J. Med. Chem. 1994, 37, 1569–1571

CCK-A-Selective Tetrapeptides Containing Lys(N^ϵ)-Amide Residues: Favorable *in Vivo* and *in Vitro* Effects of N-Methylation at the Aspartyl Residue

Michael J. Bennett, A. L. Nikkel, Bruce R. Bianchi, Thomas R. Miller, Lisa Bednarz, David G. Witte, Michael Stashko, Song Shung Wang, Paul A. Gore, Karen E. Asin, Chun-Wel Lin, Alex M. Nadzan,† and Mark W. Holladay*

Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064

Received January 11, 1994®

Previous structure–activity studies on a series of CCK-A selective tetrapeptide agonists, typified by A-71623 (Boc-Trp-Lys(CONH-Ph-*o*-Me)-Asp-(*N*-Me)Phe-NH_2), have shown that replacement of the Lys(N^ϵ-carbamoyl) substituent with N^ϵ-acyl substituents resulted in partial agonists with moderate to high affinities for the CCK-A receptor and that replacement of the C-terminal dipeptide with either (*N*-Me)Asp-Phe or (*N*-Me)Asp-(*N*-Me)Phe was highly favorable to *in vitro* and *in vivo* CCK activity. The present study demonstrates that although analogues in the ϵ-amide series that are *N*-methylated at the Phe position are weakly active or inactive in an *in vivo* rat appetite suppression assay, incorporation of (*N*-Me)Asp or (*N*-Me)Asp-(*N*-Me)Phe modifications in this series results in analogues with markedly improved *in vivo* activity. In *in vitro* assays, there is minimal effect of N-methylation pattern on binding affinity, whereas there is a trend toward improved functional activity in the phosphatidylinositol hydrolysis assay in analogues containing (*N*-Me)Asp.

Introduction

Previous reports have described the discovery and subsequent studies on a series of tetrapeptide CCK-A agonists that are characterized by the presence of a lysine residue at the putative amino acid 31 position (CCK-33 numbering).[1–4] The prototypical compound of the "Lys-(ϵ-urea) series," A-71623 (Boc-Trp-Lys(*o*-methylphenylaminocarbonyl)-Asp-(*N*-Me)Phe-NH_2), contains an arylcarbamoyl substituent at the lysine N^ϵ-position and an N^α-methyl group at the C-terminal phenylalanine residue. A-71623 possesses low nanomolar affinity for pancreatic CCK-A receptors, is nearly 1000-fold selective for binding to CCK-A over cortical CCK-B receptors, is a full agonist in amylase release and phosphatidylinositol (PI) hydrolysis assays in guinea pig pancreatic tissues, and is active in suppression of food intake in several species, including rat and monkey. A secondary series (Lys(ϵ-amide) series) also was reported that differed from A-71623 in that acyl, rather than carbamoyl, substituents were present at the lysine ϵ-position.[3] While a few compounds in the Lys(ϵ-amide) series possessed binding affinity to pancreatic CCK-A receptors as low as ca. 10 nM, in no case was there observed agonist activity in the PI assay that was >90% of the response elicited by CCK-8, and in the majority of cases the PI response was <80%. In separate structure–activity studies in the Lys(ϵ-urea) series, it was shown that analogues containing either the (*N*-Me)Asp-Phe or (*N*-Me)Asp-(*N*-Me)Phe dipeptide units at the C-terminus possessed potent *in vitro* and *in vivo* CCK-A activity, and in the case of the (*N*-Me)Asp series, improved selectivity for binding to CCK-A vs CCK-B receptors.[5,6]

Because of the favorable effects on CCK activity observed for the (*N*-Me)Asp and (*N*-Me)Asp-(*N*-Me)Phe modifications in the Lys(ϵ-urea) series, incorporation of these residues into analogues containing selected Lys(ϵ-amide) moieties was undertaken to investigate the effect of these modifications in this series. This paper reports data showing that *N*-methylation of the Asp residue in these tetrapeptides has a highly beneficial effect on appetite suppression activity *in vivo*, accompanied by a favorable, but much less profound, effect on activity *in vitro*.

