strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corp. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[20]

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed with monolayer cultures of HFF cells by a procedure similar to that referenced above for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. HSV-1 plaque reduction experiments were performed with monolayer cultures of BSC-1 cells. The assay was performed exactly as referenced above for HSV-1 titration assays except that the 0.2 mL of virus suspension contained approximately 100 PFU of HSV-1 and the compounds to be tested were dissolved in the overlay medium.

(c) Cell Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF and BSC-1 cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the incorporation of radioactive precursors into DNA, RNA, and protein as detailed elsewhere.[20]

(d) Data Analysis. Dose-response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (I_{50}) concentrations were calculated from the regression lines. The three I_{50} concentrations for inhibition of DNA, RNA, and protein synthesis were averaged to give the values reported in the tables for KB cell cytotoxicity. Samples containing positive controls (acyclovir, ganciclovir, and vidarabine) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

Acknowledgment. We thank Gary Birch, Lisa Coleman, and Gail Genzlinger for knowledgeable technical assistance and Jack Hinkley for large-scale preparation of starting materials. We also thank Connie Lopatin for expert preparation of the manuscript. This project was supported with Federal funds from the Department of Health and Human Services under Contract No. NO1-AI-42554. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Registry No. 1, 69-33-0; **1a**, 16754-83-9; **1b**, 13241-46-8; **1c**, 20371-00-0; **1d**, 42204-36-4; **1e**, 16754-82-8; **1f**, 2862-16-0; **1g**, 16754-81-7; **1h**, 60870-17-9; **1i**, 2864-21-3; **1k**, 24386-95-6; **1l**, 57024-73-4; **1n**, 24385-15-7; **1n**, 21193-80-6; **1o**, 24386-88-7; **1p**, 24386-89-8; **1q**, 24414-54-8; **1r**, 24386-90-1; **1s**, 24386-93-4; **1t**, 24386-91-2; **2**, 3680-69-1; **2a**, 81777-40-4; **3**, 115093-81-7; **4**, 115093-82-8; **5**, 115093-83-9; **6**, 86626-00-8; **7a**, 115093-84-0; **7b**, 115093-93-1; **8a**, 115093-85-1; **8b**, 115093-94-2; **9a**, 115093-86-2; **9b**, 115093-95-3; **10**, 115093-87-3; **11**, 115093-88-4; **12**, 115093-89-5; **13a**, 115093-90-8; **13b**, 22276-95-5; **14a**, 115093-91-9; **14b**, 115093-92-0; **14c**, 115093-96-4; **14d**, 115093-97-5.

N-Acylphenylalanines and Related Compounds. A New Class of Oral Hypoglycemic Agents

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N-Benzoyl-DL-phenylalanine (1) was found to possess hypoglycemic activity. A series of the analogues of compound 1 were prepared and evaluated for their blood glucose lowering activity. Both the steric effects of the phenylalanine moiety and the effects of variations in the acyl moiety were investigated. This study elucidated some of the structure-activity relationships and led to the development of *N*-(4-ethylbenzoyl)-D-phenylalanine (**34**), which was 50 times more potent than the initial compound 1.

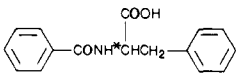
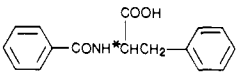
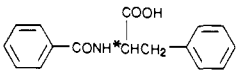
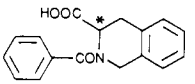
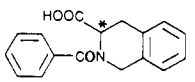
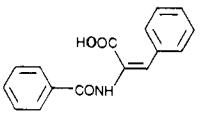
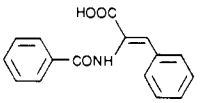
Oral therapy of non-insulin-dependent diabetes mellitus (NIDDM) largely relies on the sulfonylureas and the biguanides.[1] Although the sulfonylureas are valuable therapy for NIDDM, they do have disadvantages, e.g., hypoglycemia, and primary or secondary failure of efficacy.[2] The use of biguanides has declined because of their fetal lactic acidosis side effect.[3] To seek another type of antidiabetic drug, we screened numerous compounds in 18-h-fasted normal mice for hypoglycemic effects. In the course of this screening, we found that *N*-benzoyl-DL-phenylalanine[4] (1) exhibited a slight blood glucose lowering activity at a oral dose of 500 mg/kg.[5,6] To determine the structural requirements for possessing hypoglycemic activity and to obtain more potent compounds, the component parts of compound 1 (the acyl moiety and the phenylalanine moiety) were systematically varied.

First, the steric effects in the phenylalanine moiety were investigated. We compared *N*-benzoyl-D-phenylalanine (2) with *N*-benzoyl-L-phenylalanine (3) and found the difference in the pharmacological potency of the enantiomers. The conformationally restricted analogues, such as *N*-benzoyl-D-3-carboxy-1,2,3,4-tetrahydroisoquinoline (6), *N*-benzoyl-L-3-carboxy-1,2,3,4-tetrahydroisoquionline (7), (*Z*)-α-benzamidocinnamic acid (11), and (*E*)-α-benzamidocinnamic acid (12), were synthesized to examine the conformational effect on activity. Secondly, the acyl moiety of compound 1 was varied in order to elucidate the effects of the acyl moiety on biological activity.

