# Articles

## Somatostatin Receptor-Binding Peptides Labeled with Technetium-99m: Chemistry and Initial Biological Studies

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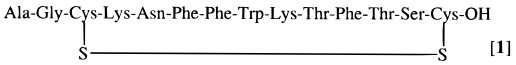
*Received February 13, 1995*⊗

The synthesis of peptides which possess a high affinity for the somatostatin receptor and contain a chelator for the radionuclide technetium-99m is described. The target compounds were designed such that they would form stable, oxotechnetium(V) chelate complexes in which the site of metal coordination was well defined and remote from the receptor-binding region. Oxorhenium(V) chelate complexes of these peptides were prepared as nonradioactive surrogates for the technetium complexes. Peptide oxorhenium complexes and Tc-99m complexes eluted closely upon HPLC analysis. The receptor-binding affinities of both the free and rhenium-coordinated species were measured *in vitro*. The binding affinities of the free peptides ($K_i$'s in the 0.25−10 nM range) compared favorably with [DTPA]octreotide ($K_i$ = 1.6 nM), which, as the indium-111 complex, is already approved for somatostatin receptor (SSTR)-expressing tumor imaging in the United States and Europe. Furthermore, the rhenium-coordinated peptides had binding affinities which, in many cases, were higher than those of the corresponding free peptides, with several complexes having a $K_i$'s of 0.1 nM. Some of the more potent SSTR-binding peptides were labeled with technetium-99m and assessed in an *in vivo* study with tumor-bearing rats. The $^{99m}$Tc-labeled peptides prepared in this study should be useful as SSTR-expressing tumor-imaging agents due to their high SSTR-binding affinities, ease of preparation, and, because they are low molecular weight peptides, expected pharmacokinetics characterized by rapid tracer excretion from the body resulting in high-contrast images.

### Introduction

Somatostatin (somatotropin release-inhibiting factor, SRIF) is a cyclic peptide which was initially isolated from the hypothalamus[1] and has been shown to have an inhibitory effect on the secretion of many hormones, including growth hormone. Since the initial discovery, several related somatostatin peptides have been identified, with the tetradecapeptide compound **1** designated as somatostatin 14 (SRIF-14). These peptides are widely distributed throughout the body and are found in the gut, various exocrine and endocrine glands, and most organs.[2] There are at least five subtypes of the SRIF receptor (somatostatin-type receptor or SSTR), and subtypes SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 have been cloned.[3] Most tumors of neuroendocrine origin express receptors for SRIF to a much greater extent than normal tissue.[4,5] To the extent that it has been examined, the SSTRs on tumor cells belong predominantly to the SSTR2 subtype.[3c] The list of the types of tumors found to express SSTRs currently includes tumors of the amine-precursor-uptake-and-decarboxylation (APUD) cell system including small cell lung carcinoma (accounting for 25% of all malignant lung cancers) (57%), endocrine pancreatic tumors (89%), metastatic carcinoids (87%), GH-producing pituitary adenomas (98%), paragangliomas (92%), and also certain breast tumors (20% of all), lymphomas (87%), astrocytomas (82%), meningiomas (98%), and some colorectal cancers (12%). Values given in parentheses are percentages of tumor samples tested and found to be SSTR positive.[5] Although it has been shown to have an inhibitory effect on various tumors, the use of **1** for the treatment of cancer is hampered by its short *in vivo* half-life of about 3 min.[6]



[1]

Analogs of somatostatin have been synthesized which incorporate D-amino acids to prolong *in vivo* half-life by inhibiting the action of amino- and carboxypeptidases. Somatuline (BIM-23014C, compound **2**)[7] is a cyclic octapeptide which has been shown to inhibit the growth of tumors of the human small cell lung carcinoma (SCLC) cell line NCI-H69 in an animal model. It has been approved for use in France and is currently in phase III clinical trials in the United States. Octreotide (compound **3**) is another cyclic octapeptide which has been shown to be 2000 times more effective than **1** in the suppression of growth hormone secretion in the rat 1 h postadministration.[8] Compounds **2** and **3** both bind preferentially to the SSTR2 receptor subtype.[3c] Derivatives of octreotide have been labeled with the $\gamma$-emitting radionuclides $^{123}$I ([$^{123}$I]Tyr$^3$-octreotide) and $^{111}$In ([$^{111}$In][DTPA]octreotide **4**), and these radiotracers have been successfully used to detect somatostatin receptor-positive tumors by $\gamma$ scintigraphy.[9a]

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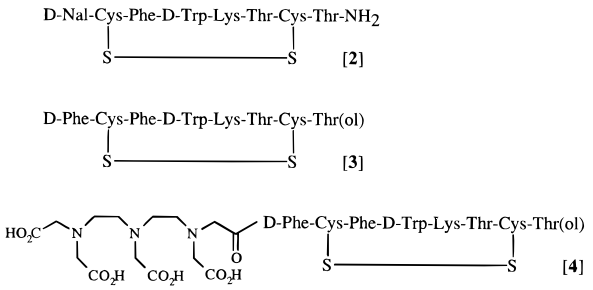
⊗ Abstract published in *Advance ACS Abstracts*, February 1, 1996.

0022-2623/96/1839-1361$12.00/0 

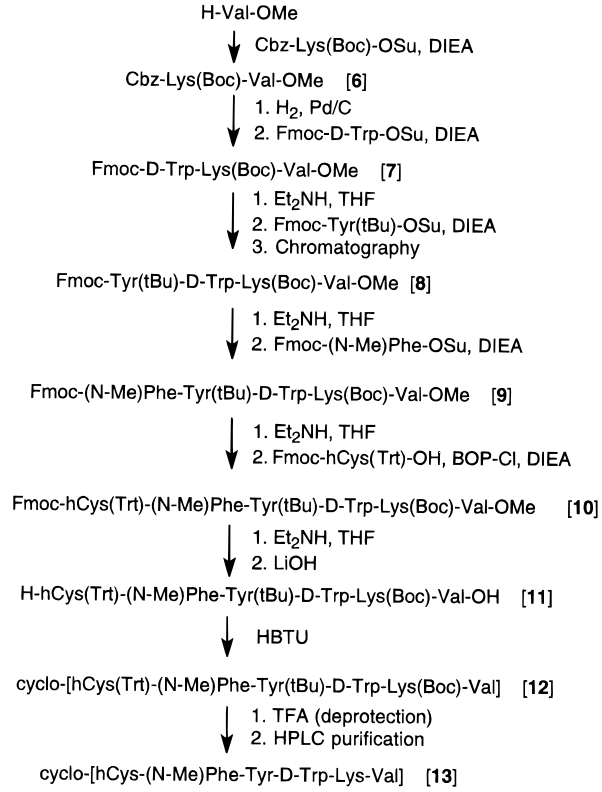



The $^{111}$In-labeled agent **4** has been approved in the United States and Europe and is now marketed under the trade name Octreoscan. Despite the success of Octreoscan as an imaging agent,[9b] a somatostatin analog incorporating the radionuclide $^{99m}$Tc would be more desirable. As an isotope for radioisotopic imaging, $^{99m}$Tc is preferred over $^{111}$In because it yields greater photon flux per unit of radiation dose delivered to the patient. It also has a convenient 6 h physical half-life ($t_{1/2}$($^{111}$In) = 60 h), is relatively inexpensive, and is available 24 h a day as a solution of [$^{99m}$Tc]pertechnetate ($^{99m}TcO_4$) in normal saline from an in-house $^{99}$Mo/$^{99m}$Tc generator.

Although several attempts have been made to label somatostatin analogs with $^{99m}$Tc, a successful clinical candidate has not been produced to date. The ideal agent would possess a chelator for technetium covalently attached to an SSTR-binding compound. Furthermore, this agent would be stable to the chemistry involved in its chelation of $^{99m}$Tc using an instant kit formulation. The use of SRIF analogs which are cyclized via a disulfide bond is problematic in this regard. $^{99m}$Tc is normally available in the 7+ oxidation state as $^{99m}TcO_4^-$. In order to form a stable chelate complex, the technetium must be reduced to the 5+ oxidation state, and stannous ion is normally used as the reductant. However, stannous ion can also reduce disulfide bonds[10] and consequently may severely reduce the SSTR-binding affinity of disulfide-cyclized molecules. We chose to address this problem by designing molecules incorporating high-affinity SSTR-binding peptides not possessing a reductively labile disulfide bond and possessing peptide sequences which form effective technetium chelators.[11] Because all nuclides of technetium are radioactive, oxorhenium complexes of these chelators were prepared to serve as substitutes for technetium complexes when assessing the binding affinity of analogs in an *in vitro* assay. It should be mentioned that the oxorhenium complexes of these peptides (when in the radioactive $^{186}$Re or $^{188}$Re form) may be useful for the radiotherapy of SSTR-expressing tumors.

It has been shown that the residues Phe-D-Trp-Lys-Thr of **2** and **3** closely approximate the key binding elements in the 7−10 segment of the receptor-bound hormone.[12] Cyclic analogs which incorporate this pharmacophore for somatostatin receptors have been prepared in which the ring is formed in a "head-to-tail" fashion where the N-terminal amino group is linked to the C-terminal carboxyl group via an amide bond. One such analog is the very high affinity SSTR-binding cyclic peptide MK-678 (compound **5**).[13,14] Recognizing that this approach to constraining the pharmacophore in a nonreducible cyclic structure fit our needs, we synthesized a novel modification of this structure which incorporated a site for attaching a chelator for $^{99m}$Tc (or Re) while maintaining the potent biological activity of these analogs. The details of their synthesis and biological activity *in vitro* and *in vivo* are described herein.

**Scheme 1.** Preparation of Cyclic Receptor-Binding Synthon




cyclo-[Phe-(N-Me)Ala-Tyr-D-Trp-Lys-Val] [5]

## Chemistry

The cyclic receptor-binding portion was synthesized in solution as outlined in Scheme 1. Starting with valine, methyl ester, the amino acids were added sequentially as their succinimide esters to the free amine of the growing peptide chain. The intermediates were carried through without purification up to the tetrapeptide **8**, which was purified by silica gel flash chromatography. The synthesis was continued as before, with BOP-Cl reagent[15] used for the difficult coupling of Fmoc-hCys(Trt)-OH to the N-terminal *N*-methylphenylalanine. Once the N-terminal Fmoc group and the C-terminal methyl ester were removed, the resulting crude hexapeptide was cyclized using HBTU reagent.[16] Finally the side chain-protecting groups were removed with trifluoroacetic acid using water, ethanedithiol, and triisopropylsilane[17] as scavengers to yield **13**, which was purified by preparative reversed-phase HPLC.

