Benextramine–Neuropeptide Y Receptor Interactions: Contribution of the Benzylic Moieties to [^{3}H]Neuropeptide Y Displacement Activity

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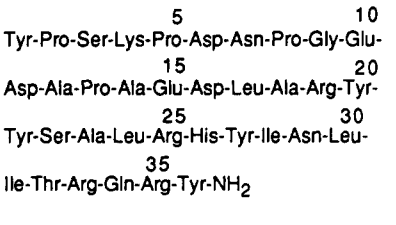
Analogs of *N,N'*-bis[6-[(2-methoxybenzyl)amino]hex-1-yl]cystamine (benextramine, BXT, **2**) were synthesized using solution-phase peptide synthesis methodology and analyzed for activity in displacing specifically bound 1 nM *N*-[*propionyl*-^{3}H]neuropeptide Y([^{3}H]NPY) from benextramine-sensitive neuropeptide Y (NPY) binding sites in rat brain. Our new synthetic approach to these analogs began with the acylation of cystamine with the N-hydroxysuccinimide ester of *tert*-butyloxycarbonyl (*t*-Boc) protected 6-aminohexanoic acid, followed by deprotection of the t-Boc groups with 4 N HCl in dioxane. Acylation of this symmetric diamine with *N*-hydroxysuccinimide esters of appropriately substituted benzoic acids, followed by reduction of the resultant tetraamides with diborane in refluxing THF, afforded the target compounds. The BXT analog lacking the benzylic group (i.e., compound **11**) had no [^{3}H]NPY displacement activity at concentrations up to 1.4×10^{-3} M. The 9-fold range in activities observed for the ortho, meta, and para regioisomers of the methoxy, chloro, and hydroxy benextramine analogs at benextramine-sensitive NPY rat brain binding sites does not differ from the range of potencies observed at α-adrenoceptors. However, the order of potencies at [^{3}H]NPY sites differs from the order of potencies at α-adrenoceptors, with the *m*-methoxyphenyl (**9a**), *m*-hydroxyphenyl (**10b**), and 2-naphthyl (**9f**) analogs being the most active at [^{3}H]NPY binding sites. The present results demonstrate the importance of the benzylic moiety for BXT's NPY antagonist activity, and suggest that the BXT binding site on the NPY receptor is significantly distinct from that on the α-adrenoceptor.

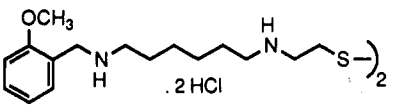
Introduction

Neuropeptide Y (NPY, **1**, Chart I), a 36 amino acid peptide hormone and neurotransmitter, was originally discovered by Tatemoto and co-workers[1] during the screening of porcine brain for peptides containing C-terminal tyrosine amides. NPY was initially recognized as a member of the pancreatic polypeptide family,[2] and was subsequently shown to have a wide distribution in both the mammalian central and peripheral, autonomic nervous systems. In the periphery, NPY is costored and coreleased with sympathetically-derived norepinephrine, potently induces vasoconstriction,[3] and inhibits synpathetic neuronal firing by a presynaptic mechanism.[4] In the central nervous system (CNS), NPY actions are more varied, and involvement in the regulation, control, or maintenance of autonomic tone, eating and drinking behavior, and in the modulation of hormone release, have been postulated.[5] However, lack of a selective NPY antagonist has hampered evaluation of the role of NPY's central and peripheral-mediated actions. Furthermore, the selective role of NPY in increasing vascular resistance without affecting cardiac output[6] has highlighted the clinical need for the development of NPY receptor antagonists as antihypertensives.

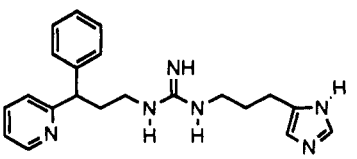
Chart I



Neuropeptide Y (NPY, **1**)



Benextramine (BXT, **2**)



He 90481 (**3**)

Like many other peptide hormones and neuropeptides, design and synthesis of potent and useful peptidergic NPY receptor antagonists appears to be a difficult task as is evidenced by the availability of only a few such antagonists

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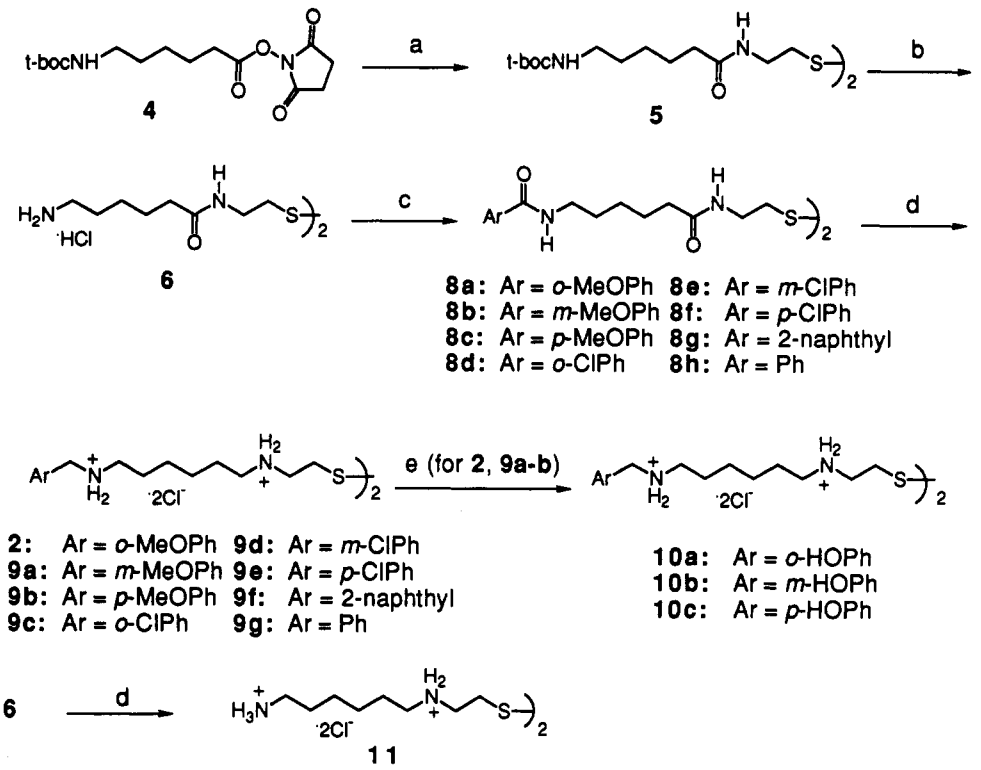
Scheme I[a]



[a] Reagents: (a) cystamine, DIEA; (b) 4 N HCl/dioxane; (c) (aroyloxy)succinimide (**7a–f**) or ArCOCl, DIEA; (d) diborane/THF; (e) 48% HBr.

reported to date.[7] Moreover, the peptide ligands, in general, suffer from some serious limitations, such as metabolic lability and poor oral absorption. Additionally, peptide-derived antagonists often show partial agonist rather than full antagonist activity.[8] Thus, although NPY_{18-36} is a pure antagonist at Y_3-like NPY receptor populations, it maintains partial agonist activity at Y_1-like, and full agonist potency at Y_2-like, NPY receptor populations.[7]

Clearly, an approach to overcome the above problems lies in the development of non-peptidal ligands. Recent work from our laboratory has shown that *N,N'*-bis-[6-[(2-methoxybenzyl)amino]hex-1-yl]cystamine, benextramine (BXT, **2**, Chart I), designed by Melchiorre and co-workers[9] as an α-adrenoceptor antagonist, produces a long-acting antagonism of NPY's pressor activity, and irreversibly inhibits [^{3}H]NPY binding to a subpopulation of NPY binding sites in rat brain membranes.[10] Subsequently, the activity of BXT in displacing [^{125}I]NPY from Y_2 receptors in porcine hippocampus was reported by Hexum and co-workers.[11] The only other non-peptide NPY antagonist known to date is He 90481 (**3**, Chart I),[12] a potent H_2-receptor agonist. However, the usefulness of these ligands as NPY antagonists is rather limited because of their low activity (micromolar range) and nonspecificity for NPY receptors. Nevertheless, an understanding of the molecular interactions between these antagonists and the NPY receptors will provide chemical insights into the BXT–NPY receptor interaction that can be applied towards the development of more selective and potent non-peptidergic NPY antagonists. The goal of our present investigation was to develop a more versatile procedure for the synthesis of BXT analogs, and to determine the importance of the outer benzylic groups of BXT on its displacement of [^{3}H]NPY from rat brain membranes.

