collected, washed, and dried to give 0.2 g (60%) of **43**.

(2) **By Reduction of 18.** Compound 18 (500 mg, 2 mmol) was added portionwise to a stirred and cooled mixture of Zn dust (320 mg, 5 mmol), $CuSO_4 \cdot 5H_2O$ (2 mg), water (0.5 mL), and 20% NaOH (1 mL). The mixture was stirred in the cold for $^1/_2$ h, diluted with water (2 mL), and heated at 90 °C for 1 h. The Zn was filtered off, and the filtrate was concentrated to about half its volume and acidified (concentrated HCl) in the cold to pH 3. The crude 4-chloro-2-(hydroxymethyl)benzoic acid thus obtained was cyclized by heating in concentrated HCl (1 mL) at 90 °C for $^1/_2$ h. The solid was collected, washed well with water, and dried to give **43** (55%). The spectral data (NMR, IR, MS) of the 5-chlorophthalide prepared in this way are identical with those of the authentic phthalide obtained as described above. No depression was observed in a mixture melting point.

Pharmacology. For saludiuretic screening, all test compounds were prepared as a solution or as a suspension in saline and fed with a stomach tube to male "Sabra" rats (weight 170–200 g) of the Hebrew University, Jerusalem. The animals received 50 mg/kg in a loading volume of 25 mL of saline/kg of body weight. Eight rats were used for testing each compound. Four test compounds and two controls (furosemide or saline, eight rats each) were tested simultaneously on each experimental day. The rats were starved 18 h prior to the feeding and, following administration of the test compounds, were immediately placed in metabolic cages (two animals/cage) in a sound-proof 22 ± 1 °C, well-ventilated, 12:12 h illuminated room. No food or water was supplied during the experimental period. The urine was collected into plastic graduate measuring cylinders (each containing 2 drops of toluene), measured, and analyzed 5 and 24 h after administration of the compound. pH was measured immediately upon collection of the urine. The samples were centrifuged at 2000 rpm, and chlorides were titrated, after acidification with HNO_3, by $Hg(NO_3)_2$ in the presence of diphenylcarbazone as indicator. Na^+ and K^+ were determined by atomic absorption with a Perkin-Elmer Model 403 spectrophotometer.

For antihypertensive screening, groups of six Sprague–Dawley rats, of 250–350 g of body weight, with a systolic blood pressure (BP) of minimum 170 mmHg, were treated daily subcutaneously with 10 mg/kg doca and saline. When the BP reached 170 mmHg (usually after 3–4 weeks of doca/saline treatment), the test compounds were given orally in doses of 25 mg/kg in 5 mL/kg of 1% methylcellulose. BP was determined by the tail-cuff method prior to and at 1, 4, and 24 h following administration. Hydralazine (4 mg/kg), hydrochlorothiazide (25 mg/kg), and furosemide (25 mg/kg) served as positive controls.

Summary statistics (mean and standard deviation) for all compounds tested were calculated, and comparisons between compounds analyzed were made by the Student's *t* test for independent samples.

Acknowledgment. Diuresis and doca/saline experiments were conducted by Dr. J. Shani at the School of Pharmacy, The Hebrew University of Jerusalem, Israel. SHR experiments were peformed at the Israel Institute for Biological Research, Ness-Ziona, Israel. The assistance of S. Fenster in the statistical significance calculations is gratefully acknowledged.

Registry No. **1a**, 100448-25-7; **1b**, 100448-26-8; **1c**, 100448-27-9; **1d**, 100448-28-0; **1e**, 100448-29-1; **2a**, 100448-31-5; **2b**, 100448-32-6; **2c**, 100448-33-7; **2d**, 100448-34-8; **3a**, 100448-36-0; **3b**, 100448-37-1; **3c**, 100448-38-2; **3d**, 100448-39-3; **3e**, 100448-40-6; **4a**, 100448-41-7; **4b**, 100448-42-8; **4c**, 100448-43-9; **4d**, 100448-44-0; **16**, 2736-23-4; **17**, 3861-99-2; **18**, 100448-45-1; **19**, 89-20-3; **20**, 7147-90-2; **21**, 6015-57-2; **22**, 5566-48-3; **23**, 100448-46-2; **24a**, 100448-47-3; **24b**, 100448-48-4; **24c**, 100448-49-5; **24d**, 100448-50-8; **25a**, 100448-51-9; **25b**, 100448-52-0; **25c**, 100448-53-1; **25d**, 100448-54-2; **26**, 100448-30-4; **27**, 100448-35-9; **36**, 100448-55-3; **37**, 100448-56-4; **40**, 100448-57-5; **41**, 100448-58-6; **43**, 54109-03-4; furfurylhydrazine, 6885-12-7; 4-chloro-2-(hydroxymethyl)benzoic acid, 100448-59-7; hydrazine, 302-01-2; methylhydrazine, 60-34-4; benzylhydrazine, 555-96-4; [*m*-(trifluoromethyl)phenyl]hydrazine, 368-78-5; thiophenol, 108-98-5; 2,4-dichlorobenzoic acid, 50-84-0; *N*,*N*-dimethylformamide dimethyl acetal, 4637-24-5; 4-chloro-2-methylbenzoic acid, 7499-07-2; CuCN, 544-92-3.

Modified Di- and Tripeptides of the C-Terminal Portion of Oxytocin and Vasopressin as Possible Cognition Activation Agents

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A number of peptides and modified peptides were synthesized and studied for their ability to reverse electroconvulsive shock-induced amnesia in rodents. A few of these peptides were selected for secondary evaluation in tests of short-term memory in rats and aged rhesus monkeys. A number of the peptides and modified peptides were active in the amnesia reversal test. In selected secondary tests, however, the chosen compounds failed to show significant activity in enhancing memory. New methods for preparing methyleneamino and methyleneoxy isosteres of peptides are reported. Other modified peptides also included methylenethio, methylenesulfonyl, and ethylene isosteres in place of the normal peptide amide bond.

The ability of peptides to influence cognitive functions has been the subject of several recent investigations.[1-5] de Wied has shown that lysine vasopressin delays the extinction of active avoidance behavior in the intact rat and Walter et al. have reported on the protective effects of neurohypophyseal hormones and analogues and C-terminal fragments of these hormones on the amnestic action of puromycin in mice.

An interest in the area of pharmacological treatments for cognitive dysfunctions or impairments has been actively pursued in these laboratories for some time. Previous reports covered the activity of 3-(aryloxy)pyridines[6] and

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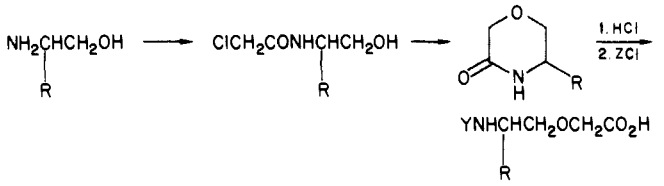
Scheme I[a]



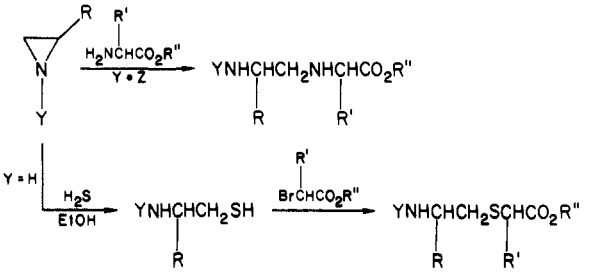
[a] Y = H, Z.

N-[(disubstituted amino)alkyl]-2-oxo-1-pyrrolidineacetamides with emphasis on pramiracetam (CI-879).[7] Our purpose in the present study was, first, to confirm the previous reports that peptides indeed do reverse amnesia and, second, to develop an orally active modified peptide modeled after one of the more active peptide fragments.

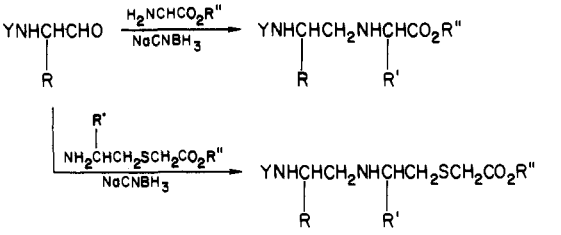
By a modified peptide, we are referring to those types of structures in which the normal peptide amide bond is replaced with an isosteric bond such as methyleneamino, methylenethio, methyleneoxy, ethylene, ketomethylene, etc. Recently, considerable attention has been focused on this approach with the hope of finding nonenzymatically degradable, orally active peptides. A certain amount of progress has been made in this direction. An ethylene isostere of an enkephalin gave enhanced biological activity.[8] Szelke et al.[9] have reported that isosteric replacement of the Leu-Leu bond in angiotensinogen resulted in a compound possessing renin-inhibiting properties. Almquist et al.[10] prepared a Leu-enkephalin with a ketomethylene isostere between Tyr-Gly and Gly-Gly. Hudson et al.[11] made a Met-enkephalin with $-CH_2NH-$ and $-CH_2CH_2-$ replacements in these positions. A review of this area has appeared.[12] Admittedly, there is no solid proof that isosteric peptides will be orally active and only further work will answer this question. Of equal, or perhaps of more importance, is the half-life of the modified peptide, a longer half-life being desirable since most peptides have short half-lives. Unless more satisfactory delivery systems for peptides are forthcoming, the use of many peptides as drugs will continue to remain a problem.

Chemistry. A number of modified peptides were prepared. The original intent was to model the isosteres after the most active of the peptides, but synthetic problems with lysine resulted in mostly Pro-Leu-Gly type compounds being prepared. The peptides in Table I were prepared by standard methods of solution synthesis, solid-phase synthesis, or combinations of these. The synthetic methods for preparing peptide isosteres are less well known. Some of these methods are very limited and produce isosteres that require Gly to be a component. Reductive amination via an imine generated from an amino acid aldehyde and an amino acid ester (Scheme III) can be successfully used, but yields are low and the reaction success is dependent on the amino acids used. Racemization is a recurring problem. Equally as good for certain compounds was the ring opening of aziridines with amino

Scheme II



Scheme III[a]



[a] Y = Z, BOC, Tos.

esters or with H_2S, giving methyleneamino or methylenethio isosteres (Scheme II). We have found that aziridines derived from amino acids can be successfully opened with amino acid esters or hydrogen sulfide to give methyleneamino or methylenethio isosteres. This reaction does have limitations. The amine opening is carried out in refluxing ethanol so the amino ester must be such that it cannot form a diketopiperazine. These reactions are shown in Scheme II. One side reaction that was observed with the aziridine ring opening was the alkylation of the secondary amine **77** to give the dialkyl derivative, which on removal of the benzyloxycarbonyl groups cyclized to **55**. We have also prepared methyleneoxy isosteres by a novel method (Scheme I). Since most amino acid alcohols can now be obtained, this method is applicable for making nearly all modified dipeptides with a C-terminal Gly. In the original synthesis we were limited to making methyleneoxy isosteres with glycine C-terminal, but more recent work has overcome this restriction. The same applies to the ethylene isostere **28** and **29**. A Phe[CH=CH] Phe peptide has been recently obtained.[15]

Pharmacology. Initially, compounds were tested for their ability to reverse electronconvulsive shock (ECS) induced amnesia in rodents.[6,7] Briefly, animals were placed into a small, highly illuminated area that was connected to a large, darkened compartment through an opening in the center of the wall. On entry into the darkened compartment (all four feet into the chamber) footshock was applied through the grid floor until the animal returned to the lighted area or 3 s had elaspsed. Training continued in rats until the animal remained in the lighted compartment for 60 s. Footshock was delivered only once in tests using mice. Two hours after training, ECS (20 mA for 1 s) was delivered transcorneally through saline-soaked electrodes. All animals were required to exhibit maximal hindlimb extension. Two hours after training, drugs were administered intramuscularly (im) at selected doses. One hour after drug treatment, animals were placed into the lighted area, and latency to enter the darkened compartment was recorded. If animals remained in the safe area

