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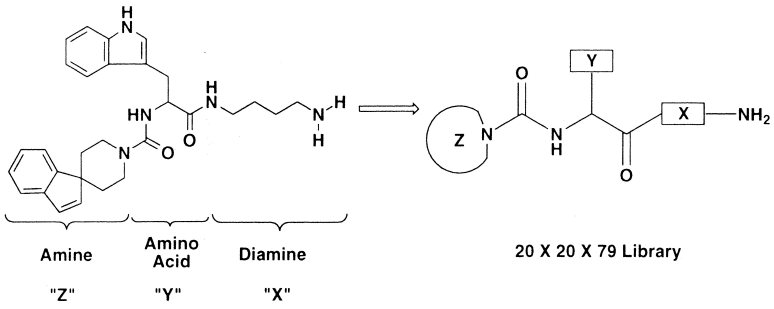

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A Combinatorial Approach toward the Discovery of Non-Peptide, Subtype-Selective Somatostatin Receptor Ligands

Scott C. Berk,*,† Susan P. Rohrer,*,‡ Sylvia J. Degrado,† Elizabeth T. Birzin,‡ Ralph T. Mosley,† Steven M. Hutchins,† Alexander Pasternak,§ James M. Schaeffer,‡ Dennis J. Underwood,† and Kevin T. Chapman†

Department of Molecular Design and Diversity, R123-242, Department of Biochemistry and Physiology, R80T-136, and Department of Medicinal Chemistry, R50G-336, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

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The tetradecapeptide somatostatin is widely distributed throughout the body and is thought to be involved with a variety of regulatory functions. Recently, five human somatostatin receptors (hSSTR1-5) have been cloned and characterized. Several selective peptidal agonists of the hSSTR receptors are known, and we sought to apply this information to the design of novel non-peptide small molecule ligands for each receptor. Initial computational methods identified a 200 nM murine SSTR2 active compound via a database search of our sample collection. A combinatorial library was designed around the structural class of the compound with the goal of rapidly developing this initial lead into the desired subtype-selective small molecules in order to characterize the pharmacology of each of the receptor subtypes. The library was synthesized using the resin-archive, iterative deconvolution format. The total number of unique compounds in the library was expected to be 131 670, present in 79 mixtures of 1330 or 2660 compounds per mixture. Through sequences of screening and mixture deconvolution, the components of selective and highly active (K_i = 50 pM to 200 nM) non-peptide small molecule ligands for somatostatin subtypes 1, 2, 4, and 5 were identified. In addition to discovering compounds with the desired activity and selectivity, useful structure/activity information was generated which can be used in the design of new compounds and second-generation combinatorial libraries.

Introduction

Somatostatin is a widely distributed tetradecapeptide[1−3] with multiple functions including modulation of secretion of growth hormone, insulin, glucagon, and gastric acid.[3−7] The mechanism of action of somatostatin is mediated via a high affinity membrane associated receptor. Recently, five human somatostatin receptors (hSSTR1-5) have been cloned and characterized.[8−11] All five receptors are members of the G-protein coupled receptor family.[12] Structure−function studies with a large number of peptidal analogues have shown that the Trp[8]-Lys[9] dipeptide of somatostatin is necessary for high affinity binding[13] and have facilitated the development of potent analogues including SMS 201-955 (sandostatin or octreotide), which is used clinically for the treatment of acromegaly and certain endocrine tumors.[14−16] The availability of the cloned somatostatin receptors and a large number of structural analogues has led to the identification of moderately subtype-selective peptide agonists.[9,17−19]

In an effort to discover novel small molecule somatostatin receptor modulators, database searching techniques were employed to screen the Merck compound sample collection. The database probe was based on a modeled conformation of the cyclic peptide c(Pro[6]-Tyr[7]-D-Trp[8]-Lys[9]-Thr[10]-Phe[11]),[13] in which the superscript numbers indicate the location of the amino acids in somatostatin. This potent agonist is the Tyr[7] analogue of the corresponding Phe[7] cyclic peptide which has been the subject of extensive structure−activity relationship and conformational studies[13,20−22] which identified a βII′ turn around the Trp[8]-Lys[9] and essential amino acids Phe[7], Trp[8], Lys[9], and Phe[11]. It was also known from this work that the peptide backbone is not required for activity, and this fact is illustrated in carbohydrate-[23,24] and benzodiazepine-based[25] somatostatin ligands. Using the side chains of the Tyr[7]-D-Trp[8]-Lys[9] in the modeled hexapeptide as the probe, our proprietary 3D similarity search engine, SQ,[26] was employed to search a database of 3D models of compounds[27] in the Merck compound sample collection. Of the 75 compounds selected as most similar and tested in the murine SSTR2 assay, L-264,930 was identified as a high affinity (K_i = 200 nM) ligand (see Figure 1).

Combinatorial chemistry has emerged as a powerful tool for the identification of novel medicinal chemistry leads[28,29] and for the process of structure-based lead refinement.[30] Compound L-264,930 can easily be divided into three simple parts (Figure 1) and lends itself well to solid-phase combinatorial synthesis. Herein, we describe a large combinatorial library patterned after the lead compound with the goal of discovering highly potent and selective ligands for each of the somatostatin receptor subtypes. In preparing the library,

† Department of Molecular Design and Diversity.
‡ Department of Biochemistry and Physiology.
§ Department of Medicinal Chemistry.

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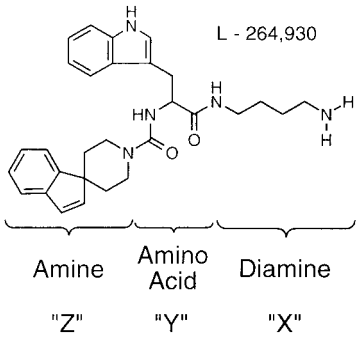


Figure 1. Disconnection of the lead molecule into three sets of subunits used in the combinatorial library.

we chose a resin-archive, iterative deconvolution format.[31] This strategy uses a standard solid-phase "mix and split" protocol[32] to synthesize the initial library mixtures. After the addition of each set of subunits to the growing molecules, but before the resin is mixed, a small portion of resin from each reactor vessel is saved to be used as a starting point in future deconvolution steps. By producing complex mixtures in this way, we were able to efficiently present a large number of compounds to each of the receptor binding assays as a relatively small number of testable entities. It was our hope that each receptor subtype would select for different library mixtures, leading us down deconvolution pathways where activity and selectivity would increase as mixture complexity decreased. This hope has been borne out with the discovery of subtype-selective agonists for somatostatin receptor subtypes 1,2, 4, and 5.[33−37]