Table 1. Physical Data for New Compounds

compd	formula	anal.	FAB+ MS *m/e*	FAB+ MS adduct
2	$C_{45}H_{55}N_7O_{10}\cdot 2.6H_2O$	CHN	854	M + H+
			876	M + Na+
3	$C_{46}H_{57}N_7O_{10}\cdot 1.7H_2O\cdot 0.5HOAc$	CHN	868	M + H+
			890	M + Na+
5	$C_{44}H_{54}N_8O_9\cdot H_2O\cdot 0.3HOAc$	CHN	839	M + H+
6	$C_{45}H_{56}N_8O_9\cdot 0.8H_2O\cdot 0.2HOAc$	CHN	853	M + H+
			875	M + Na+
7	$C_{43}H_{53}N_7O_9S\cdot 1.1H_2O\cdot 0.3HOAc$	CHN	844	M + H+
			866	M + Na+
8	$C_{43}H_{53}N_7O_9S\cdot 0.6HOAc\cdot 0.3NH_4OAc$	CHN	844	M + H+
			866	M + Na+
9	$C_{44}H_{55}N_7O_9S\cdot 0.1H_2O$	CHN	857[a]	(M – H)-

[a] FAB-.

Results and Discussion

Binding affinities and functional activities *in vitro* and activity in suppression of food intake *in vivo* are presented in Table 2, which also includes data for A-71623 for reference. While a trend toward lower affinities for pancreatic receptors is discernible when the Asp-(*N*-Me)-Phe dipeptide unit in **1**, **4**, and **7** is replaced by (*N*-Me)-Asp-Phe (**2**, **5**, and **8**) or (*N*-Me)Asp-(*N*-Me)Phe (**3**, **6**, and **9**), the changes are small and in most cases not significant. In contrast, the N-methylation pattern does influence selectivity for CCK-A vs CCK-B receptors, since, consistent with previous findings,[5,8] the presence of an *N*-methyl substituent on the Asp residue has the effect of reducing affinity for CCK-B receptors. Thus, selectivities for binding to pancreatic CCK-A over CCK-B receptors for (*N*-Me)Asp-containing compounds **2**, **3**, **5**, **6**, **8**, and **9** range from 130- to over 1500-fold compared to the 4.5–170-fold selectivities observed for the Asp-(*N*-Me)Phe-containing analogues. In the PI hydrolysis assay, the latter group of

* Address correspondence to: Mark W. Holladay, Ph.D., Dept. 47W, AP10-3, Abbott Park, IL 60064.
† Current address: Ligand Pharmaceuticals, Inc., 9393 Towne Centre Dr., San Diego, CA 92121.
® Abstract published in *Advance ACS Abstracts*, April 1, 1994.

0022-2623/94/1837-1569$04.50/0 © 1994 American Chemical Society

Table 2. *In Vitro* and in *Vivo* Activity of CCK Tetrapeptide Analogues[a]

Boc-Trp-Lys(N^{ϵ}-X)-(N-R^1)Asp-(N-R^2)Phe-NH_2

no.	X	R^1	R^2	IC_{50} (nM) pancreas	IC_{50} (nM) cortex	PI hydrolysis % max[b]	suppression of food intake ED_{50} (nmol/kg, ip)
A-71623	$CONHC_6H_4$-*o*-Me	H	CH_3	3.7 ± 0.85[c]	4300 ± 770[c]	100[c]	3.6[d]
1		H	CH_3	4.2 ± 1.3[e]	710 ± 38[e]	76[e]	413 (268–636)
2		CH_3	H	5 ± 1	5810 ± 1700	101 ± 4	18 (15–21)
3		CH_3	CH_3	9 ± 2	13600 ± 1470	91 ± 4	62
4		H	CH_3	28 ± 10[e]	128 ± 9[e]	88 ± 3	2518 (1123–5649)
5		CH_3	H	41 ± 6	10400 ± 1140	98 ± 4	36 (23–55)
6		CH_3	CH_3	50 ± 10	22700 ± 2460	94 ± 4	16 (13–20)
7		H	CH_3	18 ± 6	731 ± 99	84 ± 2	1400 (538–3643)
8		CH_3	H	35 ± 6	4577 ± 1200	95 ± 5	12 (9–16)
9		CH_3	CH_3	32 ± 14	10900 ± 709	93 ± 2	22 (16–31)

[a] All values for new compounds represent an average of at least three determinations. [b] Percent stimulation at a dose of 10^{-7} M relative to the maximal response elicited by CCK-8. [c] Data from ref 2. [d] Data from ref 4. [e] Data from ref 3.

compounds elicited functional responses ranging from 76 to 88%. Although the differences are small, a consistent trend toward improved functional activity is apparent in analogues containing the (*N*-Me)Asp residue, such that at least compounds **2**, **5**, and **8** could reasonably be viewed as full agonists in this assay.

