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Synthesis and structure–activity relationships of novel dipeptides and reduced dipeptides as ligands for melanocortin subtype-4 receptor

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Dedicated to the memory of David W. Robertson in recognition of his outstanding accomplishments in drug discovery and research.

Abstract—A series of benzylic piperazines (e.g., **4** and **5**) attached to an 'address element', the dipeptide H-D-Tic-D-*p*-Cl-Phe-OH, **3** has been identified as ligands for the melanocortin subtype-4 receptor (MC4R). We describe herein the structure–activity relationship (SAR) studies on the N-terminal residue of the 'address element'. Several novel dipeptides and reduced dipeptides with high MC4R binding affinities and selectivity emerged from this SAR study.

The five melanocortin receptors (MCR) form a subfamily of G-protein coupled receptors (GPCRs).[1,2] The melanocortins [e.g., adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), β-MSH, and γ-MSH] are agonist peptide ligands for these receptors and they are derived from post-translational processing of proopiomelanocortin (POMC).[3] The melanocortins and their corresponding receptors support diverse physiological functions including feeding behavior and erectile function.[4–7]

Selective small molecule melanocortin agonists have been reported by several research groups.[7,8] We have recently reported the identification of a series of novel aryl and benzylic piperazines (e.g., **1** and **2**), which have potent and selective MC4R agonist activity.[9,10] These molecules can be described as being composed of a GPCR privileged structure coupled to a dipeptide 'address element' as shown in Figure 1. We have reported structure–activity relationships regarding the privileged structures utilizing a fixed H-D-Tic-D-*p*-Cl-Phe-OH dipeptide address element.[9,10] While we have shown that chemical modifications of the privileged structure can provide significant dynamic range of biological activities for these series, we wondered if modification of the address element would provide additional opportunities for increases in potency and modifications of overall physicochemical properties.[11] We herein report an effort to develop new address elements focusing on the replacements of the N-terminal residue.

Syntheses of N-terminal residues derived from (2,3-dihydro-1*H*-isoindol-1-yl)-acetic acid are described in Scheme 1. Commercially available 2-bromobenzylamine hydrochloride was protected with Boc_2O to give intermediate **7**, which was then subjected to a Heck reaction with methyl acrylate in the presence of TEA and a catalytic amount of dichlorobis (triphenylphosphine) palladium (II) to yield **8**. Deprotection of **8** allowed the

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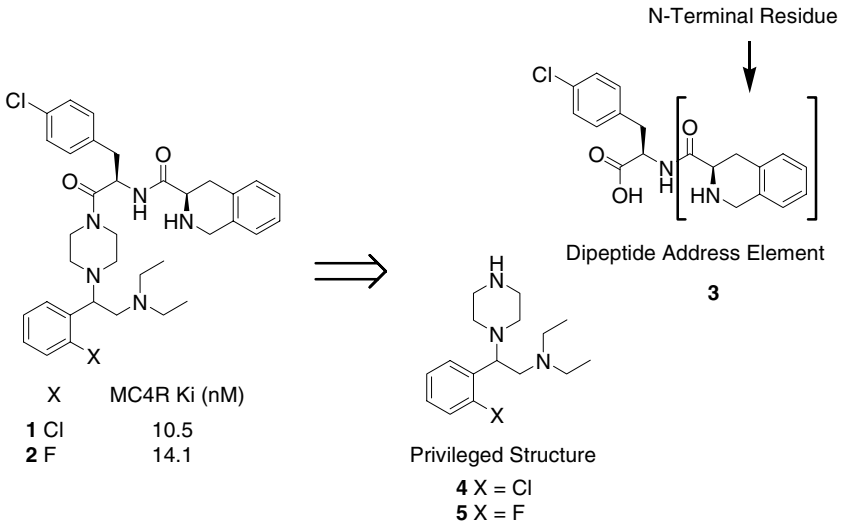
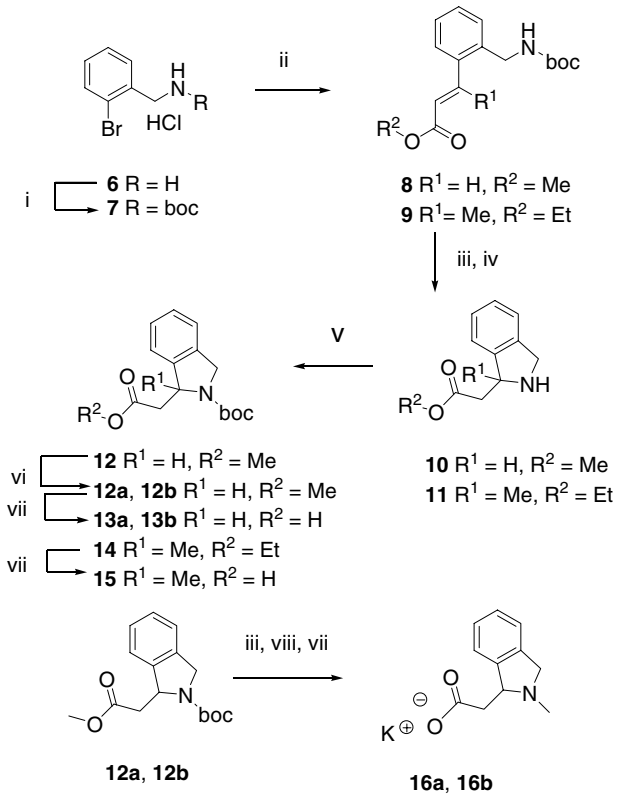


