Development of Potent and Selective CCK-A Receptor Agonists from Boc-CCK-4: Tetrapeptides Containing Lys(N^{ϵ})-Amide Residues

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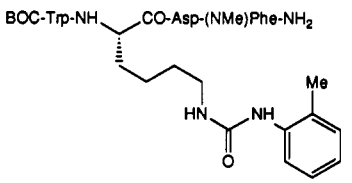
A series of Boc-CCK-4 derivatives represented by the general structure Boc-Trp-Lys(N^{ϵ}-COR)-Asp-Phe-NH_2, where R is an aromatic, heterocyclic, or aliphatic group, are potent and selective CCK-A receptor agonists. These amide-bearing compounds complement the previously described urea-based tetrapeptides (Shiosaki et al. *J. Med. Chem.* **1991**, *34*, 2837–2842); structure–activity studies revealed parallel as well as divergent trends between these two series. A significant correlation was observed between pancreatic binding affinity and the resonance constant *R* of the phenyl substituent in one particular series of derivatives. Sulfation of phenolic amides appended onto the ϵ-amino group of the lysine did not affect affinity for the CCK-A receptor in contrast to the 500-fold increase in binding potency observed upon sulfation of CCK-8, suggesting that the lysine appendage and the sulfated tyrosine in CCK-8, both key structural elements that impart high affinity for the CCK-A receptor, are interacting differently with the receptor. The amide-bearing tetrapeptides are full agonists relative to CCK-8 in stimulating pancreatic amylase release while being partial agonists in eliciting phosphoinositide (PI) hydrolysis. Both effects were blocked by selective CCK-A receptor antagonists.

Cholecystokinin (CCK), originally isolated from porcine intestine as a 33-amino acid peptide, is distributed widely in peripheral tissues and is responsible for a number of physiological actions including gallbladder contraction and secretion of pancreatic enzymes.[1,2] CCK is found also in the central nervous system (CNS) and studies suggest that it may have a role there as a neurotransmitter or neuromodulator.[3] Although multiple molecular forms of CCK have been identified, the full spectrum of biological activity can be elicited potently by the C-terminal octapeptide CCK-8 (Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2). Sulfation of tyrosine-27 (based on CCK-33 numbering) is a critical feature of the octapeptide for potent biological activity since the desulfated analogue CCK-8-DS (Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2) is roughly 500-fold weaker in binding to the peripheral receptor. In addition, the C-terminal tetrapeptide Boc-CCK-4 (Boc-Trp-Met-Asp-Phe-NH_2) is able to elicit the peripheral actions of CCK-8 but is over 1000-fold less potent in binding to the peripheral receptor.[4]

The predominant CCK receptor found in peripheral tissues such as the pancreas has been labeled CCK-A,[5] and its activation is responsible for the secretory and contractile actions attributed to CCK as well as its anorexogenic effects through activation of receptors on peripheral vagal afferent fibers.[6] The CCK receptors in the CNS are labeled CCK-B[5] and possess similar ligand binding characteristics as the peripheral gastrin receptor. These two CCK receptors can be differentiated by their relative affinity for CCK and its fragments as well as a number of synthetic ligands, both peptides and nonpeptides, that are highly selective for the CCK-A receptor, including MK-329 (devazepide),[7] CR-1409,[8] and A-71378,[9] and those that are selective for the CCK-B receptor, including CI-998,[10] L-365,260,[11] and A-63387.[12]

We recently reported a novel series of Boc-CCK-4 derivatives in which the methionine was replaced with a lysine whose ϵ-amino group had been incorporated into various aromatic and aliphatic ureas.[13] In contrast to Boc-CCK-4, which is 70-fold more selective for the CCK-B receptor, these tetrapeptides are potent and selective agonists for the CCK-A receptor. A-71623 (1), a prototypic urea-bearing tetrapeptide, possesses high affinity (IC_{50} = 3.7 nM) for the CCK-A receptor and is greater than 1000-fold selective over the CCK-B receptor (Table I).[14]



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In addition, many of the tetrapeptides in this series are full agonists in stimulating pancreatic amylase secretion

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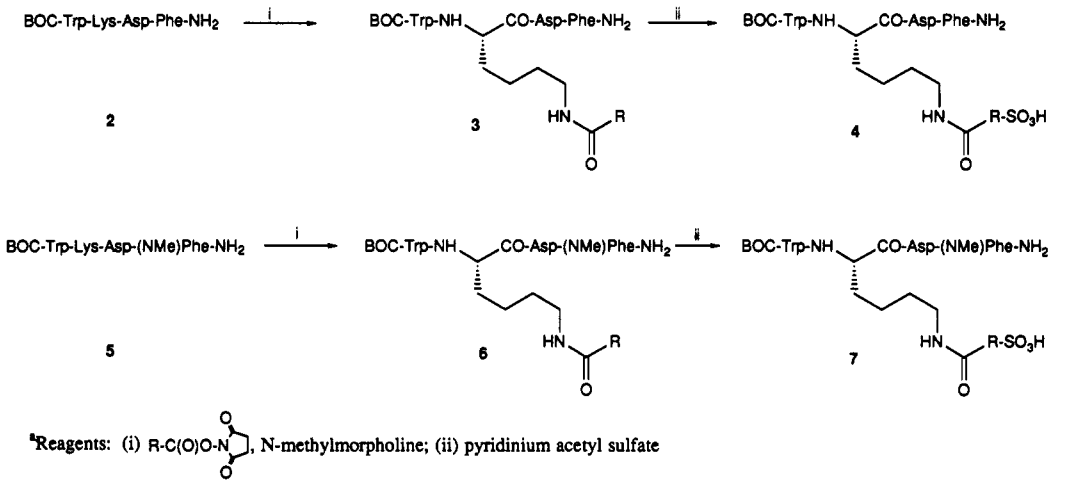
Table I. Binding and Functional Data of Boc-CCK-4 and A-71623

	IC_{50}, nM[a,b]		EC_{50}, nM[a,c]	PI[d]
	pancreas	cortex	amylase	% max
Boc-CCK-4	1800 ± 630 (5)	25 ± 4.5 (6)	2600 ± 950 (5)	40
A-71623	3.7 ± 0.85 (8)	4500 ± 770 (4)	0.39 ± 0.06 (12)	100

[a] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ± SE are indicated for those compounds with three or more determinations. [b] IC_{50} was determined as the concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [c] EC_{50} was determined in the amylase release assay as concentration of peptide that produce 50% of maximal response. [d] Indicates percent response of peptide at concentration of 10^{-4} M in PI hydrolysis relative to maximal response elicited by CCK-8.

Scheme I[a]



[a] Reagents: (i) R-C(O)O-N(succinimide), N-methylmorpholine; (ii) pyridinium acetyl sulfate

and phosphoinositide (PI) hydrolysis relative to CCK-8 in guinea pig pancreatic acini. A-71623 was demonstrated to inhibit food intake in rodents and monkeys.[15] These actions can be potently inhibited by selective CCK-A antagonists. Detailed structure–activity studies about the pendent N,N′-disubstituted urea function demonstrated its importance for CCK-A receptor recognition and activation. Substituting the urea with various mono and bicyclic aromatic groups as well as some aliphatic residues afforded tetrapeptides with high affinity and selectivity for the CCK-A receptor. Removing the urea, i.e. Boc-Trp-Lys-Asp-Phe-NH_2, or replacing it with a singly substituted urea, i.e. Boc-Trp-Lys(N^{ϵ}-$CONH_2$)-Asp-Phe-NH_2, produced compounds that were very poor or inactive ligands for the CCK-A receptor.

