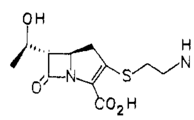
Inhibition of the Mammalian β-Lactamase Renal Dipeptidase (Dehydropeptidase-I) by (Z)-2-(Acylamino)-3-substituted-propenoic Acids

Donald W. Graham,* Wallace T. Ashton,* Louis Barash, Jeannette E. Brown, Ronald D. Brown, Laura F. Canning, Anna Chen, James P. Springer, and Edward F. Rogers*

Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065. *Received July 31, 1986*

The title enzyme deactivates the potent carbapenem antibiotic imipenem in the kidney, producing low antibiotic levels in the urinary tract. A series of (Z)-2-(acylamino)-3-substituted-propenoic acids (3) are specific, competitive inhibitors of the enzyme capable of increasing the urinary concentration of imipenem in vivo. Many of the compounds were prepared in one step from an α-keto acid and a primary amide. The optimum R^2 groups are 2,2-dimethyl, -dichloro, and -dibromocyclopropyl. With R^2 = 2,2-dimethylcyclopropyl (DMCP), a wide variety of R^3 groups including alkyl, oxa- and thiaalkyl, and alkyl groups containing acidic, basic, and neutral substituents give effective inhibitors with K_i values of 0.02–1 μM and a range of pharmacokinetic properties. By resolution of enantiomers and X-ray crystallography, the enzyme-inhibitory activity of the DMCP group was found to reside with the 1S isomer. The cysteinyl compound 176 (cilastatin, MK-0791) has the desired pharmacological properties and has been chosen for combination with imipenem.

The discovery[1] of thienamycin (1) with its novel structure, breadth of spectrum, high potency, and β-lactamase stability was a major advancement in β-lactam research. The problem of chemical instability that precluded commercial development of thienamycin was solved with the synthesis[2] of the N-formimidoyl derivative (2, imipenem)



1, R = H (thienamycin)
2, R = CH=NH (imipenem)

which also has enhanced antibacterial activity[3] in addition to improved chemical stability. In vivo evaluation of these antibiotics indicated that they were extensively metabolized in mammals.[4] Particularly disturbing was the finding that in man urinary recoveries frequently were less than 10% of the administered dose.[5] Since the plasma half-lives were acceptable, renal metabolism was suspected. On the basis of biochemical experiments, Kropp and co-workers[4] established that the deactivation of these antibiotics was due to the enzyme renal dipeptidase (dehydropeptidase-I, DHP-I, EC 3.4.13.11),[6,7] functioning as a β-lactamase. The enzyme resides in the brush-border microvilli of the proximal renal tubule[8] and has access to antibiotic both in the glomerular filtrate and during tubular secretion. It was a matter of concern that because of this "postexcretory metabolism"[9] effective antibiotic concentrations would not be maintained in the urine although plasma levels would be sufficient to treat systemic infections. It is ironic indeed that these carbapenem antibiotics which are resistant to the *microbial* β-lactamases that plague classical penicillin and cephalosporin therapy should be susceptible to deactivation by a *mammalian* β-lactamase, thereby limiting their effectiveness against urinary tract infections. One solution to this problem would be to develop an inhibitor of renal dipeptidase suitable for combination with imipenem to protect the antibiotic during renal passage. This paper describes the development of such an inhibitor. Another approach to the problem would be to modify the imipenem structure such that renal dipeptase susceptibility is eliminated while antimicrobial potency is retained. Progress using this approach has been described recently.[10] A number of carbapenem and penem antibiotics related to thienamycin also are susceptible to deactivation by renal dipeptidase.[4,9]

Campbell and colleagues[11] have shown that porcine renal dipeptidase is a metalloprotease of MW 94 000 containing two Zn atoms. Recently the same group reported[12] that human renal dipeptidase is made up of four MW 59 000 subunits each containing a Zn atom. Neither the crystal structure nor the primary sequence of either enzyme is known. The enzyme is a specific dipeptidase that requires an L-amino acid at the N-terminus but will accept L-, D-, or dehydro amino acids at the C-terminus.[6] The resemblance of thienamycin and imipenem to dehydrodipeptides presumably accounts for their acceptance as substrates. Aside from inorganic anions (PO_4^{3-}, CN^-) and Zn chelators, the only inhibitors of renal dipeptidase reported are nucleotides such as CTP and GTP ($K_i \sim 0.4$ mM).[13]

In a directed search for potential inhibitors, Kahan and co-workers of this laboratory screened compounds containing modified dehydrodipeptide structures against renal dipeptidase. They found that (Z)-2-benzamido-2-butenoic acid (59) was a moderately effective inhibitor both in vitro and in vivo.[9] In this paper we describe the synthesis and renal dipeptidase inhibitory activity of a substantial

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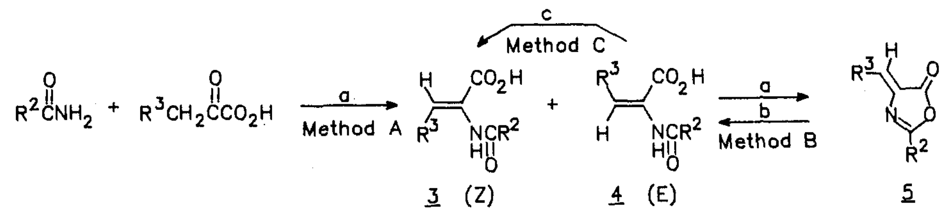
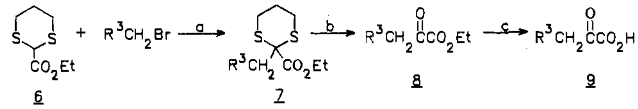
Scheme I



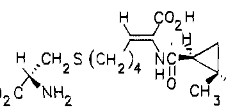
a. Δ, toluene. b. NaOH. c. Δ, I_2.

Scheme II



a. NaH, DMF, toluene. b. NBS, MeCN, H_2O. c. HBr, HOAc or NaOH.

number of the compounds that were prepared during an extensive synthetic program undertaken to explore this lead. Compound **59** proved to be a prototype of a class of compounds of general structure **3**, which are specific, competitive inhibitors of renal dipeptidase. One of the compounds of this type, **176**, was found to have the requisite chemical and pharmacological properties and has been selected for combination with imipenem.



176 (cilastatin)

Chemistry

Most of the target compounds in this study were prepared by a one-step reaction between an α-keto acid and a primary amide (Scheme I).[14] Nearly all the butenoates in Table I were prepared from commercially available α-ketobutyric acid and the appropriate amide (method A).[15] Many of the 3-substituted 2-(2,2-dimethylcyclopropanecarboxamido)propenoic acids listed in Table II were prepared from 2,2-dimethylcyclopropanecarboxamide (**11**)[16] and the appropriate α-keto acid (method A1). The α-keto acids were prepared either from the dithiane **6** by Eliel's method[17] (Scheme II) or by the Claisen condensation procedure of Schreiber.[18] As can be seen in Tables I–III, the yields for the α-keto acid–amide reaction usually were poor. However, the reaction gave the target compounds in one step from readily available starting materials and rarely failed. Additional experimental aspects of the reaction are discussed at the end of method A in the Experimental Section. In a few cases (**60**, **61**, **66**), the α-keto acid–amide reaction was continued until the azlactone **5** was formed.[19] The azlactone was isolated and hydrolyzed back to the butenoate (method B).

The *Z* stereochemistry (**3**) is assigned to the major product from the α-ketobutyrate–amide reaction (Table I) on the basis of th NMR data of Srinivasan et al.[20] These workers found that the position of the β-methyl doublet was the best indicator of stereochemistry with the *Z* isomer (**3**) absorbing at higher field than the *E* isomer (**4**). Crude products from shorter reaction times frequently contained varying amounts of the *E* isomer (**4**) having a low field β-methyl doublet. In one case (**72**), such an *E*–*Z* mixture was isomerized to the pure *Z* isomer with I_2 (method C). The compounds in Tables II and III were assigned the *Z* stereochemistry by analogy. In one instance a *Z* compound (**139**) was photochemically isomerized to an *E*–*Z* mixture from which the pure *E* isomer **241** was isolated by chromatography. The allylic CH_2 in **241** absorbs at lower field than in **139**.

Although some of the compounds with functionality in R^3 (Table II) were prepared from the appropriate functionalized α-keto acid, it was more convenient to prepare most of these compounds from the ω-bromoalkyl intermediates **12**. These important intermediates were prepared from **11** and the ω-bromo α-keto acids **10**. As summarized in Scheme III, the bromide in **12** could be displaced easily by a variety of S, N, and O nucleophiles to form many of the inhibitors in Tables II and III. Most important was the reaction with the cysteinyl dianion to give cysteinyl thioether derivatives[21] (method F).

Compounds obtained by nucleophilic displacement on **12** could be transformed further. Thus the primary amino compounds obtained by method H could be acylated (method J), carbamoylated (**208**), and converted into amidines (method M) or a 2-aminoimidazolidine (**194**). Displacement with $EtOCS_2K$ gave the O-ethyl dithiocarbonate **211**, which was cleaved[22] to give the mercapto compound **212**. The methylthio **156** was oxidized to the sulfone **157**.

Attempted displacement of the bromide in **188** by phthalimide with K_2CO_3 in hot DMF gave a neutral product that did not contain the phthalimido group. Treatment of **188** with just K_2CO_3 in hot DMF gave the same product. Spectral data indicated that the product was the macrodilide **13** (Scheme IV). Formation of this 18-membered dilactone in moderate yield (48%) is of interest since conditions favoring cyclization such as high dilution or slow addition were not used. This fairly facile cyclization of **188** compared to the corresponding reaction with 8-bromooctanoic acid[23] probably is due to the rigid α,β-unsaturated acid moiety which reduces the degrees of conformational freedom in **188**. Base hydrolysis cleaved

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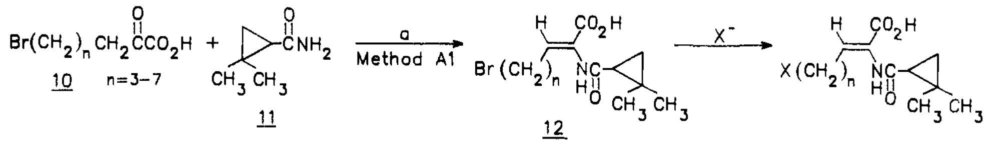
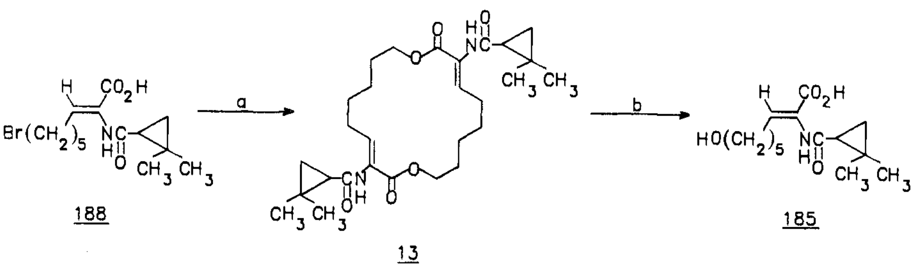
Scheme III



a. Δ, toluene.

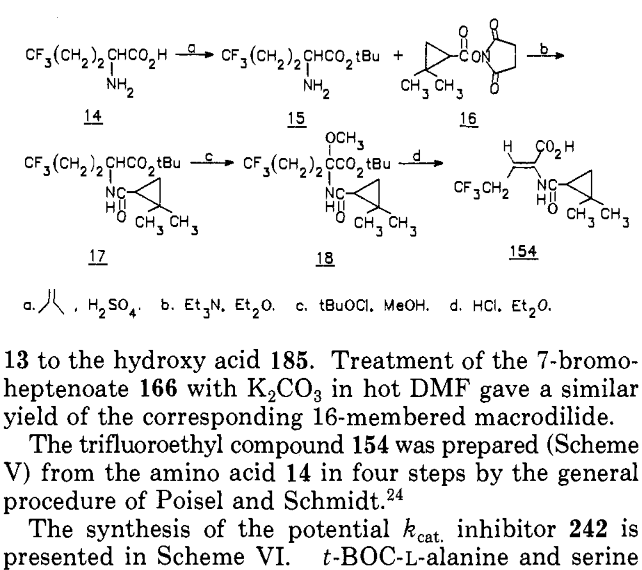
X	Method/Compd
RS	E. F. 164. 211
R_2N	H. I. K
R_3N^+	L
CH_3CO_2	186
CH_3O	187
CN	G
SO_3^-	171

Scheme IV



a. K_2CO_3, DMF. b. LiOH.

Scheme V



a. , H_2SO_4. b. Et_3N, Et_2O. c. tBuOCl, MeOH. d. HCl, Et_2O.

13 to the hydroxy acid 185. Treatment of the 7-bromoheptenoate 166 with K_2CO_3 in hot DMF gave a similar yield of the corresponding 16-membered macrodilide.

The trifluoroethyl compound 154 was prepared (Scheme V) from the amino acid 14 in four steps by the general procedure of Poisel and Schmidt.[24]

The synthesis of the potential $k_{cat.}$ inhibitor 242 is presented in Scheme VI. *t*-BOC-L-alanine and serine methyl ester were condensed and dehydrated to the protected dehydrodipeptide 19 in a one-pot reaction with a carbodiimide and CuCl.[25] After conversion of the methyl ester 20 to the *tert*-butyl ester 21, the chlorination–dehydrochlorination procedure of Richards et al.[26] gave the protected 3-chloro dehydro dipeptide 22 as a mixture of *E* and *Z* isomers. Deprotection and ion-exchange purification gave 242 containing 16% of the *E* isomer.

Results

Structure–Activity Relationships. The structures, chemical data, and enzyme-inhibitory activity for the compounds synthesized are presented in Tables I–III. The enzyme-inhibitory activity is expressed as a K_i (μM) determined by the effect of the test compound on the renal dipeptidase mediated hydrolysis of glycyldehydrophenylalanine (**3**, $R^2 = CH_2NH_2$, R^3 = Ph)[6] by using the assay procedure described in the Experimental Section.

Initial work focused on R^2 variations utilizing the readily accessible (*Z*)-2-(acylamino)butenoic acids (**3**, $R^3 = CH_3$), and the results are given in Table I. As will be discussed below, the contributions of R^2 and R^3 are roughly additive so this approach is not misleading. Following the (*Z*)-2-benzamido-2-butenoic acid (**59**) lead, a number of other aryl and heteroaryl groups (**60–67**) were tested as replacements for phenyl with unimpressive results. Better activity was noted when R^2 was *n*-alkyl, especially C_3–C_{11} (**32–40**). Even better activity was found with branched alkyls, principally β-methyls (**44, 47–49, 53, 54**). Although one α-CH_3 compound (**49**) was very active, other α-methyl compounds (**43, 45**), larger α-groups (**50, 51**), and γ-substituents (**46**) all were less effective. Elimination of the α-H with α-substituents (**45**) or a double bond (**56, 57, 72**) reduced activity greatly. In compounds **73–90** various kinds of functionality were introduced at different positions on a straight or branched alkyl chain. In general, the activity of these compounds did not deviate greatly from that of the analogous unsubstituted compound despite the wide variation in size (C_6H_5), charge (CO_2H), lipophilicity (amide), and electronegativity (Cl) of the substituents. Small (C_3 and C_4) cycloalkyl (**91, 92**) R^2 groups were very active while larger cycloalkyl (**93–96**) and alkylcycloalkyl (**97–106**) compounds were less so. By far the most active R^2 substituents are the closely related 2,2-dimethyl, -dichloro, and -dibromocyclopropyl compounds (**113, 126, 127**). Of the analogues (**107–131**) synthesized to explore this activity, only the 2-methyl-2-ethyl compound **119** approached the activity of **113**. Noteworthy is the substantial loss in activity of the tetramethyl compound **118**, the spiro analogue **124**, the 1-methyl compound **129**, and the cyclobutyl analogues **130** and **131**.

The 2,2-dimethylcyclopropyl (DMCP) group was chosen as the preferred R^2, and the results of variation of the R^3

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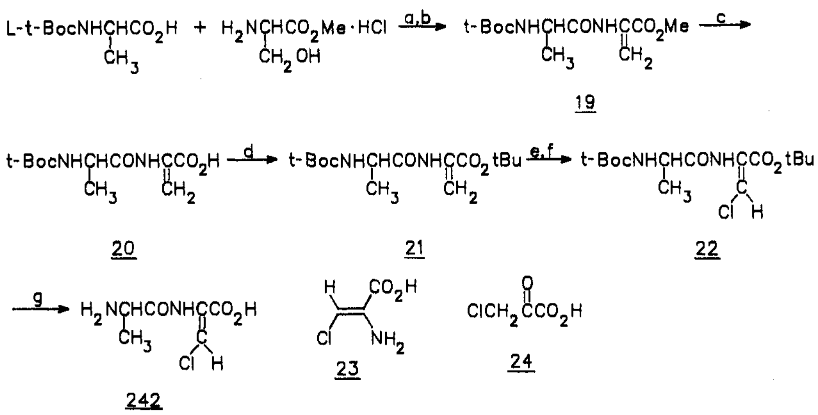
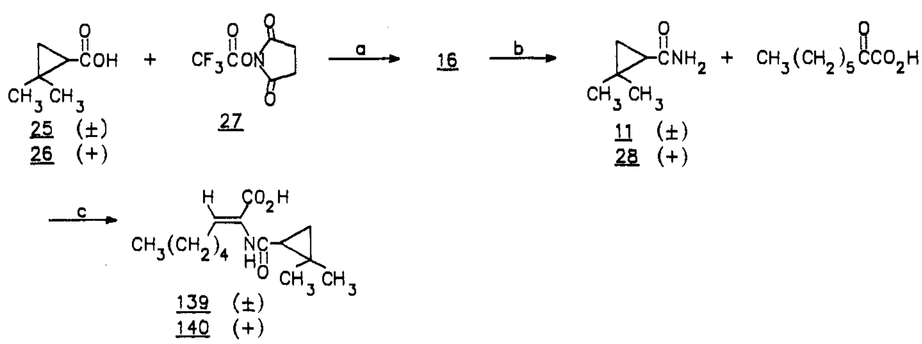
Scheme VI



a. $Me_2N(CH_2)_2N{=}C{=}NEt\cdot HCl$, Et_3N, CH_2Cl_2. b. $Me_2N(CH_2)_2N{=}C{=}NEt\cdot HCl$, CuCl. c. NaOH, MeOH. d. iPrN=C(OtBu)–NHiPr, DMF. e. Cl_2, CCl_4. f. DABCO, MeCN. g. TFA.