The most striking effect of *N*-methylation pattern is found in the results for suppression of food intake *in vivo*. Whereas Asp-(*N*-Me)Phe-containing analogues **1**, **4**, and **7** showed comparatively weak *in vivo* activity under the assay conditions, the (*N*-Me)Asp-Phe and (*N*-Me)Asp-(*N*-Me)Phe analogues suppressed food intake with ED_{50} values ranging from 12 to 62 nmol/kg. Compared to Boc-Trp-Lys(*o*-methylphenylaminocarbonyl)-(*N*-Me)Asp-Phe-NH_2, the most potent Lys(ϵ-urea) compound reported thus far in reduction of food intake in rats,[5] compound **8**, the most potent of the Lys(ϵ-amide) series, is ca. 7-fold less active.

On the basis of the results presented here and previously,[8] it is tempting to postulate that activity in suppression of food intake is related to the ability of compounds to stimulate a full or nearly full agonist response in the PI assay. However, such interpretations may be suggested only with caution, since it is clear from the present study that small differences in PI activity can be associated with markedly different *in vivo* responses. Moreover, the molecular structure and effector mechanisms for the receptors responsible for PI hydrolysis and suppression of food intake are not necessarily identical, and the role of pharmacokinetic and pharmacodynamic considerations as well as species differences must be considered. In *in vitro* experiments using tissue homogenates, representative members of analogues containing (*N*-Me)Asp-Phe or (*N*-Me)Asp-(*N*-Me)Phe possessed roughly equivalent stabilities to degradation by rat kidney, liver, and plasma as those containing Asp-(*N*-Me)Phe. The extent to which these results are representative of *in vivo* conditions remains to be established.

In summary, a trend in the structure–activity relationships of tetrapeptide CCK-A agonists has been identified that suggests that, at least in the case of analogues containing Lys(ϵ-amide) residues, the (*N*-Me)Asp and (*N*-Me)Asp-(*N*-Me)Phe modifications lead to markedly improved appetite-suppressant activity *in vivo* together with an upward trend in the ability to elicit phophatidylinositol hydrolysis *in vitro*. It is possible that these results will be applicable to enhancing the CCK-A-mediated activities in other series of CCK-related peptides.

Experimental Section

^{1}H-NMR spectra were obtained at 300 MHz and are expressed as ppm downfield from a tetramethylsilane internal standard. Preparative HPLC was carried out on a Gilson instrument using a Vydac C_{18} column (22 × 250 mm) using gradient mixtures of CH_3CN and 50 mM NH_4OAc (pH 4.5) at a flow rate of 14 mL/min, monitored at 280 nm. Elemental analysis data were consistent with theoretical values to within ±0.4%. Analytical HPLC of all final compounds showed ≥95% homogeneity (Vydac C_{18} column with eluent monitoring carried out at 254 and 280 nm). The procedures presented below are representative of those used for preparation of all final compounds.

Methods. Peptides were prepared by methods analogous to those described previously.[3,5] Briefly, tetrapeptides were assembled using standard solution-phase methods, with Lys and Asp side chains protected by Cbz and benzyl, respectively. Following hydrogenolysis of the protecting groups, acylation at the Lys ϵ-position was carried out using the appropriate *N*-succinimidyl ester. Preparation and physical data for compounds 1 and 4 are described in ref 3; physical data for new compounds are collected in Table 1.

In vitro binding and functional assays[7,8] and the *in vivo* appetite suppression assay[8] were conducted as described previously. Briefly, *in vitro* binding data were determined as IC_{50} for half maximal inhibition of [^{125}I]Bolton-Hunter-CCK-8 in guinea pig pancreas (CCK-A receptors) or cortex (CCK-B receptors), and functional activity was determined as percent maximal stimulation of phosphatidylinositol hydrolysis in guinea pig pancreatic acini (CCK-A receptors) relative to that elicited by CCK-8. Appetite suppression data are based on intakes of liquid diet by food-deprived rats for 1 h following ip injection of the drug.