Figure 1. Disconnective analysis of MC4R active leads.

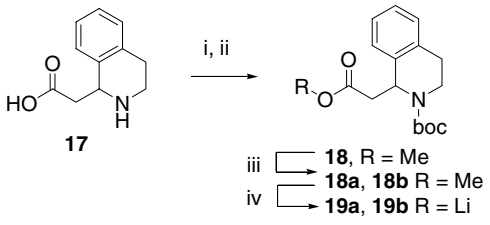


Scheme 1. Reagents: (i) K_2CO_3, *t*-Boc_2O, THF/H_2O (1:1); (ii) $CH_2CHCOOMe$ (for **8**) or $CH_3CHCHCOOEt$ (for **9**), TEA, $(Ph_3P)_2PdCl_2$, DMF; (iii) TFA, CH_2Cl_2; (iv) $NaHCO_3$, CH_2Cl_2; (v) DIEA, *t*-Boc_2O, CH_2Cl_2; (vi) Chiralcel OD column; (vii) 1 N NaOH, MeOH or $KOSi(Me)_3$, THF; (viii) 37% aq HCOH, $NaB(OAc)_3H$, CH_2Cl_2.

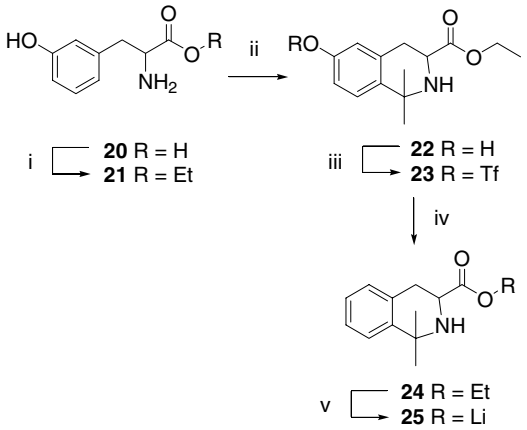
compound to undergo an intramolecular Michael addition affording isoindoline **10** as a racemic mixture, which was then protected with Boc_2O providing **12**. The racemic mixture was resolved efficiently by chiral chromatography with a Chiralcel OD column (elution: 10/90, IPA/heptane) to give enantiomers **12a** (isomer 1, first eluting isomer; 100% ee) and **12b** (isomer 2, second eluting isomer; 99.0% ee). The absolute configuration of **12a** or **12b** was not elucidated; rather their identity was tracked by their order of elution. Both enantiomers **12a** and **12b** were hydrolyzed with NaOH providing **13a** and **13b**, respectively. Alternatively, enantiomers **12a** and **12b** were each converted to the N-methyl derivatives **16a** (isomer 1) and **16b** (isomer 2), respectively, by deprotection, reductive amination with formaldehyde, and hydrolysis.

An example of a 1-methyl substituted isoindoline was prepared in an analogous fashion starting from **7** and ethyl crotonate. The racemic isoindoline **15** was then coupled to D-*p*-Cl-Phe-OMe (**26**) as shown in Scheme 4. The resulting diastereomers were deprotected and separated by reversed-phase HPLC (elution: 90/10 to 65/35, H_2O/CH_3CN over 30 min) to give **27a** (isomer 1, first eluting isomer) and **27b** (isomer 2, second eluting isomer). The diastereomers **27a** and **27b** were each protected and hydrolyzed to **28a** and **28b**, respectively.

