Tetrapeptide CCK Agonists: Structure–Activity Studies on Modifications at the N-Terminus

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We had reported earlier[1] on a novel series of potent and selective tetrapeptide cholecystokinin-A (CCK-A) agonists of the general structure Boc-Trp-Lys[ϵ-Y]-Asp-N(R)PheNH$_2$ [Y = amides, ureas; R = H, Me] that were potent anorectic agents in rats. In an effort to optimize the potency, selectivity, stability, and efficacy of our lead candidate A-71623 [R = Me, Y = *o*-tolylaminocarbonyl; Tac] toward development of a clinical candidate, we have explored a series of analogues in which the N-terminal Boc functionality was systematically replaced with various amides, ureas, carbamates, and sulfonamides of differing size, hydrophobicity, and stereoelectronic properties. In general, these analogues maintained good potency and selectivity for the CCK-A receptor (guinea pig pancreas), as well as potent anorectic activity in rats. Those analogues exhibiting equal or superior activity compared to A-71623 but differing physicochemical properties may represent superior drug candidates.

Cholecystokinin (CCK) is a brain–gut peptide that acts as a neurotransmitter/neuromodulator in the central and the peripheral nervous systems. The multiple peripheral actions of this peptide include regulation of pancreatic and bile secretions, gall bladder contractions, and gastric emptying, whereas in the central nervous system (CNS) CCK may modulate the dopaminergic and enkephalinergic systems.[2] These actions are mediated by multiple receptors, designated as CCK-A (alimentary) and CCK-B (brain) receptors, both of which are found in the CNS and periphery, and are readily distinguished by their differential affinity for various ligands. Evidence indicates that most of the gastrointestinal effects of CCK are mediated through the A-type receptor, whereas it appears that the various central effects of cholecystokinin are mediated by both CCK-A and CCK-B receptors. It has been shown that CCK and CCK agonists can regulate food intake in animals, including rats and primates (including man),[3] and that this anorectic effect is mediated via the CCK-A receptor located on vagal afferent fibers.[4] Thus, CCK agonists represent novel anorectic agents which may have fewer problems than those associated with existing therapies for weight control.

We have shown previously that our novel series of tetrapeptides are potent and selective CCK-A agonists with good anorectic activity in rats.[1,8] A detailed investigation of various Lys-ϵ-N-urea and amide analogues of these tetrapeptides indicated that A-71623, Boc-Trp-Lys-[Tac]-Asp-N(Me)Phe-NH$_2$, had a good profile in terms of potency and receptor selectivity. However, this compound had low oral bioavailability (~1%) as well as potential problems with long-term (shelf) storage related to the chemical lability of the Boc group. In addition, although many modifications had been made on other areas of the molecule, the structure–activity relationship (SAR) at the N-terminus remained unexplored. Derivatization of peptides at the N- or C-terminus has frequently been carried out in attempts to improve the activity, bioavailability, duration of action, and/or physicochemical properties of potential drug candidates, often with much success.[5] This paper describes the results of N-terminal modifications on A-71623 which were undertaken to optimize the potency, selectivity, efficacy, and physicochemical properties of this series in order to identify a potential drug candidate for a novel CCK-based anorectic agent.

Methods

The tetrapeptide precursor H-Trp-Lys(Tac)-Asp(β-OBn)-N(Me)Phe-NH$_2$ was made by standard solution-phase peptide coupling of Boc-Lys(Tac)-OH to Asp(β-OBn)-N(Me)Phe-NH$_2$,[6] followed by deprotection, coupling with Boc-Trp-OH, and removal of the Boc group (see the Experimental Section). The N-terminal ureas and carbamates were, in general, prepared by reacting the tetrapeptide H-Trp-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH$_2$ with an isocyanate or chloroformate, respectively. The N-terminal amides were synthesized by reacting the amine with the corresponding acid chloride, carbodiimide (EDCI) coupling with a carboxylic acid, or via reaction with an anhydride. Removal of the benzyl protecting group was effected via hydrogenolysis using 10% Pd–C in various solvents. The analytical and physical data for compounds **1–24** are shown in Table 1.

