Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 133–137

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationship of Linear Peptide Bu-His-DPhe-Arg-Trp-Gly-NH_2 at the Human Melanocortin-1 and -4 Receptors: Histidine Substitution

Adrian Wai-Hing Cheung,* Waleed Danho, Joseph Swistok, Lida Qi, Grazyna Kurylko, Karen Rowan, Mitch Yeon, Lucia Franco, Xin-Jie Chu, Li Chen and Keith Yagaloff

Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110, USA

Received 10 July 2002; accepted 16 September 2002

Abstract—Systematic substitution of His[6] residue using non-selective hMC4R pentapeptide agonist (Bu-His[6]-DPhe[7]-Arg[8]-Trp[9]-Gly[10]-NH_2) as the template led to the identification of Bu-Atc[6](2-aminotetraline-2-carboxylic acid)-DPhe[7]-Arg[8]-Trp[9]-Gly[10]-NH_2 which showed moderate selectivity towards hMC4R over hMC1R. Further SAR studies resulted in the discovery of Penta-5-BrAtc[6]-DPhe[7]-Arg[8]-Trp[9]-Gly[10]-NH_2 and Penta-5-Me_2NAtc[6]-DPhe[7]-Arg[8]-Trp[9]-Gly[10]-NH_2 which are potent hMC4R agonists and are inactive in hMC1R, hMC3R and hMC5R agonist assays.
© 2002 Published by Elsevier Science Ltd.

In the last decade, five human melanocortin receptor subtypes (hMC1R–hMC5R) have been cloned and characterized.[1] The melanocortin receptors belong to the superfamily of G-protein coupled receptors (GPCRs) mediating a wide range of physiological functions: pigmentation (MC1R), glucocorticoid production (MC2R), food intake and energy expenditure (MC3R and MC4R) as well as exocrine gland function (MC5R).[1] Our laboratories are interested in the identification of potent and selective human melanocortin-4 receptor (hMC4R) agonists for the treatment of obesity.[2]

As previously reported, our lead pentapeptide **1** (Bu-His[6]-DPhe[7]-Arg[8]-Trp[9]-Gly[10]-NH_2, α-MSH numbering) is a potent hMC4R agonist (EC_{50} = 20 nM), selective against hMC3R (no agonist activity at 50 μM) and hMC5R (no agonist activity at 50 μM) but not selective against hMC1R (EC_{50} = 10 nM).[3] In an extensive structure–activity relationship (SAR) study of pentapeptide **1**, we systematically replaced each of the five amino acids of peptide **1** by other coding or non-coding amino acids in an effort to dial out hMC1R activity and to maintain or improve hMC4R activity. We previously reported our results in replacing Arg[8] residue of pentapeptide **1**;[3] this report summarizes our initial effort in replacing the His[6] residue of peptide **1**.

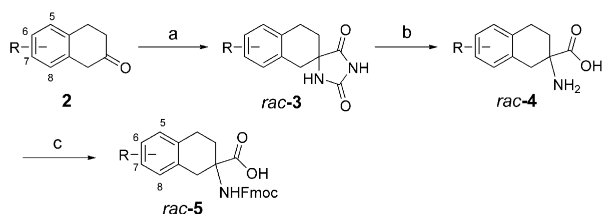
All new peptides and NDP-MSH were synthesized on solid phase from suitably protected amino acids using standard Fmoc methodology.[4] The crude peptides were purified to homogeneity using reversed-phase HPLC and characterized by fast atom bombardment mass spectroscopy. Peptide α-MSH; amino acids Fmoc-His(Trt)-OH, Fmoc-Ala-OH and racemic Fmoc-Atc-OH were purchased from commercial sources.

Synthesis of racemic Fmoc-protected substituted Atc (2-aminotetraline-2-carboxylic acid) amino acids 5. Various substituted β-tetralones **2** were converted into the corresponding racemic hydantoins **3** by Bucherer–Bergs reaction (Scheme 1).[5] Basic hydrolysis of hydantoins **3** gave racemic amino acids **4**, which were then Fmoc protected under standard conditions to give amino acids **5**.

