On-Line Synthesis of Pseudopeptide Library Incorporating a Benzodiazepinone Turn Mimic: Biological Evaluation on MC1 Receptors

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Alpha melanocyte stimulating hormone (α-MSH) is a widely distributed hormone. This tridecapeptide exhibits various biological activities mediated through different receptors. α-MSH binds to the melanocortin-1 receptor (MC1-R), mainly expressed in keratinocytes and melanocytes, inducing melanogenesis and anti-inflammatory processes. The central His-Phe-Arg-Trp tetrapeptide sequence of α-MSH is known to form a turn in the bioactive conformation. To find new potent analogs of α-MSH, we decided to introduce non-peptide building blocks in the α-MSH sequence. Molecular modeling studies showed that two amino acids of the central core sequence could be replaced by the benzodiazepinone building block without loosing the β-turn conformation. Benzodiazepines are well-known pharmacophores exhibiting a wide scope of biological activities and are described as constrained dipeptide mimics templates. Although numerous synthetic pathways leading to benzodiazepinones have been described in literature, no methodology has 1,4-benzodiazepine-2,5-diones building blocks bearing a free carboxylic acid function and a protected amino function suitable for incorporation into peptide sequences. In this study, we report the synthesis of peptides with a benzodiazepinone moiety obtained directly during the course of solid-phase peptide synthesis (SPPS). This "on-line" strategy leads to the generation of a 54-member pseudo-peptide library of α-MSH analogs. After LC/MS purification, binding assays were performed on the MC1 receptor leading to the discovery of several micromolar ligands.

Introduction

Alpha melanocyte stimulating hormone (α-MSH) is a tridecapeptide hormone exhibiting various biological activities mediated through different receptors. These melanocortin receptors belong to the superfamily of seven transmembrane-spanning G-protein-coupled receptors and stimulate the cAMP signal transduction pathway.[1] The first known function of α-MSH is the photoprotection induced by stimulation of the biosynthesis of melanine in the skin and animal fur. The role of α-MSH has recently been shown in physiological phenomenons such as satiety, inflammation, homeostasis, sexual behavior, steroidogenesis and exocrine functions.[2] Among the five receptor subtypes of α-MSH, the melanocortin-1 receptor (MC1-R), also expressed in pituitary gland and immune cells, is mostly prevalent in the skin. Mainly expressed by keratinocytes and melanocytes, MC1-R activation leads to melanogenesis[1,3] and anti-inflammatory processes.[4]

The melanocortin receptor agonist α-MSH is a 13 amino acid linear peptide that is post-translationally processed to include the N-terminal acetyl and C-terminal amide moieties.[1,3] It contains the His-Phe-Arg-Trp tetrapeptide sequence which is crucial for the ligand selectivity and stimulation of the melanocortin receptors.[5] Moreover, the central tetrapeptide of α-MSH is known to form a turn in the bioactive conformation.[7] During the past few years, analogues of α-MSH have been synthesized and characterized by our group[6] and others.[8] The introduction of modifications within the peptidic sequence included several truncations, N- and C-terminal modifications, cyclization, and substitutions with other amino acids.

V. J. Hruby's group first synthesized cyclic constrained peptides to induce a β-turn conformation in α-MSH, leading to the discovery of the potent agonist MTII (Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2).[9] More recently, Zhang et al. described the synthesis of disubstitued azabicyclononane amino acid to mimic the β-turn Phe−Arg fragment within melanotropin peptides.[10]

Benzodiazepines are well-known pharmacophores exhibiting a wide scope of biological activities,[11] but they are also described as constrained templates such as dipeptide or β-turn mimetics. As an example, four amino acids had been replaced by a benzodiazepine in cyclic octapeptide, retaining a double β-turn conformation.[12] This strategy had been successfully applied in our group to the synthesis of a selective bradykinin

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antagonist, replacing two consecutive amino acids by benzothiazepinone.[13] In this piece of work, we focused our attention on the synthesis of 1,4-benzodiazepine-2,5-dione building blocks and their insertion into α-MSH analogues in place of two amino acids. Preliminary molecular modeling and minimization studies showed that introduction of such constrained building blocks did not drastically modify the folding of the peptide chain.

Five sequences of α-MSH analogues, including NDP-MSH (Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2),[8] melitane (Ac-Nle-Ala-His-D-Phe-Arg-Trp-NH_2),[14] and other hit compounds discovered in our laboratory (CHT05-19, Ac-Nle-Ala-Arg-D-Phe-Arg-Trp-NH_2; CHT05-26, Ac-Nle-Ala-His-D-Phe-Arg-Nal-NH_2; JMV 2437, Palm-His-D-Phe-Arg-Trp-NH_2),[6] were selected to start this study.

Results and Discussion

Preliminary Molecular Modeling Studies. Using literature structural data,[15,16] we first modeled the cyclic analogue MTII (Ac-Nle^4-c[Asp^5-His^6-D-Phe^7-Arg^8-Trp^9-Lys^{10}]-NH_2 and the central sequence (residues 5−9) of α-MSH (Ac-Ser^1-Tyr^2-Ser^3-Met^4-Glu^5-His^6-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2) and NDP-MSH[8] (Ac-Ser^1-Tyr^2-Ser^3-Nle^4-Glu^5-His^6-D-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2). Core sequences including residues 6 and 7 were chosen because of their involvement in type II β-turn conformation, according to NMR and molecular modeling studies performed by Ying et al.[16] We decided to evaluate the impact of the replacement of these two consecutive residues by amino acid benzodiazepinone building blocks **1a** (Figure 1). Amide bonds were added to **1a** to mimic Cα and peptide bonds of amino acids at each side of the benzodiazepine building block. Structure **1b** (with $n = 0-4$) was obtained and the low-energy conformation was calculated. Following the successful strategy used in our group for the conception of Bradykinin analogues,[13] we superimposed the low-energy conformation of **1b** to residues 6 and 7 in the modeled central core (residues 5−9) of the three α-MSH analogues.

Carbonyl and Cα of Glu^5 in NDP-MSH(5−9) and α-MSH(5−9), and Asp^5 in MTII were superimposed, respectively, with the carbonyl (*a*) and carbon (*b*) of benzodiazepinone **1b** (Figure 1); while the nitrogen and Cα of Arg^8 of the three analogues were superimposed, respectively, with nitrogen (*d*) and carbon (*c*) of **1b**. Among the structures tested, molecule {**1b**, $n = 1$}, based on the 3-aminomethyl-2,5-dioxo-1,2,3,5-tetrahydro-1,4-benzodiazepin-4-yl)-acetic acid building block (AMB) gave rather interesting results. The rms values of 0.424, 0.688, and 0.421 Å were obtained by superimposition of Ac-AMB-NHMe {**1b**, $n = 1$} with NDP-MSH(5−9) (green), with α-MSH(5−9) (orange), and with MTII (purple), respectively (Figure 2). It is worth noting that, as expected, no correct superimposition can be obtained with the longer alkyl chain (i.e., $n = 4$) because of the flexibility the aliphatic arm.