Preparation of N-terminal residues containing a tetrahydroisoquinoline moiety started from commercially available (1,2,3,4-tetrahydroisoquinolin-1-yl)-acetic acid **17** as outlined in Scheme 2. Ester formation and nitrogen protection afforded **18** in good yield. This material was separated into enantiomers **18a** (isomer 1, first eluting isomer; 100% ee) and **18b** (isomer 2, second eluting isomer; 100% ee) on a Chiralcel OD column (elution: 20/80, IPA/heptane). Each enantiomer was then hydrolyzed with LiOH to give **19a** and **19b**, respectively.



Scheme 2. Reagents: (i) HCl, MeOH; (ii) *t*-Boc_2O, THF; (iii) Chiralcel OD column; (iv) LiOH, 1,4-dioxane/H_2O.

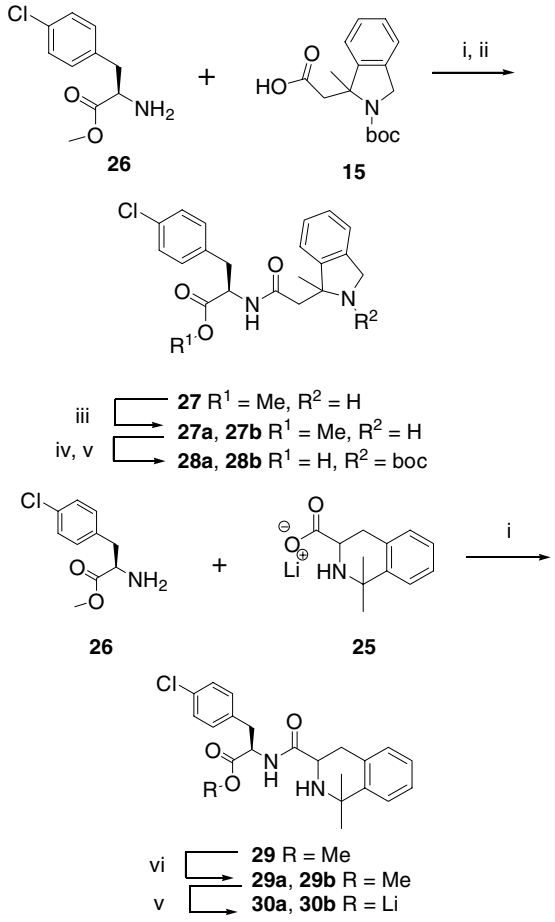


Scheme 3. Reagents: (i) $SOCl_2$, EtOH; (ii) acetone; (iii) Tf_2O, TEA, CH_2Cl_2; (iv) H_2, 5% Pd/C, acetone/toluene; (v) LiOH, H_2O, THF.

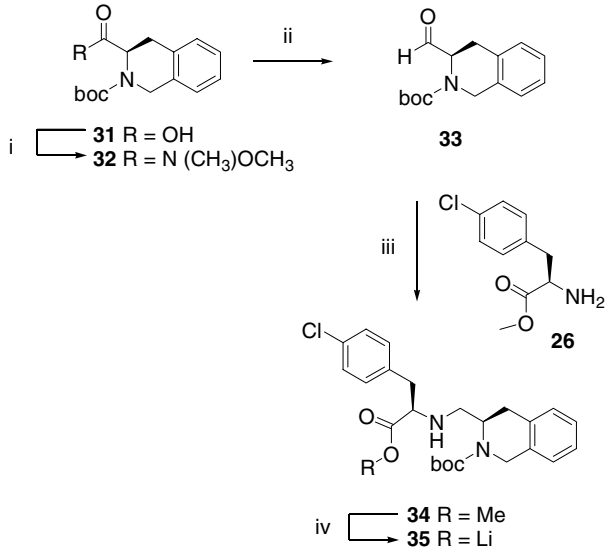
A 1,1-dimethyl substituted tetrahydroisoquinoline was prepared as outlined in Scheme 3. Esterification of *m*-tyrosine **20** in the presence of thionyl chloride and ethanol gave the ester **21**, which was heated under reflux in acetone to generate intermediate **22**. The phenol was converted to the corresponding triflate **23** with triflic anhydride. Deoxygenation of this intermediate was achieved catalytically with hydrogen in the presence of 5% Pd/C at 50 psi affording the desired intermediate **24**. Hydrolysis of **24** with LiOH gave the lithium salt **25**. The racemic mixture **25** was then coupled to D-*p*-Cl-Phe-OMe (**26**). The resulting diastereomers **29** were separated by flash chromatography (linear gradient, 40 ml/min 10–50% EtOAc/hexane for 25 min and 50% EtOAc/hexane for 7 min) providing **29a** (isomer 1, first eluting isomer) and **29b** (isomer 2, second eluting isomer) as outlined in Scheme 4. Notable is the fact that the nitrogen contained in these molecules is sufficiently hindered as to preclude acylation under a variety of standard conditions. Diastereomers **29a** and **29b** were each hydrolyzed to **30a** and **30b**, respectively.

