divinylbenzene, 0.75 mmol of Cl/g of resin) and BAH(HCl)-resin (0.3524 mmol of NH_2/g of resin) were obtained from *Bachem* Fine Chemicals Inc., if not otherwise stated, and were used without further purification. DCC[I]) was purified by dissolving the commercial product (*Pierce* Chemicals) in ether; the insoluble material was removed by filtration and the ether was evaporated *in vacuo*. All solvents and reagents used for solid-phase synthesis were of 'analytical reagent' quality and were redistilled before use. TLC. was performed on *Merck* precoated silica gel plates (type G60 – F254) in the solvent systems BAW, BAWP, BIWCl. The spots were visualized with UV. fluorescence, ninhydrin [19], *Pauly* reagent, or a modified *Reindel-Hoppe* procedure [20]. Gel electrophoresis was performed with a *Beckman* model R electrophoresis apparatus using *Merck* precoated cellulose plates (F254, thickness 0.10 mm). The buffer used for electrophoresis was prepared by diluting glacial acetic acid (59 ml) and formic acid (36 ml) to 2 l with distilled water (pH 2.1). Migrations are indicated by the ratio of the distance of migration of the peptides to that of L-arginine HCl (m_{Arg}). Peptide samples for amino acid analyses were hydrolyzed during 24 h at 110° in 6N HCl + 0.2% phenol in vacuum-sealed tubes. The amino acid analysis were performed on a *Technicon* TSM analyzer equipped with an *Autolab* integrator and are acknowledged to Dr. *P. Schiller* of the Institute for Clinical Research in Montreal. All mixtures of liquids are given in *v/v*.

Peptide synthesis was carried out with a *Burrel* shaker and glass reaction vessels [21] using procedures previously described by *Park et al.* [22]. The manual operations for stepwise solid-phase synthesis are shown in *Table 1*. *N-a*-Boc protection was used for all the amino acids. This group was removed, prior to the next coupling, by reacting with a 33% TFA/CH_2Cl_2 solution for 20 min. A 5% solution of DEA in CH_2Cl_2 was used for neutralisation. The free base reacted with the symmetrical anhydride (according to the method used by *Lemaire et al.* [15]) of the next amino acid; a 3-fold excess of the Boc-amino acid was used for each coupling. The formation of the symmetrical anhydride of Boc-Car is incomplete, so its coupling to the peptide had to be carried out by equilibration of the peptide-resin with Boc-Car followed by DCC addition. The completion of every coupling was checked after the reaction (step 12 in *Table 1*) by the procedure of *Kaiser et al.* [23]. Peptide-amides were formed by coupling the first Boc-amino acid to a BHA(HCl)-resin. Simultaneous cleavage of the peptide from the resin and of the side-chain protecting groups was performed in liquid HF/anisole 5:1 for 30 min at

−20°, followed by 60 min at 0°, using a Kel-F/Teflon apparatus from *Protein Research Foundation*, Osaka, Japan [24] and are acknowledged to Dr. *S. St-Pierre* of our department. The peptides were extracted from the resin with 2N acetic acid and lyophilized.

Peptide purification. – Crude peptides were purified after cleavage with gel filtration. Three different types of mediums were utilized: Sephadex LH 20, G 15, G 25. LH 20 chromatography was carried out with a 2.9×85 cm column (MeOH equilibrated). Columns of 3×73 cm or 3×116 cm (G 15) and of 1×82 cm or 3×85 cm (G 25) were used. These columns were equilibrated with degassed 0.2M acetic acid. For partition chromatography G 25 was equilibrated with the aqueous layer of the degassed 2 phase system BAW 4:1:5 and eluted with the organic layer. The columns were run at a 25 ml/h flow rate, by gravity or by using a *Buchler* peristaltic pump. The 5-ml fractions containing the peptides were detected by spotting 10-µl aliquots from each tube on TLC. plates and were revealed with ninhydrin, *Reindel-Hoppe* or *Pauly* reagent (angiotensins).

The homogeneity of each purified peptide was verified by 3 TLC. systems (BAW, BAWP, BIWCl), and by electrophoresis. Results are summarized in *Table 3*. The values for amino acid compositions of all the peptides **1–13** are presented in *Table 4*.

