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4-ACETAMIDOPHENYL ESTERS AND 4-ACETAMIDOANILIDES OF L-ARGININE, *p*-GUANIDINO-L-PHENYLALANINE, L-LYSINE, N^2-[D-FRUCTOS-3-*O*-YL AND D-GLUCOS-3-*O*-YL]ACETYL-L-LYSINE AS POTENTIAL ACROSIN INHIBITORS[1]

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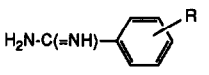
Abstract: Syntheses of 4-acetamidophenyl esters and 4-acetamidoanilides of L-lysine, *p*-guanidino-L-phenylalanine, L-leucyl-L-lysine, L-leucyl-L-leucyl-L-lysine, N^2-[(1,2-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl)acetyl]-L-lysine, N^2-[(1,2-*O*-isopropylidene-ß-D-fructopyranos-3-*O*-yl)acetyl]-L-lysine are reported. 4-Acetamidophenyl L-argininate was too unstable to be isolated but L-arginin-4-acetamidoanilide was prepared. These amino acid and peptide derivatives proved to be enantiomerically pure and were characterized by their ^1H and ^{13}C chemical shifts which were established by means of multipulse 1D and 2D NMR techniques. Preliminary evaluation showed these compounds to be relatively weak inhibitors of human acrosin and bovine trypsin.

Introduction

Acrosin (E.C. 3.4.21.10) is an essential enzyme released from the acrosomes of mammalian spermatozoa.[2] This protease, perhaps in synergistic combination with other acrosomal enzymes, is implicated in one of the crucial phases of mammalian fertilization, namely sperm penetration of the zona pellucida, the innermost vestment of the ovum.[2-6] This spermatozoan enzyme is an endopeptidase that has been characterized as "trypsin-like" based on its inhibitor sensitivity and its ability to catalyze the hydrolysis of natural and synthetic esters and amides of arginine and lysine.[4] Its physical and kinetic properties, however are significantly different from those of other serine proteases. One notable structural difference from trypsin is the carbohydrate content of acrosin. Considerable efforts have been expended to discover compounds which would inhibit acrosin but not other members of the trypsin family of serine proteases. Such specific inhibitors would help to better define acrosin's role in fertilization and, in addition, could provide an attractive non-hormonal contraceptive.[5,7]

In developing potential selective inhibitors of a trypsin-like serine protease,[8] binding interactions (i.e., ionic, hydrophobic, hydrogen bonding) between the substrate or inhibitor and the enzyme's primary (S_1) and secondary binding sites (S_3, S_2, S_1', ...)[9] must be considered. Since acrosin and trypsin have been found to possess similar primary binding sites, secondary interactions must assume a pivotal role in realizing acrosin's specificity between similar substrates or inhibitors.

Efforts to prepare selective acrosin inhibitors based mainly on primary binding interactions have met with limited success. Numerous benzamidines **1**, phenylguanidines **2**, and substituted aminoacyl derivatives **3**, {where A can be amidine [H_2N-C(=NH)] or guanidine [H_2N-C(=NH)-NH], R an electron-donating or withdrawing substituent and X is an ester (OR) or amide (NRR')} have been prepared and found to provide several potent inhibitors of trypsin-like serine proteases.[10]



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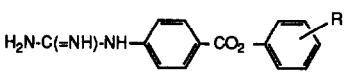

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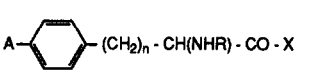

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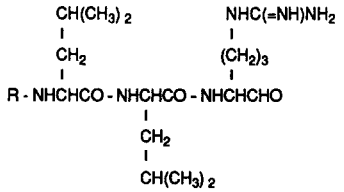
For those compounds (**1** - **3**) tested for acrosin inhibition, relatively few have demonstrated selective inhibition of acrosin over trypsin.[11] The most selective acrosin inhibitors among these general classes thus far reported are 2-(4-toluenesulfonamido)-5-[4-(carboxamidino)phenyl]pentanilide (K_i of 8.0 x 10^{-8} M for boar acrosin, 4.1 x 10^{-6} M for bovine trypsin) and 6-carboxamidinoindole (K_i of 3.9 x 10^{-7} M for boar acrosin and 2.2 x 10^{-5} M for bovine trypsin).[10h,11c]

Esters of 3- and 4-guanidinobenzoic acid inhibit many trypsin-like serine proteases, including acrosin.[7,12-15] Human acrosin and human trypsin are inhibited about equally by aryl 4-guanidinobenzoates **2** irrespective of the nature or ring position of R.[7] One of the most potent and least toxic analogues is the 4-acetamidophenyl ester (**2**, R = 4-$NHCOCH_3$, I_{50} for human acrosin of 8 nM).[7b] The lack of specificity demonstrated by these compounds is attributed to the apparent similarity in the primary substrate binding sites in acrosin and trypsin. Thus, **2** acts as a pseudo-irreversible inhibitor of trypsin-like serine proteases forming a typical enzyme-substrate complex followed by relatively fast acylation of the serine hydroxyl leading to an *O*-*p*-guanidinobenzoylated enzyme.[16] Structurally related amides can react similarly, but their rate of acylation is comparatively slow and this step becomes rate-limiting.[8f,10q,17] Because of their relatively fast acylation, aryl esters are much less discriminatory in detecting secondary sub-site binding interactions, as compared to the analogous amides.[18] Once the *O*-acyl-enzyme is formed, relatively weak secondary binding interactions would not be expected to significantly alter the steady state hydrolysis of the acylated enzyme.

Peptidyl halomethanes, and in particular arginyl chloromethanes [Xaa-Yaa-Arg-CH_2Cl] are effective inhibitors of trypsin-like serine proteases.[19] This class of inhibitors has proven extremely useful in defining subtle secondary interactions between the enzyme and inhibitors. Since these analogues react by initial formation of a typical enzyme-substrate complex, the reactivities of these inhibitors reflects their ability to interact with the enzyme in the same manner as with substrates. Examination of the inhibitory potencies within a series of peptidyl chloromethanes has demonstrated that enhanced reactivity and selectivity for acrosin over several other members of the trypsin family of serine proteases is realized with branched chain amino acids at the P_2 and P_3 positions.[19f] Consistent with these findings is the potent, reversible inhibition of acrosin observed with the naturally occurring tripeptide argininals, the leupeptins [4, (R = acetyl or propionyl)-L-leucyl-L-leucyl-L-argininal] and antipain (5, {S-[(1-carboxy-2-phenylethyl)carbamoyl]-L-arginyl-L-valyl-L-argininal].[20a,21]



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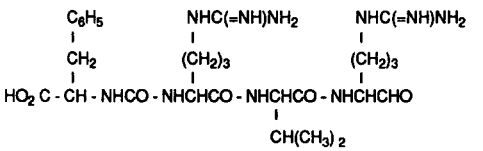

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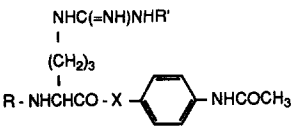
Human acrosin is selectively inhibited by certain monosaccharides and related polyols.[22] The mechanism by which these compounds exert their inhibitory action is not yet understood. Speculation that the aldehyde or ketone functionality of the sugars is interacting with the active site serine hydroxyl seems unlikely since methyl-α-D-mannoside is more potent than D-mannose, and certain polyols (glucitol, inositol, mannitol) are also good inhibitors. At lower inhibitory concentrations (10-60 mM), D-fructose displays a pattern of inhibition consistent with that of a classical competitive inhibitor, while at higher concentrations it behaves as a noncompetitive inhibitor, evidenced by a lowering of the apparent V_m. This complex pattern of inhibition suggests that acrosin may possess allosteric properties and that the fructose-acrosin interaction may produce a different enzyme form. Human trypsin is relatively insensitive to the modulatory effects of fructose[22a] further establishing that acrosin possesses a unique inhibitor selectivity profile.[22b] Although inhibitory concentrations of fructose are present in human ejaculates,[22c] it is unlikely that it exerts significant effects on fertilization as it is probably removed during transport of the spermatozoa through the female genital tract. Fructose may, however, modulate acrosin activity immediately after ejaculation, preventing premature destabilization of the acrosomal content.[22d]

Current work

In developing new acrosin inhibitors, cognizance has been taken that acrosin and trypsin catalyze the hydrolysis of arginine and lysine esters and amides.[23] To incorporate some of the structural features inherent in prior active aryl guanidinobenzoates and leupeptins, 4-acetamidophenyl esters of L-arginine, L-lysine, and *p*-guanidino-L-phenylalanine, L-leucyl-L-lysine and L-leucyl-L-leucyl-L-lysine were initially synthesized and evaluated. It was further intended to compare the activities of some of these esters with those of the corresponding 4-acetamidoanilides, which are closely related in size and electronic disposition. Since acrosin is an endopeptidase, the *N*-terminus of these amino acid and peptide derivatives {**3** [A = NHC(=NH)NH], **6** and **8**} would bear suitable protective groups R, such as *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Z), or acetyl (Ac). It was further intended to replace the N^2-acyl group of some of these L-lysine derivatives with a glycosyl derivative *via* a glycolyl bridge (*O*-CH_2-CO).

The synthesis of 4-acetamidophenyl esters of arginine proved to be frustrating. Esterification of N^{α},N^{ω}-diprotected arginines with 4-acetamidophenol furnished the corresponding esters (**6**, X = O), but these products were unstable, readily cyclizing to lactams **7**.[24,25] Bis-protection of the guanidino group limits this cyclization, thus tris-($N^{\alpha},N^{\delta},N^{\omega}$-benzyloxycarbonyl)-L-arginine[26] was successfully esterified with 4-acetamidophenol, however, hydrogenolysis of the Z groups in the resultant ester was followed by rapid lactamization (as monitored by ^{1}H NMR). By comparison, the corresponding anilide was stable. N^2-Boc-N^G-nitroarginine condensed with 4-acetamidoaniline to form **6** (where R = Boc, R' = NO_2, X = NH) which upon hydrogenolysis provided the argininanilide **6e**, which was isolated as the 4-toluenesulfonate. In addition, the related guanidino ester, derived from *p*-guanidino-L-phenylalanine was prepared. N^2-*tert*-Butoxycarbonyl-*p*-guanidino-L-phenylalanine[10l] [**3a**, A = $H_2NC(C{=}NH)NH$, n = 1, R = Boc, X = OH] was readily esterified by 4-acetamidophenol to furnish **3b** [A = $H_2NC(=NH)NH{\cdot}TsOH$, n = 1, R = Boc, X = 4-$OC_6H_4NHCOCH_3$].

Since lysine may be substituted for arginine at the P_1 site of a potential acrosin inhibitor, we directed our efforts to the synthesis of 4-acetamidophenyl esters and anilides of L-lysine **8**. Aryl lysinates (**8**, X = O) and corresponding anilides (**8**, X = NH) are not prone to cyclize to seven-membered lactams. In order to emulate the structures of the leupeptins, we synthesized several analogous aryl esters and anilides of L-leucyl-L-lysine and L-leucyl-L-leucyl-L-lysine, **9** and **10**.



