# Synthesis and Biological Evaluation of Cholecystokinin Analogs in Which the Asp-Phe-$NH_2$ Moiety Has Been Replaced by a 3-Amino-7-phenylheptanoic Acid or a 3-Amino-6-(phenyloxy)hexanoic Acid

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Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester (JMV180), an analog of the C-terminal octapeptide of cholecystokinin (CCK-8), shows interesting biological activities behaving as an agonist at the high-affinity CCK binding sites and as an antagonist at the low-affinity CCK binding sites in rat pancreatic acini. Although we did not observe any major hydrolysis of the ester bond of Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester in our *in vitro* studies, we were aware of a possible and rapid cleavage of this ester bond during *in vivo* studies. To improve the stability of Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester, we decided to synthesize analogs in which the ester bond would be replaced by a carba ($CH_2$-$CH_2$) linkage. We synthesized the 3-amino-7-phenylheptanoic acid ($\beta$-homo-Aph) with the *R* configuration in order to mimic the Asp-2-phenylethyl ester moiety and the 3-amino-6-(phenyloxy)hexanoic acid (H-$\beta$-homo-App-OH), an analog of H-$\beta$-homo-Aph-OH in which a methylene group has been replaced by an oxygen. (*R*)-$\beta$-Homo-Aph and (*R*)-H-$\beta$-homo-App-OH were introduced in the CCK-8 sequence to produce Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-$\beta$-homo-Aph-OH and Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-$\beta$-homo-App-OH. Both compounds were able to recognize the CCK receptor on rat pancreatic acini ($IC_{50}$ = 12 ± 8 nM and 13 ± 5 nM, respectively), on brain membranes ($IC_{50}$ = 32 ± 2 nM and 57 ± 5 nM, respectively), and on Jurkat T cells ($IC_{50}$ = 75 ± 15 nM and 65 ± 21 nM, respectively). Like Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester, both compounds produced maximal stimulation of amylase secretion ($EC_{50}$ = 6 ± 2 nM and 4 ± 2 nM, respectively) with no decrease of the secretion at high concentration indicating that these compounds probably act as agonists at the high-affinity peripheral CCK-receptor and as antagonists at the low-affinity CCK-receptor. Replacing the tryptophan by a D-tryptophan in such analogs produced full CCK-receptor antagonists. All these analogs might be more suitable for *in vivo* studies than Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester.

## Introduction

We reported earlier on the synthesis and biological activities of synthetic analogs of cholecystokinin (CCK) in which the C-terminal primary amide group was deleted. Compound Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ (JMV180), in which the phenylalanine residue was replaced by a 2-phenylethyl alcohol[1] and both methionines in position 28 and 31 were substituted by norleucines (a substitution that proved not to influence biological activity), appeared to exhibit partial agonist activity of CCK. The dose–response curve for CCK-stimulated enzyme secretion is biphasic. With increasing doses of CCK the amylase secretion increases up to a maximum and then decreases at higher concentrations. Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester does not give any decrease in amylase stimulation at supramaximal concentrations in rat pancreatic acini[2] but shows a plateau of maximal stimulation. It has been hypothesized that Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester interacts with both low- and high-affinity peripheral CCK-binding sites and functions as an agonist at the high-affinity binding sites and as an antagonist at the low-affinity binding sites.[3-5] Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester has been extensively used to determine the relations existing between occupation of each class of CCK binding sites and their linkage to various transduction systems and resulting biological activities.[6-15] Interestingly, replacing the L-tryptophan by a D-tryptophan in Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester produced Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ which proved to be a full and potent CCK receptor antagonist.[16] Although we did not observe any hydrolysis of the ester bond in the *in vitro* biological studies of these analogs, we were aware of a possible cleavage of this ester bond in the course of *in vivo* biological studies. In fact, when Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester or the corresponding D-tryptophan analog was used in *in vivo* studies, higher doses than expected from *in vitro* studies had to be used to obtain biological activity.[17,18] We also observed that although the corresponding 2-phenylethylamide analog Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-NH-$CH_2$-$CH_2$-$C_6H_5$ was less potent in *in vitro* studies than Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester, it was equally potent in *in vivo* studies.[18] We thus decided to investigate the replacement of the ester bond by a "carba" linkage ($CH_2CH_2$). This modification implied the synthesis of 3-amino-7-phenyl-

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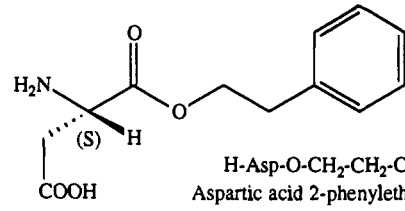
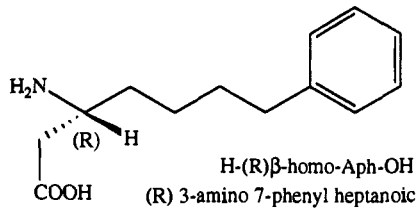
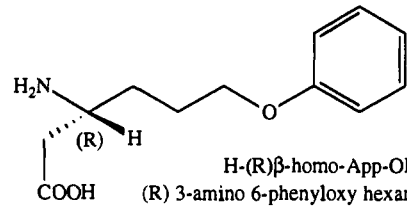
**Chart I.** Chemical Structure of (*R*)-3-Amino-7-phenylheptanoic Acid (H-β-homo-Aph-OH) and of (*R*)-3-Amino-6-(phenyloxy)hexanoic Acid (H-β-homo-App-OH)




