A Screening Library for Peptide Activated G-Protein Coupled Receptors. 1. The Test Set

Karine Lavrador,[†] Brian Murphy,[‡] John Saunders,*[,†] Scott Struthers,[‡] Xiaochuan Wang,[†] and John Williams[†]

Chemistry and Biology Departments, Neurocrine Biosciences, 12790 El Camino Real, San Diego, California 92130

Received April 16, 2004

One subset of the G-protein coupled receptor (GPCR) superfamily is that which is activated by a peptide carrying an obligatory positively charged residue (GPCR−PA$^+$). This subclass is exemplified by receptors for melanocortins, GnRH, galanin, MCH, orexin, and some chemokine receptors variously involved in eating disorders, reproductive disorders, pain, narcolepsy, obesity, and inflammation. Using the methods described in this study, a region of chemical property space enriched in GPCR ligands was identified. This information was used to design and synthesize a "test" library of 2025 single, pure compounds to sample portions of this property space associated with GPCR−PA$^+$ ligands. The library was evaluated by high-throughput screening against three different receptors, rMCH, hMC4, and hGnRH, and found to be highly enriched in active ligands (4.5−61-fold) compared to a control set of 2024 randomly selected compounds. In addition, the analysis suggested that about 7000 compounds will be necessary to complete the sampling of this GPCR−PA$^+$ ligand-rich region and to better define its borders.

Introduction

Stimulation of receptors linked to G-protein activation represents a primary mechanism by which cells sense changes in their external environment and convey that information to the cytosol through various effector mechanisms.[1,2] Historically, GPCR's have represented a "gold mine" for drug discovery, and over 30% of currently approved prescription medicines act as either agonists or antagonists at such sites[3]. With some notable exceptions, most successes have been achieved by drugs interacting at receptors for the simpler ligands, particularly the monoamines; the purpose of this study was to facilitate the design and synthesis of new drugs that compete with the more complex peptide ligands.[4]

The GPCRs have been classified[1] into five categories (A, rhodopsin-like; B, secretin-like; C, metabotropic glutamate-like; D, fungal pheromone; E, c-AMP) on the basis of extensive phylogenetic studies, and these categories have been further subdivided into subclasses on the basis of the level of protein sequence homology (e.g., A−I, melanocortin; B−II, parathyroid). Those receptors that are activated by peptide ligands do not neatly fall into one of these subcategories but are distributed within subclasses A and B. The current study was focused on receptors within these subclasses that are activated by positively charged peptide ligands exemplified by receptors for melanocortins (MC, A−I), gonadotropin releasing hormone (GNRH, A−III), bradykinin (A−IV), melanin concentrating hormone (MCH, A−V), calcitonin gene related peptide (CGRP, B−I), and vasoactive intestinal peptide (VIP, B−III). The increased interest in GPCRs activated by endogenous peptides is a reflection of the growing number of gene sequences that have now been assigned to such receptors and also the belief that the design of non-peptide ligands may now be a tractable problem, thereby overcoming the well-documented limitations of peptides themselves as drugs.[5] In addition, many of the new orphan GPCRs may turn out to be opportunities for therapeutic intervention and those that are activated by peptides would naturally fall into this subcategory.

To date, strategies for the discovery of non-peptide ligands[4,6] have included high-throughput screening of random, large compound collections and/or combinatorial libraries, progressive replacement of amide bonds in fragments of peptides thought to be critical for binding, and the synthesis of novel templates putatively mimicking a presumed secondary structural feature, such as a β-turn mimetic. While all of these approaches are valid and have had some notable successes, they do not offer a systematic approach for the family of receptors under review. Central to the current study is the concept that the "property space" associated for ligands for peptide-activated GPCRs is definable and forms only a small fragment of what is referred to as a "druglike" space. Here we describe in detail how this space may be characterized and hence associated with a designed combinatorial "test" library consisting of around 2000 molecule that is highly enriched in ligands to these receptors. Furthermore, we show that the designed library performed significantly better than a random set of equal size selected from our corporate compound collection when tested against three relevant receptors. It is essential to appreciate that the objective of this test set, and eventually of the completed library, is to define an area of drug space for GPCR−PA$^+$. Once this has been defined, specific areas can subsequently be highly populated with novel molecules targeting a specific receptor in an iterative approach. Therefore, it is not anticipated that potent molecules for a given receptor will be found directly in this library.

* Corresponding author. Tel: 858 617 7637. E-mail: jsaunders@neurocrine.com.
[†] Chemistry Department.
[‡] Biology Department.

10.1021/jm040084c CCC: $27.50
Published on Web 12/01/2004

Computational Chemistry Methods

We have defined "drug space" computationally by an analysis of drugs and druglike molecules known in the literature and representative, biologically active "in-house" compounds selected from over 20 historical discovery programs at Neurocrine; collectively this set totals 81 560 and the space it defines is termed "NBI-drug space" (NBI-DS). To delineate GPCR−PA⁺ property space as a portion of the property space occupied by all drugs, a database composed of 630 molecules active against over 40 distinct GPCRs was constructed from data available in the literature and from Neurocrine's proprietary compound database. So as not to populate the set with the vast number of ligands described for the "classical" monoaminergic receptors, which were the subject of intense study during the 1970−1990 era, or for specific, well-studied GPCR−PA⁺ (e.g. somatostatin[7]), only representative structures for each receptor were included.

Using the DiverseSolutions software package, BCUT metrics[8,9] were calculated for NBI-DS. Examination of different combinations of metrics allowed us to select a five-dimensional space (H-bond donor and acceptor, two metrics of polarizability, and charge) in which the compounds are widely distributed. To accelerate the analysis of large virtual libraries, where spatial locations of millions of compounds must be calculated and compared, we have typically employed cell-based methods. With this approach, each of the five axes is divided into 10 bins resulting in the partitioning of the entire space into 100 000 individual cells. Comparing the location or index of the occupied cells can be used to measure the diversity/similarity between compounds or collections of compounds. Numbers of compounds within a given cell or range of cells is also a convenient measure of sampling density.

Importantly, we have introduced the concept of analyzing neighboring cells which, in the context of five-dimensional space, requires that each occupied cell has $3^5 - 1 = 242$ neighbors. Empirically, this is important for the following reasons: (1) The objective of the screening library is to (almost) guarantee multiple hits against any GPCR−PA⁺ target; with drug space for a specific receptor so defined, subsequent, more dense sampling focused on this space will lead to the nanomolar ligands required of drug candidates. Thus, while highly active molecules reside within a few cells or even a single cell, we have shown that progressively less active compounds (but still active!) are layered in neighboring cells. (2) Different levels of receptor promiscuity may determine the volume of drug space for a given receptor; it is well-established, for example, that the dopamine D_4 receptor is highly promiscuous based on the ease with which antagonists can be made. (3) Compounds residing in one cell may lie at, or close to, the boundaries of that cell so that compounds in the nearest neighboring cell may actually be closer than if they were located at the extremities of the occupied cell.

The locations of compounds known to be active against GPCRs activated by positively charged peptide ligands projected into three of the five dimensions of this diversity space is shown graphically in Figure 1A. Analysis of their cell occupancies together with other categories of ligands in the full five-dimensional space

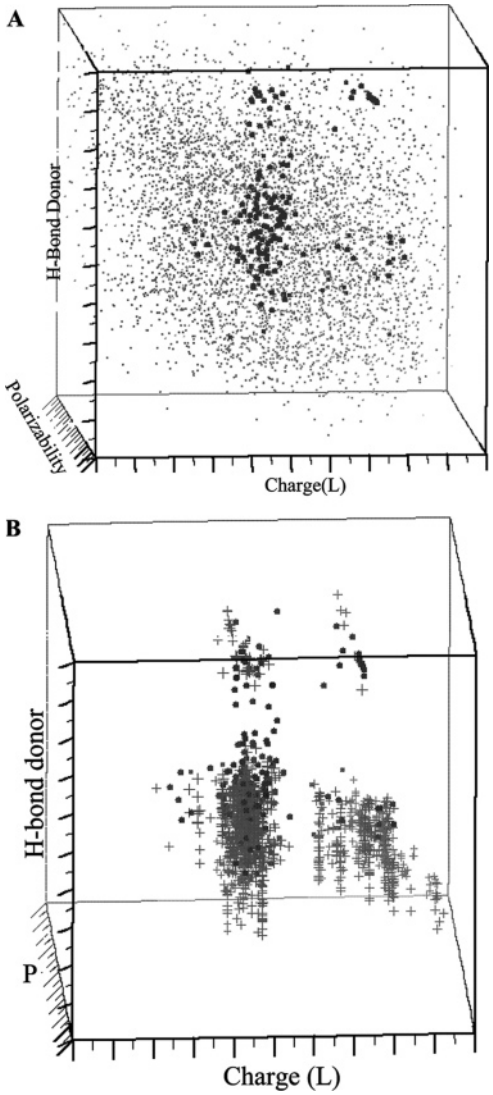


Figure 1. (A) Set of known non-peptide ligands for GPCR−PA⁺ (filled circles) mapped into three dimensions of BCUT space vs the WDI−NBI (gray dots; representative sampling only). (B) Projection of the test set (crosses) into drug space using the BCUT methodology each compared to GPCR−PA⁺ space (filled circles). (P = polarizability axis).

