96. Angiotensin-II Analogues. I: Synthesis and Incorporation of the Halogenated Amino Acids 3-(4′-Iodophenyl)alanine, 3-(3′,5′-Dibromo-4′-chlorophenyl)alanine, 3-(3′,4′,5′-Tribromophenyl)alanine, and 3-(2′,3′,4′,5′,6′-Pentabromophenyl)alanine[1])

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Summary

The synthesis of the polyhalogenated phenylalanines Phe(3′,4′,5′-Br_3) (**3**), Phe(3′,5′-Br_2-4′-Cl) (**4**) and DL-Phe(2′,3′,4′,5′,6′-Br_5) (**9**) is described. The trihalogenated phenylalanines **3** and **4** are obtained stereospecifically from Phe(4′-NH_2) by electrophilic bromination followed by *Sandmeyer* reaction. The most hydrophobic amino acid **9** is synthesized from pentabromobenzyl bromide and a glycine analogue by phase-transfer catalysis. With the amino acids **4**, **9**, Phe(4′-I) and D-Phe, analogues of [1-sarcosin]angiotensin II ([Sar[1]]AT) are produced for structure-activity studies and tritium incorporation. The diastereomeric pentabromo peptides L- and D-**13** are separated by HPLC. and identified by catalytic dehalogenation and comparison to [Sar[1]]AT (**10**) and [Sar[1], D-Phe[8]]AT (**14**).

Introduction. – In the past few years, structure-activity studies with biologically active compounds have been increasingly carried out in quantitative terms. Especially the effects of hydrophobicity, sterical requirements and also other chemical parameters like electronegativity have been taken into account [2]. With peptides several studies have been carried out in order to predict the influence of a single amino acid and its parameters on the biological properties. Strong affinity changes were observed if the hydrophobicity of certain amino acids was increased in peptides like the enkephalins or the kinins. The principle of very hydrophobic amino acids was well demonstrated on examples like L-*o*-carboranylalanine [3] (Car), L-adamantylalanine [4] (Ada) and others. During studies on the C-terminal

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position of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) [1] with Car in the position 8 [5], important increases of duration of action concomitant with a serious drop in affinity were observed.

Car has an almost isotropic spherical side chain which has a core considerably thicker than a normal phenyl ring. Therefore we believe that steric hindrance is the reason for affinity loss, and enhanced lipophilicity is responsible for the increased duration of action.

Chlorine, bromine and especially iodine as substituents strongly increase the lipophilicity of a phenyl ring and maintain the planarity of this system without increasing substantially its thickness. We therefore intended to design halogenated phenylalanine analogues with increasing lipophilicity and to incorporate them into angiotensin II in the hope to increase the duration of action without loosing the high affinity of angiotensin II to its hormone receptor. Another potential of halogenated aromatic amino acids is their function as precursors for halogenated peptides. Halogen substituents are easily exchanged against tritium by catalytic dehydrohalogenation, introducing 30 Ci/mmol for every tritium incorporated. This method has some decisive advantages over unsaturated peptide precursors: Tritiation is for technical reasons not always exhaustive, and unlabeled or only partially labeled peptides have to be effectively removed from the desired product, otherwise low specific activities or, even worse, altered binding kinetics are the consequence of heterogeneously contaminated labels.

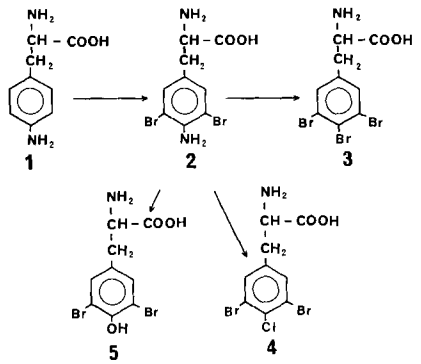
The frequently used 3,4-didehydroproline [6] and other unsaturated amino acid analogues [7] have very similar physicochemical properties compared to the saturated analogues. Therefore effective purification can be very difficult, even by HPLC. On the other hand, halogenated phenylalanines are much more lipophilic than phenylalanine itself; already monoiodination of simple peptides can increase the retention time twofold on isocratic HPLC. Therefore, separation of starting material and product is easily possible. The relatively low specific radioactivity of tritium makes it also interesting to use a precursor with a very high halogen content.

We therefore prepared several halogenated analogues of phenylalanine and incorporated them into the C-terminal position of [Sar^1, Val^5]AT. As internal reference also [Sar^1, D-Phe^8]AT (**14**) was synthesized.

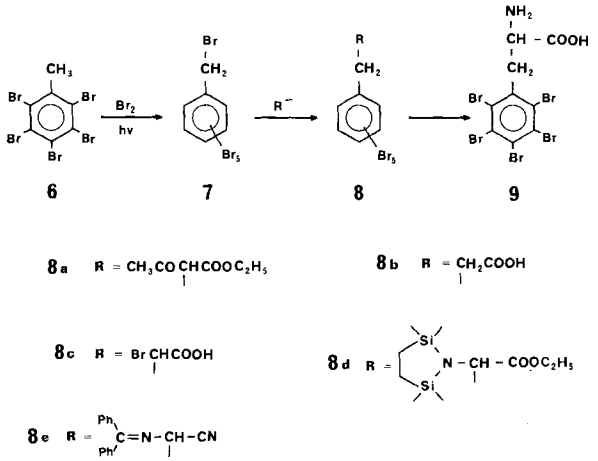
Syntheses. – Monohalogenated phenylalanines are easily accessible by the *Sandmeyer* reaction on L-4′-aminophenylalanine (**1**), and 4′-chloro-, 4′-bromo-, or 4′-iodophenylalanines are even commercially available. The trihalogenated species **3** and **4** were obtained in a similar manner (see *Scheme 1*): **1** was brominated with bromine in acetic acid, and the produced **2** was subjected to a *Sandmeyer* reaction. The tribromo analogue **3** was obtained in very small quantities, the low yield was probably due to steric factors, and was accompanied by the diazonium degradation by-product 3′,5′-dibromotyrosine (**5**). The alternative dibromo-chloro analogue **4** was obtained in somewhat better yields and has been preliminarily reported [8].

