# Application of a Novel Design Paradigm to Generate General Nonpeptide Combinatorial Templates Mimicking β-Turns: Synthesis of Ligands for Melanocortin Receptors

Thomas R. Webb,*[,†,#] Luyong Jiang,[†] Sergey Sviridov,[‡] Ruben E. Venegas,[‡] Anna V. Vlaskina,[‡] Douglas McGrath,[†] John Tucker,[†] Jian Wang,[†] Alain Deschenes,[§] and Rongshi Li[†]

*ChemBridge Research Labs, Inc., ChemBridge Corporation, 16981 Via Tazon, San Diego, California 92127, and Chemical Computing Group, Suite 910-1010 Sherbrooke Street W, Montreal, Quebec, Canada H3A 2R7*

*Received December 2, 2006*

We report the further application of a novel approach to template and ligand design by the synthesis of agonists of the melanocortin receptor. This design method uses the conserved structural data from the three-dimensional conformations of β-turn peptides to design rigid nonpeptide templates that mimic the orientation of the main chain C-α atoms in a peptide β-turn. We report details on a new synthesis of derivatives of template **1** that are useful for the synthesis of exploratory libraries. The utility of this technique is further exemplified by several iterative rounds of high-throughput synthesis and screening, which result in new partially optimized nonpeptide agonists for several melanocortin receptors.

## Introduction

With the expansion in the application of library methods in medicinal chemistry and chemical biology, there is a growing need for methods that generally allow for the design of novel templates suited for the synthesis of targeted libraries (TLs) directed toward specific subsets of protein families. The discovery of druglike nonpeptide agonists at receptors with native peptide ligands is important in medicinal chemistry[1] because nonpeptide druglike compounds that mimic bioactive peptides are desirable for a large number of protein targets that are involved in many disease states.[2−10] We have previously reported a practical and novel method for the design of templates that are suitable for synthetic elaboration to yield TLs of small molecule compounds with druglike properties that exhibit peptide pharmacophores.[11,12] In this report, we delineate an improved stepwise process for the design of new β-turn-like templates and demonstrate the generality of this method by the synthesis of agonists of the melanocortin type-4 receptor (MC4R).[13,14]

Melanocortin receptors are G-protein-coupled receptors and are composed of five known subtypes (MC1R−MC5R). The first melanocortin receptor (MC1R) was identified in melanocytes;[15] subsequently, the other subtypes were identified and found to be expressed in different tissue types: MC2R (adrenal gland),[16] MC3R (brain, gut, and heart),[17] MC4R (brain),[18] and MC5R (brain).[19] The receptors in the melanocortin family mediate a large number of physiologic functions in humans including pigmentation, sexual function, and energy balance.[20] The MC4R receptor has attracted significant attention as a potential target for obesity because a clear relationship has been established between mutations of MC4R and severe obesity in humans.[21] Progress in the discovery of CNS-active ligands for the melanocortin receptors as potential therapeutics has been the subject of numerous reviews.[22]

Overall much of the progress in the discovery of small molecule nonpeptide *agonists* at G-protein-coupled receptors (GPCRs) with peptide ligands has relied on the stepwise modification of peptide analogs containing modified α-amino acids.[1−10,14] In general, this approach yields compounds with physical properties that limit their application as orally active, blood−brain barrier-penetrating drug leads.[24] An alternate approach is to develop small molecule templates (molecular scaffolds) that mimic the size and shape of the main chain of peptide β-turns that can also be combinatorially arrayed with the substituents which, in turn, may mimic the properly oriented interactions of the side-chains of a specific bioactive β-turn peptide. Despite the need and the interest in this problem, there have been only a few examples of the use of templates that do not include α-amino acids in the template.[9]

As part of our work on the development of new and practical methods for the design of templates that would be useful to medicinal and combinatorial chemists, we have recently published some of our initial results toward the development of such an approach.[11,12] As we have previously reported,[11] the starting point in the development of our approach were the critical insights reported by Garland and Dean.[25] These authors observed that cluster analysis and recombination of the experimentally observed positioning

* To whom correspondence should be addressed. E-mail: thomas.webb@stjude.org.
† ChemBridge Research Labs.
‡ ChemBridge Corporation.
§ Chemical Computing Group.
# Current address: Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, MS 1000, 332 North Lauderdale, Memphis, TN 38105.

10.1021/cc0601581 CCC: $37.00 
Published on Web 04/13/2007

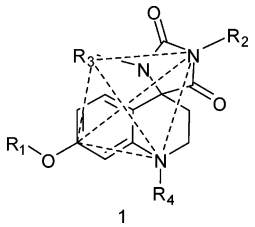
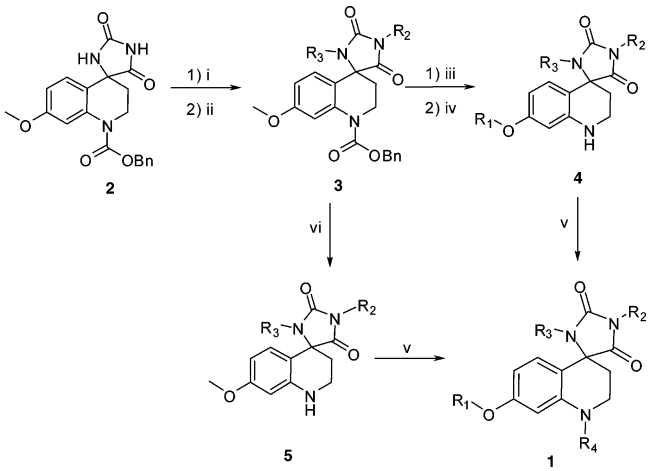


**Figure 1.** Template **1** is shown with dashed bonds to indicate the Garland−Dean triangles (connections of atoms that mimic the C-α atom positions in peptide β-turns).