Table 1. Composition of the GPCR Training, Test, and Random Sets

ligand set	no. of ligands	no. of cells occupied	occupied + neighbor cells	% drug space (NBI-DS)
all drugs + NBI (NBI-DS)	81560	8506	91080	91
all GPCR	630	397	19225	19
monoamines	201	165	8727	9
monoacids	35	34	2853	3
GPCR-PA⁺	111	97	6437	6
GPCR-PA⁻	106	79	4050	4
random set	2024	1169	31098	31
random basic set	1401	352	15174	15
test set	2025	506	10692	11

is presented in Table 1. The various GPCR ligand classes occupy only small regions of this chemical space as compared to the original 81 560 compounds used to define its extent. Thus, GPCR ligands occupy 397 cells, whereas the broader collection, the NBI drug space set, occupies 8506 cells. If neighboring cells are included, all GPCR ligands occupy only 19% of drug space; in

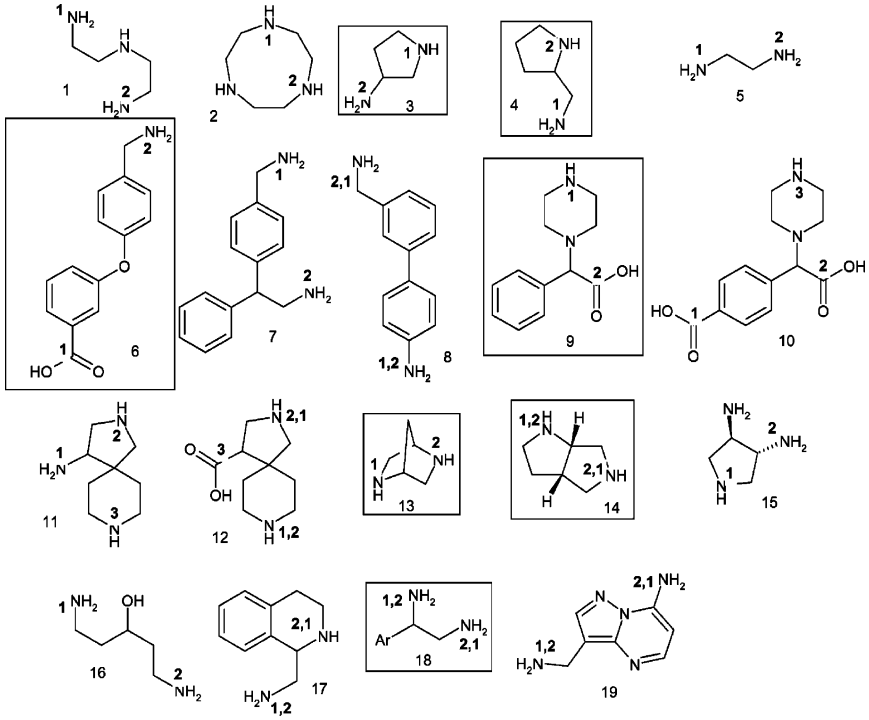


Figure 2. Templates selected for virtual library enumeration (points of monomer attachment are via NH or COOH groups). Numbers located near to the reacting functional group indicate an association with a specific monomer listed in Table 2. In some instances, because of symmetry in the template, this nomenclature is redundant.

addition, GPCR−PA^+ ligands form a distinct subspace (6%). An important goal of this preliminary investigation was then to evaluate whether these smaller regions of GPCR and GPCR−PA^+ space reflect a true mapping of properties common to these receptor classes and are predictive for designing new ligands or are simply due to the smaller numbers of compounds used to define the regions, compared to the overall space.

Virtual Library Enumeration. There were 19 templates in the Neurocrine template database with at least one basic center in the central scaffold (Figure 2) that were selected for computational analysis so that this key feature would be present in all of the final molecules. Electronic lists of commercially available reagents (alkyl halides, aldehydes, carboxylic acids, and sulfonyl halides) that serve as side chains in each of these templates were prepared using the Available Chemicals Directory (ACD). Reagents with reactive functionality that could not be blocked with a protecting group, potentially toxic moieties (polyaromatics, Michael acceptors, etc.), or reagents that would give rise to compounds in the virtual library with molecular weights greater than 600 were discarded from the set. Clusters within these lists were established so that a subset of each reagent list could be selected, since the virtual molecules were stored as structural data (sd) files and electronic storage space was limited. Combination of templates and reagents lists yielded virtual molecules that spanned the NBI-drug space. The reagent lists would be fully expanded in the event of a positive hit from the screening process (i.e. as part of the optimization process). The virtual libraries were assembled with the aid of a commercially available computer program (Afferent Structure 3.0) to yield a virtual collection summarized in Table 2.

The five BCUT metrics discussed above were calculated for all of the virtual compounds (Table 2) in order to evaluate their location relative to the GPCR−PA^+ space defined above. Virtual libraries selected for synthesis by the design algorithm were first explored synthetically by generation of a small (typically 20 compound) trial library, and synthetic failure resulted in rejection of the template. A substitute template that closely mirrored the drug space defined by the rejected templates was then selected. Of the 19 templates (Figure 2) that were subjected to computational analysis, seven were utilized in library production (boxed in Figure 2) to form the basis of the 2025 member test set.

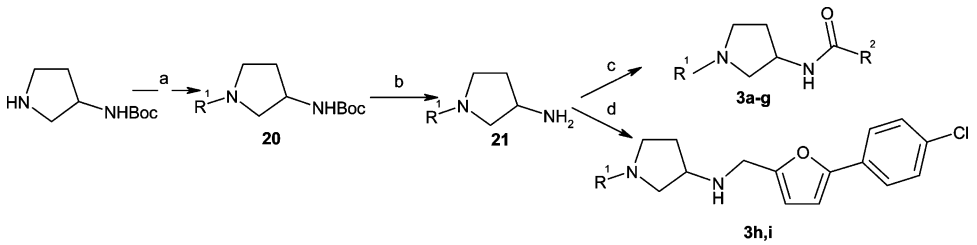
Synthetic Chemistry. The design principle for the test set required that a basic nitrogen atom, which will be predominantly protonated at physiological pH, be present in each molecule. As a result, the synthetic chemistry was restricted to reactions that preserved at least one basic nitrogen atom in the original core. Combinatorial libraries were prepared around templates **3**, **4**, **6**, **9**, **13**, **14**, and **18** utilizing synthetic procedures analogous to those described in Schemes 1 and 2. The template was orthogonally protected and synthesized on a 20-g scale. Side chain reagents were then reacted with the unprotected functional group and the intermediates were purified via automated flash chromatography.[10] The final compounds were then purified and characterized by automated preparative high performance liquid chromatography coupled to a mass spectrometer.[11] Each was prepared on a 10-mg scale and only compounds that were greater than 85% pure were screened to avoid misinterpretation of biological data. Validated hits were purified to >95% purity prior to K_i determination.

Table 2. Summary of the Virtual Library

template	available monomer 1	NBI-VL monomer 1	available monomer 2	NBI-VL monomer 2	available monomer 3	NBI-VL monomer 3	virtual library
1	RCHO	666	RCl	459	NA		305694
2	RCl	459	RCHO	666	NA		305694
3a	RCl	459	RCO2H	634	NA		291006
3b	RCO2H	634	RCl	459	NA		291006
3c	RCl	459	RCHO	666	NA		305694
4	RCOCl	282	RCl	459	NA		129438
5	RCl	459	RCHO	666	NA		305694
6	RNH2	719	RCl	459	NA		330021
7	RCHO	666	RCOCl	282	NA		187812
8	RCHO	666	RCOCl	282	NA		187812
9	RCHO	666	RNH2	719	NA		478854
10[a]	RNH2	80	RNH2	80	RCO2H	100	640000
11[a]	RCO2H	100	RCl	100	RCHO	80	800000
12a[a]	RCl	100	RCO2H	100	RNH2	80	800000
12b[a]	RCO2H	100	RCHO	80	RNH2	80	800000
12c[a]	RCl	100	RCHO	80	RNH2	80	640000
13	RCO2H	634	RCHO	666	NA		422244
14	RCl	459	RCO2H	634	NA		291006
15	RCO2H	634	RCl	459	NA		291006
16	RSO2Cl	158	RCl	459	NA		72522
17	RCO2H	634	RCHO	666	NA		422244
18	RCO2H	634	RCHO	666	NA		422244
19	RCHO	666	RCl	459	NA		305694
						total	9025685

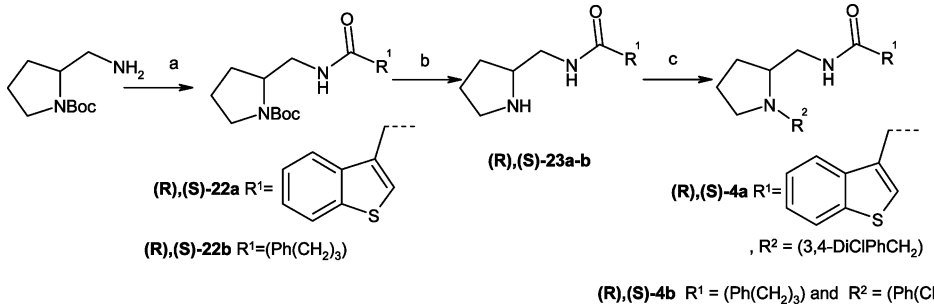
[a] Template has three positions of diversity.

Scheme 1. Compounds Derived from Template **3** and Synthetic Routes[a]



[a] Reagents: (a) R^1CHO, $Na(OAc)_3BH$, DCE, rt, 4 h; or (2-bromoethyl)benzene or 1-bromo-3,5,5-trimethylhexane, DMF, Hunig's base, 40°C, 12 h; (b) TFA, DCM, 1 h, rt; (c) R^2CO_2H, EDCI, HOBT, DMF/NMP, rt, 12 h; (d) 5-(4-chlorophenyl)-2-furaldehyde, $Na(OAc)_3BH$, DCE, rt, 12 h.

Scheme 2. Synthetic Chemistry Associated with Template **4**[a]



[a] Reagents: (a) R^1CO_2H, EDCI, HOBT, DCM, rt, 4 h, 40−85%; (b) TFA, DCM, rt, 1 h, 100%; (c) R^2Br, Hunig's base, 40 °C, 16 h, 50−85%.

Synthetic procedures for two templates (**3**, **4**), which afforded several of the hits for the MCH1−R, are discussed in more detail. Derivatives of template **3** were prepared (Scheme 1) from the monoprotected diamine 3-Boc amino pyrrolidine. Orthogonal protection of the primary amine was required in order to conduct a selective *N*-alkylation of the secondary amine, and the Boc protecting group was chosen due to the fact that it is removed rapidly under acidic conditions at room temperature. The intermediates **20a** and **20b** were prepared by reductive amination of 3-Boc amino pyrrolidine with the corresponding aldehydes in the presence of diisopropylethylamine (3 equiv) and sodium triacetoxyborohydride.[12] The intermediates **20c** and **20d** were respectively prepared by N-alkylation of 3-Boc amino pyrrolidine with (2-bromoethyl)benzene and 1-bromo-3,5,5-trimethylhexane in the presence of diisopropylethylamine in DMF at 40 °C. Deprotection of **20a**−**d** was accomplished by treatment with trifluoroacetic acid in dichloromethane, and then the intermediate primary amines **21a**−**c** were reacted with the corresponding carboxylic acids in the presence of EDCI and HOBT to yield the final compounds exemplified by **3a**−**3g**. Dichloroethane solutions of amines **21b** and **21d** were added to the corresponding aldehyde in the presence of diisopropylethylamine (3 equiv) and sodium triacetoxy-

borohydride. The resultant mixtures were agitated at room temperature for 12 h to yield the diamines **3h** and **3i**.