Peptides representing the portion of the molecule designed to serve as a chelator were synthesized by a standard solid-phase protocol[18] using Rink amide resin[19] as the support. All couplings were performed with HBTU reagent. In those sequences which did not contain an arginine, the cysteine sulfhydryl was protected with a trityl group. These peptides were cleaved from the resin using TFA/water (95:5) with simultaneous removal of the *tert*-butyl-based protecting groups

**Table 1.** Chloroacetyl Intermediates from Solid-Phase Peptide Synthesis

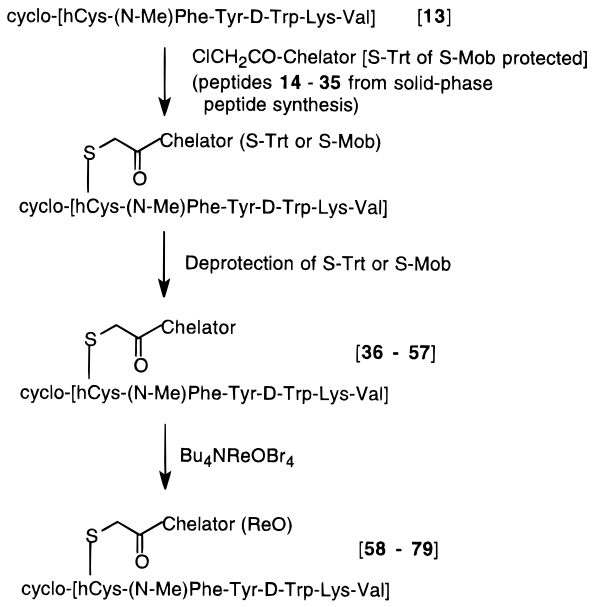
$ClCH_2CO$-Peptide

| peptide | compd |
|---|---|
| -Cys(Trt)-Gly-Cys(Trt)-OH | **14** |
| -Cys(Trt)-Gly-Cys(Trt)-$NH_2$ | **15** |
| -(ϵ-Lys)-Gly-Cys(Trt)-$NH_2$ | **16** |
| -Lys-(ϵ-Lys)-Gly-Cys(Trt)-$NH_2$ | **17** |
| -Gly-Gly-Cys(Trt)-Lys-$NH_2$ | **18** |
| -(ϵ-Lys)-Gly-Cys(Trt)-Lys-$NH_2$ | **19** |
| -Gly-Gly-Cys(Mob)-Arg-$NH_2$ | **20** |
| -(ϵ-Lys)-Lys-Cys(Trt)-$NH_2$ | **21** |
| -Gly-Gly-Cys(Trt)-Lys-Lys-$NH_2$ | **22** |
| -Gly-Gly-Cys(Trt)-Orn-$NH_2$ | **23** |
| -Gly-Gly-Cys(Trt)-Orn-Asp-Orn-$NH_2$ | **24** |
| -(ϵ-Lys)-Gly-Cys(Trt)-Lys-Lys-$NH_2$ | **25** |
| -Lys-Lys-Cys(Trt)-$NH_2$ | **26** |
| -Lys-Lys-Cys(Trt)-Lys-$NH_2$ | **27** |
| -Gly-Gly-Cys(Trt)-Lys-Lys-Lys-$NH_2$ | **28** |
| -Gly-Gly-Cys(Mob)-Arg-Arg-$NH_2$ | **29** |
| -Gly-Gly-Cys(Mob)-Arg-Lys-$NH_2$ | **30** |
| -Gly-Gly-Cys(Mob)-Arg-Asp-$NH_2$ | **31** |
| -Gly-Gly-Cys(Trt)-Orn-Asp-$NH_2$ | **32** |
| -Gly-Gly-Cys(Trt)-Lys-Asp-Lys-Asp-$NH_2$ | **33** |
| -(δ-Orn)-Gly-Cys(Trt)-Lys-$NH_2$ | **34** |
| -(β-Dap)-Lys-Cys(Trt)-Lys-$NH_2$ | **35** |

and, by adapting the workup conditions, with selective reprotection of the thiol group(s). This was accomplished by removing the TFA of the cleavage mixture *in vacuo* followed by dissolution of the crude peptide in chloroform, which was also removed *in vacuo*. After several such treatments with chloroform, all of the residual acid was removed with concomitant reattachment of the trityl group to the cysteine SH. This strategy did not work well when Pmc-protected arginine was present in the sequence. In these cases the cysteine SH was 4-methoxybenzyl (Mob) protected and the arginine-containing peptides were cleaved from the resin using TFA/water/thioanisole/ethanedithiol/triethylsilane (95:5:5:2.5:2), which removed all protecting groups except the *S*-Mob group.

The above strategies were employed to produce compounds **14**−**35** (Table 1). These peptides, which were capped with a chloroacetyl group at the N-terminus, were coupled to **13** in a carbonate buffer (pH = 10) as shown in Scheme 2. This reaction produced pharmacophore−chelator conjugates in which the cysteines of the chelator portion were either trityl or methoxybenzyl protected. For those compounds which were *S*-trityl protected, the trityl group was removed with TFA/water/triisopropylsilane (10:0.5:0.3). For those compounds which were *S*-Mob protected, the Mob group was removed with TFA/$BF_3$·$OEt_2$/*m*-cresol (10:1:1). The final products **36**−**57** were purified by reversed-phase preparative chromatography (≥95% pure) and confirmed by mass spectral analysis. Oxorhenium(5+) complexes were prepared by reacting peptides **36**−**57** with $Bu_4NReOBr_4$[20] in DMF to produce compounds **58**−**79**. The resulting compounds were also purified by reversed-phase chromatography (≥95% pure) and found to be different, with longer analytical HPLC retention times, from the uncomplexed peptides by coinjection. All rhenium complexes were also confirmed by mass spectral analysis. The technetium-99m complexes of compounds **40**−**43**, **45**, and **57** were also prepared by reacting these peptides with [$^{99m}$Tc]glucoheptonate in a buffered solution. The radiochemical purity of these

**Scheme 2.** Preparation of SSTR-Binding Analogs Containing a Chelator




complexes was measured by analytical HPLC. The peptide−rhenium complexes had retention times which were very close to those of the technetium complexes, thus supporting the use of rhenium complexes as surrogates for the Tc-99m complexes.

## Biology

Peptides **4**, **4** ($^{111}$In complex), **36**−**44**, **56**, **57**, and rhenium complexes **58**−**79** were tested for SSTR-binding affinities using AR42J cells (see the Experimental Section for details), which express mostly SSTR2 receptors. Compounds were assayed by incubating equal aliquots of cell membrane with [$^{125}$I][Tyr-11]SRIF-14 and the compound to be assayed at a final concentration of $10^{-11}$−$10^{-6}$ M. All compounds were tested in duplicate at five different concentrations. SSTR binding was assessed from determinations of free vs cell membrane-bound radioactivity. Analysis of the data gave inhibition constants ($K_i$) via Hill plots.[21] The results are presented in Table 2.

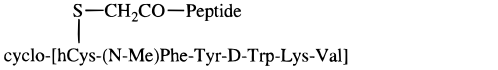
A comparison was also made of compounds **40** and **42** with BIM-23014C and MK-678 in a receptor subtype specific assay. Cell membrane preparations were prepared by homogenization of the appropriate cells (human SSTR1, mouse SSTR3, human SSTR4 transfected into CHO-DG44 cells) in buffer (see the Experimental Section for details). The assays were again performed by incubating equal aliquots of cell membrane with [$^{125}$I]-[Tyr-11]SRIF-14 and the compound to be assayed as described above. The results are presented in Table 3.

The technetium-99m complexes of compounds **40**−**43**, **45**, and **57** were also administered to tumor-bearing rats and the resulting tumor uptake and biodistribution measured and compared to the $^{111}$In complex of compound **4**. The results are presented in Table 4.

## Results and Discussion

Our goal in this work was to synthesize $^{99m}$Tc-labeled compounds which had high affinity for somatostatin receptors. These analogs were designed to contain a chelator for the radionuclide $^{99m}$Tc and designed such that metal coordination could be accomplished easily

**Table 2.** Binding Affinities of SST Analogs and Corresponding Rhenium Complexes




| peptide | compd | $K_i$ (nM)[a] | ReO complex | $K_i$ (nM)[a] |
|---|---|---|---|---|
| -Cys-Gly-Cys-OH | **36** | 1.8 | **58** | 20 |
| -Cys-Gly-Cys-$NH_2$ | **37** | 1.5 | **59** | 1.7 |
| -($\epsilon$-Lys)-Gly-Cys-$NH_2$ | **38** | 2.0 | **60** | 0.9 |
| -Lys-($\epsilon$-Lys)-Gly-Cys-$NH_2$ | **39** | 0.7 | **61** | 0.5 |
| -Gly-Gly-Cys-Lys-$NH_2$ (P587) | **40** | 2.5 | **62** | 0.2 |
| -($\epsilon$-Lys)-Gly-Cys-Lys-$NH_2$ | **41** | 4.2 | **63** | 0.3 |
| -Gly-Gly-Cys-Arg-$NH_2$ (P617) | **42** | 0.3 | **64** | 0.2 |
| -($\epsilon$-Lys)-Lys-Cys-$NH_2$ | **43** | 2.2 | **65** | 0.4 |
| -Gly-Gly-Cys-Lys-Lys-$NH_2$ | **44** | 0.3 | **66** | 0.1 |
| -Gly-Gly-Cys-Orn-$NH_2$ | **45** | | **67** | 0.2 |
| -Gly-Gly-Cys-Orn-Asp-Orn-$NH_2$ | **46** | | **68** | 0.6 |
| -($\epsilon$-Lys)-Gly-Cys-Lys-Lys-$NH_2$ | **47** | | **69** | 0.5 |
| -Lys-Lys-Cys-$NH_2$ | **48** | | **70** | 0.5 |
| -Lys-Lys-Cys-Lys-$NH_2$ | **49** | | **71** | 0.3 |
| -Gly-Gly-Cys-Lys-Lys-Lys-$NH_2$ | **50** | | **72** | 0.3 |
| -Gly-Gly-Cys-Arg-Arg-$NH_2$ | **51** | | **73** | 0.1 |
| -Gly-Gly-Cys-Arg-Lys-$NH_2$ | **52** | | **74** | 0.1 |
| -Gly-Gly-Cys-Arg-Asp-$NH_2$ | **53** | | **75** | 1.7 |
| -Gly-Gly-Cys-Orn-Asp-$NH_2$ | **54** | | **76** | 2.1 |
| -Gly-Gly-Cys-Lys-Asp-Lys-Asp-$NH_2$ | **55** | | **77** | 1.8 |
| -($\delta$-Orn)-Gly-Cys-Lys-$NH_2$ | **56** | 10 | **78** | 0.1 |
| -($\beta$-Dap)-Lys-Cys-Lys-$NH_2$ (P829) | **57** | 10 | **79** | 0.3 |
| [DTPA]octreotide | **4** | 1.6 | $^{111}$In complex | 1.2 |

[a] Binding affinity for the SSTRs on AR42J cells using $^{125}$I-labeled SRIF-14 as ligand.

