Peptoid NPhe⁴ in AGRP-Based c[Pro¹-Arg²-Phe³-Phe⁴-Xxx⁵-Ala⁶-Phe⁷-DPro⁸] Scaffolds Maintain Mouse MC4R Antagonist Potency

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ABSTRACT: The melanocortin receptors are involved in numerous physiological functions and are regulated by agonists derived from the proopiomelanocortin gene transcript and two endogenous antagonists, agouti and agouti-related protein (AGRP). The key binding and functional determinant of AGRP, an MC3R and MC4R antagonist, is an Arg-Phe-Phe tripeptide sequence located on an exposed hexapeptide (Arg-Phe-Phe-Asn-Ala-Phe) loop. It has previously been observed that cyclizing this sequence through a DPro-Pro motif (c[Pro¹-Arg²-Phe³-Phe⁴-Asn⁵-Ala⁶-Phe⁷-DPro⁸]) resulted in a macrocyclic scaffold with MC4R antagonist activity, with increased MC4R potency when a diaminopropionic acid (Dap) residue is substituted at position 5. In this report, a series of 11 single-peptoid substitutions were performed in the AGRP-derived macrocycles. While most peptoid substitutions decreased MC4R antagonist potency, it was observed that NPhe⁴ (compounds 4 and 11) or NDb⁵ (diaminobutyric acid, compound 7) maintained MC4R antagonist potency. The NPhe⁴ substitutions also resulted in MCSR antagonist and inverse agonist activity equipotent to the parent scaffolds. These data may be used in the design of future MC4R and MCSR antagonist leads and probes that possess increased metabolic stability due to the presence of peptoid residues.

KEYWORDS: Melanocortin receptors, peptoid, AGRP, macrocycle

The melanocortin system consists of five receptors^{1–8} known to date from the GPCR superfamily, agonists including ACTH as well as α -, β -, and γ -MSH derived from the proopiomelanocortin gene transcript,⁹ and antagonists agouti and agouti-related protein (AGRP).^{10–12} The melanocortin-3 and -4 receptors (MC3R and MC4R) play a central role in the regulation of appetite, weight, and energy homeostasis.^{13–17} Since administration of MC3R and MC4R antagonist molecules increases food intake in rodents,^{16–18} developing MC3R and MC4R antagonist ligands may lead to new therapeutic lead compounds for the treatment of negative energy disorders. Since AGRP is a naturally occurring nanomolar to subnanomolar potent MC3R and MC4R antagonist (see SI Table 1 for reported inverse agonist activities),^{11,19} probes derived from AGRP may be useful in developing compounds that increase appetite to treat conditions including cachexia and anorexia.

Although potent, the relatively large size (50 residues) of the *in vivo* active form of AGRP²⁰ may limit its ability to be optimized as a therapeutic lead. An Arg-Phe-Phe tripeptide sequence within AGRP has been identified to be critical for receptor binding.²¹ Structural NMR studies performed on AGRP or a 34-residue “mini-AGRP” suggested this tripeptide sequence was part of an exposed β -hairpin loop composed of

the hexapeptide sequence Arg-Phe-Phe-Asn-Ala-Phe.^{22–24} While many AGRP truncation studies led to ligands with decreased antagonist potencies, a scaffold consisting of the active-loop hexapeptide cyclized head-to-tail through a DPro-Pro motif (c[Pro¹-Arg²-Phe³-Phe⁴-Asn⁵-Ala⁶-Phe⁷-DPro⁸]) resulted in a ligand that was 50-fold less potent than AGRP at the MC4R.²⁵ Further structure–activity relationship studies demonstrated that replacement of the Asn with a diaminopropionic (Dap) residue resulted in an octapeptide macrocycle (c[Pro¹-Arg²-Phe³-Phe⁴-Dap⁵-Ala⁶-Phe⁷-DPro⁸]) that was equipotent to AGRP at the MC4R.^{25,26} Setmelanotide, a cyclic octapeptide melanocortin agonist ligand [Ac-Arg-c(Cys-DAla-His-DPhe-Arg-Trp-Cys)-NH₂] has been reported to decrease self-reported hunger scores and decrease body weight in select patient populations,^{27,28} suggesting melanocortin

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Figure 1. Structures of the peptoid residues used in this study.