**Scheme 1**[a]



[a] Reagents: (i) $R_2X/Na_2CO_3/DMF$; (ii) $R_3X/NaH/DMF$; (iii) $BBr_3/CH_2Cl_2$; (iv) $R_1OH/PPh_3/DEAD/THF$; (v) $R_4CHO/NaBH(OAc)_3/HOAc/CH_2Cl_2$; (vi) $NH_4CHO_2/Pd-C/MeOH$.

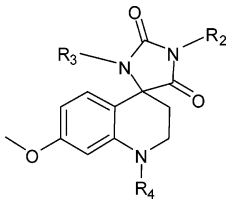
patterns yielded a triangular consensus positioning of the C-α atoms among the various β-turn types and that these triangles could be used as queries to search 3D databases to find existing compounds that match the consensus positioning of the C-α atoms of β-turns.

We have focused on the extension of the Garland−Dean approach to the *design and synthesis* of novel templates such as **1** (Figure 1), which contain multiple substitution points that match several of the Garland−Dean geometries.[11] This combination of features is rarely seen in randomly designed diverse libraries.[26] This design paradigm allows for the construction of other templates that, like **1**, are suitable for the exhibition of many of the interesting combinatorial pharmacophoric possibilities that may be observed in bioactive peptides. As an example of the utility of our design method, we report here the discovery of an MC4R "hit" (potential starting points for drug lead compound discovery) by the synthesis and functional screening of a small TL, based on template **1**, using the chemistry described in Scheme 1. We concentrated our search on compounds that are active at the MC4R subtype because druglike MC4R agonists are potential starting points for the development of drug leads for the treatment of obesity,[2,13]

## Results and Discussion

**Design of an MC4R Agonist TL Based on Template 1.** For our first exploratory synthetic iteration, we chose to prepare compounds using building blocks that could mimic some of the common interactions of the side-chains of potent *peptide* MC4R agonists, which contain the critical His-(D)-Phe-Arg-Trp sequence,[27] or the small molecule mimics that all contain an aryl group, a basic group, and a hydrophobic group.[22] Our first exploratory library was designed using building blocks that we hoped would present these interactions, although we expected that the contacts would not be optimal. Using the chemistry outlined in Scheme 1, we prepared a set of compounds **6**−**20** (Table 1) for evaluation as potential MC4R agonists, based on the β-turn template **1** (for details on the synthesis of **2**−**5** and all TL compounds see the Experimental Section and the Supporting Information). The most active compound in this first generation TL was compound **12**, which showed weak agonist activity at MC4R and contained 3-picolyl and 2-picolyl groups as substituents at $R_2$ and $R_3$, respectively. Presumably, the picolyl group could serve as both a basic group and an aryl group.

**Table 1.** First Generation MC4-Targeted Library[a]

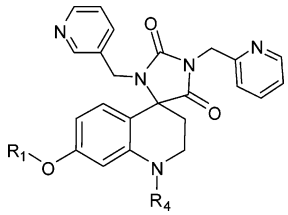


| compound | $R_2$ | $R_3$ | $R_4$ | $EC_{50}$ MC4 (μM) |
|---|---|---|---|---|
| **6** | INDE | H | Cbz | NA |
| **7** | 2-Pi | H | Cbz | NA |
| **8** | 4-Pi | H | Cbz | NA |
| **9** | INDE | Me | Cbz | >50 |
| **10** | INDE | 3-Pi | Cbz | 43 |
| **11** | 2-Pi | Me | Cbz | NA |
| **12** | 2-Pi | 3- Pi | Cbz | 28 |
| **13** | INDE | 3- Pi | H | 39 |
| **14** | INDE[H] | 3- Pi | H | >50 |
| **15** | PIPM-2 | Me | H | NA |
| **16** | 2-Pi | 3-Pi | H | NA |
| **17** | 4-Pi | 3-Pi | H | NA |
| **18** | INDE | Me | H | NA |
| **20** | INDE | Me | Ac | NA |

[a] Abbreviations: NA, not active; Ac, acetyl; Cbz, benzyloxycarbonyl; Me, methyl; INDE, indole-3-(eth-2-yl); PIPM-2, piperinemeth-2-yl; INDE[H], indoline-3-(eth-2-yl).

To explore the most active substitution pattern found in the first generation TL, a second generation TL was generated that held the best $R_2$ and $R_3$ pattern constant and modified the substitution at $R_1$ and $R_4$ to give compounds **21**−**32** (Table 2). This iteration yielded compound **26** with an $EC_{50}$ at MC4R of 5.6 μM (a 5-fold improvement) and also provided structure−activity relationship (SAR) data that suggested that large hydrophobic groups at $R_1$ were not desirable. Using this information, we prepared a third library of 199 compounds that primarily explored modifications at $R_3$, while $R_4$ was held constant as isobutyl (iBu), with some very limited modifications at $R_1$ and $R_2$ (Table 3; for a full list of the structures of the 199 compounds generated on this iteration, along with the screening data, see Supporting Information Table 1). The novel chemistry developed that allows for the high-throughput synthesis of derivatives that hold $R_4$ fixed and that was applied for this iteration is

**Table 2.** Second Generation MC4-Targeted Library[a]



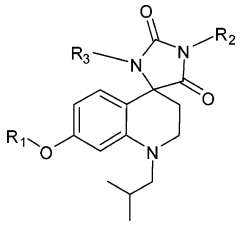
| compound | $R_1$ | $R_4$ | $EC_{50}$ MC4R ($\mu$M) |
|---|---|---|---|
| **21** | H | H | NA |
| **22** | $(Me)_2CH$ | H | NA |
| **23** | $CH_2{=}CHCH_2$ | H | NA |
| **24** | Bn | H | NA |
| **25** | Me | $Ph(CH_2)_2$ | 9.8 |
| **26** | Me | iBu | 5.6 |
| **27** | $CH_3CH_2CH_2$ | iBu | NA |
| **28** | Bn | $Ph(CH_2)_2$ | NA |
| **29** | $(Me)_2CHCH_2$ | iBu | NA |
| **30** | *p*-$ClPhCH_2$ | iBu | NA |
| **31** | *p*-$CH_3OPhCH_2$ | iBu | NA |
| **32** | $Ph(CH_2)_3$ | iBu | NA |