Scheme VII



a. pyr. b. NH_3, EtOH. c. Δ, toluene.

substituent are presented in Table II. In general, the enzyme-inhibitory activity proved to be less sensitive to variation of the R^3 substituent, and the potent activity of the DMCP group could be retained or even enhanced with a wide variety of R^3 groups. Although replacement of CH_3 with H (**133**) produced a large drop in activity, increasing the chain to *n*-decyl (**134, 135, 137, 139, 144, 146–149**) resulted in up to a fourfold increase in activity. In contrast to R^2, branched alkyl (**138, 142, 143**) and cycloalkyl (**151–153**) were not improvements over *n*-alkyl. The remaining compounds in Table II contain functionalized alkyl chains. To a remarkable degree the activity of the DMCP group is unaffected by these diverse substituents although certain trends are noticeable. Acidic (anionic) groups (**158, 164, 165, 171, 184**) usually increase activity substantially, while basic (cationic) groups (**170, 191–194, 203, 221–223**) decrease activity particularly on alkyl groups below C_6. The activity of groups containing both basic and acidic functions (**172, 174, 196**) is roughly an average of the two groups alone. The substituted pyridylthio and phenylthio compounds **180–183** are the most potent class of R^3 substituents.

Certain information can be deduced from the miscellany of R^2 and R^3 groups presented in Table III. First the requirement that R^3 be at least CH_3 can be seen from the significant loss of activity when R^3 is H, Cl, or OCH_3 (**236–238**). Also the deleterious effect of a 3(*E*)-substituent (R^4) can be seen by the low activity of both the disubstituted compounds **239** and **240** as well as the *E* isomer **241**. Finally the additivity between the R^2 and R^3 substituents noted above can be exemplified by comparison of compounds **229, 44** and **139, 113**. Thus R^3 = *n*-pentyl is roughly twice as active as $R^3 = CH_3$ when R^2 is either isobutyl or DMCP. Other examples are compounds **234**, **126** and **203**, **113**—$R^3 = (CH_3)_3N^+(CH_2)_5$ is approximately half as active as $R^3 = CH_3$ when R^2 is either 2,2-dichloro- or 2,2-dimethylcyclopropyl.

Stereochemistry of the 2,2-Dimethylcyclopropyl (DMCP) Group. Since the preferred DMCP group has an asymmetric center, it was of interest to determine the effect of its chirality on the enzyme-inhibitory activity. Both the butenoic (**113**) and octenoic (**139**) acids were resolved with (+)- and (−)-*threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol and (+)- and (−)-ephedrine, respectively. In both cases the enzyme-inhibitory activity residues almost entirely with the (+) enantiomers (**114**, **140**).

The absolute configuration of these active enantiomers was established as *S* as follows (Scheme VII). The resolution of 2,2-dimethylcyclopropanecarboxylic acid[16] (**25**) via (+)- and (−)-α-methylbenzylamine has been reported[27] with $[\alpha]^{20}_D$ −71.7° and +65.0° (*c* 1.0, $CHCl_3$) given for the two isomers. In addition, partial asymmetric synthesis of **26** has been accomplished,[28] the product, $[\alpha]^{20}_D$ +6.6° (MeOH), being assigned the *S* configuration on the basis of the Brewster conformational asymmetry calculations. We found that conversion of **25** to the (−)-quinine salt followed by successive recrystallizations from MeOH–H_2O to constant rotation of the free acid afforded the (+) isomer (**26**), $[\alpha]^{20}_D$ +142° (*c* 1.0, $CHCl_3$), +132° (*c* 1.0, MeOH).

An X-ray diffraction study on the (−)-quinine salt of **26** was carried out to establish the absolute configuration.

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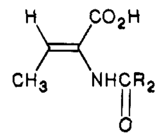
Table I. (*Z*)-2-(Acylamino)-2-butenoic Acids



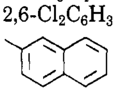
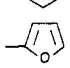
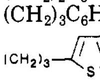
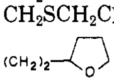
compd	R_2	method[a]	mp, °C	crystn solvent[b]	yield, %	formula	anal.[c]	K_i, μM or (% inhibn at 100 μM)
29	CH_3		151–155[d]					(12)
30	CH_2CH_3	A	149–150.5	I	26	$C_7H_{11}NO_3$	C, H, N	(43)
31	$-(CH_2)_4-$	A	239–241 dec	II	40	$C_{14}H_{20}N_2O_6$	C, H, N	10
32	$(CH_2)_2CH_3$	A	141–142	III	23	$C_8H_{13}NO_3$	C, H, N	30
33	$(CH_2)_3CH_3$	A	153–154	IV	18	$C_9H_{15}NO_3$	C, H, N	32
34	$(CH_2)_4CH_3$	A	141–142	IV	23	$C_{10}H_{17}NO_3$	C, H, N	(54)
35	$(CH_2)_5CH_3$	A	142	I	8	$C_{11}H_{19}NO_3$	C, H, N	(49)
36	$(CH_2)_6CH_3$	A	141–142	IV	23	$C_{12}H_{21}NO_3$	C, N; H[e]	26
37	$(CH_2)_7CH_3$	A	145–147	IV	30	$C_{13}H_{23}NO_3$	C, H, N	32
38	$(CH_2)_8CH_3$	A	145–146	V	11	$C_{14}H_{25}NO_3$	C, H, N	(58)
39	$(CH_2)_9CH_3$	A	141–142	IV	19	$C_{15}H_{27}NO_3$	C, H, N	19
40	$(CH_2)_{10}CH_3$	A	141–142	IV	24	$C_{16}H_{29}NO_3$	C, H, N	35
41	$(CH_2)_{12}CH_3$	A	149–151	IV	34	$C_{18}H_{33}NO_3$	C, H, N	(45)
42	$(CH_2)_{16}CH_3$	A	140–141	IV	25	$C_{22}H_{41}NO_3$	C, N; H[f]	(12)
43	$CH(CH_3)_2$	A	184–184.5	IV	11	$C_8H_{13}NO_3$	C, H, N	(31)
44	$CH_2CH(CH_3)_2$	A	176	VI	27	$C_9H_{15}NO_3$	C, H, N	6
45	$C(CH_3)_3$	A	182–183.5	IV	14	$C_9H_{15}NO_3$	C, H, N	(0)
46	$(CH_2)_2CH(CH_3)_2$	A	164–165	IV	13	$C_{10}H_{17}NO_3$	C, H, N	14
47	$CH_2CH(CH_3)CH_2CH_3$	A	168.5–169.5	IV	27	$C_{10}H_{17}NO_3$	C, H, N	10
48	$CH_2C(CH_3)_3$	A	191–192	IV	47	$C_{10}H_{17}NO_3$	C, H, N	20
49	$CH(CH_3)CH(CH_3)_2$	A	197–198	V	34	$C_{10}H_{17}NO_3$	C, H, N	3.3
50	$CH(CH_2CH_2CH_3)_2$	A	185–186	IV	53	$C_{12}H_{21}NO_3$	C, H, N	(13)
51	$CH(CH_2CH_3)(CH_2)_3CH_3$	A	160–162	IV	50	$C_{12}H_{21}NO_3$	C, H, N	(33)
52	$(CH_2)_4CH(CH_3)_2$	A	146.5–147	VII	7	$C_{12}H_{21}NO_3$	C, H, N	(57)
53	$CH_2CH(CH_3)CH_2C(CH_3)_3$	A	136–138	IV	21	$C_{13}H_{23}NO_3$	C, N; H[g]	4.4
54	$CH_2CH(CH_3)(CH_2)_3CH(CH_3)_2$	A	144–145	IV	55	$C_{14}H_{25}NO_3$	C, H, N	4
55	$(CH_2)_2CH{=}CH_2$	A	143–145	IV	17	$C_9H_{13}NO_3$	C, H, N	(57)
56	$CH{=}C(CH_3)_2$	A	183–184	VIII	30	$C_9H_{13}NO_3$	C, H, N	28
57	$CH{=}CHCH{=}CHCH_3$	A	198–199	VIII, V	11	$C_{10}H_{13}NO_3$	H, N; C[h]	(0)
58	$(CH_2)_8CH{=}CH_2$	A	135–137	IV	27	$C_{15}H_{25}NO_3$	C, H, N	34
59	C_6H_5[i]							70
60	$4\text{-}CH_3C_6H_4$	B	199–202	IX		$C_{12}H_{13}NO_3$	C, H, N	(2.5)
61	$4\text{-}OCH_3C_6H_4$	B	201–203	X	60	$C_{12}H_{13}NO_4$	H, N; C[j]	(0)
62	$2\text{-}ClC_6H_4$	A	207–209	XII	22	$C_{11}H_{10}ClNO_3$	C, H, N, Cl	25
63	$3\text{-}ClC_6H_4$	A[k]	188–190	XII	34	$C_{11}H_{10}ClNO_3$	C, H, N, Cl	22
64	$4\text{-}ClC_6H_4$	A[k]	227 dec	XIV	7	$C_{11}H_{10}ClNO_3$	C, H; N[l]	(7)
65	$2,6\text{-}Cl_2C_6H_3$	A[k]	223.5–225.5	I	11	$C_{11}H_9Cl_2NO_3$	C, H, N	(0)
66		B	212–215	X	98	$C_{15}H_{13}NO_3$	C, H, N	(3)
67		A	140–141.5	XI	9	$C_9H_9NO_4$	C, H, N	(57)
68	$CH_2C_6H_5$	A[m]	195–197	XII	6	$C_{12}H_{13}NO_3$	C, H, N	(21)
69	$(CH_2)_2C_6H_5$	A	188–189	V	18	$C_{13}H_{15}NO_3$	C, H, N	(30)
70	$(CH_2)_3C_6H_5$	A	130–132	IV	49	$C_{14}H_{17}NO_3$	C, H, N	11
71		A	132–135	IV	55	$C_{12}H_{15}NO_3S$	C, H, N	10
72	$CH{=}CHC_6H_5$	A, C	198.5–201	IV	27[n]	$C_{13}H_{13}NO_3$	C, H, N	(0)
73	CH_2Cl		166–168[o]					(52)
74	$CH_2OC_6H_5$	A[k]	127.5–128	IV	37	$C_{12}H_{13}NO_4$	C, H, N	(3)
75	CH_2NH_2		290 dec[p]					(6)
76	$CH_2NHCO(CH_2)_2CH_3$	D	164	XII	18	$C_{10}H_{16}N_2O_4$	C, H, N	(5)
77	$CH_2NHCOCH_2CH(CH_3)_2$	D	144	XII	25	$C_{11}H_{18}N_2O_4$	C, H, N	(5)
78	$CH_2SCH_2CH_3$	A[q]	114–115.5	IV	7	$C_8H_{13}NO_3S$	C, H, N	31
79		A	133.5–134	XI	19	$C_{11}H_{17}NO_4$	C, H, N	(51)
80	$(CH_2)_3CO_2CH_2CH_3$	A	102–103	VII	9	$C_{11}H_{17}NO_5$	C, H, N	39
81	$(CH_2)_3CON(CH_3)_2$	A[r]	137.5–139	VIII	26	$C_{11}H_{18}N_2O_4$	C, H, N	(46)
82	$(CH_2)_3NHCOCH_3$	A	155–157	XII	20	$C_{10}H_{16}N_2O_4\cdot 0.25H_2O$	C, H, N	102
83	$(CH_2)_3NHCOCH_2CH(CH_3)_2$	A	132–135	XII	18	$C_{13}H_{22}N_2O_4$	C, H, N	52
84	$(CH_2)_4CN$	A[s]	102–109[t]	XI	5	$C_{10}H_{14}N_2O_3\cdot 0.125H_2O$	C, H, N	25
85	$(CH_2)_4CO_2CH_2CH_3$	A	123–124	VII	18	$C_{12}H_{19}NO_5$	C, H, N	18
86	$(CH_2)_5Br$	A	132–135	IV	8	$C_{10}H_{16}BrNO_3$	C, H, N	19
87	$(CH_2)_5NHCOCH_2CH_3$	A	108–110	XII	12	$C_{13}H_{22}N_2O_4\cdot 0.25H_2O$	C, H, N	41
88	$CH_2CH(CH_3)(CH_2)_2OCH_3$	A	105–107	IV	21	$C_{11}H_{19}NO_4$	C, H, N	9
89	$CH_2CH(CH_3)(CH_2)_3C(OH)(CH_3)_2$	A	115–117	IV	20	$C_{14}H_{25}NO_4$	C, N; H[u]	6.7
90	$(CH_2)_8CO_2H$	A[k]	155–158	XII	2	$C_{14}H_{23}NO_5$	C, H, N	28

Table I (Continued)

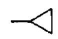
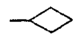
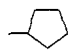
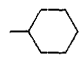
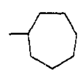
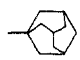
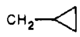
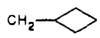
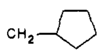
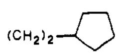
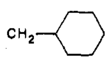
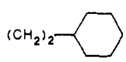
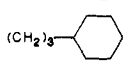
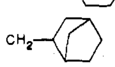
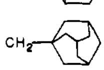
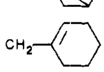
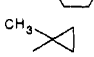
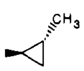
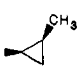
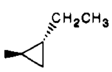
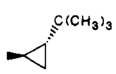
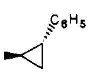
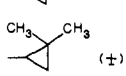
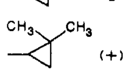
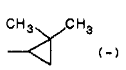
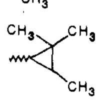
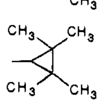
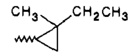
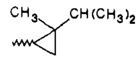
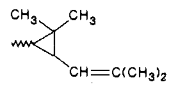
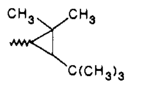
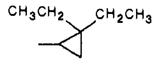
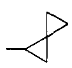
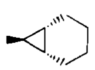
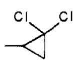
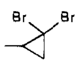
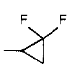
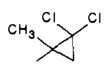
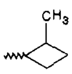
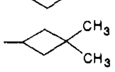
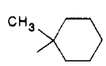
compd	R_2	method[a]	mp, °C	crystn solvent[b]	yield, %	formula	anal.[c]	K_i, μM or (% inhibn at 100 μM)
91		A	212–213	I	51	$C_8H_{11}NO_3$	C, H, N	3
92		A	153–155	I	3	$C_9H_{13}NO_3$	C, H, N	6.2
93		A	205.5–206	XIV	34	$C_{10}H_{15}NO_3$	C, N; H[v]	30
94		A	208–209	I	18	$C_{11}H_{17}NO_3$	C, H, N	15
95		A	206–207	I	18	$C_{12}H_{19}NO_3$	C, H, N	12
96		A	205–206	I	40	$C_{15}H_{21}NO_3$	C, H, N	(3)
97		A	142–143	IV	9	$C_9H_{13}NO_3$	C, H, N	22
98		A	155–156	IV	17	$C_{10}H_{15}NO_3$	C, H, N	6.6
99		A	168–169	VIII	2	$C_{11}H_{17}NO_3$	C, H, N	9.8
100		A	184–185	VIII	28	$C_{12}H_{19}NO_3$	C, H, N	(56)
101		A	176–177	VIII	29	$C_{12}H_{19}NO_3$	C, H, N	25
102		A	180.5–181	VII	10	$C_{13}H_{21}NO_3$	C, H, N	(54)
103		A	156–157	IV	10	$C_{14}H_{23}NO_3$	C, H, N	30
104		A[k]	182.5–183.5	IV, V	15	$C_{13}H_{19}NO_3$	C, H, N	(54)
105		A[k]	153–155	IV	36	$C_{16}H_{23}NO_3$	C, H, N	(33)
106		A	163–165	IV	18	$C_{12}H_{17}NO_3$	C, H, N	(50)
107		A[k]	144–145	IV	3	$C_9H_{13}NO_3$	H, N; C[w]	(16)
108		A	178–180	IV	6	$C_9H_{13}NO_3$	C, H, N	1.7
109		A	167–168	V	5	$C_9H_{13}NO_3$	C, H, N	12
110		A	184	IV	16	$C_{10}H_{15}NO_3$	C, H, N	1.6
111		A	189–190	IV	45	$C_{12}H_{19}NO_3$	C, H, N	6.5
112		A	196–197.5	V	40	$C_{14}H_{15}NO_3$	C, H, N	5
113		A	142–143.5	XII	27	$C_{10}H_{15}NO_3$	C, H, N	0.4
114			145.5–146.5[x]	IV		$C_{10}H_{15}NO_3$	C, H, N	0.19
115			145.5–146.5[y]	IV				19.8
116		A	238	VIII	21	$C_{10}H_{15}NO_3$	C, H, N	4.6
117		A	159–161	XII	42	$C_{11}H_{17}NO_3$	C, H, N	7.2
118		A	201–202	IV	53	$C_{12}H_{19}NO_3$	C, H, N	(10)