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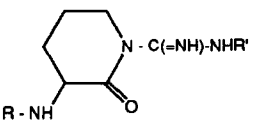

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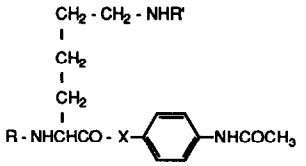

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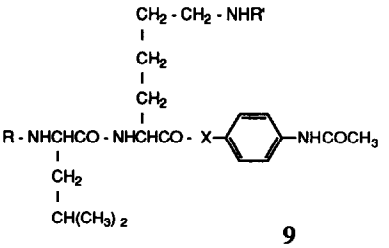

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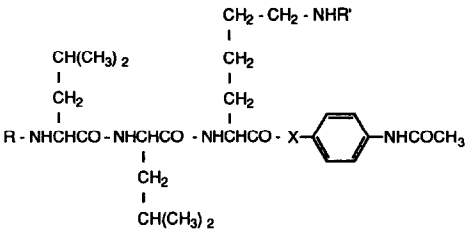

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a,	R = Boc	R' = Z	X = O
b,	R = Boc	R' = H · TsOH	X = O
c,	R = H · TFA	R' = Z	X = O
d,	R = Boc	R' = Z	X = NH
e,	R = Boc	R' = H · TsOH	X = NH
f,	R = H · TFA	R' = Z	X = NH
g,	R = Ac	R' = Z	X = NH
h,	R = Ac	R' = H · TsOH	X = NH
i,	R = Z	R' = Boc	X = NH
j,	R = Z	R' = H · TsOH	X = NH

Essentially, carbodiimide-assisted condensations were employed to prepare the required esters, anilides and peptides.[27] Although DCC[28] was used initially, it was sometimes difficult to free the required peptide from accompanying and relatively insoluble *N,N'*-dicyclohexylurea and *N*-acylureas. The use of EDC[1c] has the advantage that the corresponding ureas are easily separated from neutral products by mild aqueous acid extraction. Certain auxiliary reagents, e.g. HOBt,[1c,29] DMAP,[1c,30] and others, proved essential in these condensations by significantly increasing the rates of reaction and effectively decreasing the formation of by-products, as well as limiting racemization. For example, in the absence of DMAP, the condensation of Boc-Lys(Z) with ArOH gave the requisite ester in poor yield (15-20%) and was accompanied by significant amounts of *N*-acyl urea byproducts.

Condensation of Boc-Lys(Z) with 4-acetamidophenol in the presence of EDC and catalytic amounts of DMAP furnished Boc-Lys(Z)-OAr (**8a**) in good yield. Hydrogenation of **8a** released the 6-amino group and **8b** was isolated as the 4-toluenesulfonate. Trifluoroacetic acid (TFA) deprotection of the Boc group of **8a** provided the trifluoroacetate **8c**. Due to the sensitivity of aryl esters towards hydrolysis and condensations, the tripeptide ester **10a** was made by a (2 + 1) segment condensation, thereby exposing the aryl ester to a minimum number of steps. Coupling of Boc-Leu with Leu-OMe·HCl in the presence of EDC, HOBt and NMM[1c] provided Boc-Leu-Leu-OMe[47] (**11**) which was hydrolyzed under mild conditions to Boc-Leu-Leu (**12**). Condensation of **12** with the base from **8c** afforded **10a** which was hydrogenolyzed to the required tripeptidyl esters, **10b**.

The ^{13}C NMR spectrum of **10a** attested to its purity. Additional signals would have appeared had the enantiomeric integrity of the α-methine of the leucyl or lysyl residues been destroyed. This premise was tested using the analogous tripeptidyl methyl ester **13**. Under our standard conditions, Boc-Leu-Leu

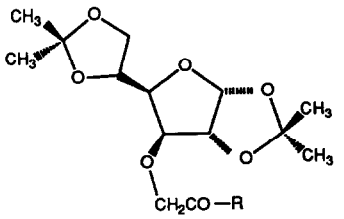
(12) was coupled with commercially available Lys(Z)-OMe·HCl to provide stereochemically pure Boc-Leu-Leu-Lys(Z)-OMe (13). However, when such a coupling was carried out in the presence of excess triethylamine at somewhat higher temperatures, racemization took place to give Boc-L-Leu-D,L-Leu-L-Lys(Z)-OMe. Partial epimerization was evident as an additional methyl ester signal from the diastereomeric ester appeared in its ^1H NMR spectrum in $CDCl_3$ and additional signals were present in its ^{13}C NMR spectrum. The Z group of 13 was reduced to provide **14**.

Due to the inherent stability of the 4-acetamidoanilides of lysine, conventional elongation from the C-terminus was used in the preparation of analogous peptides. Coupling of Boc-Lys(Z) with 4-acetamidoaniline led to **8d** which was appropriately deprotected to form either **8e** or **8f**. Acetylation of **8f** provided **8g**, which in turn was transformed to the N^2-acetyl derivative **8h**. Similarly, **8i** was converted to the N^2-Z-protected lysinanilide **8j**. Coupling of **8f** with Boc-Leu formed **9d** which was deprotected with H_2/Pd to provide one of the target compounds, **9e**. Deprotection of **9d** with TFA provided **9f**, which was coupled with Boc-Leu to provide **10d**. Reduction of **10b** yielded **10e**.

In an effort to further explore the kinetic effects of monosaccharides on acrosin activity, an N-terminal glycosyl derivative was attached *via* a small bridge to the N^2 of lysyl 4-acetamidophenyl esters and amides. The majority of synthetic and naturally-occurring glycopeptides have the carbohydrate attached to an amino acid or peptide either at the anomeric carbon or to a ring carbon *via* a lactyl ether.[31] A notable example of the latter type is the important naturally occurring glycopeptide, muramyl dipeptide {2-acetamido-3-*O*-[(R)-1-carboxyethyl-L-alanyl-D-isoglutamine]-2-deoxy-D-glucose, MDP}. For the work reported here, the protected L-lysine 4-acetamidophenyl esters and anilides were attached not through the anomeric carbon, but by means of an ether linking the 3-OH group of appropriate D-glucose and D-fructose derivatives *via* a glycolylamide (-*O*-CH_2-CO-NH). By attaching the glycolyl bridge to a ring OH rather than at the anomeric center, more stable derivatives (ether *vs* acetal) are obtained.

D-Glucose and D-fructose were protected as their bis-isopropylidene acetals[32,33] leaving the 3-OH group available for bridging to an amino acid or peptide *via* a glycolyl spacer. The reaction of D-glucose with acetone in the presence of sulfuric acid provided 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (15).[33] Alkylation of the 3-OH group with ethyl bromoacetate in the presence of sodium hydride in tetrahydrofuran (THF) furnished the ether ester **16a**[34], if excess sodium hydride was decomposed with ethanol at the end of the reaction. Interestingly, if methanol is used for this purpose,[34] facile ester interchange takes place and the methyl ester **16b** is isolated. Alkaline hydrolysis of **16a** or **16b** leads to the acid **16c**. Coupling of **16c** with N^6-(benzyloxycarbonyl)-L-lysin-4-acetamidoanilide furnished **16d**.

Acetalization of D-fructose with acetone and sulfuric acid as catalyst yields the kinetic product, 1,2:4,5-di-*O*-isopropylidene-ß-D-fructopyranose (17)[32a] which was alkylated by ethyl bromoacetate to provide **18a**. Hydrolysis of **18a** gave acid **18c** which was converted by 4-acetamidoaniline to anilide, **18f**. Coupling of **18c** with the 4-acetamidophenyl ester **8c** or anilide **8f** provided **18g** and **18d**, respectively.



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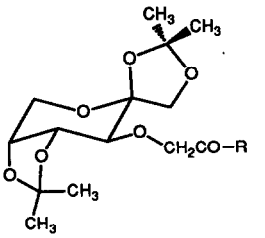

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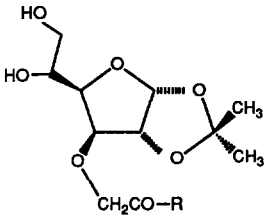

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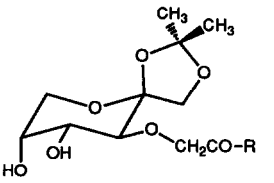

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where **a**, R = OCH_2CH_3 **d**, R = Lys(Z)-NHAr **g**, R = Lys(Z)-OAr
b, R = OCH_3 **e**, R = Lys-NHAr·TsOH **h**, R = Lys-OAr·TsOH
c, R = OH **f**, R = NHAr
and Ar = 4-acetamidophenyl

Our original intent was to completely deprotect **16d, 18d, 18f** and **18g**. Although the lysyl N^6-Z group was hydrogenolyzed readily, it was not possible to remove both acetals without complete destruction of the target molecules. Attempted hydrolyses of both acetals under a variety of relatively mild conditions such as, acetic, oxalic, trifluoroacetic, hydrochloric, sulfuric, and 4-toluenesulfonic acids[35], cation-exchange resins[36] and Lewis acids[37], resulted in partial cleavage of the glycolyl amide, as evidenced by the appearance of the amino component in the reaction mixture (^{1}H NMR). The unusual lability of these glycolylamides is attributed to a facile acid-catalyzed intramolecular nucleophilic acyl displacement of the amide due to the close proximity of one of the neighboring ring hydroxyls once released from the acetal.[38]

Selective cleavage of one of the acetals was readily accomplished, prior to removal of the Z group. Preferential hydrolysis of sugar acetals is well documented and in general the one which incorporates an anomeric oxygen atom is hydrolyzed slower than others.[32c,e] The order of deprotection (Z vs acetal) was independent of the observed lability of the glycolylamide. Experimentally, the reaction mixture was most readily purified if the acetal was removed before hydrogenolysis of the Z group. The partially protected glycosyl derivatives hydrogenolyzed sluggishly and required higher than normal pressure (60 psi). Selective hydrolysis of the 5,6-isopropylidene group of **16d**, by 60% acetic acid at 45 °C[39], followed by catalytic hydrogenolysis afforded **19e** in 77% yield. Similarly, the 4,5-isopropylidene group was removed cleanly from **16d, 18f** and **18g**, as above, followed by reduction to yield **19e, 20f** and **20h**, respectively, in excellent yields.

Structures of all compounds were confirmed through detailed analyses of their ^{1}H and ^{13}C NMR spectra (Tables 1-4) to ensure their configurational and stereoisomeric purity. Appropriate (in particular, 2D) NMR experiments were used to establish chemical shifts. Reviews pertaining to NMR techniques in establishing the structure of peptides,[40] carbohydrates and glycopeptides[41] are available. Furthermore, the mass spectra of the peptides and carbohydrates were consistent with the assigned structures.[42]

DISCUSSION OF BIOLOGICAL TESTING

The compounds reported in Table A were examined for their ability to inhibit the amidase (BAPNA) activity of partially purified human acrosin[48,7b,49] and bovine trypsin. With the exception of **20f** (which lacks a basic amino acid necessary for primary specificity and was found to be devoid of activity), these anilides and esters were found to be weak inhibitors of acrosin displaying no apparent selectivity over trypsin. The relatively poor activity found suggests that these compounds are acting as competitive substrates for the enzymes. The lack of selectivity for acrosin and the good trypsin activity by the fructosyl derivative **20h** suggests that this compound is not interacting with the enzymes in the manner by which the previously reported monosaccharides selectively inhibit acrosin.

TABLE A. INHIBITION OF ACROSIN AND TRYPSIN AMIDASE ACTIVITY

% Inhibition (S.E.M., n)[a]

	Acrosin		Trypsin	
Compound[b]	[I]=2.0 mM	[I]=0.5 mM	[I]=2.0 mM	[I]=0.5 mM
8b	22 (3,2)		49 (3,3)	19 (2,2)
10b	43 (2,3)		83 (3,3)	39 (4,2)
14	48 (2,3)		77 (2,3)	
3b	32 (3,2)		26 (2,2)	
6e	34 (2,2)		23 (1,2)	
88h	42 (2,3)		36 (3,2)	
8e	28 (4,2)		45 (2,2)	19 (3,2)
8j	64 (2,3)		62 (1,3)	33 (3,2)
9e	46 (2,2)	16 (4,2)	60 (2,4)	21 (1,3)
10e	66 (1,3)	33 (1,3)	69 (3,3)	37 (2,2)
20f	5 (1,4)		0 (0,3)	
20e		5 (2,3)		13 (4,3)
19e	28 (3,3)		60 (4,3)	32 (2,3)
20h	63 (1,3)	26 (2,3)	88 (3,3)	54 (2,2)

[a] % Inhibition in the presence of inhibitor; (S.E.M.: standard error of the mean; n: number of determinations).
[b] All compounds, excedpt **20f**, were tested as the 4-toluenesulfonates.

