New $sst_{4/5}$-Selective Somatostatin Peptidomimetics Based on a Constrained Tryptophan Scaffold

Debby Feytens,[†] Renzo Cescato,[‡] Jean Claude Reubi,[‡] and Dirk Tourwé*,[†]

Department of Organic Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050, Brussels, Belgium, and Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Berne, Switzerland

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The synthesis and biological evaluation of four peptidomimetic analogs of somatostatin based on a constrained Trp residue, 3-amino-indolo[2,3-*c*]azepin-2-one (Aia), are reported. It is shown that dipeptidomimetics with a D-Aia-Lys sequence, functionalized with *N*- and *C*-terminal aromatic substituents, display a good selectivity for both sst_4 and sst_5. This study allowed us to identify a new highly potent sst_5 agonist with good selectivity over the other receptors, except versus sst_4.

Introduction

Somatostatin (SRIF[a], H-Ala[1]-Gly[2]-[Cys[3]-Lys[4]-Asn[5]-Phe[6]-Phe[7]-Trp[8]-Lys[9]-Thr[10]-Phe[11]-Thr[12]-Ser[13]-Cys[14]]-OH), **1**, was originally isolated from mammalian hypothalamus as a potent inhibitor of growth hormone secretion.[1] It is now known that it is widely distributed throughout the endocrine and central nervous systems and peripheral tissues. SRIF has multiple functions, such as modulation of growth hormone, insulin, glucagon, and gastric acid secretion.[2−4] The effects of SRIF are mediated by five G protein-coupled receptors, termed sst_{1-5}.[5] Structure−activity relationship (SAR) studies on a large number of SRIF analogs revealed that the Phe[7]-Trp[8]-Lys[9] sequence is critical for biological recognition.[6,7] Several cyclic hexa- and octapeptide analogs containing a D-Trp[8]-Lys[9] sequence (numbering of the residues follows that of native SRIF) have been developed,[8,9] including octreotide **2** (Figure 1), which is clinically used for the treatment of endocrine tumors and acromegaly.[10,11] Extensive structural studies by NMR[6,12] and also by X-ray diffraction[13] indicated that a type-II′ β-turn conformation with D-Trp[8] and Lys[9] at the i+1 and i+2 position, respectively, was present. A close proximity of the D-Trp[8] side chain to that of Lys[9], as indicated by a high field shift of the Lys $C^{\gamma}H_2$ in the NMR spectra, was a requisite for high potency.[14] Despite the successful clinical development of small cyclic SRIF analogs such as octreotide, lanreotide, and vapreotide,[15,16] extensive research toward nonpeptide analogs that are selective for each receptor subtype was carried out over the past decade.[17−19] The critical side chains of the Phe[7]-Trp[8]-Lys[9] sequence were displayed successfully on a variety of nonpeptide scaffolds **3**−**8** (Figure 1).[17,18,20−25]

The Merck group used the SRIF pharmacophore model to select members of a library that was obtained by derivatizing privileged structures with capped amino acids. Screening of a library of only seventy-five members identified L-264,930 **9** (Figure 2), which was further optimized by using the D-Trp stereochemistry and extending the amine chain length to that of Lys, which resulted in a potent and sst_2 selective ligand.[26−28]

This lead structure was used to build further libraries that were screened for affinity to all of the five SRIF receptor subtypes.[26] Selective peptidomimetics for each of the receptor subtypes were found. Our attention was drawn by the series of analogs containing a β-MeTrp residue. Whereas the (2*R*,3*S*) isomer **10** was a potent and selective sst_2 ligand, the (2*R*,3*R*) isomer was much less potent.[28] This is in agreement with the requirement for a *trans*-conformation of χ_1 of the Trp sidechain, which is favored by the (3*S*) methyl substituent.[27−29]

We have recently developed a synthesis of a new type of Trp analog in which the side chain conformation is fixed by formation of a seven-membered ring (Figure 3).[30] The resulting 3-amino-indolo[2,3-*c*]azepin-2-one (Aia) constrains χ_1 to trans and gauche(+), but χ_2 is also fixed ($\pm 5°$ and $\pm 70°$).

