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(54) **Title:** NOVEL MODULATORS OF MELANOCORTIN RECEPTORS

(57) **Abstract:**

NOVEL MODULATORS OF MELANOCORTIN RECEPTORS

CROSS REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/017,137 filed June 25, 2014, and claims priority to U.S. Patent Application No. 14/300,991 filed on June 10, 2014, the specification(s) of which is/are incorporated herein in their entirety by reference.

GOVERNMENT SUPPORT

[0002] This invention was made with the government support under Grant Nos. R01 DK 017420 and R01 GM108040 awarded by the national institutes of health (NIH). The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates to a series of N-methylated variations on a cyclic peptide, in particular to a series of N-methylated variations on the SHU9119 peptide. Methyl groups are added to a variety of places on a parmacophore of a base compound, and the agonizing and antagonizing properties of the resulting compounds are examined.

BACKGROUND OF THE INVENTION

[0004] Melanocortin receptors, MC1-5R are a family of five receptor compounds that have varying effects related to melanin. All, but melanocortin-2, is capable of being activated by different types of melanocyte stimulating hormone (Yang). Prior to the invention, it has been difficult to target the receptors, except for MC2R, independently of one another. The key difference between this compound and similar compounds is that the present invention is specific to individual types of melanocortin receptors (Grimes, et. al). Each methylated form of the invention grants a different effect to certain melanocortin receptors, either agonizing them or antagonizing them. This would potentially reduce the amount of side effects incurred by the treatments involving the present invention.

[0005] The melanocortin system[1-3] remains a challenging target for rational peptide and peptidomimetic design as the 3D-topographical requirements for specific melanocortin receptor subtype recognition have not been fully elucidated.[4-7]

Nevertheless, the numerous multifaceted physiological functions of the five known subtypes of human melanocortin receptors (hMC1-5R), including skin pigmentation,[8,9] control of the immune system,[10,11] erectile function,[12,13] blood pressure and heart rate,[14,15] control of feeding behavior and energy homeostasis,[3,6,16-21] modulation of aggressive/defensive behavior,[22,23] and mediation of pain,[24-26] continue to provide a strong stimulus for further development of potent and selective melanocortin agonists and antagonists.

[0006] Until recently, much of this work was focused on the hMC4R due to its direct involvement in the regulation of feeding behavior and energy homeostasis,[3,6,16-21] as well as sexual behavior.[1,6,12,13,27] The hMC3 receptor has been suggested to play a complementary role in weight control.[20,21,28] Although the full scope of physiological functions of this receptor is yet to be unraveled, the current understanding based on the observed stimulation of food intake by peripheral administration of an MC3R-selective agonist[29] and MC3R agonist-induced inhibition of spontaneous action of Pro-opiomelanocortin (POMC) neurons[30] suggests that hMC3R is an inhibitory autoreceptor on POMC neurons.[3] In addition, development of selective ligands for the hMC1 and hMC5 receptors is receiving increasing attention lately due to the roles of these receptors in regulation of pain, skin pigmentation,[6,7] control of the immune system (hMC1R)[8,11] and; in regulating exocrine gland function[31] and coordinating central and peripheral signals for aggression (hMC5R).[22,23]

[0007] On the other hand, development of selective ligands to melanocortin system bears intrinsic challenges due to conserved amino acid sequences and their structural similarity contained in the 7 transmembrane GPCR fold.[1-3,32,33] Unlike other protein targets, hMCRs, known to be the smallest GPCRs have separate natural agonist and antagonist molecules for functional regulation.[1-3,32,33] This aspect of hMCRs imposes a second dimension on melanocortin ligands for achieving selectivity not only to receptor subtype but also between the required agonistic and antagonistic properties. Designing such molecules which possess both functional selectivity and hMCR subtype selectivity from a four residue hMCR recognition sequence[34,35] His-Phe-Arg-Trp is very demanding and necessitates to experiment with every possible molecule designing tool in peptide chemistry.

[0008] Great efforts have been made in the last decade to develop selective melanotropin peptides by following various general approaches,[36-39] which include (a) D-amino acid scan/unnatural amino acid substitutions in linear α-, β- and γ-melanocortin stimulating hormone (MSH)-derived sequences,[40,41] (b) hybridizations of the native MSH sequences with each other and with sequences of other bioactive peptides,[42-44] (c) implementation of various global and local conformational constraints *via* peptide cyclization and employment of constrained amino acids,[45-52] (d) manipulation of steric factors that influence receptor-ligand interaction,[49,53-55] (e) construction of small molecules based on β-turn peptidomimetics and "privileged structure" scaffolds, [55-59] and (f) multiple *N*-methylation.[60]

[0009] Application of several of these strategies has resulted in the development of two cyclic peptides, i. Ac-Nle^4-c[Asp^5, D-Nal (2')^7, Lys^{10}]α-MSH(4-10)-NH$_2$ (SHU9119, D-Nal (2')^7 henceforth is abbreviated as nal^7),[48] a potent agonist for the hMC1R and hMC5R and a potent antagonist for the hMC3R and hMC4R; ii. Ac-Nle^4-c[Asp^5, D-Phe^7, Lys^{10}]α-MSH(4-10)-NH$_2$ (MT-II), a universal agonist for all the hMCRs.[46] The two ligands, although lacking exclusive receptor subtype selectivity, have been extensively used in understanding the combined functional aspects of hMCRs. The complexity of the melanocortin system can be envisaged from the amino acid sequences of SHU9119 and MT-II. Although these two ligands differ in side chain of a single amino acid (nal^7 Vs. D-Phe^7 in SHU9119 and MT-II, respectively) and possess a structural similarity,[61,62] they give rise to entirely contrasting functional responses with melanocortins. The distinctness in activity of these two similar ligands has been attributed mainly to the differences in the disposition of functional side chains around the peptide backbone.

[0010] Noticeable variations in the activity of SHU9119 have been reported with any changes even in the chemical constitution of nal^7 aromatic side chain, signifying the sensitivity of hMCRs towards their ligands.[48] Several amino acid substitution studies have been carried out on MT-II and SHU9119 to modify the structure.[36,63] Replacement of His^6 in SHU9119 with conformationally constrained amino acids resulted in selective agonists for hMC5R and antagonists for hMC3R and hMC4R.[64,65] This lead to the understanding that the conformational restriction at His^6 and around nal^7 may help in receptor discrimination. Among the other studies carried

out, it has also been shown that the activity of melanocortin ligands is sensitive to the side chain χ conformation.[66] Such outcomes suggest melanocortin ligand designs in which side chain disposition is altered while retaining the functional moieties unchanged. Furthermore, a proper insight into the structure driven responses of melanocortin subtypes requires a systematic perturbation of ligand conformation.

[0011] To accomplish this, the N-methylation strategy[67-69] is applied to the melanocortin ligands. Recently, N-methylation of backbone amide NHs of peptides has been shown to substantially improve the physicochemical, structural and biological features of peptides.[67-73] N-methylation stands as the simplest way to include conformational restraints into the peptide backbone as it introduces steric restrictions, allows for cis-peptide bonds, and prevents hydrogen bond formation. As these effects make N-methylated amino acids often act as turn promoting moieties (akin to prolines),[67,73] the strategy helps in generating secondary structural features in peptides without changing the constituent peptide sequence. The specific impact of N-methylation on melanocortin peptide ligands has not been investigated systematically until this strategy is used on MT-II to develop completely selective hMC1R agonists.[60] Interestingly, in contrast to the parent MT-II, some of the N-methylated MT-II peptides possessed antagonistic activity, meaning a switch in functional selectivity while the amino acid sequence remained unchanged.

[0012] Based on these intriguing aspects and being deficient of melanocortin ligands exhibiting improved biological activity and pharmacokinetic profiles, the present invention is considered to modulate the peptide conformation and the functional side chain disposition of SHU9119 peptide by N-methylation of the backbone amide NHs. In search of a more selective melanocortin peptides and also to gain further insight into the intricate correlations among amino acid sequence, conformation and activity-selectivity, a combinatorial library consisting of N-methylated-SHU9119 analogues with single and multiple N-methylation in the core cyclic sequence of SHU9119 has been designed, synthesized and characterized in vitro. Syntheses were performed in analogy to the synthesis of the library of N-methylated MT-II peptides.[60]

[0013] Any feature or combination of features described herein are included within the scope of the present invention provided that the features included in any such

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combination are not mutually inconsistent as will be apparent from the context, this specification, and the knowledge of one of ordinary skill in the art. Additional advantages and aspects of the present invention are apparent in the following detailed description and claims.

SUMMARY OF THE INVENTION

[0014] Melanocortin receptors (hMC1R, hMC3R, hMC4R and hMC5R), due to their structural similarity have been challenging targets to develop ligands that are explicitly selective to each of their subtypes, which is further complicated by the presence of separate biological agonist and antagonist responses for the receptor system demanding for function specific ligands too. To modulate the conformational preferences of the melanocortin ligands and improve the biofunctional agonist-antagonistic activities and selectivities, the present invention applies a backbone N-methylation approach on SHU9119, a non-selective cyclic peptide antagonist at hMC3R and hMC4R. Systematic N-methylated derivatives of SHU9119, with all possible backbone N-methylation combinations have been synthesized and examined for their binding and functional activities towards melanocortin receptor subtypes 1, 3, 4 and 5 (hMCRs).

[0015] Several N-methylated analogues which have selective and potent agonists or antagonists activity for the hMC1R or hMC5R; or selective antagonist activity for the hMC3R are discovered from the library. The selective hMC1R ligands show strong binding for human melanoma cells. The present invention features the first universal antagonist for all subtypes of hMCRs, which will be of critical importance to modulate the melanocortin system with endogenous agonists. Structure Activity Relation (SAR) studies of selective ligands and chimeric human melanocortin 3 receptor lead to new insights for hMC3R selectivity. No correlation could be seen between the SHU9119 and the previous MT-II libraries, with respect to the N-methylation pattern and switching of agonistic or antagonistic activities.

[0016] Conformational studies in solution of the selected ligands have revealed that the peptides have an anti-parallel β-sheet structure and the pharmacophore (His[6]-nal[7]-Arg[8]-Trp[9]) occupies a βII′-turn like region with the turn centered about nal[7]-Arg[8]. The analogues with different selectivities however showed distinct differences in the

spatial arrangement of individual amino acid side chains. The docking studies do not show any interactions between the *N*-methyl groups and the receptors, implying that the role of *N*-methyl groups is primarily to reorganize the conformation of the peptide backbone. The present invention signifies the sensitivity of the melanocortin system to subtle changes in preferred backbone conformation and orientation of the functional groups in ligands to define the agonistic and antagonistic activity for a given peptide sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 shows a structure of SHU9119 peptide. The arrows indicate the sites of *N*-methylation in analogues synthesized with all combinations examined systematically.

[0018] FIG. 2a shows the amino acid sequences for SHU9119 (1) and *N*-methylated SHU9119 analogues (peptides 2-32; with the site of *N*-methylation highlighted in bold font. FIGs. 2b to 2e respectively are histograms showing the results for IC50 from binding assay (black bars) and EC50 from cAMP assay (light grey bars) at hMC1R, hMC3R, hMC4R and hMC5R. For a convenient comparison, the IC_{50} and EC_{50} are plotted in $\log_{10}$ scale with error bars also represented. However, the absolute IC_{50} and EC_{50} values are indicated above each bar. For more numeric details including the binding efficiency and ligand induced functional activity compared to MTII (Act%) are shown in Table 4 and Table 5. IC_{50}= concentration of peptide at 50% specific binding (N=4) and EC_{50} = Effective concentration of peptide that was able to generate 50% maximal intracellular cAMP accumulation (N=4).