Synthesis of substituted β-tetralones. For 8-(**7**) and 6-(**9**) substituted β-tetralones, Burckhalter–Campbell synthesis starting from the corresponding *ortho*-(**6**) or *para*-(**8**) substituted phenylacetic acids is preferred (Scheme 2).[6] For R = bromine and chlorine, Burckhalter–Campbell reaction on *meta*-substituted (**10**) phenylacetic acid

*Corresponding author. Fax: +1-973-235-7239; e-mail: adrian.cheung@roche.com

0960-894X/03/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0960-894X(02)00830-2



Scheme 1. (a) KCN, $(NH_4)_2CO_3$, ethanol, H_2O, 80 °C, 85–100% yield; (b) $Ba(OH)_2.H_2O$, H_2O, 120 °C, 75–95% yield; (c) Fmoc-OSu, CH_3CN, H_2O, Et_3N, rt or Fmoc-Cl, 10% Na_2CO_3, dioxane, rt, 70–95% yield.

gave a separable mixture of 5-(**11**) and 7-(**12**) substituted β-tetralones.[7]

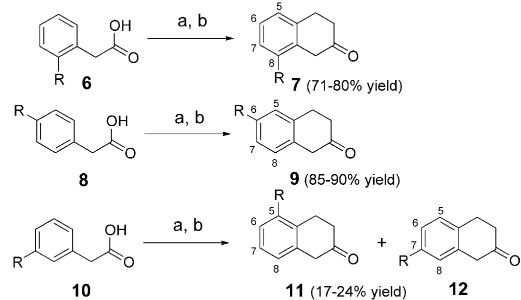
As the evolving structure–activity relationship (vide infra) indicated that 5-substituted Atc gave more hMC4R selective peptides, two regioselective protocols of preparing 5-substituted β-tetralones (**11**) were employed. For β-tetralones with chlorine, bromine, methyl, ethyl and isopropyl substituents at the 5-position (Scheme 3), the key step in the synthesis involved a rhodium-catalyzed intramolecular Buchner reaction of aryl diazo ketones (**14**),[8] which in turn were prepared from the corresponding *ortho*-substituted phenyl propanoic acid (**13**).[9]

For β-tetralones with methoxy (**15**), ethoxy (**16**), isopropoxy (**17**) and dimethylamino (**18**) substituents at the 5-position, the appropriate 1,6-disubstituted naphthalene was reduced using the method of Cornforth (Scheme 4).[10]

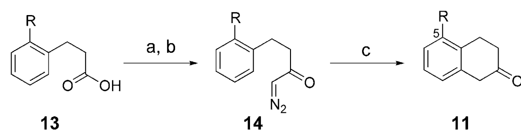
Agonist assays were performed using HEK293 cells transfected with hMC1R–hMC5R as reported in detail elsewhere.[4,11] The EC_{50} values reported in Tables 1–3 are the average of at least two separate experiments. Binding assays were performed using radiolabeled NDP-MSH as reported in detail elsewhere.[11] The IC_{50} values reported in Table 4 are the average of at least two separate experiments.

As shown in Table 1, the lead pentapeptide **1** (Bu-His-DPhe-Arg-Trp-Gly-NH_2) is a potent hMC4R agonist (EC_{50} = 20 nM) but is not selective against hMC1R (EC_{50} = 10 nM). For comparison purpose, known linear peptide agonist NDP-MSH[12] was determined in our assays to have EC_{50} values of 0.5 nM (hMC1R) and 1 nM (hMC4R) while α-MSH showed EC_{50} values of 0.8 nM (hMC1R) and 25 nM (hMC4R). When His^6 in peptide **1** was replaced with Ala, the resulting peptide **19** showed a 9-fold drop (the standard error in our assays is about 2-fold) in potency at hMC4R and a 23-fold drop in potency at hMC1R, compared with peptide **1**. As reported by Yang et al.,[13] the same His^6 to Ala substitution using linear peptide NDP-MSH as the template resulted in a 4-fold drop in agonist potency at hMC4R.