Library Design

The solid-phase synthesis of the library molecules is indicated in Scheme 1. The diamine **X** subunit is first coupled to a resin-bound, acid-cleavable 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB) handle through a carbamate linkage.[38] The Fmoc-protected amino acid **Y** subunit is then added to the amine to form an amide bond using the standard peptide coupling reagent, diisopropyl carbodiimide (DIC) with 3% added *N*,*N*′-(dimethylamino)-pyridine (DMAP), followed by Fmoc deprotection. Next, the **Z** subunit is installed through a urea linkage,[39] and the product is removed from the resin by treatment with glacial acetic acid at 45 °C under nitrogen. We developed these mild cleavage conditions in order to avoid unwanted decomposition of indole-containing library members which occurred when using standard trifluoroacetic acid-based cleavage cocktails.[40]

The library was prepared as a set of 79 mixtures of compounds containing all combinations of 20 **X** subunits and 20 **Y** subunits using the "mix and split" technique with resin archiving after each step. The basis set (see Figure 2) was chosen to produce a large number of analogues of L-264,930. Note that the lead compound itself is also included in the library as an internal reference (library member **Z14-Y2-X5**). The **X** subunits consist of diamines (and one amino-alcohol) in which the spacing between the two functional groups varies from four to six atoms. Using rapid analogue synthesis, we found that, for hSSTR2, the optimal spacing for the diamine was five atoms.[33,34] Rings and branching groups were included to induce conformational constraints on the positioning of the free amine in the final molecules. Some of the asymmetric or pro-chiral diamines led to multiple regio- and stereoisomers in the final molecules (**X4**, **X10**, **X12**, and **X14)**. During the course of preparing model compounds while developing the library synthesis, we found that other asymmetric diamines react regioselectively. For example, since alkylamines are better nucleophiles than arylamines, subunit **X3** attaches to the resin solely through the alkyl amino group, leading to a single regioisomer. Similarly, secondary amines are much more reactive than primary amines (**X9** and **X19**), and simple primary amines react faster than amines bearing an alpha substituent (**X13**, **X16**, and **X17**). These subunits also give rise to single regioisomers. The asymmetric diamine **X15** was monoprotected prior to the library synthesis with an allyloxycarbonyl (Alloc) group, so that only one amine group is able to attach to the resin. The Alloc group was then removed before the next step of the synthesis.

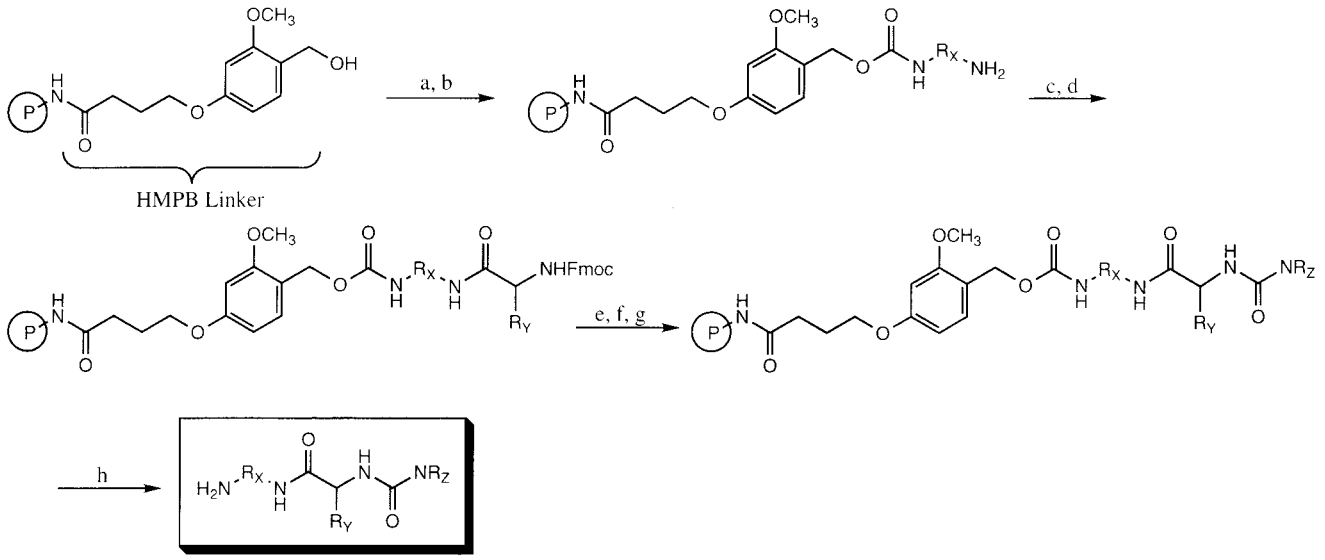
The **Y** subunits are all α-amino acids. Six of the 20 subunits are tryptophan derivatives; the indole nucleus was determined to be important to binding based on structure−activity relationships determined from the large series of cyclic hexapeptide somatostatin ligands[13,20−22] and also from the rapid analogue syntheses.[33,34] The same studies also suggest that an aromatic group is necessary in this position for maintained potency, and most of the remaining subunits reflect this bias. The **Y** subunits were added as racemic mixtures in all cases except for **Y1**, **Y2**, **Y17**, **Y18**, and **Y19**. The latter three were only available as single enantiomers. **Y1** and **Y2** represent the two enantiomers of tryptophan, and we reasoned that keeping these two isomers separate was important for comparing novel library mixture activity with the activity of mixtures containing close analogues of L-264,-930.

Selection of the **Z** subunits was biased toward piperidines and piperazines to mimic the spiroindenylpiperidine structure from the lead compound. Almost all of the **Z** subunits contain aromatic rings, in line with modeling studies of both the cyclic hexapeptides and L-264,930. All but three of these subunits are amines, added to the **Y** subunit through a urea linkage. The final three **Z** subunits were reacted directly with the amine from the **Y** subunit to produce a sulfonamide (**Z77**) or a carbamate (**Z78** and **Z79**). The total number of unique **X-Y-Z** combinations in the library is 20 × 20 × 79, or 31 600. However, when stereo- and regioisomers are taken into account, the actual number of unique library members expected is 131 670 (38 × 35 × 99 entities arising from isomers of the **X**, **Y**, and **Z** subunits, respectively). Depending on the isomeric complexity of the **Z** subunit, each of the 79 initial library mixtures is expected to contain either 1330 or 2660 total members.

