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A Solid-Phase Approach to Mouse Melanocortin Receptor Agonists Derived from a Novel Thioether Cyclized Peptidomimetic Scaffold

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Abstract: The solid-phase synthesis of a novel thioether cyclized peptidomimetic scaffold, displaying functionality at the *i* to *i* + 3 positions, is reported. The thioether bridge is formed *on-bead* by an intramolecular reaction between a chloroacetylated reduced peptide bond and the free thiol from a cysteine. The crude products were obtained in moderate to very high purity. A series of 19 compounds were prepared and tested for agonist activity at the mouse melanocortin receptors 1, 3, 4, and 5 (mMC1-5R). From these results, several compounds were identified as having low micromolar agonist activity at the mMC1R and mMC4R. The former is involved in skin pigmentation and animal coat coloration. The latter is involved in the regulation of appetite and food intake and is currently a drug target for potential treatment of obesity. The most potent compound **1n** with the pharmacophore motif "His-DPhe-Arg-Trp" was identified as having an EC_{50} value of 165 nM at mMC1R, 7600 nM at mMC3R, 650 nM at mMC4R, and 335 nM at mMC5R. In addition, some of the compounds showed moderate selectivity for the mMC1R.

Introduction

In recent years, the design of cyclic peptidomimetics, which are conformationally constrained to mimic peptide and protein surface structures, has been pursued with significant efforts.[1−9] Such peptidomimetic scaffolds can be used to constrain pharmacophore elements in space, which can minimize entropy loss upon interaction with the receptor, presumably leading to higher binding affinity than that observed for the linear peptide. This can provide potential drug leads, as well as a better understanding of molecular recognition. One of the pharmaceutically interesting biological targets to which the peptidomimetic approach has been applied recently[10] is the family of melanocortin receptors.[11−14] The melanocortin peptides, the natural ligands to the melanocortin receptors, include α-, β-, γ-melanocyte stimulating hormones (MSH) and adrenocorticotropin (ACTH). They are a class of neuropeptides that are involved in skin pigmentation,[11,15] animal coat coloration,[11] obesity syndrome,[16,17] energy homeostasis,[18,19] and adrenocortial steroidogenesis,[11] and they are known to act via G-protein coupled melanocortin receptors.[11−13,17] All of these hormones possess a central His-Phe-Arg-Trp motif, which constitutes the receptor contact residues and is referred to as the "message" sequence.[10,20,21] It was hypothesized in the 1980s that the bioactive

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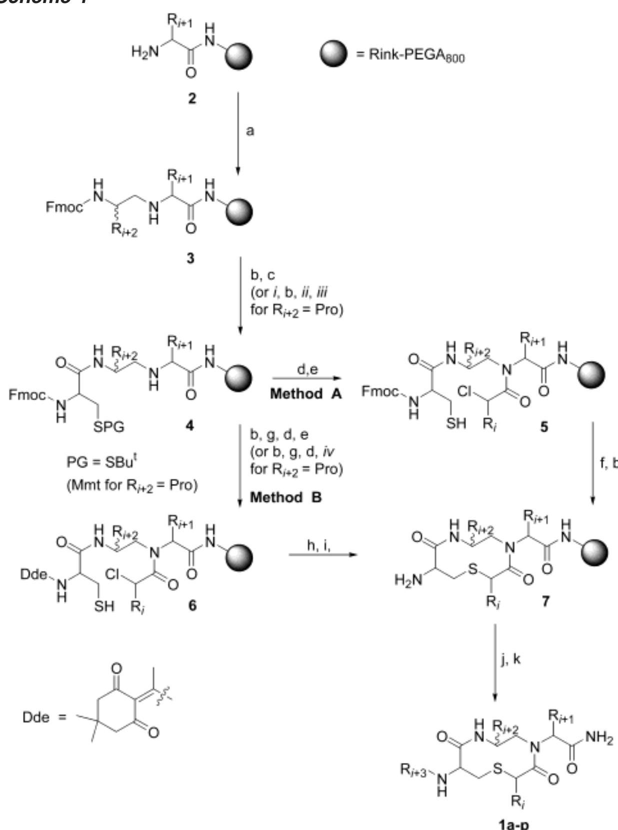
Scheme 1



Reagents and conditions: (a) Fmoc-AA-H, $NaBH_3CN$, AcOH, DMF; (b) 20% piperidine in DMF; (c) Fmoc-Cys(SBu^t)-OH, TBTU, NEM, DMF; (d) $(ClCH_2CO)_2O$, NEM, DCM or PhCHClCOCl, DIPEA, DCM; (e) Bu_3P/H_2O/THF; (f) NEM, DMF, Δ; (g) 2-acetyldimedone, DMF; (h) DBU, DMF; (i) 3% hydrazine in DMF; (j) $R_{i+3}CHO$, $NaBH_3CN$, AcOH, DMF or $R_{i+3}CO_2H$, TBTU, NEM, DMF; (k) TFA:TIPS 95:5; (*i*) Alloc-Cl, DIPEA, DCM; (*ii*) Fmoc-Cys(Mmt)-OH, HATU, NEM, DMF; (*iii*) $(Ph_3P)_4Pd$, $CHCl_3$/NEM/AcOH; (*iv*) DCM:TFA:TIPS 90:3:7.

conformation involves a β-turn containing this message sequence,[18,22] which has been supported by recent studies with a small molecule β-turn mimic.[10] Because the melanocortin-3 and -4 receptors are involved in the regulation of appetite and feeding behavior,[16,17,19,23,24] they presently constitute an important drug target for design of selective therapeutics for potential treatment of eating disorders (i.e., obesity and anorexia).

The present report describes a solid-phase approach to the peptidomimetic scaffold **1** (Scheme 1, Table 1), which is based on standard Fmoc peptide chemistry. A key feature in the design is the use of mild *on-bead cyclization* conditions, which leaves the compound attached to the resin for further synthetic manipulation or for use in *on-bead* screening. The heterocyclic structure displays functionality at the *i* to *i* + 3 positions, and all side chains are introduced via commercial Fmoc amino acid derivatives and easily accessible precursors. The synthetic scheme combines diversity with accessibility and speed which makes this scaffold suitable for automated parallel synthesis and combinatorial chemistry. A series of these compounds were prepared and tested for agonist activity at the mouse melanocortin receptors to explore the biological potential of scaffold **1**.

Table 1. Synthesis of Scaffold **1**[a]

entry	R_i	R_{i+1}[b]	R_{i+2}[b]	R_{i+3}	purity[c] (crude)	method
1a	H	Nap	D-Lys(Boc)	Nap	58	B
1b	H	Nap	Lys(Boc)	Nap	64	B
1c	H	Nap	Arg(Boc)$_2$	Nap	55	B
1d	H	Phe	D-Lys(Boc)	Nap	66	B
1e	H	Phe	Lys(Boc)	Nap	75	B
1f[d,e]	H	Trp(Boc)	Pro	Nap	55	B
1g[d,e]	H	Phe	Pro	Nap	54	B
1h	H	Tyr(Boc)	D-Lys(Boc)	Nap	73	B
1i	H	Tyr(Boc)	Lys(Boc)	Nap	83	B
1j[f]	H	Trp(Boc)	D-Lys(Boc)	Nap	71	B
1k	H	Trp(Boc)	Lys(Boc)	Nap′-CO	88	B
1l	Ph	Trp(Boc)	Lys(Boc)	Nap	51	B
1m[g]	H	Tyr(Boc)	Leu	Nap	85	B
1n	H	Trp(Boc)	Arg(Boc)$_2$	AcHis(Trt)DPhe	70	B
1o	H	Arg(Pmc)	Phe	Nap	57[h]	A
1p	H	Trp(Boc)	Lys(Boc)	Nap	75	A

[a] Nap = 1-naphthyl-CH_2; Nap′-CO = 2-naphthoyl. [b] The three letter code refers to the side chain of the amino acid. The L-amino acid derivatives were used unless otherwise noted. The temporary, acid labile protecting groups used during synthesis are shown is parentheses. [c] Combined isomeric purity, determined by RP-HPLC at 215 nm. [d] The reduced bond was temporarily protected with Alloc. [e] Fmoc-Cys(Mmt)-OH used instead of Fmoc-Cys(SBu^t)-OH. [f] Contains a D-Cys. [g] Reductive alkylation performed for 5.5 h. [h] Purity determined prior to introduction of R_{i+3}.