We performed molecular modeling studies, replacing residues involved in the β-turn, His^6 and D-Phe,^{7} with AMB in MTII cyclic analogue sequence. The chimera compound [AMB$^{6-7}$]-MTII was minimized and superimposed with MTII yielding a rms value of 0.081 Å. (Figure 3).

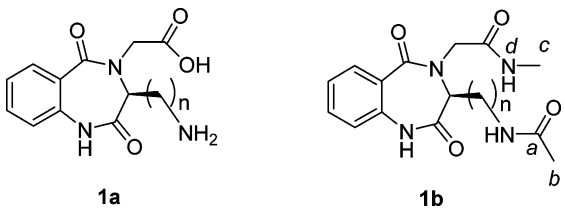


Figure 1. Amino acid benzodiazepinone building blocks.

The modified ligand side chains keeps the same orientation. The introduction of the benzodiazepinone moiety seems to have little effect on the general backbone conformation. Of course, His^6 and Phe^7 side chains are not displayed by the benzodiazepinone moiety, and additional functional groups are probably needed on the benzodiazepinone template to keep optimal interactions with the melanocortin receptors. However, these preliminary studies showed that the Dap-containing benzodiazepine moiety ($n = 1$) could be an appropriate candidate to mimic the β-turn described in the α-MSH analogues.

Chemistry. Abundant literature related to the supported synthesis of benzodiazepines can be found. Synthetic strategies belongs to three main routes: anchoring the benzodiazepine through the benzenic cycle,[16] cyclization−cleavage from the support,[18] and N-anchoring on the diazepine.[19] However, to our knowledge, no route can produce benzodiazepine with both an acid and amine function such as 3-aminomethyl-2,5-dioxo-1,2,3,5-tetrahydro-1,4-benzodiazepin-4-yl)-acetic acid (AMB). The simplest way to use benzodiazepinone template **1a** as an amino acid in a peptide sequence was to develop a strategy enabling direct preparation of the benzodiazepine moiety during the course of the solid-phase peptide synthesis (SPPS). Automated SPPS is routinely performed using Fmoc/tBu[20] protocols and TFA-labile linkers. To be easily applicable on synthesizers, our strategy had to be fully compatible with Fmoc/tBu SPPS conditions. Briefly, benzodiazepinone was obtained by cyclization of two building blocks: an N-substitued amino acid benzyl ester with a protected amino group side chain and a substituted anthranilic acid, obtained from reduction of its *o*-nitrobenzoic acid derivative. Model compound **2**, [2-(2,3-dihydro-3-isobutyl-2,5-dioxo-1H-benzo[1,4]diazepin-4(5H)-yl)acetamide], was synthesized on Fmoc−Rink amide polystyrene SynPhase lanterns (Mimotopes, Pty) (Scheme 1). Each reaction step was optimized using parallel arrays of lanterns. Several parameters including reaction time, solvent, temperature, concentration, and reactants were varied to determine the best experimental procedure, summarized in Scheme 1.

After Fmoc removal of the Rink amide linker, acylation with bromoacetic acid, and activation with DIC/HOBt, the supported bromo intermediate was produced. Substitution with leucine benzyl ester was complete in 24 h. In some experiments, we noticed that the nature of α-amino salt was very important. Indeed, the use of the amino acid ester hydrochloride led to bromo−chloro exchange on the solid support and to incomplete substitution. Acylation of the secondary amine was performed with ortho nitrobenzoic acid chloride at 60 °C in the presence of triethylamine. The effectiveness of the secondary amine acylation was determined by LC/MS analysis of the cleaved compound and by

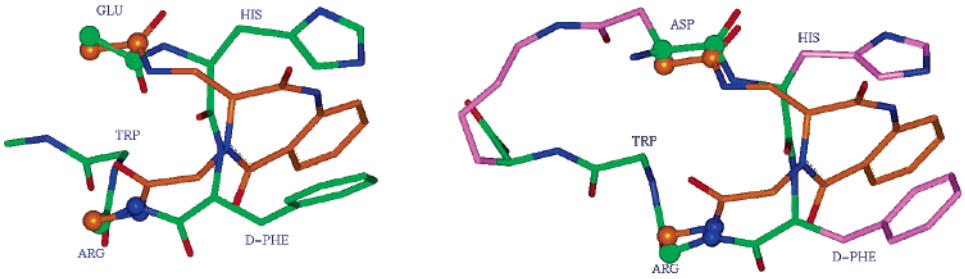


Figure 2. (left) Superimposition of Ac-AMB-NHMe (orange) with NDP-MSH (green). (right) Surperimposition Ac-AMB-NHMe (orange) with MTII (purple).

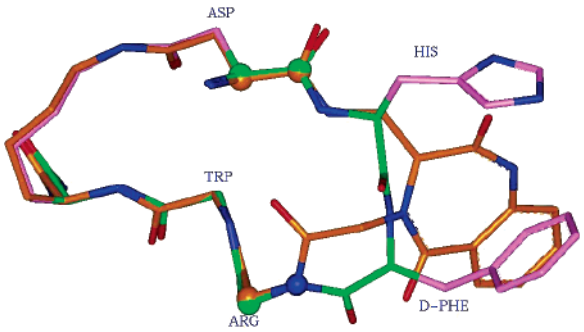


Figure 3. Superimposition of [AMB$^{6-7}$]-MTII (orange) with MTII (green).

a colorimetric Chloranil test[19] performed on a slice of the lantern. Reduction of the aromatic group with tin chloride was concomitant with cyclization. Heating was required to drive the reduction to completion within 16 h. LC/MS analysis of the reaction medium in acidic conditions confirmed that no premature cleavage of Rink amide linker occurred.

ESI+ LC/MS analysis was performed on compound **2** which was obtained in 93% purity determined by UV integration at 214 nm.

Library Synthesis. A 54-member library has been designed to demonstrate the feasibility of our synthetic route to peptides incorporating a benzodiazepinone moiety. Five starting sequences of α-MSH analogues were chosen: (i) NDP MSH[8] the tridecapeptide reference agonist of MC1-R, (ii) Melitane,[14] Ac-Nle-Ala-His-D-Phe-Arg-Trp-NH_2, a hexapeptide agonist of α-MSH, (iii) CHT05-19, Melitane analogue, Ac-Nle-Ala-Arg-D-Phe-Arg-Trp-NH_2, (iv) CHT05-26, Ac-Nle-Ala-His-D-Phe-Arg-Nal-NH_2, a lead compound discovered in our laboratory during primary screening of a tetrapeptide library on MC1-R, and (v) JMV 2437, Palm-His-D-Phe-Arg-Trp-NH_2, a patented N-palmitoylated tetrapeptide[22] which showed the same binding affinity for the MC1-R as α-MSH on melanoma cell line M2Gen.