Library Screening and Deconvolution

The primary assay for mixture evaluation was a ligand binding assay using radiolabeled somatostatin and membranes isolated from Chinese hamster ovary (CHO) cells stably expressing cloned human somatostatin receptors for each receptor subtype.[35] A semiautomated procedure was

Scheme 1[a]



[a] Reagents and conditions: (a) *p*-nitrophenylchloroformate, DIEA, THF/CH_2Cl_2 (1:1), 6 h; (b) diamine or amino alcohol (**X**), DIEA, DMF, 16 h; (c) archive resin; mix and redistribute resin; (d) Fmoc-amino acid (**Y**), DIC, 3% DMAP, DMF, 2 × 3 h; (e) archive resin; mix and redistribute resin; (f) 20% piperidine in DMF, 30 min; (g) *p*-nitrophenylchloroformate, DIEA, THF/CH_2Cl_2 (1:1), 40 min; (g) amine (**Z**), DIEA, DMF, 20 min; (h) HOAc, 35−40 °C, 22 h.

established for the rapid testing of compounds using a 96 well plate format. To expedite the screening of both the initial library mixtures and the deconvolution mixtures, percent inhibition data was first determined at two or three concentrations (10, 1, and 0.1 μM if mixtures were still active at 1 μM). Only a small subset of the mixtures (usually the two to five most active) in each set were then titrated to determine K_i values. In general, only this small number of mixtures showed any activity at all (>50% inhibition at 1 μM for subtype 2, >50% inhibition at 10 μM for the other subtypes). The most active initial library mixtures in each of the somatostatin receptor subtype binding assays are presented in Table 1. On the whole, the library binds much more strongly to the subtype 2 receptor than to the other subtypes. We focused on this subtype for our initial deconvolution experiments. Not surprisingly, one of the most active library mixtures in the hSSTR2 assay (mixture **Z14**, containing the spiroindenylpiperidine subunit) is the one which contains L-264,930. We chose to deconvolute this mixture in addition to the mixture containing the benzimidazolonylpiperidine **Z8** subunit, which was equally active.

First-round deconvolutions were performed by starting with a portion of the "**Y** archive" resins from the initial library synthesis and adding the desired **Z** subunit to each of the 20 archive samples. This produced 20 new mixtures where the **Z** and **Y** subunits are defined and the **X** subunit is scrambled. The most active wells from the **Z8** and **Z14** deconvolutions are shown in Table 2. All of the most active **Y** subunits were tryptophan derivatives. The **Z14** deconvolution mixtures are more active than the **Z8** deconvolution mixtures in all cases except for the **Z8-Y4** mixture. Because this synergistic combination of the benzimidazolonylpiperidine **Z8** and β-methyltryptophan **Y4** subunits led to the best activity, we further deconvoluted this mixture starting from the "**X** archive" resins. This second-round deconvolution produced 20 sets of regio- and stereoisomers, the most active of which are summarized in Table 3. As expected, the most active members generally contained diamines separated by five carbon atoms.[33,34]

The most active well, **Z8-Y4-X13**, is a mixture of two diastereomers arising from the two enantiomers of (2*RS*,3*SR*)-β-methyltryptophan (subunit **Y4**) used in the library synthesis. Resynthesis of the individual diastereomers starting with pure enantiomers of β-methyltryptophan led to the identification of the most active stereoisomer, the structure and activity profile of which is shown in Figure 3.

Our success with compound **Z8-(2*R*,3*S*)Y4-X13** led us to focus on another active well from the second-round deconvolution. **Z8-Y4-X4** is a mixture of eight compounds. This stereochemical complexity arises from the combination of the two enantiomers of β-methyltryptophan with the *cis*/*trans*-1,3-diaminocyclohexane **X4** subunit. Although *cis* **X4** is *meso*, differential functionalization of this diamine leads to the generation of two additional stereogenic centers in the molecule. Resynthesis of the individual stereoisomers of this mixture starting from enantiomerically pure β-methyltryptophan and pure *cis* and *trans* 1,3-diaminocyclohexane provided mixtures of two diastereomers. The most active of these sets of diastereomers were separated chromatographically to identify the highly potent and selective compound shown in Figure 4. The absolute configuration of this molecule was determined by an alternative synthesis beginning with enantiomerically pure fragments of known chirality.[34]

We next focused on obtaining a subtype 5 selective compound. We chose to deconvolute the aminobenzolactam **Z29** subunit, which was the most active mixture in the hSSTR5 assay. While this mixture was also moderately active in the hSSTR2 assay (K_i = 2938 nM), we expected to derive subtype selectivity from the **X** and **Y** portions of the molecule based on our experiences with the subtype 2 compounds. Testing of the **Z29** deconvolution mixtures in both the hSSTR5 and hSSTR2 assays identified the 5-fluorotryptophan **Y3** subunit as the sole active **Y** subunit (824

Figure 2. Basis set for the library.

Table 1. Activity of Initial Library Mixtures in Each of the Human Somotostatin Receptor Binding Assays

assay	mixture	activity(K_i, nM)
hSSTR1	**Z13**	1709
hSSTR1	**Z37**	2591
hSSTR1	**Z73**	2900
hSSTR2	**Z8**	45
hSSTR2	**Z14**	52
hSSTR2	**Z22**	119
hSSTR3	**Z14**	2664
hSSTR3	**Z76**	1873
hSSTR4	**Z13**	1300
hSSTR4	**Z24**	2100
hSSTR4	**Z46**	1500
hSSTR5	**Z29**	2117
hSSTR5	**Z76**	2400

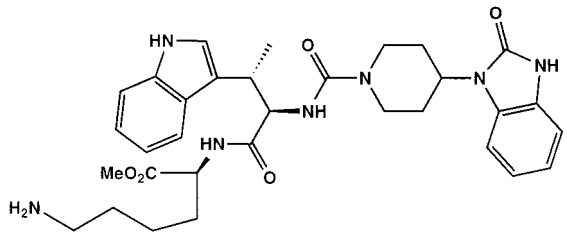
Table 2. Activity of the **Z8** and **Z14** Deconvolutions in the hSSTR2 Assay

mixture	activity(K_i, nM)
Z8-Y2	69
Z8-Y3	69
Z8-Y4	5.6
Z8-Y5	90
Z14-Y2	50
Z14-Y3	25
Z14-Y4	7.1
Z14-Y5	68

nM vs hSSTR5, 931 nM vs hSSTR2). While subtype 2 selectivity was still an issue at this stage, this deconvolution pathway appeared to be the most promising. The most active mixtures produced in the deconvolution of the **Z29-Y3** mixture are presented in Table 4, indicating a preference for the simple C-6 and C-7 diamines (**X6** and **X7**) and lysine methyl ester (**X13**). Since both the **Z** and **Y** subunits were added as racemates, these active mixtures each contain four stereoisomers. Resynthesis of the active compounds using enantiomerically pure versions of aminobenzolactam **Z29**, followed by chromatographic separation of the resulting pair of diastereomers, identified the subtype 5 selective com-

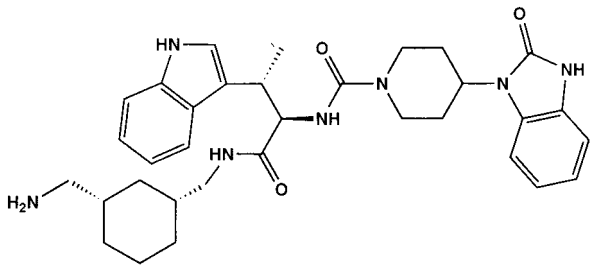
Table 3. Activity of the **Z8-Y4** Deconvolution in the hSSTR2 Assay

mixture	no. of compds	activity(K_i, nM)
Z8-Y4-X1	2	4.7
Z8-Y4-X4	8	4.4
Z8-Y4-X13	2	1.2
Z8-Y4-X14	4	5.8
Z8-Y4-X18	2	2.8



Assay	K_i (nM)
hSSTR1	6360
hSSTR2	0.2
hSSTR3	n/d
hSSTR4	767
hSSTR5	1400

Figure 3. Structure and activity profile for subtype 2 selective compound **Z8-(2*R*,3*S*)Y4-X13**.