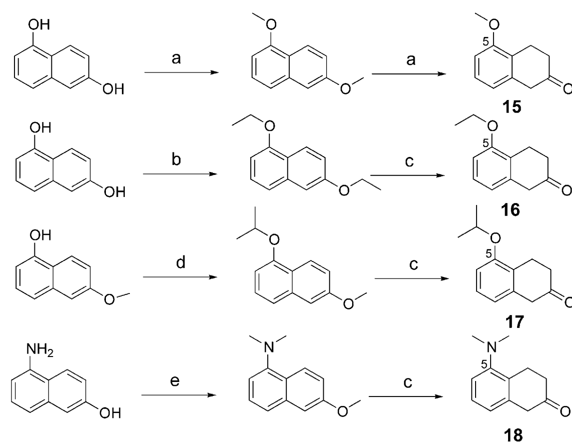
Encouraged by the modest potency of peptide **19**, dozens of analogues of the general structure Bu-X-$DPhe^7$-Arg^8-Trp^9-Gly^{10}-NH_2, in which X is a coding or noncoding amino acid,[14] were prepared and tested in both hMC1R and hMC4R agonist assays. The great majority of these His-substituted pentapeptides suffered a modest to substantial drop in hMC4R efficacy (data not shown). In addition, none of the above His-substituted pentapeptides, except the ones containing Atc (2-aminotetraline-2-carboxylic acid), showed any significant selectivity towards hMC4R over hMC1R (data not shown). When racemic Atc (**5**, R=H, Scheme 1) was used in place of His^6, two separable diastereomers **20** and **21** were obtained.[15] Peptides **20** and **21** suffered a 147- and 15-fold drop in hMC4R agonist potency, compared with peptide **1**; more importantly however, peptide **21** showed about 15-fold hMC4R selectivity over hMC1R. Peptides **22** and **23**, made by extending the *N*-cap of peptides **20** and **21** from *n*-butanoyl (Bu-) to *n*-pentanoyl (Penta-), showed slight improvement in agonist potency at both hMC1R and hMC4R, compared with peptides **20** and **21**. Individual preparation of peptides **22** and **23** using optically pure (L)- and



Scheme 2. (a) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C to rt; (b) $AlCl_3$, ethene gas, 0 °C.



Scheme 3. (a) $SOCl_2$, toluene, reflux or $(COCl)_2$, DMF, CH_2Cl_2, 0 °C to rt; (b) CH_2N_2, ether, 0 °C to rt, 60–82% yield for two steps; (c) $Rh(OAc)_2$, CH_2Cl_2, reflux, 76–100% yield.



Scheme 4. (a) Ref 10(a); (b) K_2CO_3, EtI, DMF, 35 °C, 65% yield; (c) Na, ethanol, reflux; H_2O, ethanol, *p*-TSA, reflux, 30–67% yield; (d) Cs_2CO_3, $BrCH(CH_3)_2$, DMF, 40 °C, 86% yield; (e) K_2CO_3, MeI, acetone, reflux, 94% yield.