TABLE 1A: ^{1}H CHEMICAL SHIFTS OF PEPTIDE 4-ACETAMIDOPHENYL ESTERS

Group	Protons	8a	8b	8c	10a	10b	13	14
Leu[a]	NH-2				6.91	6.99	6.94	6.94
	H-2				3.99	3.98	3.98	3.94
	H-3				1.39	1.38	1.40	1.42
	H-4				1.57	1.55	1.64	1.59
	H-5				0.83	0.85[b]	0.86[b]	0.85
	H-5'				0.86	0.85	0.86	0.89
Leu[a]	NH-2				7.80	7.78	7.76	7.75
	H-2				4.45	4.43	4.99	4.31
	H-3				1.44	1.44	1.44	1.48
	H-4				1.62	1.63	1.64	1.63
	H-5				0.83	0.85	0.86	0.85
	H-5'				0.86	0.85	0.86	0.89
Lys	NH-2	7.45	7.46	8.67	8.46	8.51	8.24	8.29
	H-2	4.12	4.12	4.32	4.35	4.35	4.17	4.20
	H-3	1.80	1.72	1.97	1.78	1.75	1.67	1.62
	H-4	1.44	1.38	1.50	1.36	1.58	1.38	1.30
	H-5	1.45	1.59	1.48	1.44	1.59	1.41	1.57
	H-6	3.03	2.78	3.07	3.04	2.79	2.97	2.76
	NH-6	7.29	7.67	7.33	7.29	7.78	7.26	7.73
OAr[c]	CH_3	2.05	2.04	2.07	2.05	2.04		
	Phenyl[d]	7.01	7.00	7.14	7.01	6.99		
	Phenyl[e]	7.67	7.61	7.69	7.62	7.61		
	NH	10.02	10.03	10.16	10.02	10.06		
Boc	CH_3	1.41	1.41		1.38	1.38	1.37	1.38
Z	CH_2	5.02		5.03	5.02		5.00	
	Phenyl[f]	7.35		7.35	7.35		7.34	
TsOH	CH_3		2.29			2.29		2.29
	Phenyl		7.19			7.19		7.12
	Phenyl		7.51			7.51		7.48
OMe	CH_3						3.60	3.61

TABLE 1B: ^{1}H CHEMICAL SHIFTS OF PEPTIDE 4-ACETAMIDOANILIDES

Group	Protons	8d	8e	8f	9d	9e	9f	10d	10e	8g	8h	8i	8j
Leu[a]	NH-2							6.95	6.98				
	H-2							3.96	3.97				
	H-3							1.40	1.40				
	H-4							1.62[g]	1.58				
	H-5							0.85[b]	0.85				
	H-5'								0.87				
Leu[a]	NH-2				6.96	6.96	8.20	7.81	7.84[h]				
	H-2				3.99	3.99	3.58	4.36	4.35				
	H-3				1.40	1.46	1.57	1.45	1.48				
	H-4				1.57	1.61	1.67	1.62	1.63				
	H-5				0.85	0.86	0.88	0.85	0.85				
	H-5'				0.87	0.88	0.91		0.87				
Lys	NH-2	6.96	6.99	8.33	7.87	7.93	8.76	7.97	8.05[h]	8.12	8.16	7.50	7.32
	H-2	4.00	4.03	3.95	4.37	4.41	4.43	4.32	4.35	4.32	4.35	4.09	4.11
	$H\text{-}3_A$	1.59	1.54	1.82	1.64	1.61	1.66	1.65	1.60	1.60	1.52	1.58	1.60
	$H\text{-}3_B$	-	1.64	-	-	1.72	-	-	1.72	-	1.62	1.66	1.68
	H-4	1.32	1.31	1.36	1.28	1.33	1.38	1.28	1.31	1.29	1.32	1.32	1.37
	H-5	1.39	1.56	1.44	1.38	1.55	1.45	1.41	1.55	1.40	1.51	1.39	1.54
	H-6	2.98	2.76	3.00	2.97	2.73	3.00	2.98	2.78	2.98	2.75	2.89	2.76
	NH-6	7.24	3.42	7.25	7.23	3.34	7.26	7.24	3.62	7.26	3.50	6.78	7.68
NHAr[c]	CH_3	2.02	2.01	2.04	2.02	2.02	2.02	2.01	2.02	2.02	2.02	2.01	2.02
	Phenyl[f]	7.50	7.50	7.56	7.50	7.51	7.51	7.50	7.52	7.51	7.52	7.50	7.52
	NH	9.86	9.88	10.01	9.89	9.91	9.92	9.88	9.90	9.90	9.90	9.87	9.91
	NH	9.88	9.90	10.51	9.95	10.01	9.94	9.92	9.93	9.97	9.99	9.93	9.98
Boc	CH_3	1.38	1.38		1.37	1.38		1.37	1.38			1.36	
Z	CH_2	5.00		4.99	4.99		4.99	4.99		4.99		5.03	5.04
	Phenyl[f]	7.33		4.99	7.33		7.34	7.34		7.34		7.36	7.36
TsOH	CH_3		2.29				2.29		2.29		2.29		2.28
	Phenyl		7.13			7.13			7.14		7.14		7.13
	Phenyl		7.52			7.51			7.53		7.52		7.52
Acetyl	CH_3									1.86	1.88		

[a]In peptides containing more than one leucyl residue, assignments of H-5 and H-5' are interchangable. [b]Center of overlapping signals. [c]Ar is 4-acetamidophenyl. [d]Assigned to H-2' (H-6'). [e]Assigned to H-3' (H-5'). [f]Narrow multiplet for aromatic proton signals. [g]Center of overlapping cross-peaks in H,H-COSY spectra. [h]Assignments may be interchanged.

TABLE 2A: ^{13}C CHEMICAL SHIFTS OF PEPTIDE 4-ACETAMIDOPHENYL ESTERS

Group	Carbons	8a	8b	11	12	8c	10a	10b	13	14
Leu[a]	C-2			52.53	52.75		52.77	52.83	52.84	52.87
	C-3			39.78	40.84		40.72	40.63	40.74	40.65
	C-4			24.10	24.12		23.97	23.97	23.94	23.94
	C-5			21.14	21.29		21.61	21.57	21.63	21.62
	C-5'			22.88	22.83		22.97	22.94	22.98	22.96
Leu[a]	C-2			51.72	49.99		50.38	50.48	50.41	50.55
	C-3			40.64	40.35		41.20	41.10	41.23	41.06
	C-4			24.12	24.10		24.25	24.22	24.26	24.25
	C-5			21.74	21.61		21.73	21.63	21.73	21.66
	C-5'			22.88	22.88		23.01	23.01	23.08	23.07
Lys	C-2	53.96	53.69			52.01	52.33	52.10	51.93	51.71
	C-3	30.13	29.81			29.78[b]	30.18	29.79	30.42	30.14
	C-4	22.85	22.40			21.62	22.98	22.27	22.70	22.28
	C-5	29.03	26.49			28.90[b]	29.02	26.49	29.00	26.45
	C-6	40.06	38.58			39.87	39.89	38.57	39.75	38.66
OAr[c]	CH_3	23.94	23.90			23.90	23.94	23.93		
	C-1'	145.61	145.52			144.79	145.54	145.46		
	C-2'	121.65	121.57			121.53	121.57	121.55		
	C-3'	119.89	119.91			120.04	119.85	119.83		
	C-4'	137.11	137.08			137.66	137.11	137.10		
	C=O	168.28	168.26			168.42	168.24	168.26		
Boc	CH_3	28.19	28.15	28.17	28.10		28.15	28.14	28.15	28.15
	C	78.44	78.49	77.88	77.92		78.01	78.05	78.07	78.07
	C=O	155.78	155.70	155.21	155.11		155.29	155.27	155.28	155.28
Z	CH_2	65.18				65.21	65.17		65.13	
	C-1'[d]	137.30				137.66	137.29		137.28	
	C-2'[d]	127.77				127.75	127.77		127.74	
	C-3'	128.38				128.35	128.38		128.38	
	C=O	156.20				156.32	156.14		156.09	
TsOH	CH_3		20.77				20.76		20.78	
	C-1'[e]		137.84				137.80		137.97	
	C-2'[f]		125.47				125.47			125.50
	C-3'		128.12					128.10		128.16
	C-4'		145.32					145.38		145.16
Carbonyl	C=O[g]	171.87	171.66	172.71	172.39	168.81	170.91	170.79	172.09	172.24
	C=O			172.90	173.94		172.21	172.24	172.18	172.24
	C=O						172.49	172.48	172.38	172.26
OMe	CH_3			49.97					51.83	51.80
CF_3CO_2H	CF_3					117.08				
	C=O					160.17				

TABLE 2B: ^{13}C CHEMICAL SHIFTS OF PEPTIDE 4-ACETAMIDOANILIDES

Group	Carbons	8d	8e	8f	9d	9e	9f	10d	10e	8g	8h	8i	8j
Leu[a]	C-2							52.75	52.89				
	C-3							40.47	40.58				
	C-4							23.93	24.26				
	C-5							21.49	21.52				
	C-5'							22.83	22.93				
Leu[a]	C-2				52.88	52.92	50.73	50.74	50.89				
	C-3				40.65	40.62	40.26	40.80	40.82				
	C-4				24.21	24.26	23.46	24.09	24.01				
	C-5				21.42	21.49	21.86	21.58	21.67				
	C-5'				22.94	22.65	22.73	23.08	23.18				
Lys	C-2	54.92	54.88	52.96	52.99	52.92	53.60	53.19	53.21	53.24	53.24	55.19	55.26
	C-3	31.50	31.30	30.91	31.96	31.73	31.89	31.75	31.26	31.74	31.56	31.44	31.29
	C-4	22.79	22.59	21.59	22.53	22.41	22.63	22.63	22.66	22.71	23.89	22.82	22.54
	C-5	29.06	26.90	29.00	29.07	26.71	29.23	29.04	26.79	29.07	26.82	29.10	26.65
	C-6	39.84	38.66	39.98	40.11	38.65	40.06	40.09	39.41	40.02	38.69	39.37	38.69
NHAr[c]	CH_3	23.77	23.86	23.89	23.84	23.87	23.88	23.80	23.88	23.78	23.89	23.72	23.91
	C-1'[e]	134.16	134.24	133.24	134.00	134.04	134.03	134.06	134.10	134.08	134.16	134.01	134.19
	C-2'[f]	119.26	119.31	119.50	119.25	119.29	119.29	119.19	119.30	119.18	119.30	119.19	119.33
	C-3'	119.50	119.53	120.05	119.55	119.59	119.56	119.45	119.55	119.54	119.68	119.48	119.63
	C-4'	134.75	134.83	135.70	134.94	134.99	135.05	134.83	134.98	134.78	134.94	134.67	134.95
	C=O	167.83	167.93	167.12	167.89	167.96	167.97	167.85	167.98	167.85	167.99	167.95	168.02

TABLE 2B CONTINUED: ^{13}C CHEMICAL SHIFTS OF PEPTIDE 4-ACETAMIDOANILIDES

Group	Carbons	8d	8e	8f	9d	9e	9f	10d	10e	8g	8h	8i	8j
Boc	CH_3	28.14	28.20		28.10	28.17		28.06	28.16			28.16	
	C	77.93	78.04		78.02	78.07		78.01	78.08			77.35	
	C=O	155.35	155.45		155.34	155.38		155.22	155.32			155.57	
Z	CH_2	65.03		65.18	65.06		65.15	65.02		65.02		65.37	65.49
	C-1'	137.21		137.26	137.21		137.25	137.17		137.12		136.88	136.98
	C-2'[d]	127.62		127.75	127.65		127.73	127.62		127.58		127.63	127.79
	C-3'	128.25		128.36	128.25		128.35	128.23		128.26		128.26	128.37
	C=O	155.98		156.14	156.01		156.12	155.97		155.98		156.96	156.10
TsOH	CH_3		20.78			20.77				20.79		20.79	20.08
	C-1'[e]		145.32			145.38				145.15		145.25	145.16
	C-2'[f]		125.47			125.49				125.40		125.41	125.21
	C-3'		128.12			128.11				128.18		128.18	128.22
	C-4'		137.86			137.82				138.01		137.96	138.04
Carbonyl	C=O[g]	170.90	170.90	168.16	170.15	170.09	168.95	169.96	169.96	169.26	169.41	170.64	170.64
	C=O				172.46	172.55	169.67	171.87	172.00	170.51	170.49		
	C=O							172.33	172.49				
Ac	CH_3									22.37	22.49		
CF_3CO_2H	CF_3			116.13			116.11						
	C=O			159.20			159.23						

[a]In peptides containing more than one leucyl residue, assignments of C-5 and C-5' are interchangable. [b]Assignments may be interchanged. [c]Ar is 4-acetamidophenyl. [d]Signals from C-2'(C-6') and C-4' are overlapping. [e]Assignment of C-1' and C-4' may be interchanged. [f]Assignment of C-2'(C-6') and C-3'(C-5') may be interchanged. [g]These carbonyl carbon signals were not assigned.

