Potential Antiviral Agents. Carbobenzoxy Di- and Tripeptides Active against Measles and Herpes Viruses

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A large number of carbobenzoxy dipeptides, several tripeptides, and a number of alkyl, cycloalkyl, aryl, and heterocyclic amide derivatives of carbobenzoxy-L- and -D-phenylalanine have been synthesized. Many of the peptides were found to be active against measles and herpes viruses.

During the course of an investigation into the synthesis of biologically active peptides, it was found that an intermediate dipeptide, carbobenzoxy-L-phenylalanylnitro-L-arginine,[1] displayed significant activity against measles virus propagated by tissue culture in plastic cups. The unusual activity displayed by this simple dipeptide was of considerable interest to us, since peptides of this size have been devoid of any biological activity.[2] Since that initial finding, we have made a detailed study of the synthesis and biological activity (Table I) of a large number of carbobenzoxy dipeptides, several carbobenzoxy tripeptides, and some amide derivatives of carbobenzoxyphenylalanine.

We initially assumed that the nitroarginine portion of the molecule was mainly responsible for its biological activity, since it could be viewed as an unnatural amino acid. The synthesis of several nitroarginine dipeptides (Table II) and their lack of antiviral activity led us to the preparation of a somewhat random series of dipeptides containing carbobenzoxy-L- and -D-phenylalanine. A number of these compounds had high levels of antiviral activity *in vitro* against measles virus and a few were active against herpes virus. The importance of the carbobenzoxy group as an integral part of the active molecule was soon verified. Dipeptides without a protecting group had very weak or no activity, and substitution of other commonly used peptide-protecting groups also afforded inactive compounds. Expansion of the synthetic effort to encompass a large number of carbobenzoxy dipeptides containing D-amino acids and a limited series of carbobenzoxy tripeptides was carried out. A variety of simple amide derivatives of carbobenzoxy-L- and -D-phenylalanine was prepared, since it was evident that this fragment was of vital importance for antimeasles activity and, also, as a possible lead into other types of antiviral activity.

Chemistry.—The procedure used for the synthesis of the majority of the dipeptides was the mixed-anhydride method which has found wide application in the preparation of simple peptides because of its simplicity.[3] The product of this reaction, the carbobenzoxy dipeptide methyl ester, was treated with methanolic NaOH to remove the ester group. All of the amide derivatives of carbobenzoxy-L- and -D-phenylalanine (Table III) were obtained using the mixed-anhydride method. The *p*-nitrophenyl ester method[4] was frequently employed. The azide and the dicyclohexylcarbodiimide methods and Woodward's[5] reagent K were employed occasionally. Typical procedures for these methods are to be found in the Experimental Section. The use of DL-amino acids was kept to a minimum since stereochemical–activity relationships would be difficult to interpret, and the eventual synthesis of the separate isomers would be inevitable.

Biological Results.[6]—Carbobenzoxy peptides showing antiviral activity in the plastic cup or plaque-reduction tests are listed in Table I.[7] With three exceptions, all of the peptides active against measles have D-phenylalanine in the molecule, and most of them have an adjacent carbobenzoxy function. It is of interest that both carbobenzoxy-D- and -L-phenylalanines were found to be inactive. The quantitative activity differences among this group of compounds are small, and it is probable that they all act by the same mechanism. A few of the peptides, active against measles, were also active against the distemper virus in embryonated eggs. The most active compounds found against distemper were carbobenzoxy-D-phenylalanylnitroarginine and carbobenzoxy-D-phenylalanyl-D-alanine. The similarity of the two viruses may account for this, but it is apparent that the structural modifications are more restricted for activity in the distemper test.

The dipeptide methyl esters were routinely tested and a few of them were active against herpes. Structure–activity relationships with the herpes virus are difficult to assess. The correlation between the plastic cup and the plaque test is questionable. At best, it can be inferred that the sulfur-containing amino acids are the most interesting in this series. Three peptides were found to have significant activity in the plastic cup test against simian B virus. Carbobenzoxyphenylalanylnitroarginine had a virus rating of 1.4, its D-phenylalanine isomer 3.1, and carbobenzoxy-D-phenylalanyl-D-phenylalanine was 2.9 in this test. The activity could not be confirmed in the plaque test.

(1) K. Hofmann, W. D. Peckham, and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).

(2) Glutathione, anserine, and carnosine could be possible exceptions. The tetrapeptide from gastrin was reported some time after our initial observation.

(3) E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, Inc., New York, N. Y., 1965, p 85.

(4) M. Bodanszky, *Nature*, **175**, 685 (1955).

(5) R. B. Woodward, R. A. Olofson, and H. J. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).

(6) A more detailed description of the biological effects of some of the peptide on measles virus is in preparation by F. A. Miller, G. J. Dixon, G. Arnett, J. R. Dice, W. A. Rightsel, F. M. Schabel, Jr., and I. W. McLean, Jr.

(7) We are indebted to Dr. W. A. Rightsel and F. A. Miller for the test results reported here. For a detailed explanation of these tests the reader should see J. Ehrlich, H. J. Sloan, F. A. Miller, and H. E. Machamer, *Ann. N. Y. Acad. Sci.*, **130**, 5 (1965). In brief, the higher the virus rating (plastic cup test) the more active the compound (these virus ratings are an average of several tests). The measles and herpes plaque reduction tests are expressed as per cent reduction of plaque-forming units.

