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Preparation of human Melanocortin-4 receptor agonist libraries: Linear peptides X-Y-DPhe^7-Arg^8-Trp(or 2-Nal)^9-Z-NH_2

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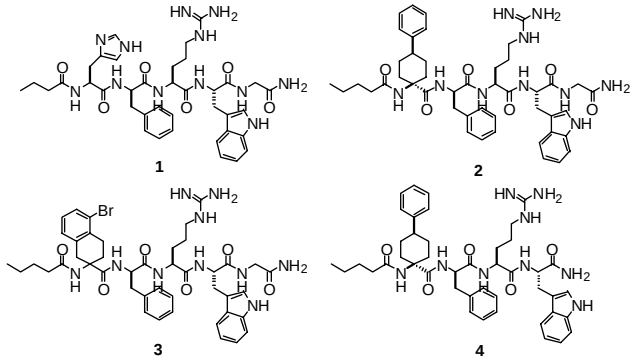
Abstract—Two libraries of hMC4R agonists, **X**-**Y**-DPhe^7-Arg^8-2-Nal^9-**Z**-NH_2 and **X**-**Y**-DPhe^7-Arg^8-Trp^9-**Z**-NH_2, totaling 185 peptides were prepared using Irori radiofrequency tagging technology and Argonaut Quest 210 Synthesizer, where **X** stands for N-caps, **Y** for His^6 surrogates and **Z** for Gly^{10} surrogates. As a result of this study, His-modified pentapeptides with Trp were found to be more hMC4R potent than the corresponding 2-Nal analogs, novel N-caps and Gly surrogates were identified and 19 new peptides which are potent hMC4R agonists (EC_{50} 1–15 nM) and selective against hMC1R were discovered.

In the last decade, five human melanocortin receptor subtypes (hMC1R–hMC5R) have been cloned and characterized.[1] The melanocortin receptors are G-protein coupled receptors (GPCRs) which mediate a wide range of physiological functions including pigmentation (MC1R), glucocorticoid production (MC2R), food intake and energy expenditure (MC3R and MC4R) as well as exocrine gland function (MC5R).[1] Recently, it was suggested that MC4R also plays a role in sexual function.[2] To better understand the physiological functions of the melanocortin receptors in different animal species, potent and highly subtype selective agonists and/or antagonists are required. The identification of these pharmacological agents remains one of the key challenges in the melanocortin field.

We previously reported the use of potent but non-selective hMC4R peptide agonist **1** (Bu-His^6-DPhe^7-Arg^8-Trp^9-Gly^{10}-NH_2) as the template in which each of its five amino acid residues was systematically replaced by other coding or non-coding amino acids.[3–5] The above structure–activity relationship studies of peptide **1** showed that there are relatively limited opportunities at the DPhe, Arg, and Trp sites to improve hMC4R potency and selectivity of peptide **1**.[3,5] On the other hand, we found that the use of phenyl-containing rigid templates such as Apc[6] and 5-BrAtc[4] led to pentapeptides **2** (Penta-Apc-DPhe-Arg-Trp-Gly-NH_2) and **3** (Penta-5-BrAtc-DPhe-Arg-Trp-Gly-NH_2) with good hMC4R potency and selectivity against hMC1R (Table 1).



Removal of Gly from peptide **2** gave peptide **4** (Penta-Apc-DPhe-Arg-Trp-NH_2) which drastically reduced both hMC4R potency and selectivity (Table 1). The importance of the Gly residue, together with the SAR data at the DPhe, Arg, and Trp residues, led us in the present work to concentrate on mixing and matching His and Gly residues in an attempt to further improve the potency and selectivity of peptides **2** and **3**. A library of the general structure **X**-**Y**-DPhe-Arg-2-Nal-**Z**-NH_2 (15 **Y** × 11 **Z** = 165 members) was prepared where **X**

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Table 1. Agonist activity of the various linear peptides at the human melanocortin receptors