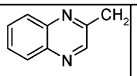
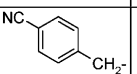
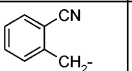
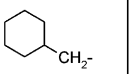
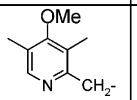
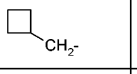
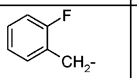
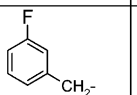
[a] Abbreviations: NA, not active; 2-Pi, 2-picolyl (pyridyl-2-methyl), 3-Pi, 3-picolyl; iBu, $Me_2CHCH_2$.

described in Scheme 2 and the Supporting Information. This TL yielded a small number of compounds (e.g., **33**−**43**) that were confirmed (see the Experimental Section) to be MC4R agonists that are as active as or more active than **26**. Table 3 also includes the inactive but structural related compounds **44**−**46** as examples of the diversity of this iteration and of the sensitivity of the observed SAR. Compounds **25** and **26** showed selective agonism for the MC3 and the MC4 receptors when they were subjected to similar assays for agonist activity with the related MC subtypes (MC1, MC2, MC3, and MC5), as well as with the unrelated receptor neuropeptide Y (subtype 5) and the somatostatin (subtype 2) receptors (see Table 4 and Supporting Information). This suggests that compounds could be developed based on this scaffold that have even higher selectivity for MC4. The potential for highly selective MC4 agonism is one important consideration in the selection of leads for obesity.[22]

In summary, we present another example of application of our previously published template **1** that supports the proposal that this template (and the method that was used to design it) have broad application in the design of compounds that mimic the important pharmacophores of $\beta$-turn peptides.[11,19] Using this approach, we have found new agonists and antagonists for MC4R, and we further demonstrate the utility of this method of template and TL design. The four points of substitution of template **1** could readily allow for the preparation of on the order of $10^8$ analogs, and yet with only the exploration of several hundred compounds, we have been able to discover and partially optimize designed TL screening hits with a 40-fold improvement of activity to yield submicromolar full agonists of MC4R (see Supporting Information). The application of this method to other target families should allow for the facilitation of the discovery of other hits (molecular starting points for medicinal chemistry optimization), as well as new small molecule tool compounds

**Table 3.** Examples of the Third Generation MC4-Targeted Library of 199 Compounds[a]

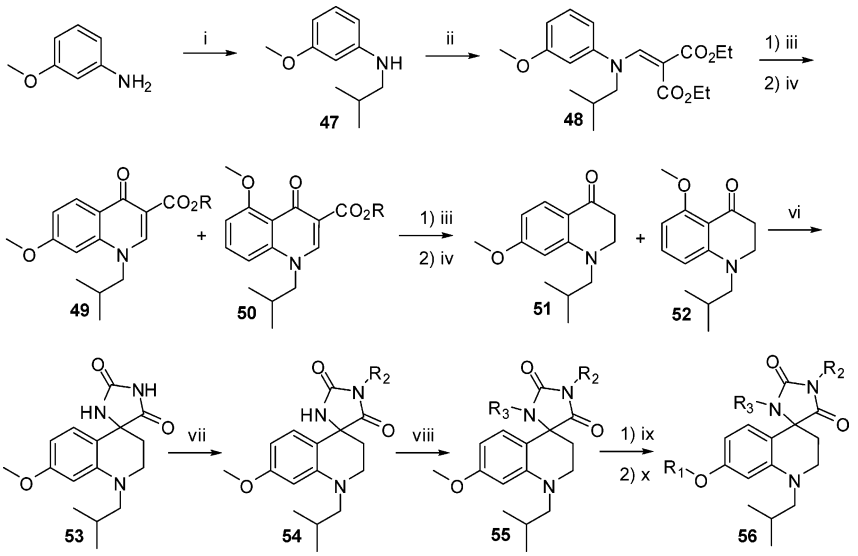


| Compound | $R_1$ | $R_2$ | $R_3$ | $EC_{50}$ MC-4 ($\mu$M) |
|---|---|---|---|---|
| **33** | Me |  | 3-Pi | 0.7 |
| **34** | Me |  | 3-Pi | 1.0 |
| **35** | Et | 2-Pi | 3-Pi | 1.2 |
| **36** | Me |  | 3-Pi | 1.6 |
| **37** | iPr | 2-Pi | 3-Pi | 2.0 |
| **38** | Me |  | 3-Pi | 2.1 |
| **39** | Me | 4-Pi | 3-Pi | 2.5 |
| **40** | Me |  | 3-Pi | 2.5 |
| **41** | Me |  | 3-Pi | 4.9 |
| **42** | Me | 2-Pi | 4-Pi | 6.4 |
| **43** | Me |  | 3-Pi | 6.6 |
| **44** | Me | 2-Pi | 2-Pi | NA |
| **45** | Me |  | 3-Pi | NA |
| **46** | Me | 3-Pi |  | NA |

[a] The full list of 199 compounds and screening data can be found in Supporting Information Table 1. Abbreviations: NA, not active; 2-Pi, 2-picolyl (pyridyl-2-methyl), 3-Pi, 3-picolyl; 4-Pi, 4-picolyl. For full details, see the Supporting Information. The chemistry utilized for the preparation of these TL is Described in Scheme 2.

