Modification of Receptor Selectivity and Functional Activity in Cholecystokinin Peptoid Ligands†

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Hybrid analogs of the cholecystokinin A (CCK-A) receptor selective tetrapeptide agonist Boc-Trp-Lys(Tac)-Asp-MePhe-NH_2 (**1**, A-71623) and the CCK-B receptor selective antagonists PD-135118 (**2**) and CI-988 (**3**) were prepared. Incorporation of the Lys(Tac) side chain into **2** produced a moderately potent antagonist of CCK-8 in the isolated guinea pig gallbladder (GPGB). Incorporation of the Lys(Tac) side chain into **3** produced the novel agonist analog **7** (EC_{50} = 28 nM in the GPGB) with excellent affinity for both human CCK-A (IC_{50} = 12 nM) and CCK-B (IC_{50} = 17 nM) receptors. Analog **7** was a full agonist (EC_{50} = 3.5 nM) for calcium mobilization on CHO-K1 cells expressing hCCK-A receptors but a partial agonist on CHO-K1 cells expressing hCCK-B receptors, eliciting a weak agonist reponse (EC_{50} = 2800 nM) and antagonizing CCK-8-induced calcium mobilization (K_B = 20 nM). Despite this unusual in vitro profile, analog **7** was a potent anorectic agent in rats (ED_{50} = 30 nmol/kg) following intraperitoneal administration.

Cholecystokinin (CCK) is a gastrointestinal hormone and neurotransmitter first isolated from porcine intestine.[1] CCK is released from intestinal endocrine cells in response to nutrient ingestion[2] and serves to integrate the digestion of nutrients through the induction of gallbladder contraction, pancreatic secretion, and delayed gastric emptying.[3,4] Exogenous CCK has also been shown to decrease meal size in a variety of species, including lean[5] and obese[6] humans. Two G-protein-coupled 7TM receptor subtypes have been characterized for CCK. CCK-A receptors predominate in the periphery (gallbladder, pancreas, pyloric sphincter, and vagal afferent fibers) but are also found in discrete regions of the brain.[3] CCK-B or gastrin receptors predominate in the brain and gastric glands.[7] The satiety effect of CCK requires an intact vagal afferent nerve[8] and appears to be mediated by peripheral CCK-A receptors.[9]

While a variety of endogenous molecular forms of CCK have been identified, the C-terminal octapeptide (CCK-8, H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2) appears to be the minimal sequence required for biological activity.[4] The high molecular weight, acid lability of the Tyr(SO_3H) residue, metabolic instability, and lack of receptor selectivity of CCK-8 have provided the impetus for numerous chemical modifications.[10]

Recently, two unique series of CCK analogs were reported, both derived from the CCK-B receptor selective agonist Boc-CCK-4 (Boc-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2). Replacement of the Met^{31} residue of Boc-CCK-4 with side chain-substituted Lys derivatives[11,12] produced potent CCK-A receptor selective agonists, as exemplified by **1** (A-71623; Figure 1). This tetrapeptide reduced feeding in rats,[13,14] mice, dogs, and monkeys[15] but was devoid of oral activity.

The CCK-B receptor selective dipeptoid antagonists **2** (PD-13518) and **3** (CI-988) were also developed from Boc-CCK-4. Analog **3** was reported to be orally active in mice.[17] Despite potent CCK-B receptor selective antagonist activity, **3**[18] and related analogs[19,20] retained weak CCK-A agonist activity.

Our search for an orally active analog of **1** led us to evaluate **2** and **3** as templates for the design of novel CCK-A agonists. Since incorporation of the Lys(Tac) residue into Boc-CCK-4 provided a remarkable enhancement of CCK-A receptor selectivity, we proposed substitution of this critical pharmacophore into peptoids **2** and **3**. We now report the synthesis of hybrid peptoid analogs **4**–**7** (Figure 2) in which the (*S*)-benzyl side chain of **2** or the (*R*)-phenyl side chain of **3** is replaced with the *N*-butyl-*N*-*o*-tolylurea side chain from **1**. In this first approach, the stereochemistry of the substituted diaminoethane bridge was preserved, but both the L- and D-α-MeTrp analogs were prepared.

Methods

L-α-MeTrp and D-α-MeTrp were prepared by enzymatic resolution of α-MeTrp-OCH_3.[21] N-α-Adoc-L-α-MeTrp, N-α-Adoc-D-α-MeTrp, and **3** were prepared according to published literature methods.[17]

Treatment of N-α-Boc-L- or -D-Lys with o-tolyl isocyanate (Scheme 1) provided the N-ε-substituted ureas **8** and **9**. The amino acids were converted to the α-hydroxymethyl derivatives **10** and **11** by lithium aluminum hydride reduction of the intermediate mixed anhydrides.[17,22] Azidomethyl derivatives **12** and **13** were prepared by reaction of the intermediate mesylates of **10** and **11** with sodium azide. The low overall yields for these derivatives reflect the thermal instability of the mesylates.[23]

N-α-Boc-L-lysine azidomethyl derivative **12** was deprotected (Scheme 2) with HCl/dioxane to provide the α-amino α-azidomethyl intermediate which was coupled

† Abbreviations: 2-Adoc, (2-adamantyloxy)carbonyl; Boc, (*tert*-butyloxy)carbonyl; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, *N*-hydroxybenzotriazole; NAA, Ac-Trp-Lys(Tac)-Asp-MePheNH_2; Tac, (*o*-tolylamino)carbonyl; 7TM, seven transmembrane.

