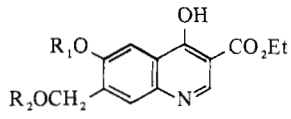
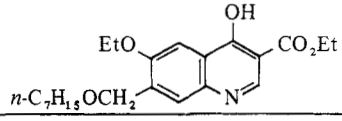
Table III



Compd	R_1	R_2	Yield purified, %	Mp, °C	Formula[a]	Weight ratio (day 5/day 0)[b] 0.01%	Weight ratio (day 5/day 0)[b] 0.001%	Oocyst count (day 7)[c] 0.01%	Oocyst count (day 7)[c] 0.001%	Fecal score (day 5)[d] 0.01%	Fecal score (day 5)[d] 0.001%
81	Me	Et	23	293.5	$C_{16}H_{19}NO_5$	1.18		1;1;0;0	1;1	1;1;1;1	3;3
82	Me	*i*-Bu	17	248	$C_{18}H_{23}NO_5$	1.17		1;1;1;1		2;1;2;3	
83	Me	*n*-C_7H_{15}	27	+300	$C_{21}H_{29}NO_5$	1.25		1;1;1;1	2;2;2;2	0;1;1;1	2;2;3;2
84	Me	*n*-C_8H_{17}	25	230	$C_{22}H_{31}NO_5$	1.25		0;0;0	3;2;2	1;1;1;1	2;2;1;3
85	Me	$CH_2C_6H_5$	29	231	$C_{21}H_{21}NO_5$	1.17		1;2;1;2		2;2;1;2	
86	Et	*i*-Bu	17	246	$C_{19}H_{25}NO_5$	1.24		1;2;2	2;2	1;1;1;0	1;2
87	Et	*n*-C_6H_{13}	31	222	$C_{21}H_{29}NO_5$	1.20		1;1;1;1	1;1;3;3	1;1;1;1	3;2;3;3
88	Et	*n*-C_7H_{15}	47	227	$C_{22}H_{31}NO_5$	1.24	1.23	0;0;1;1	0;0;2;2	1;0;1;1	1;1;1;1
89	Et	*n*-C_8H_{17}	31	226	$C_{23}H_{33}NO_5$	1.26	1.23	0;0;0;0	0;0;1;1	0;1;1;1	1;1;2;1
90	Et	*n*-C_9H_{19}	12	225	$C_{24}H_{35}NO_5$	1.22		1;1;0;0	0;0;2;2	1;1;0;0	1;1;3;3
91	Et	*n*-$C_{11}H_{23}$	31	222	$C_{26}H_{39}NO_5$	1.21		1;1;1;1	1;1;1;1	1;1;1;1	1;1;1;2
92	Et	$CH_2CH_2C_6H_5$	56	229	$C_{23}H_{25}NO_5$	1.25		1;1;2;2	1;1;2;2	0;0;2;1	2;1;1;2
93	*n*-Bu	*n*-Pr	33	227	$C_{20}H_{27}NO_5$	1.19		1;1;1;1	1;1;2;2	1;2;1;1	1;1;2;1
94	*n*-Bu	*n*-C_7H_{15}	28	217	$C_{24}H_{35}NO_5$	1.20		1;1;1;1	1;1;2;2	2;1;1;1	2;2;1;1
95	*n*-Bu	*n*-C_8H_{17}	32	215	$C_{25}H_{37}NO_5$	1.20		0;0;1;1	1;1;1;1	0;1;1;0	1;1;1;3
96	*n*-Bu	*n*-C_9H_{19}	25	213	$C_{26}H_{39}NO_5$	1.17		0;0;1;1	1;1;2;2	1;1;1;1	1;1;1;1
97	*n*-C_7H_{15}	Et	32	221	$C_{22}H_{31}NO_5$	1.18		1;2;2;2	1;1	1;3;1;1	3;3
98	*n*-C_7H_{15}	$CH_2C_6H_5$	27	219	$C_{27}H_{33}NO_5$	1.18		1;1;1;1	1;1;1;1	1;2;0;1	1;1;2;3
99	*n*-$C_{10}H_{21}$	Et	31	217	$C_{25}H_{37}NO_5$	1.30		1;1;1;1	1;1;1;1	0;0;1;1	1;1;1;1
100	*n*-$C_{10}H_{21}$	*n*-Pr	43	213	$C_{26}H_{39}NO_5$	1.19		2;1	3;3	3;1	3;3
101	*n*-$C_{10}H_{21}$	*n*-C_5H_{11}	23	205	$C_{28}H_{43}NO_5$	1.20		2;2;1;1	1;1;1;1	2;1;1;2	1;1;2;2
1a	Decoquinate (ethyl 6-*n*-decyloxy-7-ethoxy-4-hydroxy-3-quinoline-carboxylate)					1.24		0;0;0;0	0;0;1;1	1;0;1;1	1;1;1;0
1b	Nequinate (methyl 7-benzyloxy-6-*n*-butyl-4-hydroxy-3-quinoline-carboxylate)					1.24	1.19	0;0;1;0	0;1;2;1	0;1;1;0	0;0;2;1
1c	Amquinate (methyl 7-diethylamino-4-hydroxy-6-*n*-propyl-3-quinolinecarboxylate)					1.22		0;1;1;1	1;1;3;1	0;0;1;0	1;2;3;1

