APhA
January 1993
Volume 82, Number 1

JOURNAL OF PHARMACEUTICAL SCIENCES

A publication of the American Pharmaceutical Association

ARTICLES

In the Search for New Anticancer Drugs. XXIV: Synthesis and Anticancer Activity of Amino Acids and Dipeptides Containing the 2-Chloroethyl- and [*N'*-(2-Chloroethyl)-*N'*-Nitroso]-aminocarbonyl Groups

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Received October 11, 1991, from the *University of Wisconsin-Milwaukee, Department of Chemistry, Post Office Box 413, Milwaukee, WI 53201.* Accepted for publication May 27, 1992.

Abstract □ A series of L,L- (**42**, **44**, **46**, and **60**) and D,D- (**43**, **45**, **47**, and **61**) dipeptide derivatives composed of phenylglycine, phenylalanine, homophenylalanine, and valine and containing a 2-chloroethylamino group at the C-terminus and an *N'*-(2-chloroethyl)-*N'*-nitroso-aminocarbonyl group at the N-terminus of the dipeptides were prepared. The dipeptide derivatives (**42**–**47**, **60**, and **61**) were first evaluated in vivo for their anticancer activities against the murine lymphocytic leukemia P388. Compounds **42**, **44**, **46**, and **60** possessed activities ranging from 46 to 111 percent increase in life span (%ILS), whereas **43** was marginal (%ILS = 31) and **45**, **47**, and **61** were inactive. In general, the L,L-series exhibited low to good activity (%ILS = 46–111), whereas the corresponding D, D-series, except for **43** (%ILS = 31), was devoid of activity. The analogously structured monoamino acid derivatives of L-alanine (**74**), L-phenylalanine (**75**), and L-aspartic acid (**76**) exhibited higher activity against P388 than the dipeptide derivatives (i.e., 481, 297, and 481 %ILS, respectively). The more active representatives of dipeptides (i.e., **42**, **44**, and **60**) and the amino acids derivatives **74**–**76** were then tested in vivo against the murine lymphoid leukemia L1210. Compounds **42**, **44**, and **60** exhibited either low or marginal activity (i.e., the %ILS values were 46, 31, and 26, respectively). Compounds **74**, **75**, and **76** possessed low to moderate activity, as evidenced by the %ILS values of 56, 48, and 64, respectively. The %ILS parameters obtained against the P388 and L1210 tumor lines were correlated with the corresponding lipophilicities, and there is a trend towards higher activity with concomitant decrease in hydrophobicity.

A number of clinically important alkylating anticancer drugs,[1,2] such as chlorambucil, melphalan, nitrogen mustard (Mustargen), cyclophosphamide (Endoxan), ifosfamide, carmustine, lomustine (CCNU), semustine (MeCCNU), streptozotocin, Cymerin,[3] and the experimental drug chlorozotocin[1,4] contain either *N*-nitroso, *N-bis*(2-chloroethyl), or *N*-nitroso-2-chloroethylamino groups.[1,2] Furthermore, in some cases, the 2-chloroethylamino group can be converted in vitro and in vivo into an aziridine type of electrophile. Hence, the alkylating clinical drug thiotepa[2,5] and various analogues[2,5] can be considered to be related to this class of compounds.

A tremendous effort has been made[1–34] to elucidate in vitro and in vivo the mechanisms of action of various *N*-nitroso, *N-bis*(2-chloroethyl), and *N*-nitroso-*N*-(2-chloroethyl)amino compounds and to develop more active and less toxic drugs containing these moieties. Among the many classes of compounds under investigation, the *N*-nitroso and *N*-nitroso-*N*-(2-chloroethyl) ureas derived from carbohydrates,[1–6,9,14,15,19,26,33] amino acids and peptides,[8,12,14–19,22–24,28,30–32] steroid–amino acid conjugates,[16,22,25,31] and aminoxyl radicals[5,20,21,23,26,29] unfold promising avenues for further explorations.

In the last decade, a number of monoamino acid and oligopeptide derivatives containing either 2-chloroethylamino[34] or *N*-(2-chloroethyl)-*N*-nitrosoaminocarbonyl(*N*-2-chloroethyl-*N*-nitrosocarbamoyl) moieties have been synthesized and evaluated[8,12,14–19,22–24,28,30–32,34] in vivo for anticancer activity. A series of monoamino acid derivatives containing in the same molecule the 2-chloroethylamino group at C-terminus and the *N'*-(2-chloroethyl)-*N'*-nitroso-aminocarbonyl at the N-terminus were investigated.[15,32] Cystamine, containing two *N*-(2-chloroethyl)-*N*-nitroso-aminocarbonyl groups, was also studied.[14,24] However, to the best of our knowledge, no analogously structured dipeptide derivatives have been investigated.

The present study is devoted to the evaluation of some dipeptides containing both the 2-chloroethylamino group at the C-terminus and the *N'*-(2-chloroethyl)-*N'*-nitroso-aminocarbonyl group at the N-terminus and to the comparison of these dipeptide derivatives with analogously structured monoamino acid derivatives. Furthermore, because the L-amino acids participate in transport through mammalian cell membranes,[35,36] it was of interest to establish whether the L,L-dipeptide derivatives would have a superior anticancer activity than the analogously structured D,D-derivatives and whether the activity in vivo could be correlated with the presumed facility of permeation through cell membranes by