(1) Sarges, R. *Prog. Med. Chem.* **1981**, *18*, 191.
(2) Cherner, R. *Topics in Medicinal Chemistry*; Rabinowitz, J. L., Myerson, R. M., Eds.; Wiley: New York, 1968; Vol. 2, p 195.
(3) Sarges, R. *Prog. Med. Chem.* **1981**, *18*, 206.
(4) Available from Sigma Chemical Co.
(5) Seto, Y.; Fukuma, M.; Dan, K.; Toyoshima, S. *Jpn. J. Pharmacol.* **1982**, *32* (Suppl.), 269.
(6) Seto, Y.; Dan, K.; Fukuma, K.; Fukushima, K.; Fujita, H. *Jpn. J. Pharmacol.* **1987**, *43* (Suppl.), 152.

0022-2623/88/1831-2092$01.50/0

Table I. Steric Modification of *N*-Benzoylphenylalanines

compd	structure	confign at *C	mp, °C	$[\alpha]_D$,[a] deg	yield, %	formula[b]	method[c]	hypoglycemic activity[d]
1[e]		DL						500
2		D	154–155	−6.8[f]	83	$C_{16}H_{15}NO_3 \cdot H_2O$	A	150
3[e]		L						inact.[g]
6		D	171–172	+0.3[h]	79	$C_{17}H_{15}NO_3$	A	inact.[g]
7		L	168–169.5	−0.6[h]	71	$C_{17}H_{15}NO_3$	A	inact.[g]
11			197–198		83	$C_{16}H_{13}NO_3$		inact.[g]
12			243–244		73	$C_{16}H_{13}NO_3$		inact.[g]
tolbutamide								25

[a] In MeOH, *c* 1.0. [b] Analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [c] A, acid chloride method. [d] Lowest dose in mg/kg causing a 20% blood glucose decrease (fasting mice, po). [e] Available from Sigma Chemical Co. [f] At 25 °C. [g] Less than 20% blood glucose decrease at a dose of 250 mg/kg. [h] At 23 °C.

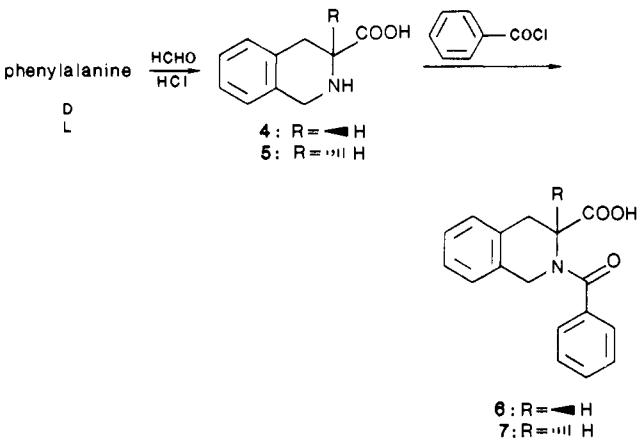
Some of these investigations provided valuable information concerning the structure–activity relationships and led to more potent compound **34**.

Chemistry

The N-acylated derivatives of phenylalanine were prepared by the acid chloride method or active ester method. The acid chloride method coupled the appropriate acid chloride to L- or D-phenylalanine in an acetone–aqueous sodium hydroxide solution without racemization of the phenylalanine moiety.[7] The solution was continually maintained at an alkaline pH by the addition of extra alkali in order to neutralize the hydrogen chloride. The acid chlorides were prepared from the corresponding carboxylic acids with thionyl chloride. In the case of compounds **24–26**, the acid chloride, acylprolyl chloride, undergoes racemization. Therefore, compounds **24–26** were synthesized by the active ester method, which was to couple the corresponding carboxylic acids to the D-phenylalanine methyl ester with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu).[8,9] The carboxylic compounds that have a nucleophilic site (e.g., tertiary amine) also could lead to the target compounds by using the active ester method without any side reaction.

In order to obtain D-3-carboxy-1,2,3,4-tetrahydroisoquinoline (**4**), the condensation of D-phenylalanine with formaldehyde was attempted.[10,11] Some racemization occurred during the conversion of D-phenylalanine to compound 4. The analysis of the product by high-performance liquid chromatography (HPLC) with DAICEL Chiralpak WH showed that the ratio of D- to L-3-carboxy-1,2,3,4-tetrahydroisoquinoline was 83:17. Compound 4 (99.5% enantiomeric purity) was purified by crystallization from ethanol–water (9:1). L-3-Carboxy-1,2,3,4-tetrahydroisoquinoline (**5**) (99.0% enantiomeric purity) was synthesized from L-phenylalanine by using the same procedure. The *N*-benzoyl derivatives of D- and L-3-carboxy-1,2,3,4-tetrahydroisoquinoline (**6** and **7**) were prepared by the reaction with benzoyl chloride (Scheme I).