Esterification of Boc-amino-acids to the resin. – One mmol of dry Boc-amino acid Cs-salt, which was prepared according to *Gisin* [25], was coupled with one equiv. of chloromethylated resin [25]. The substitution degree was measured with the picric acid test [26] and in the case of Car-resin by elemental analysis of boron. The following substitution degrees were (mmol/g): Boc-Phe 0.4, Boc-Car 0.28, 0.35 and 0.43, Boc-Leu 0.4, Boc-Arg(Tos) 0.19.

The name, primary structure chemical formula and molecular weight of the peptides **1–13** are presented in *Table 2*.

H-Arg-Pro-Pro-Gly-Car-Ser-Pro-Phe-OH ([Car^5]-octa(1–8)-BK, **1***).* The peptide **1** was synthesized from 1 g (0.4 mmol Cl/g) Boc-Phe-resin and the following protected L-amino acids: Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Car-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH. After HF-cleavage, **1** was purified on Sephadex-LH-20(MeOH)- and G-15(0,25M AcOH)-column chromatography.

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Car-OH ([Car^8]-octa(1–8)-BK, **2***).* The peptide **3** was synthesized from 1.05 g (0.35 mmol Cl/g) Boc-Car-resin and the following protected L-amino acids: Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Phe-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH. After HF-cleavage **2** was purified on Sephadex-LH-20(MeOH)- and G-15(0.2M AcOH)-column chromatography.

H-Arg-Pro-Pro-Gly-Car-Ser-Pro-Car-OH ([Car^{5,8}]-octa(1–8)-BK, **3***).* The peptide **3** was synthesized from 1 g (0.43 mmol Cl/g) Boc-Car-resin and the following protected L-amino acids: Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Car-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH. After HF-cleavage **3** was purified on Sephadex-LH-20(MeOH)- and G-15(0.2M AcOH)-column chromatography.

H-Arg-Pro-Pro-Gly-Car-Ser-Pro-Phe-Arg-OH ([Car^5]-BK, **4***).* The peptide **4** was synthesized from 2.93 g (0.19 mmol Cl/g) Boc-Arg(Tos)-resin and the following protected L-amino acids: Boc-Phe-OH, Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Car-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH. After HF-cleavage **4** was purified on Sephadex-G-15(0.2M AcOH)- and LH-20(MeOH)-column chromatography.

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Car-Arg-OH ([Car^8]-BK, **5***).* The peptide **5** was synthesized from 2.93 g (0.19 mmol Cl/g) Boc-Arg(Tos)-resin and the following protected L-amino acids: Boc-Car-OH, Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Phe-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH. After HF-cleavage **5** was purified on Sephadex-G-15(0.2M AcOH)- and LH-20(MeOH)-column chromatography.

H-Lys-Arg-Pro-Pro-Gly-Car-Ser-Pro-Leu-OH ([Lys^0, Car^5, LeuOH^8]-octa(1–8)-BK, **6***).* The peptide **6** was synthesized from 1.0 g (0.4 mmol Cl/g) Boc-Leu-resin and the following protected L-amino acids: Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Car-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH, Boc-Lys(Boc)-OH. After HF-cleavage **6** was purified on Sephadex-G-15(0.2M AcOH)-column chromatography. Further purification (LH 20, G 25 (BAW)) did not raise the biological activity [13] of the already chromatographically pure product.

H-Lys-Arg-Pro-Pro-Gly-Car-Ser-Pro-Leu-NH$_2$ ([Lys^0, Car^5, LeuNH$_2^8$]-octa(1–8)-BK, **7***).* The peptide **7** was synthesized from 1.09 g (0.35 mmol NH_2/g) BHA (HCl)-resin and the following protected L-amino acids: Boc-Leu-OH, Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Car-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH, Boc-Lys(Boc)-OH. After HF-cleavage **7** was purified on Sephadex-G-15(0.2M AcOH)- and LH-20 (MeOH)-column chromatography.