TABLE I

CARBOBENZOXY DI- AND TRIPEPTIDES ACTIVE AGAINST MEASLES AND HERPES VIRUSES

Compd[a]	Mp, °C	Method[b]	Formula	N, % Calcd	N, % Found	Virus rating[c]	Measles plaque test[c] Undiluted	3.2 ×	10 ×	32 ×	100 ×
Z-Phe-(NO2)Arg[d]	174–176	A	$C_{23}H_{28}N_6O_7$	16.79	16.81	2.2	100	100	90	41	7
Z-D-Phe-(NO2)Arg	143–145	A	$C_{23}H_{28}N_6O_7$	16.79	16.89	2.1	80	52	0	..	...
Z-DL-Phe-(NO2)Arg	137–139	C	$C_{23}H_{28}N_6O_7$	16.79	16.96	2.3	99	70	11	10	...
Z-Phe-(NO2)-D-Arg	135–137	C	$C_{23}H_{28}N_6O_7$	16.79	16.76	2.4	...	...	...	..	...
Z-D-Phe-Ala	116–118	A	$C_{20}H_{22}N_2O_5$	7.56	7.67	1.5	100	78	81	79	53
Z-D-Phe-D-Ala	165–167	A	$C_{20}H_{22}N_2O_5$	7.56	7.59	1.2	80	47	28	7	16
Z-D-Phe-D-Leu[e]	138–139	C	$C_{23}H_{28}N_2O_5$	6.79	6.88	0.95	100	90	68	57	0
Z-D-Phe-Met	115–117	A	$C_{22}H_{26}N_2O_5S \cdot 0.5H_2O$	6.39	6.39	1.5	97	93	78	40	98
Z-D-Phe-D-Met	106–109	A	$C_{22}H_{26}N_2O_5S$	6.51	6.30	2.8	100	100	79	42	+15
Z-Phe-D-Phe	100–102	C	$C_{26}H_{26}N_2O_5 \cdot 0.5H_2O$	6.15	5.98	0.5	100	100	33	0	0
Z-D-Phe-D-Phe[f]	156–157	A	$C_{26}H_{26}N_2O_5$	6.28	6.44	1.7	98	98	99	84	27
Z-D-Phe-Ser	136–138	A	$C_{20}H_{22}N_2O_6$	7.25	7.23	2.9	80	24	+5	17	22
Z-D-Phe-D-Ser	155–157	A	$C_{20}H_{22}N_2O_6$	7.25	7.12	2.3	98	88	82	61	66
Z-D-Phe-D-Thr	168–170	A	$C_{21}H_{24}N_2O_6$	7.00	7.01	1.6	94	10	0	0	...
Z-D-Phe-D-Trp	160–163	A	$C_{28}H_{27}N_3O_5$	8.66	8.67	1.3	100	87	44	0	0
Z-D-Phe-D-Tyr	168–170	A	$C_{26}H_{26}N_2O_6$	6.06	6.03	3.6	100	100	100	0	0
Z-D-Phe-D-Val	144–146	C	$C_{22}H_{26}N_2O_5$	7.03	7.13	1.1	90	92	46	25	0
Z-D-Phe-Phe-D-Ala	85–90	C	$C_{28}H_{30}N_3O_6$	8.33	8.13	1.8	53	40	35	..	...
Z-D-Phe-Phe-(NO2)Arg	105–107	A, C	$C_{32}H_{39}N_7O_9$	14.76	14.70	2.4	100	100	100	94	66
Z-Phe-D-Phe-D-Tyr	183–185	A, C	$C_{35}H_{36}N_3O_7$	6.89	6.93	0.7	95	71	43	..	...
Z-D-Phe-Phe-D-Val	95–100	C	$C_{31}H_{38}N_3O_6$	7.69	7.92	1.5	98	87	50	35	1
							Herpes plaque test[c]				
Z-D-Asn-Ala	188–189	C	$C_{15}H_{19}N_3O_6$	12.45	12.38	0.2	44	50	42	22	23
Z-D-Phe-Ala						0.6	82	65	60	52	26
Z-γAbu-(S-Bzl)Cys	82–85	B	$C_{22}H_{26}N_2O_5S$	6.51	6.48	0	89	68	67	7	14
(NO2)Z Arg-(S-Bzl)Cys	170–172	B	$C_{24}H_{30}N_6O_7S$	15.38	15.34	0.8	24	63	30	0	0
Di Z-DL-Lys-(S-Bzl)Cys-OCH3	139–141	B	$C_{33}H_{39}N_3O_7S$	6.77	7.12	0	75	26	59	0	...
Di Z-DL-Lys-(S-Bzl)-D-Cys-OCH3	153–155	B	$C_{33}H_{39}N_3O_7S$	6.77	6.98	0.2	66	...	64	12	0
Z-D-Phe-(S-Bzl)Cys	75–78	A	$C_{27}H_{28}N_2O_5S$	5.69	5.79	0.6	94	80	55	47	24
Z-D-Phe-(S-Bzl)-D-Cys	86–89	A	$C_{27}H_{28}N_2O_5S$	5.69	5.49	0	60	26	26	19	...
Z-Ser-(S-Bzl)Cys	137–139	C	$C_{21}H_{24}N_2O_5S$	6.48	6.34	0	36	7	12	22	...
Z-D-Ser-(S-Bzl)Cys	157–159	C	$C_{21}H_{24}N_2O_5S$	6.48	6.51	0	77	23	19	16	...
Z-Trp-(S-Bzl)Cys	65–70	B	$C_{29}H_{29}N_3O_5S$	7.90	8.21	0	Toxic	51	12	7	0
Z-Tyr-(S-Bzl)Cys-OCH3	135–138	B	$C_{28}H_{30}N_2O_6S$	5.36	5.51	0	88	73	58	62	51
Z-Val-(S-Bzl)Cys	168–170	B	$C_{23}H_{28}N_2O_5S$	6.30	6.27	0.1	100	55	0	6	0
Z-Val-(S-Bzl)-D-Cys	91–94	A	$C_{23}H_{28}N_2O_5S$	6.30	6.38	0	67	37	23	0	...
Z-D-Phe-(S-Et)Cys	73–77	A	$C_{22}H_{26}N_2O_5S$	6.51	6.72	0	47	64	56	41	0
Z-D-Phe-(S-Me)Cys	112–117	A	$C_{21}H_{24}N_2O_5S$	6.73	6.86	0	72	74	74	69	...
Z-Phe-DL-ethionine-OCH3	126–128	C	$C_{24}H_{30}N_2O_5S$	6.11	6.06	0.75	80	76	0	..	...
Z-Pro-Gly-ONp	142–143	A	$C_{21}H_{21}N_3O_7$	9.83	10.00	1.1	66	28	16	37	24
Z-Phe-Ile-OCH3	104–105	A	$C_{24}H_{30}N_2O_5$	6.57	6.57	0.7	66	61	46	69	...
Z-Pro-Met-OCH3	107–109	C	$C_{19}H_{26}N_2O_5S$	7.10	7.13	0.4	90	35	42	41	30
Z-D-Phe-Met						0.3	68	52	14	0	0
(NO2)Z-D-Phe-D-Met-OCH3	130–132	B	$C_{23}H_{27}N_3O_7S$	8.58	8.79	0.5	62	66	54	66	...
Z-(S-Bzl)Cys-D-Phe	166–168	A	$C_{27}H_{28}N_2O_5S$	5.69	5.74	0	Toxic	Toxic	63	48	...
Z-D-Pro-D-Phe	120–122	A	$C_{22}H_{24}N_2O_5$	7.05	7.10	0.6	85	72	64	30	19
Z-D-Asn-D-Ser-OCH3	198–200	C	$C_{16}H_{21}N_3O_7$	11.44	11.57	0.3	27	42	53	50	...
Z-D-Asn-Val-OCH3	187–189	C	$C_{18}H_{25}N_3O_6$	11.08	11.28	0.4	54	49	39	7	6
Z-DL-Glu-Val	203–206	C	$C_{18}H_{25}N_3O_6$	11.08	11.07	0.8	85	30	16	0	...
Z-Phe-D-Val-OCH3	135–136	A	$C_{23}H_{28}N_2O_5$	6.79	6.79	1.1	85	47	49	49	34

[a] The methyl esters were prepared but are omitted here except for active compounds; Z = carbobenzoxy, Bzl = benzyl, ONp = *p*-nitrophenyl; for amino acid symbols used see *J. Biol. Chem.*, **241**, 2491 (1966). Amino acids are the L configuration unless otherwise designated. [b] See Experimental Section for methods used. [c] See ref 6. [d] Reference 1. [e] Y. Noda, *Nippon Kagaku Zasshi*, **80**, 411 (1959). [f] This peptide was reported as an oil: Z. J. Vejdělek, *Collection Czech. Chem. Commun.*, **15**, 929 (1951).

None of the peptides tested was active against other viruses such as polio, rabies, or influenza. The nonpeptide amide derivatives were all inactive regardless of stereochemistry.[8] A few tripeptides possessed antimeasles activity. The *in vivo* antiviral activity of carbobenzoxyphenylalanylnitroarginine has been investigated.[9] The serum from rats or rabbits receiving the drug by injection or by oral administration was found to be active against measles virus in the plaque test. Oral administration to monkeys did not produce active serum. Injection into monkeys resulted in severe tissue irritation.

Experimental Section

Melting points, taken with the Thomas-Hoover capillary melting point apparatus, are corrected.

General Procedures for the Preparation of the Compounds Listed in Tables I and II. Method A.—To a cold (−10°), stirred solution of 7 g (0.021 mole) of carbobenzoxy-D-tryptophan in 100 ml of CH_2Cl_2 was added 2.1 g (0.021 mole) of Et_3N followed by 2.3 g (0.021 mole) of ethyl chloroformate. The solution was stirred 20 min at −10°, and a solution of 3 g (0.021 mole) of D-alanine methyl ester hydrochloride and 2.1 g (0.021 mole) of Et_3N in 100 ml of CH_2Cl_2 at 0° was added. The solution was stirred for 3 hr at 0° and at room temperature overnight. The solution was washed with H_2O, 5% $NaHCO_3$ solution, dilute HCl, and H_2O and dried. The filtered solution was evaporated to a small volume, and petroleum ether (bp 35–60°) was added giving carbobenzoxy-D-tryptophyl-D-alanine methyl ester which was recrystallized from CH_2Cl_2–petroleum ether; yield 8 g (90%), mp 79–81°.

Anal. Calcd for $C_{23}H_{25}N_3O_5$: C, 65.23; H, 5.95; N, 9.92. Found: C, 65.21; H, 5.91; N, 9.80.