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Table I. Chemical and Biological Data of Di- and Tripeptides

compound	$[\alpha]^{25}_D$, deg (c 1, MeOH)	formula	anal.	reversal of electroconvulsive shock induced amnesia in rodents[a] dose, mg/kg im	% reversal rats	% reversal mice
1, Arg-vasopressin	−21.2[b]	$C_{46}H_{65}N_{15}O_{12}S_2$		0.10	60	
				0.010	100	
				0.001	0	
2, Lys-vasopressin	−25.2[b]	$C_{46}H_{65}N_{13}O_{12}S_2$		0.10	56	67
				0.010	20	58
				0.001	20	58
3, Pro-Lys-Gly-NH_2	−60.5[c]	$C_{13}H_{25}N_5O_3 \cdot 0.8H_2CO_3$	C, H, N	0.10	78	39
				0.010	78	48
				0.001	78	100
4, Z-Pro-Lys-GlyNH_2	−55.3	$C_{21}H_{31}N_5O_5 \cdot HOAc \cdot {}^1/_2H_2O$	C, H, N	0.10		63
				0.010		100
				0.001		9
5, BOC-Pro-Lys-Gly-NH_2	−59	$C_{18}H_{33}N_5O_5 \cdot H_2O \cdot {}^1/_3CHCl_3$	Cl,[d] C, H, N	0.10		33
				0.010		17
				0.001		25
6, Z-Pro-Lys(BOC)-Gly-NH_2	−43.8	$C_{26}H_{39}N_5O_7$	C, H, N	0.10	0	
				0.010	0	
				0.001	50	
7, BOC-Pro-Lys(Z)-Gly-NH_2	−38.1	$C_{26}H_{39}N_5O_7 \cdot {}^1/_2H_2O$	C, H, N	0.10		0
				0.010		63
				0.001		91
8, Z-Pro-Lys(BOC)-Gly-OCH_3	−45	$C_{27}H_{40}N_4O_8$	C, H, N	0.10		0
				0.010		54
				0.001		91
9, Z-Pro-Lys-Gly	−52.9	$C_{21}H_{30}N_4O_6 \cdot CF_3CO_2H \cdot 1 \cdot {}^1/_2H_2O$	C, H, N	0.10		79
				0.010		79
				0.001		100
10, Pro-Lys(Z)-Gly-NH_2	−17.6	$C_{21}H_{31}N_5O_52CF_3CO_2H \cdot 3 {}^1/_2H_2O$	H,[e] C, N	0.10	60	100
				0.010	40	92
				0.001	60	66
11, BOC-Lys(Z)-Gly-NH_2	−3.7	$C_{21}H_{32}N_4O_6$	C, H, N	0.10	16	35
				0.010	47	53
				0.001	33	48
12, Z-Lys(BOC)-Gly-NH_2	−5.9[c]	$C_{21}H_{32}N_4O_6$	C, H, N	0.10		33
				0.010		25
				0.001		33
13, Pro-Leu-Gly-NH_2	−49.4	$C_{13}H_{24}N_4O_3 \cdot {}^1/_2H_2O$	C, H, N	0.10	43	42
				0.010	43	33
				0.001	14	42
14, Z-Pro-Leu-Gly-NH_2	−74	$C_{21}H_{30}N_4O_5$	C, H, N	0.10		100
				0.010		47
				0.001		93
15, Pro-Arg-Gly-NH_2	−53.5[c]	$C_{13}H_{25}N_7O_3 \cdot H_2CO_3 \cdot {}^3/_4H_2O$	C, H, N	0.10		30
				0.010		0
				0.001		30
16, BOC-Pro-Arg-Gly-NH_2	−50.4	$C_{18}H_{33}N_7O_5 \cdot H_2O \cdot {}^1/_4CHCl_3$	C, H, N, Cl	0.10		71
				0.010		43
				0.001		0
17, BOC-Pro-Arg(NO_2)-Gly-NH_2	−42.8	$C_{18}H_{32}N_8O_7 \cdot {}^1/_2CHCl_3$	N,[f] C, H	0.10		33
				0.010		20
				0.001		44
18, BOC-Arg(NO_2)-Gly-NH_2	−3.0[g]	$C_{13}H_{25}N_7O_6$	C, H, N	0.10		73
				0.010		53
				0.001		33
19, Arg-Gly-NH_2	+8.3[c]	$C_8H_{18}N_6O_2 \cdot H_2CO_3$	C,[h] H, N	0.10		27
				0.010		20
				0.001		51

[a] Except for one or two compounds, replicate experiments were not done. Criteria established with control animals was such that the results obtained with test compounds were taken as having definitive value. [b] 0.5 HOAC. [c] H_2O. [d] Calcd 7.75, found 7.20. [e] Calcd 6.04, found 5.22. [f] Calcd 21.05, found 22.22. [g] DMF. [h] Calcd 36.98, found 36.48.

for 60 s, they were recorded as having retained the avoidance response.

Comparison of results obtained from tests of peptides in mice vs. rats did not appear to be unacceptably divergent, and since extreme difficulties in running the mouse test were encountered, most testing was done in the rat model.

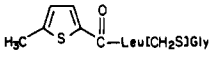
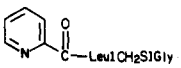
These data were analyzed by using the normal approximation to the binomial distribution. According to an established criteria, 75% of ECS-control animals must exhibit amnesia (enter the darkened compartment in less than 60 s). With this constraint 40% or greater reversal of amnesia was significantly different from ECS-control ($p < 0.05$) and the compound was rated as active.

Compounds selected for their ability to reverse ECS-induced amnesia were tested for effects on performance of rats on a delayed alternation task.[13] Briefly, male hooded rats ranging in age from 4 months to 3 years were used. These animals were drug- and test-sophisticated and were food deprived 22 h prior to testing. Groups of 15–20

Table II. Chemical and Biological Data of Modified Di- and Tripeptides

compound[a]	$[\alpha]^{25}_D$, deg (MeOH)	formula	anal.	reversal of electroconvulsive shock induced amnesia in rodents[b] dose, mg/kg im	% reversal rats	% reversal mice
20, Z-Pro[CH_2O]Gly	−52.7	$C_{15}H_{19}NO_5$	C, H, N	0.10	50	
				0.010	40	
				0.001	40	
				1	37	
				10	62	
				100	62	
21, Z-Pro[CH_2O]Leu-Gly	−79	$C_{21}H_{30}N_2O_6$	C, H, N	0.10	43	
				0.010	71	
				0.001	29	
22, Z-Pro[CH_2O]CH_2COGly	−41.7	$C_{17}H_{22}N_2O_6$	C, H, N	0.10	57	
				0.010	43	
				0.001	14	
23, Z-Pro[CH_2O]CH_2COGly-NH_2	−41	$C_{17}H_{23}N_3O_5$	C, H, N	0.10	20	
				0.010	33	
				0.001	56	
24, Z-Pro[CH_2O]Leu-Gly-NH_2	−76.9	$C_{21}H_{31}N_3O_5$	C, H, N	0.10	0	
				0.010	0	
				0.001	33	
25, Z-Pro-Leu[CH_2O]Gly	−69.6	$C_{21}H_{30}N_2O_6$	C, H, N	0.10	0	
				0.010	0	
				0.001	29	
26, Z-Pro-Leu[CH_2O]Gly-NH_2	−55.3	$C_{21}H_{31}N_3O_5$	C, H, N	0.10	14	
				0.010	57	
				0.001	42	
27, Z-Pro-Phe[CH_2O]Gly-NH_2	−45.1	$C_{24}H_{29}N_3O_5$	C, H, N	100	25	71
				10	42	59
				1	25	20
				0.10	0	
				0.010	11	
				0.001	11	
28, Z-Pro-Leu[CH=CH]Gly-NH_2	−73.0	$C_{22}H_{31}N_3O_4$	C, H, N	0.10	44	
				0.010	77	
				0.001	33	
29, <Glu-Leu[CH=CH]Gly-NH_2	−39.1	$C_{14}H_{23}N_3O_3$	C, H, N	0.10	44	
				0.010	0	
				0.001	11	
30, Z-Pro[CH_2NH]Leu-Gly-NH_2	−62.6	$C_{21}H_{32}N_4O_4$	C, H, N	100	60	57[c]
				10	60	67
				1	80	85
				0.10	0	
				0.010	0	
				0.001	11	
31, Z-Pro-Leu[CH_2NH]Gly-NH_2	−55.2	$C_{21}H_{32}N_4O_4 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N	100	0	
				10	0	
				1	0	
				0.10	43	
				0.010	43	
				0.001	43	
32, <Glu-Leu[CH_2NH]Gly	−4.3	$C_{13}H_{23}N_3O_4 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N, Cl	100	50	
				10	0	
				1	66	
				0.10	33	
				0.010	33	
				0.001	33	
33, Z-Pro[CH_2NH]Leu[CH_2NH]Gly	−7.2	$C_{21}H_{33}N_3O_4 \cdot 1^3/_4HCl \cdot {}^1/_3H_2O$	C, H, N, Cl	100	40	
				10	0	
				1	10	
				0.10	14	
				0.010	20	
				0.001	0	
34, Tos-Pro[CH_2NH]Leu[CH_2NH]Gly	−69.1	$C_{20}H_{33}N_3O_4S \cdot 2HCl$	C, H, N	50	0	
				10	80	
				1	20	
				0.10	40	
				0.010	0	
				0.001	0	
35, Tos-Pro[CH_2NH]Leu-Gly	−61.0	$C_{20}H_{31}N_3O_5S \cdot 1.4HCl$	C, H, N	0.10	0	
				0.010	33	
				0.001	33	
36, Tos-Pro-Leu[CH_2NH]Gly	−120.5	$C_{20}H_{31}N_3O_5S \cdot HCl$	C, H, N	0.10	17	
				0.010	33	
				0.001	17	
37, Tos-Pro[CH_2NH]-*D*-Leu-Gly-NH_2	−79.4	$C_{20}H_{32}N_4O_4S \cdot {}^3/_4H_2O$	H,[d] C, N	100	87	
				10	62	
				1	75	
				0.10	34	
				0.010	0	
				0.001	0	

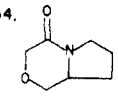
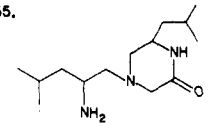
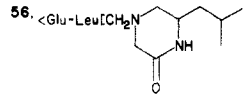
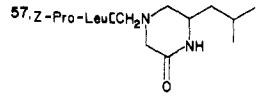
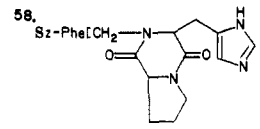
Table II (Continued)