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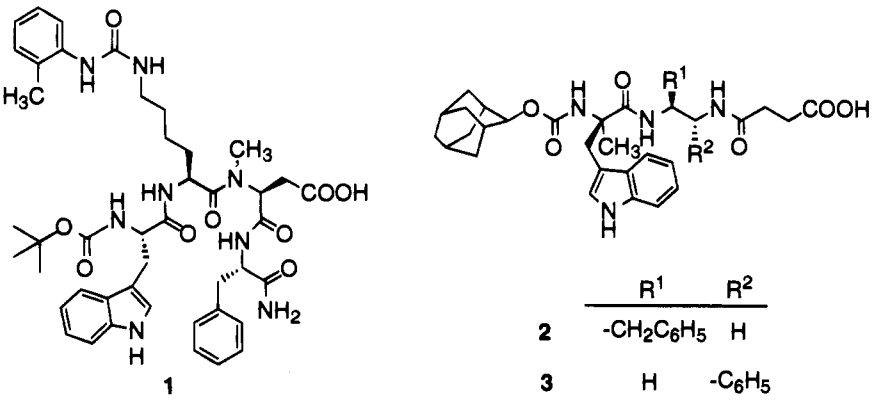


Figure 1. Structures of 1 (A-71623), **2** (PD-135118), and **3** (CI-988).

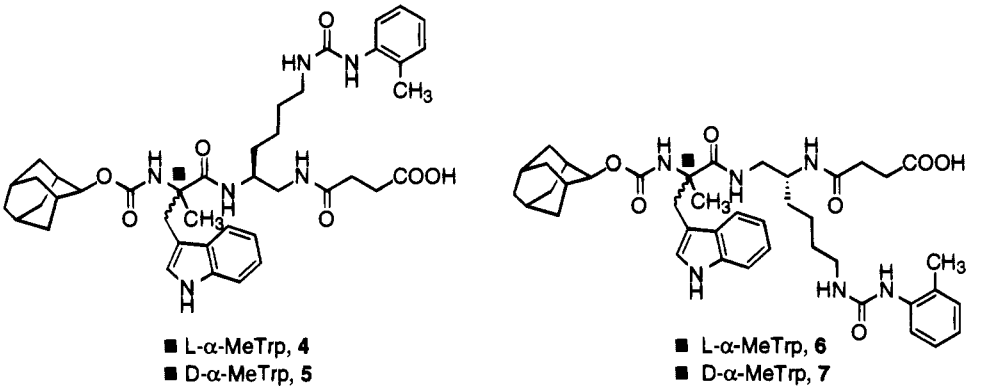
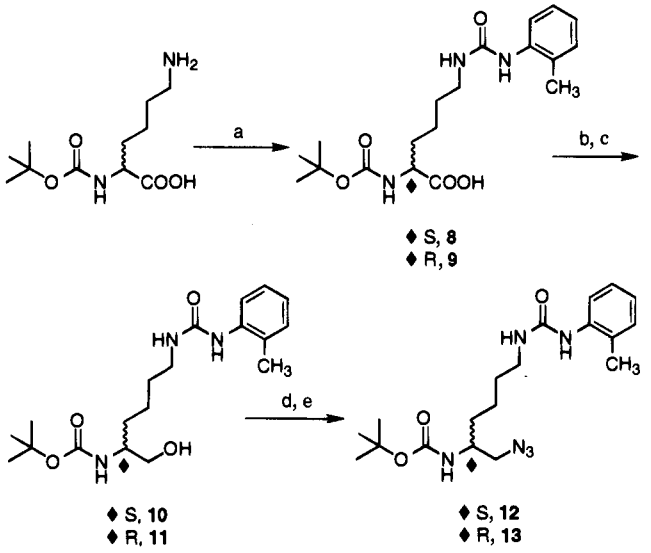


Figure 2. Target hybrid analogs based on **2** (analogs 4 and **5**) and **3** (analogs **6** and **7**).

Scheme 1[a]

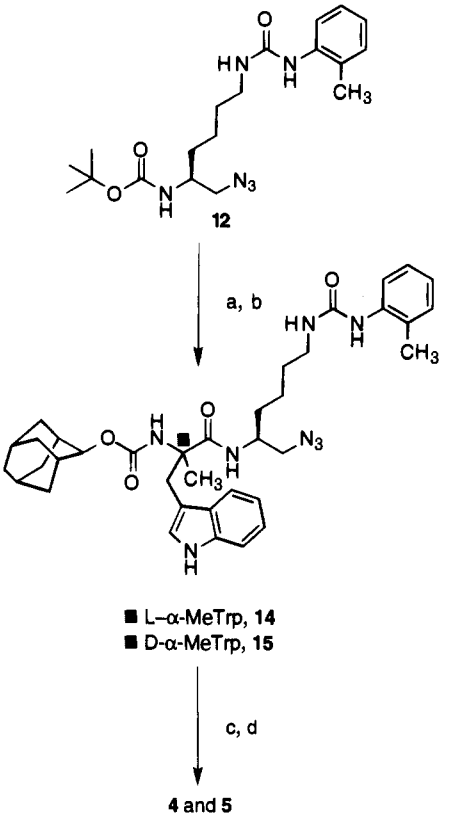


[a] (a) *o*-Tolyl isocyanate, NaOH, H_2O; (b) isobutyl chloroformate, DIEA, THF, 0 °C; (c) LAH, ether, 0–25 °C; (d) methanesulfonyl chloride, CH_2Cl_2, 0 °C; (e) NaN_3, DMF, 80 °C.

with either N-α-Adoc-L-α-MeTrp or N-α-Adoc-D-α-MeTrp to give **14** and **15**. Catalytic reduction of the azide, followed by treatment with succinic anhydride provided the L-Lys-derived target molecules **4** and **5** after RP-HPLC purification.

N-α-Boc-D-lysine azidomethyl derivative **13** was reduced (Scheme 3) to the β-amino intermediate which was coupled with either N-α-Adoc-L-α-MeTrp or N-α-Adoc-D-αMeTrp to give **16** and **17**. Treatment with HCl/dioxane followed by reaction with succinic anhydride provided the D-Lys-derived target molecules **6** and **7** following RP-HPLC purification.