The target compounds were tested at both the CCK-A receptor (guinea pig pancreas) and CCK-B receptor (guinea pig cortex) as previously described[7] using [^{125}I]Bolton–Hunter CCK-8 as the radioligand. Functional activity of these analogues in stimulating PI hydrolysis were performed as previously described.[8] The activity of analogues in feeding behavior was measured in rats as previously described.[9]

Results and Discussion

The moieties appended onto the N-terminus of the tetrapeptide were strategically chosen to represent groups with widely varying sizes and polarities. The HPLC retention times for these tetrapeptides vary from 9.44 min (compound **20**, Table 1) to over 15 min (compounds **8**, **16**,

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Table 1. Analytical and Physical Properties of Tetrapeptides R-Trp-Lys(Tac)-Asp-N(Me)-Phe-NH_2

compd	R	formula	method[a]	Anal.[b]	MS	HPLC[c]
1	*tert*-butyloxycarbonyl	$C_{44}H_{56}N_8O_9 \cdot H_2O$	A	C, H, N	841 $(M + H)^+$	12.87
2	t-BuNHCO	$C_{44}H_{57}N_9O_8 \cdot 0.5H_2O \cdot 0.5HOAc$	B	C, H, N	840 $(M + H)^+$	12.50
3	t-BuCH_2CO	$C_{45}H_{58}N_8O_8 \cdot H_2O \cdot 0.5HOAc$	C	C, H, N	839 $(M + H)^+$	12.91
4	acetyl	$C_{41}H_{50}N_8O_8 \cdot 2H_2O$	E	C, H, N	783 $(M + H)^+$	10.22
5	benzyloxycarbonyl	$C_{47}H_{54}N_8O_{11} \cdot 3H_2O$	D	C, H, N	875 $(M + H)^+$	12.83
6	MeOCO	$C_{41}H_{50}N_8O_9 \cdot 1.5H_2O$	D	C, H, N	799 $(M + H)^+$	10.79
7	MeNHCO	$C_{41}H_{57}N_9O_8 \cdot 1.4H_2O \cdot 1.4HOAc$	B	C, H, N	888 $(M + H)^+$	10.10
8	2-adamantyloxycarbonyl	$C_{50}H_{62}N_8O_9 \cdot 2H_2O \cdot 0.6HOAc$	D	C, H, N	902 $(M - NH_2)^+$	15.63
9	benzoyl	$C_{46}H_{52}N_8O_8 \cdot 1.5H_2O$	E	C, H, N	845 $(M + H)^+$	12.24
10	phenylOCO	$C_{46}H_{52}N_8O_9 \cdot 2.5H_2O$	D	C, H, N	861 $(M + H)^+$	12.75
11	phenylNHCO	$C_{46}H_{53}N_9O_8 \cdot 1.8H_2O \cdot 1.8HOAc$	B	C, H, N	860 $(M + H)^+$	12.42
12	phenacetyl	$C_{47}H_{54}N_8O_8$	C	HRMS[d]	859 $(M + H)^+$	12.41
13	3-COOH-propionyl	$C_{43}H_{52}N_8O_{10} \cdot H_2O$	E	C, H, N	931 $(M + H)^+$	10.75
14	methylsulfonyl	$C_{40}H_{50}N_8O_9S \cdot 1.5H_2O$	F	C, H, N	841 $(M + H)^+$	10.55
15	diphenylpropionyl	$C_{54}H_{60}N_8O_8 \cdot H_2O$	G	C, H, N	971 $(M + Na)^+$	14.67
16	1-adamantyloxycarbonyl	$C_{50}H_{62}N_8O_9 \cdot 2H_2O$	D	C, H, N	919 $(M + H)^+$	15.35
17	isobutyloxycarbonyl	$C_{44}H_{56}N_8O_9 \cdot 0.5H_2O \cdot 0.5HOAc$	A	C, H, N	841 $(M + H)^+$	12.99
18	isopropyloxycarbonyl	$C_{43}H_{54}N_8O_9 \cdot 1.5HOAc$	A	C, H, N	827 $(M + H)^+$	12.07
19	propionyl	$C_{42}H_{52}N_8O_8 \cdot 0.5H_2O \cdot HOAc$	C	C, H, N	797 $(M + H)^+$	10.72
20	2-carboxybenzoyl	$C_{47}H_{52}N_8O_{10} \cdot 2.5H_2O \cdot 0.5HOAc$	E	C, H, N	875 $(M + H)^+$	9.44
21	*N*,*N*-dimethylGly	$C_{43}H_{56}N_9O_8 \cdot 1.5H_2O \cdot HOAc$	G	C, H, N	826 $(M + H)^+$	10.15
22	1-adamantylacetyl	$C_{51}H_{64}N_8O_8$	G	HRMS[e]	917 $(M + H)^+$	15.06
23	pivaloyl	$C_{51}H_{62}N_8O_8 \cdot H_2O$	E	C, H, N	825 $(M + H)^+$	12.19
24	trifluoroacetyl	$C_{41}H_{47}F_3N_8O_8 \cdot H_2O \cdot 0.4HOAc$	E	C, H, N	836 $(M + H)^+$	11.68[f]