The ultimate amino acid (pentabromophenyl)alanine **9**, was inaccessible by direct halogenation of phenylalanine and had to be synthesized in a different way: pentabromotoluene (**6**) was photohalogenated in the α-position, a step which proceeded without problems and gave only monohalogenation in high yield. Due

Scheme 1



Scheme 2



to steric hindrance, no dibrominated product was observed. Pentabromobenzyl bromide (**7**) was used as the building block for the amino acid synthesis according to *Scheme 2*. Several methods were tried for the introduction of this side chain into an amino-acid precursor, but due to steric hindrance probably, the classical amino-malonic ester synthesis did not give any product, and with the smaller carbanion from ethyl acetoacetate (see *Scheme 2*) only small yields of **8a** were observed. Functionalization attempts of pentabromobenzyl bromide with *Grignard* reagent, with butyllithium or even metallic alkali under various appropriate conditions failed also. Luckily, newer amino-acid synthons were just becoming available: *N, N*-(2,5-dimethyl-2,5-disilahexane-2,5-diyl)glycine ethyl ester (= ethyl 2,2,5,5-tetramethyl-1-aza-2,5-disilolane-1-acetate) [9] and the *Schiff*base ((diphenylmethylidene)amino)acetonitrile [10]. With these synthons (pentabromophenyl)-alanine (**9**) was obtained, especially ((diphenylmethylidene)amino)acetonitrile gave reasonable yields (49% over all steps to Boc-**9**). This alkylating reaction was performed with aqueous KOH-solution and phase transfer catalysis which was

much less cumbersome than the lithium diisopropylamide procedure employed for the intermediate **8d**.

The free amino acid **9** was obtained by acid hydrolysis of the N- and C-terminal protecting groups from **8d** or **8e** but not characterized fully, because the product was highly insoluble in the usual solvents. The racemic amino acid **9** was therefore directly converted into the *N-t*-butoxycarbonyl derivative Boc-**9** which permitted satisfactory purification, analysis and peptide synthesis. The properties of the halogenated phenylalanines are summarized in *Table 1* and *2*.

For peptide synthesis, the C-terminal amino acids to the desired AT analogues, Boc-Phe(4′-I), Boc-Phe(3′,5′-Br_2-4′-Cl) (Boc-**4**), Boc-DL-Phe(2′,3′,4′,5′,6′-Br_5) (Boc-**9**) and Boc-D-Phe were esterified to the solid polystyrene support and the synthesis carried out by the solid phase method. The completed peptides were cleaved from the side chain protecting groups and the solid support by treatment with liquid anhydrous hydrogen fluoride and purified either by gel filtration and partition chromatography or by gel filtration and reversed phase high pressure liquid chromatography. The latter technique permitted the separation of the diastereomeric (pentabromophenyl)alanine-containing peptides L-**13** and D-**13**. The properties of the [Sar^1]AT analogues are summarized in *Table 3*.

Catalytic tritiation of the halogenated analogues **11**, **12** and **13** produced AT analogues of 30, 90 and 150 Ci/mol, respectively, in high yields.

The new AT analogues were tested in two bioassays [11], the *in vitro* test rabbit aorta strip, and *in vivo* on the rat blood pressure. All three analogues **11**–**13** have

Table 1. *Physicochemical properties of halogenated phenylalanines*[a])

	TLC.-Rf			Parameters		
	A	B	C	Π	σ	MR.
Phe	0.579±0.022	0.264±0.010	0.468±0.008	1.96	0.00	25.36
Phe(4′-I)	0.473±0.027	0.311±0.025	0.473±0.015	3.08	0.18	38.27
Phe(3′,5′-Br_2-4′-Cl) (**4**)	0.568±0.044	0.395±0.029	0.557±0.010	4.29	1.01	46.06
Phe(3′,4′,5′-Br_3) (**3**)	0.610±0.010	0.426±0.020	0.579±0.005	4.54	1.01	48.91
Phe(2′,3′,4′,5′-Br_5) (**9**)	0.691±0.009	0.425±0.021	0.624±0.020	6.26	1.47	64.61
Ada	0.586±0.018	0.382±0.006	0.582±0.017	3.3	–	40.63
Car	0.629±0.017	0.317±0.027	0.615±0.013	*ca.* 4.0	*ca.* 2	*ca.* 45

[a]) For the solvent systems A, B, and C, see *Exper. Part*, Π is the hydrophobicity coefficient, σ is the *Hammett* factor and MR. is the molecular refractory index. Π, σ, and MR. are calculated from [2] and are only for the benzyl side chain. The values added for Car [3] and Ada [4] are for comparison with other highly lipophilic amino acids.