Chemistry

Although a convenient method for the synthesis of benextramine and its analogs was reported by Melchiorre and co-workers in 1978,[9] we have developed a new methodology for the synthesis of these compounds. Unlike the method of Melchiorre, our method avoids the use of the highly toxic starting material thiirane for the introduction of the ethylene disulfide moiety. Instead, our method uses the readily available cystamine for this purpose. In addition, we envisioned that a synthetic methodology involving amide reduction would be more versatile in allowing the synthesis of BXT analogs in which the inner (i.e., cystamine) and outer (i.e., benzylic) amines could be incorporated selectively. In our synthetic protocol, the amino functionality in 6-aminohexanoic acid was first protected by a *tert*-butyloxycarbonyl (*t*-Boc) group following the method of Stewart and Young;[13] reaction of this protected amine with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) afforded the stable active ester **4**. As outlined in Scheme I, cystamine was then coupled with ester **4** in the presence of diiso-

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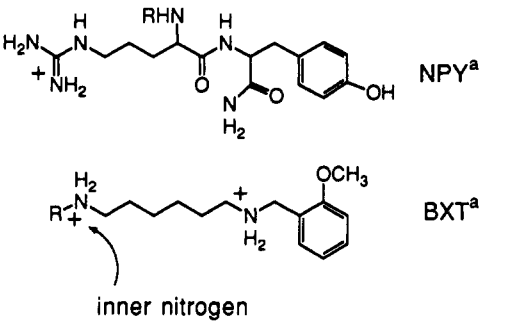
propylethylamine (DIEA) to give the diamide disulfide **5**. The *t*-Boc protecting groups were removed with 4 N HCl in dioxane to afford the intermediate diamine **6**. Alternatively, the *t*-Boc protecting groups can be removed in a 20-fold excess of 50% trifluoroacetic acid (TFA) in CH_2Cl_2, although the TFA salt must be converted to the free base prior to the next condensation. This diamine was then coupled to the *N*-hydroxysuccinimide esters **7a–f** (or the acid chloride of the appropriately substituted aromatic acid) affording the corresponding tetraamido disulfide derivatives **8a–h**. Where necessary, the active esters were synthesized by the reaction of the DCC-activated aromatic acid with *N*-hydroxysuccinimide. Our initial attempts to reduce the amido functionalities of **8a** with $LiAlH_4$ to the corresponding target compound **2** were not successful as the yield of the tetraamine was very low due primarily to the difficulties associated with purifying the desired product. However, reduction using diborane in THF in a manner analogous to the method of Brown and Heim[14] was found to be very clean, affording the desired amine in good yield, although the reaction time for this reduction was increased from 6 to 18 h to ensure complete reduction of the tetraamide. The diborane reduction was subsequently followed for the reduction of tetraamides **8b–h** to afford the corresponding tetraamines **9a–g**.[15] The hydroxy analogs **10a–c** were prepared by cleavage of the methyl group from the corresponding methoxybenextramine analog using 48% HBr following the literature method of Ueda and co-workers.[16] The benextramine analog lacking the benzyl moiety (i.e., compound **11**) was prepared by the direct diborane reduction of the diamide **6**. Overall yields of the BXT analogs using cystamine as starting material are comparable to, but not better than, yields reported by Melchiorre and co-workers.[9]

Results and Discussion

The importance of the C-terminal tail region (i.e., Arg^{33}-Gln^{34}-Arg^{35}-Tyr^{36}) of NPY in its binding to brain and peripheral NPY receptors has been demonstrated in several laboratories.[17,18] We noticed some striking similarities between the non-peptide BXT molecule and the C-terminal tail region of NPY; for example (a) they are both extended, polycationic structures containing an aromatic ring (phenolic moiety of Tyr^{36} in the case of NPY and methoxybenzyl moiety in the case of BXT), and (b) the distance between the positively charged inner N-atom and the phenyl group in BXT is 8 atoms, the same as that between the positively charged guanidinium group of Arg^{35}



[a] R = rest of the structure in each case.

Figure 1. Hypothetical structural relationships between benextramine's inner nitrogen and phenyl ring and NPY's Arg^{35} guanidinium group and Tyr^{36} phenolic ring. The inner (i.e., cystamine) nitrogen of BXT is identified by an arrow.

and the phenolic moiety of Tyr^{36} in NPY (see also Figure 1 for this hypothetical structural relationship). These chemical similarities between BXT and the C-terminal region of NPY raised the possibility of common hydrophobic and ionic interactions at the NPY receptors for these chemically different classes of molecules, and thereby the importance of the benzylic moiety of BXT for its activity at the NPY benextramine-sensitive binding sites.

As mentioned above, BXT has a rather low activity (57 μM) at the NPY receptor. Nevertheless, it provided us with a "lead" for further structural modifications. In this preliminary structure–activity study we investigated the effects of the following manipulations of the aromatic ring of BXT with respect to its activity at the BXT-sensitive rat brain NPY binding sites: (a) the effect of truncating the aromatic moiety, (b) the effect of electronic modifications (i.e., the addition of electron withdrawing or electron donating groups), and (c) the effect of increasing steric bulk. In addition, we took advantage of the fact that each of these modifications substantially decreases BXT's adrenergic activity.[9,19,20]

The IC_{50} for each compound in the displacement of specifically bound 1 nM [^{3}H]NPY from benextramine-sensitive rat brain binding sites as calculated from nonlinear regression analysis of the concentration-dependent [^{3}H]NPY displacement data, and the activity of each compound relative to benextramine at both rat brain NPY binding sites and α-adrenoceptors, are compiled in Table I. The selectivity of these compounds at the benextramine-sensitive versus insensitive NPY rat brain binding sites was analyzed using a modification of the "paired tube" assay.[21] The results for NPY, **9f**, and **9g** are given in Figure 2. NPY, a nonselective ligand, displaced approximately 90% of specifically bound [^{3}H]NPY from both the "native" and 500 μM benextramine-treated membrane preparations at a concentration of 5 times its IC_{50} (i.e., 10 nM). However, although **9f** and **9g** significantly displaced approximately 60% of [^{3}H]NPY specifically bound to "native" membrane preparations at concentrations equal to 10 times their respective IC_{50}'s, they did not significantly displace

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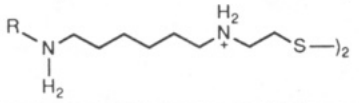
Table I. Comparison of [^{3}H]NPY Inhibitory Activity of Benextramine Analogs with That of Benextramine, and with Their Corresponding Relative α-Blockade Activity



compd	R	IC_{50}[a,b]	relative activity: NPY receptor[c]	relative activity: α-adrenoceptor[d]
10b	*m*-HOPhCH$_2$	20.3 ± 3.89[e]	2.8	0.24[f]
9a	*m*-MeOPhCH$_2$	21.5 ± 2.1[e]	2.7	0.72[h]
9f	2-naphthyl-CH$_2$	31.3 ± 2.9[e]	1.8	0.27[g]
9e	*p*-ClPhCH$_2$	37.8 ± 3.7	1.5	0.50[g]
9b	*p*-MeOPhCH$_2$	44.1 ± 6.08	1.3	0.81[h]
9d	*m*-ClPhCH$_2$	46.7 ± 5.52	1.2	0.46[f]
10a	*o*-HOPhCH$_2$	50.6 ± 14.6	1.1	0.36[f]
2	*o*-MeOPhCH$_2$	57.6 ± 7.1	1.0	1.00
9c	*o*-ClPhCH$_2$	79.9 ± 15.5	0.7	0.44[f]
10c	*p*-HOPhCH$_2$	161.5 ± 21[e]	0.4	0.14[h]
9g	PhCH$_2$	191 ± 21[e]	0.3	0.90[f]
11	H	>1400[e]	<0.04	0.27[h]

[a] The IC_{50} ± SE represents the concentration (in μM) producing half-maximal displacement of [^{3}H]NPY specific binding from benextramine-sensitive NPY binding sites in rat brain membranes. [b] Inactive compounds include tolazoline, phenoxybenzamine, prazosin, cystine, glutathione disulfide, and cystamine. [c] Relative potency in displacing 1 nM [^{3}H]NPY from benextramine-sensitive rat brain NPY sites with respect to BXT, calculated by dividing the IC_{50} of BXT by the IC_{50} of each compound. [d] Relative potency of each analog at α-adrenoceptors relative to that of benextramine as measured by its relative ability to antagonize the effect of epinephrine on the contraction of the electrically stimulated rat vas deferens. [e] IC_{50} statistically different from benextramine's IC_{50} (paired *t*-test); $p < 0.05$. [f] Data from ref 22. [g] Data from ref 19. [h] Data from ref 9.