H-Asp-O-$CH_2$-$CH_2$-$C_6H_5$
Aspartic acid 2-phenylethyl ester




H-(R)β-homo-Aph-OH
(R) 3-amino 7-phenyl heptanoic acid




H-(R)β-homo-App-OH
(R) 3-amino 6-phenyloxy hexanoic acid

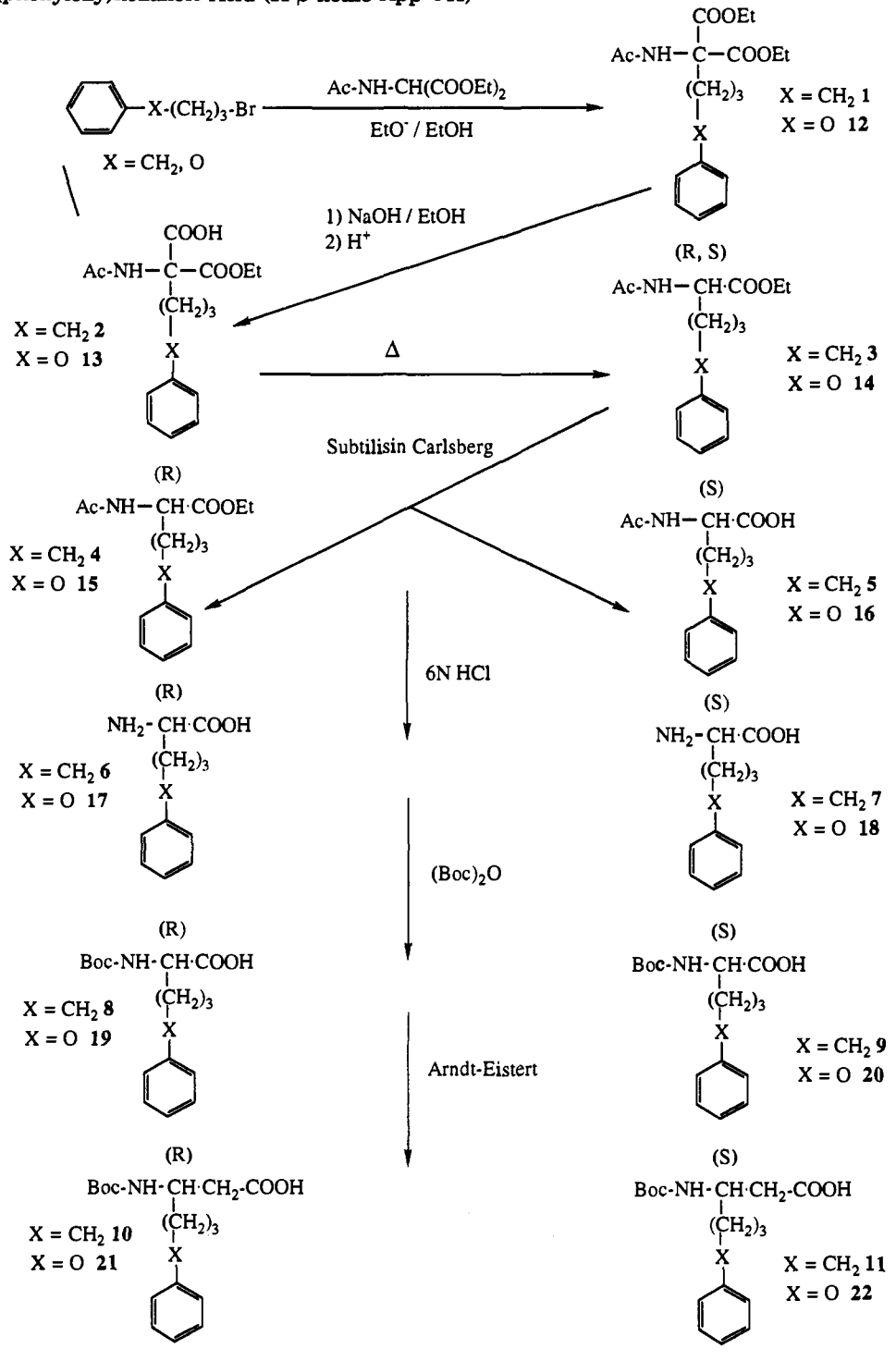
heptanoic acid (H-β-homo-Aph-OH) with the *R* configuration, in order to mimic the Asp-O-$CH_2CH_2$-$C_6H_5$ moiety (Chart I). We also synthesized an analog of the above β-amino acid by replacement of a methylene group by an oxygen, i.e., 3-amino 6-(phenyloxy)hexanoic acid (H-β-homo-App-OH), in order to investigate the influence of the amino acid side chain lipophilicity. Since the synthetic pathway of both H-β-homo-Aph-OH and H-β-homo-App-OH led to their *R* and *S* isomers, we undertook the synthesis of compounds Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (**47**), Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*S*)-β-homo-Aph-OH (**48**), Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-App-OH (**49**), Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*S*)-β-homo-App-OH (**50**), Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-Aph-OH (**51**), Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(**S**)-β-homo-Aph-OH (**52**), Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-App-OH (**53**), and Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-App-OH (**54**) and evaluated their biological activities.

## Results and Discussion

**Chemistry.** In order to mimic the Asp-O-$CH_2$-$CH_2$-$C_6H_5$ moiety of the parent molecules Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ and Boc-Tyr($SO_3$-H)-Met-Gly-D-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$, we had to obtain the two amino acids 3-amino-7-phenylheptanoic acid (H-β-homo-Aph-OH) and 3-amino-6-(phenyloxy)-hexanoic acid (H-β-homo-App-OH). As shown in Chart I, both amino acids should have the *R* configuration to retain the spatial arrangement of the aspartyl residue. As a total, stereospecific synthesis of those compounds was a difficult and expensive approach, we decided to synthesize and enzymatically resolve 2-amino-6-phenylhexanoic acid (Aph) and 2-amino-5-(phenyloxy)pentanoic acid (App) and homologate their Boc-derivatives by an Arndt–Eistert reaction to obtain Boc-β-homo-Aph-OH and Boc-β-homo-App-OH, respectively. The synthetic pathway leading to Boc-(*R*)-β-homo-Aph-OH and Boc-(*S*)-β-homo-Aph-OH is outlined in Scheme I. The procedure used for the amino acid synthesis and resolution was developed by Rao et al.[19] Reaction of 1-bromo-4-phenylbutane with the sodium salt of diethyl acetamidomalonate in absolute ethanol led to compound **1**, which was hemisaponified in ethanol by 1 molar equiv of sodium hydroxide to afford the monoester **2**. Decarboxylation occurred at 130 °C *in vacuo* without solvent to lead to the racemic ethyl ester of 2-acetamido-6-phenylhexanoic acid (**3**). Resolution of the racemic mixture with Subtilisin Carlsberg (Sigma) afforded in excellent yield ethyl 2-acetamido-6-phenylhexanoate (**4**) with the probable *R* configuration and 2-acetamido-6-phenylhexanoic acid (**5**) with the probable *S* configuration. Treatment of compounds **4** and **5** with 6 N HCl produced (*R*)- and (*S*)-2-amino-6-phenylhexanoic acids (**6** and **7**). *N-tert*-Butyloxycarbonylation of **6** and **7** followed by and Arndt–Eistert homologation led, respectively, to (*R*)-2-[(*tert*-butyloxycarbonyl)amino]-7-phenylheptanoic acid (**10**) and (*S*)-2-[(*tert*-butyloxycarbonyl)amino]-7-phenylheptanoic acid (**11**). Synthesis of the β-homo-App analogs proceeded similarly from 3-phenoxypropyl bromide and homologation of (*R*)- and (*S*)-2-amino-5-(phenyloxy)pentanoic acid (App). Obtention of the peptides described in this work followed the general procedure described in Scheme II for the synthesis of the CCK analog Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (**47**). DBU-mediated esterification of (*R*)-2-[(*tert*-butyloxycarbonyl)amino]-7-phenylheptanoic acid, [Boc-(*R*)-β-homo-Aph-OH] (**10**), afforded Boc-(*R*)-β-homo-Aph-OBzl (**23**), which was deprotected with trifluoroacetic acid and subsequently coupled to Boc-Nle with BOP[20] to lead to Boc-Nle-(*R*)-β-homo-Aph-OBzl (**27**). Acidolytic deprotection followed by BOP coupling to Z-Trp afforded compound **31**, which was hydrogenated and reacted with Boc-Tyr-Nle-Gly-OSu[1] to lead to the hexapseudopeptide Boc-Tyr-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (**39**). Sulfation of the tyrosine residue according to the usual procedure (pyridine–$SO_3$ complex in a mixture of DMF and pyridine)[1] led to compound Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (**47**). Physical and analytical data of the synthetic intermediates are given in Table I, those of the final compounds in Table II. All compounds were identified by $^1H$ NMR at 360 MHz and mass spectrometry. As an example, $^1H$ NMR data in DMSO-$d_6$ of compound **47** are given in Table III.