Boc-Trp-Lys(2-thienylacryloyl)-Asp-(*N*-Me)Phe-NH_2 (7). Boc-Trp-Lys-Asp-(*N*-Me)Phe-NH_2 acetate salt[3] (114 mg, 0.15 mmol) in DMF (1.5 mL) was treated with *N*-methylmorpholine (NMM) (0.033 mL, 0.30 mmol) and *N*-succinimidyl 3-(2-thienyl)-acrylate (37 mg, 0.15 mmol), prepared as described previously.[3] Acid–base workup gave the crude product (no observable

solubility in saturated aqueous $NaHCO_3$). HPLC purification was performed using a gradient of 20–60% CH_3CN over 40 min. Fractions containing pure product were combined and lyophilized twice to afford 70 mg (56%) of the product as a fluffy white solid. ^1H NMR (DMSO-d_6, selected data for two conformers, ca. 2:1): δ 1.29 (s, Boc), 2.74 (s, N-Me), 2.94 (s, N-Me), 4.12 (m, α-H), 4.19 (m, 2 α-H), 4.28 (m, α-H), 4.45 (m, α-H), 4.87 (m, α-H), 4.96 (m, α-H), 5.13 (dd, *J* = 6, 11 Hz, α-H).

Boc-Lys(Cbz)-(*N*-Me)Asp(OBn)-Phe-NH_2 (10). A solution of Boc-Lys(Cbz)-OH (2.0 g, 5.25 mmol) in CH_2Cl_2 (5.0 mL) at 0 °C was treated with 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) (504 mg, 2.63 mmol) and stirred for 1 h. The solution was then added to a precooled (0 °C) solution of HCl (*N*-Me)Asp(OBn)-Phe-NH_2[8] (1.0 g, 2.39 mmol) and NMM (0.30 mL, 2.63 mmol) in DMF (2.0 mL), and the mixture was allowed to warm to room temperature over 3 h and stir overnight. The mixture was concentrated under reduced pressure, and the residual solution was diluted with EtOAc, washed successively with saturated aqueous $NaHCO_3$, saturated aqueous $KHSO_4$, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product, which was subjected to flash chromatography (HOAc/hexane/EtOAc, 2:25:73). Fractions containing pure product as judged by TLC were combined, diluted with H_2O, and lyophilized to afford 1.48 g (83%) of a white fluffy solid. MS (FAB^+): *m/e* 746 $(M + H)^+$. ^1H NMR (DMSO-d_6, selected data for two conformers, ca. 3:2): δ 1.33 (s, Boc, major conformer), 1.43 (s, Boc, minor conformer), 2.07 (s, N-Me, minor conformer), 2.35 (s, N-Me, major conformer), 4.13 (m, α-H, major conformer), 4.50 (m, α-H, major conformer), 5.22 (m, α-H, major conformer), 4.97–5.05 (benzylic protons, both conformers).

Boc-Trp-Lys(Cbz)-(*N*-Me)Asp(OBn)-Phe-NH_2 (11). A solution of compound **10** (400 mg, 0.54 mmol) in CH_2Cl_2 (3.3 mL) was treated with trifluoroacetic acid (2.7 mL) at room temperature. After 1 h, the solution was concentrated and the residue was triturated with diethyl ether to give a solid. The resulting tripeptide amine salt in DMF (2.0 mL) was extended to the protected tetrapeptide by a symmetrical anhydride procedure with workup in an analogous fashion to that described for preparation of **10**. The crude product was purified by flash chromatography (EtOAc/hexane, 2:1) to afford 369 mg (74%) of a white solid after lyophilization. MS (FAB^+): *m/e* 932 $(M + H)^+$, 954 $(M + Na)^+$. ^1H NMR ($CDCl_3$, selected data for two conformers, ca. 2:1): δ 1.43 (s, Boc), 1.46 (s, Boc), 2.15 (s, N-Me), 2.18 (s, *N*-Me), 4.44 (m, α-H), 4.58 (m, α-H), 4.84 (m, α-H), 5.35 (m, α-H), 5.02–5.17 (benzylic protons).

Boc-Trp-Lys-(*N*-Me)Asp-Phe-NH_2 Acetate (12). Compound 11 (346 mg, 0.37 mmol) in DMF (3.0 mL) was stirred overnight under 1 atm of H_2 in the presence of 10% Pd/C (150 mg). The catalyst was removed by filtration and rinsed with MeOH. The filtrate was concentrated, H_2O/HOAc (ca. 20:1) was added, and the resultant suspension was lyophilized to afford 224 mg (79%) of the acetate salt as a cream-colored solid. MS (FAB^+): *m/e* 708 $(M + H)^+$. ^1H NMR (DMSO-d_6, selected data): δ 1.32 (s, Boc), 2.08 (s, N-Me), 4.19–4.38 (m, 2 α-H), 4.29 (m, α-H), 5.31 (br d, *J* = 11 Hz, α-H).