The synthesis of the active compounds derived from 2-aminomethylpyrrolidine template **4** is outlined in Scheme 2. Acylation of the monoprotected diamines (*R*)- and (*S*)-2-aminomethyl-1-Boc-pyrrolidine with the corresponding carboxylic acids in DMF in the presence of EDCI and HOBT at room temperature for 12 h yielded the intermediate amides (*R*)- and (*S*)-**22a,b**. Deprotection of (*R*)- and (*S*)-**22a,b** was accomplished with TFA in dichloromethane, and the product secondary amines (*R*)- and (*S*)-**23a,b** were then reacted with either 3,4-dichlorobenzyl bromide or phenethyl bromide in 3 equiv of diisopropylethylamine and *N*-methylpyrrolidinone as solvent to provide (*R*)- and (*S*)-**4a,b**.

Results

Screening Results. The 2025 compounds in the test set were arrayed in 96-well plates and dissolved in DMSO to a standard concentration of 15 μM. A "random set" of 2024 compounds was selected from Neurocrine's corporate screening collection and used as a control library. For practical convenience, it was possible to retrieve and assay only plates of compounds rather than discrete samples for the "random set". Each of 23 random plates selected contained 88 compounds per plate. We were concerned that the designed set may generate more hits than the random set, simply because the ubiquitous presence of a positive charge in the former may bias the outcome. For that reason, we also wished to compare the designed set's performance against a second set that was still random other than having an obligatory basic center. Fortuitously, we were in a position to make this comparison because all compounds made at Neurocrine are simultaneously assayed in all current screens, since we expect some "cross-talk" between the GPCRs within the area of interest. Thus, as before, compounds (not plates on this occasion) were selected at random from a list of over 30 000 compounds bearing a basic center. The caveat here is that compounds originally designed as ligands for either of the three receptors employed in this study were deselected from the pick for that receptor, which resulted in a 1401 member "random basic" set. Both the test and random sets of compounds were simultaneously tested for activity in three high-throughput screens (HTS) against receptors of high therapeutic potential. The melanocortin-4 receptor (MC4−R, obesity[13,14]), melanin concentrating hormone receptor (MCH1−R, obesity and depression[15,16]), and gonadotropin-releasing hormone receptor (GnRH−R, endometriosis, uterine fibroids, prostate cancer[17,18]) are members of the class A subclass of GPCRs activated by peptides bearing a positive charge. Compounds were screened in singlet at 15 μM in radioligand binding assays in standard HTS mode in 96-well format. Each plate contained six "total" wells containing no compounds and six "background" wells containing an excess of a cognate competitor to define specific binding for each plate (total cpm − backgound cpm). The Z' factor of each assay plate was approximately 0.5, verifying the suitability of the assays for HTS.[19] Compounds that displaced 50% or more of the specifically bound radioligand were designated as a "hit" and their activity was confirmed by repeating them in duplicate on a separate day. Compounds that displaced 50% or more of the specifically bound radioligand in the follow-up assay were defined as "confirmed hits" (Table 3). Active compounds were then subjected to 12-point dose−response curve analysis to determine binding affinities (typical curves are exemplified for MCH1−R in Figure 3) and the resulting K_is from templates **3** and **4** are listed in Table 4. A second random set of 1401 compounds, which all contain a basic nitrogen, was selected from Neurocrine's corporate collection and used as a basic/random control library. This random basic set was tested under identical conditions to the test and random sets.

Table 3. Distribution of Confirmed Hits for HTS for the Test, Random, and Random Basic Sets[a]

	confirmed hits			hit rate (%)		
receptor	test set	random set	random basic set	test set	random set	random basic set
hMC4	61	1	5	3.00	0.05	0.35
rMCH1	123	6	5	6.10	0.30	0.35
GnRH	9	2	1	0.44	0.10	0.07

[a] Confirmed hits represent compounds that displaced 50% or more binding in both the original HTS and the confirmation assay. The hit rate was determined as (no. confirmed hits/total no. of compounds tested) × 100. The test set, random set, and random basic set contained 2025, 2024, and 1401 compounds, respectively.

As an initial proof of concept for the design process, the number of hits identified in the designed set was significantly greater than those obtained from the random sets for each of the three receptors (Table 3). Hit enrichment rates ranged from 4.5-fold (GnRH−R) to 61-fold (MC4−R) when compared with the random set. Although the random basic set picked up more hits than the random set for MC4, the designed set was still more impressive, even allowing for the smaller size of the former. Thus, for MC4−R, the 2025 compound test set gave a number of hits that would have required screening more than 120000 compounds of a typical corporate collection. The absolute number of hits varied from 9 for the GnRH receptor to 123 for the MCH1 receptor.

On the basis of cell occupancy (Table 1), it is clear that the designed set (506 and 10 692 cells occupied and occupied plus neighboring cells, respectively) is less diverse than the random set (1169 and 31098). This was verified by a standard diversity/similarity analysis, whereby MACCS keys were used as the fingerprint and Tanimoto coefficients used as the similarity measure. The method was used to calculate the similarity of every possible pair of compounds in each set with the result that the designed set was least diverse (mean similarity 0.56), the random set most diverse (0.33), and the random basic set (0.48) somewhere between the two.

Structure−Activity Observations: MCH1−R. The current library was not designed to establish structure−activity predictions for each receptor; the intent was more that the set should provide a starting point from which such predictions can be made. Since many of the highest affinity ligands that bound the MCH receptor identified from this library originated from templates **3** and **4** (Table 4), some observations may be made. SNAP-7941,[16] a known selective and highly potent

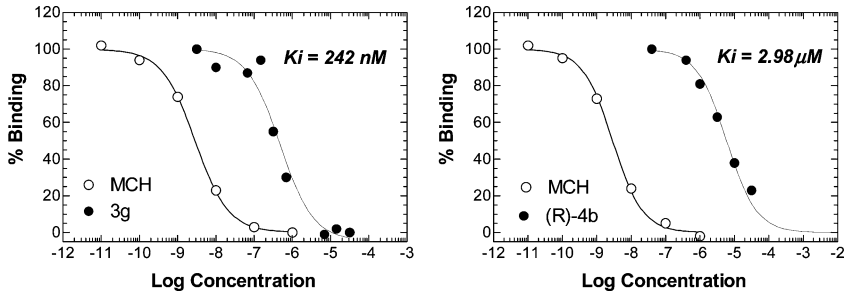
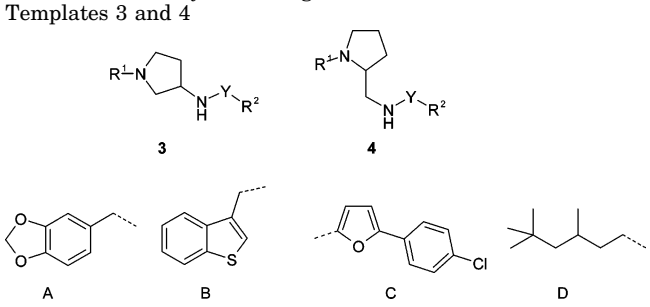


Figure 3. Representative dose−response curves of MCH−R hits from the test set. The K_i values are derived from the IC_{50} values of inhibition using the Cheng−Prusoff equation.

Table 4. Summary of Binding Data for MCH Hits from Templates 3 and 4



compd	Y	R^1	R^2	MCH1-R binding, K_i (μM)[a]
SNAP-7941				0.002 (±0.001)[b]
3a	CO	$PhCH_2$	$PhCH_2$	>10
3b	CO	$PhCH_2CH_2$	$PhCH_2$	>10
3c	CO	3,4-di-Cl-$C_6H_3CH_2$	$PhCH_2$	0.98
3d	CO	3,4-di-Cl-$C_6H_3CH_2$	$PhCH_2CH_2$	>10
3e	CO	3,4-di-Cl-$C_6H_3CH_2$	4-PhO-$C_6H_4CH_2$	0.51
3f	CO	3,4-di-Cl-$C_6H_3CH_2$	A	0.63
3g	CO	3,4-di-Cl-$C_6H_3CH_2$	B	0.36
3h	CH_2	Ph_2CHCH_2	C	1.7
3i	CH_2	D	C	5.0
(*R*)-4a	CO	3,4-di-Cl-$C_6H_3CH_2$	B	9.8
(*S*)-4a	CO	3,4-di-Cl-$C_6H_3CH_2$	B	6.0
(*R*)-4b	CO	$PhCH_2CH_2$	$PhCH_2CH_2CH_2$	3.1
(*S*)-4b	CO	$PhCH_2CH_2$	$PhCH_2CH_2CH_2$	>10

[a] Value is an average of three determinations unless reported otherwise. [b] $n = 7$.

MCH1−R ligand, was used as a positive reference in the biological assays for K_i determination. Benzyl (**3a**) and phenethyl (**3b**) substituents at the secondary nitrogen of the amino amide derivative of template **3** did not result in a compound that bound MCH1−R, but the addition of chlorine atoms at the 3 and 4 positions of the benzyl group did result in a compound with moderate affinity for the MCH1 receptor (**3c**, $K_i = 0.98$ μM). Increasing the distance between the core and the phenyl group at R^2 did not improve the biological activity, as seen in compound **3d**, but increased binding affinity was observed when an additional phenoxy substituent was appended to the R^2 phenyl group (**3e**, $K_i = 0.51$ μM). Other related high-affinity MCH1−R compounds from the library incorporate a bicyclic moiety, as illustrated in compounds **3f** and **3g**, which possess K_i values of 0.63 and 0.36 μM, respectively.

Reduction of the amide bond in template **3** also resulted in diamines that are moderate affinity ligands for MCH1−R. Two diamine derivatives, **3h** ($K_i = 1.7$ μM) and **3i** ($K_i = 5$ μM), share an arylfuranyl group that is one atom closer to the core in the R^2 region of the template, and the R^1 region is significantly more lipophilic than the corresponding region in the amino amide derivatives. This suggests that the optimal relationship between side chain structure and MCH1−R affinity of the template **3** diamines will be distinct to that of aforementioned amino amides **3a**−**g**.