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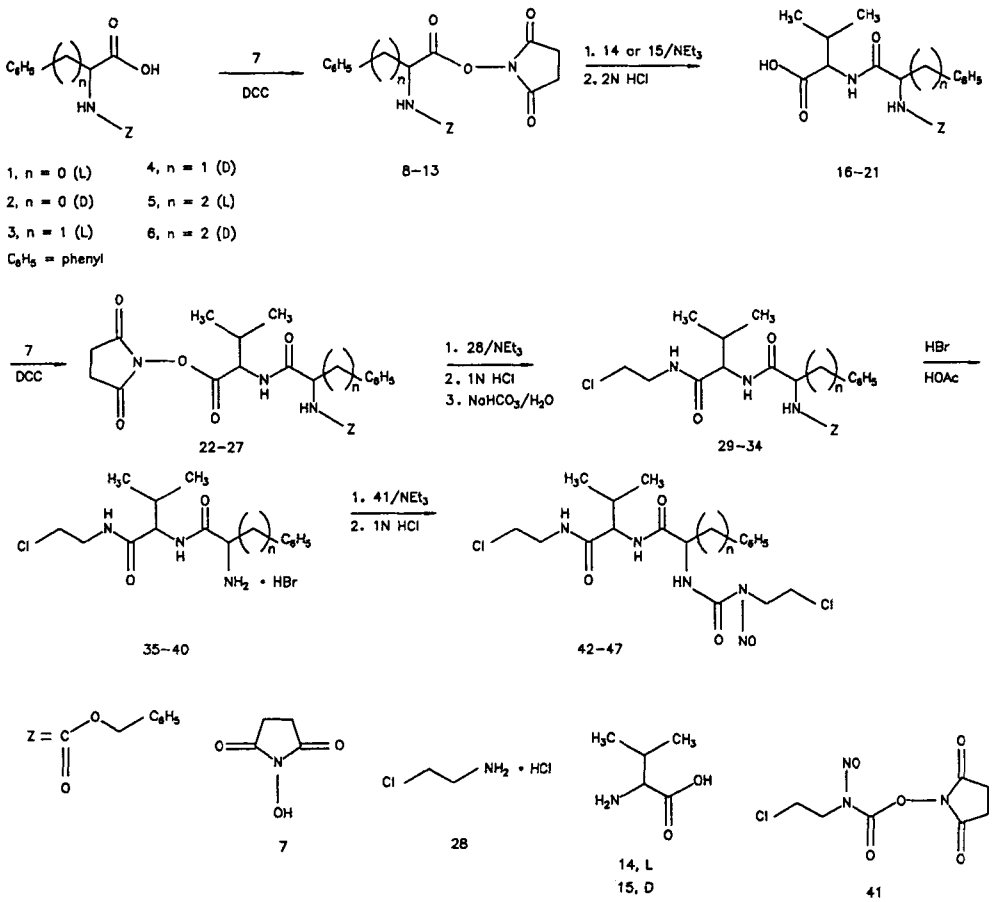
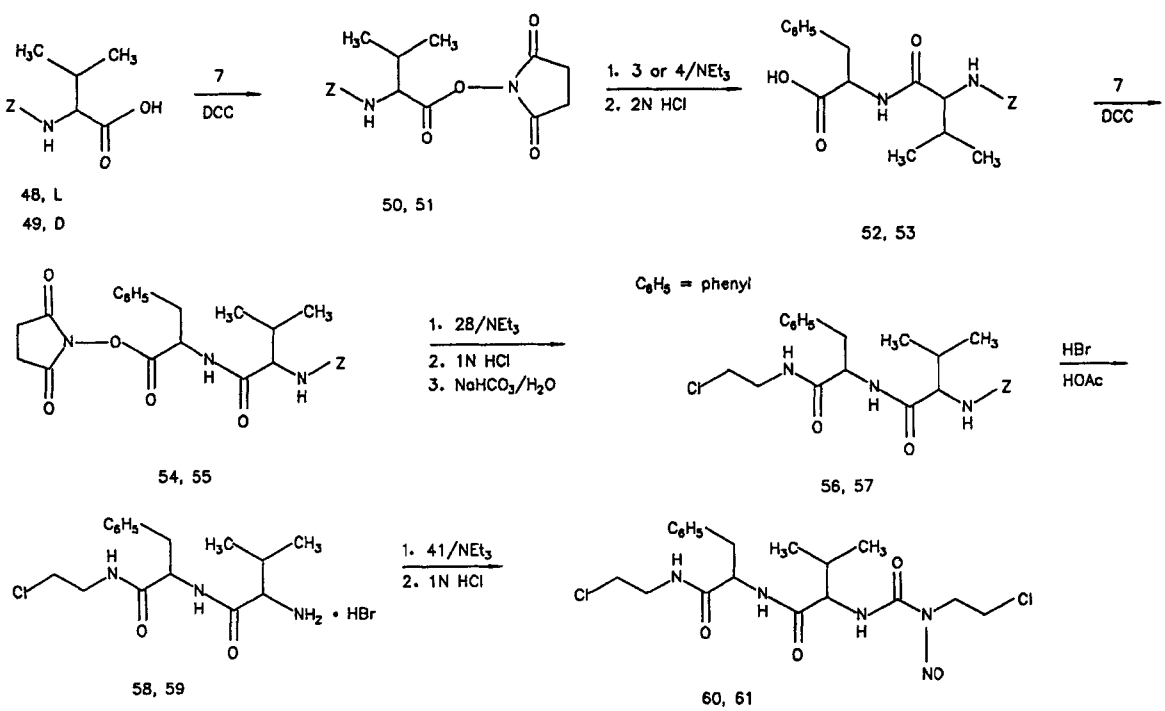


Scheme I



Scheme II

Table I—Physical Properties of *N*-Succinimide Derivatives

Compound	Yield, %	mp (dec), °C	Molecular Formula[a]	MS: *m/e*[b]
8	74	115–117	$C_{20}H_{18}N_2O_6$ (382.37)	383 (M^++1,20), 339 (M^++1)-44,100
9	64	116–118	$C_{20}H_{18}N_2O_6$ (382.37)	383 (M^++1,18), 339 (M^++1)-44,100
10	78	125–128	$C_{21}H_{20}N_2O_6$ (396.39)	397 (M^++1,15), 353 (M^++1)-44,100
11	62	125–127	$C_{21}H_{20}N_2O_6$ (396.39)	397 (M^++1,16), 353 (M^++1)-44,100
12	72	131–134	$C_{22}H_{22}N_2O_6$ (410.43)	411 (M^++1,16), 367 (M^++1)-44,100
13	57	132–135	$C_{22}H_{22}N_2O_6$ (410.43)	411 (M^++1,19), 367 (M^++1)-44,100
50	62	118–121	$C_{17}H_{20}N_2O_6$ (348.35)	349 (M^++1,14), 305 (M^++1)-44,100
51	51	119–122	$C_{17}H_{20}N_2O_6$ (348.35)	349 (M^++1,14), 305 (M^++1)-44,100

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.5%; M_r values are given in parentheses. [b] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1.

Table II—Physical Properties of the Dipeptide Derivatives

Compound	Yield, %	mp (dec), °C	Molecular Formula[a]	MS: *m/e*[b]
16	46	126–128	$C_{21}H_{24}N_2O_5$ (384.43)	385 (M^++1,100), 341 (M^++1)-44,80
17	38	127–129	$C_{21}H_{24}N_2O_5$ (384.43)	385 (M^++1,100), 341 (M^++1)-44,78
18	48	138–141	$C_{22}H_{26}N_2O_5$ (398.46)	399 (M^++1,100), 355 (M^++1)-44,85
19	33	139–142	$C_{22}H_{26}N_2O_5$ (398.46)	399 (M^++1,100), 355 (M^++1)-44,78
20	43	145–148	$C_{23}H_{28}N_2O_5$ (412.48)	413 (M^++1,100), 369 (M^++1)-44,78
21	30	144–147	$C_{23}H_{28}N_2O_5$ (412.48)	413 (M^++1,100), 369 (M^++1)-44,80
52	31	128–131	$C_{22}H_{26}N_2O_5$ (398.46)	399 (M^++1,100), 355 (M^++1)-44,80
53	28	130–133	$C_{22}H_{26}N_2O_5$ (398.46)	399 (M^++1,100), 355 (M^++1)-44,70

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%; M_r values are given in parentheses. [b] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1.

measurements of lipophilicity in vitro. Preliminary results of this study[32] were reported.

Results and Discussion

Syntheses—The incorporation of the chloroethylamino moiety and 2-chloroethylnitrosourea moiety into each of the amino acids of the dipeptides was achieved according to Schemes I and II. Thus, the L,L- and D,D-phenylglycyl-valyl (**42** and **43**), L,L- and D,D-phenylalanyl-valyl (**44** and **45**), and L,L- and D,D-homophenylalanyl-valyl (**46** and **47**) derivatives were synthesized according to Scheme I, whereas the L- and D-valyl-phenylalanyl derivatives **60** and **61** were prepared according to Scheme II. The *N*-succinimidyl derivatives **8–13** were prepared by condensing the corresponding carbobenzoxy-protected amino acids **1–6** with *N*-hydroxy succinimide (**7**) in the presence of dicyclohexylcarbodiimide (Table I). The corresponding *N*-succinimidyl derivatives (**8–13**) were reacted with L- and D-valine (**14** and **15**) to give the dipeptide derivatives **16–21** (Table II). The *N*-succinimidyl derivatives **22–27** of the dipeptides **16–21** were synthesized by condensing *N*-hydroxysuccinimide (**7**) with the corresponding dipeptide derivatives **16–21** in the presence of dicyclohexylcarbodiimide (Table III). The incorporation of the 2-chloroethylamino group into the C-terminus of **22–27** was achieved by reacting 2-chloroethylamine hydrochloride (**28**) with the dipeptide derivatives **22–27** in the presence of triethylamine (Table IV). The carbobenzoxy group was removed from **29–34** by the standard deprotection method with a mixture of hydrobromic and acetic acids. The resulting hydrobromide salts **35–40** were purified by recrystallization (Table V). Finally, the incorporation of the *N'*-nitroso-*N'*-(2-chloroethyl)-aminocarbonyl group into the N-terminus of the dipeptide derivatives **35–40** was achieved by reacting the transfer reagent, *N'*-hydroxysuccinimide-*N*-(2-chlorethyl)nitroso-carbamate (**41**), with the corresponding dipeptide salts (**35–40**) in the presence of triethylamine to give the chloroethylnitrosourea compounds **42–47** (Table VI). The valyl-phenylalanyl derivatives **60, 61** were synthesized according to Scheme II in an analogous way to the preparation of **42–47** (Scheme I). The intermediates **50** and **51** to **58** and **59** and the final products **60** and **61** were synthesized in a similar way to that of the intermediates **8–13** to **35–40** and **42–47** (Tables I–VI).