[a] All compounds were analyzed for C, H, and N. Compound **97**, C: −0.54; **99**, C: −0.64; **100**, C: +0.47. [b] Refer to chemotherapy. [c] 0, no oocysts in feces; 1, 0–5 × 10^4 oocyst/g of feces; 2, 5 × 10^4–1 × 10^5 oocyst/g of feces; 3, 10^5–2 × 10^5 oocyst/g of feces; 4, more than 2 × 10^5 oocyst/g of feces. [d] 0, normal feces; 1, soft to normal feces; 2, fluid droppings with some mucous casts; 3, slimy, greyish, mucoid diarrhea.

Table IV. Chemotherapeutical Results of



Eimeria strain	Treatment	Mean weight ratio: Noninfected chicks	Infected chicks	Infected chicks treated with 0.01% of **88**	Infected chicks treated with 0.001% of **88**	Infected chicks treated with 0.001% of **1b**
Acervulina[a]	Simultaneous	1.33	1.04	1.24	1.23	1.19
	Prophylactic	1.60	1.16		1.50	1.50
Brunetti[b]	Simultaneous	1.42	1.14	1.40	1.42	1.41
	Prophylactic	1.65	1.14		1.66	1.62
Tenella[c]	Simultaneous	1.34	1.19	1.53	1.51	1.49
	Prophylactic	1.78	1.33	1.79	1.74	1.72

[a] Results of the 5th day. [b] Results of the 6th day. [c] Results of the 7th day.

filtered and triturated with Me_2CO for 1 hr. The precipitate was collected and dried *in vacuo* to give 5.5 g (47%) of **88**, mp 227°. *Anal.* ($C_{22}H_{31}NO_5$) C, H, N.

Acknowledgment. The authors are indebted to Mr. F. Sels for the C, H, and N analyses and to Mr. P. Demoen for the other analyses. The work described in this publication is part of a program supported by a grant from the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (IWONL).

References

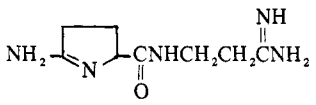
(1) C. F. Spencer, A. Engle, Chia-Nien Yu, R. C. Finch, E. S. Watson, F. F. Ebetino, and C. A. Johnson, *J. Med. Chem.*, **9**, 934 (1966).
(2) R. A. Bowie, J. P. Cavins, M. S. Grant, A. Hayes, W. G. M. Jones, and J. F. Ryley, *Nature (London)*, **214**, 1349 (1967).
(3) S. J. Ball, M. Davies, J. N. Hodgson, J. M. S. Lucas, E. W. Parnell, B. W. Sharp, and D. Warburton, *Chem. Ind. (London)*, 56 (1968).
(4) R. H. Mizzoni, F. Goble, E. Konopka, J. Gelzer, J. Szanto, D. C. Maplesden, J. E. Brown, J. Boxer, G. Zaunius, J. B. Ziegler, and G. de Stevens, *J. Med. Chem.*, **13**, 870 (1970).