We also prepared an address element with an amine linkage between the N-terminal residue and the D-*p*-Cl-Phe instead of an amide linkage (Scheme 5). Commercially available D-*N*-Boc-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid **31** was converted to the Weinreb amide **32** in the presence of EDC–HOBT. Lithium aluminum hydride reduction gave the aldehyde **33**, which was then coupled to D-*p*-Cl-Phe-OMe (**26**) by reductive amination. Hydrolysis of **34** with lithium hydroxide provided the lithium salt **35**, which was used in the final peptide coupling without further protection of the amino group.

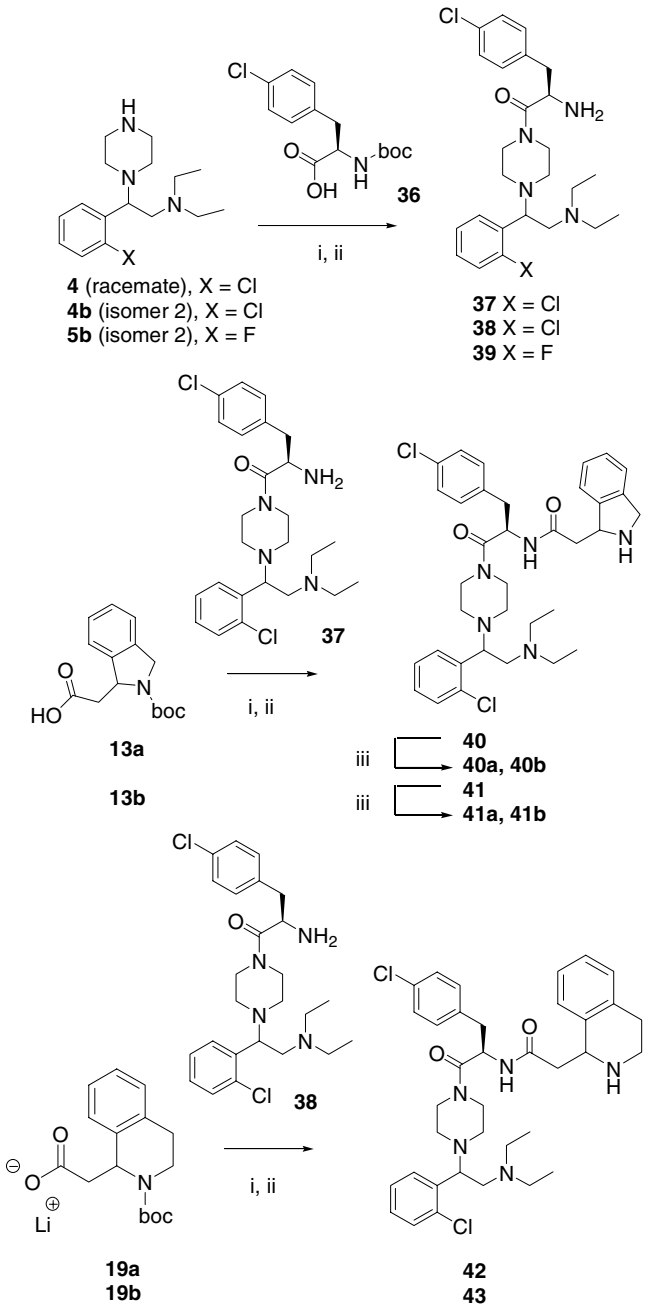
Since the resolved *o*-chloro and *o*-fluoro substituted privileged structures **4** and **5** provided compounds with good MC4R binding affinity (Fig. 1), they were chosen for use in this report.[10,12] Final compounds were prepared as described in Scheme 6. The racemic mixture **4** was coupled with Boc-D-*p*-Cl-Phe-OH **36** and deprotected to give a mixture of diastereomers **37**, which was further coupled with **13a** providing **40**. Reversed-phase



Scheme 4. Reagents: (i) EDC, HOBT; (ii) TFA, CH_2Cl_2; (iii) HPLC; (iv) *t*-Boc_2O, CH_2Cl_2; (v) LiOH, THF, H_2O or NaOH, MeOH; (vi) flash chromatography.