TABLE 3A: ^{1}H CHEMICAL SHIFTS OF GLUCOSE DERIVATIVES

Group	Protons	15	16b	16c	16d	19e
Glucofuranose	H-1	5.82	5.85	5.84	5.88	5.85
	H-2	4.40	4.70	4.70	4.72	4.68
	H-3	3.96	3.92	3.89	3.97	3.93
	H-4	3.80	4.08	4.07	4.05	4.01
	H-5	4.22	4.27	4.26	4.31	3.71
	$H\text{-}6_A$	3.94	3.82	3.79	3.87	3.43
	$H\text{-}6_B$	-	4.01	3.98	4.05	3.60
Isopropylidene	CH_3	1.23	1.25	1.25	1.29	1.26
	CH_3	1.27	1.28	1.28	1.34	1.40
	CH_3	1.32	1.32	1.33	1.36	-
	CH_3	1.38	1.39	1.39	1.41	-
Glycolyl CH_2	H_A		4.25	4.13	4.05	4.08
	H_B		-	-	4.16	4.19
Lys	NH-2				7.68	8.44
	H-2				4.47	4.38
	$H\text{-}3_A$				1.56	1.73
	$H\text{-}3_B$				1.74	-
	H-4				1.29	1.40
	H-5				1.39	1.56
	H-6				2.99	2.78
	NH-6				7.24	3.34
NHAr[a]	CH_3				2.03	2.01
	Phenyl[b]				7.52	7.50
	NH				9.89	9.88
	NH				10.06	10.01
Z	CH_2				5.00	
	Phenyl[b]				7.34	
TsOH	CH_3					2.29
	Phenyl				7.11	
	Phenyl					7.47
Ring alcohol	OH-3	5.37				
	OH-5					5.29
	OH-6					4.56
OMe	CH_3		3.67			
Acid	CO_2H			9.12		

TABLE 3B: ^{1}H CHEMICAL SHIFTS OF FRUCTOSE DERIVATIVES

Group	Protons	17[c]	18a[c]	18c[c]	18f	20f	18g	18d	20h	20e
Fructofuranose	H-1_A	3.99	3.96	3.96	3.85	3.85	3.79	3.84	3.83	3.83
	H-2_B	4.19	4.50	4.42	4.38	4.25	4.30	4.22	4.19	4.10
	H-3	3.66	3.64	3.64	3.62	3.67	3.56	3.59	3.54	3.53
	H-4	4.15	4.37	4.36	4.24	3.80	4.18	4.17	3.61	3.68
	H-5	4.21	4.20	4.21	4.24	3.78	4.20	4.21	3.74	3.70
	H-6_A	4.01	4.00	4.01	3.96	3.55	3.92	3.95	3.58	3.58
	H-6_B	4.16	4.13	4.12	-	3.76	-	-	3.74	3.74
Isopropylidene	CH_3	1.37	1.34	1.36	1.30	1.37	1.27	1.27	1.35	1.39
	CH_3	1.44	1.43	1.43	1.37	1.40	1.36	1.40	1.38	1.39
	CH_3	1.51	1.48	1.50	1.40	-	1.39	1.41	-	-
	CH_3	1.53	1.49	1.50	1.42	-	1.42	1.45	-	-
Glycolyl CH_2	H_A		4.35	4.42	4.37	4.31	4.28	4.24	4.36	4.16
	H_B		4.56	4.56	-	-	-	-	4.17	4.28
Lys	NH-2						7.99	7.62	8.21	7.88
	H-2						4.47	4.49	4.51	4.47
	H-3_A						1.74	1.58	1.70	1.52
	H-3_B						-	1.72	1.81	-
	H-4						1.36	1.32	1.37	1.31
	H-5						1.39	1.42	1.60	1.49
	H-6						3.01	2.99	2.80	2.72
	NH-6						7.27	7.23	3.43	3.40
OAr[a]	CH_3						2.04		2.04	
	Phenyl[d]						7.01		7.04	
	Phenyl[e]						7.60		7.61	
	NH						10.16		10.04	
NHAr[a]	CH_3				2.01	2.02		2.03		2.01
	Phenyl[b]				7.50	7.52		7.52		7.50
	NH				9.53	9.87		9.90		9.88
	NH				9.89	9.89		10.09		
Z	CH_2						5.00	4.99		
	Phenyl[b]						7.34	7.34		
TsOH	CH_3								2.29	2.29
	Phenyl							7.13	7.11	
	Phenyl								7.50	7.47
Ring alcohol	OH-3	2.52								
	OH-4					5.60			5.36	5.26
	OH-5					4.87			4.84	4.80
OEt	CH_2		4.18							
	CH_3		1.26							
Acid	CO_2H			9.40						

[a]Ar is 4-acetamidophenyl. [b]Narrow multiplet for aromatic proton signals. [c]Recorded in $CDCl_3$. [d]Assigned to H-2' (H-6'). [e]Assigned to H-3' (H-5').

TABLE 4: ^{13}C CHEMICAL SHIFTS OF GLUCOSE AND FRUCTOSE DERIVATIVES

Group	Carbons	15	16b	16c	16d	19e	17[a]	18a[a]	18c[a]	18f	20f	18g	18d	20h	20e
Glucofuranose	C-1	104.54	104.60	104.62	104.63	104.83									
	C-2	84.98	81.96	82.05	81.53	81.23									
	C-3	73.15	82.52	82.42	82.43	82.23									
	C-4	80.94	80.11	80.15	80.23	79.09									
	C-5	72.24	72.52	72.62	72.28	69.03									
	C-6	66.05	67.09	65.70	66.16	63.13									
Fructopyranose	C-1						72.28	71.73	71.80	71.15	71.42	71.27	71.34	71.64[k]	71.49
	C-2						104.53	104.11	104.01	103.94	105.48	104.29	103.90	105.50	105.36
	C-3						70.28	76.00	76.66	77.33	78.81	77.81	77.42	78.80	78.26
	C-4						77.29	77.58	77.16	76.41	70.12	76.92	76.53	70.22	70.25
	C-5						73.34	73.91	73.86	73.23	69.04	73.48	73.21	69.08	69.02
	C-6						60.72	60.07	60.26	59.76	64.88	59.92	59.77	65.07	64.83
Isopropylidene	CH_3	25.24	25.25	25.25	25.12	26.13	25.97	25.87	25.87	26.00	26.21	26.36	26.07	26.33	26.23
	CH_3	26.04	26.03	26.11	26.00	22.62	26.27	26.19	26.09	26.19	26.45	26.60	26.22	26.33	26.28
	CH_3	26.57	26.46	26.51	26.59	-	26.41	26.78	26.57	26.31	-	26.64	26.29	-	-
	CH_3	26.07	26.58	26.65	26.63	-	27.96	28.01	27.83	27.75	-	28.23	27.85	-	-
	C	107.86	108.00	107.87	108.38	110.91	109.43	108.99	109.39	108.38	110.73	108.98	108.43	110.73	110.48
	C	110.61	111.12	110.89	110.89	-	111.88	111.99	112.21	111.16	-	111.73	111.25	-	-
Glycolyl	CH_2		65.72	67.12	68.60	67.56		67.34	67.25	70.12	71.88	70.07	70.08	71.39[k]	71.49

TABLE 4 CONTINUED: ^{13}C CHEMICAL SHIFTS OF GLUCOSE AND FRUCTOSE DERIVATIVES

Group	Carbons	15	16b	16c	16d	19e	17[a]	18a[a]	18c[a]	18f	20f	18g	18d	20h	20e
Lys	C-2				52.73	53.20						52.24	52.43	51.63	52.38
	C-3				31.93	31.18						30.89	32.37	30.51	31.96
	C-4				22.70	22.73						22.82	22.46	22.56	22.33
	C-5				29.08	26.90						29.31	29.15	26.33	26.94
	C-6				40.12	38.91						40.37	40.13	40.18	38.65
OAr[b]	CH_3											24.17		23.98	
	C-1'											145.91		145.18	
	C-2'											122.08		121.88	
	C-3'											120.42		120.09	
	C-4'											137.27		137.17	
NHAr[b]	CH_3				23.86	23.90				23.75			23.87		23.86
	C-1'[c]				133.94	133.99				133.47			133.94		133.96
	C-2'[d]				119.27	119.34				119.43			119.30		119.27
	C-3'				119.76	119.84				119.50			119.73		119.77
	C-4'				135.05	135.05				135.10			135.06		135.04
Z	CH_2				65.10							65.63	65.12		
	C-1'				137.24							137.51	137.25		
	C-2'[e]				127.29							128.15	127.70		
	C-3'				127.69							128.80	128.31		
	C=O				156.06							156.70	156.06		
TsOH	CH_3					20.81								20.96	20.75
	C-1'[c]					145.45								145.61	145.25
	C-2'[d]					125.53								125.68	125.48
	C-3'					128.14								128.39	128.06
	C-4'					137.84								138.30	137.31
OMe	CH_3		51.52												
OEt	CH_2							60.59							
	CH_3							14.18							
Other	C=O[f]		170.25	171.33	167.93	168.07		170.44	174.28	167.53	168.29	169.11	167.95	168.66	167.96
	C=O				168.75	169.99				167.92	168.60	170.20	168.79	170.66	169.90
	C=O				169.89	170.06						171.31	169.75	170.94	169.72

[a]Recorded in $CDCl_3$. [b]Ar is 4-acetamidophenyl. [c]Assignment of C-1' and C-4' may be interchanged. [d]Assignment of C-2'(C-6') and C-3'(C-5') may be interchanged. [e]Signals from C-2'(C-6') and C-4' are overlapping. [f]These carbonyl carbon signals were not assigned.

Experimental Section

Amino acid derivatives were purchased from Sigma Chemical Company (St. Louis, MO) or Chemical Dynamics Corp. (South Plainfield, NJ) and checked for purity (NMR and TLC). Solvents and reagents were purified just before use: THF was distilled from $LiAlH_4$, pyridine from CaH_2, NMM and triethylamine from KOH pellets, CH_2Cl_2 and EtOAc from P_2O_5, and DMF from ninhydrin (*in vacuo*). Hydrated reagents (e.g. protected amino acids, HOBt, TsOH) were dried azeotropically with toluene, prior to use. "Petroleum ether" refers to that fraction, bp 30-60 °C. Evaporation of liquids, '*in vacuo*', implies vacuum distillation at room temperature by means of a rotary evaporator using a water or oil pump. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. Microanalyses were determined by Microtech Labs, Skokie, IL, or Midwest Microlab, Indianapolis, IN. Special combustion techniques (powdered tin, 980 °C in pure O_2) were required for some of the peptides to avoid "residues". Optical rotations were determined on a Perkin Elmer 241 Polarimeter. Mass spectra were recorded from a Varian MAT 112 S Spectrometer or Finnigan MAT 4510 Quadrapole Spectrometer. Ions with $m/z < 40$ and relative intensities < 10% of the base peak are not reported, unless deemed significant. Thin layer chromatograms (TLC) were run on silica gel 60F-254 precoated plates. Spots were visualized by UV, I_2, or by ninhydrin in EtOH. Compounds with Boc groups were sprayed first with TFA-CH_2Cl_2 (1:1, to liberate the amino group) and then with ninhydrin. Flash chromatography[43] utilized silica gel (40μm) and for reverse flash chromatography bonded phase octadecyl (C_{18}) silica gel (40μm) (J T Baker) was used. Vessels were protected with aluminum foil during reactions involving light-sensitive 4-acetamidoaniline.