compound[a]	$[\alpha]^{25}_D$, deg (MeOH)	formula	anal.	reversal of electroconvulsive shock induced amnesia in rodents[b] dose, mg/kg im	% reversal rats	% reversal mice
38, BOC-Pro[CH_2NH]-*D*-Lys(Z)-Gly-NH_2	−13.5	$C_{26}H_{41}N_5O_6 \cdot {}^1/_4CH_2Cl_2$	H,[e] C, N	0.1	16	
				0.010	33	
				0.001	16	
39, Pro[CH_2NH]-*D*-Lys(Z)-Gly-NH_2	−7.9	$C_{21}H_{33}N_5O_4 \cdot 2CF_3CO_2H$	C,[f] H, N, F	100	83	
				10	16	
				1	16	
				0.10	33	
				0.010	16	
				0.001	0	
40, Z-Pro[CH_2NH]Leu[CH_2S]Gly	−16.1	$C_{21}H_{32}N_2O_4S$	C, H, N, S	100	100	
				10	0	
				1	60	
				0.10	60	
				0.010	80	
				0.001	20	
41, Z-Pro[CH_2NH]Leu[CH_2S]Gly-NH_2	−5.9	$C_{21}H_{33}N_3O_3S \cdot HCl \cdot {}^1/_4H_2O$	S,[g] C, H, N	0.10	25	
				0.010	25	
				0.001	45	
42, Z-Pro-Leu[CH_2S]Gly	−18.0	$C_{21}H_{30}N_2O_5S \cdot 0.1CH_2Cl_2$	C, H, N, S	0.10	14	69
				0.010	57	77
				0.001	57	69
43, Z-Pro(4-CO)-Leu[CH_2S]Gly	−32.5	$C_{21}H_{28}N_2O_6S$	C, H, N, S	10	80 0[c]	
				1	100 17	
				0.10	83 17	
				0.010	50	
				0.001	17	
44, Z-Pro-Leu[CH_2SO_2]Gly		$C_{21}H_{30}N_2O_7S$	C, H, N, S	0.10	0	
				0.010	0	
				0.032	66	
				0.010	100	
				0.001	50	
45, Z-<Glu-Leu[CH_2S]Gly	+0.26	$C_{21}H_{28}N_2O_6S$	C, H, N, S	100	0	
				10	0	
				1	0	
				0.10	0	
				0.010	33	
				0.001	33	
46, Z-Pro-Leu[CH_2S]Gly-NH_2	−30.3	$C_{21}H_{31}N_3O_4S \cdot {}^1/_2H_2O$	C, H, N, S	0.10	50	
				0.010	33	
				0.001	66	
				0.0001	0	
47, Z-Pro[CH_2S]Lys(Z)	−34	$C_{27}H_{34}N_2O_6SNa$	C, H, N	0.10	45	
				0.010	12	
				0.001	45	
48, Bz(4-OMe)-Pro-Leu[CH_2S]Gly	−38.1	$C_{21}H_{30}N_2O_5S$	C, H, N, S	0.10	60 12[c]	
				0.010	0 50	
				0.001	40 12	
49, Z-Pro[CH_2S]Leu-NH-C_3H_7	−35.5	$C_{22}H_{34}N_2O_3S$	C, H, N, S	0.10	20	
				0.010	20	
				0.001	60	
50, Z-Pro[CH_2S]Leu-NH$CH_2CH_2SCH_2CH_3$	−21.5	$C_{23}H_{36}N_2O_3S_2$	C, H, N, S	0.10	0	
				0.010	0	
				0.001	33	
51, 	+57.3	$C_{14}H_{21}NO_3S_2$	C, H, N, S	0.10	20	
				0.010	60	
				0.001	60	
52, 	+48.3	$C_{14}H_{20}N_2O_3S \cdot HCl \cdot 0.2H_2O$	C, H, N, S	0.10	14	
				0.010	0	
				0.001	57	
53, Z-Pro-NHC(CH_2OH)H$(CH_2)_4$NHZ	−28.4	$C_{27}H_{35}N_3O_6$	C, H, N	100	0	
				10	20	
				1	0	
				0.10	40	
				0.010	40	
				0.001	50	

[a] All amino acids L unless otherwise designated. Z = benzyloxycarbonyl, BOC = *tert*-butoxycarbonyl, Bz = benzoyl, Tos = tosyl. The moiety in brackets, e.g., [CH_2O], indicates that the amide bond, CONH has been replaced with that function. The structure of compound **20** therefore would be Z-Pro[CH_2O]CH_2CO_2H. [b] Except for one or two compounds, replicate experiments were not done. Criteria established with control animals was such that the results obtained with test compounds were taken as having definitive value. [c] Oral. [d] Calcd 7.82, found 7.28. [e] Calcd 7.73, found 7.34. [f] Calcd 46.37, found 45.84. [g] Calcd 7.90, found 8.67.

animals were used at each dose of the selected compounds. Discrete-trial, operant procedures were used to assess short-term memory with use of a two-choice, delayed-alternation problem. This tastk required the animal to alternate lever-press responses between two levers on successive trials. Accuracy of performance as a function of the intertrial interval (ITI) was used as the measure of retention. Generally, performance on this task was above 90% correct with ITIs of less than 30 s. A progressive decline in accuracy occurred as the ITI is lengthened until

Table III. Cyclic Modified Peptides

compound	$[\alpha]^{25}_D$, deg (MeOH)	formula	anal.	reversal of electroconvulsive shock induced amnesia in rodents		
				dose, mg/kg im	% reversal rats	% reversal mice
54. 		$C_7H_{11}NO_2$	C, H, N	100		63[a]
				10		77
				1		58
				0.10	33	
				0.010	33	
				0.001	0	
55. 		$C_{14}H_{29}N_{30}\cdot 1.7HCl$	C,[b] Cl,[c] H, N	100	24	
				10	0	
				1	20	
				0.10	66	
				0.010	83	
				0.001	50	
				0.0001	82	
56. 	−21.3	$C_{19}H_{34}N_4O_3\cdot HCl\cdot H_2O$	H,[d] C, N	0.10	0	
				0.010	0	
				0.001	0	
57. 	−25.5	$C_{27}H_{42}N_4O_4\cdot HCl\cdot {}^1/_2H_2O$	C, H, N	100	80	
				10	0	
				1	0	
				0.10	50	
				0.010	17	
				0.001	17	
58. 		$C_{27}H_{29}N_5O_3\cdot {}^1/_4CHCl_3$	C, H, N	100	83	
				10	30	
				1	50	
				0.10	43	
				0.010	0	
				0.001	0	

[a] Oral. [b] Calcd 52.98, found 52.52. [c] Calcd 18.99, found 18.02. [d] Calcd 8.86, found 8.09.

responding fell to chance (50% correct) with ITIs greater than 120 s. Selected compounds were administered intramuscularly 30 min prior to testing. Each dose level was studied in an independent group and was compared to the immediately preceding control session in the same animals.

One modified peptide, Z-Pro[CH_2NH]Leu-Gly-NH_2, **30**, was tested at 0.1, 1.0, 10.0, and 100.0 μg/kg (im) for its ability to improve performance of aged and young rhesus monkeys in a delyaed response test. These procedures are described in detail elsewhere.[14] Briefly, a stimulus was presented on one of nine panels arranged in a 3 × 3 matrix. Recall of the spatial position of the stimulus was tested following retention intervals of various lengths. Drug effects were evaluated by comparing the percent correct and the average delay attained under drug and control conditions by using an analysis of variance with repeated measures.

Results and Discussion

Lysine vasopressin (LVP) and arginine vasopressin (AVP) and several peptides related to the C-terminal portion of these two hormones were tested for their effects on memory. Pro-Leu-Gly-NH_2 and Z-Pro-Leu-Gly-NH_2 were also tested. Several of these peptides showed significant activity in reducing electroconvulsive shock-induced amnesia in rodents. These results confirm earlier observations obtained with puromycin-induced amnesia.

We also found very little difference between the activity of LVP and AVP. Z-Pro-Leu-Gly-NH_2 (**14**) was about twice as active as the unprotected peptide **13** and was one of our most active compounds. Peptides containing Lys had significant activity also.

There was not a large difference in activity between the Z peptide and the non-Z **3** and **4**. However, other structural changes in the Lys peptides were significant. Replacement of Z with BOC gave an inactive compound **5**. The terminal amide was not essential since the free acid **9** had good activity. A BOC group on the N^{ϵ}-Lys appeared to be detrimental, **6** and **8**, but a Z in this position increased activity, **7**. Dipeptides of Lys-Gly **11** and **12** were weakly active. In general, peptides containing Arg were uniformly less active and it was decided not to pursue the synthesis of modified peptides containing this amino acid for the present.

With all of the peptides investigated, there appeared to be a "window" of activity in which compounds were active at a fairly narrow dose level. This window occurred at various dose levels, which make structure–activity comparisons difficult. For example, choosing the most active compound from **4**, **9**, and **10** would be problematical, at best.

The modified peptides were tested mainly in the rat for their ability to reverse electroconvulsive shock-induced amnesia, and several of these were significantly active. One of the most consistently active compounds was **20**, but some caution must be used in ascribing its activity to its structural relationship to a tripeptide since it does have some resemblance to pramiracetam.[7] The other methyleneoxy isosteres in this series, **21–27**, are more easily defined. The compounds with this isosteric replacement between the Pro and Leu are more active than those between the Leu and Gly. This can also be said for a single methylene amino isostere **30** and **31**. A doubly modified methyleneamino isostere, **33**, was nearly inactive; a tosyl group replacing a Z increased activity slightly, **34**. This contrasts with other tosyl replacements that were of doubtful value, e.g., **35**. In compound **37**, it is possible that the D-Leu contributed to its activity sufficiently to overcome the negative effect of the tosyl group. In contrast to the low activity of the double methyleneamino compound, the mixed methyleneamino, methylenethio peptide **40** was very active. The methylenethio isosteres were of some interest. The majority of those prepared had low to moderate activity, **42–52**. The sulfone **44** had good activity

at low doses only (window effect), and the 4-ketoproline **43** was active at most doses but was inactive orally.

The activity of some of the cyclic compounds in Table III was surprising. Compound **54** was quite active orally in the mouse but inactive im in the rat, which is an anomalous result. Compound **55** was very active at low doses.

From a comparison of the peptides containing isosteric amide bond replacements with the parent peptides, it can be concluded in general that the isosteres were somewhat less active. However, this is very dependent upon the type of isostere and its position in the peptide. Also in some cases this comparison is difficult since not all the parent peptides were prepared.

In order to further study the effect of peptides and the modified peptides on short-term memory, five compounds, **3**, **9**, **10**, **13**, and **30**, were selected for evaluation in the rodent delayed alternation test. The compounds were given im and three of them showed very weak activity in this test, **9**, **10**, and **13**. Compound **30** was also tested in the rhesus monkey for effects upon delayed-response performance at four dose levels (0.1, 1.0, 10.0, and 100 μg/kg, im) administered 30 min before testing. Statistical analyses indicated that **30** did not significantly facilitate short-term memory in either rats or aged rhesus monkeys.

Our conclusions from these tests were that, although some of the peptides and modified peptides reverse amnesia, there were no significant effects in tests of short-term memory. However, it is certainly possible that a different type of learning and memory test would give different results.

Experimental Section

Melting points were determined in a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were within ±0.4% unless otherwise indicated. Infrared spectra were recorded on a Digilab FTS-14 pulsed Fourier-transform spectrophotometer. The NMR spectra were recorded on a Varian EM-390, Varian XL-200, or a IBM WP100SY instrument. The IR and NMR spectra were compatible with the assigned structures. Rotations were recorded on a Perkin-Elmer Model 141 polarimeter. TLC was done on precoated sheets (silica gel 60F 254, Merck). Silica gel chromatography was done with Kieselgel 60 (70–230 mesh or 230–400 mesh for flash). Protected amino acids were purchased from Bachem, Inc. or Chemical Dynamics.

Methyl *N*-[*N*^{6}-[(1,1-Dimethylethoxy)carbonyl]-*N*^{2}-[1-[(phenylmethoxy)carbonyl]-L-prolyl]-L-lysyl]glycinate. Z-Pro-Lys(BOC)-Gly-OMe (8). A solution of 8 g (0.0177 mol) of Z-Lys(BOC)-Gly-OMe[16] and 1.14 g of citric acid in 50 mL of MeOH was hydrogenated at 50 psi with 0.5 g of 20% Pd/C for 18 h. The catalyst was removed and the filtrate evaporated in vacuo to a gum. The gum was dissolved in 50 mL of DMF and cooled to 10 °C, and 2.5 g (0.0177 mol) of 1*H*-benzotriazol-1-ol (HOBT), 5 mL of Et_3N, 4.5 g (0.0177 mol) of Z-Pro, and 3.8 g (0.0177 mol) of DCC were added. The solution was stirred for 2 days at 25 °C, filtered, and evaporated. Hexane gave a solid; 6 g (62%), mp 103–105 °C, $[\alpha]^{25}_D$ −45° (*c* 1, MeOH). Anal. ($C_{27}H_{40}N_4O_8$) C, H, N.