We chose to replace several pairs of consecutive amino acids within each peptide analogue with benzodiazepinone moiety (denoted benzo [*n*, R] according to the number of carbons, *n*, in the side chain of amino acid ester chemset **4** and the aromatic substituent R of reagent chemset **6**). For the NDP-MSH analogs, the [His^6−D-Phe^7] dipeptide was replaced by benzodiazepinone to yield chemset **10** and [D-Phe^7−Arg^8] substitution led to chemset **11**. The same substitution approach was used for other analogs leading to nine different peptidyl sequences (Table 1). It should be emphasizeded that NMR studies on some examples of α-MSH analogs revealed that the *β*-turn was induced by the [His^6−D-Phe^7] and not by the [D-Phe^7−Arg^8] dipeptidyl sequence.[16] Nevertheless, we decided to synthesize both types of benzo-substituted analogs.

The benzodiazepinone template synthesis was performed using a chemset of two amino acid esters bearing Fmoc amino-protected side chains (reagent chemset **4,** Figure 4) combined with a chemset of three nitrobenzoic acid derivatives (chemset **6,** Figure 5). Although molecular modeling superimpositions stated that benzodiazepinone bearing flexible alkyl arm {**1b**, $n = 4$} is not suitable for mimicking the *β*-turn, we decide to include H-Lys(Fmoc)-OBzl in reagent chemset **4** to validate our hypothesis. All these combinations generate $2 \times 3 \times 9 = 54$ library members.

To quickly manage the library construction, we chose a split and pool approach on SynPhase lanterns.[23] Lanterns were attached to transponder bearing a RF (radio frequency) tag. After each synthetic step, the lanterns were manually sorted using an antenna and were pooled into the separate vials containing the respective building blocks, using the TranSort software. The library of 54 peptides incorporating the benzodiazepinone moiety was generated according to the reaction sequence presented in Scheme 2, using optimized conditions as mentioned previously. After the synthesis, all the lanterns were cleaved separately in individual polypropylene vials. An aliquot of each library member was submitted to LC/MS analyses, and the remaining solutions were lyophylized.

Crude products were obtained in overall yields of 40−60%, calculated on the basis of the initial loading of the lanterns. Forty-eight compounds were detected by LC/MS analysis with an average crude purity of 45%. Analytical data are available as Supporting Information in Table A.

All 48 detected compounds were purified by preparative LC/MS ESI automated system (Waters Micromass) before being submitted to biological binding assays on the MC1 receptor. Positive mode was chosen and fraction triggering was set up on monocharged and dicharged ions. Crude compounds were solubilized in 1 mL of water/acetonitrile (50/50, v/v) containing 0.1% TFA and were injected for reverse-phase liquid chromatography. Fractions containing expected ions were pooled together, freeze-dried, and submitted again to LC/MS analysis. The purity percent was based on relative peak absorbance at 214 nm on the UV spectra. Analytical data are available as Supporting Information in Table A. Low purification yields (15%) were obtained because of relatively high value of minimum intensity threshold (MIT) chosen for ion detection (MIT = 8×10^5).

Scheme 1. Benzodiazepinone Synthesis on SynPhase Lanterns

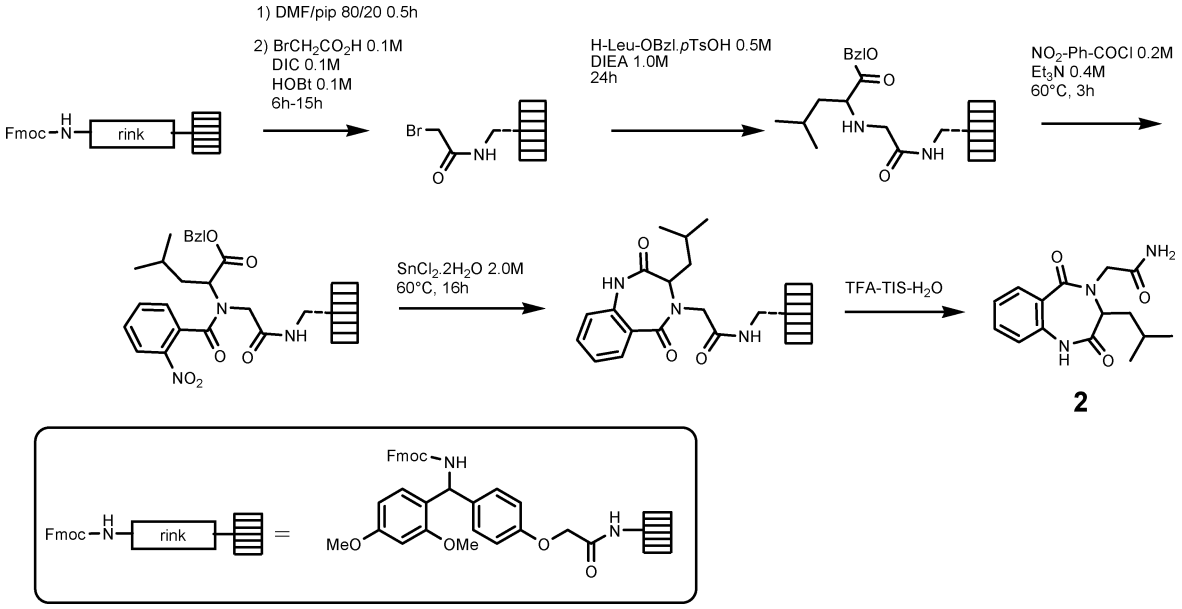


Table 1. Peptide Sequences Incorporating the Benzodiazepinone Moiety.

reference α-MSH Agonist		chemset
NDP-MSH	Ac-Ser-Tyr-Ser-Nle-Glu-**benzo[*n*, R]**-Arg-Trp-Gly-Lys-Pro-Val-NH_2	**10**
Ac-Ser-Tyr-Ser-Nle-Glu-His-**benzo[*n*, R]**-Trp-Gly-Lys-Pro-Val-NH_2	**11**	
JMV 2437	Palm-**benzo[*n*, R]**-Arg-Trp-NH_2	**12**
Palm-His-**benzo[*n*, R]**-Trp-NH_2	**13**	
Melitane	Ac-Nle-Ala-**benzo[*n*, R]**-Arg-Trp-NH_2	**14**
Ac-Nle-Ala-His-**benzo[*n*, R]**-Trp-NH_2	**15**	
Arg^3-Melitane	Ac-Nle-Ala-Arg-**benzo[*n*, R]**-Trp-NH_2	**16**
CHT05-26	Ac-Nle-Ala-**benzo[*n*, R]**-Arg-Nal-NH_2	**17**
Ac-Nle-Ala-His-**benzo[*n*, R]**-Nal-NH_2	**18**	

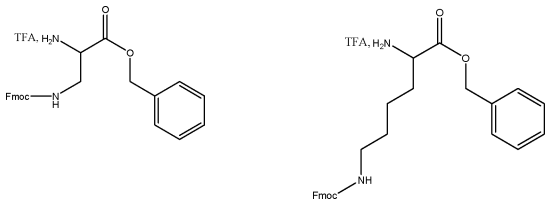


Figure 4. Chemset **4**{*1*−*2*}.