Peptide	Amino acid sequence	hMC4R EC_{50} (nM)[a]	hMC1R EC_{50} (nM)[a]
1	Bu-His-DPhe-Arg-Trp-Gly-NH_2[b]	20	10
2	Penta-**Apc**-DPhe-Arg-Trp-Gly-NH_2	2	25% at 50 μM
3	Penta-**5-BrAtc**-DPhe-Arg-Trp-Gly-NH_2 (2nd isomer)[c]	35	50% at 50 μM[e]
4	Penta-**Apc**-DPhe-Arg-Trp-NH_2	130	1300
5	Bu-His-DPhe-Arg-**2-Nal**-Gly-NH_2	25	14
6	Bu-**Apc**-DPhe-Arg-2-Nal-$\mathbf{Z}^2$-NH_2	44	25% at 10 μM[e]
7	Bu-**Apc**-DPhe-Arg-2-Nal-$\mathbf{Z}^3$-NH_2	220	25% at 10 μM[e]
8	Bu-**Apc**-DPhe-Arg-2-Nal-$\mathbf{Z}^4$-NH_2	90	25% at 10 μM[e]
9	Bu-**Apc**-DPhe-Arg-2-Nal-$\mathbf{Z}^8$-NH_2	110	25% at 10 μM[e]
10	Bu-**Apc**-DPhe-Arg-2-Nal-$\mathbf{Z}^9$-NH_2	39	50% at 10 μM[e]
11	Bu-**Apc**-DPhe-Arg-2-Nal-$\mathbf{Z}^{10}$-NH_2	85	15% at 50 μM[e]
12	Bu-**5-BrAtc**-DPhe-Arg-2-Nal-$\mathbf{Z}^9$-NH_2 (mixture)[d]	72	60% at 50 μM
13	Penta-**Apc**-DPhe-Arg-2-Nal-Gly-NH_2	50	60% at 50 μM[e]
14	Penta-**5-BrAtc**-DPhe-Arg-2-Nal-Gly-NH_2 (mixture)[d]	200	2900

[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. The EC_{50} values are the average of at least two separate experiments.
[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.[13]
[d] Tested as a 1:1 mixture of diastereomers.
[e] Not tested for antagonist activities.

stands for N-caps, **Y** for His surrogates, and **Z** for Gly surrogates. In an attempt to discover novel hMC4R potent and selective peptides that do not contain Apc ($\mathbf{Y}^1$) and 5-BrAtc ($\mathbf{Y}^2$), $\mathbf{Y}^3$-$\mathbf{Y}^{15}$ (Fig. 1) were also chosen as His surrogates in this library. Amino acids $\mathbf{Y}^3$-$\mathbf{Y}^9$ gave pentapeptides (Bu-**Y**-DPhe-Arg-Trp-Gly-NH_2) with moderate or good hMC4R potency but with no or low hMC4R selectivity (data not shown); it is thought that by matching them with different Gly surrogates, the resulting peptides might possess improved hMC4R agonist potency or selectivity. $\mathbf{Y}^{10}$-$\mathbf{Y}^{15}$ all contained phenyl rings (similar to Apc and 5-BrAtc) and were chosen to act as hybrids of His surrogates and N-caps.

The impact of replacing Gly^{10} in linear peptides on hMC4R agonist activities, to the best of our knowledge, has not been systematically studied. Our choice of Gly surrogates (**Z**) was based on the SAR of dozens of peptides with the structure Bu-His-DPhe-Arg-Trp-**Z**-NH_2. Although none of the above Gly-modified pentapeptides showed any significant selectivity toward hMC4R over hMC1R (data not shown), $\mathbf{Z}^1$-$\mathbf{Z}^{11}$ (Fig. 2) were chosen for the library because they gave pentapeptides Bu-His-DPhe-Arg-Trp-**Z**-NH_2 with good or excellent hMC4R potency (data not shown). It is thought that by matching them with different His surrogates (**Y**), the resulting peptides might possess improved hMC4R agonist potency or selectivity. For the first library, 2-Nal was used in place of Trp because of its higher chemical stability and at the time of library construction, 2-Nal and Trp were thought to be interchangeable based on the comparable binding and agonist activities of peptide **5** (Bu-His-DPhe-Arg-**2-Nal**-Gly-NH_2) and peptide **1** (Bu-His-DPhe-Arg-**Trp**-Gly-NH_2).[5] This view was shared by Haskell-Luevano et al., who stated that