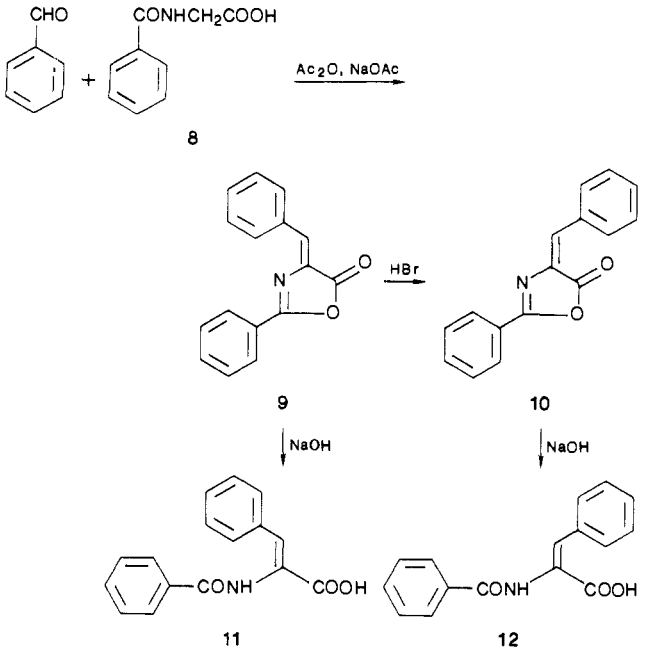
Scheme I



Scheme II shows the preparation of dehydrophenylalanine derivatives.[12,13] (*Z*)-4-Benzoyl-2-phenyl-Δ^2-oxazolin-5-one (**9**) was prepared by the reaction of hippuric

(7) Bayer, H. O.; Gotthardt, H.; Huisgen, R. *Chem. Ber.* **1970**, *103*, 2356.
(8) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.
(9) Weygand, F.; Hoffmann, D.; Wünsch, E. *Z. naturforsch.* **1966**, *21*, 426.
(10) Julian, P. L.; Karpel, W. J.; Magnani, A.; Meyer, E. W. *J. Am. Chem. Soc.* **1948**, *70*, 180.
(11) Hein, G. E.; Niemann, C. *J. Am. Chem. Soc.* **1962**, *84*, 4487.
(12) Brown, J. M.; Chaloner, P. A. *J. Am. Chem. Soc.* **1980**, *102*, 3040.
(13) Vineyard, B. D.; Knowles, W. S.; Sabacky, M. J.; Bachman, G. L.; Weinkauff, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 5946.

Scheme II



acid[14] (8) and benzaldehyde in acetic anhydride. The *Z* azlactone **9** was isomerized to the *E* azlactone **10**, which contained a small amount (5%) of the *Z* isomer **9**, with hydrogen bromide in acetic acid. (*Z*)- and (*E*)-α-benzamidocinnamic acid (**11** and **12**) were prepared from the corresponding azlactones by hydrolysis with aqueous sodium hydroxide. The geometric purity of **11** (99.8%) and **12** (98.7%) was analyzed by HPLC with YMC-A311 ODS.

Results and Discussion

The steric effects (the configuration at the asymmetric carbon and the conformation) of the phenylalanine derivatives on hypoglycemic activity are shown in Table I. The pharmacological data of the optically active forms of compound **1** were compared. *N*-Benzoyl-D-phenylalanine (**2**) exhibited 3 times the potency of compound **1**, while *N*-benzoyl-L-phenylalanine[15] (**3**) was inactive. From the results, the *R* configuration was considered to be essential for hypoglycemic activity. The requirement of *R* configuration was confirmed by examining a series of analogues (**16** and **17** in Table II, **34–37** in Table III). The decrease in activity of the conformationally restricted analogues, such as the tetrahydroisoquinoline derivatives (**6** and **7**) and dehydrophenylalanine derivatives (**11** and **12**), made it clear that these analogues do not possess the appropriate conformation for showing good hypoglycemic activity. It will be necessary to synthesize the other conformationally restricted analogues in order to assess the appropriate conformational requirements for this activity. The above investigation elucidated that the biological activity of phenylalanine derivatives was dependent on their three-dimensional structure.

The effects of the variations of acyl groups are shown in Table II. The benzoyl compound (**2**) exhibited good efficacy at a dose of 150 mg/kg, but the benzyl compound (**13**) did not. To change the amide structure to an amine structure caused a loss in activity. We noticed that *N*-(cyclohexylcarbonyl)-D-phenylalanine (**14**) was 1.5 times more potent than compound **2** and deduced that either the flat structure (benzene ring) or the availability of π-electrons was not necessary for the acyl moiety. Therefore,

(14) Available from Aldrich Chemical Co.
(15) Available from Sigma Chemical Co.