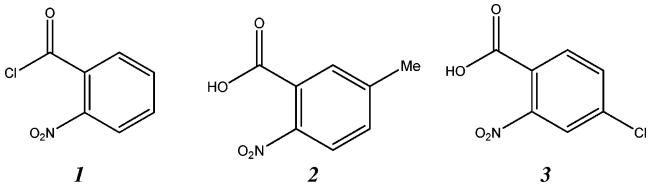


Figure 5. Chemset **6**{*1*−*3*}.

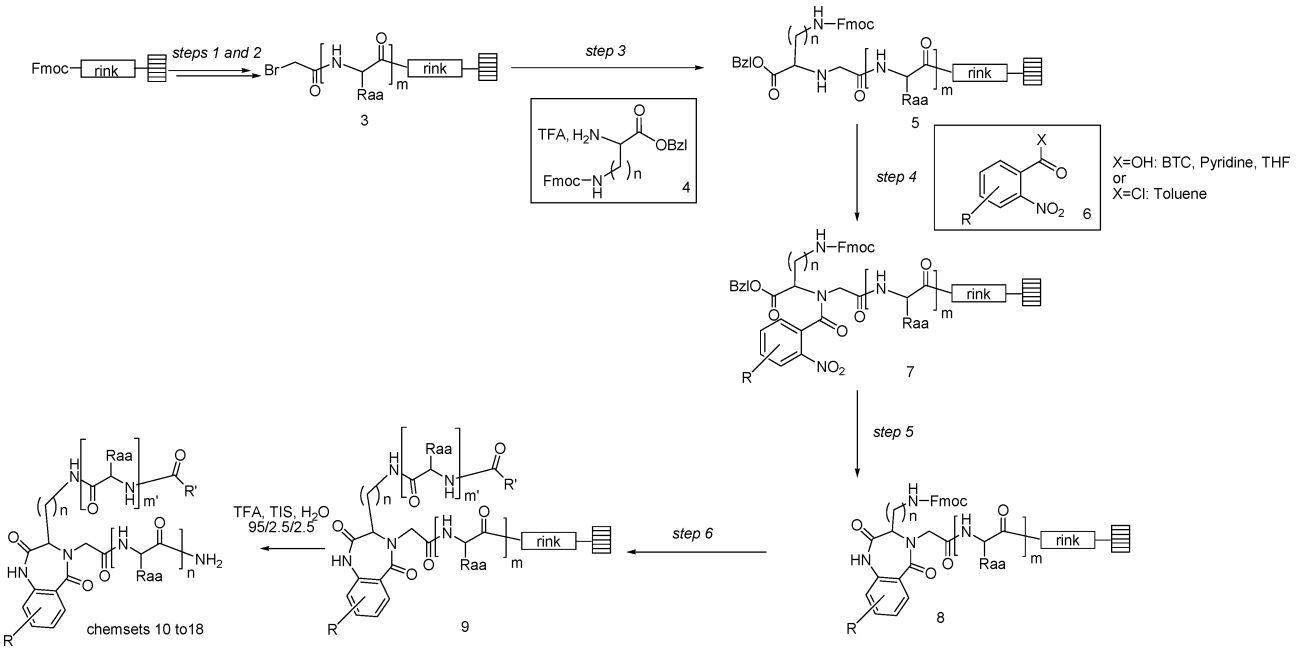
Finally, 33 purified compounds (HPLC purity above 85%) were submitted to biological assays.

Biological Evaluation. Thirty-three compounds were tested in vitro on radioligand binding tests for human melanocortin MC1 receptors. The human DNA for the MC1 receptor was transiently expressed in Cos-1 cells for competitive receptor-binding assays using [^{125}I]NDP-MSH as a radioligand. Three concentrations of each peptide in the library were tested in triplicate. IC_{50} values were obtained after analysis of the competition curves using the GraphPad Prism software.

At the same time, the α-MSH reference was examined. The results obtained for this standard ($IC_{50} = 9.2 \times 10^{-10}$ M) were consistent with those reported in the literature.[24] The IC_{50} values of the compounds are presented in Table 2.

While 22 compounds did not bind to MC1-R receptors at 10^{-5} M, compound **17**{*1,2*} had an IC_{50} of 0.9 μM. These moderate results confirm that replacement of two important residues within the core tetrapeptidic sequence ([His-D-Phe-Arg-Trp] or [His-D-Phe-Arg-Nal]) of α-MSH analogues, such as NDP-MSH or Melitane by a non-peptidic template (omitting side-chain functionalities), has a bad effect. In general, the IC_{50} values obtained are not low enough to accurately discuss the SAR of library compounds. However, an interesting result was obtained by comparison of chemset **14** (derived from Melitane) and chemset **17** (derived from CHT05-26, a lead compound we have discovered during our primary screening). Melitane differs from the CHT05-26 sequence by substitution of its C-terminal Trp^6 by the nonnatural amino acid 2-naphtylalanine (Nal). During our primary screening campaign, we measured the cAMP induction resulting from activation of MC1 receptor on mouse melanoma cell line S91. CHT05-26 had an EC_{50} of 0.3 nM, while Melitane was five times less active with an EC_{50} of

Scheme 2. Library Synthesis[a]



[a] Reagents and conditions: (step 1) Fmoc SPPS in 9 separate vials for the first part of peptide chain (see Table 1, chemsets **10**−**18**). HBTU and DIEA in NMP were used for coupling steps. (step 2) Double coupling, $BrCH_2CO_2H$, DIC, HOBT, 9 and 2 h, room temp. (step 3) Sort and combine procedure, reagent chemset **4**, DMF, DIEA, 24 h, room temp. (step 4) Sort and combine procedure, reagent chemset **6**. For R = H, *o*-nitrobenzoic acid chloride in THF was used. For R = *m*Me or *p*Cl, free *o*-nitrobenzoic acid derivatives were used with BTC/pyridine mixture in toluene. (step 5) Lanterns were pooled together. $SnCl_2 \cdot 2\ H_2O$, 2 M, 60 °C, 22 h. (step 6) DMF/piperidine 80/20, 30 min then sort and combine procedure (see Table 1), followed by Fmoc SPPS. (step 7) Chemsets **10**, **11**, **14**−**18** (R′ = CH_3) Ac_2O/DCM 1/1, 10 min, (×2), room temp. Chemsets **12** and **13** (R′ = $CH_3(CH_2)_{14}$) palmitic acid, HBTU, DIEA, DMF, 6 h, room temp.