We now report the use of this new scaffold in the design of novel receptor selective SRIF peptidomimetics.

Results

Synthesis. The conformationally constrained D-Trp-Lys analogs were prepared as shown in Scheme 1.

Boc-2′-formyl-D-tryptophan **11** was prepared by SeO_2 oxidation of 2-(*tert*-butoxycarbonyl)-2,3,4,9-tetrahydro-1*H*-beta-carboline-3-carboxylic acid (Boc-D-Tcc).[30] Reductive amination with mono-Fmoc-protected diaminopentane and $NaBH_3CN$, immediately followed by cyclization using EDC/pyridine, yielded **14**. Although the cyclization reaction was very slow (2−3 weeks), no side compounds were formed and the final compounds were obtained in very pure form. After Boc-deprotection, a coupling with phenylacetic acid or 3,3′-diphenylpropionic acid was performed leading to **16** and **17**. Final removal of the Fmoc protecting group with 1% 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) in DMF in the presence of dithiothreitol (DTT)[31] gave the SRIF mimetics **20** and **21**, which were purified by semipreparative HPLC. Similarly, aldehyde **11** was reacted with Lys(Cbz)NHBn and $NaBH_3CN$ to yield **13**, which was cyclized to the dipeptide mimetic Boc-D-Aia-Lys(Cbz)-NHBn **15** in 30% overall yield. The latter was transformed into the phenylacetyl and diphenylpropionyl amides, as described above, and the resulting compounds **22** and **23** were also purified by HPLC.

Determination of Somatostatin Receptor Affinity Profiles. The compounds were tested for their ability to bind to the five human sst receptor subtypes in complete displacement experiments using the universal SRIF radioligand [^{125}I]-[Leu[8], D-Trp[22], Tyr[25]]-SRIF-28. CHO-K1 and CCL39 cells stably expressing the human sst_1−sst_5 receptors were grown as described

* To whom correspondence should be addressed. Tel.: 32-2-629-3295. Fax: 32-2-629-3304. E-mail: datourwe@vub.ac.be.

[†] Vrije Universiteit Brussel.

[‡] University of Berne.

[a] Abbreviations: SRIF, somatostatin; Aia, 3-amino-indolo[2,3-*c*]azepin-2-one; DTT, dithiothreitol; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; NMM, *N*-methylmorpholine; IBMX, 3-isobutyl-1-methylxanthine; Boc-D-Tcc, 2-(*tert*-butoxycarbonyl)-2,3,4,9-tetrahydro-1*H*-beta-carboline-3-carboxylic acid

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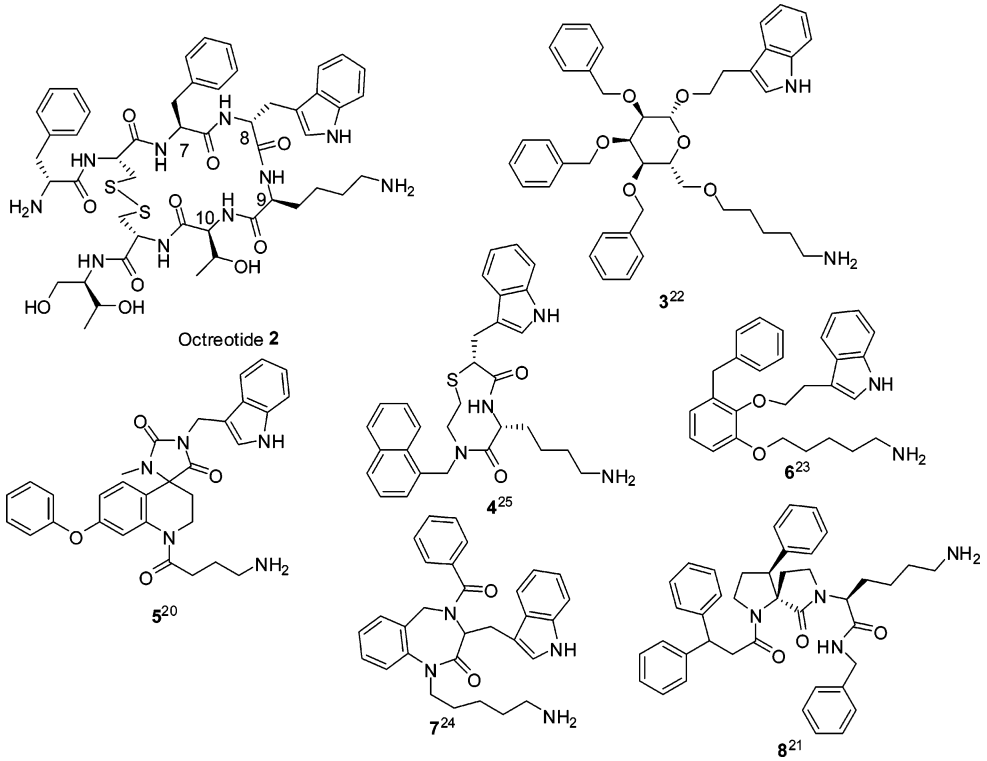