Table I (Continued)

compd	R_2	method[a]	mp, °C	crystn solvent[b]	yield, %	formula	anal.[c]	K_i, μM or (% inhibn at 100 μM)
119		A	131–132	IV	12	$C_{11}H_{17}NO_3$	C, H, N	0.45
120		A	183–184	IV	22	$C_{12}H_{19}NO_3$	C, H, N	3
121		A	174–175	IV	18	$C_{14}H_{21}NO_3$	C, H, N	(15)
122		A	140–142	IV	6	$C_{14}H_{23}NO_3$	C, H, N	(44)
123		A	129–130	IV	10	$C_{12}H_{19}NO_3$	C, H, N	0.86
124		A	189–190.5	XII	32	$C_{10}H_{13}NO_3$	C, H, N	1.6
125		A[z]	256	XV	8	$C_{12}H_{17}NO_3$	C, H, N	4.2
126		A	188–189.5	V	18	$C_8H_9Cl_2NO_3$	C, H, N, Cl	0.08
127		A[k]	191–192	V	23	$C_8H_9Br_2NO_3$	C, H, N, Br	0.03
128		A[k]	199–200	V	12	$C_8H_9F_2NO_3$	C, H, N, F	2.1
129		A[k]	138–141	V	37	$C_9H_{12}Cl_2NO_3$	C, H, N, Cl	164
130		A	172–174.5	XII	19	$C_{10}H_{15}NO_3$	C, H, N	4.7
131		A[k]	171–172	XVI	59	$C_{11}H_{17}NO_3$	C, H, N	11.2
132		A	164–165	IV	27	$C_{12}H_{19}NO_3$	C, H, N	(11)

[a] See Experimental Section. Procedures for compounds without method designation are given separately. [b] I = 1,1,2,2-tetrachloroethane; II = DMF–HOAc; III = toluene–diisopropyl ether; IV = toluene; V = CH_3NO_2; VI = toluene–diethyl ketone–diisopropyl ether; VII = toluene–diethyl ketone; VIII = methyl ethyl ketone; IX = acetone–H_2O; X = dioxane–H_2O; XI = diisopropyl ketone; XII = EtOAc; XIII = EtOH–H_2O; XIV = diethyl ketone; XV = methyl isovalerate; XVI = CH_3CN. [c] Analyses were within ±0.4% of theory except where indicated. [d] Lit. mp 159–160 °C (Price, V. E.; Greenstein, J. P. *Arch. Biochem.* **1948**, *18*, 383). [e] H: calcd, 9.31; found, 9.91. [f] H: calcd, 11.24; found, 11.84. [g] H: calcd, 9.54; found, 10.07. [h] C: calcd, 61.53; found, 61.07. [i] Reference 19. [j] C: calcd, 61.27; found, 60.74. [k] *p*-Toluenesulfonic acid catalyst added. [l] N: calcd, 5.85; found, 6.40. [m] Trichloroethylene used as reaction solvent. [n] Overall yield for two steps. [o] Literature mp 170–172 °C (Flavin, M.; Slaughter, C. *J. Biol. Chem.* **1969**, *244*, 1434. [p] Literature mp >270 °C (see footnote *o*). [q] Product obtained by concentration of reaction mixture and trituration of residue with Et_2O to give solid. [r] Product obtained by decantation of reaction solvent, extraction of residual oil with EtOAc, and gradual evaporation of extracts to give crystals. [s] Product obtained by decantation of reaction solvent and trituration of residual oil with diethyl ketone to give solid. [t] Softened >90 °C. [u] H: calcd, 9.29; found, 9.74. [v] H: calcd, 7.67; found, 8.14. [w] C: calcd, 59.00; found, 58.49. [x] $[\alpha]^{24}_D$ +98.5° (*c* 0.35, $CHCl_3$). [y] $[\alpha]^{24}_D$ −97.2° (*c* 0.35, $CHCl_3$). [z] Methyl isovalerate used as reaction solvent.

Figure 1 is a computer-generated perspective drawing from the final X-ray coordinates showing the absolute stereochemistry of *S* for C-1. This confirms the assignment made by application of Brewster's rules.[28]

In order to establish the absolute configuration of **140**, **26** was reacted (Scheme VII) with *N*-(trifluoroacetoxy)succinimide (**27**)[29] to give **16**, which upon reaction with NH_3 gave the *S*-(+) amide **28**. Condensation of **28** with 2-oxooctanoic acid yielded **140**, $[\alpha]^{20}_D$ +79.0° (*c* 0.50, $CHCl_3$), corresponding to the active isomer obtained by resolution of **139**. Thus, by relation to **26**, the absolute configuration of **140** is established as *S*. Reaction of **28** with other α-keto acids allowed the preparation of a variety of inhibitors (Table II) containing the active (*S*)-DMCP group.

(29) Sakakibara, S.; Inukai, N. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 1979.

***$K_{cat.}$* Inhibitor.** The potential $k_{cat.}$ inhibitor (suicide substrate[30]) **242** was designed with the expectation that it would be hydrolyzed by renal dipeptidase and in so doing generate 3-chlorodehydroalanine (**23**) and its hydrolysis product, 3-chloropyruvate (**24**), at the active site. 3-Chloropyruvate is a potent electrophile and is known to be an enzyme alkylating agent,[31] while the electrophilic nature of **23** is speculative since the compound is unknown. When exposed to renal dipeptidase, **242** proved to be an excellent substrate (better than glycyldehydrophenylalanine). However, it showed only weak competitive inhibition, and no time-dependent deactivation of the enzyme that is characteristic of suicide substrates was de-

(30) Walsh, C. *Tetrahedron* **1982**, *38*, 871.

(31) Barnett, J. E. G.; Kolisis, F. *Biochem. J.* **1974**, *143*, 487. Osman, A. M.; Yamano, T.; Morino, Y. *Biochem. Biophys. Res. Commun.* **1976**, *70*, 153.

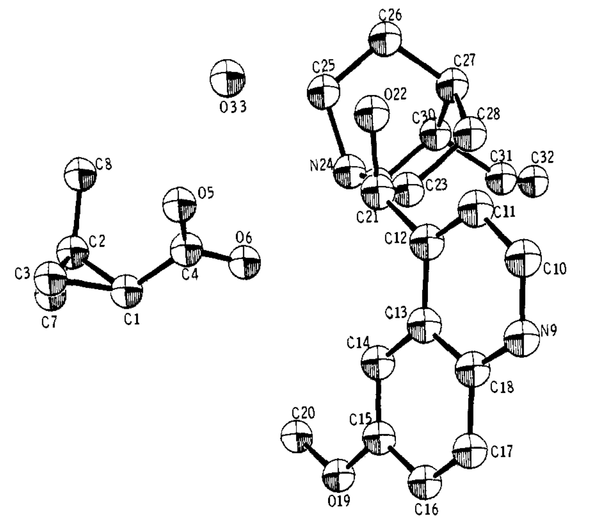


Figure 1. A computer-generated drawing of the (−)-quinine salt of **26** derived from the X-ray coordinates with hydrogens omitted for clarity.

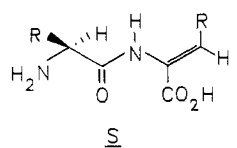
tected. Possibly **23** is not sufficiently electrophilic to react with enzyme nucleophiles, and its hydrolysis to **24** is slow compared to its release from the enzyme. Alternatively it could be that **23** and/or **24** do not encounter any enzyme nucleophiles before being released.

Discussion and Conclusions

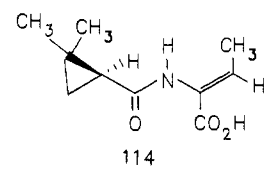
The work presented here is concerned only with variations at R^2 and R^3 in **3**. As will be reported elsewhere, changes in other parts of **3** such as the double bond (cyclopropanation, reduction), carboxyl (replacement with tetrazole or phosphonate), or NH (methylation) resulted in substantial losses in activity.

The enzyme-inhibitory activity of **3** is much more sensitive to variations in R^2 than in R^3. Of the many variants in Table I, the DMCP (**113**) and closely related dichloro (**126**) and dibromo (**127**) groups are by far the most potent substituents. The most active open chain R^2 groups are isobutyl (**44**) and 1-methylisobutyl (**49**), both close structurally to the DMCP group. Although various substituted cyclopropanecarboxylic acids have been found to mimic natural amino acids[32] and have been used as competitive enzyme inhibitors[33] and suicide substrates,[34] the DMCP group has not previously been used in enzyme inhibitors. The basis for the strong inhibitory activity of these compounds is unknown. A pilot Hansch QSAR study[35] with 16 R^2 substituents including **44**, **113**, and **126** (a 2000-fold range in K_i) indicated no correlation between enzyme-inhibitory activity and the usual Hansch parameters[36] plus an indicator variable for a cyclopropyl ring. Clearly the DMCP and related groups possess the correct size and shape to bind tightly to the enzyme. The stringent size and shape requirements at this binding site are revealed by the substantial loss in activity of compounds such as the spiropentane **124**, the tetramethylcyclopropyl **118**, and the 1-methyl compound **129**. The location and nature of this binding site cannot be specified until the X-ray crystal structure of the enzyme is available.

If one considers an inhibitor such as **114** as a desaminodipeptide analogue and superimposes the active (*S*)-DMCP group on the N-terminal L-amino acid of a dipeptide substrate (S), then the ring CH_2 and $(CH_3)_2C$



S



114

correspond to the NH_2 and side chain of the amino acid, respectively. Although there are examples of successful CH_2 for peptide NH replacement in a carboxypeptidase A substrate[37] and an angiotensin converting enzyme inhibitor,[38] we are unaware of any examples of a small ring CH_2 for NH_2 replacement in peptide-like enzyme inhibitors. The substrate specificity of renal dipeptidase has not been studied extensively particularly for N-terminal residues. However, it is known[13] that Ala-Gly, Leu-Gly, and Phe-Gly are hydrolyzed at relative rates of 1, 0.73, and 0.23, respectively, suggesting a steric restriction at the side-chain binding site. This might explain the decreased activity of inhibitors with larger $C(CH_3)_2$ replacements such as **119** and **120**. The decreased hydrolysis rate of dipeptides with a methylated amino[6] group suggests a similar steric restriction at the ring CH_2 and rationalizes the decreased activity of tri- and tetrasubstituted cyclopropanes such as **117**, **118**, **121**, and **122**. It would be of interest to see if replacement of the N-terminal amino acid by the (*S*)-DMCP group in substrates of other Zn-containing aminopeptidases such as leucine aminopeptidase or aminopeptidase B would result in good inhibitors of those enzymes.

As can be seen in Table II, a large number of diverse substituents can be tolerated in R^3 without substantially affecting the potent activity of the DMCP group. This behavior is possibly related to the broad tolerance the enzyme shows for the nature and configuration of the side chain of the analogous C-terminal amino acid of its dipeptide substrate. Although it is clear that the α,β-unsaturated acid moiety and the allylic CH_2 of the R^3 chain are important for the binding of **3**, the extended portions of R^3 are probably not in as close contact with the enzyme. Although portions of R^3 within a few bond lengths of the double bond are apparently in sufficient proximity to a positively charged enzyme group to influence moderately the binding of anionic (stronger) and cationic (weaker) functions, the indifference of the enzyme to chirality (**174** ≡ **175**) and functionality in the extended portions of R^3 support this hypothesis.

It is of interest that while the propionamido inhibitor **30** has low activity, its head-to-head oxidative dimer, the adipamide **31**, is quite effective. The possibility that **31** may combine simultaneously with the active sites in two subunits of renal dipeptidase is considered elsewhere.[39] In contrast, the analogous pair of compounds (**135**, **145**) in which the R^3 groups are joined (in **145**) are nearly equally active, offering still more evidence for the lack of contact of the extended portions of R^3 with the enzyme.

A number of the inhibitors in Tables II and III were evaluated in several species including the chimpanzee to

(32) Umezawa, H.; Muraoka, Y.; Fujii, A.; Naganawa, H.; Takita, T. *J. Antibiot.* **1980**, *33*, 1079.
(33) O'Leary, M. H.; DeGooyer, W. J.; Dougherty, T. M.; Anderson, V. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1320.
(34) Ghisla, S.; Wenz, A.; Thorpe, C. In Enzyme Inhibitors; Broadbeck, U., Ed.; Verlag Chemie: Weinheim, 1980; p 43.
(35) Hansch, C. In *Structure–Activity Relationships*; Cavallito, C. J., Ed.; Pergamon: Oxford, 1973; Vol. 1, p 75.
(36) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(37) Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1979**, *101*, 3946.
(38) Almquist, R. G.; Chao, W.-R.; Ellis, M. E.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392.
(39) Rogers, E. F. *J. Theor. Biol.* **1984**, *109*, 471.

determine their pharmacokinetic parameters as well as their ability to protect imipenem against renal metabolism.[40] In this respect the broad tolerance for substituents in R^3 proved useful since it made available potent inhibitors with a range of pharmacological properties. Two compounds were found to have the desired pharmacokinetic properties for combination with imipenem. First the octenoate **140**[41] was chosen. Although it was found to be effective in protecting imipenem in humans,[42] it caused local irritation when injected repeatedly at high doses in animals. The cysteinyl compound **176**, although slightly less potent than **140**, did not cause irritation upon injection and was found to restore effective urinary levels of imipenem in humans.[43] Because of these desirable properties **176** (USAN name, cilastatin[43]) was selected for combination with imipenem. Preclinical studies on imipenem, cilastatin, and the combination have been summarized and discussed by Kahan et al.[9]

During metabolism studies on cilastatin in which drug concentration was determined by enzyme-inhibitory activity, urinary recoveries greater than 100% were consistently found in the chimpanzee and in humans.[9] The active metabolite was identified[44] as *N*-acetylcilastatin (**177**). Although nearly twice as active as cilastatin, the short half-life (0.5 h) of **177** prevented its use with imipenem.

Besides protecting imipenem from renal dipeptidase, cilastatin has the additional property of being able to protect laboratory animals from the acute proximal tubular necrosis caused by very high doses of imipenem. It has been proposed that this protection against renal toxicity is due to the ability of cilastatin to compete with imipenem for entry into the tubular epithelium.[9] Thus cilastatin offers the possibility of providing an additional margin of safety if this nephrotoxicity is also a problem in humans.

Sepharose-bound cilastatin has been used in the affinity column purification of human[12] and porcine[4] renal dipeptidase.

In conclusion, this paper reports the development of a class of potent in vivo active renal dipeptidase inhibitors based on general structure **3**. In addition to being reversible, competitive inhibitors,[12,45,46] they are highly specific. Thus no significant inhibitory activity was observed at 10 000 times (1 mM) the renal dipeptidase K_i levels with the following enzymes: hog kidney acylase-I, bovine pancreas carboxypeptidase-A and -B, rat lung angiotensin-converting enzyme, β-lactamases from *Bacillus cereus*, *Enterobacter cloacae*, and *Escherichia coli* R10, porcine membrane-bound Zn metalloendopeptidase (enkephalinase), collagenase, gelatinase, and human PMN elastase.[9,45,47,48] This specificity is reassuring with respect to the safety of these inhibitors. No significant toxicity is expected from inhibition of renal dipeptidase itself during the short span of most antibiotic therapy because the role the enzyme plays in renal physiology, scavenging dipeptides in the glomerular filtrate, is secondary and probably not unique.[8] One of the inhibitors, **176** (cilastatin), was selected for combination with imipenem where it improves both the efficacy and safety margin of the antibiotic although it lacks any antibacterial activity itself.[49]

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover apparatus. ^1H NMR spectra were obtained with a Varian T-60, T-60A, or XL200 FT spectrometer in Me_2SO-d_6 unless noted otherwise. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si (DSS in D_2O). The broad peak centered at δ 1.5 for $(CH_2)_n$ ($n > 2$) is omitted. Mass spectra were determined on a Varian MAT 731 instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Analytical TLC was performed on Analtech silica gel GF plates (250 μm). Spots were visualized with UV light or I_2. Preparative TLC was carried out on Analtech Uniplates silica gel GF (20 × 20 cm, 1000 μm). Preparative HPLC purification was performed on a Waters Prep LC 500 apparatus. Elemental analyses were performed by J. Gilbert and his associates, Merck Sharp and Dohme Research Laboratories.

