Antineoplastic Agents. 1. N-Protected Vinyl, 1,2-Dihaloethyl, and Cyanomethyl Esters of Phenylalanine[1a]

Larry J. Loeffler,* Ziaodin Sajadi,[1b] and Iris H. Hall

Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.
Received January 3, 1977

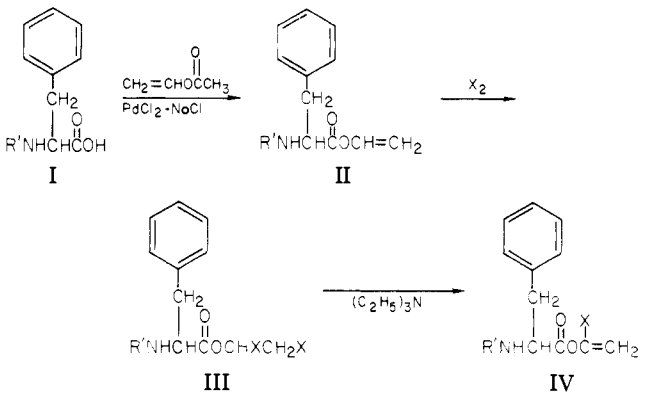
A series of N-protected vinyl, 1,2-dihaloethyl, and cyanomethyl esters of phenylalanine was synthesized and these compounds were evaluated for antitumor activity against the growth of Ehrlich ascites carcinoma in CF_1 male mice (33 mg/kg/day), Walker 256 carcinosarcoma in Sprague–Dawley male rats (2.5 mg/kg/day), and P388 lymphocytic leukemia in DBA/2 mice (20 mg/kg/day). Structure–activity relationships were evaluated and acute toxicity studies (LD_{50} determinations) in male CF_1 mice were also carried out on selected compounds. Carbobenzoxy-L-phenylalanine vinyl ester (**5**), *N*-carbobenzoxy-L-phenylalanine 1,2-dibromoethyl ester (**12**), and *N*-carbobenzoxy-L-phenylalanine cyanomethyl ester (**8**) were found to be very potent inhibitors of Ehrlich ascites tumor growth at nontoxic doses cited above. Compounds **5** and **12** also tripled survival time in the Walker 256 system. LD_{50} values for compounds **5**, **12**, and **8** were >2000 mg/kg (>6.15 mmol/kg), 74 mg/kg (0.15 mmol/kg), and 150 mg/kg (0.44 mmol/kg), respectively.

A number of research groups in recent years have reported antineoplastic activity in animal test systems for certain N-protected amino acid derivatives. Derivatives of L-phenylalanine (such as its *N*-carbobenzoxy derivative) have been reported to exhibit antitumor activity, an observation of particular interest because of the probable low toxicity of metabolites of this natural amino acid.[2,3] Troll et al.[4] have reported that certain *N*-acylamino acid derivatives, originally prepared as inhibitors or substrates of proteases, effectively inhibited carcinogenesis initiated in mouse skin by 7,12-dimethylbenz[*a*]anthracene and promoted by croton oil or its active principal, phorbol ester. Among these were tosylphenylalanyl chloromethyl ketone (TPCK),[5] tosyllysyl chloromethyl ketone (TLCK),[6] and tosylarginine methyl ester (TAME).[6] Schnebli and Burger[7] have reported that five amino acid analogues, including TPCK and TLCK, selectively inhibited the growth of Swiss SV-3T3 transformed tissue culture cells (but not untransformed cells) in a dose-dependent manner. The nature of the possible relationship between antineoplastic activity and protease inhibition has also been the subject of considerable discussion found elsewhere in the literature.[3,8-11]

Such observations concerning the antitumor activity of the amino acid derivatives cited above have stimulated our interest in determining the structure–antineoplastic activity relationships among certain amino acid derivatives. In this paper, we report the synthesis, antitumor testing, and toxicity evaluation of a number of stable but reactive vinyl, dihaloethyl, and cyanomethyl esters of L-phenylalanine which contain a variety of N-protecting groups.

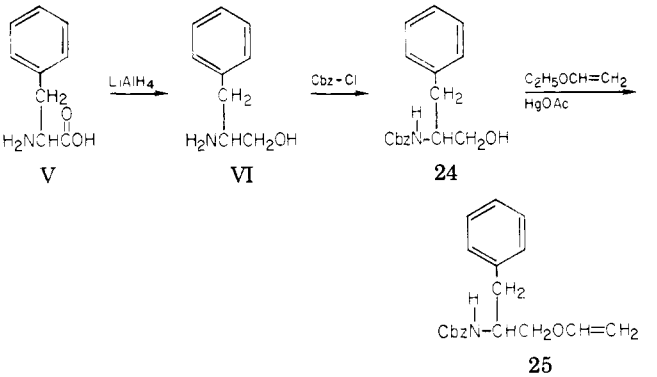
Chemistry. The synthesis of the new phenylalanine derivatives listed in Table I proceeded in a straightforward manner as indicated in Schemes I–III. A wide variety of N-acylated derivatives of phenylalanine (Scheme I, structure I) have been reported and many are of special interest in peptide synthesis.[12] In contrast, only a few vinyl esters of amino acids have been reported. These were initially prepared in order to explore their use as active esters for peptide synthesis.[13-15] For the vinyl esters of interest to us, a transesterification process was employed using the N-protected amino acid, vinyl acetate, and a palladium chloride–sodium chloride complex as a catalyst.[16] Yields of 70–80% were realized in most cases, after purification procedures. Vinyl esters prepared (**1**, **3**, and **5**–**7**) appear to be stable in a dry atmosphere for periods up to several months. Dibromo- and dichloroethyl esters **12**, **13**, and **15** or similar derivatives have not been reported in the literature to date. These proved to be surprisingly stable crystalline compounds, obtained in 80–85% yield

Scheme I. Synthesis of Vinyl and Halogenated Esters of L-Phenylalanine[a]



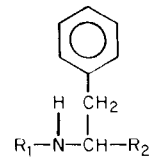
[a] R' = carbobenzoxy (Cbz), benzoyl, tosyl, acetyl, cinnamoyl, propargyl, *N*-Cbz-L-phenylalanyl; X = Cl, Br.