Table 2. *Elemental analyses*

Substance	No.	Formula	MW.		C	H	N	Cl	Br
Boc-Phe(3′,4′,5′-Br_3)	Boc-**3**	$C_{14}H_{16}Br_3NO_4$	502.02	Calc.	33.50	3.21	2.79	–	47.75
				Found	33.25	3.45	2.91		47.98
Boc-Phe(3′,5′-Br_2-4′-Cl)	Boc-**4**	$C_{14}H_{16}Br_2ClNO_4$	457.56	Calc.	36.75	3.52	3.06	7.75	34.95
				Found	36.66	3.63	3.35	7.82	35.12
DL-Boc-Phe(2′,3′,4′,5′,6′-Br_5)	Boc-**9**	$C_{14}H_{14}Br_5NO_4$	659.82	Calc.	25.49	2.14	2.12	–	60.55
				Found	25.49	2.38	2.07		60.26

Table 3. *Physicochemical properties and biological activities of [1-sarcosin]angiotensin II analogues*

	No.	MW.[a])	TLC.-Rf D	TLC.-Rf E	RP.-TLC.	Yield	Amino-acid analysis[c])						Rabbit aorta[c]) pD_2	Rabbit aorta[c]) pA_2	Rabbit aorta[c]) a^E	Rabbit aorta[c]) R.A.	Rat R.B.P.[d])
[Sar^1]AT	**10**	1048.22	0.405	0.428	0.534	–	Arg	Val	Tyr	His	Pro	Phe	8.67	–	1.0	100%	100%
[Sar^1, Phe(4′-I)^8]AT	**11**	1174.12	0.428	0.452	0.324	46%	1.10	2.02	0.94	0.94	1.02	–	8.57	–	0.83	79%	27%
[Sar^1, Phe(3′,5′-Br_2-4′-Cl)^8]AT	**12**	1240.47	0.440	0.476	0.034	33%	1.05	1.90	1.02	0.97	1.07	–	7.58	–	0.47	8%	12%
[Sar^1, Phe(2′,3′,4′,5′,6′-Br_5)^8]AT	L-**13**	1442.73	0.440	0.476	0.000	59%[b])	1.14	1.96	1.01	0.94	1.08	–	–	7.83	0.0	14%	< 0.1%
[Sar^1, D-Phe(2′,3′,4′,5′,6′-Br_5)^8]AT	D-**13**	1442.73	0.440	0.488	0.007								–	7.78	0.0	13%	< 0.1%
[Sar^1, D-Phe^8]AT	**14**	1048.22	0.404	0.434	0.547	46%	0.99	1.92	1.01	0.97	1.07	1.03	–	8.18	0.0	32%	< 0.1%
[Sar^1, Car^8]AT [4]			0.452	0.488	0.149										0.15	12%	0.4%

[a]) Monoacetate.

[b]) Yield of diastereomeric mixture, from which 25% of L-**13** and 28% of D-**13** were separated. Amino-acid analysis: Sarcosine was always present, but because of the very weak absorbance it was not quantified (between His and NH_3). Phe(4′-I) and Phe(3′,5-Br_2-4′-Cl) were seen but not quantified at the end of the spectrum, both between His and NH_3, the first close to His, the second close to NH_3; Phe(Br_5) was too lipophilic to be seen.

[c]) Biological activities: pD_2 is the negative log of the dose of agonist that produces half maximal contraction; pA_2 is the negative log of the dose of an antagonist, that reduces the response of a double dose of AT to that of a single dose. a^E is the intrinsic activity (full agonist $a^E = 1$, pure antagonist $a^E = 0.0$). R.A. is the relative affinity and includes the antagonists (pA_2 compared to $pD2$). Standard errors of pA_2 or pD_2 are always less than 5% and at least 6 determinations for each value have been carried out.

[d]) R.B.P. is the relative blood pressure potency in %.

AT-specfic actions on the two bioassays, and the antagonistic properties increase with increasing halogen content (see *Table 3*) of the side chain.

Discussion. – In the biological test it was shown that [Sar^1]AT is still the most potent agonist, however, [Sar^1, D-Phe^8]AT is a pure antagonist, comparable to the classical antagonists [Sar^1, Ala^8]AT or [Sar^1, Leu^8]AT [11]. If halogen substituents are introduced on the aromatic ring of Phe^8, the lipophilicity Π and the molecular refractory index MR. increase, and the agonistic behavior decreases ($a^E < 1.0$). Parallel to this we also observe enhanced retention on RP.-TLC. and enhanced Rf in the solvent system E. A third phenomenon which is very difficult to quantify in this biological system is the duration of action. We observe both *in vivo* and *in vitro* a longer duration of action of **11** if compared to **10**, an effect we attribute to the increased lipophilicity. Due to the partial agonistic effect of **12**, no equally clear answer is possible. However, if we compare the inhibitors **14** with D-**13**, an increase of duration is clearly visible: An inhibitory dose of **14** in rabbit aorta assay is 100% reversible in 90 min, a similar dose of D-**13** specifically suppresses the angiotensin II responsiveness of this tissue for at least 6 hours, even stronger than [Sar^1, Car^8]AT [4]. Compared to [Sar^1, Car^8]AT, L-**13** and also D-**13** have a 100fold higher affinity. Car has similar hydrophobicity and bulk as **4**, and is surpassed in both by **9**, reflected by the Π and MR. values in *Table 1*. However, the flat nature of **4** and **9** compared to the spherical nature of Car might much better accomodate with the AT receptor and would explain the relatively high affinity even of the pentabromo analogue **13**. We feel therefore that the new amino acid **9** will have great possibilities if bulky, very hydrophobic amino acids are needed.