Table III—Physical Properties of *N*-Succinimide Derivatives of the Dipeptides

Compound	Yield, %	mp (dec), °C	Molecular Formula[a]	MS: *m/e*[b]
22	52	147–150	$C_{25}H_{27}N_3O_7$ (481.50)	367 (M^+-114,100), 240 (M^+-241,29)
23	41	146–150	$C_{25}H_{27}N_3O_7$ (481.50)	367 (M^+-114,100), 240 (M^+-241,32)
24	54	158–160	$C_{26}H_{29}N_3O_7$ (495.53)	381 (M^+-114,100), 382 (M^+-113,25), 254 (M^+-241,27)
25	37	157–160	$C_{26}H_{29}N_3O_7$ (495.53)	381 (M^+-114,100), 382 (M^+-115,30), 254 (M^+-241,27)
26	53	164–167	$C_{27}H_{31}N_3O_7$ (509.55)	395 (M^+-114,100), 268 (M^+-241,27)
27	34	164–166	$C_{27}H_{31}N_3O_7$ (509.55)	395 (M^+-114,100), 268 (M^+-241,30)
54	46	143–147	$C_{26}H_{29}N_3O_7$ (495.53)	382 (M^+-113,30), 381 (M^+-114,100)
55	42	145–148	$C_{26}H_{29}N_3O_7$ (495.53)	382 (M^+-113,24), 381 (M^+-114,100)

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%; M_r values are given in parentheses. [b] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1.

Difficulties were encountered in all series during the isolation and purification processes because of the formation of semisolids. Attempts to use flash chromatography frequently resulted in the decomposition of target compounds. Hence, cumbersome repeated recrystallization procedures were used to obtain pure compounds. The yields of compounds in the D-series tended to be lower than those of the corresponding L-series. All intermediates and final products were checked for uniformity by thin-layer chromatography, and analyzed by micro combustion (C,H,N) and mass spectrometry.

The incorporation of the 2-chloroethylamino moiety into the C-terminus and the 2-chloroethylnitroso carbonyl moiety into the N-terminus of the amino acids was achieved according to Scheme III. Although the target compounds **74–76** were previously described,[15] difficulties have been experienced in reproducing the procedures. Hence, more detailed experimental results are now reported. The succinimidyl derivatives **65–67** were prepared by condensing the corresponding carbobenzoxy-protected amino acids with the *N*-hydroxysuccin-

Table IV—Physical Properties of Protected Dipeptides Containing Chloroethylamino Moiety

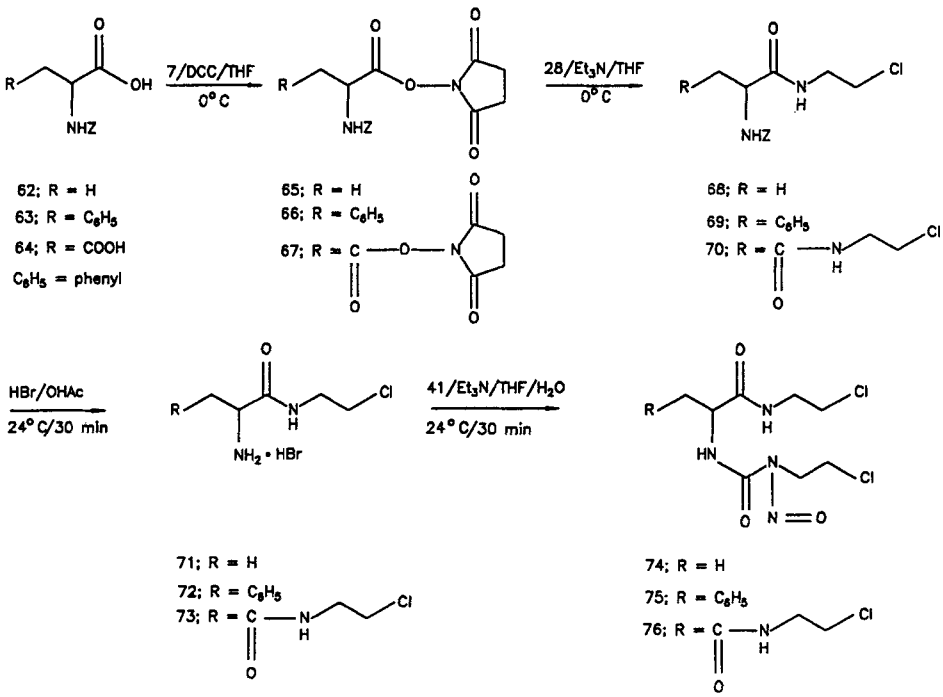
Compound	Yield, %	mp (dec), °C	Molecular Formula[a]	MS: *m/e*[b]
29	47	185–189	$C_{23}H_{28}N_3O_4Cl$ (445.94)	447 (M^++2,6), 446 (M^++1,16), 411 (M^+-34,22), 410 (M^+-35,100)
30	41	185–188	$C_{23}H_{28}N_3O_4Cl$ (445.94)	447 (M^++2,8), 446 (M^++1,18), 411 (M^+-34,16), 410 (M^+-35,100)
31	47	199–201	$C_{24}H_{30}N_3O_4Cl$ (459.97)	462 (M^++3,5), 461 (M^++2,4), 460 (M^++1,14), 425 (M^+-34,27), 424 (M^+-35,100)
32	40	199–201	$C_{24}H_{30}N_3O_4Cl$ (459.97)	462 (M^++3,6), 461 (M^++2,7), 460 (M^++1,16), 425 (M^+-34,28), 424 (M^+-35,100)
33	42	206–210	$C_{25}H_{32}N_3O_4Cl$ (473.99)	475 (M^++2,4), 474 (M^++1,14), 439 (M^+-34,27), 438 (M^+-35,100)
34	38	206–210	$C_{25}H_{32}N_3O_4Cl$ (473.99)	475 (M^++2,4), 474 (M^++1,16), 439 (M^+-34,30), 438 (M^+-35,100)
56	41	182–186	$C_{24}H_{30}N_3O_4Cl$ (459.97)	462 (M^++3,5), 460 (M^++1,14), 424 (M^+-35,100)
57	36	185–189	$C_{24}H_{30}N_3O_4Cl$ (459.97)	462 (M^++3,6), 460 (M^++1,17), 424 (M^+-35,100)

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%; M_r values are given in parentheses. [b] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1.

Table V—Physical Properties of Dipeptides Containing Chloroethylamino Moiety

Compound	Yield, %	mp (dec), °C	Molecular Formula[a]	MS: *m/e*[b]
35	64	210–216	$C_{15}H_{23}N_3O_2ClBr$ (392.73)	314 (M^+-78,22), 313 (M^+-79,14), 312 (M^+-80,68), 276 (M^+-116,100)
36	52	210–215	$C_{15}H_{23}N_3O_2ClBr$ (392.73)	314 (M^+-78,30), 313 (M^+-79,12), 312 (M^+-80,70), 276 (M^+-116,100)
37	62	226–228	$C_{16}H_{25}N_3O_2ClBr$ (406.76)	370 (M^+-36,5), 328 (M^+-78,26), 327 (M^+-79,15), 326 (M^+-80,75), 290 (M^+-116,100)
38	50	227–229	$C_{16}H_{25}N_3O_2ClBr$ (406.76)	370 (M^+-36,4), 328 (M^+-78,22), 327 (M^+-79,12), 326 (M^+-80,71), 290 (M^+-116,100)
39	58	230–234	$C_{17}H_{27}N_3O_2ClBr$ (420.78)	384 (M^+-36,6), 342 (M^+-78,24), 341 (M^+-79,11), 340 (M^+-80,71), 304 (M^+-116,100)
40	47	230–235	$C_{17}H_{27}N_3O_2ClBr$ (420.78)	384 (M^+-36,7), 342 (M^+-78,23), 341 (M^+-79,10), 340 (M^+-80,74), 304 (M^+-116,100)
58	48	209–214	$C_{16}H_{25}N_3O_2ClBr$ (406.76)	328 (M^+-78,26), 290 (M^+-116,100)
59	34	210–215	$C_{16}H_{25}N_3O_2ClBr$ (406.76)	328 (M^+-78,30), 290 (M^+-116,100)

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%; M_r values are given in parentheses. [b] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1.