Results and Discussion

Synthesis of Scaffold 1. Method A. The chemistry was developed on PEGA-resin,[25,26] using the Rink amide linker. Introduction of the first side chain was done by coupling of an Fmoc-protected amino acid, using standard peptide chemistry to give **2** (Scheme 1). The second side chain was incorporated via a reductive alkylation, using $NaBH_3CN$ together with the Fmoc-protected amino aldehyde, prepared either by $LiAlH_4$ reduction of the corresponding Weinreb amide,[27−29] by DIBAL reduction of the ester, or by Dess−Martin periodinane[30−32] oxidation of the amino alcohol (Scheme 2).[33−35]

The reductive alkylation proceeds smoothly to the monoalkylated product **3** at room temperature in 3 h, as indicated by a negative Kaiser test.[36] Racemization of the *i* + 2 α-carbon was observed. It is not clear whether this is due to racemization during preparation of the aldehyde or during formation of the

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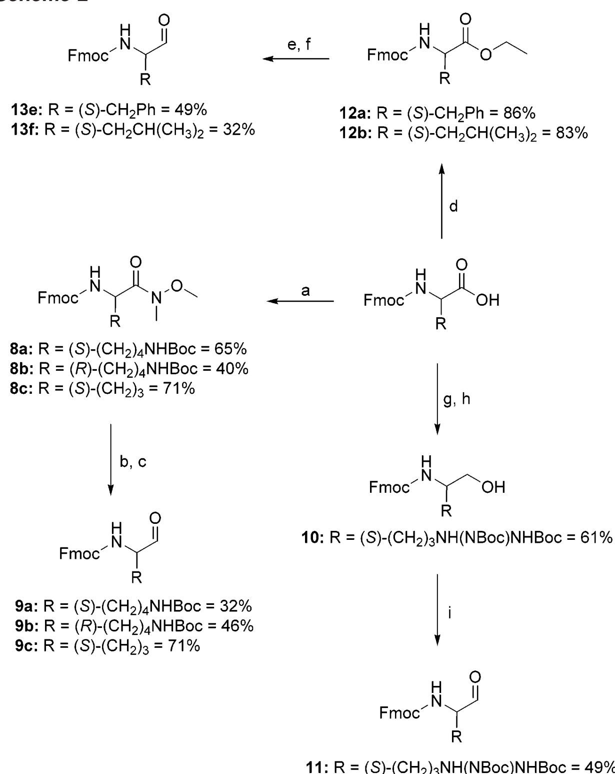
Scheme 2



Reagents and conditions: (a) DCC, DhbtOH, MeNH(OMe)·HCl, DIPEA, THF; (b) $LiAlH_4$, THF, −78 °C; (c) H_2O; (d) EtOH, H_2SO_4, Δ; (e) DIBAL-H, DCM, −78 °C; (f) Rochelle's salt (saturated aqueous potassium sodium tartrate); (g) Bu^iOCOCl, NEM, THF; (h) $NaBH_4$, THF/H_2O; (i) Dess−Martin periodinane, DCM.

C−N bond; however, it is well-known that amino aldehydes are configurationally labile, and, in particular, they are reported to racemize upon silica chromatography and during storage.[37,38] Dialkylation was only observed in significant amounts (>5%) upon longer reaction times (>6 h) or when performing double couplings. However, attempts to use Gly at the $i + 1$ and/or $i + 2$ positions resulted in the dialkylation product being the major product. The tendency for Gly to give dialkylation has been reported earlier, both in solution[39] and on solid support.[40] Following reductive alkylation, the primary amine was liberated and then selectively acylated with Fmoc-Cys(SBu^t)-OH using TBTU activation to give **4**. The sterically hindered secondary amine was not acylated to any extent detectable by HPLC or MS analysis under these conditions (3 equiv of acid, 4 h). However, when it was subsequently reacted with the much less bulky chloroacetic anhydride in dichloromethane, quantitative acylation was achieved within 30 min, as indicated by a negative chloranil test for secondary amines.[41] When using Arg(Pmc) in the sequence in connection with a large excess of acylating agent, byproducts with a mass corresponding to dichloroacetylation were detected in significant amounts (>20%). This is presumably due to acylation of the unprotected nitrogen in the guanidino group, as reported earlier.[42] The problem could be

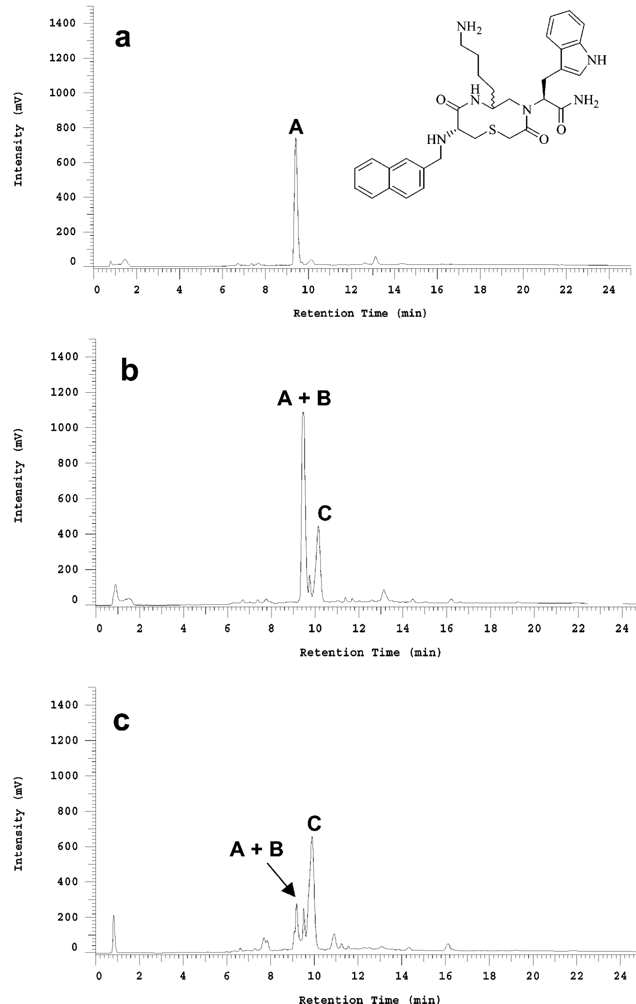


Figure 1. RP-HPLC of crude **1p** after treatment with 95% TFA for (a) 0.5 h; (b) 3 h; (c) 24 h. Peak A is a mixture of $i + 2$ epimeric *C*-terminal amides, whereas peaks B and C are the corresponding $i + 2$ epimeric C-terminal acids.

avoided by using $Arg(Boc)_2$ instead. The *tert*-butylthio cysteine protection group was removed with Bu_3P to give the cyclization precursor **5**.[43] Following the deprotection of cysteine, quantitative cyclization to form the thioether bridge was achieved by gentle heating (55−60 °C) with *N*-ethylmorpholine in DMF for 7 h, yielding **7**. No dimers or oligomers were detected by HPLC or MS. After cyclization, the Fmoc group was removed, and the primary amine was then reductively alkylated with 1-naphthaldehyde. The products **1o** and **1p** were cleaved from the resin by a 2 h treatment with a 95% TFA/triisopropylsilane 95:5 mixture, and analyzed by HPLC and MS.

Crude **1p** was obtained in 68% yield as a mixture of three components (**1p-A**, **1p-B**, **1p-C**) in a 12:1:6 ratio, with a combined purity of 75%, as determined by HPLC with detection at 215 nm (Figure 1). The combined overall yield of the three analytically pure components after preparative HPLC purification was 28% on the basis of resin loading. MALDI-TOF MS analysis of the individual components revealed that the MH^+

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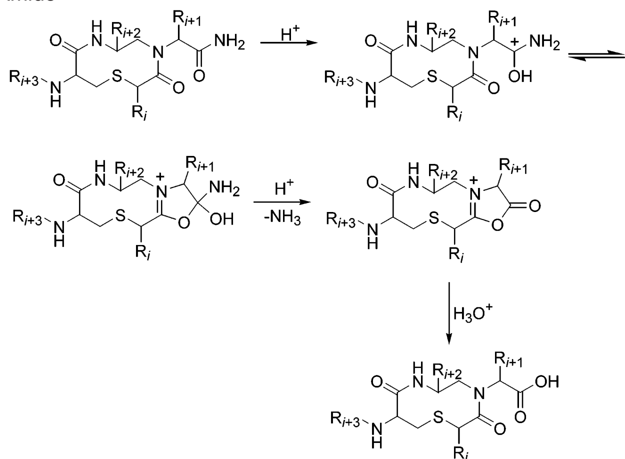
(43) When bromoacetic anhydride was used instead of chloroacetic anhydride for acylation of the reduced bond, significant amounts of unidentified higher mass byproducts were detected by HPLC and MALDI-TOF MS analysis. This is presumably due to nucleophilic substitution of the more labile bromine atom by Bu_3P, followed by subsequent side reactions of the formed adduct.

Scheme 3. Proposed Mechanism for Hydrolysis of the *C*-Terminal Amide



species of both **1p-B** and **1p-C** were 1 mass unit heavier than the MH^+ of **1p-A**, as determined with an accuracy of ±0.05 by calibrating against bradykinin as an internal standard. It was observed that the **1p-A**:**1p-B**:**1p-C** ratio changed to 3:1:6 upon prolonged cleavage with TFA mixture for 24 h, and at the same time **1p-A** appeared as two isobaric, slightly overlapping peaks. Therefore, it was assumed that **1p-B** and **1p-C** are $i + 2$ epimeric *C*-terminal acids, formed by postcleavage hydrolysis of the two $i + 2$ epimeric *C*-terminal amides presumably contained in **1p-A**. This was supported by the absence of the *C*-terminal amide protons in the 1D proton spectrum of **1p-C**. *N*-Alkylated peptide bonds are known to be more labile toward TFA cleavage than their unalkylated counterparts, due to the increased inductive effect of the alkyl substituent.[44] The evidence presented herein indicated that hydrolysis proceeds via an equilibrium involving an oxazolone-like intermediate. The mechanism for the accelerated hydrolysis of the *C*-terminal amide in scaffold **1** is proposed in Scheme 3.