Table 1. Analytical Data for Peptides Synthesized in this Study

Peptide	Sequence	Retention Time (min) ^a		M (calc)	mass spectral analysis (M + 1)	Purity %
		System 1	System 2			
1	c[Pro-Arg-Phe-Phe-Dap-Ala-Phe-DPro]	17.5	28.5	948.5	949.4	>98%
2	c[Pro-NArg-Phe-Phe-Dap-Ala-Phe-DPro]	16.1	24.9	948.5	949.4	>96%
3	c[Pro-Arg-NPhe-Phe-Dap-Ala-Phe-DPro]	16.4	25.6	948.5	949.6	>99%
4	c[Pro-Arg-Phe-NPhe-Dap-Ala-Phe-DPro]	17.8	27.2	948.5	949.6	>97%
5	c[Pro-Arg-Phe-Phe-Dap-NAla-Phe-DPro]	16.5	25.9	948.5	949.5	>99%
6	c[Pro-Arg-Phe-Phe-Dap-Ala-NPhe-DPro]	17.1	27.0	948.5	949.5	>99%
7	c[Pro-Arg-Phe-Phe-NDab-Ala-Phe-DPro]	17.7	26.4	962.5	963.7	>97%
9	c[Pro-NArg-Phe-Phe-Asn-Ala-Phe-DPro]	16.9	26.8	976.5	977.6	>98%
10	c[Pro-Arg-NPhe-Phe-Asn-Ala-Phe-DPro]	17.5	27.1	976.5	977.7	>99%
11	c[Pro-Arg-Phe-NPhe-Asn-Ala-Phe-DPro]	19.5	28.8	976.5	977.6	>99%
12	c[Pro-Arg-Phe-Phe-Asn-NAla-Phe-DPro]	18.6	28.3	976.5	977.7	>97%
13	c[Pro-Arg-Phe-Phe-Asn-Ala-NPhe-DPro]	17.8	27.4	976.5	977.7	>99%

^aPeptide retention times (min) are reported for solvent system 1 (10% acetonitrile in 0.1% trifluoroacetic acid/water and a gradient to 90% acetonitrile over 35 min) and solvent system 2 (10% methanol in 0.1% trifluoroacetic acid/water and a gradient to 90% methanol over 35 min). An analytical Vydac C18 column (Vydac 218TP104) was used with a flow rate of 1.5 mL/min. The peptide purity was determined by HPLC at a wavelength of 214 nm.

peptide ligands of this size may be optimized for therapeutic effects *in vivo*.

One strategy to improve the stability of peptide ligands is to replace amino acids with peptoid residues, in which an amino acid side chain is appended to the nitrogen atom of a glycine residue instead of the α -carbon. Pioneered by Zuckermann,^{29,30} such oligo-N-substituted glycine chains have been demonstrated to possess increased proteolytic stability compared to the corresponding all-L amino acid peptides.³¹ Melanocortin scaffolds with peptoid substitutions have been reported to possess nanomolar agonist activity (Ac-Gly-Phe-NPhe-Arg-Trp-Gly-NH₂ at the MC4R)³² and subnanomolar binding affinity (Ac-c[Cys-His-DPhe-Cys]-N^α-guanidinybutyl-Trp-NH₂ at the MC4R),³³ although a general trend for decreased agonist activity has been reported for peptoid scans of the melanocortin agonist tetrapeptide Ac-His-DPhe-Arg-Trp-NH₂³⁴ or heptapeptide Ac-Nle-Gly-Lys-DPhe-Arg-Trp-Gly-NH₂³⁵ sequences. Work with a tripeptoid scaffold based upon the Arg-Phe-Phe active sequence of AGRP yielded a compound with micromolar binding affinity that was able to dose-dependently decrease α -MSH cAMP production at the MC4R using micromolar concentrations, a 1000-fold decrease compared to AGRP.³⁶

While peptoid substitutions in melanocortin ligands have trended toward decreased potency, with noted exceptions, it has been observed that incorporation of peptoid residues into a protegrin I antibiotic peptide permitted the peptide–peptoid

ligand cyclized head-to-tail through a DPro-Pro motif to adopt a regular β -hairpin conformation.³⁷ Due to an observed β -hairpin conformation observed in the NMR structures of AGRP,^{22–24} it was hypothesized that incorporation of peptoid residues into the AGRP octapeptide macrocyclic scaffold cyclized through a DPro-Pro motif might modulate potency. Using commercially available Fmoc-NPhe, Fmoc-NArg, and Fmoc-NAla (Fmoc-sarcosine) amino acids (Figure 1), single peptoid substitutions were investigated in the c[Pro¹-Arg²-Phe³-Phe⁴-Dap⁵-Ala⁶-Phe⁷-DPro⁸] scaffold. To investigate the Dap position, an Fmoc-NDab (diaminobutyric acid, one methylene unit longer compared to Dap) residue was incorporated. Comparable peptoid insertions were also made in the c[Pro¹-Arg²-Phe³-Phe⁴-Asn⁵-Ala⁶-Phe⁷-DPro⁸] scaffold to investigate how peptoid substitutions affected a similar set of compounds.