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Experimental Part

NMR. spectra were recorded on a *Varian T60* spectrometer, chemical shifts are indicated in ppm against tetramethylsilane, *s* = singulet, *d* = doublet, *t* = triplet, *qa* = quadruplet, *m* = multiplet. Melting points were determined in open capillaries and are uncorrected. Elemental analyses have been carried out by *Galbraith Laboratories* (Knoxville, Tennessee, U.S.A.). UV. spectra were recorded on a *Beckman 25* spectrophotometer; λmax in nm, logε in parentheses. IR. spectra were carried out on a *Perkin-Elmer 457* instrument with nujol suspension on NaCl windows; absorptions in cm^{-1}. Mass spectra (*m/z*) were recorded on *H5-30* double beam instrument of AEI, England. Optical rotations were measured in a *Zeiss-OLD* polarimeter in 0.5% solutions in EtOH. Analytical HPLC. was carried out on a *Waters M45* instrument with a gradient of 0–45% CH_3CN in 0.25 N $AcONH_4$ (pH 5.0) and 2.5% 2-propanol on a *μbondapack C-18* column (4 × 300 mm).

L-(*t*-Butoxycarbonyl)amino acids, peptide reagents, chloromethylated resin (copolystyrene – 1% divinylbenzene, 0.75 mmol of Cl/g of resin) were obtained from *Bachem Feinchemikalien AG*, Switzerland, if not otherwise stated, and were used without further purification. DCC was purified by dissolving the commercial product *(Aldrich Chemicals)* in Et_2O; the insoluble material was removed by filtration, and the Et_2O was evaporated *in vacuo*. All reagents used for solidphase synthesis were of analytical reagent quality and solvents were redistilled before use. TLC. was performed on *Merck* precoated silica gel plates (type *G60-F254*) in the solvent systems A–F; A: 2-propanol/conc. aq. ammonia 3:1; B: butanol/0.05 M $AcONH_4$ 2:1, pH 7.0; C: butanol/AcOH/H_2O/4:1:1; D: butanol/AcOH/H_2O 5:2:3; E: butanol/AcOH/H_2O/pyridine 30:6:20:12; F: $CHCl_3$/AcOH/CH_3OH 95:5:3; RP.-TLC. was

performed on *Whatman* reversed phase plates *KC 18* in the solvent system 0.5M $AcONH_4$ with 40% of CH_3CN and 7% of 2-propanol. The spots were visualized with UV. fluorescence, ninhydrin or *Pauly* reagent. All mixtures of liquids are given in *v/v*. Peptide samples for amino-acid analyses were hydrolyzed during 24 h at 110° in TFA/conc. hydrochloric acid 1:1 with 1% of β-mercaptopropionic acid in vacuum-sealed tubes. The amino-acid analyses were performed on a *Beckman 119 CL* analyzer. – Abbreviations are: AT: angiotensin II, DCC: dicyclohexylcarbodiimide, RP.-TLC.: reversed phase TLC., HF: anh. hydrogen fluoride, DEA: diisopropylethylamine, Tos: *p*-toluenesulfonyl, Br-Bzl: (3′-bromobenzyl), Boc: *t*-butoxycarbonyl, Boc_2O: di-*t*-butyl carbonate, DMF: dimethylformamide, TFA: trifluoroacetic acid, r.t.: room temperature.

Preparation of L-*3-(4′-Amino-3′,5′-dibromophenyl)alanine* (**2**, *Phe(3′,5′-Br₂-4′-NH₂)*). To a solution of L-4′-aminophenylalanine (**1**, Phe(4′-NH_2); 6 g, 33 mmol) (from our laboratories) in 150 ml of AcOH/H_2O 4:1, 18.1 g (113 mmol) of Br_2 were slowly added under vigourous stirring at r.t. Stirring was continued for 90 min, then the mixture slowly heated to 75° for 20 min and cooled to r.t. The dark brown mixture was extracted with AcOEt/Et_2O 5:3 and the org. phase rejected. The aq. phase was evaporated to dryness and desalted on ion exchange resin *Dowex 50 W-8* [12] yielding 6.4 g (57%) of brown powder. Rf (ninhydrin) 0.64 (A), 0.38 (B), or 0.54 (C). – ^{1}H-NMR. ((D)TFA): 7.8 (2 H); 5.0 (*m*, 1 H); 3.3 (*m*, 2 H). This product was not further characterized and directly used for the next step. Catalytic hydrogenation of **2** produced **1**, as shown by TLC.

Preparation of L-*3-(3′,4′,5′-Tribromophenyl)alanine* (**3**, *Phe(3′,4′,5′-Br₃)*. *Sandmeyer* catalyst: To 25 ml of 1M $CuSO_4$ were added subsequently at r.t. and under stirring 3.86 g (37.5 mmol) of NaBr and 25 ml of freshly prepared 0.5M Na_2SO_3. The white precipitate was filtered off and washed twice with cold H_2O, dissolved in 20 ml of 12M HBr and kept under N_2 until use.

