Brief Articles

Synthesis and Human Neurotensin Receptor Binding Activities of Neurotensin(8−13) Analogues Containing Position 8 α-Azido-*N*-alkylated Derivatives of Ornithine, Lysine, and Homolysine

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A series of neurotensin(8−13) (NT) analogues were synthesized through intermediates in which the N-terminal Arg(8) was replaced by various ω-bromo-2(*S*)-azido residues spanning 3−5 methylene units in side-chain length. Subsequent nucleophilic substitution of the ω-bromo groups with ammonia, methylamine, dimethylamine, or trimethylamine provided peptides containing N-terminal 2(*S*)-azido residues containing primary through quaternary *N*-alkylated side chains corresponding in length to ornithine, Lys, and homolysine. The synthetic procedure appears applicable for incorporation of a wide variety of amine-containing nonnatural amino acids into peptides. The particular modifications were intended to enhance physiochemical properties of NT(8−13) responsible for human NT 1 receptor (hNTR) binding, overall lipophilicity, and stability that may influence the potency of the peptides in vivo. When the peptides were tested for hNTR binding, the affinities in each series followed the order primary > secondary > tertiary > quaternary amine with the homolysine side-chain length being favored. All analogues possess binding affinities between acetyl-NT(8−13) and NT(8−13) indicating that the sterically less bulky α-azido may be inherently preferable to the acetyl group for N-terminal protection. This study extends the strategy of modifying amine-containing drugs through alkylations to peptide modification. The set of alkylated side chains also offers a new method of steric selection between receptor subtypes and could be used to modify the properties and biological effects of any peptide that contains cationic residues.

Introduction

The C-terminal binding fragment of neurotensin (NT), NT(8−13) (Arg(8)-Arg(9)-Pro(10)-Tyr(11)-Ile(12)-Leu(13)), is the minimal structural element able to bind and trigger biological effects at human NT receptors (hNTRs).[1] Since the peptide possesses an atypical neuroleptic profile in the brain,[2] there is substantial interest in developing bioavailable NT(8−13) derivatives as alternatives for the treatment of schizophrenia. Although NT(8−13) possesses high receptor binding affinity, it is rapidly degraded by peptidase action and does not cross the blood−brain barrier to a sufficient degree to permit peripheral administration. A successful strategy to increase biological half-lives of peptides has been to produce analogues with N-terminal alterations to prevent inactivation of the peptide through aminopeptidase-catalyzed removal of the N-terminal residues. For example, in NT derivatives, substitution of the Arg(8)-Arg(9) amide with a reduced peptide bond has enhanced stability in the NT(8−13) analogue [Lys(8)ψ[CH_2NH]Lys(9)]NT(8−13) while maintaining comparable agonist activity.[3] In comparison, the *N*-α-methylated analogue NT-1 (Figure 1) is stable and reportedly crosses the blood−brain barrier.[4] This likely results from the peptide's enhanced lipophilicity in comparison to NT(8−13), which is known to enhance the potential for blood−brain barrier access.[5] It is, however, 3−25 times less potent as a NT agonist than NT(8−13) depending on the type of assay in which the peptides are evaluated.[6]

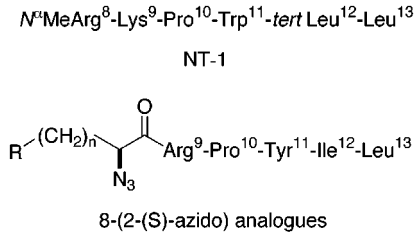

Figure 1. Structural comparison of NT(8−13) analogues.