Scheme III

Table VI—Physical Properties of Dipeptides Containing Chloroethylamino Moiety and Chloroethylnitroso Moiety

Compound	Yield, %	mp (dec), °C	Molecular Formula[a]	MS: *m/e*[b]
42	32	203–207	$C_{18}H_{25}N_5O_4Cl_2$ (446.33)	411 (M^+-35,6), 381 (M^+-65,54), 346 (M^+-100,21), 345 (M^+-101,100)
43	28	203–206	$C_{18}H_{25}N_5O_4Cl_2$ (446.33)	411 (M^+-35,7), 381 (M^+-65,60), 346 (M^+-100,20), 345 (M^+-101,100)
44	34	218–220	$C_{19}H_{27}N_5O_4Cl_2$ (460.36)	425 (M^+-35,4), 395 (M^+-65,50), 387 (M^+-73,17), 360 (M^+-100,23), 359 (M^+-101,100)
45	27	218–221	$C_{19}H_{27}N_5O_4Cl_2$ (460.36)	425 (M^+-35,4), 395 (M^+-65,46), 387 (M^+-73,12), 360 (M^+-100,23), 359 (M^+-101,100)
46	31	222–225	$C_{20}H_{29}N_5O_4Cl_2$ (474.38)	439 (M^+-35,5), 409 (M^+-65,60), 374 (M^+-100,24), 373 (M^+-101,100)
47	25	222–224	$C_{20}H_{29}N_5O_4Cl_2$ (474.38)	439 (M^+-35,6), 409 (M^+-65,60), 374 (M^+-100,22), 373 (M^+-101,100)
60	30	202–206	$C_{19}H_{27}N_5O_4Cl_2$ (460.36)	425 (M^+-35,6), 395 (M^+-65,62), 360 (M^+-100,26), 359 (M^+-101,100)
61	22	204–207	$C_{19}H_{27}N_5O_4Cl_2$ (460.36)	425 (M^+-35,7), 395 (M^+-65,51), 360 (M^+-100,18), 359 (M^+-101,100)

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%; M_r values are given in parentheses. [b] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1.

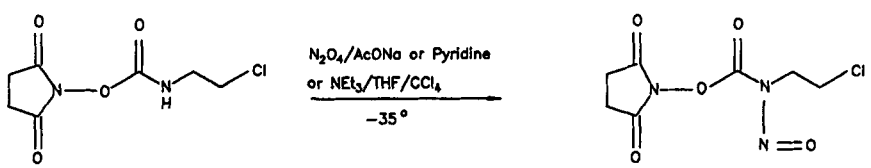
Table VII—Physical Properties of *N*-Succinimide Derivatives 65–67

Compound	Yield, %	mp (dec), °C	Lit.[46] mp (dec), °C
65	87	122–124	123–123.5
66	53	139–141	140–140.5
67	52	114–116	—[a]

[a] Not applicable.

imide (**7**) in the presence of DCC (Table VII). The succinimidyl derivatives (**65–67**) were condensed with 2-chloroethylamine salt (**28**) in the presence of triethylamine to give **68–70** (Table VIII). The carbobenzoxy group was removed from **68–70** by the standard deprotection method with a mixture of hydrobromic and acetic acids. The resultant hydrobromide salts **71–73** were extremely hygroscopic and could not be analyzed by combustion. Therefore, **71–73** were used directly in the next step. Thus, these compounds were reacted with the transfer reagent **41** in the presence of triethylamine to give the end products **74–76**. The isolation and purification of **74–76** presented difficulties because of formation of semisolid residues. Although on the basis of NMR analyses **74–76** appeared to have the desired purity, the microanalyses were not entirely satisfactory (Table IX). Attempts to further improve the quality of **74–76** by flash chromatography resulted in decompositions of **74–76** (Table IX). The important transfer reagent **41** was prepared by the nitrosation of the succinimidyl chloroethyl carbamate **77** with dinitrogen tetroxide with either homogenous reaction mixtures with pyridine or triethylamine as bases, or a heterogenous mixture with sodium acetate (Scheme IV). The reaction was monitored by ^{1}H NMR observing the CH_2Cl shift from 3.71 ppm (**77**) to 4.25 ppm (**41**). The reaction requires two equivalents of dinitrogen tetroxide, not one equivalent as was erroneously reported[29] in literature.

Mass Spectrometry—The most abundant peak in the mass spectra of **9**, **11**, and **13**, beside the molecular ion, the (M^++1)-44 peak, was attributed to elimination of carbon



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Scheme IV

Table VIII—Physical Properties of 2-(Chloroethylamide) of Carbobenzyloxy Amino Acids 68–70

Compound	Yield, %	mp (dec), °C[a]	Lit. mp (dec), °C	Molecular Formula[b]	MS: *m/e*[c]
68	42	114–116 (2-propanol:pet. ether, 1:4)	—[d]	$C_{13}H_{17}ClN_2O_3$ (284.74)	285 (M^++1,2), 249 (M^+-35,1), 178 (M^+-106,69), 134 (M^+-150,100)
69	69	138–140 (ethyl acetate)	138–140[e]	$C_{19}H_{21}ClN_2O_3$ (360.83)	361 (M^++1,2), 325 (M^+-35,4), 254 (M^+-106,24), 210 (M^+-150,100)
70	64	174–176 (2-propanol)	174–176[e]	$C_{16}H_{21}Cl_2N_3O_4$ (390.26)	391 (M^++1,2), 284 (M^+-106,1), 240 (M^+-150,1)

[a] Recrystallization solvent is given in parentheses. [b] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N within ±0.4%); M_r values are given in parentheses. [c] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1. [d] Not applicable. [e] Reference 15.

Table IX—Physical Properties of Amino Acids 74–76 Containing Chloroethylamino Moiety and Chloroethylnitroso Moiety

Compound	Yield, %	mp (dec), °C	Lit. mp (dec), °C[a]	Molecular Formula[b]	MS: *m/e*[c]	^{1}H NMR[d]
74	81	111–113	112–114	$C_8H_{14}Cl_2N_4O_3$ (285.13)	286 (M^++1,14), 250 (M^+-35,4), 248 (M^+-37,1), 207 (M^+-78,5), 179 (M^+-106,3)	7.44 (d, 1H, N*H*CH, exch with D_2O), 6.40 (brs, 1H, N*H*CH_2, exch with D_2O). 4.60 (dd, 1H, C*H*), 4.17 (t, 2H, CH_2Cl), 3.63 (d, 4H, CH_2Cl, CH_2N(N=O), 3.48 (t, 2H, CH_2NH), 1.57 (d, 3H, CH_3).
75	83	92–94	93–95	$C_{14}H_{18}Cl_2N_4O_3$ (361.22)	362 (M^++1,21), 326 (M^+-35,1), 283 (M^+-78,4), 255 (M^+-106,32), 253 (M^+-108,100)	7.48 (d, 1H, N*H*CH, exch with D_2O), 7.13–7.37 (m, 5H, Ph), 5.97 (brs, 1H, N*H*CH_2 exch with D_2O), 4.74 (dd, 1H, C*H*), 4.13 (t, 2H, CH_2Cl), 3.40–3.60 (m, 6H, CH_2Cl, CH_2NH, CH_2N(N=O), 3.20 (q, 2H, PhCH_2).
76	92	155–157	156–157	$C_{11}H_{18}Cl_3N_4O_4$ (390.66)	391 (M^++1), 355 (M^+-35,1), 312 (M^+-78,1), 284 (M^+-106,4), 282 (M^+-108,9)	7.46 (d, 1H, N*H*CH, exch with D_2O), 6.20 (brs, 1H, N*H*CH_2, exch with D_2O), 6.00 (brs, 1H, N*H*CH_2 exch with D_2O), 4.66 (dd, 1H, C*H*), 4.07 (t, 2H, CH_2Cl), 4.07 (t, 2H, CH_2Cl), 3.36–3.63 (m, 10H, C*H*'s), 2.48 (d, 2H, CH_2CO).