***N*-[*N*-[1-[(Phenylmethoxy)carbonyl]-L-prolyl]-L-lysyl]-glycine. Z-Pro-Lys-Gly (9).** To a solution of 4 g (0.0073 mol) of **8** in 25 mL of MeOH was added 10 mL of 2 N NaOH. After 1 h, the MeOH was evaporated, 10 mL of 2 N HCl was added, and the solution was extracted with EtOAc. The EtOAc was dried and evaporated to a solid. The solid was taken up in 30 mL of trifluoroacetic acid (TFA) and kept 15 min at 25 °C. The solution was evaporated and then gave a white solid; 2.7 g (85%), $[\alpha]^{25}_D$ −52.9° (*c* 1, MeOH). Anal. $C_{21}H_{30}N_4O_6 \cdot 2CF_3CO_2H$) C, H, N.

1-[(1,1-Dimethylethoxy)carbonyl]-L-prolyl-*N*^{5}-[imino(nitroamino)methyl]-L-ornithylglycinamide. BOC-Pro-Arg(NO_2)-Gly-NH_2 (17). This peptide was prepared by solid-phase synthesis on a 2% Merrifield resin with use of standard procedures. The product was obtained as a white solid; $[\alpha]^{25}_D$ −42.8° (*c* 1, MeOH). Anal. ($C_{18}H_{32}N_8O_7 \cdot 0.5CHCl_3$) C, H; N: calcd, 21.06; found, 22.22.

1-[(1,1-Dimethylethoxy)carbonyl]-L-prolyl-L-arginylglycinamide. BOC-Pro-Arg-Gly-NH_2 (16). Catalytic hydrogenation of peptide **17** in MeOH gave **16** as a cream solid; $[\alpha]^{25}_D$ −50.4° (*c* 1, MeOH). Anal. ($C_{18}H_{33}H_7O_5 \cdot H_2O$) C, H, N.

(*S*)-2-Chloro-*N*-[1-(hydroxymethyl)-3-methylbutyl]acetamide (59). A solution of chloroacetyl chloride (25.64 g, 0.227 mol) in 57 mL of acetone was added dropwise with stirring to a solution of L-leucinol (26.63 g, 0.227 mol), and NaOAc (37.24 g, 0.454 mol) in a mixture of 340 mL of acetone and 170 mL of H_2O at 0–5 °C. The mixture was stirred and allowed to reach room temperature over 2 h, the solvent was evaporated in vacuo, and the residue was suspended in 250 mL of $CHCl_3$ and washed with H_2O. The $CHCl_3$ layer was separated, dried over Na_2SO_4, evaporated in vacuo, and the residue was purified by chromatography using silica gel and eluting with CH_2Cl_2–MeOH (95:5) to give **59**, 28 g (64%); $[\alpha]^{25}_D$ −32.6° (*c* 0.52, MeOH). Anal. ($C_8H_{16}ClNO_2$) C, H, N.

(*S*)-5-(2-Methylpropyl)-3-morpholinone (60). Compound **59** (26.34 g, 0.136 mol) was dissolved in 450 mL of THF, the solution was cooled to 0–5 °C, and 7.8 g (0.195 mol) of 60% NaH in mineral oil dispersion was slowly added. The mixture was allowed to reach room temperature and was stirred for 14 h. H_2O (10 mL) was added, the solvents were evaporated in vacuo, and the residue was suspended in 250 mL of $CHCl_3$ and washed with 100 mL of H_2O and then a saturated aqueous solution of NaCl. The $CHCl_3$ layer was separated, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by chromatography using silica gel and eluting with CH_2Cl_2–MeOH (95:5) to give **60**; 5.1 g (24%), mp 69–70 °C, $[\alpha]^{25}_D$ −13.7° (*c* 1.11, MeOH). Anal. ($C_8H_{15}NO_2$) C, H, N.

(*S*)-[[4-Methyl-2-[[(phenylmethoxy)carbonyl]amino]-pentyl]oxy]acetic Acid (61). Compound **60** (3 g, 0.019 mol) was suspended in a solution of 50 mL of concentrated HCl and 50 mL of H_2O, and the mixture was refluxed for 4 h, cooled to room temperature, and extracted with 200 mL of CH_2Cl_2. The aqueous layer was separated and evaporated in vacuo, the residue dissolved in 200 mL of H_2O, and the pH adjusted with a 10% aqueous solution of NaOH to pH 10–10.5. The solution was cooled to 5 °C and 3.73 g (0.022 mol) of benzyl chloroformate added dropwise while the pH was maintained at pH 10 with a 10% aqueous solution of NaOH. The mixture was stirred 1 h and extracted with 100 mL of diethyl ether. The aqueous layer was separated and the pH was adjusted to pH 3–4 by the addition of a 10% aqueous solution of HCl. The mixture was extracted with CH_2Cl_2. The CH_2Cl_2 layer was separated, washed with a saturated aqueous solution of NaCl, separated, dried over Na_2SO_4, and evaporated, and the residue was purified by chromatography using silica gel and eluting with CH_2Cl_2–MeOH (95:5) to give **61**; 5 g (85%), $[\alpha]^{25}_D$ −30.5° (*c* 0.55, MeOH). Anal. ($C_{16}H_{23}NO_5$) C, H, N.

(*S*)-Phenylmethyl [1-[(2-Amino-2-oxoethoxy)methyl]-3-methylbutyl]carbamate (62). Methyl chloroformate (0.87 g, 0.009 mol) was added dropwise with stirring to a solution of 2.52 g (0.008 mol) of **61** and 0.93 g (0.009 mol) of Et_3N in 100 mL of CH_2Cl_2 at −5 to 10 °C. The mixture was stirred for 30 min at −5 °C, allowed to reach 0 °C, and saturated with NH_3 for 5 min. The mixture was allowed to reach room temperature and stirred for 1 h, the solvent was evaporated in vacuo, the residue was dissolved in 200 mL of CH_2Cl_2 and washed with a saturated aqueous solution of NaCl, and the CH_2Cl_2 layer was separated, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by chromatography using silica gel and eluting with CH_2Cl_2–MeOH (95:5) to give **62**; 1.8 g (73%), mp 78–79 °C, $[\alpha]^{25}_D$ −22.1° (*c* 1.15, MeOH). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[(2-Amino-2-oxoethoxy)methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate (26).** A stirred suspension of 1.58 g (0.0051 mol) of **62** and 0.3 g of 20% Pd/C in a 100 mL of MeOH was exposed to H_2 gas for 15 min, the suspension was purged with N_2 gas and filtered, and the solvent was evaporated in vacuo at 30 °C. The residue was dissolved in 100 mL of CH_2Cl_2, the

(16) Ten Kortenaar, P. B. W.; Wilkerson, W. W.; Boggs, N. T.; Madar, D. A.; Koehler, K. A.; Hiskey, R. C. *Int. J. Peptide Protein Res.* **1980**, *16*, 440.

Table IV. Intermediates

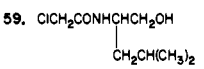
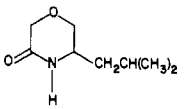
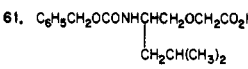
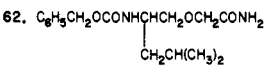
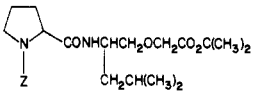
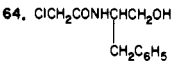
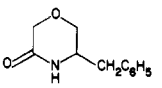
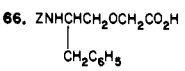
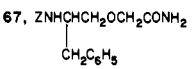
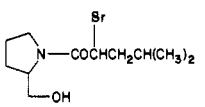
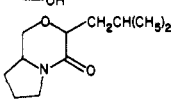
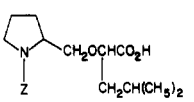
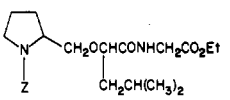
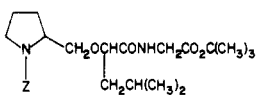
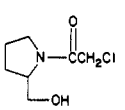
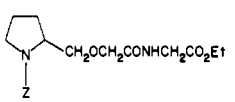
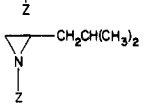
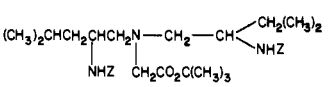
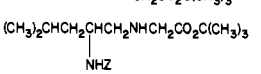
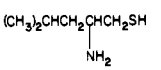
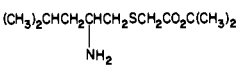
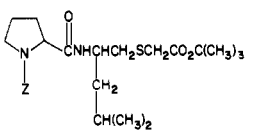
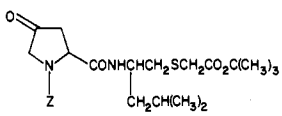
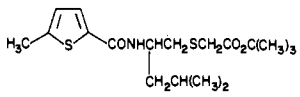
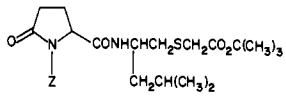
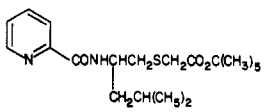
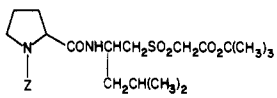
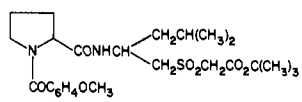
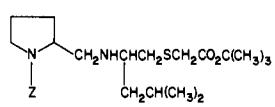
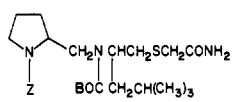
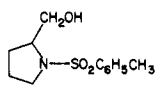
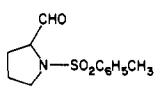
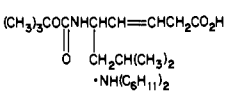
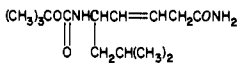
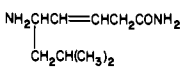
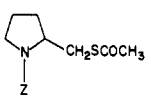
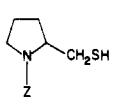
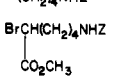
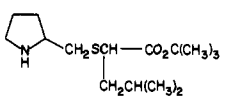
compound	mp, °C	formula	anal.	$[\alpha]^{25}_D$, deg (MeOH)
59. 		$C_8H_{16}ClNO_2$	C, H, N	−32.6
60. 	69–70	$C_8H_{15}NO_2$	C, H, N	−13.7
61. 		$C_{16}H_{23}NO_5$	C, H, N	−30.5
62. 	78–79	$C_{16}H_{24}N_2O_4$	C, H, N	−22.1
63. 		$C_{25}H_{38}N_2O_6$	C, H, N	−62.8
64. 	83–82	$C_{11}H_{14}ClNO_2$	C, H, N	−25.9
65. 	95–98	$C_{11}H_{13}NO_2$	C, H, N	
66. 	96–100	$C_{19}H_{21}NO_5$	C, H, N	
67. 	135–137	$C_{19}H_{22}N_2O_4$	C, H, N	
68. 	79–82	$C_{11}H_{20}BrNO_2$	C, H, N	−54.0
69. 	61–65	$C_{11}H_{19}NO_2$	C, H, N	−73.3
70. 		$C_{19}H_{27}NO_5$	C, H, N	−78.7
71. 		$C_{23}H_{34}N_2O_6$	C, H, N	−72.7
72. 		$C_{25}H_{38}N_2O_6$	C, H, N	−70.2
73. 		$C_7H_{12}ClNO_2$	C, H, N	−62.2
74. 		$C_{19}H_{28}N_2O_6$	C, H, N	−37.3
75. 		$C_{14}H_{19}NO_2$	C, H, N	−46.1 ($CHCl_3$)
76. 	107–108	$C_{34}H_{51}N_3O_6$	C, H, N	−16.3
77. 	50–52	$C_{20}H_{32}N_2O_4$	C, H, N	−10.0
78. 		$C_6H_{15}NS$	C, H, N, S	+35.5
79. 		$C_{12}H_{25}NO_2S$	C, H, N, S	+46.7
80. 		$C_{25}H_{38}N_2O_5S$	C, H, N, S	−11.6