**Table 3.** SSTR Subtype Comparison

| | *in vitro* binding affinities: $K_i$ (nM) | | | |
|---|---|---|---|---|
| receptor | BIM-23014C (**2**) | MK-678 (**5**) | compd **40** (P587) | compd **42** (P617) |
| SSTR1 | 789 | >1000 | 19 | 79 |
| SSTR2 | 0.3 | 0.2 | 1.0 | 0.9 |
| SSTR3 | 5.6 | 12 | 89 | 152 |
| SSTR4 | >1000 | >1000 | 68 | 36 |
| SSTR5 | 0.10 | 5.5 | | |
| $\mu$-opioid | 2.2 | >10000 | >10000 | 5425 |

using a standard kit formulation.[22] Furthermore, SSTR-binding affinity of the pharmacophore would not be affected by the radiolabeling process. The bioactive core chosen for this work was a cyclic hexapeptide which did not contain any reducible disulfide bonds. A convergent synthetic strategy was employed where this cyclic portion was attached, via sulfhydryl alkylation, to several different cysteine-protected peptide chelators containing a chloroacetyl moiety. This efficient reaction produced, after cysteine deprotection, analogs **36**−**57**. Because only radioactive nuclides of technetium exist, rhenium, which is very similar to technetium in its (5+) oxo coordination chemistry,[23] was used in the preparation of metal complexes to serve as surrogates for the corresponding technetium complexes in *in vitro* assays. Several different chelating systems were examined. These chelators included (see Figure 1) bisamide bisthiols, such as are provided by the sequence -Cys-Gly-Cys- (compounds **36**, **37**), and triamide thiols, such as are provided by the sequence -Gly-Gly-Cys- (compounds **40** (P587), **42**, **44**−**46**, **48**−**55**). Both of these types of chelators are expected to form anionic oxotechnetium (5+) complexes.[24] A novel diamide−amine−thiol chelator system of the type -($\epsilon$-Lys)-Gly-Cys- was also prepared (compounds **38**, **39**, **41**, **43**, **47**, **56**, **57** (P829)). In this system it is postulated that the α-amine of lysine (or ornithine or diaminopropionic acid) becomes part of the coordination complex, while the $\epsilon$-amine ($\delta$-amine for ornithine or $\beta$-amine for diaminopropionic acid) is used to form an amide bond to the next amino acid (or other carboxy-containing moiety) in the sequence. This system is similar to the triamide thiols above in that the metal chelator contains three nitrogens and one sulfur. However, unlike the triamide thiols, one of the nitrogens is a less readily ionizable primary amine. We expect metal coordination by this amine to be through its lone pair electrons, therefore forming neutral oxotechnetium(5+) complexes as shown in Figure 1. The use of the strategy described above insured that the chelator could be incorporated unambiguously at known positions during synthesis so that the effect of the chelated metal on the binding affinity of the molecule could be altered in a rational manner.

*In vitro* SSTR binding results are shown in Table 2. Most compounds had high affinity to the SSTR, with inhibition constants ($K_i$'s) in the low-nanomolar or subnanomolar range. This compares favorably with [DTPA]octreotide **4** ($K_i$ = 1.6 nM) and [$^{111}$In][DTPA]-octreotide ($K_i$ = 1.2 nM). Changes in the distance of the chelated metal from the cyclic bioactive core seemed to have little effect on *in vitro* activity. Interestingly, for our series of compounds, in almost every case examined the rhenium complex had a higher binding affinity than the uncoordinated species. An exception to this phenomenon was compound **36** where the affinity of the coordinated species was 1 order of magnitude less than that of the parent compound. This may be due to the negative charge of the carboxy-terminal cysteine. Compounds **53**−**55**, which contain a negative charge in the form of an aspartic acid side chain carboxyl, also exhibit decreased binding in relation to the rest of the compounds in this family. Whether the chelated metal was anionic or neutral also appeared to have some effect on this phenomenon. The affinity for the complexed species was much greater than that of the free peptide for compounds **41**, **43**, **56**, and **57**, which we had postulated would form neutral complexes. This discovery provides an advantage in that the affinity of the radiolabeled imaging agent can be higher than that of the excess unlabeled peptide (the molar ratio of carrier peptide to radiolabeled peptide is usually ≥10000:1). This produces an effectively higher specific activity of the radiolabeled imaging agent than would normally be the case and means that the unlabeled peptide should be at a competitive disadvantage with the radiolabeled peptide for the SSTRs.

Compounds **40** (P587) and **42** were also examined in receptor subtype specific assays, and the binding was compared to that of the potent SSTR binders BIM-23014C (**2**) and MK-678 (**5**). As seen in Table 3, both **2** and **5** were very potent for SSTR2 receptors and moderately potent for SSTR3 receptors. The affinity of these compounds was markedly decreased for both SSTR1 and SSTR4 receptors. Compounds **40** and **42** showed a much broader range of activity although they had higher affinity for the SSTR2 receptor.

Once it was determined that negative charge in the region of the chelator had a deleterious effect on receptor-binding affinity, changes were made in the location of positively charged residues in an attempt to alter the biodistribution of $^{99m}$Tc-labeled peptides. Technetium-99m complexes of compounds **40**−**43**, **45**, and **57** and the $^{111}$In complex of compound **4** were admin-

**Table 4.** Biodistribution of Technetium-99m Complexes

| | | | % ID/g | | % ID | | | | |
|---|---|---|---|---|---|---|---|---|---|
| compd[a] | RCP[b] (%) | $t_R$[c] (min) | tumor[d] | blood | kidneys | liver | GI[e] | tumor/blood[f] | tumor/muscle[g] |
| **40** (3) | 99 | 15.0 | 2.75 | 0.50 | 21.6 | 8.3 | 27.9 | 5.6 | 30.2 |
| (P587) | | | (0.50) | (0.16) | (1.7) | (0.3) | (5.2) | (0.9) | (2.7) |
| **41** (3) | 99 | 14.7 | 1.28 | 0.28 | 16.9 | 16.0 | 15.9 | 4.6 | 13.8 |
| | | | (0.36) | (0.08) | (4.1) | (1.1) | (1.6) | (0.2) | (1.1) |
| **42** (3) | 99 | 15.1 | 3.54 | 0.28 | 14.1 | 9.3 | 27.3 | 11.1 | 40.3 |
| (P617) | | | (0.63) | (0.03) | (2.6) | (0.8) | (1.3) | (0.7) | (1.1) |
| **43** (3) | 99 | 14.5 | 0.57 | 0.37 | 12.1 | 14.5 | 16.3 | 1.6 | 4.9 |
| | | | (0.04) | (0.57) | (7.2) | (6.2) | (3.9) | (0.2) | (0.4) |
| **45** (3) | 99 | 7.0 | 1.48 | 0.60 | 20.4 | 9.2 | 38.1 | 2.4 | 18.6 |
| | | | (0.70) | (0.06) | (0.3) | (0.6) | (0.4) | (1.0) | (7.0) |
| **57** (3) | 99 | 7.0 | 4.88 | 0.29 | 32.7 | 10.0 | 8.4 | 21.0 | 67.9 |
| (P829) | | | (0.36) | (0.02) | (2.5) | (0.1) | (0.5) | (11.1) | (26.4) |
| **4** ($^{111}$In complex) (9) | | | 2.43 | 0.11 | 5.3 | 1.0 | 6.0 | 22.0 | 73.0 |
| | | | (0.74) | (0.04) | (0.3) | (0.1) | (1.2) | (5.9) | (15.2) |

[a] Data refer to the $^{99m}$Tc complex of the indicated compound number. Number of rats per study in parentheses. Letter + numbers in parentheses refer to potential clinical candidates (Diatide compound code). [b] Radiochemical purity of $^{99m}$Tc complex as measured by HPLC (see Experimental Section). [c] Analytical HPLC retention time of $^{99m}$Tc complex. Compounds **40**−**43** were run at 0−100% B/A over 20 min using a Waters Delta-Pak C18 column, 5 $\mu$m, 39 × 150 mm. Compounds **45** and **57** were run at 0−100% B/A over 10 min using a Waters Nova-Pak Radial Compression C18 column, 4 $\mu$m, 8 × 100 mm (A = 0.1% TFA in water, B = 0.1% TFA in 90% acetonitrile/water). [d] Percent injected dose contained in tumor (see Experimental Section). Standard deviation values appear beneath in parentheses. [e] Percent injected dose contained in gastrointestinal tract (stomach, without contents; small intestine, duodenum, jejunum, and ileum, all with contents; large intestine, cecum and colon, all with contents). [f] Tumor to blood ratio based on % ID/g values at 90 min. [g] Tumor to muscle ratio based on % ID/g values at 90 min. Nontarget values were obtained from the contralateral leg muscle.

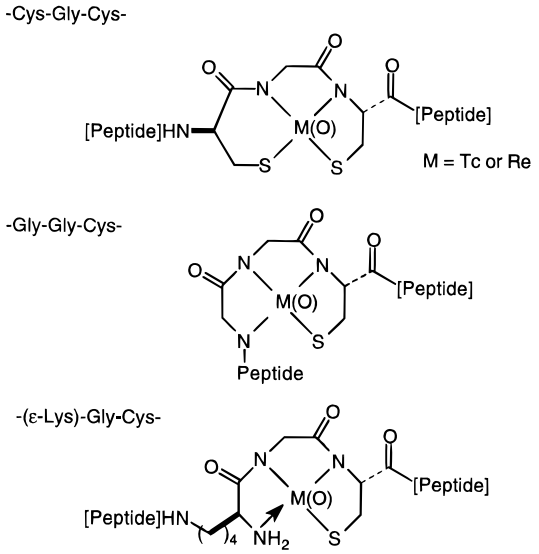



**Figure 1.** Peptidyl-chelating systems.

istered to tumor-bearing rats, and their tumor uptake and biodistribution were measured. As illustrated in Table 4, the location of the positively charged residues lysine or arginine and the proximity of the technetium complex to the cyclic pharmacophore had a pronounced effect on biodistribution. Several of the more potent compounds had high gastrointestinal uptake compared to [$^{111}$In]Octreoscan. Compound **57** (P829), whose tumor uptake and biodistribution was similar to the $^{111}$In complex of compound **4**, was chosen for clinical evaluation.