Figure 1. Structure of octreotide and of various SRIF mimetics.

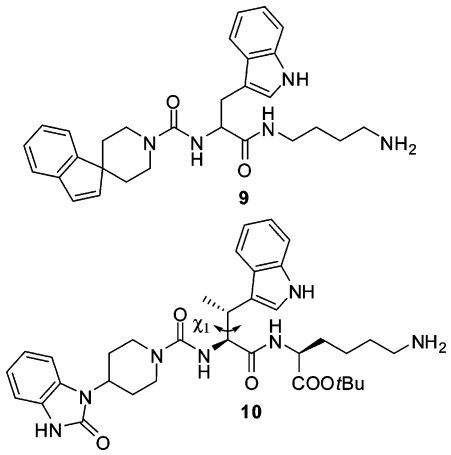


Figure 2. Structure of SRIF mimetics developed by Merck.

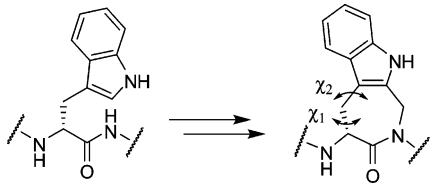


Figure 3. Constraining the tryptophan residue by cyclization.

previously.[32] Cell membrane pellets were prepared and receptor autoradiography was done as described in detail previously.[32] SRIF-28 was run in parallel as control. IC_{50} values were calculated after quantification of the data using a computer-assisted image processing system. The data are shown in Table 1.

Effect on cAMP Formation. The effect of the sst_5 selective analog **22** on forskolin-stimulated cAMP formation was performed on sst_5-transfected CHO-K1 cells, as described previously.[33,34] cAMP formation was determined using a scintillation proximity assay (SPA) system. cAMP data were expressed as percentages of stimulation over the nonstimulated level. The EC_{50} value of 13.0 ± 4.2 nM was derived from concentration−response curves of three independent experiments.[33,34]