(for use in the elucidation of biological or biochemical mechanisms), for other protein family subtypes. We have developed a formal computational approach that facilitates the design of new templates and ligands by chemists that will be published in the near future. It should also be noted that this method has broader potential application, including the use of information derived solely from the interactions of well-defined pharmacophores, which we are in the process of developing. Progress on the development of these techniques will be reported in the near future.

**Scheme 2.** Larger TLs[a]



[a] (i) *i*-PrCHO, $H_2$, Raney-Ni/EtOAc; (ii) EtOCH=C(COOEt)$_2$, heat; (iii) $POCl_3$, heat; (iv) NaOH/MeOH−$H_2O$; (v) $NaBH_4$/EtOH; (vi) NaCN, $(NH_4)_2CO_3$/EtOH, heat; (vii) $R_2$-Hal, $Na_2CO_3$/DMF; (viii) $R_3$-Hal, NaH/DMF or $R_2$-OH, DIAD/$Ph_3P$; (ix) DCM/$BBr_3$, 0 °C; (x) NaHMDS/THF, then $R_1$-X.

**Table 4.** $EC_{50}$ Data Using GeneBlazer Assays[28,30] ($\mu$M)[a]

| compound | MC1R | MC2R | MC3R | MC4R | MC4 antagonist[b] | MC5R | NPY5R | SST2R |
|---|---|---|---|---|---|---|---|---|
| **25** | NA | NA | 19 | 9.8 | NA | NA | NA | NA |
| **26** | NA | NA | 5.6 | 5.6 | NA | NA | NA | NA |

[a] Abbreviations: NA, not active; MCXR, melanocortin subtype-X receptor; NPY5R, neuropeptide Y subtype-5 receptor; SST2R, somatostatin subtype-2 receptor; $EC_{50}$ or $IC_{50}$ greater than 40 $\mu$M. [b] Geneblazer Antagonist Assay[28,30]

## Experimental Section

**Experimental Chemistry. General Methods for the Compounds in Tables 1 and 2.** Thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254. Separation by column chromatography was performed on Merck silica gel 60 for flash chromatography. Nuclear magnetic resonance ($^1$H NMR) spectra were recorded on a Brucker AMX 300. Chemical shifts were expressed in parts per million ($\delta$) with tetramethylsilane (TMS) as an internal standard: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, bs = broad singlet. Low-resolution mass spectra (ESI) were obtained with PE SCIEX API 150 EX. High-performance liquid chromatography (HPLC) was carried out using a dual-pump Gilson system and Speedrod Rp-18e (50 mm × 4.6 mm, MerckKGaA); the solvents were acetonitrile (A) and water (B), each containing 0.1% trifluoroacetic acid (TFA), and the flow rate was 4.5 mL min$^{-1}$ (A starting from 5% to 95% in 1.8 min, ELSD detector, Sedex, 75 °C). Chemicals and solvents were purchased from Aldrich Chemical Co. and were used without further purification.

**General Methods for Larger Libraries.** Thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254. Separation by column chromatography was performed on Merck silica gel 60 for flash chromatography. Nuclear magnetic resonance spectra were recorded on a Bruker Avance DRX 400 operating at 400.13 MHz for protons equipped with a 5 mm inverse multinuclear gradient probehead. Chemical shifts were expressed in part per million ($\delta$): s = singlet, d = doublet, dd = doublet of doublets. t = triplet, m = multiple, bs = broad singlet. Low-resolution mass spectra (ESI) were obtained with 1100LCMSD VL Series (Agilent Technologies, Santa Clara, CA) spectrometer. High-performance liquid chromatography (HPLC) was carried out using a 1100 quarternary pump and 1100 diode array detector (Agilent Technologies, Santa Clara, CA). An evaporative light-scattering detector PL-ELS-1000 (Polymer Laboratories, UK) was connected via passive splitter. Hi-Q 5um 50 mm × 4.6 mm C18 (Peeke Scientific, Redwood City, CA) was used. Solvents were water (A) and acetonitrile (B), each containing 0.1% TFA: flow rate = 3.75 mL/min, B starting from 2.5 to 100% in 2.3 min, hold 100% B up to 2.8 min. Chemicals and solvents were purchased from Aldrich Chemical Co. and used without further purification.

**Purity and Characterization of the Library Components.** All final compounds described in this work were purified. Unless otherwise mentioned, all libraries were purified by HPLC. After purification, all compounds met the following minimum purity requirements: >85% purity by *both* total ion current and evaporative light-scattering detection. After purification, the average purity by this method was >95% for all selected samples. All compounds were also characterized by mass spectral analysis with the requirement that the expected parent mass was >20% of the base peak. A subset of compounds was also subjected to NMR analysis (see below and Supporting Information). An additional subset of compounds was subjected to high-resolution mass spectral analysis, which gave the correct molecular formula in every case (see Supporting Information Table 2).

**General Procedures.** Compound **2** can be prepared as previously described for 7-phenoxy-3,4-dihydro-2H-quinoline-1-carboxylic acid benzyl ester-4-spiro-5′-imidazolidine-2′,4′-dione.[11] For general method descriptions, full reaction protocols, procedures, and additional compound characterization data, see the Supporting Information. High-resolution mass spectral data for a representative set of compounds can be found in Supporting Information Table 3.

**Synthesis of Derivatives of 3 (7-Methoxy-3,4-dihydro-2H-quinoline-1-carboxylic acid benzyl ester-4-spiro-5′-3′-(alkyl or arylalkyl)-imidazolidine-2′,4′-dione). Step i of Scheme 1.** $Na_2CO_3$ (0.954 g, 9 mmol) was added to a solution of **2** (1.143 g, 3.0 mmol) in anhydrous DMF (15 mL), followed by an alkyl or arylalkyl halide (0.541 mg, 3.3 mmol). The reaction was stirred in an oil bath at 45 °C for 48 h. The reaction mixture was quenched with water and extracted with EtOAc (3 × 150 mL). The combined extracts were washed with water (3 × 100 mL), dried with anhydrous $Na_2SO_4$, and evaporated. Chromatography using EtOAc/hexanes/MeOH gave the monoalkylated intermediate (∼50−90% yield, 7-methoxy-3,4-dihydro-2H-quinoline-1-carboxylic acid benzyl ester-4-spiro-5′-3′-[alkyl or arylalkyl]-1′-(alkyl′ or arylalkyl′)-imidazolidine-2′, 4′-dione).