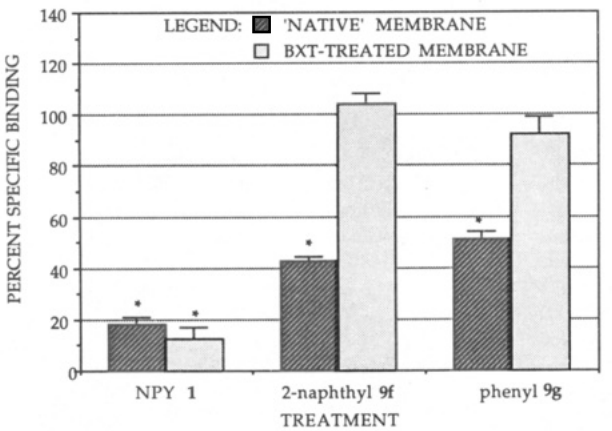


Figure 2. Selectivity profiles for NPY, **9f**, and **9g** in the displacement of 1 nM [^{3}H]NPY from native and 500 μM benextramine-treated rat brain membrane preparations. The data are presented as % specific binding in the presence of 10 nM NPY, 313 μM **9f**, and 1.9 mM **9g** relative to the control assays performed in the absence of compound. The binding data represent the average of at least triplicate determinations (error bars represent standard error of the mean). In these membrane preparations the benextramine-sensitive binding site population represents about 60% of the total binding site population. Nonspecific binding in the benextramine-treated and native membrane preparations are not statistically different. (*) Specific binding in the presence of compound statistically different from specific binding in corresponding control assays (paired *t*-test); $p < 0.05$.

[^{3}H]NPY from the benextramine-treated membranes at an identical concentration. These results demonstrate that in the concentration range studied these benextramine analogs, but not NPY, are selective for the benextramine-sensitive population (about 60% of the total binding sites) observed in the rat brain membranes, and they are unable to significantly displace [^{3}H]NPY from the benextramine-insensitive sites.

As might have been predicated from the possible common hydrophobic interactions for NPY and BXT (see above), analog **11** lacking the aromatic rings did not exhibit any appreciable activity at rat brain NPY binding sites, indicating the necessity of the aromatic moiety of BXT for its activity at the benextramine-sensitive rat brain NPY binding sites. Additionally, this finding also suggests that the disulfide moiety of BXT is not sufficient in itself to explain BXT's NPY antagonistic activity. In this regard, we have also tested the activities of cystamine, glutathione disulfide, and cystine, and none of these disulfides have observable in vitro activity in displacing [^{3}H]NPY from rat brain membranes.

With regards to the ring-substitution pattern, removing the *o*-methoxy substituent (i.e., compound **9g**) significantly reduced activity by 3-fold, whereas switching the electron donating methoxy group from the ortho position in BXT to the para position as in compound **9b** had no effect on BXT's [^{3}H]NPY displacement activity. However, a similar switch from the ortho to meta position affording compound **9a** resulted in a significant 2.7-fold increase in activity. In the case of the electron withdrawing chloro group, the meta or para regioisomers (i.e., compounds **9d** and **9e**, respectively) appear to be tolerated as compared to BXT. However, substitution of the *o*-methoxy group (in BXT) by its chloro counterpart affording compound **9c** resulted in a slight (but not significant) decrease in activity. Further, the *m*-hydroxy analog (i.e., **10b**) was as active as its *m*-methoxy counterpart **9a**, whereas the *o*-hydroxy analog (i.e., **10a**) exhibited an activity identical to that of BXT. Interestingly, the *p*-hydroxy analog (i.e., compound **10c**) exhibited a significantly lower activity compared to both its *p*-methoxy counterpart and to BXT itself (also see below). Some early trends that emerge from these structural studies of BXT vis-a-vis NPY's benextramine-sensitive binding sites are as follows: (1) an aromatic moiety is required for the interaction of BXT with NPY binding sites that corresponds to the benzylic moiety of BXT, (2) either an ion–dipole or a dipole–dipole interaction appears to be favored between the oxygen moiety at the meta position of BXT and its counterpart at the NPY binding sites, and this interaction, if any, is of the acceptor type, as suggested by an identical activity of the *m*-hydroxy versus the *m*-methoxy analog (i.e., compounds **9a** and **10b**, respectively), and/or the hydrophobic area on NPY sites that binds the aromatic moiety of BXT appears to favor an electron rich environment as indicated by a significant 2- to 3-fold increase in activity of the *m*-hydroxy and *m*-methoxy analogs over that of the corresponding *m*-chloro analog, and (3) bulk on the ortho position of the aromatic rings of BXT appears to be less favorable compared to that on the meta position (e.g., compare the IC_{50} of the *o*-Cl analog **9c** versus the *m*-Cl analog **9d** and that of the *m*-CH_3O analog **9a** vs BXT).

In accordance with the possibility of a common hydrophobic interaction between the phenolic moiety of Tyr^{36} in NPY and the phenyl moiety of BXT, we first visualized that a *p*-hydroxy substituent on the phenyl ring resulting in compound **10c** would better mimic the tyrosine moiety of NPY, and should therefore exhibit better activity compared to its *o*- or *m*-OH analogs (i.e., compounds **10a** and **10b**, respectively). In contrast to this notion, **10c**

exhibited more than a 3- and 7-fold lower activity than its *o*- and *m*-analogs, respectively. We are unable to explain this anomaly, which in part, may be due to a different mode of binding of BXT and NPY at the NPY receptors. Nevertheless, in view of the fact that the *p*-methoxy analog (i.e., compound **9b**) showed equal activity as that of BXT and 4-fold higher activity than the phenyl analog (i.e., **9g**), there appears to be a hydrophobic interaction at the NPY benextramine-sensitive sites that corresponds to the para position of the aromatic moiety of BXT. This is further substantiated by a significant 3-fold decrease in activity on replacement of the *p*-methoxy in **9b** by its *p*-OH counterpart (i.e., **10c**).

As mentioned above, the preliminary results suggest regions of bulk tolerance on NPY binding sites that corresponds to the meta and para positions of the benzylic moiety of BXT. In order to gain further insight into this hydrophobic region, synthesis and pharmacological evaluation of the 2-naphthyl tetraamine disulfide (2-naphthyl-BXT, **9f**) was undertaken. Although, the activity of this compound was found to be only 2-fold higher than that of BXT (Table I), the result substantiates the presence of a large hydrophobic region on NPY binding sites that corresponds to the phenyl moiety of BXT.

Another important finding of this investigation is the fact that except for the *o*-chloro and *p*-hydroxy analogs of BXT (i.e., compounds **9c** and **10c**, respectively), the *o*-, *m*-, and *p*-substituted analogs as well as the 2-naphthyl-BXT exhibit either an equal or higher potency than BXT in inhibiting [^{3}H]NPY specific binding to benextramine-sensitive sites. However, all of these *o*-, *m*-, and *p*-methoxy/chloro/hydroxy-substituted BXT analogs and 2-naphthyl-BXT (i.e., compound **9a–f**, **10a–c**) are significantly less active than BXT in their respective α-blockade activity (see Table I), and the analogs of BXT lacking the benzyl groups are inactive at [^{3}H]NPY binding sites but are active at the α-adrenoceptor.[9,19,22] This suggests that the binding site for BXT on the NPY receptor is distinct from that on α-adrenoceptors. This is further supported by the observation that α-adrenoceptor antagonists (such as tolazoline, phenoxybenzamine, and prazosin) do not exhibit any significant displacement of [^{3}H]NPY in these rat brain membrane assays.