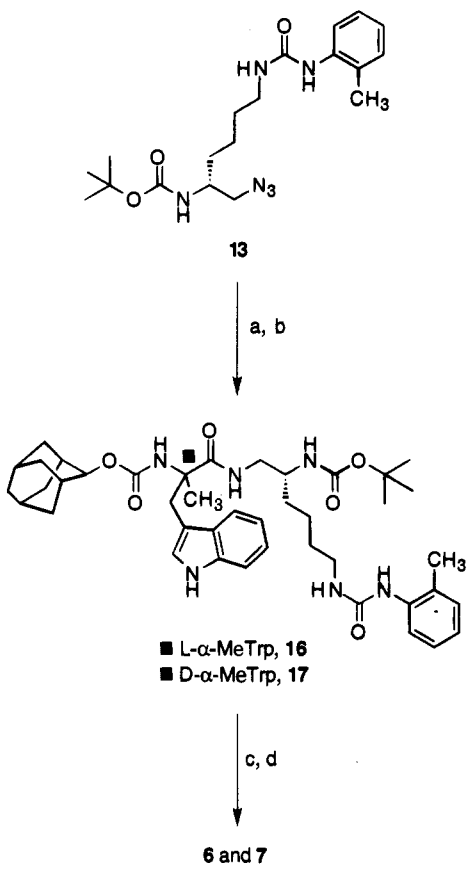
Scheme 2[a]



[a] (a) 4 N HCl in dioxane; (b) 2-Adoc-L-α-MeTrp or 2-Adoc-D-α-MeTrp, EDC, HOBt, Et_3N; (c) H_2, Pd/C; (d) succinic anhydride, Et_3N.

Compounds **4**–**7** were purified to homogeneity (>98%) by preparative RP-HPLC, and the lyophiles were char-

Scheme 3[a]



[a] (a) H_2, Pd/C; (b) 2-Adoc-L-α-MeTrp or 2-Adoc-D-α-MeTrp, EDC, HOBt, Et_3N; (c) 4 N HCl in dioxane; (d) succinic anhydride, Et_3N.

acterized by analytical RP-HPLC, ^{1}H-NMR spectroscopy, and high-resolution mass spectrometry. Analogs were evaluated for functional activity on the isolated guinea pig gallbladder (GPGB)[24] and on CHO-K1 cells stably expressing the human CCK-A (hCCK-A) or CCK-B (hCCK-B) receptor. Receptor binding affinities were measured on membrane preparations from the stably transfected CHO-K1 cells.[24] The in vitro biological data for compounds **4**–**7** and the comparative data for CCK-8, the *N*-acetyl derivative of **1** (NAA, Ac-Trp-Lys(Tac)-Asp-MePhe-NH_2),[24–26] and **3** are reported in Tables 1 and 2. Anorectic activity was evaluated following ip or po administration in rats conditioned to a liquid diet and fasted for 2 h (Table 3).

Results

As expected,[18,20] **3** induced weak contraction of the isolated GPGB at the highest concentration tested (30 μM) but did not block the response to CCK-8 in this tissue. For the analogs derived from **2**, the D-α-MeTrp analog **5** was a moderately potent antagonist of CCK-8 in the GPGB, while the L-α-MeTrp analog **4** was inactive as either an agonist or antagonist of CCK-8. For the analogs derived from **3**, both the D- and L-α-MeTrp analogs were full agonists in the GPGB, but the D-α-MeTrp analog **7** was 30-fold more potent than the L-α-MeTrp analog **6**. The agonist activity of **7** was blocked with the CCK-A receptor selective antagonist MK-329[27] (1 μM) but not with the CCK-B receptor selective antagonist L365,260[28] (1 μM).

The peptoid antagonist **3** was a potent CCK-B receptor selective ligand for the human receptors used in this study. The rank order of hCCK-A receptor affinities for analogs **4**–**7** paralleled the potency (pEC_{50} or pK_B) measured in the GPGB. D-α-MeTrp analogs **5** and **7** were more potent than L-α-MeTrp analogs **4** and **6** on both hCCK-A and hCCK-B receptors. Antagonist analog **5** had moderate CCK-A receptor affinity with modest CCK-A receptor selectivity. Agonist analog **7** had potent affinity for both hCCK-A and hCCK-B receptors.

The abilities of CCK-8, NAA, and **7** to influence intracellular calcium were evaluated on the stably transfected CHO cells expressing the hCCK-A or hCCK-B receptor and loaded with FURA2-AM. CCK-8 and NAA were full agonists on both cell lines. In contrast, analog **7** was a potent, full agonist on the CHO cells expressing the hCCK-A receptor but a partial agonist on the CHO cells expressing the hCCK-B receptor, eliciting only 49% of the CCK-8-induced response. Furthermore, **7** potently blocked the CCK-8 concentration–response curve on the CHO cells expressing the hCCK-B receptor, with a pK_B (Table 2) equivalent to the hCCK-B receptor affinity of this compound (Table 1).

Analog **7** was equipotent to CCK-8 in the rat anorexia assay (Table 3) and 3-fold less potent than NAA following ip administration. Neither NAA nor **7** (1 μmol/kg) were able to achieve the same maximal inhibition of feeding as CCK-8 (0.5 μmol/kg), and neither compound was orally active (up to 10 μmol/kg). While disappointing, the lack of oral activity of **7** was not surprising in light of recent report[29] that the oral bioavailability of **3** was less than 1% in rats.

Discussion

Incorporation of the Lys(Tac) side chain into **2** provided the moderately potent CCK antagonist **5**. Incorporation of the Lys(Tac) side chain into **3** provided the potent CCK-A agonist **7** which, compared to the parent peptoid **3**, had 100-fold increased hCCK-A receptor affinity and 100-fold decreased hCCK-B receptor affinity. Corresponding to these changes in receptor affinity, the weak CCK-A agonist[18–20] activity of **3** was enhanced and the potent CCK-B antagonist activity[17] was reduced. Since an intact C-terminal phenylalanine residue has been shown to be critical for both the CCK-A agonist activity of CCK-8[30] and the CCK-B agonist activity of CCK-4,[31] the unique pharmacological profile of **7** is remarkable. To our knowledge, **7** represents the smallest biologically active CCK-A agonist reported to date.