**Step ii**. NaH (61 mg, 2.56 mmol) was added portionwise to a solution of product **3** from above (0.64 mmol) dissolved in anhydrous DMF (5 mL). The suspension was stirred for 30 min. To this solution, the alkyl′ or arylalkyl′ halide (0.77 mmol) was added; the reaction was then stirred at room temperature for 2 h. The reaction mixture was quenched with water and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with water (3 × 50 mL), dried with anhydrous $Na_2SO_4$, and evaporated. Chromatography using EtOAc/hexanes 1:1 gave compound **3** (∼50−90% yield).

**Step iii.** One milliliter of $BBr_3$ was added slowly, using a syringe, to a solution of **7** (0.2 mmol) in 2 mL of $CH_2Cl_2$ stirred at 0 °C. The mixture was stirred at 0 °C for 30 min; then it was stirred at room temperature for 2 h. Ten milliliters of cold ether was added, and the precipitate was washed twice with 5 mL of cold ether. The mixture was dissolved in 5 mL of $H_2O$, adjusted to pH 10 by the addition of 0.5 N $Na_2CO_3$, and then extracted with EtOAc. The organic phase was dried ($K_2CO_3$) and concentrated to dryness to afford the precursor to **4** ($R_1$ = H) in ∼90−95% yield.

**Step iv.** This material was then treated as follows: a portion (0.1 mmol) was dissolved in 3 mL anhydrous DMF, then treated with solid anhydrous $Na_2CO_3$ (53 mg, 5 equiv), followed by addition of the alkyl bromide (0.2 mmol); this mixture was then stirred at room temp for 16 h. Evaporation of the solvent and purification via silica column chromatography gave **4** in ∼30−40% yield.

**Synthesis of Fully Substituted Derivatives 1. Step v of Scheme 1.** A solution of **4** or **5** (0.1 mmol) in 3 mL of dichloroethane was treated with isobutylaldehyde (0.3 mmol). This mixture was treated with 1 M $NaBH(OAc)_3$ in $CHCl_3$ (0.9 mmol) and stirred for 16 h. Evaporation of all solvents and purification via silica column chromatography gave the final products (derivatives of **1**) in 30−50% yield.

**Synthesis of Derivatives of 4 (7-Alkyloxy-3,4-dihydro-2H-quinoline-1-(alkyl″ or arylalkyl″)-4-spiro-5′-3′-[alkyl or arylalkyl]-imidazolidine-2′,4′-dione). Step vi of Scheme 1.** A well stirred solution of **4** (0.154 mmol) and ammonium formate (116 mg, 1.84 mmol) in 6 mL of MeOH in a capped vial was treated with solid 10% Pd/C (0.1 g). The mixture was heated at 60 °C for 1 h with stirring. When TLC showed that starting material had disappeared, the mixture was filtered through a Celite bed, washed with MeOH, and concentrated under reduced pressure to afford **5** (90 mg, 100% yield).

**Library of 1′-Isobutyl-7′-methoxy-1-(2-picolyl)-2-R-2′,3′-dihydro-1′H,2H,5H-spiro[imidazolidine-4,4′-quinoline]-2,5-diones (55 $R_2$ = 2-Pi, $R_3$ = variable). (**See Scheme 2 and protocols in Supporting Information.) The syntheses were carried out in 6 × 8 test tube blocks in capped vials. A set of solutions of compound **54** ($R_1$ = 2-Pi; 80 μM) in THF (400 μL) was treated with a suspension of hexane-washed NaH (240 μM) in DMF (600 uL) under a dry nitrogen atmosphere in a glove box. The suspensions were vigorously stirred for 30 min. Then a solution of the appropriate $R_3$-Hal (130 μM) in a mixture of THF−DMF, 1:1 (600 μL), was added, and the mixtures were stirred at 50 °C overnight. Excess NaH was neutralized with AcOH (∼50 uL), and the resulting reaction mixtures were evaporated to dryness. The residues were dissolved in MeOH, placed on small Dowex-$H^+$ columns, and washed with MeOH, and the product was eluted with 30% $Et_2NH$/MeOH. The mixtures were evaporated, and the residues were purified by Prep LCMS.

**Library of 1′-Isobutyl-7′-methoxy-1-R-2-(3-picolyl)-2′,3′-dihydro-1′H,2H,5H-spiro[imidazolidine- 4,4′-quinoline]-2,5-diones (55 $R_2$ = 3-Pi; $R_3$ = variable).** The synthesis was carried out in 6 × 8 test tube blocks. **Alkylhalides.** A solution of halide, $R_3$-Hal, (160 μM) in DMF (300 μL), $K_2CO_3$ (180 μM), and water (25 uL) were added to a solution of compound **53** (80 μM) in DMF (200 μL). The mixture was allowed to stir at 70 °C overnight. Then the reaction mixture was diluted with MeCN (3 mL) and filtered through Celite. The filtrate was evaporated, and the residue was dissolved in MeOH, placed on Dowex-$H^+$, and washed with MeOH; the product was desorbed by 30% $Et_2NH$/MeOH. The mixture was evaporated, and the residue was distributed between DCM−5% NaOH using Celite to separate the aqueous phase. DCM was evaporated, and the residue was purified by Prep LCMS. **Alcohols.** A solution of $PPh_3$ (120 μM) in THF (300 μL) and a solution of $R_3$-OH (120 μM) in THF (300 uL) were added to a solution of compound **53** (80 μM) in THF (200 μM). PhMe (1.5 mL) was added, and the mixture was evaporated to dryness. The residue was dissolved in THF (0.5 mL), and a solution of DIAD (120 μM) in THF (300 uL) was added. The mixture was stirred at room temperature overnight. THF was evaporated, and the residue was dissolved in MeOH, placed on Dowex-$H^+$, and washed with MeOH; the product was desorbed by 30% $Et_2NH$/MeOH. The mixture was evaporated, and the residue was distributed between DCM−5% NaOH using Celite to separate the aqueous phase. DCM was evaporated, and the residue was purified by Prep LCMS. After purification, 40 μM aliquots of each compound obtained in the previous step were sampled and dissolved

