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Design and Pharmacology of Peptoids and Peptide–Peptoid Hybrids Based on the Melanocortin Agonists Core Tetrapeptide Sequence

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Abstract—A series of *N*-substituted glycine oligomers (peptoids) and peptide–peptoid hybrids were synthesized based on the Ac-His-Phe-Arg-Trp-NH_2 tetrapeptide template. The compounds were pharmacologically characterized at the mouse melanocortin receptors (MC1R, MC3R–MC5R) for agonist activity.

The melanocortin (MC) receptor system belongs to the G-protein coupled receptor (GPCR) superfamily and consist of five receptor isoforms identified to date, designated MC1R–MC5R, and both endogenous agonists and antagonists exists.[1] This receptor family appears to be involved in many important physiological activities, such as cognitive functions, adrenal function, hair and pigment coloration, sexual function, lipid production, and energy and weight homeostasis.[2] There is considerable interest in the MC3R and MC4R due to participation of these receptors in weight and energy homeostasis.[3–5] Melanocortin based agents will facilitate additional in vitro and in vivo characterization of this receptor family, and due to the association of this receptor system in a vast array of physiological functions, MC ligands may potentially find therapeutic application.

Proteins and peptides are involved in a variety of biological activities. The prevalence and diversity of peptides, along with their participation in crucial physiological functions, has sparked a renewed interest in using peptides and peptide derivatives as therapeutic agents.[6] Although peptides have been used extensively to characterize receptor systems, the development of peptides into pharmaceutical products is generally limited due to poor bioavailability. Considerable effort has gone into improving the pharmacological properties of peptides, mainly by increasing enzymatic stability while preserving the chemical moieties required for functional activity.[6,7]

Peptoids represent a class of oligomeric compounds that closely mimic the natural structure of peptides (Fig. 1) and possess increased enzymatic stability, as compared with homologous peptides.[8,9] Peptoids are polymeric compounds composed of *N*-substituted glycine (NSG) residues, and advances in the synthetic strategy (Scheme 1) used to prepare peptoid oligomers make them an attractive class of peptidomimetics.[10] The synthetic efficiency and the diversity that can be achieved make peptoids ideally suited for combinatorial chemistry, and indeed novel ligands for various GPCR systems have been discovered from screening large and diverse peptoid libraries.[11,12] Although novel ligands for the MC1R have been discovered using combinatorial methods,[12] little is known regarding the comparative biological activities of analogous peptoids and peptides at the cloned melanocortin receptors.

Novel melanocortin receptor ligands that possess enhanced properties, as compared with peptide ligands, may prove to be beneficial for in vitro, and especially in vivo, characterization of this important receptor system. Based on the observation that novel ligands have been discovered from screening large diverse combinatorial

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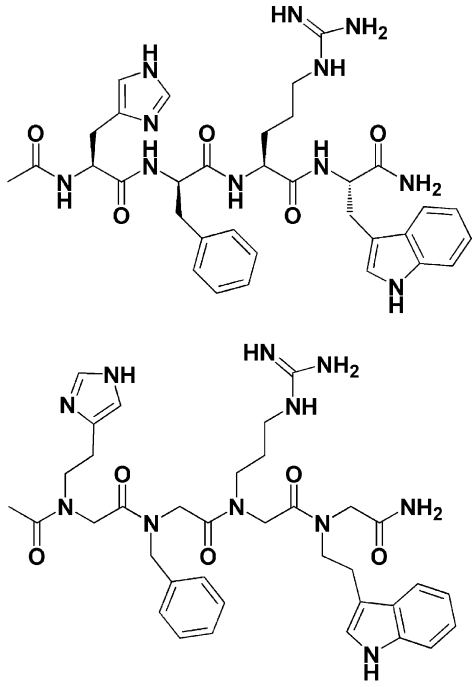
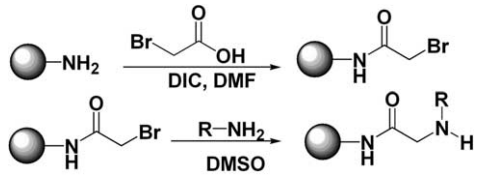


Figure 1. Structural comparison of peptide **1** and peptoid **9**.

collections of peptoid oligomers, investigations were initiated to determine if tetrameric *N*-substituted glycines could be modeled after melanocortin tetrapeptides and retain similar functional activity at the melanocortin receptors. The endogenous melanocortin agonists, adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), β-MSH and γ-MSH, all contain a His-Phe-Arg-Trp tetrapeptide sequence that is essential for biological activity, and it is this sequence that was used to model the collection of peptoid and peptide–peptoid hybrid tetramers presented herein.