Starting Materials. The amides used to prepare the compounds in Tables I and III were commercially available or were prepared from the known acids, acid chlorides, or nitriles by standard procedures, except for three amides (for **80**, **88**, and **128**), whose synthesis is described in the Supplementary Material. The ω-bromo α-keto acids (**10**) used to make the ω-bromoalkyl compounds **12** (**159**, **166**, **167**, **188**, **189**, **218**, **220**, and **233**) were prepared by using the α-keto ester synthesis of Eliel and Hartmann[17] followed by HBr–HOAc hydrolysis (Scheme II). For example, 7-bromo-2-oxoheptanoic acid (for compounds **166** and **167**) was prepared: To a well-stirred suspension of NaH (60% oil dispersion, 24.0 g, 0.6 mol) in toluene (720 mL) was added over 1 h a solution of 1,5-dibromopentane (276 g, 1.2 mol) and ethyl 1,3-dithiane-2-carboxylate[17] (**6**; 115 g, 0.6 mol) in DMF (240 mL) while the temperature was maintained at 25–30 °C with a cooling bath. The suspension was stirred at room temperature for 16 h, then transferred to a separating funnel with ether (1 L), washed with H_2O (4 × 500 mL), dried ($MgSO_4$), and evaporated in vacuo to give 354 g of a yellow oil containing the alkylated dithiane **7** [$R^3 = (CH_2)_4Br$], 1,5-dibromopentane, and mineral oil. The crude material was dissolved in CH_3CN (100 mL) and added over 70 min to a well-stirred suspension of *N*-bromosuccinimide (856 g, 4.8 mol) in CH_3CN (1.6 L) and H_2O (400 mL) while the temperature was kept below 20 °C with a cooling bath. After being stirred at 15 °C for 15 min, the red solution was poured into ice-cold CH_2Cl_2–hexane (1:1, 4 L) and extracted with cold saturated $NaHSO_3$ (2 × 1 L) and cold H_2O (1 × 1 L). The nearly colorless solution was cautiously (vigorous CO_2 evolution) washed with cold 20% Na_2CO_3 (2 × 1 L,) and H_2O (1 L) and dried ($MgSO_4$). Evaporation in vacuo gave 279 g of crude bromo keto ester **8** [$R^3 = (CH_2)_4Br$] containing 1,5-dibromopentane and mineral oil. The crude bromo keto ester was heated at 90 °C

(40) Kropp, H.; Sundelof, J. G.; Bohn, D. L.; Kahan, F. M. *Improved Urinary Tract Bioavailability of MK-0787 (N-Formimidoyl-Thienamycin) When Coadministered with Inhibitors of Renal Dehydropeptidase-I*, presented at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, Sept 1980, Abstract 270 and unpublished work.

(41) The Na salt of **140** has been designated MK-0789.

(42) Norrby, S. R.; Alestig, K.; Bjornegard, B.; Burman, L. A.; Ferber, F.; Huber, J. L.; Jones, K. H.; Kahan, F. M.; Kahan, J. S.; Kropp, H.; Meisinger, M. A. P.; Sundelof, J. G. *Antimicrob. Agents Chemother.* **1983**, *23*, 300.

(43) The mono Na salt of **176** has been designated MK-0791.

(44) Liesch, J. M., MSDRL, unpublished work.

(45) Kropp, H.; Sundelof, J. G.; Hajdu, R.; Kahan, F. M. *Metabolism of Thienamycin and Related Carbapenem Antibiotics by the Renal Dipeptidase: Dehydropeptidase-I*, presented at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, Sept 1980, Abstract 272.

(46) Kim, H. S.; Campbell, B. J. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1638.

(47) We express our appreciation to H. Kropp, J. G. Sundelof, and R. A. Mumford and Drs. M. Zimmerman, T. Y. Lin, and E. H. Ulm for these enzyme-inhibition results.

(48) Recently cilastatin was found (Köller, M.; Brom, J.; Raulf, M.; König, W. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 974) to inhibit (IC_{50} = 0.24 μM) a renal "leukotriene D_4-dipeptidase". Whether this enzyme is renal dipeptidase or not was not determined. Cilastatin was much less active against the leukotriene D_4-dipeptidase activity in other tissues such as liver, lung, serum, and polymorphonuclear granulocytes.

(49) A preliminary account of part of this work was presented at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, Sept 1980; Abstract 271.

(internal temperature) for 75 min with concentrated HBr (47–49%, 290 mL) and HOAc (580 mL). The dark mixture was evaporated in vacuo (bath ~50 °C) until nearly all of the HOAc was removed. The residue was dissolved in ether (1 L), washed with H_2O (3 × 200 mL), and extracted with saturated $NaHCO_3$ (4 × 200 mL). The combined $NaHCO_3$ extracts were washed with ether (3 × 150 mL) and acidified with concentrated HCl. The precipitated oil was extracted with ether (3 × 200 mL). The ether extracts were washed with H_2O (1 × 150 mL) and saturated NaCl solution (1 × 150 mL) and dried ($MgSO_4$). Evaporation of the solvent in vacuo gave 93.9 g (70%) of 7-bromo-2-oxoheptanoic acid (**10**, *n* = 4) as a brown oil: NMR ($CDCl_3$) δ 2.97 (t, *J* = 7 Hz, 2 H, CH_2CO), 3.42 (t, *J* = 7 Hz, 2 H, CH_2Br), 9.68 (br s, 1 H, CO_2H). 2,11-Dioxododecanedioic acid (for **145**), 5,5-dimethyl-2-oxohexanoic acid (for **143**), 2-oxo-6-heptenoic acid (for **150**), and 2-oxo-3-cyclopropanepropionic acid (for **151**) were prepared by the above procedure except that the molar ratio of **6** to bromide was 1:1 (2:1 for the diacid). Also for the last two acids, the keto ester was hydrolyzed with base (5% KOH, room temperature, 2 h). 5-(Methylthio)-2-oxopentanoic acid (for **156**) was prepared from methyl 4-(methylthio)butyrate.[52] 5-Methoxy-2-oxopentanoic acid (for **155**), 6-methoxy-2-oxohexanoic acid (for **160**), and 7-methoxy-2-oxoheptanoic acid (for **168**) were prepared from the corresponding ω-methoxy norester by the procedure of Schreiber[18] (H_2SO_4 hydrolysis).

Method A. (*Z*)-2-(3-Methylbutyramido)-2-butenoic Acid (**44**). A solution of 1.07 g (10.5 mmol) of 2-ketobutyric acid and 0.71 g (7 mmol) of isovaleramide in 15 mL of toluene was stirred under reflux for 5 h with collection of H_2O in a small Dean–Stark trap. The solid that crystallized on cooling was collected on a filter and washed with toluene followed by CH_2Cl_2. Recrystallization from diisopropyl ketone gave 0.32 g (25%) of **44** as colorless crystals: mp 175 °C (slight preliminary softening); TLC in 4:1 toluene–HOAc; NMR δ 0.92 (d, *J* = 6 Hz, 6 H, $CH(CH_3)_2$, 1.65 (d, *J* = 7 Hz, 3 H, CH_3CH=), 2.1 (m, 2 H, $COCH_2CH$), 6.52 (q, *J* = 7 Hz, 1 H, CH_3CH=), 8.92 (br s, 1 H, NH). Anal. ($C_9H_{15}NO_3$) C, H, N.

The reaction time for other amides varied from 2 to 20 h. Shorter reaction times increased the risk of contamination with the *E* isomer. For unreactive amides, addition of 5 mol % *p*-toluenesulfonic acid reduced the reaction time to 1–2 h. Longer reaction times led to reduced yields due to formation of azlactone **5** and other side products. Other solvents such as trichloroethylene and methyl isovalerate proved useful in a few cases. If the product did not precipitate from the cooled reaction mixture, evaporation of the solvent and recrystallization or base extraction (method A1) were successful in many cases. Column or thin-layer chromatography with silica gel using toluene–HOAc (4:1) or toluene-EtOAc–HOAc (75:25:2) could be used for isolation and purification of the products. Most of these amide-α-keto acid condensations were carried out only once, and the product yields were not optimized.

Method A1. (+)-(*Z*)-7-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic Acid (**167**). A mixture of 7.5 g (0.066 mol) of **28**, 22.3 g (0.1 mol) of **10** (*n* = 4), and 225 mL of toluene was heated under reflux for 9 h with removal of H_2O by a modified Dean–Stark trap containing molecular sieves (4A). The cooled mixture was diluted with toluene (100 mL) and extracted with 10% aqueous K_2CO_3 (4 × 100 mL). The combined extracts were washed with ether (2 × 100 mL) and acidified to pH 3.5 with concentrated HCl. The oily precipitate was extracted with ether (3 × 150 mL). The combined extracts were washed with H_2O (2 × 100 mL) and saturated brine and dried ($MgSO_4$). Evaporation of the ether under reduced pressure gave 16.2 g of an oil that was crystallized from 50 mL of CH_3NO_2 at 0 °C to give 5.8 g (28%) of **167**: mp 86–88 °C; NMR δ 0.5–1.0 (m, 2 H, cyclopropyl CH_2), 1.09, 1.12 (s, 6 H, cyclopropyl CH_3), 1.8–2.3 (m, 2 H, CH_2CH=), 3.51 (t, *J* = 6 Hz, 2 H, CH_2Br), 6.37 (t, *J* = 7 Hz, 1 H, CH_2CH=), 9.10 (br s, 1 H, NH); $[\alpha]^{25}_D$ +72.8° (*c* 0.5, $CHCl_3$). Anal. ($C_{13}H_{20}BrNO_3$) C, H, N, Br. Additional product could be recovered from the mother liquors.

Compound **189** was prepared by this procedure. The racemates **159**, **166**, **188**, **218**, and **220** crystallized soon after acidification and were filtered, washed with H_2O, and recrystallized.

Method B. (*Z*)-2-(4-Methylbenzamido)-2-butenoic Acid (**60**). A mixture of 0.68 g (5 mmol) of *p*-toluamide, 1.53 g (15 mmol) of 2-ketobutyric acid, 200 mg of *p*-toluenesulfonic acid, and 20 mL of toluene was stirred under reflux for 6 h with removal of H_2O by a modified Dean–Stark trap containing molecular sieves (4A). The cooled reaction mixture was poured onto a column of silica gel (40 g) and eluted with hexane (200 mL). Elution with 10% EtOAc in hexane gave 102 mg (10%) of **5** (R = 4-$CH_3C_6H_5$) as a colorless glass: R_f 0.8 (10% EtOAc in hexane); NMR ($CDCl_3$) δ 2.24 (d, *J* = 7 Hz, 3 H, CH_3CH=), 2.43 (s, 3 H, Ar CH_3), 6.72 (q, *J* = 7 Hz, 1 H, CH_3CH=), 7.32 (d, *J* = 8 Hz, 2 H, 3′,5′-Ar H), 8.00 (d, *J* = 8 Hz, 2 H, 2′,6′-Ar H).

A 92-mg sample of **5** (R = 4-$CH_3C_6H_4$) was heated at 50 °C for 1 h with 6 mL of acetone and 2 mL of 0.5 M HCl. The precipitate resulting from evaporation in vacuo was filtered, washed with H_2O, and dried to give 82 mg of **60** as colorless crystals: mp 199–203 °C; NMR δ 1.72 (d, *J* = 7 Hz, 3 H, CH_3CH=), 2.38 (s, 3 H, Ar CH_3), 6.68 (q, *J* = 7 Hz, 1 H, CH_3CH=), 7.28 (d, *J* = 8 Hz, 2 H, 3′,5′-Ar H), 7.85 (d, *J* = 8 Hz, 2 H, 2′,6′-Ar H), 9.32 (br s, 1 H, NH). Anal. ($C_{12}H_{13}NO_3$) C, H, N.

Method C. (*Z*)-2-*trans*-Cinnamamido-2-butenoic Acid (**72**). A mixture of 0.23 g (1.0 mmol) of isomerically impure 2-*trans*-cinnamamido-2-butenoic acid (*Z*–*E* ratio approximately 2:1 by NMR and TLC) prepared by method A, 2.5 mg of iodine, and 10 mL of chlorobenzene was stirred at reflux under N_2 for 16.5 h. The solid that separated on cooling was isolated to yield 0.11 g (48%) of **72** as light tan crystals: mp 198.5–201 °C partial dec; TLC in 4:1 toluene–HOAc; NMR δ 1.71 (d, *J* = 7 Hz, 3 H, CH_3CH=), 6.58 (q, *J* = 7 Hz, 1 H, CH_3CH=), 6.77 (d, *J* = 16 Hz, 1 H, $COCH$=CH), 7.3–7.8 (m, 6 H, Ar H, Ar CH=), 9.29 (br s, 1 H, NH). Anal. ($C_{13}H_{13}NO_3$) C, H, N.

Method D. (*Z*)-2-[[2-[(3-Methyl-1-oxobutyl)amino]-1-oxoethyl]amino]-2-butenoic Acid (**77**). Isovaleryl chloride (1.20 g, 10 mmol) was added dropwise to a solution of **75** (1.46 g, 10 mmol) and NaOH (1.6 g, 40 mmol) in H_2O (4 mL) at 0 °C. After being stirred for 1 h in the cold, the mixture was acidified with concentrated HCl and diluted with H_2O (5 mL). The solid was filtered, dried, and recrystallized (EtOAc) to give 600 mg (25%) of **77**: mp 144 °C; NMR δ 0.89 (d, *J* = 6 Hz, 6 H, $CH(CH_3)_2$), 1.66 (d, *J* = 7 Hz, 3 H, CH_3CH=), 2.06 (br s, 2 H, CH_2CH), 3.83 (d, *J* = 6 Hz, 2 H, CH_2NH), 6.56 (q, *J* = 7 Hz, 1 H, CH_3CH=), 8.08 (br t, *J* = 6 Hz, 1 H, CH_2NH), 8.97 (br s, 1 H, NH). Anal. ($C_{11}H_{18}N_2O_4$) C, H, N.

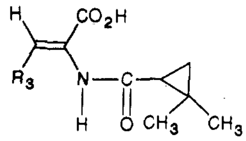
Method E. (*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(methylthio)-2-octenoic Acid (**213**). A solution of 332 mg (1 mmol) of **174** and $NaSCH_3$ [prepared from 162 mg (3 mmol) of $NaOCH_3$ and CH_3SH] in 5 mL of CH_3OH was heated under reflux for 30 min in a N_2 atmosphere. Most of the CH_3OH was removed in vacuo. The residue was partitioned between dilute HCl and ether. The ether was washed with H_2O and brine and dried ($MgSO_4$). The residue after evaporation of the ether was recrystallized from ether–hexane to give 178 mg (60%) of **213**: mp 82–84 °C; NMR δ 0.7–1.1 (m, 2 H, cyclopropyl CH_2), 1.07, 1.10 (s, 6 H, cyclopropyl CH_3), 2.00 (s, 3 H, CH_3S), 6.40 (t, *J* = 7 Hz, 1 H, CH_2CH=), 9.10 (br s, 1 H, NH). Anal. ($C_{15}H_{25}NO_3S$) C, H, N, S.

Compounds **161**, **214**, **215**, and **217** were prepared from the appropriate mercaptan by this procedure except that the **217** reaction was carried out at room temperature for 24 h. Compounds **173** and **179** were prepared with 1 N NaOH (3 equiv) for 3 h at room temperature. Compounds **163**, **180**, **181**, **182**, and **183** were prepared with 1 M Na_2CO_3 (2 mol) at room temperature for 20 h.

Method F. (*Z*)-7-[(2*R*)-(2-Amino-2-carboxyethyl)thio]-2-[(1*S*)-2,2-dimethylcyclopropanecarboxamido]-2-heptenoic Acid (**176**). To a magnetically stirred suspension of 1.20 g (5 mmol) of L-cystine in 50 mL of liquid NH_3 in a N_2 atmosphere was added 0.56 g (24 mg-atoms) of Na in small pieces. After the blue color faded, a solution of 3.18 g (10 mmol) of **167** in 15 mL of CH_3OH was added rapidly. Another 25 mL of CH_3OH was added to dissolve the gummy precipitate. The solution was

(50) Burke, D. E.; Cook, J. M.; LeQuesne, P. W. *J. Am. Chem. Soc.* **1973**, *95*, 546.
(51) Kamel, M.; Kimpenhaus, W.; Buddrus, J. *Chem. Ber.* **1976**, *109*, 2351.
(52) Yamada, S.; Sakurai, S. *J. Biochem. (Tokyo)* **1957**, *44*, 557.