Table 1. Agonist activity of the His[6] modified pentapeptides at the human melanocortin receptors

Peptide	Amino acid sequence	hMC4R EC_{50} (nM)[a]	hMC1R EC_{50} (nM)[a]
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2	25	0.8
NDP-MSH	Ac-Ser-Tyr-Ser-Nle-Glu-His-DPhe-Arg-Trp-Gly-Lys-Pro-Val-NH_2	1	0.5
1	Bu-**His**-DPhe-Arg-Trp-Gly-NH_2[b]	20	10
19	Bu-**Ala**-DPhe-Arg-Trp-Gly-NH_2	180	230
20	Bu-**Atc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)[c]	2940	5640
21	Bu-**Atc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)[c]	290	4380
22	Penta-(D)**Atc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)	1000	1950
23	Penta-(L)**Atc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)	45	830
24	Penta-**5-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)	33	75% @ 50 μM[e]
25	Penta-**5-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)	35	50% @ 50 μM[e]
26	Penta-**6-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)	50% @ 50 μM[e]	4630
27	Penta-**6-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)	60% @ 50 μM[e]	3380
28	Penta-**7-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)[d]	40% @ 50 μM[e]	0% @ 50 μM[e]
29	Penta-**8-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)	0% @ 50 μM[e]	1710
30	Penta-**8-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)	0% @ 50 μM[e]	2910

[a]Concentration of peptide at 50% maximum cAMP accumulation or the % of cAMP accumulation (relative to NDP-MSH) observed at the highest peptide concentration tested.
[b]Bu stands for $CH_3CH_2CH_2C(=O)$ and Penta stands for $CH_3CH_2CH_2CH_2C(=O)$.
[c]1st isomer and 2nd isomer refer to the order in which the two diastereomers eluted under our HPLC conditions.[15]
[d]Tested as a 1:1 mixture of diastereomers.
[e]Not tested for antagonist activities.

Table 2. Agonist activity of pentapeptides containing various substituted Atc amino acids at the human melanocortin receptors

Peptide	Amino acid sequence	hMC4R EC_{50} (nM)[a]	hMC1R EC_{50} (nM)[a]
24	Penta-**5-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)[b,c]	33	75% @ 50 μM[e]
25	Penta-**5-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)[c]	35	50% @ 50 μM[e]
31	Penta-**5-ClAtc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)	600	1400
32	Penta-**5-ClAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)	130	890
33	Penta-**5-MeOAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)[d]	90	1480
34	Penta-**5-EtOAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)	120	990
35	Penta-**5-*i*PrOAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)	130	680
36	Penta-**5-MeAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)	35	1220
37	Penta-**5-EtAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)	24	195
38	Penta-**5-*i*PrAtc**-DPhe-Arg-Trp-Gly-NH_2 (mixture)	13	295
39	Penta-**5-Me_2NAtc**-DPhe-Arg-Trp-Gly-NH_2 (1st isomer)	16	1850
40	Penta-**5-Me_2NAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)	2	940

[a]Concentration of peptide at 50% maximum cAMP accumulation or the % of cAMP accumulation (relative to NDP-MSH) observed at the highest peptide concentration tested.
[b]Penta stands for $CH_3CH_2CH_2CH_2C(=O)$.
[c]1st isomer and 2nd isomer refer to the order in which the two diastereomers eluted under our HPLC conditions.[15]
[d]Tested as a 1:1 mixture of diastereomers.
[e]Not tested for antagonist activities.

(D)-Atc[5] unambiguously confirmed that peptide **22** contains (D)-Atc and peptide **23** contains (L)-Atc.

Peptide **23** is similar to α-MSH in hMC4R agonist potency but it is significantly more selective towards hMC4R compared with α-MSH. To further improve the potency and selectivity of peptide **23** towards hMC4R, substitutents were systematically introduced into the four positions of the phenyl ring of Atc. Peptides with 6-BrAtc (**26** and **27**), 7-BrAtc (**28**) and 8-BrAtc (**29** and **30**) surprisingly were devoid of hMC4R activity. Peptides containing 5-BrAtc, **24** and **25**, showed good hMC4R potency (33 and 35 nM) and were unable to induce 100% hMC1R stimulation even at 50 μM concentration. Other 6-, 7-, or 8-substituted Atc analogues investigated include 6-ClAtc, 7-MeOAtc and 8-HOAtc; none of the pentapeptides derived from these amino acids is as potent or hMC4R selective as peptides **24** and **25** (data not shown).