1.8 nM. The influence of 2-naphtylalanine substitution is confirmed by the results obtained with chemsets **14** and **17**. No binding was observed for compounds of chemset **14** and micromolar affinities were obtained for most of most of the compounds of chemset **17**. For example, **17**{*1,2*} exhibits an IC_{50} of 0.9 μM, while compound **14**{*1,2*} does not even bind to MC1 receptor at 10^{-5} M, indicating the significant role of Nal.

Substitution of the [D-Phe−Arg] dipeptide of NDP−MSH sequence with the benzodiazepinone template produced inactive compounds (chemset **11**), while replacement of [His−D-Phe] dipeptide of NDP-MSH gave poor but significant MCR1 binding (chemsets **10**). These results are consistent with literature studies establishing the presence of a β-turn induced by the [His−D-Phe] sequence and not by the [D-Phe−Arg] sequence. The same observation can be made by comparison of the CHT05-26 derivatives (chemsets **17** and **18**). Replacement of [D-Phe−Arg] by benzodiazepinone (chemset **17**), except for the weakly active compound **18**{*1,2*}, led to inactive compounds. In an opposite manner, when the benzodiazepinone template replaced the [His−D-Phe] sequence, the most active ligands of the library (chemset **17**) are obtained with an IC_{50} in the micromolar range.

Compounds with a short ($n = 1$) amino alkyl chain on the benzodiazepinone core, obtained with diaminoproprionic acid reagent **4**{*1*}, are more potent than analogues with longer ($n = 4$) amino alkyl chain, obtained with the lysine building block (reagent **4**{*2*}). Compounds **18**{*1,2*} and **12**{*1,1*} have a weak but significant IC_{50}, while no binding is detected with compounds **18**{*2,2*} and **12**{*2,1*}. The same observation can be done by examining compound **17**{*2,2*}, which has an IC_{50} three times smaller than that of **17**{*1,2*}. This result can be explained by the β-turn structure that is easier to induce using a benzodiazepinone template, obtained with the Dap building block, rather than a benzodiazepinone synthesized with the flexible and longer side chain of lysine. The only exception is for the Dap-containing compound **17**{*1,1*}, which binds to MC1-R with less affinity than the Lys-containing analogues **17**{*2,1*−*3*}. The low binding of **17**{*1,1*} ($IC_{50} > 10^{-5}$M) is difficult to explain, especially when one compares this compound with analogue **17**{*1,2*} ($IC_{50} = 9.5 \times 10^{-7}$M). Indeed, these two compounds only differ by a methyl group in position 7 on the benzodiazepinone moiety. Moreover, the data collected are not sufficient to discuss the influence of aromatic ring substitution. For example, compounds **16**{*1,3*} and **18**{*1,2*} are the only binding compounds in their chemset, but they do not share the same substituent. To be relevant, this study should require the synthesis of focused library expanding the diversity of chemset **6**.

Conclusions

In this work, we developed an efficient approach to the on-line solid-phase synthesis of a peptide incorporating a benzodiazepinone template, and we synthesized a focused compound library targeted on MC1 receptors.

The chemistry applied allowed the introduction of diverse building blocks during the synthesis of benzodiazepinone and could be easily implemented to introduce further diversity on the aryl group or the nitrogen atom of the diazepine cycle. The library compounds were purified by an automated LC/MS system.

Table 2. MC1-R Binding Results

compound	sequence	IC_{50} (M)
α-MSH	Ac-Ser-Tyr-Ser-Nle-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2	9.2×10^{-10}
10{*1,1*}	Ac-Ser-Tyr-Ser-Nle-Glu-benzo[$n = 1$, R = H]-Arg-Trp-Gly-Lys-Pro-Val-NH_2	$>10^{-5}$
10{*1,2*}	Ac-Ser-Tyr-Ser-Nle-Glu-benzo[$n = 1$, R = 7-Me]-Arg-Trp-Gly-Lys-Pro-Val-NH_2	$>10^{-5}$
10{*2,1*}	Ac-Ser-Tyr-Ser-Nle-Glu-benzo[$n = 4$, R = H]-Arg-Trp-Gly-Lys-Pro-Val-NH_2	$>10^{-5}$
11{*1,1*}	Ac-Ser-Tyr-Ser-Nle-Glu-His-benzo[$n = 1$, R = H]-Trp-Gly-Lys-Pro-Val-NH_2	no binding
11{*1,2*}	Ac-Ser-Tyr-Ser-Nle-Glu-His-benzo[$n = 1$, R = 7-Me]-Trp-Gly-Lys-Pro-Val-NH_2	no binding
11{*2,1*}	Ac-Ser-Tyr-Ser-Nle-Glu-His-benzo[$n = 4$, R = H]-Trp-Gly-Lys-Pro-Val-NH_2	no binding
JMV 2437	Palm-His-D-Phe-Arg-Trp-NH2	5.42×10^{-8}
12{*1,1*}	Palm-benzo[$n = 1$, R = H]-Arg-Trp-NH_2	1.1×10^{-5}
12{*1,2*}	Palm-benzo[$n = 1$, R = 7-Me]-Arg-Trp-NH_2	no binding
12{*2,1*}	Palm-benzo[$n = 4$, R = H]-Arg-Trp-NH_2	no binding
12{*2,2*}	Palm-benzo[$n = 4$, R = 7-Me]-Arg-Trp-NH_2	no binding
13{*2,1*}	Palm-His-benzo[$n = 4$, R = H]-Trp-NH_2	no binding
Melitane	Ac-Nle-Ala-His-D-Phe-Arg-Trp- NH_2	1.62×10^{-9}
14{*1,2*}	Ac-Nle-Ala-benzo[$n = 1$, R = 7-Me]-Arg-Trp-NH_2	no binding
14{*1,3*}	Ac-Nle-Ala-benzo[$n = 1$, R = 8-Cl]-Arg-Trp-NH_2	no binding
14{*2,1*}	Ac-Nle-Ala-benzo[$n = 4$, R = H]-Arg-Trp-NH_2	no binding
14{*2,2*}	Ac-Nle-Ala-benzo[$n = 4$, R = 7-Me]-Arg-Trp-NH_2	no binding
15{*1,1*}	Ac-Nle-Ala-His-benzo[$n = 1$, R = H]-Trp-NH_2	no binding
15{*1,3*}	Ac-Nle-Ala-His-benzo[$n = 1$, R = 8-Cl]-Trp-NH_2	no binding
15{*2,1*}	Ac-Nle-Ala-His-benzo[$n = 4$, R = H]-Trp-NH_2	no binding
15{*2,2*}	Ac-Nle-Ala-His-benzo[$n = 4$, R = 7-Me]-Trp-NH_2	no binding
15{*2,3*}	Ac-Nle-Ala-His-benzo[$n = 4$, R = 8-Cl]-Trp-NH_2	no binding
CHT05-19	Ac-Nle-Ala-Arg-D-Phe-Arg-Trp-NH_2	7.62×10^{-9}
16{*1,1*}	Ac-Nle-Ala-Arg-benzo[$n = 1$, R = H]-Trp-NH_2	no binding
16{*1,3*}	Ac-Nle-Ala-Arg-benzo[$n = 1$, R = 8-Cl]-Trp-NH_2	7.4×10^{-6}
16{*2,1*}	Ac-Nle-Ala-Arg-benzo[$n = 4$, R = H]-Trp-NH_2	no binding
16{*2,2*}	Ac-Nle-Ala-Arg-benzo[$n = 4$; R = 7-Me]-Trp-NH_2	no binding
CHT05-26	Ac-Nle-Ala-His-D-Phe-Arg-Nal-NH_2	6.86×10^{-10}
17{*1,1*}	Ac-Nle-Ala-benzo[$n = 1$, R = H]-Arg-Nal-NH_2	$>10^{-5}$
17{*1,2*}	Ac-Nle-Ala-benzo[$n = 1$; R = 7-Me]-Arg-Nal-NH_2	9.5×10^{-7}
17{*2,1*}	Ac-Nle-Ala-benzo[$n = 4$, R = H]-Arg-Nal-NH_2	1.6×10^{-6}
17{*2,2*}	Ac-Nle-Ala-benzo[$n = 4$, R = 7-Me]-Arg-Nal-NH_2	3×10^{-6}
17{*2,3*}	Ac-Nle-Ala-benzo[$n = 4$, R = 8-Cl]-Arg-Nal-NH_2	1.9×10^{-6}
18{*1,1*}	Ac-Nle-Ala-His-benzo[$n = 1$, R = H]-Nal-NH_2	no binding
18{*1,2*}	Ac-Nle-Ala-His-benzo[$n = 1$, R = 7-Me]-Nal-NH_2	$>10^{-5}$
18{*2,2*}	Ac-Nle-Ala-His-benzo[$n = 4$, R = 7-Me]-Nal-NH_2	no binding
18{*2,3*}	Ac-Nle-Ala-His-benzo[$n = 4$, R = 8-Cl]-Nal-NH_2	no binding