The instability of the *C*-terminal amide was general for compounds **1a**–**p**; however, all of the different components present in the crude mixtures could usually only be detected during preparative HPLC, because they did not always separate well in the analytical HPLC. It was later realized that the hydrolysis could be reduced to less than 5% by using *neat* TFA for short periods of time (30 min), preferably using lyophilized resin.

Method B. Although the cyclization conditions initially developed in method A worked satisfactorily, an alternative room-temperature approach was desired, because this is much more convenient, in particular in connection with parallel synthesis of a large number of compounds. However, lowering of the reaction temperature required the use of a stronger base to ensure quantitative cyclization, which in turn required replacement of the base-sensitive Fmoc group. The Dde[45] protection group proved highly suitable for this purpose. It was introduced selectively onto the primary amine[46] of **4** after Fmoc cleavage. Acylation with chloroacetic anhydride or racemic α-chlorophenylacetyl chloride[47] and treatment with Bu_3P gave the cyclization precursors **6**, which were cyclized quantitatively with DBU in DMF in less than 2 h at room temperature, as indicated by a negative Ellman test for free thiols.[48] The Dde group was removed again with 3% hydrazine in DMF to give **7**. The products **1a**–**n** were obtained upon alkylation or acylation and cleaved as in method A. The overall crude purity was moderate to very high. When using Fmoc-Pro-H as the $i + 2$ component in the synthesis, it was necessary to use the more powerful activating agent HATU to obtain complete acylation (from **3** to **4**). However, under these forcing conditions, the reduced bond in **3** had to be temporarily protected with the Alloc group to avoid partial (>10%) acylation in the subsequent step.[49] The Alloc group was introduced in **3** prior to removal of Fmoc. The highly acid-labile Mmt group was used as S-protection instead of the *tert*-butylthio, due to observed cleavage of the disulfide bond during Alloc deprotection with $(Ph_3P)_4Pd$. Thus, coupling of Fmoc-Cys(Mmt)-OH after deprotection of **3** with piperidine and subsequent removal of Alloc with $(Ph_3P)_4Pd$ gave **4**. Treatment with piperidine and acylation of the primary amine with 2-acetyldimedone were performed as above. The reduced bond was then acylated as above, and the Mmt group was removed with dilute TFA, although this required a slightly higher concentration than that reported earlier,[50] presumably due to the polar and proton accepting nature of the PEGA resin. The precursor **6** was cyclized as described above to give **7**.

Design of Melanocortin Agonists. A series of putative melanocortin agonists were designed on the basis of the knowledge gained from prior studies.[10,51,52] These results further supported a hypothesis dating back to the 1980s, which suggests that the minimal sequence required to elicit measurable biological response is the tripeptide "Phe-Arg-Trp", presumably displayed in a β-turn conformation. In a recent report from Haskell-Luevano and co-workers,[10] a library of small molecule β-turn mimics was screened for agonist activity at the melanocortin receptors. It was found that β-turn mimics containing naphthylalanine, Phe, or Trp in the $i + 1$ and $i + 3$ positions, and D-Lys, D-Arg, or D-Pro in the $i + 2$ position, gave the best agonists with values in the micromolar range at the mMC1R. These results were consistent with a previous model obtained from homology molecular modeling, proposing two hydrophobic and one electrostatic pocket for binding to the mMC1R.[22] However, these compounds were about 200-fold less potent than the linear tetrapeptide fragment Ac-His-DPhe-Arg-Trp-NH_2, identified in a recent study[52] to have nanomolar agonist activity at the mMC1-5R, indicating that a fourth residue is required for potency in the same vicinity as the natural ligand α-MSH.

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Table 2. Functional Activity of Compounds **1a**−**p** at the Mouse Melanocortin Receptors[a]

entry	mMC1R EC_{50} (μM)	mMC3R EC_{50} (μM)	mMC4R EC_{50} (μM)	mMC5R EC_{50} (μM)
α-MSH	0.00107 ± 0.00027	0.00248 ± 0.00071	0.0034 ± 0.00076	0.00126 ± 0.00043
Ac-His-DPhe-Arg-Trp-NH_2	0.0256 ± 0.0047	0.195 ± 0.0446	0.0102 ± 0.00144	0.00346 ± 0.00033
1a	2.7 ± 0.58	>100	>100	39.2 ± 7.6
1b	6.8 ± 1.6	slight @ 100 μM	8.7 ± 7.7	16.5 ± 6.1
1c	5.7 ± 2.5	>100	>100	slight @ 100 μM
1d	6.1 ± 1.5	>100	>100	>100
1e	46.2 ± 13	>100	>100	29.3 ± 5.7
1f	4.5 ± 2.5	slight @ 100 μM	slight @ 100 μM	slight @ 100 μM
1g	slight @ 100 μM	>100	>100	slight @ 100 μM
1h	18.8 ± 4.2	>100	74.9 ± 19.3	20.0 ± 2.6
1i	40.7 ± 16.1	>100	>100	>100
1j	10.8 ± 4.7	slight @ 100 μM	12.1 ± 3.3	slight @ 100 μM
1k-A	32.1 ± 6.3	>100	>100	>100
1k-B	41.6 ± 5.7	>100	>100	>100
1l	7.1 ± 1.9	>100	>100	slight @ 100 μM
1m	10.4 ± 3.4	37.1 ± 3.1	35.9 ± 7.5	15.7 ± 3.7
1n	0.164 ± 0.022	7.6 ± 1.89	0.65 ± 0.126	0.335 ± 0.106
1o	3.48 ± 1.89	slight @ 100 μM	>100	20.9 ± 5.9
1p-A	6.4 ± 0.2	29.3 ± 5.5	15.2 ± 6.0	16.1 ± 2.1
1p-B	10.4 ± 6.3	>100	>100	54.3 ± 19.9
1p-C	2.35 ± 0.68	25.4 ± 7.6	14.6 ± 2.6	6.0 ± 0.6

[a] Values reflect assay results of RP-HPLC purified material. The indicated errors represent the standard error of the mean determined from at least four independent experiments. Two molecules of TFA were included in the calculations. Except for **1k** and **1p** all compounds were tested as a C-terminal amide/acid mixture. In addition, all compounds except **1p-B** and **1p-C** are presumably partially racemized at the $i + 2$ position. "Slight @ 100 μM" denotes that some agonist response was observed at 100 μM but not enough to determine an EC_{50} value. The values for α-MSH (Ac-SYSMEHFRWGKPV-NH_2) were determined in parallel, and the values for Ac-His-DPhe-Arg-Trp-NH_2 were taken from a previous report.[52]

The present report based the peptidomimetic design on the pharmacophore motif "aromatic-Lys/Arg/Pro-aromatic", varying the identity of the aromatic residues between naphthyl, phenyl, hydroxyphenyl, and indole, and the $i + 2$ stereochemistry. Stereochemical modifications at the $i + 1$ position were not included, because it has been shown recently in tetrapeptides to result in decreased potency.[52] In addition, the effects of introducing a fourth His residue in the scaffold were explored (entry **1n**) on the basis of the knowledge from recent studies,[52] which identified the tetrapeptide Ac-His-DPhe-Arg-Trp-NH_2 as the most potent minimal sequence at the melanocortin receptors.