Peptoid oligomers were synthesized using the submonomer approach outlined by Zuckerman and colleagues (Scheme 1).[10] Peptide–peptoid hybrids were synthesized using a suitable combination of: (1) standard Fmoc solid-phase peptide synthesis for amino acid residues[13,14] and (2) the submonomer approach for *N*-substituted glycine residues. The required *N*-substituted glycine residues, NhHis, NPhe, NhTrp, and NNal(1′), were synthesized in a straightforward manner outlined in Scheme 1 and did not require orthogonal protection of functional groups. When the NLys residue was required (**7**), N-Boc-1,4-diaminobutane was used as the amine submonomer. The Boc protecting group was removed under the acidic cleavage conditions used to remove the oligomers from the Rink-amide-MBHA resin. When a NArg or NhArg residue was required in the oligomeric sequence (**5**, **8**, and **9**), a suitable synthetic strategy was designed and employed (Scheme 2). Traditionally in solid-phase peptoid synthesis an acid liable solid support such as Rink-amide-MBHA resin is used, which permits cleavage from the resin using acidic conditions such as 95% trifluoroacetic acid (TFA). Unlike the traditional acid liable polymeric supports, *p*-methyl-benzhydrylamine resin (*p*-MBHA) was chosen

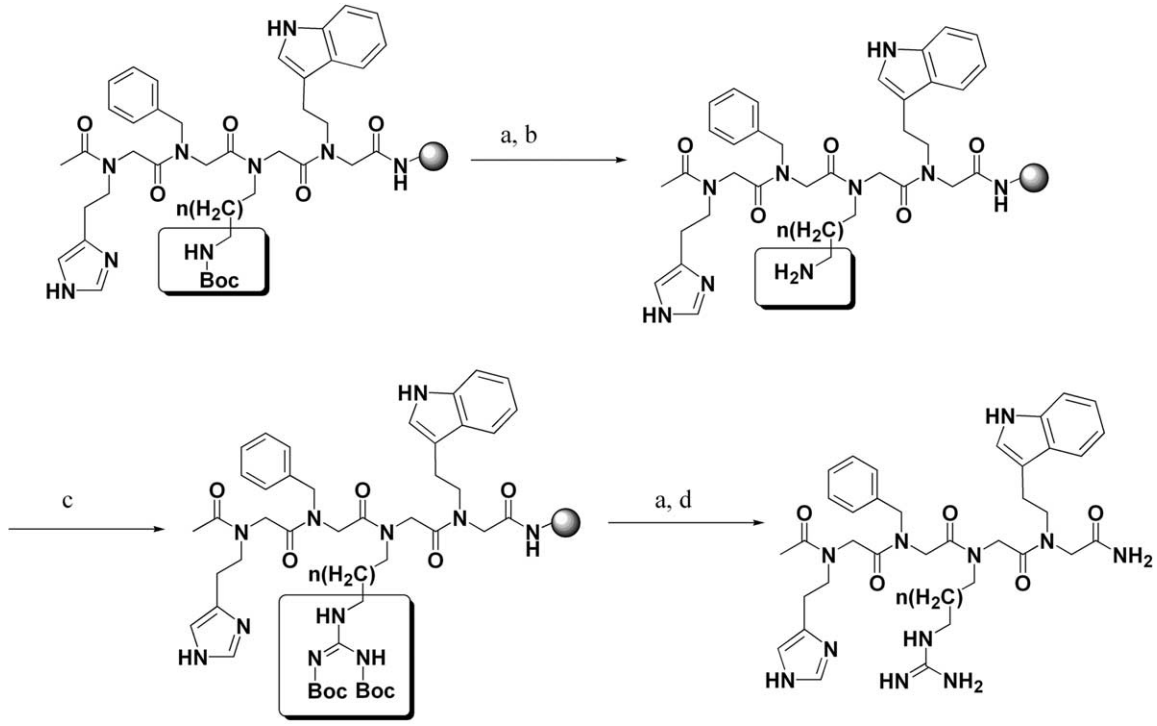


Scheme 1. The submonomer approach to peptoid synthesis.