Table II. (*Z*)-3-Substituted-2-(2,2-dimethylcyclopropanecarboxamido)propenoic acids



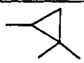
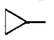
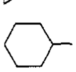
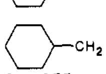
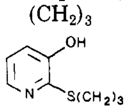
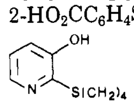
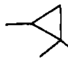
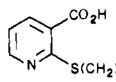
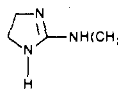
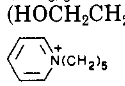
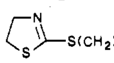
compd	R_3	 config	method[a]	mp, °C	recrystn solvent[b]	yield, %	formula	anal.	K_i, μM
133	H	*RS*	A	122–123	I	5	$C_9H_{13}NO_3$	C, H, N	52
134	CH_3CH_2	*RS*	A	154.5–155.5	II	30	$C_{11}H_{17}NO_3$	C, H, N	0.18
135	$CH_3(CH_2)_2$	*RS*	A	122–123	III	10	$C_{12}H_{19}NO_3$	C, H, N	0.11
136	$(CH_3)_2CH$	*RS*	A	164–166	I	27	$C_{13}H_{21}NO_3$	C, H, N	0.54
137	$CH_3(CH_2)_3$	*RS*	A	94–95	IV	24	$C_{13}H_{21}NO_3$	C, H, N	0.11
138	$(CH_3)_2CHCH_2$	*RS*	A	145–147	XXIV	35	$C_{13}H_{21}NO_3$	C, H, N	0.23
139	$CH_3(CH_2)_4$	*RS*	A	104–105	I	10	$C_{14}H_{23}NO_3$	C, H, N	0.17
140	$CH_3(CH_2)_4$	*S*[c]		95–96.5			$C_{14}H_{23}NO_3$	C, H, N	0.08
141	$CH_3(CH_2)_4$	*R*[d]		94.5–96.5					8.8
142	$(CH_3)_2CH(CH_2)_2$	*RS*	A	115–116	V	20	$C_{14}H_{23}NO_3$	C, H, N	0.15
143	$(CH_3)_3CCH_2$	*RS*	A	138.5–140	IV	28	$C_{14}H_{23}NO_3$	C, H, N	0.34
144	$CH_3(CH_2)_5$	*RS*	A	111–113	I	11	$C_{15}H_{25}NO_3$	H, N; C[e]	0.16
145	$-(CH_2)_6-$	*RS*	A	188–190	VI	9	$C_{24}H_{36}N_2O_6\cdot 0.5H_2O$	C, H; N[f]	0.092
146	$CH_3(CH_2)_6$	*RS*	A	108–110	I	13	$C_{16}H_{27}NO_3$	C, H, N	0.096
147	$CH_3(CH_2)_7$	*RS*	A	95–96	VII	48	$C_{17}H_{29}NO_3$	C, H, N	0.11
148	$CH_3(CH_2)_8$	*RS*	A	91–92	IV	52	$C_{18}H_{31}NO_3$	C, H, N	0.11
149	$CH_3(CH_2)_9$	*RS*	A	98–99	VII	25	$C_{19}H_{33}NO_3$	C, H, N	0.14
150	$CH_2{=}CH(CH_2)_2$	*RS*	A	88–90	II	39	$C_{13}H_{19}NO_3$	C, H, N	0.23
151		*RS*	A	158–159	II	42	$C_{12}H_{17}NO_3$	C, H, N	0.44
152		*RS*	A	149–150.5	II	40	$C_{15}H_{23}NO_3$	C, H, N	0.40
153		*RS*	A	146–148	II	31	$C_{16}H_{25}NO_3$	C, H, N	0.15
154	CF_3CH_2	*S*		139–142			$C_{11}H_{14}F_3NO_3$	C, H, N	0.24
155	$CH_3O(CH_2)_2$	*RS*	A	*g*	I	19	$C_{12}H_{19}NO_4$	C, H, N	0.32
156	$CH_3S(CH_2)_2$	*RS*	A	65–67	VIII	5	$C_{12}H_{19}NO_3S$	C, H, N	0.12
157	$CH_3SO_2(CH_2)_2$	*RS*		148–149			$C_{12}H_{19}NO_5S$	C, H, N, S	0.50
158	$HO_2C(CH_2)_2$	*RS*	A[h]	163–165	VI	8	$C_{12}H_{17}NO_5$	C, H, N	0.14
159	$Br(CH_2)_3$	*RS*	A1	118–120	I	5	$C_{12}H_{18}BrNO_3$	C, H, N	0.38
160	$CH_3O(CH_2)_3$	*RS*	A	78–80	VIII	33	$C_{13}H_{21}NO_4$	C, H, N	0.28
161	$CH_3O_2CCH_2S(CH_2)_3$	*RS*	E	133–135	XXIII	71	$C_{15}H_{23}NO_5S$	C, H, N	0.28
162	L-$HO_2CCH(NH_2)CH_2S$-$(CH_2)_3$	*RS*	F	120–132 dec	IX	68	$C_{15}H_{24}N_2O_5S\cdot 0.5H_2O$	C, H, N, S	0.27
163		*RS*	E	*g*		40	$C_{17}H_{22}N_2O_4S\cdot 0.25H_2O$	C, H, N	0.13
164	$Na^{+-}HO_3PCH_2S(CH_2)_3$	*RS*		*g*			$C_{13}H_{21}NNaO_6PS\cdot 1^1/_3H_2O$	C, H, N	0.22
165	$HO_2C(CH_2)_3$	*RS*	A[h]	114–115	X	18	$C_{13}H_{19}NO_5$	C, H, N	0.048
166	$Br(CH_2)_4$	*RS*	A1	124–125	I	36	$C_{13}H_{20}BrNO_3$	C, H, N	0.15
167	$Br(CH_2)_4$	*S*[i]	A1	86–88	I	28	$C_{13}H_{20}BrNO_3$	C, H, N, Br	
168	$CH_3O(CH_2)_4$	*RS*	A	102–104	III	35	$C_{14}H_{23}NO_4$	C, H, N	0.16
169	$NC(CH_2)_4$	*RS*	G	150	XI	46	$C_{14}H_{20}N_2O_3$	C, H, N	0.21
170	$(CH_3)_2N(CH_2)_4$	*RS*	H	158–161	XVII	71	$C_{15}H_{26}N_2O_3\cdot 0.25H_2O$	C, H, N	3.45
171	$Na^{+-}O_3S(CH_2)_4$	*RS*		*g*			$C_{13}H_{20}NNaO_6S\cdot 0.75H_2O$	C, H, N	0.087
172	$HO_2CCH_2N(CH_3)$-$(CH_2)_4$	*RS*	I	*g*	*j*	12	$C_{16}H_{26}N_2O_5\cdot 0.5AcOH\cdot 0.40H_2O$	C, H, N	0.56
173	$HO_2CCH_2S(CH_2)_4$	*RS*	E	119–121	I	91	$C_{15}H_{23}NO_5S$	C, H, N, S	0.13
174	L-$HO_2CCH(NH_2)CH_2S$-$(CH_2)_4$	*RS*	F	*g*	*j*	71	$C_{16}H_{26}N_2O_5S\cdot 0.25H_2O$	C, H, N, S	0.21
175	D-$HO_2CCH(NH_2)CH_2S$-$(CH_2)_4$	*RS*	F	*g*	*j*	75	$C_{16}H_{26}N_2O_5S\cdot 0.25H_2O$	C, H, N, S	0.19
176	L-$HO_2CCH(NH_2)CH_2S$-$(CH_2)_4$	*S*[k]	F	*g*	*j*	63	$C_{16}H_{26}N_2O_5S$	C, H, N, S	0.11
177	L-$Na^{+-}O_2CCH(NHCOCH_3)$-$CH_2S(CH_2)_4$	*S*[l]	J	*g*	IX	68	$C_{18}H_{27}N_2NaO_6S\cdot H_2O$	C, H, N, S	0.06
178	L-$HO_2CCH(NHCH_3)$-$CH_2S(CH_2)_4$	*S*[m]	F	144–148	IX	54	$C_{17}H_{28}N_2O_5S\cdot 0.5H_2O$	C, H, N, S	0.15
179	$HO_2CCOCH_2S(CH_2)_4$	*RS*	E	*n*		78	$C_{16}H_{23}NO_6S\cdot ^2/_3H_2O$	C, H, N	0.16
180	$C_6H_5S(CH_2)_4$	*RS*	E	100–104	I		$C_{19}H_{25}NO_3S\cdot 0.75H_2O$	C, H, N	0.10
181	2-$HO_2CC_6H_4S(CH_2)_4$	*RS*	E	163–167	I	21	$C_{20}H_{25}NO_5S\cdot 0.75H_2O$	C, H, N	0.09
182		*S*[o]	E	188.5–189	XII	65	$C_{18}H_{24}N_2O_4S$	C, H, N, S	0.02

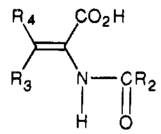
Table II (Continued)

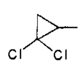
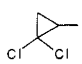
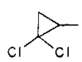
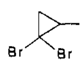
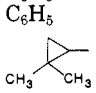
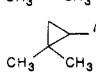
compd	R_3	 config	method[a]	mp, °C	recrystn solvent[b]	yield, %	formula	anal.	K_i, μM
183		*S*	E	*g*		50	$C_{19}H_{24}N_2O_5S\cdot0.25H_2O$	C, H, N	0.04
184	$HO_2C(CH_2)_4$	*RS*	A[h]	165–167	VI	28	$C_{14}H_{21}NO_5$	C, H, N	0.058
185	$HO(CH_2)_5$	*RS*		137–139			$C_{14}H_{23}NO_4$	C, H, N	0.23
186	$CH_3CO_2(CH_2)_5$	*RS*		59–62			$C_{16}H_{25}NO_5$	C, H, N	0.22
187	$CH_3O(CH_2)_5$	*RS*		71–72			$C_{15}H_{25}NO_4$	C, H, N	0.18
188	$Br(CH_2)_5$	*RS*	A1	149–151	XIV	55	$C_{14}H_{22}BrNO_3$	C, H, N, Br	0.27
189	$Br(CH_2)_5$	*S*[p]	A1	100.5–101.5	I	44	$C_{14}H_{22}BrNO_3$	C, H, N, Br	
190	$NC(CH_2)_5$	*RS*	G	113–115	XVI	38	$C_{15}H_{22}N_2O_3\cdot0.25H_2O$	C, H, N	0.082
191	$H_2N(CH_2)_5$	*RS*	H	*g*	XVII	78	$C_{14}H_{24}N_2O_3\cdot H_2O$	C, H, N	1.00
192	$(CH_3)_2N(CH_2)_5$	*RS*	H	101–112	XIX	71	$C_{16}H_{28}N_2O_3\cdot H_2O$	C, H, N	1.28
193	$(C_2H_5)_2N(CH_2)_5$	*RS*	H	78–81	XV	77	$C_{18}H_{32}N_2O_3$	C, H, N	0.86
194		*RS*		126–129			$C_{17}H_{28}N_4O_3$	C, H, N	0.84
195	$HO_2CCH_2N(CH_3)$-$(CH_2)_5$	*RS*	I	*g*	XVII	27	$C_{17}H_{28}N_2O_5\cdot2H_2O$	C, H, N	0.29
196	$(HO)_2P(O)CH_2NH$-$(CH_2)_5$	*RS*	K	125–130	XVI	33	$C_{15}H_{27}N_2O_6P\cdot H_2O$	C, H, N, P	0.40
197	$(HO)_2P(O)(CH_2)_2NH(CH_2)_5$	*RS*	K	*g*	XVI	96	$C_{16}H_{29}N_2O_6P\cdot0.5H_2O$	C, H, N, P	0.58
198	D,L-$(HO)_2P(O)CH$-$(CH_3)NH(CH_2)_5$	*RS*	K	136–139	XVI	29	$C_{16}H_{29}N_2O_6P$	C, H, N, P	0.28
199	D,L-$(HO)_2P(O)CH$-$(CH_3)NH(CH_2)_5$	*S*[q]	K	143–145	XVI	54	$C_{16}H_{29}N_2O_6P$	C, H, N	0.16
200	$(HO)_2P(O)C(CH_3)_2$-$NH(CH_2)_5$	*S*[r]	K	162–165	XVI	49	$C_{17}H_{31}N_2O_6P$	C, H, N, P	0.18
201	$CH_2{=}CHCH_2NH$-$(CH_2)_5$	*RS*	H	163–165	XVII	92	$C_{17}H_{28}N_2O_3\cdot C_2H_5OH$	H, N; C[s]	0.87
202	$HC{\equiv}CCH_2NH(CH_2)_5$	*RS*	H	164–166 dec	XVIII	66	$C_{17}H_{26}N_2O_3$	C, H, N	0.74
203	$(CH_3)_3N^+(CH_2)_5$	*RS*	L	220–222 dec	XIX	77	$C_{17}H_{30}N_2O_3$	C, H, N	1.10
204	$(CH_3)_3N^+(CH_2)_5$	*S*[t]	L	225–227 dec	XIX	71	$C_{17}H_{30}N_2O_3$	C, H, N	0.65
205	$(HOCH_2CH_2)(CH_3)_2N^+(CH_2)_5$	*RS*	L	185–188	XX	55	$C_{18}H_{32}N_2O_4\cdot1.25H_2O$	C, H, N	0.91
206		*RS*	L	*g*	*j*	4	$C_{19}H_{26}N_2O_3\cdot2H_2O$	H, N; C[u]	1.09
207	$CH_3CONH(CH_2)_5$	*RS*	J	*g*	XXI	79	$C_{16}H_{26}N_2O_4\cdot0.20CH_3COCH_3$	C, H, N	0.23
208	$H_2NCONH(CH_2)_5$	*RS*		*g*			$C_{15}H_{25}N_3O_4\cdot{}^2/_3H_2O$	C, H, N	0.30
209	$H_2NCH{=}N(CH_2)_5$	*RS*	M	160–162 dec	XVII	77	$C_{15}H_{25}N_3O_3\cdot{}^1/_3H_2O$	C, H, N	0.78
210	$H_2NC(CH_3){=}N(CH_2)_5$	*RS*	M	143–145	XX	60	$C_{16}H_{27}N_3O_3\cdot0.75H_2O$	C, H, N	0.72
211	$CH_3CH_2OCS_2(CH_2)_5$	*RS*		88–91			$C_{17}H_{27}NO_4S_2$	C, H, N, S	0.044
212	$HS(CH_2)_5$	*RS*		128–130			$C_{14}H_{23}NO_3S$	C, H, N, S	0.17
213	$CH_3S(CH_2)_5$	*RS*	E	82–84	XI	60	$C_{15}H_{25}NO_3S$	C, H, N, S	0.15
214	$CH_3O_2CCH_2S(CH_2)_5$	*RS*	E	*g*	XIII	60	$C_{17}H_{27}NO_5S$	C, H, N, S	0.20
215	$H_2NCOCH_2S(CH_2)_5$	*RS*	E	125–126	VI	55	$C_{16}H_{26}N_2O_4S$	H, N, S; C[v]	0.25
216	L-$HO_2CCH(NH_2)CH_2S(CH_2)_5$	*RS*	F	174–177 dec	*j*	54	$C_{17}H_{28}N_2O_5S\cdot{}^1/_3H_2O$	C, H, N, S	0.23
217		*RS*	E	86–90	I	4	$C_{17}H_{26}N_2O_3S_2\cdot0.036CH_3NO_2$	C, H, N, S	0.15
218	$Br(CH_2)_6$	*RS*	A1	129–131	I	57	$C_{15}H_{24}BrNO_3$	C, H, N, Br	0.16
219	$HO_2CCH_2N(CH_3)$-$(CH_2)_6$	*RS*	I	*g*	XVII	51	$C_{18}H_{30}N_2O_5\cdot0.5H_2O$	C, H, N	0.40
220	$Br(CH_2)_7$	*RS*	A1	96–97	IV	71	$C_{16}H_{26}BrNO_3$	C, H, N, Br	0.11
221	$H_2N(CH_2)_7$	*RS*	H	110–120	XXII	58	$C_{16}H_{28}N_2O_3$	C, H, N	0.81
222	$(CH_3)_2N(CH_2)_7$	*RS*	H	168–172	IV	43	$C_{18}H_{32}N_2O_3\cdot0.5H_2O$	C, H, N	0.52
223	$(CH_3)_3N^+(CH_2)_7$	*RS*	L	*g*	XV	29	$C_{19}H_{34}N_2O_3\cdot H_2O$	C, H, N	0.57
224	$(C_6H_5CH_2)(CH_3)_2N^+(CH_2)_7$	*RS*	L	190–193 dec	XVII	83	$C_{25}H_{38}N_2O_3\cdot0.5H_2O$	H, N; C[w]	0.45
225	C_6H_5	*RS*	A	167–168	VI	17	$C_{15}H_{17}NO_3$	C, H, N	0.62
226	$C_6H_5(CH_2)_2$	*RS*	A	131–132	I	33	$C_{17}H_{21}NO_3$	C, H, N	0.33

[a] See Experimental Section. Procedures for compounds without method designation are given separately. [b] I = CH_3NO_2, II = toluene, III = toluene–diisopropyl ether, IV = ether–petroleum ether, V = toluene–cyclohexane, VI = EtOAc, VII = hexane, VIII = ether–cyclohexane, IX = MeOH–ether, X = EtOAc–hexane, XI = ether–hexane, XII = CH_3NO_2–acetic acid, XIII = MeOH–chloroform, XIV = CH_3CN, XV = EtOH–ether–acetone, XVI = H_2O, XVII = EtOH–H_2O, XVIII = tetrahydrofuran–hexane, XIX = EtOH–acetone, XX = MeOH–H_2O, XXI = acetone–H_2O, XXII = acetone–ether, XXIII = petroleum ether, XXIV = toluene–diisopropyl ketone. [c] $[\alpha]^{20}_D$ +77.7° (*c* 0.51, $CHCl_3$). [d] $[\alpha]^{20}_D$ −78.0° (*c* 0.48, $CHCl_3$). [e] C: calcd, 67.38; found, 66.88. [f] N: calcd, 6.12; found, 5.71. [g] Amorphous solid. Indistinct melting point. [h] Methyl isovalerate used as reaction solvent. [i] $[\alpha]^{25}_D$ +72.8° (*c* 0.5, $CHCl_3$). [j] Purified by ion-exchange chromatography (see Experimental Section). [k] $[\alpha]^{25}_D$ +17.6° (*c* 0.5, MeOH), +14.2° (*c* 0.5, 0.1 N HCl). [l] $[\alpha]^{25}_D$ −9.8° (*c* 0.5, 0.1 N NaOH). [m] $[\alpha]^{25}_D$ +35.0° (*c* 0.5, 0.1 N HCl). [n] Oil. A mixture of enolic isomers. [o] $[\alpha]^{27}_D$ +34.3° (*c* 0.5, CH_3OH). [p] $[\alpha]^{25}_D$ +66.2° (*c* 0.5, $CHCl_3$). [q] $[\alpha]^{25}_D$ +12.5° (*c* 0.6, H_2O). [r] $[\alpha]^{25}_D$ +30.5° (*c* 0.24, H_2O). [s] C: calcd, 64.37; found, 64.96. [t] $[\alpha]^{25}_D$ +58.0° (*c* 0.5, CH_3OH). [u] C: calcd, 61.26; found, 61.73. [v] C: calcd, 56.11; found, 55.61. [w] C: calcd, 70.89; found, 71.34.

allowed to warm to room temperature over 1 h and then warmed to 45 °C for 15 min. The residue from evaporation in vacuo was dissolved in 30 mL of H_2O and applied to a 4.8 × 12 cm column of Dowex 50W-X8 (100–200 mesh, H^+), which was eluted with 500 mL of H_2O (until eluate no longer acidic) and 500 mL of 4% NH_4OH. The residue from evaporation of the NH_4OH was dissolved in 40 mL of H_2O and applied to a bed of 40 mL of AG1-X8 (200–400 mesh, OAc^-) in a 60-mL sintered glass funnel.