In summary, our investigation reveals (a) the necessity of the aromatic moiety of BXT for its activity at the NPY binding sites, (b) a comparatively large hydrophobic region, perhaps, in association with an electron-rich character on the NPY binding sites vis-a-vis the aromatic moiety of BXT, and (c) a distinct binding site for BXT on NPY receptors as compared to its binding site on α-adrenoceptors. These findings deserve due consideration in future development of BXT analogs as non-peptidergic NPY receptor antagonists. It is of importance to be mentioned here that 2-naphthyl-BXT (**9f**) exhibits only 27% adrenergic activity as compared to BXT. Taking advantage of this fact, we are currently investigating further structural modification of 2-naphthyl-BXT, particularly with regards to the importance of the inner (i.e., cystamine) and outer (i.e., benzylic) amines and the disulfide bond, hopefully leading to more potent and selective NPY antagonists.

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Experimental Section

Krebs-Ringer bicarbonate buffer, bacitracin, and BSA were purchased from Sigma Chemical Co. (St. Louis, MO), *N*-[*propionyl*-^{3}H]neuropeptide Y ([^{3}H]NPY) was purchased from Amersham (Arlington Heights, IL), and all other solvents and reagents were obtained commercially and used without further purification unless otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Brucker AM 500, QE 300, or a Varian XL 300 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. IR spectra were recorded on a Bowmem Fourier transform spectrometer. Positive-ion fast atom bombardment mass spectra (FABMS) were obtained on a VG Analytical ZAB spectrometer with glycerol as matrix. Microanalyses were performed on a Hewlett-Packard Model 185B CHN analyzer at the University of Kansas by Mr. Nguyen. Methylene chloride was dried by distillation over phosphorus pentoxide. THF was dried by distillation over sodium. Analytical TLC was performed on 0.25-mm silica gel plates (Sigma T6270). Flash column chromatography was carried out with silica gel (Merck, grade 60, 230–400 mesh).

6-[(*tert*-Butyloxycarbonyl)amino]hexanoic Acid *N*-Hydroxysuccinimide Ester (4). 6-(*t*-Boc-amino)hexanoic acid was prepared by dropwise addition of di-*tert*-butyl dicarbonate (18.3 g, 83.8 mmol) in *t*-BuOH (100 mL) to a stirred solution of 6-aminohexanoic acid (10.0 g, 76.2 mmol) in *t*-BuOH (80 mL) and NaOH (3 g dissolved in 8 mL water). An additional 100 mL of *t*-BuOH was added for smooth stirring. After allowing the reaction mixture to stir for 12 h, the resultant turbid product was diluted with water (40 mL) and extracted with hexane (3 × 60 mL). The aqueous phase was cooled to 0–5 °C, ethyl acetate (60 mL) was added, and the reaction mixture was acidified with 1 N H_2SO_4 to pH 2. The aqueous phase was separated and extracted with ethyl acetate (3 × 50 mL). The combined organic phase was washed with water (3 × 40 mL) and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded 15.7 g (89%) of **3** as a pale yellow viscous oil: IR (Nujol) 3669–3227 (COOH, NH), 1710 (CO), 1699 (CONH) cm^{-1}; NMR ($CDCl_3$) δ 10.42 (br s, 1 H, COOH), 4.63 (br s, 1 H, CONH), 3.14–3.12 (m, 2 H, CH_2N), 2.33 (t, J = 7.8 Hz, 2 H, $COCH_2$), 1.71–1.61 (m, 2 H, CH_2), 1.56–1.24 (m, 13 H, CH_2, CH_2 and $C(CH_3)_3$).

The activated ester **4** was prepared by dropwise addition of a solution of DCC (3.6 g, 17.5 mmol) in methylene chloride (100 mL) to a stirred solution of 6-(*t*-Boc-amino)hexanoic acid (3.7 g, 15.9 mmol) and *N*-hydroxysuccinimide (2 g, 17.5 mmol) in methylene chloride (90 mL) at 0–5 °C. The reaction mixture was allowed to stir for 1 h at 0–5 °C and then placed in the refrigerator at 0 °C for 12 h. The precipitated material was removed by filtration, and the filtrate was evaporated under reduced pressure to give a white amorphous residue. Recrystallization of the crude product from ether–hexane afforded 3.6 g (69%) of **4** as white shining flakes: mp 87–89 °C; IR (KBr) 3389 (NH), 1826 (CO), 1789 (CO), 1694 (CONH), 1214 (COC) cm^{-1}; NMR ($CDCl_3$) δ 4.62 (br s, 1 H, CONH), 3.13–3.11 (m, 2 H, CH_2N), 2.83 (s, 4 H, $COCH_2CH_2CO$), 2.61 (t, J = 7.3 Hz, 2 H, $COCH_2$), 1.79–1.64 (m, 2 H, CH_2), 1.49–1.43 (m, 4 H, CH_2, CH_2), 1.41 (s, 9 H, $C(CH_3)_3$).

***N*,*N*′-Bis[6-[(*tert*-butyloxycarbonyl)amino]hexanoyl]cystamine (5).** A solution of **4** (4.3 g, 13.1 mmol) in methylene chloride (40 mL) was added dropwise to a stirred suspension of cystamine dihydrochloride (1.0 g, 6.6 mmol) in methylene chloride (40 mL) and DIEA (4.5 mL, 26.3 mmol) under a N_2 atmosphere. After allowing the reaction mixture to stir for 6 h, the precipitated solid was removed by filtration, and the filtrate was washed successively with 0.5 N citric acid (3 × 60 mL), water (2 × 60 mL), a saturated solution of sodium bicarbonate (3 × 40 mL), and water (2 × 40 mL), and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave a viscous oil. After triturating the oil with hexane (100 mL) for 5 h, the resulting solid was collected by filtration and washed with hexane to give a crude amorphous product (mp 72–75 °C). Recrystallization of the crude from 2-propanol–anhydrous ether–hexane afforded 2.5 g (66%) of **5** as a white solid: mp 76–80 °C; IR (KBr) 1818 (CO), 1697 (CONH) cm^{-1}; NMR ($CDCl_3$) δ 6.59

(br s, 2 H, CONH), 4.71 (br s, 2 H, CONH), 3.60–3.53 (m, 4 H, CH_2N), 3.12–3.07 (m, 4 H, CH_2N), 2.85 (t, J = 7.3 Hz, 4 H, $COCH_2$), 2.67 (t, J = 6.8 Hz, 4 H, CH_2), 1.68–1.61 (m, 4 H, CH_2), 1.53–1.30 (m, 8 H, CH_2, CH_2), 1.44 (s, 18 H, $C(CH_3)_3$).

N,N'-Bis(6-aminohexanoyl)cystamine Dihydrochloride (6). A solution of 4 N HCl in dioxane (10 mL) was added at once to a suspension of **5** (0.99 g, 1.71 mmol) in anhydrous ether (30 mL) under a N_2 atmosphere, and the resulting mixture was allowed to stir for 50 min. The precipitated hydrochloride salt was collected by filtration, triturated with anhydrous ether (2 × 100 mL), and filtered to obtain a white solid product. After removing the traces of HCl gas from this solid under high vacuum, 0.42 g (53%) of **6** was obtained as a white amorphous hygroscopic solid. The product was used for the next step without further purification: IR (KBr) 3324 (NH), 1695 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.21–7.80 (m, 6 H, D_2O exchangeable, NH_3^+), 5.05 (br s, 2 H, CONH), 3.33–3.29 (m, 4 H, CH_2N), 2.78–2.73 (m, 4 H, CH_2N), 2.65 (t, J = 6.9 Hz, 4 H, CH_2), 2.09 (t, J = 6.8 Hz, 4 H, $COCH_2$), 1.56–1.48 (m, 8 H, CH_2, CH_2), 1.31–1.26 (m, 4 H, CH_2).