Interpretation of Biological Results. Compounds **1a**−**p** were tested for agonist activity at the mouse melanocortin receptors, using a β-galactosidase bioassay. The pharmacological data are summarized in Table 2, and the structures of compounds **1a**−**p** are shown in Figure 2. Generally, compounds **1a**−**p** are most active at the mMC1R and least active at the mMC3R. The selectivity varies, but in particular entry **1f** with Trp $i + 1$, Pro $i + 2$, and naphthyl $i + 3$ is 15−50-fold selective for mMC1R as compared to the other three receptors. Interestingly, the close analogue with Phe instead of Trp at $i + 1$ (entry **1g**) shows only slight agonist activity at all of the receptors. Also, entries **1c** (naphthyl $i + 1$, Arg $i + 2$, naphthyl $i + 3$) and **1d** (Phe $i + 1$, D-Lys $i + 2$, naphthyl $i + 3$) both show at least a 15-fold selectivity for mMC1R. There seems to be a slight preference at the mMC1R for the D configuration at the $i + 2$ position (entries **1a**/**1b**, **1d**/**1e**, and **1h**/**1i**), which is consistent with previous results from a library of small molecule β-turn mimics.[10] When comparing **1a**/**1b**, **1d**/**1e**, and **1h**/**1i**, there is also a slight preference at mMC1R for naphthyl $i + 1$. The fact that **1f** is an active compound suggests that an electrostatic interaction in the $i + 2$ position is not a requirement for receptor activation, which is further validated by the activity of **1m** with Leu $i + 2$. Inverting the pharmacophore motif "aromatic-basic-aromatic" to "aromatic-aromatic-basic" (entry **1o**) does not result in loss of activity. In fact, **1o** is one of the most potent and selective mMC1R agonists evaluated in this report, which taken together with the results for **1f** and **1m** indicates that the aromatic residues are most important for binding, as reported recently.[53] Substitution of H for Phe at the i position (entries **1l** and **1p-A**) results in an overall increased selectivity at mMC1R while maintaining approximately the same potency, indicating that this position is potentially important for receptor selectivity. However, it must be kept in mind that a mixture (**1l**) and a pure amide (**1p-A**) are being compared. The most potent compound identified in this study is **1n** with EC_{50} values at least 10-fold lower at mMC1R, mMC4R, and mMC5R as compared to all of the other compounds in Table 2, which clearly demonstrates the importance of the fourth His residue. However, **1n** does not display significant selectivity, and further studies are needed to determine if perhaps selectivity can be increased by substitution with, for example, conformationally constrained aromatic residues at this position similar to a recent report.[53]

Conclusion

We have developed a methodology for the solid-phase synthesis of a novel 10-membered heterocyclic peptidomimetic scaffold, displaying diversity at up to four positions. The synthetic scheme, which is based on readily available precursors, yields crude products with moderate to very high purity and enables rapid parallel synthesis of a large number of diverse structures. The compounds showed low micromolar to high nanomolar agonist activity at the mMC1-5R. Additionally, some showed moderate selectivity for the mMC1R. To our knowledge, this study has identified the most potent small molecule peptidomimetic ligand (**1n**) to the mouse melanocortin receptors

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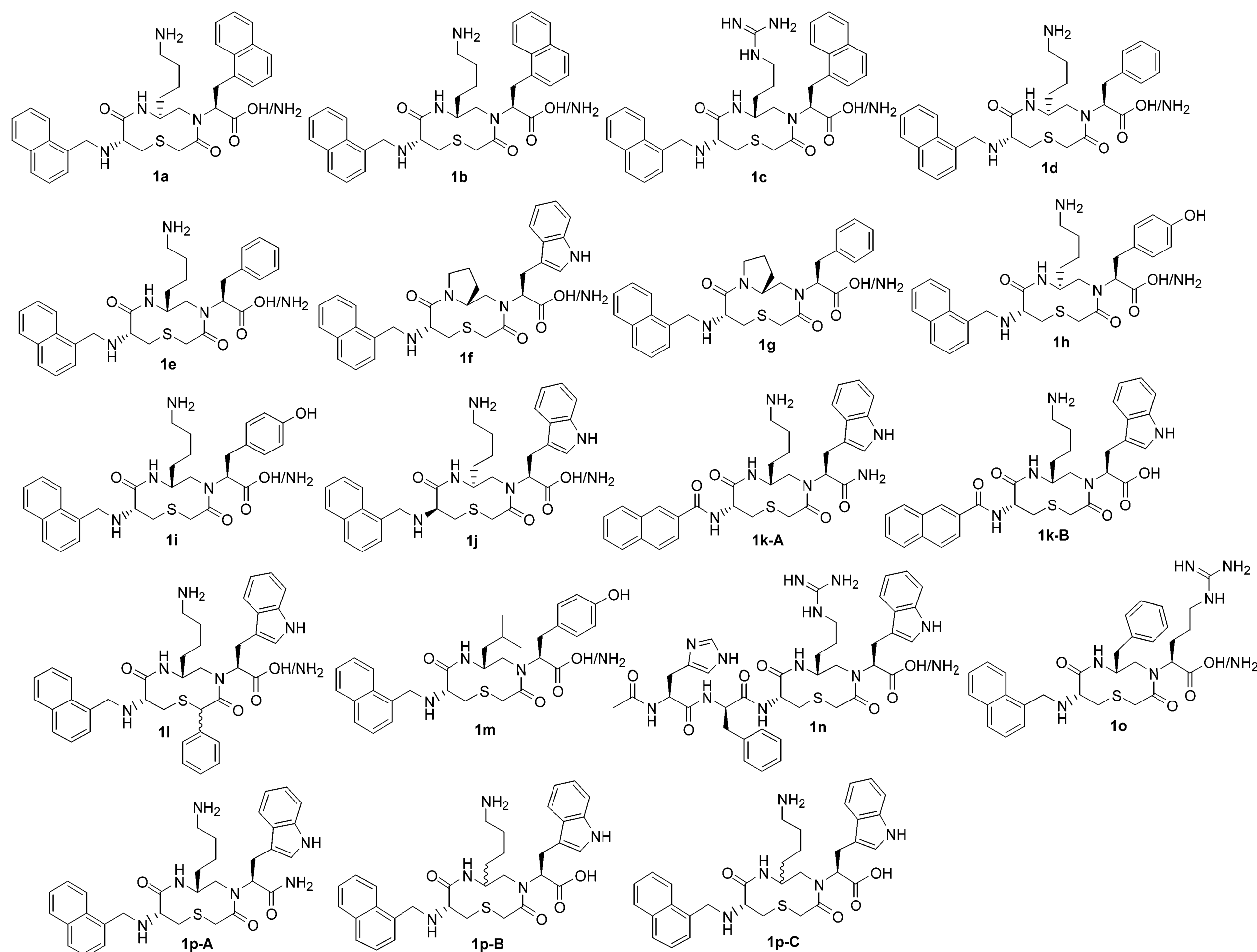

Figure 2. Structures of compounds **1a**−**p**. The designated stereochemistry at the $i + 2$ position is based on the configuration of the starting material; however, partial racemization was observed. "OH/NH_2" indicates a mixture of amide and acid.

reported to date with an EC_{50} value of 165 ± 22 nM at the mMC1R involved in skin pigmentation, 7600 ± 1890 nM at the mMC3R involved in energy homeostasis, 650 ± 126 nM at the mMC4R involved in regulation of appetite and feeding behavior, and 335 ± 106 nM at the mMC5R involved in exocrine gland regulation. These results should be useful in the further design of potent and selective non-peptide ligands to the melanocortin receptors. In addition, the methodology developed herein should be viewed as a general tool for introducing conformational constraints in a peptide or peptidomimetic attached to a solid support for the use in, for example, *on-bead* screening, and scaffold **1** can potentially be used as a versatile tool in small molecule drug discovery.

Experimental Section

The following abbreviations were used throughout the text. Alloc, allyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; DCC, *N*,*N*-dicyclohexylcarbodiimide; DCM, dichloromethane; Dde, N^{ϵ}-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl; DhbtOH, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; DIBAL, diisobutylaluminumhydrid; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HATU, *N*-(9-*N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; Mmt, 4-methoxytrityl; NEM, *N*-ethylmorpholine; Pfp, pentafluorophenyl; PEGA, poly(ethylene glycol)-poly(acryl amide) copolymer; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Rink amide linker, *p*-[(*R*,*S*)-α-[1-(9*H*-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid; SBu^t, *tert*-butylthio; TBTU, *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)-methylene]-*N*-methylmethan-aminium tetrafluoroborate *N*-oxide; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, triisopropylsilane.

Reagents and General Methods. All solvents were HPLC grade. Anhydrous solvents were obtained by storing over 4 Å activated molecular sieves. Degassed solutions were obtained by bubbling with Ar for 10 min. 2-Acetyldimedone was prepared according to a previous procedure.[54] All other starting materials were purchased from commercial suppliers and used without further purification. Solid-phase reactions run at room temperature were performed in flat-bottom polyethylene syringes equipped with sintered Teflon filters (50 μm pores), Teflon tubing, Teflon valves for flow control, and suction to drain the syringes from below. Fmoc deprotection was performed with 20% piperidine in DMF (2 + 10 min). TBTU-couplings were performed by dissolving the acid (3 equiv) in DMF with NEM (4 equiv), followed by addition of TBTU (2.88 equiv). The resulting solution was preactivated for 10 min before use (reaction time 2 h). HATU couplings were done likewise; however, preactivation was only 2 min. Pfp esters were coupled with DhbtOH (1 equiv) present. The disappearance of the bright yellow color indicated complete capping of the resin-bound amino groups. Solid-phase reactions were generally run in an amount of solvent that was enough to cover the resin (0.1−0.15 M). Resin loadings were determined by Fmoc cleavage and optical density