[a] See the Experimental Section general methods: (A) 1+3 peptide coupling; (B) isocyanate + tetrapeptide; (C) acid chloride + tetrapeptide; (D) haloformate + tetrapeptide; (E) anhydride + tetrapeptide; (F) sulfonyl chloride + tetrapeptide; (G) carboxylic acid + EDCI + tetrapeptide. [b] Elemental analyses were within ±0.4% of theoretical values. [c] Vydac C-18 RP HPLC column, 20–80% MeCN/50 mM NH_4OAc; flow rate = 1 mL/min. [d] Calcd 859.4143, measured 859.4143. [e] Calcd 917.4925, measured 917.4918. [f] Vydac C-18 RP HPLC column, 30–80% MeCN/50 mM NH_4OAc; flow rate = 1 mL/min.

Table 2. Biological Data for N-Terminal Tetrapeptides R-Trp-Lys(Tac)-Asp-N(Me)Phe-NH_2

compd	R	guinea pig binding IC_{50} (nM)[a,b] pancreas	guinea pig binding IC_{50} (nM)[a,b] cortex	ratio (cortex/pancreas)	PI[c] (% max., guinea pig)	FI(ED_{50})[d,e] (nmol/kg; rat)
1	*tert*-butyloxycarbonyl	3.0 ± 0.49 (41)	4700 (19) ± 93.5	1600	110 ± 2.05 (3)	4
2	t-BuNHCO	5.4 ± 1.46 (3)	3500 ± 807.3	600	98 ± 2.98 (4)	38
3	t-BuCH_2CO	1.4 ± 0.23 (3)	5200(4) ± 293.8	3600	100 ± 2.29 (4)	7
4	acetyl	4.8 ± 0.72 (6)	6400(5) ± 197.6	1300	93 ± 2.42 (6)	12
5	benzyloxycarbonyl	6.2 ± 1.22 (3)	4800(3) ± 901.5	800	103 ± 1.69 (5)	10
6	MeOCO	3.9 ± 0.76 (5)	5200(3) ± 717.4	1300	103 ± 3.89 (3)	4
7	MeNHCO	5.9 ± 0.73 (3)	4800(3) ± 63.6	800	94 ± 2.54 (4)	25
8	2-adamantyloxycarbonyl	11 ± 4.41 (3)	7400(4) ± 372.4	700	105 ± 4.27 (3)	230
9	benzoyl	1.3 ± 0.29 (3)	4600(3) ± 487.5	3400	95 ± 1.26 (5)	64(2)
10	phenylOCO	4.1 ± 1.31 (3)	6500(3) ± 821.4	1600	94 (3)	210
11	phenylNHCO	3.0 ± 0.93 (3)	3000(3) ± 483.8	1000	98 ± 2.75 (3)	61
12	phenylacetyl	18 ± 2.72 (3)	7600(3) ± 398.6	420	85 (4)	ND
13	3-carboxypropionyl	13 ± 1.69 (3)	6400(3) ± 682.5	490	91 ± 1.23 (4)	15
14	methylsulfonyl	41 ± 10.31 (4)	47% (3) ± 1.4%[f]	N/A	83 ± 2.74 (4)	900
15	diphenylpropionyl	2.2 ± 0.38 (3)	6100(3) ± 920.5	2800	100 ± 2.58 (4)	83
16	1-adamantyloxycarbonyl	17.0 ± 0.87 (3)	49% (3) ± 9.9%[f]	N/A	100 ± 6.78 (3)	78
17	isobutyloxycarbonyl	2.3 ± 0.35 (3)	6100(3) ± 845.0	2600	100 ± 1.53 (3)	5(2)
18	isopropyloxycarbonyl	1.6 ± 0.11 (3)	4500(3) ± 277.6	2800	100 ± 2.78 (3)	3
19	propionyl	2.0 ± 0.16 (3)	5700(3) ± 429.8	2800	93 ± 1.99(3)	6
20	2-carboxybenzoyl	100 ± 29.27 (3)	2500(3) ± 355.1	25	11 ± 6.53 (3)	ND
21	*N*,*N*-dimethylGly	11 ± 0.28 (3)	35% (3) ± 6.6%[f]	N/A	96 ± 2.54 (4)	7
22	1-adamantylacetyl	19 ± 5.22 (3)	41% (3) ± 3.3%[f]	N/A	95 ± 2.05 (5)	ND
23	pivaloyl	1.1 ± 0.21 (3)	4700(4) ± 885.6	4100	98 ± 3.81 (4)	4
24	trifluoroacetyl	1.7 ± 0.24 (4)	42% (3) ± 5.9%[f]	N/A	99 ± 2.57 (3)	8