[0019] FIGs. 3a to 3c show stereo views of the NMR structure of peptides 15 (3a, pink); 17 (3b, green) and the overlay of the β-turn like His[6]-nal[7]-Arg[8]-Trp[9] region from peptides 15 and 17 (3c). The ribbons along the backbones highlight the secondary structural features. For a clearer view, the non-polar hydrogens in FIG. 3a and 3b, whereas all the hydrogens and main chain in FIG. 3c are not shown.

[0020] FIGs. 4a to 4c shows Ramachandran plots that represent the (Φ,Ψ) dihedral space of constituent amino acids and corresponding secondary structural information, generated for (4a) peptides 15 and 17 based on their NMR structures;

(4b) for peptides MT-II and SHU9119. FIG. 4c is a graph showing the site specific variations in the dihedral space of the above four peptides.

[0021] FIG. 5a shows ligands in contact with residues from the chimeric hMC3R. 1) F295 is contacted by MT-II in >80% of results, with less than 60% in SHU9119 /Peptide 17 and about 20% in Peptide 15, indicate that F295 is important for the agonist activity of hMC3R. 2) D154/D158 are contacted >83%, but almost 100% by each ligand, indicate that D154/D158 are important for the binding to the hMC3R; 3) H298 abolishes binding affinity in bioassay, but highest contact rate is 40%, usually closer to 20%. 4) E131 may play more important role in SHU9119 binding with 50-60 contact rates, whereas in MT-II has the lowest rate (35%). D-Phe-Arg-Trp is essential to binding with highest contact % rates. FIG. 5b shows docking results on interaction of peptide ligands with hMC3R showing the amino acid wise cumulative contacts with residues of the hMC3R. Arg[8] has the highest contacts at hMC3R with nearly all four peptides except SHU9119. D-Nal(2')[7] show more contacts at hMC3R compared to the rest of the three peptides since it is agonist of hMC1R and hMC5R; antagonist at hMC3R and hMC4R. Trp[9] also has very high contacts (>40) at hMC3R (SHU9119, peptide 15 and 17) except for universal agonist MT-II, indicating that Trp[9] plays an important role of the hMC3R antagonist activity.

[0022] FIG. 6 shows docking results showing the peptide ligands binding with hMC3R. Six potential binding sites of hMC3R (E131, D154, D158, L165, F295, H298) are chosen to interact with the peptide ligands. Cluster represents unique docked conformation for two sets of docking runs for each ligand. Two runs multiplied by nine clusters equals18 total conformations.

[0023] FIG. 7 shows names of the dihedral angles for the Lys10-Asp5 side chain cyclized region (as shown for peptide 17) of SHU9119.

[0024] FIG. 8a shows histograms showing the backbone dihedral angle distribution in fMD (with restraints) trajectory of 15. The vertical red lines in each graph indicate the corresponding dihedral angle values as measured from the input DG structure. FIG. 8b shows histograms showing the backbone dihedral angle distribution in fMD

(without restraints) trajectory of 17. The vertical red lines in each graph indicate the corresponding dihedral angle values as measured from the input DG structure.

[0025] FIG. 9a shows overlay of the dihedral angles measured for 15 from its DG structure and the mean structure of the most dominant cluster represented in corresponding MD calculations. FIG. 9b shows overlay of the dihedral angles measured for 17 from its DG structure and the mean structure of the most dominant cluster represented in corresponding MD calculations.

[0026] FIG. 10a shows $\chi 1$ dihedral angles distribution for side chains of peptide 15 as calculated from the corresponding MD trajectory. FIG. 10b shows $\chi 1$ dihedral angles distribution for side chains of peptide 17 as calculated from the corresponding MD trajectory.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0027] Referring now to FIGs. 1- 10, the present invention features a backbone *N*-methylation approach on SHU9119, a non-selective cyclic peptide antagonist at hMC3R and hMC4R. Systematic *N*-methylated derivatives of SHU9119, with all possible backbone *N*-methylation combinations have been synthesized and examined for their binding and functional activities towards melanocortin receptor subtypes 1, 3, 4 and 5 (hMCRs). Each methylated form of the present invention grants a different effect to certain melanocortin receptors, either agonizing them or antagonizing them. This would potentially reduce the amount and severity of side effects that treatments involving the invention would incur.

[0028] As defined herein, the term "agonist" refers to compound that enhances a response. The agonist binds to the same site as the endogenous compound and produces the same type of signal, usually of equal or greater magnitude than the endogenous agent.

[0029] As defined herein, the term "antagonist" refers to compound that diminishes a response. The antagonist binds to the same site as the endogenous compound and diminishes or blocks the signal generated by the endogenous agent.

[0030] As defined herein, the term "*N*-methylation" refers to a form of alkylation wherein a methyl group, CH_3, replaces the hydrogen atom of the NH moiety in an amino acid.

[0031] As defined herein, the terms "therapeutically effective amount" means the amount of a compound that, when administered to a mammal for treating a condition, is sufficient to effect such treatment for the condition. The "therapeutically effective amount" will vary depending on the compound, the condition and its severity and body factors such as age, weight, etc., of the mammal to be treated.

[0032] As defined herein, the terms "Treating" or "treatment" of a condition includes: (1) preventing the condition, i.e., causing the clinical symptoms of the condition not to develop in a mammal that may be exposed to or predisposed to the condition but does not yet experience or display symptoms of the condition; (2) inhibiting the condition, i.e., arresting or reducing the development of the condition or its clinical symptoms; or (3) relieving the condition, i.e., causing regression of the condition or its clinical symptoms.

[0033] As used herein, the terms "those defined above" and "those defined herein" when referring to a variable incorporates by reference the broad definition of the variable as well as any narrow and/or preferred, more preferred and most preferred definitions, if any.

[0034] As used herein, the term "*NMe*" preceding any three-letter abbreviation for an amino acid, i.e. (*NMe*)Lys or (*NMe*)Trp, denotes the *N*-methylated form of the amino acid. As used herein, the term "Nle" refers to a Norleucine.

[0035] Methods described herein may be equivalently represented in a Swiss-type format. As a non-limiting example, "a method for treating a disease Y using compound X" may have a Swiss-type equivalent of "the use of compound X in curing disease Y". It is to be understood that other Swiss-type formats may be acceptable.

[0036] The specification(s) of U.S. Patent Application No. 14/300,991 filed on June 10, 2014 is/are incorporated herein in their entirety by reference. For example, paragraphs [00100] to [00186] in pages 25-49 of the specification are incorporated

herein in their entirety by reference. Moreover, any of the peptide-ligands from SEQ. ID NO. 4, SEQ. ID NO. 5, SEQ. ID NO. 9, SEQ. ID NO. 11, SEQ. ID NO. 18, and SEQ. ID NO. 19 of U.S. Patent Application No. 14/300,991 are incorporated herein by reference. For example, SEQ. ID NO. 18, and SEQ. ID NO. 19 of U.S. Patent Application No. 14/300,991 are incorporated herein by reference: H-Tyr-Val-Leu-Gly-His-DPhe-Arg-DNal(2')-Asp-Arg-Phe-Gly-NH$_2$ (SEQ ID NO:18) and H-Tyr-Val-ILe-Gly-His-DPhe-Arg-DNal(2')-Asp-Arg-Phe-Gly-NH$_2$ (SEQ ID NO:19).

[0037] *N*-methylated derivatives of SHU9119: Peptide Sequence List

[0038] Peptide 1: Ac-Nle-c[Asp-His-nal-Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 1)

[0039] Peptide 2: Ac-Nle-c[Asp-His-nal-Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 2)

[0040] Peptide 3: Ac-Nle-c[Asp-His-nal-Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 3)

[0041] Peptide 4: Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 4)

[0042] Peptide 5: Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 5)

[0043] Peptide 6: Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 6)

[0044] Peptide 7: Ac-Nle-c[Asp-His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 7)

[0045] Peptide 8: Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 8)

[0046] Peptide 9: Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 9)

[0047] Peptide 10: Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 10)

[0048] Peptide 11: Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 11)

[0049] Peptide 12: Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 12)

[0050] Peptide 13: Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 13)

[0051] Peptide 14: Ac-Nle-c[Asp-His-(*NMe*)nal-(*NMe*)Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 14)

[0052] Peptide 15: Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 15)

[0053] Peptide 16: Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 16)

[0054] Peptide 17: Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 17)

[0055] Peptide 18: Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 18)

[0056] Peptide 19: Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 19)

[0057] Peptide 20: Ac-Nle-c[Asp-His-(*NMe*)nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 20)

[0058] Peptide 21: Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 21)

[0059] Peptide 22: Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH$_2$ (SEQ. NO. 22)

[0060] Peptide 23: Ac-Nle-c[Asp-His-(*NMe*)nal-(*NMe*)Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 23)

[0061] Peptide 24: Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 24)

[0062] Peptide 25: Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-(*NMe*)Trp-Lys]-NH$_2$ (SEQ. NO. 25)

[0063] Peptide 26: Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-(*NMe*)Arg-Trp-Lys]-NH$_2$ (SEQ. NO. 26)

[0064] Peptide 27: Ac-Nle-c[Asp-His-(NMe)nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂; (SEQ. NO. 27)

[0065] Peptide 28: Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂; (SEQ. NO. 28)

[0066] Peptide 29: Ac-Nle-c[Asp-(NMe)His-(NMe)nal-Arg-(NMe)Trp-(NMe)Lys]-NH₂; (SEQ. NO. 29)

[0067] Peptide 30: Ac-Nle-c[Asp-(NMe)His-(NMe)nal-(NMe)Arg-Trp-(NMe)Lys]-NH₂; (SEQ. NO. 30)

[0068] Peptide 31: Ac-Nle-c[Asp-(NMe)His-(NMe)nal-(NMe)Arg-(NMe)Trp-Lys]-NH₂; (SEQ. NO. 31)

[0069] Peptide 31: Ac-Nle-c[Asp-(NMe)His-(NMe)nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂; (SEQ. NO. 32)

[0070] Embodiment 1: The present invention features a modified melanocortin receptor modulator, the modulator comprising Ac-Nle-c[Asp-His-nal-Arg-Trp-Lys]-NH₂ ("SHU9119"), wherein at least one amino acid within the c[Asp-His-nal-Arg-Trp-Lys] portion of SHU9119 is N-methylated.

[0071] Embodiment 2: The present invention features a method of modulating a melanocortin receptor, said method comprising administering to a mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient for stimulating melanin synthesis in said mammal. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, an hMC5 receptor, or a combination thereof.

[0072] Embodiment 3: The present invention features a method of modulating a melanocortin receptor for regulating a weight of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to bring about a determined satiation. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the melanocortin receptor is an hMC3 receptor, an hMC4 receptor, or a combination thereof.

[0073] Embodiment 4: The present invention features a method of modulating a hMC4 receptor for stimulating sexual functions of a mammal, said method

comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to bring about an enhanced and sustained sexual arousal. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal.

[0074] Embodiment 5: The present invention features a method of modulating a melanocortin receptor for regulating pain sensed by a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to bring about sufficient pain relief. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

[0075] Embodiment 6: The present invention features a method of modulating a melanocortin receptor for regulating skin pigmentation of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to bring about a desired skin coloration. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

[0076] Embodiment 7: The present invention features a method of modulating the hMC1 receptor for controlling the immune system of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to bring about stimulation of an immune response. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal.