To a solution of 5 g (0.0118 mole) of the above dipeptide ester in 50 ml of MeOH was added 7 ml (0.014 mole) of 2 *N* NaOH. The solution was kept for 1 hr a 25° and diluted with 100 ml of H_2O, then 7 ml of 2 *N* HCl. The carbobenzoxy-D-tryptophyl-D-

(8) It is of interest that a recent patent has claimed carbobenzoxy-L-phenylalanylcytosamine to be active against herpes virus: The Upjohn Co., Netherlands Patent 6,510,258 (1966).

(9) J. R. Dice, W. A. Rightsel, F. M. Schabel, Jr., and I. W. McLean, Jr., *Ann. N. Y. Acad. Sci.*, **130**, 24 (1965).

TABLE II

SYNTHETIC CARBOBENZOXY DI- AND TRIPEPTIDES

Compd[a]	Mp, °C	Method[b]	$[\alpha]^{25-26}_D$, deg[c]	Formula	Calcd, %			Found, %		
					C	H	N	C	H	N
Z-Asn-D-Ala	193–195	C	−4.2 (1)	$C_{15}H_{19}N_3O_6$	53.41	5.68	12.45	53.32	5.64	12.49
Z-D-Asn-D-Ala	220–221	C	+13.9 (3)	$C_{15}H_{19}N_3O_6$	53.41	5.68	12.45	53.59	5.65	12.36
Z-(S-Bzl)Cys-D-Ala	109–111	A	−25.3 (2)	$C_{21}H_{24}N_2O_5S$	60.56	5.81	6.73	60.59	5.70	6.71
Z-Gln-D-Ala	180–181	C	...	$C_{16}H_{21}N_3O_6$	54.70	6.03	11.96	54.49	6.31	11.79
Z-DL-Gln-D-Ala	135–140	A	−1.5 (1)	$C_{16}H_{21}N_3O_6$	54.70	6.03	11.96	54.74	6.21	11.86
Z-D-(α-Ph)Gly-D-Ala	152–155	A	−23 (1)	$C_{19}H_{20}N_2O_6$	64.03	5.66	7.86	63.43	5.82	7.91
Z-Ile-D-Ala	161–163	C	+12.7 (1)	$C_{17}H_{24}N_2O_5$	60.69	7.19	8.33	60.52	6.93	8.48
Z-D-Leu-D-Ala	145–148	A	+31.5 (3)	$C_{17}H_{24}N_2O_5$	60.69	7.19	8.33	60.46	6.90	8.31
Z-Leu-D-Ala	97–101	A	−12 (4)	$C_{17}H_{24}N_2O_5$	60.69	7.19	8.33	60.95	7.08	8.42
Z-Met-D-Ala	169–170	A	−3.3 (1)	$C_{16}H_{22}N_2O_5S$	54.22	6.26	7.90	53.97	6.38	7.83
Z-D-Met-D-Ala	161–163	A	+5.0 (1)	$C_{16}H_{22}N_2O_5S \cdot 0.5H_2O$	52.88	6.38	7.71	53.05	6.36	7.67
Z-Phe-D-Ala	116–117	A	−1.8 (4)	$C_{20}H_{22}N_2O_5$	64.83	5.99	7.56	64.89	5.82	7.66
Z-Pro-D-Ala	Oil	C	−33.3 (3)	$C_{16}H_{20}N_2O_5$	59.99	6.30	8.75	59.70	6.77	8.45
Z-D-Ser-D-Ala	157–158	A	+13.9 (4)	$C_{14}H_{18}N_2O_6$	54.19	5.85	9.03	53.97	5.81	8.98
Z-Trp-D-Ala	75–80	A	−26.3 (2)	$C_{22}H_{23}N_3O_5$	64.54	5.67	10.27	64.09	5.70	10.18
Z-D-Trp-D-Ala	149–150	A	+18.3 (1)	$C_{22}H_{23}N_3O_5$	64.54	5.67	10.27	64.41	5.48	10.11
Z-Val-D-Ala	160–161	A	+18.3 (4)	$C_{16}H_{22}N_2O_5$	59.78	6.88	8.69	59.81	7.01	8.88
Z-D-Val-D-Ala	174–175	A	+5 (2)	$C_{16}H_{22}N_2O_5$	59.78	6.88	8.69	59.46	7.00	8.73
Z-D-Phe-δ-Ava	118–120	C	+7.5 (1)	$C_{22}H_{26}N_2O_5$	66.31	6.57	7.03	66.27	6.47	7.00
Z-D-Ala-(NO$_2$)Arg	120–121	A	−10 (1)	$C_{17}H_{24}N_6O_7$	48.11	5.70	19.80	47.64	5.65	20.10
Z-Asp-NH$_2$-(NO$_2$)Arg	224–225	A	...	$C_{15}H_{18}N_7O_8$	46.25	5.39	20.98	46.31	5.42	21.38
Z-Ile-(NO$_2$)Arg	156–159	C	+0.5 (1)	$C_{20}H_{30}N_6O_7$	51.49	6.49	18.02	51.07	6.33	18.03
Z-Met-(NO$_2$)Arg	140–143	C	−3.9 (1)	$C_{19}H_{28}N_6O_7S$	47.09	5.83	17.35	47.21	5.64	17.07
Z-D-Phe-(NO$_2$)-D-Arg	105–110	A	+3.0 (3)	$C_{23}H_{26}N_6O_7$	55.20	5.61	16.79	55.35	5.60	16.61
Z-Trp-(NO$_2$)Arg	68–75	A	−17.5 (1)	$C_{25}H_{29}N_7O_7 \cdot 0.5H_2O$	54.74	5.51	17.87	54.44	5.85	17.58
Z-*p*-Chloro-D-Phe-(NO$_2$)Arg	125–128	C	+2.8 (1)	$C_{23}H_{27}ClN_6O_7$	51.63	5.09	15.71	51.74	5.16	15.16
Z-D-(α-Ph)Gly-(NO$_2$)Arg	90–95	A	−14.9 (1)	$C_{23}H_{26}N_6O_7$	54.31	5.39	17.28	54.13	5.49	17.38
Bz-Phe-(NO$_2$)Arg	115–120		+3.4 (1)	$C_{22}H_{26}N_6O_6$	56.16	5.57	17.86	55.98	6.18	17.70
Pht-Phe-(NO$_2$)Arg	188–190		−111 (1)	$C_{23}H_{24}N_6O_7$	55.64	4.88	16.93	55.43	4.93	16.95
BOC-Phe-(NO$_2$)Arg	115–120		−8.5 (1)	$C_{20}H_{30}N_6O_7$	51.49	6.49	18.02	51.38	6.60	18.05
Trt-Phe-(NO$_2$)Arg	135–140		+1.6 (1)	$C_{34}H_{36}N_6O_6$	67.09	5.97	13.83	66.93	6.02	13.68
Bzl$_2$-Phe-(NO$_2$)Arg	97–104		−3.8 (3)	$C_{29}H_{34}N_6O_6$	63.72	6.28	15.47	63.91	6.47	15.21
Tos-Phe-(NO$_2$)Arg	120–125		+5.13 (1)	$C_{22}H_{28}N_6O_7S$	50.75	5.42	16.14	51.13	5.74	16.28
Z-Ile-D-Asp	75–90	A	+17.9 (1)	$C_{18}H_{24}N_2O_7$	58.83	6.36	7.36	57.05	6.74	7.16
Z-DL-Gln-D-Asp	142–144	C	+4.4 (1)	$C_{17}H_{21}N_3O_8$	51.64	5.36	10.63	52.00	5.53	10.34
Z-Met-D-Asp	125–130	A	−21 (3)	$C_{17}H_{22}N_2O_7S$	51.11	5.57	7.01	51.09	5.67	6.75
Z-D-Phe-D-Asp	64–72	A	+13.2 (2)	$C_{21}H_{22}N_2O_7$	60.86	5.35	6.76	60.63	5.64	6.58
Z-Phe-D-Asp	60–68	A	−7.4 (1)	$C_{21}H_{22}N_2O_7$	60.86	5.35	6.76	61.02	5.38	6.78