Diazotation: 2 g (5.9 mmol) of **2** were dissolved in 200 ml of 2N HBr under stirring. The temp. was lowered to 0°, and during 10 min a solution of 488 mg of $NaNO_2$, in 20 ml of H_2O was added. After 5 min, a first iodine-starch test was positive and solid sulfamic acid was added slowly, until a subsequent iodine-starch test became negative. After this, 15 ml of the above catalyst solution was added and the temp. increased to 40° for 30 min. Gas evolved vividly from the mixture, and a tar-like layer was formed which was filtered. The reaction mixture was evaporated to dryness and desalted on ion-exchange resin *Dowex 50 W-8* [12]. After lyophilization, a brownish amino-acid powder was collected (798 mg, 35%). On TLC. two major spots were observed: Rf 0.61 and 0.21 (A); Rf 0.43 and 0.39 (B); Rf 0.58 and 0.53 (C). This mixture was not further characterized and converted directly to the Boc-derivative.

Preparation of N-(t-*Butoxycarbonyl)-3-(3′,4′,5′-tribromophenyl)alanine* (*Boc-***3**, *Boc-Phe(3′,4′,5′-Br₃)*). The above amino-acid powder was dissolved in 2 ml of 1N NaOH, diluted to 10 ml with H_2O and to 16 ml with *t*-BuOH. Under magnetic stirring at r.t., 472 mg of Boc_2O were added, and the pH was maintained over 10 by subsequent additions of 1N NaOH for 24 h. Ice and 20 ml of AcOEt were added, and the mixture was carefully acidified to pH 2 with solid $NaHSO_4$. The extraction was repeated, the org. phases pooled and dried over anh. Na_2SO_4. Evaporation yielded a yellowish oil (944 mg) which contained 2 major compounds. TLC. (F): Rf 0.36 and 0.43 and a yellow impurity at 0.49. Three repetitive chromatographies on a *Lobar*, prepacked silica gel column (size A, *Merck*), eluted with gradients of $CHCl_3$ and CH_3OH produced 2 homogeneous fractions: 154 mg (5.2%) of Boc-**3**, m.p. 144–145° and 136 mg (5.3%) of Boc-**5**, m.p. 143–144°, mixed m.p. 135–140°[3]). – ^{1}H-NMR. ($CDCl_3$): Boc-**3**: 7.4 (*s*, 2 H); 4.5 (*m*, 1 H); 3.1 (*m*, 2 H); 1.4 (*s*, 9 H). Boc-**5**: 7.3 (*s*, 2 H); 6.7 (br., 1 H); 4.5 (*m*, 1 H); 3.1 (*m*, 2 H); 1.4 (*s*, 9 H). – MS. (Boc-**3**): 505, 503, 501 and 499 (< 1. M^+); 388, 386, 384, and 382 (100, ratio 12.5:36:37:13, M^+ – Boc – OH).

Preparation of L-*3-(3′,5′-Dibromo-4′-chlorophenyl)alanine* (**4**, *Phe(3′,5′-Br₂-4′-Cl)*). The catalyst was prepared as above with 30 ml of 1M $CuSO_4$, 3.46 g of NaCl, 60 ml of fresh 0.5M Na_2SO_3, and 20 ml of conc. hydrochloric acid. To a solution of 6.4 g (18.8 mmol) of **2** in 800 ml of 6N HCl at 0° were added slowly and under vigorous stirring 1.5 g (22.6 mmol) of $NaNO_2$ in 120 ml of H_2O. After 10 min, a first iodine-starch test was positive, and excess nitrite was destroyed with solid sulfamic acid, as above. The catalyst solution was slowly added and stirring continued in the thawing ice bath overnight. The mixture was heated to 55°, filtered and evaporated to dryness. The crude amino acid was isolated with

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[3]) Mixed m.p. of Boc-**5** and synthetic Boc-Tyr(3′,5′-Br_2) 143–144°, of Boc-**3** and synthetic Boc-Tyr(3′,5′-Br_2) 136–140°. Catalytic hydrogenation [13] of Boc-**3** produced Boc-Phe (as shown by TLC.), and Boc-**5** yielded Boc-Tyr.

Dowex 50-W8 [12] as 4.3 g of brown powder, impure on TLC., main spot with Rf 0.57 (A), 0.40 (B) or 0.56 (C). This product was not further characterized and directly converted to the Boc-protected derivative.

Preparation of N-*(t-Butoxycarbonyl)-3-(3',5'-dibromo-4'-chlorophenyl)alanine (Boc-***4**, *Boc-Phe(3',5'-Br$_2$-4'-Cl)).* The above product, presumably 12 mmol, was dissolved in a mixture of 173 ml of H_2O, 104 ml of *t*-BuOH and 24 ml of 1N NaOH at RT. Under stirring, 3.1 g (14.4 mmol) of Boc_2O was added within 16 h, and the pH was maintained above 9 with 2N Na_2CO_3. The crude product was isolated by careful acidification to pH 2 and extraction as above. Purification was carried out by several subsequent chromatographies on *Lobar* prepacked silica gel columns, (size B, *Merck*) giving Boc-**4** as white crystals (1.944 g, 22.6%), m.p. 173–175°, $[\alpha]_D = +2.40$. TLC. (F): Rf 0.44 (ninhydrine)[4]). – UV. (ethanol): 281 (2.98). – ^{1}H-NMR. ($CDCl_3$): 7.46 (*s*, 2 H); 7.22 (*m*, 1 H); 4.50 (*m*, 1 H); 3.05 (*m*, 2 H); 1.40 (*s*, 9 H). – MS.: 457 (*ca.* 1, M^+); 344, 342, 340, and 338 (100, ratio 11.2:27.9:43.6:17.3, $M^+ - 117$).