In an effort to optimize the substitution at the 5-position of Atc, a series of halo- (chloro-), alkoxy- (methoxy-, ethoxy-, isopropoxy-), alkyl- (methyl-, ethyl- and isopropyl) and dimethylamino-Atc containing peptides were prepared (peptides **31**–**40**, Table 2). Although a number of the above peptides showed equal or improved hMC4R potency compared with 5-BrAtc containing peptides **24** and **25**, none of them was able to dial out hMC1R activity as effectively as peptides **24** and **25**. For peptides **31**–**40**, the hMC1R potencies are spread out over only 9-fold (195–1850 nM), while their hMC4R potencies are spread out over 300-fold (2–600 nM), indicating a stronger influence of 5-substitution of Atc on hMC4R than on hMC1R agonist activity. It is reasonable to speculate that further fine-tuning of 5-substituted Atc could yield more potent and/or more selective hMC4R agonists. One of the more interesting peptides discovered from this initial optimization study is peptide **40**, which is as potent as NDP-MSH towards hMC4R (2 nM vs 1 nM, within the 2-fold experimental error), >450-fold selective against hMC1R and inactive in hMC3R and hMC5R agonist assays (vide infra).

Table 3. Agonist activity of pentapeptides containing various 5-substituted Atc amino acids at the human melanocortin receptors

Peptide	hMC1R EC_{50} (nM)[a]	hMC3R EC_{50} (nM)[a]	hMC4R EC_{50} (nM)[a]	hMC5R EC_{50} (nM)[a]
1	10	0% @ 50 μM[b]	20	0% @ 50 μM[b]
24	75% @ 50 μM[b]	0% @ 50 μM[b]	33	0% @ 50 μM[b]
25	50% @ 50 μM[b]	0% @ 50 μM[b]	35	32% @ 50 μM[b]
39	1850	0% @ 50 μM[b]	16	24% @ 50 μM[b]
40	940	0% @ 50 μM[b]	2	0% @ 50 μM[b]

[a]Concentration of peptide at 50% maximum cAMP accumulation or the % of cAMP accumulation (relative to NDP-MSH) observed at the highest peptide concentration tested.
[b]Not tested for antagonist activities.

Table 4. Binding affinity of pentapeptides containing various 5-substituted Atc amino acids at the human melanocortin receptors

Peptide	hMC1R IC_{50} (nM)[a]	hMC3R IC_{50} (nM)[a]	hMC4R IC_{50} (nM)[a]	hMC5R IC_{50} (nM)[a]
1	580	4000	150	13,300
24	4900	Not determined	200	Not determined
25	5100	Not determined	105	Not determined
39	21,700	Not determined	150	Not determined
40	25,500	Not determined	25	Not determined

[a]Concentration of peptide at 50% radiolabeled NDP-MSH displacement.

The most hMC4R selective peptides described above were tested in hMC3R and hMC5R agonist assays and the results are shown in Table 3. As discussed previously, the lead pentapeptide **1** (Bu-His-DPhe-Arg-Trp-Gly-NH_2) was inactive in both hMC3R and hMC5R agonist assays. This lack of agonist activity in hMC3R and hMC5R is maintained when His[6] of **1** was replaced by 5-BrAtc (peptides **24** and **25**) and 5-Me_2NAtc (peptides **39** and **40**). The above peptides were also tested in hMC1R–hMC5R binding assays (Table 4). In hMC4R, the binding affinities of the peptides, within limits of experimental error, track with their agonist activities. However, in hMC1R, the order of binding affinity is **1** > **24**, **25** > **39**, **40** while the order of agonist activity is **1** > **39**, **40** > **24**, **25**. It is unclear why peptides **24** and **25** are better binders but weaker agonists than peptides **39** and **40** at hMC1R.