[a] Concentration of the peptide that inhibited 50% of the specific binding. [b] Number of determinations are in parentheses. [c] Percent response of peptide at a concentration of 10^{-4} M in PI hydrolysis relative to maximal response elicited by CCK-8. [d] Dose of peptide (ip) required to reduce food intakes by 50%. [e] ND = not determined. [f] Percent inhibition of specific [^{125}I]Bolton–Hunter CCK-8 binding at 10^{-5} M.

22), demonstrating the large physicochemical differences between these analogues. In addition, most of these tetrapeptides would be expected to have a better shelf-life than A-71623, which possesses the labile *tert*-butyloxycarbonyl (Boc) moiety.

In general, most of the N-terminal modified analogues retained high affinity and selectivity for the CCK-A receptor (Table 2). The most potent analogues, compounds **3**, **9**, **11**, **15**, **17**, **18**, **19**, **23**, and **24**, had binding affinities comparable or slightly better than A-71623. However, a few compounds, notably **14** and **20**, had somewhat lower affinity for the pancreatic receptor (41 and 100 nM, respectively). In general, the pancreatic receptor appears to have a greater tolerance for larger hydrophobic groups than for the more polar methyl sulfonamide or carboxylate groups of **14** and **20**, although the carboxylate-containing analogue **13** had fairly good binding affinity. In contrast to results found by the Parke-Davis group in developing their dipeptoid CCK-B selective antagonists,[10] appending the 2-adamantyloxycarbonyl moiety at the N-terminus of A-71623 afforded no improvement in CCK-A or CCK-B receptor affinity. A

number of analogues had better receptor selectivity than 1 (cortex IC_{50}/pancreas IC_{50} = 1600), the largest being 4100 for compound **23**. Due to its low CCK-A binding affinity, compound **20** showed modest receptor selectivity, with a ratio of only 25.

The N-terminal modifications seemed to have a greater effect on the agonist efficacy of these compounds as measured by their ability to stimulate PI hydrolysis. None of the compounds demonstrated greater intrinsic activity than 1, although most had close to 100% activity. The most notable exception is compound **20**, which showed a maximal stimulation of only 11%. Several other analogues (e.g. **12** and **14**) had maximal activity less than 90%. In general, the carbamates (with the notable exception of **10**) and ureas tended to have greater intrinsic activity than the amides, which tended to have less than full agonism. In cases where isosteric replacements were made the carbamates tended to show the highest % PI, e.g. comparing compounds **1**, **2**, and **3** (t-Bu-X-CO, X = O, NH, and CH_2; % PI = 110, 98, and 100, respectively) and **6**, **7** and **19** (Me-X-CO, X = O, NH and CH_2; % PI = 103, 94, and 93, respectively), although this trend is not statistically significant. However, analogues **10**, **11**, and **12** did not show this trend (C_6H_5-X-CO, X = O, NH, and CH_2; % PI = 94, 98, and 85, respectively). These trends were not found in binding affinity for the pancreatic receptor.