The biological evaluation of the purified library members, with respect to their affinity for melanocortin receptor type 1, led to the identification of several micromolar ligands. The results obtained are consistent with literature data concerning the presence of a β-turn structure at the [His−D-Phe] dipeptide in the α-MSH analogues.

Experimental Section

Materials. All solvents were obtained from Acros and were used without purification. PA−Rink amide linker SynPhase lanterns with 8 μmol loading, radiofrequency tags, and TranSort software were provided from Mimotopes, Pty, Clayton, Australia. Fmoc-protected amino acids are used with appropriate side-chain protection including Fmoc-His(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ser(tBu)-OH, and Fmoc-Glu(OtBu)-OH. Amino acids and the HBTU reagent were purchased from Senn Chemicals. Palmitic acid, acetic anhydride, and other reagents were purchased from Aldrich and Lancaster.

Adenosine 5′-triphosphate disodium salt (ATP), $CaCl_2$, chloramine T, EGTA, glutathione, HEPES, KCl, K_2HPO_4, KH_2PO_4, $MgCl_2$, NaCl, Na_2HPO_4, NaH_2PO_4, and 1,10-phenanthroline were purchased from Sigma (St Louis, MO). NaOH was purchased from Carlo Erba Reagenti (Italy). ^{125}I-Na was purchased from Amersham (Buckinghamshire, U.K.). Dulbecco's modified Eagles' medium (DMEM), glutamine, penicillin−streptomycin, and trypsin-EDTA were purchased from Cambrex (Verviers, Belgium). Minimun essential medium (MEM) and fetal bovine serum (FBS) were purchased from Invitrogen (Groningen, The Netherlands). Cos-1 cells were purchased from ATCC. Bovine serum albumin (BSA) fraction V was purchased from Euromedex (France).

α-MSH and [Nle,^{4} D-Phe^7]-α-MSH (NDP-MSH) were synthesized in our laboratory.

The MC1-R cDNA cloned into the pcDNA3 was a gift from Dr. Celia Jiménez-Cervantes Frigols (University of Murcia, Spain).

The following abbreviations were used: BTC, bistrichloromethylcarbonate; $CHCl_3$, chloroform; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIEA, diisopropylethylamine; DMF, dimethylformamide; TFA, trifluoroacetic acid; HBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; Nal,

2-naphtylalanine; Pbf, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; TIS, triisopropyl silane. The other abbreviations used were recommended by the IUPAC-IUB Commission (*Eur. J Biochem*. **1984**, *138*, 9−37).

LC/MS Analysis. Samples were prepared in an acetonitrile/water (50/50 v/v) mixture containing 0.1% TFA. The LC/MS system consisted of a Waters Alliance 2690 HPLC, coupled to a Micromass (Manchester, UK) Platform II spectrometer (electrospray ionization mode, ESI+). All the analyses were carried out using a C18 Xterra MS 21 × 3.0 mm column. A flow rate of 500 μL/min and a gradient of (0−100)% B over 5 min were used: eluent A, water/0.1% TFA; eluent B, acetonitrile/0.1% TFA. Positive-ion electrospray mass spectra were acquired at a solvent flow rate of 100−500 μL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 400 to 1400 *m*/*z* in 0.1 s intervals; 10 scans were summed up to get the final spectrum.

LC/MS Purification. Samples were prepared in an acetonitrile/water (50/50 v/v) mixture containing 0.1% TFA. The LC/MS autopurification system consisted of a binary pump Waters 2525, an injector/fraction collector Waters 2676, coupled to a Waters Micromass ZQ spectrometer (electrospray ionization mode, ESI+). All the purifications were carried out using a Waters Symmetry Shield C18 19 × 100 mm, 5 μm particle size, column. A flow rate of 20 mL/min and a gradient of 0−60% B over 20 min were used: eluent A, water/0.1% TFA; eluent B, acetonitrile/0.1% TFA. Positive-ion electrospray mass spectra were acquired at a solvent flow rate of 204 μL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 100 to 1000 *m*/*z* in 0.1 s intervals; 10 scans were summed up to get the final spectrum. Collection control trigger is set on single protonated and diprotonated ion with a MIT (minimum intensity threshold) of 8×10^5.

Standard Fmoc-Deprotection Protocol. The Fmoc-deprotection step was carried out by immersing the lanterns in a mixture of piperidine and DMF (20/80, v/v) for 60 min. A 200 mL standard flask, equipped with a drilled top was used. After removal of the deprotection solution, the lanterns were washed following the standard washing protocol.

Standard Fmoc SPPS Coupling Protocol. DMF solutions containing Fmoc aminoacid, HBTU, and DIEA were freshly prepared in a standard Schott flask before coupling ([Fmoc-AA-OH] = 120 mM, [HBTU] = 120 mM, [DIEA] = 240 mM). The lanterns were immersed for 2 h in the coupling solution at room temperature. The solution was decanted, and the lanterns were washed following the standard washing procedure. The procedure described above was repeated.