In summary, our study shows[16–18] that rigid non-basic histidine surrogates such as Atc could lead to excellent hMC4R selectivity in linear peptides. Pentapeptides containing 5-BrAtc and 5-Me_2NAtc are potent hMC4R agonists and are inactive in hMC1R, hMC3R and hMC5R agonist assays. Further modification of Atc amino acid, incorporation of 5-substituted Atc into cyclic peptides and in vivo studies using Atc-containing linear/cyclic peptides would be reported in due course.

Acknowledgements

The authors are grateful to Roche Physical Chemistry Department for spectroscopic measurements and interpretations. We would also like to thank Dr. Ramakanth Sarabu and Dr. Jefferson Tilley for critical reading of the manuscript.

References and Notes

1. Cone, R. D., Ed. *The Melanocortin Receptors*; Humana: Totowa, 2000.
2. MacNeil, D. J.; Howard, A. D.; Guan, X.; Fong, T. M.; Nargund, R. P.; Bednarek, M. A.; Goulet, M. T.; Weinberg, D. H.; Strack, A. M.; Marsh, D. J.; Chen, H. Y.; Shen, C.-P.; Chen, A. S.; Rosenblum, C. I.; MacNeil, T.; Tota, M.; MacIntyre, E. D.; Van der Ploeg, L. H. T. *Eur. J. Pharmacol.* **2002**, *440*, 141.
3. Cheung, A. W.-H.; Danho, W.; Swistok, J.; Qi, L.; Kurylko, G.; Franco, L.; Yagaloff, K.; Chen, L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2407.
4. (a) Chen, L.; Cheung, A. W.-H.; Chu, X.-J.; Danho, W.; Swistok, J.; Yagaloff, K. A. WO 0174844, 2001; CAN 135:304143. (b) Chen, L.; Cheung, A. W.-H.; Chu, X.-J.;

Danho, W.; Swistok, J.; Wang, Y.; Yagaloff, K. A. WO 0218437, 2002; CAN 136:217052.

5. Obrecht, D.; Spiegler, C.; Schonholzer, P.; Muller, K.; Heimgartner, H.; Stierli, F. *Helv. Chim. Acta* **1992**, *75*, 1666.

6. (a) Burckhalter, J. H.; Campbell, J. R. *J. Org. Chem.* **1961**, *26*, 4232. (b) Tschaen, D. M.; Abramson, L.; Cai, D.; Desmond, R.; Dolling, U.-H.; Frey, L.; Karady, S.; Shi, Y.-J.; Verhoeven, T. R. *J. Org. Chem.* **1995**, *60*, 4324.

7. (a) Stjernlof, P.; Elebring, T.; Andersson, B.; Svensson, A.; Svensson, K.; Ekman, A.; Carlsson, A.; Wikstrom, H. *Eur. J. Med. Chem.* **1993**, *28*, 693. (b) Rosowsky, A.; Battaglia, J.; Chen, K. K. N.; Modest, E. J. *J. Org. Chem.* **1968**, *33*, 4288.

8. (a) McKervey, M. A.; Tuladhar, S. M.; Twohig, M. F. *J. Chem. Soc., Chem. Commun.* **1984**, 129. (b) Kennedy, M.; McKervey, M. A.; Maguire, A. R.; Tuladhar, S. M.; Twohig, M. F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1047. (c) Cordi, A. A.; Lacoste, J.-M.; Hennig, P. *J. Chem. Soc., Perkin Trans. 1* **1993**, 3.

9. 3-(2-Methylphenyl) and 3-(2-chlorophenyl) propanoic acid are commercially available. 3-(2-Bromophenyl) propanoic acid was prepared from 2-bromobenzyl bromide according to the procedures of Cooke, M. P., Jr.; Widener, R. K. *J. Org. Chem.* **1987**, *52*, 1381. 3-(2-Ethylphenyl) and 3-(2-isopropylphenyl) propanoic acid were prepared from the corresponding 1-alkyl-2-iodobenzene in three steps [Step 1: $Pd(OAc)_2$, methyl acrylate, tri(*o*-tolyl)phosphine, Et_3N, 100 °C; Step 2: Mg, CH_3OH, 10–15 °C; Step 3: KOH, ethanol, water, reflux; overall yield for three steps: 50% for 2-Et and 64% for 2-*i*Pr].