The ability of these analogues to suppress food intake in our feeding paradigm in rat varied greatly from ~3–4 to >900 nmol/kg, and there appears to be very little correlation between either receptor affinity, % PI hydrolysis, or the ED_{50}. In addition, there appears to be little correlation between the in vitro stability of these peptides and their anorectic activity (data not shown).[11] Interestingly, the ureas as a class showed poor suppression of food intake, whereas other analogues with similar binding affinity and % PI hydrolysis showed much better activity. The overall lack of any correlation between in vitro and in vivo activity could be due to many factors, including species differences (guinea pig versus rat), in vivo half-life, bioavailability, etc. Other groups have also seen a breakdown in the correlation between pancreatic receptor binding and potency in amylase secretion with food intake.[12] Good efficacy in % PI hydrolysis may be a necessary prerequisite, but no guarantee, for efficacy in suppression of food intake. It is also possible that the pancreatic receptor is somewhat different than those found on the afferent vagal nerve which mediate the satiety effects of CCK.

In summary, a series of N-terminally modified tetrapeptide CCK-A agonists have been synthesized and tested in an effort to optimize the biological activity and physicochemical properties of these novel anorectic agents. Although it is not known what the ideal physicochemical properties for a CCK peptide agonist are, these analogues represent a pool of potential clinical candidates which vary widely in their hydrophobicity and water solubility, as well as perhaps in their biodistribution and metabolism. Thus, a large number of these analogues not only maintained good potency and selectivity for the CCK-A receptor, as well as good suppression of food intake in rats, but also have a wide range of physicochemical properties (e.g. log *P*, water solubility, good chemical stability) that should allow for the selection of a clinical candidate with the most favorable combination of efficacy, duration of action, stability, and therapeutic ratio.

Experimental Section

All solvents and reagents were reagent grade unless otherwise noted. Most of the amino acids were purchased from Aldrich Chemical Co. or Sigma Chemical Co. ^{1}H-NMR spectra were generally taken in DMSO-d_6 and recorded at 300 or 500 MHz and are reported in ppm downfield from tetramethylsilane or 3-(trimethylsilyl)propionic acid, sodium salt (D_2O). Elemental analyses were obtained from Abbott Laboratories Analytical Department, North Chicago, IL; Midwest Laboratories, Indianapolis, IN; or Robertson Laboratories, Madison, NJ. Chromatographic purifications were carried out using flash chromatography (60-mesh silica, 0.04–0.063 mm, E. Merck) or preparative HPLC (Rainin Dynamax-60A 83-221-C C-18 reverse-phase column). Analytic HPLC was performed using a Vydac C-18 reverse-phase column, monitoring at 254 and 280 nM. HPLC solvents were generally either acetonitrile/50 mM ammonium acetate or acetonitrile/0.1% aqueous trifluoroacetic acid, run either isocratically or as a gradient. All final compounds, unless otherwise noted, were analyzed by ^{1}H-NMR and mass spectroscopy, elemental analysis, and analytical HPLC, and in general had a chemical purity >95%. Abbreviations: TFA = trifluoroacetic acid; DMF = *N*,*N*-dimethylformamide; DIEA = diisopropylethylamine; Bodansky solution = H_2O/pyridine/acetic acid, 55:100:30, v:v:v; EDCI = 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; NMM = *N*-methylmorpholine.

t-BOC-Lys(Tac)-OH. To a solution of Boc-Lys-OH (1.0 g, 4.1 mmol) in H_2O (5 mL), dioxane (7 mL), and 2 N NaOH (2 mL) at 0 °C were added *o*-tolyl isocyanate (1.12 g, 8.4 mmol) in portions along with additional 2 N NaOH (4.2 mL, 8.4 mmol). The mixture was stirred and allowed to warm to ambient temperature and then made basic with additional aqueous NaOH. The solution was washed with EtOAc and acidified with aqueous $KHSO_4$, and the aqueous layer was extracted with EtOAc, dried (Na_2SO_4), and concentrated. The residue was crystallized from ether/hexane to afford 1.2 g of product: MS (CI) *m/e* 380 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.30–1.55 (m, 3H), 1.70–1.90 (m, 3H), 2.28 (s, 3H), 3.22 (m, 2H), 4.29 (m, 1H), 4.90 (br m, 2H), 5.23 (br d, J = 7.5 Hz, 1H), 7.10–7.31 (m, 4H).