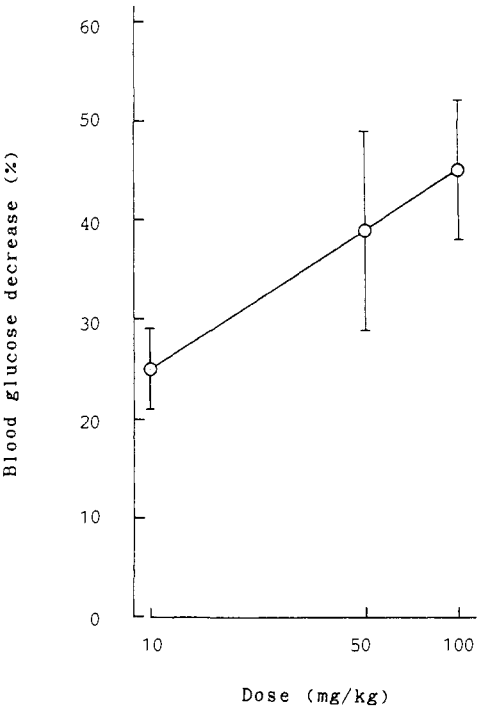
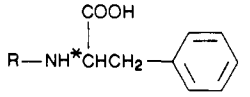


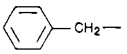
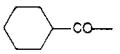
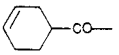
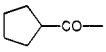
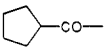
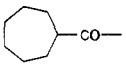
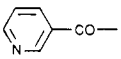
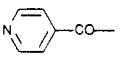
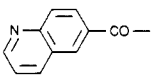
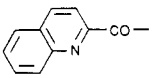
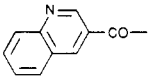
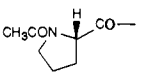
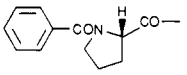
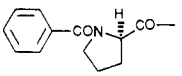
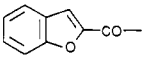
Figure 1. Dose–response curve of **34** on blood-lowering activity. The blood glucose decrease (percent) was measured at 60 min after oral administration of 10, 50, and 100 mg/kg of **34**. Each value represents the mean with the SE (n = 5).

we examined the influence of variations in the ring size of the acyl groups. Compound **14** and *N*-(cyclohexenylcarbonyl)-D-phenylalanine (**15**) (six-membered ring) were 1.5 times more potent than compound **2**. *N*-(Cyclopentylcarbonyl)-D-phenylalanine (**16**) (five-membered ring) was 3 times more active than compound **2**. *N*-(Cycloheptylcarbonyl)-D-phenylalanine (**18**) (seven-membered ring) did not exhibit hypoglycemic activity. These data showed that the effect of the acyl moiety depended on its size. It was necessary for the size of the acyl moiety to be smaller than a seven-membered ring. Pyridylcarbonyl compounds (**19** and **20**) and quinolylcarbonyl compounds (**21–23**) were less active than the benzoyl compound (**2**) and the naphthoyl compound (**31**). The introduction of a nitrogen atom into the acyl moiety caused the decrease in their activity. N-Substituted prolyl compounds (**24–26**) were inactive. The benzofuranylcarbonyl compound (**27**) was active.

The effects of the substituents on the benzoyl ring of compound **2** are shown in Table III. The study on the kind and position of substituents was undertaken. The alkyl substituents at the ortho and/or meta position on the benzoyl ring (**29–33**) caused a decrease in their activity. However, the introduction of a methyl substituent at the para position on the benzoyl ring (**28**) enhanced the hypoglycemic potency. The effect of the para substituent was dependent on its size and form. Although compound **34** was remarkably more potent than compound **28**, *N*-(4-*n*-butylbenzoyl)-D-phenylalanine (**40**) was less potent than the nonsubstituted compound (**2**). The branched alkyl substituents (such as isopropyl and *tert*-butyl) were more effective than the straight alkyl substituents (such as *n*-propyl and *n*-butyl). We presume that the difference in the effects was caused by the difference of the length of the acyl moiety (between the nitrogen atom and the end of the acyl moiety). Among the para alkyl groups (**28**, **34**, **36**, **38**, **39**, and **40**), the *p*-ethyl compound (**34**) showed the best efficacy at a 10 mg/kg dose. The dose–response curve of compound **34** is shown in Figure 1. The alkoxy sub-

Table II. N-Substituted Phenylalanines



compd	R	confign at *C	mp, °C	$[\alpha]_D$, deg	yield, %	formula[a]	method[b]	hypoglycemic activity[c]
13		D	252–253	−17.9[d]	82	$C_{16}H_{17}NO_2$	A	inact.[e]
14		D	170–171	−42.6[f]	65	$C_{16}H_{21}NO_3 \cdot H_2O$	A	100
15		D	100–101	−12.6[g]	41	$C_{16}H_{19}NO_3$	A	100
16		D	111–112.5	−35.2[g]	61	$C_{15}H_{19}NO_3$	A	50
17		L	115–117	+30.1[h]	69	$C_{15}H_{19}NO_3$	A	inact.[e]
18		D	109–110	−8.6[h]	71	$C_{17}H_{23}NO_3$	A	inact.[e]
19		D	179–180	+46.9[f]	63	$C_{15}H_{14}N_2O_3$	B	inact.[e]
20		D	170–172	+44.0[f]	59	$C_{15}H_{14}N_2O_3$	B	inact.[e]
21		D	297–299	+24.0[i]	44	$C_{19}H_{16}N_2O_3$	B	inact.[e]
22		D	191–192	+25.2[i]	62	$C_{19}H_{16}N_2O_3$	B	inact.[e]
23		D	235–236	+26.7[i]	50	$C_{19}H_{16}N_2O_3$	B	inact.[e]
24		D	155–157	−66.8[h]	69	$C_{16}H_{20}N_2O_4$	B	inact.[e]
25		D	147–149	−74.8[h]	65	$C_{21}H_{22}N_2O_4$	B	inact.[e]
26		D	209–210	+28.6[h]	70	$C_{21}H_{22}N_2O_4$	B	inact.[e]
27		D	114–116	+89.6[g]	68	$C_{18}H_{15}NO_4$	A	100