Synthesis and Antiviral Activity of Homologs of Noformycin

Guy D. Diana,* Urano J. Salvador, Ethel S. Zalay, and Francis Pancic

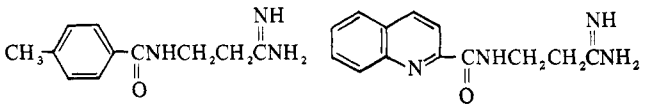
Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received February 28, 1973*

In the past two decades, several papers have appeared on the antiviral activity of noformycin[1–7] **(1)** obtained from a culture of *Nocardia formica.* Among the viruses reportedly



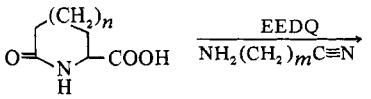
1

susceptible to this compound were swine influenza and sk poliomyelitis. In our laboratories, noformycin has also exhibited *in vitro* activity against equine rhinovirus, human rhinovirus type 2, and parainfluenza type 3. In view of its broad spectrum of activity, we became interested in preparing homologs in the hope of retaining activity and lowering toxicity. Ueda, *et al.*,[8] reported on the synthesis of compounds possessing the amidinoethyl carboxamide side chain, with compounds **2** and **3** in their series being the most active.

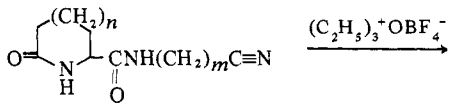


2 3

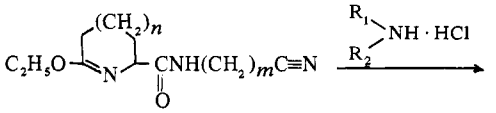
However, no biological data have been published. Our intention was to make slight modifications of the noformycin structure. We recently developed a synthesis of noformycin† which lends itself to the preparation of homologs of type **8**.



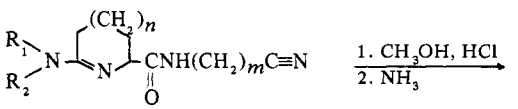
4



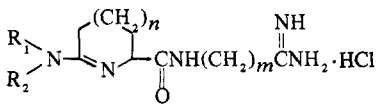
5



6



7



8

(±)-2-Piperidone-6-carboxylic acid (**4**, $n = 1$) was prepared from α-aminoadipic acid according to the procedure of Greenstein.[9] In our synthesis of noformycin, we originally utilized DCC as the amidating agent. For the preparation of **5**, however, we subsequently found that *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was a superior reagent for this reaction. The reaction of **5** with a slight excess of triethyloxonium fluoroborate produced **6**. This selective ethylation of lactams in the presence of secondary amides was also recently reported by Kato, *et al.*[10]

Imino esters **6** were converted to the cyclic amidines **7** *via* reaction with the appropriate amine hydrochlorides in refluxing ethanol. Conversion of nitrile **7** to the amidines **8** was achieved by the Pinner synthesis.[11]

† G. D. Diana, unpublished results.

Biology. Antiviral effects of the analogs were evaluated in a tissue culture system of a serially propagated human amnion cell line (CATR) established at Sterling-Winthrop Research Institute and grown in stationary tubes.

Human rhinovirus type 2 was selected as the challenge virus, using 100 $TCID_{50}$'s per tube. The virus was added to 3-day-old cultures and permitted to absorb for 1 hr at 35° After the virus absorption, appropriate concentrations of the compounds were added to the tubes in maintenance medium of M – 199 + 5% inactivated fetal calf serum. The cultures were then incubated at 32° for 5 days, and the presence or absence of viral CPE (cytopathic effect) was read.