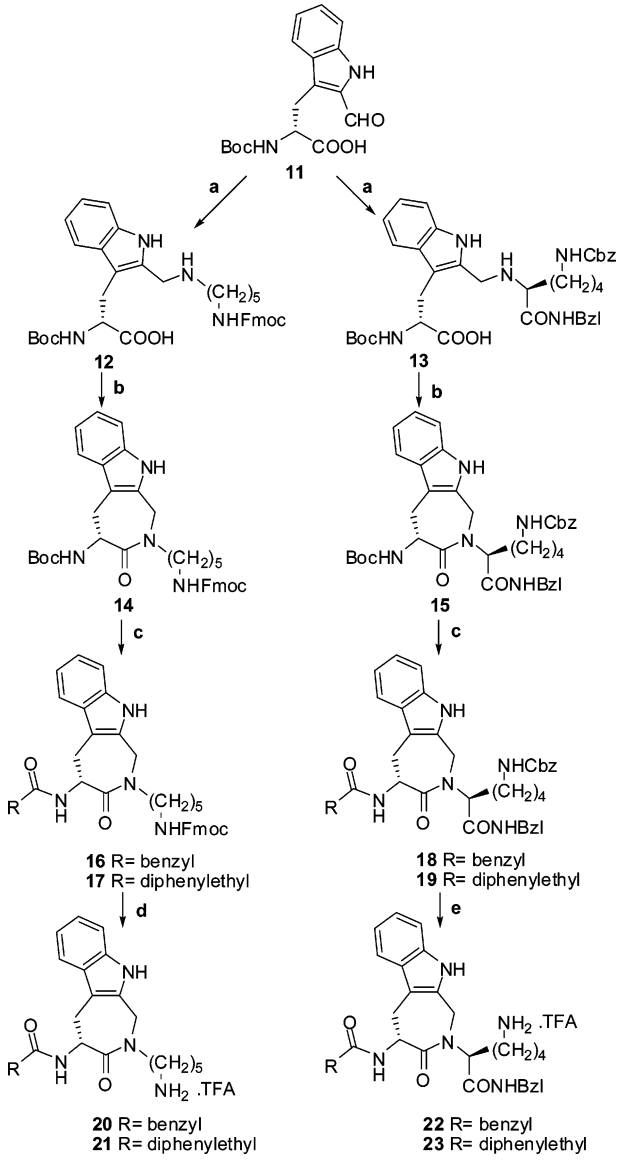
NMR Study. The two most potent analogs **22** and **23** were studied by ^1H NMR and ^{13}C NMR spectroscopy. All signals were assigned using DEPT, HMQC, HMBC, and COSY spectra and NOEs were detected using NOESY spectra. The temperature dependence of the amide NH signals was studied in DMSO solution, and NH signals in $CDCl_3$ and DMSO were compared for compounds **22** and **23**. The obtained data are collected in Table 2.

The large temperature and solvent dependence of the amide protons does not support the formation of a β-turn, which is stabilized by the presence of an intramolecular hydrogen bond.

The Lys $C^{\gamma}H_2$s are found at $\delta = 0.59$ and 1.05 ppm for **18** and at $\delta = 0.57$ and 0.96 ppm for **19**, which are upfield compared to the normal value (1.38 ppm for an inactive analog[35]).

Discussion. The fixation of the tryptophan side chain in the D-Trp-Lys sequence by formation of an indolo[2,3-*c*]azepin-2-one ring system and attachment of aromatic substituents at the *N*- and *C*-terminus provided new SRIF mimetics with a high affinity for sst_4 and sst_5. These compounds also have moderate affinity for sst_2. Compounds lacking the lysine carboxylic amide were less potent and displayed a slight selectivity for sst_4, whereas compound **22** containing the lysine benzylamide displayed low nM affinity for both sst_4 and sst_5. Analog **23** is less potent than **22** but has a better sst_5 selectivity. The 1,5-diaminopentane chain was selected because it has the same length as the lysine side chain and it was shown to provide the optimal distance between the amino groups for sst_2 affinity.[27] Potency was better for the phenylacetyl *N*-terminal analogs than for the diphenylpropionyl analogs. These aromatic substituents were introduced to target the receptor pockets occupied by Phe[6] and Phe[10] of the native ligand. The most potent analog **22** has

Scheme 1. Synthesis of **20**−**23**[a]



[a] Reagents and conditions: (a) RNH_2, CH_2Cl_2, NMM, pH 6, $MgSO_4$, $NaBH_3CN$, 2 h; (b) EDC, pyridine, CH_3CN/H_2O 1:1, high dilution, 2 to 3 weeks; (c) (i) 0.5% H_2O in TFA/CH_3CN 2:1, 1 h; (ii) RCOOH, EDC, CH_2Cl_2, NEt_3, 18 h; (d) 1% DBU in DMF, DTT, THF, 2 h; (e) H_2, Pd/C, EtOH, 18 h.

an IC_{50} value of 1.2 nM for the sst_5 and behaves as a full agonist in the forskolin-stimulated cAMP assay with an EC_{50} value of 13.0 nM.