NMR spectra were recorded in approximately 0.2 M solutions in DMSO-d_6 at 20°C (unless otherwise specified) on a Varian XL-300 spectrometer operating at 299.943 MHz for proton and 75.429 MHz for carbon. Chemical shifts (δ) are compiled in Tables 1-4, in parts per million downfield from internal tetramethylsilane. Abbreviations used are s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br for broad signals. Chemical shifts for AA'XX' spin systems are reported from the center of each half of the symmetrical multiplet. Homonuclear shift-correlated (COSY) spectra were obtained using Jeener's 2-pulse sequence.[44] A read pulse of 60° and a relaxation delay of 1 s were used. Data matrices were generally 512 x 1024 points with one level of zero filling in the F_1 dimension to a final matrix of 1024 x 1024 points. A pseudo-echo gaussian weighting function was used in both dimensions. The spectra were displayed in the absolute value mode and symmetrized.[45] Heteronuclear shift correlated (HETCOR) spectra were obtained using the pulse sequence described,[46] with a 1 s relaxation delay. Data matrices were generally 128 x 512 points with one level of zero filling in the F_1 dimension. A pseudo-echo gaussian weighting function was used in both dimensions.

GENERAL PROCEDURES

Method A: Synthesis of Aryl Esters, Anilides and Peptides from EDC-Mediated Condensations. The diimide, solvent, temperatures, and work-up for these reactions are the same, but auxiliary reagents, and reaction times were varied. Reactions were checked at suitable intervals by either TLC and/or NMR. The acid, the amine or phenol, NMM (when a base was required), and auxiliary reagents were stirred at 0-5 °C in CH_2Cl_2-DMF (1:1) until the majority of the reagents had dissolved (ca. 15 min). EDC was added and the mixture stirred at 0-5 °C (1 h), then at room temperature (4 h). Solvents were removed, *in vacuo,* at 25-30 °C and the residue was dissolved in EtOAc (150 mL). After washing successively with 5% aq. citric acid (3 x 100 mL), 5% aq. $NaHCO_3$ (3 x 100 mL) and brine (2 x 50 mL), the extract was dried ($MgSO_4$), evaporated, *in vacuo*, and the product purified.

Method B: Cleavage of Boc Groups by TFA. Boc derivatives were dissolved in cold 70% aq. TFA (7.5 mL/ mmol) and stirred (0-5 °C) until the reactions were complete as followed by the disappearance of starting material (TLC, ca. 1.5 h). Solvents were removed, *in vacuo*, and the resultant *trifluoroacetates* triturated with ether and dried, *in vacuo*, (KOH pellets).

Method C: Hydrogenolysis of *N*-Benzyloxycarbonyl Groups. To a solution of the carbamate in HOAc (30 mL/ mmol, under N_2) was added 10% palladium on carbon (10% by weight of compound). The mixture was hydrogenated (Parr apparatus, 40 psi H_2 at 25 °C) until the reaction was complete (TLC, ca. 1 h). The catalyst was filtered (millipore filter paper), washed with HOAc and the filtrate concentrated to about one-half, *in vacuo*, (20-25 °C). Anhydrous TsOH (1 molar equivalent) was then added and the remaining solvent removed, *in vacuo*. The 4-*toluenesulfonates* were dried, *in vacuo*, (P_2O_5) and purified further by recrystallization and/or reverse phase column chromatography.

***N*^{2}-(*tert*-Butoxycarbonyl)-*N*$^\omega$-nitro-L-arginin-4-acetamidoanilide.** Boc-Arg(NO_2) (3.00 g, 7.8 mmol), $ArNH_2$ (4.68 g, 31 mmol), DMAP (0.38 g, 3.1 mmol) and EDC (1.79 g, 9.3 mmol) were reacted as outlined in Method A. The product was purified by flash chromatography. Elution with EtOAc and HOAc (0-2%) yielded a yellow gummy solid (2.11 g, 60%), softens 100, mp 128-130 °C; R_f 0.59 (THF); $[\alpha]_D^{20}$ -4.0° (*c* 1.0, EtOH); ^{1}H NMR: δ Boc CH_3 (1.39), Arg [7.03 (NH), 4.03 (H-2), 1.65 (H-3) 1.56 (H-4), 3.15 (H-5), guanidine NH's (7.93, 8.50)], ArNH [2.02 (CH_3), 7.50 (phenyl, narrow complex AA'BB' m), 9.87, 9.90 (NH's)]; ^{13}C NMR: δ Boc [28.23 (CH_3), 78.17 (C), 154.5 (C=O)], Arg [54.70 (C-2), 29.30 (C-3), 25.16 (C-4), 40.33 (C-5), 160.0 (guanidine), 170.8 (C=O)], ArNH [23.93 (CH_3), 134.2, 134.9 (C-1 or C-4), 119.4, 119.6 (C-2 or C-3), 168.0 (C=O)]. *Anal.* Calcd for $C_{19}H_{29}N_7O_6 \cdot 0.5H_2O$: C, 49.55; H, 6.57; N, 21.29. Found: C, 49.60; H, 6.58; N, 21.09.

N^2-(*tert*-Butoxycarbonyl)-L-arginin-4-acetamidoanilide 4-Toluenesulfonate (6e). Reduction of Boc-Arg(NO_2)-NHAr (500 mg, 1.11 mmol) according to Method C yielded an oil from which crystallized (aq. 2-PrOH) a colorless hygroscopic solid (610 mg, 95%), mp (softening at 130) 165-167 °C; R_f 0.81 (reverse phase, 2% EtOAc); $[\alpha]_D^{20}$ -2.0° (*c* 0.5, HOAc); ^1H NMR: δ Boc CH_3 (1.39), Arg [7.09 (NH), 4.05 (H-2), 1.61 (H-3) 1.56 (H-4), 3.07 (H-5), guanidine NH's (7.40, 8.46)], ArNH [2.01 (CH_3), 7.50 (phenyl, narrow m, AA'BB'), 9.90, 9.99 (NH's)], TsOH [2.29 (CH_3), 7.13, 7.48 (phenyl, AA'XX', m); there were few significant changes from the ^{13}C NMR data of starting nitro compound, the guanidinium carbon being at 157.0 ppm. *Anal.* Calcd for $C_{26}H_{38}N_6O_7S \cdot H_2O$: C, 52.33; H, 6.76; N, 14.08. Found: C, 52.45; H, 6.96; N, 13.89.

4-Acetamidophenyl N^2-(*tert*-Butoxycarbonyl)-N^6-(benzyloxycarbonyl)-L-lysinate (8a). Boc-Lys(Z) (1.90 g, 5.0 mmol], ArOH (3.80 g, 25 mmol), and DMAP (0.24 g, 2.0 mmol) were dissolved in CH_2Cl_2-DMF (1:1, 10 mL) and cooled (0-5 °C). EDC (1.00 g, 5.2 mmol) was added and the mixture stirred at 0-5 °C (1 h), then at 25 °C (3 h). Solvents were removed, *in vacuo*, the residue dissolved in $CHCl_3$ (200 mL) and the product isolated according to Method A. Recrystallization from EtOAc-petroleum ether furnished a colorless solid (1.69 g, 66%), mp 139-140 °C; R_f 0.60 (EtOAc); $[\alpha]_D^{20}$ -8.0° (*c* 1.0, $CHCl_3$); CIMS (NH_3), *m/z* (relative intensity) 531 [12, $(M + NH_4)^+$], 414 (34), 380 (19), 354 (22), 320 (51), 303 (56), 280 (41), 265 (26), 246 (23), 235 (75), 229 (27), 218 (27), 170 (100), 152 (31). *Anal.* Calcd for $C_{27}H_{35}N_3O_7$: C, 63.14; H, 6.87; N, 8.18. Found: C, 63.08; H, 6.92; N, 8.22.

4-Acetamidophenyl N^2-(*tert*-Butoxycarbonyl)-L-lysinate 4-Toluenesulfonate (8b). Hydrogenolysis of **8a** (500 mg, 0.97 mmol) according to Method C produced an oil from which crystallized (2-PrOH-Et_2O) colorless hygroscopic crystals (470 mg, 87%), mp 149-151 °C; R_f 0.29 (reverse phase, $CHCl_3$); $[\alpha]_D^{20}$ -7.2° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{26}H_{37}N_3O_8S \cdot 0.5H_2O$: C, 55.69; H, 6.83; N, 7.50. Found: C, 55.87; H, 6.92; N, 7.34.

N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucine (12). Boc-Leu (1.1 g, 5 mmol), Leu-OMe·HCl (0.91 g, 5 mmol), NMM (0.55 g, 4.75 mmol), HOBt (0.68 g, 5 mmol) and EDC (1.05 g, 5.1 mmol) were reacted (Method A) in CH_2Cl_2 (15 mL) to provide **11** (1.51 g, 84%) which was recrystallized from EtOAc-petroleum ether, mp 138-140 °C, lit[47] mp 140-141, 141-142 °C; R_f 0.45 ($CHCl_3$); $[\alpha]_D^{20}$ -47.0° (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 302(2), 186 (9), 130 (57), 86 (100), 57 (73), 44 (18). A solution of **11** (1.50 g, 4.20 mmol) was saponified with 1 N NaOH (4.8 mmol) in MeOH (42 mL) at room temperature (ca. 2 h) until **11** had disappeared (TLC). The reaction mixture was diluted with water (85 mL), partially concentrated, *in vacuo*, (25-30 °C), cooled (0-5 °C), and acidified to pH 3.0 with 1 N HCl. After extraction ($CHCl_3$, 3 x 100 mL), the extract was dried ($MgSO_4$) and evaporated, *in vacuo*. Recrystallization from aq. MeOH yielded colorless crystals of **12** (1.43 g, 99%), mp 115-118 °C, R_f 0.12 (EtOAc); $[\alpha]_D^{20}$ -37.9° (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 271 (6), 186 (25), 130 (85), 86 (100), 57 (68), 44 (11), 43 (16), 41 (16). *Anal.* Calcd for $C_{17}H_{32}N_2O_5$: C, 59.26; H, 9.36; N, 8.13. Found: C, 58.91; H, 9.25; N, 8.03.

Methyl N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-N^6-(benzyloxycarbonyl)-L-lysinate (13). Boc-Leu-Leu (0.52 g, 1.50 mmol), was reacted (Method A) with Lys(Z)-OMe·HCl (0.33 g, 1.0 mmol), HOBt (0.20 g, 1.50 mmol), NMM (0.10 g, 0.99 mmol) and EDC (0.27 g, 1.50 mmol) in CH_2Cl_2 (8 mL) and DMF (2 mL) at 0-5°C. The product was recrystallized from aq. MeOH to yield **13** (0.52 g, 84%) as colorless crystals; mp 132-134° C; R_f 0.82 (EtOAc); $[\alpha]_D^{20}$ -44.7° (*c* 1.0, EtOH); CIMS (NH_3), *m/z* (rel intensity) 638 [41, $(M + NH_4)^+$)], 621 (14, MH^+), 521 (53), 487 (100), 387 (42), 361 (52), 344 (25), 297 (45), 244 (29), 186 (22), 169 (32), 136 (36). *Anal.* Calcd for $C_{32}H_{52}N_4O_8$: C, 61.91; H, 8.44; N, 9.02. Found: C, 62.02; H, 8.40; N, 9.08.