In general there were fewer and less active compounds derived from template **4** versus template **3**, perhaps suggesting that the extra conformational freedom is unfavorable entropically. Where a direct comparison can be made, such as between **3g** ($K_i = 0.36$ μM) and **(*S*)-4a** ($K_i = 6.0$ μM), almost a 20-fold difference in activity is observed. A slight improvement in MCH1−R binding is realized when the R^1 and R^2 substituents are phenethyl and phenpropyl, respectively [**(*R*)-4b**, $K_i = 3.1$ μM]. The difference in the electronic nature and shape of the side chains as well as a difference in the absolute stereochemistry of the template [**(*R*)-4a** and **(*S*)-4b** are less active than the corresponding enantiomers] again suggests distinct SAR within this subseries of MCH1−R ligands.

Using these "hints" of SAR, the synthesis of more focused libraries is underway; it is clear that both templates **3** and **4** could be used as starting points for further lead optimization. The designed library adds yet another dimension; however, actives are spread across more than one series of compounds (i.e. derived from several templates). This facet strengthens the resulting computational model, the next step being to derive 3-D pharmacophores and further increases the chance of being able to optimize activity below 10 nM following further iterations of design, synthesis, and assay. In the context of lead optimization, it is important to note that the set contains 1036 and 864 mono- and diamines, respectively (the remainder are triamines), and since all the hits are within these two classes, these properties should not present any difficulties.

Discussion

While the random set was shown to occupy 1169 cells totaling 31098 when neighboring cells were included, the test designed library (Figure 1B) physically occupied 506 and, with neighbors, 10692 cells, respectively. The screening hits from each of the three receptors have been projected into GPCR−PA^+ space (Figure 4) noting, of course, that we are unable to visualize simultaneously the remaining two dimensions. Of the 193 total hits, it was observed that MCH1−R, MC4−R, and GnRH−R respectively occupied 73, 35, and 7 cells, and with the exception of one GnRH−R hit, all are located in cells occupied by GPCR−PA^+ or their neighbors. This is very suggestive that GPCR−PA^+ space does indeed exist and

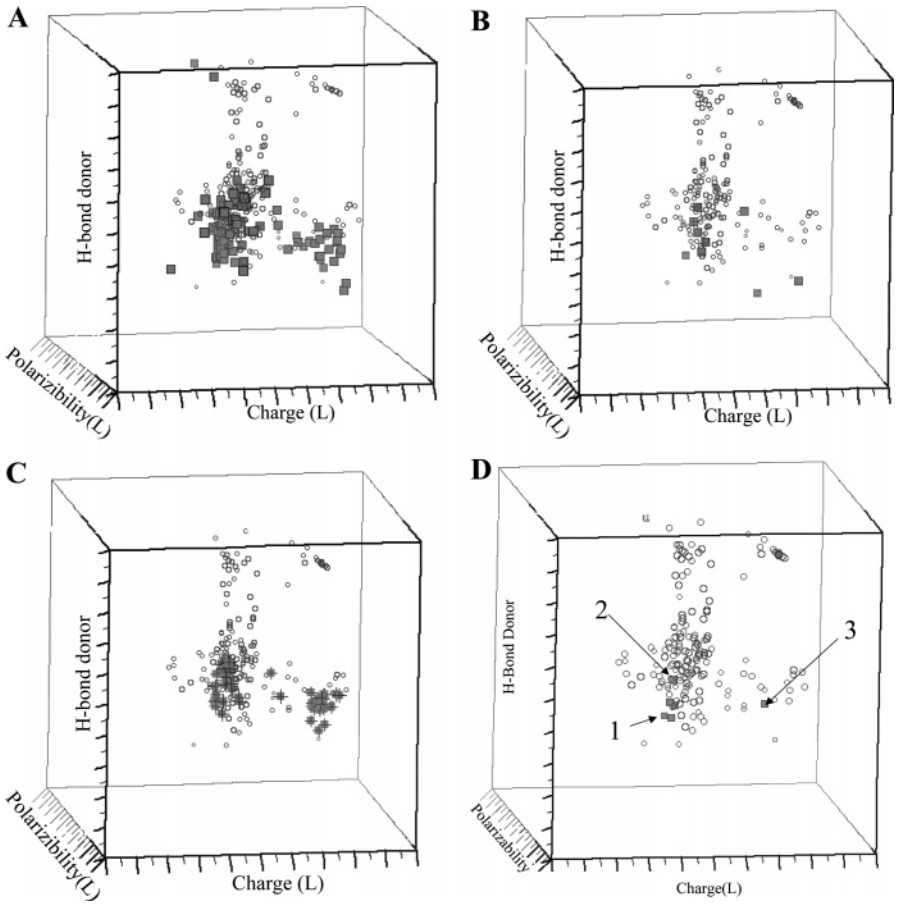


Figure 4. Screening hits (squares) from the test and random sets projected into drug space against a background of GPCR−PA^+ ligands (open circles). (A) MCH−R hits from the test set; (B) GnRH−R hits from the test set; (C) MC4−R hits from the test set; (D) hits from the random set [(1) region of GnRH random hits; (2) MC4, and (3) MCH)].

can assist ligand design for this subclass of receptors. Of the 73 cells occupied by the hits for MCH1−R, 15 and two overlapped with MC4−R and GnRH−R, respectively. For the MC4 receptor, 35 occupied cells had two cells in common with GnRH−R. Two cells had hits for all three receptors, and this information may be relevant for receptor selectivity. The GnRH hit referred to above as an "exception" is a consequence of the convenience of matrix synthesis, which results in several compounds being prepared that fall outside of the desired space. In contrast, the small number of hits from the random set arises from a significant, albeit low, occupation of GPCR−PA^+ space.

The data presented above show that a test library of 2025 compounds synthesized on the basis of their location within a GPCR−PA^+ ligand-enriched region of drug space is highly enriched (4.5−61-fold) in ligands for three receptors of this subfamily; the absolute hit rate ranged from 0.45% to 6%. If one postulates the existence of a discrete region of chemistry space that is enriched in ligands for this class of receptor, as is suggested by our preliminary data, then this variability in hit rate and enrichment ratio could be due to uneven sampling of either compounds or receptors in these experiments. As was discussed above, we observed that individual receptors appear to prefer compounds that reside in discrete subregions. It may be that the population of compounds in the initial set was not evenly distributed into regions of chemical space preferred by each of the three test receptors. Alternatively, the three receptors selected may have intrinsic characteristics (such as the number, size, shape, or complexity of potential ligand binding pockets) that result in greater or lesser degrees of ligand selectivity. Further, by querying the library with only three receptors, each of which can detect ligands only from their cognate subregion, one would not expect to delineate the entire range of the GPCR−PA^+ ligand-enriched region.

The design and synthesis of the completed library are now underway to attempt to fill, wherever possible, the remaining unoccupied cells together with their neighbors. What is already certain is that the task cannot be completed by another round of matrix-based parallel synthesis; rather a cherry-picking approach will be adopted so as to avoid the synthesis of large numbers of compounds falling outside the desired space and to avoid overpopulating cells already covered. It is expected that around 7000 compounds will be the minimum required to cover the relevant space (GPCR−PA^+ occupied plus neighboring cells = 6437) although, with some redundancy, this may be significantly larger. On the other hand, initial analysis suggests that it may not be possible to occupy some neighboring cells with real molecules, and this will reduce the number of compounds to be made.

Experimental Section

Synthetic Chemistry. General Methods. Proton NMR spectra were obtained with a Bruker 500 MHz spectrometer at Numega Labs. Inc., San Diego, CA, and chemical shifts are

reported in parts per million (δ) downfield from tetramethylsilane as internal standard. Matrix-assisted laser desorption/ionization (MALDI) FTMS experiments were performed on an IonSpec FTMS mass spectrometer at The Scripps Research Institute, San Diego, CA. Samples were irradiated with a nitrogen laser operated at 337 nm, and the laser beam was controlled by a variable attenuator and focused on the sample target. A Sciex API-150EX mass spectrometer operating in electrospray positive ionization mode was used to obtain [MH$^+$] ion of the target molecules. Optical activity was measured at 589 nm, 25 °C, using a Perkin-Elmer 341 polarimeter. Key intermediates were synthesized in normal laboratory glassware and purified by automated flash chromatography (Jones Chromatography, Lakewood, CO). Parallel synthesis experiments were performed under a nitrogen atmosphere using a liquid handler (Gilson 215, Middleton, WI) to deliver each solution into a deep-well microtiter plate with 96 750-μL glass inserts. The plates were agitated on a vortexing heating block (J-Kem, St. Louis, MO). Evaporation of the volatiles in vacuo was performed using a Savant Discovery SpeedVac. Purification of final compounds was conducted on a prep-LCMS Dionex system, using an Alpha C18 30 × 75 mm reverse phase column at a flow rate of 45 mL/min. The mobile phase was a gradient of 95/5 to 5/95 A/B, where A = H_2O−0.1% TFA, and B = CH_3-CN−0.1% TFA. All compounds after purification were reanalyzed on a reverse phase HPLC−MS system (DIONEX with ESI+ ionization mode) and shown to be at least 85% pure on the basis of both UV wavelengths (220, 254 nm) and total ion current (TIC) from the mass spectrum.

[1-(3,4-Dichlorobenzyl)pyrrolidin-3-yl]carbamic Acid *tert*-Butyl Ester (20a). To a solution of 3-(*tert*-butoxycarbonylamino)pyrrolidine (1.5 g, 8.06 mmol, 1 equiv) and *N,N*-diisopropylethylamine (2.80 mL, 2.0 equiv) in dichloroethane (20 mL) was added 3,4-dichlorobenzaldehyde (1.455 g, 8.06 mmol). The solution was stirred at room temperature for 10 min and then $NaBH(OAc)_3$ (2.570 g, 12.09 mmol) was added. The resultant mixture was allowed to stir for an additional 10 h and then the reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (10 mL). The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by automated flash chromatography (gradient elution: 30% EtOAc in hexane to 60% EtOAc in hexane) to afford [1-(3,4-dichlorobenzyl)pyrrolidin-3-yl]carbamic acid *tert*-butyl ester as an oil (1.80 g, 66.0%). MALDI−FTMS calcd for $C_{16}H_{22}Cl_2N_2O_2$: 345.1131 [MH$^+$]. Found: 345.1136. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 1.43 (s, 9H), 1.58 (m, 1H), 2.27 (m, 2H), 2.59 (m, 1H), 2.75 (m, 1H), 3.53 (m, 2H), 4.16 (s, 1H), 4.82 (s, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.41 (s, 1H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 28.6, 32.8, 50.0, 52.5, 59.0, 61.0, 79.6, 128.1, 130.5, 130.7, 131.2, 132.6, 139.2, 155.5.