Assay	K_i (nM)
hSSTR1	3640
hSSTR2	0.04
hSSTR3	283
hSSTR4	2800
hSSTR5	3300

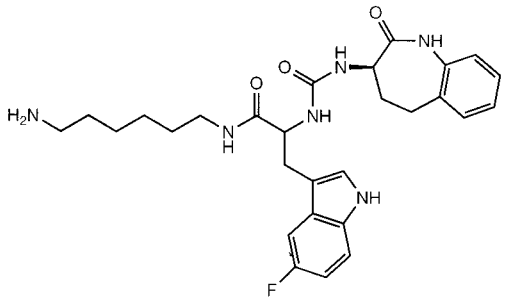
Figure 4. Structure and activity profile for subtype 2 selective compound **Z8-(2*R*,3*S*)Y4-(1*S*,3*R*)X4**.

Table 4. Activity of the **Z29-Y3** Deconvolution in the hSSTR5 and hSSTR2 Assays

mixture	hSSTR5 activity(K_i, nM)	hSSTR2 activity(K_i, nM)
Z29-Y3-X6	416	2300
Z29-Y3-X7	833	5400
Z29-Y3-X13	307	2300

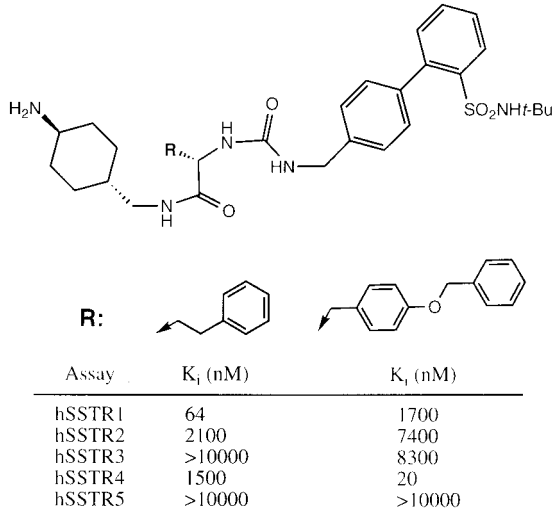
pound, **(*R*)Z29-Y3A-X7** (absolute stereochemistry at the 5-fluorotryptophan center not determined), shown in Figure 5. While the compound **(*R*)Z29-Y3A-X13** was more active in the hSSTR5 assay, it was not at all selective against hSSTR2 (58 nM vs hSSTR5, 37 nM vs hSSTR2).

Using a similar strategy, we were able to identify subtype-selective compounds for hSSTR1 and hSSTR4.[36] Biphenylmethylamine **Z13** was the most active library mixture for both of these subtypes. However, the first-round **Z13** deconvolution data identified the appropriate **Y** subunits for each receptor (mixture **Z13-Y8**: 640 nM vs hSSTR1, 51% inhibition at 5 μM vs hSSTR4; mixture **Z13-Y18:** 46%



Assay	K_i (nM)
hSSTR1	>10000
hSSTR2	4000
hSSTR3	>10000
hSSTR4	>10000
hSSTR5	170

Figure 5. Structure and activity profile for subtype 5 selective compound **(*R*)Z29-Y3A-X7**.



Assay	K_i (nM)	K_i (nM)
hSSTR1	64	1700
hSSTR2	2100	7400
hSSTR3	>10000	8300
hSSTR4	1500	20
hSSTR5	>10000	>10000

Figure 6. Structure and activity profile for subtype 1 selective compound **Z13-(*S*)Y8-X15** and subtype 4 selective compound **Z13-(*S*)Y18-X15**.

inhibition at 5 μM vs hSSTR1, 246 nM vs hSSTR4). Final deconvolutions of the appropriate mixtures identified the compounds shown in Figure 6. The only structural difference between these two compounds arises from their **Y** subunits. The subtype 1 selective compound contains homophenylalanine **Y8**. Replacing this fragment with the benzyltyrosine **Y18** eliminates most of the subtype 1 activity and enhances binding of the compound to the subtype 4 receptor by a factor of 75.

Deconvolution of the initial subtype 3 active wells (spiroidenylpiperidine **Z14** and 2-phenylindole derivative **Z76**) did not identify a preferred **Y** subunit. Instead, the deconvoluted wells were all of similar, moderate activity. Thus, a subtype 3 compound was not pursued with this library.[35]

Discussion

The results of this study establish the use of large combinatorial libraries as a powerful method for discovering highly potent and selective compounds across a range of receptor subtypes. We have demonstrated that the iterative deconvolution strategy worked well to identify potent, non-

peptide ligands for four out of the five known somatostatin receptors. For each receptor subtype, the screening of the entire set of complex (>1000 member) initial library mixtures identified an appropriate deconvolution pathway by selecting a small subset of active library wells. In subsequent mixture deconvolutions, the success of a particular pathway was readily evidenced by the selection of a smaller subset of active wells with enhanced activity over the original parent well. This selection process occurred for somatostatin receptor subtypes 1, 2, 4, and 5, eventually leading to the desired active ligands. For subtype 3, deconvolution led to mixtures which were relatively equipotent and not substantially more active than the parent mixture. It is noteworthy that the final, rigorously purified compounds are in some cases considerably more active than one would expect from the assay data of the crude deconvolution mixtures, even when stereochemical complexity is taken into account. We have attributed this observation to two major factors: contamination of the cleaved mixtures by poly(ethylene glycol) from the Tentagel resins used to synthesize the library and possible degradation of the **X** resin archives over time by the reaction of the amine groups with CO_2 to form unreactive carbonates. Both of these effects would serve to dilute the effective concentration of the desired library members in the crude mixtures, leading to decreased apparent activity. Since these dilution effects should be consistent accross library mixtures, selecting compounds for further resolution and purification based on their relative activity is still relevant.

The subunits for the library were chosen by a lead-based design strategy derived from L-264,930, a 200 nM mSSTR2 agonist. It was desirable to explore a large amount of chemical space around this initial structural class in order to answer as many questions about the structural requirements of each receptor subtype as possible. Part of the power of the iterative deconvolution combinatorial method is that one does not have to synthesize the entire set of compounds individually to obtain this information.