Scheme II. Synthesis of L-Phenylalaninol Derivatives



by direct halogenation of the vinyl ester. An attempt was made to prepare a monobromoethyl ester **14** from its precursor dibromo ester **12** through the action of triethylamine. A single oily product was obtained and purified and identified unambiguously as the 1-bromovinyl isomer by IR and NMR spectral methods; however, the product proved to be unstable at room temperature. Vinyl ethers of phenylalaninol such as compound **25** or other similar amino alcohol derivatives have not been reported previously in the literature. Cyanomethyl esters **2**, **4**, **8**–**10**, **20**, **23**, and **28** (Table I) were prepared in good yield through the action of chloroacetonitrile on the N-protected amino acid in the presence of triethylamine. As reported for other similar compounds prepared for use in peptide

Table I. Physical Properties of N-Protected L-Phenylalanine Vinyl Esters, Dihaloethyl Esters, and Related Compounds



No.	Confign	R_1	R_2	Mp, °C	Crystn solvent	Yield,[a] %	$[\alpha]^{24}_D$, deg	Formula	Analyses
1	L	Tosyl	$-C(=O)OCH=CH_2$	98–100	$CHCl_3$–hexane	20		$C_{18}H_{19}NO_4S$	C, H, N, S
2	L	Tosyl	$-C(=O)OCH_2CN$	95–97	*i*-PrOH	75	−12.8[b]	$C_{18}H_{18}N_2O_4S$	C, H, N, S
3	L	Benzoyl	$-C(=O)OCH=CH_2$	86–88	C_6H_6	20		$C_{18}H_{17}NO_3$	C, H, N
4	L	Benzoyl	$-C(=O)OCH_2CN$	112	*i*-PrOH	75	+22.2[b]	$C_{16}H_{16}N_2O_3$	C, H, N
5	L	Cbz[c]	$-C(=O)OCH=CH_2$	*e*	Oil	75–80	−13.4[d]	$C_{19}H_{19}NO_4$	C, H, N
6	D	Cbz	$-C(=O)OCH=CH_2$	*e*	Oil	80	+11.2[b]	$C_{19}H_{19}NO_4$	C, H, N
7	DL	Cbz	$-C(=O)OCH=CH_2$	*e*	Oil	80		$C_{19}H_{19}NO_4$	C, H, N
8	L	Cbz	$-C(=O)OCH_2CN$	53–55	*i*-PrOH	70	+5.2[b]	$C_{19}H_{18}N_2O_4$	C, H, N
9	D	Cbz	$-C(=O)OCH_2CN$	54–56	*i*-PrOH	75	−6.4[b]	$C_{19}H_{18}N_2O_4$	C, H, N
10	DL	Cbz	$-C(=O)OCH_2CN$	95–97[g]	*i*-PrOH	80		$C_{19}H_{18}N_2O_4$	C, H, N
11	L	Cbz	$-C(=O)OCH_2CH_3$	*e*	Oil	70	+43.9[b]	$C_{19}H_{21}NO_4$	C, H, N
12	L	Cbz	$-C(=O)OCHBrCH_2Br$	78–80	$CHCl_3$	80	−43.5[d]	$C_{19}H_{19}Br_2NO_4$	C, H, N, Br
13	D	Cbz	$-C(=O)OCHBrCH_2Br$	82–84	$CHCl_3$	75	+46.0[b]	$C_{19}H_{19}Br_2NO_4$	C, H, N, Br
14	L	Cbz	$-C(=O)OCBr=CH_2$	u[e,h]	Oil	10–15		$C_{19}H_{18}BrNO_4$	C, H, N, Br
15	L	Cbz	$-C(=O)OCHClCH_2Cl$	74–75	EtOAc–ligroine	70	−14.8[b]	$C_{19}H_{19}Cl_2NO_4$	C, H, N, Cl
16	L	Cbz	$-C(=O)OCH_2CH=CH_2$	*e*	Oil	75	+14.7[d]	$C_{20}H_{21}NO_4$	C, H, N
17	DL	Cbz	$-C(=O)OCH_2CH=CH_2$	*e*	Oil	80		$C_{20}H_{21}NO_4$	C, H, N
18	L	Cbz	$-C(=O)OCH_2C\equiv CH$	62–64	$CHCl_3$–ligroine	70	+13.6[b]	$C_{20}H_{19}NO_4$	C, H, N
19	L	Propargyl	$-C(=O)OCH_2CH_3$	*e*	Oil	68–70	+12.4[b]	$C_{14}H_{17}NO_2$	C, H, N
20	L	Acetyl	$-C(=O)OCH_2CN$	120–121		70	+20.1[b]	$C_{13}H_{14}N_2O_3$	C, H, N
21	L	Cinnamoyl	$-C(=O)OCH_2CH_3$	88–90	$CHCl_3$–ligroine	70	+166.2[b]	$C_{20}H_{20}NO_3$	C, H, N
22	L	Cinnamoyl	$-C(=O)OH$	159–160	$CHCl_3$–ligroine	80	+35.2[d]	$C_{18}H_{17}NO_3 \cdot H_2O$	C, H, N
23	L	Cinnamoyl	$-C(=O)OCH_2CN$		EtOAc–ligroine	105–107	+64.5[b]	$C_{20}H_{18}N_2O_3$	C, H, N
24	L	Cbz	$-CH_2OH$	88–90	$CHCl_3$–ligroine	80	−29.6[b]	$C_{17}H_{19}NO_3$	C, H, N
25	L	Cbz	$-CH_2OCH=CH_2$	57–58	$CHCl_3$	50	−28.0[b,f]	$C_{19}H_{21}NO_3$	C, H, N
26	LL	Cbz-L-phenylalanyl	$-C(=O)OCH=CH_2$	124–125	$CHCl_3$–ligroine	80	+8.5[b]	$C_{28}H_{28}N_2O_5$	C, H, N
27	LL	Cbz-L-phenylalanyl	$-C(=O)OCHBrCH_2Br$	137–138	EtOAc–ligroine	70	+128.0[b]	$C_{28}H_{28}Br_2N_2O_5$	C, H, N
28	LL	Cbz-L-phenylalanyl	$-C(=O)OCH_2CN$	148–150	EtOAc–ligroine	80	+24.7[d]	$C_{28}H_{27}N_3O_5$	C, H, N

[a] Yield for the last synthetic step. [b] c 1, $CHCl_3$. [c] Cbz = carbobenzoxy (benzyloxycarbonyl). [d] c 1, MeOH. [e] Oily, purified by column chromatography. [f] $[\alpha]^{22}_D$. [g] See ref 29. [h] Unstable.