[0077] Embodiment 8: The present invention features a method of modulating a melanocortin receptor for treating atherosclerosis in a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to reduce atherosclerotic plaque inflammation and to improve vascular endothelial function. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, or a combination thereof.

[0078] Embodiment 9: The present invention features a method of modulating a melanocortin receptor for treating skin cancer in a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to prevent proliferation of cancer cells. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the skin cancer is caused by over-exposure to ultraviolet radiation. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

[0079] Embodiment 10: The present invention features a method of modulating a melanocortin receptor for treating a skin disease in a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Embodiment 1 in a therapeutically effective amount sufficient to bring about a prevention of symptoms or a cessation of the skin disease. In some embodiments, administration of the modified melanocortin receptor modulators is by oral, nasal, or topical means, or by injection to the mammal. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof. In some embodiments, the skin disease is a skin pigmentation disorder.

[0080] Embodiment 11: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a

melanocortin receptor for stimulating melanin synthesis. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, an hMC5 receptor, or a combination thereof.

[0081] Embodiment 12: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a melanocortin receptor for regulating weight by bringing about a determined satiation. In some embodiments, the melanocortin receptor is an hMC3 receptor, an hMC4 receptor, or a combination thereof.

[0082] Embodiment 13: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating an hMC4 melanocortin receptor for stimulating sexual functions by bringing about an enhanced and sustained sexual arousal.

[0083] Embodiment 14: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a melanocortin receptor for regulating pain by bringing about sufficient pain relief. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

[0084] Embodiment 15: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a melanocortin receptor for regulating skin pigmentation by bringing about a desired skin coloration. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

[0085] Embodiment 16: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating an hMC1 receptor for controlling an immune system by bringing about a stimulation of an immune response.

[0086] Embodiment 17: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a melanocortin receptor for treating atherosclerosis by reducing atherosclerotic plaque

inflammation and improving vascular endothelial function. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, or a combination thereof.

[0087] Embodiment 18: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a melanocortin receptor for treating skin cancer by preventing proliferation of cancer cells. In some embodiments, the skin cancer is caused by over-exposure to ultraviolet radiation. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

[0088] Embodiment 19: The present invention features the use of the modified melanocortin receptor modulator according to Embodiment 1 for modulating a melanocortin receptor for treating a skin disease by bringing about a prevention of symptoms or a cessation of the skin disease. In some embodiments, the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof. In some embodiments, the skin disease is a skin pigmentation disorder.

[0089] In some embodiments, the modulator according to Embodiment 1 is a member selected from a group consisting of the following N-methylated derivatives of SHU9119:

(SEQ. No. 3)

Ac-Nle-c[Asp-His-nal-Arg-(NMe)Trp-Lys]-NH$_2$;

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-Trp-(NMe)Lys]-NH$_2$;

(SEQ. No. 9)

Ac-Nle-c[Asp-His-(NMe)nal-Arg-Trp-(NMe)Lys]-NH$_2$;

(SEQ. No. 10)

Ac-Nle-c[Asp-(NMe)His-nal-Arg-Trp-(NMe)Lys]-NH$_2$;

(SEQ. No. 13)

Ac-Nle-c[Asp-(NMe)His-nal-Arg-(NMe)Trp-Lys]-NH$_2$;

(SEQ. No. 15)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-Trp-Lys]-NH$_2$;

(SEQ. No. 16)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH$_2$;

(SEQ. No. 17)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 19)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 28)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$; or

(SEQ. No. 32)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$.

[0090] In some embodiments, a selective hMC1 receptor agonist is a member selected from a group consisting of the following *N*-methylated derivatives of SHU9119:

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 10)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 15)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-Lys]-NH$_2$; or

(SEQ. No. 28)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$.

[0091] In some embodiments, the highly selective hMC1 receptor antagonist is:

(SEQ. No. 32)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$.

[0092] In some embodiments, the selective hMC3 receptor antagonist is a member selected from a group consisting of the following *N*-methylated derivatives of SHU9119:

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 15)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-Lys]-NH$_2$; or

(SEQ. No. 17)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$.

[0093] In some embodiments, a highly selective hMC5 receptor agonist is:

(SEQ. No. 3)

Ac-Nle-c[Asp-His-nal-Arg-(*NMe*)Trp-Lys]-NH$_2$.

[0094] In some embodiments, a highly selective hMC5 receptor antagonist is a member selected from a group consisting of the following *N*-methylated derivatives of SHU9119:

(SEQ. No. 9)

Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH$_2$; or

(SEQ. No. 13)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-Lys]-NH$_2$.

[0095] In some embodiments, a selective universal antagonist for an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, or an hMC5 receptor is a member selected from a group consisting of the following *N*-methylated derivatives of SHU9119:

(SEQ. No. 16)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH$_2$; or

(SEQ. No. 19)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$.

[0096] **SAR studies of *N*Me-SHU9119 analogues**

[0097] Effects of *N*-methylation on SHU9119 were evaluated on stably cloned hMCRs (hMC1R, hMC3R, hMC4R, and hMC5R) cell lines (HEK 293 cells) via competition binding assays and functional activity assays (cAMP assay). Competitive binding assays with [^{125}I]-[Nle^4,D-Phe^7]-α-MSH (NDP-α-MSH) on whole cells, and [^{3}H]-adenylate cyclase on PKA were tested along with the application of standard MT-II molecules (Ac-Nle-c[Asp^5, D-Phe^7, Lys^6]-α-MSH(4-10)NH$_2$ and SHU9119. The results are shown in Figure 2 (also refer to Tables 4 and 5). The pharmacological characterization for the binding efficiencies greater than 70% are defined as competitive binding, binding efficiency less than 70% are non-competitive binding. The pharmacological characterization for the potency of ligand used MTII as a

standard full agonist is Act% equal to 100%. Ligands induced functional activity (cAMP level) compared to MTII generated cAMP are defined as Act%. Act% less than 30% are defined as No Activity; Act% between 30-50% is Partial Antagonist; Act% between 50-80% is Partial Agonist; and Act% greater than 80% is Agonist.

[0098] The first group of peptides are mono-N-methylated derivatives of SHU9119 (peptides 2-6) Figure 1 and 2a. In some embodiments, the competitive binding assays show that the whole profile of the mono-N-methylation at each position of the pharmacophore does not have a remarkable influence on the binding affinity towards hMCRs except for the hMC4R for N-methylation at the position 7 (NMe-nal^7, peptide 5) implying that NMe-nal^7 causes a drastical loss of binding affinity at the hMC4R. In some embodiments, cAMP assays show that peptide 3 with a NMe-Trp^9 lead to a greater loss of functional activity (cAMP activity) at the hMC1R compared to the NMe-nal^7 analogue 5, except at the hMC5R. Therefore, the single N-methylated derivatives of SHU9119 at the Trp^9 position (Peptide 3) leads to a selective agonist for the hMC5R.

[0099] The second group of peptides are di-N-methylated derivatives in the core sequence of SHU9119 (peptides 7-16) Figure 1 and 2a. In some embodiments, the first subgroup is NMe-Lys^{10}+NMeXaa (Peptides 7-10, Xaa implies the rest of the amino acids in the cyclic structure of SHU9119 considered for N-methylation, Figure 1, Figure 2a). In some embodiments, the results show that any site of N-methylation combined with NMe-Lys^{10} (Peptides 7-10, Figure 2) retains the same binding behavior as the first group for the hMC1R, hMC3R, hMC5R and loses the binding affinities 1-2 order of magnitude at the hMC4R; Peptide 9 loss the binding affinities towards all subtypes of melanocortin receptors due to the NMe-nal^7. It is interesting to note that when both the positively charged amino acids (Arg^8 and Lys^{10}) are N-methylated at the peptide bond (peptide 8), the binding affinity for the hMC4R decreases by 3 orders of magnitude but increases at the hMC3R. Therefore, in some embodiments, peptide 8 becomes a potent antagonist of the hMC3R and a potent agonist for hMC1R. In some embodiments, this observation agrees with the previous studies using chimeric human melanocortin 4 receptor and truncation studies demonstrating that Arg^8 is critical for the binding towards the hMC4R.[57,58] In some

embodiments, the *N*-methylation of Arg[8] changed the Φ, Ψ space and reduced the flexibility of the charged side chain group, which in turn reduced the binding affinity towards the receptor. In some embodiments, when *N*Me-nal[7] is included (Peptide 9), binding affinity is drastically lost at the hMC1R, hMC3R, and hMC4R. Hence, in some embodiments, Peptide 9, a very selective antagonist for the hMC5R is discovered.

[00100] The second subgroup of di-*N*-methylated SHU9119 analogues is *N*Me-Trp[9]+*N*MeXaa (peptides 7, 11-13). In some embodiments, peptide 7 retains the same binding affinities and functional activities as the mono-*N*-methylated derivatives of SHU9119. In some embodiments, peptide 11-13 all show antagonist activity based on the EC_{50} and Act%. In some embodiments, in the third subgroup consisting of *N*Me-Arg[8]+*N*MeXaa (peptides 8, 11, 14-15), the combination with *N*Me-Arg[8] shows no significant changes in the binding behavior compared to the mono-*N*-methylated compounds, except for the hMC4R, as mentioned above. cAMP agonist activity at hMC5R is lost when it is combined with *N*Me-His. Interestingly, when *N*Me-Arg[8] is combined with *N*Me-nal[7], the hMC1R, hMC3R, hMC4R all show partial agonistic activities (peptide 14).

[00101] Among the fourth subgroup of *N*Me-nal[7]+*N*MeXaa peptides (peptides 9, 12, 14 and 16), the combination with *N*Me-nal[7] showed a significant loss of the binding at all receptor subtypes. In some embodiments, the fifth and last of the di-*N*-methylated SHU9119 compounds is *N*Me-His[6]+*N*MeXaa (peptides 10, 13, 15 and 16). In some embodiments, when *N*Me-His[6] is involved in di-*N*-methylated analogues, the agonist activity at the hMC5R is diminished compared to the first subgroup. In some embodiments, when the di-*N*-methylated combination of *N*Me-His[6] and *N*Me-Trp[9] is used, the binding affinity is often reduced by one order of magnitude and the functional cAMP activities are lost at the hMC1R as was observed with the mono-*N*-methylated compounds (Peptides 7, 12 and 13). In some embodiments, when two *N*-methylated aromatic residues (*N*Me-His[6] and *N*Me-nal[7], Peptide 16) are used as a combination, all of the agonist activities are lost and most of the binding affinities and cAMP activities are greatly diminished. However, in some embodiments, the combination of *N*Me-His[6] with the positive charged residues *N*Me-Lys[10] and *N*Me-

Arg[6] increased selective agonist activities at the hMC1R (Peptides 10 and 15). In some embodiments, peptide 15 has a 6 fold selective potent antagonist activity at the hMC3R compared to that at hMC4R. In some embodiments, peptide 16 with all full binding efficiency towards all hMCRs is a universal antagonist for all subtype of melanocortin receptors. In some embodiments, the fifth subgroup has also presented a moderate selective antagonist for the hMC5R (Peptide 13). Generally, in some embodiments, when the aromatic amino acids are involved in dimethylation (Peptide 16), most of the binding affinities and cAMP activities are greatly diminished. In some embodiments, peptide 16 is a universal antagonist for the hMCRs.