In addition to furnishing the required activity, we were able to use the mixture screening information to make important assumptions about the structure/activity relationships for each receptor subtype, mirroring information derived from rapid analogue synthesis of many single compounds[33,34] as well as identifying completely novel active structures. It is obvious from the data, for example, that the subtype 2 receptor prefers a tryptophan derivative in the **Y** position of this series of compounds. Further, the optimum spacing between amine groups of the diamine **X** position was established to be roughly five atoms. We were able to discover a synergistic combination of **Z** and **Y** subunits through examination of the library data. Molecules containing both the benzimidazolonylpiperidine (**Z8**) and (2*RS*,3*SR*)-*β*-methyltryptophan (**Y4**) subunits bind more tightly to hSSTR2 than would be expected from the activity of compounds containing only one of these pieces. We also found that adding conformational constraints to the diamine **X** subunits substantially increased both potency and selectivity of the subtype 2 compound. Compounds containing lysine methyl ester **X13** were more active than the lead structure, which contained no branching groups on the diamine chain. Installation of the appropriate stereoisomer of conformationally rigid 1,3-cyclohexanediamine **X4** led to even higher activity and much improved selectivity. Information uncovered from this library led to an increase in potency of 4 orders of magnitude and a 3 orders of magnitude increase in selectivity over L-264,930.

This study has also shown that by screening libraries and their deconvolution mixtures in multiple receptor subtype assays a desired selectivity profile can be optimized. In the case of subtypes 2 and 5, each successive deconvolution pointed not only toward more active compounds but also toward compounds which bound more specifically to the appropriate receptor. In the case of subtypes 1 and 4, the most active **Z** and **X** subunits were shown to be identical. However, deconvolution of the initial active mixture clearly showed a preference for different **Y** subunits by each receptor. Library mixtures may also be tested in other assays to screen out unwanted side effects or to select for other desirable properties. Since the entire population of compounds is synthesized up front, any new assay which becomes available can effectively query this set.

The use of combinatorial chemistry to furnish potent small molecule leads has been previously established. Somewhat less has been reported about the ability of these techniques to refine a moderate active into a useful and pharmaceutically relevant advanced lead, as well as the utility of screening large mixtures across a range of receptor subtypes. This study is significant in that it shows the successful use of combinatorial technology and lead-based library design to produce an extremely potent and specific hSSTR2 ligand from an active lead structure. The same library, when queried by the other receptor subtypes, led to the identification of potent and subtype-selective ligands for hSSTR1, 4, and 5. These compounds are among the first small molecules reported with such broad potency and selectivity profiles.[23−25,33−37] The power and efficiency of the "mix and split" technology have also been demonstrated. When coupled with resin archiving and iterative deconvolution, this method represents one of the fastest ways to produce and screen hundreds of thousands of compounds, especially in the absence of fully automated chemical synthesis, ultrahigh throughput screening, or specialized tagging techniques. Screening of initial library mixtures of >1000 members per mixture not only led to fruitful deconvolution pathways for four of the five receptor subtypes but also uncovered useful structure/activity relationships which were exploited in later library designs and more classical medicinal chemistry approaches.[35] By quickly identifying subtype-selective small molecules, this combinatorial strategy has produced a clear and useful path toward the understanding of the pharmacological effects associated with the somatostatin receptor subtypes. Moreover, these techniques should be directly applicable to a host of other receptor and enzyme families.

Experimental Section

Library Synthesis. Step 1. HMPB Resin. Rapp Tentagel NH_2 resin (100 g, 0.29 mmol/g loading, 29 mmol equiv) was added to a 2 L round-bottom flask with 1.2 L of

dimtheylacetamide (DMA). 4-(4-Hydroxymethyl-3-methoxyphenoxy)butyric acid (27.8 g, 116 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (22.2 g, 116 mmol) were added to the slurry and stirred at room temperature until a Kaiser amine test of a portion of the resin was negative (48 h). The resin was washed with 4 × 1 L each of DMF, THF, CH_2Cl_2, *i*-PrOH, and CH_2Cl_2 and then dried under vacuum overnight. Resin loading was determined from an Fmoc echo experiment to be 0.20 mmol/g.

Step 2. Addition of X Subunits. HMPB resin (3.6 g, 0.20 mmol/g, 0.72 mmol) was swelled in each of 20 fritted polypropylene tubes with THF/CH_2Cl_2 (1:1). The solvent was drained, and then diisopropylethylamine (DIEA) (18 mL, 0.75 M in THF/CH_2Cl_2 (1:1), 13.5 mmol) and *p*-nitrophenyl chloroformate (18 mL, 0.75 M in THF/CH_2Cl_2 (1:1), 13.5 mmol) were added, venting the tubes after addition. The reaction mixtures were agitated for 6 h, the tubes were drained, and the resins were washed with 2 × 40 mL of THF/CH_2Cl_2 (1:1). The appropriate **X** subunit (36 mL, 0.25 M in DMF, 9 mmol) was then added, along with DIEA (9 mmol) as a co-reagent. The mixtures were agitated at room temperature overnight (16 h), the tubes were drained, and the resins were washed with 4 × 40 mL of DMF. The resins for subunits **X13**, **X16**, and **X17** were also washed with glacial acetic acid to remove insoluble salts. To remove the allyloxycarbonyl protecting group, the **X15**-bound resin was first washed with 3 × 20 mL of CH_2Cl_2, and then 1,3-dimethylbarbituric acid (36 mL, 0.25 M in CH_2Cl_2, 9 mmol) and $Pd(PPh_3)_4$ (1.8 g, 1.6 mmol) were added and the reaction vessel was agitated at room temperature for 3 h. After the solvent was drained, the resin was washed with 3 × 40 mL each of CH_2Cl_2, CH_2Cl_2/acetic acid (1:1), and DMF. A portion (1 g) of each of the **X** resins was archived by adding 30% DMF/1,2-dichloroethane (DCE) to a total volume of 30 mL and removing 8.3 mL of the isopyncnic slurry. The remaining resins were mixed together in 30% DMF/DCE for 2.5 h. The mixed resin slurry was redistributed equally into 20 fritted tubes. The tubes were drained and the resins washed with 20 mL of DMA.

Step 3. Addition of the Y Subunits. The resins were washed with 3 × 20 mL of DMF. The appropriate **Y** subunit (10 mL, 0.25 M in DMF, 5.2 mmol) was added, followed by DIC/3% DMAP (10 mL, 0.52 M in DMF, 5.2 mmol DIC, 0.16 mmol DMAP). The tubes were agitated at room temperature for 3 h, and the solutions were then drained. The resins were washed with 2 × 20 mL of DMF, and the acylation was repeated. The resins were then washed with 3 × 20 mL of DMF. A portion (1 g) of each of the **Y** resin mixtures was archived by adding 30% DMF/1,2-dichloroethane to a total volume of 20 mL and removing 7.7 mL of the isopyncnic slurry. The remaining resins were mixed together in 30% DMF/DCE for 2.5 h. The mixed resin slurry was redistributed equally into 79 fritted tubes. The tubes were drained and the resins washed with 3 × 5 mL of DMA.