[a] Reference 15. [b] The M_r values are given in parentheses; microanalyses (%): **74** (calcd: C, 33.68; H, 4.91; N, 19.64; found: 34.54, 4.86, 18.68); **75** (calcd: C, 46.53; H, 4.98; N, 15.51; found: 47.44, 5.30, 15.15); **76** (calcd: C, 33.80; H, 4.60; N, 17.92; found: 35.03, 4.67, 17.52). [c] Relative percent intensities of the peaks; chemical ionization; molecular ion M^++1. [d] The ^{1}H NMR spectra were recorded in $CDCl_3$.

dioxide (Table I). The most abundant peak in the mass spectra of **16**, **18**, and **20**, beside the molecular ion, the (M^++1)-44 peak, was also attributed to elimination of carbon dioxide (Table II). The most abundant peak in the mass spectra of **22–27**, beside the molecular ion, the (M^+-114) peak, was attributed to the elimination of *N*-oxysuccinimidyl moiety (Table III). The most abundant peak in the mass spectra of **29–34**, beside the molecular ion, the (M^+-35) peak, was attributed to the elimination of chlorine (Table IV). The most abundant peaks in the mass spectra of **35–40**, the (M^+-78), (M^+-80) and (M^+-116) peaks, were attributed to the elimination of $HNCH_2CH_2Cl$, bromine, and simultaneous elimination of chlorine and hydrogen bromide (Table V). The most abundant peaks in the mass spectra of **42–47**, the (M^+-35) and (M^+-100) peaks, were attributed to the sequential loss of chlorine and O=C–N(NO)CH_2CH_2 moieties (Table VI). Analogous mass spectral fragments were observed for **50**, **51–58**, **59**, and the final products **60** and **61** (Tables I–VI). The most abundant peaks in the mass spectra of **68–70**, the (M^+-35) and (M^+-106) peaks, were attributed to the elimination of chlorine and 2-chloroethyl-aminocarbonyl moieties, respectively (Table VIII). The most abundant fragments in the mass spectra of **74–76**, the (M^+-35) (M^+-78), and (M^+-106) peaks, were attributed to the sequential loss of chlorine, $HNCH_2CH_2Cl$, and HNC(O)CH_2CH_2Cl moieties, respectively (Table IX).

Biological Evaluations—Acute toxicities of **42–47** and **60**, **61** were obtained with Swiss male mice at 50-, 100-, 150-, and 200-mg/kg doses, and those of **74–76** were obtained at 100-, 200-, 300-, and 500-mg/kg doses. Compounds **42–47** and **60**, **61** possessed acute toxicity at 100-, 150-, and 200-mg/kg doses, and **74–76** possessed acute toxicity at 300- and 500-mg/kg doses. The anticancer activity was evaluated in vivo against the murine lymphocytic leukemia P388 in accordance with the National Cancer Institute protocol.[37] Compounds **42–47** and **60**, **61** were tested in vivo against P388 at doses of 20 and 40 mg/kg per day. In the L,L-series, **42** exhibited the highest activity, with a %ILS of 111, followed by **44** (%ILS = 81), **60** (%ILS = 69), and **46** (%ILS = 46). In the case of the D,D-series (**43**, **45**, **47**, **61**), except for **43** (%ILS = 31), no pronounced anticancer activity was observed. Thus, the natural analogues of the dipeptides [i.e., the L,L-series (**42**, **44**, **46**, and **60**)] possessed anticancer activities with a %ILS ranging from 46 to 111 at 40-mg/kg per day doses (Table X). The corresponding synthetic analogues in the D,D-series (**43**, **45**, **47**, and **61**) exhibited either none or marginal anticancer properties, with a %ILS ranging between 2 and 31 at 40-mg/kg per day doses (Table VII). However, in both series, the phenylglycyl-valyl dipeptide derivatives exhibited the highest activity, (i.e., the %ILS of the L,L- and D,D-derivatives were 111 and 31, respectively). The analogously structured monoamino acid derivatives of L-alanine (**74**), L-phenylalanine (**75**), and L-aspartic acid (**76**) possessed much higher activities against the P388 tumor than any of the dipeptide derivatives. Thus, the %ILS values at optimum dose for **74**, **75**, and **76** were 481, 297, and 481, respectively (Table X). The more active **42**, **44**, **60**, and **74–76** in the P388 test were further investigated in vivo against the lymphoid leukemia L1210 in accordance with the protocol of the National Cancer Institute.[37] Compounds **42**, **44**, and **60** possessed either low or marginal activities (i.e., the %ILS values were 46, 31, and 26, respectively; Table XI). Compounds **74**, **75**, and **76** exhibited somewhat higher but, nevertheless, low-to-moderate activities, with %ILS values of 56, 48, and 64, respectively (Table XI). This result is in contrast to previously reported[15] high activities. However, in the previous report,[15] a different test procedure was used than that[37] in the present work.

It has been shown[5,8,9,29,33] that to pursue a rational and systematic approach for development of anticancer drugs, a correlation between %ILS and lipophilicity parameters [partition coefficient (*P*)] can be rewarding. We hypothesized that on the basis of a considerable spread and sequence of lipophilicities of the parent amino acids (Asp < Ala < Val < Phe)[38,39] that were used in this project, an analogous spread and sequence may result in the target compounds. However, although the sequence of lipophilicities was basically retained, the introduction of highly hydrophobic *N*-2-chloroethyl and *N*-nitroso-*N*-2-chloroethyl moieties caused a compression of lipophilicities of the target compounds into a narrow range of log *P* of ~3.2–3.5. Nevertheless, a trend toward higher activities can be associated with structures possessing lower hydrophobicities. Thus, in P388 tumor lines (Table X), the following sequence was obtained (%ILS, log *P*): **76** (481, 3.15), **74** (481, 3.22) > **75** (297, 3.25) > **42** (111, 3.32) > **44** (81, 3.46) > **60** (69, 3.47). Analogously, in L1210 tumor lines (Table XI), the following sequence was obtained: **76** (64,

Table X—Anticancer Activities of Amino Acid and Dipeptide Derivatives against P388 Lymphocytic Leukemia in CD_2F_1 Male Mice