Table III. 3-Substituted-2-(acylamino)propenoic acids



compd	R_2	R_3	R_4	method[a]	mp, °C	recryst solvent[b]	formula	anal.	K_i, μM or (% inhibn at 100 μM)
227	$HOCH_2$	C_6H_5	H		172–173		$C_{11}H_{11}NO_4$	C, H, N	(4)
228	$(CH_3)_2CHCH_2$	CH_3CH_2	H	A	128.5–129.5	*c*	$C_{10}H_{17}NO_3$	C, H, N	3.2
229	$(CH_3)_2CHCH_2$	$CH_3(CH_2)_4$	H	A	126–128	III	$C_{13}H_{23}NO_3$	C, H, N	2.6
230	$(CH_3)_2CHCH_2$	$HO_2C(CH_2)_2$	H	A[d]	127–128	I	$C_{11}H_{17}NO_5$	C, H, N	2.7
231		$HO_2C(CH_2)_2$	H	A[d]	119–120	II	$C_{10}H_{13}NO_5$	C, H, N	5.0
232		$CH_3(CH_2)_4$	H	A[e]	135–136.5	III	$C_{12}H_{17}Cl_2NO_3$	C, H, N, Cl	0.06
233		$Br(CH_2)_5$	H	A1[e]	159–160.5	III	$C_{12}H_{16}BrCl_2NO_3$	C, H, N, Cl; Br[f]	
234		$(CH_3)_3N^+(CH_2)_5$	H	L	183–185 dec	IV	$C_{15}H_{24}Cl_2N_2O_3\cdot C_2H_5OH$	C, H, N	0.18
235		$CH_3(CH_2)_2$	H	A	178–179	III	$C_{10}H_{13}Br_2NO_3$	C, H, N, Br	0.015
236	C_6H_5	H	H		125–127[g]				(9)
237	C_6H_5	Cl	H		*h*				(36)
238	C_6H_5	CH_3O	H		216–217 dec[i]				(23)
239	C_6H_5	CH_3	CH_3		228[j]			C, H, N	(4)
240		CH_3	CH_3	A	168–169	III	$C_{11}H_{17}NO_3$	H, N; C[k]	(57)
241	 [l]	H	$CH_3(CH_2)_4$		86–87.5		$C_{14}H_{23}NO_3$	C, H, N	11.2[m]
242	L-CH(CH$_3$)-NH$_2$[n]	Cl	H		*o*		$C_6H_9ClN_2O_3\cdot {}^2/_3H_2O$	C, H, N	(6)

[a] See Experimental Section. Procedures for compounds without method designation are given separately. [b] I = EtOAc, II = H_2O, III = CH_3NO_2, IV = EtOH–ether. [c] Purified by chromatography. [d] Methyl isovalerate used as reaction solvent. [e] *p*-Toluenesulfonic acid catalyst added. [f] Br: calcd, 21.42; found, 20.92. [g] Lit. mp 122 °C dec (ref 14a). [h] Strukov, I. T. *Zh. Obshch. Khim.* **1957**, *27*, 432. [i] Literature mp 203–204 °C dec (Schulz, W. *Chem. Ber.* **1953**, *86*, 1010). [j] Literature mp 216–218 °C (Kochetkov, N. K.; Khomutar, R. M.; Budovski, E. I.; Karpeiskii, M. Y.; Severin, E. S. *Zh. Obshch. Khim.* **1959**, *29*, 4069). [k] C: calcd, 62.54; found, 62.04. [l] S configuration, $[\alpha]^{25}{}_D$ +72.2° (*c* 1, 0.1 N methanolic HCl). [m] 1% *Z* isomer. [n] $[\alpha]^{24}{}_D$ +25.3° (*c* 2, 1 N HCl). [o] Amorphous solid.

The resin was washed with 300 mL of H_2O and 350 mL of 2.5 M HOAc. Evaporation of the acidic eluate under reduced pressure followed by evaporation of several portions of H_2O and C_2H_5OH gave 2.24 g (63%) of **176** as an amorphous solid: homogeneous by TLC (*n*-BuOH, HOAc, H_2O; 4:1:1); $[\alpha]^{25}{}_D$ +17.6° (*c* 0.5, CH_3OH), +14.2° (*c* 0.5, 0.1 N HCl); NMR (D_2O, NaOD) δ 0.8–1.05 (m, 2 H, cyclopropyl CH_2), 1.13, 1.19 (s, 6 H, cyclopropyl CH_3), 2.0–2.2 (m, 2 H, CH_2CH=), 2.58 (t, 2 H, SCH_2), 2.80 (m, 2 H, cysteinyl CH_2), 3.43 (m, 1 H, cysteinyl CH), 6.47 (t, *J* = 7 Hz, 1 H, CH_2*CH*=). Anal. ($C_{16}H_{26}N_2O_5S$) C, H, N, S.

Compounds **162**, **174**, **175**, and **216** were prepared with the appropriate cystine by this procedure. Compound **178** was prepared from L-thiazolidine-4-carboxylic acid.

Method G. (*Z*)-8-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic Acid (190). A mixture of 3.32 g (10 mmol) of **188**, 1.0 g (20 mmol) of NaCN, and 20 mL of Me_2SO was heated at 80 °C for 15 min, cooled, poured into 200 mL of ice/H_2O, acidified with concentrated HCl, and extracted with EtOAc (2×) and ether (2×). The combined extracts were washed with H_2O (2×) and dried ($MgSO_4$). The residue obtained by evaporation of the solvent under reduced pressure was chromatographed on silica gel with a Waters Prep 500 apparatus with toluene–HOAc (4:1) to remove a polar impurity. Aqueous extracts of the fractions containing pure product were concentrated, and the solid was filtered and dried to give 1.08 g (38%) of **190**: mp 113–115 °C; NMR δ 0.5–1.0 (m, 2 H, cyclopropyl CH_2), 1.11, 1.13 (s, 6 H, cyclopropyl CH_3), 1.8–2.2 (m, 2 H, CH_2CH=), 2.47 (t, 2 H, CH_2CN), 6.36 (t, *J* = 7 Hz, 1 H, CH_2*CH*=), 9.07 (br s, 1 H, NH). Anal. ($C_{15}H_{22}N_2O_3\cdot 0.25H_2O$) C, H, N.

Method H. (*Z*)-8-(Dimethylamino)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic Acid (192). A solution of 664 mg (2 mmol) of **188** in 10 mL of 40% aqueous $(CH_3)_2NH$ was kept at room temperature for 4 h and then applied to a 3.5 × 20 cm column of Dowex 50W-X4 (100–200 mesh, H^+) resin. The column was first eluted with H_2O until the effluent was no longer acidic and then with 300 mL of 2 N NH_4OH. The basic eluate was evaporated under reduced pressure and several portions of H_2O were added and evaporated. The residue was dissolved in 3 mL of C_2H_5OH, filtered, and added dropwise to 200 mL of rapidly stirred acetone. The gummy precipitate solidified after stirring for 2 days. The solid was filtered, washed with acetone, and dried to give 445 mg (71%) of **192** as hygroscopic crystals: mp 101–112 °C; homogeneous by TLC (silica gel; *n*-BuOH, HOAc, H_2O; 4:1:1); NMR (D_2O) δ 0.7–1.1 (m, 2 H, cyclopropyl CH_2), 1.12, 1.18 (s, 6 H, cyclopropyl CH_3), 1.9–2.3 (m, 2 H, CH_2CH=), 2.83 (s, 6 H, $(CH_3)_2N$), 3.12 (t, 2 H, CH_2N), 6.45 (t, *J* = 7 Hz, 1 H, CH_2*CH*=). Anal. ($C_{16}H_{28}N_2O_3\cdot H_2O$), C, H, N.

Method I. (*Z*)-8-[(Carboxymethyl)methylamino]-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic Acid (195). A solution of 3.32 g (10 mmol) of **188**, 1.0 g (11.3 mmol) of sarcosine, and 3.5 g (34 mmol) of Na_2CO_3 in 30 mL of H_2O was heated at 80 °C for 2 h. After cooling and dilution with 50 mL of H_2O, the mixture was acidified with concentrated HCl, decanted from a little gum, and extracted (2×) with EtOAc. The aqueous solution was applied to a 40-mL bed of Dowex 50W-X8 (200–400 mesh, H^+) resin, eluted with H_2O until the effluent was no longer acidic, and then eluted with 400 mL of 2 N NH_4OH. The basic eluate was evaporated in vacuo. The residue was dissolved in 20 mL of H_2O and applied to a 40-mL bed of AG1-X8 (200–400 mesh, AcO^-) resin. The bed was washed with H_2O (500 mL) followed by 0.6 M HOAc (500 mL). The acidic eluate was evaporated in vacuo, and several portions of H_2O were added and evaporated.

The residue was dissolved in 20 mL of C_2H_5OH and added dropwise to 400 mL of rapidly stirred ether. The solid was filtered, washed with ether, and dried to give 1.01 g (27%) of **195** as an amorphous solid: homogeneous by TLC (silica gel; *n*-BuOH, HOAc, H_2O; 4:1:1); NMR (D_2O) δ 0.7–1.1 (m, 2 H, cyclopropyl CH_2), 1.11, 1.18 (s, 6 H, cyclopropyl CH_3), 2.0–2.4 (m, 2 H, CH_2CH=), 2.88 (s, 3 H, NCH_3), 3.1 (m, 2 H, CH_2N), 3.70 (s, 2 H, HO_2CCH_2N), 6.78 (t, J = 7 Hz, 1 H, CH_2CH=). Anal. ($C_{17}H_{28}N_2O_5{\cdot}2H_2O$) C, H, N.

Method J. Sodium (*Z*)-7-[[(2*R*)-2-(Acetylamino)-2-carboxyethyl]thio]-2-[(1*S*)-2,2-dimethylcyclopropanecarboxamido]-2-heptenoate (177). A 7.14-g (20 mmol) sample of **176** was suspended in 45 mL of H_2O and the pH adjusted to 9.0 with 50% NaOH. The solution was cooled in an ice bath and 4.0 mL (40 mmol) of Ac_2O was added dropwise while the pH was kept between 9 and 11 with 50% NaOH. After acidification with concentrated HCl and extraction with EtOAc (4×), the extracts were washed with H_2O (2×) and saturated brine and dried ($MgSO_4$). The residue (7.57 g) after evaporation of the solvent was stirred with 1.59 g of $NaHCO_3$ in 70 mL of H_2O for 1 h at room temperature. The cloudy solution was extracted with EtOAc (3×) and evaporated under reduced pressure. After evaporation of several portions of 2-propanol, the residue was dissolved in 30 mL of CH_3OH, filtered, and added dropwise to 300 mL of rapidly stirred ether. After stirring for several hours, the precipitate was filtered, washed with ether (4×), and dried to give 5.97 g (68%) of **177** as an amorphous solid: homogeneous by TLC (toluene–HOAc, 4:1); $[\alpha]^{25}_D$ −9.8° (*c* 0.5, 0.1 N NaOH); NMR δ 0.5–1.1 (m, 2 H, cyclopropyl CH_2), 1.12, 1.18 (s, 6 H, cyclopropyl CH_3), 2.03 (s, 3 H, CH_3CO), 2.58 (t, 2 H, CH_2S), 2.92 (dd, 2 H, cysteinyl CH_2), 4.32 (dd, H, cysteinyl CH), 6.42 (t, J = 7 Hz, 1 H, CH_2CH=). Anal. ($C_{18}H_{27}N_2NaO_6S{\cdot}H_2O$) C, H, N, S.

Method K. (+)-(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-[(1-phosphonoethyl)amino]-2-octenoic Acid (199). A solution of **189** (16.45 g, 49 mmol), DL-1-aminoethylphosphonic acid (6.26 g, 50 mmol), and NaOH (8.3 g, 210 mmol) in H_2O (150 mL) was heated at 60 °C for 48 h. The cooled reaction mixture was acidified to pH 6.5 with concentrated HCl and applied to a 5 × 32 cm column of Dowex 50W-X8 (200–400 mesh, H^+) resin. Elution first with H_2O (3 L) and then 0.24 M NH_4OH (2.8 L), evaporation in vacuo of the basic fractions containing the product, and recrystallization of the residue from H_2O (charcoal) gave 9.98 g (54%) of **199**: mp 143–145 °C; homogeneous by TLC (*n*-BuOH, HOAc, H_2O; 4:1:1); $[\alpha]^{25}_D$ +12.5° (*c* 0.6, H_2O); NMR (D_2O) δ 0.8–1.05 (m, 2 H, cyclopropyl CH_2), 1.12, 1.20 (s, 6 H, cyclopropyl CH_3), 1.44 (dd, J_{HH} = 7.0 Hz, J_{HP} = 13 Hz, 3 H, CH_3CHP), 2.24 (m, 2 H, CH_2CH=), 3.2 (m, 2 H, CH_2N), 3.3–3.42 (m, 1 H, NCHP), 6.86 (t, J = 7 Hz, 1 H, CH_2CH=). Anal. ($C_{16}H_{29}N_2O_6P$) C, H, N.

Compounds **196**, **197**, **198**, and **200** were prepared by this procedure.

Method L. (*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(trimethylammonio)-2-octenoate (203). A solution of 996 mg (3 mmol) of **188** in 15 mL of 25% aqueous $(CH_3)_3N$ was kept at room temperature for 2 h, poured onto a 2 × 10 cm column of AG2-X8 (100–200 mesh, OH^-) resin, and eluted with H_2O. The basic effluent (200 mL) was evaporated in vacuo, and several portions of H_2O were added and evaporated. The residue was dissolved in 20 mL of C_2H_5OH, filtered, and diluted with 600 mL of acetone. After the mixture was allowed to stand overnight, the precipitate was filtered, washed with acetone (3×), and dried to give 720 mg (77%) of **203** as hygroscopic crystals: mp 220–222 °C dec; homogeneous by TLC (*n*-BuOH, HOAc, H_2O; 4:1:1); NMR δ 0.6–1.1 (m, 2 H, cyclopropyl CH_2), 1.11, 1.15 (s, 6 H, cyclopropyl CH_3), 3.07 (s, 9 H, $(CH_3)_3N^+$), 3.33 (t, 2 H, CH_2N), 6.44 (t, J = 7 Hz, 1 H, CH_2CH=). Anal. ($C_{17}H_{30}N_2O_3$) C, H, N.

Compounds **204**, **205**, **206**, and **223** were prepared from the appropriate amine by this method except that the **206** reaction time was 2 days. Compound **224** was prepared with 50% methanolic *N*,*N*-dimethylbenzylamine heated under reflux for 4 h. After ion-exchange chromatography in CH_3OH, the basic effluent was concentrated, and the amine was removed by partitioning between ether and H_2O. Evaporation of the H_2O and crystallization from C_2H_5OH–ether gave **224**.

Method M. (*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(formimidoylamino)-2-octenoic Acid (209). To a solution of **191** (350 mg, 1.3 mmol) in H_2O (10 mL) at room temperature was added in small portions benzyl formimidate hydrochloride (947 mg, 5.9 mmol) while the pH was kept at 8–9 with 2.5 N NaOH. After 30 min the cloudy solution was extracted with ether (3×) and chromatographed on a 2 × 25 cm column of AG50W-X4 (Na^+, 200–400 mesh) resin with H_2O. Fractions containing **209** were pooled, evaporated in vacuo, and chromatographed on a 2 × 25 cm column of AG1-X8 (HCO_3^-, 200–400 mesh) resin with H_2O. Fractions containing pure **209** were evaporated in vacuo, and the residue was dissolved in C_2H_5OH (4 mL), filtered, and added dropwise to 200 mL of rapidly stirred ether. The solid was filtered, washed with ether, and dried to give 243 mg (83%) of **209** as a colorless powder: mp 160–162 °C dec; NMR (D_2O) δ 0.5–1.1 (m, 2 H, cyclopropyl CH_2), 1.11, 1.17 (s, 6 H, cyclopropyl CH_3), 1.9–2.3 (m, 2 H, CH_2CH=), 3.32 (t, 2 H, CH_2N), 6.46 (t, J = 7 Hz, 1 H, CH_2CH=), 7.79 (s, 1 H, NCH=N). Anal. ($C_{15}H_{25}N_3O_3{\cdot}{}^1/_3H_2O$) C, H, N.