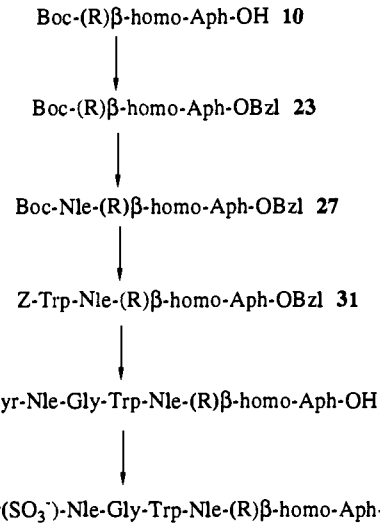
**Biological Activity.** All pseudopeptides described above (**47–54**) were tested for their ability to stimulate *in vitro* amylase release from rat pancreatic acini (Table IV) and to inhibit the binding of [$^{125}$I]BH-CCK-8 (Bolton–Hunter derivative of the C-terminal octapeptide of cholecystokinin) to isolated rat pancreatic acini (peripheral CCK-receptor, CCK-A), to guinea pig brain membranes, and to Jurkat cells, representative of central CCK-receptors (CCK-B).[21,22] They were compared in the same experiments with the potent CCK analog, Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-Phe-$NH_2$ (Boc-[Nle$^{28}$,Nle$^{31}$]-CCK-7).[23,24] The results are listed in Table IV. We included in this table the biological results of Boc-Tyr($SO_3H$)-Nle-

**Scheme I.** Synthesis of (*R*)- and (*S*)-3-Amino-7-phenylheptanoic Acid (H-β-homo-Aph-OH) and of (*R*)- and (*S*)-3-Amino-6-(phenyloxy)hexanoic Acid (H-β-homo-App-OH)




Gly-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ (JMV180), a CCK agonist that does not exhibit inhibition of amylase secretion at supramaximal concentrations, and Boc-Tyr($SO_3$H)-Nle-Gly-DTrp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ (JMV179), a potent CCK receptor antagonist. As expected, the CCK analogs **47–54** were able to inhibit binding of labeled CCK-8 to rat pancreatic acini with potencies varying from about 12 to 42 nM. The agonist compounds with a L-tryptophan (**47–50**) were in general slightly more potent than the antagonist analogs having a D-tryptophan (**51–54**). These results indicate that in CCK analogs lacking the C-terminal amide function and expressing only part of the CCK response on amylase secretion from rat pancreatic acini, replacement of the L-tryptophan by a D-tryptophan does not dramatically affect the affinity for the CCK receptor in contrast with what occurs with CCK-8 and full CCK agonists.[25] On the other hand, the stereochemistry of the tryptophan residue is crucial for the biological activity, with replacement of the L-tryptophan by a D-tryptophan in such analogs leading to full CCK-receptor antagonists. (*R*) and (*S*) analogs were almost equally potent (Table IV), suggesting that in these CCK analogs the configuration of the residue mimicking the aspartic acid is not crucial for recognizing the CCK-receptor. On stimulation of amylase secretion from rat pancreatic acini, compounds **47**, **49**, and **50** behaved like Boc-Tyr($SO_3$H)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester, exhibiting maximal response with no decrease in the response at high concentrations (Figure

**Scheme II.** Synthesis of Boc-Tyr($SO_3^-$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (47)




1). Interestingly, compound **47** having a (*R*)-β-homo-Aph ($IC_{50}$ = 6 ± 2 nM) and **49** having a (*R*)-β-homo-App ($IC_{50}$ = 4 ± 2 nM) are more potent on amylase secretion than their corresponding (*S*) isomers **48** ($IC_{50}$ = 50 ± 25 nM) and **50** ($IC_{50}$ = 20 ± 8 nM), although they are almost equally potent in inhibiting binding of labeled CCK-8 to rat pancreatic acini ($IC_{50}$ from 12 ± 8 nM to 16 ± 7 nM), suggesting that they might be more potent at the CCK binding site responsible for amylase secretion. Unexpectedly, compound **48** produced only 75% of the maximal response (Table IV). None of the D-tryptophan analogs **51**, **53**, or **54** were able to stimulate amylase secretion. However, they were potent in inhibiting CCK-8-stimulated amylase secretion, the (*R*) isomers being the most potent ($IC_{50}$ = 0.5 ± 0.2 μM for compound **51** and 1 ± 0.5 μM for compound **53**) (Table IV). Interestingly, the CCK analog **52** was a partial agonist, exhibiting only 20% of the maximal response on amylase secretion. It corresponds to the partial agonist **48** in which the L-tryptophan has been replaced by a D-tryptophan. This observation is in accordance with previous results showing that replacement of L-tryptophan by D-tryptophan resulted in full CCK-receptor antagonists only in analogs where the L-tryptophan was fully efficacious in stimulating amylase secretion with no decrease of the maximal response at high concentrations.[25,26] As expected, the analogs **47** to **50** were less potent in inhibiting binding of labeled CCK-8 to guinea pig brain membranes and to Jurkat T cells than CCK-8, both expressing CCK-B/gastrin receptors, their potencies varying from 32 ± 2 to 250 ± 53 nM (Table IV). Compounds having a D-tryptophan (compounds **51–54**) were far less active, being in the micromolar range. These results are in accordance with the observation that the C-terminal tetrapeptide is more sensitive to modifications as far as the CCK-B receptor is concerned.

## Conclusion

This paper presents the synthesis and biological evaluations of potent analogs of Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester in which the C-terminal ester bond has been replaced by a carba bond. (*R*) compounds having a L-tryptophan in their sequence behave similarly to Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester, producing maximal response on amylase secretion with no decrease at high concentrations. Although the (*S*) isomer containing a β-homo-App was fully efficacious and has the same activity profile as Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester on amylase secretion from rat pancreatic acini, the (*S*) isomer with a β-homo-Aph was unable to produce maximal response. When L-tryptophan was replaced by D-tryptophan in fully efficacious analogs (e.g., (*R*) isomer compounds or the (*S*) isomer with a β-homo-App), full CCK-receptor antagonists were obtained. However, when L-tryptophan was replaced by D-tryptophan in analogs unable to produce maximal response on amylase secretion (e.g., the (*S*) isomer with β-homo-Aph), partial agonists of decreased efficacy were obtained. These results probably reflect different interactions of these analogs at both low- and high-affinity CCK-binding sites, both sites having different structural requirements. On the other hand, these analogs might be suitable for *in vivo* studies.