## Conclusions

We designed and synthesized high-affinity SSTR-binding peptides containing sequences which form strong chelates with $^{99m}$Tc. The peptides synthesized were designed such that stable, metal-coordinated derivatives could be prepared where the site of metal coordination was well defined and the SSTR-binding affinity of the peptide was not compromised. Rhenium complexes of these compounds were prepared as $^{99m}$Tc complex surrogates and found, in most cases, to have even higher receptor-binding affinity than their parent peptides. The $^{99m}$Tc complexes of the peptides described hold promise to serve as useful SSTR-expressing tumor-imaging agents due to their high receptor-binding affinity, ease in preparation, and, because they are low molecular weight peptides, expected rapid pharmacokinetics. Several $^{99m}$Tc-labeled peptides were administered to tumor-bearing rats and the tumor uptake and biodistribution of these peptides measured. It was found that these $^{99m}$Tc-labeled peptides localized in tumors. Compound **57** (P829) displayed a biodistribution similar to that of $^{111}$In-labeled octreotide and was selected for investigation in clinical studies.

## Experimental Section

Symbols and abbreviations generally follow the IUPAC-IUB recommendations as published in *Int. J. Pept. Protein Res.* (**1984**, *24*, 9−37). Amino acids used were of the L-configuration unless stated otherwise. All protected amino acids used (except *N*-Fmoc-*S*-tritylhomocysteine and *N*-α-Boc-*N*-β-Fmoc-diaminopropionic acid) were purchased from either Bachem, CA, Novabiochem, or Advanced ChemTech and used as is. *N*-α-Boc-*N*-β-Fmoc-diaminopropionic acid (Dap) was prepared according to literature precedent.[26] *N*-Fmoc-*S*-tritylhomocysteine was prepared as indicated below. Other chemicals and solvents were purchased from either J.T. Baker, Advanced ChemTech, or Aldrich and used as is. Aldrich SureSeal solvents were used when anhydrous conditions were necessary. When indicated, an automated Applied Biosystems 431A peptide synthesizer was used. For manual peptide synthesis, the reaction vessel used was purchased from Safe-Lab, Santee, CA. Analytical HPLCs were performed on a Waters system using a Delta-Pak C18 column (300 Å, 5 $\mu$m, 3.9 × 150 mm) at a flow rate of 1.2 mL/min or on a Waters system using a radial compression Nova-Pak C18 column (4 $\mu$m, 8 × 100 cm) at a flow rate of 3.0 mL/min. Preparative HPLCs were performed on a Waters LC-4000 system using a 4 × 32.5 cm C18 Delta-Pak preparative HPLC column. For normal-phase chromatographic separations, Merck grade 9385 silica gel was used with a mesh of 230−400. $R_f$ values were determined with silica gel TLC plates (Kieselgel 60 $F_{254}$, 0.25 mm layer thickness; Merck). $^1$H-NMR spectra were obtained on a Varian Gemini 200 spectrometer at 200 MHz using TMS as an internal standard. Fast atom bombardment mass spectra (FABMS) were obtained by M-Scan using a VG Analytical ZAB

2-SE instrument or by Scripps Research Institute using a VG ZAB VSE instrument. Electrospray mass spectra were obtained at Scripps Research Institute using an API III Pe Sciex triple-quadrupole mass spectrometer.

***S*-Tritylhomocysteine.** Methionine (100 g, 0.67 mol) was placed in a 5-L three-necked round bottom flask equipped with a mechanical stirrer and a cold finger condenser cooled to −78 °C with dry ice/acetone. The reaction flask was flushed with argon and cooled with a dry ice/acetone bath. Anhydrous ammonia gas was condensed in the flask until the methionine was completely dissolved. The dry ice bath was removed, and 55.7 g (2.42 mol) of sodium metal was added portionwise to the refluxing ammonia solution until the reaction mixture maintained its blue color; 47.3 g (0.61 mol) of ammonium acetate was added in portions to quench the reaction and the ammonia evaporated overnight under a stream of argon. The resulting thick solid was broken up with a spatula and then ground with a mortar and pestle. It was then combined with 182.75 g (0.70 mol) of triphenylmethanol and this mixture placed under an atmosphere of argon. With cooling by using a water bath, 500 mL of chloroform was added followed by the addition of 1000*g* of TFA. The reaction mixture was stirred for 2.5 h, and the solvents were removed *in vacuo*, the final traces being removed under high vacuum at 42 °C; 1 L of distilled water was added and the solution cooled in a water bath at 10 °C; 211.5 g of sodium hydroxide was added portionwise until a pH of 13 was achieved. The resulting white precipitate was filtered off and washed with distilled water. The solid was suspended in 1 L of water, and the pH was adjusted to 4.0 by the addition of solid citric acid; 1 L of ethyl ether was added, and the solid was filtered and washed with water followed by ether. Thoroughly drying the solid under high vacuum at 60 °C yielded 188.24 g of *S*-tritylhomocysteine as the product in 74.5% yield: $^1$H NMR ($CD_3OD$) $\delta$ 1.88 (2H, m), 2.39 (2H, t, $J$ = 7.6 Hz), 3.40 (2H, t, $J$ = 5.6 Hz), 7.1−7.5 (15H, m); $^{13}$C NMR ($CD_3OD$) $\delta$ 28.97, 31.62, 55.38, 67.81, 127.59, 128.71, 130.48, 145.86, 173.10.

***N*-α-Fmoc-*S*-tritylhomocysteine.** A three-necked round bottom flask equipped with a mechanical stirrer was charged with 108.8 g (1.03 mol) of sodium carbonate in 750 mL of water, and 161.3 g (0.425 mol) of *S*-tritylhomocysteine in 1 L of dioxane was added. After the solution became homogeneous, 143.7 g (0.425 mol) of *N*-[(9-fluorenylmethoxycarbonyl)-oxy]succinimide (FmocOSu) in 1.25 L of anhydrous dioxane was added to the reaction mixture over 5 min. The reaction mixture was stirred for 3 h followed by adding citric acid until a pH of 4 was reached; 1.5 L of ethyl acetate was added, and the layers were separated in a separatory funnel. The organics were washed with saturated sodium chloride (2 × 400 mL). The solution was concentrated *in vacuo* to yield the crude product as an oil. This was taken up in 2 L of ethyl ether and washed with water (3 × 500 mL) and saturated NaCl (2 × 250 mL) to remove residual *N*-hydroxysuccinimide. The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield an oil. The product was obtained as a foam (176.22 g, 68.7%) after further drying under high vacuum: $^1$H NMR ($CDCl_3$) $\delta$ 1.53 (1H, m), 1.80 (1H, m), 2.28 (2H, m), 4.19 (3H, m), 4.39 (2H, m), 4.88 (1H, d, $J$ = 7.6 Hz), 7.10−7.75 (23H, m), 8.22 (1H, br s); $^{13}$C NMR ($CDCl_3$) $\delta$ 27.90, 31.46, 47.18, 53.19, 67.18, 67.20, 119.96, 124.96, 126.72, 127.08, 127.73, 127.90, 129.60, 141.32, 143.69, 144.62, 155.99, 176.09.

**Preparation of *N*-Hydroxysuccinimide Esters.** *N*-Hydroxysuccinimide esters of *N*-α-Cbz-*N*-ε-Boc-lysine, *N*-α-Fmoc-D-tryptophan, *N*-Fmoc-*O*-*tert*-butyltyrosine, and *N*-Fmoc-*N*-methylphenylalanine were prepared by reacting 1.0 equiv of amino acid with 1.1 equiv of *N*-hydroxysuccinimide in the presence of 1.1 equiv of diisopropylcarbodiimide in dry THF. In a representative experiment, 100 mmol of *N*-α-Cbz-*N*-ε-Boc-lysine was dissolved in 250 mL of dry tetrahydrofuran (THF); 110 mmol of *N*-hydroxysuccinimide was added, and the reaction mixture was cooled with an ice water bath; 110 mmol of diisopropylcarbodiimide (DIC) was added, and the reaction mixture was stirred for 2 h. The progress of the reaction was monitored by thin layer chromatography (TLC). When the reaction was judged complete, the reaction mixture was filtered through a medium frit sintered glass funnel. The collected precipitate was washed with 50 mL of dry THF, and the combined filtrates were concentrated *in vacuo* to yield crude product as a white paste.

This paste was taken up in 200 mL of 10% ethyl acetate/ethyl ether, and 300 mL of hexanes was added. The solution was cooled at 4 °C for 2 h. The resulting precipitate was filtered and washed twice with 100 mL of hexanes. Drying the resulting solid *in vacuo* yielded pure hydroxysuccinimide ester as the product in 99% yield. The product was one spot by TLC analysis in two solvent systems ($R_f$ = 0.95 in 4:1:1 butanol/acetic acid/water and 0.78 in 90:8:2 chloroform/methanol/acetic acid).

TLC characteristics for the other hydroxysuccinimide esters are as follows: Fmoc-D-Trp-OSu ($R_f$ = 0.68 in 90:8:2 chloroform/methanol/acetic acid and 0.25 in 1:1 ethyl acetate/hexanes), Fmoc-Tyr(tBu)-OSu ($R_f$ = 0.76 in 90:8:2 chloroform/methanol/acetic acid and 0.27 in 1:1 ethyl acetate/hexanes), and Fmoc-(N-Me)Phe-OSu ($R_f$ = 0.85 in 90:8:2 chloroform/methanol/acetic acid and 0.54 in 1:1 ethyl acetate/hexanes).

**Cbz-Lys(Boc)-Val-OMe (6).** Valine, methyl ester, hydrochloride (17.43 g, 104.5 mmol) was reacted with diisopropylethylamine (DIEA) (33.1 mL, 190 mmol) in 500 mL dry THF. *N*-α-Cbz-*N*-ε-Boc-lysine, hydroxysuccinimide ester (53.73 g, 95 mmol) was added portionwise, and the reaction mixture was stirred at room temperature. The progress of the reaction was monitored by TLC (product $R_f$ = 0.43 in 1:1 EtOAc/hexane). When the reaction mixture was judged complete, the solvents were removed *in vacuo*. The crude product was taken up in ethyl acetate and washed with 5% citric acid, saturated sodium bicarbonate, and saturated sodium chloride. The organic layer was dried over magnesium sulfate and filtered and the filtrate concentrated *in vacuo*. The resulting foam was dissolved in 300 mL of ethyl ether, and 400 mL of hexanes was added. After cooling the solution for 3 h at 4 °C, the resulting solid was filtered, washed twice with hexanes, and dried under high vacuum to yield Cbz-Lys(Boc)-Val-OMe (44.46 g, 90.07 mmol) in 95% crude yield.