Boc-Trp-Lys(2-thienylacryloyl)-(*N*-Me)Asp-Phe-NH_2 (8). Treatment of compound **12** (88 mg, 0.12 mmol) with *N*-succinimidyl 3-(2-thienyl)acrylate (29 mg, 0.12 mmol) and subsequent purification of the crude product under conditions analogous to those used for preparation of compound **7** afforded 60 mg (62%) of the title compound as a fluffy white solid. ^1H NMR (DMSO-d_6) (two conformers ca. 1:1): δ 1.04–1.75 (m, 15 H, includes 1.31 (s)), 2.09–2.17 (m, 2.5 H, includes 2.14 (s)), 2.34–2.56 (m, 2.5 H, partially obscured, includes 2.38 (s)), 2.68–2.97 (m, 3 H), 3.01–3.44 (m, 3 H, partially obscured), 4.19 (m, 0.5 H), 4.29–4.38 (m, 1 H), 4.44 (m, 0.5 H), 4.53 (m, 0.5 H), 4.84 (m, 0.5 H), 5.14 (dd, *J* = 5, 10 Hz, 0.5 H), 5.21 (m, 0.5 H), 6.36–6.44 (m, 1 H), 6.72 (br d, *J* = 10 Hz, 0.5 H), 6.87 (d, *J* = 10 Hz, 0.5 H), 6.92–7.59 (m, 16 H), 7.98 (br d, *J* = 9 Hz, 0.5 H), 8.04–8.13 (m, 1.5 H), 8.26–8.55 (m, 1 H), 10.78 (br s, 0.5 H), 10.87 (br s, 0.5 H).

Boc-Trp-Lys(2-thienylacryloyl)-(*N*-Me)Asp-(*N*-Me)Phe-NH_2 (9). The title compound was prepared from the HCl salt of H-(NMe)Asp(OBn)(NMe)Phe-NH_2[5] by procedures analogous to those used for preparation of **8** from HCl (*N*-Me)Asp(OBn)-Phe-NH_2. **Boc-Lys(Cbz)-(*N*-Me)Asp(OBn)-(*N*-Me)Phe-NH_2 (13)**: The crude product was triturated with EtOAc/EtOH/hexane (ca. 1:1:20) to afford the product as a white solid in quantitative yield. MS (FAB^+): *m/e* 760 $(M + H)^+$, 782 $(M + Na)^+$. ^1H NMR (DMSO-d_6, selected data for two conformers, ca. 2:1): δ 1.32 (s, Boc, major conformer), 1.37 (s, Boc, minor conformer), 2.02 (s, N-Me, major conformer), 2.66 (s, N-Me, minor conformer), 2.71 (s, N-Me, major conformer); 2.94 (s, N-Me, minor conformer), 4.02 (m, α-H, major conformer), 4.02 (m, α-H, minor conformer), 4.14 (t, *J* = 6 Hz, α-H, minor conformer), 4.33 (t, 5 Hz, α-H, minor conformer), 5.36 (dd, *J* = 5, 12 Hz, α-H, major conformer), 5.60 (dd, *J* = 6, 12 Hz, α-H, major conformer). **Boc-Trp-Lys-(Cbz)-(*N*-Me)Asp(OBn)-(*N*-Me)Phe-NH_2 acetate (14)**: yield 58%. MS (FAB^+): *m/e* 945 (weak $M + H)^+$, 929 $(M + H - NH_2)^+$. ^1H NMR (DMSO-d_6, selected data): δ 1.27 (br s, 9 H, Boc), 1.97 (br s, 3 H, N-Me), 2.73 (br s, 3 H, N-Me), 4.17 (m, α-H), 4.42 (m, α-H), 4.98 (br d, *J* = 3 Hz, 2 H, benzylic), 5.03 (br s, 2 H, benzylic), 5.36 (dd, *J* = 6, 12 Hz, α-H), 5.61 (m, α-H). **Boc-Trp-Lys-(*N*-Me)Asp-(*N*-Me)Phe-NH_2 acetate (15)**: yield 86%. MS (FAB^+): *m/e* 722 $(M + H)^+$, 744 $(M + Na)^+$. ^1H NMR (MeOH-d_4, two conformers): δ 1.27–1.46 (m, 12 H, includes 1.40 (br s)), 1.50–1.71 (m, 3 H), 2.03–2.17 (m, 4 H, includes 2.11 (br s, N-Me, major conformer)), 2.58–3.25 (m, 8 H, partially obscured by solvent, includes 2.86 (s with shoulder, N-Me for major and minor conformers), 2.98 (s, minor conformer)), 3.38 (d, *J* = 6 Hz, 1 H), 4.43 (d, *J* = 6 Hz, 1 H), 4.28 (m, 1 H), 4.41 (m, 1 H), 5.31 (m, 1 H), 5.54 (m, 1 H), 6.93–7.38 (m, 9 H), 7.38 (br d, *J* = 9 Hz, 1 H), 7.97 (br s, minor conformer). **Compound 9.** HPLC purification was performed using a gradient of 20–55% CH_3CN over 40 min. Yield: 59 mg (68%) as a fluffy white solid. ^1H NMR (DMSO-d_6, selected data): δ 1.22 (s, Boc), 1.96 (s, N-Me), 2.68 (s, N-Me), 4.11 (m, α-H), 4.34 (m, α-H), 5.27 (dd, *J* = 5, 13 Hz, α-H), 5.43 (dd, *J* = 5, 10 Hz, α-H).