H-Arg-Pro-Pro-Gly-Car-Ser-Pro-Leu-NH^2 ([Car^5, LeuNH$_2^8$]-octa(1–8)-BK, **8***).* The peptide **8** was synthesized from 1.69 g (0.35 mmol NH_2/g) BHA (HCl)-resin and the following protected L-amino

Table 1. *Procedure for the solid-phase synthesis of the peptides*

Step	Reagents and operations[a])	Mixing time, min	Step	Reagents and operations[a])	Mixing time, min
1	CH_2Cl_2 wash[b])	10	7	CH_2Cl_2 wash (3×)	5
2	CH_2Cl_2 wash (2×)	2	8[c])	Neutralization: DEA/CH_2Cl_2 1:19 (3×)	5
3	Deprotection prewash TFA/CH_2Cl_2 1:2	5	9	CH_2Cl_2 wash (3×)	5
4	Deprotection: TFA/CH_2Cl_2 1:2	20	10	Symmetrical anhydride of Boc-amino acid in CH_2Cl_2	60
5	CH_2Cl_2 wash	5	11	2-Propanol wash (3×)	5
6	2-Propanol wash	5	12[d])	CH_2Cl_2 wash (3×)	5

[a]) Starting with the Boc-amino acid polymer suspended in CH_2Cl_2.
[b]) The quantity of this solvent was adjusted to the amount of resin (30 ml for 1 g of resin).
[c]) In the case of BHA (HCl) – resins (amide-forming) the synthesis started at step 8 with 15% TEA/CH_2Cl_2 as neutralization solvent.
[d]) Aliquots were taken at this point for the ninhydrin test [19]: if negative, the next cycle was started with the next amino acid residue at point 1; if positive, or slightly positive steps 8 to 13 were repeated with the same excess of Boc-amino acid.

Table 2. *Primary structure of compounds* **1–13**

No	Name	Primary structure												Formula	Mol.-Weight
		0	1	2	3	4	5	6	7	8	9	10	11		
1	[Car^5]-octa(1–8)-BK		H-Arg	Pro	Pro	Gly	Car	Ser	Pro	Phe-OH				$C_{40}H_{66}B_{10}N_{11}O_{10}$	969.14
1	[Car^8]-octa(1–8)-BK		H-Arg	Pro	Pro	Gly	Phe	Ser	Pro	Car-OH				$C_{40}H_{66}B_{10}N_{11}O_{10}$	969.14
3	[$Car^{5,8}$]-octa(1–8)BK		H-Arg	Pro	Pro	Gly	Car	Ser	Pro	Car-OH				$C_{36}H_{72}B_{20}N_{11}O_{10}$	1035.24
4	[Car^5]-BK		H-Arg	Pro	Pro	Gly	Car	Ser	Pro	Phe	Arg-OH			$C_{46}H_{78}B_{10}N_{15}O_{11}$	1125.32
5	[Car^8]-BK		H-Arg	Pro	Pro	Gly	Phe	Ser	Pro	Car	Arg-OH			$C_{46}H_{78}B_{10}N_{15}O_{11}$	1125.32
6	[Lys^0,Car^5,Leu-OH^8]-octa(1–8)-BK	H-Lys	Arg	Pro	Pro	Gly	Car	Ser	Pro	Leu-OH				$C_{43}H_{80}B_{10}N_{13}O_{11}$	1063.29
7	[Lys^0.Car^5.Leu-NH_2^8]-octa(1–8)-BK	H-Lys	Arg	Pro	Pro	Gly	Car	Ser	Pro	Leu-NH_2				$C_{43}H_{81}B_{10}N_{14}O_{10}$	1062.31
8	[Car^5,Leu-NH_2^8]-octa(1–8)-BK		H-Arg	Pro	Pro	Gly	Car	Ser	Pro	Leu-NH_2				$C_{37}H_{69}B_{10}N_{12}O_9$	934.13
9	[Car^1,Leu^5]-Enk		H-Car	Gly	Gly	Phe	Leu-OH							$C_{24}H_{43}B_{10}N_5O_6$	605.74
10	[Car^7]-octa(4–11)-SP					H-Pro	Gly	Gly	Car	Phe	Gly	Leu	Met-NH_2	$C_{42}H_{73}B_{10}N_{11}O_{10}S$	1032.28
11	[Car^8]-octa(4–11)-SP					H-Pro	Gly	Gly	Phe	Car	Gly	Leu	Met-NH_2	$C_{42}H_{73}B_{10}N_{11}O_{10}S$	1032.28
12	[Sar^1,Car^4]-AT_{II}		H-Sar	Arg	Val	Car	Val	His	Pro	Phe-OH				$C_{44}H_{75}B_{10}N_{13}O_9$	1038.27
13	[Sar^1,Car^8]-AT_{II}		H-Sar	Arg	Val	Tyr	Val	His	Pro	Car-OH				$C_{44}H_{75}B_{10}N_{13}O_{10}$	1054.27