t-BOC-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH$_2$. To a solution of Boc-Lys(Tac)-OH (1.36 g, 3.57 mmol) in THF (10 mL) under nitrogen was added NMM (385 uL, 3.50 mmoL) and the solution cooled to −15 °C. Isobutyl chloroformate (450 μL, 3.53 mmoL) was added, followed 10 min later by triethylamine (500 μL, 3.57 mmoL) and a solution of H-Asp(OBn)-(NMe)Phe-NH_2, hydrochloride salt[6] (1.50 g, 3.57 mmol) in DMF (5 mL), then the reaction mixture allowed to gradually warm to room temperature. After 3 h the solvent was removed in vacuo, the residue was dissolved in EtOAc, and the organic layer was washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated to afford the crude product. Chromatographic purification (silica, 2% MeOH/$CHCl_3$) afforded the pure product as a white solid (1.97 g, 74%): MS (FAB^+) *m/e* 745 $(M + H)^+$, 767 $(M - Na)^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) δ 1.35 (s, 9H), methyl singlets 2.15, 2.16 (s, 3H); 2.72, 2.88 (3H); α protons 3.84, 4.65, 4.90, 4.98–5.12 (m, 3).

H-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH$_2$, TFA Salt. To a solution of Boc-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (297 mg, 0.4 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added trifluoroacetic acid (3 mL), and the reaction mixture was stirred for 2.5 h at room temperature. The reaction mixture was then concentrated in vacuo and lyophilized from MeOH/H_2O to afford the product as a white powder (263 mg, 86%): MS (FAB^+) *m/e* 645 $(M + H)^+$, 628 $(M - NH_2)^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) methyl singlets δ 2.13, 2.15 (s, 3H); 2.74, 2.92 (3H); α protons 4.65, 4.77, 4.97–5.18 (m, 3).

General Procedure A: Coupling of an N-Terminal Protected Amino Acid with H-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH$_2$. Boc-Trp-Lys(Tac)-Asp-N(Me)Phe-NH$_2$ (1). To a solution of the TFA salt of H-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (2.27 g, 3.0 mmol) and Boc-Trp-OH (913 mg, 3 mmol) in DMF (12 mL) at ambient temperature was added NMM (395 μL, 3.6 mmol) and HOBt (405 mg, 3 mmol), followed by the addition of EDCI (632 mg, 3.3 mmol). After stirring overnight

10% aqueous citric acid (150 mL) was added to the reaction mixture and the precipitate collected by filtration. The precipitate was washed with water (2 × 50 mL) and dried at 40 °C in vacuo to give 2.72 g (97% yield) of the protected tetrapeptide as a white solid: MS (FAB$^+$) m/e 931 (M + H)$^+$, 914 (M − NH_2)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) methyl singlets δ 2.15 (s, 3H); 2.74, 2.89 (3H); α protons 4.22, 4.28, 4.66, 4.88, 4.98–5.12 (m, 2); 10.78 (br s, 1).

Hydrogenation of a portion of this material (240 mg, 0.258 mmoL) in EtOH (15 mL) using 10% Pd–C under H_2 (1 atm) followed by filtration through Celite and evaporation of the solvent afforded the title compound as a white solid (179 mg, 82%): MS (FAB$^+$) m/e 841 (M + H)$^+$, 863 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 2:1) δ methyl singlets: 2.08, 2.09 (3H), α protons 4.14, 4.23, 4.59, 4.82, 4.92, 5.97 (4H), indole NH 10.70, 10.73 (1H). Anal. Calcd for $C_{44}H_{56}N_8O_9 \cdot 1.0H_2O$: C, 61.52; H, 6.81; N, 13.04. Found: C, 61.45; H, 6.93; N, 13.06.