Table IV (Continued)

compound	mp, °C	formula	anal.	$[\alpha]^{25}_D$, deg (MeOH)
81. 	89–91	$C_{25}H_{36}N_2O_6S$	C, H, N, S	+33.2
82. 	81–83	$C_{18}H_{19}NO_3S_2$	C, H, N, S	+56.5
83. 		$C_{25}H_{36}N_2O_6S$	C, H, N, S	+13.0
84. 		$C_{18}H_{25}N_2O_3S$	C, H, N, S	+57.9
85. 		$C_{25}H_{38}N_2O_7S$	C, H, N, S	−38.3
86. 	117–121	$C_{25}H_{38}N_2O_5S$	C, H, N, S	−30
87. 		$C_{25}H_{40}N_2O_4S \cdot {}^1/_3CHCl_3$	C, H, N, S	−21.4
88. 		$C_{26}H_{41}N_3O_5S$	N,[a] C, H, S	+16.6
89. 	90–91	$C_{12}H_{17}NO_3S$	C, H, N	−89.4
90. 	139–140	$C_{11}H_{15}NO_3S$	C, H, N	−121.0
91. 	126–128	$C_{26}H_{48}N_2O_4$	C, H, N	−18.0
92. 	88–94	$C_{14}H_{26}N_2O_3$	C, H, N	−20.0
93. 		$C_9H_{18}N_2O \cdot HCl \cdot {}^3/_4H_2O$	H,[b] C, N	+20.9
94. 		$C_{15}H_{19}NO_3S \cdot {}^1/_6DIAD$	C, H, N	
95. 		$C_{13}H_{17}NO_2S \cdot {}^1/_8DIAD$	C, H, N	
96. 		$C_{14}H_{18}BrNO_4 \cdot 0.1HBr$	C, H, N	
97. 		$C_{15}H_{20}BrNO_4$	C, H, N	
98. 		$C_{23}H_{31}NO_5S$	C, H, N, S	

[a] Calcd 8.27, found 7.72. [b] Calcd 9.38, found 8.65.

solution was cooled to 0 °C, and 1.3 g (0.0051 mol) and Z-Pro, 0.78 g (0.0051 mol) of HOBT, and 1.1 g (0.0051 mol) of DCC were added. The mixture was allowed to reach room temperature, stirred for 14 h, and filtered, the solvent evaporated in vacuo, and the residue dissolved in 200 mL of CH_2Cl_2 and washed successively with a 5% aqueous solution of Na_2CO_3, 10% aqueous solution of citric acid, and a saturated aqueous solution of NaCl. The CH_2Cl_2 layer was separated, dried over Na_2SO_4, and evaporated in vacuo and the residue purified by chromatography using silica gel and eluting with CH_2Cl_2–MeOH (95:5), yielding **26**; 1.1 g (53%), mp 141–148 °C, $[\alpha]^{25}_D$ −55.3° (*c* 1.31, MeOH). Anal. ($C_{21}H_{31}N_3O_5$) C, H, N.

[S-(R*,R*)]-Phenylmethyl 2-[[[1-[[2-(1,1-Dimethylethoxy)-oxoethoxy]methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate (63). Compound **61** (1.75 g, 0.0057 mol) was dissolved in 50 mL of CH_2Cl_2, the solution was placed in a pressure vessel and cooled under N_2 gas to 5 °C, 0.5 mL of concentrated H_2SO_4 was added, the mixture was recooled to 5 °C, and 10 mL of isobutylene was added. After 65.5 h the mixture was poured with stirring over a mixture of 1.35 g (0.0098 mol) of K_2CO_3, 50 mL of H_2O, 50 g of ice, and 50 mL of CH_2Cl_2 at such a rate that the temperature did not exceed 15 °C. The CH_2Cl_2 layer was separated, washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by chromatography using silica gel and eluting with CH_2Cl_2–MeOH (98:2), affording the intermediate *tert*-butyl ester. A stirred suspension of 0.92 g (0.0025 mol) of the intermediate *tert*-butyl ester and 0.2 g of 20% Pd/C in 100 mL of MeOH was exposed to H_2 gas for 15 min, the suspension was purged with N_2 gas and filtered, and the solvent evaporated in vacuo at 50 °C. The residue was dissolved in 100 mL of CH_2Cl_2, the solution was cooled to 0 °C, and 0.63 g (0.0025 mol) of Z-Pro, 0.4 g (0.0025 mol) of HOBT, and 0.52 g of DCC were added. The mixture was allowed to reach room temperature, stirred for 14 h, and filtered, the solvent evaporated in vacuo, and the residue dissolved in 200 mL of CH_2Cl_2 and washed successively with a 5% aqueous solution of Na_2CO_3, 10% aqueous solution of citric acid, and a saturated aqueous solution of NaCl. The CH_2Cl_2 layer was separated, dried over Na_2SO_4, evaporated in vacuo, and eluted with CH_2Cl_2–MeOH (98:2), giving **63**; 0.9 g (34%), $[\alpha]^{25}_D$ −62.8° (*c* 0.56, MeOH). Anal. ($C_{25}H_{38}N_2O_6$) C, H, N.

[S-(R*,R*)]-Phenylmethyl 2-[[[1-[(Carboxymethoxy)-methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate (25). Compound **63** (0.6 g, 0.0013 mol) was dissolved in 20 mL of TFA at 0 °C. The mixture was allowed to stand 15 min at 0 °C and then allowed to reach room temperature over 30 min, and the solvent was evaporated in vacuo at 25 °C. The residue was dissolved in 100 mL of CH_2Cl_2 and washed with a saturated aqueous solution of NaCl. The CH_2Cl_2 layer was separated, dried over Na_2SO_4, and evaporated in vacuo and the residue purified by chromatograpy using silica gel and eluting with CH_2Cl_2–MeOH (95:5), affording **25**; 0.4 g (75.5%), mp 118–120 °C, $[\alpha]^{25}_D$ −69.6° (*c* 0.52, MeOH). Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

[S-(R*,R*)]-Phenylmethyl 2-[[[1-[(2-Amino-2-oxoethoxy)ethyl]-2-phenylethyl]amino]carbonyl]-1-pyrrolidinecarboxylate (27). This compound was obtained from intermediates **64**–**66** via Scheme I in 41.5% yield; mp 137–142 °C, $[\alpha]^{25}_D$ −45.1° (*c* 1.09, MeOH). Anal. ($C_{24}H_{29}N_3O_5$) C, H, N.

[S-(R*,R)]-Phenylmethyl 2-[[1-[[(Carboxymethyl)-amino]carbonyl]-3-methylbutoxy]methyl]-1-pyrrolidinecarboxylate (21). Hydrolysis of **72** with TFA at 0 °C for 30 min and subsequent chromatography on silica gel with CH_2Cl_2–MeOH (95:5) gave the desired product in 71% yield; $[\alpha]^{25}_D$ −79° (*c* 0.34 MeOH). Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

[S-(R*,R*)]-Phenylmethyl 2-[[1-[[(2-Amino-2-oxoethyl)-amino]carbonyl]-3-methylbutoxy]methyl]-1-pyrrolidinecarboxylate (24). A solution of 2 g (0.0046 mol) of **71** in 200 mL of MeOH saturated with NH_3 was kept 14 h at 25 °C. Evaporation and silica gel chromatography afforded 1.6 g (86%) of **24**; $[\alpha]^{25}_D$ −76.9° (*c* 0.46 MeOH). Anal. $C_{21}H_{31}N_3O_5$) C, H, N.

Tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-4(3*H*)-one (54). A solution of 12 g (0.068 mol) of **73** in 500 mL of THF was treated with NaH and gave 4.4 g (46%) of the desired product, mp 63.5–66 °C. Anal. ($C_7H_{11}NO_2$) C, H, N.

(S)-Phenylmethyl 2-[(Carboxymethoxy)methyl]-1-pyrroldinecarboxylate (20). From 5 g (0.0354 mol) of **54** there was obtained after HCl hydrolysis and then acylation with benzyl chloroformate 8 g (77%) of **20**; $[\alpha]^{25}_D$ −52.7° (*c* 1, MeOH). Anal. ($C_{15}H_{19}NO_5$) C, H, N.

(S)-Phenylmethyl 2-[[2-[(2-Amino-2-oxoethyl)amino]-2-oxoethoxy]methyl]-1-pyrrolidinecarboxylate (23). A 4-g (0.0106 mol) sample of **74** with NH_3–MeOH yielded 3 g (81%) of **23**; mp 109.5–111 °C, $[\alpha]^{25}_D$ −41.0° (*c* 0.57, MeOH). Anal. ($C_{17}H_{23}N_3O_5$) C, H, N.

(S)-Phenylmethyl 2-[[2-[(Carboxymethyl)amino]-2-oxoethoxy]methyl]-1-pyrrolidinecarboxylate (22). Treatment of 3.87 g (0.0102 mol) of **74** with 1 equiv of 50% NaOH in MeOH for 4 h afforded 1.75 g (49%) of product, $[\alpha]^{25}_D$ −41.7° (*c* 0.58, MeOH). Anal. ($C_{17}H_{22}N_2O_6$) C, H, N.

(S)-Phenylmethyl 2-(2-Methylpropyl)-1-aziridinecarboxylate (75). To a solution of 24.3 g (0.0245 mol) of (*S*)-2-(2-methylpropyl)aziridine[17] in 500 mL of ether at 0 °C was added 34.2 mL (0.0245 mol) of Et_3N followed by 40.4 mL of benzyl chloroformate dropwise over 30 min. After 1 h at 0 °C, the ether solution was filtered and evaporated and the residue distilled; bp 105–112 °C (0.6 mm), yield 51.7 g (90.5%), $[\alpha]^{25}_D$ −46.1° (*c* 1, $CHCl_3$). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

Aziridine Ring Opening. With Amines. To a refluxing solution of 15.1 g (0.115 mol) of Gly-O-*tert*-butyl in 200 mL of absolute EtOH was added dropwise over 1 h 24.2 g (0.104 mol) of **75** in 50 mL of absolute EtOH. The solution was refluxed for 2 days, the solvent removed, and the residue chromatographed on silica gel with $CHCl_3$–EtOAc (3:1). A fast-moving component was isolated and was recrystallized from hexane; 14.3 g, mp 107–108 °C, $[\alpha]^{25}_D$ −16.3° (*c* 1, MeOH). This product was identified as **[S-(R*,R*)]-1,1-dimethylethyl *N*,*N*-bis[4-methyl-2-[[(phenylmethoxy)carbonyl]amino]pentyl]glycinate (76).** A slow-moving fraction from the silica gel gave 17.6 g of an oil, which crystallized; mp 50–52 °C, $[\alpha]^{25}_D$ −10.0° (*c* 1, MeOH). This proved to be the expected product **77**.

Aziridine Ring Opening. With H_2S. To a solution of 27 g (0.79 mol) of H_2S in 100 mL of absolute EtOH at −78 °C was added in small portions 8.99 g (0.09 mol) of (*S*)-2-(2-methylpropyl)aziridine. The stirred solution was allowed to warm to 25 °C, and the solvent was evaporated, leaving a yellow oil, which crystallized. Silica gel chromatography using CH_2Cl_2–MeOH (97:3) gave 6.63 g (55%) of (*S*)-2-amino-4-methyl-1-pentanethiol (**78**); $[\alpha]^{25}_D$ +35.5° (*c* 1, MeOH). Anal. ($C_6H_{15}NS$) C, H, N, S.

[S-(R*,R*)]-Phenylmethyl 2-[[[1-[[(2-Amino-2-oxoethyl)amino]methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate (31). The carbobenzoxy group was removed from **77** with use of 20% Pd/C–H_2 at 50 psi in MeOH. To a solution of 1.34 g (0.0058 mol) of the crude amino ester, 1.45 g (0.0058 mol) of Z-Pro, 0.786 g of HOBT in 40 mL of THF at 0 °C was added 1.21 g (0.0058 mol) of DCC. The mixture was stirred 18 h at 25 °C, filtered, and evaporated. The product was purified by chromatography over silica gel, eluting with $CHCl_3$–MeOH (98:2), and yielded 1.77 g of [*S*-(*R**,*R**)]-phenylmethyl 2-[[[2-[[[2-(1,1-dimethylethoxy)-2-oxoethyl]-amino]methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate. The ester was added to a solution of 50 mL of MeOH saturated with HCl gas. After the solution was allowed to stand for 4 h at 25 °C and 18 h at 0 °C, the solvent was removed, leaving 1.14 g of the methyl ester as an oil. The oil was dissolved in 50 mL of MeOH at 0 °C and saturated with NH_3 gas. The flask was stoppered and let stand 18 h. The solvent was removed and the residue chromatographed on silica gel with $CHCl_3$–MeOH (9:1). The product, **31** (0.86 g, 37%), was converted to the hydrochloride salt; $[\alpha]^{25}_D$ −55.2° (*c* 1.16, MeOH). Anal. ($C_{21}H_{32}N_4O_4$·HCl·0.25H_2O) C, H, N.