[1-(2,2-Diphenylethyl)pyrrolidin-3-yl]carbamic Acid *tert*-Butyl Ester (20b). This compound was prepared according to the procedure described above using diphenylacetaldehyde in place of 3,4-dichlorobenzaldehyde (2.5 g, 84.5%). MALDI−FTMS calcd for $C_{23}H_{30}N_2O_2$: 367.2380 [MH$^+$]. Found: 367.2362. ^{1}H NMR (500 MHz, methanol-d_4): δ 1.40 (s, 9H), 1.50 (m, 1H), 2.10 (m, 1H), 2.40 (m, 1H), 2.48 (m, 1H), 2.68 (m, 1H), 2.77 (m, 1H), 3.12 (d, J = 7.6 Hz, 2H), 3.31 (m, 1H), 3.98 (m, 1H), 4.16 (t, J = 7.6 Hz, 1H), 7.14−7.30 (aromatic, 10H). ^{13}C NMR (500 MHz, methanol-d_4): δ 28.8, 32.6, 50.8, 52.0, 54.4, 62.1, 62.6, 80.2, 127.5, 129.2, 129.6, 145.3, 158.0.

[1-(3,5,5-Trimethylhexyl)pyrrolidin-3-yl]carbamic Acid *tert*-Butyl Ester (20c). To a solution of 3-(*tert*-butoxycarbonylamino)pyrrolidine (1.5 g, 8.06 mmol, 1 equiv) and *N,N*-diisopropylethylamine (2.80 mL, 2.0 equiv) in anhydrous DMF (20 mL) was added 1-bromo-3,5,5-trimethylhexane (1.75 g, 8.46 mmol, 1.05 equiv). The reaction mixture was stirred at 40 °C for 12 h, and after cooling to room temperature, the solvent was removed under reduced pressure. The crude product was purified by automated flash chromatography (gradient elution: 30% EtOAc in hexane to 60% EtOAc in hexane with 5% methanol) to afford [1-(3,5,5-trimethylhexyl)pyrrolidin-3-yl]-carbamic acid *tert*-butyl ester (1.3 g, 52.0%). MALDI−FTMS calcd for $C_{18}H_{36}N_2O_2$: 312.5 [MH$^+$]. Found: 313.2841. ^{1}H NMR (500 MHz, methanol-d_4): δ 0.92 (s, 9H), 0.94 (m, 1H), 0.96 (d, J = 6.5 Hz, 3H), 1.12 (AB, J = 5.8 and 14.0 Hz, 1H), 1.28 (AB, J = 2.4 and 14.0 Hz, 1H), 1.48 (s, 9H), 1.50 (m, 4H), 2.20 (m, 1H), 2.50 (m, 4H), 2.69 (m, 1H), 2.86 (m, 1H), 4.08 (m, 1H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.2, 28.7, 29.0, 30.4, 31.9, 32.3, 38.8, 50.6, 52.5, 54.0, 55.5, 55.7, 61.4, 80.2, 158.0.

1-(3,4-Dichlorobenzyl)pyrrolidin-3-ylamine, TFA Salt (21a). To a stirred solution of [1-(3,4-dichlorobenzyl)pyrrolidin-3-yl]carbamic acid *tert*-butyl ester **20a** (350 mg, 1.0 mmol) in CH_2Cl_2 (2 mL) was added trifluoroacetic acid (2 mL). The resultant solution was stirred at room temperature for 2 h and the CH_2Cl_2/TFA solvent was removed under reduced pressure. The residue was coevaporated with CH_2Cl_2, dissolved in MeOH (10 mL), and purified by prep-LCMS to yield the desired TFA salt as a colorless liquid (211.6 mg, 86.4%). MALDI−FTMS calcd for $C_{11}H_{14}Cl_2N_2$: 245.0607 [MH$^+$]. Found: 245.0596. ^{1}H NMR (500 MHz, methanol-d_4): δ 2.08 (m, 1H), 2.52 (m, 1H), 3.13 (m, 1H), 3.29 (m, 1H), 3.42 (m, 2H), 4.04 (m, 1H), 4.20 (m, 2H), 4.88 (s, 2H), 7.42 (dd, J = 8.2 Hz, 1.9 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 1.9 Hz, 1H). ^{13}C NMR (500 MHz, methanol-d_4): δ 30.0, 50.0, 53.7, 57.4, 58.3, 131.0, 132.3, 133.2, 134.1, 144.5.

1-(2,2-Diphenylethyl)pyrrolidin-3-ylamine, TFA Salt (21b). The compound was prepared according to the procedure given immediately above using **20b** in place of **20a** (176.0 mg, 66.0.%). MALDI−FTMS calcd for $C_{18}H_{22}N_2$: 267.1856 [MH$^+$]. Found: 267.1851. ^{1}H NMR (500 MHz, methanol-d_4): δ 2.07 (m, 1H), 2.49 (m, 1H), 3.38 (m, 1H), 3.57 (m, 1H), 3.64 (m, 1H), 4.00 (m, 3H), 4.48 (t, J = 7.8 Hz, 1H), 4.86 (s, 1H), 7.24−7.31 (aromatic, 10H). ^{13}C NMR (500 MHz, methanol-d_4): δ 29.6, 49.6, 49.8, 55.2, 58.2, 60.9, 128.8, 129.0, 130.3, 130.4, 141.9, 142, 157.

1-(3,5,5-Trimethylhexyl)pyrrolidin-3-ylamine, TFA Salt (21c). The compound was prepared according to the procedure given immediately above using **20c** in place of **20a** (255.0 mg, 81.5%). MALDI−FTMS calcd for $C_{13}H_{28}N_2$: 213.2325 [MH$^+$]. Found: 213.2320. ^{1}H NMR (500 MHz, methanol-d_4): δ 0.93 (s, 9H), 0.97 (m, 1H), 1.05 (d, 3H), 1.16 (AB, J = 6.0 and 14.0 Hz, 1H), 1.27 (AB, J = 3.3, 3.4, 1.3 Hz, 1H), 1.62 (m, 2H), 1.75 (m, 1H), 2.2 (m, 1H), 2.65 (m, 1H), 3.30 (m, 3H), 3.6 (m, 1H), 4.15 (m, 1H), 4.89 (m, 3H).

***N*-(1-Benzylpyrrolidin-3-yl)-2-phenylacetamide (3a).** To a solution of phenylacetic acid (40 mg, 0.3 mmol) in NMP (1.5 mL) was added a solution of commercially available 1-benzyl-pyrrolidin-3-ylamine (44 mg, 0.25 mmol) and *N,N*-diisopropylethylamine (130.0 μL, 0.75 mmol) in DMF (1.0 mL). This followed by a solution of EDCI (86 mg, 0.45 mmol) and HOBT (59 mg, 0.45 mmol) in NMP (1.0 mL). The reaction mixture was stirred at room temperature for 16 h and then purified by prep-LCMS to give the desired product as the TFA salt (62.0 mg, 85.0%). MALDI−FTMS calcd for $C_{19}H_{22}N_2O$: 295.1805 [MH$^+$]. Found: 295.1808. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 1.77 (m, 1H), 2.12 (m, 1H), 2.41 (m, 1H), 2.89 (m, 1H), 3.02 (m, 1H), 3.36 m, 1H), 3.46 (s, 2H), 3.70 (m, 1H), 4.08 (m, 1H), 4.26 (m, 1H), 4.79 (m, 1H), 7.25 (m, 5H), 7.43 (m, 5H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 30.6, 43.5, 47.2, 52.6, 58.8, 59.0, 115.7, 118.0, 127.1, 128.8, 128.9, 129.2, 129.4, 129.8, 130.4, 130.6, 135.2, 171.7.

***N*-(1-Phenethylpyrrolidin-3-yl)-2-phenylacetamide (3b).** The compound was prepared according to the procedure described for compound **3a** using 1-phenethylpyrrolidin-3-ylamine[20] in place of 1-benzylpyrrolidin-3-ylamine (54.9 mg, 70%). MALDI−FTMS calcd for $C_{20}H_{24}N_2O$: 309.1961 [MH$^+$]. Found: 309.1968. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 2.13 (m, 1H), 2.43 (m, 1H), 2.81 (m, 1H), 2.96 (m, 1H), 3.07 (m, 2H), 3.24 (m, 2H), 3.49 (s, 2H), 3.63 (m, 1H), 3.83 (m, 1H), 4.22 (m, 2H), 7.15−7.52 (aromatics, 10H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 24.0, 30.6, 43.5, 47.5, 53.5, 57.2, 60.2, 127.2−135.8, 171.8

***N*-[1-(3,4-Dichlorobenzyl)pyrrolidin-3-yl]-2-phenylacetamide (3c).** To a solution of phenylacetic acid (40 mg, 0.3 mmol) in NMP (1.5 mL) was added a solution of 1-(3,4-dichlorobenzyl)pyrrolidin-3-ylamine **21a** (73 mg, 0.25 mmol)

and *N,N*-diisopropylethylamine (130.0 μL, 0.75 mmol) in DMF (1.0 mL). This was followed by a solution of EDCI (86 mg, 0.45 mmol) and HOBT (59 mg, 0.45 mmol) in NMP (1.0 mL). The reaction mixture was stirred at room temperature for 16 h and then purified by prep-LCMS to give the desired product as the TFA salt (63.5 mg, 70.0%). MALDI−FTMS calcd for $C_{19}H_{20}Cl_2N_2O$: 363.1025 [MH^+]. Found: 363.1026. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 2.12 (m, 1H), 2.44 (m, 1H), 2.87 (m, 1H), 3.03 (m, 1H), 3.34 d, *J* = 12.5 Hz, 1H), 4.17 (d, *J* = 12.5 Hz, 1H), 4.88 (m, 1H), 7.26 (m, 5H), 7.34 (dd, *J* = 8.2 Hz, 1.7 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.54 (s, 1H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 30.4, 43.4, 47.2, 52.9, 57.5, 59.3, 127.2, 128.8, 129.4, 129.7, 131.8, 132.4, 134.0, 135.1, 135.2, 171.8.