Table 3. *Characterization of compounds* **1–13**

Number		Thin layer Chromatography Rf-values			Electrophoresis	Yield
		BIWCl	BAW	BAWP	m_{Arg}	%
1	[Car^5]-octa(1–8)-BK	0.35	0.30	0.44	0.51	14.3
2	[Car^8]-octa(1–8)-BK	0.30	0.30	0.42	0.43	14.1
3	[Car^{5,8}]-octa(1–8)-BK	0.34	0.28	0.48	0.29	18.2
4	[Car^5]-BK	0.29	0.19	0.31	0.56	42.4
5	[Car^8]-BK	0.27	0.19	0.31	0.50	46.0
6	[Lys^0,Car^5,LeuOH^8]-octa(1–8)-BK	0.23	0.06	0.43	0.42	30.1
7	[Lys^0,Car^5,LeuNH$_2^8$]-octa(1–8)-BK	0.24	0.05	0.47	0.70	11.3
8	[Car^5,LeuNH$_2^8$]-octa(1–8)-BK	0.32	0.27	0.54	0.57	12.2
9	[Car^1,Leu^5]-enkephalin	0.63	0.75	0.67	0.0	7.0
10	[Car^7]-octa(4–11)-SP	0.38	0.32	0.60	0.15	7.1
11	[Car^8]-octa(4–11)-SP	0.38	0.37	0.61	0.15	5.0
12	[Sar^1,Car^4]-AT$_{II}$	0.27	0.44	0.59	0.44	14.4
13	[Sar^1,Car^8]-AT$_{II}$	0.26	0.47	0.56	0.44	77.5

Table 4. *Ratio of amino acids (on an acid hydrolysate) of the peptides* **1–13**[a)][b)]

No	Analogues	Arg	Gln	Gly[a)]	His	Leu	Lys	Met[c)]	Phe	Pro	Sar[d)]	Tyr	Val
1	[Car^5]-octa(1–8)-BK	1.09	–	1.37	–	–	–	–	1.16	2.85	–	–	–
2	[Car^8]-octa(1–8)-BK	1.20	–	1.67	–	–	–	–	0.98	2.85	–	–	–
3	[Car^{5,8}]-octa(1–8)-BK	1.17	–	2.43	–	–	–	–	–	2.85	–	–	–
4	[Car^5]-BK	2.52	–	0.94	–	–	–	–	1.01	3.0	–	–	–
5	[Car^8]-BK	2.36	–	1.02	–	–	–	–	0.99	3.0	–	–	–
6	[Lys^0,Car^5,LeuOH^8]-octa(1–8)-BK	1.05	–	1.23	–	–	1.05	–	1.06	2.94	–	–	–
7	[Lys^0,Car^5,LeuNH$_2^8$]-octa(1–8)-BK	0.95	–	1.20	–	–	0.95	–	1.17	2.97	–	–	–
8	[Car^5,LeuNH$_2^8$]-octa(1–8)-BK	1.14	–	1.01	–	–	–	–	1.09	3.00	–	–	–
9	[Car^1,Leu^5]-enkephalin	–	–	2.38	–	1.08	–	–	0.92	–	–	–	–
10	[Car^7]-octa(4–11)-SP	–	2.0	1.10	–	0.96	–	0.63	0.95	0.95	–	–	–
11	[Car^8]-octa(4–11)-SP	–	2.0	1.10	–	0.96	–	0.54	0.94	0.90	–	–	–
12	[Sar^1,Car^4]-AT$_{II}$	1.07	–	–	1.07	–	–	–	1.18	1.01	1.60	–	1.90
13	[Sar^1,Car^8]-AT$_{II}$	0.95	–	–	1.11	–	–	–	–	0.89	1.21	0.95	2.31

a) The amino acid Car does not appear as such in the normal spectrum [6]. Several contaminations are observed after hydrolysis. Under the hydrolysis conditions used, these contaminations were minimal and only Gly was increased.