(S)-1,1-Dimethylethyl [(2-Amino-4-methylpentyl)thio]-acetate (79). To 200 mL of liquid NH_3 was added 2.66 g (0.020 mol) of **78** followed by 3.95 g (0.020 mol) of 1,1-dimethylethyl bromoacetate. The NH_3 was allowed to evaporate, H_2O was added, and the mixture was extracted with ether. The ether was dried and evaporated, and the residual oil was chromatographed on silica gel using CH_2Cl_2–MeOH (98:2), affording 2.11 g (43%) of product; $[\alpha]^{25}_D$ +46.7° (*c* 1, MeOH). Anal. ($C_{12}H_{25}NO_2S$) C, H, N, S.

[S-(R*,R*)]-Phenylmethyl 2-[[[1-[[[2-(1,1-Dimethylethoxy)-2-oxoethyl]thio]methyl]-3-methylbutyl]amino]-carbonyl]-1-pyrrolidinecarboxylate (80). A mixture of 7.51 g (0.03 mol) of **79**, 7.61 g (0.03 mol) of Z-Pro, 4.08 g (0.03 mol) of HOBT, and 6.34 g (0.03 mol) of DCC was kept at 0 °C for 3 days, filtered, and evaporated, and the residue was taken up in ether. The ether solution was washed with $NaHCO_3$ solution, 10% citric acid solution, dried, and evaporated to a white solid, **80**; 11 g (77%), $[\alpha]^{25}_D$ −11.6° (*c* 1.2, MeOH). Anal. ($C_{25}H_{38}N_2O_5S$) C, H, N, S.

[2S-(R*,R*)]-Phenylmethyl 2-[[[1-[[(Carboxymethyl)-thio]methyl]-3-methylbutyl]amino]carbonyl]-1-

(17) Tsuboyama, S. *Bull. Soc. Chem. Jpn.* **1962**, *35*, 1004.

pyrrolidinecarboxylate (42). To 10 mL of TFA (0 °C) was added 1.8 g (0.0038 mol) of **80**. The solution was allowed to warm to 25 °C and evaporated in vacuo and the residue purified on silica gel, eluting with CH_2Cl_2–MeOH (98:2), to give 0.92 g (56%) of **42**; $[\alpha]^{25}_D$ −18.0° (*c* 1, MeOH). Anal. ($C_{21}H_{30}N_2O_5S\cdot0.1CH_2Cl_2$) C, H, N, S.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(2-Amino-2-oxoethyl)thio]methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate (46).** To a solution of 4.59 g (0.010 mol) of **42** in 100 mL of CH_2Cl_2 at −5 °C were added 1.1 g Et_3N and 0.99 g (0.01 mol) of methyl chloroformate. The solution was cooled to −15 °C and NH_3 gas bubbled into the solution for 1 h. The solution was kept 18 h at 25 °C, filtered, washed with 10% Na_2CO_3 solution and 10% citric acid solution, dried, and evaporated to an oil. The oil was chromatographed on silica gel, using CH_2Cl_2–MeOH (98:2), to give 0.92 g (21%) of **46**; $[\alpha]^{25}_D$ −30.3° (*c* 1, MeOH). Anal. ($C_{21}H_{31}N_3O_4S\cdot0.5H_2O$) C, H, N, S.

[*S*-(*R,*R**)]-*N*-[4-Methyl-2-[[(5-oxo-2-pyrrolidinyl)carbonyl]amino]pentyl]glycine Hydrochloride (32).** Reaction of the amino ester derived from **77** (1.54 g, 6.68 mmol) with 890 mg (6.68 mmol) of 5-oxo-L-proline, 904 mg (6.68 mmol) of HOBT, and 1.4 g (6.68 mmol) of DCC gave the *tert*-butyl ester of **32**. The *tert*-butyl ester was removed with TFA and the product converted to hydrochloride **32**; 730 mg (33%), mp 94–125 °C, $[\alpha]^{25}_D$ −4.3° (*c* 1, MeOH). Anal. ($C_{13}H_{23}N_3O_4\cdot HCl\cdot0.25H_2O$) C, H, N, Cl.

[*S*-(*R,*R**)]-4-(2-Amino-4-methylpentyl)-6-(2-methylpropyl)-2-piperazinone (55).** Hydrogenation of 8.15 g of **76** over 20% Pd/C in methanol gave an oil (3.13 g, 89%), which was converted to hydrochloride **55**, mp 163–173 °C. Anal. ($C_{14}H_{29}N_3O\cdot1.7HCl$) Calcd: C, 52.98; Cl, 18.99. Found: C, 52.52; Cl, 18.02; H, N.

[2*S*-[2*R,4[*R**(*R**)]]]-Phenylmethyl 2-[[[3-Methyl-1-[[3-(2-methylpropyl)-5-oxo-1-piperazinyl]methyl]butyl]amino]carbonyl]-1-pyrrolidinecarboxylate Hydrocloride (57).** Reaction of 2.0 g (0.0078 mol) of the **55** free amine with 1.95 g (0.0078 mol) of Z-Pro, 1.05 g (0.0078 mol) of HOBT, and 1.63 g (0.0078 mol) of DCC in THF afforded after conversion to its hydrochloride salt in ether solution 1.69 g (41%) of **57**; mp 95–120 °C, $[\alpha]^{25}_D$ −25.5° (*c* 1, MeOH). Anal. ($C_{27}H_{42}N_4O_4\cdot HCl\cdot0.5H_2O$) C, H, N.

[2*S*-[2*R,4[*R**(*R**)]]]-*N*-[3-Methyl-1-[[3-(2-methylpropyl)-5-oxo-1-piperazinyl]methyl]butyl]-5-oxo-2-pyrrolidinecarboxamide Hydrochloride (56).** 5-Oxo-L-proline (1.08 g, 8.34 mmol) and the amine from **55** (2.13 g, 8.34 mmol) with HOBT and DCC gave an oil, which in ether afforded the hydrochloride **56**; mp 77–110 °C, 25% yield; $[\alpha]^{25}_D$ −21.3° (*c* 1, MeOH). Anal. ($C_{19}H_{34}N_4O_3\cdot HCl\cdot H_2O$) Calcd: H, 8.86. Found: H, 8.09; C, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(Carboxymethyl)thio]methyl]-3-methylbutyl]amino]carbonyl]-4-oxo-1-pyrrolidinecarboxylate (43).** Coupling of Z-Pro (4-CO) with **79** (DCC–HOBT) gave **81**. The *tert*-butyl ester was removed with TFA, yielding **43** in 19% yield; $[\alpha]^{25}_D$ −32.5° (*c* 1, MeOH). Anal. ($C_{21}H_{28}N_2O_6S$) C, H, N, S.

(*S*)-[[4-Methyl-2-[[(5-methyl-2-thienyl)carbonyl]amino]pentyl]thio]acetate (51): prepared in the manner described for **43** from compound **82**, $[\alpha]^{25}_D$ +57.3° (*c* 0.52, MeOH). Anal. ($C_{14}H_{21}NO_3S_2$) C, H, N, S.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(Carboxymethyl)thio]methyl]-3-methylbutyl]amino]carbonyl]-5-oxo-1-pyrrolidinecarboxylate (45).** The DCC–HOBT coupling of **74** with Z-5-oxo-L-proline gave **83**, which on treatment with TFA produced **45**; $[\alpha]^{25}_D$ +0.26° (*c* 0.54, MeOH). Anal. ($C_{21}H_{28}N_2O_6S$) C, H, N, S.

(*S*)-[[4-Methyl-2-[(2-pyridinylcarbonyl)amino]pentyl]thio]acetate hydrochloride (52): prepared from **84**, which was obtained from a DCC–HOBT reaction of **79** with 2-pyridinecarboxylic acid; $[\alpha]^{25}_D$ +48.3° (*c* 1, MeOH). Anal. ($C_{14}H_{20}N_2O_3S\cdot HCl\cdot0.2H_2O$) C, H, N, S.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(Carboxymethyl)sulfonyl]methyl]-3-methylbutyl]amino]carbonyl]-1-pyrrolidinecarboxylate (44).** Refluxing 2.54 g (0.0053 mol) of **46** for 24 h in $CHCl_3$ with 3 equiv of *m*-chloroperoxybenzoic acid gave **85**. Treatment of **85** with TFA gave **44** in 38% yield. Anal. ($C_{21}H_{30}N_2O_7S$) C, H, N,S.

[*S*-(*R,*R**)]-[[2-[[[1-(4-Methoxybenzoyl)-2-pyrrolidinyl]carbonyl]amino]-4-methylpentyl]thio]acetate (48).** A 5-g (0.02 mol) sample of **79** and 5.52 g (0.022 mol) of 1-(4-methoxybenzoyl)-L-proline with HOBT–DCC gave 3.5 g (36%) of **86**. The *tert*-butyl ester was removed with TFA, giving **48** in 99% yield; $[\alpha]^{25}_D$ −38.1° (*c* 1, MeOH). Anal. ($C_{21}H_{30}N_2O_5S$) C, H, N, S.

(*S*)-*N*-[[1-[(Phenylmethoxy)carbonyl]-2-pyrrolidinyl]methyl]-L-leucylglycinamide (30). To a stirred solution of 12.17 g (0.065 mol) of leucylglycinamide in 600 mL of dry THF was added 120 g of 5A molecular sieves (dried at 400 °C) and 17.5 g (0.067 mol) of Z-prolinal. The mixture was stirred overnight, the solvent evaporated, and the residue dissolved in 500 mL EtOH. A crystal of bromcresol green and then 8.17 g (0.13 mol) of $NaCNBH_3$ were added. The color of the solution was adjusted to green with EtOH saturated with HCl gas. The mixture was stirred for 2 days at 25 °C and then 119 g of solid citric acid was carefully added. The solvent was evaporated, the residue extracted with EtOAc, and the EtOAc washed with H_2O and 10% Na_2CO_3 solution, dried, and evaporated. The residue (13 g) was chromatographed on silica gel with $CHCl_3$–MeOH (95:5). The product was obtained as a white solid; 8.3 g (32%), mp 98–99 °C, $[\alpha]^{25}_D$ −62.6° (*c* 1, MeOH). Anal. ($C_{21}H_{32}N_4O_4$) C, H, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(Carboxymethyl)thio]methyl]-3-methylbutyl]amino]methyl]-1-pyrrolidinecarboxylate (40).** Compound **40** was obtained in 44% yield from **87** with TFA. Compound **87** was prepared from Z-prolinal and **79** in 80% yield. Anal. ($C_{21}H_{32}N_2O_4S$) C, H, N, S.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(2-Amino-2-oxoethyl)thio]methyl]-3-methylbutyl]amino]methyl]-1-pyrrolidinecarboxylate Hydrochloride (41).** Compound **40** was reacted with di-*tert*-butyl dicarbonate and then with isobutyl chloroformate to generate a mixed anhydride, which with NH_3 gas gave **88**. HCl removed the BOC group, giving **41**; $[\alpha]^{25}_D$ −5.9° (*c* 1, MeOH). Anal. ($C_{21}H_{33}N_3O_3S$) Calcd: S, 7.90. Found: S, 8.67; C, H, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-[[(Carboxymethyl)amino]methyl]-3-methylbutyl]amino]methyl]-1-pyrrolidinecarboxylate (33).** Removal of the benzyloxycarbonyl group from **77** and reaction with Z-prolinal and $NaCNBH_3$ gave the desired double modified tripeptide *tert*-butyl ester. The *tert*-butyl group was removed with TFA. The resulting oil was converted to the hydrochloride in CH_2Cl_2, yielding a white solid; $[\alpha]^{25}_D$ −7.2° (*c*, 1, MeOH). Anal. ($C_{21}H_{33}N_3O_4\cdot HCl$) C, H, N, Cl.