Since CCK-A receptor potency and activity appears to involve an appropriate appendage extending off of the lysine side chain in this tetrapeptide series, we investigated whether functional groups other than ureas would serve as an acceptable pharmacophore. We were also interested in addressing whether any structural correlation existed between the lysine side-chain moiety and the sulfated tyrosyl group in the CCK-8-based peptides, both key residues that impart potent CCK-A receptor affinity. We therefore investigated a series of CCK-4 derivatives bearing an amide function at the ϵ-amino of lysine. Phenolic derivatives in this amide series were sulfated subsequently to determine if there were any significant effects on CCK-A receptor binding. In addition, detailed structure–activity studies were conducted on the amides to determine if any trends paralleling our previously described urea-based tetrapeptides were present.

Methods

The tetrapeptides were prepared as outlined in Scheme I. The parent tetrapeptide **2** was prepared via standard solution phase peptide chemistry as previously described.[13] The side-chain amino group of the lysine in tetrapeptide **2** was allowed to react with the *N*-hydroxysuccinimide ester of various carboxylic acids, prepared by condensing the acid with *N*-hydroxysuccinimide in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), to form the desired amide-substituted compounds **3**. No reaction of the free β-carboxyl of the aspartic acid was observed under the coupling conditions. Subsequent sulfation of the phenolic group was conducted using pyridinium acetyl sulfate (PAS)[16] to yield **4**. *N*-Methylphenylalanine ((NMe)Phe) was incorporated into tetrapeptide **5** under similar peptide coupling conditions, and reaction with various *N*-hydroxysuccinimide esters provided the (NMe)Phe derivatives **6**. Subsequent sulfation with PAS yielded **7**.

The compounds were tested in a receptor binding assay described previously using guinea pig pancreas and cortex as tissues containing the CCK-A and CCK-B receptors, respectively, and [^{125}I]-Bolton–Hunter CCK-8 ([^{125}I]BH-CCK-8) as the radioligand.[9] Protocols to assess the ability of these compounds to stimulate amylase release and

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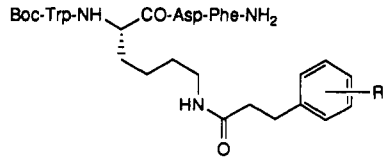
Table II. Physical Properties and Biological Data of Hydrocinnamoyl and Substituted Hydrocinnamoyl Tetrapeptides **3** and **4**



no.	R	mp, °C	formula	anal.[a]	IC_{50}, nm[b,c] pancreas	IC_{50}, nm[b,c] cortex	EC_{50}, nM[b,d] amylase	PI[b,e] % max
3a	H	175–179	$C_{44}H_{55}N_7O_9 \cdot 2AcOH$	C,H,N	310 ± 76 (6)	5900 ± 1000 (3)	850 (2)	49 ± 6.6 (3)
3b	3-OH	145–163	$C_{44}H_{55}N_7O_{10} \cdot 1.5H_2O$	C,H,N	400 ± 40 (3)	~3000	160 (1)	46 ± 6.3 (6)
3c	4-OH	147–170	$C_{44}H_{55}N_7O_{10} \cdot H_2O$	C,H,N	120 ± 36 (5)	6100 ± 170 (3)	92 (2)	77 ± 4.9 (4)
4a	3-OSO_3H	181–185	$C_{44}H_{55}N_7O_{13}S \cdot NH_4OH \cdot 0.5H_2O$	C,H,N	180 ± 19 (3)	~10000	69 (1)	50 ± 8.9 (3)
4b	4-OSO_3H	180–186	$C_{44}H_{55}N_7O_{13}S \cdot NH_4OH$	C,H,N	76 ± 8.5 (5)	4800 (2)	98 (2)	68 ± 4.9 (5)
3d	4-Cl	201–205	$C_{44}H_{54}N_7O_9Cl \cdot 1.5H_2O$	C,H,N	410 ± 90 (3)	7000 (1)	170 (2)	51 ± 4.9 (4)
3e	4-OMe	193–194	$C_{45}H_{57}N_7O_{10} \cdot 0.5H_2O$	C,H,N	140 ± 50 (4)	>10,000	74 (2)	70 ± 8 (3)
3f	4-F	114–124	$C_{44}H_{54}N_7O_9F \cdot H_2O$	C,H,N	250 ± 36 (6)	9900 (1)	450 (1)	68 ± 7.4 (3)
3g	4-CF_3	100–110	$C_{45}H_{54}N_7O_9F_3 \cdot 0.5H_2O$	C,H,N	390 ± 76 (8)	19000 (1)	agonist[f]	77 (1)
3h	4-Me	193–195	$C_{45}H_{57}N_7O_9$	C,H,N	290 ± 100 (3)	~10000	240 (1)	62 ± 10 (3)
3i	3,4-di-OH	143–145	$C_{44}H_{55}N_7O_{11} \cdot H_2O \cdot AcOH$	C,H,N	160 ± 48 (4)	4400 (2)	300 (1)	44 (2)

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ± SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [d] EC_{50} was determined in the amylase release assay as concentration of peptide that produce 50% of maximal response. [e] Indicates percent response of peptide at concentration of 10^{-4} M in PI hydrolysis relative to maximal response elicited by CCK-8. [f] Indicates the peptide elicited release of amylase at a concentration of 30 uM.

phosphoinositide (PI) breakdown in guinea pig pancreas have been described.[9] The compounds were evaluated at concentrations between 10^{-5} and 10^{-9} M in the amylase assay and at 10^{-4} M for the PI assay. Each determination was performed in duplicate with less than 5% sample variability. Additional determinations were conducted on selected compounds.

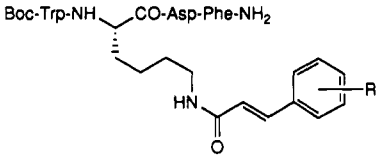
Results and Discussion

As reported, the development of this novel series of CCK-A selective tetrapeptides was initiated by our finding that a CCK-4 derivative, Boc-Trp-Lys(Cbz)-Asp-Phe-NH_2, had modest affinity (IC_{50} = 510 nM) for the pancreatic receptor but, in contrast to Boc-CCK-4, was 10-fold selective for the CCK-A receptor. Lack of biological activity by the free amino (Boc-Trp-Lys-Asp-Phe-NH_2) and the *N*-acetyl (Boc-Trp-Lys(Ac)-Asp-Phe-NH_2) derivatives suggested that an appropriate appendage extending from the lysine ε-amino group was necessary for CCK-A receptor recognition. The hydrocinnamoyl derivative **3a**, prepared as an isosteric replacement of the Cbz group, had similar potency for the CCK-A receptor as the original carbamoyl compound (Table II). Substitution at the 2- or 3-position of the phenyl ring in the hydrocinnamoyl moiety with hydroxyl, e.g. **3b**, had little effect on binding while the 4-hydroxyl derivative **3c** improved pancreatic affinity over **3a** by 2-fold. This improvement in binding potency suggested that the phenolic group extending from **3c** might be interacting in a similar manner within the CCK-A receptor as the phenolic group of tyrosine-29 in CCK-8. Should such a common means of interaction be involved, then sulfation of the phenolic group in the hydrocinnamoyl derivatives would be expected to significantly improve its affinity for the CCK-A receptor as had been clearly established for CCK-8. Sulfation of the isomeric phenols produced **4a** and **4b** whose affinity for the peripheral receptor was improved by roughly 2-fold over their respective unsulfated analogues, far less than the 500-fold improvement observed upon sulfation of CCK-8. This lack of a significant enhancement in binding potency upon sulfation suggests that the phenols of the lysine residue and the tyrosine of CCK-8 are interacting differently with the receptor, possibly through interactions at distinct sites on the receptor or the greater contributions for binding provided by the other residues in CCK-8 upon sulfation.