in THF (300 $\mu$L). A suspension of hexane-washed NaH (120 $\mu$M) in DMF (300 uL) was added in a dry nitrogen atmosphere in a glove box. The mixture was vigorously stirred for 30 min. Then a solution of 3-chloromethylpyridine (60 $\mu$M) in a mixture of THF and DMF, 1:1 (300 $\mu$L), was added, and the mixture was stirred at 50 °C overnight. Excess NaH was neutralized with AcOH (~50 $\mu$L), and the reaction mixtures were evaporated to dryness. The residue was dissolved in MeOH, placed on small Dowex-$H^+$ columns, and washed with MeOH, and the product was eluted by 30% $Et_2NH$/MeOH. The mixture was evaporated, and the residue was purified by Prep LCMS.

**Compounds of Type 56 (Scheme 2 and Supporting Information). Step viii.** A solution of **55** (0.2 mmol) in 2 mL of DCM was stirred at 0 °C, and then it was treated (dropwise) with 1 mL of $BBr_3$. The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. This mixture was then treated with 10 mL of cold ether, and the precipitate was washed twice with 5 mL of cold ether. The residue was dissolved in 5 mL of $H_2O$, adjusted to pH 10 with 0.5 N $Na_2CO_3$, and extracted with EtOAc. The combined organic extracts were dried with $Na_2SO_4$, filtered, and concentrated to give the crude intermediate, which was used directly for the next step.

**Step (ix).** $NaN(Si(CH_3)_3)_2$ (16 mg, 4 equiv) was added to a solution of the product from above in anhydrous THF (1 mL). The suspension was stirred for 30 min. To this solution, $R_1$-X (1.2 equiv) was added, and the reaction mixture was then stirred at room temperature for 16 h. The reaction mixture was then quenched with water and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with water (3 × 10 mL), dried with anhydrous $Na_2SO_4$, filtered, evaporated, and purified by prep LCMS.

**Representative Synthetic Procedures: Specific Examples. 7-Methoxy-3,4-dihydro-2H-quinoline-1-carboxylic Acid Benzyl Ester-4-spiro-5′-3′-[2-picolyl]-imidazolidine-2′,4′-dione (7).** $Na_2CO_3$ (0.954 g, 9mmol) was added to a solution of **2** (1.143 g, 3.0 mmol) in anhydrous DMF (15 mL), followed by 2-picolyl chloride (HCl salt) (0.541 mg, 3.3 mmol). The reaction mixture was stirred in an oil bath at 45 °C for 48 h. The reaction mixture was quenched with water and extracted with EtOAc (3 × 150 mL). The combined extracts were washed with water (3 × 100 mL), dried with anhydrous $Na_2SO_4$, and evaporated. Chromatography using EtOAc/hexanes/MeOH, 6:4:0.5, gave **7** (0.84 g, 59% yield). $^1$H NMR ($CDCl_3$): $\delta$ 8.52 (d, $J$ = 4.3 Hz, 1H), 7.62 (t, $J$ = 1.8 Hz, 1H), 7.36 (m, 7H), 7.29 (m, 2H), 7.20 (m, 1H), 6.63 (dd, $J$ = 2.6 and 8.7 Hz, 1H), 6.18 (s, 1H), 5.23 (s, 2H), 4.84 (s, 2H), 4.23 (m, 1H), 3.98 (m, 1H), 3.66 (s, 3H), 2.37 (m, 1H), 2.17 (m, 1H). ESI-MS: $m/z$ 473 $[M + H]^+$.

**7-Methoxy-3, 4-dihydro-2H-quinoline-1-carboxylic Acid Benzyl Ester-4-spiro-5′-3′-[2-picolyl]-1′-(3-Picolyl)-imidazolidine-2′,4′-dione (12).** NaH (2.56 mmol) was added portionwise to a solution of **7** (0.64 mmol) in anhydrous DMF (5 mL). The suspension was stirred for 30 min. To this solution, 3-picolyl chloride (HCl salt) (126 mg, 0.77 mmol) was added; the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with water and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with water (3 × 50 mL), dried with anhydrous $Na_2SO_4$, and evaporated. Chromatography using EtOAc/hexanes, 1:1, gave **12** (57% yield). $^1$H NMR ($CDCl_3$): $\delta$ 8.54 (d, $J$ = 4.8 Hz, 1H), 8.48 (dd, $J$ = 1.5 and 4.8 Hz, 1H), 8.42 (d, $J$ = 1.5 Hz, 1H), 7.64 (td, $J$ = 7.8 and 1.8 Hz, 1H), 7.51 (dt, $J$ = 7.8 and 1.8 Hz, 1H), 7.41−7.25 (m, 6H), 7.26 (m, 2H), 7.15 (m, 2H), 7.10 (d, $J$ = 8.6 Hz, 1H), 6.60 (dd, $J$ = 8.8 and 2.6 Hz, 1H), 5.25 (s, 2H), 4.91 (s, 2H), 4.74 (d, $J$ = 16 Hz, 1H), 4.12 (m, 1H), 4.12 (m, 2H), 3.71 (s, 3H), 2.23 (m, 1H), 1.95 (m, 1H). ESI-MS: $m/z$ 564 $[M + H]^+$.