(+)- and (−)-(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-2-octenoic Acid (140, 141). A. A suspension of 20.24 g (80 mmol) of **139** and 13.20 g (80 mmol) of *l*-ephedrine in 400 mL of H_2O and 120 mL of EtOH was heated on a steam bath until all of the material dissolved. The solid that crystallized on cooling to near room temperature was collected on a filter and washed with Et_2O. This salt was successively recrystallized six times from 10:3 H_2O–EtOH. At each step a 200-mg sample was withdrawn and converted to the free acid for determination of optical rotation, which did not change significantly during the final two crystallizations. The remaining salt (3.23 g) was partitioned between 100 mL of CH_2Cl_2 and 100 mL of 1 N HCl. After being washed with an additional 100 mL of 1 N HCl, the CH_2Cl_2 phase was dried over $MgSO_4$, filtered, and concentrated. The residual solid was collected on a filter and washed with petroleum ether (bp 30–60 °C) to yield 1.86 g of **140** as colorless crystals: mp 95–96.5 °C; $[\alpha]^{20}_D$ +77.7° (*c* 0.51, $CHCl_3$); NMR δ 0.6–1.9 (m, 12 H, $CH_3(CH_2)_3$), cyclopropyl H's), 1.07, 1.11 (s, 6 H, cyclopropyl CH_3), 1.9–2.3 (m, 2 H, CH_2CH=), 6.41 (t, J = 7 Hz, 1 H, CH_2CH=), 9.09 (br s, 1 H, NH). Anal. ($C_{14}H_{23}NO_3$) C, H, N.

The mother liquor from the initial crystallization above (after filtering off a small second crop, which was discarded) was acidified with 100 mL of 2.5 N HCl and extracted with 250 mL of CH_2Cl_2. The organic phase was washed with 1 N HCl, then dried, and filtered. Concentration of the filtrate gave 5.94 g of a white solid, $[\alpha]^{20}_D$ −58.7° (*c* 0.49, $CHCl_3$). This material (5.89 g, 23.3 mmol) was treated with 3.84 g (23.3 mmol) of *d*-ephedrine. The resulting salt was successively crystallized four times from 10:3 H_2O–EtOH and then converted to the free acid as described for the (+) isomer to give 2.93 g of **141** as colorless crystals, mp 94.5–96.5 °C; $[\alpha]^{20}_D$ −78.0° (*c* 0.48, $CHCl_3$).

B. A solution of 1.04 g (0.92 mmol) of **28** and 2.62 g (1.66 mmol) of 2-oxooctanoic acid in 20 mL of toluene under N_2 was stirred at reflux for 17 h with collection of liberated H_2O in a Dean–Stark trap. The cooled solution was concentrated in vacuo, and the residual oil was stirred with pentane in a stoppered flask for 3 days. The semisolid was collected on a filter and dissolved in a small amount of MeCN. Evaporation of this solution in air gave a granular solid. Recrystallization from $MeNO_2$ yielded 0.73 g (31%) of **140** as colorless crystals: mp 96–97.5 °C; $[\alpha]^{20}_D$ +79.2° (*c* 0.50, $CHCl_3$); homogeneous by TLC in 4:1 toluene–AcOH and 98:1:1 CH_2Cl_2–MeOH–AcOH. The NMR spectrum was identical with those of the racemic compound **139** and **140** made by resolution of the racemate as described above.

***tert*-Butyl 2-Amino-5,5,5-trifluoropentanoate (15).** A mixture of 8.55 g (50 mmol) of **14**,[53] 55 mL of dioxane, 5.5 mL of 98% H_2SO_4 (added at 0 °C), and 55 mL of liquid isobutylene (added at −70 °C) in a pressure bottle was shaken for 24 h at room temperature under autogenous pressure. After removal of excess isobutylene, the mixture was partitioned between Et_2O and cold 1 N NaOH. The Et_2O phase was shaken with cold 0.5 N HCl. The aqueous layer was made strongly basic with 2.5 N NaOH and extracted with Et_2O. The Et_2O solution was dried ($MgSO_4$), filtered, and concentrated to give 5.95 g (52%) of **15** as a colorless oil; TLC in 9:1 $CHCl_3$–MeOH; IR (Nujol) 1730 cm^{-1}; NMR ($CDCl_3$) δ 1.52 (s, 9 H, CH_3), 1.6–2.6 (br m, 4 H, CH_2CH_2), 3.37

(53) Babb, R. M.; Bollinger, F. W. *J. Org. Chem.* **1970**, *35*, 1438.

(m, 1 H, NCHCO). Anal. ($C_9H_{16}F_3NO_2$) C, H, N, F.

***tert*-Butyl 2-[(*S*)-2,2-Dimethylcyclopropanecarboxamido]-5,5,5-trifluoropentanoate (17).** A mixture of 5.69 g (25 mmol) of **15**, 5.27 g (25 mmol) of (±)-**16**, 2.83 g (28 mmol) of Et_3N, and 50 mL of Et_2O was stirred in a stoppered flask at room temperature for 6 days. The mixture was partitioned between 200 mL of H_2O and 400 mL of additional Et_2O. The Et_2O phase was washed with 2 × 200 mL of 0.5 N HCl, then with 2 × 200 mL of 0.5 N NaOH (shaken thoroughly in order to hydrolyze any remaining *N*-(acyloxy)succinimide), and finally with 200 mL of H_2O. The dried Et_2O solution was filtered and concentrated to yield 7.29 g (90%) **17** as a pale yellow oil, which gradually crystallized: mp 60–65 °C; TLC in hexane–EtOAc; NMR ($CDCl_3$) δ 0.6–0.9 (m, 2 H, cyclopropyl CH_2), 1.17 (s, 9 H, $C(CH_3)_3$), 1.51 (s, 6 H, cyclopropyl CH_3), 1.6–2.6 (br m, 4 H, CH_2CH_2), 4.6 (m, 1 H, NCHCO), 6.2 (br s, 1 H, NH). Anal. ($C_{15}H_{24}F_3NO_3$) C, H, N.

***tert*-Butyl 2-[(*S*)-2,2-Dimethylcyclopropanecarboxamido]-2-methoxy-5,5,5-trifluoropentanoate (18).** A solution of 3.23 g (10 mmol) of **17** in 15 mL of MeOH was treated in semidarkness with 1.59 mL (1.45 g, 13.3 mmol) of *tert*-butyl hypochlorite. The resulting solution (protected from light and maintained under a drying tube) was stirred in an ice bath as 18 mL (13.5 mmol) of 0.75 M NaOMe in MeOH was added over 10 min, accompanied by precipitation of NaCl. After an additional 20 min, the mixture was concentrated, and the residue was partitioned between Et_2O and H_2O. Evaporation of the dried, filtered Et_2O solution gave after vacuum drying 3.22 g (91%) of colorless crystals: mp 106–110 °C; TLC in 4:1 hexane–EtOAc (pair of spots due to diastereomers); NMR ($CDCl_3$) δ 0.6–0.9 (m, 2 H, cyclopropane CH_2), 1.19 (s, 9 H, $C(CH_3)_3$), 1.54 (s, 6 H, cyclopropyl CH_3), 1.6–3.0 (br m, 4 H, CH_2CH_2), 3.25 (s, 3 H, OCH_3), 6.70 (br s, 1 H, NH). Anal. ($C_{16}H_{26}F_3NO_4$) C, H, N.

(*Z*)-2-[(*S*)-2,2-Dimethylcyclopropanecarboxamido]-5,5,5-trifluoro-2-pentenoic Acid (154). To a solution of 1.06 g (3.0 mmol) of **18** in 10 mL of Et_2O was added 10 mL of Et_2O saturated with anhydrous HCl. The solution was stirred at room temperature under a drying tube. After 1 day an additional 10 mL of Et_2O saturated with HCl was added, and stirring was continued for a second day. The solution was concentrated under a stream of N_2. The residue was taken up in 10 mL of Et_2O and filtered to remove a small amount of insoluble solid. The filtrate was shaken with 10 mL of saturated $NaHCO_3$ solution. The solid that precipitated upon cautious acidification of the aqueous phase with 2.5 N HCl was collected on a filter and washed with dilute HCl to give 226 mg (28%) of **154** as a white solid: mp 139–142 °C; TLC in 8:1 toluene–AcOH; NMR δ 0.6–1.1 (m, 2 H, cyclopropyl CH_2), 1.08, 1.13 (s, 6 H, cyclopropyl CH_3), 1.66 (dd, J = 8 Hz, 5 Hz, 1 H, COCH), 2.8–3.5 (br m, 2 H, CF_3CH_2CH=), 6.13 (t, J = 7 Hz, 1 H, CH_2CH=), 9.43 (br s, 1 H, NH). Anal. ($C_{11}H_{14}F_3NO_3$) C, H, N.

(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-6-[(phosphonomethyl)thio]-2-hexenoic Acid Sodium Salt (164). A solution of H_2N^+=$C(NH_2)SCH_2PO_3H$[54] (104 mg, 0.61 mmol) in 2.0 N NaOH (1.2 mL, 2.4 mmol) was first warmed at 55 °C for 15 min in a N_2 atmosphere and then **159** (152 mg, 0.5 mmol) was added. The solution was warmed at 55 °C for 1 h, applied to a 2 × 8 cm column of AG50W-X4 (H^+, 100–200 mesh) resin, and eluted with H_2O. The acidic eluate (50 mL) was applied to a 2 × 18 cm column of Amberlite XAD-2 (20–60 mesh) resin and eluted with H_2O until the effluent was no longer acidic (~150 mL). The acidic effluent (55 mL) from elution with 30% THF in H_2O was evacuated to remove THF, the pH was adjusted to 3.5 with dilute NaOH, and it was lyophilized to give 120 mg of a colorless fluff. This was dissolved in C_2H_5OH (1.5 mL), filtered, and added dropwise to 50 mL of rapidly stirred ether. The precipitate was quickly filtered, washed with ether, and dried to give 90 mg (45%) of **164** as a hygroscopic, amorphous solid: homogeneous by TLC (*n*-BuOH, HOAc, H_2O; 4:1:1), NMR (D_2O) δ 0.8–1.1 (m, 2 H, cyclopropyl CH_2), 1.15, 1.19 (s, 6 H, cyclopropyl CH_3), 2.1–2.3 (m, 2 H, CH_2CH=), 2.65 (t, 2 H, SCH_2), 2.65 (d, J = 14 Hz, 2 H, PCH_2S), 6.76 (t, J = 7 Hz, 1 H, CH_2CH=). Anal. ($C_{13}H_{21}NNaO_6PS\cdot1^1/_3H_2O$) C, H, N.

(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-7-sulfo-2-heptenoic Acid Sodium Salt (171). A mixture of **166** (636 mg, 2 mmol), $NaHCO_3$ (185 mg, 2.2 mmol), and Na_2SO_3 (277 mg, 2.2 mmol) in 2:1 H_2O–C_2H_5OH (4 mL) was heated at 70 °C in a N_2 atmosphere for 4 h with stirring. The cooled solution was applied to a 2 × 20 cm column of AG50W-X4 (H^+, 100–200 mesh) resin and eluted with H_2O. The acidic effluent (100 mL) was applied to a 3 × 20 cm column of Amberlite XAD-2 (20–60 mesh) resin. Elution with H_2O gave a strongly acidic (~300 mL) and a weakly acidic (~400 mL) fraction. The strongly acidic effluent (~200 mL) from elution with 20% THF in H_2O was combined with the weakly acidic aqueous fraction and evacuated to remove THF, and the pH was adjusted to 3.0 with dilute NaOH and lyophilized to give 500 mg of a glassy solid. This was dissolved in CH_3OH (4 mL), added dropwise to 160 mL of rapidly stirred ether. The precipitate was filtered, washed with ether, and dried to give 465 mg (68%) of **171** as a hygroscopic, amorphous solid: homogeneous by TLC (*n*-BuOH, HOAc, H_2O; 4:1:1); NMR (D_2O) δ 0.8–1.1 (m, 2 H, cyclopropyl CH_2), 1.12 1.18 (s, 6 H, cyclopropyl CH_3), 2.20 (q, J = 7 Hz, 2 H, CH_2CH=), 2.91 (t, J = 7 Hz, 2 H, $CH_2SO_3^-$), 6.81 (t, J = 7 Hz, 1 H, CH_2CH=). Anal. ($C_{13}H_{20}NNaO_6S\cdot0.75H_2O$) C, H, N.

(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-hydroxy-2-octenoic Acid (185). A mixture of **188** (996 mg, 3 mmol), K_2CO_3 (414 mg, 3 mmol), and DMF (15 mL) was heated at 85 °C with stirring for 20 min. After cooling, ice (15 g) was added, and the slurry was stirred for 30 min, filtered, and dried to give 833 mg of crude **13**, which was purified by chromatography on silica gel with 5% MeOH–$CHCl_3$ and recrystallization (toluene) to give 363 mg (48%) of pure macrodilide **13**: mg 220–223 °C; homogeneous by TLC (5% MeOH–$CHCl_3$); mass spectrum, *m/e* 502 (M^+); NMR δ 0.6–1.0 (m, 4 H, cyclopropyl CH_2), 1.10, 1.13 (s, 12 H, cyclopropyl CH_3), 2.0–2.4 (m, 4 H, CH_2CH=), 4.16 (br s, 4 H, CH_2O_2C), 6.45 (t, J = 7 Hz, 2 H, CH_2CH=), 9.17 (br s, 2 H, NH). Anal. ($C_{28}H_{42}N_2O_6$) C, H, N.

A suspension of **13** (420 mg, 0.84 mmol) in CH_3OH (25 mL) containing 0.3 M LiOH (8.4 mL, 2.4 mmol) was heated under reflux under N_2 for 20 min. The clear solution was cooled, and 2 mL of AG50W-X8 (H^+, 200–400 mesh) resin was added. After stirring for several minutes, the resin was filtered and washed with water (4×). The residue after evaporation in vacuo slowly crystallized over several weeks. Recrystallization (CH_3OH–$CHCl_3$) gave 305 mg (68%) of **185**: mp 137–139 °C; homogeneous by TLC (toluene–HOAc, 4:1); NMR δ 0.5–1.0 (m, 2 H, cyclopropyl CH_2), 1.07, 1.10 (s, 6 H, cyclopropyl CH_3), 1.9–2.2 (m, 2 H, CH_2CH=), 3.39 (t, 2 H, CH_2OH), 6.39 (t, J = 7 Hz, 1 H, CH_2CH=), 9.13 (br s, 1 H, NH). Anal. ($C_{14}H_{23}NO_4$) C, H, N.

(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methoxy-2-octenoic Acid (187). A solution of **188** (332 mg, 1 mmol) and Na (56 mg, 2.43 g-atom) in CH_3OH (5 mL) was heated under reflux for 1 h in a N_2 atmosphere. After evaporation in vacuo the residue was dissolved in H_2O, acidified with dilute HCl, and extracted with ether (3×). The extracts were washed with H_2O and saturated brine and dried ($MgSO_4$). Evaporation of the solvent in vacuo and recrystallization of the residue from ether–hexane gave 140 mg (50%) of **187**: mp 71–72 °C; homogeneous by TLC (toluene–HOAc, 4:1); NMR δ 0.6–1.0 (m, 2 H, cyclopropyl CH_2), 1.08, 1.12 (s, 6 H, cyclopropyl CH_3), 1.9–2.3 (m, 2 H, CH_2CH=), 3.20 (s, 3 H, OCH_3), 3.30 (t, 2 H, CH_2O), 6.37 (t, J = 7 Hz, 1 H, CH_2CH=), 9.07 (br s, 1 H, NH). Anal. ($C_{15}H_{25}NO_4$) C, H, N.

(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-[[ethoxy(thiocarbonyl)]thio]-2-octenoic Acid (211). A mixture of **188** (664 mg, 2 mmol) and $C_2H_5OCS_2K$ (640 mg, 4 mmol) in acetone (8 mL) was heated under reflux in a N_2 atmosphere for 2 h with stirring. Most of the acetone was removed in vacuo, and the residue was dissolved in H_2O (30 mL) and acidified with dilute HCl. The precipitate was extracted with $CHCl_3$ (4×), and the extracts were washed with H_2O, dried ($MgSO_4$), and evaporated in vacuo. Crystallization of the residue from ether–hexane gave 453 mg (71%) of **211**: mp 88–91 °C; homogeneous by TLC (toluene–HOAc, 4:1); NMR δ 0.5–1.1 (m, 2 H, cyclopropyl CH_2), 1.07, 1.10 (s, 6 H, cyclopropyl CH_3), 1.33 (t, J = 7 Hz, 3 H, OCH_2CH_3), 1.8–2.1 (m, 2 H, CH_2CH=), 3.12 (t, 2 H, SCH_2), 4.63 (q, J = 7 Hz, 2 H, OCH_2CH_3), 6.39 (t, J = 7 Hz, 1 H, CH_2CH=),

(54) Ivasyuk, N. V.; Shermergorn, I. M. *Zh. Obshch. Khim.* **1971**, *41*, 2199.