Toxicity of each analog to the cell system was observed simultaneously in a parallel test. The cells were treated with identical concentrations of the compound in the absence of virus. Only those concentrations which produced no visible toxic effect upon microscopic examination at the end of 5 days were considered for evaluation. The lowest concentration of the compound which completely inhibited viral CPE was designated as MIC (minimum inhibitory concentration).

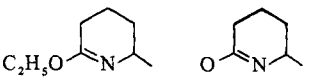
The antiviral activity "*in vitro*" is summarized in Tables I-III. The only compounds which exhibited any appreciable activity against human rhinovirus type 2 were **7b**, **7e**, **8a**, and **8d**. However, when compared to noformycin, these compounds were significantly less active. The most interesting observation is that the slightest modification of the noformycin structure drastically lowers the activity (Table III). This leads one to speculate that noformycin fulfills a very rigid structural requirement for activity.

Experimental Section

Melting points were taken on a Fisher-Jones melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of the theoretical values. Analyses were performed by Instranal Laboratories, Rensselaer, N. Y.

Amides of Table I. *N*-(2-Cyanoethyl)-6-oxopipecolamide (5a). To a solution of 12.8 g (0.09 mol) of 2-piperidone-6-carboxylic acid and 24.2 g (0.099 mol) of EEDQ in 250 ml of EtOH was added 6.3 g (0.09 mol) of β-aminopropionitrile in 5 ml of EtOH. The mixture was heated to 50° for 2.5 hr and concentrated to dryness and the residual gum was stirred with 600 ml of Et_2O. A solid gradually formed as the flask was cooled. The material was collected and recrystallized from 2-propanol. Material (14.2 g) was obtained which melted at 118–120°. *Anal.* ($C_9H_{13}N_3O_2$) C, H, N.

***N*-(2-Cyanoethyl)-6-ethoxy-2,3,4,5-tetrahydropicolinamide (6) (n, m = 1).** To a solution of triethyloxonium fluoroborate prepared from 11.1 g (0.12 mol) of epichlorohydrin and 20.7 g (0.144 mol) of BF_3 etherate in 500 ml of CH_2Cl_2 was added 19.5 g (0.1 mol) of **5a**. The suspension was stirred overnight, during which time the solid dissolved and an oil separated. To the mixture was added 20 g of 50% aqueous K_2CO_3. After the addition was complete, the solid was removed by filtration, the filtrate was dried, and the solid obtained after removal of the solvent was recrystallized from EtOAc: yield 16.1 g; mp 118–120°; mass spectrum *m/e* 223, abundant fragments at 126 (structure a) and 98 (structure b). *Anal.* ($C_{11}H_{17}N_3O_2$)

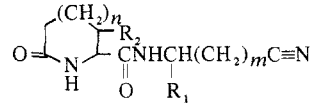


a b

C, H, N. In many cases, the imino esters could not be purified and were used directly.

Amidines of Table II. (±)-6-Amino-*N*-(2-cyanoethyl)-2,3,4,5-tetrahydropicolinamide Hydrochloride (7f). A suspension of 11 g (0.05 mol) of **6** (n, m = 1) and 3.74 g (0.07 mol) of NH_4Cl in 125 ml of MeOH was refluxed for 7 hr. The solution was concentrated to dryness and the residual solid recrystallized from 2-propanol after removal of the undissolved NH_4Cl; 10.7 g was obtained, mp 154–156°. *Anal.* ($C_9H_{14}N_4O \cdot HCl$) C, H, N.