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measurements at 290 nm, using a calibration curve. Reactions at elevated temperatures were conducted in a sealed Nunc tube, or in glass Reacti Vials. Routine NMR data were aquired on a Bruker Avance DRX 250. Chemical shifts are reported in ppm downfield, relative to internal solvent peaks (2.49 for DMSO-d_6, 7.25 for $CDCl_3$). Coupling constants J are reported in Hz. For 2D NMR experiments, approximately 8 mg of the sample was dissolved in 600 μL of DMSO-d_6 (new Aldrich ampule). All 2D spectra were recorded on a Bruker DRX-600 MHz spectrometer. The TOCSY experiment was performed with a spinlock of 80 ms. Unless otherwise noted, the MS data reported were obtained on low-resolution instruments. ES-MS spectra were obtained on a Fisons VG Quattro 5098 MS in the positive mode, unless otherwise noted. LC-MS spectra were obtained in the positive mode on a Hewlett-Packard MSD1100 apparatus. MALDI-TOF spectra were recorded on a Bruker Reflex III MALDI-TOF MS, using α-cyano-4-hydroxycinnamic acid as matrix. High-resolution MALDI-TOF MS (HRMS) spectra were recorded at University of Odense, Denmark on a 4.7 T Ionspec FT-ICR mass spectrometer, using 2,5-dihydroxybenzoic acid as matrix and internal reference. TLC plates used were Merck silica gel $60F_{254}$ on aluminum. Visualization was achieved with UV light when applicable, or developed by Mo-staining. Column chromatography was performed on silica 60H (230−400 mesh). Analytical HPLC was performed on (A) a Waters system (490E detector at 215 and 280 nm, two 510 pumps with gradient controller and a Zorbax RP-18 column, 300 Å, 0.45 × 50 mm) or (B) a Merck-Kitachi D7000 system (L-4250 UV−vis detector 215 nm, L-6250 Intelligent pump,). Eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile/water 9:1) were used in a linear gradient (0% B → 100% B in 25 min). Retention times refer to the designated system. Semipreparative and preparative HPLC were performed on a Waters 600E system (Waters 991 photodiode array detector at 215 and 280 nm, FOXY fraction collector) connected to a Millipore Delta Pak RP-18 column (25 × 200 mm, or 47 × 300 mm), using eluents A (0.1% TFA in water) and B (0.1% TFA in $MeCN/H_2O$ 9:1) in a linear gradient, starting with 85% A and 15% B, with a slope of 0.5%/min and a flow of 20 mL/min.

General Procedure for Preparation of Weinreb Amides (5−10 mmol Scale). Modifications were made of a previous procedure for the *Z*-protected glycine derivative.[55] The Fmoc-protected amino acid was dissolved in dry THF (100 mL) and cooled to 0 °C in an ice bath. DCC (1 equiv), DhbtOH (1 equiv), and *N*,*O*-dimethylhydroxylamine hydrochloride (1 equiv) were added, followed by DIPEA (1 equiv). The resulting bright yellow solution was stirred overnight, filtered, and taken to dryness in vacuo. The residue was redissolved in ethyl acetate, washed with saturated aqueous $NaHCO_3$ (×4), 1 M HCl (×2), and brine, and dried ($MgSO_4$). Evaporation usually gave an oil, which was coevaporated with diethyl ether and dried to give a white solid. The crude products were purified by column chromatography.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ϵ-(*tert*-butyloxycarbonyl)-lysine *N*-Methoxy-*N*-methylamide (8a). This compound is described previously;[56] however, only HPLC analysis was provided. Chromatographed on silica, using petroleum ether/ethyl acetate 1:2. White solid. Yield: 65%. $R_f = 0.54$. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.29−1.87 (m, 6H), 3.11−3.15 (br, 2H), 3.25 (s, 3H), 3.80 (s, 3H), 4.22−4.27 (t, J = 6.85, 1H), 4.39−4.41 (d, J = 7.08, 2H), 4.60 (br, 1H), 4.76 (br, 1H), 5.53−5.57 (d, J = 8.93, 1H), 7.30−7.46 (m, 4H), 7.61−7.65 (m, 2H), 7.77−7.80 (d, J = 7.38, 2H). ^{13}C NMR (62.9 MHz, $CDCl_3$, rotameric signals given in parentheses): δ 22.9, 28.8, 29.9, 32.5(32.8), 40.6, 47.6, 51.1, 62.0, 66.6(67.4), 79.4, 119.8(120.3), 125.1(125.6), 126.7, 127.5, 128.1, 141.7, 144.2(144.4), 156.4(156.6), 157.3, 173.1. LC-MS: mass calcd for $C_{28}H_{37}N_3O_6Na$ 534.6 (MNa^+). Found 534.2. HPLC purity > 99%, R_t (B, no TFA) = 17.79.

(*R*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ϵ-(*tert*-butyloxycarbonyl)-lysine *N*-Methoxy-*N*-methylamide (8b). Cromatographed on silica as above. White solid. Yield: 40%. This compound was pure by TLC. The NMR and MS data were identical to those of **8a**.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-proline *N*-Methoxy-*N*-methylamide (8c). Chromatographed on silica, using petroleum ether/ethyl acetate 1:3. White solid. $R_f = 0.45$. Yield: 71%. ^{1}H NMR (250 MHz, $CDCl_3$): δ (two rotamers in a 1:2 ratio; rotameric signals given in parentheses) 1.70−2.19 (m, 4H), 3.11(3.00) (s, 3H), 3.38−3.65 (m, 2H), 3.68(3.36) (s, 3H), 4.06−4.52 (m, 3H), 4.66−4.71 (m, 1H), 7.15−7.31 (m, 4H), 7.47−7.66 (m, 4H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ (rotameric signals given in parentheses) 23.5(24.7), 30.0(31.0), 47.2(47.7), 57.2(57.4), 61.5(61.7), 67.2(67.8), 120.2, 125.4(125.5), 127.4, 127.9(128.0), 141.7, 144.6(144.7), 154.9(155.3), 173.2. ES-MS: mass calcd for $C_{22}H_{25}N_2O_4$ 381.17 (MH^+). Found 381.33. HPLC purity > 90%, R_t (B) = 15.32.

General Procedure for Reduction of Weinreb Amides (5−10 mmol Scale).[28] The Weinreb amide was dissolved in dry THF (20 mL) and added dropwise to a suspension of $LiAlH_4$ (2 equiv) in THF (30 mL) at −78 °C. The suspension was stirred at −78 °C for 1−2 h, or when judged complete by TLC, and then quenched at −78 °C with water (3 mL). $MgSO_4$ was added, and the solution was filtered. The solvent was removed in vacuo to give an oil or a sticky solid. Trituration with diethyl ether usually gave a white solid, which was essentially pure and could be used directly or purified by column chromatography.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ϵ-(*tert*-butyloxycarbonyl)-lysinal (9a).[57] Chromatographed on silica using petroleum ether/ethyl acetate 1:1. White solid. Yield: 32%. $R_f = 0.48$. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.43 (s, 9H), 1.48−2.04 (m, 6H), 3.11 (m, 2H), 4.20−4.56 (m, 4H), 5.46 (br, 1H), 7.26−7.43 (m, 4H), 7.59−7.67 (d, J = 7.22, 2H), 7.75−7.78 (d, J = 7.25, 2H), 9.58 (s, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 22.51, 28.82, 30.22, 40.20, 47.60, 60.50, 67.41, 79.5, 120.41, 125.46, 127.50, 128.15, 141.74, 144.18, 156.60, 199.81. ES-MS: mass calcd for $C_{26}H_{33}N_2O_5$ 453.23 (MH^+). Found 453.31.

(*R*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ϵ-(*tert*-butyloxycarbonyl)-lysinal (9b). The crude product was triturated with diethyl ether and used without further purification. Yield: 46%. TLC (petroleum ether/ethyl acetate 1:1) showed only minor amounts of dibenzofulvene as the only impurity. NMR and MS data were identical to those of **9a**.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-prolinal (9c). The crude product was purified by column chromatography (petroleum ether/ethyl acetate 1:3) to give a clear oil, which could not be crystallized. Yield: 71%. $R_f = 0.45$. ^{1}H NMR ($CDCl_3$, 250 MHz): δ (two rotamers in a 1:1 ratio; rotameric signals given in parentheses) 1.59−2.02 (m, 4H), 3.33−3.44 (m, 2H), 3.61(3.85) (m, 1H), 4.07−4.46 (m, 4H), 7.15−7.67 (m, 8H), 9.12(9.44) (s, 1H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (rotameric signals in parentheses) 24.0, 24.9, 27.0, 28.2, 47.1(47.3), 49.4, 65.2(65.7), 67.7(67.9), 120.4, 125.1(125.2), 127.1, 128.1, 141.8, 144.1(144.3), 155.0(155.7), 200.1(200.3). ES-MS: mass calcd for $C_{20}H_{20}NO_3$ 322.14 (MH^+). Found 322.23. HPLC purity > 95%, R_t (B) = 15.40.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ϵ-(*tert*-butyloxycarbonyl)-*N*-γ-(*tert*-butyloxycarbonyl)-arginol (10). Minor modifications were made of a previous procedure for the preparation of Fmoc amino alcohols.[58] (*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ϵ-(*tert*-butyloxycarbonyl)-*N*-γ-(*tert*-butyloxycarbonyl)-arginine (1 g, 1.68 mmol) was dissolved in THF (15 mL) and cooled in an ice bath. NEM (212 μL, 1 equiv) was added, followed by isobutylchloro formate (250 μL, 1.1 equiv). After 10 min, the precipitated *N*-ethylmorpholinium hydrochloride was filtered off and washed with THF. The combined filtrate and washings were recooled to 0 °C, and a solution of $NaBH_4$ (100 mg, 1.5 equiv) in water (1 mL) was added dropwise, carefully. The