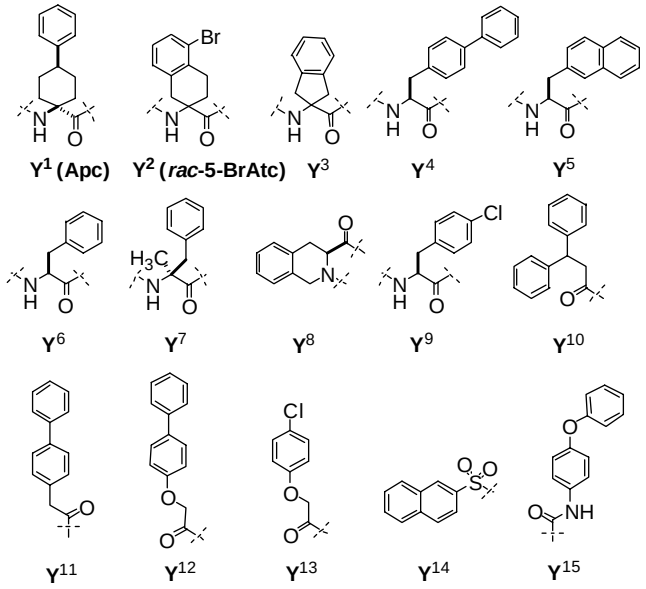

Figure 1. Structures of $\mathbf{Y}^1$-$\mathbf{Y}^{15}$.

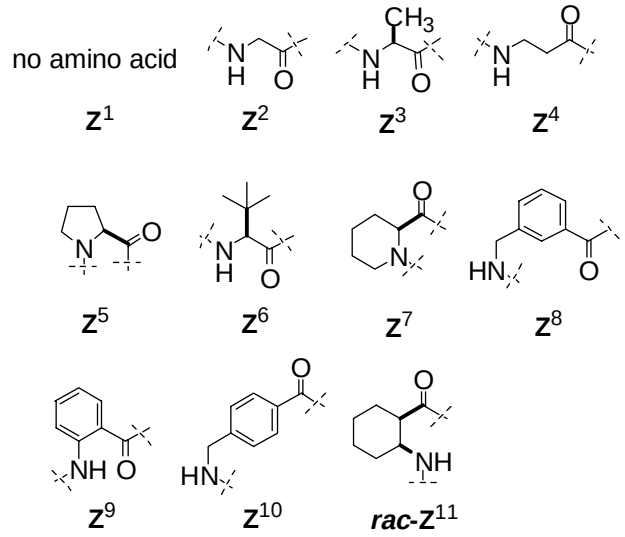

Figure 2. Structures of $\mathbf{Z}^1$-$\mathbf{Z}^{11}$.

"the chemically reactive Trp indole side chain may be replaced with the non-reactive naphthyl moiety in the design of peptide and non-peptide melanocortin receptor ligands, as long as the naphthyl ring is in the correct orientation (1′ vs. 2′)".[7] 1-Nal was not pursued in this study because Bu-His-DPhe-Arg-**1-Nal**-Gly-NH_2 was much less potent than the corresponding 2-Nal analog, peptide **5**.[5]

165 peptides of the general structure **X**-**Y**-DPhe-Arg-2-Nal-**Z**-NH_2 were prepared using Irori radiofrequency tagging technology[8] as described in Ref. 9. The 165 purified peptides were initially screened in hMC1R and hMC4R agonist assays at a single peptide concentration of 100 nM using HEK293 cells transfected with hMC1R and hMC4R as reported in detail elsewhere.[10,11] In the hMC4R assay, less than 10% of the library achieved more than 30% stimulation at 100 nM concentration. Only those hits (peptides **6**–**12**) from the one-point agonist assays which showed both good hMC4R potency (>30% activation at 100 nM) and selectivity[12] were fully titrated to generate EC_{50} values (Table 1).