In this work, preparation and initial evaluation of a series of NT(8−13) analogues featuring novel modifications of the N-terminal Arg(8) residue are reported. The modifications were designed to enhance the physiochemical parameters of the peptide to maintain receptor binding while increasing overall lipophilicity that ultimately may translate into higher in vivo activity. The general structure of these peptides is provided in Figure 1. All peptides feature substitution of an α-azido group for the N-terminal α-amino group. This modification was intended to hinder aminopeptidase action while enhancing overall lipophilicity and thus potential for blood−brain barrier access.[5] This free amino group is

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not essential for receptor binding since it is present in an amide bond in the parent peptide. In addition, the peptides feature substitution of the Arg(8) side chain with alkylated derivatives of L-Orn, L-Lys, and L-homolysine (L-Hlys). Peptides in which L-Orn and L-Lys were substituted for Arg(8) in NT(8−13) have been shown to maintain high receptor binding affinity.[7] These modifications were designed to further increase the peptides' overall lipophilicity while exploring electrostatic and steric requirements for receptor binding at this site. Of considerable interest is that, although proteins are known to be methylated posttranslationally in vivo[8] and the properties of a number of nonpeptidic drugs are improved through amine alkylation,[9] this strategy has not been applied to any degree in peptide design. The relative binding of all new compounds to hNTR 1 (the "high-affinity" NT receptor) demonstrates the validity of the structural changes, which indicates that the strategies utilized may be applicable to the modification of any peptide of potential therapeutic interest that contains important cationic residues.

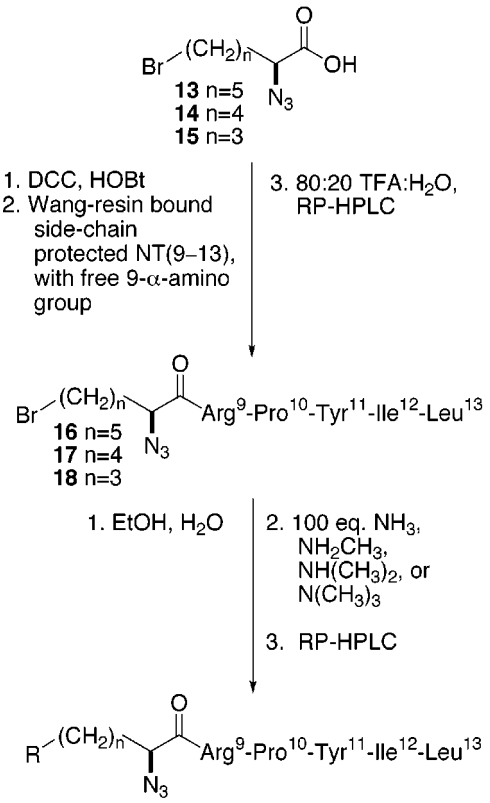
Results

Chemistry. The specific structures of the NT(8−13) analogues prepared for this work, peptides **1**−**12**, are shown in Scheme 1. It was thought that these nonnatural cationic side chains could be prepared routinely using standard solid-phase peptide synthetic procedures, with condensation of the appropriately protected alkylated amino acid with resin-bound NT(9−13). Accordingly, the *ω*-bromo-2(*S*)-azido acids **13**−**15**, synthesized previously in this laboratory,[10] were readily converted to *N*-*ω*-alkylated analogues of Orn, Lys, and Hlys, respectively, and *N*-α-Fmoc derivatized in preparation for solid-phase peptide synthesis. It was found, however, that solubility incompatibilities and stability problems due to the inability to protect the side chains of the *N*-α-Fmoc tertiary and quaternary alkylated amino acids hindered facile peptide bond condensation.

A simpler and more efficient route (Scheme 1) evolved from incorporation of **13**−**15** directly into the N-terminal position of NT(9−13) through DCC−HOBt-mediated condensation to produce intermediates **16**−**18** (yields ca. 50%). These intermediates then were treated with 100 equiv of the requisite amine nucleophile in aqueous ethanol and stirred overnight at ambient temperature and pressure to produce the alkylation patterns in **1**−**12**. After solvent removal, purification by RP-HPLC resulted in product yields of 80−90% from **16**−**18**. At this point the azido group could be reduced to the α-amino functionality if desired, but for the current work, it was retained as a more lipophilic group expected to be aminopeptidase-resistant. Notably, the choice of nucleophilic substitution of the *ω*-bromo-2(*S*)-azido acids **13**−**15** enables unequivocal definition of the alkylation state of the resulting amine, in contrast to the classical treatment of amines with alkyl halides which can lead to polyalkylations.[11] Overall, this parallel method for the synthesis of **1**−**12** was efficient in that side-chain protections were not necessary and only three brominated intermediates (**16**−**18**) needed to be synthesized and purified to produce the desired products (and potentially many others) by nucleophilic substitutions.