## Experimental Section

Melting points were taken on a Büchi apparatus in open capillary tubes. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Le Service de Microanalyses de l'ENSCM (Montpellier, France). Ascending TLC was performed on precoated plates of silica gel 60 F 254 (Merck) using the following solvent systems (by volume): A, AcOEt/hexane, 1:5; B, AcOEt/hexane, 3:7; C, AcOEt/hexane, 5:5; D, AcOEt/hexane, 7:3; E, AcOEt; F, AcOEt/hexane/acetic acid, 50:50:1; G, chloroform/methanol/acetic acid, 120:10:5; H, chloroform/methanol/acetic acid, 85:10:5; I, AcOEt/pyridin/acetic acid/water, 80:20:5:10, J, *n*-butanol/acetic acid/water, 3:1:1. Peptide derivatives were located with UV light (254 nm), charring reagent, or ninhydrin. Column chromatographies were performed with silica gel 60, 60–229 mesh, ASTM (Merck). HPLC purifications were run on a Merck/Hitachi instrument on a Beckman Ultrasphere ODS (10 mm) 21.2- × 150-mm column at a flow rate of 7 mL/min of a mixture of (A) ammonium acetate 0.05 M, pH 6.5, and (B) methanol, with an UV detection at 279 nm. $^1H$ NMR spectra were recorded as DMSO-$d_6$ solutions at 305 K on a AMX 360 Bruker spectrophotometer with chemical shifts reported in δ relative to the signal set to 2.5 ppm. Mass spectra were recorded on a JEOL JMS DX 100 and DX 300 spectrometer in a FAB positive mode. Amino acids and derivatives were purchased from Bachem (Switzerland), Subtilisin Carlsberg from Sigma (St. Louis, MO). All reagents and solvents were of analytical grade. The following abbreviations were used: DMF, dimethylformamide; HOBT, 1-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; DIEA, *N*,*N*-diisopropylethylamine; BOP, (benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate; NMM, *N*-methylmorpholine; IBCF, isobutylchloroformiate; TFA, trifluoroacetic acid; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene. Other abbreviations used were those recommended by the IUPAC-IUB Commission (*Eur. J. Biochem.* 1984, *138*, 9–37).

**Diethyl (4-Phenylbutyl)acetamidomalonate (1).** Sodium (5.47 g, 237 mmol) was dissolved in absolute ethanol (500 mL). After the mixture was cooled to 0 °C diethyl acetamidomalonate (50 g, 230 mmol) was added, and after 5 min of stirring, 4-phenylbutyl bromide (48.1 g, 226 mmol) in absolute ethanol (300 mL) was added. The reaction mixture was refluxed for 6 h, cooled to room temperature, and poured into a well-stirred mixture of aqueous 0.05 M potassium hydrogenosulfate (2000 mL) and hexane (1000 mL). The precipitate which formed was collected by filtration, thoroughly washed with water and hexane, and dried in vacuo to afford the title compound. Yield 63 g (79%). Physical and analytical data are given in Table I.

**Ethyl (4-Phenylbutyl)acetamidomalonate (2).** To a solution of compound **1** (63 g, 180 mmol) in 95% ethanol (200 mL) was added a 1 M sodium hydroxide solution (190 mL). After 30 min of stirring at room temperature, the reaction mixture was diluted with water (1000 mL) and extracted with ether (3 × 300 mL). The aqueous phase was acidified with 1 M potassium hydrogenosulfate and extracted with ethyl acetate (3 × 200 mL). The organic extracts were washed with 1 M potassium hydrogenosulfate and brine, dried over sodium sulfate, and concentrated *in vacuo* to lead to a white solid that was recrystallized