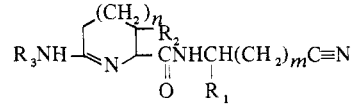
Table I



Compd no.	n	m	R_1	R_2	Mp, °C	Crystn solvent[a]	Yield, %	Formula	Analyses	Antiviral activity against HRV type 2,[b] μg/ml
5a	1	1	H	H	118–120	A	58	$C_9H_{13}N_3O_2$	C, H, N	Inactive
5b	0	0	H	H	114–116	A	77	$C_7H_9N_3O_2$	C, H, N	Inactive
5c	0	1	H	H	114–115	A	72	$C_8H_{11}N_3O_2$	C, H, N	Inactive
5d	0	3	H	H	78–80	B	78	$C_{10}H_{15}N_3O_2$	C, H, N	Inactive
5e	0	1	H	CH_3	159–163	A	67	$C_9H_{13}N_3O_2$	C, H, N	Inactive

[a]Crystallization solvents: 2-propanol = A; acetone–ethyl acetate = B; ethanol = C; 2-propanol–ether = D; methanol–water = E; DMF–water = F; methanol–ether = G; ethanol–water = H; 2-propanol–water = I; methanol = J. [b]HRV = human rhinovirus.

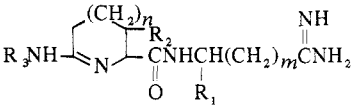
Table II



Compd no.	n	m	R_1	R_2	R_3	Mp, °C	Crystn solvent[a]	Yield, %	Formula	Analyses	Antiviral activity against HRV type 2,[b] μg/ml
7a	0	0	H	H	H	169–171	C	60	$C_7H_{10}N_4O\cdot HCl$	C, H, N	Inactive
7b	0	1	H	H	H	138.5–139.5	A	60	$C_8H_{12}N_4O\cdot HCl$	C, H, N	100
7c	0	2	H	H	H	144–146	A	65	$C_9H_{14}N_4O\cdot HCl\cdot 0.5H_2O$	C, H, N	Inactive
7d	0	3	H	H	H	129–130	A	37	$C_{10}H_{16}N_4O\cdot HCl$	C, H, N	Inactive
7e	0	1	H	CH_3	H	159–162	A	92	$C_9H_{14}N_4O\cdot HCl$	C, H, N	100
7f	1	1	H	H	H	154–156	A	54	$C_9H_{14}N_4O\cdot HCl$	C, H, N	Inactive
7g	0	1	H	H	CH_3	141–143	A	66	$C_9H_{14}N_4O\cdot HCl$	C, H, N	Inactive
7h	0	1	H	H	C_2H_5	127–129	D	70	$C_{10}H_{16}N_4O\cdot HCl$	C, H, N	Inactive
7i	0	1	H	H	n-C_3H_7	103–105	D	77	$C_{11}H_{18}N_4O\cdot HCl$	C, H, N	Inactive
7j	0	1	CH_3	H	H	190–192	A	20	$C_9H_{14}N_4O\cdot HCl$	C, H, N	200

[a]See footnote *a*, Table I. [b]HRV = human rhinovirus.

Table III



Compd no.	n	m	R_1	R_2	R_3	Mp, °C	Crystn solvent[a]	Yield, %	Formula	Analyses	Antiviral activity against HRV type 2,[b] μg/ml
8a	0	0	H	H	H	120–121	C	50	$C_7H_{13}N_5O\cdot HCl\cdot H_2O$	C, H, N	50
8b	0	2	H	H	H	248 dec	E	39	$C_9H_{17}N_5O\cdot H_2SO_4\cdot H_2O$	C, H, N	Inactive
8c	0	3	H	H	H	239–241	F	25	$C_{10}H_{19}N_5O\cdot H_2SO_4$	C, H, N	Inactive
8d	1	1	H	H	H	245–247	G	89	$C_9H_{17}N_5O\cdot HCl$	C, H, N	100
8e	0	1	CH_3	H	H	257–260	E	23	$C_9H_{17}N_5O\cdot H_2SO_4$	C, H, N	Inactive
8f	0	1	H	CH_3	H	260 dec	H	26	$C_9H_{17}N_5O\cdot H_2SO_4\cdot H_2O$	C, H, N	Inactive
8g	0	1	H	H	CH_3	107–109	I	27	$C_9H_{17}N_5O\cdot 2HCl\cdot H_2O$	C, H, N	Inactive
8h	0	1	H	H	C_2H_5	254–256	J	18	$C_{10}H_{21}N_5O\cdot H_2SO_4\cdot 0.5H_2O$	C, H, N	Inactive
8i	0	1	H	H	n-C_3H_7	259–260	C	49	$C_{11}H_{21}N_5O\cdot H_2SO_4$	C, H, N	Inactive
Noformycin											1.5