[a] Analyzed for C, H, and N; analytical results were within 0.4% of the theoretical values. [b] A, acid chloride method; B, active ester method. [c] Lowest dose in mg/kg causing a 20% blood glucose decrease (fasting mice, po). For potency of reference compound (tolbutamide), refer to Table I. [d] In 0.1 N aqueous NaOH, *c* 1.0, at 31 °C. [e] Less than 20% blood glucose decrease at a dose of 250 mg/kg. [f] In MeOH, *c* 1.0, at 25 °C. [g] In MeOH, *c* 0.5, at 22 °C. [h] In MeOH, *c* 1.0, at 23 °C. [i] In 1 N aqueous NaOH, *c* 1.0, at 25 °C.

stituents (**41–43**) exhibited decreased activity.

This study made some of the structural requirements at the acyl moiety clear and also showed the stereospecificity in the association of the phenylalanine moiety with whatever receptor these compounds bind. The best compound (**34**), which was 50 times more potent than our initial lead compound **1**, was discovered during the course of this work. Studies on mechanism of action using **34** and more extensive structural modifications are in progress.

Experimental Section

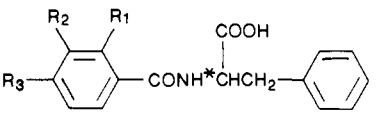
All the melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Analytical data for the compounds listed in the tables obtained for specified elements are within ±0.4% of the theoretical values unless otherwise denoted. Optical rotations were measured on a JASCO DIP-140 digital polarimeter with a 10-cm cell. IR spectra were measured as KBr tablets with a JASCO IR-810 infrared spectrophotometer. NMR spectra of $CDCl_3$, CD_3OD, $(CD_3)_2SO$, or $(CD_3)_2CO$ solutions [internal $(CH_3)_4Si$, δ 0] were recorded on a Varian EM-390 spectrometer.

***N*-Benzoyl-D-phenylalanine (2). Acid Chloride Method.** Both a solution of benzoyl chloride (10 g, 71 mmol) in acetone and 2 N aqueous sodium hydroxide were added to a solution of D-phenylalanine (11.7 g, 71 mmol) in a mixture of 1 N aqueous sodium hydroxide (80 mL) and acetone (80 mL) at such a rate as to maintain an alkaline pH in order to neutralize the hydrogen chloride. The reaction mixture was stirred until no further pH decrease was observed. The solution was acidified with dilute hydrochloric acid to pH 2–3 and vigorously stirred. The resulting solid was collected and dried. Crystallization from aqueous methanol yielded 15.8 g (83%): mp 154–155 °C (lit.[16] mp 142–143 °C); $[\alpha]^{25}_D$ −6.8° (*c* 1.0, methanol) (lit.[16] $[\alpha]^{24.5}_D$ −19.8° (*c* 8.8, 0.4 N sodium hydroxide). Anal. ($C_{16}H_{15}NO_3 \cdot H_2O$) C, H, N.

D-3-Carboxy-1,2,3,4-tetrahydroisoquinoline (4). A mixture of D-phenylalanine (75 g, 455 mmol), 36% formalin (170 mL), and concentrated hydrochloric acid (575 mL) was heated at 100 °C for 1 h with vigorous stirring. After the addition of another 75 mL of formalin and 150 mL of concentrated hydrochloric acid,

(16) Schuller, W. H.; Niemann, C. *J. Am. Chem. Soc.* **1951**, *73*, 1644.