Peptides were synthesized using standard Fmoc techniques,^{38,39} as previously described.^{25,26,40,41} Due to incomplete coupling following the addition of the Fmoc-NPhe residue at select positions, the synthetic procedure was modified as noted in the experimental section. Peptides were purified to greater than 95% purity in two diverse solvent systems (Table 1), and the correct molecular weight was determined by ESI-MS or MALDI-MS (University of Minnesota Mass Spectrometry Lab). Purified cyclic peptides were dissolved in DMSO (10^{−2} M) for pharmacological characterization at the mouse MC1R, MC3R, MC4R, and MC5R using the AlphaScreen cAMP

Table 2. Pharmacology of AGRP Macrocylic Analogues at the Mouse Melanocortin Receptors^a

Peptide	Sequence	mMC1R		mMC3R		mMC4R		mMCSR	
		EC ₅₀ (nM)	pA ₂	EC ₅₀ (nM)	pA ₂	EC ₅₀ (nM)	pA ₂	EC ₅₀ (nM)	pA ₂
NDP-MSH		0.010 ± 0.002		0.09 ± 0.01		0.9 ± 0.1		0.11 ± 0.01	
AGRP ^b	c[Pro-Arg-Phe-Phe-Dap-Ala-Phe-DPro]	30% @ 100 μM	8.7 ± 0.1	20% @ 100 μM	8.7 ± 0.1	>100,000	8.6 ± 0.1	Inverse Agonist –10%	7.5 ± 0.1
	c[Pro-NArg-Phe-Phe-Dap-Ala-Phe-DPro]	>100,000	6.5 ± 0.3	>100,000	6.5 ± 0.3	>100,000	5.5 ± 0.2*	>100,000	
	c[Pro-Arg-Phe-Phe-Dap-Ala-Phe-DPro]	Partial Agonist 80% NDP-MSH 110 ± 50	6.9 ± 0.3	>100,000	6.9 ± 0.3	>100,000	7.5 ± 0.1*	Partial Agonist 70% NDP-MSH 550 ± 40	
	c[Pro-Arg-Phe-NPhe-Dap-Ala-Phe-DPro]	Partial Agonist 60% NDP-MSH 2,700 ± 700	7.1 ± 0.1	>100,000	7.1 ± 0.1	>100,000	9.0 ± 0.1*	Inverse Agonist –10%	7.4 ± 0.1
	c[Pro-Arg-Phe-Phe-Dap-NAla-Phe-DPro]	>100,000	5.6 ± 0.1*	>100,000	5.6 ± 0.1*	>100,000	6.1 ± 0.1*	>100,000	
	c[Pro-Arg-Phe-Phe-Dap-Ala-NPhe-DPro]	>100,000	5.5 ± 0.2*	>100,000	5.5 ± 0.2*	>100,000	6.6 ± 0.1*	Inverse Agonist –10%	
	c[Pro-Arg-Phe-Phe-NDab-Ala-Phe-DPro]	60% @ 100 μM	6.9 ± 0.1	>100,000	6.9 ± 0.1	>100,000	8.4 ± 0.2	Inverse Agonist –10%	
Asn ^c	c[Pro-Arg-Phe-Phe-Asn-Ala-Phe-DPro]	25% @ 100 μM	6.3 ± 0.1	>100,000	6.3 ± 0.1	>100,000	8.2 ± 0.1	Inverse Agonist –10%	6.4 ± 0.2
	c[Pro-NArg-Phe-Phe-Asn-Ala-Phe-DPro]	Partial Agonist 50% NDP-MSH 3,100 ± 300	<5.5	>100,000	<5.5	>100,000	<5.5	>100,000	
	c[Pro-Arg-NPhe-Phe-Asn-Ala-Phe-DPro]	240 ± 60	5.8 ± 0.1	>100,000	5.8 ± 0.1	>100,000	<5.5	Inverse Agonist –10% @ 100 μM	
	c[Pro-Arg-Phe-NPhe-Asn-Ala-Phe-DPro]	Partial Agonist 70% NDP-MSH 5,600 ± 200	6.0 ± 0.1	>100,000	6.0 ± 0.1	>100,000	8.4 ± 0.1	Inverse Agonist –10%	6.1 ± 0.1
	c[Pro-Arg-Phe-Phe-Asn-NAla-Phe-DPro]	1,000 ± 200	<5.5	>100,000	<5.5	>100,000	<5.5	>100,000	
	c[Pro-Arg-Phe-Phe-Asn-Ala-NPhe-DPro]	85% @ 100 μM	<5.5	60% @ 100 μM	<5.5	>100,000	<5.5	>100,000	