[00102] The third group of peptides are the tri-N-methylated derivatives in the core sequence of SHU9119 (peptides 17-26) Figure 1 and 2a. In some embodiments, except for a few cases of dramatically increased selectivity, this group of peptides in general have shown significantly reduced binding affinities, which might be due to the hindrance caused by the multiple N-methylations that are involved; but in a few cases, there was dramatic increase in selectivity. In some embodiments, peptide 17 with the N-methylations at the positions of Arg[8]-Trp[9]-Lys[10] lead to selective partial agonistic activity at the hMC4R and selective antagonist activities at the hMC3R (20 times more potent compare to the hMC4R). In some embodiments, the combination of NMe-nal[7], NMe-Trp[9] and NMe-Lys[10] (Peptide 18) show full agonist activity at the hMC1R and partial agonist at the hMC4R. In some embodiments, the combination of NMe-His[6], NMe-Trp[9] and NMe-Lys[10] (Peptide 19) show the good binding to all hMCRs but no activities to all hMCRs. Hence, in some embodiments, Peptide 19 is another potent universal antagonist. In some embodiments, the Peptide 20 also shows almost a universal antagonist activity as well (with very weak activity at the hMC3R).

[00103] In some embodiments, the combination of NMe-nal[7] with positive charged residue NMe-His[6], NMe-Lys[10] (Peptide 22); NMe-nal[7], NMe-Arg[8], NMe-Trp[9] (Peptide 23), showed all functional activities are abolished, and the combination of NMe-His[6], NMe-Trp[9] and NMe-Arg[8] (Peptide 24) showed selective partial agonist activity at the hMC1R and hMC5R. In some embodiments, this is an observation in consistence with earlier MT-II N-methylation studies.[60] In some embodiments, in this group, peptides 19-20 are antagonists or partial antagonist towards all hMCRs with reduced

binding affinities compared to mono-*N*-methylated group of peptides (Peptide 2-6). In some embodiments, peptide 21 retains agonist activity at the hMC5R. It is noticed that, when *N*Me-nal[7] is involved, nearly all of the binding affinities are lowered (Peptides 20, 22, 23, 25, 26).

[00104] The 4th group of peptides has five different tetra-*N*-*methylated* SHU9119 derivatives (peptides 27-31) Figure 1 and 2a. In some embodiments, the studies show that the sites of *N*-methylation, when combined with *N*Me-nal[7], reduce the binding affinity at all hMCRs. In some embodiments, only peptide 28 retains nanomolar binding at all hMCRs while cAMP activity show agonist activities at the hMC1R and hMC5R and antagonist activities at hMC3R and hMC4R, much like SHU9119. In some embodiments, peptide 28 exhibited a 0.5 nM binding to hMC4R, which is at least 16 folds stronger than its binding to other receptors and also the strongest binding exhibited by any other peptide in this library to any of the four discussed receptors.

[00105] In some embodiments, the final penta-*N*-*methylated* compound 32 shows good binding at the hMC1R with an IC_{50} of 68 nM and poor efficacy at the hMC5R (37%); and micromolar binding at the hMC3R and hMC4R. In some embodiments, in the cAMP assay, the analogue was inactive at all hMCRs. Thus, in some embodiments, a highly selective hMC1R antagonist is discovered.

[00106] **Comparison of *N*-methylation on MT-II and SHU9119**

[00107] Earlier *N*-methylation studies on MT-II demonstrated that *N*-methylation of cyclized melanotropins increased the selectivities for hMCRs. For example, *N*-methylation of MT-II lead to the most selective agonist of hMC1R, which is also a specific biomarker for melanoma cells.[60] In some embodiments, the present results showed that *N*-methylation of SHU9119 derivatives also leads to selective agonists and antagonists towards various hMCRs, but with a much broader spectrum of selectivity compared to *N*-methylated MT-II. In some embodiments, the present invention features selective hMC1R agonists (peptide 8, 10, 15), and a most selective highly potent hMC1R agonist (peptide 32); selective antagonists for the hMC3R (peptide 8, 15, 17), and highly selective hMC5R agonists (peptide 3) and antagonists (peptide 9 and 13). Finally, in some embodiments, the present invention

features two unique universal antagonists (peptide 16 and 19) of all four hMCRs which is an important advancement for examining the biological roles of the endogenous agonist MSH system.

[00108] Direct comparison of binding and activity values, respectively, for the same methylation patterns shows that N-methylation influences structural changes of MT-II and SHU9119 in different ways. Influencing both compounds in a parallel manner leading to similar changes in binding and activity properties for each methylation pattern is probably not to be expected because the direct difference between MT-II and SHU9119 involves substitution of D-Phe^7 by a bulky nal^7. Since the bulky amino acid D-Nal(2') is presented in SHU9119 but not in MT-II, any N-methylation on these aromatic amino acids and combination with other N-methylated amino acids might have induced different conformational changes, which can dramatically change binding and functional selectivity properties. Introducing N-methylation on amide NHs of aromatic amino acids can be expected to re-orient the backbone conformation and side chain stacking (aromatic groups), all of which are important factors for receptor subtypes selectivity in the melanocortin receptors.

[00109] The following discussion is presented for purposes of illustration and description and is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate,

[00110] **Binding Assays of Selective hMC1R Ligands at Human Melanoma Cells**
[00111] The above discussed SAR studies revealed that peptides 15 and peptide 28 are quite good selective agonists of the hMC1R. The present invention further investigates their binding and cAMP activities in human melanoma cells (A375). As shown in Table 1, these two peptides have also shown nanomolar binding affinities for the A375 melanoma cell.

Table 1. Binding and cAMP activities of selected peptides at human melanoma cells (A375).

Peptide	Sequence	IC₅₀	Binding Efficiency	EC₅₀	Act %
Peptide 15	Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-Lys]-NH₂	14.8±2	100	22 ± 2.5	NA
Peptide 28	Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂	8.7±1	100	20±2	NA
MT-II	Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH₂	6.7 ± 2.9	100 ± 10	110 ± 0.6	100
SHU9119	Ac-Nle-c[Asp-His-nal-Arg-Trp-Lys]-NH₂	100 ± 96	80 ± 34	3.3 ± 2.6	NA

[00112] NMR Conformational Studies

[00113] To understand the conformational changes brought in by the *N*-methylation of SHU9119 and for a better interpretation of the different SAR (structure-activity relationship) between hMC3R and hMC4R, two representative peptides, 15 and 17 which both are antagonists at the hMC3R, selective agonist of hMC1R for peptide 15; selective partial agonist of hMC4R for peptide 17, are selected for structural characterization by NMR and MD. Thus obtained structural information has been further used for docking studies with hMC3R homology model.

[00114] NMR studies for the two peptides have been carried out in DMSO-d_6 solvent. The corresponding NMR spectroscopic structures in solution have been calculated by using distance geometry (DG)[74] program with NOE derived distances and $^3J_{H}$-coupling constant supported dihedral angles as structural restraints. In addition, the obtained structures are further analyzed by free or restrained (using the above derived NMR restraints) simulated annealing molecular dynamics (MD) calculations on GROMACS program[75-77] by using the DG derived NMR structure as the starting conformation. The analysis resulted in a preferred backbone conformation (Figure 3) for each of the peptides exempting the side chain cyclized region consisting of the Lys^{10}-Asp^4 residues.

[00115] A single set of ^1H chemical shift resonances (in DMSO-d_6 at 300K) with explicit ^3J_H multiplicities and a good dispersion of backbone NH and Hα chemical shifts observed for these two peptides in the respective NMR spectra are indicative

of a single predominant conformation and absence of slowly exchanging conformations on the time scales of NMR.[78] The final structures for both the peptides presented a *trans* configuration for all the peptide bonds (Figure 3). Temperature coefficients ($\Delta\delta/\Delta T$); calculated for the backbone NHs over a temperature range of 295 K to 315 K, Table 8) which are in the order < -3.00 ppb/K for all the NHs in both the peptides suggests their solvent accessibility and non-involvement in strong hydrogen bonding.

Table 2. The (Φ,Ψ) dihedral angles at each of the constituent amino acids as measured from the NMR structures of the following peptides.

Compound	Nle[4]		Asp[5]		His[6]		D-Phe[7]/nal[7]	
	Φ	Ψ	Φ	Ψ	Φ	Ψ	Φ	Ψ
Peptide 15	-85.7	122.3	-78.1	113.1	-119.5	36.4	103.4	-84.9
Peptide 17	152.9	52.1	96.3	164.4	-78.7	51.9	104.1	-155.0
MTII	-111.0	131.0	-85.0	113.0	-108	109.0	84.0	0.0
SHU9119	-109.0	163.0	-155.0	179.0	-90.0	49.0	82.0	-6.0
MTII(NMe)	-101.0	109.1	71.4	143.7	-97.8	78.4	96.1	-125.9

Table 2. *continued*

Compound	Arg[8]		Trp[9]		Lys[10]	
	Φ	Ψ	Φ	Ψ	Φ	Ψ
Peptide 15	-112.8	28.4	-90.3	6.5	-69.2	-52.8
Peptide 17	-107.4	88.9	-144.0	51.7	-169.5	69.3
MTII	-122.0	90.0	-77.0	108.0	-101.0	103.0
SHU9119	-99.0	117.0	-79.0	111.0	-90.0	-68.0
MTII(NMe)	-135.2	80.4	-119.8	62.6	-113.7	0.4

[00116] For a comparative analysis of the conformations of 15, 17 and MT-II and SHU9119 related to α-MSH, the (Φ,Ψ) dihedral space of all the peptides are represented as Ramachandran plots[79] (Figure 4). Exempting the Φ-Nle[4], Ψ-Nle[4], and Φ-Asp[5] which are external to the cyclic core of the peptides, the (Φ,Ψ) dihedral space for the cyclic region have mostly occupied the (-+ quadrant) of the Ramachandran plot corresponding to the β-sheet population. The (Φ,Ψ) angles for

nal[7] residues fall in (+- quadrant) diagonally opposite to the (-+ quadrant) which is meaningful considering its opposite stereo configuration. Although the (Φ,Ψ) distribution for basic MT-II and SHU9119 peptides is more centered in the β-sheet region, a downward shift of the overall distribution towards the overlapping β-sheet and α-helical regions is observed for 15 and 17. None of the sequential (Φ,Ψ) pattern matched perfectly with any of the standard β-turn types.[80,81] However, the dihedral angles $(\Phi,\Psi) = (-78.7, 51.9)$ for His[6] in peptide 17 are characteristic of an inverted γ-turn centered around it which facilitates for nal[7]-NH-Asp[5]-CO hydrogen bonding. In support of this, a temperature coefficient of -3.00 ppb/K for nal[7]-NH signifies a lower solvent accessibility and participation in a moderate hydrogen bonding. In contrary, the specific turn structure is disrupted in 15 as evident from the $(\Phi,\Psi) = (-119.5, 36.4)$ about His[6]. This might be due to the N-methylation of the His[6]-NH which brings in steric restriction factors and prevents an orientation of Asp[5]-His[6] peptide bond that is appropriate for nal[7]-NH-Asp[5]-CO hydrogen bonding. In agreement is the temperature coefficient (-4.20 ppb/K) for nal[7]-NH in 15 proving its higher solvent accessibility in comparison to the corresponding nal[7]-NH in 17.