Table II. Effect of Phenylalanine Derivatives on Ehrlich Ascites Tumor Growth in Mice. Acute Toxicity Evaluation in Mice

No.	Compd[a]	N[c]	Survival at 7th day	Ascrit (packed cell vol)	Ascites vol, mL	% inhibn	Toxicity, LD_{50}, mmol/kg
	0.05% Tween 80	78	77/78	32.75 ± 7.87[b]	4.1 ± 1.24[b]		
29	L-Phe-OCH=CH_2·HCl	6	4/6	27	2.2	41	
30	L-Phe-OCH_2CN·HCl	6	5/6	38.5	2.5	9	
31	*N*-Tos-L-Phe-OH	7	7/7	41.7	0.5	84	
1	*N*-Tos-L-Phe-OCH=CH_2	6	5/6	19.2	3.8	56.8	
2	*N*-Tos-L-Phe-OCH_2CN	6	4/6	3.4	0.4	97	0.73
32	*N*-Benzoyl-L-Phe-OH	7	7/7	29.2	1.3	73	
3	*N*-Benzoyl-L-Phe-OCH=CH_2	6	6/6	9.8	2.1	56.8	
4	*N*-Benzoyl-L-Phe-OCH_2CN	6	5/6	13.2	1.8	47	0.36
33	*N*-Cbz-L-Phe-OH[g]	6	6/6	4.7	0.6	75	0.84
34	*N*-Cbz-D-Phe-OH	6	6/6	28	4.3	0	
35	*N*-Cbz-DL-Phe-OH	6	5/6	30.5	2.6	52	
5	*N*-Cbz-L-Phe-OCH=CH_2	6	6/6	0.4	0.1	99.9	>6.15
6	*N*-Cbz-D-Phe-OCH=CH_2	6	6/6	32.5	3.6	36	1.54
7	*N*-Cbz-DL-Phe-OCH=CH_2	6	6/6	29	1.3	58	1.54
8	*N*-Cbz-L-Phe-OCH_2CN	6	6/6	0.1	0.2	99.9	0.44
9	*N*-Cbz-D-Phe-OCH_2CN	6	5/6	35.0	1.0	72	0.08
10	*N*-Cbz-DL-Phe-OCH_2CN	6	6/6	53	0.4	86	0.76
11	*N*-Cbz-L-Phe-OCH_2CH_3	6	6/6	40	1.5	57	1.53
12	*N*-Cbz-L-Phe-$OCHBrCH_2Br$	6	6/6	1.4	0.0	100	0.15
13	*N*-Cbz-D-Phe-$OCHBrCH_2Br$	6	6/6	0.0	0.0	100	0.31
15	*N*-Cbz-L-Phe-$OCHClCH_2Cl$	6	6/6	8.2	0.8	93	1.26
16	*N*-Cbz-L-Phe-$OCH_2CH=CH_2$	6	6/6	30.0	2.6	24	
17	*N*-Cbz-DL-Phe-$OCH_2CH=CH_2$	6	6/6	39.7	0.6	71	
18	*N*-Cbz-L-Phe-$OCH_2C\equiv CH$	6	5/6	25	3.3	54	
19	*N*-Propargyl-L-Phe-OC_2H_5	6	6/6	32	3.7	16	
20	*N*-Acetyl-L-Phe-OCH_2CN	6	6/6	39	3.9	16	
21	*N*-Cinnamoyl-L-Phe-OC_2H_5	6	6/6	28	5.0	0	
22	*N*-Cinnamoyl-L-Phe-OH	6	6/6	22	2.1	58	0.74
23	*N*-Cinnamoyl-L-Phe-OCH_2CN	6	6/6	38	2.8	27	
36	L-Phenylalaninol	7	6/7	18.7	0.6	92	0.50
24	*N*-Cbz-L-phenylalaninol	6	6/6	35	1.3	55	
25	L-2-(*N*-Cbz)amino-3-phenyl-1-propanol vinyl ether	6	4/6	40	1.4	47	
26	*N*-Cbz-L-Phe_2-OCH=CH_2	7	6/7	37.5	2.1	43	
28	*N*-Cbz-L-Phe_2-OCH_2CN	6	6/6	33.5	1.5	52	
27	*N*-Cbz-L-Phe_2-$OCHBrCH_2Br$	6	6/6	46.0	0.2	92	0.78
	Benzaldehyde	6	6/6	49	0.8	69	
	TPCK[d]	6	5/6	0.05	0.01	100	0.21
	Melphalan[e]	6	6/6	3	0.1	99	
	6-MP[d]	6	6/6	0.3	0.7	99.6	
	Uracil mustard						0.015[f]
	Chlorambucil						0.061[f]

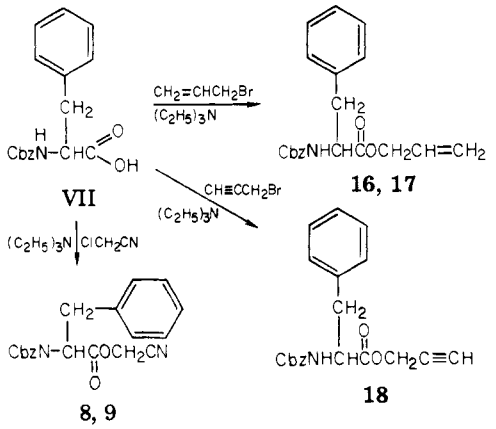
[a] See Table I for structural equivalent. [b] Mean and standard deviation on the control value for volume was 4.12 ± 1.24 and ascrit (total packed cell volume) was 32.75 ± 7.87 at 7 days. [c] *N* is the number of animals per group. [d] Sigma Chemical Co. [e] Wellcome Research Laboratories, Research Triangle Park, N.C. [f] Merck Index, 8th ed. [g] Cbz = carbobenzoxy.

synthesis, the products were found to be crystalline solids with well-defined melting points and underwent no detectable decomposition upon prolonged storage in a dry atmosphere. Allyl and propargyl esters listed in Table I (16–18) were prepared in an analogous manner (Scheme III).