Preparation of 2,3,4,5,6-pentabromobenzyl bromide (**7**). In a 2-neck flask)condenser and septum), 42.7 g (87.8 mmol) of pentabromotoluene (**6**, *ICN Pharmaceuticals*, Plainview, New York) were dissolved in 800 ml of boiling CCl_4. Under irradiation by two mercury medium pressure lamps (*JC-PAR-38, Westinghouse*, 100 W each), 15.4 g (96.6 mmol) of Br_2 were slowly added through the septum. After 1 h, the evolution of HBr had completely ceased, the mixture was slowly cooled to r.t. (crystallization started), evaporated and recrystallized from a minimum of hot CCl_4: 48.8 g (98%) of **7**, m.p. 180.5–182°. TLC. (petroleum ether): Rf 0.46, homogeneous. – ^{1}H-NMR. ($CDCl_3$): 4.9 (*s*).

Attempts to functionalize **7**. – *a)* Under several conditions [14], 1.76 g (3.11 mmol) of **7** were reacted with 0.85 mol-equiv. of sodium salt of diethyl acetaminomalonate, but only unchanged **7** was recovered.

b) In 20 ml of dry E_2O or THF, chips of Mg were preactivated with I_2 or small quantities of benzyl chloride and brought into contact with **7**, but never any typical *Grignard* reaction was observed with **7**.

c) To 20 ml of distilled toluene saturated with **7** were added either 1.5 mol-equiv. of BuLi-solution or Li or even Na, but no reaction was observed in any case.

Preparation of ethyl 2-(2',3',4',5',6'-pentabromobenzyl)acetoacetate (**8a**). In a two-neck flask (condenser, septum), 15 g of K and 500 ml of *t*-BuOH under N_2 were refluxed until all K had disappeared. Through the septum were injected 40.27 g (309.5 mmol) of ethyl acetoacetate. After heating under reflux for another 15 min, 70 g (123.8 mmol) of **7** in 500 ml of toluene were added, and refluxing was continued for 24 h. The solvent was evaporated, the residue resuspended in H_2O, neutralized with 1N HCl and extracted twice with hot toluene. The org. phase was dried over anh. $MgSO_4$, filtered and evaporated. The crude product was recrystallized from benzene/petroleum ether yielding 24.8 g (31%) of **8a**, m.p. 125°, sharp. TLC. (petroleum/toluene 2:3): Rf 0.145. – ^{1}H-NMR. ($CDCl_3$): 4.36–4.0 (*m*, 2 H); 3.81 (*s*, 2 H and 1 H); 2.27 (*s*, 3 H); 1.33–1.10 (*t*, 3 H). – ^{13}C-NMR. (*Bruker HFX-10*, 22.6 MHz); 13.91 and 62.02 (CH_3CH_2O); 28.99 (CH_3CO); 39.30 (C(*a*)); 57.34 (C(2)); 127.62, 128.21, 129.27, 129.36 (C(2'), C(3'), C(4'), C(5'), C(6')); 140.76/140.86 (C(1')); 168.81 (CH_3CO); 200.68/200.73 (*C*OOEt). The product was not further characterized.

Preparation of 3-(2,3,4,5,6-pentabromophenyl)propionic acid (**8b**). A solution of 5 g (8.13 mmol) of **8a** in a minimum of toluene was mixed with 100 ml of EtOH and 35 ml of 6N NaOH, stirred at r.t. for 2 days, and filtered. The filtrate was mixed with 200 ml of H_2O and extracted with 200 ml of toluene/AcOEt 1:1. The aq. phase was acidified to pH 3 with 1N HCl and extracted again. The org. phases were pooled, dried over anh. $MgSO_4$ and evaporated. The residue was recrystallized from hot toluene: 3.96 g (89.2%) of homogeneous **8b**, m.p. 245° (dec.). – IR. (nujol): 1410, 3400–2600, 1700 (acid).

For 5 h 200 mg of **8b** were hydrogenated in 25 ml of 90% AcOH over 100 mg of Pd/C. Extraction and workup gave 50 mg of a product identical to hydrocinnamic acid *(Aldrich)* (NMR., TLC., m.p. 47–49°).

Synthesis of **9** *by a-halogenation of* **8b** *and ammonolysis.* A mixture of 1 g (1.83 mmol) of **8b** with 20 ml of freshly distilled $SOCl_2$ and 10 ml of CCl_4 was refluxed for 30 min. A solution of 400 mg of *N*-bromosuccinimide in 10 ml of CCl_4, and 1 ml of conc. aq. hydrobromic acid were added and refluxed for additional 3 h. After cooling, the mixture was evaporated, resuspended with 50 ml of

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[4]) Catalytic hydrogenation [13] of Boc-**4** (5 mg) yielded a product identical to synthetic Boc-Phe (m.p., TLC., a_D).

thawing ice, extracted twice with $CHCl_3$, the org. phase dried over $MgSO_4$ and evaporated. This residue was not further characterized and immediately subjected to ammonolysis in 2 ml of DMF together with 50 mg of $(NH_4)_2CO_3$ and 4 ml of conc. ammonia. The flask was tightly closed and kept at 60° for 4 days. After evaporation and treatment with strong acid ion exchange resin, 15 mg of brown powder resulted. On TLC. several ninhydrin-positive spots were visible with a major spot at Rf 0.43 (B) or 0.63 (C). No further characterization was carried out due to the impurities and the excessively small yield.