2-Methoxybenzoic Acid N-Hydroxysuccinimide Ester (7a). The ester was prepared from 2-methoxybenzoic acid using the same procedure as described for the synthesis of **4**. Recrystallization of the crude product from methylene chloride–hexane afforded 11.0 g (68%) of **7a** as white crystals: mp 178–180 °C (lit.[23] mp 82–84 °C); IR (KBr) 1800, 1775 (CO), 1718 (CO) cm^{-1}.

3-Methoxybenzoic Acid N-Hydroxysuccinimide Ester (7b). The ester was obtained from 3-methoxybenzoic acid using the same procedure as described above for the synthesis of **4**. Recrystallization of the crude material from methylene chloride–hexane afforded 6 g (72%) of **7b**: mp 98–99 °C (lit.[23] mp 95–96 °C); IR (KBr) 1802, 1775 (CO), 1739 (CO) cm^{-1}.

4-Methoxybenzoic Acid N-Hydroxysuccinimide Ester (7c). The ester was prepared from 4-methoxybenzoic acid using the same procedure as described above for the synthesis of **4**. Recrystallization of the crude solid from methylene chloride–2-propanol afforded 2.7 g (81%) of **7c**: mp 144–146 °C (lit.[23] mp 141–142 °C); IR (KBr) 1792, 1776 (CO), 1719 (CO) cm^{-1}.

2-Chlorobenzoic Acid N-Hydroxysuccinimide Ester (7d). The ester was prepared from 2-chlorobenzoic acid using the same procedure as described above for the synthesis of **4** in 74% yield: mp 172–174 °C (methylene chloride–hexane, lit.[23] mp 174 °C); IR (KBr) 1804, 1777 (CO), 1746 (CO) cm^{-1}.

4-Chlorobenzoic Acid N-Hydroxysuccinimide Ester (7e). The ester was prepared from 4-chlorobenzoic acid in 70% yield using the same procedure as described above for the synthesis of **4**: mp 205–207 °C (methylene chloride–hexane, lit.[23] mp 123–125 °C); IR (KBr) 1818, 1799 (CO), 1755 (CO) cm^{-1}.

2-Naphthoic Acid N-Hydroxysuccinimide Ester (7f). The ester was prepared from 2-naphthoic acid using the same procedure as described above for the synthesis of **4**. The crude product was recrystallized from 2-propanol–CH_2Cl_2–hexane to afford 3.5 g (74%) of **7f** as white crystals: mp 163–164 °C (lit.[23] mp 165 °C); IR (KBr) 1807, 1770 (CO), 1736 (CO) cm^{-1}.

General Method for the Preparation of the Tetraamido Disulfide Derivatives (8a–h) As Illustrated by N,N'-Bis[6-(2-methoxybenzamido)hexanoyl]cystamine (8a). A solution of **7a** (0.33 g, 1.32 mmol) in methylene chloride (7 mL) was added dropwise to a suspension of **6** (0.3 g, 0.66 mmol) in methylene chloride and DIEA (0.56 mL, 3.3 mmol) under a N_2 atmosphere. The reaction mixture was allowed to stir for 16 h and then washed in succession with 0.5 N citric acid (3 × 20 mL), water (3 × 20 mL), a saturated solution of $NaHCO_3$ (3 × 10 mL), and water (3 × 10 mL). The organic phase was dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give a viscous oily residue. Purification of the crude residue by flash chromatography (silica gel, 1 × 7 in., CH_2Cl_2–CH_3OH 9.6:0.4) gave 0.28 g (66%) of **8a** as a semisolid residue: IR (KBr) 3314 (NH), 1681 (CONH), 1660 (CONH) cm^{-1}; NMR ($CDCl_3$) δ 8.14 (d, J = 7.8 Hz, 2 H, ArH), 7.91 (t, J = 5.1 Hz, 2 H, CONH), 7.42 (t, J = 7.5 Hz, 2 H, ArH), 7.30–6.95 (m, 6 H, ArH and CONH), 3.95 (s, 6 H, OCH_3), 3.52–3.47 (m, 8 H, NCH_2, NCH_2), 2.79 (t, J = 6.3 Hz, 4 H, $COCH_2$), 2.23 (t, J = 6.0 Hz, 4 H, SCH_2), 1.73–1.57 (m, 8 H, CH_2, CH_2) and 1.45–1.37 (m, 4 H, CH_2).

N,N'-Bis[6-(3-methoxybenzamido)hexanoyl]cystamine (8b). This compound was prepared using the same procedure as described for **8a** by the condensation of **7b** (0.92 g, 3.7 mmol) with **6** (0.84 g, 1.8 mmol). The crude product obtained after workup was purified by flash chromatography (silica gel, 1 × 7 in., CH_2Cl_2–CH_3OH 9.6:0.4) to give 0.7 g (58%) of **8b** as white solid residue: mp 114–116 °C; IR (KBr) 3334 (NH), 1638 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.42 (t, J = 3.0 Hz, 2 H, CONH), 7.95 (t, J = 3.0 Hz, 2 H, CONH), 7.42–7.32 (m, 6 H, ArH), 7.08–7.02 (m, 2 H, ArH), 3.83 (s, 6 H, OCH_3), 3.35–3.20 (m, 8 H, NCH_2, NCH_2), 2.75 (t, J = 6.0 Hz, 4 H, $COCH_2$), 2.08 (t, J = 6.0 Hz, 4 H, SCH_2), 1.60–1.48 (m, 8 H, CH_2, CH_2) and 1.35–1.28 (m, 4 H, CH_2).

N,N'-Bis[6-(4-methoxybenzamido)hexanoyl]cystamine (8c). This compound was prepared by the condensation of **7c** with **6** following the general method described above for the synthesis of **8a** except that DMF was used instead of methylene chloride as solvent. Recrystallization of the crude product from ethyl acetate–hexane afforded 0.5 g (59%) of **8c** as a white solid: mp 171–174 °C; IR 3304 (NH), 1630 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.26 (t, 2 H, CONH), 7.95 (t, 2 H, CONH), 7.81 (d, J = 8.4 Hz, 4 H, ArH), 6.97 (d, J = 8.4 Hz, 4 H, ArH), 3.82 (s, 6 H, OCH_3), 3.36–3.25 (m, 8 H, NCH_2, NCH_2), 2.76 (t, J = 6.0 Hz, 4 H, $COCH_2$), 2.12 (t, J = 6.0 Hz, 4 H, SCH_2), 1.56–1.48 (m, 8 H, CH_2, CH_2), 1.27–1.23 (m, 4 H, CH_2).

N,N'-Bis[6-(2-chlorobenzamido)hexanoyl]cystamine (8d). This compound was prepared using the same procedure as described for **8a** by the condensation of **7d** (1.1 g, 4.4 mmol) with **6** (1.0 g, 2.2 mmol). Recrystallization of the crude product from ethyl acetate–hexane afforded 0.9 g (62%) of **8d** as a white solid: mp 124–127 °C; IR (KBr) 3287 (NH), 1642 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.38 (s, 2 H, CONH), 7.99 (s, 2 H, CONH), 7.45–7.38 (m, 8 H, ArH), 3.33–3.17 (m, 8 H, NCH_2, NCH_2), 2.76 (t, J = 6.0 Hz, 4 H, $COCH_2$), 2.06 (t, J = 6.3 Hz, 4 H, SCH_2), 1.55–1.48 (m, 8 H, CH_2, CH_2), 1.33–1.31 (m, 4 H, CH_2).