(*S*)-1-[(4-Methylphenyl)sulfonyl]-2-pyrrolidinemethanol (89). Reaction of (*S*)-L-pyrrolidine-2-methanol with *p*-toluenesulfonyl chloride in H_2O–acetone at 10 °C gave an oil in 83% yield, which crystallized from ether–cyclohexane; mp 90–91 °C, $[\alpha]^{25}_D$ −89.4° (*c*, 1, MeOH). Anal. ($C_{12}H_{17}NO_3S$) C, H, N.

(*S*)-1-[(4-Methylphenyl)sulfonyl]-2-pyrrolidinecarboxaldehyde (90). Oxidation of **89** with either oxalyl chloride–DMF or pyridine–SO_3 in Me_2SO gave the aldehyde in excellent yield (95–96%) as a solid; mp 139–140 °C, $[\alpha]^{25}_D$ −121° (*c* 1, MeOH). Anal. ($C_{12}H_{15}NO_3S$) C, H, N.

[*S*-(*R,*R**)]-*N*-[4-Methyl-2-[[[1-[(4-methylphenyl)sulfonyl]-2-pyrrolidinyl]methyl]amino]pentyl]glycine Dihydrochloride (34).** The benzyloxycarbonyl group was removed from compound **77** with 20% Pd/C–H_2 and then treated with di-*tert*-butyl dicarbonate to give the BOC derivative. The resulting compound was reacted with **90** and $NaCNBH_3$ as previously described to give the desired *tert*-butyl ester. The BOC and *tert*-butyl ester were removed with TFA, and the resulting oil was converted to the hydrochloride salt **34**; mp 138 °C dec, $[\alpha]^{25}_D$ −69.1° (*c* 1, MeOH). Anal. ($C_{20}H_{33}N_3O_4S\cdot2HCl$) C, H, N.

[*S*-(*R,*R**)]-*N*-[4-Methyl-2-[[[1-[(4-methylphenyl)sulfonyl]-2-pyrrolidinyl]carbonyl]amino]pentyl]glycine Hydrochloride (36).** This compound was prepared by reacting *N*-tosylproline with the same intermediate **90** was reacted with in the previous example (**34**). The protecting groups were removed as before, yielding **36**; mp 175 °C dec, $[\alpha]^{25}_D$ −120.5° (*c* 1, MeOH). Anal. ($C_{20}H_{31}N_3O_5S\cdot HCl$) C, H, N.

(*S*)-*N*-[*N*-[[1-[(4-Methylphenyl)sulfonyl]-2-pyrrolidinyl]methyl]-L-leucyl]glycine Hydrochloride (35). Reductive amination of **77** with **90** and $NaCNBH_3$ gave the desired ester, which after treatment with TFA and then HCl gas in ether afforded **35**; mp 148 °C dec, $[\alpha]^{25}_D$ −61.0° (*c* 1, MeOH). Anal. ($C_{23}H_{31}N_3O_5S\cdot HCl$) C, H, N.

(*S*)-*N*-[[1-[(4-Methylphenyl)sulfonyl]-2-pyrrolidinyl]methyl]-D-leucylglycinamide (37). Reductive amination of **90** with D-leucylglycinamide followed by silica gel chromatography with $CHCl_3$–MeOH (90:10) gave **37** as an oil in 49% yield; $[\alpha]^{25}_D$ −79.4° (*c* 1, MeOH). Anal. ($C_{20}H_{32}N_4O_4S$) Calcd: H, 7.82. Found: H, 7.28; C, N.

(*S*)-*N*-[[1-[(1,1-Dimethylethoxy)carbonyl]-2-pyrrolidinyl]methyl]-N^5-[(phenylmethoxy)carbonyl]-D-lysylglycinamide (38). BOC-prolinal and D-lysylglycinamide with $NaCNBH_3$ gave **38**; $[\alpha]^{25}_D$ −13.5° (*c* 1, MeOH). Anal. ($C_{26}H_{41}N_5O_6$) Calcd: H, 7.73. Found: H, 7.24; C, N. Removal of the BOC group with TFA gave **39**; $[\alpha]^{25}_D$ −7.9° (*c* 1, MeOH). Anal. ($C_{21}H_{33}N_5O_4 \cdot 2CF_3CO_2H$) Calcd: C, 46.37. Found: C, 45.04; H, N, F.

[*S*-(*E*)]-5-[[(1,1-Dimethylethoxy)carbonyl]amino]-7-methyl-3-octenoic Acid (91). Under N_2, a suspension of 84.0 g (0.185 mol) of [3-(trimethylsilyl)-1-propyne]triphenylphosphonium bromide in 1 L of THF was cooled to −80 °C and treated dropwise with 88.3 mL (0.185 mol) of a 2.1 M solution of *n*-butyllithium in hexane. After the mixture was stirred for 45 min at −80 °C, a solution of 39.9 g (0.185 mol) of BOC-L-leucinal in 600 mL of THF was added. After stirring at −80 °C for 30 min, the mixture was allowed to warm to room temperature over 2 h. The mixture was concentrated to one-half volume under reduced pressure and poured into 1 L of H_2O. The mixture was extracted with ether and then two times with petroleum ether. The combined organic extracts were washed with saturated NaCl solution and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in petroleum ether and the insoluble triphenylphosphine oxide removed by filtration. Removal of the solvent under reduced pressure gave the crude product. After chromatography on silica gel, eluting with $CHCl_3$, there was obtained 34.2 g of [*S*-(*E*)-1,1-dimethylethyl [1-(2-methylpropyl)-5-(trimethylsilyl)-2-penten-4-ynyl]carbamate sufficiently pure to use in the following reaction.

Under N_2, a solution of 316 mL (0.316 mol) of borane (1 N in THF) was cooled in ice and treated dropwise with a solution of 64 mL (0.732 mol) of cyclohexene in 890 mL of THF. After stirring at 0 °C for 35 min, the solution was treated dropwise with a solution of 27.94 g (0.0903 mol) of [*S*-(*E*)-1,1-dimethylethyl [1-(2-methylpropyl)-5-(trimethylsilyl)-2-penten-4-ynyl]carbamate in 110 mL of THF. After stirring for 0.5 h, the solution was treated dropwise with 112 mL of MeOH and 157 mL of 2 N NaOH and then over 0.5 h with 102 mL of 30% H_2O_2 while the temperature was maintained below 10 °C. The solution was then stirred for 0.5 h and poured into 2.2 L of H_2O containing 112 mL of 2 N NaOH. After extraction three times with ether, the pH was adjusted to 2.0 and the solution extracted three times with ether. The combined ether extracts were washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent under reduced pressure and chromatography on silica gel, eluting with $CHCl_3$–MeOH (95:5), gave 10.7 g of [*S*-(*E*)]-5-[[(1,1-dimethylethoxy)carbonyl]amino]-7-methyl-3-octanoic acid as an oil suitable for use in subsequent reactions. The product was characterized by converting a small amount to the *N*-cyclohexylcyclohexanamine salt; mp 126–128 °C, $[\alpha]^{25}_D$ −18.0° (*c* 1.02, methanol). Anal. ($C_{26}H_{48}N_2O_4$) C, H, N.

[*S*-(*E*)]-1,1-Dimethylethyl [5-Amino-1-(2-methylpropyl)-5-oxo-2-pentenyl]carbamate (92). A solution of 6.0 g (0.0221 mol) of [*S*-(*E*)]-5-[[(1,1-dimethylethoxy)carbonyl]-amino]-7-methyl-3-octenoic acid in 60 mL of THF was cooled in ice and treated with 2.44 mL (0.022 mol) of 4-methylmorpholine followed by the dropwise addition of 2.9 mL (0.022 mol) of isobutyl chloroformate. After 5 min at 0 °C, 6.0 mL of concentrated NH_4OH was added and the solution allowed to stir at 0 °C for 1 h. The solvent was removed under reduced pressure and the residue taken up in EtOAc, washed with H_2O, 1 N citric acid, saturated $NaHCO_3$, and then with saturated NaCl solution. After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with $CHCl_3$–MeOH (9:1). There was obtained 3.5 g of **92** as an oil, which solidified on standing. The material was suitable for use in the following reaction. A small sample, recrystallized from toluene–hexane had mp 85–89 °C, $[\alpha]^{25}_D$ −20.0° (*c*, 1.04, methanol). Anal. ($C_{14}H_{26}N_2O_3$) C, H,N.

[*S*-(*E*)]-5-Amino-7-methyl-3-octenamide Hydrochloride (93). A solution of 6.23 g (0.023 mol) of **92** in 60 mL of TFA was stirred at room temperature for 1 h. The TFA was removed under reduced pressure, the residue taken up in CH_2Cl_2, and the solvent removed again. The residue was taken up in CH_2Cl_2 and HCl gas bubbled into the solution for a few minutes. The solvent was then removed under reduced pressure. The residue was taken up in a small amount of CH_2Cl_2 and added dropwise to excess ether. The solid was collected and washed with ether. There was obtained 4.14 g of **93** suitable for use in the following reaction $[\alpha]^{25}_D$ +20.9° (*c* 1.1, methanol). Anal. ($C_9H_{18}N_2O \cdot HCl \cdot 0.75H_2O$) Calcd: H, 9.38. Found: 8.65; C, N.

[*S*-(*R,*R**-(*E*))]-Phenylmethyl 2-[[[5-Amino-1-(2-methylpropyl)-5-oxo-2-pentenyl]amino]carbonyl]-1-pyrrolidinecarboxylate (28).** To a suspension of 2.0 g (9.7 mmol) of **93** in 50 mL of THF was added 2.42 g (9.7 mmol) of Z-Pro and 1.31 g (9.7 mmol) of HOBT. The mixture was cooled in ice and treated with 1.35 mL (9.7 mmol) of Et_3N followed by a solution of 2.0 g (9.7 mmol) of DCC in 10 mL of THF. The solution was kept at 0 °C for 1 h and then at room temperature overnight. The solution was then filtered and the filtrate concentrated under reduced pressure. The residue was taken up in EtOAc and washed with H_2O, 1 N citric acid, saturated $NaHCO_3$, and then with saturated NaCl. After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, eluting with $CHCl_3$–MeOH (95:5). After combining the appropriate fractions, there was obtained 1.54 g of **28**; mp 140–142 °C, $[\alpha]^{25}_D$ −73.0° (*c* 1.03, methanol). Anal. ($C_{22}H_{31}N_3O_4$) C, H, N.

[*S*-(*R,*R**-(*E*))]-*N*-[5-Amino-1-(2-methylpropyl)-5-oxo-2-pentenyl]-5-oxo-2-pyrrolidinecarboxamide (29).** This compound was obtained in the same manner as **28** from 2.1 g (0.01 mol) of **93** and 1.32 g (0.01 mol) of 5-oxo-L-proline, 1.03 g of **29**; mp 150–152 °C, $[\alpha]^{25}_D$ −39.1° (*c* 1, MeOH). Anal. ($C_{14}H_{23}N_3O_3$) C, H, N.

(*S*)-Phenylmethyl 2-(Mercaptomethyl)-1-pyrrolidinecarboxylate (95). To a solution of 33.6 g (0.0128 mol) of triphenylphosphine in 150 mL of THF at 0 °C was added 25.2 mL (0.128 mol) of diisopropyl azodicarboxylate (DIAD) over 10 min. The solution was stirred 0.5 h at 0 °C and 15 g (0.064 mol) of Z-prolinol and 9.2 mL (0.128 mol) of ethanethioic acid in 75 mL of THF was added dropwise. After 18 h at 25 °C, evaporation gave **94** in 78% yield. To a solution of 1.7 g (0.0058 mol) of **94** in 25 mL of CH_3CN was added 5.3 g (0.058 mol) of hydrazine hydrate. The solution was heated to 50 °C for 0.5 h, evaporated, and extracted with ether. The ether solution was washed with 1 N HCl, dried, and evaporated to a purple oil, 1.4 g. Silica gel chromatography gave 1.1 g (75%) of **95**. Anal. ($C_{13}H_{17}NO_2S \cdot {}^1/_8$DIAD) C, H, N.