**7-Methoxy-3,4-dihydro-2H-quinoline-4-spiro-5′-3′-[2-picolyl]-1′-(3-picolyl)-imidazolidine-2′,4′-dione (16).** A well stirred solution of the precursor of **12** (0.154 mmol) and ammonium formate (1.84 mmol) in 6 mL of MeOH in a capped vial was treated with solid 10% Pd/C (0.1 g). The mixture was heated at 60 °C for 1 h with stirring. When TLC showed that starting material had disappeared, the mixture was filtered through a Celite bed, washed with MeOH, and concentrated under reduced pressure to afford **16** (100% yield). HPLC retention time: 0.75 min. ELSD purity: 97%. ESI-MS: $m/z$ 430 $[M + H]^+$.

**7-Methoxy-3,4-dihydro-2H-quinoline-1-isobutyl-4-spiro-5′-3′-[2-picolyl]-1′-(3-picolyl)-imidazolidine-2′,4′-dione (26).** Isobutylaldehyde (0.3 mmol, 3equiv) was to a solution of **16** (0.1mmol) in 3 mL DCE, and $NaBH(OAc)_3$ (3equiv) in $CHCl_3$ was added to the above mixture and stirred for 16 h. All solvents were evaporated off, and purification via silica column chromatography gave 30 mg (62%) of **26**. $^1$H NMR ($CDCl_3$): $\delta$ 8.53 (m, 3H), 7.68 (d, $J$ = 6 Hz, 1H), 7.63 (m, 1H), 7.35(m, 1H), 7.20 (m, 2H), 6.89 (d, $J$ = 6 Hz, 1H), 6.18 (m, 2H), 4.92 (s, 2H), 4.87 (d, $J$ = 15 Hz, 1H), 4.03 (d, $J$ = 15 Hz, 1H), 3.95 (m, 1H), 3.78 (s, 3H), 3.02 (m, 3H), 2.03 (m, 3H), 0.93 (dd, $J$ = 6 Hz, 6H). ELSD: 100%. ESI-MS: $m/z$ 486 $[M + H]^+$.

**Functional Screening of the Melanocortin Receptors. MCR4 Agonist Assay. Primary Screen.** CHO-k1 cells engineered to express the MC4 receptor[29] and the 4× CRE $\beta$-lactamase reporter[28] were plated at 5000 cells per well in a 384 well black clear bottom assay plate in 50 $\mu$l per well of DMEM supplemented with 10% fetal bovine serum. These cells were allowed to adhere to the plate for 24 h, and then the growth medium was aspirated and replaced with serum-free DMEM media. After 12 h of serum starvation, media was removed from the wells, and 25 $\mu$L of DMEM was added. To positive control wells, 5 $\mu$L of 10 nM α-MSH in DMEM was added, and to negative control wells, only DMEM was added. To test wells, compounds at 10 $\mu$M final concentration were added in 5 $\mu$L of DMEM media. Compounds were distributed to plates using a Perkin-Elmer multiprobe automated pipetting station. Cells were then incubated 4 h at 37 °C in a 5% $CO_2$ humidified cell culture incubator. Following the 4 h incubation, 7 $\mu$L of CCF2 dye stock prepared per the manufacturer's protocol was then added to each well. Plates were then incubated for 1 h at room temperature, and the fluorescence at 460 and 530 nm was measured using a BMG polarstar reader with bottom reading optics. Compounds with high ratios of 460/530 nm,

indicating agonist activity, were then flagged and selected for secondary screening.

**Secondary Screening.** To differentiate compounds that specifically agonized the MC4 receptor from those compounds that nonspecifically activated the CRE signaling pathway, putative agonist compounds were rescreened on a CHOk1 cell line engineered to express only the 4× CRE $\beta$-lactamase reporter. In this assay, forskolin at 1 $\mu$M final concentration is used as a positive control. Compounds that exhibited agonist activity greater than 80% of the α-MSH control in the primary screen and less than 20% of the forskolin control in the secondary assay were then selected for dose response followup.

**Acknowledgment.** The authors would like to thank Cynthia Jefferies and the CRL Analytical Chemistry Group for assistance in the acquisition of HPLC/MS data for some of these compounds described in this manuscript. We would also like to thank Su Hua and the CRL Bioassay Technology Group for their assistance in the acquisition of the bioassay data reported here. We would also like to thank Dr. Yang Yang for his synthetic work.

**Supporting Information Available.** Detailed experimental material including Table 1 (structures and screening results for 199 member library), Table 2 (high-resolution mass spectral results for the most-active compounds), and Supplementary Methods (additional detailed experimental for the synthesis of library compounds and melanocortin receptor assay). This material is available free of charge via the Internet at http://pubs.acs.org.

**References and Notes**

(1) (a) Ball, J. B.; Alewood, P. R. *J. Mol. Recognit.* **1990**, *2*, 55−64. (b) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bos, M.; Cook, C. M.; Fry, D.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *21*, 3041−3049. (c) Adang, A. E. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; van Staveren, C. J. *Recl. Trav. Chim. Pays−Bas* **1994**, *113*, 63−78.

(2) Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; Johnston, D. B. R.; Bakshi, R.; Palucki, B.; Weinberg, D. H.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Stearns, R. A.; Miller, R. R.; Tamvakopoulos, C.; Strack, A. M.; McGowan, E.; Cashen, D. E.; Drisko, J. E.; Hom, G. H.; Howard, A. D.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A.; Nargund, R. P. *J. Med. Chem.* **2002**, *45*, 4589−4893.

(3) Nargund, R. P.; Patchett, A. A.; Bach, M. A.; Murphy, M. G.; Smith, R. *J. Med. Chem.* **1998**, *41*, 3103−3127.