General Procedure B: Preparation of N-Terminal Ureas. (Methylaminocarbonyl)-Trp-Lys(Tac)-Asp-N(Me)-Phe-NH_2 (7). To a solution of the TFA salt of H-Trp-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (125 mg, 0.13 mmol) in DMF (2 mL) at ambient temperature was added DIEA (0.065 mL, 0.4 mmol) followed by methyl isocyanate (0.01 mL, 0.16 mmol). After stirring overnight water was added to the reaction mixture, and the precipitate was collected by filtration. The precipitate was washed with 10% aqueous citric acid solution and dried at 40 °C in vacuo to give 105 mg (88% yield) of the title compound as a white solid: MS (FAB$^+$) m/e 888 (M + H)$^+$; ^{1}H-NMR (DMSO-d_6, selected data, two conformers ca. 1:1) δ methyl singlets: 2.14, 2.15 (2 s, 3H), 2.42, 2.43 (2 s, 3H), 2.69, 2.72 (2 s, 3H).

Hydrogenation of this material followed by chromatography (EtOAc/Bodansky solution) afforded the title compound as a white solid: MS (FAB$^+$) m/e 798 (M + H)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 2:1) δ methyl singlets 2.14, 2.15 (3H), α protons 4.18, 4.24, 4.41, 4.72, 4.88, 4.92, 5.16 (4H), indole NH 10.78, 10.84 (1H).

General Procedure C: Preparation of N-Terminal Amides by Coupling with an Acid Chloride. Benzoyl-Trp-Lys(Tac)-Asp-N(Me)Phe-NH_2 (9). To a solution of the TFA salt of H-Trp-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (141 mg, 0.15 mmol), DIEA (65 μL, 0.375 mmol), and a catalytic amount of (*N,N*-dimethylamino)pyridine (~5 mg) in DMF (1 mL) was added benzoyl chloride (21 μL, 0.18 mmoL). After being stirred at room temperature for 6 h the reaction mixture was diluted with 10% citric acid and the product filtered, washed with 2-mL portions of aqueous 10% citric acid (3×) and water (2×), and then dried in vacuo to afford the product as white solid (121 mg, 86%): MS (FAB) m/e 835 (M + H)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) δ 2.12, 2.13, (2 s, 3H), 2.74, 2,89 (2 s, 3H), 4.16–4.34, 4.62–4.80, 4 .84–4.91 (α protons 4H), 4.95–5.12 (m, 2H), 10.76 (NH, indole, 1H).

Hydrogenation of this benzyl ester (93 mg, 0.1 mmoL) in MeOH (6 mL) afforded the product as a slightly yellow solid (69 mg, 81%): MS (FAB) m/e 845 (M + H)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 2:1) δ 2.1, 2.13 (2 s, 3H), 2.72, 2.93 (2 s, 3H), 4.22, 4.28, 4.68, 4.72, 4.75, 4.86, 4.98, 5.1 (α protons, 4H), 6.5 (NH, 2H), 10.71, 10.73 (NH, indole, 1H).

General Procedure D: Preparation of N-Terminal Carbamates by Coupling with a Haloformate. 2-(Adamantyloxycarbonyl)-Trp-Lys(Tac)-Asp-N(Me)Phe-NH_2 (8). To a solution of 2-adamantyl chloroformate[9] (35.4 mg, 0.165 mmol) and the TFA salt of H-Trp-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (141 mg, 0.15 mmol) in DMF (1 mL) was added DIEA (65 μL, 0.375 mmol) at ambient temperature. After 21 h of stirring, more chloroformate (~10 mg) was added, and after stirring an additional 90 min the reaction mixture was quenched with 10% aqueous citric acid (2 mL), stirred for 10 min, and then filtered and washed with more aqueous citric acid (2 × 1 mL) and water (3 × 1 mL). The white precipitate was dried to give the product as a white powder (127 mg, 85%): MS (FAB)$^+$ m/e 1010 (M + H)$^+$, 1048 (M + K)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) δ methyl singlets 2.14, 2.15 (2s, 3H), 2.74, 2.90 (2 s, 3H).

Hydrogenation of a portion of this tetrapeptide (101 mg, 0.10 mmoL) in MeOH (6 mL) afforded the product as a white solid (81.7 mg, 89%). Preparative HPLC (MeCN/50 mM NH_4OAc) of this material afforded an analytically pure sample: MS (FAB$^+$) m/e 902 (M − NH_2)$^+$, 919 (M + H)$^+$, 941 (M + H + Na)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) δ 2.6 (3H); 2.74, 2.95 (3H); α protons 4.21, 4.28, 4.54, 4.71, 4.88, 4.96, 5.15 (5H).