Standard Washing Protocol. The washing steps after the reductive amination, coupling, or deprotection steps were carried out by dipping the lanterns in DMF (3 × 5 min), MeOH (2 × 5 min), and DCM (1 × 5 min), respectively. A single 200 mL standard Schott flask, equipped with a drilled top, was used. The lanterns were allowed to air-dry for 15 min after the DCM washing.

Acylation with Bromoacetic Acid. A DMF solution containing bromoacetic acid, DIC, and HOBT was freshly prepared in a standard Schott flask ([Br-CH_2-CO_2H] = 500 mM, [DIC] = 500 mM, [HOBT] = 500 mM). The lanterns were immersed for 9 h in the solution at room temperature. The solution was decanted, and the lanterns were immersed again for an additional 2 h. Then lanterns were washed according to the standard washing procedure.

Substitution of Bromo Derivatives with Amino Acid Benzyl Esters. The lanterns were immersed in a 0.5 M DMF solution of aminoacid benzyl ester (H-Leu-Obzl, H-Dap(Fmoc)-Obzl or H-Lys(Fmoc)-Obzl) containing 1 M DIEA for 24 h at room temperature in a Schott flask. The solution was decanted, and the lanterns were submitted to the standard washing protocol.

Secondary Amine Acylation Protocol. Acylation was done either with acid chloride derivative (method a) or with carboxylic acid activated with BTC (method b).

Method a. Each lantern was treated with a solution of 2-nitrobenzoyl chloride (200 mM) and triethylamine (400 mM) in toluene at 60 °C for 3 h. The reagent solution was decanted, and the lanterns were washed following the standard washing procedure.

Method b. Each lantern was treated with a solution of 5-methyl-2-nitrobenzoic acid or 4-chloro-2-nitrobenzoic acid (230 mM), BTC (77 mM), and pyridine (760 mM) in THF at 60 °C for 3 h. The reagent solution was decanted, and the lanterns were washed following the standard washing procedure.

Standard Nitro Reduction Protocol. The acylated benzoyl nitro lanterns were treated with a suspension of 2 M $SnCl_2 \cdot H_2O$ in DMF at 60 °C for 16 h. The reagent was decanted. The lanterns were washed following the standard washing procedure.

Cleavage Protocol. A 500 μL aliquot of TFA was dispensed into each polypropylene tube of the deep 96-well plate. Cleavage was carried out for 60 min. The cleavage cocktail was removed from the tubes using a Jouan RC1010 vacuum centrifuge. Compounds were precipitated with dry diethyl ether, centrifuged, and decanted one by one. A 100 μL portion of acetonitrile/water (50/50, v/v) containing a 0.1% TFA was poured into each tube to dissolve the sample. Then the samples were frozen at −80 °C and lyophilized. Precipitation, centrifugation, and decantation were repeated twice to completely remove the remaining volatile residues.

Cell Culture and Transient Transfection. Cos-1 cells were grown in DMEM containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 25 U/mL penicillin, and 25 μg/mL streptomycin and maintained in a 37 °C humidified air environment containing 5% CO_2.

Transfection of cells were performed by electroporation using an Easyject Optima electroporator (Equibio). Ten million cells were harvested by trypsin−EDTA and centrifuged at 800 g. Cells were rinsed once with electroporation buffer ($CaCl_2$ 0.15 mM, KCl 120 mM, K_2HPO_4/KH_2PO_4 10 mM pH 7.6, $MgCl_2$ 5 mM, HEPES 25 mM pH 7.6, EGTA 2 mM pH 7.6), centrifuged, and then resuspended in the same buffer supplemented with 5 μM glutathione and 2 μM ATP in a final volume of 500 μL. Cells were electroporated with

1 μg of plasmid encoding for the wild type MC1 receptor at 250 V, 1500 μF, and no resistance. Cells were then diluted in growing medium without phenol red.

Radioligand Binding Assay. Iodinated NDP-MSH, [I^{125}](iodotyrosyl^2)-[Nle,^{4} D-Phe^7]-α-MSH, was prepared by the chloramine T method as follows: 250 μCi (2.5 μL) ^{125}I Na was added to 5 μg of NDP-MSH diluted in 2.5 μL of a 0.5 M sodium phosphate buffer (pH 7.4); 2.5 μL of a 0.5 mg/mL (in 0.5 M sodium phosphate buffer saline pH 7.4) chloramine T solution was then added for 30 s. The reaction was quenched with 100 μL of sodium metabisulfite solution (2 mg/mL in 0.5 M phosphate buffer saline pH 7.4). The reaction mixture was purified by reverse-phase HPLC on a C18 column using a gradient of 0−100% acetonitrile (with 0.1% TFA) for 20 min with a flow rate of 1 mL/min. Stockage buffer (250 μL; HEPES 50 mM, BSA 4%) was added to the 500 μL fraction containing the radioiodinated peptide. The radioligand was then aliquoted and conserved at −20 °C.

Transfected cells were plated in 48-well plates at a concentration of about 3×10^4 cells per well. Then 48 h later, the cells were rinsed once with phosphate-buffered saline (PBS pH 7.4), 0.2% BSA, incubated (2 h, 37 °C) with increased concentrations of α-MSH or peptides of the library, ranging from 10^{-9} to 10^{-5} M and a fixed amount of ^{125}I-NDP-MSH, corresponding to 10^{-10} M per well, in a final volume of 250 μL of MEM, 0.2% BSA, and 0.3 mM 1,10-phenanthroline. Cells were then washed twice with PBS, 0.2% BSA, and detached from the plates with NaOH (0.1 N), and the bound radioactivity was measured in a γ counter. The results were fitted by nonlinear regression using the GraphPad Prism software (San Diego, U.S.A.).

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Supporting Information Available. ESI+ mass spectrometry data and HPLC data of crude and purified library members. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Cone, R. D.; Lu, D.; Koppula, S.; Vage, D. I.; Klungland, H.; Boston, B.; Chen, W.; Orth, D. N.; Kesterson, R. A. *Recent Prog. Horm. Res*. **1996**, *51*, 287−317. (b) Eberle, A. N. *The Melanotropins: Chemistry, Physiology, and Mechanism of Action*; Karger: Basel, Switzerland, 1988.

(2) (a) De Wield, D.; Jolles, J. *Physiol. Rev*. **1982**, *62*, 976−1059. (b) Wikberg, J. E. S. *Eur. J. Pharmacol*. **1999**, *375*, 295−310. (c) Strand, F. L. *Ann. N.Y. Acad. Sci*. **1999**, *897*, 1−16.

(3) (a) Hruby, V. J.; Sharma, S. D.; Toth, K.; Jaw, J. Y.; Al-Obeidi, F.; Sawyer, T. K.; Hadley, M E. *Ann. N.Y. Acad. Sci.* **1993**, *680*, 51−63. (b) Lerner, A.B.; McGuire, J. S. *Nature* **1961**, *189*, 176. (c) Mountjoy, K.G.; Robbins, L. S.; Mortrud, M. T.; Cone, R. D. *Science* **1992**, *257*, 1248−1251.