A type II′ β-turn conformation around the D-Trp-Lys residues is present in most sst_{2-5} subtype selective peptide analogs. An NMR study of **22** and **23** indicated that these compounds do not adopt a type-II′ β-turn conformation. This observation is in agreement with previous findings that this type of amino-azepinones do not adopt turn conformations but rather prefer an extended conformation.[36] However, the upfield position of the Lys $C^{\gamma}H_2$ (0.59 and 0.57 ppm), which is ascribed to a close proximity of the Trp and Lys side chains in the peptidic analogs and which correlates with high receptor affinity, is also observed here. The corresponding chemical shift of the γ-methylene in L-054,522 **10** is 0.7 ppm.[28]

Conclusions. The conformational constraint of the D-Trp residue in the Aia ring system has proven to be a successful approach to obtain higly potent sst_4/sst_5 selective peptidomimetics. Apparently this type of conformational constraint of χ_1, but also of χ_2, is a basis for differentiation between sst receptor subtypes and is particularly effective for the sst_5 and sst_4. This allowed us to identify analog **22** as a highly potent sst_5 agonist with good selectivity over the other receptors, except versus sst_4. The potential therapeutic utility of selective agonists for sst_4, which is expressed in the lung, or for sst_5, which is expressed in the lymphoid system, in pancreatic beta cells, and in corticotroph adenoma cells has been mentioned.[19] The efficient synthetic method allows easy modifications of the metabolically stable peptidomimetic. Thus, further improvement can be expected for modifications of the *N*- and *C*-terminal substituents. Work along these lines is currently in progress.

Experimental Section

General. Mono-Fmoc-protected diaminopentane and Boc-Lys-(ϵ-Cbz)OH were purchased from Fluka (Bornem, Belgium). Lys-(ϵ-Cbz)NHBzl was prepared using the standard procedures for amide formation and Boc-deprotection.[37] Boc-2′-formyl tryptophan **11** was synthesized as described previously.[30]

Thin layer chromatography (TLC) was performed on plastic sheet precoated with silica gel $60F_{254}$ (Merck, Darmstadt, Germany) using specified solvent systems. Mass Spectrometry (MS) was recorded on a VG Quattro II spectrometer using electrospray (ESP) ionization (positive or negative ion mode). Data collection was done with Masslynx software. Analytical RP-HPLC was performed using an Agilent 1100 Series system (Waldbronn, Germany) with a Supelco Discovery BIO Wide Pore (Bellefonte, PA) RP C-18 column (25 cm × 4.6 mm, 5 μm) using UV detection at 215 nm. The mobile phase (system 1, water/acetonitrile; system 2, water/methanol) contained 0.1% TFA. The standard gradient consisted of a 20 min run from 3 to 97% acetonitrile (system 1) or methanol (system 2) at a flow rate of 1 mL/min. Preparative HPLC was performed on a Gilson apparatus and controlled with the software package Unipoint. The reverse phase C18-column (Discovery BIO Wide Pore 25 cm × 21.2 mm, 10 μm) was used under the same conditions as the analytical RP-HPLC, but with a flow rate of 20 mL min^{-1}. Nuclear magnetic resonance (NMR): ^1H NMR and ^{13}C NMR spectra were recorded at 250 and 63 MHz, respectively, on a Bruker Avance 250 spectrometer or at 500 and 125 MHz on a Bruker Avance II 500. Calibration was done with TMS (tetramethylsilane) or residual solvent signals as an internal standard. The solvent used is mentioned in all cases and the abbreviations used are s (singlet), d (doublet), dd (double doublet), t (triplet), q (quadruplet), br s (broad singlet), m (multiplet), cad (diaminopentane protons), Lys (Lysine protons), azep (azepinone protons), arom (aromatic protons). Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectral data were obtained using an Avatar 370 FT-IR.