Scheme 5. Reagents: (i) NH(Me)(OMe)·HCl, EDC, HOBT, DIEA, THF; (ii) $LiAlH_4$, THF; (iii) $NaBH_3CN$, NaOAc, HOAc, MeOH; (iv) LiOH, H_2O/1,4-dioxane.



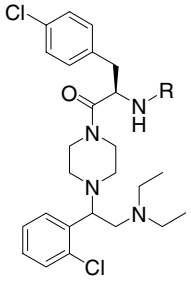
Scheme 6. Reagents: (i) EDC, HOBT; (ii) TFA, CH_2Cl_2; (iii) HPLC.

HPLC separation of **40** (elution: 90/10 to 70/30, H_2O/CH_3CN, 96 min) gave **40a** (isomer 1, first eluting isomer) and **40b** (isomer 2, second eluting isomer). Similarly, compounds **41a** (isomer 1, first eluting isomer) and **41b** (isomer 2, second eluting isomer) were prepared from **13b** and **37**. Intermediate **38**, generated from enantiomer **4b** (isomer 2)[12], was coupled to **19a** and **19b** separately to provide **42** and **43**, respectively. The rest of the final compounds were prepared using EDC/HOBT or HATU coupling and then TFA deprotection conditions. Thus, **13b** and **39** gave **44**; **16b** and **39** gave **45**; **19a** and **39** gave **46**; **39** and commercially available isoquinoline-3-carboxylic acid gave **47**; **28a** and **5b** (isomer 2) gave **48**; **30b** and **5b** gave **49**; **35** and **5** (racemate) gave **50**.

The final compounds either as TFA salt or HCl salt were tested in a radioligand binding assay to determine the competitive inhibition of ^{125}I-NDP-α-MSH binding to cloned human MC1, MC3, MC4, and MC5 receptors using membranes from stably transfected HEK293 cells.[9] The specific data obtained from the MC4R binding assay are listed in Tables 1–3 as well as Figure 1.

We first chose to examine N-terminal modifications in the *o*-Cl series **4** (Table 1). Replacement of the N-terminal tetrahydroisoquinoline with an isomeric isoindoline provided diastereomeric pairs **40a**–**b** (178, 18 nM) and **41a**–**b** (107, 5 nM). Consistent with previous observations, we found activity differences between privileged structure antipodes.[10] Comparing pairs **40a** with **40b** and **41a** with **41b** demonstrates a 9- and 21-fold difference in affinity, respectively. It appears that the impact on affinity of the N-terminal residue absolute configuration was not as significant, evidenced by comparing **40b** (18 nM) with **41b** (5 nM). We also observed similar

Table 1. Chiral optimization on the N-terminal residue and privileged structure



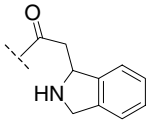
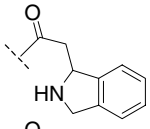
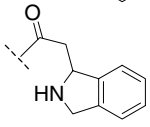
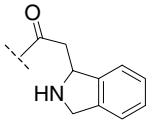
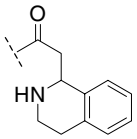
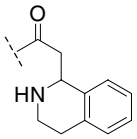
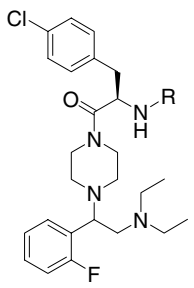
Compound	R	MC4R K_i (nM)[13]
40a		177.8
40b		18.1
41a		107.4
41b		5.3
42		30.2
43		6.4