(4) Liao, S.; Alfaro-Lopez, J.; Shenderovich, M. D.; Hosohata, K.; Lin, J.; Li, X.; Stropova, D.; Davis, P.; Jernigan, K. A.; Porreca F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1998**, *41*, 4767−4776.

(5) Okayama, T.; Wei, H.; Davis, P.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. *Pept. Sci.* **2000**, 349−352.

(6) Croston, G. E.; Olsson, R.; Currier, E. A.; Burstein, E. S.; Weiner, D.; Nash, N.; Severance, D.; Allenmark, S. G.; Thunberg, L.; Ma, J.-N.; Mohell, N.; O'Dowd, B.; Brann, M. R.; Hacksell, U. *J. Med. Chem.* **2002**, *45*, 4950−4953.

(7) 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. *Science* **1998**, *282*, 737−740.

(8) Yang, L.; Berk, S. C.; Rohrer, S. P.; Mosley, R. T.; Guo, L.; Underwood, D. J.; Arsion, B. H.; Birzin, E. T.; Hayes, E. C.; Mitra, S. W.; Parmar, R. M.; Cheng, K.; Wu, T.-J.; Butler, B. S.; Foor, F.; Pasternak, A.; Pan, Y.; Silva, M.; Freidinger, R. M.; Smith, R. G.; Chapman, K.; Schaeffer, J. M.; Patchett, A. A. Proc. *Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10836−10841.

(9) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengler, P. A.; Hamley, P.; Smith, A. B., III; Reisine, T.; Raynor, K., Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550 − 12568.

(10) Crider, M. *J. Med. Chem.* **2002**, *45*, 507−517.

(11) Chianelli, D.; Kim, Y.-C.; Lvovskiy, D.; Webb. T. R. *Bioorg. Med. Chem.* **2003**, *11*, *23*, 5059−5068.

(12) Im, I.; Webb, T. R.; Gong, Y.-D.; Kim, J.-I.; Kim, Y.-C. *J. Comb. Chem.* **2004**, *6*, 207−213.

(13) Irani, B. G.; Holder, J. R.; Todorovic, A.; Wilczynski, A. M.; Joseph, C. G.; Wilson, K. R.; Haskell-Luevano C. *Curr. Pharm. Des.* **2004**, *10*, 3443−3479.

(14) Haskell-Luevano, C.; Rosenquist, A.; Souers, A.; Khong, K. C.; Ellman, J. A.; Cone, R. D. *J. Med. Chem.* **1999**, *42*, 4380−4387.

(15) Chhajlani, V.; Wikberg, J. E. S. *FEBS Lett.* **1992**, *309*, 417−420.

(16) Mountjoy, K. G.; Robbins, L. S.; Mortrud, M. T.; Cone, R. D. *Science* **1992**, *257*, 1248−1251.

(17) Chhajlani, V.; Muceniece, R.; Wikberg, J. E. S. *Biochem. Biophys. Res. Commun.* **1993**, **1993**, *195*, 866−873.

(18) Gantz, I.; Konda, Y.; Tashiro, T.; Shimoto, Y.; Miwa, H.; Munzert, G.; Watson, S. J.; Del Valle, J.; Yamada, T. *J. Biol. Chem.* **1993**, *268*, 8246−8250.

(19) Gantz, I.; Miwa, H.; Konda, Y.; Shimoto, Y.; Tashiro, T.; Watson, S. J.; DelValle, J.; Yamada, T. *J. Biol. Chem.* **1993**, *268*, 15174−15179.

(20) Farooqi, S.; O'Rahilly S. *Endocr. Rev.* **2006**, *27*, 710−718.

(21) Vaisse, C.; Clement, K.; Durand, E.; Hercberg, S.; Guy-Grand, B.; Froguel, P. *J. Clin. Invest.* **2000**, *106*, 253−262.

(22) (a) Bednarek, M. A.; Fong, T. M. *Expert Opin. Ther. Pat.* **2004**, *14*, 327−336. (b) MacNeil, D. J.; Howard, A. D.; Guan, X.; Fong, T. M.; Nargund, R. P.; Bednarek, M. A.; Goulet, M. T.; Weinberg, D. H.; Strack, A. M.; Marsh, D. J.; Chen, H. Y.; Shen, C. P.; Chen, A. S.; Rosenblum, C. I.; MacNeil, T.; Tota, M.; MacIntyre, E. D.; Van der Ploeg, L. H. *Eur. J. Pharmacol.* **2002**, *440*, 141−57.

(23) Bondebjerg, J.; Xiang, Z.; Bauzo, R. M.; Haskell-Luevano, C.; Meldal, M. A. *J. Am. Chem. Soc.* **2002**, *124*, 11046−11055.

(24) Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235−249.

(25) Garland S. L.; Dean, P. M. *J. Comput.-Aided Mol. Des.* **1999**, *13*, 469−483.

(26) Webb, T. R. in *Methods and Principles in Medicinal Chemistry*; Kubinyi, H., Müller, G., Eds.; Wiley-VCH: Weinheim, Germany, 2004; Chapter 11.

(27) Al-Obeidi, F.; Hadley, M. E.; Pettitt, B. M.; Hruby, V. J. *J. Am. Chem. Soc.* **1989**, *111*, 3413−3416.

(28) Hanson, B. J. *J. Biomol. Screening* **2006**, *11*, 644−651.

(29) Gantz, I.; Miwa, H.; Konda, Y.; Shimoto, Y.; Tashiro, T.; Watson. S. J.; DelValle, J.; Yamada, T. *J. Biol. Chem*. **1993**, *268*, 15174−15179.

(30) Zlokarnik, G.; Negulescu, P. A.; Knapp, T. E.; Mere, L.; Burres, N.; Feng, L.;Whitney, M.; Roemer, K.; and Tsien, R. Y. *Science* **1998**, *279*, 84−88.

CC0601581