***N*-[1-(3,4-Dichlorobenzyl)pyrrolidin-3-yl]-2-(4-phenoxyphenyl)acetamide (3e).** The compound was prepared according to the procedure described for compound **3c** using 4-phenoxyphenylacetic acid in place of phenylacetic acid (70.3 mg, 62%). MALDI−FTMS calcd for $C_{25}H_{24}Cl_2N_2O_2$: 455.1288 [MH^+]. Found: 455.1283. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 2.08 (m, 1H), 2.45 (m, 1H), 2.87 (m, 1H), 3.03 (m, 1H), 3.29 (m, 1H), 3.44 (s, 2H), 3.60 (m, 1H), 3.99 (d, *J* = 12.9 Hz, 1H), 4.12 (d, *J* = 12.9 Hz, 1H), 4.74 (m, 1H), 6.91−7.55 (aromatics, 12H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 30.6, 42.6, 47.6, 52.9, 57.7, 59.2, 118.0, 119.1, 123.5, 129.5, 129.9, 130.7, 131.7, 132.3, 133.9, 156.5, 157.3, 171.7.

2-Benzo[1,3]dioxol-5-yl-*N*-[1-(3,4-dichlorobenzyl)pyrrolidin-3-yl]acetamide (3f). The compound was prepared according to the procedure described for compound **3c**, using 3,4-methylenedioxyphenylacetic acid in place of phenylacetic acid (86.0 mg, 85.0%). MALDI−FTMS calcd for $C_{20}H_{20}Cl_2N_2O_3$: 407.0924 [MH^+]. Found: 407.0915. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 2.12 (m, 1H), 2.45 (m, 1H), 2.89 (m, 1H), 3.06 (m, 1H), 3.35 (m, 1H), 3.37 (s, 2H), 3.68 (m, 1H), 4.03 (m, 1H), 4.19 (d, *J* = 13.0 Hz, 1H), 4.80 (m, 1H), 5.90 (s, 2H), 7.12 (dd, *J* = 8.2 Hz, 1.8 Hz, 1H), 7.32−7.54 (aromatics, 5H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 30.4, 43.0, 47.3, 53.0, 57.5, 59.2, 101.2, 108.6, 109.7, 122.5, 129.6, 131.8, 132.4, 133.9, 135.2, 146.9, 148.0, 172.0.

2-Benzo[*b*]thiophen-3-yl-*N*-[1-(3,4-dichlorobenzyl)pyrrolidin-3-yl]acetamide (3g). This compound was prepared according to the procedure described for compound **3c**, using benzo[*b*]thiophene-3-acetic acid in place of phenylacetic acid (52.3 mg, 50.0%). MALDI−FTMS calcd for $C_{21}H_{20}Cl_2N_2OS$: 419.0746 [MH^+]. Found: 419.0756. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 2.04 (m, 1H), 2.38 (m, 1H), 2.77 (m, 1H), 2.97 (m, 1H), 3.24 (m, 1H), 3.47 (s, 1H), 3.55 (m, 1H), 3.72 (m, 1H), 3.88 (d, *J* = 12.8 Hz, 1H), 4.7 (d, *J* = 12.8 Hz, 1H), 4.74 (m, 1H), 7.26−7.82 (11H aromatic). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 30.4, 36.5, 48.0, 52.9, 57.5, 59.1, 122.1, 123.0, 124.4, 124.6, 125.0, 129.4, 129.6, 129.9, 131.7, 132.3, 133.9, 135.0, 138.8, 140.5, 170.6.

[5-(4-Chlorophenyl)furan-2-ylmethyl][1-(2,2-diphenylethyl)pyrrolidin-3-yl]amine (3h). To a stirred solution of 1-(2,2-diphenylethyl)pyrrolidin-3-ylamine, TFA salt **21b** (114 mg, 0.3 mmol) in dichloroethane (750 μL) in the presence of *N,N*-diisopropylethylamine (110 μL, 0.6 mmol) was added 5-(4-chlorophenyl)-2-furaldehyde (57.0 mg, 0.28 mmol). The reaction mixture was stirred at room temperature for 10 min and then $NaBH(OAc)_3$ (63 mg, 0.3 mmol) was added and the mixture was further stirred for 2 h. The reaction mixture was then diluted with water (100 μL) and the crude mixture was directly purified by prep-LCMS without any workup to give the desired diamine as a bis-TFA salt (25.6 mg, 20.0%). MALDI−FTMS calcd for $C_{29}H_{29}ClN_2O$: 457.2041 [MH^+]. Found: 457.2039. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 1.97 (m, 1H), 2.22 (m, 1H), 2.91 (m, 1H), 3.15 (m, 2H), 3.46 (s, 2H), 3.60 (m, 2H), 3.84 (m, 1H), 3.89 (s, 1H), 4.87 (t, *J* = 7.10 Hz, 1H), 6.37 (d, *J* = 2.8 Hz, 1H), 6.50 (d, *J* = 2.8 Hz, 1H), 7.20−7.53 (aromatics, 14H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 28.4, 42.6, 48.8, 53.7, 54.9, 57.0, 60.2, 106.5, 113.7, 125.3, 127.7, 127.8, 129.2, 134.0, 140.6, 140.7, 154.3, 162.7.

[5-(4-Chlorophenyl)-furan-2-ylmethyl][1-(3,5,5-trimethylhexyl)pyrrolidin-3-yl]amine (3i). This compound was prepared according to the procedure described for compound **3h**, using 1-(3,5,5-trimethylhexyl)pyrrolidin-3-ylamine, TFA salt **21c** in place of 1-(2,2-diphenylethyl)pyrrolidin-3-ylamine, TFA salt **21b** (100.0 mg, 83.7%). MALDI−FTMS calcd for $C_{24}H_{35}ClN_2O$: 403.2511 [MH^+]. Found: 403.2508. ^{1}H NMR (500 MHz, methanol-d_4): 0.92 (s, 9H), 0.97 (d, *J* = 6.5 Hz, 3H), 1.14 (AB, *J* = 6.0 Hz and 14.0 Hz, 1H), 1.23 (AB, 1H), 1.6 (m, 4H), 2.25 (m, 1H), 2.6 (m, 1H), 3.25 (m, 2H), 3.64 (m, 3H), 4.12 (m, 1H), 4.38 (m, 2H), 6.72 (d, *J* = 3.4 Hz, 1H), 6.87 (d, *J* = 3.4 Hz, 1H), 7.40 (m, 2H), 7.74 (m, 2H**).** ^{13}C NMR (500 MHz, methanol-d_4): δ 22.7, 28.7, 29.2, 30.4, 31.9, 35.9, 44.0, 52.0, 54.0, 55.2, 55.9, 56.5, 108.1, 115.6, 126.6, 130.2, 135.0, 148.0, 156.0.

(*R*)-2-[(2-Benzo[*b*]thiophen-3-yl-acetylamino)methyl]-pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester ((*R*)-22a). To a solution of benzo[*b*]thiophene-3-acetic acid (1.15 g, 6 mmol) and *N,N*-diisopropylethylamine (3.1 mL, 15 mmol) in CH_2Cl_2 (20 mL) was added (*R*)-1-*tert*-butoxycarbonyl-2-aminomethylpyrrolidine (1.0 g, 5 mmol), followed by EDCI (1.432 g, 7.5 mmol) and HOBT (1.01 g, 7.5 mmol). The reaction mixture was stirred at room temperature for 16 h, diluted with CH_2Cl_2 (25 mL), and washed with aqueous 2.0 M NaOH solution (20 mL) and then brine (20 mL). The organic extract was dried over sodium sulfate and the solvent was removed in vacuo. The residue was purified by automated flash chromatography (gradient elution: 10% EtOAc in hexane to 35% EtOAc in hexane) to yield the desired compound as a white oil (1.3 g, 71.0%). MALDI−FTMS calcd for $C_{20}H_{26}N_2O_3S$: 397.1556 [MNa^+]. Found: 397.1564. ^{1}H NMR (500 MHz, methanol-d_4): 1.43 (d, 9H), 1.72 (m, 3H), 1.84 (m, 1H), 3.17 (m, 1H), 3.26 (m, 2H), 3.43 (m, 1H), 3.78 (s, 2H), 3.85 (m, 1H), 7.36 (m, 3H), 7.85 (m, 2H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.8, 28.9, 29.7, 37.2, 42.7, 47.6, 81.2, 122.9, 123.9, 125.3, 125.6, 125.7, 125.9, 140.1, 141.9, 173.3.

(*S*)-2-[(2-Benzo[*b*]thiophen-3-yl-acetylamino)methyl]-pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester ((*S*)-22a). This compound was prepared according to the procedure described for compound **(*R*)-22a** using (*S*)-1-*tert*-butoxycarbonyl-2-aminomethylpyrrolidine in place of (*R*)-1-*tert*-butoxycarbonyl-2-aminomethylpyrrolidine (1.0 g, 53.4%). MALDI−FTMS calcd for $C_{20}H_{26}N_2O_3S$: 397.1556 [MNa^+]. Found: 397.1564. ^{1}H NMR (500 MHz, methanol-d_4): 1.44 (d, 9H), 1.72 (m, 3H), 1.84 (m, 1H), 3.18 (m, 1H), 3.27 (m, 2H), 3.45 (m, 1H), 3.78 (s, 2H), 3.85 (m, 1H), 7.36 (m, 3H), 7.86 (m, 2H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.8, 28.4, 29.5, 37.2, 42.7, 48.1, 81.2, 122.9, 124.0, 125.3, 125.6, 125.7, 125.9, 140.1, 141.9, 173.3.

(*R*)-2-[(4-Phenylbutyrylamino)methyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester ((*R*)-22b). This compound was prepared according to the procedure described for compound **(*S*)-22a** using 4-phenylbutyric acid in place of benzo[*b*]thiophene-3-acetic acid (1.6 g, 94.6%). MALDI−FTMS calcd for $C_{20}H_{30}N_2O_3$: 369.2149 [MNa^+]. Found: 369.2138. ^{1}H NMR (500 MHz, $CHCl_3$-*d*): δ 1.44 (s, 9H), 1.66 (m, 1H), 1.87 (m, 3H), 1.94 (m, 2H), 2.19 (m, 2H), 2.63 (m, 2H), 3.19 (m, 1H), 3.32 (m, 2H), 4.02 (m, 1H), 7.14−7.27 (m, 5H). ^{13}C NMR (500 MHz, $CHCl_3$-*d*): δ 24.1, 27.4, 28.6, 29.7, 35.6, 36.4, 46.4, 47.3, 56.4, 80.3, 126.0, 128.5, 128.7, 141.9, 157.0, 173.4.