Table III. N-Substituted Benzoyl Derivatives of Phenylalanine



comd	R_1	R_2	R_3	confign at *C	mp, °C	$[\alpha]_D$, deg	yield, %	formula[a]	method[b]	hypoglycemic activity[c]
28	H	H	CH_3	D	155–156	+48.0[d]	59	$C_{17}H_{17}NO_3$	A	100
29	H	CH_3	H	D	145–146	+40.6[d]	63	$C_{17}H_{17}NO_3$	A	inact.[e]
30	CH_3	H	H	D	129.5–131	+38.3[d]	64	$C_{17}H_{17}NO_3$	A	inact.[e]
31	H	$-CH=CHCH=CH-$		D	160–162	+89.0[f]	88	$C_{20}H_{17}NO_3$	A	250
32	H	$-CH_2CH_2CH_2-$		D	160–161	+52.0[f]	57	$C_{19}H_{19}NO_3$	A	250
33	$-CH=CHCH=CH-$	H		D	129–131	+16.8[g]	78	$C_{20}H_{17}NO_3$	A	inact.[e]
34	H	H	C_2H_5	D	165.5–166	+4.4[g]	68	$C_{18}H_{19}NO_3$	A	10
35	H	H	C_2H_5	L	129–130	−4.6[g]	84	$C_{18}H_{19}NO_3$	A	inact.[e]
36	H	H	$(CH_3)_2CH$	D	141–145	+25.5[h]	78	$C_{19}H_{21}NO_3 \cdot {}^1/_4H_2O$	A	25
37	H	H	$(CH_3)_2CH$	L	121–123	−29.3[g]	84	$C_{19}H_{21}NO_3 \cdot {}^1/_4H_2O$	A	inact.[e]
38	H	H	n-C_3H_7	D	117–118	+51.5[d]	71	$C_{19}H_{21}NO_3$	A	50
39	H	H	$(CH_3)_3C$	D	177–178	+51.5[h]	86	$C_{20}H_{23}NO_3$	A	25
40	H	H	n-C_4H_9	D	153–154	+48.4[d]	51	$C_{20}H_{23}NO_3$	A	250
41	H	H	CH_3O	D	85–90	+60.2[i]	58	$C_{17}H_{17}NO_4$	B	100
42	H	H	C_2H_5O	D	122–123	+58.4[j]	65	$C_{18}H_{19}NO_4$	B	100
43	H	$-OCH_2O-$		D	158–159	+62.8[j]	63	$C_{17}H_{15}NO_5$	B	100

[a] Analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [b] A, acid chloride method; B, active ester method. [c] Lowest dose in mg/kg causing a 20% blood glucose decrease (fasting mice, po). For potency of reference compound (tolbutamide), refer to Table I. [d] In MeOH, *c* 1.0, at 27 °C. [e] Less than 20% blood glucose decrease at a dose of 250 mg/kg. [f] In MeOH, *c* 0.5, at 22 °C. [g] In MeOH, *c* 1.0, at 23 °C. [h] In MeOH, *c* 1.0, at 20 °C. [i] In MeOH, *c* 0.5, at 20 °C. [j] In MeOH, *c* 1.0, at 25 °C.

the mixture was heated for 3 h. It was chilled and the solid separated by filtration. This product contained a partially racemized compound. Crystallization of the crude product from aqueous ethanol yielded 37 g (46%) of **4**: mp 328 °C dec.; $[\alpha]^{25}_D$ +167.0° (*c* 2.2, 1.4 N aqueous sodium hydroxide). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

L-3-Carboxy-1,2,3,4-tetrahydroisoquinoline (5). This compound was prepared from L-phenylalanine as described for the D-isomer and crystallized from aqueous methanol: yield 48%; mp 327 °C dec; $[\alpha]^{21}_D$ −167.0° (*c* 1.8, 1.4 N aqueous sodium hydroxide). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

Compounds **4** and **5** were analyzed by HPLC with DAICEL Chiralpak WH (25 × 0.46 cm) which was developed by a 0.5 mM copper(II) sulfate buffer at a flow rate of 2 mL/min. The retention times were 30.3 min for D enantiomer **4** and 17.4 min for L enantiomer **5**. The resolution value was 2.58. The enantiomeric purities of **4** and **5** were 99.5% and 99.0%, respectively.

(*Z*)-4-Benzylidene-2-phenyl-Δ^2-oxazolin-5-one (9). A suspension of **8** (14 g, 0.79 mol), freshly fused sodium acetate (9.5 g, 0.14 mol), and benzaldehyde (16 mL, 0.16 mol) in acetic anhydride (80 mL) was heated at a temperature carefully maintained between 85 and 95 °C for 1 h. Ethanol (100 mL) and water (100 mL) were cautiously added to the warm solution, causing the precipitation of a yellow solid. The slurry was poured into water (500 mL), and the solid was collected by filtration, washed with water and *n*-hexane, and dried. Crystallization from toluene yielded 16.5 g (85%): mp 167–168 °C. Anal. ($C_{16}H_{11}NO_2$) C, H, N.

(*E*)-4-Benzylidene-2-phenyl-Δ^2-oxazolin-5-one (10). Gaseous hydrogen bromide was bubbled for 1 h through a slurry of **9** (10 g, 40 mmol) in glacial acetic acid (100 mL) with vigorous stirring. This solution was then poured into water (200 mL), and the yellow precipitate was collected, washed with water, and dried under vacuum. The crude product contained 5% of the *Z* isomer. Crystallization from toluene yielded 8.2 g (81%): mp 142–145 °C. Anal. ($C_{16}H_{11}NO_2$) C, H, N.

The geometric purities of **9** and **10** were analyzed by ^{1}H NMR. The vinyl proton for the *E* azlactone was 8.36–7.36 ppm, which was concealed in the aromatic region, and that for the *Z* azlactone was 7.23 ppm. The geometric purities of both **9** and **10** were obtained by the ratio of the vinyl proton of the *Z* isomer to the aromatic protons. On the basis of this method, both **9** and **10** were geometrically pure.