Step 4. Addition of the Z Subunits. The resins were washed with 3 × 4 mL of DMF. Piperidine in DMF (20%, 4 mL) was added, and the tubes were agitated at room temperature for 30 min. After draining the tubes, the resins were washed with 3 × 5 mL each of DMF and THF/CH_2Cl_2 (1:1). To tubes 1−76 were added DIEA (2 mL, 0.5 M in THF/CH_2Cl_2 (1:1), 1 mmol) and *p*-nitrophenyl chloroformate (2 mL, 0.5 M in THF/CH_2Cl_2 (1:1), 1 mmol), venting the tubes after addition. The tubes were agitated at room temperature for 40 min. Subunits **Z77**, **Z78**, and **Z79** (4 mL, 0.25 M in THF/CH_2Cl_2 (1:1), 1 mmol) were added to tubes 77, 78, and 79, respectively, and the tubes were agitated at room temperature for 1 h. Meanwhile, tubes 1−76 were drained, and the resins were washed with 5 mL of THF/CH_2Cl_2 (1:1). The appropriate **Z** subunit (4 mL, 0.25 M in DMF, 1 mmol) was then added to each of these tubes in addition to DIEA (1 mmol) as a co-reagent. The tubes were then agitated at room temperature for 15 min. All 79 tubes were drained, and the resins were washed with 4 × 5 mL each of DMF and THF/CH_2Cl_2 (1:1), 2 × 5 mL each of THF and CH_2Cl_2, 4 × 5 mL each of glacial acetic acid and *i*-PrOH, 1 × 5 mL of warm methanol, and 3 × 5 mL of CH_2Cl_2. Each of the resins was then dried under a stream of nitrogen.

Step 5. Cleavage of Library Mixtures from the Resin. Glacial acetic acid (4 mL) was added to each tube under nitrogen. The tubes were sealed and heated to 40 °C for 22 h. The tubes were then drained, collecting the product into preweighed 13 × 100 glass test tubes. The resins were washed with an additional 1 mL of glacial acetic acid. The solutions were then frozen at −78 °C and lyophilized. After lyophilization was complete, each mixture was diluted to 10 mM in DMSO based on the average molecular weight of all mixture components.

First-Round Deconvolutions. Twenty-five milligrams of each of the 20 **Y** archive resins was transferred to an individual reactor vessel via an isopyncnic slurry (70:30 dichloroethane (DCE)/DMF). Removal of the Fmoc protecting groups (25% piperidine in DMF for 30 min) was followed by activation with *p*-nitrophenyl chloroformate and DIEA in 1:1 THF/DCM for 45 min as described in step 4, above. The appropriate single **Z** subunit was then added to each vessel as a 0.25 M solution in DMF with DIEA as a co-reagent. After 30 min, the resins were washed and the products were cleaved from the resin, lypohilized, and diluted as described above.

Second-Round Deconvolutions. Twenty-five milligrams of each of the 20 **X** archive resins was transferred to an individual reactor vessel via an isopyncnic slurry (70:30 DCE/DMF). The appropriate single **Y** subunit was added to each vessel as a 0.5 M solution along with the peptide coupling agents DIC and 3% DMAP in DMF for 3 h as described in step 3, above. After the mixture was washed with DMF, the peptide coupling step was repeated to ensure complete loading. Removal of the Fmoc protecting group (25% piperidine in DMF for 30 min) was followed by activation with *p*-nitrophenyl chloroformate and DIEA in 1:1 THF/DCM for 45 min as described in step 4, above. The appropriate single **Z** subunit was then added to each vessel as a 0.25 M solution in DMF with DIEA as a co-reagent. After 30 min, the resins were washed and the products were cleaved from the resin, lypohilized and diluted as described above.

Single Pure Compounds. Final library compounds were prepared from enantio- or diastereo-pure subunits using the solid-phase techniques described above. After cleavage from the resin, the materials were further purified by preparative HPLC. Compound **Z8-(2*R*,3*S*)Y4-(1*S*,3*R*)X4** was also prepared using the chiral synthetic methodology described in ref 34. All ^{1}H NMR chemical shift values were referenced relative to residual protio solvent peaks.

Compound Z8-(2*R*,3*S*)Y4-(1*S*,3*R*)X4. ^{1}H NMR (300 MHz, CD_3OD): δ 0.14 (q, $J = 9$ Hz, 1 H), 0.46 (qd, $J = 9$, 2 Hz, 1 H), 0.62 (qd, $J = 9$, 2 Hz, 1 H), 0.85−0.95 (m, 1 H), 1.02−1.13 (m, 2 H), 1.19−1.28 (m, 2 H), 1.46 (d, $J =$ 6 Hz, 3 H), 1.59−1.70 (m, 2 H), 1.74−1.82 (m, 2 H), 2.26−2.44 (m, 4 H), 2.52 (dd, $J = 6$, 10 Hz, 1 H), 2.90−3.04 (m, 3 H), 3.52 (dq, $J = 9$, 6 Hz), 4.19−4.30 (m, 2 H), 4.46 (tt, $J = 9$, 3 Hz, 1 H), 4.54 (d, $J = 8$ Hz, 1 H), 6.98−7.10 (m, 5 H), 7.12−7.20 (m, 2 H), 7.34 (d, $J = 7$ Hz, 1 H), 7.66 (d, $J = 7$ Hz, 1 H) ppm. LRMS (electrospray, positive ion mode, *m*/*z*) for $C_{33}H_{43}N_7O_3$: 586.3 (MH^+).

Compound (*R*)Z29-Y3A-X7. ^{1}H NMR (500 MHz, CD_3OD): δ 1.14−1.19 (m, 2 H), 1.27−1.32 (m, 4 H), 1.52−1.58 (m, 2 H), 1.93−2.01 (m, 1 H), 2.43−2.48 (m, 1 H), 2.67−2.72 (m, 1 H), 2.85 (t, $J = 7.3$ Hz, 2 H), 2.87−2.91 (m, 1 H), 2.97−3.05 (m, 2 H), 3.07 (dd, $J = 3.4$, 6.6 Hz, 2 H), 4.23 (dd, $J = 7.9$, 12 Hz, 1 H), 4.30 (t, $J = 6.8$ Hz, 1 H), 6.83 (td, $J = 9.1$, 2.6 Hz, 1 H), 7.04 (d, $J = 7.8$ Hz, 1 H), 7.14−7.27 (m, 6 H), 7.71 (t, $J = 5.6$ Hz, 1 H) ppm. LRMS (electrospray, positive ion mode, *m*/*z*) for $C_{28}H_{35}FN_6O_3$: 523.2 (MH^+).