Preparation of DL-*3-(2′,3′,4′,5′,6′-pentabromophenyl)alanine* (**9**, DL-*Phe(2′,3′,4′,5′,6′-Br*$_5$*)*). – *a)* In a 2-neck flask (magnetic stirrer, two septa), 4.52 g (32.4 mmol) of glycine ethyl ester were dissolved in 30 ml of CH_2Cl_2. Through one septum a N_2 inlet and outlet were installed and through the second 13.7 ml of Et_3N were added, followed by 6.1 g (27 mmol) of 1,2-bis[(chloro)dimethylsilyl]ethylene (*Petrach Systems*, Bristol, Pennsylvania) in 20 ml of CH_2Cl_2. After 2 h, the mixture was evaporated and the residue extracted twice with petroleum ether. This fraction was evaporated yielding 6.48 g or 98% of *N,N*-(2,5-dimethyl-2,5-disilahexane-2,5-diyl)glycine ethyl ester (= ethyl 2,2,5,5-tetramethyl-1-aza-2,5-disilolane-1-acetate). – ^{1}H-NMR. (CCl_4): 4.1 (*qa.* 2 H); 3.5 (*s*, 2 H); 1.3 (*t*, 3 H); 0.7 (*s*, 4 H); 0.0 (*s*, 12 H).

Under N_2, 34.4 mmol of diisopropylamine were mixed with 31.3 mmol of BuLi (1.6M in hexane). This mixture was diluted with 75 ml of freshly distilled THF and chilled to −78°. Then 6.39 g (26.1 mmol) of the above prepared glycine derivative were added. After 90 min at −78°, 14.8 g (26.1 mmol) of **7** were added, and the mixture was left overnight to warm up to r.t., and then extracted with ice cold 0.5N NH_4Cl at pH 9 and AcOEt. The org. layer containing **8d** was dried over Na_2SO_4, evaporated, and the residue was hydrolyzed in 100 ml of 6N HCl under reflux for 16 h. The acid was evaporated and the product isolated by strong acid ion exchange resin *Dowex 50 W-8* [12] followed by gel filtration on *Sephadex G10* (2 cm × 65 cm) eluted with 0.2N AcOH. A heterogeneous fraction was obtained (150 mg) with a major ninhydrin positive spot on TLC.: Rf 0.43 (B), or 0.63 (C). This fraction was not further purified nor analyzed because of the small yield.

b) At 0°, 4.1 g (18 mmol) of *N*-(diphenylmethylidene)aminoacetonitril (*Chemical Dynamics*, New Jersey), 0.5 g (1.6 mmol) of benzyltributylammonium chloride (*Aldrich*), 20 ml of 50% KOH-solution and 20 ml of toluene were stirred. Then, 12.2 g (21.6 mmol) of **7** were added over 15 min. Stirring was continued for 2 h at 0° and overnight at r.t. The mixture was extracted 3 times with CH_2Cl_2, the org. phase containing **8e** dried over $MgSO_4$, evaporated and resuspended in 100 ml of toluene. Under vigorous stirring, 200 ml of 1N HCl was added and stirring continued for 24 h. The aq. phase was separated, washed twice with CH_2Cl_2, and adjusted to 6N with conc. hydrochloric acid. After 24 h, the aq. phase was evaporated to dryness and the amino acid isolated with *Dowex 50 W-8* [12] yielding 7.3 g of brown powder. TLC.: heterogenous, major spot at Rf 0.44 (B) or 0.62 (C). The amino acid was not further purified and directly converted to Boc-**9**.

Preparation of DL-N-*(t-butoxycarbonyl)-3-(2′,3′,4′,5′,6′-pentabromophenyl)alanine (Boc-***9**, *Boc-Phe(2′,3′,4′,5′,6′-Br*$_5$*)).* The residue of the above reaction was dissolved in 300 ml of 1N NaOH/*t*BuOH 1:1. Then, 5.6 g (25 mmol) of Boc_2O were added, and the pH was maintained above 10 by occasional addition of 4N NaOH. After 2 days, ice was added, the mixture carefully acidified with $KHSO_4$ and extracted 3 times with AcOEt. The org. fractions were pooled, dried over Na_2SO_4 and evaporated to dryness. The residue was further purified by filtration over silica gel *60* (14 × 4.5 cm, 70–230 mesh, *Merck*) with $CHCl_3/CH_3OH$ 10:1. Recrystallization from CH_3OH/H_2O yielded 5.8 g (49%) of homogeneous Boc-**9**, m.p. 250° (dec.). TLC. (F): Rf 0.48. – ^{1}H-NMR. ($CDCl_3$): 9.0 (*s*, 1 H); 5.2–4.6 (*m*, 1 H); 3.8–3.6 (*m*, 2 H); 1.25 (*s*, 9 H).