[00117] It can be stated that the amino acid stretch His[6]-nal[7]-Arg[8]-Trp[9] is forming a β-turn like structure (rather than a loop which is often constituted by more amino acids) in both the peptides with a turnabout at nal[7]-Arg[8]. The turn residues are flanked by His[6] and Trp[9] amino acids on either side of the turn and are facing opposite to each other. The nucleation of turn around nal[7]-Arg[8] can be explained by the conformational preference in general of consecutively located D-configured nal[7] and N-methylated Arg[8] amino acids. Corresponding dihedral space for the turn region in both the peptides can be noted from the Table 2 which are close to type II' β turn observed for D-Pro-L-Pro standard $[(\Phi_{i+1},\Psi_{i+1}, \Phi_{i+2},\Psi_{i+2}) = (60°, -120°, -80°, 0)]$.[82,83] A separation of Cα atoms of His[6] and Trp[9] by 6.12 Å and 7.17 Å, respectively in 15 and 17 is in agreement with general definition of turns and supports the current structure.[80,81]

[00118] In both peptides, the backbone region opposite to the nal[7]-Arg[8] is comprised of the side chains of Asp[5]-Lys[10], which are linked via side chain cyclization. However, the distance between Cα atoms of Asp[5] and Lys[10] is measured to be 6.14

Å and 7.56 Å in 15 and 17, which is similar to the distance between Cα atoms of His[6] and Trp[9] in respective peptides. This suggests the propagation of nal[7]-Arg[8] into an anti-parallel β-sheet structure along the His[6] to Asp[5] and Trp[9] to Lys[10] on either side. However, a shorter distance of ~ 6 Å between the Cα atoms of oppositely located amino acids in 15 compared to ~7 Å in 17 implies the constrained nature of the former structure. Apart from this, an increase in Cα separation from His[6] and Trp[9] to Asp[5] and Lys[10] in 17 implies the broadening of the structure along the length. This increase in girth may be is a consequence of the steric restrictions imposed by the three consecutive N-methyl groups in sequential amino acids namely Arg[8], Trp[9] and Lys[10] of 17. The orientation of NMe in Arg[8] is fixed by the conformational preorganization of turn forming nal[7]-Arg[8] moiety whereas the arrangement of NMe of Trp[9] into the cyclic core is necessitated both by the turn and the steric requirements from the NMe of Lys[10]. The absence of NMe on Trp[9] for 15, therefore resulted in a narrower structure.

[00119] It is interesting to note the interplay of aromatic and Van der Waals interactions in the nal[7]-Arg[8]-Trp[9] segment of 17. A long range π-π face to face aromatic interaction between the naphthyl group of nal[7] and the indolyl group of Trp[9] is directly evidenced by the strikingly very low chemical shift (1.989 ppm), for the N-Me on Arg[8], which is directly sandwiched between the mentioned two aromatic groups. The observed chemical shift is so distinct that it is at least 0.7 ppm upfield of the chemical shift for any other NMe in both peptides. In contrast, a normal chemical shift value of 2.868 ppm for NMe of Arg[8] in 15 implies the absence of such aromatic interactions and also different orientations for the corresponding side chains. The overall twist in the backbone conformation of the two peptides leading to a completely different arrangement of functional side chains can be noted from the secondary structure representation in Figure 3c.

[00120] The structures are further validated by MD studies. Though the MD calculations without any restraints have been sufficient to further validate the DG structure for 17, peptide 15 required usage of distance restraints within the cyclic region, which is speculated to be necessitated by the more constrained bent structure obtained for 15 by DG. Analysis of the MD trajectories for inter-proton distances (in Å, Tables 10 and 11) have shown that all the measured distances are

in agreement with those calculated based on the NOE data, except for a very few distances connecting the protons in the *N*-methyl groups, the indole NH of Trp[8] or the Nle[4]-NH. For all the complying cases, the mean value for each inter-proton distance has been measured to be within the ± 10% boundaries of the corresponding distance calculated from NMR and used in DG. The observed violations in the specific cases are attributed to the presence of Nle[4]-NH in a conformationally flexible region and the exchanging nature of the indole NH of Trp[9]. The stability of the backbone conformations for both peptides is further illustrated by the conserved dihedral angle distribution in the respective MD studies (Figures 8a to 9b), which are centered in the vicinity (maximum ±30°) of dihedral angles as measured from the corresponding DG structures. However, as expected, a flexibility in the distribution is observed for the side chain cyclized region.

[00121] The χ_1 rotamer populations for the side chain conformations of all the amino acids are analyzed from the MD trajectories. They are also experimentally deduced based on the $^3J_{H\alpha\text{-}H\beta}$, heteronuclear couplings and NOE data of the stereospecifically assigned β-protons, wherever possible (see Supporting Information).[84] The absence of low $^3J_{H\alpha\text{-}H\beta}$ coupling constants and their sum > 13 Hz in each of the amino acids suggests a complete predominance of χ_1=+60° conformation for L-amino acids and χ_1=-60° conformation for D-amino acids. The discrete $^3J_{H\alpha\text{-}H\beta}$ values (but not with extreme difference of 9 Hz) observed for each residue therefore is a result of population mix mostly of χ_1=-60° (χ_1=+60° for D-amino acids) and χ_1=180° with a relative degree of preference within, which is represented also in the MD data. For Asp[5], the NMR data imply χ_1=180° and χ_1=-60° as the most predominant conformations in 15 ($J_{H\alpha\text{-}H\beta pro\text{-}R}$=4.9 Hz, $J_{H\alpha\text{-}H\beta pro\text{-}S}$=10.2 Hz) and 17 ($J_{H\alpha\text{-}H\beta pro\text{-}R}$=9.1 Hz, $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.1 Hz), respectively, which is also reproduced in the MD data. As Asp[5] side chain is cyclized with Lys[10] side chain, and forms an integral part of the cyclic core, this distinctness for Asp[5] χ_1 conformations in 15 and 17 may have been enforced on it by the conformational requirements of the cyclical core. NMR data for nal[7] (with larger $J_{H\alpha\text{-}H\beta pro\text{-}S}$=9.0 Hz and 8.7 Hz coupling constants in 15 and 17, respectively) predicts χ_1=60° as the major conformation in both the peptides. Although the MD shows a similar population distribution in 15, a higher population of χ_1=180° is shown for 17. Lys[10] in 15 has exhibited a dynamical nature with almost

equally populated χ_1 rotamers in contrast to that in 17 with a strong preference for $\chi_1 = -60°$.

[00122] These varying peptide conformations for peptides 15 and 17, as derived from NMR stand as a proof for the conformational modulation in SHU9119 backbone that is generated by the *N*-methyl groups and are the key to the differential interaction with melanocortin receptor subtypes and corresponding activity responses. The implications of these structural dissimilarities that are responsible for distinct activities at receptors are further accounted by the docking studies.

[00123] The following is a non-limiting example of the present invention, and is presented for purposes of illustration and description, and is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate,

[00124] **Docking Studies of Peptide 15 and Peptide 17**

[00125] To further understand the difference of ligand-receptor interactions between the hMC3R and the hMC4R, docking simulations of MT-II, SHU9119, and peptides 15 and 17 were performed into the homology model of the hMC3R. Docking shows good overall agreement with mutation studies. A previous study from Chen et al. had identified several residues in MC3R that directly affect agonist/antagonist binding and receptor activity[85] (Figure 5, 6). Of these residues identified, all but two (D121 and D332) were proximal to the hMC3R binding pocket in the homology model. All clusters (total 18) for each ligand were analyzed to detect contacts within 4.0 Å of the mutated amino acids (Figure 6). Each ligand interacts with D154 and D158, consistent with the study by Chen et al., which showed that the mutation of D154 or D158 leads to dramatically decreased binding affinity and receptor signaling.[85] The docking results also show that D154/D158 have the highest contact within four peptides (MT-II, SHU9119, Peptide 15, Peptide 17) and indicate that D154/D158 are critically important for the ligand binding to the hMC3R. In the majority of cases,

H298 was not in close contact with ligands, which is in contrary to the results by Chen et al. However, slight differences have also been seen between binding of MT-II and the SHU9119 N-methylated compounds: F295 was in contact with MT-II in over 80% of the clusters, whereas less than 60% of SHU9119 and peptide 17 clusters, and about 20% of peptide 15 clusters are in close proximity to F295, which indicates that F295 is important for agonist activity. These results agree with binding affinities of MT-II, SHU9119, peptide 17 and peptide 15 with 2nM, 3.7nM, 9.7nM and 15nM, respectively, at the hMC3R. The gradual decrease in contact rates with F295 at the hMC3R agrees with chimeric receptor studies from Yang's lab[85] as well and imply that F295 is critical for the agonist activity at the hMC3R. In addition, E131 did not play a significant role in MT-II binding (about 35% contact rate) but is observed to be in contact with more than half the clusters of the SHU ligands indicating that E131 is important for the antagonist activities of the SHU9119, peptides 15 and 17.

[00126] Another important aspect from the Chen et al. study is the identification of amino acid residues in the cyclic peptide ligands that were most responsible for binding and activity, showing that the -D-Phe^7-Arg^8-Trp^9- tripeptide was the smallest fragment that could still bind and activate the MC3R.[85] Each of the current ligands confirm this result. D-Phe^7 (or nal in the case of the SHU ligand), Arg^8, and Trp^9 are the residues with the highest average number of contacts (total number of contacts between ligand residue and any atom in MC3R, averaged over eighteen clusters) (Figure 5b). Slight differences exist among the ligands, as Arg^8 has the most ligand-receptor contacts for MT-II, peptide 17, and peptide 15, whereas nal^7 has the highest number of contacts for SHU9119.

[00127] Polar and aromatic interactions play important roles in ligand binding. Inspection of individual ligand-receptor complexes reveals subtle differences for MT-II, SHU9119, peptides 15 and 17. The best docked complex for the agonist MT-II (nM binding affinity (K_i), forms polar contacts with D154 and D158 through the side chains of Arg^8 (Figure 6A). In addition, polar contacts are formed between Q151 and Arg^8 and between D158 and the backbone of D-Phe^7. π-π stacking, which stabilizes interactions between aromatic amino acid side chains[67] are also present in the MT-II-MC3R complex. D-Phe^7 inserts itself most deeply into the MC3R binding pocket, forming a T-shaped stacking with the aromatic side chains of F295, F296, and F318

on TM6 and TM7. In contrast, the antagonist, SHU9119 (nM K_i) is rotated within the MC3R binding pocket. Polar interactions with D154 and D158 are still present, but are with Asp[5] and Arg[8], respectively (Figure 6B). An additional polar contact exists between the carbonyl group of the I217 backbone and the terminal amino group of SHU9119. No penetration into the deepest part of the binding pocket is present with SHU9119, but a T-shaped stacking interaction is formed between nal[7]-F318-Trp[9]. Another possible T-shaped interaction could be formed between nal[7] and either Y310 or Y314, which are located in the extracellular loop 3 (EL3), but its verification has been limited by the AutoDock Vina models which require a rigid protein backbone for the MC3R.

[00128] The methylated analogues of SHU9119, peptide 17 and 15, share some of the characteristic non-bonded interactions present for both MT-II and SHU9119 at the hMC3R. Peptide 17 (nM K_i) forms an extensive network of polar contacts with Q151, D154, and D158 through its Arg[8] side chain (Figure 6C). Additionally, there are also polar contacts existing on that side of the MC3R binding pocket between the backbone of Asp[5] and the side chains of D154 and D158 as well as between the N-terminal carbonyl and the side chain of E131. Similar to MT-II, peptide 17 also wedges the nal[7] residue into the deepest part of the binding pocket, forming a T-shaped stacking interactions with F295 and F296; however, Trp[9] has stacking interactions with F318, similar to SHU9119. Again, Y310 and Y314 could potentially form stacking interactions with Trp[9], but they lie just outside the 5 Å range typical of π-π stacking.