(*Z*)-α-Benzamidocinnamic Acid (11). The *Z* azlactone **9** (15 g, 60 mmol) was added to a mixture of methanol (300 mL) and 1 M aqueous sodium hydroxide (150 mL). The reaction mixture was stirred until **9** was completely dissolved. Methanol was then removed under reduced pressure, and concentrated hydrochloric acid was added to the aqueous solution. The resulting solid was washed with water and dried. Crystallization from aqueous methanol yielded 15.7 g (98%): mp 243–244 °C (lit.[12] mp 235–236 °C). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

(*E*)-α-Benzamidocinnamic Acid (12). This compound was prepared from 8.7 g (33 mmol) of **10** by using the method described for the *Z* isomer. Crystallization from aqueous methanol yielded 8 g (91%): mp 197–198 °C (lit.[12] mp 189–191 °C). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

The vinyl proton for the *E* acid appeared at 6.60 ppm and that for the *Z* acid at 8.06–7.15 ppm, which was concealed in the aromatic region.

The geometric purities of **11** and **12** were analyzed by HPLC with a YMC-A311 ODS (50 × 0.6 cm) which was developed by a methanol–0.04% potassium phosphate buffer (pH 7.0) (40:60) at a flow rate of 1 mL/min. The retention times were 5.3 min for the *Z* isomer **11** and 7.4 min for the *E* isomer **12**. The resolution value was 1.29. The geometric purities of *Z* isomer **11** and *E* isomer **12** were 99.8% and 98.7%, respectively.

***N*-Nicotinoyl-D-phenylalanine (19). Active Ester Method.** Nicotinic acid (3.6 g, 29 mmol) and HOSu (3.7 g, 32 mmol) were mixed in 100 mL of chloroform. DCC (6.6 g, 32 mmol) was then added. The suspension was stirred for 3 h, dicyclohexylurea was filtered, acetic acid (10 mL) was added, and the solution was stirred for another hour. The solution was washed with saturated aqueous sodium bicarbonate and water, dried with anhydrous magnesium sulfate, and filtered. D-Phenylalanine methyl ester (35 mmol) was added, and the mixture was stirred at 25 °C for 1 day. The mixture was washed with 1 N hydrochloric acid, saturated aqueous sodium bicarbonate, and watter, dried with anhydrous magnesium sulfate, filtered, and concentrated to an oil. This oil was dissolved in 50 mL of methanol and 20 mL of 2 N sodium hydroxide solution and the mixture stirred for 10 min. The methanol was evaporated, and the aqueous residue was adjusted to pH 3 with 1 N hydrochloric acid. The powdery precipitate was filtered and then washed with water. Crystallization from AcOEt yielded 4.82 g (61%): mp 179–180 °C; $[\alpha]^{25}_D$ +46.9° (*c* 1.0, 1 N aqueous sodium hydroxide). Anal. ($C_{15}H_{14}N_2O_3$) C, H, N.

Pharmacological Methods. The biological tests were carried out on groups of five ICR-CD1 mice (male, 5 weeks old, 18 h fasted). The test compounds were given orally as a suspension in 0.5% (carboxymethyl)cellulose–0.14 M sodium chloride solution. The total volume was 1 mL/kg. A control group was treated in parallel with an equal volume of 0.5% (carboxymethyl)cellulose–0.14 M sodium chloride solution. The test compounds were

applied in doses of 500, 250, 100, 50, 25, and 10 mg/kg until the blood glucose level was significantly higher than that caused by 25 mg/kg tolbutamide (more than 20% decrease in blood glucose level). After 1 h blood samples were taken from the carotid. The blood glucose content was determined enzymatically by the glucose–oxidase–peroxidase method.[17] The statistical evaluation of the results utilized the Student's t test ($p < 0.05$).

Registry No. 1, 2901-76-0; 2, 37002-52-1; 4, 103733-65-9; 5, 74163-81-8; 6, 115732-14-4; 7, 115732-15-5; 8, 495-69-2; 9, 17606-70-1; 10, 15732-43-1; 11, 26348-47-0; 12, 57427-85-7; 13, 85114-36-9; 14, 85856-40-2; 15, 105746-35-8; 16, 105746-32-5; 17, 75691-91-7; 18, 115732-16-6; 19, 74204-45-8; 20, 115732-17-7; 21, 115732-18-8; 22, 115732-19-9; 23, 115794-99-5; 24, 115732-20-2; 25, 115795-00-1; 26, 115795-01-2; 27, 105746-33-6; 28, 105746-25-6; 29, 115732-21-3; 30, 115732-22-4; 31, 86808-12-0; 32, 105746-34-7; 33, 115732-23-5; 34, 105746-24-5; 35, 115732-24-6; 36, 105746-38-1; 37, 105746-43-8; 38, 115732-25-7; 39, 105746-42-7; 40, 115732-26-8; 41, 105746-31-4; 42, 115732-27-9; 43, 115732-28-0; H-D-Phe-OH, 673-06-3; H-Phe-OH, 63-91-2; PhCHO, 100-52-7; PhCOCl, 98-88-4; 4-MeC_6H_4COCl, 874-60-2; 3-MeC_6H_4COCl, 1711-06-4; 2-MeC_6H_4COCl, 933-88-0; 4-EtC_6H_4COCl, 16331-45-6; 4-(*i*-Pr)C_6H_4COCl, 21900-62-9; 4-PrC_6H_4COCl, 52710-27-7; 4-(*t*-Bu)C_6H_4COCl, 1710-98-1; 4-BuC_6H_4COCl, 28788-62-7; 4-$MeOC_6H_4COOH$, 100-09-4; 4-$EtOC_6H_4COOH$, 619-86-3; H-D-Phe-OMe, 21685-51-8; cyclohexanoyl chloride, 2719-27-9; cyclohex-3-enoyl chloride, 932-67-2; cyclopentanoyl chloride, 4524-93-0; cycloheptanoyl chloride, 6557-86-4; nicotinic acid, 59-67-6; pyridine-4-carboxylic acid, 55-22-1; quinoline-6-carboxylic acid, 10349-57-2; quinoline-2-carboxylic acid, 93-10-7; quinoline-3-carboxylic acid, 6480-68-8; (*S*)-*N*-acetyl-2-pyrrolidinecarboxylic acid, 68-95-1; (*S*)-*N*-benzoyl-2-pyrrolidinecarboxylic acid, 5874-58-8; (*R*)-*N*-benzoyl-2-pyrrolidinecarboxylic acid, 115795-02-3; benzofuran-2-carbonyl chloride, 41717-28-6; 2-naphthoyl chloride, 2243-83-6; 2,3-dihydroindene-5-carbonyl chloride, 15497-40-2; 1-naphthoyl chloride, 879-18-5; benzodioxole-5-carboxylic acid, 94-53-1.