In 10 ml dioxane/2N $AcONH_4$ 1:1 100 mg of Boc-**9** were hydrogenated over Pd/C (50 mg). After 2 h, the catalyst was filtered off, the mixture evaporated and extracted with AcOEt. The org. solvent was evaporated and the residue treated with 200 µl of TFA during 10 min. The amino acid was precipitated by the addition of 5 ml of anh. Et_2O and decanted. The residue was identical to phenylalanine on TLC. (Rf 0.26 (B) or 0.45 (C)) and by ^{1}H-NMR. (NaOD).

Peptide synthesis, general procedures. The Boc-protected amino acid (2 mmol) was esterified by the Cs-salt method [15] with 1 mol-equiv. of chloromethylated polystyrene resin, and the substitution degree was measured with the picric acid test [16]. The N^{α}-Boc-strategy was used on a manual peptide synthesizer with glass reaction vessels according to an earlier published schedule [17]. The following protected amino acids were used: Boc-Pro, Boc-His(*im*Tos), Boc-Val, Boc-Tyr(*O*-BrBzl), Boc-Arg(N^{δ}-Tos), Boc-Sar. A sixfold excess of each amino acid was used for the formation of symmetrical

anhydrides with DCC, and the completion of every coupling was tested with a ninhydrine test. Simultaneous cleavage of the side chain protecting groups and the resin ester was performed in liquid HF with 10% of anisole for 60 min at 0°, using a *Kel-F/Teflon* home built apparatus. The HF was evaporated with a stream of N_2 and the residues of HF and anisole were removed by applying high vacuum through a NaOH-filled trap. The crude peptides were extracted from the residue with 2N AcOH and lyophilized.

Preparation of Sar-Arg-Val-Tyr-Val-His-Pro-Phe(4'-I) (**11**, *[Sar^1,Phe(4'-I)^8]AT).* To start the synthesis, 1 g of Phe(4'-I)-O-resin (0.48 mequiv./g) was used. After completion and HF cleavage, the peptide has been filtered over *Sephadex G15* (2×68 cm) with 0.2N AcOH. The peptide fractions were pooled, lyophilized and purified by partition chromatography on *Sephadex G25* (2×75 cm) with the two-phase-system butanol/AcOH/H_2O 4:1:5. The pure fractions were determined by TLC. (D and E), pooled and lyophilized to give 261 mg of homogeneous **11**. Thereof, 5 mg were hydrogenated [13]: the product was indistinguishable from [Sar^1]AT (**10**).

Preparation of Sar-Arg-Val-Tyr-Val-His-Pro-Phe(3',5'-Br_2-4'-Cl) (**12**, *[Sar^1,Phe(3',5'-Br_2-4'-Cl)^8]-AT).* At the beginning of this synthesis, 0.9 g of Phe(3',5'-Br_2-4'-Cl)-O-resin (0.52 mmol/g) were used. After HF cleavage, the peptide was purified as above giving 191 mg of pure **12**. Catalytic hydrogenation [13] produced material identical to [Sar^1]AT (**10**), both in chemical and pharmacological tests.

Preparation of Sar-Arg-Val-Tyr-Val-His-Pro-Phe(2',3',4',5',6'-Br_5) (**13**, *[Sar^1,Phe(2',3',4',5',6'-Br_5)^8][AT).* To start this synthesis, 2.21 g of DL-Phe(2',3',4',5',6'-Br_5)-O-resin (0.22 mmol-equiv./g) have been used. After completion and cleavage with HF, the crude peptide was first filtered over *Sephadex G15* as already described. The peptide containing fractions were pooled, buffered with 1N $AcONH_4$ to pH 5 and loaded onto a reversed-phase column (*Macherey-Nagel*, 30 μ, spherical *C18* modified silica gel in a 2.2×30 cm column), which was eluted with a mean pressure of 6 atm with a linear gradient of 25–60% CH_3CN in 0.25M $AcONH_4$, pH 5.0, and 7% 2-propanol. The resulting product peak was split in the middle, and each fraction was eluted again with a gradient of 25 to 45% of CH_3CN. The second half peak produced a product which precipitated upon standing. The second chromatography produced peaks with one shoulder each, the pure fractions were collected and lyophylized. Of each fraction, 20 mg were hydrogenated [13] in 1M $AcONH_4$, pH 5 (HBr formation!), and the products compared on RP.-TLC. and in pharmacological tests to [Sar^1]AT and to [Sar^1,D-Phe^8]AT. The first fraction proved to be identical to [Sar^1]AT and the second was identical to [Sar^1,D-Phe^8]AT **14**. Yields; 55 mg of [Sar^1,Phe(2',3',4',5',6'-Br_5)^8]AT (L-**13**), 59 mg of [Sar^1,D-Phe(2',3',4',5',6'-Br_5)^8]AT (D-**13**) and 303 mg of unresolved diastereoisomeric mixture **13**.

Preparation of Sar-Arg-Val-Tyr-Val-His-Pro-D-Phe (**14**, *[Sar^1,D-Phe^8]AT).* At the beginning of this synthesis, 1.0 g of D-Phe-O-resin (0.33 mmol/g) was used. After HF cleavage, the peptide was purified by gel filtration over *Sephadex G15* and a prep. reversed-phase chromatography with a gradient of 10–30% CH_3CN in 0.25N $AcONH_4$, pH 7.0, and 5% 2-propanol. After a last gel filtration over *G15*, 161 mg of pure **14** were collected.

Biological activities. The tests *in vitro* on rabbit aorta strip and *in vivo* on the rat blood pressure were carried out as recently reported [11] and the results are presented in *Table 3.*

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