Boc-L-Leu-D,L-Leu-Lys(Z)-OMe was synthesized as above, substituting triethylamine (0.20 g, 2.0 mmol) for NMM

and the reaction was carried out in DMF (10 mL) at 40 °C. The product was isolated as a tan solid, (0.58 g, 94%); mp 118-124 °C; R_f (EtOAc) 0.82; $[\alpha]_D^{20}$ -26.4° (*c* 1.0, EtOH). The ^1H NMR spectrum ($CDCl_3$) was identical to the one described for enatiomerically pure **13** except that the methyl ester signal consisted of two singlets at 3.65 and 3.68 ppm. Such an additional signal could not be detected in DMSO. The ^{13}C NMR spectrum (DMSO) contained additional signals at δ 22.51 (Lys C-4), 24.19 (Leu^2 C-4), 28.86 (Lys C-5), 30.67 (Lys C-3), 40.80 (Leu^1 C-3), 50.62 (Leu^2 C-2), 53.06 (Leu^1 C-2).

Methyl N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-L-lysinate 4-Toluenesulfonate (14). The Z group of **13** (500 mg, 0.80 mmol) was removed (Method C). The resultant oil was crystallized from aq. HOAc to afford Boc-Leu-Leu-Lys-OMe·TsOH (515 mg, 98%) as colorless, hygroscopic crystals; mp 142-144 °C; R_f 0.25 (reverse phase, EtOAc); $[\alpha]_D^{20}$ -18.4° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{31}H_{54}N_4O_9S$·HOAc: C, 55.13; H, 8.13; N, 7.79. Found: C, 54.79; H, 8.39; N, 7.51.

4-Acetamidophenyl N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-N^6-(benzyloxycarbonyl)-L-lysinate (10a). The Boc group of **8a** (1.69 g, 3.3 mmol) was cleaved (Method B) and the resultant oil crystallized from EtOAc-Et_2O to provide Lys(Z)-OAr·TFA (**8c**) (1.53 g, 88%), R_f 0.20 (EtOAc), as a hygroscopic amorphous solid which was used without further purification. To a suspension of **12** (2.07 g, 6.0 mmol), and HOBt (0.81 g, 6.0 mmol) in CH_2Cl_2 (10 mL) at 0-5 °C was added a pre-cooled solution (0-5 °C) of EDC (1.14 g, 6.0 mmol) in CH_2Cl_2-DMF (1:1 10 mL). After complete dissolution (ca. 15 min), the mixture was stirred (30 min) and then a cold solution (0-5 °C) of **8c** (2.15 g, 4.0 mmol) and NMM (0.38 g, 3.8 mmol) in CH_2Cl_2 (5 mL) was added dropwise (ca. 10 min). After 6 h at 0-5 °C, there was no further reaction (^1H NMR). Solvents were removed, *in vacuo*, the residue dissolved in $CHCl_3$ (150 mL), washed with brine (2 x 50 mL), dried ($MgSO_4$), and evaporated, *in vacuo*, The oil was flash chromatographed and the product eluted as a gum by EtOAc-Et_2O (1:1) and a gradient of MeOH (0-10%). Recrystallization from CH_2Cl_2-petroleum ether furnished colorless crystals of **10a** (1.38 g, 47%), mp 139-142 °C; R_f 0.67 (EtOAc); $[\alpha]_D^{20}$ -52.5° (*c* 1.0, EtOH); CIMS (NH_3), *m/z* (rel intensity) 606(12), 589 (10), 545 (18), 361 (37), 186 (14), 170 (100), 152 (29). *Anal.* Calcd for $C_{39}H_{57}N_5O_9$: C, 63.30; H, 7.77; N, 9.47. Found: C, 63.21; H, 7.87; N, 9.34.

4-Acetamidophenyl N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-L-lysinate 4-Toluenesulfonate (10b). Removal of the Z group from **10a** (500 mg, 0.68 mmol), (Method C) yielded an oil which was purified by reverse phase flash chromatography (CH_2Cl_2 and a gradient of 0-20% Et_2O) to give the tripeptide (390 mg, 74%) as a colorless, hygroscopic, amorphous solid; mp 164-165 °C; R_f 0.51 (reverse phase, $CHCl_3$); $[\alpha]_D^{20}$ -30.0° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{38}H_{59}N_5O_{10}S$: C, 58.66; H, 7.64; N, 9.00. Found: C, 58.65; H, 7.75; N, 9.33.

N^2-(*tert*-Butoxycarbonyl-N^6-(benzyloxycarbonyl)-L-lysin-4-acetamidoanilide (8d). An ice-cold stirred solution of Boc-Lys(Z) (1.90 g, 5.0 mmol), 4-acetamidoaniline (1.50 g, 10 mmol), DMAP (0.16 g, 1.3 mmol) was stirred in CH_2Cl_2-DMF (1:1, 10 mL) with EDC (1.00 g, 5.2 mmol) at 0-5 °C (1h), then at 25 °C (3h), Method A, furnished crystals (from EtOAc, 1.74 g, 68%) mp 165-168 °C; R_f 0.55 (EtOAc); $[\alpha]_D^{20}$ -8.5° (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 512 (7, M^+), 235 (60), 218 (13), 174 (29), 151 (16), 150 (86), 128 (11), 108 (59), 107 (40), 91 (90), 84 (35), 79 (26), 77 (15), 59 (28), 57 (26), 56 (89), 55 (42), 53 (16), 44 (97), 43 (11), 41 (100). *Anal.* Calcd for $C_{27}H_{36}N_4O_6$: C, 63.26; H, 7.08; N, 10.93. Found: C, 63.14; H, 7.21; N, 10.89.

N^2-(*tert*-Butoxycarbonyl)-L-lysin-4-acetamidoanilide 4-Toluenesulfonate (8e). Removal of the Z group of **8d** (500 mg, 0.98 mmol) by Method C provided an oil which was recrystallized from aq. EtOH (colorless hygroscopic crystals, 520 mg, 96%), mp (softens at 145) 165-166 °C; R_f 0.76 (reverse phase, EtOAc-2% HOAc); $[\alpha]_D^{20}$ +2.4° (*c* 0.5, HOAc). *Anal.* Calcd for

$C_{26}H_{38}N_4O_7S \cdot 0.5H_2O \cdot 0.5EtOH$: C, 55.64; H, 7.26; N, 9.62. Found: C, 55.78; H, 6.92; N, 9.69.

N^2-Acetyl-N^6-(benzyloxycarbonyl)-L-lysin-4-acetamidoanilide (8g). Deprotection of **8d** (1.0 g, 1.95 mmol) furnished **8f** (quantitative) as a hygroscopic amorphous solid, homogeneous to TLC [R_f 0.09 (2% HOAc in EtOAc)], which was used immediately. This trifluoroacetate was suspended in EtOAc (100 mL), washed with 5% $NaHCO_3$ (3 x 100 mL), brine (2 x 50 mL), dried ($MgSO_4$) and solvent evaporated, *in vacuo*. A solution of the amine in dry pyridine (10 mL), cooled in an ice-EtOH bath (*ca.* -10° C) was reacted with acetic anhydride (2.04 g, 20 mmol). After stirring at -10° C (2 h), then 25° (3 h), the reaction was complete (TLC). Solvents were removed, *in vacuo*, the residue dissolved in EtOAc (100 mL), washed with cold 5% citric acid (3 x 100 mL), 5% $NaHCO_3$ (3 x 100 mL), brine (2 x 50 mL), dried ($MgSO_4$). After removal of solvents, the gum was flash chromatographed. Elution with EtOAc and a gradient of THF (0-10%) yielded colorless crystals (0.64 g, 72%), soften 198, mp 227-228 °C; R_f 0.80 (THF); $[\alpha]_D^{20}$ -15.0° (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 346 (6), 304 (17), 202 (10), 150 (39), 135 (13), 134 (95), 133 (15), 126 (23), 109 (13), 108 (100), 107 (38), 106 (40), 105 (11), 93 (12), 84 (42), 80 (27), 56 (13), 53 (12), 52 (18), 44 (16), 43 (75), 42 (20). *Anal.* Calcd for $C_{24}H_{30}N_4O$: C, 63.42; H, 6.65; N, 12.33. Found: C, 63.09; H, 6.72; N, 12.00.

N^2-Acetyl-L-lysin-4-acetamidoanilide 4-Toluenesulfonate (8h). The Z group of **8g** (500 mg, 1.10 mmol) was removed (Method C). The product was purified (reverse phase flash chromatography) using CH_2Cl_2 and a gradient of petroleum ether (0-10%) and provided **8h** (470 mg, 87%) as an amorphous powder; mp 182-183 °C; R_f 0.26 (reverse phase, EtOAc-2% HOAc); $[\alpha]_D^{20}$ -13.8° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{23}H_{32}N_4O_6S$: C, 56.07; H, 6.55; N, 11.37. Found: C, 56.43; H, 6.55; N, 11.47.

N^2-(Benzyloxycarbonyl)-N^6-(*tert*-butoxycarbonyl)-L-lysin-4-acetamidoanilide (8i). To obtain the free acid, Boc-Lys(Z)-OH · $N(C_6H_{11})_2$ (2.50 g, 4.45 mmol) was partitioned between EtOAc (200 mL) and aq. $KHSO_4$ (1.00 g, 7.3 mmol, in 50 mL H_2O). The aq. phase was extracted further with EtOAc (2 x 100 mL). The EtOAc extract was washed with brine (2 x 50 mL), dried (Na_2SO_4) and evaporated, *in vacuo*. The acid was dried azeotropically with toluene, dissolved in CH_2Cl_2-DMF (1:1, 30 mL), cooled (0-5 °C) and treated with $ArNH_2$ (2.67 g, 17.8 mmol), DMAP (0.22 g, 1.8 mmol) and EDC (1.02 g, 5.3 mmol). After stirring at 0-5 °C (1 h), ambient temperature (4 h), the mixture was worked up by method A. Flash chromatography using EtOAc and a gradient of HOAc (0-2%) furnished colorless crystals (1.64 g, 72%), mp 188-189 °C; R_f 0.47 (EtOAc); $[\alpha]_D^{20}$ (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 512 (7, M^+), 348 (10), 150 (42), 134 (13), 128 (10), 108 (100), 107 (76), 91 (80), 84 (44), 79 (73), 59 (40), 57 (24), 56 (35), 44 (61), 43 (15), 41 (62). *Anal.* Calcd for $C_{27}H_{36}N_4O_6$: C, 63.26; H, 7.08; N, 10.93. Found: C, 63.01; H, 7.11; N, 10.78.

N^2-(Benzyloxycarbonyl)-L-lysin-4-acetamidoanilide 4-Toluenesulfonate (8j). The Boc group of **8i** (500 mg, 0.98 mmol) was removed according to Method B. After removal of solvents, anhydrous TsOH (0.17 g, 0.98 mmol) was added and the remaining TFA removed in a vacuum desiccator (KOH pellets). The colorless hygroscopic salt was recrystallized from 2-PrOH-Et_2O, and weighed 470 mg. (82%), mp 190-192 °C; R_f 0.71 (reverse phase, 2% EtOAc-HOAc); $[\alpha]_D^{20}$ -7.4° (*c* 0.5, EtOH). *Anal.* Calcd for $C_{29}H_{36}N_4O_7S \cdot 1.5H_2O$: C, 56.94; H, 6.43; N, 9.16. Found: C, 57.07; H, 6.82; N, 9.16.

N^2-(*tert*-Butoxycarbonyl)-L-leucyl-N^6-(benzyloxycarbonyl)-L-lysin-4-acetamidoanilide (9d). The Boc group of **8d** (2.56 g, 5.0 mmol) was deprotected by Method B to form **8f**, which was reacted with NMM (0.48 g, 4.75 mmol), Boc-Leu (1.16 g, 5.0 mmol), HOBt (0.74 g, 5.5 mmol), and EDC (1.00 g, 5.2 mmol) in CH_2Cl_2-DMF (1:1, 35 mL), according to Method A. Crude **9d** was flash chromatographed and elution with petroleum ether and a gradient of EtOAc (50-100%) yielded as a beige

waxy solid, (1.85 g, 59%), mp 166-167 °C; R_f 0.44 (EtOAc); $[\alpha]_D^{20}$ -39.8° (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 443 (22), 150 (100), 134 (14), 108 (43), 84 (23), 57 (6), 43 (17). *Anal.* Calcd for $C_{33}H_{47}N_5O_7$: C, 63.34; H, 7.57; N, 11.19. Found: C, 63.39; H, 7.65; N, 11.11.