due to the stability under mild acid conditions. The use of *p*-MBHA resin permitted the selective removal of the Boc-protecting group from the reactive amino side chain functionality using 50% TFA without loss of the oligomer from the resin. Once the Boc group was liberated from the side chain amino group, subsequent guanylation was performed to generate the appropriate NArg residue. This method has the advantage of incorporation of NArg residues in situ without need for prior preparation of large quantities of suitably protected NArg monomers, with the caveat that a stronger acidic cleavage protocol using hydroflouric acid is required to liberate the oligomer from the polymeric support. All crude peptoid and peptide–peptoid hybrid tetramers were purified to homogeneity using reversed-phase HPLC, analytically characterized in two diverse solvent systems, and all had the correct molecular weight as verified by MALDI-TOF mass spectroscopy. The amino acids Fmoc-His(Trt)-OH, Fmoc-DPhe-OH, Fmoc-Arg(Pbf)-OH and Fmoc-Trp(Boc)-OH; the monomers bromoacetic acid, *N*-Boc-1,3-diaminopropane, *N*-Boc-1,4-diaminobutane, histamine, benzylamine, tryptamine, and 1-naphthalenemethylamine; the guanylation agent *N*,*N*-bis-Boc-1-guanylpyrazole; the coupling reagents *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) and 1,3-diisopropylcarbodiimide; and all solvents were purchased from commercial sources and used without further purification.

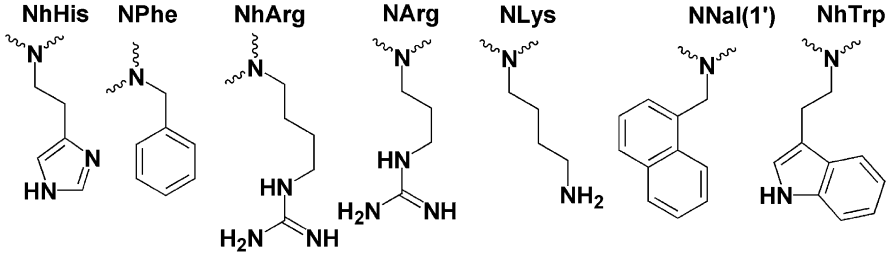
Agonist assays were performed using HEK293 cells, stably expressing mouse MC1R, MC3R-MC5R, transfected with the CRE/β-galactosidase reporter gene[15] as previously described in detail.[16] Agonist EC_{50} values reported in Table 1 represent the mean of duplicate experiments performed in three or more independent experiments. The EC_{50} 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.).

When the side chain of an amino acid residue is shifted from the chiral α-carbon to the achiral nitrogen atom of a peptoid residue, this will have effects on both biological activity as well as the physical and chemical properties of a ligand as compared with a control peptide.[17] Shifting the side chain to the amide nitrogen creates a tertiary amide, thus increasing *cis–trans* isomerism and eliminating the amide proton.[18] This shift results in increased flexibility, proteolytic stability, and lipophilicity of a oligomer but also reduces the H-bonding ability, all of which may affect biological activity and bioavailability of a peptoid ligand as compared with peptides of similar structure.[17,18]



Scheme 2. Synthesis of NSG arginine analogues: (a) 50% TFA, 2% anisole, CH_2Cl_2, rt, 1 × 5 min, 1×20 min; (b) 10% DIEA, CH_2Cl_2, rt, 2 × 2 min; (c) *N*,*N*-bis-Boc-1-guanylpyrazole, DMF, rt, 18 h; (d) *m*-cresol, thioanisole, HF, resin (0.5:0.5:9:1, v/v/v/w), 0 °C, 1 h.

Table 1. Agonist EC_{50} values of compounds used in this study and the corresponding structure of NSG residues



Compd	Sequence	EC_{50}(nM)			
		MC1R	MC3R	MC4R	MC5R
α-MSH	Ac-S-Y-S-M-E-H-F-R-W-G-K-P-V-NH_2[a]	0.6±0.1	0.8±0.1	5.4±0.6	0.4±0.1
1	Ac-His-DPhe-Arg-Trp-NH_2[a]	20±1	160±9.0	17±3	4.0±1
2	Ac-N**hHis**-DPhe-Arg-Trp-NH_2	1700±370	60%@100 μM	880±270	530±120
3	Ac-N**Phe**-DPhe-Arg-Trp-NH_2	780±120	25%@100 μM	260±15	360±87
4	Ac-His-N**Phe**-Arg-Trp-NH_2	48,000±8900	NA	20%@100 μM	40%@100 μM
5	Ac-His-DPhe-N**Arg**-Trp-NH_2	170±60	9200±1700	1100±130	160±33
6	Ac-His-DPhe-Arg-N**hTrp**-NH_2	500±160	70%@100 μM	640±90	56±4
7	Ac-N**hHis**-N**Phe**-N**Lys**-N**hTrp**-NH_2	27,000±11,000	75%@100 μM	27,200±10,600	7380±3800
8	Ac-N**Phe**-N**Phe**-N**hArg**-N**Nal(1′)**-NH_2	7600±3300	55%@100 μM	NA	20,000±8500
9	Ac-N**hHis**-N**Phe**-N**Arg**-N**hTrp**-NH_2	6600±1900	55%@100 μM	65%@100 μM	5300±2300