**Fmoc-D-Trp-Lys(Boc)-Val-OMe (7).** Cbz-Lys(Boc)-Val-OMe (41.96 g, 85.0 mmol) was taken up in 210 mL of 1% acetic acid/methanol in a 1-L round bottom flask. The solution was purged with argon, and 500 mg of 10% palladium on carbon was added. The atmosphere of the flask was evacuated and charged with hydrogen gas. This evacuation/charging procedure was repeated three times. The reaction mixture was then stirred at room temperature under an atmosphere of hydrogen. The progress of the reaction was monitored by TLC (product $R_f$ = 0.17 in $CHCl_3$/MeOH/HOAc, 90:8:2). When the starting material was no longer visible by TLC, the atmosphere of the reaction mixture was evacuated and replaced with argon. The solution was filtered through a layer of Celite, and the volatiles were removed *in vacuo*. The crude product was taken up in carbon tetrachloride (150 mL), and this was also removed *in vacuo* to remove any traces of acetic acid. Treatment with carbon tetrachloride was repeated twice more, and the crude intermediate H-Lys(Boc)-Val-OMe was dried under high vacuum for 4 h. The resulting white foam was dissolved in 200 mL of anhydrous THF, and the atmosphere of the mixture was flushed with argon gas. Fmoc-D-tryptophan, hydroxysuccinimide ester (32.0 g, 61.1 mmol) was added followed by the addition of diisopropylethylamine (29.6 mL, 170 mmol). The reaction mixture was stirred overnight under an atmosphere of argon and monitored by TLC (product $R_f$ = 0.51 in $CHCl_3$/MeOH/HOAc, 90:8:2, and 0.17 in EtOAc/hexane, 1:1).

After this time the volatiles were removed *in vacuo* on the rotory evaporator to yield an oily residue which was taken up in 500 mL of ethyl acetate. The organics were washed with 5% citric acid, saturated sodium bicarbonate, and saturated sodium chloride followed by drying over magnesium sulfate. Filtration and concentration of the filtrate *in vacuo* yielded a foam which was dissolved in 300 mL of ethyl ether with 20 mL of ethyl acetate added to totally solubilize the crude product. Hexanes (400 mL) were added to the solution of crude product, and the resulting suspension was allowed to stand at 4 °C for 4 h. The solid was filtered off and washed with

hexanes to yield the crude product as a white solid (42.9 g, 55.8 mmol) in 84.5% yield.

**Fmoc-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe (8).** Fmoc-D-Trp-Lys(Boc)-Val-OMe (42.24 g, 55.0 mmol) was dissolved in 55 mL of dry THF in a 1-L round bottom flask equipped with a stir bar. The atmosphere of the reaction mixture was flushed with argon, and diethylamine (50 mL) was added. The reaction mixture was stirred under an atmosphere of argon at room temperature for 1 h. The volatiles were removed *in vacuo* on the rotory evaporator, and the intermediate H-D-Trp-Lys(Boc)-Val-OMe was taken up in 200 mL of acetonitrile. The volatiles were again removed *in vacuo* on the rotory evaporator. This acetonitrile treatment was repeated twice more to yield an oil which was taken up in 200 mL of ethyl ether and 20 mL of ethyl acetate. Hexanes (400 mL) were added, and the resulting suspension was allowed to stand at 4 °C for 4 h. The crude intermediate was filtered off and washed with hexanes to yield a solid which was dried under high vacuum for 2 h.

This solid was placed in a 1-L round bottom flask equipped with a magnetic stir bar and dissolved in 150 mL of anhydrous THF, and the atmosphere of the mixture was flushed with argon gas. *N*-α-Fmoc-*O*-*tert*-butyltyrosine, hydroxysuccinimide ester (30.6 g, 55.0 mmol) was added followed by the addition of diisopropylethylamine (19.2 mL, 110 mmol). The reaction mixture was stirred overnight under an atmosphere of argon and monitored by TLC (product $R_f = 0.53$ in $CHCl_3$/MeOH/HOAc, 90:8:2, and 0.14 in EtOAc/hexane, 1:1).

At this time the reaction was judged to be complete, so the volatiles were removed *in vacuo* and the crude product was taken up in 500 mL of ethyl acetate. The organics were washed with 5% citric acid, saturated sodium bicarbonate, and saturated sodium chloride followed by drying over magnesium sulfate. Filtration and concentration of the filtrate *in vacuo* yielded an oil which was dissolved in 300 mL of ethyl ether with 30 mL of ethyl acetate added to totally solubilize the crude product. Hexanes (400 mL) were added to the solution of crude product, and the resulting suspension was allowed to stand at 4 °C for 4 h. The crude product was filtered off and washed with hexanes to yield a white solid (45.02 g).

This was taken up in 200 mL of chloroform and applied to a column of silica gel (1000 g of slurry packed with chloroform). The column was eluted with chloroform (2.5 L) followed by 2% methanol/chloroform (7 L). Fractions (250 mL) were collected with the product eluting in fractions 5−28. Fractions containing product were combined and the volatiles removed *in vacuo* to yield a total of 35.74 g (36.2 mmol, 65.8% yield) of compound **8**. The product was analyzed by reversed-phase HPLC (Nova-Pak column, 0−100% B/A over 10 min, 100% B for 10−15 min; solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/10% water) and found to be 98% pure with a $t_R$ of 10.24 min. FABMS indicated a molecular ion peak ($MH^+$) at 987 which corresponds to the molecular formula of $C_{56}H_{70}N_6O_{10}$ with a monoisotopic mass of 986.52.

**Fmoc-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe (9).** Fmoc-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe (35.24 g, 35.7 mmol) was dissolved in 50 mL of dry THF in a 1-L round bottom flask equipped with a stir bar. The atmosphere of the reaction mixture was flushed with argon, and diethylamine (50 mL) was added. The reaction mixture was stirred under an atmosphere of argon at room temperature for 1 h. The volatiles were removed *in vacuo* on the rotory evaporator, and the intermediate H-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe was taken up in 200 mL of acetonitrile. The solvent was again removed *in vacuo* on the rotory evaporator. The acetonitrile treatment was repeated twice more to yield an oil which was taken up in 400 mL of ethyl ether and 50 mL of ethyl acetate. Hexanes (400 mL) were added, and the resulting suspension was allowed to stand at 4 °C for 4 h. The crude intermediate was filtered off and washed with hexanes to yield a solid which was dried under high vacuum for 2 h.

This solid (24.0 g) was placed in a 1-L round bottom flask equipped with a magnetic stir bar and dissolved in 120 mL of anhydrous THF. The atmosphere of the reaction mixture was flushed with argon gas. *N*-α-Fmoc-*N*-α-methylphenylalanine, hydroxysuccinimide ester (15.64 g, 31.4 mmol) was added followed by the addition of diisopropylethylamine (10.9 mL, 62.8 mmol). The reaction mixture was stirred overnight under an atmosphere of argon and monitored by TLC (product $R_f =$ 0.63 in $CHCl_3$/MeOH/HOAc, 90:8:2, and 0.20 in EtOAc/hexane, 1:1).

At this time the reaction was judged to be complete, so the volatiles were removed *in vacuo* and the crude product was taken up in 500 mL of ethyl acetate. The organics were washed with 5% citric acid, saturated sodium bicarbonate, and saturated sodium chloride followed by drying over magnesium sulfate. Filtration and concentration of the filtrate *in vacuo* yielded an oil which was dissolved in 200 mL of ethyl ether with 30 mL of ethyl acetate added to totally solubilize the crude product. Hexanes (400 mL) were added to the solution of crude product, and the resulting suspension was allowed to stand at 4 °C for 4 h. The solid was filtered off and washed with hexanes to yield a white solid which was dried under high vacuum to yield crude product (34.06 g, 29.66 mmol) in 94.5% yield.

**Fmoc-hCys(Trt)-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe (10).** Fmoc-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe (33.30 g, 29.0 mmol) was dissolved in 50 mL of dry THF in a 1-L round bottom flask equipped with a stir bar. The atmosphere of the reaction mixture was flushed with argon, and diethylamine (50 mL) was added. The reaction mixture was stirred under an atmosphere of argon at room temperature for 1 h. The volatiles were removed *in vacuo* on the rotory evaporator, and the intermediate H-Phe(N-Me)-Tyr-(tBu)-D-Trp-Lys(Boc)-Val-OMe was taken up in 200 mL of acetonitrile. The solvent was again removed *in vacuo* on the rotory evaporator. The acetonitrile treatment was repeated twice more to yield an oil which was taken up in 400 mL of ethyl ether and 50 mL of ethyl acetate. Hexanes (400 mL) were added, and the resulting suspension was allowed to stand at 4 °C for 4 h. The crude intermediate was filtered off and washed with hexanes to yield a solid which was dried under high vacuum for 2 h.

In a separate 1-L round bottom flask equipped with a magnetic stir bar was placed Fmoc-hCys(Trt)OH (20.30 g, 33.6 mmol) which was dissolved in 70 mL of anhydrous THF. The atmosphere of the solution was flushed with argon gas, and the solution was cooled to −15 °C with an ice/acetone bath under an atmosphere of argon. Bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP-Cl; 10.3 g, 40.3 mmol) was added followed by the addition of diisopropylethylamine (7.02 mL, 40.3 mmol). The reaction mixture was stirred for 0.5 h at −15 °C under an atmosphere of argon. At this time the intermediate H-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe formed above was added followed by the addition of 20 mL of anhydrous THF and 7.02 mL (40.3 mmol) of diisopropylethylamine. The ice bath was removed, and the reaction mixture was stirred at room temperature until the reaction was complete by TLC analysis (product $R_f = 0.57$ in $CHCl_3$/MeOH/HOAc, 90:8:2, and 0.21 in EtOAc/hexane, 1:1).

At this time the volatiles were removed *in vacuo* and the crude product was taken up in 300 mL of ethyl acetate. The organics were washed with 5% citric acid, saturated sodium bicarbonate, and saturated sodium chloride followed by drying over magnesium sulfate. Filtration and concentration of the filtrate *in vacuo* yielded an oil which was dissolved in 400 mL of ethyl ether with 40 mL of ethyl acetate added to totally solubilize the crude product. Hexanes (400 mL) were added to the solution of crude product, and the resulting suspension was allowed to stand at 4 °C for 4 h. The solid was filtered off and washed with hexanes to yield a white solid which was dried under high vacuum to give crude product (42.8 g, 28.4 mmol) in 97.8% yield.

**H-hCys(Trt)-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OH (11).** Fmoc-hCys(Trt)-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val-OMe (43.7 g, 29.0 mmol) was dissolved in 60 mL of dry THF in a 1-L round bottom flask equipped with a stir bar. The atmosphere of the reaction mixture was flushed with argon, and diethylamine (50 mL) was added. The reaction mixture was stirred under an atmosphere of argon at room temperature for 1 h. The volatiles were removed *in vacuo* on the rotory evaporator, and the intermediate H-Phe(N-Me)-Tyr-

(tBu)-D-Trp-Lys(Boc)-Val-OMe was taken up in 400 mL of acetonitrile. The solvent was again removed *in vacuo* on the rotory evaporator. The acetonitrile treatment was repeated twice more to yield an oil which was taken up in 400 mL of ethyl ether and 30 mL of ethyl acetate. Hexanes (400 mL) were added, and the resulting suspension was allowed to stand at 4 °C for 4 h. The crude intermediate was filtered off and washed with hexanes to yield a yellow solid (37.02 g, 28.8 mmol) which was dried under high vacuum for 2 h.