[a]See footnote *a*, Table I. [b]HRV = human rhinovirus.

Amidines of Table III. (±)-6-Amino-*N*-(2-amidinoethyl)-2,3,4,5-tetrahydropicolinamide Dihydrochloride (8d). **7f** (14 g, 0.061 mol) was stirred with 60 ml of MeOH saturated with HCl in an ice bath for 2 hr and the resulting solution left overnight in the refrigerator. The solution was poured into 500 ml of Et_2O. The ether was decanted from the precipitated gum and the latter stirred several times with cold ether, with subsequent decantation of the ether. The gum was then dissolved in 150 ml of MeOH and 9% methanolic ammonia was added dropwise to the stirred solution until the pH was 9. The solution was left at room temperature overnight. The solid which separated was removed by filtration and the filtrate concentrated to dryness. The residual solid was recrystallized from EtOH giving 12.5 g, mp 245–247°. *Anal.* ($C_9H_{17}N_5O\cdot HCl$) C, H, N.

References

(1) D. A. Harris and H. B. Woodruff, *Antibiot. Annu.*, 609 (1953–1954).
(2) T. Fukushi, *Nippon Shokubutsu Byori Gakkaiho*, **21**, 1 (1955).
(3) R. C. Linder, H. C. Kirkpatrick, and T. E. Weeks, *Phytopathology*, **49**, 802 (1959).
(4) E. Furusawa, *Kagaku Ryoho*, **8**, 95 (1964).
(5) E. Furusawa, W. Cutting, P. Buckley, and S. Furusawa, *Proc. Soc. Exp. Biol. Med.*, **116**, 938 (1964).
(6) E. Furusawa, S. Furusawa, and W. Cutting, *Med. Pharmacol. Exp.*, **12**, 259 (1965).
(7) S. Toyoshima, Y. Seto, and K. Saito, *Nippon Kagaku Ryohogal-*

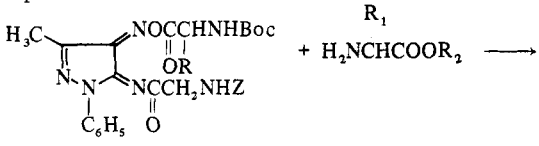
sulsai Zasshi, **14,** 457 (1966).
(8) T. Ueda, Y. Okamoto, T. Tsuji, and M. Muraska, *Chem. Pharm. Bull.*, **16,** 2355 (1968).
(9) J. P. Greenstein, S. M. Birnbaum, and M. C. Otey, *J. Amer. Chem. Soc.*, **75,** 1994 (1953).
(10) T. Kato, A. Tuksada, and T. Ueda, *Chem. Pharm. Bull.*, **20** (5), 901 (1972).
(11) A. W. Dox, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1932, p 5.

Synthesis of Bradykinin *via* Oximinopyrazoline Active Esters (OPmp)†

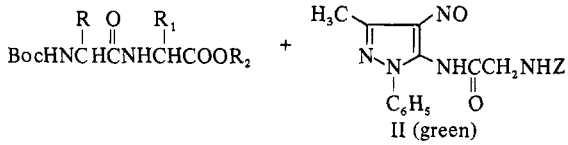
Roberto Tomatis, Roberto Ferroni, Mario Guarneri, and Carlo A. Benassi*

Istituto di Chimica Farmaceutica, Universita di Ferrara, Ferrara, Italy. *Received February 23, 1973*