[00129] The poorest binder of the above four is peptide 15, most likely due to its orientation inside the binding pocket, different from that of MT-II, SHU9119, and peptide 17. Polar contacts were again formed with Arg[8], but between the carbonyl backbone and side chains of Q151 and D154 (Figure 6D). Side chain polar contacts were only formed between Arg[8] and D158. S220 also forms a polar contact with the acetyl group of the N-terminus. F318 is close enough to create stacking interactions, but is not orthogonal or parallel to nal[7], a prerequisite for π-π stacking. Finally, Y219 could potentially form stacking interactions with Trp[9], but is rotated away from the binding pocket.

[00130] Lacking selectivity and potency in agonistic or antagonistic properties of ligands for the melanocortin receptor subtypes is still the most difficult hurdle for application of these compounds in medicine. Mono- and multiple backbone N-methylations of SHU9119 peptides were investigated to achieve selective agonists and antagonists for melanocortin receptors via the conformational modulation in peptide backbone that is imparted by N-methyl steric constraints. This also is known to improve the pharmacokinetic profile of peptides in order to be used as drug leads. It turned out that the activity and selectivity profile induced by N-methylation is very different for the SHU9119 compared to a similar approach in MT-II. This is striking as both stem peptides differ only in the exchange of one amino acid (D-Nal[7] instead of D-Phe[7] in MT-II.

[00131] The systematic N-methylation of SHU9119 lead to the most selective antagonist of hMC1R (Peptide 32), which can be used as a powerful inhibitor for the treatment of melanoma. This strategy still retains several hMC1R agonists (peptide 8, 10 and 15) as previously described.[60] In the present invention, selective agonists for hMC5R (peptide 3) and also peptides 9 and 13 showed the most selective antagonistic activity found so far. The most selective antagonist for the hMC3R (peptide 17) known so far can potentially be used to treat obesity and diabetes. Apart from this, peptides 16, 19 are universal antagonist of the hMCR system, which is important to modulate the endogenous agonist MSH system. Molecular docking studies of MT-II, SHU9119, peptide 15 and peptide 17 reveal that Arg[8] is critical for the binding towards D154, D158 at the hMC3R. The docking studies further support that the more the contact of Trp[9] is towards the F295 at the hMC3R, the higher is the agonist activity that can be expected. These results strengthen the earlier discovered D-Trp[8]-γ-MSH as a selective agonist of hMC3R. Furthermore, E131 is seen to be important for the antagonist activities of the SHU9119 as well as of peptides 15 and 17. A complete structural characterization of all the important peptides discussed here for a more detailed correlation of melanocortin ligand conformation to the observed receptor discrimination is in progress, which information shall be crucial in structure based ligand designing for hMCRs.

[00132] Manifold of results obtained from multiply N-methylated SHU9119 peptide along with that from multiply N-methylated MT-II studies helped in understanding the

finer details of the functional properties of melanocortin receptors and the bioactive conformational preferences of the ligands required by them. The studies further highlight the synthetic simplicity and impact of *N*-methylation strategy in fine tuning the conformational preferences of the ligands to achieve the desired biological effects. The studies, in general, anticipate a bright future for peptide chemistry toward the development as peptide therapeutic agents.

[00133] **Experimental Section**

[00134] The following is a non-limiting example of the present invention, and is presented for purposes of illustration and description, and is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate,

[00135] **Materials**

[00136] N^α-Fmoc-amino acids, peptide coupling reagents, Rink amide MBHA resin and solvents were reagent grade and used without further purification unless otherwise specified. These chemicals were obtained from Aldrich, Novabiochem, Iris Biotech GmbH, Merck, NeoMPS, ORPEGEN Pharma and GLS. The following amino acids were used: Fmoc-Lys(Alloc)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-nal-OH, Fmoc-His(Trt)-OH, Fmoc-Asp(OAllyl)-OH and Fmoc-Nle-OH. The polypropylene reaction vessels (syringes with frits) were purchased from B. Braun Melsungen AG. The purity of the peptides was checked by analytical reverse-phase HPLC using an Amersham Pharmacia Biotech Äkta Basic 10F with a ODS-A C18 (120 Å, 5 µm, 250 mm ×4.6 mm) column (Omnicrom YMC Europe GmbH) monitored at 220 and 254 nm and by high resolution mass spectral analysis.

[00137] **Synthesis**

[00138] The SHU9119 analogues were synthesized manually by the Fmoc-SPPS and characterized using methods described in Doedens et al.[60]

[00139] **NMR Conformational Studies**

[00140] For NMR spectroscopic studies, approximately 1.5 to 2 mg of the compound was dissolved in 125 μl of DMSO-$d6$. The required NMR spectra were recorded at 300K on Bruker 500 MHz spectrometer equipped with TXI cryoprobe. 1H-1D, TOCSY, ROESY, HSQC, and HMBC NMR experiments have been acquired for each sample. 1H-selective homonuclear decoupling experiments were carried out to measure 3JH couplings. Furthermore, to estimate the solvent shielding or hydrogen bonding strengths of NH protons, the temperature dependency of NH chemical shifts was studied by acquiring 1H-1D spectra between 295 K and 315 K in steps of 5 K increments. Mixing times of 80 ms and 100 ms were used for TOCSY and ROESY experiments, respectively. HSQC spectra were recorded with a direct proton carbon coupling constant of 140 Hz, and HMBC spectra with a long-range 1H-13C coupling constant of 7 Hz. For HSQC spectra, a 13C composite pulse decoupling was utilized. 8k (except HSQC: 1k) data points were recorded in the direct dimension, 384 and 512 (heteronuclear spectra) in the indirect dimension. For all spectra a 1.5 s relaxation delay was used after every transient. Exponential / square sine window functions were used for spectra apodization.

[00141] **Proton-proton internuclear distances for structure calculation**: ROE cross peaks in corresponding ROESY spectra recorded in various solvents were integrated by using box method in SPARKY software. These integrated volumes of ROE cross peaks were converted to proton-proton internuclear distances by linear approximation method. Thus calculated distances were then relaxed by ±10 to generate upper and lower distance bounds to account for experimental and simulation uncertainties.

[00142] **Distance Geometry (DG) Calculations**

[00143] Metric matrix DG calculations were carried out with a home-written distance geometry program utilizing random metrication. The above calculated experimental distance restraints which are more restrictive than the geometric distance bounds (holonomic restraints) were used to create the final distance matrix. 50 structures were calculated for each system. The structures were then verified and checked for violations if any with respect to the given experimental distance restraint inputs. The structures that best satisfied the distance inputs were then taken forward for MD

simulations.

[00144] **Biological Activity Assays**

[00145] **Receptor Binding Assay.** Competition binding experiments were carried out using both of cloned cell line and melanoma cells (A375, ATCC). The whole HEK293 cells stably expressing human MC1, MC3, MC4, and MC5 receptors as described before. HEK293 cells transfected with hMCRs were seeded on 96-well plates 48 hours before assay (50,000 cells/well). For the assay, the cell culture medium was aspirated and the cells were washed once with a freshly prepared MEM buffer containing 100% minimum essential medium with Earle's salt (MEM, GIBCO), and 25 mM sodium bicarbonate. Next, the cells were incubated for 40 min at 37°C with different concentrations of unlabeled peptide and labeled [^{125}I]-[Nle^4,DPhe^7]-α-MSH (Perkin-Elmer Life Science, 20,000 cpm/well, 33.06 pM) diluted in a 125 μL of freshly prepared binding buffer containing 100% MEM, 25 mM HEPES (pH 7.4), 0.2% bovine serum albumin, 1 mM 1,10-phenanthroline, 0.5 mg/L leupeptin, 200 mg/L bacitracin. The assay medium was subsequently removed, the cells were washed once with basic medium, and then lysed by the addition of 100 μL of 0.1M NaOH and 100 μL of 1% Triton X-100. The lysed cells were transferred to 12×75 mm borosilicate glass tubes, and the radioactivity was measured by a Wallac 1470 WIZARD Gamma Counter. The results are shown in Table **2**.

[00146] **Adenylate Cyclase Assay.** HEK293 cells transfected with human melanocortin receptors were grown to confluence in MEM medium (GIBCO) containing 10% fetal bovine serum, 100 units/mL penicillin and streptomycin, and 1 mM sodium pyruvate. The cells were seeded on 96-well plates 48 hours before assay (50,000 cells/well). For the assay, the cell culture medium was removed and the cells were rinsed with 100 μL of MEM buffer (GIBCO). An aliquot (100 μL) of the Earle's balanced salt solution with 5 nM isobutylmethylxanthine (IBMX) was placed in each well for 1 min at 37°C. Next, aliquots (25 μL) of melanotropin peptides of varying concentration were added, and the cells were incubated for 3 min at 37°C. The reaction was stopped by aspirating the assay buffer and adding 60 μL ice-cold Tris/EDTA buffer to each well, then placing the plates in a boiling water bath for 7 min. The cell lysates were then centrifuged for 10 min at 2,300 x g. A 50 μL aliquot of the supernatant was transferred to another 96-well plate and placed with 50 μL [^{3}H]

cAMP and 100 µL protein kinase A (PKA) buffer in an ice bath for 2-3 hours. The PKA buffer consisted of Tris/EDTA buffer with 60 µg/mL PKA and 0.1% bovine serum albumin by weight. The incubation mixture was filtered through 1.0 µm glass fiber filters in MultiScreen™-FB 96-well plates (Millipore, Billerica, MA). The total [^{3}H] cAMP was measured by a Wallac Micro β- TriLux 1450 LSC and Luminescence Counter (PerkinElmer Life Science, Boston, MA). The cAMP accumulation data for each peptide analogue was determined with the help of a cAMP standard curve generated by the same method as described above. IC_{50} and EC_{50} values represent the mean of two experiments performed in triplicate. IC_{50} and EC_{50} estimates and their associated standard errors were determined by fitting the data using a nonlinear least squares analysis, with the help of GraphPad Prism 4 (GraphPad Software, San Diego, CA). The maximal cAMP produced at 10 µM concentration of each ligand was compared to the amount of cAMP produced at 10 µM concentration of the standard agonist MT-II, and is expressed given as percentage(as % max effect) in the Table 3. The antagonist properties of the lead compounds were evaluated by their ability to competitively displace the MT-II agonist in a dose-dependent manner, at up to 10 µM.

[00147] **Data Analysis.** IC_{50} and EC_{50} values represent the mean of two experiments performed in triplicate. IC_{50} and EC_{50} estimates and their associated standard errors were determined by fitting the data using a nonlinear least squares analysis, with the help of GraphPad Prism 5 (GraphPad Software, San Diego, CA).

[00148] **Homology model** Chain R of the crystal structure of the activated β$_2$-adrenergic receptor (PDB ID 3SN6) was used as the template for secondary structural alignment in constructing the multiple sequence alignment (MSA). Seven other GPCR crystal structure sequences were included in the MSA (β$_1$-adrenergic receptors 2VT4 and 2Y00, A$_2$A receptors 2YDV, 3EML, and 3PWH, and β$_2$-adrenergic receptors 3KJ6 and 3POG). In addition to the sequence from *Homo sapiens* (RefSeq ID NP_063941), MC3R sequences (AFH58736, AAI62747, AFH58735, NP_032587, ACK98821, AFH58734, NP_001020441, AAS66720, AFK25142, ACK98822) from ten other organisms were used. ClustalX[86] was used to generate the initial MSA, and manual alignment to the most highly conserved residue in each transmembrane helix (1.50: Asn93; 2.50: Asp121; 3.50: Arg179; 4.50:

Trp206; 5.50: Met236; 6.50: Pro294; 7.50: Pro333, MC3R numbering) was carried out in SeaView.[87] 3SN6 was also chosen as the template structure for homology model construction. MODELLER[88] was used to generate a set of ten MC3R models. The model that retained alpha-helical structure for all transmembrane helices, as well as the characteristic outward helical tilt and rotation in TM5 and TM6 of activated GPCRs, was selected for further loop refinement. All extracellular loops were simultaneously optimized using MODELLER, and the model chosen for docking studies had all extracellular loops pulled away from the active site. This was chosen because previous studies had shown that extracellular loops had no effect on ligand binding to the MC3R.[89] Cytoplasmic loops were not optimized due to the fact that they are distal from the binding pocket.