Results and Discussion

Initial testing in the Ehrlich ascites carcinoma protocol in mice indicated some antineoplastic activity associated with *N*-acyl-L-phenylalanine derivatives themselves, that is, in the free acid form (Table II, compounds **31–33**). Only one reference to such activity has been found in the literature, a report that *N*-carbobenzoxy-L-phenylalanine was found to be active in the Ehrlich ascites screen.[2] Preliminary data in our test indicated that the *N*-Cbz substituent resulted in the best activity; thus *N*-Cbz-L-phenylalanine was used for further molecular modification. Preparation of the ethyl ester **11** of this compound appeared to have minimal effect on the improvement of antitumor activity. However, modification to the vinyl ester **5** or cyanomethyl ester **8** resulted in a profound inhibition of ascitic cell and fluid production. *N*-Tosyl and *N*-benzoyl vinyl ester and cyanomethyl ester derivatives were found to be much less active (**1–4**). The increased lipophilic character of the Cbz grouping relative to benzoyl or especially tosyl substituents may possibly be correlated with increased activity. Optical stereospecificity is clearly shown in the results displayed in Table II. With the free acids, the L isomer **33** was active, the D isomer **34** inactive, and the DL form **35** intermediate in activity. The same order of potency was observed with the three vinyl ester derivatives **5–7** as well as cyanomethyl esters **8–10**. It is expected that these differences should appear to be even more pronounced if dosages (mg/kg/day) of each agent were adjusted to give the same effect, rather than making a comparison of percent inhibition values for one dose for all three agents. Vinyl esters and cyanomethyl esters appeared to be quite stable chemically and optically over a period of several months when stored in a dry atmosphere, as judged by TLC and optical rotation measurements. Bromination of isomeric vinyl esters **5** and **6** afforded readily the surprisingly stable dibromoethyl esters **12** and **13**, respectively. Both of these compounds were

Scheme III. Synthesis of Cyanomethyl, Allyl, and Propargyl Esters[a]



[a] See ref 25 and 29.

maximally active in the Ehrlich test at 33 mg/kg/day; however, it is possible and probably likely that differences in activity of these enantiomers might be detected at lower dosage levels. The L-dichloroethyl ester **15** also appeared to be almost equally as active. Three unsaturated ester analogues of compound **3** were prepared for comparative purposes, compounds **16**–**18**. Both allyl and propargyl esters were found to be far less active than the vinyl ester. Interest in preparation of the propargyl ester **18** was stimulated by observations that propargyl compounds are known to isomerize in vivo to allenes, which have alkylating potential.[17] Following up on this idea, *N*-propargyl-L-phenylalanine ethyl ester **19** was also prepared but found to be inactive. Two *N*-cinnamoyl compounds **21** and **22**, with potential as acceptor molecules in nucleophilic Michael additions, were also found to be inactive. Compound **25**, an isosteric vinyl ether analogue of the active ester **5**, was prepared in order to test the requirement for an active ester. The activity of the vinyl ether **25** was quite low, in fact, lower that that of two synthetic precursor intermediates **24** and **36**. These observations support then the postulation that a reactive vinyl ester is needed for maximum activity. Three L-phenylalanine dipeptides were prepared, the vinyl ester **26**, the dibromoethyl ester **27**, and the cyanomethyl ester **28**. Compounds **27** and **28** were the most active of these; however, both were considerably less active than the amino acid derivatives **5** and **12**. Benzaldehyde has been included in Table II because of the feasibility that benzyl alcohol is a likely product of metabolism of all *N*-Cbz derivatives. This could subsequently be oxidized to the reactive molecule benzaldehyde. The compounds TPCK, 6-mercaptopurine, and melphalan have also been included in Table II as standard reference compounds, the first a known proteolytic inhibitor with reported antitumor properties[4] and the latter two are well-known clinically used antitumor agents.

The antineoplastic effect of compounds **5** and **12** was particularly striking in the Walker 256 ascites carcinosarcoma in male rats (Table III). Survival time was prolonged by a factor of about 3, comparable to the effect produced by melphalan. Again, stereospecificity of antitumor action is shown by comparison of enantiomers **12** and **13**. The cyanomethyl ester **8** was found to be inactive in the Walker test systems. All compounds tested were found to be inactive against P388 lymphocytic leukemia (Table IV).

The excellent activity exhibited by compounds **5** and **12** in both Ehrlich and Walker protocols was of particular significance after acute toxicity studies were carried out

Table III. Effect of Antitumor Agents on Walker 256 Ascites Tumor Growth

No.	Compd	*N*[c]	Days survived	T/C[a]
	0.05% Tween 80	6	7.5 ± 0.5	
5	*N*-Cbz-L-Phe-OCH=CH_2	6	18.1	226
12	*N*-Cbz-L-Phe-OCHBrCH_2Br	6	23.0	305
13	*N*-Cbz-D-Phe-OCHBrCH_2Br	6	8.7	117
8	*N*-Cbz-L-Phe-OCH_2CN	6	8.5	113
2	*N*-Tos-L-Phe-OCH_2CN	6	8.5	113
	Melphalan[b]	5	23.0	305

[a] T/C > 125 value denotes minimum significant activity. [b] Wellcome Research Laboratories, Research Triangle Park, N.C. [c] *N* is the number of animals per group.

Table IV. Effect of Antitumor Agents on P388 Lymphocytic Leukemia Growth

No.	Compd	*N*[b]	Days survived	T/C[a]
	0.05% Tween 80	6	8.1	100
5	*N*-Cbz-L-Phe-OCH=CH_2	6	8.1	100
12	*N*-Cbz-L-Phe-OCHBrCH_2Br	6	6.7	83
13	*N*-Cbz-D-Phe-OCHBrCH_2Br	6	7.5	92
8	*N*-Cbz-L-Phe-OCH_2CN	6	6.6	82
2	*N*-Tos-L-Phe-OCH_2CN	6	9.1	112
	5-FU[c]	6	11.0	136
	6-MP[c]	6	12.0	148

[a] T/C > 125 value denotes minimum significant activity. [b] *N* is the number of animals per group. [c] Sigma Chemical Co.

in mice. Values for LD_{50}'s are listed along with Ehrlich test results in Table II. Compound **5**, with an LD_{50} > 2000 mg/kg (>6.15 mmol/kg), thus exhibits an acute toxicity far above the effective antineoplastic dose utilized. Even compound **12**, with an LD_{50} of 75 mg/kg (0.15 mmol/kg) was therefore nontoxic at the therapeutic dose utilized. As indicated in Table II, acute toxicity was somewhat greater for dibromoethyl ester derivatives than for vinyl esters. For purposes of comparison, the LD_{50} values for the clinically utilized drugs chlorambucil (18.5 mg/kg, 0.061 mmol/kg) and uracil mustard (3.7 mg/kg, 0.015 mmol/kg) have been included in Table II.