The present communication reports a stepwise synthesis of bradykinin, employing oximinopyrazolineamino acid active esters (OPmp, I).[2a] These esters were allowed to react with amino acid or peptide esters according to the following equation.[2-4]



I (red)



II (green)

The synthesis was carried out *via* a stepwise procedure; protection of functional groups was planned in view of a final cleavage with HF.[5] The masking of the serine hydroxyl group could be avoided since no reaction of OPmp active esters upon such a function has been observed.[3] Very high rates (between 2 and 4 min) and satisfactory yields were achieved in the formation of all peptide bonds.

At each stage, the peptide was isolated from the nitrosoacylamine II, taking advantage of the fact that the former is insoluble in ethyl ether whereas the latter is soluble in the same solvent. After deprotection with HF at 0° for 30 min, the bradykinin trifluoride was desalted by passing its aqueous solution through a column of Amberlite CG 400; the product was then converted to the acetate form and lyophilized. The nonapeptide had optical rotation and elemental and amino acid analyses corresponding to those of the authentic product;[5,6] its biological activity was tested in comparison with a sample of bradykinin taken as standard.[7]

Experimental Section

Melting points were taken on a Tottoli capillary melting point apparatus and are uncorrected. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Times required for complete condensation to occur were 4 min for protected peptides **1, 4,** and **8** and 2 min for protected peptides **2, 3, 5, 6,** and **7.** The intermediates reported below were recrystallized from the appropriate solvents until homogeneous at tlc in two different solvent systems: 1-butanol–glacial acetic acid–water (3:1:1); ethyl acetate–pyridine–glacial acetic acid–water (60:20:6:14).

Boc-Phe-Arg(NO_2)-OBzl (1). To a solution of Arg(NO_2)-OBzl[8] (1.54 g, 5 mmol) in chloroform (60 ml), the red active ester Boc-Phe-OPmp[4] (3.20 g, 5 mmol) was added. When the solution became green, it was evaporated; the residue was triturated with ethyl ether and then crystallized from ethyl acetate–ethyl ether to yield 2.20 g (80%) of **1:** mp 156–157°; $[\alpha]^{25}_{578}$ −18.93° (*c* 0.48, $CHCl_3$) *Anal.* ($C_{27}H_{36}N_6O_7$) C, H, N.

Boc-Pro-Phe-Arg(NO_2)-OBzl (2). Compound **1** (1.66 g, 3 mmol) was allowed to react with TFA (5 ml) for 20 min at room temperature; the resulting tripeptide trifluoroacetate was precipitated and washed with ethyl ether and then dried *in vacuo* over sodium hydroxide. A solution of the salt (1.71 g, 3 mmol) in chloroform (60 ml) was treated with triethylamine (0.42 ml, 3 mmol) and Boc-Pro-OPmp[4] (1.70 g, 3 mmol). After 2 min the green solution was washed with citric acid (5%) and water and dried over Na_2SO_4 Evaporation gave a solid that was triturated with ether and crystallized from ethyl acetate–ethyl ether to give **2** (1.57 g, 80%): mp 86–89°; $[\alpha]^{25}_{578}$ −48.06° (*c* 0.69, $CHCl_3$). *Anal.* ($C_{32}H_{43}N_7O_8$) C, H, N.

Using the above procedure for the subsequent steps of cleavage and condensation, the stepwise peptide elongation was carried out by means of molar amounts of OPmp active esters[4] of the required amino acid; the following protected peptides were obtained.

Boc-Ser-Pro-Phe-Arg(NO_2)-OBzl (3): yield 81%; mp 99–100°; $[\alpha]^{25}_{578}$ −39.15° (*c* 0.75, ethyl acetate). *Anal.* ($C_{35}H_{48}N_8O_{10}$) C, H, N. This and the following intermediates were recrystallized from ethyl acetate–ethyl ether.