(*S*)-2-[(4-Phenyl-butyrylamino)methyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester ((*S*)-22b). This compound was prepared according to the procedure described for compound **(*S*)-22a** using 4-phenylbutyric acid in place of benzo[*b*]thiophene-3-acetic acid (1.3 g, 80%). MALDI−FTMS calcd for $C_{20}H_{30}N_2O_3$: 369.2149 [MNa^+]. Found: 369.2138. ^{1}H NMR (500 MHz, methanol-d_4): δ 1.46 (d, 9H), 1.87 (m, 4H), 1.91 (m, 2H), 2.20 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 7.6 Hz, 2H), 3.38 (m, 4H), 3.9 (m, 1H), 7.17−7.25 (m, 5H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.8, 28.9, 36.7, 42.5, 47.6, 58.4, 81.0, 127.1, 129.5, 129.6, 129.7, 143.0, 176.2.

(*R*)-2-Benzo[*b*]thiophen-3-yl-*N*-1-pyrrolidin-2-ylmethylacetamide ((*R*)-23a). To a stirred solution of (*R*)-2-[(2-benzo[*b*]thiophen-3-yl-acetylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*R*)-22a** (800 mg, 2.1 mmol) in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (5 mL). The resultant solution was stirred at room temperature for 2 h and

the CH_2Cl_2/TFA solvent was removed under reduced pressure. The residue was coevaporated with CH_2Cl_2, dissolved in MeOH (10 mL), and purified by prep-LCMS to yield the desired compound as TFA salt (500.0 mg, 84.8%). MALDI−FTMS calcd for $C_{15}H_{18}N_2OS$: 275.1213 [MH^+]. Found: 275.1205. ^{1}H NMR (500 MHz, methanol-d_4): δ 1.74 (m, 1H), 1.98 (m, 2H), 2.08 (m, 1H), 3.22 (t, J = 7.6 Hz, 2H), 3.49 (d, J = 5.8 Hz, 2H), 3.66 (m, 2H), 3.86 (s, 2H), 7.6 (m, 2H), 7.47 (s, 1H), 7.83 (dd, 1H, J = 1.0 and 10 Hz), 7.89 (dd, J = 1.0 and 10 Hz). ^{13}C NMR (500 MHz, methanol-d_4): δ 24.5, 28.5, 36.6, 41.9, 46.7, 62.2, 122.9, 123.9, 125.4, 125.7, 126.2, 130.5, 140.1, 141.9, 175.1.

(*S*)-2-Benzo[*b*]thiophen-3-yl-*N*-1-pyrrolidin-2-ylmethylacetamide ((*S*)-23a). This compound was prepared according to the procedure described for compound **(*R*)-23a** using (*S*)-2-[(2-benzo[*b*]thiophen-3-ylacetylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*S*)-22a** in place of (*R*)-2-[(2-benzo[*b*]thiophen-3-ylacetylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*R*)-22a** (527.0 mg, 90.0%). MALDI−FTMS calcd for $C_{15}H_{18}N_2OS$: 275.1213 [MH^+]. Found: 275.1209. ^{1}H NMR (500 MHz, methanol-d_4): δ 1.93 (m, 1H), 1.98 (m, 2H), 2.08 (m, 1H), 3.22 (t, J = 7.3 Hz, 2H), 3.49 (d, J = 5.8 Hz, 2H), 3.66 (m, 2H), 3.86 (s, 2H), 7.6 (m, 2H), 7.47 (s, 1H), 7.83 (d, 1H, J = 7.4 Hz), 7.89 (d, J = 7.4 Hz). ^{13}C NMR (500 MHz, methanol-d_4): δ 24.5, 28.5, 36.6, 41.9, 46.7, 62.2, 122.9, 123.9, 125.4, 125.7, 126.2, 130.5, 140.1, 141.9, 175.1

(*R*)-4-Phenyl-*N*-1-pyrrolidin-2-ylmethylbutyramide ((*R*)-23b). This compound was prepared according to the procedure described for compound **(*R*)-23a** using (*R*)-2-[(4-phenylbutyrylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*R*)-22b** in place of (*R*)-2-[(2-benzo[*b*]thiophen-3-ylacetylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*R*)-22a** (484.0 mg, 92.0%). MALDI−FTMS calcd for $C_{15}H_{22}N_2O$: 247.1805 [MH^+]. Found: 247.1802. ^{1}H NMR (500 MHz, methanol-d_4): δ 1.74 (m, 1H), 1.94 (m, 2H), 2.02 (m, 1H), 2.03 (m, 1H), 2.07 (m, 1H), 2.26 (t, J = 7.5 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 3.30 (m, 2H), 3.45 (m, 2H), 3.67 (m, 1H), 7.17−7.25 (m, 5H). ^{13}C NMR (500 MHz, methanol-d_4): δ 24.5, 28.6, 36.2, 36.4, 41.7, 46.7, 62.2, 127.1, 129.5, 129.6, 143.0, 177.8.

(*S*)-4-Phenyl-*N*-1-pyrrolidin-2-ylmethylbutyramide ((*S*)-23b). This compound was prepared according to the procedure described for compound **(*R*)-23a** using (*S*)-2-[(4-phenylbutyrylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*S*)-22b** in place of (*R*)-2-[(2-benzo[*b*]thiophen-3-ylacetylamino)methyl]pyrrolidine-1-carboxylic acid *tert*-butyl ester **(*R*)-22a** (447.0 mg, 85.0%). MALDI−FTMS calcd for $C_{15}H_{22}N_2O$: 247.1805 [MH^+]. Found: 247.1800. ^{1}H NMR (500 MHz, methanol-d_4): δ 1.74 (m, 1H), 2.01 (m, 5H), 2.27 (t, J = 7.3 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 3.27 (m, 1H), 3.44 (d, J = 5.8 Hz, 2H), 3.66 (m, 1H), 7.2 (m, 5H). ^{13}C NMR (500 MHz, methanol-d_4): δ 24.5, 28.5, 36.2, 36.4, 41.7, 46.7, 62.3, 127.1, 129.5, 129.6, 129.7, 142.9, 177.9.

(*R*)-2-Benzo[*b*]thiophen-3-yl-*N*-[1-(3,4-dichlorobenzyl)-pyrrolidin-2-ylmethyl]acetamide ((*R*)-4a). To a solution of (*R*)-2-benzo[*b*]thiophen-3-yl-*N*-1-pyrrolidin-2-ylmethylacetamide **(*R*)-23a** (200.0 mg, 0.72 mmol) in the presence of *N,N*-diisopropylethylamine (375 μL, 2.16 mmol) in anhydrous DMF (3.6 mL) was added 3,4-dichlorobenzyl bromide (209.0 mg, 0.87 mmol). The reaction mixture was stirred at 40 °C for 12 h, cooled to room temperature, and purified by prep-LCMS to give the desired compound as the TFA salt (243.0 mg, 78.0%). MALDI−FTMS calcd for $C_{22}H_{22}Cl_2N_2OS$: 433.0903 [MH^+]. Found: 433.0899. $[\alpha]_D$ −10.457 (*c* 1, MeOH). NMR (methanol-d_4): δ 1.84 (m, 2H), 2.04 (m, 2H), 2.23 (m, 1H), 3.13 (m, 1H), 3.26 (m, 1H), 3.46 (m, 1H), 3.68 (m, 2H), 3.89 (s, 2H), 4.05 (d, J = 12.5 Hz, 1H), 4.48 (d, J = 12.5 Hz, 1H), 7.34 (m, 3H), 7.5 (s, 1H), 7.55 (d, 1H), 7.66 (d, 1H), 7.85 (m, 2H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.1, 28.0, 36.6, 41.3, 55.3, 57.9, 70.3, 122.9, 124.0, 125.4, 125.7, 126.4, 130.4, 131.5, 132.4, 132.5, 133.8, 134.3, 135.3, 139.9, 141.9, 176.2.

(*S*)-2-Benzo[*b*]thiophen-3-yl-*N*-[1-(3,4-dichloro-benzyl)-pyrrolidin-2-ylmethyl] -acetamide ((*S*)-4a). This compound was prepared according to the procedure described for compound **(*R*)-4a** using (*S*)-2-Benzo[*b*]thiophen-3-yl-*N*-1-pyrrolidin-2-ylmethyl-acetamide **(*S*)-23a** in place of (*R*)-2-Benzo[*b*]-thiophen-3-yl-*N*-1-pyrrolidin-2-ylmethyl-acetamide **(*R*)-23a** (126.0 mg, 50.0%). MALDI−FTMS calcd for $C_{22}H_{22}Cl_2N_2OS$: 433.0903 [MH^+]. Found: 433.0912. $[\alpha]_D$ +1.769 (*c* 1, MeOH). ^{1}H NMR (methanol-d_4): δ 1.83 (m, 2H), 2.03 (m, 2H), 2.21 (m, 1H), 3.13 (m, 1H), 3.24 (m, 1H), 3.47 (m, 1H), 3.64 (m, 2H), 3.89 (s, 2H), 4.04 (d, J = 12.5 Hz, 1H), 4.51 (d, J = 12.5 Hz, 1H), 7.33 (m, 3H), 7.51 (s, 1H), 7.55 (d, 1H), 7.66 (d, 1H), 7.86 (m, 2H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.2, 28.0, 36.6, 41.4, 55.3, 58.0, 70.4, 122.9, 124.0, 125.4, 125.7, 126.4, 130.4, 131.5, 132.5, 133.8, 134.3, 139.9, 141.9, 176.3.

(*R*)-*N*-(1-Phenethylpyrrolidin-2-ylmethyl)-4-phenylbutyramide ((*R*)-4b). This compound was prepared according to the procedure described for compound (**(*R*)-4a** using (2-chloroethyl)benzene in place of 3,4-dichlorobenzyl bromide (126 mg, 50.0%). MALDI−FTMS calcd for $C_{23}H_{30}N_2O$: 351.2431 [MH^+]. Found: 351.2423. $[\alpha]_D$ +11.382 (*c* 1, MeOH). ^{1}H NMR (methanol-d_4): δ 1.93 (m, 5H), 2.2 (m, 4H), 2.64 (t, J = 7.5 Hz, 2H), 3.0.6 (m, 2H), 3.30 (m, 2H), 3.45 (m, 1H), 3.66 (m, 3H), 7.26 (m, 10H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.5, 27.9, 28.5, 33.2, 36.1, 36.4, 40.7, 55.6, 57.3, 70.1, 127.2, 128.5, 129.5, 129.6, 129.9, 130.1, 137.6, 142.8, 178.7.