Table 2. SAR of the N-terminal residue



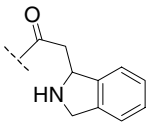
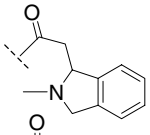
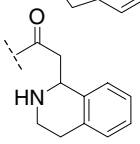
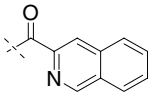
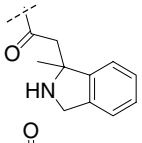
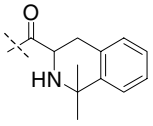
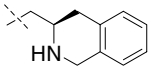
Compound	R	MC4R K_i (nM)[13]
44		7.3
45		17.3
46		10.4
47		95.6
48		73.7
49		5.8
50		40.6

Table 3. Binding affinity at hMC1R, hMC3R, hMC4R, and hMC5R[13]

Compound	MC1R K_i (μM)	MC3R K_i (μM)	MC4R K_i (μM)	MC5R K_i (μM)
44	6.0	3.4	0.0073	1.2
45	8.6	4.6	0.017	2.0
46	3.0	4.2	0.010	0.91
47	7.0	1.4	0.096	0.92
48	20	16	0.074	1.4
49	7.0	1.9	0.0058	0.42
50	5.8	7.0	0.041	0.95

results with the 1-substituted tetrahydroisoquinoline acetic acid N-terminus, which provided analogs **42** (30 nM) and **43** (6 nM). The above data suggest that each pair of N-terminal enantiomers provided final compounds with comparable MC4R binding affinity.

Further examination of the address element SAR using the *o*-fluoro substituted privileged structure **5** is described in Table 2. Only the most active diastereomers (in terms of affinity) are listed. Replacements of tetrahydroisoquinoline carboxylic acid with (2,3-dihydro-1*H*-isoindol-1-yl)-acetic acid and (1,2,3,4-tetrahydro-isoquinolin-1-yl)-acetic acid again proved to be successful, yielding compounds **44** (7 nM) and **46** (10 nM). α-Methylation of the isoindoline analog (**48**) resulted in a 10-fold loss of MC4R binding affinity relative to **44**, which may indicate that steric bulk in this position is not well tolerated. N-Methylation of (2,3-dihydro-1*H*-isoindol-1-yl)-acetic acid (**45**) is tolerated providing an MC4R affinity of 17 nM, which is comparable to that of the original tetrahydroisoquinoline analog **2**. Compound **49**, possessing 1,1-dimethyl substitution on the tetrahydroisoquinoline carboxylate residue, had good MC4R binding affinity (6 nM). Replacing the tetrahydroisoquinoline moiety of **2** with isoquinoline gave compound **47**, which had reduced MC4R binding affinity (96 nM). Reducing the amide linker of the dipeptide address element found in **2** to the amine yielded **50**, which showed 40 nM binding affinity for MC4R.

All of the compounds listed in Table 2 showed good to excellent selectivity for MC4R versus MC1R, MC3R, and MC5R ranging from 10- to 1280-fold (Table 3).

In summary, we have demonstrated that it is possible to synthesize a variety of address elements containing a basic nitrogen. Replacement of the N-terminal residue with the less basic isoquinoline moiety caused an erosion of activity, which may indicate that the basic center provides a positive interaction. Interestingly, the amide linker contained in the dipeptide address element of **3** may not be required for activity as demonstrated by compound **50**. Several pairs of N-terminal enantiomers generated final compounds with comparable MC4R binding affinity, which provided the flexibility of using both *R* and *S* enantiomers and it may provide more opportunities to change physicochemical properties of the final compounds. Identification of these additional address elements, each with their own unique physicochemical properties, should facilitate the optimization of our privileged structure based melanocortin ligands.

Acknowledgments

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11. For a related strategy, see Fotsch, C.; Han, N.; Arasasingham, P.; Bo, U.; Carmouche, M.; Chen, N.; Davis, J.; Goldberg, M. H.; Hale, C.; Hsieh, F.; Kelly, M. G.; liu, Q.; Norman, M. H.; Smith, D. M.; Stec, M.; Tamayo, N.; Xi, N.; Xu, S.; Bannon, A. W.; Baumgartner, J. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1623.
12. Compounds **1** and **2** are enantiomerically pure. The chiral chromatographic resolution of the chloro analog **4** (giving **4a** and **4b**) was accomplished with the same solvent system as reported for fluoro analog **5** in Ref. 10. The order of elution previously reported for **5** was in error with the more active enantiomer **5b** being the second eluting isomer, not the first (**5a**) as reported.
13. All of K_i values reported here are an average from two or four runs. Average error of the binding assay is 15%.