The resulting intermediate H-hCys(Trt)-Phe(N-Me)-Trp-(tBu)-D-Trp-Lys(Boc)-Val-OMe (36.64 g, 28.5 mmol) was dissolved in 50 mL of dry THF in a 1-L round bottom flask. The atmosphere was flushed with argon gas, and a solution of 1.44 g of lithium hydroxide hydrate (34.2 mmol) dissolved in 10 mL of water was added. This was followed by the addition of another 80 mL of THF to achieve solution homogeneity. The reaction mixture was stirred under argon for 4 h at room temperature. Reversed-phase HPLC analysis (Nova-Pak column, 0−100% B/A over 10 min, 100% B for 10−15 min; solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/10% water) showed that the reaction was incomplete (methyl ester $t_R$ = 10.16 min, free carboxyl $t_R$ = 10.06), so an additional 0.94 g of lithium hydroxide (22.3 mmol) in 10 mL of water was added followed by the addition of another 50 mL of THF. The reaction mixture was stirred overnight at which time HPLC analysis indicated that the reaction was complete.

At this time the volatiles were removed *in vacuo* and the crude product was taken up in 400 mL of ethyl acetate. The organics were washed with 5% citric acid and saturated sodium chloride followed by drying over magnesium sulfate. Filtration and concentration of the filtrate *in vacuo* yielded an oil which was dissolved in 300 mL of ethyl ether with 50 mL of ethyl acetate added to totally solubilize the crude product. Hexanes (400 mL) were added to the solution of crude product, and the resulting suspension was allowed to stand at 4 °C for 4 h. The solid was filtered off and washed with hexanes to yield a white solid which was dried under high vacuum to yield 35.7 g (28.05 mmol) of crude product in 98.4% yield.

***cyclo*-[hCys(Trt)-Phe(N-Me)-Tyr(tBu)-D-Trp-Lys(Boc)-Val] (12).** H-hCys(Trt)-Phe(N-Me)-Trp(tBu)-D-Trp-Lys(Boc)-Val-OH (35.0 g, 27.52 mmol) was dissolved in 3500 mL of anhydrous dimethylformamide (DMF) in a dry 5-L round bottom flask. The atmosphere was flushed with argon gas, and 61.2 mL (27.54 mmol) of a 0.45 M solution of 1:1 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/*N*-hydroxybenzotriazole (HOBt) was added via syringe. The reaction mixture was stirred for 0.5 h at room temperature under an atmosphere of argon, and 9.60 mL (55.04 mmol) of diisopropylethylamine was added via syringe. The reaction mixture was stirred at room temperature until reversed-phase HPLC analysis (Nova-Pak column, 0−100% B/A over 10 min, 100% B for 10−15 min; solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/10% water) indicated that the starting material ($t_R$ = 10.06 min) had disappeared to be replaced by product ($t_R$ = 10.40 min).

At this time the DMF was removed in vacuo to yield crude product as an oil. This was taken up in 400 mL of ether and 50 mL of ethyl acetate. Hexanes (400 mL) were added, and the solution was left standing for 4 h. The solution was decanted from the resulting gum which was triturated with hexanes until the residue solidified. The solid was collected by filtration and washed with hexanes to yield crude product (33.8 g, 26.96 mmol) in 98% yield after drying under high vacuum overnight.

***cyclo*-[Homocysteinyl-*N*-methylphenylalanyl-tyrosyl-D-tryptophanyl-lysyl-valinyl] (13).** A deprotection cocktail consisting of 200 mL of trifluoroacetic acid, 10 mL of water, 5 mL of ethanedithiol, and 4 mL of triisopropylsilane was prepared and added to *cyclo*-[hCys(Trt)-Phe(N-Me)-Trp(tBu)-D-Trp-Lys(Boc)-Val] (33.5 g, 26.7 mmol) in a 1-L round bottom flask. The reaction mixture was stirred for 1 h at room temperature at which time the mixture turned from red to a light yellow. The deprotection mixture was added slowly with stirring to 1200 mL of cold ethyl ether in a 2-L Ehrlenmeyer flask. After addition was complete, the flask was sealed and left standing at 4 °C for 4 h to complete the precipitation of crude product.

The solid was collected by filtration and washed with cold ether to yield 29.72 g of crude product after drying under high vacuum overnight. The product was purified in 15 2.0-g portions. Therefore, 2.0 g of crude peptide **10** was dissolved in 10 mL of 0.1% TFA in 1:1 acetonitrile/water, and this solution was filtered through a 0.2-$\mu$m nylon filter. The filter was washed with an additional 2 mL of 0.1% TFA in 1:1 acetonitrile/water and the total filtrate (12 mL volume) loaded directly onto a 4 × 32.5 cm C18 Delta-Pak preparative HPLC column running in 0.1% TFA/water (solvent A) at 25 mL/min. After the crude product had been completely loaded, the flow rate was increased to 75 mL/min. After 5 min, the ratio of solvent B (0.1% TFA in 90% acetonitrile/10% water) was increased from 0% to 10%. After 5 min at 10% B, solvent B was increased from 10% to 20%. After 5 min at 20% B, solvent B was increased from 20 to 30%. After 5 min at 30% B, solvent B was increased linearly from 30% to 50% over 20 min. Solvent B was then increased to 100% and held at 100% for an additional 10 min.

Fractions were collected based on effluent monitoring at 230 nm. Collected fractions were evaluated by analytical HPLC (Delta-Pak column, 30−50% B/A over 20 min, 100% B for 20−25 min; solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/10% water). Fractions containing **13** ($t_R$ = 11.28 min) with a purity of ≥95% were pooled, the acetonitrile was evaporated in vacuo, and the final aqueous solution was lyophilized to yield solid **13** as the trifluoroacetate salt. Fractions containing peptide **13** with a purity of <95% were pooled and reprocessed using the same preparative HPLC conditions outlined above. Pure material from the initial purifications and the reprocessing purifications were combined to yield 4.85 g of **13** as the trifluoroacetate salt. FABMS indicated a molecular ion peak ($MH^+$) at 855 which corresponds to the molecular formula of $C_{45}H_{58}N_8O_7S_1$ with a monoisotopic mass of 854.4.

***N*-Chloroacetyl-glycyl-glycyl-*S*-tritylcysteinyl-lysine Amide (19).** Fmoc-protected Rink resin (0.25 mmol) was placed in the reaction vessel of an automated peptide synthesizer. The standard FastMoc protocol[27] was used, and appropriately protected amino acids were added sequentially until the desired sequence had been synthesized. Briefly, the N-terminal Fmoc was removed with 20% piperidine/DMF, and the resin or resin-supported peptide was washed with *N*-methylpyrrolidinone (NMP). Coupling was affected in NMP by reacting 4 equiv (1 mmol) of amino acid derivative with 4 equiv (1 mmol) of 1:1 HBTU/HOBt solution (0.45 M in DMF) and 8 equiv (2 mmol) of DIEA. After adding the activated amino acid to the reaction vessel, the resin was agitated for an appropriate amount of time (usually 40 min). The resin was then washed with additional NMP and any unreacted amine capped with excess acetic anhydride. The sequence of addition for the synthesis of **13** was Fmoc-Lys(Boc)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, and Fmoc-Gly-OH.

After the last amino acid in the sequence had been added, the N-terminal Fmoc protecting group was once again removed as indicated above and the resulting free amine reacted with excess chloroacetic anhydride, thus placing a chloroacetyl moiety at the N-terminus. After additional washes with NMP and then methylene chloride, the resin was transferred to a round bottom flask and 10 mL of trifluoroacetic acid/water (95:5) was added. The deprotection/cleavage mixture was stirred at room temperature for 1 h and filtered through a sintered glass funnel. The resin was washed with chloroform (3 × 10 mL) which was added to the filtrate. The red filtrate was concentrated *in vacuo* on the rotory evaporator, and the resulting red paste was taken up in 30 mL of chloroform. The volatiles were once again removed *in vacuo*. This chloroform treatment was repeated (usually about five times) until the resulting crude product was no longer red. This indicated all trace amounts of TFA had been removed with concomitant reattachment of the trityl group to the free sulfhydryl.

The crude product was taken up in a minimum amount of acetonitrile (about 1 mL), and the resulting solution was

diluted with 0.1% TFA/water until the acetonitrile was ca. 20% by volume. The solution was loaded onto a preparative reversed-phase HPLC column at a flow rate of 20 mL/min. The peptide was eluted as follows at 75 mL/min (solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/water): 0−5 min, 0% B/A; 5−10 min, 0−20% B/A; 10−30 min, 20−50% B/A; 30−34 min, 50% B/A. Fractions were collected while monitoring at 230 $\lambda$ and analyzed by analytical reversed-phase HPLC (20−50% B/A over 20 min; **19** had a $t_R$ of 18.25 min). Fractions containing pure product were combined, and the acetonitrile was removed *in vacuo* on the rotory evaporator. Lyophilization yielded **19** (82 mg) as the trifluoroacetate salt. ESMS indicated a molecular ion peak at 681 which corresponds to the molecular formula of $C_{34}H_{41}N_6O_5S_1Cl_1$ with a molecular mass of 681.25. Compounds **14**−**19**, **21**−**28**, and **32**−**35** were produced by the same protocol as outlined above.

***N*-Chloroacetyl-glycyl-glycyl-*S*-(4-methoxybenzyl)cysteinyl-arginine Amide (20).** The same FastMoc protocol described for the synthesis of **19** was used in the synthesis of **20** with the sequential addition of Fmoc-Arg(Pmc)-OH, Fmoc-Cys(Mob)-OH, Fmoc-Gly-OH, and Fmoc-Gly-OH. The N-terminus was once again capped as the chloroacetate. Cleavage of the peptide from the resin and removal of all protecting groups except the *S*-methoxybenzyl group protecting cysteine was affected by treatment with 10 mL of TFA, 0.5 mL of thioanisole, 0.5 mL of water, 0.25 mL of ethanedithiol, and 0.2 mL of triisopropylsilane. After stirring for 1.5 h, the cleavage mixture was filtered and the peptide precipitated by adding the cleavage mixture to 150 mL of cold ether; 90 mg of product as the trifluoroacetate salt was collected which was used as is in the next coupling reaction. FABMS indicated molecular ion peaks ($MH^+$) at 753 and 755 which corresponded to the molecular formula of $C_{37}H_{53}N_8O_9S_2Cl_1$ with a monoisotopic mass of 752.31. Compounds **29**−**31** were produced by the same protocol as outlined above.