(*S*)-*N*-(1-Phenethylpyrrolidin-2-ylmethyl)-4-phenylbutyramide ((*S*)-4b). This compound was prepared according to the procedure described for compound (**(*S*)-4a** using (2-chloroethyl)benzene in place of 3,4-dichlorobenzyl bromide (189.0 mg, 75.0%). MALDI−FTMS calcd for $C_{23}H_{30}N_2O$: 351.2431 [MH^+]. Found: 351.2426. $[\alpha]_D$ −15.314 (*c* 1, MeOH). ^{1}H NMR (500 MHz, methanol-d_4): δ 1.36 (m, 2H), 1.93 (m, 3H), 2.13 (m, 1H), 2.24 (m, 3H), 2.64 (t, J = 7.5 Hz, 2H), 3.09 (m, 2H), 3.30 (m, 1H), 3.44 (m, 1H), 3.67 (m, 5H), 7.22 (m, 10H). ^{13}C NMR (500 MHz, methanol-d_4): δ 23.54, 28.0, 28.5, 33.2, 36.1, 36.4, 40.7, 55.6, 57.3, 70.1, 127.2, 128.5, 129.6, 137.6, 142.8, 178.7.

Computational Chemistry. Using an input SD file of the 81 560 compounds making up NBI-DS, standard 3D BCUT metrics were calculated for each member of the collection. A χ^2 approach provided by Diverse Solutions automatically chose and optimized which metric combinations would comprise the optimal chemistry space to represent maximum diversity of this set. The result was a chemical space defined by five BCUT metrics: charge, H-bond donor, H-bond acceptor, polarizability I (low eigenvalue), and polarizability II (high eigenvalue). Arbitrarily dividing each axis into 10 bins afforded 100 000 cells, so cell occupancy can be used as a measure of the diversity/similarity between the molecules based on the coordinates of each cell.

A similar process was used to describe the location of the known GPCR ligands having reported affinity for specific a GPCR (630 molecules), the training set (111 ligands for peptide-activated GPCRs), and the virtual libraries from which the test set (2025 compounds) was selected. In the case of the latter, virtual libraries for each of the 19 templates were enumerated separately using the Afferent and Project Library (MDL), and the resulting SD files contained over 9 million compounds. For reasons already described, the location of the training set (97 occupied cells) was expanded to include all neighboring cells (6437 cells). For every molecule in each virtual library, the same five BCUT metrics were calculated to give 19 cell-index files, which were then compared with the locations of molecules within the expanded training set. The cell-index files also provided information on how many cells were occupied by molecules from each virtual library, and the degree of overlap with the training set was assessed using an in house algorithm, "Cell Compare". Those virtual libraries having the highest percentage of occupied cells overlapping with the GPCR−PA$^+$ subspace were selected for synthetic trials; in all, 10 templates were thereby selected. As additional selection criteria, so as to achieve a balance between relevant space coverage and density of cell population, no more than 25 compounds per cell were selected for the test set, selection was biased to maximize the number of templates used, and finally, matrixes for parallel synthesis were chosen so as to maximize cell occupancy in GPCR−PA$^+$ subspace.

Biological Assays. MC4−R and MCH1−R binding assays were carried out with membrane preparations derived from mammalian cell lines stably transfected with human MC4 receptor (2) or rat MCH1 receptor (3). Crude membrane preparations were generated by nitrogen cavitation of cells followed by differential centrifugation. Specifically, cells were suspended in "binding buffer" and subjected to nitrogen cavitation at 900 psi on ice for 30 min. Lysed cells were centrifuged at 800*g* for 10 min, and the resultant supernatant was centrifuged at 38 000*g* for 20 min. Pellets were resuspended in the appropriate binding buffer and stored at −80 °C until use. MC4−R binding assays were carried out in 25 mM HEPES (pH 7.0), 1.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 100 mM NaCl. MCH1−R binding assays were carried out in 50 mM HEPES (pH 7.0), 10 mM $MgCl_2$, 2 mM EGTA. Binding reactions were carried out at equilibrium in 96-well format and were terminated by rapid vacuum filtration using a Packard Unifilter-96 plate harvester, over glass-fiber (GF−C) filter plates pretreated for 15 min with 0.5% polyethylenimine. Filters were washed four times with 0.2 mL of binding buffer.

GnRH receptor binding assays were carried out in whole-cell format using mammalian cells stably transfected with human GnRH receptor (4). Cells were harvested on the day of assay and assayed at 0.5×10^6 cells per well in 10 mM Hepes (pH 7.5), 150 mM NaCl, and 0.1% BSA. Binding reactions were carried out at equilibrium in 96-well format and terminated by rapid vacuum filtration over Millipore Multiscreen (GF−C) filter plates pretreated for 120 min with 0.1% polyethylenimine. Filters were washed two times with 0.2 mL phosphate-buffered saline (pH 7.4). In all binding assays, filters were dried, 0.05 mL Microscint-20 was added, and radioactivity was monitored by liquid scintillation counting.

In all binding experiments, specific binding is defined as that displaced by a known, pharmacologically relevant unlabeled competitor. The concentration of competitor was defined from competition curves for each assay where the competitor inhibited greater than 95% of tracer binding. Percent inhibition of binding for test compounds is defined as [1 − (specific binding in the presence of test compound)/(control specific binding)] × 100. Binding data for K_i determinations were analyzed by the nonlinear least squares curve-fitting program GraphPad Prism.

Acknowledgment. This work was supported in part by a grant from the National Institutes of Health (Grant Number 5R44 GM61491-03). In addition, we thank Dr. Christian Baber for help in analyzing the final data set.

Supporting Information Available: HPLC purity data and MALDI−FTMS data. Color versions of Figures 1 and 4 are available form the authors on request. This material is available free of charge via the Internet at htpp://pubs.acs.org.

References

(1) The most up to date listing can be viewed and searched on the GPCRDB home page at http://www.darmstadt.gmd.de/~gpcrdb.
(2) Watson, S.; Arkinstall, S. *The G-Protein Linked Receptor Facts-Book*; Academic Press: San Diego, CA, 1996.
(3) For a list of the Top 200 Prescription Medicines see: http://www.rxlist.com/top200.htm.
(4) Marshall, G. R. Peptide Interactions with G-Protein Coupled Receptors. *Biopolymers (Pept. Sci.)* **2001**, *60*, 246−277.
(5) Rees, D. C. Non-Peptide Ligands for Neuropeptide Receptors. *Annu. Rep. Med. Chem*. **1993**, *28*, 59−68.
(6) Sugg, E. E. Non-peptide Agonists for Peptide Receptors: Lessons from Ligands. *Annu. Rep. Med Chem*. **1997**, *32*, 277.
(7) Yang, L. Non-Peptide Somatostatin Receptor Ligands. *Annu. Rep. Med. Chem.* **1999**, *34*, 209−218.
(8) Pearlman, R. S.; Smith, K. M. Novel Software Tools for Chemical Diversity. *Perspect. Drug Discovery* **1998**, *9*, 339−353.
(9) Pearlman, R. S.; Smith, K. M. Metric Validation and the Receptor-Relevant Subspace Concept. *J. Chem. Inf. Comput. Sci*. **1999**, *39*, 28−35.
(10) Williams, J. P.; Lavrador, K. A Solution-Phase Combinatorial Synthesis of Selective Dopamine D_4 Ligands. *Comb. Chem. HTS* **2000**, *3*, 43−50.
(11) Zeng, L.; Burton, L.; Yung, K.; Shushan, B.; Kassel, D. B. Automated Analytical/Preparative High-Performance Liquid Chromatography−Mass Spectrometry System for the Rapid Characterization and Purification of Compound Libraries. *J. Chromatogr. A* **1998**, *794*, 3−13.
(12) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem*. **1996**, *61*, 3849−3862.
(13) Cone, R. D.; Lu, D.; Koppula, S.; Vage, D. I.; Klungland, H.; Boston, B.; Chen, W.; Orth, D. N.; Pouton, C.; Kesterson, R. A. The Melanocortin Receptors: Agonists, Antagonists, and the Hormonal Control of Pigmentation. *Rec. Prog. Hor. Res.* **1996**, *51*, 287−318.
(14) Goodfellow, V. S.; Saunders, J. The Melanocortin System and Its Role in Obesity and Cachexia. *Curr. Top. Med. Chem.* **2003**, *3*, 855−883.
(15) Qu, D.; Ludwig, D. S.; Gammeltoft, S.; Piper, M.; Pelleymounter, M. A.; Cullen, M. J.; Mathes, W. F.; Przypek, J.; Kanarek, R.; Maratos-Flier, E. A Role For Melanin-Concentrating Hormone in the Central Regulation of Feeding Behavior. *Nature* **1996**, *380*, 243−7.
(16) Borowsky, B.; Durkin, M. M.; Ogozalek, K.; Marzabadi, M. R.; DeLeon, J.; Lagu, B.; Heurich, R.; Lichtblau, H.; Shaposhnik, Z.; Daniewska, I.; Blackburn, T. P.; Branchek, T. A.; Gerald, C.; Vaysse, P. J.; Forray, C. Antidepressant, Anxiolytic and Anorectic Effects of a Melanin-Concentrating Hormone-1 Receptor Antagonist. *Nat. Med*. **2002**, *8*, 825−30.
(17) Sealfon, S. C.; Weinstein, H.; Millar, R. P. Molecular Mechanisms of Ligand Interaction with the Gonadotropin-Releasing Hormone Receptor. *Endocr. Rev*. **1997**, *18*, 180−205.
(18) Emons, G.; Grundker, C.; Gunthert, A. R.; Westphalen, S.; Kavanagh, J.; Versschraege, C. GnRH Antagonists in the Treatment of Gynaecological and Breast Cancers. *Endocr. Relat. Cancer* **2003**, *10*, 291−299.
(19) Zhang, J.-H.; Chung, T. D. Y.; Oldenburg, K. R. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J. Biomol. Screening* **1999**, *4*, 67−73.
(20) Ohmori, J.; Maeno, K.; Hidaka, K.; Nakato, K.; Matsumoto, M.; Tada, S.; Hattori, H.; Sakamoto, S.; Tsukamoto, S. Dopamine D3 and D4 receptors Antagonists: Synthesis and Structure−Activity Relationships of (S)-(+)-N-(1-Benzyl-3-pyrrolidinyl)-5-chloro-4-[(cyclopropylcarbonyl)amino]-2-methoxybenzamide (YM-43611) and related compounds. *J. Med. Chem.* **1996**, *39*, 2764−2772.

JM040084C