^aThe indicated errors represent the standard error of the mean determined from at least three independent experiments. The use of >100,000 indicates that the compound was examined but lacked agonist activity at up to 100 μM concentrations. A percentage denotes the percent maximal stimulatory response observed at 100 μM concentrations but not enough stimulation was observed to determine an EC₅₀ value. Partial agonist indicates partial agonist activity was observed, along with the percentage of activation relative to NDP-MSH and the EC₅₀ (compounds were considered full agonist if >90% maximal NDP-MSH signal was observed). Inverse agonist indicates that inverse agonist pharmacology was observed with the percent decrease from basal indicated. For inverse agonists, if a decrease in cAMP signal was observed without a sigmoidal dose–response curve, the percent change from basal at 100 μM concentrations is indicated. The antagonistic pA₂ values were determined using the Schild analysis and the agonist NDP-MSH. The use of <5.5 indicates that no antagonist potency was observed in the highest concentration ranged assayed (10,000, 5,000, 1,000, and 500 nM). Statistical analysis was performed using a student *t* test compared to the respective controls 1 (Dap⁵) and 8 (Asn⁵) receptor values with **p* < 0.05. ^bThe pharmacology for AGRP and macrocyclic peptide 8 has previously been reported and is included for reference.^{26,45}

Figure 2. Illustration of the agonist pharmacology of NDP-MSH, 1, 3, 4, and 10 at the mMC1R and mMC5R. The black box indicates the observed inverse agonist activity at the mMC5R.

assay. Peptides were assayed for agonist activity at all receptors. Compounds that did not possess agonist activity at the MC3R and MC4R were then assayed for antagonist activity using a Schild assay paradigm⁴² and the synthetic melanocortin agonist NDP-MSH.⁴³ For compounds that appeared to possess inverse agonist activity at the MC5R (decrease signal from basal activity), the percent change from basal activity was recorded. Compounds that decreased basal activity at the highest concentration assayed but did not result in a sigmoidal dose–response curve were reported as the percent decrease from basal activity at 100 μ M. Since the AlphaScreen assay results in a loss of signal, activity curves were normalized to baseline and maximal NDP-MSH signal for illustrative purposes.

The lead macrocyclic peptide 1 (c[Pro¹-Arg²-Phe³-Phe⁴-Dap⁵-Ala⁶-Phe⁷-DPro⁸]) possessed antagonist activity at the MC3R, MC4R, and MC5R (pA_2 = 6.5, 8.6, and 7.5, respectively), possessed inverse agonist activity at the MC5R, and resulted in partial stimulation of the MC1R and MC3R at 100 μ M concentrations. These values are similar to the previously reported antagonist potencies (pA_2 values of 6.5–7.2, 8.7–8.9, and 7.3 at the MC3R, MC4R, and MC5R, respectively) for this peptide,^{26,44,45} and the inverse agonist pharmacology has previously been reported at the MC5R.^{26,45,46} While substitution of NArg², NAla⁶, and NPhe⁷ (2, 5, and 6, respectively) resulted in no agonist activity at the MC1R, MC3R, and MC4R, substitution of NPhe³ (3) or NPhe⁴ (4) generated ligands that partially stimulated the MC1R (Table 2, Figure 2 for 3). Peptoid residues in this scaffold generated MC3R antagonist potencies (pA_2 = 5.5–7.1) in a similar range to the lead compound 1 (pA_2 = 6.5). While four of the five peptoid substitutions (NArg², NPhe³, NAla⁶, and NPhe⁷) decreased MC4R antagonist potency greater than 10-fold compared to the lead 1 (pA_2 = 8.6), substitution of NPhe⁴ (4) resulted in a pA_2 value of 9.0, the most potent observed in this study (Figure 3). At the MC5R, two compounds did not possess agonist activity at the concentrations assayed (2 and 5), one compound partially stimulated the MC5R to 70% the maximal signal of NDP-MSH (3, Figure 2), and two compounds possessed inverse agonist activity at the MC5R (4 [Figures 2 and 3] and 6). Due to MC4R antagonist potency and the MC5R inverse agonist activity, 4 was also evaluated for antagonist activity at the MC5R using a Schild assay paradigm and was found to be an equipotent antagonist (pA_2 = 7.4, Figure 3) compared to the 1 lead ligand at the MC5R.