Z-D-Phe-Asp	60–65	A	+5.2 (1)	$C_{21}H_{22}N_2O_7$	60.86	5.35	6.76	60.49	5.70	6.73
Z-Pro-D-Asp	60–65	A	−46.2 (4)	$C_{17}H_{20}N_2O_7$	56.04	5.53	7.69	55.60	5.79	7.36
Z-DL-Pro-D-Asp	65–70	A	−0.4 (3)	$C_{17}H_{20}N_2O_7$	56.04	5.53	7.69	56.02	5.61	7.68
Z-Val-D-Asp	65–75	A	−17.1 (3)	$C_{17}H_{24}N_2O_7$	55.72	6.05	7.64	55.81	6.36	7.59
Z-D-Ala-(S-Bzl)Cys	115–117	A	−3 (3)	$C_{21}H_{24}N_2O_5S$	60.54	5.82	6.73	60.48	5.75	6.65
Z-D-Asn-(S-Bzl)Cys	161–163	C	−11.2 (3)	$C_{22}H_{25}N_3O_6S$	57.50	5.49	9.15	57.29	5.57	9.03
Z-D-Leu-(S-Bzl)Cys	75–77	B	−6.1 (1)	$C_{24}H_{30}N_2O_5S$	62.88	6.59	6.11	62.87	6.62	6.23
DI Z-DL-Lys-(S-Bzl)-D-Cys	115–119	B	+9.2 (1)	$C_{32}H_{37}N_3O_7S$	63.24	6.13	6.94	63.31	6.26	7.34
Z-D-Trp-(S-Bzl)Cys	65–70	A	+13.1 (2)	$C_{29}H_{29}N_3O_5S$	65.52	5.50	7.90	65.22	5.43	7.88
Z-D-Val-(S-Bzl)Cys	95–100	B	−30.4 (1)	$C_{23}H_{28}N_2O_5S$	62.14	6.35	6.30	61.89	6.33	6.29
Z-Phe-(S-Me)Cys	111–116	A	−19 (2)	$C_{21}H_{24}N_2O_5S$	60.54	5.82	6.73	60.82	5.91	6.82
Z-D-Phe-(S-Allyl)Cys	75–80	A	+2.3 (1)	$C_{23}H_{26}N_2O_5S \cdot 0.5H_2O$	60.16	6.03	6.21	61.21	6.08	6.31
Z-Phe-(S-Et)Cys	73–78	A	−20 (1)	$C_{22}H_{26}N_2O_5S$	61.38	6.09	6.51	61.09	6.13	6.54
Z-Phe-D-Glu	163–168	A	−11.2 (1)	$C_{22}H_{24}N_2O_7$	61.67	5.65	6.54	62.11	5.67	6.46
Z-D-Phe-Glu	147–155	A	+2.4 (1)	$C_{22}H_{24}N_2O_7$	61.67	5.65	6.54	62.10	5.85	6.52
Z-D-Phe-D-Glu	168–171	A	+14.6 (1)	$C_{22}H_{24}N_2O_7$	61.67	5.65	6.54	61.72	5.59	6.35
Z-D-Phe-Gly	149–151	A	+21.2 (2)	$C_{19}H_{20}N_2O_5$	64.03	5.66	7.86	63.82	5.38	8.02
Z-D-Phe-D-His	210–211	A	+4.3 (1)	$C_{25}H_{24}N_4O_5$	63.30	5.55	12.84	62.57	5.81	12.87
Z-D-Phe-His	165–168	A	+38 (3)	$C_{25}H_{24}N_4O_5$	63.30	5.55	12.84	62.65	5.54	12.77
Z-Phe-D-His	171–172	A	−24.9 (2)	$C_{25}H_{24}N_4O_5$	63.30	5.55	12.84	63.22	5.48	12.61
Z-D-Asn-D-Ile	188–189	C	−4.7 (1)	$C_{18}H_{25}N_3O_6$	56.98	6.64	11.08	57.22	6.65	11.06
Z-D-Asn-Ile	187–188	C	+16.8 (3)	$C_{18}H_{25}N_3O_6$	56.98	6.64	11.08	57.05	6.55	11.10
Z-D-Phe-D-Ile	150–152	A	−4.6 (2)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.94	6.75	6.76
Z-D-Phe-Ile	110–112	A	+22.6 (1)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.88	6.90	6.64
Z-Phe-D-Ile	107–109	A	−23.1 (1)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.95	6.88	6.92
Z-D-Phe-DL-Iva	144–145	A	+16.2 (3)	$C_{23}H_{28}N_2O_5$	66.31	6.58	7.03	66.40	6.60	6.87
Z-Phe-DL-Iva	145–146	C	−15.7 (3)	$C_{22}H_{26}N_2O_5$	66.31	6.57	7.03	66.21	6.48	7.02
Z-D-Asn-D-Leu	178–180	C	+5 (1)	$C_{18}H_{25}N_3O_6$	56.98	6.64	11.08	56.88	6.67	11.03
Z-D-Asn-Leu	188–190	C	+15.1 (3)	$C_{18}H_{25}N_3O_6$	56.98	6.64	11.08	57.26	6.52	10.92
Z-D-Met-D-Leu	80–83	A	+12.3 (1)	$C_{19}H_{28}N_2O_5S$	57.55	7.12	7.07	57.03	6.79	6.89
Z-Phe-D-Leu	113–115	A	+2.2 (4)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.87	6.94	6.71
Z-Ala-D-Met	91–93	A	+13.1 (1)	$C_{16}H_{22}N_2O_5S$	54.22	6.26	7.90	54.37	6.30	7.91
Z-D-Ala-D-Met	112–114	A	+0.5 (1)	$C_{16}H_{22}N_2O_5S$	54.22	6.26	7.90	54.33	6.18	8.05
Z-(NO$_2$)Arg-D-Met	133–135	A	+2.9 (1)	$C_{19}H_{28}N_6O_7S$	47.10	5.83	17.35	47.23	5.91	17.18
Z-Asn-D-Met	163–165	C	+4.1 (1)	$C_{17}H_{23}N_3O_6S$	51.38	5.84	10.58	51.40	5.60	10.11
Z-D-Asn-D-Met	183–185	C	+9.8 (1)	$C_{17}H_{23}N_3O_6S$	51.38	5.81	10.58	51.33	5.87	10.50
Z-D-Asn-Met	157–159	C	−5.8 (1)	$C_{17}H_{23}N_3O_6S$	51.38	5.84	10.58	51.22	5.77	10.41
Z-(S-Bzl)Cys-D-Met	100–103	A	−13.3 (3)	$C_{29}H_{32}N_2O_5S_2$	57.96	5.93	5.89	57.66	6.00	5.80
Z-Gly-D-Met	110–112	A	+3.9 (1)	$C_{16}H_{20}N_2O_5S$	52.93	5.92	8.24	52.80	6.00	8.33
Z-His-D-Met	117–120	E	−4.8 (1)	$C_{19}H_{24}N_4O_5S \cdot 0.5H_2O$	53.11	5.87	13.05	52.79	5.66	12.89
Z-Hyp-D-Met	118–120	C	−22.4 (1)	$C_{18}H_{24}N_2O_6S$	54.53	6.10	7.07	54.29	5.95	7.11
Z-Ile-D-Met	123–125	C	+18.3 (1)	$C_{19}H_{28}N_2O_5S$	57.55	7.12	7.07	57.76	7.30	7.09
Z-Leu-D-Met	98–100	C	+1.7 (1)	$C_{19}H_{28}N_2O_5S$	57.55	7.12	7.07	57.63	7.21	6.90
Z-D-Leu-D-Met	95–99	A	+7.1 (1)	$C_{19}H_{28}N_2O_5S$	57.55	7.12	7.07	57.48	6.96	6.82
DI Z-Lys-D-Met	113–115	D	+2.4 (1)	$C_{27}H_{36}N_3O_7S$	59.92	6.46	7.70	59.68	6.46	7.90
Z-Met-D-Met	106–108	A	+4.3 (1)	$C_{18}H_{26}N_2O_5S_2$	52.14	6.32	6.76	52.33	6.31	6.92
Z-D-Met-D-Met	118–120	A	+9.66 (1)	$C_{18}H_{26}N_2O_5S_2$	52.14	6.32	6.76	52.24	6.31	6.77