Peptide Characterizations and hNTR Binding

Scheme 1



8-(2-(S)-Azido-Homolysyl) NT(8–13) Analogues

1: n=5, R=NH_2

2: n=5, R=$NHCH_3$

3: n=5, R=$N(CH_3)_2$

4: n=5, R=$N(CH_3)_3$

8-(2-(S)-Azido-Lysyl) NT(8–13) Analogues

5: n=4, R=NH_2

6: n=4, R=$NHCH_3$

7: n=4, R=$N(CH_3)_2$

8: n=4, R=$N(CH_3)_3$

8-(2-(S)-Azido-Ornithyl) NT(8–13) Analogues

9: n=3, R=NH_2

10: n=3, R=$NHCH_3$

11: n=3, R=$N(CH_3)_2$

12: n=3, R=$N(CH_3)_3$

Assays. After synthesis, the structures of NT(8−13) analogues **1**−**12**, NT(8−13), acetyl-NT(8−13), [Hlys(8)]-, [Lys(8)]-, and [Orn(8)]NT(8−13), NT(9−13), and bromoazido intermediates **16**−**18** were characterized by mass spectrometry. All compounds exhibited correct molecular weights (Table 1) and sequential breakdown patterns (data not shown). The peptides were assayed by RP-HPLC to assess purity and demonstrate that the

Table 1. Binding Affinity Comparison and Analytical Data

peptide	relative affinity[a]	t_R[c] (min)	MW found[d] (calcd)
NT(8−13)	100[b] ± 12.7	22.1	817.0 (817.0)
acetyl-NT(8−13)	15 ± 3.7	23.2	859.2 (859.0)
[Hlys(8)]NT(8−13)	106 ± 10.2	21.6	803.0 (803.0)
1	74 ± 3.4	25.1	828.8 (829.0)
2	54 ± 10.7	25.4	843.0 (843.0)
3	35 ± 6.4	25.6	856.8 (857.1)
4	34 ± 13.8	25.6	872.0 (872.1)
[Lys(8)]NT(8−13)	64 ± 7.3	21.7	789.0 (789.0)
5	57 ± 4.2	24.5	815.0 (815.0)
6	55 ± 15.5	24.9	829.0 (829.0)
7	39 ± 0.6	25.1	843.0 (843.0)
8	32 ± 6.4	25.1	858.0 (858.1)
[Orn(8)]NT(8−13)	63 ± 18.9	21.6	775.0 (775.0)
9	41 ± 13.6	24.1	800.8 (801.0)
10	29 ± 8.7	24.3	814.8 (815.0)
11	24 ± 9.5	24.4	829.0 (829.0)
12	22 ± 1.5	24.6	844.0 (844.1)
NT(9−13)	0.4 ± 0.084	22.4	660.8 (660.8)
16	0.6 ± 0.078	36.2	893.3 (892.9)
17	2.2 ± 0.25	34.4	879.3 (878.9)
18	2.1 ± 0.18	32.9	865.3 (864.9)

[a] Binding data were obtained by assaying the ability of an analogue to compete with [^{125}I-Tyr(3)]NT (0.15 nM) for human NT (Leu^{194}) receptors as described in the Experimental Section. Assays were performed in triplicate and are the geometric means reported relative to NT(8-13) with ±SE given as a percentage of the geometric mean. [b] The measured K_d which corresponds to 100% relative affinity for NT(8−13) was 5.4 nM. This compares well to the literature value of Cusack et al.[7] (12 nM). [c] Analytical RP-HPLC retention times were obtained using system 1 in the Experimental Section. [d] A mass spectrum for each analogue was obtained.

azido-containing peptides were more lipophilic than the N-terminal amino standards. As indicated in Table 1, all α-azido-*N*-alkylated peptides exhibited longer retention times and thus increased lipophilicity; the uncharged 8-position *ω*-bromo compounds **16**−**18** are drastically more lipophilic. Within the 8-position *N*-alkylated series, greater lipophilicity was exhibited with extent of alkylation, as expected.