As shown in Table 1, the most hMC4R potent and selective peptides from the first library, peptides **6**–**12**, all contain either Apc ($\mathbf{Y}^1$) or 5-BrAtc ($\mathbf{Y}^2$), the two optimized His surrogates. Although 143 peptides without either Apc or 5-BrAtc were prepared, none of them met our stringent criteria of hMC4R potency and selectivity, thus demonstrating the challenges in discovering hMC4R potent and selective peptides. Peptide **6**, which differs from peptide **2** in having 2-Nal instead of Trp and *n*-butanoyl (Bu-) N-cap instead of *n*-pentanoyl (Penta), is about 20-fold less potent at hMC4R and inactive at hMC1R, compared to peptide **2** (the standard error in our assays is about 2-fold). Slight modification of Gly ($\mathbf{Z}^2$) in peptide **6** to Ala ($\mathbf{Z}^3$) and homoAla ($\mathbf{Z}^4$) gave peptides **7** and **8**, respectively, which displayed hMC1R and hMC4R agonist potencies within 5-fold of those of peptide **6**. It is intriguing that peptides **9**–**11**, containing Gly surrogates with aromatic rings ($\mathbf{Z}^8$-$\mathbf{Z}^{10}$), are comparable in hMC1R and hMC4R potencies to peptides **6**–**8**. Peptide **12**, with 5-BrAtc as His surrogate and $\mathbf{Z}^9$ as Gly surrogate, is comparable to peptide **3** in hMC1R and hMC4R agonist activities. Of the 22 peptides bearing either Apc or 5-BrAtc, only seven (peptides **6**–**12**) met our cut-off criteria of hMC4R agonist potency and selectivity, clearly highlighting the influence of Gly surrogates on the pentapeptides' overall hMC4R potency and selectivity.

As mentioned earlier, when the first library was constructed, 2-Nal and Trp were believed to be interchangeable based on the comparable hMC1R and hMC4R activities of peptides **1** and **5**. Later on, as more Apc or 5-BrAtc containing linear peptides were prepared, it became apparent that His-modified peptides bearing Trp are more potent at hMC4R compared to their 2-Nal counterparts. For example, peptide **2** (Penta-Apc-DPhe-Arg-**Trp**-Gly-NH_2) is 25-fold more potent as a hMC4R agonist compared to peptide **13** (Penta-Apc-DPhe-Arg-**2-Nal**-Gly-NH_2); similarly, peptide **3** (Penta-5-BrAtc-DPhe-Arg-**Trp**-Gly-NH_2) is about 6-fold more potent as a hMC4R agonist compared to peptide **14** (Penta-5-BrAtc-DPhe-Arg-**2-Nal**-Gly-NH_2).

To test the hypothesis that His-modified pentapeptides with Trp are more potent than the corresponding 2-Nal analogs, a follow-up library was carried out in which peptides **6**–**12** were modified by substituting 2-Nal with Trp. At the same time, to study the influence of N-caps on the peptides' biological activities, 3 N-caps ($\mathbf{X}^1$-$\mathbf{X}^3$, Fig. 3) were also incorporated into the second library giving a total of 21 possible peptides. As $\mathbf{X}^1$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^2$-NH_2 is structurally very similar to peptide **2**, it was removed from the list giving a revised total of 20 peptides.

These peptides (**X**-**Y**-DPhe-Arg-Trp-**Z**-NH_2) were prepared using a Quest 210 Synthesizer[14] as described in Ref. 15. The agonist activities of the 19 purified peptides (one was lost during purification) from the second library are shown in Table 2.