In order to evaluate if a peptoid residue would be tolerated at the fifth position, a commercially available NDab residue was inserted, yielding peptide–peptoid macrocyclic ligand 7.

Figure 3. Illustration of the antagonist pharmacology of 4 at the mMC3R, mMC4R, and mMC5R. The black box indicates the observed inverse agonist activity at the mMC5R.

This compound possessed similar pharmacology to the lead ligand (1), resulting in partial activation of the MC1R at the highest concentrations assayed, submicromolar antagonist potency at the MC3R (pA_2 = 6.9), nanomolar antagonist potency at the MC4R (pA_2 = 8.4), and inverse agonist activity at the MC5R (Table 2).

A similar set of peptoid substitutions using NArg, NPhe, and NAla (sarcosine) was also investigated in the c[Pro-Arg-Phe-Phe-Asn-Ala-Phe-DPro] scaffold (8 in Table 2). At the MC1R, two compounds resulted in full agonist activity (10 [Figure 1] and 12, with EC_{50} values of 240 and 1,000 nM, respectively), two compounds were partial agonists (9 and 11), and one compound resulted in partial activation of the receptor at the highest concentrations assayed (13). Two compounds possessed micromolar antagonist potencies at the MC3R (10

and 11), while three (9, 12, and 13) did not possess measurable MC3R antagonist activity in the concentrations assayed. One substitution, NPhe⁴ (11) possessed antagonist potency at the MC4R in the concentration range assayed (pA_2 = 8.4). The corresponding NPhe⁴ substitution in the Dap-scaffold (4) also resulted in the most potent MC4R antagonist compound in that scaffold. While three peptoid substitutions (9, 12, and 13) did not produce a response at the MC5R at up to 100 μ M concentrations, one compound produced a decreased response from basal at the 100 μ M concentrations (10, Figure 2), and another (11) produced an inverse agonist response comparable to the lead ligand (1). As a comparison to the NPhe⁴, Dap⁵ 4 peptide, the antagonist potency of NPhe⁴, Asn⁵ 11 was measure at the MC5R (Table 2). This compound possessed submicromolar antagonist potency at the MC5R (pA_2 = 6.1), 20-fold decreased potency compared to 4 but similar to the reported value for 8 (pA_2 = 6.4).⁴⁵

While past peptoid scans based upon agonist melanocortin sequences have resulted in decreased potencies,^{34,35} the present experiments demonstrate that positions 4 and 5 of the AGRP-derived macrocyclic scaffold c[Pro¹-Arg²-Phe³-Phe⁴-Xxx⁵-Ala⁶-Phe⁷-DPro⁸] may be substituted with peptoid residues and maintain MC4R antagonist potency compared to the lead sequence. Substitution of NPhe at the Phe⁴ resulted in the most potent MC4R antagonist assayed with Dap at position 5 (4). The equivalent NPhe⁴ substitution when Asn was at position 5 (11) was the only Asn⁵ peptide-peptoid macrocycle to possess antagonist potency at the MC4R and was equipotent to previous reports of the amino acid only form (reported pA_2 values at the MC4R range from 7.7 to 8.2).^{25,26,44} A previous report inverting the stereochemistry at this position in the macrocyclic scaffold, resulting in DPhe⁴, decreased antagonist potency greater than 10-fold.⁴¹ It therefore appears that the loss of stereochemistry alone does not result in the increased potency observed for the NPhe substitutions. Antagonist potency at the MC4R is also maintained when a hPhe (the Phe side chain is extended by one methylene unit) is inserted into position 4.^{25,44} The extra length of the side chain with a hPhe⁴ substitution may permit additional conformations of the side-chain that position the aromatic ring for more favorable interactions with the receptor. Shifting the side-chain from the α -carbon to the nitrogen may better position the NPhe⁴ aromatic ring in analogous conformations. When a Tic residue was utilized in the fourth position in the octapeptide scaffold possessing an Asn⁵, no antagonist activity was observed at the MC3R or MC4R with concentrations up to 10 μ M.²⁵ Since Tic is a constrained version of NPhe, this suggests conformational flexibility at the fourth position is important to maintain MC4R antagonist potency for N-substituted residues. Substitution of an NDab residue in position 5 (7) also maintained MC4R antagonist potency compared to the lead ligand 1. This supports prior studies that a charge is important at this position, although the length of side chain, stereochemistry, and distribution of the charge (amine versus guanidinium group) are less important than the charge.^{25,44} A computational model has suggested that this position of full length AGRP may be in close proximity to the Asp¹⁸⁹ residue of the MC4R,¹⁹ which may explain why a basic residue at this position is important for antagonist potency.