N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-lysin-4-acetamidoanilide 4-Toluenesulfonate (9e). The Z group of **9d** (500 mg, 0.80 mmol) was hydrogenolyzed (Method C) and the colorless hygroscopic salt (480 mg, 90%) recrystallized from EtOH, softens 174, mp 201-203 °C; R_f 0.23 (reverse phase, EtOAc); $[\alpha]_D^{20}$ -19.4° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{32}H_{49}N_5O_8S \cdot H_2O$: C, 56.36; H, 7.54; N, 10.27. Found: C, 56.59; H, 7.91; N, 10.16.

N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-N^6-(benzyloxycarbonyl)-L-lysin-4-acetamidoanilide (10d). The Boc group of **9d** (1.19 g, 1.9 mmol) was removed (Method B) to provide Leu-Lys(Z)-NHAr·TFA (**9f**, quantitative) as a hygroscopic amorphous homogeneous (TLC, NMR) solid which was used immediately and without further purification; R_f 0.18 (EtOAc-2% HOAc). The amino group of **9f** was neutralized, *in situ*, by NMM (0.18 g, 1.78 mmol) and coupled (Method A) with Boc-Leu (0.44 g, 1.9 mmol), in the presence of HOBt (0.32 g, 2.1 mmol) and EDC (0.38 g, 1.98 mmol), in CH_2Cl_2-DMF (1:1, 15 mL). After flash chromatography using EtOAc and a gradient of HOAc (0-2%), the tripeptide was obtained as colorless crystals (0.79 g, 56%), mp 203-204 °C; R_f 0.38 (EtOAc); $[\alpha]_D^{20}$ -52.2° (*c* 1.0, EtOH); EIMS, *m/z* (rel intensity) 225 (38), 157 (25), 151 (22), 150 (100), 134 (13), 129 (12), 108 (35), 107 (19), 86 (15), 84 (60), 69 (15), 43 (37), 41 (20). *Anal.* Calcd for $C_{39}H_{58}N_6O_8$: C, 63.39; H, 7.91; N, 11.37. Found: C, 63.05; H, 7.85; N, 11.28.

N^2-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-L-lysin-4-acetamidoanilide 4-Toluenesulfonate (10e). Removal of the Z group of **10d** (500 mg, 0.68 mmol, Method C) yielded an oil which was purified by reverse phase flash chromatography using EtOAc and a gradient of CH_2Cl_2 (5-10%) to give a tan, hygroscopic powder (480 mg, 91%), mp 209-210 °C; R_f 0.87 (reverse phase, 2% HOAc-EtOAc); $[\alpha]_D^{20}$-24.6° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{38}H_{60}N_6O_9S \cdot 0.5H_2O$: C, 58.06; H, 7.82; N, 10.69. Found: C, 58.30; H, 7.92; N, 10.52.

4-Acetamidophenyl N^2-(*tert*-Butoxycarbonyl)-*p*-guanidino-L-phenylalaninate 4-Toluenesulfonate (3b). A stirred ice-cold solution of N^2-(*tert*-butoxycarbonyl)-*p*-guanidino-L-phenylalanine (**3a**[10i,10t], 0.50 g, 1.55 mmol), ArOH (0.48 g, 3.20 mmol) anhydrous TsOH (0.27 g, 1.57 mmol), and DMAP (0.08 g, 0.64 mmol) in CH_2Cl_2-DMF (1:1, 7 mL) was reacted with EDC (0.34 g, 1.77 mmol). The oily product was flash chromatographed and the product eluted by using EtOAc-$CHCl_3$ (1:1) and a gradient of EtOH (0-10%). The gummy salt was further purified by Soxhlet extraction with Et_2O (to remove ArOH) to provide **3b** (0.17 g, 33%) as a colorless hygroscopic powder, mp 163-165 °C; R_f 0.60 (reverse phase, EtOAc); ^1H NMR: δ Boc CH_3 (1.36), Phe [2.96 (H-3), 3.87 (H-2), 5.96 (NH), 7.20, 7.35 (phenyl, centers of AA'XX' m), guanidine NH's (7.39, 9.69), ArO [2.03 (CH_3), 6.95, 7.60 (phenyl, AA'XX' m), 10.05 (NH)], TsOH [2.29 (CH_3), 7.12, 7.48 (phenyl, AA'XX' m]; ^{13}C NMR: δ Boc [28.21 (CH_3), phenyl [133.9, 135.6 (C-1 or C-4), 124.2, 130.5 (C-2, or C-3), 155.8 (Gu), 171.1 (C=O)], ArNH [23.88 (CH_3), 145.5 (C-1), 121.5 (C-2), 119.9 (C-3), 137.1 (C-4), 168.3 (C=O)], TsOH [20.78 (CH_3), 138.3, 144.7 (C-1) or C-4), 125.5, 128.2 (C-2 or C-3)]. *Anal.* Calcd for $C_{30}H_{37}N_5O_8S \cdot H_2O$: C, 55.80; H, 6.09; N, 10.84. Found: C, 55.86; H, 6.19; N, 11.02.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-carboxymethyl-α-D-glucofuranose (16c). Sodium hydride (60% in mineral oil, 3.74 g, 93.5 mmol) was added, in portions, to a pre-cooled (0-5 °C) solution of **15**[33] (12.1 g, 46.5 mmol) in anhydrous THF (145 mL). After the initial reaction had subsided, the stirred mixture was refluxed (30 min), cooled (0-5 °C) and ethyl bromoacetate (39.0 g, 232 mmol) was added dropwise (colorless precipitate). The mixture was stirred at 0-5 °C (3 h) and then 18h at room

temperature. Methanol (50 mL) was added to decompose excess sodium hydride and the mixture concentrated, *in vacuo*. The oil was dissolved in CH_2Cl_2 (200 mL), washed with ice cold water (3 x 100 mL), dried and evaporated, *in vacuo*. Unreacted ethyl bromoacetate was distilled (bp 82 °C, 1 Torr) and **16b** (10.7 g, 69%) was obtained as beige crystals (from petroleum ether), mp 101-103 °C; R_f 0.21 [petroleum ether-EtOAc (9:1)] ; $[\alpha]_D^{20}$ -6.1° (*c* 1.0, EtOH); CIMS (CH_4), *m/z* (rel intensity) 333 (40, MH^+), 317, (14), 275 (49), 217 (100), 199 (36), 185 (14), 127 (88). *Anal.* Calcd for $C_{15}H_{24}O_8$: C, 54.21; H, 7.27. Found: C, 54.26; H, 7.07. The ethyl ester (**16a**) has previously been as an oil, bp 154-154.5 °C at 2.0 Torr.[33]

A dispersion of **16b** (3.8 g, 11.4 mmol) in 1 N NaOH solution (35 mL) was refluxed (1.5 h). After cooling to 0-5 °C, $CHCl_3$ (75 mL) was added and the aqueous phase acidified to pH 1.5 by the addition of ice cold 1 N HCl. Further extracts with $CHCl_3$ (3 x 100 mL) were combined, dried ($MgSO_4$) and evaporated, *in vacuo*, to afford **16c** (3.45 g, 95%) as colorless gum (agrees with lit[33]) which was used without further purification; R_f 0.31 ($CHCl_3$); EIMS, *m/z* (rel intensity) 303 (19), 245 (11), 101 (78), 85 (15), 81 (11), 73 (14), 59 (38), 55 (17), 43 (100).

***N*²-[(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl)acetyl]-*N*⁶-(benzyloxycarbonyl)-L-lysin-4-acetamidoanilide (16d).** This amide was prepared according to Method A starting from **16c** (1.59 g, 5.0 mmol), Lys(Z)-NHAr·TFA [obtained after deprotection of Boc-Lys(Z)-NHAr (2.56 g, 5.0 mmol)], NMM (0.50 g, 5.0 mmol), HOBt (0.74 g, 5.5 mmol), and EDC (1.00 g, 5.2 mmol) in a 1:1 mixture of DMF-CH_2Cl_2 (20 mL). Purification by flash chromatography using a 1:1 mixture of petroleum ether-Et_2O and a gradient of EtOAc (0-10%) yielded **16d** (1.99 g, 56%) as a hygroscopic powder, mp 156-158 °C; R_f 0.76 (THF); $[\alpha]_D^{20}$ -45.2° (*c* 0.5, EtOH); EIMS *m/z* (rel intensity) 712 (M^+, 1), 151 (23), 150 (100), 128 (10), 127 (10), 108 (82), 107 (55), 101 (13), 91 (26), 84 (15), 79 (42), 77 (21), 51 (15), 43 (40). *Anal.* Calcd for $C_{36}H_{48}N_4O_{11}\cdot 0.5H_2O\cdot 0.5Et_2O$: C, 60.14; H, 7.17; N, 7.38. Found: C, 60.09; H, 7.46; N, 7.34.

***N*²-[(1,2-*O*-Isopropylidene-α-D-glucofuranos-3-*O*-yl)acetyl]-L-lysin-4-acetamidoanilide 4-Toluenesulfonate (19e).** For selective removal of the 5,6-acetal, **16d** (500 mg, 0.70 mmol) was stirred in 60% aqueous HOAc (10 mL) at 45 °C until the reaction was complete (TLC, ^1H NMR, ca. 3.5 h). Solvents were removed, *in vacuo*, the oil dissolved in HOAc (5 mL) and palladium on carbon (10%, 60 mg, under N_2) was added. The mixture was hydrogenated (Parr, 60 psi of H_2, room temperature) until complete (^1H NMR, ca. 5h). The catalyst was filtered (millipore filter paper) and washed with HOAc. After partial concentration of the filtrate, *in vacuo*, anhydrous TsOH (120 mg, 0.70 mmol) was added, and the remaining solvent removed, *in vacuo*. The resulting oil was crystallized from aq. 2-PrOH to produce **19e** (385 mg, 77%) as colorless, hygroscopic crystals; mp 250 °C (decomp); R_f 0.67 [reverse phase, EtOAc-HOAc (2%)]; $[\alpha]_D^{20}$ -14.6° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{32}H_{46}N_4O_{12}S\cdot 0.5H_20$: C, 53.39; H, 6.58; N, 7.78. Found: C, 53.31; H, 6.80; N, 7.77.

***Dicyclohexylammonium* Salt of 1,2:4,5-Di-*O*-isopropylidene-3-*O*-carboxymethyl-ß-D-fructopyranose (18c).** 1,2:4,5-Di-*O*-isopropylidene-ß-D-fructopyranose (**17**) was prepared in 34% yield by the method of Brady[32a] from ß-D-fructose, mp 116-118 °C, lit[8] mp, 118-119 °C; R_f 0.84 (EtOAc); $[\alpha]_D^{20}$ -155° (*c* 1.0, acetone), identical to lit. value; EIMS *m/z* (rel intensity) 245 (32), 185 (12), 144 (17), 127 (19), 117 (53), 100 (20), 85 (46), 75 (21), 69 (26), 59 (96), 57 (21), 55 (16), 43 (100), 28 (27). Sodium hydride (60% in mineral oil, 2.40 g, 60 mmol) was added, in portions, to a pre-cooled (0-5 °C) solution of **17** (10.4 g, 40 mmol) in anhydrous THF (125 mL). After the initial reaction subsided, the stirred mixture was refluxed (0.5 h), cooled (0-5 °C) and ethyl bromoacetate (33.4 g, 200 mmol) was added dropwise (colorless precipitate). The mixture was stirred at 0-5 °C (3 h), then at 15 °C (16 h). EtOH was added to decompose excess NaH and the mixture concentrated, *in vacuo*. The oily residue was dissolved in CH_2Cl_2 (200 mL) washed with ice-cold water (3 x 150 mL), dried ($MgSO_4$) and evaporated, *in vacuo*. Unreacted ethyl bromoacetate was removed by vacuum distillation (bp 82 °C at 1.0 Torr) and the residual oil treated with

pentane (100 mL) and was filtered to remove unreacted **17**. Evaporation of the solvent, *in vacuo*, furnished the 1,2:4,5-di-*O*-isopropylidene-3-*O*-(ethoxycarbonyl)methyl-ß-D-fructopyranose (**18a**, 13.0 g, 94%) as a colorless oil which was used without further purification; R_f 0.92 (EtOAc); $[\alpha]_D^{20}$ -16.3° (*c* 1.0, EtOH); EIMS *m/z* (rel intensity) 331 (16), 213 (24), 185 (15), 143 (25), 127 (10), 100 (31), 97 (11), 85 (35), 83 (12), 81 (11), 71 (10), 69 (23), 68 (19), 58 (30), 57 (40), 55 (23), 43 (100), 41 (31).