**Table I.** Physical and Analytical Data of the Synthetic Intermediates

| compd | mp (°C) | $[\alpha]_D$ deg (*c*; solvent) | $R_f$[a] | anal. C, H, N |
|---|---|---|---|---|
| Ac-NH-C($CO_2Et$)$_2$-($CH_2$)$_4$-$C_6H_5$ (1) | 83–84 | | B: 0.33; C: 0.59 | $C_{19}H_{27}NO_5$ |
| Ac-NH-C($CO_2H$)($CO_2Et$)-($CH_2$)$_4$-$C_6H_5$ (2) | 118–120 | | G: 0.32; H: 0.54 | $C_{17}H_{23}NO_5$ |
| (*R,S*)-Ac-NH-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2Et$ (3) | oil | | B: 0.20; C: 0.36 | |
| (*R*)-Ac-NH-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2Et$ (4) | oil | | B: 0.20; C: 0.36 | |
| (*S*)-Ac-NH-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2H$ (5) | 120–121 | −3.7 (1.09; DMF) | G: 0.36; H: 0.60 | $C_{14}H_{19}NO_3$ |
| (*R*)-$H_2N$-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2H$ (6) | 200 (dec) | −20 (1; 50% AcOH) | J: 0.53 | $C_{12}H_{17}NO_2$ |
| (*S*)-$H_2N$-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2H$ (7) | 200 (dec) | +20 (1; 50% AcOH) | J: 0.53 | $C_{12}H_{17}NO_2$ |
| (*R*)-Boc-NH-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2H$ (8) | 56–57 | +8.3 (1.16; DMF) | G: 0.61; F: 0.81 | $C_{17}H_{25}NO_4$ |
| (*S*)-Boc-NH-CH[($CH_2$)$_4$-$C_6H_5$]-$CO_2H$ (9) | 57 | −8.3 (1.2; DMF) | G: 0.61; F: 0.81 | $C_{17}H_{25}NO_4$ |
| Boc-(*R*)-β-homo-Aph-OH (10) | 82–83 | +1.6 (1.16; DMF) | G: 0.76; F: 0.75 | $C_{16}H_{27}NO_4$ |
| Boc-(*S*)-β-homo-Aph-OH (11) | 80–81 | −1.7 (1.27; DMF) | G: 0.76; F: 0.75 | $C_{16}H_{27}NO_4$ |
| Ac-NH-C($CO_2Et$)$_2$-($CH_2$)$_3$-O-$C_6H_5$ (12) | 75–77 | | B: 0.27; C: 0.51 | $C_{16}H_{25}NO_6$ |
| Ac-NH-C($CO_2H$)($CO_2Et$)-($CH_2$)$_3$-O-$C_6H_5$ (13) | 145–147 | | G: 0.27; H: 0.46 | $C_{16}H_{21}NO_6$ |
| (*R,S*)-Ac-NH-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2Et$ (14) | oil | | B: 0.17; C: 0.31 | |
| (*R*)-Ac-NH-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2Et$ (15) | oil | | B: 0.17; C: 0.31 | |
| (*S*)-Ac-NH-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2H$ (16) | 103–105 | +2.1 (0.95; DMF) | G: 0.31; H: 0.49 | $C_{13}H_{17}NO_4$ |
| (*R*)-$H_2N$-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2H$ (17) | >250 | −20 (1; 50% AcOH) | J: 0.44 | $C_{11}H_{15}NO_3$ |
| (*S*)-$H_2N$-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2H$ (18) | >250 | +20 (0.9; 50% AcOH) | J: 0.44 | $C_{11}H_{15}NO_3$ |
| (*R*)-Boc-NH-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2H$ (19) | oil | | G: 0.63; H: 0.76 | |
| (*S*)-Boc-NH-CH[($CH_2$)$_3$-O-$C_6H_5$]-$CO_2H$ (20) | oil | | G: 0.63; H: 0.76 | |
| Boc-(*R*)-β-homo-App-OH (21) | 82–84 | +4.9 (1.03; DMF) | F: 0.70; G: 0.65 | $C_{17}H_{25}NO_5$ |
| Boc-(*S*)-β-homo-App-OH (22) | 79–81 | −4.8 (1.01; DMF) | F: 0.70; G: 0.65 | $C_{17}H_{25}NO_5$ |
| Boc-(*R*)-β-homo-Aph-OBzl (23) | 48 | +6 (1.18; DMF) | A: 0.30; B: 0.67 | $C_{25}H_{33}NO_4$ |
| Boc-(*S*)-β-homo-Aph-OBzl (24) | 41–43 | −5.6 (1.07; DMF) | A: 0.30; B: 0.67 | $C_{25}H_{33}NO_4$ |
| Boc-(*R*)-β-homo-App-OBzl (25) | 49–50 | +4.4 (1.35; DMF) | A: 0.21; B: 0.51 | $C_{24}H_{31}NO_5$ |
| Boc-(*S*)-β-homo-App-OBzl (26) | wax | −4.8 (0.79; DMF) | A: 0.21; B: 0.51 | $C_{24}H_{31}NO_5$ |
| Boc-Nle-(*R*)-β-homo-Aph-OBzl (27) | 72–74 | −5.7 (0.88; DMF) | B: 0.44; C: 0.89 | $C_{31}H_{44}N_2O_5$ |
| Boc-Nle-(*S*)-β-homo-Aph-OBzl (28) | 74–76 | −11.3 (1.18; DMF) | A: 0.10; B: 0.44 | $C_{31}H_{44}N_2O_5$ |
| Boc-Nle-(*R*)-β-homo-App-OBzl (29) | 72–73 | −2.5 (1.36; DMF) | B: 0.32; C: 0.79 | $C_{30}H_{42}N_2O_6$ |
| Boc-Nle-(*S*)-β-homo-App-OBzl (30) | 40 | −10.0(1.23; DMF) | B: 0.30; C: 0.77 | $C_{30}H_{42}N_2O_6$ |
| Z-Trp-Nle-(*R*)-β-homo-Aph-OBzl (31) | 145 | −14.2 (0.64; DMF) | C: 0.42; D: 0.78 | $C_{45}H_{52}N_4O_6$ |
| Z-Trp-Nle-(*S*)-β-homo-Aph-OBzl (32) | 132–134 | −20.0 (1.10; DMF) | C: 0.42; D: 0.79 | $C_{45}H_{52}N_4O_6$ |
| Z-Trp-Nle-(*R*)-β-homo-App-OBzl (33) | 138–140 | −12.6 (1.22; DMF) | C: 0.26; D: 0.72 | $C_{44}H_{50}N_4O_7$ |
| Z-Trp-Nle-(*S*)-β-homo-App-OBzl (34) | 131 | −16.3 (0.88; DMF) | C: 0.26; D: 0.71 | $C_{44}H_{50}N_4O_7$ |
| Z-D-Trp-Nle-(*R*)-β-homo-Aph-OBzl (35) | 143 | +12.8 (0.89; DMF) | C: 0.35; D: 0.72 | $C_{45}H_{52}N_4O_6$ |
| Z-D-Trp-Nle-(*S*)-β-homo-Aph-OBzl (36) | 140–144 | −11.1 (0.9; acetone) | C: 0.27; D: 0.67 | $C_{45}H_{52}N_4O_6$ |
| Z-D-Trp-Nle-(*R*)-β-homo-App-OBzl (37) | 132–134 | +6.7 (1.16; DMF) | C: 0.21; D: 0.65 | $C_{44}H_{50}N_4O_7$ |
| Z-D-Trp-Nle-(*S*)-β-homo-App-OBzl (38) | 147–148 | −11.0 (0.85; acetone) | C: 0.19; D: 0.62 | $C_{44}H_{50}N_4O_7$ |
| Boc-Tyr-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (39) | 150 (dec) | −6.3 (1.0; DMF) | G: 0.27; H: 0.62 | $C_{52}H_{71}N_7O_{10}$ |
| Boc-Tyr-Nle-Gly-Trp-Nle-(*S*)-β-homo-Aph-OH (40) | 184 (dec) | −12.9 (1.05; DMF) | G: 0.23; H: 0.51 | $C_{52}H_{71}N_7O_{10}$ |
| Boc-Tyr-Nle-Gly-Trp-Nle-(*R*)-β-homo-App-OH (41) | 145 (dec) | −5.7 (1.11; DMF) | G: 0.25; H: 0.57 | $C_{51}H_{69}N_7O_{11}$ |
| Boc-Tyr-Nle-Gly-Trp-Nle-(*S*)-β-homo-App-OH (42) | 145 | −10.7 (1.31; DMF) | G: 0.22; H: 0.50 | $C_{51}H_{69}N_7O_{11}$ |
| Boc-Tyr-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-Aph-OH (43) | 105 (dec) | −2.6 (1.01; DMF) | G: 0.21; H: 0.62 | $C_{52}H_{71}N_7O_{10}$ |
| Boc-Tyr-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-Aph-OH (44) | 184 (dec) | −10.1 (0.85; DMF) | G: 0.32; H: 0.67 | $C_{52}H_{71}N_7O_{10}$ |
| Boc-Tyr-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-App-OH (45) | 115 (dec) | −6.5 (0.82; DMF) | G: 0.29; H: 0.57 | $C_{51}H_{69}N_7O_{11}$ |
| Boc-Tyr-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-App-OH (46) | 82 (dec) | −10.6 (0.95; DMF) | G: 0.33; H: 0.65 | $C_{51}H_{69}N_7O_{11}$ |

[a] (A) AcOEt/hexane (1:5); (B) AcOEt/hexane (3:7); (C) AcOEt/hexane (5:5); (D) AcOEt/hexane (7:3); (E) AcOEt; (F) AcOEt/hexane/acetic acid (50:50:1); (G) chloroform/methanol/acetic acid (120:10:5); (H) chloroform/methanol/acetic acid (85:10:5); (J) butanol/acetic acid water (3:1:1).

**Table II.** Physical Data of CCK Analogs

| compd | mp, °C | $[\alpha]_D$, deg (*c*; DMF) | $t_R$, min [(A)/(B)[a]] | $R_f$ (solvent I[b]) |
|---|---|---|---|---|
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (47) | 163 (dec) | −8.1 (1.01) | 18.64 [30/70] | 0.44 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*S*)-β-homo-Aph-OH (48) | 140 (dec) | −15.5 (1.01) | 22.91 [30/70] | 0.38 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-App-OH (49) | 142 (dec) | −7.8 (1.04) | 13.49 [30/70] | 0.43 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*S*)-β-homo-App-OH (50) | 115 (dec) | −15.4 (1.11) | 14.05 [30/70] | 0.39 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-Aph-OH (51) | 135 (dec) | −0.26 (1.05) | 16.44 [30/70] | 0.45 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-Aph-OH (52) | 140 (dec) | −10.9 (0.95) | 20.85 [30/70] | 0.39 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-App-OH (53) | 157 (dec) | −8.2 (0.91) | 19.67 [33/67] | 0.38 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-App-OH (54) | 125 (dec) | −10.1 (0.99) | 25.24 [33/67] | 0.35 |

[a] (A) ammonium acetate 0.05 M, pH 6.5; (B) methanol. [b] I: AcOEt/pyridine/acetic acid/water (80:20:5:10).

in a mixture of ethyl acetate and hexane. Yield 53.2 g (92%). Physical and analytical data are given in Table I.