Other substituents were incorporated at the 4-position of the phenyl ring in the hydrocinnamoyl compound. In general, these derivatives (**3d–3h**) exhibited comparable pancreatic receptor affinities as the parent compound **3a** with the exception of the 4-methoxy analogue **3e** whose potency was comparable to the 4-hydroxy example **3c**. Incorporation of disubstitution, exemplified by the 3,4-dihydroxy compound **3i**, yielded analogues of comparable binding potencies to **3c**. All the hydrocinnamoyl derivatives maintained selectivity for the CCK-A receptor and were demonstrated to exhibit full agonist activity in stimulating amylase release and partial efficacy in PI hydrolysis.

Further improvement in CCK-A receptor affinity was obtained upon constraining the ethylene bridge in **3a** by substituting with a cinnamoyl residue to produce **3j** (Table III). A 4- to 6-fold increase in binding potency was realized in most of the cinnamoyl derivatives (**3l–3r**) over their saturated counterparts (**3b–3h**). As in the hydrocinnamoyl series, hydroxyl substitution at the 4-position produced the most potent CCK-A receptor ligand **3m** over the isomeric derivatives **3k** and **3l**. Sulfation of the phenolic derivatives **3l** and **3m** to produce **4c** and **4d**, respectively, had little or no effect upon binding affinity, further supporting the notion that the phenolic group of the tetrapeptides and the tyrosine in the octapeptides are interacting differently with the CCK-A receptor. Other substituents at the 4-position revealed compounds (**3n–3u**) that were generally equipotent or slightly less potent than the parent cinnamoyl derivative **3j**. In contrast, substitution with methyl and chloro in the phenylurea series resulted in a 7-fold improvement in binding affinity over the unsubstituted derivative.[13] A number of disubstituted cinnamoyl derivatives was also investigated. The binding affinities of most of the disubstituted compounds (**3v–3z**) were comparable to that of the parent cinnamoyl derivative **3j**, although the 2,4-dichloro analogue **3x** yielded a 2-fold weaker compound. As in the previous series of compounds, the cinnamoyl tetrapeptides exhibited binding selectivity for the CCK-A receptor and showed agonist activity in both functional assays.

Table III. Physical Properties and Biological Data of Cinnamoyl and Substituted Cinnamoyl Tetrapeptides 3 and 4



no.	R	mp, °C	formula	anal.[a]	IC_{50}, nM[b,c] pancreas	IC_{50}, nM[b,c] cortex	EC_{50}, nm[b,d] amylase release	PI[b,e] % max
3j	H	203–205	$C_{44}H_{53}N_7O_9$·AcOH	C,H,N	55 ± 20 (3)	1800 ± 190 (3)	30 (2)	52 (2)
3k	2-OH	135–138	$C_{44}H_{53}N_7O_{10}$·1.5H_2O	C,H,N	110 ± 35 (7)	210 (1)	210 (1)	54 (2)
3l	3-OH	202–205	$C_{44}H_{53}N_7O_{10}$	C,H,N	22 ± 4.8 (8)	970 (1)	59 (2)	54 ± 10 (3)
3m	4-OH	203–204	$C_{44}H_{53}N_7O_{10}$	C,H,N	16 ± 3.3 (6)	730 ± 140 (4)	3.9 (2)	76 (2)
4c	3-OSO_3H	165–170	$C_{44}H_{53}N_7O_{13}S$·NH_4OH·2H_2O	C,H,N	39 ± 13 (3)	238 (2)	25 (2)	70 ± 7.3 (4)
4d	4-OSO_3H	166–169	$C_{44}H_{53}N_7O_{13}S$·NH_4OH	C,H,N	13 ± 3.9 (7)	470 ± 54 (6)	7.2 ± 2.2 (3)	72 ± 4.7 (4)
3n	4-Cl	157–169	$C_{44}H_{52}N_7O_9Cl$·H_2O	C,H,N	71 ± 49 (3)	2900 (2)	79 (2)	66 (2)
3o	4-OMe	125–130	$C_{45}H_{55}N_7O_{10}$·2H_2O	C,H,N	30 ± 9.1 (3)	1700 ± 96 (4)	20 (2)	72 ± 8.7 (4)
3p	4-F	132–149	$C_{44}H_{52}N_7O_9F$·1.5H_2O	C,H,N	65 ± 24 (5)	1700 ± 190 (5)	19 (2)	74 ± 6.0 (3)
3q	4-CF_3	131–139	$C_{45}H_{52}N_7O_9F_3$·1.5H_2O	C,H,N	220 ± 56 (3)	5300 (1)	55 (1)	60 (1)
3r	4-Me	128–135	$C_{45}H_{55}N_7O_9$·H_2O	C,H,N	71 ± 26 (4)	1500 ± 180 (3)	52 (2)	71 ± 4.5 (3)
3s	4-Br	138–148	$C_{44}H_{52}N_7O_9Br$·H_2O·AcOH	C,H,N	140 ± 42 (4)	5100 ± 440 (3)	38 (2)	72 ± 10 (4)
3t	4-$N(Me)_2$	181–185	$C_{46}H_{58}N_8O_9$·0.5H_2O	C,H,N	120 ± 36 (4)	2100 ± 190 (3)	17 ± 3.0 (3)	49 (1)
3u	4-NO_2	145–152	$C_{44}H_{52}N_8O_{11}$·H_2O	C,H,N	57 ± 19 (8)	4400 (1)	27 (2)	64 ± 6.3 (4)
3v	3,4-di-OH	164–166	$C_{44}H_{53}N_7O_{11}$·H_2O·AcOH	C,H,N	33 ± 6.9 (3)	750 (2)	45 (2)	66 ± 5.8 (3)
3w	3,4-di-Cl	145–156	$C_{44}H_{51}N_7O_9Cl_2$·1.75AcOH·H_2O	C,H,N	49 ± 7 (7)	~5000	46 (2)	62 (2)
3x	2,4-di-Cl	191–193	$C_{44}H_{51}N_7O_9Cl_2$·1.5H_2O	C,H,N	130 ± 71 (6)	~5000	90 ± 18 (3)	86 ● 3.5 (3)
3y	3,4-di-OMe	178–185	$C_{46}H_{57}N_7O_{11}$·H_2O	C,H,N	40 ± 19 (3)	4000 (2)	17 (2)	61 ± 5.8 (7)
3z	2,4-di-OMe	136–139	$C_{46}H_{57}N_7O_{11}$·0.5H_2O	C,H,N	38 ± 26 (8)	~3000	14 (2)	82 ± 4.1 (3)

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ● SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [d] EC_{50} was determined in the amylase release assay as concentration of peptide that produced 50% of maximal response. [e] Indicates percent response of peptide at concentration of 10^{-4} M in PI hydrolysis relative to maximal response elicited by CCK-8.