(*S*)-Methyl 2-Bromo-6-[[(phenylmethoxy)carbonyl]amino]hexanoate (97). Lys (Z) (40 g, 0.14 mol) was dissolved in 120 mL of 6 M aqueous HBr at 0 °C. Sodium nitrite (10.6 g, 0.15 mol) was added in portions over 30 min with stirring. After 5 min the mixture was extracted with EtOAc and the EtOAc dried and evaporated to an orange oil, 23.2 g (**96**). Ten grams of the oil was dissolved in MeOH at 5 °C and HCl gas bubbled in for 5 min. After 3 days at 5–10 °C the solution was evaporated to give the ester as a yellow oil, 9 g (86%) (**97**). Anal. ($C_{15}H_{20}BrNO_4$) C, H, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-Carboxy-5-[[(phenylmethoxy)carbonyl]amino]pentyl]thio]methyl]-1-pyrrolidinecarboxylate Sodium Salt (47).** A 2.7-g (0.0106 mol) sample of **95** in 50 mL of THF was cooled to 0 °C and 1.7 g (0.043 mol) of NaH was added followed by 3.8 g (0.0106 mol) of **97** in 50 mL of THF. The mixture was stirred 18 h at 25 °C. The THF was evaporated, and the residue was dissolved in CH_2Cl_2, washed with 1 N HCl and 10% Na_2CO_3 solution, dried, and evaporated. The residue was chromatographed on silica gel. After combining the appropriate fractions, the ester was taken up in MeOH and hydrolyzed with 1 equiv of 1 N NaOH. Evaporation of the solvent gave **47** or the sodium salt, $[\alpha]_D^{25}$ −34° (*c* 1, MeOH): Anal. ($C_{27}H_{34}N_2O_6SNa$) C, H, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[1-(Hydroxymethyl)-5-[[(phenylmethoxy)carbonyl]amino]pentyl]amino]carbonyl]-1-pyrrolidinecarboxylate (53).** A solution of 5 g (0.0136 mol) of BOC-lysinol (Z) was stirred 15 min in 50 mL of TFA. The solution was evaporated to an oil, which solidified when treated with ether, 4.3 g, mp 91–95 °C. Reaction of the N^{ϵ}-Z-lysinol

with Z-Pro, HOBT, and DCC in CH_2Cl_2 solution gave 2.5 g (68%) of **53** as a white solid; $[\alpha]^{25}_D$ −28.4° (*c* 1, MeOH). Anal. ($C_{27}H_{35}N_3O_6$) C, H, N.

[*S*-(*R,*R**)]-Phenylmethyl 2-[[[3-Methyl-1-[(propylamino)carbonyl]butyl]thio]methyl]-1-pyrrolidinecarboxylate (49).** DL-Leucine was converted to its α-bromo derivative with $NaNO_2$–HBr in 65% yield. The *tert*-butyl ester of this acid was obtained in 50% yield with use of *tert*-butyl alcohol, DCC, and *N*,*N*-dimethyl-4-pyridinamine. Condensation of this α-bromo ester with 2-pyrrolidinemethanethiol in liquid NH_3 gave **98** in 20% yield. The pyrrolidine was then protected with benzyloxycarbonyl chloride and the *tert*-butyl ester removed with TFA. Reaction of the crude oil with 1-propylamine, DCC, and HOBT yielded an oil, which after chromatography on silica gel gave pure **49**; $[\alpha]^{25}_D$ −35.5° (*c* 1, MeOH). Anal. ($C_{22}H_{34}N_2O_3S$) C, H, N, S.

Reaction of the crude acid from above with 2-(ethylthio)ethylamine in the presence of DCC–HOBT gave **[*S*-(*R**,*R**)]-phenylmethyl 2-[[[1-[[[2-(ethylthio)ethyl]amino]carbonyl]-3-methylbutyl]thio]methyl]-1-pyrrolidinecarboxylate (50)** as a clear oil; $[\alpha]^{25}_D$ −21.5° (*c* 1, MeOH). Anal. ($C_{23}H_{36}N_2O_3S_2$) C, H, N, S.

2-[2-(Benzoylamino)-3-phenylpropyl]hexahydro-3-(1*H*-imidazol-4-ylmethyl)pyrrolo[1,2-*a*]pyrazine-1,4-dione (58). Reductive amination of benzylphenylalaninal with DL-His-Pro-OBzl and $NaCNBH_3$ in MeOH for 4 days gave a white solid, which was identified as the cyclized product **58** by NMR and MS; mp 107–122 °C. Anal. ($C_{27}H_{29}N_5O_3 \cdot {}^1/_4CHCl_3$) C, H, N.

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Registry No. 1, 113-79-1; 2, 50-57-7; 3, 55299-63-3; 4, 101249-82-5; 4 (free base), 76576-32-4; 5, 51644-73-6; 6, 58960-74-0; 7, 55878-52-9; 8, 101249-83-6; 9, 101249-85-8; 9 (free base), 101249-84-7; 10, 101249-86-9; 10 (free base), 2549-03-3; 11, 33043-27-5; 12, 5550-02-7; 13, 2002-44-0; 14, 14485-80-4; 15, 101249-87-0; 15 (free base), 47307-27-7; 16, 101249-88-1; 17, 101249-89-2; 18, 67844-86-4; 19, 101249-90-5; 19 (free base), 34367-76-5; 20, 101249-91-6; 21, 101249-92-7; 22, 101249-93-8; 23, 101249-94-9; 24, 101249-95-0; 25, 101249-96-1; 26, 101249-97-2; 27, 101249-98-3; 28, 101249-99-4; 29, 101250-00-4; 30, 101315-94-0; 31, 101250-01-5; 31 (free base), 101250-02-6; 32, 101250-03-7; 32 (free base), 101250-04-8; 32 (free base, *tert*-butyl ester), 101250-05-9; 33, 101250-06-0; 33·F_3CCO_2H, 101250-08-2; 33 (free base), 101250-07-1; 34, 101250-09-3; 34·2F_3CCO_2H, 101250-11-7; 34 (free base), 101250-10-6; 35, 101250-12-8; 35 (free base), 101250-13-9; 36, 101250-14-0; 36 (free base), 101250-15-1; 37, 101250-16-2; 38, 101250-17-3; 39, 101250-19-5; 39 (free base), 101250-18-4; 40, 101250-20-8; 41, 101250-21-9; 41 (free base), 101250-22-0; 42, 101250-23-1; 43, 101250-24-2; 44, 101250-25-3; 45, 101250-26-4; 46, 101250-27-5; 47, 101250-28-6; 47 (free acid), 101250-29-7; 48, 101250-30-0; 49, 101250-31-1; 50, 101250-32-2; 51, 101250-33-3; 52, 101250-34-4; 52 (free base), 101250-35-5; 53, 101250-36-6; 54, 101250-37-7; 55, 101250-38-8; 55 (free base), 101250-39-9; 56, 101250-40-2; 56 (free base), 101399-24-0; 57, 101250-41-3; 57 (free base), 101399-25-1; 58 (free base), 101250-42-4; 59, 101250-43-5; 60, 101250-44-6; 61, 101250-45-7; 62, 101250-46-8; 63, 101250-47-9; 64, 94193-78-9; 65, 101250-48-0; 66, 101250-49-1; 67, 101250-50-4; 68, 101315-95-1; 69, 101250-51-5; 70, 101250-52-6; 71, 101250-53-7; 72, 101250-54-8; 73, 101250-55-9; 74, 101315-96-2; 75, 101250-56-0; 76, 101250-57-1; 77, 101250-58-2; 78, 88264-65-7; 79, 101250-59-3; 80, 101250-60-6; 81, 101250-61-7; 82, 101250-62-8; 83, 101250-63-9; 84, 101250-64-0; 85, 101250-65-1; 86, 101250-66-2; 87, 101250-67-3; 88, 101250-68-4; 89, 55456-48-9; 90, 101250-69-5; 91, 91465-68-8; 92, 101250-70-8; 93, 101250-71-9; 94, 101250-72-0; 95, 101250-73-1; 96, 42990-75-0; 97, 101250-74-2; 98, 101250-75-3; Z-Lys(BOC)-Gly-OMe, 10342-52-6; H-Lys(BOC)-Gly-OMe, 45265-57-4; Z-Pro, 1148-11-4; Z-Pro-Lys(BOC)-Gly-OH, 101250-76-4; DL-His-Pro-OCH_2Ph, 101250-77-5; Z-Pro(4-CO), 64187-47-9; Lys(Z), 1155-64-2; (*S*)-$H_2NCH(i\text{-Bu})CH_2OCH_2COH$, 101250-78-6; (*S*)-$H_2NCH(i\text{-Bu})CH_2OCH_2CONH_2$, 101250-79-7; (*S*)-$PhCH_2OCONHCH(i\text{-Bu})CH_2OCH_2COOBu\text{-}t$, 101250-80-0; (*S*)-$H_2NCH(i\text{-Bu})CH_2OCH_2COOBu\text{-}t$, 101250-81-1; $(i\text{-Bu})CH(NH_2)CH_2NHCH_2COOBu\text{-}t$, 101250-82-2; (*S*-(*F**,*R**))-Z-Pro-NHCH-$(i\text{-Bu})CH_2NHCH_2COOBu\text{-}t$, 101250-83-3; (*S*-(*R**,*R**))-Z-Pro-NHCH(*i*-Bu)CH_2NHCH_2COOMe, 101250-84-4; (±)-BrCH(*i*-Bu)COOH, 42990-24-9; (±)-BrCH(*i*-Bu)$COOBu\text{-}t$, 101250-85-5; Z-$\overline{NCH_2CH_2CH_2C}HCH_2SCH(i\text{-Bu})COOH$, 101250-86-6; chloroacetyl chloride, 79-04-9; L-leucinol, 7533-40-6; benzyl chloroformate, 501-53-1; isobutylene, 115-11-7; (*S*)-2-(2-methylpropyl)aziridine, 23852-57-5; Gly-*O*-*tert*-butyl, 6456-74-2; 1,1-dimethylethyl bromoacetate, 5292-43-3; 5-oxo-L-proline, 98-79-3; Z-5-oxo-L-proline, 32159-21-0; 2-pyridinecarboxylic acid, 98-98-6; 1-(4-methoxybenzoyl)-L-proline, 101399-26-2; L-leucylglycinamide, 39705-58-3; (*S*)-Z-prolinal, 71461-30-8; di-*tert*-butyl dicarbonate, 24424-99-5; (*S*)-L-pyrrolidine-2-methanol, 23356-96-9; *p*-toluenesulfonyl chloride, 98-59-9; D-leucylglycinamide, 45082-46-0; (*S*)-BOC-prolinal, 69610-41-9; D-lysylglycinamide, 101250-88-8; [3-(trimethylsilyl)-1-propyne]triphenylphosphonium bromide, 101250-89-9; BOC-L-leucinal, 58521-45-2; [*S*-(*E*)-1,1-dimethylethyl [1-(2-methylpropyl)-5-(trimethylsilyl)-2-penten-4-ynyl]carbamate, 91547-47-6; ethanethioic acid, 507-09-5; (*S*)-BOC-Lysinol(Z), 82689-20-1; (*S*)-N^{ϵ}-Z-Lysinol, 101250-90-2; DL-Leucine, 328-39-2; (*S*)-2-pyrrolidinemethanethiol, 85657-09-6; 1-propylamine, 107-10-8; 2-(ethylthio)ethylamine, 36489-03-9; benzoylphenylalaninal, 101250-91-3; [*S*-(*E*)]-5-[[(1,1-dimethylethoxy)carbonyl]amino]-7-methyl-3-octenoic acid, 91465-67-7; (*S*)-Z-prolinol, 6216-63-3; oxytocin, 50-56-6; vasopressin, 11000-17-2.