While it is obvious that in vivo CCK-B agonist activity would be unacceptable in a therapeutic entity because of the potential for peripheral (enhanced gastric acid secretion) or central (enhanced anxiety) side effects, the desirability of a mixed CCK-A agonist–CCK-B antagonist profile in an anorectic agent is less clear. Efforts continue to explore this novel lead structure.

Experimental Section

All chemicals and solvents are reagent grade unless otherwise specified. CCK-8 and nonsulfated CCK-8 (CCK-8NS) were purchased from Sigma (St. Louis, MO). MK-329[27] and L365,260[28] were obtained from Merck & Co. (Rahway, NJ). The following solvents and reagents have been abbreviated as the following: tetrahydrofuran, THF; ethyl ether, Et_2O; dimethyl sulfoxide, DMSO; dichloromethane, DCM; trifluoroacetic acid, TFA; dimethylformamide, DMF; isobutyl chloro-

Table 1. In Vitro Profile of Analogs **3–7**

analog	GPGB[a] pEC$_{50}$	GPGB[a] % mCCK-8	GPGB[a] pK_B	pIC$_{50}$[b] CCK-A	pIC$_{50}$[b] CCK-B	B/A[c]
3		24		5.98 ± 0.11 (3)	9.86 ± 0.82 (3)	0.0001
4		IA		6.14 ± 0.18 (3)	5.15 ± 0.21 (3)	9.7
5		IA	7.0	7.22 ± 0.08 (3)	6.65 ± 0.4 (3)	3.7
6	6.08 (1)	83		6.64 ± 0.03 (3)	6.75 ± 0.32 (8)	0.8
7	7.56 ± 0.63 (4)	72		7.92 ± 0.24 (3)	7.70 ± 0.3 (3)	1.4
NAA[d]	8.8 ± 0.2 (3)	105		7.70 ± 0.04 (3)	5.30 ± 0.2 (5)	251
CCK-8	9.41 ± 0.15 (4)	100		9.41 ± 0.07 (3)	9.50 ± 0.36 (9)	0.8

[a] pEC$_{50}$, −log EC$_{50}$ ± SD, assayed in triplicate (number of determinations); % mCCK-8, contraction induced by 30 μM test compound, normalized to CCK-8 (1 μM); pK_B, single-dose pA2 against CCK-8; IA, inactive at 30 μM. [b] pIC$_{50}$, −log of the concentration displacing 50% of [^{125}I]Bolton–Hunter CCK-8 from membrane preparations isolated from CHO-K1 cells stably transfected with cDNA of human CCK-A and CCK-B receptors, ± SD; number of determinations in parentheses. [c] CCK-A receptor selectivity, IC$_{50}$(B)/IC$_{50}$(A). [d] Ac-Trp-Lys(Tac)-Asp-MePhe-NH$_2$ (24–26).

Table 2. Ca^{2+} Mobilization in Stably Transfected CHO-K1 Cell Lines Expressing the Human CCK-A (hCCK-A) or Human CCK-B (hCCK-B) Receptor

analog	hCCK-A[a] pEC$_{50}$	hCCK-A[a] % mCCK-8	hCCK-B[a,b] pEC$_{50}$	hCCK-B[a,b] % mCCK-8	hCCK-B[a,b] pK_B
7	8.5 ± 0.33 (3)	95.4	5.6 ± 0.45 (3)	49.3	7.7 ± 0.51 (4)
NAA	8.4 (1)	100	6.3 (1)	100	
CCK-8	10 ± 0.17 (4)	100	10.3 ± 0.3 (4)	100	

[a] pEC$_{50}$, −log EC$_{50}$ ± SD (number of determinations); % mCCK-8 maximal stimulation induced by 1 μM test compound, normalized to CCK-8 (1 μM). [b] pK_B, single-dose pA_2 against CCK-8 following a 60 min perfusion with **7** (300 nM).

Table 3. Anorectic Activity in Conditioned Feeder Rats (ip administration)

analog	ED$_{50}$ (nmol/kg)[a]	% max inhibition[b]
7	30	59
NAA	10	67
CCK-8	27	95

[a] Dose required to achieve half-maximal inhibition of feeding at 30 min. [b] Maximal reduction in feeding achieved with a 1 μmol/kg dose for **7** and NAA and 0.5 μmol/kg for CCK-8. Both the ED$_{50}$ and maximal inhibition represent the average of 8–10 animals, normalized to the pretreatment response (n = 1).

formate, iBuCF; *N*-hydroxysuccinimide, HOBt; ethylcarbodiimide hydrochloride, EDC; (*tert*-butyloxy)carbonyl, Boc. Reactions were monitored by thin-layer chromatography on 0.25 μm silica gel plates (60F-254; E. Merck) and visualized with UV light, 7% ethanolic phosphomolybdic acid, or *p*-anisaldehyde solution. Final compounds were purified to homogeneity (>98%) by preparative reversed phase high-pressure liquid chromatography (RP-HPLC) using a Waters Model 3000 Delta Prep column equipped with a Delta-pak radial compression cartridge (C18, 300 A, 15 μm, 47 mm × 300 mm) as the stationary phase. The mobile phase employed 0.1% aqueous TFA with acetonitrile (Burdick and Jackson) as the organic modifier. Linear gradients were used in all cases, and the flow rate was 100 mL/min (to = 5 min). Appropriate fractions were combined and lyophilized to obtain the target analogs. Analytical purity was assessed by RP-HPLC using a Waters 600E system equipped with a Waters 990 diode array spectrometer (l range 200–400 nm). The stationary phase was a Vydac C18 column (5 m, 4.6 mm × 200 mm). The mobile phases were the same as above, and the flow rate was 1.5 mL/min (to = 2.8 min). Analytical data is reported as retention time, t_R, in min (% acetonitrile/time).