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mixture was stirred for 15 min after the evolution of gas had subsided, at which time the reaction was incomplete according to TLC (petroleum ether/ethyl acetate 1:1). Another portion of $NaBH_4$ (50 mg in 1 mL of H_2O) was added, and after 30 min no starting material could be detected by TLC. The reaction mixture was diluted with water (100 mL), and the crude product was isolated by filtration. The white solid was washed with water and petroleum ether, and dried in vacuo. Recrystallization was achieved by dissolving the crude product in 96% EtOH, diluting with water until near saturation, and cooling in a freezer for 2 h. The white solid was isolated by filtration, washed with water and petroleum ether, and lyophilized to give the title compound. Yield: 592 mg (61%). R_f = 0.22. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.14−1.19 (app t, J = 7, 2H), 1.42 (br s, 18H), 1.52 (br s, 2H), 1.9−2.3 (v br, 1H), 3.31−3.68 (m, 5H), 4.11−4.16 (t, J = 6.5, 1H), 4.34−4.37 (d, J = 6.3, 2H), 5.41−5.44 (d, J = 7.3, 1H), 7.19−7.35 (m, 4H), 7.51−7.54 (m, 2H), 7.67−7.70 (d, J = 7.3, 2H), 8.30 (br s, 1H), 11.40 (s, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.6, 28.5, 28.7, 40.9, 47.7, 53.5, 65.4, 66.9, 83.6, 79.8, 121.4, 125.5, 127.5, 129.1, 141.7, 144.3, 153.7, 156.8, 157.1, 163.8. ES-MS: mass calcd for $C_{31}H_{43}N_4O_7$ 583.31 (MH^+). Found 583.42.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-*N*-ε-(*tert*-butyloxycarbonyl)-*N*-γ-(*tert*-butyloxycarbonyl)-arginal (11). General guidelines from previous procedures were used.[33−35] Compound **10** (450 mg) was dissolved in DCM (10 mL). Dess−Martin periodinane (393 mg, 2 equiv) was added, and the mixture was stirred for 30 min, at which time the reaction was incomplete according to TLC (petroleum ether/ethyl acetate 1:1). A further 100 mg of oxidant was added, and the mixture was stirred for 2 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ and 10% aqueous $Na_2S_2O_3$ (5 mL of each). After 15 min of vigorous stirring, the organic layer was washed with brine and dried with $MgSO_4$. Evaporation of the solvent gave an off-white solid. Yield: 219 mg (49%). R_f = 0.70. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.42 (br s, 18H), 1.58 (br s, 2H), 1.96 (br s, 2H), 3.32−3.40 (m, 2H), 4.11−4.17 (t, J = 6.7, 1H), 4.24−4.26 (br s, 1H), 4.31−4.38 (d, J = 6.8, 2H), 5.91−5.94 (d, J = 7.1, 1H), 7.18−7.35 (m, 4H), 7.51−7.53 (m, 2H), 7.67−7.70 (d, J = 7.3, 2H), 8.31 (br s, 1H), 9.52 (s, 1H), 11.40 (s, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 24.5, 27.1, 27.2, 38.9, 46.2, 58.9, 65.9, 78.4, 82.3, 119.0, 124.1, 126.1, 126.7, 140.3, 142.7, 152.3, 155.4, 162.3, 198.4. ES-MS: mass calcd for $C_{31}H_{41}N_4O_7$ 581.31 (MH^+). Found 581.51, 599.38 ($MH^+ + H_2O$).

General Procedure for Preparation of Fmoc Amino Esters (10 mmol Scale). The Fmoc amino acid was refluxed in absolute ethanol (50 mL) with concentrated H_2SO_4 (0.5 mL) for 4 h. Water (200 mL) was added, and the mixture was extracted three times with ethyl acetate. The combined organic phases were washed with saturated $NaHCO_3$ (aqueous) and brine and dried over $MgSO_4$. Evaporation left a white solid which was dried in vacuo. This material was used directly.

Ethyl (*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-phenylalaninate (12a). White solid. Yield: 86%. R_f (silica, petroleum ether/ethyl acetate 3:1) = 0.58. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.12−1.19 (t, J = 7.1, 3H), 3.01−3.05 (m, 2H), 4.02−4.39 (m, 5H), 4.53−4.61 (q, J = 6, 1H), 5.20−5.23 (d, J = 8, 1H), 7.01−7.03 (d, J = 5.9, 2H), 7.14−7.34 (m, 7H), 7.45−7.50 (m, 2H), 7.65−7.69 (d, J = 7.4, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 15.4, 39.6, 48.5, 56.1, 62.8, 68.2, 121.3, 126.4, 128.4, 128.5, 128.9, 129.0, 129.5, 137.1, 142.6, 145.1, 156.8, 172.8. ES-MS: mass calcd for $C_{26}H_{26}NO_4$ 416.18 (MH^+). Found 416.35. HPLC purity > 95%, R_t (B) = 18.96.

Ethyl (*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-leucinate (12b). White solid. Yield: 83%. R_f (petroleum ether/ethyl acetate 3:1) = 0.51. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.84−0.86 (m, 6H), 1.14−1.19 (t, J = 7.12, 3H), 1.33−1.69 (m, 3H), 4.05−4.14 (m, 3H), 4.28−4.35 (m, 3H), 5.21−5.24 (d, J = 8.7, 1H), 7.16−7.31 (m, 4H), 7.47−7.51 (m, 2H), 7.62−7.65 (d, J = 7.5, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 14.6, 22.3, 23.3, 25.2, 42.2, 47.6, 53.0, 61.7, 67.4, 119.9, 120.4, 121.5, 125.5, 127.5, 128.1, 141.7, 144.2, 156.4, 173.6. ES-MS: mass calcd for $C_{23}H_{28}NO_4$ 382.19 (MH^+). Found 382.42. HPLC purity > 95%, R_t (B) = 19.13.

General Procedure for DIBAL Redution of Fmoc Amino Esters (5−10 mmol Scale).[38,59] The Fmoc amino ester was dissolved in CH_2Cl_2 (50 mL) and cooled to −78 °C under Ar. Diisobutylaluminum hydride (1 M solution in CH_2Cl_2, 2.5 equiv) was added slowly via a syringe. The solution was stirred at −78 °C for 2 h and then quenched at −78 °C with a saturated solution of Rochelle's salt (5 mL). Drying over $MgSO_4$ and evaporation gave a white solid. This material was purified by column chromatography.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-phenylalaninal (13a).[57] This compound was flash chromatographed on silica using petroleum ether/ethyl acetate 4:1. Yield: 49%. R_f (petroleum ether/ethyl acetate 2:1) = 0.43. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.98−3.0 (d, J = 7.5, 2H), 4.03−4.09 (t, J = 6.8, 1H), 4.22−4.46 (m, 3H), 5.22−5.25 (d, J = 7.5, 1H), 6.97−6.99 (d, J = 7.5, 2H), 7.10−7.29 (m, 7H), 7.40−7.43 (d, J = 7.5, 2H), 7.61−7.64 (d, J = 7.5, 2H), 9.47 (s, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 25.4, 34.4, 35.8, 47.6, 61.6, 67.4, 120.5, 125.5, 127.5, 127.6, 128.2, 129.2, 129.8, 136.0, 141.8, 144.1, 156.3, 199.2. ES-MS: mass calcd for $C_{24}H_{22}NO_3$ 372.15 (MH^+). Found 372.33.

(*S*)-*N*-α-(Fluorenylmethyloxycarbonyl)-leucinal (13b).[57] This compound was flash chromatographed on silica using petroleum ether/ethyl acetate 4:1. Yield: 32%. R_f (petroleum ether/ethyl acetate 3:1) = 0.37. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.87 (d, J = 4.5, 6H), 1.27−1.34 (m, 1H), 1.56−1.67 (m, 2H), 4.09−4.15 (t, J = 6.67, 1H), 4.21−4.27 (br, 1H), 4.32−4.36 (d, J = 6.74, 2H), 5.08−5.11 (br d, J = 6.76, 1H), 7.15−7.33 (m, 4H), 7.48−7.51 (d, J = 7.1, 2H), 7.64−7.67 (d, J = 7.41, 2H), 9.47 (s, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 21.8, 23.0, 24.5, 38.0, 47.1, 58.7, 66.8, 119.9, 124.9, 127.0, 127.6, 141.2, 143.7, 156.1, 199.6. ES-MS: mass calcd for $C_{21}H_{24}NO_3$ 338.17 (MH^+). Found 338.28.