Compound	Dose, mg/kg/day[a]	Weight Change, %[b]		T/C, %[c]	T/C, %[d]	ILS, %[c]	ILS, %[d]	Survivors (survivors/total)	
		5-Day	10-Day					On Day 30	On Day 60
5-Fluorouracil	200[e]	−4.0	—[f]	179	—	79	—	0/8	—
L-Phegly-L-Val	20	−4.6	—	155	—	55	—	0/6	—
42	40	−9.1	—	211	—	111	—	0/6	—
D-Phegly-D-Val	20	−5.8	—	105	—	5	—	0/6	—
43	40	−9.6	—	131	—	31	—	0/6	—
L-Phe-L-Val	20	−5.2	—	137	—	37	—	0/6	—
44	40	−9.8	—	181	—	81	—	0/6	—
D-Phe-D-Val	20	−6.2	—	94	—	−6	—	0/6	—
45	40	−10.4	—	109	—	9	—	0/6	—
L-Homophe-L-Val	20	−5.1	—	123	—	23	—	0/6	—
46	40	−10.8	—	146	—	46	—	0/6	—
D-Homophe-D-Val	20	−6.8	—	88	—	−12	—	0/6	—
47	40	−11.2	—	102	—	2	—	0/6	—
L-Val-L-Phe	20	−5.3	—	128	—	28	—	0/6	—
60	40	−10.4	—	169	—	69	—	0/6	—
D-Val-D-Phe	20	−6.4	—	91	—	−9	—	0/6	—
61	40	−10.5	—	105	—	5	—	0/6	—
5-Fluorouracil	200[e]	−2.7	—	145	—	45	—	0/6	—
MeCCNU	30	0.0	—	279[g]	340	179[g]	240	3/3	1/5
L-Ala	20	—	−15.7	295	581	195	481	6/0	6/0
74	30	—	−29.7	211	309	111	209	2/4	2/4
	40	—	−29.7	117	—	17	—	0/6	
L-Phe	20	—	+13.5	173	—	73	—	0/6	—
75	30	+10.4	+9.0	179	—	79	—	0/5	—
	40	—	−9.4	276	397	176	297	5/1	1/5
L-Asp	20	—	+14.1	292	418	192	318	5/1	2/4
76	30	—	+6.5	295	487	195	387	6/0	3/3
	40	—	−10.8	289	581	189	481	5/0	5/0

[a] All compounds were administered in a 5% polysorbate 80 solution. [b] The average percentage weight changes on days 5 and 10 were taken as a measure of drug toxicity. [c] Results obtained on day 30. [d] Results obtained on day 60. [e] 5-Fluorouracil in a single dose of 200 mg/kg in 0.85% saline solution (Sigma Chemical) was administered on day 1, in accordance with the Protocol of the National Cancer Institute.[37] [f] —, Not determined. [g] In ref 10, T/C = 245.

3.15) > **74** (56, 3.22) > **75** (48, 3.25) > **42** (46, 3.32) > **44** (31, 3.46) > **60** (26, 3.47). These results are in general agreement with results of our preliminary communication.[32]

These results indicate that a search for less hydrophobic congeners should lead to more effective anticancer drugs. In support of this contention, it was found[19] that the replacement of one 2-chloroethyl group by a 2-hydroxyethyl group results in such a congener, the 1-(2-chloroethyl)-1-nitroso-3-(2-hydroxyethyl) urea with a log *P* value of 0.3. This compound, predictably,[5] exhibits superior activities than the clinically used nitrosourea congeners[19] against a variety of cancers.

Experimental Section

Mice—Male Swiss mice were used for acute toxicity studies (average weight, 18–21 g). The male CD_2F_1 mice for testing (average weight, 18–20 g), and the male DBA/2 mice for tumor propagation[36] (6–7-weeks-old) were supplied by Harlan Sprague-Dawley, Inc. (Indianapolis, IN). The tumor-bearing mice or vials with tumor cells were obtained from the Frederick Cancer Research Facility, Frederick, MD. Mice were fed rodent Laboratory Chow 5001 (Purina Mills, St. Louis, MO) and water ad libitum.

Drugs—Compounds were administered in a 5% polysorbate 80 solution (Sigma Chemical Company, St. Louis, MO).

Biological Evaluations—Acute toxicity tests of **42–47** and **60, 61** were obtained with Swiss male mice at 50-, 100-, 150-, and 200-mg/kg doses and for **74–76** at 100-, 200-, 300-, and 500-mg/kg doses. Compounds were evaluated in vivo against the lymphocytic leukemia P388 and against lymphoid leukemia L1210 in mice following the protocol of the National Cancer Institute.[37] The CD_2F_1 male mice (18–20 g weight), in groups of six, were inoculated intraperitoneally either with 10^6 cells of P388 or with 10^5 cells of L1210 tumor at day zero of the experiment. Compounds **42–47, 60, 61,** and **74–76** were injected intraperitoneally at doses listed in Tables XII and XI for nine successive days from day one. The animals were then observed according to the protocol[37] for 30–60 days, keeping a record of deaths and survivors. The anticancer activity was evaluated by comparing the mean survival time of treated with that of the control animals (i.e., by the T/C method, where T represents the mean survival time of the treated group and C the mean survival time of the tumor-bearing control group). The %ILS parameter was calculated by the formula $[(T - C)/C] \times 100$.

Materials—All chemicals were of the best quality available commercially. Solvents were dried using standard procedures.[40] Triethylamine was stored over solid potassium hydroxide. All amino acids, prepared[41,42] by aminopeptidase technology, were donated by DSM Research, Geleen, The Netherlands. Authentic samples of carbobenzoxy derivatives of alanine, aspartic acid, homophenylalanine, phenylalanine, phenylglycine, and valine were either prepared by literature methods,[43,44] or obtained from the United States Biomedical Corporation, Cleveland, OH. The *N*-hydroxysuccinimide esters of benzyloxycarbonylamino acids and dipeptides were synthesized by adaptation of literature methodologies.[45,46] The *N*-hydroxysuccinimide-*N*-(2-chlorethyl)-nitrosocarbamate (**41**) was synthesized by several modifications of methods reported in literature.[14,29]

Analytical Procedures—Melting points were determined on a Thomas-Hoover Apparatus (model 6406-K) with a calibrated thermometer. The ^1H NMR were recorded on either a Bruker 250 or a GE QE-300 spectrophotometer. Mass spectra were recorded on a Hewlett-Packard mass spectrometer (model 5985 GS), with methane as the

Table XI—Anticancer Activities of Amino Acid and Dipeptide Derivatives against L1210 Lymphoid Leukemia in CD_2F_1 Male Mice

Compound	Dose, mg/kg/day[a]	5-Day Weight Change, %[b]	T/C, %[c]	ILS, %[c]	Survivors on Day 30 (survivors/total)	P[d]	log P
CCNU	25	−11.00	732	632[e]	6/6	380	2.58[f]
MeCCNU	—[g]	—	360[h]	260[h]	—	—	3.30[i]
L-Phegly-L-Val	20	−5.3	111	11	0/6	2089	3.32
42	40	−9.6	146	46	0/6		
L-Phe-L-Val	20	−5.5	104	4	0/6	2896	3.46
44	40	−10.2	131	31	0/6		
L-Val-L-Phe	20	−5.8	121	21	0/6	2925	3.47
60	40	−10.9	126	26	0/6		
L-Ala	20	−3.8	122	22	0/6	1650	3.22
74	40	−7.2	156	56	0/6		
L-Phe	20	−4.2	116	16	0/6	1780	3.25
75	40	−9.1	148	48	0/6		
L-Asp	20	−4.6	129	29	0/6	1425	3.15
76	40	−9.8	164	64	0/6		

[a] All compounds were administered in a 5% polysorbate (Tween 80) solution. [b] The average percentage weight change on day 5 was taken as a measure of drug toxicity. [c] Results obtained on day 30. [d] The values of P (compound in 1 octanol/compound in water) were measured by UV spectroscopy according to a literature method.[48] [e] Result obtained on day 60. [f] In ref 49, log P = 2.83. [g] —, Not determined. [h] Reference 10. [i] Reference 49.

reactant gas. The $(M^+ + 1)$ values are reported for the molecular ion. Microanalyses were performed on a Perkin-Elmer elemental analyzer (model 240C). Thin-layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated sheets (EM reagents; layer thickness, 0.2 mm), with visualization by UV light and/or iodine chamber. Column chromatography was performed using a flash chromatography technique[47] on silica gel 60 (Fluka) finer than 230 mesh. The purity of **42–47** and **60, 61** was checked in a solvent system composed of chloroform and methanol (8:2, v/v). Purity control for monoamino acid derivatives **74–76** was checked in a solvent system composed of dichloromethane and ethyl acetate (2:1, 1:1, and 3:1, v/v, respectively). Values of P were obtained by literature methodology with UV spectrophotometry.[48,49] For measuring P, 1-octanol and water layers were presaturated with each other prior to use. The areas of the initial octanol solutions and the separate water solutions were used to compute the concentrations of the compounds in octanol and in the water layer. The values of P ([compound in 1-octanol]/[compound in water]) and log P are shown in Table XI.