Boc-Phe-Ser-Pro-Phe-Arg(NO_2)-OBzl (4): yield 82%; mp 113–116°; $[\alpha]^{25}_{578}$ −42.16° (*c* 0.53, ethyl acetate). *Anal.* ($C_{44}H_{59}O_{11}N_9$) C, H, N.

Boc-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-OBzl (5): yield 85%; mp 117–121°; $[\alpha]^{25}_{578}$ −40.39° (*c* 0.59, DMF). *Anal.* ($C_{46}H_{60}N_{10}O_{12}$) C, H, N.

Boc-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-OBzl (6): 86%; mp 119–122°; $[\alpha]^{25}_{578}$ −49.12° (*c* 0.58, DMF). *Anal.* ($C_{51}H_{67}N_{11}O_{13}$) C, H, N.

Boc-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-OBzl (7): yield 84%; mp 127–132°; $[\alpha]^{25}_{578}$ −45.89° (*c* 0.50, DMF). *Anal.* ($C_{36}H_{74}O_{12}N_{14}$) C, H, N.

Boc-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO_2)-OBzl (8): yield 79%; mp 138–142°; from chloroform–ethyl ether; $[\alpha]^{25}_{578}$ −43.57° (*c* 0.50, DMF). *Anal.* ($C_{62}H_{85}N_{17}O_{17}$) C, H, N.

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH · 3CH_3COOH (Bradykinin Triacetate). The protected nonapeptide **8** (0.134 g, 0.1 mmol) was placed in a HF reaction cylinder with anisole[5,9] (0.11 ml, 10 equiv) and HF (5 ml) was added. The mixture was allowed to react at 0° for 30 min with stirring. Excess HF was removed under reduced pressure at 0° and then with introducing a nitrogen stream. The residue of the reaction was dissolved in water (10 ml); the solution was extracted with ethyl ether to eliminate anisole, passed through a column (0.8 × 9 cm) of Amberlite CG 400 (OH^-) type II, and eluted with distilled water (about 40 ml) until the pH of the washing became 7. The eluate was collected in a flask containing acetic acid (2 ml) and lyophilized; 93 mg (70%) of product as triacetate pentahydrate was obtained: mp 162–170°; $[\alpha]^{20}D$ −81.1° (*c* 0.293, water). *Anal.* ($C_{50}H_{73}N_{15}O_{11}\cdot 3CH_3COOH\cdot 5H_2O$) C, H, N.

Its homogeneity was tested by paper electrophoresis (pH 3.5 and 8.5) and tlc (BuOH–AcOH–H_2O, 2:1:1) using ninhydrin and chlorine reagents. Amino acid analysis after hydrolysis (6 *N* HCl) gave Arg 1.96, Pro 2.96, Gly 1.00, Phe 1.94, and Ser 1.00. Biological activities in comparison with a sample of bradykinin (Sandoz), taken equal to 100, were as follows: rat uterus stimulation 100–120; guinea-pig ileum stimulation 90–100; dog hypotensive effect 100–110; rat duodenum inhibition 100–105.[7]

Acknowledgment. We are very grateful to Professor G. Bertaccini of the Istituto di Farmacologia dell'Universita di Parma for pharmacological assays. In addition we thank Sandoz Ltd, Basle, Switzerland, for a reference standard sample of bradykinin (BRS-640). This investigation was supported by the Italian Research Council (CNR).

†OPmp = 1-phenyl-3-methyl-4-oximino-5-(*N*-Z-glycyl)imino-2-pyrazoline esters. The following abbreviations are also used (see ref 1): Z = benzyloxycarbonyl; Boc = *tert*-butyloxycarbonyl; OBzl = benzyl ester; TFA = trifluoroacetic acid; DMF = dimethylformamide.

References

(1) IUPAC-IUB Commission on Biological Nomenclature, *Eur. J. Biochem.*, **1,** 375 (1967).
(2) (a) M. Guarneri, P. Giori, and C. A. Benassi, *Tetrahedron Lett.*,