Compound Z13-(*S*)Y8-X15. ^{1}H NMR (500 MHz, CD_3OD): δ 1.01 (s, 9 H), 1.05−1.12 (m, 2 H), 1.34−1.37 (m, 2 H), 1.51−1.55 (m, 1 H), 1.87−2.04 (m, 5 H), 2.63−2.70 (m, 2 H), 3.02−3.10 (m, 4 H), 4.18 (dd, $J = 5.2$, 8.6 Hz, 1 H), 4.39 (dd, $J = 16$, 23 Hz, 2 H), 7.15−7.19 (m, 3 H), 7.24−7.30 (m, 2 H), 7.36 (d, $J = 8.3$ Hz, 2 H), 7.42 (d, $J =$ 8.3, 2 H), 7.52 (td, $J = 8.0$, 1.4 Hz, 1 H), 7.60 (td, $J = 7.5$, 1.4 Hz, 1 H), 8.05 (t, $J = 5$ Hz, 1 H), 8.10 (dd, $J = 1.3$, 7.9 Hz, 1 H) ppm. LRMS (electrospray, positive ion mode, *m*/*z*) for $C_{35}H_{47}N_5O_4S$: 634.3 (MH^+).

Compound Z13-Y18-X15. ^{1}H NMR (400 MHz, CD_3OD): δ 0.99 (s, 3 H), 1.00−1.50 (m, 5 H), 1.60−2.05 (m, 5 H), 2.80−3.10 (m, 4 H), 3.61 (t, $J = 8$ Hz, 1 H), 4.30−4.45 (m, 2 H), 5.03 (s, 1 H), 6.92 (d, $J = 8.5$ Hz, 2 H), 7.15 (d, $J = 8.5$ Hz, 2 H), 7.20−7.42 (m, 10 H), 7.45−7.60 (m, 2 H), 8.09 (d, $J = 8$ Hz, 1 H) ppm. LRMS (electrospray, positive ion mode, *m*/*z*) for $C_{41}H_{51}N_5O_5S$: 726.4 (MH^+).

Receptor Ligand Binding Assays. All receptor binding assays were performed with membranes isolated from CHO cells expressing the cloned human somatostatin receptors. All five receptor assays were adapted for high throughput screening in 96 well format. The assay buffer[17] consisted of 50 mM Tris-HCl (pH 7.8) with 1 mM (ethylene-dioxy)-diethylenedinitrilotetraacetic acid (EGTA), 5 mM $MgCl_2$, leupeptin (10 μg/mL), pepstatin (10 μg/mL), bacitracin (200 μg/mL), and aprotinin (0.5 μg/mL). CHO cell membranes, radiolabeled somatostatin, and unlabeled test compounds were resuspended or diluted in this assay buffer. All assays were run in 96 well polypropylene plates. The final concentration of the radiolabeled ligand was 0.1 nM for all receptor assays. Unlabeled test compounds were examined over a range of concentrations from 0.01 to 10 000 nM. A 20 μL aliquot of 1 nM (3-[^{125}I]iodotyrosyl^{11})somatostatin-14(Tyr^{11}) or (3-[^{125}I]iodotyrosyl^{25})somatostatin-28(Leu^8, D-Trp^{22}, Tyr^{25}) was added to each well of the plate, followed by addition of a 20 μL aliquot of the unlabeled test compound and 160 μL of the CHO cell membrane suspension. Radiolabeled somatostatin-14 was used for the hSSTR1-4 assays. Radiolabeled somatostatin-28 was used for the hSSTR5 assay, since hSSTR5 has higher affinity for the longer form of the peptide. The amount of membrane protein used for each of the receptor subtypes was adjusted so that the amount of ^{125}I-somatostatin bound was roughly equivalent in each assay. The mixtures were incubated for 45 min at room temperature and then harvested onto Packard Unifilter GF/C plates pretreated with 0.1% polyethyleneimine. The plates were washed with ice-cold 50 mM Tris-HCl (pH 7.8) and dried overnight at room temperature. Microscint-20 scintillation fluid was added before the plates were sealed, and radioactivity was quantitated using a Packard Topcount Scintillation Counter.

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References and Notes

(1) Epelbaum, J. Somatostatin in the central nervous system: physiology and pathological modification. *Prog. Neurobiol.* **1986**, *27*, 63−100.
(2) Raynor, K.; Reisine, T. Somatostatin receptors. *Crit. Rev. Neurobiol.* **1992**, *16*, 273−289.
(3) Reichlin, S. Somatostatin (Part 1). *N. Engl. J. Med.* **1983**, *309*, 1495−1501.
(4) Mandarino, L.; Stenner, D.; Blanchard, W.; Nissen, S.; Gerich, J.; Ling, N.; Brazeau, P.; Bohlen, P.; Esch, F.; Guillemen, R. Selective effects of somatostatin-14, -25, and -28 on in vitro insulin and glucagon secretion. *Nature* **1981**, *291*, 76−77.
(5) Vaysse, N.; Pradayrol, L.; Chayvialle, A.; Pignal, F.; Esteve, J.; Susini, J.; Descos, F.; Ribet, A. Effects of somatostatin-14 and somatostatin-28 on bombesin-stimulated release of gastrin, insulin and glucagon in the dog. *Endocrinology* **1981**, *108*, 1843−1847.
(6) Krejs, G. J. Physiological role of somatostatin in the digestive tract: gastric acid secretion, intestinal absorption, and motility. *Scand. J. Gastroenterol.* **1986**, *21* (suppl 119), 47−53.
(7) Reichlin, S. Somatostatin (Part 2). *N. Engl. J. Med.* **1983**, *309*, 1556−1563.
(8) 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.
(9) Yasuda, K.; Rens-Domiano, S.; Breder, C. D.; Law, S. F.; Sapel, C. B.; Reisine, T.; Bell, G. Cloning of a novel somatostatin receptor, SSTR3, that is coupled to adenylyl cyclase. *J. Biol. Chem.* **1992**, *267*, 20422−20428.
(10) O'Carroll, A.-M.; Lolait, S. J.; Konig, M.; Mahan, L. C. Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. *Mol. Pharmacol.* **1992**, *42*, 939−946.
(11) Bruno, J.; Xu, Y.; Song, J.; Berelowitz, N. Molecular cloning and functional expression of a novel brain specific somatostatin receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11151−11155.
(12) Patel, Y. C.; Greenwood, M.; Warszynska, A.; Panetta, R.; Srikant, C. B. All five human somatostatin receptors (hSSTR1-5) are functionally coupled to adenylyl cyclase. *Biochem. Biophys. Res. Comm.* **1994**, *198*, 605−612.