Analysis of the binding data of the various 4-substituted hydrocinnamoyl and cinnamoyl derivatives revealed an interesting correlation between CCK-A receptor affinity and the resonance constant *R* of the 4-substituent in the former series. The resonance constant is a measure of the ability of the substituent to delocalize π electrons either into or out of an aromatic system.[17] Those substituents with the larger negative *R* values and therefore better able to donate electrons into the phenyl ring yielded the most potent compounds (Figure 1). No such correlation of affinity with *R* values or any other substituent constants either singly or as multivariables was observed in the cinnamoyl series based on analysis utilizing the R^2 procedure.[18] The mechanistic implications of this correlation observed in the hydrocinnamoyl or the differences between the hydrocinnamoyl and cinnamoyl series cannot be realized at this time due to the absence of any information regarding the structure of the CCK-A receptor. However, these findings are significant in that a degree of predictability is now available for some series of tetrapeptides.

Further structural studies indicated that shortening or lengthening the ethylene link between the aromatic ring and the amide carbonyl of the hydrocinnamoyl derivatives lowered pancreatic binding affinity. Thus, the 4-hydroxyphenylacetyl **3aa**, along with other substituted phenylacetyl examples, and the 4-phenylbutyryl **3bb** derivatives (Table IV) exhibited weak affinity for the pancreatic receptor. However, saturation of the phenyl ring in **3a** yielded an equipotent analogue to the parent compound **3cc**; a similar result was observed in the urea-bearing tetrapeptide series.[13] The isomeric naphthylamide

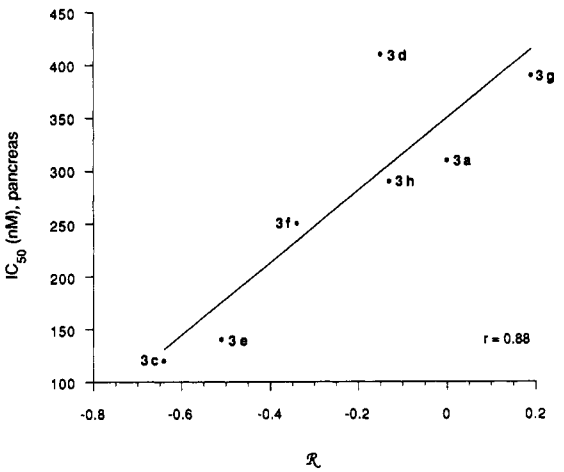
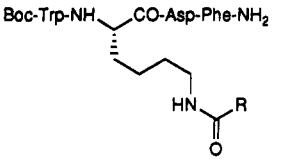


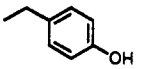
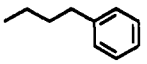
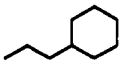
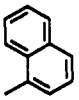
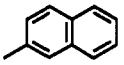
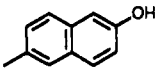
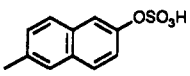
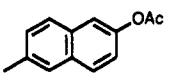
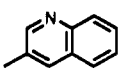
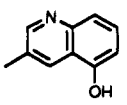
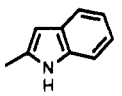
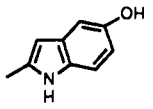
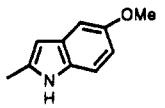
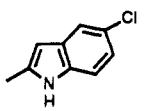
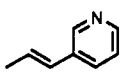
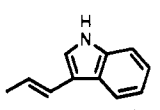
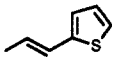
Figure 1. Correlation plot of CCK-A receptor binding affinity (IC_{50}) of 4-substituted hydrocinnamoyl tetrapeptide derivatives 3 as a function of resonance constant (*R*) of the substituent. *R* values were obtained from Hansch and Leo.[17]

derivatives **3dd** and **3ee** demonstrated a 4-fold binding preference for the 2-isomer. This result is in contrast to our previous urea series in which the 1- and 2-naphthalene derivatives were equipotent for the CCK-A receptor.[13] Introduction of a 6-hydroxyl group in the 2-naphthylamide compound **3ee** further improved binding affinity by 5-fold, yielding a very potent and selective CCK-A receptor ligand **3ff**. As observed in the hydrocinnamoyl and cinnamoyl examples, sulfation of the phenolic group in **3ff** to produce **4e** showed little improvement for CCK-A binding. The acetate derivative **3gg** possessed comparable binding to the other 6-substituted 2-naphthyl analogues. The compounds in this series maintained selectivity for the CCK-A receptor with the exception of **3aa**, which has very weak affinity for the peripheral receptor and modest binding to

(17) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979; pp 5–6, 49–51.
(18) SAS Institute, Cary, NC.

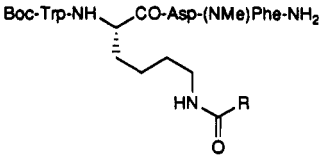
Table IV. Physical Properties and Biological Data of Amide-Substituted Tetrapeptides **3** and **4**

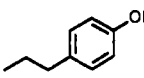
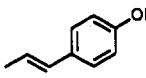
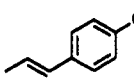
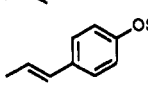
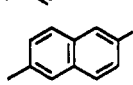
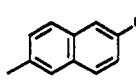
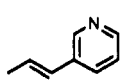


no.	R	mp, °C	formula	anal.[a]	IC_{50}, nM[b,c] pancreas	IC_{50}, nM[b,c] cortex	EC_{50}, nM[b,d] amylase	PI[b,e] % max
3aa		120–135	$C_{43}H_{53}N_7O_{10}\cdot1.5H_2O$	C,H,N	1100 ± 90 (3)	480 ± 100 (3)	agonist[f]	88 (1)
3bb		188–190	$C_{45}H_{57}N_7O_9\cdot0.5H_2O$	C,H,N	910 ± 190 (4)	>10 000	agonist	19 (2)
3cc		176–205	$C_{44}H_{61}N_7O_9\cdot H_2O$	C,H,N	310 ± 86 (3)	~10 000	agonist	40 ± 6.9 (7)
3dd		129–138	$C_{46}H_{53}N_7O_9\cdot0.5AcOH\cdot H_2O$	C,H,N	57 ± 17 (4)	4300 (1)	79 (2)	61 ± 5.2 (6)
3ee		217–219	$C_{46}H_{53}N_7O_9\cdot0.5H_2O$	C,H,N	14 ± 2.7 (7)	2700 (2)	4.0 ± 1.4 (3)	36 (2)
3ff		155–157	$C_{46}H_{53}N_7O_{10}\cdot1.5H_2O$	C,H,N	4.5 ± 0.96 (4)	1200 ± 130 (4)	3.5 ± 0.81 (5)	50 ± 7.9 (4)
4e		153–160	$C_{46}H_{53}N_7O_{13}S\cdot NH_4OH\cdot 2H_2O$	C,H,N	2.9 ± 0.38 (2)	300 (2)	3.5 (2)	49 ± 6.1 (7)
3gg		134–135	$C_{48}H_{55}N_7O_{11}\cdot H_2O$	C,H,N	8.0 ± 1.7 (5)	1400 ± 140 (4)	11 (2)	49 ± 6.0 (8)
3hh		143–145	$C_{45}H_{52}N_8O_9$	C,H,N	33 ± 2.2 (3)	200 (2)	39 (2)	46 ± 4.7 (3)
3ii		158–161	$C_{45}H_{51}N_8O_{10}\cdot H_2O$	C,H,N	7.7 ± 2 (8)	670 (1)	2.3 (2)	45 ± 6.2 (7)
3jj		155–170	$C_{44}H_{52}N_8O_9\cdot1.5H_2O$	C,H,N	39 ± 10 (4)	2100 ± 190 (4)	20 (2)	18 ± 4.0 (3)
3kk		158–160	$C_{44}H_{51}N_8O_{10}\cdot1.4AcOH\cdot H_2O$	C,H,N	14 ± 4.1 (6)	830 (1)	68 (2)	23 ± 6.5 (6)
3ll		129–134	$C_{45}H_{54}N_8O_{10}\cdot AcOH\cdot 0.5H_2O$	C,H,N	52 ± 21 (5)	~3000	16 ± 6.2 (3)	21 ± 6.0 (5)
3mm		136–139	$C_{44}H_{51}N_8O_9Cl\cdot2H_2O$	C,H,N	110 ± 62 (5)	5200 (1)	62 (2)	27 ± 6.9 (6)
3nn		129–135	$C_{43}H_{52}N_8O_9\cdot1.5H_2O$	C,H,N	21 ± 6.5 (3)	87 ± 17 (3)	38 (2)	60 ± 6.0 (5)
3oo		147–151	$C_{46}H_{54}N_8O_9\cdot1.5H_2O$	C,H,N	19 ± 3.8 (8)	4700 (1)	30 (2)	55 ± 6.7 (7)
3pp		204–206	$C_{42}H_{51}N_7O_9S\cdot H_2O$	C,H,N	50 ± 15 (3)	840 ± 110 (3)	20 (2)	66 ± 6.7 (5)