N,N'-Bis[6-(3-chlorobenzamido)hexanoyl]cystamine (8e). This compound was prepared using the same procedure as described for **8a** by the condensation of 3-chlorobenzoyl chloride (0.24 g, 1.4 mmol) with **6** (0.31 g, 0.68 mmol) in methylene chloride (40 mL) and DIEA (2 mL) under a N_2 atmosphere. The crude solid obtained after workup was recrystallized from ethyl acetate–methanol–hexane to give 0.27 g (61%) of **8e** as a white solid: mp 141–144 °C; IR (KBr) 3295 (NH), 1639 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.54 (t, J = 3.0 Hz, 2 H, CONH), 7.97 (t, J = 3.0 Hz, 2 H, CONH), 7.87 (s, 2 H, ArH), 7.79 (d, J = 7.6 Hz, 2 H, ArH), 7.56 (d, J = 8.4 Hz, 2 H, ArH), 7.47 (t, J = 7.8 Hz, 2 H, ArH), 3.35–3.21 (m, 8 H, NCH_2, NCH_2), 2.73 (t, J = 6.8 Hz, 4 H, $COCH_2$), 2.08 (t, J = 7.3 Hz, 4 H, SCH_2), 1.55–1.48 (m, 8 H, CH_2, CH_2), 1.34–1.26 (m, 4 H, CH_2).

N,N'-Bis[6-(4-chlorobenzamido)hexanoyl]cystamine (8f). This compound was prepared by the condensation of **7e** with **6** following the general method described above for the synthesis of **8a** except that DMF was used instead of methylene chloride as solvent. Recrystallization of the crude product from ethyl acetate–hexane afforded 1.2 g (55%) of **8f** as a white solid: mp 171–173 °C; IR (KBr) 3302 (NH), 1638 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.58 (t, J = 3.0 Hz, 2 H, CONH), 7.98 (t, J = 3.0 Hz, 2 H, CONH), 7.85 (d, J = 5.1 Hz, 4 H, ArH), 7.51 (d, J = 5.1 Hz, 4 H, ArH), 3.34–3.20 (m, 8 H, NCH_2, NCH_2), 2.74 (t, J = 4.0 Hz, 4 H, $COCH_2$), 2.07 (t, J = 4.5 Hz, 4 H, SCH_2), 1.53–1.48 (m, 8 H, CH_2, CH_2), 1.31–1.25 (m, 4 H, CH_2).

N,N'-Bis[6-(2-naphthylamido)hexanoyl]cystamine (8g). This compound was prepared using the same procedure as described for **8a** by the condensation of **7f** (0.72 g, 2.66 mmol) with **6** (0.61 g, 1.33 mmol). Recrystallization of the crude product from ethyl acetate–ether afforded 0.55 g (61%) of **8g** as white solid: mp 172–174 °C; IR 3298 (NH), 1637 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.61 (t, J = 3.0 Hz, 2 H, CONH), 8.42 (t, J = 3.0 Hz, 2 H, CONH), 8.01–7.90 (m, 8 H, ArH), 7.62–7.57 (m, 6 H, ArH), 3.42–3.30 (m, 8 H, NCH_2, NCH_2), 2.74 (t, J = 6.0 Hz, 4 H, $COCH_2$), 2.09 (t, J = 6.0 Hz, 4 H, S-CH_2), 1.60–1.52 (m, 8 H, CH_2, CH_2) and 1.36–1.28 (m, 4 H, CH_2).

N,N'-Bis(6-benzamidohexanoyl)cystamine (8h). This compound was prepared by using the same procedure as described

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for **8a** by the condensation of benzoyl chloride (0.24 g, 1.68 mmol) with **6** (0.37 g, 0.84 mmol). The crude product obtained after workup was purified by flash chromatography (silica gel, 1 × 6 in., CH_2Cl_2–CH_3OH 9.6:0.4) to give 0.3 g (61%) of **8h** as a semisolid residue: IR (KBr) 3334 (NH), 1652 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 8.45 (t, 2 H, CONH), 8.00 (t, 2 H, CONH), 7.82 (d, J = 8.1 Hz, 4 H, ArH), 7.49–7.42 (m, 6 H, ArH), 3.35–3.15 (m, 8 H, NCH_2, NCH_2), 2.75 (t, J = 6.7 Hz, 4 H, $COCH_2$), 2.07 (t, J = 7.3 Hz, 4 H, SCH_2), 1.53–1.46 (m, 8 H, CH_2, CH_2), 1.20–1.32 (m, 4 H, CH_2).

General Method for the Preparation of Tetraamine Disulfide Derivatives (2, 9a–g, 11) As Illustrated by *N,N'*-Bis[6-[(2-methoxybenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (2). Diborane (6.3 mL, 1 M solution in THF) was added dropwise to a suspension of **8a** (0.24 g, 0.37 mmol) in freshly distilled dry THF (10 mL) at 0–5 °C and under a N_2 atmosphere. The reaction mixture was heated at reflux for 18 h and cooled on an ice–water bath, and the excess of diborane was decomposed by careful addition of 6 M HCl (1 mL). THF was removed by distillation (oil bath, 70 °C), and the reaction mixture was saturated with careful addition of NaOH pellets at 0–5 °C. Air was bubbled through the resulting mixture for 30 min and then extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate portions were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give 0.15 g (69%) of **2** as its free base. The free base was dissolved in anhydrous ether and layered with hydrogen chloride gas to obtain the tetrahydrochloride salt as a fine precipitate. The precipitate was collected by filtration, washed with anhydrous ether, and recrystallized from absolute ethanol to afford 0.6 g of **2** as a white solid: mp 224–226 °C (lit.[9] mp 227–228 °C); IR (KBr) 3409 (NH), 1255 (COC) cm^{-1}; NMR (DMSO-d_6) δ 7.51 (d, J = 8.3 Hz, 2 H, ArH), 7.43 (t, J = 6.0 Hz, 2 H, ArH), 7.08 (d, J = 8.3 Hz, 2 H, ArH), 6.99 (t, J = 7.5 Hz, 2 H, ArH), 4.07 (t, J = 5.1 Hz, 4 H, $ArCH_2$), 3.85 (s, 6 H, OCH_3), 3.47 (t, J = 6.0 Hz, 4 H, CH_2), 3.26 (t, J = 6.0 Hz, 4 H, CH_2), 3.05 (t, J = 6.0 Hz, 4 H, CH_2), 2.99–1.95 (m, 4 H, CH_2), 1.73–1.52 (m, 8 H, CH_2, CH_2) and 1.45–1.30 (m, 8 H, CH_2, CH_2); FABMS calcd for $C_{32}H_{54}N_4O_2S_2$ m/z 591 (MH^+), found 591.

***N,N'*-Bis[6-[(3-methoxybenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (9a).** This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of **8b** (0.45 g, 0.7 mmol) in 90% yield (free base). The free base was dissolved in absolute alcohol and converted to its tetrahydrochloride salt by the dropwise addition of a solution of hydrogen chloride in anhydrous ether. The precipitate obtained was collected by filtration, washed with anhydrous ether, and recrystallized from methanol–ether to afford 0.18 g of **9a** as a white solid: mp 256–258 °C (lit.[9] mp 259–259.5 °C); IR (KBr) 3432 (NH), 1254 (COC) cm^{-1}; NMR (D_2O) δ 7.46 (t, J = 7.8 Hz, 2 H, ArH), 7.13–7.10 (m, 6 H, ArH), 4.23 (s, 4 H, $ArCH_2$), 3.88 (s, 6 H, OCH_3), 3.43 (t, J = 6.6 Hz, 4 H, CH_2), 3.13–3.03 (m, 12 H, CH_2, CH_2, CH_2), 1.77–1.68 (m, 8 H, CH_2, CH_2) and 1.44–1.40 (m, 8 H, CH_2, CH_2).

***N,N'*-Bis[6-[(4-methoxybenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (9b).** This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of **8c** (0.45 g, 0.69 mmol) in 73% yield (free base). The free base was converted to its tetrahydrochloride salt. The crude salt was recrystallized from ethanol–water to afford 0.12 g of **9b** as a white solid: mp 292–294 °C (lit.[9] mp 298–298.5 °C); IR (KBr) 3447 (NH), 1254 (COC) cm^{-1}; NMR (D_2O) δ 7.42 (d, J = 8.5 Hz, 4 H, ArH), 7.06 (d, J = 8.5 Hz, 4 H, ArH), 4.18 (s, 4 H, $ArCH_2$), 3.68 (s, 6 H, OCH_3), 3.43 (t, J = 6.7 Hz, 4 H, CH_2), 3.11–3.01 (m, 12 H, CH_2, CH_2, CH_2), 1.77–1.59 (m, 8 H, CH_2, CH_2), 1.51–1.31 (m, 8 H, CH_2, CH_2); FABMS calcd. for $C_{32}H_{54}N_4O_2S_2$ m/z 591 (MH^+), found 591.