**(*R,S*)-Ethyl 2-Acetamido-6-phenylhexanoate (3).** Decarboxylation of compound 2 (53 g, 165 mmol) occurred without solvent at 130 °C *in vacuo* in 10 min. After being cooled to room temperature, the resulting oily compound was dissolved in ethyl acetate (400 mL), washed with a saturated aqueous sodium bicarbonate solution (2 × 200 mL) and brine, dried over sodium sulfate, and concentrated *in vacuo* to lead to an oil. Yield 45 g (98%). Physical and analytical data are given in Table I.

**Resolution of (*R,S*) Ethyl 2-Acetamido-6-phenylhexanoate (3): (*R*)-Ethyl 2-Acetamido-6-phenylhexanoate (4) and (*S*)-2-Acetamido-6-phenylhexanoic Acid (5).** To a solution of compound 3 (68 g, 245 mmol) in DMSO (1000 mL) was added 1 N potassium chloride solution (200 mL) and water (800 mL). The pH of the solution/suspension was adjusted to 7.0 with 0.1 N sodium hydroxide. Subtilisin Carlsberg (600 mg) in a 0.1 N potassium chloride solution (70 mL) was added, and the mixture was stirred at 39 °C while the pH was maintained at 6.9 with a 1 N sodium hydroxide solution. After 1 h 60 mg of enzyme was added, and an additional 60 mg was added after 3 h. After 4 h 119 mL of 1 N sodium hydroxide had been added, and no modification of pH was observed upon addition of 60 mg of Subtilisin Carlsberg. The reaction mixture was then diluted with water (2000 mL) and extracted with ethyl acetate (3 × 500 mL). The organic layers were washed with water (3 × 500 mL) and brine, dried over sodium sulfate, and concentrated *in vacuo* to

Table III. $^1$H NMR Data of Compound Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (47)

| | NH | | Hα | | Hβ | | |
|---|---|---|---|---|---|---|---|
| | δ (ppm) | $^3J$ (Hz) | δ (ppm) | $^3J$ (Hz) | δ (ppm) | $^3J$ (Hz) | others δ (ppm) |
| Tyr | d 6.86 | 8.3 | 4.14 | | β 2.92 | | o, m 7.04, 7.10, |
| | | | | | β′ 2.67 | 10.6 | Boc 1.30 |
| | | | | | $^2J$ | 13.6 | |
| Nle | d 8.01 | 7.7 | 4.24 | | β 1.54 | | $CH_2$ γ, δ 1.24, |
| | | | | | | | $CH_3$ t 0.83 |
| Gly | 8.17 | | 3.73 | 5.8 | | | |
| | | | 3.64 | 5.6 | | | |
| | | | $^2J$ | 16.7 | | | |
| Trp | d 7.95 | 7.8 | 4.57 | | β 3.14 | 4.7 | NH d 10.78, s 7.14, |
| | | | | | β′ 2.95 | 8.8 | d 7.56, d 7.30, |
| | | | | | $^2J$ | 14.9 | t 7.03, t 6.94 |
| Nle | d 7.89 | 7.8 | 4.13 | | β 1.55 | | $CH_2$ γ, δ 1.24, |
| | | | | | | | $CH_3$ t 0.83 |
| (*R*)-β-homo-Aph | d 7.69 | 8.3 | 4.01 (CH), 2.30 and 2.23 ($CH_2COOH$) | | | | $CH_2$ m 1.49, $CH_2$ m 1.24, $CH_2$ m 1.48, $CH_2$ m 2.50, $C_6H_5$ m 7.30–7.0 |

Table IV. Receptor Binding and Stimulation of Amylase Secretion by CCK Analogues

| | rat pancreatic acini | | | guinea pig brain membranes | jurkat T cells |
|---|---|---|---|---|---|
| | amylase secretion | | binding | binding | binding |
| | $EC_{50}$ (nM) | $IC_{50}$ (μM) | $IC_{50}$ (nM) | $IC_{50}$ (nM) | $IC_{50}$ (nM) |
| Boc-[Nle$^{28,31}$]-CCK-7 | 0.05 ± 0.01 | | 2.3 ± 0.5 | 0.29 ± 0.05 | 0.2 ± 0.1 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ JMV 180 | 3 ± 2 (p 100%) | | 3 ± 3 | 2 ± 1 | 2 ± 1 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-NH-$CH_2$-$CH_2$-$C_6H_5$ | 20 ± 10 (p 100%) | | 30 ± 11 | 10 ± 5 | 13 ± 4 |
| Boc-Tyr($SO_3H$)-Met-Gly-D-Trp-Nle-Asp-O-$CH_2$-$CH_2$-$C_6H_5$ | antagonist | 0.3 ± 0.2 | 7 ± 5 | 500 ± 45 | 210 ± 52 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (47) | 6 ± 2 (p 100%) | | 12 ± 8 | 32 ± 2 | 75 ± 15 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*S*)-β-homo-Aph-OH (48) | 50 ± 25 (p 75%) | | 15 ± 6 | 205 ± 25 | 190 ± 23 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-App-OH (49) | 4 ± 2 (p 100%) | | 13 ± 5 | 57 ± 5 | 65 ± 21 |
| Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*S*)-β-homo-App-OH (50) | 20 ± 8 (p 95%) | | 16 ± 7 | 200 ± 60 | 250 ± 53 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-Aph-OH (51) | antagonist | 0.5 ± 0.2 | 14 ± 4 | 1150 ± 30 | 1100 ± 52 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-Aph-OH (52) | 22% at 1 mM | | 23 ± 5 | 1200 ± 120 | 1500 ± 65 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*R*)-β-homo-App-OH (53) | antagonist | 1 ± 0.5 | 18 ± 5 | 1750 ± 30 | 1800 ± 200 |
| Boc-Tyr($SO_3H$)-Nle-Gly-D-Trp-Nle-(*S*)-β-homo-App-OH (54) | antagonist | 8 ± 5 | 42 ± 12 | 1650 ± 90 | 1400 ± 180 |

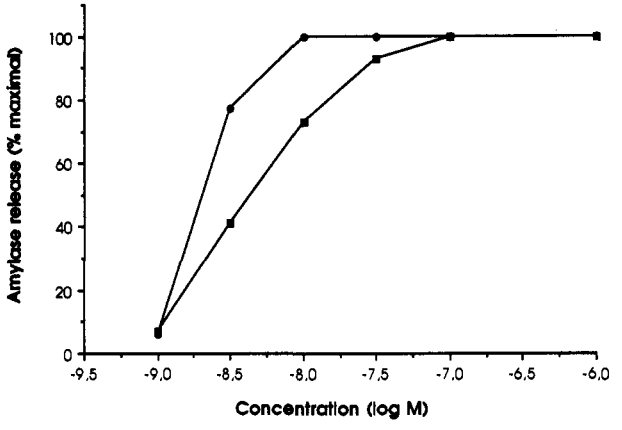



**Figure 1.** Effect of compounds Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester (JMV180) (●) and Boc-Tyr($SO_3H$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (47) (■) on amylase release from rat pancreatic acini. In each experiment, each value was determined in duplicate, and the results given are means of at least three separate experiments.

lead to (*R*)-Ethyl 2-acetamido-6-phenylhexanoate (4) as an oil. Yield 34 g (100%). Physical and analytical data are given in Table I.