***cyclo*-[*S*-(Acetyl-glycyl-glycyl-cysteinyl-lysine amide)-homocysteinyl-*N*-methylphenylalanyl-tyrosyl-D-tryptophanyl-lysyl-valinyl] (40).** **19** (62.9 mg, 0.079 mmol) and 66.7 mg (0.069 mmol) of **13** were taken up in 2.0 mL of acetonitrile and 2.0 mL of 0.15 M carbonate buffer containing ethylenediamine tetraacetic acid (EDTA) (the buffer was prepared by dissolving 12.6 g of sodium bicarbonate in 1 L of water and 5 mL of 0.1 M EDTA followed by the addition of 1 M sodium hydroxide until a pH of 10.0 was obtained as measured by a pH electrode). The reaction mixture was stirred under an atmosphere of argon overnight. The solvents were removed *in vacuo* on the rotory evaporator, and 10 mL of TFA containing 0.5 mL of water and 0.3 mL of triisopropylsilane was added to the resulting solid. The deprotection mixture was stirred for 1 h followed by removal of the volatiles on the rotory evaporator. The crude product was precipitated with 200 mL of cold diethyl ether, and the precipitated product was filtered and washed with cold ether.

The crude product was taken up in a minimum of acetonitrile and diluted with 0.1% TFA/water until the acetonitrile was ca. 20% by volume. The solution was loaded onto a preparative reversed-phase HPLC column at a flow rate of 5 mL/min. The peptide was eluted as follows at 75 mL/min (solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/water): 0−5 min, 0% B/A; 5−25 min, 20−50% B/A. Fractions were collected while monitoring at 230 $\lambda$ and analyzed by analytical HPLC (10−40% B/A over 20 min; **40** had a $t_R$ of 10.52 min). Fractions containing pure product were combined, and the acetonitrile was removed *in vacuo* on the rotory evaporator. Lyophilization yielded **40** (45 mg) as the trifluoroacetate salt. ESMS indicated a molecular ion peak at 1258 which corresponds to the molecular formula of $C_{60}H_{84}N_{14}O_{12}S_2$ with a molecular mass of 1257.5. Compounds **36**−**39**, **41**, **43**−**50**, and **54**−**57** were produced by the same protocol as outlined above and confirmed by ESMS analysis (see Supporting Information).

***cyclo*-[*S*-(Acetyl-glycyl-glycyl-cysteinyl-arginine amide)-homocysteinyl-*N*-methylphenylalanyl-tyrosyl-D-tryptophanyl-lysyl-valinyl] (42).** In a similar protocol to that outlined above for the synthesis of **40**, 78.0 mg (0.089 mmol) of **20** was coupled to 80 mg (0.082 mmol) of **13**. After stirring overnight at room temperature under an atmosphere of argon, the volatiles were removed *in vacuo* to dryness on the rotory evaporator. The crude intermediate was taken up in 15 mL of TFA, 1 mL of *m*-cresol, and 1 mL of boron trifluoride etherate and stirred for 1 h. After this time period, 200 mL of cold diethyl ether was added, and the precipitated product was filtered and washed with cold ether.

The crude product was taken up in a minimum of acetonitrile and diluted with 0.1% TFA/water until the acetonitrile was ca. 20% by volume. The solution was loaded onto a preparative reversed-phase HPLC column at a flow rate of 20 mL/min. The peptide was eluted as follows at 75 mL/min (solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/water): 0−5 min, 0% B/A; 5−25 min, 5−35% B/A. Fractions were collected while monitoring at 230 $\lambda$ and analyzed by analytical HPLC (20−50% B/A over 20 min; **42** had a $t_R$ of 13.1 min). Fractions containing pure product were combined, and the acetonitrile was removed *in vacuo* on the rotory evaporator. Lyophilization yielded **42** (67 mg) as the trifluoroacetate salt. ESMS indicated a molecular ion peak at 1285 which corresponds to the molecular formula of $C_{60}H_{84}N_{16}O_8S_2$ with a molecular mass of 1285.4. Compounds **51**−**53** were produced by the same protocol as outlined above and confirmed by ESMS analysis (see Supporting Information).

***cyclo*-[*S*-(Acetyl-glycyl-glycyl-cysteinyl-arginine amide)homocysteinyl-*N*-methylphenylalanyl-tyrosyl-D-tryptophanyl-lysyl-valinyl], Rhenium Oxide Complex (64).** **42** (30 mg, 18 $\mu$mol) and 19.1 mg (25 $\mu$mol) of $Bu_4NReOBr_4$ were taken up in 1.0 mL of anhydrous DMF under an atmosphere of argon and stirred overnight at room temperature. The DMF was removed *in vacuo* on the rotory evaporator, and the crude product was taken up in 1 mL of acetonitrile and diluted with 4 mL of 0.1% TFA/water. The solution was loaded onto a preparative reversed-phase HPLC column at a flow rate of 5 mL/min. The peptide was eluted as follows at 75 mL/min (solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in 90% acetonitrile/water): 0−5 min, 0% B/A; 5−25 min, 20−40% B/A. Fractions were collected while monitoring at 230 $\lambda$ and analyzed by analytical HPLC (20−50% B/A over 20 min; **64** had a $t_R$ of 14.1 min and was clearly differentiated from compound **42** in a coinjection experiment). Fractions containing pure product were combined, and the acetonitrile was removed *in vacuo* on the rotory evaporator. Lyophilization yielded **64** (9 mg) as the trifluoroacetate salt. ESMS indicated a molecular ion peak at 1485 which corresponds to the molecular formula of $C_{60}H_{80}N_{16}O_{13}S_2Re$ with a molecular mass of 1483.7. Compounds **58**−**63** and **65**−**79** were produced by the same protocol as outlined above and confirmed by ESMS analysis (see Supporting Information).

**SSTR Receptor-Binding Assay.** AR42J cells were cultured in Dulbecco's minimum essential medium supplemented with 10% FBS and 8 mM L-glutamine maintained in a humidified 5% $CO_2$ atmosphere at 37 °C in T-flasks. For subtype specific assays, the receptor sequences (human SSTR1, mouse SSTR3, human SSTR4) were transfected into CHO-DG44 cells. Harvested cells were homogenized in cold Tris buffer, and the homogenate was centrifuged at 39000*g*, 10 min, 4 °C. The pellet was washed once using the same buffer and then suspended in ice-cold 10 mM Tris, pH 7.7. The assay of the peptides/rhenium-labeled peptides involved incubating equal aliquots of cell membrane with [$^{125}$I][Tyr-11]SRIF[27] (Amersham Corp.; 2000 Ci/mmol, 0.05 nM, 750 000 cpm/mL) and peptide at a final concentration of $10^{-11}$−$10^{-6}$ M in 50 mM HEPES, pH 7.4, containing 1% BSA, fraction V, 5 mM $MgCl_2$, trasylol (200 kIU/mL), and phenylsulfonyl fluoride (0.02 mg/mL) for 25 min at 30 °C. Using a filtration manifold, the mixture was filtered through a poly(ethylenimine)-washed GF/C filter, and the residue was washed thrice with 5 mL of cold buffer. The pellet/filter and filtrate/washings were counted in a well counter. To assess nonspecific binding, the assay was run in the presence of 200 nM SRIF. The data provided inhibition constants via Hill plots.[21]

**Radiolabeling of Compounds 40−43, 45, and 57 with Technetium-99m.** Peptide (0.1 mg) was dissolved in 0.1 mL of 50% ethanol/water (compounds **40**, **41**, and **43**), 10% hydroxypropylcyclodextrin (compound **42**), or 0.9% saline

(compounds **45** and **57**). [$^{99m}$Tc]Glucoheptonate was prepared by reconstituting a glucoscan vial (E.I. DuPont de Nemours, Inc.; this kit contains stannous chloride as a reducing agent and glucoheptonate as a transfer ligand) with 1.0 mL of sodium [$^{99m}$Tc]pertechnetate (from a $^{99}$Mo/$^{99m}$Tc generator) containing up to 200 mCi. The vial was allowed to stand at room temperature for 15 min. A 25-$\mu$L aliquot of [$^{99m}$Tc]gluceptate from this vial was then added to the peptide solution above and the reaction allowed to proceed at room temperature for 15−30 min (for compounds **40**−**43**) or at 100 °C for 5 min (for compounds **45** and **57**). After filtration through a 0.2-$\mu$m filter, the purity of the $^{99m}$Tc-labeled peptide was assessed by analytical reversed-phase HPLC. Compounds **40**−**43** were analyzed at 0−100% B/A over 20 min using a Waters Delta-Pak C18 column, 5 $\mu$m, 39 × 150 mm. Compounds **45** and **57** were analyzed at 0−100% B/A over 10 min using a Waters Nova-Pak Radial Compression C18 column, 4 $\mu$m, 8 × 100 mm (A = 0.1% TFA in water, B = 0.1% TFA in 90% acetonitrile/water). Radioactive components were detected using an in-line radiometric detector linked to an integrating recorder. [$^{99m}$Tc]Gluceptate and sodium [$^{99m}$Tc]pertechnetate elute between 1 and 4 min under these conditions, whereas the $^{99m}$Tc-labeled peptides eluted later as one species (≥80%) as illustrated in Table 4. To compare technetium and rhenium complexes, a separate experiment was performed in which compound **42** was labeled with $^{99m}$Tc and analyzed at 20−50% B/A over 20 min using the same Delta-Pak column and eluents used above. A $t_R$ of 14.95 min was observed by radiometric detection. UV detection at 220 nM was also employed with no peak seen for $^{99m}$Tc-labeled **42** due to the very low concentrations of peptide used in the radiolabeling experiment. The sample was then spiked with the rhenium complex of **42** (compound **64**). After spiking, a radiometric $t_R$ of 14.85 min was observed for the $^{99m}$Tc complex of **42** with a nearly identical $t_R$ of 14.90 min observed at 220 nM for the rhenium complex. The chromatograms are presented as Supporting Information.

**Biodistribution Studies of Compounds 40−43, 45, 57, and $^{111}$In-Labeled 4 in Somatostatin Receptor-Positive Tumor-Bearing Rats.** The rat pancreatic tumor line CA20948 was obtained from frozen stock, prepared as a brie, and serially passaged in Lewis rats at Biomeasure. The line was passaged through the fourth generation after which the subsequent five passages had desirable SSTR expression levels averaging 80−100 fmol/mg of tumor. The tumor brie was placed in the lateral aspect of the right leg subcutaneously and allowed to grow to an average desired mass of 1−2 g (2−3 weeks).