***N,N'*-Bis[6-[(2-chlorobenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (9c).** This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of **8d** (0.6 g, 0.9 mmol) to give the free base as an oil. The free base was dissolved in absolute ethanol and layered with hydrogen chloride gas to obtain the tetrahydrochloride salt as fine precipitate. The precipitate was collected by filtration, washed with anhydrous ether, and recrystallized from methanol–ether to afford 0.3 g (44%) of **9c** as a white solid: mp 243–245 °C (lit.[9] mp 241–242 °C); IR (KBr) 3419 (NH) cm^{-1}; NMR (D_2O) δ 7.61–7.42 (m, 8 H, ArH), 4.42 (s, 4 H, $ArCH_2$), 3.46 (t, J = 6.6 Hz, 4 H, CH_2), 3.18–3.04 (m, 12 H, CH_2, CH_2, CH_2), 1.82–1.69 (m, 8 H, CH_2, CH_2) and 1.48–1.17 (m, 8 H, CH_2, CH_2); FABMS calcd for $C_{30}H_{48}Cl_2N_4S_2$ m/z 599 (MH^+), found 599. Anal. ($C_{30}H_{48}Cl_2N_4S_2$·4HCl) C, H, N.

***N,N'*-Bis[6-[(3-chlorobenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (9d).** This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of **8e** (0.2 g, 0.3 mmol) in 85% yield as its free base. The free base was dissolved in absolute ethanol and layered with hydrogen chloride gas to obtain the tetrahydrochloride salt as fine precipitate. The precipitate was collected by filtration, washed with anhydrous ether, and recrystallized from methanol–ether to afford 0.1 g of **9d** as a white solid: mp 274–276 °C (lit.[9] mp 280–281 °C); IR (KBr) 3432 (NH) cm^{-1}; NMR (D_2O) δ 7.48–7.37 (m, 8 H, ArH), 4.19 (s, 4 H, $ArCH_2$), 3.40 (t, J = 6.3 Hz, 4 H, CH_2), 3.08–2.92 (m, 12 H, CH_2, CH_2, CH_2), 1.71–1.55 (m, 8 H, CH_2, CH_2), 1.42–1.32 (m, 8 H, CH_2, CH_2) cm^{-1}; FABMS calcd for $C_{30}H_{48}Cl_2N_4S_2$ m/z 599 (MH^+), found 599.

***N,N'*-Bis[6-[(4-chlorobenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (9e).** This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of **8f** (0.46 g, 0.70 mmol) in 71% yield (free base). The free base was dissolved in absolute ethanol and layered with hydrogen chloride gas to obtain the tetrahydrochloride salt as fine precipitate. The precipitate was collected by filtration, washed with anhydrous ether, and recrystallized from methanol–water to afford 0.18 g of **9e** as a white solid: mp 292–294 °C (lit.[9] mp 295–296 °C); IR (KBr) 3550 (NH) cm^{-1}; NMR (free base) ($CDCl_3$) δ 7.28 (d, J = 8.4 Hz, 4 H, ArH), 7.23 (d, J = 8.4 Hz, 4 H, ArH), 3.74 (s, 4 H, $ArCH_2$), 3.66–3.50 (m, 4 H, NH, NH), 2.93–2.89 (m, 4 H, CH_2), 2.81–2.78 (m, 4 H, CH_2), 2.61–2.56 (m, 8 H, CH_2, CH_2), 1.73–1.65 (m, 8 H, CH_2, CH_2), 1.42–1.32 (m, 8 H, CH_2, CH_2); FABMS calcd for $C_{30}H_{48}Cl_2N_4S_2$ m/z 599 (MH^+), found 599.

***N,N'*-Bis[6-(2-naphthylamino)hex-1-yl]cystamine Tetrahydrochloride (9f).** This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of **8g** (0.4 g, 0.6 mmol). The free base was dissolved in absolute ethanol and layered with hydrogen chloride gas to obtain the tetrahydrochloride salt as a fine precipitate. The precipitate was collected by filtration, washed with anhydrous ether, and recrystallized from water–ether to afford 0.3 g (64%) of **9f** as a white solid: mp 290–292 °C (lit.[9] mp 291–293 °C); IR (KBr) 3426 (NH); NMR (D_2O) δ 8.12–7.90 (m, 8 H, ArH), 7.65–7.57 (m, 6 H, ArH), 4.25 (s, 4 H, $ArCH_2$), 3.24–2.95 (m, 16 H, CH_2, CH_2, CH_2, CH_2), 1.80–1.55 (m, 8 H, CH_2, CH_2), 1.40–1.25 (m, 8 H, CH_2, CH_2); FABMS calcd for $C_{38}H_{54}N_4S_2$ m/z 631 (MH^+), found 631. Anal. ($C_{38}H_{54}N_4S_2$·4HCl·0.25H_2O) C, H, N.

***N,N'*-Bis[6-(benzylamino)hex-1-yl]cystamine Tetrahydrochloride (9g).** This tetraamine disulfide was prepared by using the same procedure as described for the synthesis of **2** by the reduction of **8h** (0.25 g, 0.43 mmol) in 85% yield (free base). The free base was dissolved in absolute alcohol and converted to its tetrahydrochloride salt by the dropwise addition of a solution of hydrogen chloride in anhydrous ether. The precipitate obtained was collected by filtration, washed with anhydrou ether, and recrystallized from methanol–ether to afford 0.18 g of **9g** as a white solid: mp 286–289 °C (lit.[9] mp 287–288 °C); IR (KBr) 3400 (NH) cm^{-1}; NMR (D_2O) δ 7.55–7.40 (m, 10 H, ArH), 4.28 (s, 4 H, $ArCH_2$), 3.48–3.32 (m, 4 H, CH_2), 3.10–2.98 (m, 12 H, CH_2, CH_2, CH_2), 1.60–1.50 (m, 8 H, CH_2, CH_2), 1.48–1.38 (m, 8 H, CH_2, CH_2).

General Method for the Preparation of Hydroxy-Substituted Tetraamine Disulfide Derivatives (10a–c) As Illustrated by *N,N'*-Bis[6-[(2-hydroxybenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (10a). A mixture of *N,N'*-bis[6-[(2-methoxybenzyl)amino]hex-1-yl]cystamine tetrahydrochloride (**2**, 0.17 g, 0.3 mmol) and hydrogen bromide (48%, 4 mL) was heated at reflux for 4 h. After allowing to cool to room temperature, water (4 mL) was added and the solution was made basic with 12 N NH_4OH to pH 12. The free amine was extracted with ethyl acetate (3 × 20 mL) and the combined organic phase was dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give 0.14 g (83%) of the product as its free base. The amine was converted to its tetrahydrochloride salt, and the crude

salt was recrystallized from methanol–ether to give 0.08 g of the desired product as a white crystal: mp 201–203 °C (lit.[22] mp 198–199 °C); IR (KBr) 3417 (NH) cm^{-1}; NMR (DMSO-d_6) δ 10.22 (br s, 2 H, ArOH), 9.30 (br s, 4 H, NH_2^+), 8.94 (br s, 4 H, NH_2^+), 7.41 (d, J = 8.2 Hz, 2 H, ArH), 7.23 (t, J = 8.3 Hz, 2 H, ArH), 6.97 (d, J = 8.3 Hz, 2 H, ArH), 6.85 (t, J = 7.4 Hz, 2 H, ArH), 4.06 (t, J = 6.0 Hz, 4 H, $ArCH_2$), 3.21 (t, J = 6.8 Hz, 4 H, CH_2), 2.99–2.84 (m, 12 H, CH_2, CH_2, CH_2), 1.65–1.52 (m, 8 H, CH_2, CH_2), 1.32–1.22 (m, 8 H, CH_2, CH_2).