Studies on the antitumor mechanism of action of these compounds are in progress. Certain of the compounds such as the vinyl ester **5**, the 1,2-dibromoethyl ester **12**, and the cyanomethyl ester **8** could possess either a direct or a latent type reactivity toward critical bionucleophilic groups such as cysteine, lysine, histidine, guanine, etc.[18] Any of these three types of active esters might conceivably react through direct acylation of the nucleophilic moiety; alternatively, other reactive products might be generated in vivo during a process of endogenous ester hydrolysis. For example, 1,2-dibromoethyl esters such as **12** should generate α-bromoacetaldehyde upon hydrolysis. Either type of nucleophilic reactivity may possibly be related to antitumor action of these agents.

In summary, compounds such as the vinyl ester **5**, the 1,2-dibromoethyl ester **12**, and perhaps the cyanomethyl ester **8** appear to be highly promising as a result of their demonstrated activity in two in vivo test systems, and their lack of toxicity, at least as demonstrated in acute toxicity studies. Further studies are in progress, both with regard to preclinical evaluation of compounds such as **5** and **12** and in the synthesis of other potentially useful analogues.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in chloroform with a Perkin-Elmer 257 grating spectrophotometer. Nuclear magnetic resonance

spectra (NMR) were measured in deuteriochloroform with a Jeolco C60 HL spectrometer and chemical shifts reported in δ (ppm) units relative to internal tetramethylsilane: s, singlet; d, doublet; t, triplet; and m, multiplet; J values are in hertz (Hz). Data were consistent with assigned structures for all intermediates and products. Optical rotations were determined with a Perkin-Elmer 141 polarimeter with 1-dm path length. Silica gel for thin-layer chromatography (TLC) refers to Merck silica gel G. Compounds were visualized by charring with sulfuric acid (50%). Silica gel used for column chromatography refers to silicAR cc-7, 200–325 mesh. Chloroform or chloroform–ethyl acetate (4:1) was used as the eluting solvent. Elementary analyses were performed by Atlantic Microlabs, Inc., Atlanta, Ga. Palladium chloride was obtained from Engelhart Industries. Benzoyl, p-toluenesulfonyl, and carbobenzoxy chlorides were purchased from Aldrich Chemical Co. Vinyl acetate, as well as L, D, and DL isomers of phenylalanine, was obtained from Eastman Organic Chemicals.

N-Tosyl-L-phenylalanine Vinyl Ester (1). This compound was prepared by a modification of a procedure described by Weygand and Steglich.[15] A mixture of 1.50 g (4.0 mmol) of N-tosyl-L-phenylalanine,[19] 20 mL of vinyl acetate, and 20 mg of $PdCl_2$–NaCl[16] was refluxed (70–75 °C) for 5.0 h. After cooling, 35 mg of activated charcoal was added in one portion and then stirred for 5 min. The reaction mixture was filtered and solvents were removed in vacuo. The residue was dissolved in 10 mL of vinyl acetate; then 20 mg of $PdCl_2$–NaCl was added and the mixture refluxed for 5 h. After cooling at room temperature the procedure was repeated once again because the reaction was shown to be incomplete by TLC. Purification of the final product was carried out by column chromatography on silica gel [benzene–$CHCl_3$ (1:1)]. Further purification was carried out by crystallization from $CHCl_3$ and n-hexane, affording 250 mg of pure product (20–25%): mp 98–100 °C; IR 3095, 3085 (w, C=CH), 1755 (s, ester carbonyl), 1648 (s, C=C), 900 cm^{-1} (w, vinyl); NMR δ 4.7 (2 H, m, vinyl hydrogen), the other vinyl proton is masked by aromatic hydrogens.

N-Benzoyl-L-phenylalanine Vinyl Ester (2). This compound was prepared starting from N-benzoyl-L-phenylalanine[20] as described above for the N-tosyl derivative 1. The oily product was purified by column chromatography on silica gel [benzene–$CHCl_3$ (1:1)]: yield, 21%; mp 86–90 °C.

N-Carbobenzoxy-L-phenylalanine Vinyl Ester (5). The same procedure was followed as described for the preparation of N-tosyl-L-phenylalanine vinyl ester. A colorless oily product was obtained: yield 75–85%; $[\alpha]^{24}_D$ −11.0° (c 1, $CHCl_3$). The starting material, N-carbobenzoxy-L-phenylalanine, was prepared according to the procedure of Bergmann and Zervas.[21] D and DL isomers (compounds **6** and **7**) of compound **5** were prepared in an identical manner, starting from D- and DL-phenylalanine. The D isomer was a colorless oily product (yield 80%): $[\alpha]^{24}_D$ +11.2° (c 1, $CHCl_3$). The DL isomer was also an oil (yield 80%).

N-Carbobenzoxy-L-phenylalanine Ethyl Ester (11). L-Phenylalanine ethyl ester hydrochloride,[22] 4.6 g (20 mmol), was dissolved in 30 mL of water, 10% Na_2CO_3 was added until the solution was alkaline, and the free base was extracted with chloroform (3 × 15 mL). The combined chloroform extracts were dried over anhydrous $MgSO_4$. Triethylamine, 3.0 g (31 mmol), and carbobenzoxy chloride, 3.5 g (20 mmol), in 20 mL of chloroform were added dropwise simultaneously over a period of 20 min with stirring. The reaction mixture was stirred for an additional 1 h; then 20 mL of water was added, and stirring continued for 10 min. The organic layer was separated, washed with water and 0.1 N HCl, and finally dried over anhydrous $MgSO_4$. After removal of solvents in vacuo, purification of the oil was carried out by column chromatography on silica gel ($CHCl_3$). The yield of pure product was 3.80 g (70%): $[\alpha]^{24}_D$ +43.9° (c 1, $CHCl_3$).