General Procedure E: Preparation of N-Terminal Amides by Coupling with an Anhydride. Pivaloyl-Trp-Lys(Tac)-Asp-N(Me)Phe-NH_2 (23). To a solution of the TFA salt of H-Trp-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (830 mg, 1.00 mmol) and NMM (132 μL, 1.20 mmol) in DMF (3 mL) was added trimethylacetic anhydride (203 μL, 1.10 mmol). After stirring at room temperature for 35 min the reaction mixture was diluted with 10% citric acid (50 mL) and the aqueous layer extracted with EtOAc (2 × 40 mL). The combined organic layers were then washed with 10% citric acid (2 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL), and brine (2 × 15 mL), dried ($MgSO_4$), and concentrated to afford the product as a white solid (778 mg, 85%). Chromatographic purification of this material (silica, EtOAc/EtOH, 30:1) afforded analytically pure material: MS (FAB$^+$) m/e 915 (M + H)$^+$, 937 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 1:1) δ 0.96 (s, 9H), 2.11 ,2.12 (2 s, 3H); 2.72, 2.88 (2 s, 3H); α-protons 4.12–4.29, 4.43–4.53, 4.60–4.68, 4.80–4.88 (4H); 4.95–5.11 (m, 2H); 10.73 (indole NH).

General Procedure F: Preparation of N-Terminal Sulfonamides by Coupling with a Sulfonyl Chloride. (Methylsulfonyl)-Trp-Lys(Tac)-Asp-N(Me)Phe-NH_2 (14). To a solution of TFA salt of H-Trp-Lys(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (200 mg, 0.22 mmoL) in DMF (5 mL) at room temperature was added DIEA (75.5 uL, 0.5 mmoL) and methanesulfonyl chloride (25 μL, 0.3 mmol). After being stirred at room temperature overnight the reaction mixture was quenched with ice water. The white precipitate formed was washed with water and 10% citric acid solution several times and then dried in vacuo overnight to afford the crude product (150 mg). Chromatographic purification (silica, EtOAc/EtOH) afforded the pure product as a white solid (85 mg, 43%). Hydrogenation of a portion of this product (95 mg, 0.11 mmol) in DMF (7 mL) afforded the pure product (66 mg, 77%): MS (FAB$^+$) m/e 841 (M + H + Na)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 2:1) δ methyl singlet 2.15 (3H), 2.72, 2.98 (3H); α-protons 4.14, 4.21, 4.28, 4.72, 4.89, 5.18 (4H), indole NH 10.85, 10.9.

General Procedure G: Preparation of N-Terminal Amides by Coupling with a Carboxylic Acid Using EDCI/HOBt. (3,3-Diphenylpropionyl)-Trp-Lys(Tac)-Asp-N(Me)-Phe-NH_2 (15). To a solution of the TFA salt of H-Trp-Lys-(Tac)-Asp(OBn)-N(Me)Phe-NH_2 (180 mg, 0.191 mmol) and DIEA (70 μL, 0.39 mmol) in DMF (4 mL) at 0 °C was added diphenylpropionic acid (50 mg, 0.195 mmol), HOBt (55 mg, 0.4 mmol), and EDCI (50 mg, 0.25 mmol). The reaction mixture was stirred overnight at ambient temperature, the solvent was removed in vacuo and the residue was dissolved in EtOAc and washed with 10% aqueous citric acid, H_2O, saturated aqueous $NaHCO_3$, and brine. This crude product was purified by chromatography (silica, EtOAc/EtOH, 99:1) to afford the product as a white solid (85 mg, 42%). A portion of this product (80 mg, 0.076 mmol) in DMF (6 mL) was hydrogenated to afford the title compound (53 mg, 73%): MS (FAB$^+$) m/e 971 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6, selected data, two conformers ca. 2:1) δ 2.12, 2.15, (3H, CH_3), 2.7, 2.93 (3H, CH_3), 4.12, 4.18, 4.36, 4.43, 4.71, 4.88, 4.93, 5.15 (4H, α protons), 10.71, 10.75 (NH, indole).

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