TABLE II (*Continued*)

Compd[a]	Mp, °C	Method[b]	$[\alpha]^{23-26}D$, deg[c]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
Z-Phe-D-Met	118.5–119.5	A	+8 (4)	$C_{22}H_{26}N_2O_5S \cdot 0.5H_2O$	60.12	6.20	6.39	59.95	6.27	6.37
Z-D-Phe-D-Met→O	105–115	C	+10 (1)	$C_{22}H_{26}N_2O_6S \cdot 0.5H_2O$	58.01	5.98	6.15	57.78	5.76	6.28
Z-Phe-D-Met→O	140–143	C	−8 (1)	$C_{22}H_{26}N_2O_6S \cdot H_2O$	56.89	6.08	6.03	56.53	5.60	6.03
Z-D-Phe-Met→O	123–125	C	+10.5 (1)	$C_{22}H_{26}N_2O_6S \cdot H_2O$	56.89	6.08	6.03	56.99	5.64	5.98
(NO_2)Z-D-Phe-D-Met	149–151	B	+15 (1)	$C_{22}H_{25}N_3O\ S$	55.57	5.30	8.83	55.73	5.50	8.81
(CHO)-D-Phe-D-Met	155–160	A	...	$C_{15}H_{20}N_2O_4S$	55.54	6.22	8.64	55.36	6.14	8.48
Z-Pro-Met	135–137	C	−30.3 (1)	$C_{18}H_{24}N_2O_5S$	56.78	6.36	7.37	56.89	6.10	7.34
Z-Pro-D-Met	95–97	C	−25.3 (1)	$C_{18}H_{24}N_2O_5S$	56.78	6.36	7.37	56.87	6.36	7.41
Z-D-Pro-Met	98–100	A	+25 (1)	$C_{18}H_{24}N_2O_5S$	56.78	6.36	7.37	56.56	6.28	7.29
Z-D-Pro-D-Met	133–135	A	+22.3 (1)	$C_{18}H_{24}N_2O_5S$	56.78	6.36	7.37	56.56	6.42	7.39
Z-Tyr-D-Met	68–70	C	−8.2 (1)	$C_{22}H_{26}N_2O_6S \cdot 0.5H_2O$	58.01	5.98	6.15	58.19	6.14	6.15
Z-Val-D-Met	152–153	A	+22.4 (1)	$C_{18}H_{26}N_2O_5S$	56.52	6.85	7.32	56.67	6.92	7.44
Z-D-Val-D-Met	179–181	A	−1.89 (1)	$C_{18}H_{26}N_2O_5S$	56.52	6.85	7.32	56.30	6.85	7.49
Z-D-Phe-DL-Nle	129–130	C	+7.38 (3)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	67.03	6.90	6.91
Z-D-Phe-DL-Nva	141–142	C	+6.5 (3)	$C_{22}H_{26}N_2O_5$	66.31	6.58	7.03	66.52	6.69	6.95
Z-Ala-D-Phe	49–51	A	+2.6 (2)	$C_{20}H_{22}N_2O_5$	64.83	5.99	7.56	64.81	6.15	7.68
Z-D-Ala-D-Phe	56–60	A	...	$C_{20}H_{22}N_2O_5$	64.83	5.99	7.56	64.88	6.07	7.69
$Z(NO_2)$Arg-D-Phe	93–105	C	...	$C_{23}H_{28}N_6O_7$	55.20	5.64	16.79	54.95	5.72	16.85
Z-Asn-D-Phe	192–194	C	−4.3 (1)	$C_{21}H_{23}N_3O_6$	61.01	5.61	10.17	61.15	5.55	10.10
Z-D-Asn-D-Phe	215–218	C	−8.9 (3)	$C_{21}H_{23}N_3O_6$	61.01	5.61	10.17	61.05	5.48	10.15
Z-D-Asn-Phe	189–192	C	+4.5 (2)	$C_{21}H_{23}N_3O_6$	61.01	5.61	10.17	60.76	5.61	10.10
Z-Gln-D-Phe	193–195	C	−0.8 (2)	$C_{22}H_{25}N_3O_6$	61.82	5.90	9.83	61.86	5.94	10.03
Z-Hyp-D-Phe	148–150	D	−16.3 (1)	$C_{22}H_{24}N_2O_6$	64.08	5.87	6.80	63.63	5.74	6.90
Z-Ile-D-Phe	159–161	C	+8.7 (1)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.97	6.97	6.93
Z-D-Leu-Phe	55–60	A	+20 (4)	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.73	6.63	6.86
Z-D-Leu-D-Phe	oil	A	..	$C_{23}H_{28}N_2O_5$	66.97	6.84	6.79	66.96	6.86	6.70
Di Z-DL-Lys-D-Phe	118–121	C	+1.4 (1)	$C_{31}H_{35}N_3O_7$	66.30	6.28	7.48	66.33	6.28	7.62
Di Z-DL-Lys-Phe	118–120	C	+6.6 (3)	$C_{31}H_{35}N_3O_7$	66.30	6.28	7.48	66.09	6.36	7.61
Z-Met-D-Phe	123–126	A	+2.1 (4)	$C_{22}H_{26}N_2O_5S$	61.36	6.08	6.51	61.16	6.10	6.37
Z-D-Met-Phe	97–100	A	...	$C_{22}H_{26}N_2O_5S$	61.36	6.08	6.51	61.49	5.95	6.48
Z-D-Met-D-Phe	125–127	A	−5.9 (1)	$C_{22}H_{26}N_2O_5S$	61.36	6.08	6.51	61.67	5.97	6.72
Z-D-(α-Ph)Gly-D-Phe	154–157	A	−13.9 (1)	$C_{25}H_{24}N_2O_5$	69.42	5.60	6.48	69.29	5.57	6.60
Z-Pro-D-Phe	53–57	C	−15.9 (2)	$C_{22}H_{24}N_2O_5$	66.65	6.10	7.05	66.44	6.11	6.97
Z-Ser-D-Phe	137–139	C	−9.9 (2)	$C_{20}H_{22}N_2O$	62.17	5.74	7.25	62.17	5.86	7.27
Z-D-Ser-Phe	133–135	C	−19 (3)	$C_{20}H_{22}N_2O_6$	62.17	5.74	7.25	62.23	5.93	7.19
Z-D-Ser-D-Phe	138–139	E	−15.7 (1)	$C_{20}H_{22}N_2O_6$	62.17	5.74	7.25	62.46	5.91	7.30
Z-DL-Thr-D-Phe	135–138	C	−13.1 (3)	$C_{21}H_{24}N_2O_6$	63.00	6.04	7.00	63.02	6.42	7.53
Z-D-Trp-D-Phe	142–145	A	+36.2 (1)	$C_{28}H_{27}N_3O_5$	69.26	5.61	8.66	69.39	5.66	8.55
Z-D-Trp-Phe	90–100	A	+23 (2)	$C_{28}H_{27}N_3O_5$	69.26	5.61	8.66	69.37	5.62	8.74
Z-Val-D-Phe	118–120	A	+12.5 (2)	$C_{22}H_{26}N_2O_5$	66.31	6.58	7.03	66.11	6.58	7.12
Z-D-Val-Phe	132–134	A	−15 (1)	$C_{22}H_{26}N_2O_5$	66.31	6.58	7.03	65.64	6.68	7.06
Z-D-Val-D-Phe	176–178	A	−13.1 (1)	$C_{22}H_{26}N_2O_5$	66.31	6.58	7.03	66.35	6.64	7 15