The concentration of compound at 50% maximum receptor stimulation (EC_{50}), or the % of receptor stimulation at the highest concentration of compound tested (relative to control). The indicated errors represent the standard error of the mean determined from at least three independent experiments. NA denotes that no agonist activity was observed at up to 100 μM.
[a]These peptide values have been previously reported[19–23] and are included herein for reference purposes.

As previously reported,[19–23] the tetrapeptide Ac-His-DPhe-Arg-Trp-NH_2 is a potent agonist at the MC1R, MC3R–MC5R. The peptoid oligomers **7**–**9** all resulted in ligands with low potency and activity, or were devoid of functional activity at the melanocortin receptors assayed (Table 1). In attempts to determine if one specific NSG residue or a combination of NSG residues were responsible for the decreased activities, as compared with **1**, a 'peptoid scan' was performed. A peptoid scan is defined as a systematic replacement of one to several amino acid residues with corresponding NSG residues.[24] This strategy may allow one to assess the

extent a peptide sequence can be transformed into a peptoid sequence, without loss of activity.

As seen with the peptoid tetramers **7**–**9**, many of the peptide–peptoid hybrids **2**–**6** were weak agonists or had no agonist activity at the melanocortin receptors assayed (Table 1). One consideration in explaining the low activity of the peptoids and peptide–peptoid hybrids, as compared with **1**, is that the former ligands are more flexible than the analogous peptide. This increase in flexibility may result in an increase in the entropic energy associated with ligand binding to receptors.[6] The low activity of the peptoids and hybrids may result from the lack of, or the decrease in, hydrogen binding ability of the peptoid compounds. Incorporation of peptoid residues may reduce the ability to form intramolecular hydrogen bonds, and thus diminish the capability of forming regular structural motifs.[25] Although it is not known if tetrapeptide **1** adopts any well formed structural motif, it is reported in the literature that the bioactive conformation of melanocortin peptides involves a turn formation.[26] Secondary structure formations, such as tight turns, are known to be stabilized by intermolecular H-bonding.[27] Additionally, the activity of the peptoid oligomers may be explained by considering that shifting the side chain from the α-carbon to the amide nitrogen places the side chain in an unfavorable topographical position.[28] The side-chain shift could change the placement of the NSG side chain by approximately 30° in three-dimensional space, as compared with the analogous amino acid side chain.[28] For to the peptide–peptoid hybrids, the side-chain shift may also perturb the topographical orientation of the adjacent amino acid side chains, thus placing both the NSG side chain and the adjacent amino acid side chain in a three-dimensional arrangement unfavorable for melanocortin receptor activation.

In summary, we have designed and synthesized a set of peptoid and peptide–peptoid hybrid tetramers and characterized these compounds for agonist activity at the cloned mouse melanocortin MC1, MC3–MC5 receptors. The peptoid oligomers **7**–**9** had micromolar potencies or were inactive at the receptors assayed. The hybrid compounds **2**–**6** were designed to determine which of the amino acid residues could be exchanged for NSG residues without detrimental effects on agonist activity. Substitution of each amino acid residue with a peptoid residue resulted in a decrease in potency at the MC receptors assayed, although the magnitude of decrease depends on the site of substitution. The DPhe residue is well known to be important for potency at the MC receptors, and not surprisingly substitution at this site (**4**) had the largest effect on functional activity. Compound **5** (Arg substitution) was the only hybrid that retained functional activity at each of the four MC receptors, although potency was reduced as compared with HfRW peptide **1**. Although none of the compounds presented herein was as potent as the lead peptide **1**, several of the tetramers had an increase in receptor selectivity. Compounds **2**, **3**, and **6** (His and Trp residues) had increased MC4R versus MC3R selectivity as compared to peptide **1**, however it should be noted that the receptor selectivity gained is a result of a larger decrease in activity at the MC3R than at the MC4R. Additionally, compound **6** is 10-fold selective for the MC5R versus the MC1R and MC4R and is almost completely inactive at the MC3R. This study has demonstrated that peptoid tetramers have drastically reduced functional activity at the MC receptors, as compared with the analogous tetrapeptide **1**. This study has also shown that specific amino acid residues of the His-DPhe-Arg-Trp-NH_2 tetrapeptide may be exchanged for a peptoid residue at the cost of reduced potency at one or more of the MC receptors, although receptor selectivity can be gained. Additionally, although the hydrid compounds presented herein are less potent than peptide **1** they may prove to have superior in vivo properties as a result of increased enzymatic stability.