The combined aqueous phases were acidified to pH 3 with potassium hydrogen sulfate and extracted with ether (3 × 500 mL). The combined organic fractions were washed with 1 M potassium hydrogenosulfate (500 mL), water (3 × 500 mL), and brine, dried over sodium sulfate, and concentrated *in vacuo* to lead to (*S*)-2-acetamido-6-phenylhexanoic acid (5) as a white solid that was recrystallized in a mixture of ethyl acetate and hexane. Yield 27.5 g (90%). Physical and analytical data are given in Table I.

**(*R*)-2-Amino-6-phenylhexanoic Acid [(*R*)-H-Aph-OH] (6).** Compound 4 (34 g, 122 mmol) was dissolved/suspended in aqueous 6 N HCl (300 mL) and heated to reflux for 4 h. Upon cooling to 5 °C, a white precipitate formed that was collected by filtration. The wet filter cake was dissolved in water (600 mL) at 50 °C, and the pH was adjusted to 6 at 50 °C with concentrated ammonium hydroxide. The title compound precipitated as a white solid. It was collected by filtration, washed with cold water and ether, and dried *in vacuo* over phosphorous pentoxide. Yield 23 g (91%). Physical and analytical data are given in Table I.

**(*S*)-2-Amino-6-phenylhexanoic Acid [(*S*)-H-Aph-OH] (7).** Obtained as described above from (*S*)-2-acetamido-6-phenylhexanoic acid (5) (27.3 g, 109 mmol). Yield 21.8 g (96%). Physical and analytical data are given in Table I.

**(*R*)-2-[(*tert*-Butyloxycarbonyl)amino]-6-phenylhexanoic Acid [Boc-(*R*)-Aph-OH] (8).** To a solution of compound 6 (12 g, 57.9 mmol) in a mixture of 1 N aqueous solution hydroxide (58 mL) and dioxane (60 mL) was added di-*tert*-butyl dicarbonate (14.2 g, 65 mmol), and the pH was continuously adjusted to 10 by addition of 1 N aqueous sodium hydroxide. When no starting material could be detected by TLC, the reaction mixture was diluted with water (400 mL) and extracted with ether (2 × 200 mL). The expected compound precipitated upon addition of the aqueous phase into 1 M potassium hydrogenosulfate (200 mL). It was collected, thoroughly washed with water, and dried *in vacuo* over phosphorous pentoxide. Yield 17.2 g (96%). Physical and analytical data are given in Table I.

**(*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-6-phenylhexanoic Acid [Boc-(*S*)-Aph-OH] (9).** Obtained as described for compound 8. Physical and analytical data are given in Table I.

**(*R*)-2-[(*tert*-Butyloxycarbonyl)amino]-7-phenylheptanoic Acid (Boc-(*R*)-β-homo-Aph-OH) (10).** To a cold (−15 °C) solution of compound 8 (17.2 g, 55.9 mmol) in 1,2-dimethoxyethane (100 ml) were successively added under vigorous stirring NMM (6.7 mL, 61 mmol) and IBCF (8.2 mL, 61 mmol). After 5 min of stirring at −15 °C, the precipitated salt was filtered off,

the filtrate was treated with diazomethane in ether (70 mmol), and the mixture stirred at 0 °C for 30 min. It was then concentrated *in vacuo* and the residue dissolved in methanol (400 mL) and treated with silver benzoate (2.5 g, 10 mmol) in triethylamine (30 mL). After 30 min of stirring at room temperature, the solvent was concentrated *in vacuo*, the residue dissolved in AcOEt (300 mL), and the insoluble material removed by filtration. The filtrate was washed with saturated sodium bicarbonate (3 × 100 mL), water (100 mL), 1 M aqueous potassium hydrogenosulfate (3 × 100 mL), and brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was dissolved in methanol (100 mL) and treated with 1 N sodium hydroxide (60 mL, 60 mmol) at 40 °C for 30 min. Upon acidification with 1 M aqueous potassium hydrogenosulfate, the expected compound precipitated. It was collected by filtration, washed thoroughly with water and hexane, and dried *in vacuo*. Yield 15.5 g (86%). Physical and analytical data are given in Table I.

**(*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-7-phenylheptanoic Acid (Boc-(*S*)-β-homo-Aph-OH) (11).** Obtained as described for compound **10**. Physical and analytical data are given in Table I.

**(*R*)-Benzyl 2-[(*tert*-Butyloxycarbonyl)amino]-7-phenyl heptanoate (Boc-(*R*)-β-homo-Aph-OBzl) (23).** To a stirred solution of compound **10** (7 g, 21.8 mmol) in benzene (80 mL) were added DBU (3.26 mL, 21.8 mmol) and benzyl bromide (2.75 mL, 23 mmol). After 2 h of reflux, the reaction mixture was cooled to room temperature and diluted with ethyl acetate (300 mL). The resulting solution was washed with saturated sodium bicarbonate (3 × 100 mL), water (100 mL), 1 M aqueous potassium hydrogenosulfate (3 × 100 mL), and brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was filtered through a silica gel column (eluent: ethyl acetate/hexane (1:5)). Pure fractions were pooled and concentrated *in vacuo* to leave a white solid. Yield 6.9 g (77%). Physical and analytical data are given in Table I.

**Boc-Nle-(*R*)-β-homo-Aph-OBzl (27).** Compound **23** (2.0 g, 4.86 mmol) was partially deprotected with TFA (20 mL). After standing at room temperature for 30 min, the solvent was concentrated under reduced pressure at $t$ <40 °C. The residue was dried *in vacuo* over KOH pellets and added to a solution of Boc-Nle-OH (1.12 g, 4.86 mmol) in DMF (20 mL), followed by BOP (2.15 g, 4.86 mmol) and NMM (1.63 ml, 14.58 mmol), and the solution was stirred for 1 h at room temperature. The reaction mixture was diluted with ethyl acetate (100 mL). The resulting solution was washed with saturated sodium bicarbonate (3 × 50 mL), water (100 mL), 1 M aqueous potassium hydrogenosulfate (3 × 50 mL), and brine, dried over magnesium sulfate, and concentrated *in vacuo* to leave a white solid. Yield 1.80 g (70%). Physical and analytical data are given in Table I.

**Z-Trp-Nle-(*R*)-β-homo-Aph-OBzl (31).** Compound **27** (0.85 g, 1.62 mmol) was treated with TFA (10 mL) as described above. The partially deprotected peptide was dissolved in DMF (20 mL) containing Z-Trp (0.55 g, 1.62 mmol) and BOP (0.72 g, 1.62 mmol). The solution was cooled to 0 °C, and NMM (0.36 mL, 3.24 mmol) was added. After 1 h of stirring at room temperature, the expected compound precipitated upon addition of saturated aqueous sodium bicarbonate (200 mL). It was collected by filtration, washed with saturated aqueous sodium bicarbonate, water, 1 M potassium hydrogenosulfate, and water and dried *in vacuo*. Yield 0.97 g (81%). Physical and analytical data are given in Table I.