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ± SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [d] EC_{50} was determined in the amylase release assay as concentration of peptide that produced 50% of maximal response. [e] Indicates percent response of peptide at concentration of 10^{-4} M in PI hydrolysis relative to maximal response elicited by CCK-8. [f] Indicates the peptide elicited release of amylase at a concentration of 30 μM.

Table V. Physical Properties and Biological Data of (NMe)Phe-Containing Tetrapeptides 6 and 7



no.	R	mp, °C	formula	anal.[a]	IC_{50}, nM[b,c] pancreas	IC_{50}, nM[b,c] cortex	EC_{50}, nm[b,d] amylase release	PI[b,e] % max
6a		ND[f]	$C_{45}H_{57}N_7O_{10}$·AcOH·1.5H_2O	C,H,N	30 ± 10 (3)	3600 ± 640 (3)	32 (2)	77 ± 3.0 (3)
6b		152–189	$C_{45}H_{55}N_7O_{10}$·AcOH·0.5H_2O	C,H,N	4.2 ± 1.3 (7)	710 ± 38 (5)	3.0 ● 0.21 (10)	76 (2)
6c		154–175	$C_{45}H_{54}N_7O_9Cl$·1.5H_2O	C,H,N	15 ± 3.7 (3)	~3000	8.6 ± 3.8 (3)	68 ± 3.9 (5)
7a		156–215	$C_{45}H_{55}N_7O_{13}S$·NH_4OH·H_2O	C,H,N	22 ± 5.7 (8)	1000 (1)	2.9 ± 1.1 (3)	74 ± 5.4 (4)
6d		138–176	$C_{47}H_{55}N_7O_{10}$·H_2O	C,H,N	3.8 ± 0.74 (4)	730 ± 84 (3)	2.4 ± 0.26 (3)	52 (2)
6e		151–168	$C_{49}H_{57}N_7O_{11}$·1.5H_2O	C,H,N	3.4 ± 0.6 (4)	570 ± 80 (3)	2.9 ± 1.2 (3)	58 ± 4.3 (3)
6f		ND	$C_{44}H_{54}N_8O_9$·1.5H_2O	C,H,N	28 ± 10 (3)	120 (1)	28 (2)	74 (2)

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ± SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]BH-C-CK-8 in each tissue. [d] EC_{50} was determined in the amylase release assay as concentration of peptide that produced 50% of maximal response. [e] Indicates percent response of peptide at concentration of 10^{-4} M in PI hydrolysis relative to maximal response elicited by CCK-8. [f] Not determined.

the brain receptor. In addition, all the compounds possessed agonist activity in stimulating both amylase release and PI hydrolysis.

A variety of heterocyclic amides was subsequently investigated. The 3-quinolyl derivative **3hh** showed similar affinity for the CCK-A receptor as the 2-naphthyl compound **3ee** (Table IV) although the latter was ~10-fold more selective than **3hh** for the CCK-A over the CCK-B receptors. Substitution of a 5-hydroxyl in the quinoline ring provided a derivative **3ii** with 4-fold improvement in binding potency over **3hh**. The 2-indolyl analogue **3jj** possessed pancreatic binding affinity similar to that of the 1-naphthyl derivatives **3dd**. As observed in the naphthyl and quinolyl examples, introduction of a hydroxyl group onto the heterocycle provided a 3-fold more potent compound **3kk** over **3jj**. However, substitution at the 5-position with methoxy (**3ll**) did not significantly affect binding affinity from the parent compound **3jj** while the 5-chloro compound **3mm** was a 3-fold weaker derivative. A series of acryloyl derivatives such as the 3-pyridine **3nn**, 3-indole **3oo**, and 2-thiophene **3pp** yielded compounds with similar or slightly improved binding potencies over the cinnamoyl amide **3j**. All the heterocyclic examples exhibited selectivity for the CCK-A receptor and were agonists in both the amylase release and PI hydrolysis assays.

The introduction of *N*-methylphenylalanine ((NMe)-Phe) into selected derivatives had varying effects on its pancreatic affinity relative to the parent compounds. A 4-fold improvement in binding affinities was observed with the 4-hydroxyhydrocinnamoyl **6a**, the 4-hydroxycinnamoyl **6b**, and the 4-chlorocinnamoyl **6c** derivatives (Table V) compared to the parent compounds, **3c**, **3m**, and **3n**, respectively. However, this modification did not affect the potency of the sulfated phenol **7a** over the parent **4d**. The *N*-methylphenylalanine incorporation had either no effect or a slight improvement on the potency of the 2-naphthyl derivatives **6d** and **6e** over **3ff** and **3gg**, respectively. The same modification on the pyridyl derivative **6f** did not affect its binding affinity relative to the parent compound **3nn**. In general, the binding potencies of the compounds that possess high affinity (IC_{50} < 20 nM) for the CCK-A receptor were not as affected by the introduction of the *N*-methyl residue as those compounds that were weaker ligands. The introduction of N(Me)Phe into several of the potent urea-based tetrapeptides did not affect their high pancreatic affinity.[14] Selectivity for the pancreatic receptor was maintained by the incorporation of (NMe)Phe in all these examples as was the ability to stimulate amylase release and PI hydrolysis.

A detailed pharmacological evaluation of a prototypic amide-bearing tetrapeptide, represented by **6b**, has been published recently by Lin et al.[19] All the amide-bearing tetrapeptides that have been described above have similar properties to **6b** and are full agonists relative to CCK-8 in stimulating amylase release from pancreatic tissue. There appears to be a general trend correlating binding affinities with functional potencies as was observed with the urea-bearing tetrapeptides.[13] The actions of these compounds were potently inhibited by selective CCK-A

(19) Lin, C. W.; Shiosaki, K.; Miller, T. R.; Witte, D. G.; Bianchi, B. R.; Wolfram, C. A. W.; Kopecka, H.; Craig, R.; Wagenaar, F.; Nadzan, A. M. Characterization of Two Novel Cholecystokinin Tetrapeptide (30–33) Analogues, A-71623 and A-70874, That Exhibit High Potency and Selectivity for Cholecystokinin-A Receptors. *Mol. Pharmacol.* **1991**, *39*, 346–351.