(17) Huggett, A.; Nixon, D. A. *Lancet* **1957**, 368.

Synthesis and Antileukemic Activity of Bis[[(carbamoyl)oxy]methyl]-Substituted Pyrrolo[2,1-*a*]isoquinolines, Pyrrolo[1,2-*a*]quinolines, Pyrrolo[2,1-*a*]isobenzazepines, and Pyrrolo[1,2-*a*]benzazepines[1]

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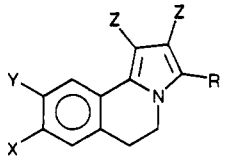
A series of bis[[(carbamoyl)oxy]methyl]-substituted pyrrole-fused tricyclic heterocycles were synthesized by using 1,3-dipolar cycloaddition reactions with a trifluoromethanesulfonate salt of an appropriate Resissert compound or with a mesoionic oxazolone intermediate. All of the bis(carbamates) were active in vivo against P388 lymphocytic leukemia with 5,6-dihydro-8-methoxy-1,2-bis(hydroxymethyl)pyrrolo[2,1-*a*]isoquinoline bis[*N*-(2-propyl)carbamate] (**3c**) showing the highest level of activity.

The design of antineoplastic agents in the "acylated vinylogous carbinolamine" class is based on the concept that these agents can act as bifunctional electrophiles in which [(carbamoyl)oxy]methyl groups serve as reactive electrophilic centers. These agents are not carbamoylating agents; instead the carbamate moieties are leaving groups in an alkyl–oxygen cleavage mechanism.[2] The reactions take place on methylenic carbons bonded directly to a heteroaromatic nucleus. The role of the heteroaromatic system is to stabilize reaction transition states, and this provides a means to control the reactivity of the two electrophilic centers. Control may be achieved through alteration of the heteroaromatic system.[3] Furthermore, if the heterocycle is not symmetrically substituted, it is possible to have different reactivities for each of the two putative electrophilic centers. The ability to control the reactivities of these bifunctional electrophiles along with the ability to exercise independent control at each center has led to the development of a number of very active compounds in this class.[3]

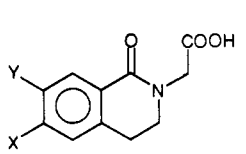
The antineoplastic activities of some pyrrolo[2,1-*a*]isoquinoline derivatives were reported in an earlier publication.[4] This paper compares a number of different tricyclic bis[[(carbamoyl)oxy]methyl] derivatives.

Chemistry

The bis(carbamates) were all prepared from the corresponding diol by treatment with 2-propyl isocyanate. The diols were synthesized from the appropriate dicarboxylic acid dister by hydride reduction. The synthesis of the diol **2b** and the bis(carbamate) **3e** have been reported.[4] The diesters **1a**, **1c**, and **1d** were prepared from the appropriately substituted 2-(1-oxo-1,2,3,4-tetrahydroisoquinolin-2-yl)acetic acid **4**. The isoquinolone precursor



1: Z = CO_2CH_3
2: Z = CH_2OH
3: Z = $CH_2OCONHCH(CH_3)_2$
a: R = X = Y = H
b: R = CH_3; X = Y = H
c: R = Y = H; X = OCH_3
d: R = H; X = Y = Cl
e: R = CH_3; X = OCH_3; Y = H



4a: X = Y = H
b: X = OCH_3; Y = H
c: X = Y = Cl

to **4a** was synthesized in a polyphosphoric acid induced

(1) Vinylogous Carbinolamine Tumor Inhibitors. 23. For part 22, see: Anderson, W. K.; Bhattacharjee, D.; Houston, D. M. *J. Med. Chem.*, in press.
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(4) Anderson, W. K.; McPherson, H. L., Jr.; New, J. S.; Rick, A. C. *J. Med. Chem.* **1984**, *27*, 1321.

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