Table 1 also provides relative hNTR receptor binding affinity of all peptides. The two standards, NT(8−13) and acetyl-NT(8−13), exhibited comparable binding to those reported previously in the literature,[12] with acetylation decreasing the relative binding affinity of NT(8−13) by almost 1 order of magnitude. While only [Hlys(8)]NT(8−13) was comparable to NT(8−13) in absolute binding efficiency, all of the other peptide analogues were well within 1 order of magnitude (22−74%) as efficient. Severe loss of binding efficiency was demonstrated with removal of the cationic 8-position residue in NT(9−13), while the 8-position *ω*-bromo-α-azido-containing peptides also exhibited poor binding.

Discussion

The NT(8−13) derivatives of interest, peptides **1**−**12** (Scheme 1), were prepared by an extension of a procedure for the synthesis of *N*-alkylated amino acids that was developed recently in our laboratory.[10] Incorporation of the appropriate *ω*-bromo-2(*S*)-azido acid into a nascent peptide chain at the site in which substitution of an *N*-alkylated side-chain amino acid is desired leads to the ability to substitute virtually any alkylated nucleophile of interest and the potential for preparation of a wide variety of novel N-terminal residue-modified peptides in a parallel fashion. In the current synthetic strategy, the *ω*-bromo group is used as an inert lipophilic "space saver" to be converted into the desired cationic side chain, and the α-azido group can be maintained as an unreactive/uncharged form of the α-amino group or reduced to the α-amino group if desired. Although not performed for the current set of experiments, further couplings to produce peptides containing internal alkylated amino acids appears straightforward, although tertiary and quaternary amines may lead to the solubility problems noted in Results. Therefore, this synthetic strategy potentially is applicable to the modification of any peptide containing cationic residues in which alteration to improve its physiochemical characteristics is desired. The parallel nature of the strategy enables a wide variety of novel peptides to be produced rapidly or in sets to undergo biological evaluation with the intent of sequentially optimizing the alkylation pattern of a given residue in an efficient fashion.

Substitution of the α-azido for the α-amino group has significance toward the pharmacokinetic properties of NT(8−13) analogues **1**−**12**, and peptides in general, in which it is incorporated. Since aminopeptidase action is a major determinant of biological half-life for peptides, α-azido substitutions are expected to increase the peptides' in vivo stabilities. This is under evaluation in our laboratory at this time. A second effect of the α-azido group is to increase the lipophilicity of the peptides by removal of a cationic charge. Traditionally, these effects have been accomplished in peptide modifications by acetylation of the N-terminus. While both changes will inhibit aminopeptidase action, the α-azido group also is more lipophilic than the acetyl group since analogues **1**−**12** have RP-HPLC retention times 1−2 min longer than acetyl-NT(8−13). This increase in lipophilicity for the azido analogues can be attributed to removal of the hydrogen-bonding ability in the acetyl amide. Most significantly, comparison of the relative binding of NT(8−13) analogues **1**−**12** to acetyl-NT(8−13) demonstrates that the α-azido is a better choice than acetylation due almost certainly to the latter group's greater steric bulk. As with acetyl-NT(8−13), some overall loss in binding affinity in the α-azido analogues, in comparison to the [Hlys(8)]-, [Lys(8)]-, and [Orn(8)]NT(8−13) standards, was observed. This indicates that a minor unfavorable steric effect is associated with the α-azido group. This effect of substituting the α-azido for α-amino group will have variable importance in different peptide−receptor interactions, depending on the nature of the active site binding motifs that are involved.