(4) Kalden, D. H.; Scholzen, T.; Brzoska, T.; Luger, T. A. *Ann. N.Y. Acad. Sci.* **1999**, *885*, 254−261.

(5) (a) Castrucci, A. M. D.; Hadley, M. E.; Sawyer, T. K.; Wilkes, B. C.; Al-Obeidi, F.; Staples, D. J.; Devaux, A. E.; Dym, O.; Hintz, M. F.; Riehm, J. P.; Rao, K. R.; Hruby, V. J. *Gen. Comp. Endocrinol.* **1989**, *73*, 157−163. (b) Haskell-Luevano, C.; Sawyer, T. K.; Hendrata, S.; North, C.; Panahinia, L.; Stum, M.; Staples, D. J.; Castrucci, A. M.D.; Hadley, M. E.; Hruby, V. J. *Peptides* **1996**, *17*, 995−1002. (c) Hruby, V. J.; Wilkes, B. C.; Hadley, M. E.; Al-Obeidi, F.; Sawyer, T. K.; Staples, D. J.; Devaux, A. E.; Dym, O.; Castrucci, A. M. D. *J. Med. Chem*. **1987**, *30*, 2126−2130.

(6) (a) Martinez, J.; Dubs, P.; Verdié, P.; Pinel, A. M.; Subra, G. Fr. Patent 04 11276, Oct 11, 2004. (b) Martinez, J.; Dubs, P.; Verdié, P.; Pinel, A. M.; Subra, G. Fr. Patent 04 11279, Oct 22,2004.

(7) Lee, J. H.; Lim, S. K.; Huh, S. H.; Lee, D.; Lee, W. *Eur. J. Biochem.* **1998**, *257*, 31−40.

(8) (a) Hruby, V. J.; Han, G. In *Melanocortin Receptors*; Cone, R. D., Ed.; Human Press Inc.: Totowa, NJ, 2000; pp 239−261. (b) Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 2801−2810. (c) Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 3073−3081. (d) Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *Peptides* **2003**, *24*, 73−82. (e) Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 5736−5744. (f) Joseph, C. G.; Sorensen, N. B.; Wood, M. S.; Xiang, Z.; Moore, M. C.; Haskell-Luevano, C. *J. Pept. Res.* **2005**, *66*, 297−307. (g) Todorovic, A.; Holder, J. R.; Bauzo, R. M.; Scott, J. W.; Kavanagh, R.; Abdel-Malek, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2005**, *48*, 3328−36. (h) Koikov, L. N.; Ebetino, F. H.; Solinsky, M. G.; Cross-Doersen, D.; Knittel, J. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2647−2650. (i) Holder, J. R.; Haskell-Luevano, C. *Med. Res. Rev.* **2004**, *24*, 325−356. Review.

(9) (a) Hadley, M. E.; Marwan, M. M.; Al-Obeidi, F.; Hruby, V. J.; Castrucci, A. M. *Pigm. Cell. Res.* **1989**, *2*, 478−484. (b) Cowell, S. M.; Lee, Y. S.; Cain, J. P.; Hruby, V. J. *Curr. Med. Chem.* **2004**, *11*, 2785−2798.

(10) Zhang, J.; Xiong, C.; Ying, J.; Wang, W.; Hruby, V. J. *Org. Lett.* **2003**, *5*, 3115−3118.

(11) (a) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kundel, K. A.; Pinger, J. P.; Hirshfield, J. *J. Med. Chem.* **1988**, *31*, 2235−2246. (b) Samanen, J. M.; Ali, F. E.; Barton, L. S.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, W.; Chen, L.; Erhard, K.; Feuerstein, G.; Heys, R.; Hwang, S-M.; Jakas, R. R.; Keenan, R. M.; Ku, T. W.; Kwon, C.; Lee, C-P.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M.; Peishoff, C. E.; Rhodes, G.; Ross, S.; Shu, A.; Simpson, R.; Takata, D.; Yellin, T. O.; Uzsinskas, I.; Venslavsky, J. W.; Yuan, C. K.; Huffman, W. F. *J. Med. Chem.* **1996**, *39*, 4867−4870.

(12) Ripka, W. C.; De Lucca, G. V.; Bach, A. C.; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *17*, 3593−3608.

(13) Bedos, P.; Amblard, M.; Subra, G.; Dodey, P.; Luccarini, J. M.; Paquet, J. L.; Pruneau, D.; Aumelas, A.; Martinez, J. *J. Med. Chem.*, **2000**, *43*, 2387−2394.

(14) Pinel, A. M. Fr. Patent FR 2835528 A1, 2002.

(15) Nikiforovich, G. V.; Sharma, S. D.; Hadley, M. E.; Hruby, V. J. *Biopolymers* **1998**, *46*, 155−167.

(16) Ying, J.; Köver, K.; Gu, X.; Han, G.; Trivedi, D.; Karavana, M. J.; Hruby, V. J. *Biopolymers* **2003**, *71*, 696−716.

(17) (a) Schwarz, M. K.; Tumelty, D.; Gallop, M. A. *Tetrahedron. Lett.* **1998**, *39*, 8397−8400. (b) Plunkett, J. M.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 2885−2893. (c) Wu, Z.; Ede, N. *J. Comb. Chem.* **2003**, *5*, 166−171.

(18) (a) Mayer, J. P.; Zhang, J.; Bjergarde, K.; Lenz, D. M.; Gaudino, J. J. *Tetrahedron Lett.* **1996**, *37*, 8081−8084. (b) Hulme, C.; Peng, J.; Morton, G.; Salvino, J. M.; Herpin, T.; Labaudiniere, R. *Tetrahedron Lett.* **1998**, *39*, 7227−7230. (c) Kennedy, A. L.; Fryer, A. M.; Josey, J. A. *Org. Lett.* **2002**, *4*, 1167−1170.

(19) (a) Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 1240−1256. (b) Boojamra, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742−5743. (c) Cheng, M.-F.; Fang, J.-M. *J. Comb. Chem.* **2004**, *6*, 99−104.
(20) Chan, W. C.; White, P. D. In *Fmoc Solid Phase Peptide Chemistry: A Practical Approach*; Hames, B. D., Ed.; Oxford University Press: Oxford, U.K., 2000.
(21) Vojkovsky, T. *Pept. Res.* **1995**, *8*, 236−237.
(22) Martinez, J.; Dubs, P.; Verdié, P.; Pinel, A. M.; Subra, G. Fr. Patent 04 05069, May, 11, 2004.
(23) Feliu, L.; Subra, G.; Martinez, J.; Amblard, M. *J. Comb. Chem.* **2003**, *5*, 356−361.
(24) Haskell-Luevano, C.; Nikiforovich, G.; Sharma, S. D.; Yang, Y. K.; Dickinson, D.; Hruby, V. J.; Gantz, I. *J. Med. Chem.* **1997**, *40*, 1738−1748.

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