N-Carbobenzoxy-L-phenylalanine 1,2-Dibromoethyl Ester (12). To a solution of 0.97 g (3.0 mmol) of N-carbobenzoxy-L-phenylalanine vinyl ester (**5**) in 45 mL of benzene, a solution of 0.47 g (3.0 mmol) of bromine in 40 mL of benzene was added dropwise over a period of 30 min with vigorous stirring. The reaction mixture was stirred for an additional 3 h at room temperature; then the solvent was removed under high vacuum. The product, shown to be impure by TLC using $CHCl_3$–ethyl acetate (2:1), was purified by column chromatography on silica [$CHCl_3$–EtOAc (2:1)]. Further purification was carried out by recrystallization from $CHCl_3$ and low-boiling ligroine to yield 1.05 g of product (70–75%): mp 78–80 °C; $[\alpha]^{24}_D$ −33.5° (c 1, MeOH); IR 1775 cm^{-1} (ester carbonyl); NMR δ 4.05 (2 H, d, $-CH_2Br$), 6.8 (1 H, t, OCHBr). The compound appeared to be stable over several months when stored in a dry atmosphere as judged by TLC. The D isomer of **12** (compound **13**) was prepared in an identical manner: yield 70–75%; mp 82–84 °C; $[\alpha]^{24}_D$ +46.0° (c 1, $CHCl_3$).

N-Carbobenzoxy-L-phenylalanine Bromovinyl Ester (14). To a solution of 0.50 g (1.0 mmol) of N-carbobenzoxy-L-phenylalanine 1,2-dibromoethyl ester (**12**) in 15 mL of $CHCl_3$ was added triethylamine (0.11 g, 1.1 mmol) in one portion. The reaction mixture was refluxed (70–75 °C) in an oil bath for a period of 30 min. After cooling to room temperature, the reaction mixture was filtered and the filtrate washed with water three times and dried over anhydrous $MgSO_4$. The oily product was shown to be impure by TLC using $CHCl_3$. Purification was carried out by column chromatography on silica gel ($CHCl_3$). The first of two fractions obtained was identified by IR as carbobenzoxy chloride. The second fraction was identified as desired product by IR and 100-MHz NMR. Since the product was extremely unstable, the elementary analysis was not obtained: overall yield 120 mg (25–28%); IR 3095, 3085 (w, C=CH), 1770 (s, ester carbonyl), 1648 (s, C=C), 935 cm^{-1} (s, vinyl); NMR δ 5.8 (1 H, d, J = 3–4 Hz, vinyl hydrogen), 7.7 (1 H, d, J = 3–4 Hz, vinyl hydrogen).

N-Carbobenzoxy-L-phenylalanine 1,2-Dichloroethyl Ester (15). To a solution of 0.97 g (3.0 mmol) of N-carbobenzoxy-L-phenylalanine vinyl ester (**5**) in 15 mL of CCl_4 in an ice bath (0–5 °C), 220 mg of dry chlorine in CCl_4 was introduced over a period of about 10 min. The mixture was allowed to warm to room temperature, 100 mg of powdered Na_2CO_3 was added, and the mixture was shaken for several minutes to remove any HCl which may have been formed. The reaction mixture was washed with water and dried over anhydrous $MgSO_4$. Removal of solvent and crystallization from EtOAc and low-boiling ligroine afforded 0.70 g (65–75%) of pure product: mp 74–75 °C; $[\alpha]^{24}_D$ −14.8° (c 1, $CHCl_3$).

N-Carbobenzoxy-L-phenylalanine Allyl Ester (16). To a solution of 3.0 g (10 mmol) of N-carbobenzoxy-L-phenylalanine in 20 mL of THF, 1.5 g (15 mmol) of triethylamine and 2.5 g (20 mmol) of allyl bromide were added simultaneously over a period of 20 min with vigorous stirring. The reaction mixture was refluxed in an oil bath (70–75 °C) for 2 h and then stirred at room temperature overnight. The organic layer was filtered, then washed with 0.1 N HCl, 5% Na_2CO_3, and water, and dried over anhydrous $MgSO_4$. Concentration at reduced pressure yielded an oily product which was purified by column chromatography on silica gel ($CHCl_3$). The yield of pure product was 2.30 g (70%): $[\alpha]^{24}_D$ +14.7° (c 1, MeOH); IR 3070, 3050 (w, C=CH), 1648 (w, C=C), 950 cm^{-1} (s, allyl). The DL isomer (**17**) of compound **16** (an oil) was prepared in an identical fashion (yield 75–80%).

N-Carbobenzoxy-L-phenylalanine Propargyl Ester (18). To a solution of 1.5 g (5.0 mmol) of N-carbobenzoxy-L-phenylalanine in 10.0 mL of ethyl acetate, 0.5 g (5.0 mmol) of triethylamine and 0.6 g (5.0 mmol) of propargyl bromide were added simultaneously over a period of 10 min with vigorous stirring. The reaction mixture was refluxed in an oil bath (70–75 °C) for 5 h and then filtered from unreacted starting material and the solvents were removed in vacuo. Starting material was recovered by extraction with 5% $NaHCO_3$ solution. After evaporation of the solvent, the product was purified by column chromatography on silica gel ($CHCl_3$) affording 560 mg of pure product (yield 72%) as an oil. Crystallization from $CHCl_3$ and low-boiling ligroine yielded 500 mg (62–65%): mp 62–64 °C; $[\alpha]^{24}_D$ +13.6° (c 1, $CHCl_3$); IR 3280 (s, C≡CH), 2150 cm^{-1} (w, C≡C); NMR δ 2.49 (1 H, t, C≡CH), 4.70 (2 H, d, $-CH_2C{\equiv}C$).

N-Propargyl-L-phenylalanine Ethyl Ester (19). L-Phenylalanine ethyl ester hydrochloride,[22] 4.6 g (20 mmol), was dissolved in 30 mL of water, 10% Na_2CO_3 was added until the solution was basic, and the free base was extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate extracts were dried over anhydrous $MgSO_4$ and filtered. Triethylamine, 3.1 g (31 mmol), and propargyl bromide, 3.6 g (40 mmol), were added simultaneously over a period of 30 min with stirring. The reaction mixture was refluxed in an oil bath (70–75 °C) for 2 h and then stirred at room temperature overnight. The reaction mixture was

filtered, washed with 0.1 N HCl, 5% of Na_2CO_3, and water, and then finally dried over anhydrous $MgSO_4$. After evaporation of the solvent in vacuo, purification was carried out by column chromatography on silica gel ($CHCl_3$). The yield of the pure product (an oil) was 2.80 g (68–70%): $[\alpha]^{24}_D$ −12.4° (c 1, $CHCl_3$); IR 3290 (s, C≡CH), 2140 cm^{-1} (w, C≡C); NMR δ 2.9 (2 H, d, $-OCH_2C{\equiv}C$).