(13) Veber, D. F. Design and discovery in the development of peptide analogues. In *Peptides, Chemistry and Biology: Proceedings of the 12th American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, 1992; pp 3−14.
(14) Lamberts, S. W. J. The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of its analogues in the treatment of human pituitary tumors. *Endocr. Rev.* **1988**, *9*, 417−436.
(15) Weckbecker, G.; Raulf, F.; Stoltz, B.; Bruns, C. Somatostatin analogues for diagnosis and treatment of cancer. *Pharmacol. Ther.* **1994**, *60*, 245−264.
(16) Chaudhry, A.; Kvols, L. Advances in the use of somatostatin in the management of endocrine tumors. *Curr. Opin. Oncol.* **1996**, *8*, 44−48.
(17) Raynor, K.; O'Carroll, A.-M.; Kong, H.; Yasuda, K.; Mahan, L.; Bell, G. I.; Reisine, T. Characterization of cloned somatostatin receptors SSTR4 and SSTR5. *Mol. Pharmacol.* **1993**, *44*, 385−392.
(18) Raynor, K.; Murphy, W.; Coy, D.; Taylor, J.; Moreau, J.-P.; Yasuda, K.; Bell, G. I.; Reisine, T. Cloned somatostatin receptors: identification of subtype selective peptides and demonstration of high affinity binding of linear peptides. *Mol. Pharmacol.* **1993**, *43*, 838−844.
(19) O'Carroll, A.-M.; Raynor, K.; Lolait, S. J.; Reisine, T. Characterization of cloned human somatostatin receptor SSTR5. *Mol. Pharmacol.* **1994**, *48*, 291−298.
(20) Mierke, D. F.; Pattaroni, C.; Delaet, N.; Toy, A.; Goodman, M.; Tancredi, T.; Motta, A.; Temussi, P. A.; Moroder, L.; Bovermann, G.; Wunsch, E. Cyclic hexapeptides related to somatostatin. *Int. J. Pept. Protein Res.* **1990**, *36*, 418−432.
(21) He, Y.-B.; Huang, Z.; Raynor, K.; Reisine, T.; Goodman, M. Synthesis and conformations of somatostatin-related cyclic hexapeptides incorporating specific α- and β-methylated residues. *J. Am. Chem. Soc.* **1993**, *115*, 8066−8072.
(22) Hocart, S. J.; Reddy, V.; Murphy, W. A.; Coy, D. H. 3-Dimensional quantitative structure−activity-relationships of somatostatin analogues. 1. Comparative molecular-field analysis of growth-hormone release-inhibiting potencies. *J. Med. Chem.* **1995**, *38*, 1974−1989.
(23) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengeler, P. A.; Hamley, P.; Smith, A. B.; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. A.; Strader, C. D. De-novo design and synthesis of somatostatin nonpeptide peptidomimetics utilizing β-D-glucose as a novel scaffolding. *J. Am. Chem. Soc.* **1993**, *115*, 12550−12568.
(24) Papageorgiou, C.; Haltiner, R.; Burns, C.; Petcher, T. J. Design, synthesis and binding affinity of a nonpeptide mimic of somatostatin. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 135−140.
(25) Papageorgiou, C.; Borer, X. A nonpeptide ligand for the somatostatin receptor having a benzodiazepinone structure. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 267−272.
(26) Mosley, R. T.; Miller, M. D.; Kearsley, S. K.; Prendergast, K.; Underwood, D. J. New lead discovery in drug development. In *Computational Medicine, Public Health and Biotechnology, (Part 1)*; Witten, M., Ed.; World Scientific Publishing: Singapore, 1995; pp 101−125.
(27) Kearsley, S. K.; Underwood, D. J.; Sheridan, R. P.; Miller, M. D. Flexibases − a way to enhance the use of molecular docking methods. *J. Comput. Aided Mol. Des.* **1994**, *8*, 565−582.
(28) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. *J. Med. Chem.* **1994**, *37*, 1385−1401.
(29) Thompson, L. A.; Ellman, J. A. Synthesis and applications of small molecule libraries. *Chem. Rev.* **1996**, *96*, 555−600.
(30) Kick, E. K.; Roe, D. C.; Skillman, A. G.; Liu, G.; Ewing; T. J. A.; Sun, Y.; Kuntz, I. D.; Ellman, J. A. Structure-based design and combinatorial chemistry yield low nanomolar inhibitors of cathepsin D. *Chem. Biol.* **1997**, *4*, 297−307.
(31) Erb, E.; Janda, K. D.; Brenner, S. Recursive deconvolution of combinatorial chemical libraries. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11422−11426.
(32) Furka, A.; Sevestyen, F.; Asgesom, M.; Dibo, G. General method for rapid synthesis of multicomponent peptide mixtures. *Int. J. Pept. Protein Res.* **1991**, *37*, 487−493.
(33) Yang, L.; Berk, S. C.; Rohrer, S. P.; Mosley, R. T.; Guo, L.; Underwood, D. J.; Arison, B. H.; Birzin, E. T.; Hayes, E. C.; Mitra. S. W.; Parmar, R. M.; Schenk, K.; Wu, T.-J.; Butler, B. S.; Foor, F.; Pasternak, A.; Pan, Y.; Silva, M.; Freidinger, R. M.; Smith, R. G.; Chapman, K.; Scaheffer, J.; Patchett, A. A. Synthesis and biological evaluation of potent peptidomimetics selective for somatostatin receptor subtype 2. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10836.
(34) Yang, L.; Guo, L.; Pasternak, A.; Mosley, R.; Rohrer, S.; Birzin, E.; Foor, F.; Cheng, K.; Schaeffer, J.; Patchett, A. A. Spiro[1H-indene-1,4′-piperidine] derivates as potent and selective non-peptide human somatostatin receptor subtype 2 (sst2) agonists. *J. Med. Chem.* **1998**, *41*, 2175.
(35) Rohrer, S. P.; Birzin, E. T.; Mosley, R. T.; Berk, S. C.; Hutchins, S. M.; Shen, D.-M.; Xiong, Y.; Hayes, E. C.; Parmar, R. M.; Foor, F.; Mitra, S. W.; Degrado, S. J.; Shu, M.; Klopp, J. M.; Cai, S.-J.; Blake, A.; Chan, W. W. S.; Pasternak, A.; Yang, L.; Patchett, A. A.; Smith, R. G.; Chapman, K. T.; Schaeffer, J. M. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* **1998**, *282*, 737.
(36) Ankersen, M.; Crider, M.; Liu, S.; Ho, B.; Andersen, H. S.; Stiden, C. Discovery of a novel non-peptide somatostatin agonist with SST4 selectivity. *J. Am. Chem. Soc.* **1998**, *120*, 1368−1373.
(37) Souers, A. J.; Virgilio, A. A.; Ellman, J. A. Synthesis and biological evaluation of β-turn mimetics that incorporate i+1, i+2 and i+3 side-chains. *Abstracts of Papers*, 213th National Meeting of the American Chemical Society; American Chemical Society: Washington, DC, 1997; p 327.
(38) Dressman, B. A.; Spangle, L. A.; Kaldor, S. W. Solid-phase synthesis of hydantoins using a carbamate linker and a novel cyclization/cleavage step. *Tetrahedron Lett.* **1996**, *37*, 937−940.
(39) Hutchins, S. M.; Chapman, K. T. A strategy for urea linked diamine libraries. *Tetrahedron Lett.* **1995**, *36*, 2583−2586.
(40) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. Preparation and application of the 5-(4-(9-fluorenylmethyloxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy)-valeric acid (PAL) handle for the solid-phase synthesis of C-terminal peptide amides under mild conditions. *J. Org. Chem.* **1990**, *55*, 3730.

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