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References and Notes

1. Cone, R. D.; Lu, D.; Koppula, S.; Vage, D. I.; Klungland, H.; Boston, B.; Chen, W.; Orth, D. N.; Pouton, C.; Kesterson, R. A. *Recent Prog. Horm. Res.* **1996**, *51*, 287 (discussion 318).
2. *The Melanocortin Receptors*; Cone, R. D., Ed.; The Humana Press Inc.: New Jersey, 2000.
3. Chen, A. S.; Marsh, D. J.; Trumbauer, M. E.; Frazier, E. G.; Guan, X. M.; Yu, H.; Rosenblum, C. I.; Vongs, A.; Feng, Y.; Cao, L. H.; Metzger, J. M.; Strack, A. M.; Camacho, R. E.; Mellin, T. N.; Nunes, C. N.; Min, W.; Fisher, J.; Gopal-Truter, S.; MacIntyre, D. E.; Chen, H. Y.; Van der Ploeg, L. H. T. *Nat. Genet.* **2000**, *26*, 97.
4. Butler, A. A.; Kesterson, R. A.; Khong, K.; Cullen, M. J.; Pelleymounter, M. A.; Dekoning, J.; Baetscher, M.; Cone, R. D. *Endocrinology* **2000**, *141*, 3518.
5. Huszar, D.; Lynch, C. A.; FairchildHuntress, V.; Dunmore, J. H.; Fang, Q.; Berkemeier, L. R.; Gu, W.; Kesterson, R. A.; Boston, B. A.; Cone, R. D.; Smith, F. J.; Campfield, L. A.; Burn, P.; Lee, F. *Cell* **1997**, *88*, 131.
6. Adessi, C.; Soto, C. *Curr. Med. Chem.* **2002**, *9*, 963.
7. Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945.
8. Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657.
9. Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Drug Develop. Res.* **1995**, *35*, 20.
10. Zuckermann, R. N. K.; Janice, M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.
11. Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S; Moos, W. H. *J. Med. Chem.* **1994**, *37*, 2678.
12. Heizmann, G.; Hildebrand, P.; Tanner, H.; Ketterer, S.; Pansky, A.; Froidevaux, S.; Beglinger, C.; Eberle, A. N. *J. Receptor Signal Transduct. Res.* **1999**, *19*, 449.
13. Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748.

14. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
15. Chen, W.; Shields, T. S.; Stork, P. J.; Cone, R. D. A. *Anal. Biochem.* **1995**, *226*, 349.
16. Haskell-Luevano, C.; Cone, R. D.; Monck, E. K.; Wan, Y. P. *Biochemistry* **2001**, *40*, 6164.
17. Kruijtzer, J. A. W.; Hofmeyer, L. J. F.; Heerma, W.; Versluis, C.; Liskamp, R. M. J. *Chem. Euro. J.* **1998**, *4*, 1570.
18. Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R. Y.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.
19. Holder, J. R.; Xiang, Z.; Bauzo, R. M.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 5736.
20. Holder, J. R.; Marques, F. F.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *Euro. J. Pharmacol.* **2003**, *462*, 41.
21. Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *Peptides* **2003**, *24*, 73.
22. Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 3073.
23. Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 2801.
24. Ruijtenbeek, R.; Kruijtzer, J. A. W.; van de Wiel, W.; Fischer, M. J. E.; Fluck, M.; Redegeld, F. A. M.; Liskamp, R. M. J.; Nijkamp, F. P. *Chembiochem* **2001**, *2*, 171.
25. Horwell, D. C. *Trends Biotechnol.* **1995**, *13*, 132.
26. Al-Obeidi, F.; O'Connor, S. D.; Job, C.; Hruby, V. J.; Pettitt, B. M. *J. Pept. Res.* **1998**, *51*, 420.
27. Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167.
28. de Haan, E. C.; Wauben, M. H. M.; Grosfeld-Stulemeyer, M. C.; Kruijtzer, J. A. W.; Liskamp, R. M. J.; Moret, E. E. *Bioorg. Med. Chem.* **2002**, *10*, 1939.