All culture reagents were supplied by Gibco BRL, Life Technologies (Grand Island, NY). Tissue standards (autoradiographic [^{125}I] microscales) were purchased from GE Healthcare, Little Chalfont, Buckinghamshire, U.K. cAMP accumulation was determined using a commercially available cAMP scintillation proximity assay (SPA) system (RPA538, GE Healthcare, Little Chalfont, Buckinghamshire, U.K.) The EC_{50} values were calculated using GraphPad Prism, version 4.0, by nonlinear regression analysis of dose response curves.

Synthesis of 12 and 13. Procedure for the Reductive Amination of 11. Aldehyde **11** (0.332 g, 1 mmol) was suspended in CH_2Cl_2 (puriss. p.a. 15 mL) and amine (hydrobromic or trifluoroacetic acid salt, 1.05 mmol, 1.05 equiv) was added. The pH was adjusted to 6 with *N*-methyl-morpholine, followed by the addition of $MgSO_4$ (20 wt %) and $NaCNBH_3$ (2.5 mmol, 2.5 equiv). The reaction was monitored with HPLC or TLC. When the reaction was completed (typically 2 h), the solvent was evaporated and the crude product was used in the cyclization reaction.

Synthesis of 14 and 15. General Procedure for the Cyclization of 12 and 13. Crude **12** or **13** was dissolved in acetonitrile/water 1:1 (80 mL). Pyridine (2 mmol, 2 equiv) was added, and the reaction mixture was cooled to 0 °C for 10 min and EDC (1.1 mmol, 1.1

Table 1. Receptor Affinities of the SRIF Peptide Mimetics

No	IC_{50}[a] (nM)				
	sst_1	sst_2	sst_3	sst_4	sst_5
SS-28	2.4 ± 0.4 (4)	2.5 ± 0.2 (4)	2.8 ± 0.3 (4)	2.3 ± 0.2 (4)	2.8 ± 0.4 (4)
20	>1000 (2)	>1000 (2)	>1000 (2)	75 ± 2 (2)	245 ± 124 (2)
21	>1000 (2)	357 ± 96	>1000 (2)	129 ± 12 (2)	318 ± 177 (2)
22	233 ± 74 (3)	83 ± 2 (3)	251 ± 16 (3)	3.3 ± 0.2 (3)	1.2 ± 0.15 (3)
23	89 ± 2 (3)	103 ± 12 (3)	694 ± 19 (3)	73 ± 8 (3)	8 ± 0.87 (3)

[a] The number of independent repetitions to obtain the mean values ± SEM are indicated between brackets.

Table 2. Solvent and Temperature Dependence of the Amide Protons in **22** and **23**

No		$\Delta\delta$ $CDCl_3 \rightarrow$ DMSO (ppm)	$\Delta\delta/\Delta T$ (ppb/K)
22	NHBzl	1.54	−5.7
	NHCOR	1.33	−5.6
23	NHBzl	1.83	−5.5
	NHCOR	1.53	−6.2

equiv) was added. After 1 h of stirring at 0 °C, the reaction was continued at rt. The reaction was monitored using HPLC, and 1 to 5 equiv of EDC and pyridine were added until the reaction was complete (2 to 3 weeks). Acetonitrile was evaporated and EtOAc (100 mL) was added. The layers were separated and the organic phase was then washed with 1 M HCl (3 × 30 mL), saturated aqueous $NaHCO_3$ (3 × 30 mL), and brine (3 × 20 mL). The organic layer was dried over $MgSO_4$. After filtration and evaporation, the crude product was purified by flash column chromatography (CH_2Cl_2−EtOAc). Yield (**14** from **11**) = 46%. Yield (**15** from **11**) = 30%.

Procedure for the Boc-Deprotection of 14 and 15. Boc-protected cyclic product (1.0 mmol) was dissolved in a solution of 5% water in TFA (10 mL) and acetonitrile was added (5 mL). The reaction was stirred for 1 h at room temperature. After completion of the reaction, the mixture was evaporated and the crude TFA-salt was used in the coupling reaction.