N,N′-Bis[6-[(3-hydroxybenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (10b). This tetraamine disulfide was obtained from **9a** (0.09 g, 0.15 mmol) in 80% yield (free base) following the above method. The amine was converted to its tetrahydrochloride salt. The crude salt was recrystallized from methanol–ether to obtain 0.06 g of the desired product: mp 195–197 °C (lit.[22] mp 194–195 °C); IR (KBr) 3454 (NH) cm^{-1}; NMR (DMSO-d_6) δ 9.65 (br s, 2 H, ArOH), 8.70 (br s, 8 H, NH_2^+, NH_2^+), 7.34 (d, J = 7.5 Hz, 2 H, ArH), 6.97–6.80 (m, 6 H, ArH), 4.05 (t, J = 6.0 Hz, 4 H, $ArCH_2$), 3.26 (t, J = 6.0 Hz, 4 H, CH_2), 3.13–3.05 (m, 4 H, CH_2), 3.02–2.86 (m, 8 H, CH_2, CH_2), 1.70–1.62 (m, 8 H, CH_2, CH_2), 1.38–1.28 (m, 8 H, CH_2, CH_2).

N,N′-Bis[6-[(4-hydroxybenzyl)amino]hex-1-yl]cystamine Tetrahydrochloride (10c). This tetraamine disulfide was prepared following the above method from **9b** (0.37 g, 0.63 mmol) in 86% yield as its free base. The amine was converted to its tetrahydrochloride salt. The crude salt was recrystallized from methanol–ether to give 0.14 g of the desired product as a white crystal: mp 208–210 °C (lit.[22] mp 210–211 °C); IR (KBr) 3395 (NH) cm^{-1}; NMR (DMSO-d_6) δ 9.70 (br s, 2 H, ArOH), 9.23 (br s, 4 H, NH_2^+), 9.06 (br s, 4 H, NH_2^+), 7.34 (d, J = 9.0 Hz, 4 H, ArH), 6.79 (d, J = 9.0 Hz, 4 H, ArH), 3.98 (t, J = 6.0 Hz, 4 H, $ArCH_2$), 3.26–3.10 (m, 8 H, CH_2, CH_2), 2.92–2.78 (m, 8 H, CH_2, CH_2), 1.70–1.58 (m, 8 H, CH_2, CH_2) and 1.40–1.30 (m, 8 H, CH_2, CH_2); FABMS calcd for $C_{30}H_{50}N_4O_2S_2$ m/z 563 (M + H^+), found 563.

N,N′-Bis(6-aminohex-1-yl)cystamine Dihydrochloride (11). This tetraamine disulfide was prepared using the same procedure as described for the synthesis of **2** by the reduction of the free base of **6** (0.3 g, 0.66 mmol). The amine obtained was dissolved in absolute ethanol and layered with hydrogen chloride gas to obtain the tetrahydrochloride salt as fine precipitate. The precipitate was collected by filtration, washed with anhydrous ether, and recrystallized from methanol–ether to afford 0.17 g (52%) of **11** as a white solid: mp 273–274 °C (lit.[9] mp 271–271.5 °C); IR (KBr) 3502 (NH) cm^{-1}; NMR (DMSO-d_6) δ 9.14 (br s, 6 H, D_2O exchangeable, 2 NH_3^+), 8.08 (br s, 4 H, D_2O exchangeable, 2 NH_2^+), 3.41 (t, J = 5.85 Hz, 4 H, CH_2), 3.12–2.70 (m, 6 H, CH_2, CH_2, CH_2), 1.63–1.34 (m, 8 H, CH_2, CH_2, CH_2, CH_2).

Radioligand Binding Assays. Rat brain membranes from male Sprague–Dawley rats (250–300 g) were prepared by a previously reported method.[10] Membrane homogenates (1.2–1.6 mg protein/mL) were incubated with 1.0 nM [^{3}H]NPY in the presence of 10^{-3} to 10^{-7} M benextramine analog in 500 μL of assay buffer (Krebs-Ringer bicarbonate buffer containing 50 mM phosphate, 0.1% BSA, 0.05% bacitracin, pH 7.4) at 25 °C for 90 min in siliconized polyethylene culture tubes; controls were incubated similarly with [^{3}H]NPY in the absence (total binding) and presence (nonspecific binding) of 1 μM NPY. After incubation, the membranes were collected by filtration of the assay mixture through siliconized, polyethylenimine-pretreated Whatman GF/A glass microfiber filters, and the filters were washed with 3 × 3 mL of assay buffer. The filters were dried at 55 °C for 90 min, and the radioactivity remaining on the filters was quantitated by scintillation counting in 8 mL of Scintiverse II (Fisher) using a Packard 1900 TR scintillation spectrometer. The IC_{50} values were calculated by fitting the concentration-dependent [^{3}H]NPY displacement data to a competitive binding isotherm according to the method of Galitzky et al.[24] and Williams et al.[25] using the program MINSQ (Micromath, Salt Lake City, UT), assuming (see below) that the observed displacement is from the benextramine-sensitive binding sites only.

The selectivity of NPY and the synthetic benextramine analogs at benextramine-sensitive versus insensitive NPY binding sites in rat brain was evaluated using modification of the "paired tube" assay.[21] Rat brain homogenate (as prepared above) was incubated with 1 nM [^{3}H]NPY in the absence (defined as "native" membrane preparation) or presence (defined as "benextramine-treated" membrane preparation) of 500 μM benextramine, and in the absence (total binding) or presence (nonspecific binding) of 1 μM NPY in a total volume of 0.50 mL of assay buffer in siliconized polyethylene culture tubes; NPY and benextramine analogs were similarly incubated at a concentration 5 or 10 times their respective IC_{50}'s in both the native and benextramine-treated membrane preparations. The assay mixtures were incubated for 90 min at 25 °C, and the total and nonspecific binding to both the "native" and benextramine-treated membrane preparations were evaluated as described above. Specific binding is defined as total binding minus nonspecific binding in each membrane preparation. The selectivity was analyzed by comparing the percent displacement of [^{3}H]NPY specific binding by each compound from the total binding site population in the "native" membrane preparation versus the percent specific displacement from the binding sites remaining (i.e., the benextramine-insensitive binding sites) after treatment with 500 μM benextramine.

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Registry No. **1**, 82785-45-3; **2**·2HCl, 142947-55-5; **2** (free base), 69790-18-7; **3**, 6404-29-1; **4**, 51513-80-5; **5**, 142947-56-6; **6**·2HCl, 142947-57-7; **6** (free base), 137048-18-1; **7a**, 60586-99-4; **7b**, 68388-07-8; **7c**, 30364-57-9; **7d**, 68388-08-9; **7e**, 68388-09-0; **7f**, 56374-47-1; **8a**, 142947-58-8; **8b**, 142947-59-9; **8c**, 142947-60-2; **8d**, 142947-61-3; **8e**, 142947-62-4; **8f**, 142947-63-5; **8g**, 142947-64-6; **8h**, 142947-65-7; **9a**·2HCl, 142947-66-8; **9a** (free base), 77799-97-4; **9b**·2HCl, 142947-67-9; **9b** (free base), 77799-98-5; **9c**·2HCl, 142947-68-0; **9c** (free base), 142947-77-1; **9d**·2HCl, 142947-69-1; **9d** (free base), 97783-50-1; **9e**·2HCl, 142947-70-4; **9e** (free base), 97783-51-2; **9f**·2HCl, 142947-71-5; **9f** (free base), 97783-72-7; **9g**·2HCl, 142947-72-6; **9g** (free base), 142947-78-2; **10a**·2HCl, 142947-73-7; **10a** (free base), 97125-00-3; **10b**·2HCl, 142947-74-8; **10b** (free base), 142947-79-3; **10c**·2HCl, 142947-75-9; **10c** (free base), 142947-80-6; **11**·2HCl, 142947-76-0; **11** (free base), 68536-04-9; $H_2N(CH_2)_5COOH$, 60-32-2; $(H_2NCH_2CH_2S)_2$·2HCl, 56-17-7; 2-$MeOC_6H_4COOH$, 579-75-9; 3-$MeOC_6H_4COOH$, 586-38-9; 4-$MeOC_6H_4COOH$, 100-09-4; 2-ClC_6H_4COOH, 118-91-2; 4-ClC_6H_4COOH, 74-11-3; 3-ClC_6H_4COCl, 618-46-2; PhCOCl, 98-88-4; 2-naphthoic acid, 93-09-4.

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