Supplementary Material Available: Experimental data for **2**, **3**, **5**, and **6** (2 pages). Ordering information is given on any current masthead page.

References

(1) Shiosaki, K.; Lin, C. W.; Kopecka, H.; Tufano, M. D.; Bianchi, B. R.; Miller, T. R.; Witte, D. G.; Nadzan, A. M. Boc-CCK-4 Derivatives Containing Side-Chain Ureas as Potent and Selective CCK-A Receptor Agonists. *J. Med. Chem.* **1991**, *34*, 2837–2842.

(2) 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.* **1990**, *39*, 346–351.

(3) Shiosaki, K.; Lin, C. W.; Kopecka, H.; Craig, R. A.; Bianchi, B. R.; Miller, T. R.; Witte, D. G.; Stashko, M.; Nadzan, A. M. Development of Potent and Selective CCK-A Receptor Agonists from Boc-CCK-4: Tetrapeptides Containing Lys(Nᵋ)-Amide Residues. *J. Med. Chem.* **1992**, *35*, 2007–2014.

(4) Asin, K. E.; Bednarz, L.; Nikkel, A. L.; Gore, P. A., Jr.; Nadzan, A. M. A-71623, a Selective CCK-A Receptor Agonist, Suppresses Food Intake in the Mouse, Dog, and Monkey. *Pharmacol. Biochem. Behav.* **1992**, *42*, 699–704.

(5) Holladay, M. W.; Kopecka, H.; Miller, T. R.; Bednarz, L.; Nikkel, A. L.; Bianchi, B. R.; Witte, D. G.; Shiosaki, K.; Lin, C. W.; Asin, K. E.; Nadzan, A. M. Tetrapeptide CCK-A Agonists: Effect of Backbone N-Methylations on In Vitro and In Vivo CCK Activity. *J. Med. Chem.* **1994**, *37*, 630–635.

(6) Holladay, M. W.; Bennett, M. J.; Lin, C. W.; Asin, K. E.; Lico, I.; Martin, Y. C.; Hutchins, C. W.; Bianchi, B. R.; Miller, T. R.; Witte, D. G.; Bednarz, L.; Nikkel, A. L.; Nadzan, A. M. Structure-activity and Computer Modeling Studies on Potent and Selective CCK-A Agonist Tetrapeptides Containing (N-Me)Asp or (N-Me)Asp-(N-Me)Phe. In *Peptides 1992: Proceedings of the Twenty-second European Peptide Symposium*; Schneider, C. H., Eberle, A. N., Eds.; Escom: Leiden, 1993; pp 617–618.

(7) Lin, C. W.; Holladay, M. W.; Barrett, R. W.; Wolfram, C. A. W.; Miller, T. R.; Witte, D.; Kerwin, J. F., Jr.; Wagenaar, F.; Nadzan, A. M. Distinct Requirements for Activation at CCK-A and CCK-B/Gastrin Receptors: Studies with a C-Terminal Hydrazide Analogue of Cholecystokinin Tetrapeptide (30–33). *Mol. Pharmacol.* **1989**, *36*, 881–886.

(8) Holladay, M. W.; Bennett, M. J.; Tufano, M. D.; Lin, C. W.; Asin, K. E.; Witte, D. G.; Miller, T. R.; Bianchi, B. R.; Bednarz, L. M.; Nadzan, A. M. Synthesis and Biological Activities of CCK Heptapeptide Analogues. Effects of Conformational Constraints and Standard Modifications on Receptor Subtype Selectivity, Functional Activity in Vitro, and Appetite Suppression in Vivo. *J. Med. Chem.* **1992**, *35*, 2919–2928.