A dispersion of **18a** (2.7 g, 7.8 mmol) in 1 N NaOH solution (30 mL) was refluxed (1.5 h), cooled to 0-5 °C, acidified to pH 1.5 by the addition of ice cold 1 N HCl and extracted with $CHCl_3$ (4 x 100 mL). The extract was dried ($MgSO_4$) and evaporated, *in vacuo*, to yield a heavy colorless oil (1.85 g, 5.8 mmol) which was dissolved in ether (100 mL). Dicyclohexylamine (1.05 g, 5.8 mmol) was added and solvents removed, *in vacuo*, and the salt recrystallized from EtOAc to provide a colorless solid (2.75 g, 71%); mp 178-180 °C; R_f 0.53 [EtOAc-HOAc (2%)]; $[\alpha]_D^{20}$ -43.8° (*c* 1.0, EtOH); CIMS (CH_4), *m/z* (rel intensity) 500 (14, MH^+), 424 (10), 387 (11), 376 (18), 359 (59), 301 (27), 261 (40), 243 (18), 210 (14), 182 (100), 127 (27). *Anal.* Calcd for $C_{26}H_{45}NO_8$: C, 62.50; H, 9.09; N, 2.80. Found: C, 62.80; H,9.49; N, 2.62.

1,2:4,5-Di-*O*-isopropylidene-3-*O*-[*N*-(4-acetamidophenyl)carboxamido]methyl-ß-D-fructopyranose (18f). To an ice-cold solution of the acid (**18c**, 1.5 g, 3.0 mmol), $ArNH_2$ (1.80 g, 12.0 mmol), DMAP (0.15 g, 1.2 mmol) in 1:1 DMF-CH_2Cl_2 (20 mL) was added EDC (0.63 g, 3.3 mmol) was added. After stirring at 0-5 °C (1 h), 25 °C (3 h), the reaction was complete (^1H NMR), and the solvents were removed, *in vacuo*. The semi-solid was dissolved in EtOAc (150 mL), washed with cold 5% aqueous citric acid (3 x 100 mL), 5% aqueous $NaHCO_3$ (3 x 100 mL), and brine (2 x 50 mL). The organic layer was dried ($MgSO_4$) and evaporated, *in vacuo*. The resulting solid was flash chromatographed on silica gel with petroleum ether-EtOAc (1:1) and a gradient of HOAc (0-4%) to provide **18f** (1.03 g, 76%) as an amorphous hygroscopic powder; mp 172-173 °C; R_f 0.68 (EtOAc); $[\alpha]_D^{20}$ -110.0° (*c* 0.5, EtOH); EIMS *m/z* (rel intensity) 450 (M^+, 80), 435 (5), 361 (14), 263 (14), 209 (20), 208 (26), 192 (68), 191 (25), 167 (18), 166 (11), 150 (29), 149 (27), 143 (54), 121 (16), 120 (16), 108 (16), 107 (47), 106 (14), 85 (32), 60 (13), 59 (42), 43 (100), 41 (16). *Anal.* Calcd for $C_{22}H_{30}N_2O_8 \cdot 1.0H_2O$: C, 56.40; H, 6.89; N, 5.98. Found: C, 56.43; H, 6.55: N, 6.10.

1,2-*O*-Isopropylidene-3-*O*-[*N*-(4-acetamidophenyl)carboxamido]methyl-ß-D-fructopyranose (20f). A stirred solution of **18f** (360 mg, 0.8 mmol) in 60% aqueous HOAc (10 mL) was heated at 45 °C until the reaction was complete (TLC, ^1H NMR, ca. 3 h). After removal of the solvents, *in vacuo*, the solid obtained was recrystallized from 2-PrOH-Et_2O to furnish **13f** (320 mg, 97%) as an amorphous hygroscopic powder; mp 213-214 °C; R_f [EtOAc-HOAc (2%)] 0.29; $[\alpha]_D^{20}$ -165° (*c* 0.5, EtOH); EIMS *m/z* (rel intensity) 411 (MH^+, 11), 410 (M^+, 83), 317 (10), 192 (44), 150 (29), 149 (20), 108 (24), 107 (49), 103 (29), 80 (11), 59 (38), 57 (11), 45 (22), 43 (100), 41 (10). *Anal.* Calcd for $C_{19}H_{26}N_2O_8 \cdot 0.5$ 2-PrOH: C, 55.89; H, 6.86; N, 6.36. Found: C, 55.72; H, 7.06; N, 6.47.

4-Acetamidophenyl N^2-[(1,2:4,5-Di-*O*-isopropylidene-ß-D-fructopyranos-3-*O*-yl)acetyl]-N^6-(benzyloxycarbonyl)-L-lysinate (18g). To an ice-cold solution of **18c** (1.59 g, 5.0 mmol), Lys(Z)-OAr·TFA (from Boc-Lys(Z)-OAr, 2.56 g, 5.0 mmol), NMM (0.5 g, 5.0 mmol), HOBt (0.74 g, 5.5 mmol) in 1:1 DMF-CH_2Cl_2 (20 mL) was added EDC (1.00 g, 5.2 mmol) according to Method A. Purification by flash chromatography using a 1:1 mixture of EtOAc-$CHCl_3$ and a gradient of HOAc (0-4%) afforded **18g** (2.30 g, 64%) as a hygroscopic powder, mp 102-104 °C; R_f 0.67 (EtOAc); $[\alpha]_D^{20}$ -50.5° (*c* 1.0, EtOH); EIMS: *m/z* (rel intensity) 712 (M^+, 1), 151 (19), 150 (100), 134 (11), 108 (46), 107 (29), 91 (32), 84 (13), 79 (40), 77 (21), 59 (14), 57 (11), 51 (11), 43 (41). *Anal.* Calcd for $C_{36}H_{47}N_3O_{12} \cdot HOAc \cdot 0.5H_2O$: C, 58.30; H, 6.70; N, 5.37. Found: C, 58.01; H, 6.32; N, 5.54.

4-Acetamidophenyl N^2-[(1,2-*O*-Isopropylidene-ß-D-fructopyranos-3-*O*-yl)acetyl]-L-lysinate 4-Toluenesulfonate (20h).

Removal of the 4,5-acetal and the Z group of **18g** (500 mg, 0.70 mmol) was carried out as described for the preparation of **19e**. Reverse phase flash chromatography, using $CHCl_3$ and a gradient of petroleum ether (0-10%), was employed to obtain **20h** (420 mg, 84%) as a colorless hygroscopic powder, mp 182-184 °C; R_f 0.88 [reverse phase, EtOAc-HOAc (2%)]; $[\alpha]_D^{20}$ -31.8° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{32}H_{45}N_3O_{13}S \cdot 1.5H_2O$: C, 52.01; H, 6.55; N, 5.69. Found: C, 51.98; H, 6.20; N, 5.37.

N^2-[(1,2:4,5-Di-*O*-isopropylidene-ß-D-fructopyranos-3-*O*-yl)acetyl]-N^6-benzyloxycarbonyl-L-lysin-4-acetamidoanilide (18d). Coupling of **18c** (1.59 g, 5.0 mmol) with Lys(Z)-NHAr·TFA [obtained from Boc-Lys(Z)-NHAr (2.56 g, 5.0 mmol)], in the presence of NMM (0.5 g, 5.0 mmol), HOBt (0.74 g, 5.5 mmol), and EDC (1.00 g, 5.2 mmol) in 1:1 DMF-CH_2Cl_2 (20 mL) was carried out by Method A. Flash chromatography using EtOAc-Et_2O (1:1) and a gradient of (0-4%) HOAc furnished **18d** (2.63 g, 74%) as a hygroscopic powder, mp 132-135 °C; R_f 0.29 [EtOAc-HOAc (2%)]; $[\alpha]_D^{20}$ -69.8° (*c* 0.5, EtOH); EIMS, *m/z* (rel intensity) 712 (M^+,1), 151 (19), 150 (100), 134 (11), 108 (46), 107 (29), 91 (32), 84 (13), 79 (40), 77 (21), 59 (14), 57 (11), 51 (11), 43 (41). *Anal.* Calcd for $C_{36}H_{48}N_4O_{11} \cdot 0.5H_2O$: C, 59.90; H, 6.84; N, 7.76. Found: C, 59.82; H, 6.75; N, 7.58.

N^2-[(1,2-*O*-Isopropylidene-ß-D-fructopyranos-3-*O*-yl)acetyl]-L-lysin-4-acetamidoanilide Toluenesulfonate (20e). Deprotection of **18d** (500 mg, 0.70 mmol) was achieved as described for the preparation of **19e**. The resulting oil was purifid by reverse phase flash chromatography and eluted by ether to afford **20e** (425 mg, 85%) as a colorless, hygroscopic powder; mp 205-207 °C; R_f 0.52 [reverse phase, EtOAc-HOAc (2%)]; $[\alpha]_D^{20}$ -46.6° (*c* 0.5, HOAc). *Anal.* Calcd for $C_{32}H_{46}N_4O_{12}S \cdot Et_2O$: C, 55.08; H, 7.19; N, 7.14. Found; C, 55.48; H, 6.96; N, 7.44.

Enzyme Inhibition Studies

Human acrosin was isolated and partially purified according to the procedure of Goodpasture and coworkers.[48] Bovine pancreatic trypsin (type I) was obtained from Sigma Chemical Co. (St. Louis, Mo.). Acrosin and trypsin activity was measured spectrophotometrically at 410 nm by following the rate of hydrolysis of N-α-benzoyl-D,L-arginine 4-nitroanilide (BAPNA) at 25 °C. The acrosin assay media contained 100-150 µL of enzyme extract and the following concentrations of reagents; 50 mM NaH_2PO_4 buffer, pH 7.5, 300 mM NaCl, 0.1 mM BAPNA, 2.0 mM of the appropriate test compound and water to a total volume of 1.0 mL. The trypsin assays utilized 0.4-0.8 µg of enzyme and the same concentrations of reagents as the acrosin assays except that NaCl was omitted. All test compounds were solubilized with DMSO. The final concentration of DMSO in the assay media (4%) was found to increase the initial velocity of acrosin catalyzed BAPNA hydrolysis by 8%. The initial velocity of the control (i.e. with no test compound) was, therefore, measured in the presence of 4% DMSO.

References and Notes

1. (a) Taken from the PhD Dissertation of RAC, University of Illinois at Chicago, March 1989; (b) Abbreviations for amino acids and peptides are those suggested by IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9). (c) Other abbreviations are: acetyl (Ac), 4-acetamidoaniline ($ArNH_2$), 4-acetamidophenol (ArOH), *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Z), dicyclohexylcarbodiimide (DCC), 1-ethyl-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), 4-(dimethylamino)pyridine (DMAP), *N*-methylmorpholine (NMM), methanol (MeOH), ethanol, (EtOH), 2-propanol (2-PrOH), ether (Et_2O), ethyl acetate (EtOAc), tetrahydrofuran (THF), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), trifluoroacetic acid (TFA), 4-toluenesulfonic acid (TsOH).

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