^{1}H-NMR spectra were recorded on either a Varian VXR-300 or Varian Unity-300 instrument. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Low-resolution mass spectra (MS) were recorded on a JEOL JMS-AX505HA, JEOL SX-102, or SCIEX-APIiii spectrometer. High-resolution mass spectra were recorded on a AMD-604 (AMD Electra GmbH) high-resolution double-focusing mass spectrometer (Analytical Instrument Group, Raleigh, NC). Mass spectra were acquired in the positive ion mode under electrospray ionization (ESI) or fast atom bombardment (FAB) methods.

2-(*S*)-[[(*tert*-Butyloxy)carbonyl]amino]-6-(3-*o*-tolylureido)hexanoic Acid (8). *o*-Tolyl isocyanate (13.5 mL, 133.2 mmol) was added to 6-amino-2-(*S*)-[[(*tert*-butyloxy)carbonyl]-amino]hexanoic acid (N-α-Boc-L-lysine; 26.7 g, 108.8 mmol) in 1 N aqueous sodium hydroxide (110 mL). The reaction mixture was stirred for 15 h, acidified to pH 2 with 1 N aqueous HCl, and extracted into ethyl acetate (6 × 50 mL). The combined organic extracts were washed with brine (1 × 30), dried ($MgSO_4$), and concentrated in vacuo to give the titled compound (38 g, 92%) which was used without further purification: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.1 Hz, 1H), 7.54 (s, 1H), 7.01 (m, 3H), 6.83 (t, J= 7.3 Hz, 1H), 6.51 (s, 1H), 3.83 (m, 1H), 3.05 (s, 2H), 2.15 (s, 3H), 1.36 (m, 13H); MS (FAB) m/z 380.4 (MH$^+$); R_f = 0.06 (10% methanol/DCM).

2-(*R*)-[[(*tert*-Butyloxy)carbonyl]amino]-6-(3-*o*-tolylureido)hexanoic Acid (9). **9** was prepared in the same way as **8** from 6-amino-2-(*R*)-[(*tert*-butyloxy)carbonyl]amino]hexanoic acid (N-α-Boc-D-lysine; 5.0 g, 20.5 mmol) to give the crude titled compound (7.93 g, 100%) which was used without further purification: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.81 (d, J = 8.06 Hz, 1H), 7.58 (s, 1H), 7.08 (m, 3H), 6.83 (t, J = 7.33 Hz, 1H), 6.51 (t, J = 4.88 Hz, 1H), 3.83 (m, 1H), 3.05 (d, J = 5.13 Hz, 2H), 2.15 (s, 3H), 1.36 (s, 13H); MS (EI) m/z 380 (MH$^+$); R_f = 0.06 (10% methanol/DCM).

1-[5-(*S*)-[(*tert*-Butyloxy)carbonyl]amino]-6-hydroxyhexyl]-3-*o*-tolylurea (10). Isobutyl chloroformate (15.6 mL, 120 mmol) and diisopropylethylamine (21.4 mL, 120 mmol) were added to **8** (38g, 100 mmol) in THF (750 mL) cooled to 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then added dropwise to 1 N lithium aluminum hydride (LAH) in Et_2O (200 mL) and stirred for 24 h at room temperature. Excess LAH was quenched by the procedure of Fieser and Fieser,[32] and the reaction mixture was filtered through a pad of Celite. The filtrate was washed with brine (1 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo to give the titled compound (31.7 g, 87%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 7.8 Hz, 1H), 7.09 (m, 2H), 6.97 (m, 2H), 6.52 (s, 1H), 5.69 (s, 1H), 5.13 (m, 1H), 3.51 (m, 3H), 3.12 (m, 2H), 2.16 (s, 3H), 1.42 (m, 14H); MS (FAB) m/z 366.1 (MH$^+$); R_f = 0.06 (10% methanol/DCM).

1-[5-(*R*)-[[(*tert*-Butyloxy)carbonyl]amino]-6-hydroxyhexyl]-3-*o*-tolylurea (11). 11 was prepared in the same way as **10** from **9** (1.98 g, 5.22 mmol) to give the titled alcohol (1.0 g, 52%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.1 Hz, 1H), 7.52 (s, 1H), 7.01–7.08 (m, 2H), 6.79–6.84 (m, 1H), 6.42–6.50 (m, 2 H), 4.54 (m, 1H), 3.15–3.29 (m, 2H), 3.04 (m, 2H),

2.13 (s, 3H), 1.34 (s, 9H), 1.29–1.52 (m, 6H); MS (FAB) m/z 366 (MH^+); R_f = 0.60 (10% methanol/DCM).

1-[6-Azido-5-(*S*)-[[(*tert*-butyloxy)carbonyl]amino]hexyl]-3-*o*-tolylurea (12). A. Methanesulfonyl chloride (8.07 mL, 104.3 mmol) in DCM (80 mL) was added dropwise to a solution of **10** (31.7 g, 86.9 mmol), DMAP (500 mg, 4.1 mmol), and triethylamine (13.9 mL, 104.3 mmol) in DCM (175 mL) cooled to 0 °C. The reaction mixture was stirred at room temperature for 2.25 h and then diluted with DCM (100 mL), washed with aqueous citric acid (2 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo to give methanesulfonic acid 2-(*S*)-[[(*tert*-butyloxy)carbonyl]amino]-6-(3-*o*-tolylureido)-hexyl ester after purification by recrystallization from ethyl acetate/hexane (18.38 g, 46%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.1 Hz, 1H), 7.54 (s, 1H), 7.01–7.09 (m, 2H), 6.80–6.91 (m, 2H), 6.50–6.51 (m, 1H), 3.99–4.10 (m, 2H), 3.63 (s, 1H), 3.13 (s, 3H), 3.04–3.07 (m, 2H), 2.14 (s, 3H), 1.3 (s, 9H), 1.25–1.49 (m, 6H); MS (FAB) m/z 444.1 (MH^+); R_f = 0.37 (5% methanol/DCM).