Z-D-Asn-Pro	55–60	C	...	$C_{17}H_{21}N_3O_6$	56.19	5.82	11.57	56.17	5,87	11.55
Z-D-Phe-Pro	55–61	A	−35 (2)	$C_{22}H_{24}N_2O_5$	66.65	6.10	7.05	66.33	5.99	7.12
Z-D-Phe-DL-Pro	55–62	A	−10 (1)	$C_{22}H_{24}N_2O_5$	66.65	6.10	7.05	66.69	6.14	6.66
Z-Ala-D-Ser	153–155	A	−20.5 (3)	$C_{14}H_{18}N_2O_6$	54.19	5.85	9.03	54.17	6.04	9.15
Z-Asn-D-Ser	172–174	C	−11.8 (1)	$C_{15}H_{19}N_3O_7$	50.99	5.42	11.89	50.80	5,38	11 93
Z-D-Asn-D-Ser	199–202	C	−11.9 (1)	$C_{15}H_{19}N_3O_7$	50.99	5.42	11.89	50.91	5.14	11.99
Z-D-Asn-Ser	180–183	C	+15 (1)	$C_{15}H_{19}N_3O_7$	50.99	5.42	11.89	51.06	5.37	11.87
Z-Gln-D-Ser	160–161	A	−3.9 (2)	$C_{16}H_{21}N_3O_7$	52.31	5.76	11.44	51.84	5.64	11.61
Z-Gly-D-Ser	121–122	C	−5.5 (1)	$C_{13}H_{16}N_2O_6$	52.72	5.45	9.45	52.66	5.63	9.37
Z-Ile-D-Ser	167–168	C	+1.8 (1)	$C_{17}H_{24}N_2O_6$	57.93	6.86	7.95	57.56	6.96	7.84
Z-Leu-D-Ser	50–55	A	−15.3 (1)	$C_{17}H_{24}N_2O_6$	57.93	6.86	7.95	57.50	6.98	7.93
Z-Met-D-Ser	162–163	A	−21.2 (3)	$C_{16}H_{22}N_2O_6S$	51.89	5.99	7.56	51.75	6.15	7.72
Z-Phe-D-Ser	134–136	A	−28.5 (1)	$C_{20}H_{22}N_2O_6$	6 .17	5.74	7.25	62.04	5.87	7.04
Z-Pro-D-Ser	117–120	C	−32.5 (1)	$C_{16}H_{20}N_2O_6$	57.14	5.98	8.33	56.77	6.15	8.32
Z-D-Ser-D-Ser	190–192	C	−23.5 (1)	$C_{14}H_{18}N_2O_7$	51.53	5.57	8.59	51.60	5.46	8.51
Z-Trp-D-Ser	Oil	A	−45.1 (1)	$C_{22}H_{23}N_3O_6$	62.11	5.46	9.88	61.71	5.50	9.53
Z-D-Val-D-Ser	174–176	A	−17 (1)	$C_{18}H_{22}N_2O_6$	56.78	6.55	8.28	56.70	6.38	8.30
Z-D-Asn-D-Thr	213–214	C	−10.5 (1)	$C_{16}H_{21}N_3O_7$	52.31	5.76	11.43	52.38	5.78	11.28
Z-Phe-D-Thr	Oil	A	−38.5 (1)	$C_{21}H_{24}N_2O_6$	63.00	6.04	7.00	63.34	6.27	7.05
Z-D-Phe-Thr	53–60	A	+37.4 (2)	$C_{21}H_{24}N_2O_6 \cdot 0.5H_2O$	61.61	6.16	6.84	62.05	6,18	6.82
Z-Pro-D-Thr	148–150	C	−56.6 (3)	$C_{17}H_{22}N_2O_6$	58.27	6.33	7.99	57.99	6.42	7.98
Z-D-Asn-D-Trp	223–225	C	−18 (1)	$C_{23}H_{24}N_4O_6$	61.06	5.35	12.39	60.94	5.30	12.48
Z-Phe-Trp	137–142	A	−10 (3)	$C_{28}H_{27}N_3O_5$	69.26	5.61	8.66	69.21	5.31	8.77
Z-Phe-D-Trp	127–128	A	−3.9 (2)	$C_{28}H_{27}N_3O_5$	69.26	5.61	8.66	69.09	5.34	8.76
Z-D-Phe-Trp	172–174	A	−1.0 (3)	$C_{28}H_{27}N_3O_5$	69.26	5.61	8.66	69.98	5.86	8.54
Z-DL-Pro-D-Trp	188–190	A	+17 (4)	$C_{24}H_{26}N_3O_5$	66.04	6.01	9.63	66.11	5.80	9.51
Z-D-Ala-DL-Tyr	50–55	A	−4.5 (1)	$C_{20}H_{22}N_2O_6$	62.17	5.74	7.25	62.14	5.96	6.86
Z-D-Asn-DL-Tyr	173–175	C	+4.1 (1)	$C_{21}H_{23}N_3O_7$	58.87	5.40	9.79	58.61	5.32	9.70
Z-D-Met-DL-Tyr	Oil	A	+12.1 (4)	$C_{22}H_{26}N_2O_6S \cdot 0.5H_2O$	58.01	5.98	6.15	58.09	5.90	6.10
Z-D-Phe-Tyr	109–112	A	+8.3 (1)	$C_{26}H_{26}N_2O_6$	67.51	5.67	6.06	67.41	5.87	5.95
Z-D-Val-DL-Tyr	90–95	A	+12.8 (3)	$C_{22}H_{26}N_2O_6$	63.7	6.33	6.76	63.97	6.38	6.69
Z-Ala-D-Val	Oil	A	−10.9 (3)	$C_{18}H_{22}N_2O_5$	59.78	6.88	8.69	59.74	7.01	8.66
Z-D-Ala-D-Val	149–150	A	+23 (4)	$C_{16}H_{22}N_2O_5$	59.78	6.88	8.69	59.69	6.66	8.69
Z-Asn-D-Val	195–197	C	−12.4 (3)	$C_{17}H_{23}N_3O_6$	55.88	6.34	11.50	56.18	6.50	11.53
Z-D-Asn-Val	196–198	C	−2.8 (2)	$C_{17}H_{23}N_3O_6$	55.88	6.34	11.50	55.77	6.10	11 46
Z-D-Asn-D-Val	197–199	A	+8 (3)	$C_{17}H_{23}N_3O_6$	55.88	6.34	11.50	55.65	6.27	11.53
Z-(S-Bzl)Cys-D-Val	50–55	A	−34 (1)	$C_{23}H_{28}N_2O_5S$	62.14	6.36	6.30	62.00	6.50	6. 1
Z-Gln-D-Val	206–207	A	−2.8 (1)	$C_{18}N_{25}N_3O_6$	56.98	6.64	11.08	57.16	6.62	11.14
Z-Hyp-D-Val	62–68	D	−10 (1)	$C_{18}H_{24}N_2O_6$	59.33	6.64	7.69	59.47	6.75	7.71
Z-Ile-D-Val	153–155	C	+2 (1)	$C_{19}H_{28}N_2O_5$	62.62	7.74	7.69	62.51	7.71	7.54
Di Z-DL-Lys-D-Val	115–120	C	−4.4 (1)	$C_{27}H_{35}N_3O_7$	63.14	6.87	8.18	62.85	6.74	8.37
Z-Met-D-Val	125–127	A	−14.2 (1)	$C_{18}H_{26}N_2O_5S$	56.52	6.85	7.32	56.74	6.79	7.44
Z-Phe-D-Val	146–148	C	−15.1 (1)	$C_{22}H_{28}N_2O_5$	66.31	6.58	7.03	66.28	6.54	6.84
Z-D-Phe-Val	1471–49	C	+22 (1)	$C_{42}H_{25}N_2O_5$	66.31	6.58	7.03	66.37	6.73	7.21
Z-DL-Pro-D-Val	Oil	A	+10.8 (3)	$C_{18}H_{24}N_2O_6$	62.05	6.94	8.04	61.90	7.19	7.83