Peptides **6**–**12**, bearing 2-Nal, showed hMC4R EC_{50} values within the range of 39–220 nM. Peptides **17**, **20**, **23**, **26**, **29**, and **31**, which only differ from peptides **6**–**12** in having Trp instead of 2-Nal, displayed much improved hMC4R EC_{50} values within the range of 1–15 nM, validating our 2-Nal/Trp non-equivalency hypothesis. Specifically, peptide **17** ($\mathbf{X}^1$-Apc-DPhe-Arg-**Trp**-$\mathbf{Z}^3$-NH_2) is over 70-fold more potent as a hMC4R agonist compared to peptide **7** ($\mathbf{X}^1$-Apc-DPhe-Arg-**2-Nal**-$\mathbf{Z}^3$-NH_2); similarly, peptide **23** ($\mathbf{X}^1$-Apc-DPhe-Arg-**Trp**-$\mathbf{Z}^8$-NH_2) is over 50-fold more potent as a hMC4R agonist compared to peptide **9** ($\mathbf{X}^1$-Apc-DPhe-Arg-**2-Nal**-$\mathbf{Z}^8$-NH_2). It is interesting that Apc containing peptides, peptides **15**–**30**, despite the variations in N-caps and Gly surrogates, all have EC_{50} values within a narrow range of 1–5 nM at hMC4R. Peptides **31**–**33**, bearing 5-BrAtc, possessed hMC4R EC_{50} values of 11–15 nM; separation of these three peptide mixtures into individual diastereomers should give more hMC4R potent peptides. None of the new peptides described in this paper was characterized in hMC1R binding or antagonist assays and we cannot rule out the possibility of hMC1R antagonist activities of some of these peptides.

In summary, two libraries of pentapeptides, **X**-**Y**-DPhe-Arg-2-Nal-**Z**-NH_2 and **X**-**Y**-DPhe-Arg-Trp-**Z**-NH_2, totaling 185 peptides were prepared using Irori radiofrequency tagging technology and Argonaut Quest 210 Synthesizer, in which N-caps, His and Gly residues were modified. As a result of this study, His-modified pentapeptides with Trp were found to be more hMC4R potent than the corresponding 2-Nal analogs, novel N-caps and Gly surrogates were identified, and 19 new

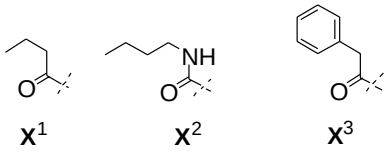

Figure 3. Structures of $\mathbf{X}^1$-$\mathbf{X}^3$.

Table 2. Agonist activity of the various linear peptides at the human melanocortin receptors

Peptide	Amino acid sequence	hMC4R EC_{50} (nM)[a]	hMC1R EC_{50} (nM)[a]
15	$\mathbf{X}^2$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^2$-NH_2	2	55% at 10 μM[c]
16	$\mathbf{X}^3$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^2$-NH_2	2	30% at 10 μM[c]
17	$\mathbf{X}^1$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^3$-NH_2	3	25% at 50 μM[c]
18	$\mathbf{X}^2$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^3$-NH_2	2	25% at 50 μM[c]
19	$\mathbf{X}^3$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^3$-NH_2	3	30% at 10 μM[c]
20	$\mathbf{X}^1$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^4$-NH_2	5	50% at 50 μM[c]
21	$\mathbf{X}^2$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^4$-NH_2	2	50% at 50 μM[c]
22	$\mathbf{X}^3$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^4$-NH_2	5	45% at 10 μM[c]
23	$\mathbf{X}^1$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^8$-NH_2	2	50% at 50 μM[c]
24	$\mathbf{X}^2$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^8$-NH_2	2	60% at 10 μM[c]
25	$\mathbf{X}^3$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^8$-NH_2	2	60% at 10 μM[c]
26	$\mathbf{X}^1$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^9$-NH_2	2	60% at 10 μM[c]
27	$\mathbf{X}^2$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^9$-NH_2	2	75% at 10 μM[c]
28	$\mathbf{X}^3$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^9$-NH_2	3	75% at 10 μM[c]
29	$\mathbf{X}^1$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^{10}$-NH_2	1	50% at 10 μM[c]
30	$\mathbf{X}^3$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^{10}$-NH_2	1	70% at 10 μM[c]
31	$\mathbf{X}^1$-5-BrAtc-DPhe-Arg-Trp-$\mathbf{Z}^9$-NH_2 (mixture)[b]	15	65% at 50 μM[c]
32	$\mathbf{X}^2$-5-BrAtc-DPhe-Arg-Trp-$\mathbf{Z}^9$-NH_2 (mixture)[b]	15	50% at 10 μM[c]
33	$\mathbf{X}^3$-5-BrAtc-DPhe-Arg-Trp-$\mathbf{Z}^9$-NH_2 (mixture)[b]	11	50% at 10 μM[c]