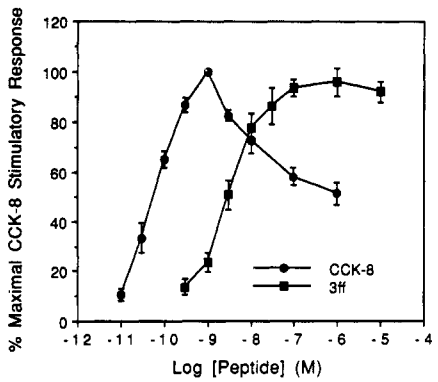


Figure 2. Comparison of the abilities of CCK-8 and **3ff** to stimulate PI hydrolysis in guinea pig pancreatic acini. The experimental protocols were as described previously.[9] Results shown are the mean ± SEM of three or more experiments conducted in duplicate. The amount of inositol phosphates was increased from 160 ● 40 dpm to 3700 ● 700 dpm in the presence of 100 nM CCK-8 (n = 4).

antagonists. However, the amide-bearing tetrapeptides, such as **3ff**, do not exhibit a high-dose inhibition of amylase release (Figure 2) and are partial agonists in eliciting PI hydrolysis relative to CCK-8 when tested in guinea pig pancreatic tissue. With several exceptions, the amide-based tetrapeptides elicited <80% of the PI response relative to CCK-8 and the indole-bearing amides **3jj–3mm** as a class exhibited lower efficacies in promoting PI hydrolysis. These properties are in contrast to the potent urea-based tetrapeptides such as A-71623 that are also full agonists in eliciting amylase release yet exhibit reduced secretion at supramaximal doses and provoke full agonist responses in PI hydrolysis relative to CCK-8. The mechanisms responsible for the difference in activation of the receptor between these two classes of tetrapeptides are not understood at this time. The absence of the high-dose inhibition by the amide-bearing tetrapeptides in guinea pig amylase release appears to be related to their lower efficacy in activating PI hydrolysis. The relationship between PI hydrolysis and high-dose inhibition of amylase release is currently being investigated by us in various rodent species using both the urea- and amide-based tetrapeptide agonists. The results and discussions of this study will be reported in due time.

In summary, we have investigated a series of CCK-4 derivatives bearing various amides in the lysine side chain that are potent and selective agonists for the CCK-A receptor. Structurally, this series compliments the urea-based tetrapeptides that have been reported previously.[13] Examples of parallel as well as divergent structure–activity trends between these two tetrapeptide series have emerged. These compounds will contribute to our continuing studies directed towards modeling the two classes of CCK-A selective tetrapeptides against other classes of CCK ligands. We eventually hope to understand the structural correlations amongst the diverse classes of both peptide and nonpeptide CCK ligands.

Experimental Section

Solvents and other reagents were reagent grade and used without further purification unless otherwise noted. Amino acids and *tert*-butoxycarbonyl (Boc) protected amino acids were purchased from Bachem, Inc., Torrance, CA or Sigma Chemical Co., St. Louis, MO. The activated esters (*N*-hydroxysuccinimide) of Boc protected amino acids were purchased from Chemical Dynamics, South Plainfield, NJ. Melting points are uncorrected and were obtained on a Buchi capillary melting point apparatus. ^{1}H-NMR spectra were recorded at 300 or 500 MHz and expressed as ppm downfield from tetramethylsilane (TMS) as an internal standard. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck) using the following solvent system: ethyl acetate–pyridine–acetic acid–water (260:20:6:11). Pyridine used as the chromatography solvent was freshly distilled from barium oxide. Preparative reverse-phase HPLC chromatography was performed on a Beckman Dual Pump 110B solvent delivery module using a Gilson Holochrom variable wavelength UV detector at 280 nm. The column was a Vydac-C_{18} (3 × 28 cm) and the solvents used were A, 0.05 M ammonium acetate at pH 4.5, and B, acetonitrile. A linear gradient beginning with 5% B and ending with 40% B over 30 min was employed. Elemental analyses were performed by the Abbott Laboratories Analytical Department, North Chicago, IL, and are within ±0.4% of calculated values unless otherwise noted. The following abbreviations have been used: Cbz, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole. Other peptide and amino acid abbreviations and conventions used are those recommended by the IUPAC-IUB Commission (*Biochem. J.* **1984**, *219*, 345–373).

General Procedure for the Preparation of Amide-Substituted Tetrapeptide 3. Boc-Trp-Lys[N^{ϵ}-(4-hydroxycinnamoyl)]-Asp-Phe-NH_2 (3m). A solution of 4-hydroxycinnamic acid (300 mg, 1.83 mmol), *N*-hydroxysuccinimide (252 mg, 2.19 mmol), and EDCI (385 mg, 2.00 mmol) in methylene chloride (20 mL) was stirred at ambient temperature for 18 h. The solvent was removed in vacuo, and the residue chromatographed on silica gel (ethyl acetate–hexane, 1:1) to yield 280 mg (59%) of the *N*-hydroxysuccinimide ester as a white solid: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.85 (br s, 4 H), 6.68 (d, J = 15 Hz, 1 H), 6.83 (d, J = 15 Hz, 1 H), 7.68 (d, J = 8 Hz, 2 H), 7.85 (d, J = 8 Hz, 2 H). To a solution of Boc-Trp-Lys-Asp-Phe-NH_2 (**2**)[13] (120 mg, 0.17 mmol) in DMF (20 mL) cooled to 0 °C were added 4-hydroxycinnamic acid *N*-hydroxysuccinimide ester (60 mg, 0.23 mmol) and *N*-methylmorpholine (20 mg, 0.20 mmol). The mixture was stirred overnight with warming to ambient temperature. The DMF was removed in vacuo, and the residue was chromatographed on silica gel using ethyl acetate–pyridine–acetic acid–water (42:3.3:1:1.8). The solvents were removed in vacuo, and the residue was dissolved in aqueous acetone and lyophilized to yield 96 mg (67%) of **3mm** as a white flocculent powder: MS (FAB+) m/e 840 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.12–1.68 (m, 6 H), 1.32 (br s, 9 H), 2.38–2.68 (m, 4 H), 2.82–3.19 (m, 4 H), 4.19–4.31 (m, 2 H), 4.32–4.41 (m, 1 H), 4.45–4.53 (m, 1 H), 6.41 (d, J = 15 Hz, 1 H), 6.76 (d, J = 8 Hz, 2 H), 6.92–7.45 (m, 13 H), 7.58 (d, J = 7 Hz, 1 H), 7.92–8.05 (m, 3 H), 8.25 (br d, J = 7 Hz, 1 H), 10.82 (br s, 1 H). Anal. Calcd for $C_{44}H_{53}N_7O_{10}$: C 62.92, H 6.36, N 11.67; found: C 63.24, H 6.43, N 11.64.