b) Serine was not found in the spectrum under the hydrolysis conditions used; this was confirmed with reference peptides containing Ser.

c) Oxidation loss during hydrolysis.

d) Sar is very close to ammonia and it is not separated in the integrated spectrum.

acids; Boc-Leu-OH, Boc-Pro-OH, Boc-Ser(Bzl)-OH, Boc-Car-OH, Boc-Gly-OH, Boc-Arg(Tos)-OH. After HF-cleavage **8** was purified on Sephadex-G-15(0.2M AcOH)-column chromatography.

H-Car-Gly-Gly-Phe-Leu-OH ([Car^1,Leu^5]-Enk, **9***).* The peptide **9** was synthesized from 1.0 g (0.4 mmol Cl/g) Boc-Leu-resin and the following L-amino acids: Boc-Phe-OH, Boc-Gly-OH, Boc-Car-OH. After HF-cleavage **9** was purified on LH-20(MeOH)-, G-15(0.2M AcOH)- and LH-20(MeOH)-column chromatography.

H-Pro-Glu-Glu-Car-Phe-Gly-Leu-Met-NH$_2$ ([Car^7]-octa(4–11)-SP, **10***).* The peptide **10** was synthesized from 1.0 g (0.35 mmol NH_2/g) BHA(HCl)-resin and the following protected L-amino acids: Boc-Met-OH, Boc-Leu-OH, Boc-Gly-OH, Boc-Phe-OH, Boc-Car-OH, Boc-Glu-OH, Boc-Pro-OH.

After HF-cleavage **10** was purified on G-15(0.2M AcOH)- and G-25(0.2M AcOH)-column chromatography.

H-Pro-Glu-Glu-Phe-Car-Gly-Leu-Met-NH_2 ([Car^8]-octa(4–11)-SP, **11***).* The peptide **11** was synthesized from 1.0 g (0.35 mmol NH_2/g) BHA (HCl)-resin and the following protected L-amino acids: Boc-Met-OH, Boc-Leu-OH, Boc-Gly-OH, Boc-Car-OH, Boc-Phe-OH, Boc-Glu-OH, Boc-Pro-OH. After HF-cleavage **11** was purified on LH-20(MeOH)-, G-15(0.2M AcOH)- and G-25(0.2M AcOH)-column chromatography.

H-Sar-Arg-Val-Car-Val-His-Pro-Phe-OH ([Sar^1, Car^4]-AT_{II}, **12***).* The peptide **12** was synthesized from 0.88 g (0.4 mmol Cl/g) Boc-Phe-OH-resin and the following protected L-amino acids: Boc-Pro-OH, Boc-His(Tos)-OH, Boc-Val-OH, Boc-Car-OH, Boc-Arg(Tos)-OH, Boc-Sar-OH. After HF-cleavage **12** was purified on LH-20(MeOH)-, G-15(0.2M AcOH)- and G-25(BAW)-column chromatography.

H-Sar-Arg-Val-Tyr-Val-His-Pro-Car-OH ([Sar^1, Car^8]-AT_{II}, **13***).* The peptide **13** was synthesized from 1.0 g (0.28 mmol Cl/g) Boc-Car-OH-resin and the following protected L-amino acids: Boc-Pro-OH, Boc-His(Tos)-OH, Boc-Sar-OH. After HF-cleavage **13** was purified on G-15(0.2M AcOH)-column chromatography. Further purification (LH 20, G 15 (BAW)) did not raise the biological activity [12] of the already chromatographically pure product.

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