[00149] *Ligand construction.* The 2-D MT-II structure was obtained in .sdf format from PubChem[4] and converted to three-dimensions and minimized in Avogadro.[90] SHU9119, peptide **15**, and peptide **17** structures were obtained based on NMR data from the Hruby and Kessler labs.

[00150] *Docking protocol.* Receptors and ligands were prepared for docking runs using the AutoDockTools4 (ADT) graphical user interface.[91] ADT was used to add hydrogen atoms, assign partial charges and atom types, and merge non-polar hydrogen atoms into their respective heavy-atom bonded partners for the MC3R homology model. Default settings were used to define rotatable bonds in each of the ligands to allow flexibility. The AutoDock/Vina plug-in for PyMOL[92] was used to set parameters for the docking search space, which was a cube 33.75 Å in each direction, centered on the MC3R binding pocket.

[00151] AutoDock Vina[93] was used to perform molecular docking of ligands into the MC3R binding pocket. Default settings were used, unless otherwise specified. Each Vina run produced nine clusters, and each ligand was run twice, for a total of eighteen clusters per ligand. Results were visualized in PyMOL using the AutoDock/Vina plug-in and analyzed for protein-ligand contacts with python scripts. AutoDock4[93] was used to calculate the binding energy and inhibition constants of each cluster from Vina.

[00152] **EMBODIMENTS**

[00153] The following are non-limiting embodiments of the present invention, and is presented for purposes of illustration and description, and is not intended to limit the invention to the form or forms disclosed herein.

[00154] Embodiment 1: A modified melanocortin receptor modulator, the modulator comprising Ac-Nle-c[Asp-His-nal-Arg-Trp-Lys]-NH$_2$, wherein at least one amino acid within the c[Asp-His-nal-Arg-Trp-Lys] sequence is *N*-methylated.

[00155] Embodiment 2: The modified melanocortin receptor modulator of Embodiment 1, wherein the modulator is selected from a series of *N*-methylated derivatives of a SHU9119 amino acid chain, said series comprising:

(SEQ. No. 3)

Ac-Nle-c[Asp-His-nal-Arg-(*NMe*)Trp-Lys]-NH$_2$;

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 9)

Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 10)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 13)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-Lys]-NH$_2$;

(SEQ. No. 15)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-Lys]-NH$_2$;

(SEQ. No. 16)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH$_2$;

(SEQ. No. 17)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 19)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$;

(SEQ. No. 28)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$; or

(SEQ. No. 32)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH$_2$.

[00156] Embodiment 3: The modified melanocortin receptor modulator of Embodiment 1, wherein a selective hMC1 receptor agonist is selected from:

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-Trp-(NMe)Lys]-NH₂;

(SEQ. No. 10)

Ac-Nle-c[Asp-(NMe)His-nal-Arg-Trp-(NMe)Lys]-NH₂;

(SEQ. No. 15)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-Trp-Lys]-NH₂; or

(SEQ. No. 28)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂.

[00157] Embodiment 4: The modified melanocortin receptor modulator of Embodiment 1, wherein a highly selective hMC1 receptor antagonist is:

(SEQ. No. 32)

Ac-Nle-c[Asp-(NMe)His-(NMe)nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂.

[00158] Embodiment 5: The modified melanocortin receptor modulator of Embodiment 1, wherein a selective hMC3 receptor antagonist is selected from:

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-Trp-(NMe)Lys]-NH₂;

(SEQ. No. 15)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-Trp-Lys]-NH₂;   or

(SEQ. No. 17)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂.

[00159] Embodiment 6: The modified melanocortin receptor modulator of Embodiment 1, wherein a highly selective hMC5 receptor agonist is:

(SEQ. No. 3)

Ac-Nle-c[Asp-His-nal-Arg-(NMe)Trp-Lys]-NH₂.

[00160] Embodiment 7: The modified melanocortin receptor modulator of Embodiment 1, wherein a highly selective hMC5 receptor antagonist is selected from:

(SEQ. No. 9)

Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH₂; or

(SEQ. No. 13)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-Lys]-NH₂.

[0001] Embodiment 8: The modified melanocortin receptor modulator of Embodiment 1, wherein a selective universal antagonist for an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, or an hMC5 receptor is selected from:

(SEQ. No. 16)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH₂; or

(SEQ. No. 19)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂.

[0002] Table 3. HPLC and Mass data for the *N*-methylated SHU9119 peptides. Amino acids with *N*-methyl groups are highlighted in grey.

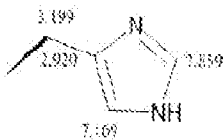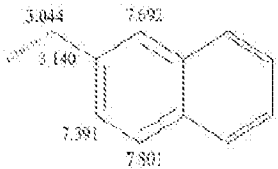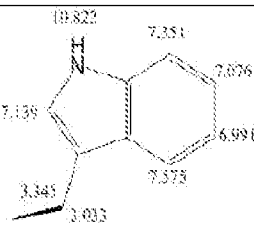| #	Sequence							ESI-MS $(M+H)^+$ [g/mol]	HPLC 10-100% [min]	HPLC 10-50% [min]	mass [mg]
1	Nle	D	H	nal	R	W	K	1074.7	13.58	26.70	5.1
2	Nle	D	H	nal	R	W	K	1088.8	16.16	28.13	5.1
3	Nle	D	H	nal	R	W	K	1088.8	14.80	24.52	5.5
4	Nle	D	H	nal	R	W	K	1088.8	13.71	22.73	5.3
5	Nle	D	H	nal	R	W	K	1088.8	14.07	23.80	5.2
6	Nle	D	H	nal	R	W	K	1089.1	15.82	27.57	5.3
7	Nle	D	H	nal	R	W	K	1102.7	15.86	27.28	5.8
8	Nle	D	H	nal	R	W	K	1102.7	13.89	23.08	5.3
9	Nle	D	H	nal	R	W	K	1102.7	14.43	24.21	5.6
10	Nle	D	H	nal	R	W	K	1102.6	15.71	27.28	5.1
11	Nle	D	H	nal	R	W	K	1102.6	14.02	22.90	5.8
12	Nle	D	H	nal	R	W	K	1102.7	14.09	23.52	5.0
13	Nle	D	H	nal	R	W	K	1102.6	15.52	26.33	5.3
14	Nle	D	H	nal	R	W	K	1102.7	13.93	23.28	5.2
15	Nle	D	H	nal	R	W	K	1102.7	13.93	23.12	5.3
16	Nle	D	H	nal	R	W	K	1102.7	15.25	25.94	5.5
17	Nle	D	H	nal	R	W	K	1116.7	13.80	22.75	5.6
18	Nle	D	H	nal	R	W	K	1116.7	14.35	24.51	5.3
19	Nle	D	H	nal	R	W	K	1116.7	15.98	27.65	5.2
20	Nle	D	H	nal	R	W	K	1116.7	13.98	23.27	5.3
21	Nle	D	H	nal	R	W	K	1116.7	14.17	23.77	5.2
22	Nle	D	H	nal	R	W	K	1116.7	14.49	24.48	5.7
23	Nle	D	H	nal	R	W	K	1116.7	13.91	23.05	5.3
24	Nle	D	H	nal	R	W	K	1116.7	13.69	22.57	5.8
25	Nle	D	H	nal	R	W	K	1116.7	14.87	25.08	5.2
26	Nle	D	H	nal	R	W	K	1116.7	14.00	23.34	5.6
27	Nle	D	H	nal	R	W	K	1130.7	14.35	24.57	5.4
28	Nle	D	H	nal	R	W	K	1130.7	13.97	22.95	5.4
29	Nle	D	H	nal	R	W	K	1130.7	15.05	25.65	5.5
30	Nle	D	H	nal	R	W	K	1130.8	14.45	24.09	5.7
31	Nle	D	H	nal	R	W	K	1130.7	13.40	22.05	5.5
32	Nle	D	H	nal	R	W	K	1144.7	14.17	23.73	5.4

[0003] Table 4. Binding assay results of *N*-methylated SHU9119 analogues at hMCRs. Amino acids with N-methyl groups are highlighted in grey. IC50 = concentration of peptide at 50% specific binding (N=4). NB = 0% of 125I-NDP-α-MSH displacement observed at 10 μM. Percent Binding Efficiency = maximal % of 125I-NDP-α-MSH displacement observed at 10 μM.

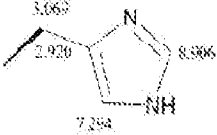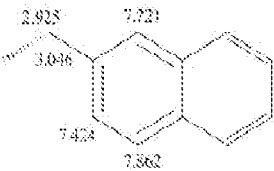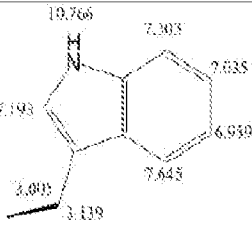| #	sequence							hMC1R IC50,nM	hMC1R Bind-Effic	hMC3R IC50,nM	hMC3R Bind-Effic	hMC4R IC50,nM	hMC4R Bind-Effic	hMC5R IC50,nM	hMC5R Bind-Effic
1	Nle	D	H	nal	R	W	K	8.7±0.9	100	3.74±0.5	97	2.4±0.2	98	6.9±1.2	98
2	Nle	D	H	nal	R	W	K	12.6±1.8	100	23±4	97	18.2±2.1	97	6.1±1.1	98
3	Nle	D	H	nal	R	W	K	192±20	77	20.4±3	77	16.2±2	70	18.8±2.3	77
4	Nle	D	H	nal	R	W	K	1.4±0.15	90	47.4±5.2	85	6.35±1.0	90	3.41±0.8	96
5	Nle	D	H	nal	R	W	K	18.7±0.38	80	27.4±2.1	80	1390±310	90	27.7±3.4	90
6	Nle	D	H	nal	R	W	K	106±21	90	3.2±0.5	90	65.9±19	90	5±0.8	90
7	Nle	D	H	nal	R	W	K	175±20	86	17.8±3	95	111±13	89	15±1.8	94
8	Nle	D	H	nal	R	W	K	3.8±0.5	80	1.8±0.1	90	1010±1.1	90	6±1.0	90
9	Nle	D	H	nal	R	W	K	3110±456	82	795±80	90	1380±110	101	85±10	87
10	Nle	D	H	nal	R	W	K	196±16	90	24.9±2.1	89	134±15	90	13±0.12	90
11	Nle	D	H	nal	R	W	K	111±20	89	19.7±2	93	59.4±8	89	16.46±2.1	93
12	Nle	D	H	nal	R	W	K	916±120	92	412±60	92	32.2±5	88	2280±230	71
13	Nle	D	H	nal	R	W	K	1050±110	88	3860±410	95	244±31	90	81±9	86
14	Nle	D	H	nal	R	W	K	4.8±0.9	90	65±11	90	507±120	90	35.2±5	90
15	Nle	D	H	nal	R	W	K	4.8±0.7	90	15.3±2.8	80	98±4.1	90	6.33±0.9	90
16	Nle	D	H	nal	R	W	K	2520±320	86	547±70	80	1700±340	90	1870±300	90
17	Nle	D	H	nal	R	W	K	246±45	80	9.7±1.3	90	203±7	80	6.06±1	90
18	Nle	D	H	nal	R	W	K	246±24	80	147±23	90	549±155	90	592±70	80
19	Nle	D	H	nal	R	W	K	243±33	74	106±13	90	100±8	79	268±31	91
20	Nle	D	H	nal	R	W	K	256±35	84	8±11	86	722±81	81	39.7±5	90
21	Nle	D	H	nal	R	W	K	16.7±2.2	9	2.5±0.6	94	3.96±1	76	2.82±0.9	90
22	Nle	D	H	nal	R	W	K	824±100	68	13.7±2	38	8740±841	59	388±41	70
23	Nle	D	H	nal	R	W	K	1330±200	70	2220±242	61	89.9±10	36	159±19	67
24	Nle	D	H	nal	R	W	K	9.4±1.2	86	51±9	78	1070±110	76	871±91	84
25	Nle	D	H	nal	R	W	K	1740±210	68	986±110	90	505±111	67	571.5±61	93
26	Nle	D	H	nal	R	W	K	1070±169	95	335±42	82	787±82	68	115±13	92
27	Nle	D	H	nal	R	W	K	1220±123	94	1270±210	92	188±21	37	1510±159	32
28	Nle	D	H	nal	R	W	K	9.8±1.2	93	20±3	88	0.5±0.02	64	8.1±1	89
29	Nle	D	H	nal	R	W	K	1460±163	42	1670±193	97	304±35	27	4290±521	64
30	Nle	D	H	nal	R	W	K	1390±150	87	8050±985	93	1790±190	24	1050±120	24
31	Nle	D	H	nal	R	W	K	1810±210	89	657±79	94	949±85	8	177±21	75
32	Nle	D	H	nal	R	W	K	68.4±8	91	5820±300	84	3140±270	19	257±34	37