General Procedure for the Preparation of Sulfated Amide-Substituted Tetrapeptide 4. Boc-Trp-Lys[N^{ϵ}-(4-(sulfooxy)cinnamoyl)]-Asp-Phe-NH_2 (4d). To a solution of **3m** (50 mg, 0.06 mmol) in DMF (4 mL) and pyridine (4 mL) was added pyridinium acetyl sulfate (131 mg, 0.60 mmol). The reaction mixture was stirred at ambient temperature for 18 h, then poured into water (50 mL), and neutralized to pH 7.0–7.5 with 1 M NaOH solution. The mixture was concentrated in vacuo, and the residue was suspended in methanol and filtered. The filtrate was evaporated in vacuo, and the residue was chromatographed via preparative reverse-phase HPLC (acetonitrile–0.05 M ammonium acetate, pH 4.5). Lyophilization of solvents yielded 35 mg (64%) of **4d** as a white flocculent powder: MS (FAB–) m/e 918 $(M - H)^-$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.12–1.18 (m, 6 H), 1.32 (br s, 9 H), 2.45–2.75 (m, 4 H), 2.82–3.24 (m, 4 H), 4.21–4.32 (m, 2 H), 4.38–4.44 (m, 1 H), 4.49–4.58 (m, 1 H), 6.51 (d, J = 15 Hz, 1 H), 6.78 (br s, 1 H), 6.92–7.46 (m, 16 H), 7.58 (br d, J = 7 Hz, 1 H), 7.82 (br d, J = 7 Hz, 1 H), 7.92 (br d, J = 7 Hz, 1 H), 8.01 (br s, 1 H), 8.27 (br s, 1 H), 10.72 (br s, 1 H). Anal. Calcd for $C_{44}H_{53}N_7O_{13}S{\cdot}NH_4OH$: C 53.81, H 6.26, N 11.41; found: C 53.81, H 5.79, N 11.24.

Boc-Trp-Lys-Asp-(NMe)Phe-NH_2 (5). a. Cbz-(NMe)Phe, Sodium Salt. A suspension of Cbz-L-Phe (24.97 g, 83.4 mmol), *p*-toluenesulfonic acid (1.0 g, 5.26 mmol), and paraformaldehyde (5.00 g, 166 mmol) in 600 mL of toluene was heated at reflux in a Dean–Stark condensor until the reaction became homogeneous. The reaction solution was cooled to ambient temperature and diluted with EtOAc (300 mL). The organic layer was washed with

water (3 × 150 mL) and dried over $MgSO_4$. The solvent was filtered and evaporated in vacuo to yield a white solid (25.94 g). The solid was dissolved in chloroform (400 mL) to which triethylsilane (40 mL, 250 mmol) and trifluoroacetic acid (360 mL, 5 mol) were subsequently added.[20] The solution was allowed to stir for 20 h. The reaction was quenched by the addition of crushed ice (1 L). The layers were separated, and the aqueous fraction was further extracted with chloroform (3 × 300 mL). The combined chloroform extracts were washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo to yield an oil that was added to a stirring solution of saturated $NaHCO_3$. The resulting precipitate was filtered, washed with Et_2O, and dried in a vacuum oven at 50 °C: MS (DCI/NH_3) m/e 314 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 2.74 (s, 1.5 H), 2.77 (s, 1.5 H), 3.28 (m, 2 H), 4.52 (dd, J = 12.0, 3.6 Hz, 0.5 H), 4.61 (dd, J = 12.0, 3.6 Hz, 0.5 H), 4.79 (d, J = 12 Hz, 0.5 H), 4.86 (d, J = 12 Hz, 0.5 H), 4.89 (d, J = 12 Hz, 0.5 H), 4.98 (d, J = 12 Hz, 0.5 H), 7.04–7.32 (m, 10 H). **b. Cbz-(NMe)Phe-NH_2.** To a solution of Cbz-(NMe)Phe sodium salt (20.6 g) and *N*-methylmorpholine (7.01 g) in THF (500 mL) cooled to −10 °C was added isobutyl chloroformate (9.5 g) dropwise over 2 min. After stirring for 5 min, a solution of aqueous NH_4OH (12 mL) was added. The reaction mixture was allowed to stir for an additional 15 min at −10 °C, and then allowed to warm to ambient temperature. The product was precipitated with the addition of water, collected, and dried to yield 18 g of a white solid: MS (CI/NH_3) m/e 313 $(M + H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 2.82–2.97 (m, 1 H), 3.13–3.28 (m, 1 H), 3.35 (s, 3 H), 4.75–5.05 (m, 3 H), 7.13–7.55 (m, 10 H). **c. (NMe)Phe-NH_2.** A solution of Cbz-(NMe)Phe-NH_2 (7.4 g, 55.8 mmol) in 500 mL of MeOH was shaken with 20% Pd–C (8.7 g) under 4 atm of H_2. The catalyst was filtered, and solvent was removed under vacuo to yield 9.35 g (94%) of a solid: MS (DCI/NH_3) m/e 279 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 2.18 (s, 3 H), 2.67 (dd, J = 13.5, 7.5 Hz, 1 H), 3.08 (dd, J = 7.8, 6.0 Hz, 1 H), 7.24 (m, 5 H), 7.01 (br s, 1 H). **d. Boc-Asp(OBzl)-(NMe)Phe-NH_2.** To a 0 °C solution of Boc-Asp(OBzl)-OH (39.9 g, 122 mmol) in methylene chloride (350 mL) was added EDCI (11.6 g, 61 mmol) in methylene chloride (150 mL) over 5 min. After stirring for 1 h at 0 °C, a solution of (NMe)Phe-NH_2 (8.33 g) in methylene chloride (300 mL) was added. The reaction was stirred an additional 2 h at 0 °C, and then allowed to warm to ambient temperature and stand for 18 h. The solvent was removed in vacuo, and the residue was dissolved in EtOAc and washed with 1 M H_3PO_4 (3 × 75 mL), followed by washings with saturated $NaHCO_3$ solution (2 × 75 mL). After washing with brine and drying over Na_2SO_4, the solvent was evaporated in vacuo to yield 25 g of the protected dipeptide as a white solid: MS (DCI/NH_3) 484 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 1.33 (s, 4 H), 1.35 (s, 5 H), 2.74 (s, 1.5 H), 2.79–2.89 (m, 2 H), 2.92 (s, 1.5 H), 3.09–3.28 (m, 2 H), 4.43 (m, 1 H), 4.86 (dd, J = 11.5, 3.0 Hz, 1 H), 4.96–5.03 (m, 2 H), 6.95–7.48 (m, 13 H). **e. Boc-Lys(Cbz)-Asp(OBzl)-(NMe)Phe-NH_2.** To a solution of Boc-Asp(OBzl)-(NMe)Phe-NH_2 (14.55 g, 30.1 mmol) in glacial acetic acid (15 mL) was added a solution of 1.4 N HCl (anhydrous) in glacial acetic acid (60 mL). The solution was stirred for 30 min at ambient temperature and then quenched with the addition of ether (200 mL). The resulting precipitate was collected, washed with fresh ether, and dried under vacuo to yield 12.27 g of a white powder as the hydrochloride salt of the dipeptide. Boc-Lys(Cbz)-OH (1.49 g, 3.92 mmol) was dissolved in THF (35 mL) and cooled to −12 °C to which was added *N*-methylmorpholine (0.432 mL, 3.92 mmol) followed by isobutyl chloroformate (0.384 mL, 3.96 mmol). After stirring at −12 °C for 5 min, the hydrochloride salt of the dipeptide (1.37 g, 3.27 mmol) in THF (10 mL) and DMF (0.5 mL) was added, followed 3 min later by addition of *N*-methylmorpholine (0.432 mL, 3.92 mmol). The reaction was allowed to stir for 1 h at −12 °C, then warmed to ambient temperature, and allowed to stand for 20 h. The THF was removed in vacuo, and the resulting residue was dissolved in EtOAc (200 mL) and washed with 1 N H_3PO_4 (3 × 100 mL), saturated $NaHCO_3$ solution (3 × 100 mL), and brine. After drying over Na_2SO_4, the solvent was evaporated in vacuo, and the residue was chromatographed on silica gel using 5% MeOH in $CHCl_3$ to yield 2.06 g (84%) of a white solid: MS (FAB+) m/e 746 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 1.12–1.43 (m, 4 H), 1.38 (s, 9 H), 2.12–2.23 (m, 2 H), 2.74 (s, 1.5 H), 2.78–2.98 (m, 2 H), 2.91 (s, 1.5 H), 3.11 (m, 2 H), 3.25 (dd, J = 15.0, 6.0 Hz, 2 H), 3.83 (m, 1 H), 4.67 (m, 1 H), 4.89 (m, 1 H), 5.03 (m, 4 H), 6.80 (m, 1 H), 6.98–7.46 (m, 15 H), 8.12 (d, J = 9.0 Hz, 1 H), 8.30 (s, 2 H), 8.42 (d, J = 6.0 Hz, 1 H). **f. Boc-Trp-Lys(Cbz)-Asp(OBzl)-(NMe)Phe-NH_2.** To a solution of Boc-Lys(Cbz)-Asp(OBzl)-(NMe)Phe-NH_2 (3.26 g, 4.38 mmol) in glacial acetic acid (10 mL) cooled to 0 °C was added a 1.4 N solution of HCl (anhydrous) in glacial acetic acid (20 mL). The reaction was warmed to ambient temperature and allowed to stir for 30 min. The acetic acid solution was frozen and lyophilized to yield 2.98 g (91%) of a white flocculent solid. The hydrochloride salt (2.24 g, 3.29 mmol), Boc-Trp-OH (1.10 g, 3.62 mmol), and HOBt (0.755 g, 4.94 mmol) were dissolved in methylene chloride (40 mL) and DMF (5 mL). The solution was cooled to 0 °C and *N*-methylmorpholine (0.398 mL, 3.62 mmol) and EDCI (0.723 g, 3.78 mmol) were added. The reaction was allowed to warm to ambient temperature and stirred for 20 h. The solvents were removed in vacuo, and the resulting residue was taken up in EtOAc (300 mL) and washed with 1 N H_3PO_4 (3 × 75 mL), a solution of saturated $NaHCO_3$ (3 × 75 mL), and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was flash chromatographed on silica gel using a 5% MeOH in $CHCl_3$ solution as the eluent to yield 2.45 g (80%) of a white solid: MS (FAB+) m/e 932 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 1.28 (s, 9 H), 0.9–1.58 (m, 6 H), 2.18 (dd, J = 18.0, 9.0 Hz, 0.5 H), 2.53–2.58 (m, 0.5 H), 2.73 (s, 0.5 H), 2.91 (s, 2.5 H), 2.79–2.99 (m, 3 H), 3.01–3.18 (m, 2 H), 3.01–3.18 (m, 2 H), 4.21 (m, 2 H), 4.67 (m, 1 H), 4.87 (dd, J = 10.5, 3.0 Hz, 1 H), 4.97–5.14 (m, 4 H), 6.82 (t, J = 6.0 Hz, 1 H), 6.92–7.48 (m, 19 H), 7.60 (t, J = 6.0 Hz, 2 H), 7.74 (d, J = 9 Hz, 1 H), 7.92 (d, J = 7 Hz, 1 H), 8.30 (d, J = 9.0 Hz, 1 H), 8.60 (d, J = 6.0 Hz, 1 H), 10.78 (br s, 1 H). **(5).** A solution of Boc-Trp-Lys(Cbz)-Asp(OBzl)-(NMe)Phe-NH_2 (1.98 g, 2.13 mmol) and 10% Pd–C (0.8 g) in glacial acetic acid (50 mL) was stirred under 1 atm of hydrogen for 3 h. The reaction mixture was filtered, and the solvent volume was reduced via evaporation in vacuo to 1–2 mL. Diethyl ether (80 mL) was added to the acetic acid solution to precipitate the product which was collected and dried to yield 1.18 g (78%) of a white solid: MS (FAB+) m/e 708 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 1.31 (s, 9 H), 1.12–1.43 (m, 4 H), 1.51 (m, 2 H), 2.18 (m, 1 H), 2.56 (m, 1 H), 2.70 (m, 3 H), 3.02 (s, 1 H), 2.75–3.15 (m, 5 H), 4.22 (m, 2 H), 4.89 (m, 1 H), 5.24 (dd, J = 10.5, 4.5 Hz, 1 H), 6.77 (d, J = 7.5 Hz, 1 H), 6.93–7.25 (m, 10 H), 7.32 (d, J = 7.5 Hz, 1 H), 7.60 (m, 2 H), 7.85 (m, 1 H), 7.93 (d, 7.5 Hz, 1 H), 8.00 (d, J = 7.5 Hz, 1 H), 10.88 (m, 1 H).