Among NT(8−13) analogues, [Lys(8)ψ[CH$_2$NH]Lys(9)]-NT(8−13) (Figure 1) and the *N*-α-methylated compound NT-1 (Figure 1) are most closely related to **1**−**12** in that all analogues were designed for enhanced stability through inhibition of aminopeptidase degradation. The current analogues may have the most potential for use in vivo, based on the following considerations. These compounds are more lipophilic than [Lys(8)ψ[CH$_2$NH]-Lys(9)]NT(8−13) and NT-1 as evidenced by the following comparison. Each analogue demonstrated an increase of about 3 min in RP-HPLC retention time (Table 1) compared to the standards NT(8−13) and [Orn(8)]-, [Lys(8)]-, and [Hlys(8)]NT(8−13), which is primarily attributable to loss of the N-terminal charge. Each methyl or methylene addition within this series adds approximately 15 s to retention time, with the homolysine group of five methylene units being the most

lipophilic. Under the conditions in which the RP-HPLC runs were performed, a 1-min retardation of elution time corresponded to approximately a 10-fold difference in octanol/water partitioning coefficient (data not shown); hence the range in relative lipophilicities within the set of analogues **1**−**12** is fairly broad. This could be a distinct advantage for the current set of compounds as there may be an optimal degree of lipophilicity that allows for sufficient barrier access in the absence of undesired sequestering in other lipophilic sites of the blood and various tissues.[5] Most importantly, all new analogues exhibited comparable binding affinities to NT(8−13) and [Lys(8)ψ[CH_2NH]Lys(9)]NT(8−13) (which does not cross the blood−brain barrier) and significantly better than NT-1 (which does). The abilities of analogues **1**−**12** to cross the blood−brain barrier will be evaluated shortly.

While analogues **1**−**12** all possess subnanomolar binding affinities to hNTR 1 (Table 1), the relative binding affinities reveal important information about the structure of the peptide−receptor interaction. The order of affinity for position 8 amino groups was primary > secondary > tertiary > quaternary, irrespective of side-chain length. Therefore it is evident that addition of alkyl functionalities decreases the interaction at the receptor by either steric or electrostatic means since all of the subgroups follow the same order. With respect to side-chain length, the homolysine side chain provides binding similar to that of the native guanidino group, indicating that it places the localized charge in a similar location to that of the resonating guanidino group of native Arg(8). In general, increasing the overall chain lengths at this position led to overall moderate increases in binding efficiencies across the homologous series, which indicates that the binding site for this residue in the hNTR is fairly elastic. The binding affinities of the brominated analogues **16**−**18** also were evaluated; removal of charge in these analogues results in a 98−99% loss in binding affinity. This demonstrates that alkylation of the cationic side chains results in only minimal losses in binding efficiency through adverse steric interactions as long as the cationic nature of the residue is maintained. The minor losses in binding efficiencies with side-chain alkylations parallel the effect of the α-azido group, which exhibits only a 25−35% loss in affinity at the hNTR when substituted for the α-amino group. Thus, while these analogues may exhibit minor decreases in overall potency compared to the standards, this may be tolerable with the gains in lipophilic character (and potential for blood−brain barrier access) and overall stability when the relative in vivo potencies are evaluated.

Previous to this work, utilization of *N*-alkyl groups has proven essential in optimizing selectivity, potency, lipophilicity/pharmacokinetics, and stability of a number of drug entities; this work extends the strategy to peptide-based compounds. Interestingly, this strategy is one employed in nature as methylation is a standard component of posttranslational modifications of proteins.[8] Cellular enzymes use *S*-adenosyl-L-methionine as the methyl donor in *O*-esterification of aspartyl residues, *N*-methylation of the imidazolidine side chain of histidine, and *N*-methylation of the ϵ-*N* of lysine. The enzyme responsible for alkylation of lysyl residues, protein methylase III, is unique in that it can effectively add three methyl groups on a single side chain in some proteins[13,14] or alkylate with one to three methyl substituents in others.[15] The role of alkylations on peptide activity is not fully understood, but it appears that these posttranslational modifications serve to balance overall peptide activity or serve as markers for degradative processes. The current modification strategy thus can be considered biomimetic.