As previously observed for the macrocyclic scaffolds, a Dap substitution at the fifth position resulted in more potent MC4R antagonist potency compared to Asn.^{41,44} Peptoid replacement

at the Arg², Phe³, Ala⁶, or Phe⁷ position all resulted in no observed antagonist activity at the MC4R when Asn was in the fifth position, while micromolar to submicromolar antagonist potency was observed when Dap was in the fifth position. For the NPhe⁴ peptoid substitution, Dap in the fifth position remained more potent than Asn (pA_2 = 9.0 vs 8.4, respectively). These data support the previous observations that Dap increases MC4R antagonist potency and is useful to generate more potent MC4R antagonist ligands.

Unlike the observed activity at the MC4R, the two ligands that were observed to possess full MC1R agonist activity (10 and 12) had an Asn⁵. The equivalent Dap⁵ macrocycles with NPhe³ (3) and NAla⁶ (5) resulted in partial MC1R agonism (80% the maximal NDP-MSH signal) or no agonist activity at up to 100 μ M concentrations, respectively. A D-amino acid scan of the Arg-Phe-Phe tripeptide sequence did not result in a similar agonist activity pattern at the MC1R.⁴¹ This observed MC1R agonism may suggest a peptoid substitution pattern that may be exploited in generated selective, potent MC1R agonist ligands.

Similar to prior reports, inverse agonist activity was observed for select ligands at the MC5R.^{26,41,46} Due to the increased MC4R antagonist potency, three compounds were examined for antagonist potency using a Schild experimental design at the MC5R, the parent 1 and the NPhe⁴ containing 4 and 11. The parent 1 was previously reported to possess nanomolar MC5R antagonist potency (pA_2 = 7.3),⁴⁵ similar to the value observed in the present study (pA_2 = 7.5). The NPhe⁴, Dap⁵ 4 resulted in an equipotent MC5R antagonist (pA_2 = 7.4) to 1, indicating that peptoid substitution at this position was not detrimental to MC5R antagonism activity. A greater than 10-fold decrease in MC5R potency was observed comparing the parent 1 to the NPhe⁴, Asn⁵-containing 11, similar to the drop previously observed between the Dap and Asn containing scaffolds without peptoid substitution (pA_2 = 7.3 and 6.4, respectively).⁴⁵ These data suggest that the NPhe⁴ substitution does not negatively impact functional activity at the MC5R and may be useful in the future design of MC5R ligands that may be more enzymatically stable.

In conclusion, a series of single peptoid residues at Arg², Phe³, Phe⁴, Ala⁶, and Phe⁷ were substituted into macrocyclic peptide scaffolds possessing a Dap⁵ or Asn⁵ derived from the purported β -hairpin active loop of AGRP. Peptoid substitutions at the Arg², Phe³, Ala⁶, and Phe⁷ positions resulted in decreased MC4R antagonist potency, while equipotent activity was observed for NPhe⁴ substitution. Additionally, insertion of a basic peptoid residue (NDab) at position 5 also maintained MC4R antagonist activity, suggesting the fourth and fifth positions within the AGRP-derived macrocyclic scaffold are amenable to peptoid substitution. The NPhe⁴ substitutions also resulted in MC5R antagonist activity equipotent to the amino acid substituted macrocycles, suggesting the NPhe⁴ peptoid substitution was tolerated at the MC5R. These data may be useful in the future design of MC4R and MC5R antagonist ligands that may have increased enzymatic stability and serve as leads in the design of new probe molecules to interrogate the activity of the different melanocortin receptors *in vivo*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmchemlett.9b00641>.

Discussion of AGRP inverse agonism, protocols for peptide synthesis, biological assays, and data analysis. (PDF)

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Author Contributions

MDE and CHL designed the research, MDE synthesized the compounds, KTF performed the biological assays, KTF and MDE analyzed the data, and MDE wrote the manuscript with the assistance of CHL.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AGRP, agouti-related protein; MCR, melanocortin receptor; MSH, melanocyte stimulating hormone; POMC, proopiomelanocortin; RP-HPLC, reverse-phase high-pressure liquid chromatography

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