Synthesis of 16, 17, 18, and 19. General Procedure for the Amide Formation. To a solution of TFA-salt (0.35 mmol) in CH_2Cl_2 (10 mL) were added phenyl acetic acid (53 mg, 0.39 mmol, 1.1 equiv) or 3,3′-diphenyl propionic acid (88 mg, 0.39 mmol, 1.1 equiv), NEt_3 (54 μL, 0.39 mmol, 1.1 equiv), and EDC·HCl (74 mg, 0.39, 1.1 equiv). The reaction was stirred at room temperature overnight and then washed with HCl (1 M, 3 × 10 mL), saturated $NaHCO_3$ (3 × 10 mL), and brine (3 × 10 mL). The organic layers were combined and dried over $MgSO_4$. After filtration and evaporation, the crude compounds were purified by flash column chromatography (CH_2Cl_2−EtOAc). Yield (**16** from **14**) = 71%. Yield (**17** from **14**) = 76%. Yield (**18** from **15**) = 62%. Yield (**19** from **15**) = 52%.

Synthesis of 20 and 21. General Procedure for the Fmoc-Deprotection. Fmoc-protected product (0.23 mmol) was dissolved in THF (25 mL). DTT (0.35 g, 2.3 mmol, 10 equiv) and DBU in DMF (1% solution, 100 μL, 0.007 mmol, 0.03 equiv) were added, and the mixture was stirred at room temperature for 4 h. The reaction mixture was evaporated and the crude compound was purified by semipreparative RP-HPLC. Yield (**20**) = 35%. Yield (**21**) = 76%.

Synthesis of 22 and 23. General Procedure for the Cbz-Deprotection. Cbz-protected product (0.17 mmol) was dissolved in EtOH (15 mL) and Pd/C (30 wt %) and HCl (17 μL, 0.17 mmol, 1 equiv) were added. The mixture was hydrogenated in a Parr apparatus at 30 psi of H_2 overnight. The catalyst was filtered off and the mixture was evaporated. The crude product was purified by semipreparative RP-HPLC. Yield (**22**) = 50%. Yield (**23**) = 55%.

Determination of Somatostatin Receptor Affinity Profiles. CHO-K1 and CCL39 cells stably expressing human sst_1−sst_5 receptors were grown as described previously.[32] Cell membrane pellets were prepared and receptor autoradiography was done on 20 μm thick pellet sections (mounted on microscope slides), as described in detail previously.[32] For each of the tested compounds, complete displacement experiments were done with the universal SRIF radioligand [^{125}I]-[Leu^8, D-Trp^{22}, Tyr^{25}]-SRIF-28 using increasing concentrations of the unlabeled compounds ranging from 0.1 to 1000 nmol/L. SRIF-28 was run in parallel as control using the same increasing concentrations. IC_{50} values were calculated after quantification of the data using a computer-assisted image processing system. Tissue standards containing known amounts of isotopes, cross-calibrated to tissue-equivalent ligand concentrations, were used for quantification.[32]

Effect on cAMP Formation. The effect of **22** on forskolin-stimulated cAMP formation was performed on CHO-K1 cells stably expressing sst_5, as described previously.[33,34] sst_5-Expressing cells were subcultured in 96-well culture plates at 2 × 10^4 cells per well and grown for 24 h. Culture medium was removed from the wells and fresh medium (100 μL) containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) was added to each well. Cells were incubated for 30 min at 37 °C. Medium was then removed and replaced with fresh medium containing 0.5 mM IBMX, with or without 10 μM forskolin and various concentrations of compounds. Cells were incubated for 30 min at 37 °C. After removal of the medium, cells were lysed and cAMP formation was determined using a commercially available cAMP scintillation proximity assay (SPA) system, according to the instructions of the manufacturer (RTA538, GE Healthcare, Little Chalfont, U.K.). cAMP data were expressed as percentages of stimulation over the nonstimulated level. The EC_{50} values (the agonist concentration causing 50% of its maximal effect) were determined using GraphPad Prism, version 4.0, by nonlinear regression analysis of dose response curves.[33,34] The experiment was performed three times. Each concentration point represents the mean of triplicate wells.

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Supporting Information Available: Characterizations of compounds **14**−**23**. HPLC spectra of compounds **20**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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