Syntheses—*Preparation of N-Succinimide Derivatives (8–13): A General Procedure*—Dicyclohexylcarbodiimide (2.06 g, 10.0 mmol) was added in one portion to a stirred solution of the corresponding carbobenzoxy-protected amino acid (**1–6**, 10.0 mmol) and *N*-hydroxysuccinimide (**7**, 1.16 g, 10.0 mmol) in tetrahydrofuran (THF, 20 mL) at 0 °C. The reaction mixture was stirred for 6 h at 24 °C and the separated solid, *N,N'*-dicyclohexylurea, was collected by filtration. The solid was successively washed with THF (4 mL) and diethyl ether (3 × 10 mL). The combined washings and filtrate were concentrated on a rotating evaporator at 50 °C and 20 torr. The resulting oily, thick liquid was triturated with diethyl ether and a solid material was formed. Collection of this solid by filtration, and washing with diethyl ether (3 × 5 mL), and repeated recrystallization from ethyl acetate gave the corresponding *N*-succinimidyl derivatives **8–13**. The purity control by TLC (silica gel; chloroform and methanol, 9:1, v/v) indicated one single spot (yields and analytical data are presented in Table I).

Preparation of the Dipeptide Compounds (16–21): A General Procedure—Valine (either **14** or **15**, 5.0 mmol) in THF (10 mL) was added to a stirred solution of the corresponding *N*-succinimide derivative (**8–13**, 5.0 mmol) and triethylamine (0.51 g, 5.0 mmol) in deionized water (3 mL) at 24 °C. The reaction mixture was stirred for 30 min at 24 °C, then the solvent was removed on a rotating evaporator at 24 °C and 20 torr. Deionized water (20 mL) was added to the resulting solid, and the pH of the solution was lowered to ~2.0 with hydrochloric acid (2 N). This solution was extracted with ethyl acetate (3 × 30 mL), the combined organic extracts were dried over magnesium sulfate, and the solid was filtered. The filtrate was evaporated on a rotating evaporator at 24 °C and 20 torr. Repeated recrystallization of the resulting solid from ethyl acetate and hexane yielded the corresponding dipeptide; that is, **16–21**. The purity control by TLC (silica gel; chloroform and methanol, 9:1, v/v) indicated one single spot (yields and analytical data are presented in Table II).

Preparation of N-Succinimidyl Derivatives of the Peptide Compounds 22–27: A General Procedure—Dicyclohexylcarbodiimide (0.52 g, 2.5 mmol) was added in one portion to a stirred solution of the corresponding dipeptide (**16–21**, 2.5 mmol) and *N*-hydroxysuccinimide (**7**, 0.29 g, 2.5 mmol) in THF (10 mL) at 0 °C. The reaction mixture was stirred for 4 h at 24 °C and the separated solid, *N,N'*-dicyclohexylurea, was collected by filtration. The solid was successively washed with THF (4 mL) and diethyl ether (2 × 2 mL). The combined washings and filtrate were concentrated on a rotating evaporator at 24 °C and 20 torr. Repeated recrystallization of the resulting solid from ethyl acetate yielded the corresponding dipeptide; that is, **22–27**. The purity control by TLC (silica gel; chloroform and methanol, 9:1, v/v) indicated one single spot (yields and analytical data are shown in Table III).

Preparation of Protected Dipeptides 29–34 Containing the Chloroethylamino Moiety: A General Procedure—A solution of triethylamine (2.5 mmol) in THF (5 mL) was added to a stirred solution of the corresponding transfer reagents **22–27** (2.0 mmol) and 2-chloroethylamine hydrochloride (**28**, 0.29 g, 2.5 mmol) in THF (10 mL) and deionized water (10 mL). The reaction mixture was stirred for 3 h at 5 °C. To this reaction mixture, deionized water (50 mL) was added, and the reaction mixture was extracted with ethyl acetate (3 × 45 mL). The combined organic extracts were successively washed with a hydrochloric acid solution (1 N, 3 × 30 mL), a saturated aqueous sodium bicarbonate solution (3 × 30 mL), and a saturated aqueous sodium chloride solution (3 × 30 mL), and then dried over magnesium sulfate. The solid was collected by filtration, and the filtrate was evaporated to dryness on a rotating evaporator at 24 °C and 20 torr. Repeated recrystallization of the resulting solids from ethyl acetate and hexane yielded the pure products **27–34**. The purity control by TLC (silica gel; chloroform and methanol, 9:1, v/v) indicated one single spot (yields and analytical data are shown in Table IV).

Preparation of Dipeptide Compounds 35–40 Containing the Chloroethylamino Moiety: A General Procedure—Concentrated hydrobromic acid (6 mL) was added to a stirred solution of the corresponding dipeptide derivative **29–31** (1.0 mmol) in glacial acetic acid (3 mL), and the reaction mixture was stirred for 30 min at 24 °C. Diethyl ether (50 mL) was added to the reaction mixture and a gummy solid was formed. The solution was decanted and the gummy solid was triturated with diethyl ether (25 mL). The resulting solid compound was separated by filtration and washed with diethyl ether (5 mL). Repeated recrystallization of the solid from absolute ethanol and diethyl ether gave the pure product **35–40** (yields and analytical data are shown in Table V).

Preparation of the Dipeptides 42–47 Containing the Chloroethyl-

amino Moiety and the Chloroethylnitroso Moiety: A General Procedure—A solution of *N'*-hydroxysuccinimide-*N*-(2-chloroethyl)-nitrosocarbamate (**41**, 0.5 mmol) was added to a stirred solution of the corresponding dipeptide **35–40** (0.5 mmol) and triethylamine (0.5 mmol) in deionized water (2 mL) and THF (2 mL) at 24 °C. The reaction mixture was stirred for 30 min, then deionized water (25 mL) was added and this reaction mixture was extracted with ethyl acetate (3 × 25 mL). The combined ethyl acetate extracts were successively washed with hydrochloric acid solution (1 N, 3 × 20 mL) and a saturated aqueous sodium chloride solution (3 × 20 mL) and dried over magnesium sulfate. The solid was removed by filtration, and the filtrate was concentrated on a rotating evaporator at 24 °C and 20 torr. Repeated recrystallizations of the crude product from diethyl ether yielded the corresponding pure product **42–47**. Purity control by TLC (silica gel; chloroform and methanol, 8:2, v/v) indicated one single spot (yields and analytical data are presented in Table VI).

Preparation of 50 and 51—Starting from either **48** or **49** and *N'*-hydroxysuccinimide (**7**), a similar procedure to that for the preparation of **15–20** derivatives was adopted (yields and analytical data are shown in Table I).

Preparation of 52 and 53—Starting from either **50** or **51** (D or L) and phenylalanine (**3** or **4**), a similar procedure to that for the preparation of **16–21** was adopted (yields and analytical data are shown in Table II).

Preparation of 54 and 55—Starting from **52** and **53** and *N'*-hydroxysuccinimide (**7**), a similar procedure to that for the preparation of **16–21** was adopted (yields and analytical data are shown in Table III).

Preparation of 56 and 57—Starting from either **54** or **55** and 2-chloroethylamine (**28**), a similar procedure to that for the preparation of **29–34** was adopted (yields and analytical data are shown in Table IV).

Preparation of 58 and 59—Starting from either **56** or **57**, a similar procedure to that for the preparation of **35–40** was adopted (yields and analytical data are shown in Table V).