B. Sodium azide (1.2 g, 18.5 mmol) was added to methanesulfonic acid 2-(*S*)-[[(*tert*-butyloxy)carbonyl]amino]-6-(3-*o*-tolylureido)hexyl ester (4.2 g, 9.23 mmol) in DMF (20 mL). The reaction mixture was heated at 80 °C for 1.5 h, poured into ice water (100 mL), and extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were washed with water (4 × 20 mL), dried ($MgSO_4$), and concentrated in vacuo to give the titled compound (2.50 g, 69%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.78 (d, J = 8.1 Hz, 1H), 7.53 (s, 1H), 7.06 (m, 2H), 6.83 (m, 2H), 6.49 (s, 1H), 3.45 (m, 1H), 3.21 (d, J= 5.8 Hz, 1H), 3.04 (s, 2H), 2.14 (s, 3H), 1.36 (m, 17H); MS (FAB) m/z 391.1 (MH^+); R_f = 0.15 (50% ethyl acetate/hexane).

1-[6-Azido-5-(*R*)-[[(*tert*-butyloxy)carbonyl]amino]hexyl]-3-*o*-tolylurea (13). 13 was prepared in two steps as described for **12** from 11 (4.00 g, 10.96 mmol) to give the azide (0.68 g, 16% overall): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 7.8 Hz, 1H), 7.53 (s, 1H), 7.02–7.09 (m, 2H), 6.80–6.88 (m, 2H), 6.49 (m, 1H), 3.52 (s, 1H), 3.22 (d, J = 5.9 Hz, 2H), 3.04 (m, 2H), 2.14 (s, 3H), 1.23–1.43 (m, 6H), 1.34 (s, 9H); MS (FAB) m/z 391 (MH^+); R_f = 0.22 (50% hexanes/ethylacetate).

2-[[(Adamantan-2-yloxy)carbonyl]amino-*N*-[1-(*S*)-(azidomethyl)-5-(3-*o*-tolylureido)pentyl]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionamide (14). A. A solution of 4 N HCl in dioxane (10 mL) was added to **12** (2.50 g, 6.40 mmol). The reaction mixture was stirred for 15 h at room temperature and then diluted with Et_2O (150 mL), and the resultant gum was triturated with Et_2O (2 × 20 mL) to give 1-(5-(*S*)-amino-6-azidohexyl)-3-*o*-tolylurea (2.26 g, 100%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.3 (bs, 2H), 8.95 (m, 2H), 7.20 (m, 2H), 6.97 (m, 1H), 3.80 (m, 2H), 3.41 (bs, 1H), 3.23 (bs, 2H), 2.29 (s, 3H), 1.678 (bs, 2H), 1.59 (bs, 4H); MS (FAB) m/z 291.2 $(MH)^+$; R_f = 0.26 (10% methanol/DCM).

B. Triethylamine (0.45 mL, 3.4 mmol), 2-[[(adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionic acid (283 mg, 0.71 mmol), HOBt (115 mg, 0.85 mmol), and EDC (163 mg, 0.85 mmol) were added to 1-(5-(*S*)-amino-6-azidohexyl)-3-*o*-tolylurea (280 mg, 0.85 mmol) in DCM (5 mL). The resulting mixture was stirred at room temperature for 15 h and then diluted with DCM (20 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), 1 N aqueous HCl (2 × 10 mL), and brine (1 × 10 mL). The organic layer was separated and dried ($MgSO_4$). Concentration in vacuo gave the titled compound (323 mg, 68%): ^{1}H-NMR (DMSO-d_6) δ 10.88 (s, 1H), 7.79 (d, J = 8.0, 1H), 7.47 (m, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.04 (m, 4H), 6.86 (m, 3H), 6.75 (s, 1H), 6.50 (m, 1H), 4.68 (s, 1H), 4.51 (m, 1H), 3.86 (m, 1H), 3.63 (s, 1H), 3.04 (m, 3H), 2.14 (s, 3H), 1.95–1.12 (m, 24H); MS (FAB) m/z 669.3 (MH^+); R_f = 0.66 (10% methanol/DCM).

2-[[(Adamantan-2-yloxy)carbonyl]amino]-*N*-[1-(*S*)-(azidomethyl)-5-(3-*o*-tolylureido)pentyl]-3-(1*H*-indol-3-yl)-2-(*R*)-methylpropionamide (15). 15 was prepared in the same way as **14** from 1-(5-(*S*)-amino-6-azidohexyl)-3-*o*-tolylurea (638 mg, 2.0 mmol) and 2-[[(adamantan-2-yloxy)carbonyl]-amino]-3-(1*H*-indol-3-yl)-2-(*R*)-methylpropionic acid (930 mg, 2.34 mmol) to give the titled compound (1.0 g, 75%): ^{1}H-NMR (DMSO-d_6) δ 10.88 (s, 1H), 7.80 (m, 1H), 7.54 (s, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.28 (m, 2H), 7.23–6.80 (m, 7H), 6.50 (m, 1H), 4.67 (m, 1H), 4.50 (d, J = 2.5 Hz, 1H), 3.62 (s, 1H), 3.10 (m, 3H), 2.14 (s, 3H), 2.06–1.13 (m, 24H); MS (FAB) m/z 669.3 (MH^+); R_f = 0.52 (10% methanol/DCM).