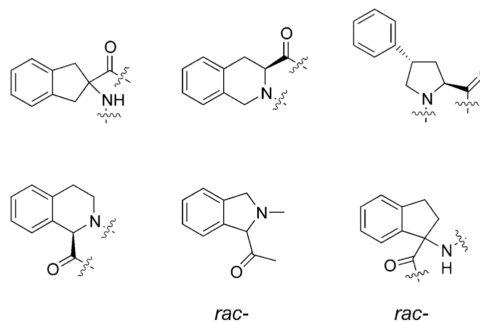
10. (a) Cornforth, J. W.; Cornforth, R. H.; Robinson, R. *J. Chem. Soc* **1942**, 689. (b) Manitto, P.; Speranza, G.; Monti, D.; Fontana, G.; Panosetti, E. *Tetrahedron* **1995**, *51*, 11531.

11. Benoit, S. C.; Schwartz, M. W.; Lachey, J. L.; Hagan, M. M.; Rushing, P. A.; Blake, K. A.; Yagaloff, K. A.; Kurylko, G.; Franco, L.; Danho, W.; Seeley, R. J. *J. Neurosci.* **2000**, *20*, 3442.

12. Sawyer, T. K.; Sanfillippo, P. J.; Hruby, V. J.; Engel, M. H.; Heward, C. B.; Burnett, J. B.; Hadley, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5754.

13. Yang, Y.; Fong, T. M.; Dickinson, C. J.; Mao, C.; Li, J.; Tota, M. R.; Mosley, R.; Van der Ploeg, L. H. T.; Gantz, I. *Biochemistry* **2000**, *39*, 14900.

14. Atc related non-coding amino acids used in our study include the following:



15. The diastereomeric mixture of pentapeptides containing racemic unsubstituted or substituted Atc are generally separable using reversed-phase high performance liquid chromatography (HPLC) on a Vydac C_{18} column. Gradient elution (10% buffer B to 60% buffer B) was carried out over 90 min at a flow rate of 8 mL/min using 0.1% TFA/H_2O (buffer A) and 0.1% TFA/CH_3CN (buffer B) with UV detection at 280 nm.

16. After our discovery that histidine surrogates such as 5-BrAtc could give rise to excellent hMC4R selectivity, Hruby et al. independently proposed that "the position 6 of the synthetic melanocortin ligands is important for enhancing potency and selectivity at MC3 and MC4 melanocortin receptors". Hruby, V. J.; Grieco, P.; Balse, P.; Han, G.; Weinberg, D.; McNeil, T. *Abstract of Papers*, 217th National Meeting of the American Chemical Society, Anaheim, CA; American Chemical Society: Washington, DC, 1999; Abstract MEDI-231.

17. A portion of this work was previously presented. Danho, W.; Swistok, J.; Cheung, A.; Chu, X-J.; Wang, Y.; Chen, L.; Bartkovitz, D.; Gore, V.; Qi, L.; Fry, D.; Greeley, D.; Sun, H.; Guenot, J.; Franco, L.; Kurylko, G.; Rumennik, L.; Yagaloff, K. *Abstracts of Papers*, 2nd International/17th American Peptide Symposium; Kluwer Academic: The Netherlands, 2001; p 701.

18. During the preparation of this manuscript, a report appeared in which 17 histidine surrogates were introduced into a Ac-His^6-$DPhe^7$-Arg^8-Trp^9-NH_2 tetrapeptide template and the resulting peptides were characterized in mouse melanocortin receptors. Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 2801.