[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. The EC_{50} values are the average of at least two separate experiments.
[b] Tested as a 1:1 mixture of diastereomers.
[c] Not tested for antagonist activities.

peptides which are potent hMC4R agonists (EC_{50} 1–15 nM) and selective against hMC1R were discovered. Further characterization of the new peptides disclosed herein will be the subject of future communications.

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References and notes

1. (a) *The Melanocortin Receptors*; Cone, R. D., Ed.; Humana: Totowa, 200; (b) Boyce, R. S.; Duhl, D. M. *Curr. Opin. Invest. Drugs* **2004**, *5*, 1063.
2. Martin, W. J.; MacIntyre, D. E. *Eur. Urol.* **2004**, *45*, 706.
3. Arg substitution 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. His substitution Cheung, A. W.-H.; Danho, W.; Swistok, J.; Qi, L.; Kurylko, G.; Rowan, K.; Yeon, M.; Franco, L.; Chu, X.-J.; Chen, L.; Yagaloff, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 133.
5. DPhe and Trp substitution Danho, W.; Swistok, J.; Cheung, A. W.-H.; Kurylko, G.; Franco, L.; Chu, X.-J.; Chen, L.; Yagaloff, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 649.
6. (a) Chu, X-J.; Bartkovitz, D.; Danho, W.; Swistok, J.; Cheung, A. W-H.; Kurylko, G.; Rowan, K.; Yeon, M.; Franco, L.; Chen, L.; Yagaloff, K. *Bioorg. Med. Chem. Lett.* in press; (b) Sun, H.; Greeley, D. N.; Chu, X.-J.; Cheung, A.; Danho, W.; Swistok, J.; Wang, Y.; Zhao, C.; Chen, L.; Fry, D. C. *Bioorg. Med. Chem.* **2004**, *12*, 2671; (c) 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. In *Proceedings of the 2nd International/17th American Peptide Symposium*; Lebl, M., Houghten, R., Eds.; Kluwer Academic Publishers: The Netherlands, 2001, p 701.
7. Holder, J. R.; Haskell-Luevano, C. *Med. Res. Rev.* **2004**, *3*, 325.
8. For more information about this technology, see: www.irori.com.
9. H_2N-Rink resin (0.54 mmol/g loading, 100–200 mesh) in 10 separate reaction vessels labeled $\mathbf{Z}^2$ through $\mathbf{Z}^{11}$ was coupled to the corresponding Fmoc-**Z**-OH using DIC/HOBT activation and then Fmoc deprotected. The above operation was skipped for $\mathbf{Z}^1$ (without Gly surrogate). To the resulting 11 resins (H_2N-**Z**-resin) in eleven separate reaction vessels were sequentially added amino acids Fmoc-2-Nal-OH; Fmoc-Arg(Pmc)-OH and Fmoc-DPhe-OH using standard Fmoc methodology and DIC/HOBT activation. The loading of the resins (Fmoc-DPhe-Arg-2-Nal-**Z**-resin) was determined by Fmoc cleavage/UV study to be in the range of 0.30–0.35 mmol/g. Trifluoroacetic acid (TFA) cleavage of small samples of all eleven resins showed the desired products (Fmoc-DPhe-Arg-2-Nal-**Z**-NH_2) by LC/MS analysis. A unique radiofrequency tag was added to each of 165 Macrokans™. Using the Irori Synthesis Manager program, a 2-step 11×15 library was generated and this program was used to manage the work flow of the subsequent steps. Approximately 50 mg of Fmoc-deprotected resin $\mathbf{Z}^1$ (H_2N-DPhe-Arg-2-Nal-$\mathbf{Z}^1$-resin) was loaded into each of 15 encoded Macrokans. Resins $\mathbf{Z}^2$ through $\mathbf{Z}^{11}$ were in turn loaded into the remaining encoded Macrokans. Guided by Synthesis Manager, all the Macrokans (containing resins $\mathbf{Z}^1$ through $\mathbf{Z}^{11}$) were sorted and placed in fifteen reaction vessels containing $\mathbf{Y}^1$ through $\mathbf{Y}^{15}$. $\mathbf{Y}^1$-$\mathbf{Y}^{13}$ were coupled using DIC/HOBT activation, while sulfonyl chloride $\mathbf{Y}^{14}$ and isocyanate $\mathbf{Y}^{15}$ were used in the presence of Hunig's base. Macrokans containing $\mathbf{Y}^1$-$\mathbf{Y}^9$ were pooled for washing,