**Boc-Tyr-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (39).** Compound **31** (0.752 g, 1.01 mmol) was hydrogenated for 3 h in a mixture of DMF, acetic acid, and water (25:3:3) (60 mL) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off and the filtrate concentrated under reduced pressure to leave a residue which crystallized upon trituration with ether. It was collected, washed with ether, and dried *in vacuo* over KOH pellets. It was added to a solution of Boc-Tyr-Nle-Gly-Osu (0.50 g, 0.91 mmol) in DMF (10 mL) and the solution was stirred for 3 h at room temperature. The expected compound precipitated upon addition of aqueous 1 M potassium hydrogenosulfate (100 mL). It was collected by filtration, washed with 1 M potassium hydrogenosulfate and water, and dried *in vacuo*. Yield 0.81 g (93%). Physical and analytical data are given in Table I.

**Boc-Tyr($SO_3^-$)-Nle-Gly-Trp-Nle-(*R*)-β-homo-Aph-OH (47).** To a solution of compound **39** (0.250 g, 0.26 mmol) in a mixture of DMF (2 mL) and pyridine (2 mL) was added $SO_3$–pyridine complex (1.5 g). After overnight stirring at room temperature, the solvents were concentrated under reduced pressure, and the excess of complex was hydrolyzed with water (10 mL) for 30 min, while the pH was maintained around 7–8 by addition of 10% aqueous sodium carbonate. The solution was then acidified to pH 5 by addition of 1 M aqueous potassium hydrogenosulfate and extracted with n-BuOH (3 × 20 mL). The organic phases were washed with water and concentrated under reduced pressure to leave a solid residue which was triturated with ether, collected, and dried *in vacuo*. It was purified by silica gel chromatography (eluent: 80:20:5:10) to afford compound **47** as a white solid. Yield 0.220 g (81%). It was finally purified by HPLC and lyophylized. Physical and analytical data are given in Table I.

$^1$H NMR data of compounds **47–54** are available as supplementary material.

**Biological Evaluations.** Male guinea pigs (280–300 g) were obtained from le Centre d'Elevage d'Animaux de Laboratoire (Ardenay, France); male Wistar rats (180–200 g) were from Effa-Credo (Saint Germain l'Arbresle, France). Hepes was from Boehringer-Mannheim; purified collagenase was from Serva (Garden City Park, NY); soybean trypsin inhibitor was from Sigma (St Louis, MO); Eagle's basal amino acid medium (100 times concentrated) was from GIBCO (Grand Island, NY); essential vitamin mixture (100 times concentrated) was from Microbiological Associates (Bethesda, MD); bovine plasma albumin (fraction V) was from Miles Laboratories Inc. (Elkhart, IN); Phadebas amylase test was from Pharmacia Diagnostics (Piscataway, NJ); and $^{125}$I-labeled *N*-succinimidyl-3-(4-hydroxyphenyl)propionyl-CCK-8 ([$^{125}$I]BH-CCK-8) was from Amersham Corp. (Buckinghamshire, UK). Unless otherwise stated, the standard incubation solutions contained 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM $NaH_2PO_4$, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mg/mL of bacitracin, 0.2% (w/v) albumin, 0.03% (w/v) soybean trypsin inhibitor, 1% (v/v) essential amino acid mixture, and 1% (v/v) essential vitamin mixture. The incubation solution was equilibrated with 95% $O_2$, 5% $CO_2$ as the gas phase. Dispersed acini from rat pancreas were prepared according the previously described modifications[27] of the method of Peikin et al.[28] Guinea pig brain membranes were prepared following the procedures described by Pelaprat et al.[29] Amylase release was measured using the procedure already described.[30] Briefly, acini were resuspended in the standard incubation solution complemented with 1% bovine serum albumin, 1 mM calcium, and 5 mM theophylline containing about 1 mg of protein/mL, and samples (1 mL) were incubated at 37 °C for 30 min. Amylase activity was determined by the method of Ceska et al.[31] using the Phadebas reagent. Amylase release was measured as the difference of amylase activity at the end of incubation that was released into the extracellular medium, with and without secretagogue, and expressed as the percentage of maximal stimulation obtained with Boc-[Nle$^{28}$,Nle$^{31}$]-CCK-7 (40 ± 5% of the total amylase contained in the acini) minus the basal amylase secretion (10 ± 2% of the total amylase contained in the acini) obtained without secretagogue.

**Binding Studies.** Binding of [$^{125}$I]BH-CCK-8 to rat pancreatic acini was performed as previously described.[30] Briefly, samples (0.5 mL containing ≈ 1 mg/mL protein) were incubated with the appropriate peptide concentrations for 30 min at 37 °C in the presence of 10 pM of [$^{125}$I]BH-CCK-8 plus various concentrations of BOC-[Nle$^{28}$,Nle$^{31}$]-CCK-7. After centrifugation at 10,000$g$ for 10 min and washings, the radioactivity associated with the acinar pellet was measured. Values are expressed as the percentage of the value obtained with labeled CCK-8 alone. The specific activities of the ligand used in our experiments was 2000 Ci/mmol. Acini from three rat pancreata were suspended in 100 mL of standard incubation solution. Specific binding in the absence of any unlabeled CCK-peptide was 13 ± 3% of the total radioactivity present in the sample. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle$^{28}$,Nle$^{31}$]-CCK-7 and was always less than 15% of the total binding. Results are the means of at least four independent experiments in duplicate.

Binding of $^{125}$I-CCK-8 to guinea pig membranes was performed according to Pelaprat et al.[29] The buffer used was 50 mM (Tris)·HCl, 5 mM $MgCl_2$, 0.1 mg/mL of bacitracin, pH 7.4 (Tris–$MgCl_2$–bacitracin buffer). Briefly, displacement experiments were performed by incubation of 1 mL of brain membranes (approximately 0.5 mg of protein) in the presence of 20 pM [$^{125}$I]-BH-CCK-8 for 60 min at 25 °C in the presence of various concentrations of Boc-[Nle$^{28}$,Nle$^{31}$]-CCK-7 or peptide analog in a total volume of 1 mL. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle$^{28}$,Nle$^{31}$]-CCK-7 and was always less than 25% of the total binding. Total binding was about 10% of the total radioactivity contained in the sample. Results are the means of at least four independent experiments in duplicate.

Binding of $^{125}$I-CCK-8 to JURKAT cells was performed according to Lignon et al.[21] Cells were harvested by centrifugation at 1500*g* for 5 min and washed twice with the binding incubation medium consisting of 24.5 mM HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM $NaH_2PO_4$, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mg/mL of bacitracin, 0.01% (w/v) soybean trypsin inhibitor, 1% (v/v) essential amino acid mixture, and 1% (v/v) vitamin mixture. Binding studies were carried out at 37 °C for 60 min, using 10 pM [$^{125}$I]-BH-CCK-8, plus appropriate drug concentrations, in a final volume of 500 μL containing $4 \times 10^6$ cells/mL. The reaction was stopped by addition of fresh standard medium plus 2% BSA and centrifugation 10 min at 10000*g*. Nonspecific binding was measured in the presence of 1 μM CCK-8 and was always less than 10% of total binding. Results are means of at least three separate experiments in duplicate.

**Supplementary Material Available**: Tables of analytical and $^1$H NMR data (9 pages). Ordering information is given on any current masthead page.

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