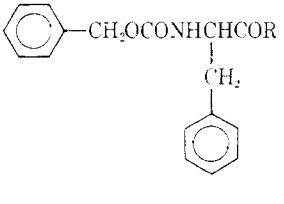
TABLE II (*Continued*)

Compd[a]	Mp, °C	Method[b]	$[\alpha]^{23-25}D$, deg[c]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
Z-Trp-D-Val	105–110	A	−36.1 (1)	$C_{24}H_{25}N_3O_5 \cdot 0.5H_2O$	63.28	5.85	9.22	63.80	6.17	9.17
Z-D-Phe-Phe-Phe	185–190	A, C	−6.2 (1)	$C_{36}H_{32}N_2O_5$	70.82	5.95	7.08	70.80	6.07	6.93
Z-D-Phe-D-Phe-D-Tyr	190–200	A, C	+16.8 (3)	$C_{36}H_{32}N_2O_7$	68.97	5.79	6.89	69.07	5.91	6.98
Z-D-Phe-D-Phe-D-Val	192–195	A, C	+21.7 (1)	$C_{31}H_{34}N_2O_6 \cdot 0.5H_2O$	67.10	6.54	7.58	66.90	6.65	7.90
Z-D-Phe-D-Phe-D-Ser	120–130	C	+15.9 (3)	$C_{29}H_{30}N_2O_7$	65.29	5.86	7.88	61.90	5.78	7.89

[a] Methyl esters were prepared but are omitted; Z = carbobenzoxy, Phl = phthalyl, Trt = trityl, Tos = tosyl, Bz = benzoyl, Bzl = benzyl; amino acids are L configuration unless otherwise designated. [b] See Experimental Section for methods used. [c] (1) *c* 1, DMF; (2) *c* 2, DMF; (3) *c* 1, MeOH; (4) *c* 2, MeOH.

TABLE III

AMIDE DERIVATIVES OF CARBOBENZOXY-L- AND -D-PHENYLALANINE



Phe isomer	R-Amide	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
D	3-Amino-1,2,4-triazole	246–248	$C_{19}H_{19}N_5O_3$	62.45	5.24	19.17	62.22	5.07	19.24
D	Cyclopropylamine	172–174	$C_{20}H_{22}N_2O_3$	70.97	6.55	8.28	70.70	6.40	8.16
D	3-Aminopropanol	131–133	$C_{20}H_{24}N_2O_4$	67.39	6.80	7.87	67.17	6.86	7.92
D	3-Amino-1,2-propanediol	135–138	$C_{20}H_{24}N_2O_5$	64.51	6.50	7.52	63.92	6.37	7.52
D	N-Aminomorpholine	184–186	$C_{21}H_{25}N_3O_4$	65.78	6.57	10.96	65.88	6.77	11.09
D	2-Aminopyridine	134–135	$C_{22}H_{21}N_3O_3$	70.38	5.64	11.19	70.15	5.60	11.34
L	Tetrahydrofurfurylamine	119–121	$C_{22}H_{26}N_2O_4$	69.08	6.85	7.33	68.83	6.52	7.10
D	2-Amino-5-(2-NO_2-furfuryl)-1,3,4-thiadiazole	233–235	$C_{28}H_{19}N_5O_6S$	55.97	3.89	14.20	55.77	3.57	14.50
D	Aniline	164–167	$C_{23}H_{22}N_2O_3$	73.76	5.93	7.48	73.79	6.11	7.53
D	*m*-Aminobenzenesulfonamide	158–160	$C_{24}H_{23}N_3O_5S$	60.92	5.12	9.27	61.06	5.20	9.32
D	1-Aminocyclopentanecarboxylic acid	Oil	$C_{28}H_{26}N_2O_5$	67.27	6.37	6.82	67.31	6.66	6.69
D	Cyclohexylamine	163–165	$C_{23}H_{28}N_2O_3$	72.60	7.42	7.36	72.37	7.60	7.46
D	ω-Aminocaproic acid	120–123	$C_{23}H_{28}N_2O_5$	66.96	6.84	6.79	66.78	6.94	6.84
L	D-Glucosamine	208–210	$C_{23}H_{28}N_2O_8$	59.98	6.13	6.08	59.91	6.17	6.00
D	*n*-Hexylamine	126–128	$C_{23}H_{30}N_2O_3$	72.21	7.91	7.32	72.37	7.82	7.25
L	2-Amino-5-chlorobenzoxazole	189–193	$C_{24}H_{20}ClN_3O_4$	64.07	4.49	9.34	63.90	4.50	9.35
D	*m*-Aminobenzotrifluoride	135–137	$C_{24}H_{21}F_3N_2O_3$	65.15	4.78	6.31	64.94	4.76	6.32
D	*p*-Aminobenzonitrile	168–170	$C_{24}H_{21}N_3O_3$	72.18	5.30	10.52	72.17	5.15	10.37
D	2-Aminobenzimidazole	239–240	$C_{24}H_{22}N_4O_3$	69.54	5.35	13.52	69.65	5.11	13.70
L	5-Aminoindazole	195–198	$C_{24}H_{22}N_4O_3$	69.54	5.35	13.52	69.48	5.34	13.27
L	Cycloheptylamine	154–157	$C_{25}H_{30}N_2O_3$	73.06	7.67	7.10	72.96	7.61	7.14
D	N-(*n*-Aminopropyl)morpholine	105–108	$C_{24}H_{31}N_3O_4$	67.74	7.34	9.88	67.01	7.34	9.86
D	*p*-Aminophenylthioacetic acid	145–148	$C_{25}H_{24}N_2O_5S$	64.64	5.21	6.03	64.80	5.32	6.23
L	*p*-Methoxybenzylamine	154–156	$C_{25}H_{26}N_2O_4$	71.77	6.26	6.70	72.09	6.46	6.74
L	Cyclooctylamine	132–133	$C_{26}H_{32}N_2O_3$	73.50	7.90	6.86	73.70	7.61	6.94
D	8-Aminoquinoline	117–119	$C_{26}H_{23}N_3O_4$	73.38	5.45	9.87	73.28	5.53	9.87
L	Tryptamine	143–146	$C_{26}H_{25}N_3O_3$	73.05	5.90	9.83	73.13	6.12	9.67
D	*p*-Aminohippuric acid	197–199	$C_{26}H_{25}N_3O_6 \cdot 0.5H_2O$	64.46	5.40	8.67	64.41	5.39	8.64
D	1-Aminoadamantane	130–131	$C_{27}H_{32}N_2O_3$	74.96	7.46	6.48	75.07	7.59	6.54
D	3-Aminocarbazole	235–238	$C_{27}H_{23}N_3O_3$	75.14	5.44	9.07	74.79	5.33	9.64
D	Cyclodecylamine	176–178	$C_{29}H_{36}N_2O_3$	74.96	8.68	6.03	74.95	8.78	6.05
L	Aminodiphenylmethane	179–180	$C_{30}H_{28}N_2O_3$	77.56	6.08	6.03	77.13	6.46	6.24

alanine which separated was removed, dried, and recrystallized from EtOAc–cyclohexane; yield 4 g (84%), mp 149–150°.

Method B.—To a suspension of 5 g (0.019 mole) of S-benzylcysteine methyl ester hydrochloride in 50 ml of CH_2Cl_2 was added 1.9 g (0.019 mole) of Et_3N. The mixture was filtered after 5 min and to the filtrate was added 4.8 g (0.019 mole) of carbobenzoxy-D-valine and 4 g (0.019 mole) of dicyclohexylcarbodiimide. The mixture was kept overnight at 25°; the precipitate was removed and the filtrate was washed with 5% $NaHCO_3$ solution, H_2O, and dilute HCl, then dried and evaporated to a small volume. Petroleum ether was added giving a white solid which was recrystallized from EtOAc–petroleum ether; yield 7 g (80%), mp 139–140°.

Anal. Calcd for $C_{24}H_{30}N_2O_5S$: C, 62.86; H, 6.59; N, 6.11. Found: C, 63.05; H, 6.57; N, 6.17.