2-[[(Adamantan-2-yloxy)carbonyl]amino]-*N*-[2-(*R*)-[[(*tert*-butyloxy)carbonyl]amino]-6-(3-*o*-tolylureido)hexyl]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionamide (16). A. A solution of **13** (0.68 g, 1.7 mmol) in ethanol (10 mL) and 10% Pd/C (68 mg) was stirred under 1 atm of hydrogen for 12 h. The solids were removed by filtration through a pad of Celite, and the filtrate was concentrated in vacuo. Purification by flash chromatography (SiO_2, 10%, methanol/DCM) gave 1-[6-amino-5-(*R*)-[[(*tert*-butyloxy)carbonyl]amino]hexyl]-3-*o*-tolylurea (0.59 g, 95%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.06 Hz, 1H), 7.53 (s, 1H), 7.02–7.09 (m, 2H), 6.82 (t, J = 7.3 Hz, 1H), 6.48–6.50 (m, 2H), 3.31 (s, 3H), 3.0 (m, 2H), 2.43 (d, J = 6.1 Hz, 2H), 2.14 (s, 3H), 1.35 (s, 9H), 1.23–1.56 (m, 6H); MS (ESI) m/z 365 (MH^+); R_f = 0.15 (10% methanol/DCM).

B. 1-Hydroxybenzotriazole (0.68 mg, 0.50 mmol), 2-[[(adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-(2*S*)-methylpropionic acid (0.20 g, 0.50 mmol), and EDC (0.96 mg, 0.50 mmol) were added to 1-[6-amino-5-(*R*)-[[(*tert*-butoxy)carbonyl]-amino]hexyl]-3-*o*-tolylurea (0.18 g, 0.50 mmol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 24 h and then diluted with ethyl acetate (100 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 30 mL), 1 N aqueous HCl (2 × 30 mL), and brine (1 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo to give the titled compound (0.28 g, 75%): ^{1}H-NMR ($CDCl_3$) δ 8.52 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.45 (m, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.20–7.05 (m, 6H), 7.00 (s, 1H), 6.88 (s, 1H), 6.58 (s, 1H), 5.40 (s, 1H), 4.80 (m, 2H), 3.85 (s, 1H), 3.20 (m, 5H), 2.23 (s, 3H), 2.04–1.19 (m, 33H); MS (FAB) m/z 743.1 (MH^+); R_f = 0.54 (10% methanol/DCM, 0.1% NH_4OH).

2-[[(Adamantan-2-yloxy)carbonyl]amino]-*N*-[2-(*R*)-[[(*tert*-butyloxy)carbonyl]amino]-6-(3-*o*-tolylureido)hexyl]-3-(1*H*-indol-3-yl)-2-(*R*)-methylpropionamide (17). 17 was prepared in the same way as **16** from **13** (0.18 g, 0.50 mmol) and 2-[[(adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-(2*R*)-methylpropionic acid (0.20 g, 0.50 mmol) to give the titled compound (0.29 g, 79%): ^{1}H-NMR ($CDCl_3$) δ 8.52 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.46 (m, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.19–7.05 (m, 6H), 7.00 (s, 1H), 6.85 (s, 1H), 6.43 (s, 1H), 5.40 (s, 1H), 4.80 (m, 2H), 3.85 (s, 1H), 3.50 (m, 5H), 3.39 (s, 2H), 3.18 (m, 3H), 2.23 (s, 3H), 2.00–1.28 (m, 32H); MS (FAB) m/z 743.1 (MH^+); R_f = 0.42 (10% methanol/DCM, 0.1% NH_4OH).

***N*-[2-[[2-(*S*)-[[(Adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionyl]amino]-6-(3-*o*-tolylureido)hexyl]succinamic Acid (4). A.** A solution of 14 (323 mg, 0.56 mmol) in ethanol (2 mL) and 10% palladium on carbon (35 mg) was stirred under a hydrogen atmosphere for 15 h. The solids were removed by filtration through a pad of Celite. The Celite pad was washed with ethyl acetate, and the combined filtrates were concentrated in vacuo to give 2-[[(adamantan-2-yloxy)carbonyl]amino]-*N*-[1-(*S*)-(aminomethyl)-5-(3-*o*-tolylureido)pentyl]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionamide (191 mg, 90%): MS (FAB) m/z 643.2 $(MH)^+$; R_f = 0.13 (10% methanol/DCM, 0.1% NH_4OH).

B. Triethylamine (0.10 mL, 0.74 mmol) and succinic anhydride (30 mg, 0.30 mmol) were added to 2-[[(adamantan-2-yloxy)carbonyl]amino]-*N*-[1-(*S*)-(aminomethyl)-5-(3-*o*-tolylureido)pentyl]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionamide (191 mg, 0.50 mmol) in DCM (2 mL). The resulting mixture was sonicated for 30 min and then concentrated in vacuo. The residue was purified by RP-HPLC (30–60%, over 30 min) to give the titled compound (87 mg, 39%): analytical RP-HPLC (30–60%, over 30 min) t_R = 15 min; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.88 (s, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.62 (s, 1H), 7.54 (s, 1H), 7.35 (m, 2H), 7.28 (d, J = 8.0 Hz, 1H), 7.12–6.72 (m, 7H), 6.48 (s, 1H), 4.68 (s, 1H), 3.42 (m, 2H), 3.08 (m, 5H), 2.39 (m, 2H), 2.28 (m, 2H), 2.14 (s, 3H), 1.93 (m, 3H), 1.82–1.62 (m, 7H), 1.54–1.10 (m, 9H); HRMS calcd for $C_{41}H_{54}N_6O_7$ (MH^+) 743.4132, obsd (MH^+) 743.41082.