Fmoc-deprotection, and N-capping with butyric anhydride. Macrokans containing $\mathbf{Y}^{10}$-$\mathbf{Y}^{15}$ were pooled for washing only. The Macrokans were sorted using Synthesis Manager, the resins in each Macrokan were cleaved with 95% trifluoroacetic acid, and the cleavage products were transferred to bar-coded scintillation vials. The above crude peptides were purified using reversed-phase HPLC and the purified samples all had purities >95% by LC/MS analysis.

10. (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.
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. Although Bu-5-BrAtc-DPhe-Arg-2-Nal-$\mathbf{Z}^2$-NH_2 showed 30% activation at hMC4R at 100 nM, it also showed 26% activation at hMC1R at 100 nM and was not further profiled due to poor selectivity. Similarly, Bu-5-BrAtc-DPhe-Arg-2-Nal-$\mathbf{Z}^4$-NH_2 displayed 32% activation at hMC4R and 40% activation at hMC1R at 100 nM and was not further profiled.
13. The diastereomeric mixture of linear peptides containing racemic substituted Atc is 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.
14. For more information about the Quest 210 Synthesizer, see: www.argotech.com.
15. ~200 mg of H_2N-Rink resin (0.51 mmol/g loading, 200-400 mesh) was added to each of twenty Quest 210 teflon reaction tubes. The above tubes were then mounted onto the twenty ports of the Quest 210 Synthesizer (Argonaut Technologies) and the port locations of the tubes served as the identifier of the resins. To the resins (H_2N-resin) were sequentially added amino acids Fmoc-**Z**-OH; Fmoc-Trp-OH; Fmoc-Arg(Pmc)-OH; Fmoc-DPhe-OH and Fmoc-**Y**-OH using standard Fmoc methodology and DIC/HOBT activation. The reagents were added manually *via* syringes while agitation and washings of the resins were automated. After coupling with Fmoc-**Y**-OH, the resins were Fmoc deprotected and N-capped with either $\mathbf{X}^1$ and $\mathbf{X}^2$ in the presence of Hunig's base or $\mathbf{X}^3$ using DIC/HOBT activation. The resins in each tube were cleaved with 60% trifluoroacetic acid in methylene chloride (with a small amount of water and triethylsilane to suppress reduction of Trp) and the cleavage products were transferred to bar-coded scintillation vials. The above crude peptides were purified using reversed–phase HPLC and were all judged to be >95% pure by LC-MS analysis. One peptide, $\mathbf{X}^2$-Apc-DPhe-Arg-Trp-$\mathbf{Z}^{10}$-NH_2, was lost during purification and was not re-synthesized.