Carbobenzoxy-D-valine-S-benzylcysteine was obtained as a colorless solid, 4.5 g (92%), mp 95–100°, by hydrolysis of the methyl ester as described in A.

Method C.—To a cold (5°) solution of 7.3 g (0.027 mole) of carbobenzoxy-D-asparagine in 50 ml of DMF was added 3.9 g (0.028 mole) of *p*-nitrophenol and 5.8 g (0.028 mole) of dicyclohexylcarbodiimide. The mixture was kept at 5° for 5 hr and filtered. To the filtrate was added a filtered solution prepared from 5 g (0.027 mole) of D-isoleucine methyl ester hydrochloride and 2.7 g (0.027 mole) of Et_3N in 50 ml of DMF. The reaction mixture was allowed to stand 18 hr at 25°, then evaporated to a small volume. The residue was taken up in 100 ml of EtOAc, and the solution, washed with H_2O, 5% Na_2CO_3 solution, and dilute HCl, was dried and evaporated to *ca.* 30 ml. Petroleum ether was added, and the resulting solid was removed and recrystallized from EtOAc–MeOH–Et_2O to give 5.5 g (52%) of carbobenzoxy-D-asparaginyl-D-isoleucine methyl ester, mp 164–165°.

Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 57.99; H, 6.92; N, 10.68. Found: C, 58.11; H, 7.05; N, 10.82.

Saponification of the above methyl ester gave, in 74% yield, carbobenzoxy-D-asparaginyl-D-isoleucine, mp 188–189°.

Alternative Method C.—A cold (0°) mixture of 5.6 g (0.022 mole) of D-phenylalanine methyl ester hydrochloride and 2.2 g (0.022 mole) of Et_3N in 75 ml of DMF was filtered, and to the filtrate was added 9 g (0.022 mole) of O-acetyl-N-carbobenzoxyserine *p*-nitrophenyl ester.[10] The solution was kept 2 days at 25° and evaporated to an oil which was taken up in EtOAc. This solution was washed with H_2O, 5% $NaHCO_3$ solution, and dilute HCl, then dried and evaporated to a solid. The product, O-acetyl-N-carbobenzoxyseryl-D-phenylalanine methyl ester was recrystallized from EtOAc–petroleum ether, 7.5 g (77%), mp 132–133°, $[\alpha]^{23}D$ +4.4 (*c* 2, DMF).

Anal. Calcd for $C_{25}H_{26}N_2O_7$: C, 62.43; H, 5.93; N, 6.33. Found: C, 62.44; H, 5.96; N, 6.52.

The methyl ester and O-acetyl groups were removed using an excess of 2 *N* NaOH in MeOH affording carbobenzoxyseryl-D-phenylalanine in 72% yield, mp 137–139°.

Method D.—To a cold (5°) solution of 7.9 g (0.03 mole) of carbobenzoxyhydroxyproline in 150 ml of MeCN was added 3 g of Et_3N and 7.5 g (0.03 mole) of Woodward's[5] reagent K. The mixture was stirred 1 hr at 5°, and 5 g (0.03 mole) of D-valine methyl ester hydrochloride and 3 g of Et_3N were added. The mixture was stirred 48 hr at 25°. The solvent was evaporated, the residue was taken up in EtOAc, and the solution was washed with H_2O, 5% $NaHCO_3$ solution, and dilute HCl and dried. Evaporation of the solvent left an oil which would not crystallize: yield 8.4 g (74%).

Anal. Calcd for $C_{19}H_{26}N_2O_6 \cdot H_2O$: C, 57.55; H, 7.12; N, 7.06. Found: C, 57.28; H, 7.00; N, 6.63.

Hydrolysis of the above methyl ester with NaOH in MeOH gave carbobenzoxyhydroxyprolyl-D-valine in 80% yield, mp 62–68°.

Method E.—To a cold (7°) solution of 6 g (0.0238 mole) of carbobenzoxy-D-serine hydrazide in 50 ml of glacial HOAc and 30 ml of 1 *N* HCl was added over 15 min, 1.7 g (0.025 mole) of $NaNO_2$ in 5 ml of H_2O. After an additional 5 min, the solution was diluted with 200 ml of ice-H_2O and extracted with cold (−5°) EtOAc. The EtOAc solution was washed with ice-H_2O several times and with cold 5% Na_2CO_3 solution until neutral and dried. To this filtered solution at 0° was added a cold (0°) mixture of 5.5 g (0.024 mole) of D-phenylalanine methyl ester hydrochloride and 2.5 g of Et_3N in 50 ml of DMF. The solution was kept 24 hr at 5° and washed with 5% $NaHCO_3$ solution, and dilute HCl then dried and the solvent was evaporated. Et_2O–cyclohexane was added producing a colorless solid, 4.5 g (46%), mp 77–78°.

Anal. Calcd for $C_{21}H_{24}N_2O_6$: C, 62.99; H, 6.04; N, 7.00. Found: C, 62.73; H, 6.23; N, 6.93.

The methyl ester was removed in the usual manner giving carbobenzoxy-D-seryl-D-phenylalanine, mp 138–139°.

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(10) E. D. Nicolaides and H. A. DeWald, U. S. Patent 3,164,614 (1965).

Nitrofuryl Heterocycles. VII.[1] 4-Amino-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidines

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Fifty-two 1-alkyl-4-amino-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine derivatives were prepared and were found to possess excellent antibacterial activity. The most active compound was the 4-bis(2-hydroxypropyl)amino-1-methyl derivative, which showed an oral ED_{50} of about 2 mg/kg against *Staphylococcus aureus* infections in mice.

In a previous paper in this series[2] it was shown that the attachment of a condensed pyrimidine ring system at the 2 position of the nitrofuran ring would give compounds possessing exceptional antibacterial activity. That paper described the antibacterial activity of numerous 4-amino-2-(5-nitro-2-furyl)quinazoline derivatives. The present paper is concerned with the synthesis and biological evaluation of derivatives of another condensed pyrimidine system, 1-alkyl-4-amino-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine.

Chemistry.—The excellent procedure of Taylor and Borror[3] for condensing nitriles with 5-amino-4-cyanopyrazoles (**1**) in the presence of base to yield 6-substituted 4-aminopyrazolo[3,4-*d*]pyrimidines (**2**) was adapted to include the reaction of 2-furonitrile with **1**. The desired nitrofuran derivatives were then prepared by mixed-acid nitration of **2**. Unfortunately, this short synthesis was not applicable to the preparation of 4-substituted amino derivatives. These compounds were prepared with little difficulty, however, by the synthesis devised by Cheng and Robins.[4] The reactions are summarized in Scheme I.

Thus, aminocyanopyrazole (**1**) was acylated with 2-furoyl chloride to give amide **3** which was cyclized in hot, alkaline, peroxide solution to pyrazolopyrimidinone (**4**). Mixed-acid nitration of **4** gave the nitrofuryl derivative **5** in excellent yield. The assignment of the keto form to the oxygen function in position 4 of compounds **4** and **5**, rather than the frequently reported tautomeric hydroxy form, was based on the observation that carbonyl absorption occurred at 1650–1670 cm^{-1} in the infrared. Chlorination of **5** with PCl_5 in $POCl_3$ gave the 4-chloro compounds **6**. Displacement of the chlorine atom in **6** with a variety of amines proceeded smoothly in DMF solution to give the amino derivatives **10–46**, **49–55**, and **58** listed in Table I. Displacement of the halogen in **6** with ammonia in aqueous DMF gave **7–9**, identical by mixture melting points and infrared spectra with those obtained by the nitration of **2**.

Biological Screening Results.—The *in vitro* and

(1) For the previous paper in this series see H. R Snyder, Jr., *J. Med. Chem.*, **10**, 737 (1967).

(2) H. A. Burch, *ibid.*, **9**, 408 (1966).

(3) E. C. Taylor and A. L. Borror, *J. Org. Chem.*, **26**, 4967 (1961).

(4) C. C. Cheng and R. K. Robins, *ibid.*, **23**, 191 (1958).