***N*-2-[[2-(*S*)-[[(Adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-2-(*R*)-methylpropionyl]amino]-6-(3-*o*-tolylureido)hexyl]succinamic Acid (5). 5** was prepared in

the same way as 4 from **15** (1.0 g, 1.50 mmol) to give the titled compound (50 mg, 45% for two steps): analytical RP-HPLC (30–60%, over 30 min) t_R = 14.9 min; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.85 (s, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.49 (s, 1H), 7.41 (m, 2H), 7.24 (d, J = 8.0 Hz, 1H), 7.02 (m, 2H), 6.88 (m, 2H), 6.78 (m, 1H), 6.43 (bs, 1H), 4.64 (s, 1H), 3.28 (d, J = 14.5 Hz, 1H), 3.10 (d, J = 14.5 Hz, 1H), 2.37 (m, 2H), 2.26 (m, 2H), 2.10 (s, 3H), 1.93 (m, 4H), 1.72 (m, 8H), 1.31 (bm, 10H); HRMS calcd for $C_{41}H_{54}N_6O_7$ (MH^+) 743.4132, obsd (MH^+) 743.41217.

***N*-[1-[[[2-(*R*)-[[(Adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionyl]amino]methyl]-5-(3-*o*-tolylureido)pentyl]succinamic Acid (6). A.** HCl (4 N) in dioxane (5 mL) was added to **16** (0.28 g, 0.38 mmol), the solution was stirred for 1 h at room temperature and then diluted with Et_2O (50 mL), and the resultant gum was triturated to give 2-[(adamantan-2-yloxy)carbonyl]-*N*-[2-(*R*)-amino-6-(3-*o*-tolylureido)hexyl]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionamide as the hydrochloride salt (197 mg, 82%): MS (FAB) m/z 643.1 (MH^+); R_f = 0.13 (10% methanol/DCM).

B. Triethylamine (0.11 mL, 0.78 mmol) and succinic anhydride (31 mg, 0.31 mmol) were added to 2-[(adamantan-2-yloxy)carbonyl]-*N*-[2-(*R*)-amino-6-(3-*o*-tolylureido)hexyl]-3-(1*H*-indol-3-yl)-2-(*S*)-methylpropionamide (197 mg, 0.31 mmol) in DCM (2 mL). The resulting mixture was sonicated for 30 min, concentrated in vacuo, and purified by RP-HPLC (36–54%, over 30 min) to give the titled compound (65 mg, 28%): analytical RP-HPLC (36–54%, over 30 min) t_R = 29.7 min; R_f = 0.27 (10% methanol/DCM, 0.1% HOAc); ^{1}H-NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 1H), 7.20–6.90 (m, 6H), 3.88 (s, 1H), 3.38 (m, 2H), 3.19–3.0 (m, 4H) 2.58 (m, 24H); HRMS calcd for $C_{41}H_{54}N_6O_7$ (MH^+) 743.4132, obsd (MH^+) 743.41298.

***N*-[1-[[[2-(*R*)-[[(Adamantan-2-yloxy)carbonyl]amino]-3-(1*H*-indol-3-yl)-2-(*R*)-methylpropionyl]amino]methyl]-5-(3-*o*-tolylureido)pentyl]succinamic Acid (7). 7** was prepared in the same way as **6** from **17** (0.29 g, 0.39 mmol) to give the titled compound (69 mg, 15% overall): analytical RP-HPLC (36–54%, over 30 min) t_R = 28.9 min; R_f = 0.27 (10% methanol/DCM, 0.1% NH_4OH); ^{1}H-NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 1H), 7.20–6.90 (m, 6H), 3.88 (s, 1H), 3.38 (m, 2H), 3.19–3.0 (m, 4H) 2.58 (m, 24H); HRMS calcd for $C_{41}H_{54}N_6O_7$ (MH^+) 743.4132, obsd (MH^+) 743.4149.

Biological Assays. *Intracellular Calcium Measurements.* CHO-K1 cells stably transfected with hCCK-A or hCCK-B receptor were grown on glass coverslips to 75–90% confluency. The cells were loaded for 50 min in serum-free culture medium containing 5 mM FURA2-AM and 2.5 mM probenecid. A JASCO CAF-102 calcium analyzer was used to measure changes in intracellular calcium concentration by standard ratiometric techniques using excitation wavelengths of 340 and 380 nm. Cells were perfused with increasing concentrations of CCK-8 (n = 3), NAA (n = 1), or compound **7** (n = 3) until a plateau in the 340/380 ratio was achieved. A washout/recovery period of at least 10 min was allowed between successive stimulations. The maximal response was normalized to the maximal response induced by CCK-8. ED_{50}'s were calculated at the concentration required to induce half-maximal response. In addition to the agonist concentration–response curves, the CHO-K1 cells expressing the human CCK-B receptor were perfused for 1 h with compound **7** (300 nM, n = 4), and then concentration–reponse curves were acquired for CCK-8 and the single concentration K_B was calculated according to the formula

$$K_B = \frac{[\text{test compound}]}{(\text{CR-1})}$$

where CR is the fold shift of the CCK-8 concentration–response curve in the presence of the antagonist.

Anorexia Assay. Male Long-Evans rats (225–300 g) were conditioned for 2 weeks to consume a palatable liquid diet (Bio-Serve F1657, Frenchtown, NJ) after a 2 h fast. On pretreatment day, rats were fasted (100 min) and injected (ip or oral gavage) with drug vehicle (propylene glycol, PG, 1 mL/kg) and an oral preload of saline (0.9%, 8 mL/kg). Liquid diet access was provided 20 min later, and consumption was measured at 30, 90, and 180 min. To qualify for the drug treatment study, rats had to consume at least 8 mL of liquid diet within the first 30 min on the pretreatment day. The next day, following the 100 min deprivation, rats (8–10 animals/dose) were treated (ip or po) with vehicle (PG, 1 mL/kg) or various doses (0.001–10 mmol/kg) of test compound dissolved in PG (1 mL/kg) immediately followed by the saline oral preload. Food access was again provided 20 min later, and food intake was measured at 30, 90, and 180 min. All food intake data were normalized for each rat to the respective values from the pretreatment day. Potency and efficacy data were collected at 30 min.

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