Conclusion

In summary, two types of modifications intended to enhance peptide stability and potential for increased barrier access—conversion of the *N*-α-amino group to an *N*-α-azido moiety and alkylation of cationic side chains—were explored in the context of NT(8−13) binding to hNTR 1. Both modifications led to more lipophilic analogues with comparable binding efficiencies to the parent peptide. The parallel mode of synthesis allows for quick optimization of sterics and electrostatics at cationic positions and hence appears to be applicable to peptide design in general. Relative stability and blood−brain barrier access of analogues **1**−**12** in comparison to NT(8−13) standards and other active NT analogues are under investigation, with results to be reported in due time.

Experimental Section

Starting Materials. *p*-Benzyloxybenzyl alcohol, resin-bound L-*N*-α-Fmoc-leucine, L-*N*-α-Fmoc-isoleucine, L-*N*-α-Fmoc-(*t*-Bu)-tyrosine, L-*N*-α-Fmoc-proline, L-*N*-α-Fmoc-(Pmc)-arginine, L-*N*-α-Fmoc-(*t*-Boc)-ornithine, and L-*N*-α-Fmoc-(*t*-Boc)-lysine were purchased from Bachem. L-*N*-α-Fmoc-(*t*-Boc)-homolysine was prepared previously in this laboratory.[16] Acetyl-NT(8−13) was purchased from Bachem. Ammonia (29.6% in H_2O) was purchased from Mallinckrodt. Methylamine (40% in H_2O), dimethylamine (40% in H_2O), and trimethylamine (25−27% in H_2O) were obtained from Aldrich. Other reagents and solvents were analytical reagent grade.

Peptide Synthesis. The leader portion of the peptide was synthesized in bulk using *p*-benzyloxybenzyl alcohol solid-phase methodology on a manual shaker[17] and stored in fully protected form for use in small-scale couplings. Final resin loading of the pentapeptide was calculated and confirmed by quantitative ninhydrin test[18] of the position 9 Fmoc-deprotected α-amino groups. Aliquots of the resin-bound pentamer (12.5 μmol) were weighed for couplings in a small-scale synthesizer[19] following general washing protocol. Standard peptides NT(8−13) and [Orn(8)]-, [Lys(8)]-, and [Hlys(8)]NT(8−13) were prepared by activating 50 μmol of the requisite diprotected amino acids with 50 μmol each of HOBt and DCC. Reactions were monitored with the Kaiser test[20] and recoupled as necessary. The N-terminal Fmoc groups were removed with 20% piperidine in DMF prior to cleavage from the resin. The halogenated peptide intermediates **16**−**18** were prepared in a similar fashion using the required ω-bromo-2(*S*)-azido acids **13**−**15**. Cleavage from the resin was effected with 300 μL of H_2O:TFA (20:80) (scavengers were not employed due to the lability of the halogen groups) for 3 h. The acidic solution was collected in a test tube and the resin was washed twice with the same solution. To complete final deprotection of the Arg(9) Pmc group, solutions were stirred vigorously for an additional 3 h in the stoppered test tubes. The crude peptides were concentrated and purified by preparative RP-HPLC.

Nucleophilic Substitution. Analogues **16**−**18** were dissolved in 0.5 mL of EtOH. The required amine nucleophiles, ammonia, methylamine, dimethylamine, or trimethylamine (100 equiv in H_2O), were added and the mixtures were stirred overnight in stoppered test tubes. The reaction mixtures were then concentrated prior to purification by RP-HPLC, giving **1**−**12**.

Purification. Preparative RP-HPLC was performed on a Waters dual pump system in combination with a Vydac (10 × 250 mm, 300 Å, 5 μm) semipreparative column. The mobile phase consisted of 0.1% trifluoroacetic acid in water (solvent

A) and 0.084% trifluoroacetic acid in acetonitrile (solvent B). Purification of intermediates **16**−**18** was performed at a flow rate of 3 mL/min. Linear gradients of 15% to 70% B over 55 min were used and the effluent was detected by UV absorbance at 254 nm. All standard peptides and final products **1**−**12** were purified using a linear gradient of 2% to 50% B over 55 min.

Characterization of NT(8−13) Analogues. Data for peptide characterization are provided in Table 1. All peptides were analyzed in two separate analytical HPLC systems to assess purity and lipophilic character. A mass spectrum for each analogue was obtained using electrospray conditions since this method was mild enough to detect the intact azido group.