8.98 (br s, 1 H, NH). Anal. ($C_{17}H_{27}NO_4S_2$) C, H, N, S.

(*Z*)-2-(2,2-Dimethylcyclopropanecarboxamido)-8-mercapto-2-octenoic Acid (212). A solution of 211 (373 mg, 1 mmol) in $H_2NCH_2CH_2NH_2$ (1 mL) was kept at room temperature for 5 h in a N_2 atmosphere, poured onto ice–10% H_2SO_4, and extracted with EtOAc (5×). The extracts were washed with H_2O, dried ($MgSO_4$), and evaporated in vacuo. The residue was recrystallized from toluene to give 101 mg (38%) of **212**: mp 128–130 °C; homogeneous by TLC (toluene–HOAc, 4:1); NMR δ 0.5–1.1 (m, 2 H, cyclopropyl CH_2), 1.08, 1.11 (s, 6 H, cyclopropyl CH_3), 1.9–2.3 (m, 2 H, CH_2CH=), 2.52 (m, 2 H, CH_2S), ~3.2 (br s, 1 H, SH), 6.35 (t, J = 7 Hz, 1 H, CH_2CH=), 9.11 (br s, 1 H, NH). Anal. ($C_{14}H_{23}NO_3S$), C, H, N, S.

(*E*)-2-(2,2-Dimethylcyclopropanecarboxamido)-2-octenoic Acid (241). A solution of **140** (Na salt) (15.06 g) in H_2O (1 L) was photolyzed in a Rayonet apparatus with 254-nm lamps for 12 h. The solution was acidified with concentrated HCl, extracted with CH_2Cl_2 (5×), and dried ($MgSO_4$). The residue (13.5 g, ~35% *E* isomer) after evaporation in vacuo was chromatographed on silica gel in a Waters Associates System 500 Prep LC apparatus with toluene–EtOAc (3.5:1 + 2% HOAc). Fractions containing the pure *E* isomer (eluted first) were evaporated in vacuo, and the residue (3.75 g, 97% *E* isomer) was recrystallized from CH_2Cl_2–hexane to give **241**: mp 86–87.5 °C; 1% *Z* isomer by LC; $[\alpha]^{25}_D$ +53.9° (c 1.0, 0.1 N methanolic HCl); NMR δ 0.5–1.0 (m, 2 H, cyclopropyl CH_2), 0.85 (t, 3 H, CH_2CH_3), 1.06, 1.09 (s, 6 H, cyclopropyl CH_3), 2.29 (q, 2 H, CH_2CH=), 5.82 (t, J = 7 Hz, 1 H, CH_2CH=), 9.44 (s, 1 H, NH). Anal. ($C_{14}H_{23}NO_3$) C, H, N.

NMR spectrum of the *Z* isomer (**140**): δ 0.6–0.9 (m, 2 H, cyclopropyl CH_2), 0.87 (t, 3 H, CH_2CH_3), 1.09, 1.14 (s, 6 H, cyclopropyl CH_3), 2.06 (q, 2 H, CH_2CH=), 6.38 (t, J = 7 Hz, 1 H, CH_2CH=), 9.12 (s, 1 H, NH).

(*Z*)-*N*-L-Alanyl-3-chloro-2,3-didehydroalanine (242). To a mixture of *N*-α-*t*-BOC-L-alanine (3.78 g, 20 mmol), DL-serine methyl ester hydrochloride (3.11 g, 20 mmol), and $(C_2H_5)_3N$ (2.8 mL, 20 mmol) in CH_2Cl_2 (80 mL) cooled in an ice bath was added $(CH_3)_2N(CH_2)_3N{=}C{=}NC_2H_5{\cdot}HCl$ (4.21 g, 22 mmol). After the mixture was stirred for 2 h in the ice bath and 18 h at room temperature, another 4.21 g of the carbodiimide and CuCl (400 mg) were added, and the suspension was stirred at room temperature for 2 days. The mixture was extracted with H_2O (2×), and the aqueous extracts were washed with CH_2Cl_2 (2×). Hydroquinone (~3 mg) was added to the combined CH_2Cl_2 phases (*all subsequent manipulations to 237 were carried out in the presence of traces of hydroquinone to inhibit polymerization*). After drying ($MgSO_4$) and evaporation in vacuo, the residue was filtered through a bed of silica gel with hexane–EtOAc (1:1) and evaporated in vacuo to give 4.05 g (75%) of **19**[55] as a colorless syrup: homogeneous by TLC (hexane–EtOAc, 2:1); NMR δ 1.41 (d, J = 7 Hz, 3 H, $CHCH_3$), 1.45 (s, 9 H, $(CH_3)_3C$), 3.82 (s, 3 H, CO_2CH_3), 4.25 (m, 1 H, $CHCH_3$), 5.00 (br d, 1 H, CON*H*), 5.92 (d, J = 1.5 Hz, 1 H, *t*-NC=CH), 6.61 (s, 1 H, *c*-NC=CH), 8.41 (br s, 1 H, $NHCO_2$); $[\alpha]^{25}_D$ −56.2° (c 1.0, $CHCl_3$).

A solution of **19** (4.00 g, 14.7 mmol) in CH_3OH (22 mL) and 1 N NaOH (19 mL) was kept at room temperature in a N_2 atmosphere for 2 h. Most of the CH_3OH was evaporated in vacuo, ice–H_2O (30 mL) was added, and the solution was extracted with EtOAc, acidified with 10% citric acid, saturated with NaCl, and extracted with EtOAc (5×). The extracts were washed with saturated NaCl, dried ($MgSO_4$), and evaporated in vacuo to give 3.48 g (92%) of **20** as a syrup; homogeneous by TLC (toluene–HOAc, 4:1).

A solution of **20** (1.04 g, 4 mmol) and *N,N′*-diisopropyl-*O*-*tert*-butylisourea[56] (4.00 g, 20 mmol) in DMF (10 mL) was kept at room temperature for 40 h and evaporated in vacuo. The residue was triturated with EtOAc (60 mL), and the urea was filtered. The filtrate was washed with cold 10% citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. The acid and base extracts were back-extracted with CH_2Cl_2 (3×). The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo. The residue was chromatographed on silica gel with hexane–EtOAc (2:1), and fractions containing pure product were evaporated in vacuo to give 1.13 g (90%) of **21** as a colorless oil; homogeneous by TLC (hexane–EtOAc, 1:1); NMR δ 1.41 (d, J = 7 Hz, 3 H, $CHCH_3$), 1.48 (s, 9 H, $CO_2C(CH_3)_3$), 1.53 (s, 9 H, $NCO_2C(CH_3)_3$), 3.9–4.5 (m, 1 H, $CHCH_3$), 5.07 (br, d, 1 H, CONH), 5.81 (d, J = 1.5 Hz, *t*-NC=CH), 6.50 (s, 1 H, *c*-NC=CH), 8.42 (br s, 1 H, $NHCO_2$).

Cl_2 was passed through a solution of **21** (0.95 g, 3 mmol) in CCl_4 (12 mL) at 15 °C until a permanent yellow color developed (~1 min). After being kept at room temperature for 15 min, the solution was evaporated in vacuo (bath temperature 40 °C). The residual oil was dissolved in CH_3CN (12 mL) and cooled to 15 °C, and 1,4-diazabicyclo[2.2.2]octane (0.38 g, 3.4 mmol) was added. After being stirred at room temperature for 1.5 h, the amine hydrochloride was filtered and washed with a little CH_3CN. The filtrate was evaporated in vacuo, and the residue was purified by preparative TLC (silica gel, 2:1 hexane–EtOAc) to give 0.72 g (69%) of a mixture of *Z* and *E* isomers of **22** (*Z*–*E* ~ 9:1): NMR (*Z* isomer δ 1.42 (d, J = 7 Hz, 3 H, $CHCH_3$), 1.47 (s, 9 H, $CO_2C(CH_3)_3$), 1.51 (s, 9 H, $NCO_2C(CH_3)_3$), 4.0–4.5 (m, 1 H, $CHCH_3$), 5.15 (br d, 1 H, CONH), 6.88 (s, 1 H, =CHCl), 7.9 (br s, 1 H, $NHCO_2$); NMR (*E* isomer) δ 1.39, 1.56, 7.84 (=CHCl), 8.4.

A solution of **22** (0.72 g) in CF_3CO_2H (8 mL) was kept at room temperature for 2 h. The residue after evaporation of the CF_3CO_2H in vacuo was chromatographed on AG1-X8 (OAc^-, 200–400 mesh) ion-exchange resin with 0.1 M HOAc. Fractions containing the product were lyophilized. The fluffy residue was dissolved in CH_3OH (10 mL) and added dropwise to 150 mL of rapidly stirred ether. Filtration and drying gave 0.30 g (71%) of **242** as an amorphous solid containing 16% of the *E* isomer: NMR (D_2O) δ 1.61 (d, J = 7 Hz, 3 H, $CHCH_3$), 4.22 (q, J = 7 Hz, 1 H, $CHCH_3$), 6.98 (s, 1 H, =CHCl); NMR (*E* isomer) δ 1.59 (d, $CHCH_3$), 6.50 (s, =CHCl); $[\alpha]^{24}_D$ +25.3° (c 2, 1 N HCl). Anal. ($C_6H_9ClN_2O_3{\cdot}{}^2/_3H_2O$) C, H, N.

(+)-2,2-Dimethylcyclopropanecarboxylic Acid (26). A mixture of 26.0 g (228 mmol) of **25**,[16] 39.0 g (114 mmol) of quinine monohydrate, 45.6 mL (114 mmol) of 2.5 N NaOH, 60 mL of MeOH, and 14.4 mL of H_2O was heated on a steam bath until all of the material dissolved. The oil that separated on cooling was induced to crystallize after vigorous scratching. The mixture was reheated until all but a few crystals had dissolved and was then allowed to cool slowly. The large crystals thus obtained were successively recrystallized seven times from 1:1 MeOH–H_2O (approximately 3 mL/g). At each step a 100-mg sample was withdrawn and converted to the free acid for determination of optical rotation, which was essentially unchanged during the final two crystallizations. The resulting salt (10.3 g) was partitioned between 75 mL of $CHCl_3$ and 37.5 mL of H_2O made strongly basic with 50% NaOH. After being washed with an additional 37.5 mL of $CHCl_3$, the H_2O layer was made strongly acidic with concentrated HCl and extracted with 75 mL of CH_2Cl_2 in two portions. The combined CH_2Cl_2 fractions were dried (Na_2SO_4), filtered, and concentrated (≥100 mm, warm water bath) to give 2.49 g of **26** as a nearly colorless oil, $[\alpha]^{20}_D$ +142° (c 1.01, $CHCl_3$), +132° (c 1.01, MeOH).

(+)-*N*-[[(2,2-Dimethylcyclopropyl)carbonyl]oxy]succinimide (16). A solution of 1.71 g (15 mmol) of **26** and 3.80 g (18 mmol) of **27**[29] in 9 mL of dry pyridine was stirred at room temperature for 1.5 h. The cloudy mixture was treated with 60 mL of H_2O and stirred in an ice bath for an additional 1.5 h. The precipitated solid was collected on a filter, washed with H_2O, and dried. Combination with a smaller second crop gave a total yield of 2.94 g (93%) of **16** as colorless crystals: mp 87–88 °C; $[\alpha]^{20}_D$ +128.5° (c 1.0, $CHCl_3$); TLC in 2:1 hexane–EtOAc; NMR ($CDCl_3$) δ 1.1–1.3 (m, 2 H, cyclopropyl CH_2), 1.28 (s, 6 H, CH_3), 1.78 (dd, J = 8 Hz, 6 Hz, 1 H, $COCHCH_2$), 2.83 (s, 4 H, CH_2CH_2). Anal. ($C_{10}H_{13}NO_4$) C, H, N.

(+)-2,2-Dimethylcyclopropanecarboxamide (28). A suspension of 2.53 g (12 mmol) of **16** in 20 mL of 3 M NH_3 in EtOH was stirred in a stoppered flask with cooling in an ice bath. After 45 min the solid was removed by filtration and washed with small volumes of EtOH. The combined filtrate and washings were concentrated, and the residual solid was dissolved in 30 mL of EtOAc. This solution was washed with saturated Na_2CO_3 solution, dried ($MgSO_4$), and filtered. Concentration of the filtrate yielded 1.13 g (83%) of **28** as colorless crystals: mp 136–137.5 °C; $[\alpha]^{20}_D$

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+101.4° (c 1.0, $CHCl_3$); TLC in EtOAc; NMR (Me_2SO-d_6-$CDCl_3$) δ 0.5–1.0 (m, 2 H, CH_2), 1.17 (s, 6 H, CH_3), 1.50 (dd, J = 8 Hz, 6 Hz, 1 H, COCH), 6.6, 7.3 (v br s, each 1 H, NH). Anal. ($C_6H_{11}NO$) C, H, N. The racemic amide 11 melted at 175–177 °C (lit.[16] mp 177–177.5 °C).

Crystal Structure of (-)-Quinine Salt of (+)-2,2-Dimethylcyclopropanecarboxylic Acid (26). Suitable crystals of the (-)-quinine salt of **26** formed from a methanol-water mixture with space group symmetry of $P2_1$ and cell constants of a = 6.840 (1) Å, b = 18.238 (4) Å, c = 10.608 (2) Å, and β = 107.74 (1) Å for Z = 2 and a calculated density of 1.203 g/cm³. Of the 1764 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 1684 were observed ($I > 3\sigma(I)$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[57] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.044. A molecule of water was found cocrystallized in the asymmetric unit. No abnormally short intermolecular contacts were noted. The positions for the atoms in the vinyl group refined poorly; therefore, the geometry for this group differs from standard values. Three tables containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material.

Renal Dipeptidase Inhibition Assay. Assays were run in 1-mL reaction mixtures containing 50 mM MOPS pH 7.1 buffer, 5 μg of a solubilized renal dipeptidase preparation, and ≤0.1 mM inhibitor candidate. The enzyme preparation corresponds to the 50–75% ammonium sulfate fraction of Campbell[4,6] and had 0.174 unit specific activity. After 5 min of equilibration at 37 °C, glycyldehydrophenylalanine (K_m = 0.6 mM[6]) was added to give a concentration of 50 μM. The rate of hydrolysis of this substrate was computed from the decrease in absorption at 275 nm over a 10-min period following addition, during which time first-order kinetics was obeyed. Inhibitor activity, I_{50}, was computed from the relation $I_{50} = I(V/V_o - V)$, where V_o is the rate in the absence of inhibitor and V is the rate in the presence of concentration I of inhibitor. Since the substrate concentration in these assays was $\ll K_m$, I_{50} is equivalent to K_i for these inhibitors. Identical I_{50} values were found for substrates tested with thienamycin as substrate. The K_i values for most of the compounds in Tables I–III were determined only once and have an estimated accuracy of ±10%. Kim and Campbell[46] found that 113 showed reversible, competitive inhibition of pure porcine renal dipeptidase with a K_i of 0.67 ± 0.04 μM using glycyldehydrophenylalanine as substrate. Recently Campbell et al.[12] reported a K_i of 0.73 ± 0.02 μM for cilastatin (**176**) when tested against pure human renal dipeptidase with imipenem as substrate.

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Supplementary Material Available: Synthetic procedures for compounds 114, 115, 157, 186, 194, 208, 227, and the following amides: ethyl 5-amino-5-oxopentanoate (for **80**), 5-methoxy-3-methylpentanamide (for **88**), and 2,2-difluorocyclopropanecarboxamide (for **128**). Tables of the atomic positional and thermal parameters, bond angles for the (-)-quinine salt of **26** (11 pages). Ordering information is given on any current masthead page.

Notes

Synthesis and Antiviral Evaluation of Carbocyclic Analogues of 2-Amino-6-substituted-purine 3′-Deoxyribofuranosides

Y. Fulmer Shealy,* C. Allen O'Dell, and Gussie Arnett

Kettering-Meyer Laboratories, Southern Research Institute, Birmingham, Alabama 35255-5305. *Received July 1, 1986*

Carbocyclic analogues of 2-amino-6-substituted-purine 3′-deoxyribofuranosides were synthesized by beginning with (±)-(1α,3α,4β)-3-amino-4-hydroxycyclopentanemethanol and 2-amino-4,6-dichloropyrimidine. The route parallels the earlier syntheses of the corresponding ribofuranoside and 2′-deoxyribofuranoside analogues. The 2-amino-6-chloropurine, guanine, and 2,6-diaminopurine derivatives and the analogous 8-azapurines were prepared. The analogue (3′-CDG) of 3′-deoxyguanosine is active in vitro against a strain of type 1 herpes simplex virus (HSV-1) that induces thymidine kinase and is modestly active against a thymidine kinase inducing strain of type 2 HSV. 3′-CDG is not active against a strain of HSV-1 that lacks the thymidine kinase inducing capacity, whereas the carbocyclic analogue of 2-amino-6-chloropurine 3′-deoxyribofuranoside is active against that strain. The carbocyclic analogue of 2,6-diaminopurine 3′-deoxyribofuranoside displayed modest activity in vitro against influenza virus.

Previously, we described the synthesis of carbocyclic analogues of ribofuranosides[1,2] (**1** and **2**, R = OH) and of 2′-deoxyribofuranosides[3] (**1** and **2**, R = H) of 2-amino-6-substituted-purines. Lee and Vince[4] reported the synthesis of carbocyclic analogues of some arabinofuranosyl

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