***N*-Cinnamoyl-L-phenylalanine Ethyl Ester (21).** A solution of 3.20 g (20 mmol) of L-phenylalanine ethyl ester hydrochloride in 25 mL of pyridine (0 °C) was added dropwise to a solution of 2.9 g (20 mmol) of cinnamoyl chloride in 30 mL of ether during 30 min. The reaction mixture was stirred overnight at room temperature. To the mixture, 20 mL of ether and 25 mL of water were added, and the mixture was stirred for 1 h. The organic layer was separated from the aqueous layer and washed three times with water in order to remove pyridinium hydrochloride. The organic layer was dried over anhydrous magnesium sulfate and filtered and the solvent was removed under low pressure, yielding 4.5 g of product (70–75%). Further purification was carried out by column chromatography on silica gel ($CHCl_3$ and low-boiling ligroine): mp 88–90 °C; $[\alpha]^{24}_D$ +166.2° (c 1, $CHCl_3$); IR 1630 cm^{-1} (w, C=C); NMR δ 6.4 (2 H, d, J = 10 Hz, cis -HC=CH-).

***N*-Cinnamoyl-L-phenylalanine (22).** A mixture of 0.97 g (3.0 mmol) of *N*-cinnamoyl-L-phenylalanine ethyl ester (21) in 10 mL of an ethanolic solution of 1 N NaOH was stirred at room temperature overnight. The reaction mixture was acidified with 2 N HCl in an ice bath, 30 mL of EtOAc added, and the organic layer separated, washed with water, and dried over anhydrous $MgSO_4$. The solvent was removed under low pressure, yielding 0.71 g of product (80%), mp 156–158 °C. Purification was carried out by recrystallization from $CHCl_3$ and low-boiling ligroine: mp 159–160.5 °C; $[\alpha]^{24}_D$ +35.2° (c 1, MeOH).

L-Phenylalanine Vinyl Ester Hydrochloride (29). This compound was prepared from **5** essentially by the procedure described by Weygand et al.[16] The yield was 0.95 g (70–75%), mp 155–157 °C (reported 159–160 °C).

***N*-Carbobenzoxy-L-phenylalaninyl-L-phenylalanine Vinyl Ester (26).** This new compound was prepared from *N*-carbobenzoxy-L-phenylalanine and **29** via the general method described by Weygand et al.[16] Purification was carried out by column chromatography on silica gel ($CHCl_3$). Final purification was carried out by recrystallization from $CHCl_3$ and low-boiling ligroine: mp 124–125 °C; $[\alpha]^{24}_D$ +8.5° (c 1, $CHCl_3$).

***N*-Carbobenzoxy-L-phenylalanyl-L-phenylalanine 1,2-Dibromoethyl Ester (27).** The same procedure was followed as described for preparation of *N*-carbobenzoxy-L-phenylalanine 1,2-dibromoethyl ester 12. Purification was carried out by column chromatography on silica gel with $CHCl_3$ as the eluting solvent, yielding pure product (65–70%). Recrystallization was carried out from $CHCl_3$ and low-boiling ligroine: mp 137–138 °C; $[\alpha]^{24}_D$ +128.0° (c 1, $CHCl_3$). The compound appears to be stable over several months when stored in a dry atmosphere as judged by TLC.

L-Phenylalaninol (36). This compound was prepared by the procedure of Karrer et al.[23] The yield was 50–55%, mp 86–88 °C (reported 85–88 °C).

***N*-Carbobenzoxy-L-phenylalaninol (24).** The same procedure was followed as described above for the preparation of *N*-carbobenzoxy-L-phenylalanine. The yield was 75–80%, mp 89–90 °C. Recrystallization was carried out using $CHCl_3$ and low-boiling ligroine: $[\alpha]^{24}_D$ −29.6° (c 1, $CHCl_3$).

2-*N*-Carbobenzoxyamino-3-phenyl-1-propyl Vinyl Ether (25). This compound was prepared using conditions described by Church et al.[24] for the preparation of other vinyl ethers. To a solution of 570 mg (1.5 mmol) of *N*-carbobenzoxy-L-phenylalaninol in 50 mL of ethyl vinyl ether (freshly distilled from sodium) was added 50 mg of mercuric acetate, which had been recrystallized from ethanol, and 0.1 mL of glacial acetic acid. After stirring for 3 h at room temperature (32 °C), the reaction mixture was diluted with an equal volume of petroleum ether (bp 30–60 °C), washed with 10 mL of 5% aqueous potassium hydroxide, and dried over anhydrous potassium carbonate. The solvents were removed in vacuo and the residue was chromatographed on silica gel [$CHCl_3$–benzene (2:5)]. The yield of product was 250 mg (45–50%): mp 57–58 °C; $[\alpha]^{24}_D$ −28.0° (c 1, $CHCl_3$); IR 3070, 3050 (w, C=CH), 1630 (s, C=C), 960, 835 cm^{-1} (vinyl).

Cyanomethyl Esters. All cyanomethyl esters (Table I) were prepared from the corresponding acids through the action of chloroacetonitrile and triethylamine in ethyl acetate, according to a procedure described by Morozoa et al.[25] Products were recrystallized from ethyl acetate. Yields, melting points, and optical rotations are shown in Table I.