1. Analytical HPLC. System 1: RP-HPLC analysis was performed on a Waters dual pump HPLC system equipped with a Bakerbond (C18, 4.6 mm × 250 mm) column. The solvent system consisted of 0.1% TFA in water (solvent A) and 0.1% TFA in 83% acetonitrile (solvent B). Samples (approximately 10−30 μg) were eluted with a linear gradient from 5% to 50% B over 30 min at a flow rate of 1 mL/min and detected by UV absorbance at 220 nm.

System 2: RP-HPLC analysis was performed using an ABI chromatograph (model 130A, Applied Biosystems) equipped with an Aquapore 300 column (C8, 2.1 × 30 mm). The solvent system consisted of 0.1% TFA in water (solvent A) and 0.1% TFA in 80% acetonitrile (solvent B). Approximately 1−2 μg of peptide were injected automatically and separated at a flow rate of 100 μL/min. Usually 60-min gradients from 5% to 50% B were employed and UV absorbance of the effluent was recorded at 230 nm. All peptides were of greater than 95% purity in both systems.

2. Mass Spectroscopy. A mass spectrum for each analogue was obtained using electrospray conditions on a Finnigan LCQ instrument. The tandem mass spectrum of the $[M + 2H]^{2+}$ ion for each analogue gave correct sequential data.

3. Radioligand Binding Assays. General procedure: Binding affinity assays for each NT(8−13) standard and analogues **1**−**12** were performed on hNTR 1 (Leu194) produced in CHO Cells from New England Nuclear (Boston, MA). Membrane preparations (0.5 mL) were diluted with 7.0 mL of incubation buffer (0.2% BSA in 50 mM Tris-HCl, pH 7.4) at 4 °C. The radioligand [^{125}I-Tyr(3)]NT diluted in 10 μL of incubation buffer (amount necessary to produce a concentration of 0.15 nM for competition assays) was introduced to each of nine Eppendorf tubes. Next, after serial dilution through the appropriate concentration range, 10 μL of a NT(8−13) analogue was added to each of the nine Eppendorf tubes. These were cooled to 4 °C at which time 150 μL of diluted membranes was added and allowed to incubate for 60 min at 4 °C. Incubation of the samples was terminated by dilution with 50 mM Tris-HCl, pH 7.4, buffer (0.5 mL) followed by rapid filtration through Whatman GF/A filters (25-mm diameter presoaked in 0.3% poly(ethylenimine)) using a Millipore 1225 sampling vacuum manifold. Each filter was immediately washed nine times with 3 mL of ice-cold 50 mM Tris-HCl, pH 7.4, and placed in tubes for gamma counting. Assays were performed in triplicate and are the geometric means reported relative to NT(8−13) ± SE. Nonspecific binding was determined in the presence of 100 nM of each analogue and was found to be 1−2% of total binding in each assay.

Modified procedure: Assays for brominated peptides **16**−**18** were modified slightly to lower the probability of nucleophilic substitution of the halogen functionalities by high concentrations of buffer amines. The incubation buffer used was 0.02% BSA in 25 mM HEPES, pH 7.4, and the wash buffer employed was 25 mM HEPES, pH 7.4. NT(9−13) was used in both buffer systems for standardization. Nonspecific binding was determined in the presence of 20 μM of each ligand.

Abbreviations: all natural amino acids used are of L-configuration; BSA, bovine serum albumin; *t*-Bu, *tert*-butyl; *t*-Boc, *tert*-butoxycarbonyl; CHO, Chinese hamster ovary; DCC, 1,3-dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HPLC, high-performance liquid chromatography; hNTR, human neurotensin receptor (Leu194); HOBt, 1-hydroxybenzotriazole; NT, neurotensin; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; RP, reversed-phase; Tris-HCl, tri(hydroxymethyl)aminomethane hydrochloride.

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Supporting Information Available: 400-MHz ^{1}H NMR spectra for one analogue of the new class of peptides, the methylated peptide **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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