The $^{99m}$Tc peptide preparations of compounds **40**−**43**, **45**, and **57** used in the animal studies resulted in solutions of 800 $\mu$g of peptide/mL with an activity of 20−30 mCi/mL. Dosing solutions were prepared by dilution of the parent stock at a ratio of 1:30 to obtain a dosing solution of 0.6−0.8 mCi/mL. The rats (mass range = 248−320 g) were administered $^{99m}$Tc-labeled peptides intravenously via the lateral tail vein at a dose volume of 1 mL/kg. The $^{111}$In complex of compound **4** was prepared as follows: $^{111}$In chloride was prepared in citrated acid (1 mL of 0.2 M HCl containing 25 mg of trisodium citrate) to a final activity of 2 mCi/mL. Compound **4** was dissolved in 0.9% saline at a concentration of 1 mg/mL, and 10 $\mu$L of this solution was added to the $^{111}$In in citrated acid and incubated at room temperature for 15 min. Doses of the $^{111}$In-labeled peptide were made by dilution of the stock solution to 1:20 (final concentration = 100 $\mu$Ci/mL) and delivering 1.0 mL/kg.

The animals were imaged 90 min after injection and then sacrificed. Blood, tumor, and other tissues were subsequently harvested at necropsy. Tissues were counted in a $\gamma$-well counter against dilutions of stock dosing solutions. Percent injected dose and percent injected dose per gram were calculated using appropriate decay corrections.

**Supporting Information Available:** Electrospray mass spectral data for compounds **36**−**79** and chromatograms from the coinjection of compound **64** with the $^{99m}$Tc complex of compound **42** (2 pages). Ordering information is given on any current masthead page.

## References


(1) Brazeau, P.; Vale, W.; Burgus, R.; Ling, N.; Butcher, M.; Rivier, J.; Guillemin, R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* **1973**, *179*, 77−79.

(2) Parmar, H.; Phillips, R. H.; Lightman, S. L. In *Somatostatin Analogues: Mechanism of Action: Peptides in Oncology*; Hffken, K., Ed.; Springer-Verlag: Heidelberg.

(3) (a) Rens-Domiano, S.; Law, S. F.; Yamada, Y.; Seino, S.; Bell, G. I.; Reisine, T. Pharmacological properties of two cloned somatostatin receptors. *Mol. Pharmacol.* **1992**, *42*, 28−34. (b) Yamada, Y.; Post, S. R.; Wang, K.; Tager, H. S.; Bell, G. I.; Seino, S. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 251−255. (c) Raynor, K.; Coy, D. C.; Reisine, T. Analogues of somatostatin bind selectively to brain somatostatin receptor subtypes. *J. Neurochem.* **1992**, *59*, 1241−1250.

(4) Lamberts, S. W. J.; Koper, J. W.; Reubi, J.-C. Potential Role of Somatostatin Analogues in the Treatment of Cancer. *Eur. J. Clin. Invest.* **1987**, *17*, 281.

(5) Lamberts, S. W. J.; Krenning, E. P.; Reubi, J.-C. The Role of Somatostatin and Its Analogs in the Diagnosis and Treatment of Tumors. *Endocrine Rev.* **1991**, *12*, 450−482.

(6) Wangberg, B.; Nilsson, O.; Theodørsson, E.; Dahlstrøm, A.; Ahlman, H. The effect of a somatostatin analogue on the release of hormones from human midgut carcinoid tumor cells. *Br. J. Cancer* **1991**, *64*, 23−28.

(7) Moreau, S. C.; Murphy, W. A.; Coy, D. H. Comparison of somatuline (BIM-23014) and somatostatin on endocrine and exocrine activities in the rat. *Drug. Dev. Res.* **1991**, *22*, 79−93.

(8) Bauer, W.; Briner, U.; Doepfner, W.; Haller, R.; Huguenin, R.; Marbach, P.; Petcher, T. J.; Pless, J. SMS 201-995: A potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci.* **1982**, *31*, 1133−1140.

(9) (a) Bakker, W. H.; Krenning, E. P.; Reubi, J.-C.; et al. [$^{111}$In-DTPA-D-Phe]- Octreotide, A Potential Radiopharmaceutical for Imaging of Somatostatin Receptor-Potsitive Tumors: Synthesis, Radiolabeling and *in vitro* Validation. *Life Sci.* **1991**, *49*, 1593−1601. (b) Krenning, E. P.; Kwekkeboom, D. J.; Bakker, W. H.; et al. Somatostain Receptor Scintigraphy with [$^{111}$In-DTPA-D-Phe$^1$]- and [$^{123}$I-Tyr$^3$]-Octreotide: The Rotterdam Experience with More Than 1000 Patients. *Eur. J. Nucl. Med.* **1993**, *20*, 716−731. Dörr, U.; Räth, U.; Sautter-Bihl, M.; Guzman, G.; Bach, D.; Adrian, H.; Bihl, H. Improved Visualization of Carcinoid Liver Metastases by Indium-111 Pentetreotide Scintigraphy Following Treatment with Cold Somatostatin Analogue. *Eur. J. Nucl. Med.* **1993**, *20*, 431−433.

(10) Paik, C. H.; Phan, L. N. B.; Hong, J. J.; et al. The Labeling of High Affinity Sites of Antibodies with $^{99m}$Tc. *Int. J. Nucl. Med. Biol.* **1985**, *12*, 3−8.

(11) Dean, R. T.; McBride, W.; Buttram, S. Technetium-99m labeled peptides for imaging. Patent WO 93/10747, 1993.

(12) Veber, D. F. In *Peptides - Synthesis-Structure-Function: Proceedings of the Seventh American Peptide Symposium*; Rich, D., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; pp 685−694.

(13) Veber, D. F.; Saperstein, R.; Nutt, R. F.; Freidinger, R. M.; Brady, S. F.; Curley, P.; Perlow, D. S.; Paleveda, W. J.; Colton, C. D.; Zacchei, A. G.; Tocco, D. J.; Hoff, D. R.; Vandlen, R. L.; Gerich, J. E.; Hall, L.; Mandarino, L.; Cordes, E. H.; Anderson, P. S.; Hirschmann, R. A super active cyclic hexapeptide analog of somatostatin. *Science* **1984**, *34*, 1371−1378.

(14) Spanevello, R. A.; Hirschmann, R.; Raynor, K.; Reisine, T.; Nutt, R. Synthesis of Novel, Highly Potent Cyclic-Hexapeptide Analogs of Somatostatin. Potential Application of Orthogonal Protection for Affinity Chromatography. *Tetrahedron Lett.* **1991**, *32*, 4675−4678.

(15) van der Auwera, C.; Anteunix, M. J. O. N,N′-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl); a superb reagent for coupling at and with iminoacid residues. *Int. J. Pept. Protein Res.* **1987**, *29*, 574−588.

(16) Courtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. O-Benzotriazolyl-N,N,N′,N′-tetramethyluronium Hexafluorophosphate as Coupling Reagent for the Synthesis of Peptides of Biological Interest. *Synthesis* **1984**, 572−574.

(17) (a) Pearson, D. A.; Baker, M. L.; Stone, C. A. Trialkylsilanes as scavengers in peptide synthesis. *Tetrahedron Lett.* **1989**, *30*, 2739−2742. (b) Chan, W. C.; Bycroft, B. W. Deprotection of Arg(Pmc)-containing peptides using TFA-trialkylsilane-methanol-EMS: Application to the synthesis of a prepeptide of nisin. In *Peptides (Proc. 12th Am. Peptide Symp., June 16−21, 1991)*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, 1992; pp 613−614.

(18) Bodansky, M. *Principles of peptide synthesis*, 2nd ed.; Springer-Verlag: Berlin, 1993; pp 1−329.

(19) (a) Rink, H. Solid-phase synthesis of protected peptide fragments using a trialkoxydiphenyl-methyl ester resin. *Tetrahedron Lett.* **1987**, *28*, 3787−3790. (b) Story, S. C.; Aldrich, J. V. Preparation of protected peptide amides using the Fmoc chemical protocal. *Int. J. Pept. Protein Res.* **1992**, *39*, 87−92.

(20) Cotton, F. A.; Lippard, S. Chemical and structural studies of rhenium (V) oxyhalide complexes. II. M[$ReX_4O$] and M[$ReX_4$-OL] complexes from $KReO_4$. *J. Inorg. Chem.* **1966**, *5*, 9−16.

(21) Bylund, D. B.; Yamamura, H. I. In *Methods in Neurotransmitter Receptor Analysis*; Yamamura, H. I., et al., Eds.; Raven Press: New York, 1990; pp 1−35.

(22) An example of a standard kit formulation would be one in which $^{99m}TcO_4$ (from a $^{99}Mo/^{99m}Tc$ generator) is added to a mixture of peptide to be labeled, reducing agent (usually $SnCl_2$), transfer ligand, and buffer. After a short (15−30 min at room temperature or 5 min at 100 °C) incubation period, the kit is assayed for $^{99m}$Tc-labeled peptide and pertechnetate content. The solution of radiolabeled peptide is then used directly.

(23) Thomas, R. W.; Estes, G.; Edler, C.; Deutsch, E. Technetium radiopharmaceutical development. 1. Synthesis, characterization, and structure of dichloro[hydrotris(1-pyrazolyl)borato]-oxotechnetium (V). *J. Am. Chem. Soc.* **1979**, *101*, 4581−4185.

(24) (a) Kasina, S.; Fritzberg, A. R.; Johnson, D. L.; Eshima, D. Tissue distribution of technetium-99m-diamide dimercaptide complexes and potential use as renal pharmaceuticals. *J. Med. Chem.* **1986**, *29*, 1933−1940. (b) Fritzberg, A. R.; Kasina, S.; Eshima, D.; Johnson, D. L. Synthesis and biological evaluation of technetium-99m $MAG_3$ as a hippuran replacement. *J. Nucl. Med.* **1986**, *27*, 111−116.

(25) Stanfield, C. F.; Felix, A. M.; Danho, W. The Preparation of N-a-t-Boc-2,3-Diaminopropionic acid (DPR) and of N-a-t-Boc-2,4-Diaminobutyric Acid Derivatives Suitable for Solid-phase Peptide Synthesis. *Org. Prep. Proc. Int.* **1990**, *22*, 597−603.

(26) *User's Manual for the Applied Biosystems Model 431A Peptide Synthesizer*, Version 2.00; January 1992; pp 3−50.

(27) Taylor, J. E.; Coy, D. H.; Moreau, J.-P. High Affinity Binding of [$^{125}$I-Tyr$^{11}$]Somatostatin-14 to Human Small Cell Lung Carcinoma (NCI-H69). *Life Sci.* **1988**, *43*, 421−427.

JM950111M