Pharmacological Studies. Ehrlich Ascites Screen. The synthetic compounds were tested for antitumor activity in the Ehrlich ascites carcinoma in CF_1 male mice using a procedure described by Piantadosi et al.,[26] with certain modification. Seven days after tumor transplantation, donor mice were sacrificed, ascites fluid was collected and diluted with isotonic saline, an aliquot was placed in a hemocytometer chamber, and the number of cells/cm^3 was calculated. Then 10^6 cells were injected ip into each test animal using an 18-gauge needle. 6-Mercaptopurine and melphalan were used as internal standards in the test. After 7 days the inoculated mice were sacrificed, and the ascitic fluid was collected. The volume (mL) of the ascitic fluid was measured and the total packed ascites cell volume for each group was measured utilizing nonheparinized capillary tubes centrifuged at 3000 rpm for 3–5 min. The control (Tween 80) (C) value for the volume of tumor was 4.12 ± 1.24 (SD) mL and for ascrit (total packed cell volume) was 32.75 ± 7.87 at 7 days. Percent inhibition of tumor growth was calculated by the following formula for the treated animals (T).

$$100 - \left[\frac{\text{vol}_T \times \text{ascrit}_T}{\text{vol}_C \times \text{ascrit}_C}\right] \times 100 = \%\ \text{inhibition}$$

Any compound that exhibited 60% inhibition of tumor growth was considered significantly active.

Walker 256 Ascites Carcinosarcoma Screen. In this test, 10^6 tumor cells were implanted ip into 75 ± 10 g Sprague–Dawley male rats. Test compounds dissolved in 0.05% Tween–H_2O were injected ip at 2.5 mg/kg/day and the day of death was recorded. Treated/control (T/C) values were calculated from the average survival time for each group according to the NIH protocol.[27] Melphalan was used as the internal standard in this screen.

Lymphocytic Leukemia P388 Screen. The lymphocytic leukemia P388 test was carried out in DBA/2 male mice (20 g). In this screen, 10^6 cells were implanted on day 0. The test compounds were administered ip at 20 mg/kg/day. T/C values were calculated according to the NIH protocol.[27] 5-Fluorouracil was used as the internal standard in this test.

Acute Toxicity Testing. LD_{50} Determination. The LD_{50} values of active antitumor compounds were determined in male CF_1 mice (25–30 g) using a dose range of 10 mg to 2 g/kg according to the procedure described by Litchfield and Wilcoxon.[28]

Acknowledgment. The authors wish to acknowledge support for a part of this work under Institutional Grant IN-15-P from the American Cancer Society. The remainder of the work was supported under U.S. Public Health Service Research Grant RR05760 from the NIH to the School of Pharmacy, University of North Carolina.

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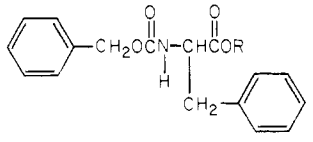
Antineoplastic Agents. 2. Structure–Activity Studies on N-Protected Vinyl, 1,2-Dibromoethyl, and Cyanomethyl Esters of Several Amino Acids

Larry J. Loeffler,* Ziaodin Sajadi, and Iris H. Hall

Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.
Received January 3, 1977

Previously reported work on N-protected activated esters of phenylalanine has been extended to include N-protected vinyl, dibromoethyl, and cyanomethyl esters of several other amino acids. These compounds have been synthesized and evaluated in Ehrlich ascites carcinoma, Walker 256 carcinosarcoma, and P388 lymphocytic leukemia tests. Among compounds tested were derivatives of tyrosine, tryptophan, glycine, leucine, proline, aspartic acid, glutamic acid, 4-aminobutyric acid, and 6-aminocaproic acid. Compounds of greatest potential interest from this study are *N*-carbobenzoxyglycine 1,2-dibromoethyl ester and *N*-carbobenzoxy-L-leucine 1,2-dibromoethyl ester. Both compounds were highly active in Ehrlich ascites test systems (33 mg/kg/day). The glycine derivative was also active in the Walker 256 test (2.5 mg/kg/day). Values for LD_{50}'s in mice were 148 mg/kg (0.37 mmol/kg) and 225 mg/kg (0.50 mmol/kg) for glycine and leucine derivatives, respectively; therefore, these compounds do not appear to be toxic at effective dose levels.

Our interest in certain N-protected amino acid active esters as potential antineoplastic agents has been discussed previously.[1] In the course of structure–activity studies described there, several active compounds were discovered, among them the vinyl and 1,2-dibromoethyl esters of *N*-carbobenzoxy-L-phenylalanine (Ia and Ib, respectively).



Ia, R = $-CH=CH_2$
b, R = $-CHBrCH_2Br$

These compounds were found to be active vs. Ehrlich ascites carcinoma (33 mg/kg/day) and Walker 256 carcinosarcoma (2.5 mg/kg/day). Of particular interest was the fact that these dose levels are considerably below toxic levels as indicated by acute toxicity studies in mice. The LD_{50} values for Ia and Ib were >2000 mg/kg (>6.15 mmol/kg) and 74 mg/kg (0.15 mmol/kg), respectively.

These previous studies on phenylalanine derivatives have now been extended to include vinyl, 1,2-dibromomethyl, and cyanomethyl esters of a number of other amino acids.

Chemistry. Vinyl and 1,2-dibromoethyl esters of N-protected derivatives of tyrosine, tryptophan, glycine, leucine, proline, aspartic acid, glutamic acid, 4-aminobutyric acid, and 6-aminocaproic acid were prepared from the corresponding acids by methods previously described.[1] Yields and pertinent physical information for new compounds are included in Table I. Cyanomethyl and allyl esters were prepared in a straightforward manner from the corresponding acids according to literature methods involving the reaction of the acid with chloroacetonitrile or allyl bromide and the base, triethylamine.[1,2] Propargyl esters were prepared in a similar manner utilizing propargyl bromide.[1,3] New compounds are listed in Table I. All compounds appeared to be stable for a period of at least several months when stored in a dry atmosphere, as judged by TLC, melting point, and optical data.

Biological Testing. Compounds were evaluated in three in vivo antitumor protocols: Ehrlich ascites carcinoma, Walker 256 ascites carcinosarcoma, and lymphocytic leukemia P388 (see Tables II–IV). In addition, acute toxicity testing was completed in mice in order to determine LD_{50} values for all compounds (Table II). The details of all of these test systems have been described previously.[1]

Results and Discussion

For purposes of comparison with new compounds, results of Ehrlich ascites testing on several previously reported phenylalanine derivatives have been included in Table II (**31–37**). Initially, derivatives of two other aromatic L-amino acids, tyrosine and tryptophan, were prepared and tested. *N*-Carbobenzoxy (Cbz) vinyl and cyanomethyl esters of both of these amino acids were