Solid-Phase Synthesis of Peptidomimetic Scaffold 1. Method A. Fmoc-Rink-amide linker was coupled onto lyophilized $PEGA_{800}$ resin via TBTU-activation. The resin was washed with DMF (×6) and DCM (×5), and then lyophilized overnight. The loading was measured to 0.27 mmol/g. The Fmoc group was removed, and the resin was washed with DMF (×5). The first amino acid was coupled either as the Pfp ester or via TBTU-activation, followed by washing with DMF (×5). The Fmoc group was removed, and the resin was washed with DMF (×5). $NaBH_3CN$ (6 equiv) and glacial acetic acid (18 equiv) were added in DMF, followed by the Fmoc amino aldehyde (3 equiv). The reaction was monitored, using a Kaiser test (reaction time usually 3−4 h). The resin was then washed with 96% ethanol (×3), DCM (×3), and DMF (×5). The Fmoc group was removed, and the resin was washed with DMF (×5). Fmoc-Cys(SBu^t)-OH was then coupled via TBTU-activation, followed by washing with DMF (×5) and DCM (×5). Chloroacetic anhydride (25 equiv) and NEM (25 equiv) were added in DCM. After 30 min, the resin was washed with DMF (×2), DCM (×5), and THF (×2) and then treated with Bu_3P (100 equiv) in THF:saturated sodium acetate (aqueous) 19:1 for 1 h. The resin was washed with 96% ethanol (×2), DCM (×5), and DMF (×5), suspended in degassed, dry DMF with NEM (5 equiv), and then heated to 55−60 °C for 7 h. The resin was washed with DMF (×5). The Fmoc group was removed, and the resin was washed with DMF (×5). $NaBH_3CN$ (6 equiv), glacial acetic acid (18 equiv), and 1-naphthaldehyde (3 equiv) were added in DMF. After 20 h, a Kaiser test was negative. The resin was washed with DMF (×5) and DCM (×5), and the product was cleaved from the resin by treatment with 95% TFA/TIPS 95:5 (a little DCM was added to obtain a homogeneous solution) for 2 h. The resin was washed five times with 95% TFA, three times with glacial acetic acid, and finally twice with 96% ethanol. The combined washings were concentrated in vacuo and then lyophilized to give an oil. Diethyl ether was added, and an off-white product was isolated. The crude products **1o** and **1p** were purified on preparative HPLC.

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Method B. After coupling of Fmoc-Cys(SBu^t)-OH as in method A, the Fmoc group was removed, and the resin was washed with DMF (×5). 2-Acetyldimedone (5 equiv) was added in DMF, and the resin was left overnight or until a Kaiser test was negative (usually 6 h). The reduced bond was then acylated with either chloroacetic anhydride (5 equiv) and NEM (5 equiv) in DCM for 30 min or racemic α-chlorophenylacetyl chloride (5 equiv) and DIPEA (5 equiv, added first) in DCM for 30 min. Deprotection of the thiol was then performed Bu_3P (100 equiv) in THF:H_2O 19:1 for 1 h. The resin was washed with DCM (×5) and DMF (×5). Cyclization was performed in DMF by addition of DBU (5 equiv). The reaction was monitored using the Ellman test for free thiols (reaction time usually 2 h). The resin was washed with DMF (×5), treated with 3% hydrazine in DMF (3 × 3 min), and washed with DMF (×5). The final reductive alkylation and subsequent cleavage were performed as in method A.

Method B for R_{i+2} = Pro. After the reductive alkylation (performed as in method A), the reduced bond was capped with Alloc-Cl (5 equiv) and DIPEA (5 equiv) in DCM for 30 min. The Fmoc group was removed, and the resin was washed with DMF (×5). Fmoc-Cys(Mmt)-OH was then coupled via HATU-activation, followed by washing with DMF (×5) and DCM (×5). The Alloc group was subsequently removed by treatment with $(Ph_3P)_4Pd$ (3 equiv) under Ar in a degassed solution of $CHCl_3$/AcOH/NEM 92.5:5:2.5 for 4 h. The resin was washed with DCM (×5) and DMF (×5). The Fmoc group was removed, and the resin was washed with DMF (×5). 2-Acetyldimedone (5 equiv) was added in DMF, and the resin was left overnight or until a Kaiser test was negative (usually 6 h). The reduced bond was then acylated with chloroacetic anhydride (5 equiv) and NEM (5 equiv) in DCM for 30 min. Removal of the Mmt group was then performed by four successive treatments (10 min each, wash with DCM between) with DCM:neat TFA:TIPS 90:3:7. The resin was washed with DCM (×5) and DMF (×5). Cyclization was performed in DMF by addition of DBU (5 equiv). The reaction was monitored using the Ellman test (reaction time usually 2 h). The resin was washed with DMF (×5), treated with 3% hydrazine in DMF (3 × 3 min), and washed with DMF (×5). The final reductive alkylation and subsequent cleavage were performed as in method A.

Characterization of Compounds 1a−p. Compounds **1a**−**p** were obtained as solids after preparative HPLC. Except for **1k** and **1p**, all were isolated as a mixture of amide and acid isomers. Both HPLC purity and MS values reflect an average of the mixture. HPLC retention times (in min) are given for each detectable component in the mixture with area percentages given in parentheses. **1p-A** was also characterized by NMR, with complete assignments based on 2D DQF-COSY, TOCSY, HSQC, and HMBC experiments. The ^{1}H NMR spectrum of **1p-A** at 303 K showed the presence of several slowly exchanging distinct conformers, as evident from multiple isomeric resonances. Heating the sample slowly to 343 K resulted in a gradual coalescence of the isomeric resonances.

1a: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 4 mg (12%). HPLC purity > 95%. R_t (A) = 12.0(63), 12.35(33). HRMS (MALDI): exact mass calcd for $C_{35}H_{42}N_5O_3S$ 612.3003 (MH^+). Found 612.2997.

1b: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 5.7 mg (17%). HPLC purity >99%. R_t (A) = 12.06(95), 12.77(1), 12.94(3). HRMS (MALDI): exact mass calcd for $C_{35}H_{42}N_5O_3S$ 612.3003 (MH^+). Found 612.2992.

1c: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 4.1 mg (12%). HPLC purity > 95%. R_t (A) = 12.22(96). HRMS (MALDI): exact mass calcd for $C_{35}H_{41}N_7O_3SNa$ 662.2844 (MNa^+). Found 662.2872.

1d: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 1.4 mg (5%). HPLC purity > 95%. R_t (A) = 10.97(91), 11.56(5). HRMS (MALDI): exact mass calcd for $C_{31}H_{39}N_5O_3SNa$ 584.2666 (MNa^+). Found 584.2667.

1e: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 3.9 mg (13%). HPLC purity > 95%. R_t (A) = 10.84(17), 11.09(7), 11.51(73). HRMS (MALDI): exact mass calcd for $C_{31}H_{39}N_5O_3SNa$ 584.2666 (MNa^+). Found 584.2644.

1f: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 2.6 mg (8%). HPLC purity > 99%. R_t (A) = 13.39(13), 14.09(86). HRMS (MALDI): exact mass calcd for $C_{32}H_{35}N_5O_3SNa$ 592.2353 (MNa^+). Found 593.2218 (corresponds to acid).

1g: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 1.5 mg (5%). HPLC purity > 95%. R_t (A) = 14.06(96). HRMS (MALDI): exact mass calcd for $C_{30}H_{34}N_4O_3SNa$ 553.2244 (MNa^+). Found 554.2105 (corresponds to acid).

1h: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 1.3 mg (4%). HPLC purity > 99%. R_t (A) = 9.58(20), 9.78(62), 10.18(17). HRMS (MALDI): exact mass calcd for $C_{31}H_{40}N_5O_4S$ 578.2796 (MH^+). Found 578.2802.

1i: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 3.3 mg (11%). HPLC purity > 98%. R_t (A) = 9.49(4), 9.74(34), 10.0(61). HRMS (MALDI): exact mass calcd for $C_{31}H_{39}N_5O_4SNa$ 600.2615 (MNa^+). Found 601.2440 (corresponds to acid).

1j: Prepared from 200 mg of Fmoc-Rink-PEGA resin. Yield: 6.8 mg (21%). HPLC purity > 95%. R_t (A) = 10.95(96). HRMS (MALDI): exact mass calcd for $C_{33}H_{40}N_6O_3SNa$ 623.2537 (MNa^+). Found 623.2741.

1k-A: Prepared from 500 mg of Fmoc-Rink-PEGA resin. This compound was prepared according to method B. After removal of the Dde group and wash with DMF (×5), 2-naphthoic acid was coupled via TBTU for 3 h. Wash with DMF (×5) and DCM (×5). Cleavage was then performed as described in method A. Yield: 20 mg (24%). HPLC purity > 95% (contains <5% B isomer). R_t (A) = 13.02(95). HRMS (MALDI): exact mass calcd for $C_{33}H_{38}N_6O_4SNa$ 637.2573 (MNa^+). Found 637.2583.

1k-B: Yield: 15 mg (18%). HPLC purity > 96%. R_t (A) = 13.56(97). HRMS (MALDI): exact mass calcd for $C_{33}H_{39}N_5O_5SNa$ 638.2414 (MNa^+). Found 638.2387.