Preparation of 60 and 61—Starting from either **58** or **59** and *N'*-hydroxysuccinimide-*N*-(2-chloroethyl)nitrosocarbamate (**41**), a similar procedure to that for the preparation of **42–47** was adopted (yields and analytical data are shown in Table VI).

N'-Hydroxysuccinimide Esters of Benzyloxycarbonyl Amino Acids (65–67): A General Procedure—Dicyclohexylcarbodiimide (2.06 g, 10 mmol) was added in one portion to a stirred solution of carbobenzyloxy-protected amino acids (**62–64**, 10 mmol) and *N*-hydroxysuccinimide (**7**, 1.15 g, 10 mmol) in dry THF (20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at 24 °C for 16 h. The separated solid was collected by filtration and washed successively with THF (5 mL) and diethyl ether (3 × 10 mL). The combined washings and filtrate were concentrated on a rotating evaporator at 35 °C and 20 torr. The resulting oily residue was triturated with diethyl ether and a solid material was obtained. Collection of this solid by filtration, washing with diethyl ether (2 × 10 mL), and recrystallization from a mixture of ethyl acetate and diethyl ether (v/v; 2:1) gave the corresponding *N*-succinimidyl derivatives **65–67**. The purity control by TLC (silica gel; chloroform and methanol, 9:1, v/v) indicated one spot (yields and analytical data are presented in Table VII).

2-(Chloroethylamide) of Carbobenzyloxy Amino Acids (68–70): A General Procedure—Triethylamine (0.51 g, 5 mmol) was added to a stirred mixture of corresponding *N*-succinimidyl derivatives **65–67** (5 mmol) and chloroethylamine hydrochloride (**28**, 0.57 g, 5 mmol) in THF (20 mL) and deionized water (2 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C and then for 16 h at 24 °C. Water (50 mL) was added to the reaction mixture, and the resulting solution was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed successively with a 10% aqueous citric acid solution (2 × 20 mL), water (2 × 20 mL), saturated aqueous bicarbonate solution (2 × 20 mL), and water (2 × 20 mL). The organic layer was separated, dried over anhydrous magnesium sulfate, and filtered, and the filtrate was concentrated on a rotating evaporator at 35 °C and 20 torr. Repeated recrystallizations of the resulting solid from appropriate solvents yielded the pure products **67–70**. The purity control by TLC (silica gel; chloroform and methanol, 9:1, v/v) indicated one single spot (yields and analytical data are shown in Table VIII).

2-(Chloroethylamide) of Amino Acids (71–73): A General Procedure—A solution of corresponding Z-amino acids **68–70** (10 mmol) in 30% hydrobromic acid in acetic acid (30% by wt, 10 mL) was stirred at 24 °C for 30 min. Diethyl ether (50 mL) was added to the mixture and a gummy solid was obtained. The solution was decanted and the gummy solid was triturated with diethyl ether (50 mL). The resulting highly hydroscopic solid was used in the next step without further purification.

[(2-Chloroethyl)nitrosocarbamoyl]amino acid (2-chloroethylamide] (74–76): A General Procedure—A solution of transfer agent (**41**, 1 mmol) in THF (5 mL) was added to a stirred solution of the corresponding amino acid amide (**71–73**, 1 mmol) and triethylamine (1 mmol) in deionized water (4 mL) and THF (5 mL) at 24 °C. The reaction mixture was stirred for 0.5 h at 24 °C. Deionized water (25 mL) was added to the reaction mixture, and the resulting solution was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed successively with hydrochloric acid (1 N, 2 × 20 mL) and a saturated aqueous sodium chloride solution (2 × 20 mL), and dried over anhydrous magnesium sulfate and filtered. Concentration of the filtrate gave crude products which on recrystallization from a mixture of ethyl acetate and petroleum ether (v/v, 2:1) yielded products **74–76**. Repeated recrystallization of **74–76** failed to improve the quality of the products. However, the purity control by TLC (silica gel; ethyl acetate and dichloromethane, 1:2, v/v) indicated one single spot. Attempts to improve the microanalytical data of products **74–76** by flash chromatography on silica gel caused decomposition of the products (yields and analytical data are shown in Table IX).

N'-Hydroxysuccinimide-N-(2-chloroethyl)-N-nitrosocarbamate (41): Method A—Anhydrous sodium acetate (9.86 g, 122 mmol) was added to a stirred solution of **77**[29] (2.2 g, 10 mmol) in dry THF (20 mL). The mixture was cooled to −35 °C with dry ice–acetone. To this cooled and stirred mixture, a solution of dinitrogen tetraoxide (1.84 g, 20 mmol) in dry carbon tetrachloride (20 mL) was added in a dropwise manner over 30 min. After the addition, the reaction mixture was stirred for 1 h at −35 °C, during which time the color of the mixture became blue, and then for 0.5 h at 5 °C, during which time the color changed from blue to green and finally to yellow. The reaction mixture was added to a mixture of methylene chloride (20 mL) and ice water (5 °C, 10 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (2 × 20 mL). The combined organic extracts were washed with a saturated aqueous sodium bicarbonate solution (2 × 20 mL) and then with water (2 × 20 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 35 °C and 20 torr gave the crude product. Repeated recrystallization of the crude product from a mixture of methylene chloride and petroleum ether (bp, 40–60 °C; v/v, 1:1) gave 2.05 g (82%) of **41** as a yellow crystalline solid; mp (dec) 105–107 °C [lit.[29] mp (dec) 104–106 °C]; ^{1}H NMR (CD_3COCD_3): δ (TMS) 4.25 (t, 2H, CH_2Cl), 3.71 (t, 2H, CH_2NNO), 3.00 (s, 4H, $-CH_2CH_2-$); MS: *m/e* 250 (M^+ + 1, 19), 185 (M^+ − 64, 5), 145 (M^+ − 104, 70), 116 (M^+ − 133, 100).

Anal.—Calcd for $C_7H_8ClN_3O_5$ (249.60): C, 33.66; H, 3.20; N, 16.83. Found: 33.69, 3.12, 16.64.

Method B—Anhydrous pyridine (1.6 g, 20 mmol) or triethylamine (2 g, 20 mmol) was added to a stirred solution of **77** (2.2 g, 10 mmol) in dry THF (20 mL). The mixture was cooled to −35 °C with dry ice–acetone. To this cooled and stirred solution, a solution of dinitrogen tetroxide (1.84 g, 20 mmol) in dry carbon tetrachloride (20 mL) was added in a dropwise manner over 30 min. After the addition, the reaction mixture was stirred for 1 h at −35 °C, during which time the color changed from blue to green and finally to yellow. The reaction mixture was added to a mixture of methylene chloride (20 mL) and ice water (5 °C, 10 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (2 × 20 mL). The combined organic extracts were washed with a saturated sodium bicarbonate solution (2 × 20 mL) and then with water (2 × 10 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 35 °C and 20 torr gave the crude product. Repeated recrystallizations of the crude product from a mixture of methylene chloride and petroleum ether (bp, 40–60 °C; v/v, 1:1) gave 2.25 g (90%) of **41** as a yellow crystalline solid; mp (dec) 106–108 °C [lit.[29] mp (dec) 104–106 °C). In the case of triethylamine, the yield of **41** was 62% and the microanalyses (C, H, and N) were within ±0.4% of the calculated values.

Anal.—Calcd for $C_7H_8ClN_3O_5$ (249.60): C, 33.66; H, 3.20; N, 16.83. Found: 33.91, 3.19, 16.39.

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Acknowledgments

We thank DSM Research (Geleen, The Netherlands) for support of this work and a generous gift of amino acids, Mr. Klaus Purgstaller for technical assistance, Ms. Nancita R. Lomax (The Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) for a sample of MeCCNU, and The Frederick Cancer Facility, NCI (Frederick, MD) for the P388 and L1210 tumor lines.