General Procedure for the Preparation of (NMe)Phe Amides 6. Boc-Trp-Lys[N^{ϵ}-(4-hydroxycinnamoyl)]-Asp-(NMe)Phe-NH_2 (6b). Tetrapeptide **6b** was prepared by reacting **5** in an analogous manner as described in the preparation of tetrapeptide **3m**. Compound **6b**: mp 152–189 °C; MS (FAB+) m/e 854 $(M + H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.05–1.62 (m, 6 H), 1.29 (br s, 9 H), 2.26–2.54 (m, 2 H), 2.69–3.26 (m, 9 H), 4.15–5.20 (m, 4 H), 6.42 (d, J = 16 Hz, 1 H), 6.72–6.85 (m, 3 H), 6.90–7.65 (m, 14 H), 7.79–8.00 (m, 2 H), 8.24 (br d, 1 H), 8.59 (m, 1 H), 9.82 (br s, 1 H). Anal. Calcd for $C_{45}H_{57}N_7O_{10}\cdot CH_3COOH\cdot 0.5H_2O$: C 61.09, H 6.65, N 10.61; found: C 60.66, H 6.26, N 11.00.

General Procedure for the Preparation of Sulfated (NMe)Phe Amides 7. Boc-Trp-Lys[N^{ϵ}-(4-(sulfooxy)-cinnamoyl)]-Asp-(NMe)Phe-NH_2 (7a). Tetrapeptide **7a** was prepared in an analogous manner to tetrapeptide **4d**. Compound **7a**: mp 156–215; MS (FAB−) m/e 932 $(M - H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.04–1.43 (m, 15 H), 2.02–2.12 (m, 1 H), 2.34–2.42 (m, 1 H), 2.73–2.94 (m, 5 H), 3.03–3.32 (m, 4 H), 4.16–4.30 (m, 2 H), 4.61–4.70 (m, 1 H), 4.84–4.92 (m, 1 H), 4.93–5.02 (m, 1 H), 5.09–5.17 (m, 1 H), 6.43–6.57 (m, 2 H), 6.78–6.84 (m, 1 H), 6.93–7.52 (m, 12 H), 7.58–7.64 (m, 1 H), 7.80–7.93 (m, 1 H), 8.00–8.09 (m, 2 H), 8.27–8.31 (m, 1 H), 8.59–8.63 (m, 1 H). Anal. Calcd for $C_{45}H_{55}N_7O_{13}S\cdot H_2O\cdot NH_4OH$: C 54.85, H 6.31, N 11.23; found: C 54.61, H 6.08, N 10.85.

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