1l: Prepared from 1 g of Fmoc-Rink-PEGA resin. Yield: 54 mg (30%). HPLC purity > 95%. R_t (A) = 12.33(2), 12.50(5), 12.68(10), 13.10(4), 13.23(79). HRMS (MALDI): exact mass calcd for $C_{39}H_{44}N_6O_3SNa$ 699.3094 (MNa^+). Found 699.3088.

1m: Prepared from 100 mg of Fmoc-Rink-PEGA resin. Yield: 3.0 mg (20%). HPLC purity > 99%. R_t (A) = 14.48(99). HRMS (MALDI): exact mass calcd for $C_{31}H_{38}N_4O_4SNa$ 585.2506 (MNa^+). Found 586.2320 (corresponds to acid).

1n: Prepared from 400 mg of Fmoc-Rink-PEGA resin. This compound was prepared according to the procedure in method B. After removal of the Dde group and wash with DMF (×5), Fmoc-D-Phe-OH was coupled via TBTU. The resin was washed with DMF (×5), and Fmoc was removed. Wash with DMF (×5). Fmoc-His(Boc)-OPfp was then coupled. Wash with DMF (×5), and removal of Fmoc. Wash with DMF (×5). The terminal amino group was capped with acetic anhydride (10 equiv) in DMF for 30 min. The resin was washed with DMF (×3) and DCM (×3), and the product was cleaved as described in method A. Yield: 7.3 mg (8%). HPLC purity > 98%. R_t (A) = 10.40(6), 10.69(68), 11.13(6), 11.39(19). HRMS (MALDI): exact mass calcd for $C_{39}H_{50}N_{12}O_6SNa$ 837.3589 (MNa^+). Found 837.3593.

1o: Prepared from 100 mg of Fmoc-Rink-PEGA resin. Yield: 4.2 mg (26%). HPLC purity > 99%. R_t (A) = 12.49(99). HRMS (MALDI): exact mass calcd for $C_{31}H_{39}N_7O_3SNa$ 612.2727 (MNa^+). Found 612.2721.

1p-A: Prepared from 1.57 g of Fmoc-Rink-PEGA resin. Yield: 64 mg (25%). HPLC purity > 98%; R_t (A) = 11.13(98). ^{1}H NMR (600 MHz, DMSO-d_6; the numbers given in parentheses refer to Figure 3): δ 1.20−1.60 (m, 6H; H16, H17, H18), 2.73−2.83 (m, 2H; H19), 3.10−3.12 (d, J = 13.71, 1H; H37b), 3.26−3.28 (dd, J = 15.19, 4.8, 1H; H4b), 3.37−3.41 (dd, J = 11.31, 4.8, 1H; H36b), 3.56−3.58 (d, J = 15.20, 1H; H4a), 3.68−3.71 (dd, J = 14.10, 5.36, 1H; H14b), 3.75−3.77 (d, J = 11.31, 1H; H36a), 3.80−3.85 (dd app t, J = 14.10, 11.66, 1H; H14a), 4.13−4.15 (d, J = 13.71, 1H; H37a), 4.31 (br, 1H; H15),

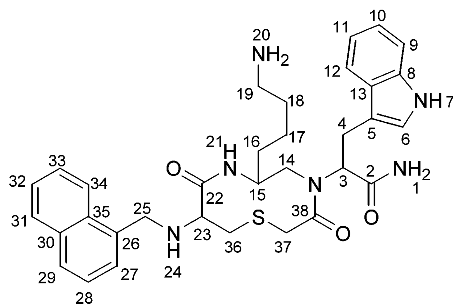

Figure 3. Structure of **1p-A** showing the numbering used for assignment of ^{1}H NMR resonances.

4.38−4.41 (m, 2H; H3, H23), 4.58−4.64 (m, 2H; H25a, H25b), 6.94−7.01 (m, 2H; 1a, H11), 7.05−7.11 (m, 2H; H6, H10), 7.32−7.37 (m, 2H; H1b, H9), 7.55−7.68 (m, 5H; H27, H28, H29, H32, H33), 7.77−7.84 (m, 3H; H12, H20a, H20b), 7.86−7.88 (d, $J = 10.2$, 1H; H21), 8.00−8.03 (app t, 1H; contains two isomeric signals of H31), 8.22−8.23 (d, $J = 8.48$, 0.5H; H34), 8.28−8.29 (d, $J = 7.68$, 0.5H; isomeric signal of H34), 9.16−9.38 (br, 1H; contains two isomeric signals of H24; not present in CD_3OD), 10.84 (s, 0.5H; H7; not present in CD_3OD), 10.87 (s, 0.5H; isomeric signal of H7). ^{13}C NMR (600 MHz, DMSO-d_6): δ 22.0 (C17a + C17b), 24.2 (C36a + C36b), 26.2 (C18a), 26.4 (C18b), 29.5 (C4a + C4b), 31.4 (C16a + C16b), 34.0 (C37a + C37b), 38.3 (C19a + C19b), 43.2 (C14a + C14b), 46.5 (C25a + C25b), 50.0 (C15), 58.4 (C23), 59.2 (C3), 60.2 (isomeric signal of C23), 109.7 (C5), 110.8 (isomeric signal of C5), 111.1 (C9), 118.0 (C11), 118.1 (C12), 118.7 (isomeric signal of C12), 120.7 (C10), 123.0 (C6), 123.3 (isomeric signal of C6), 123.6 (C34), 125.1 (C28), 126.1 (C32), 126.6 (C33), 127.1 (C13), 127.4 (C26), 128.4 (C31), 129.7 (isomeric signal of C31), 129.8 (C27), 129.8 (C29), 129.8 (isomeric signal of C33), 131.5 (C35), 133.2 (C30), 158.1 (C38), 165.1 (C22), 171.1 (C2). HRMS (MALDI) exact mass calcd for $C_{33}H_{40}N_6O_3SNa$ 623.2781 (MNa^+). Found 623.2773.

1p-B: Yield: 2 mg (0.8%). HPLC purity > 95%. R_t (A) = 11.23(96). MALDI-TOF MS: mass calcd for $C_{33}H_{42}N_5O_4S$, 602.27 (MH^+). Found 602.23, 624.23 (MNa^+).

1p-C: Yield: 6 mg (2.5%). HPLC purity > 98%. R_t (A) = 11.84(98). HRMS (MALDI): exact mass calcd for $C_{33}H_{41}N_5O_4SNa$, 624.2615 (MNa^+). Found 624.2587.

Cell Culture and Transfection. Briefly, HEK-293 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum and seeded 1 day prior to transfection at 1 to 2 × 10^6 cell/100-mm dish. Melanocortin receptor DNA in the pCDNA$_3$ expression vector (20 μg) was transfected using the calcium phosphate method. Stable receptor populations were generated using G418 selection (1 mg/mL) for subsequent bioassay analysis.

***β*-Galactosidase Bioassay.** HEK-293 cells stably expressing the melanocortin receptors were transfected with 4 μg of CRE/β-galactosidase reporter gene as previously described.[10,60] Briefly, 5000−15 000 posttransfection cells were plated into 96-well Primera plates (Falcon) and incubated overnight. Forty-eight hours posttransfection, the cells were stimulated with 100 μL of peptide (10^{-4}−10^{-12} M) or forskolin (10^{-4} M) control in assay medium (DMEM containing 0.1 mg/mL BSA and 0.1 mM isobutylmethylxanthine) for 6 h. The assay media was aspirated, and 50 μL of lysis buffer (250 mM Tris-HCl pH = 8.0 and 0.1% Triton X-100) was added. The plates were stored at −80 °C overnight. The plates containing the cell lysates were thawed the following day. Aliquots of 10 μL were taken from each well and transferred to another 96-well plate for relative protein determination. To the cell lysate plates was added 40 μL of phosphate-buffered saline with 0.5% BSA to each well. Subsequently, 150 μL of substrate buffer (60 mM sodium phosphate, 1 mM $MgCl_2$, 10 mM KCl, 5 mM β-mercaptoethanol, 200 mg of ONPG) was added to each well, and the plates were incubated at 37 °C. The sample absorbance, OD_{405}, was measured using a 96-well plate reader (Molecular Devices). The relative protein was determined by adding 200 μL of 1:5 dilution Bio Rad G250 protein dye:water to the 10 μL cell lysate sample taken previously, and the OD_{595} was measured on a 96-well plate reader (Molecular Devices). Data points were normalized to both the relative protein content and the nonreceptor dependent forskolin stimulation.

Data Analysis. EC_{50} values represent the mean of duplicate experiments performed in quadruplet, or more independent experiments. EC_{50} estimates and their associated standard errors were determined by fitting the data to a nonlinear least-squares analysis using the PRISM program (v3.0, GraphPad Inc.).

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Supporting Information Available: RP-HPLC chromatograms of compounds **1a−p**, 600 MHz ^{1}H NMR spectra of **1p-A**, and a figure showing agonist pharmacological analysis of purified **1f** and **1n** at the melanocortin receptor subtypes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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