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[0004] TABLE 5. cAMP assay results of *N*-methylatedSHU9119 analogues at hMCRs. Amino acids with *N*-methyl groups are highlighted in grey. EC50 = Effective concentration of peptide that was able to generate 50% maximal intracellular cAMP accumulation (*N*=4). Percent Activity = % of cAMP produced at 10 µM ligand concentration, in relation to MT-II. NA = 0% cAMP accumulation observed at 10 µM. The peptides were tested at a range of concentration from 10-10 to 10-5 M.

#	sequence							hMC1R EC50,nM	%max	hMC3R EC50,nM	%max	hMC4R EC50,nM	%max	hMC5R EC50,nM	%max
1	Nle	D	H	nal	R	W	K	14.8±2.0	96	>10000	NA	>10000	NA	7	100
2	Nle	D	H	nal	R	W	K	5.0±0.8	83	>10000	NA	>10000	NA	60±5	100
3	Nle	D	H	nal	R	W	K	>10000	29	>10000	17	>10000	19	189±14	100
4	Nle	D	H	nal	R	W	K	50.8±8	100	>10000	NA	>10000	NA	1060±100	100
5	Nle	D	H	nal	R	W	K	2210±310	100	>10000	NA	>10000	NA	>10000	NA
6	Nle	D	H	nal	R	W	K	115±12	83	>10000	NA	>10000	NA	>10000	100
7	Nle	D	H	nal	R	W	K	36±4	13	163±13	19	152±30	29	1560±210	100
8	Nle	D	H	nal	R	W	K	2.1±0.8	100	>10000	NA	>10000	NA	446±50	100
9	Nle	D	H	nal	R	W	K	563±70	9	709±87	22	2400±290	16	>10000	NA
10	Nle	D	H	nal	R	W	K	315±40	100	>10000	NA	>10000	NA	>10000	NA
11	Nle	D	H	nal	R	W	K	1560±170	64	463±52	19	>10000	19	480±61	100
12	Nle	D	H	nal	R	W	K	1171±21	13	9.92±1	28	264±31	21	>2000	NA
13	Nle	D	H	nal	R	W	K	1160±52	3	16±0.8	34	2100±231	31	>2000	NA
14	Nle	D	H	nal	R	W	K	759	76	13.2±0.9	78	35.5±3.9	73	>10000	NA
15	Nle	D	H	nal	R	W	K	3.7±0.8	96	>10000	NA	>10000	NA	>10000	NA
16	Nle	D	H	nal	R	W	K	>10000	NA	>10000	NA	>10000	NA	>10000	NA
17	Nle	D	H	nal	R	W	K	>10000	NA	>10000	NA	5±0.9	67	>10000	NA
18	Nle	D	H	nal	R	W	K	214±35	100	>10000	NA	197±29	58	>10000	NA
19	Nle	D	H	nal	R	W	K	>3000	13	11.±1	14	9080±110	19	1.1±0.1	30
20	Nle	D	H	nal	R	W	K	>3000	22	1810±190	58	138±15	31	>3000	NA
21	Nle	D	H	nal	R	W	K	23	36	2370±180	35	1570±170	52	239±20	100
22	Nle	D	H	nal	R	W	K	>1000	25	1790±213	34	946±78	28	>5000	NA
23	Nle	D	H	nal	R	W	K	276±32	17	1.47±0.5	19	2180±178	45	>2000	NA
24	Nle	D	H	nal	R	W	K	1970±213	80	802±91	21	1000±120	41	838±90	69
25	Nle	D	H	nal	R	W	K	117±13	46	753±89	30	6590±912	18	62.5±8	40
26	Nle	D	H	nal	R	W	K	366±41	32	1130±134	17	0.8±0.05	55	1680±192	26
27	Nle	D	H	nal	R	W	K	341±91	35	3930±410	29	152±19	46	>10000	NA
28	Nle	D	H	nal	R	W	K	28±3	85	116±41	34	67.8±9	20	0.8±0.1	100
29	Nle	D	H	nal	R	W	K	58±11	52	5180±589	37	840±93	47	1450±14	NA
30	Nle	D	H	nal	R	W	K	273±31	34	770±77	25	1110±141	51	618±83	NA
31	Nle	D	H	nal	R	W	K	679±98	64	1950±200	41	485±57	55	1520±19	NA
32	Nle	D	H	nal	R	W	K	25.7±7	23	1460±150	26	1450±182	50	>10000	NA

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[0005] **Table 6**: Chemical shift assignment of **15** in DMSO-*d*6 at 300 K.

Residue	NH/NMe	Hα	Hβ	Hγ	Hδ	Hε/NHε	Hζ/Others
Nle⁴ (norleucine)	7.869 $J_{NH-H\alpha}$=8.2 Hz	4.211 $J_{NH-H\alpha}$=8.2 Hz $J_{H\alpha-H\beta pro-S}$=4.7 Hz $J_{H\alpha-H\beta pro-R}$=9.3 Hz	1.473 (*pro-S*) 1.373 (*pro-R*) $J_{H\alpha-H\beta pro-S}$=4.7 Hz $J_{H\alpha-H\beta pro-R}$=9.3 Hz	1.252	1.252	0.854 (Hε)	1.886 (protons of N-terminus COMe)
Asp⁵	8.296 $J_{NH-H\alpha}$=7.0 Hz	4.835 $J_{NH-H\alpha}$=7.0 Hz $J_{H\alpha-H\beta pro-R}$=4.9 Hz $J_{H\alpha-H\beta pro-S}$=10.2 Hz	2.797 (*pro-S*) 2.534 (*pro-R*) $J_{H\alpha-H\beta pro-R}$=4.9 Hz $J_{H\alpha-H\beta pro-S}$=10.2 Hz $J_{H\beta pro-R-H\beta pro-S}$=14.8 Hz				
NMe-His⁶	2.978	5.355 $J_{H\alpha-H\beta}$=4.5 Hz $J_{H\alpha-H\beta'}$=11.1 Hz	3.199 (β) 2.920 (β') $J_{H\alpha-H\beta}$=4.5 Hz $J_{H\alpha-H\beta'}$=11.1 Hz $J_{H\beta-H\beta'}$=16.1 Hz				
nal⁷ (D-Naphthyl Alanine)	8.438 $J_{NH-H\alpha}$=8.3 Hz	5.001 $J_{NH-H\alpha}$=8.3 Hz $J_{H\alpha-H\beta pro-R}$=5.7 Hz $J_{H\alpha-H\beta pro-S}$=9.0 Hz	3.140 (*pro-R*) 3.044 (*pro-S*) $J_{H\alpha-H\beta pro-R}$=5.7 Hz $J_{H\alpha-H\beta pro-S}$=9.0 Hz $J_{H\beta pro-R-H\beta pro-S}$=14.1 Hz				

Residue	NH/NMe	Hα	Hβ	Hγ	Hδ	Hε/NHε	Hζ/Others
NMe-Arg⁸	2.868	4.305 (broad)	1.881 (β) 1.660 (β')	1.273 1.181	2.960 $J_{H\delta-H\epsilon}$=5.7 Hz	7.740 $J_{H\delta-H\epsilon}$=5.7 Hz	
Trp⁹	7.602 $J_{NH-H\alpha}$=7.4 Hz	4.427 $J_{NH-H\alpha}$=7.4 Hz $J_{H\alpha-H\beta}$=4.5 Hz $J_{H\alpha-H\beta'}$=8.7 Hz	3.345 (β) 3.033 (β') $J_{H\alpha-H\beta}$=4.5 Hz $J_{H\alpha-H\beta'}$=8.7 Hz $J_{H\beta-H\beta'}$=14.8 Hz		7.139 $J_{H\delta-H\epsilon}$=2.5 Hz	10.822 $J_{H\delta-H\epsilon}$=2.5 Hz	
Lys¹⁰	7.816 $J_{NH-H\alpha}$=7.4 Hz	4.071 $J_{NH-H\alpha}$=7.4 Hz $J_{H\alpha-H\beta}$=4.0 Hz $J_{H\alpha-H\beta'}$=9.5 Hz	1.694 (β) 1.694 (β') $J_{H\alpha-H\beta}$=4.0 Hz $J_{H\alpha-H\beta'}$=9.5 Hz	1.386 1.281	1.476	3.317 (ε) 3.107 (ε') $J_{H\delta-H\epsilon}$=5.7 Hz $J_{H\delta-H\epsilon'}$=5.7 Hz	8.353 $J_{H\epsilon-H\zeta}$=5.7 Hz $J_{H\epsilon-H\zeta'}$=5.7 Hz 7.188 & 6.977 (protons of CONH₂ protection)

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[0006] **Table 7**: Chemical shift assignment of **17** in DMSO-*d*6 at 300 K.

Residue	NH/NMe	Hα	Hβ	Hγ	Hδ	Hε/NHε	Hζ/Others
Nle[4] (norleucine)	7.897 $J_{NH\text{-}H\alpha}$=8.2 Hz	4.301 $J_{NH\text{-}H\alpha}$=8.2 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.0 Hz $J_{H\alpha\text{-}H\beta pro\text{-}R}$=8.6 Hz	1.629 (*pro-S*) 1.449 (*pro-R*) $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.0 Hz $J_{H\alpha\text{-}H\beta pro\text{-}R}$=8.6 Hz $J_{H\beta pro\text{-}R\text{-}H\beta pro\text{-}S}$=13.7 Hz	1.260	1.260	0.869 (Hε)	1.870 (protons of N-terminus COMe)
Asp[5]	8.020 $J_{NH\text{-}H\alpha}$=6.9 Hz	4.545 $J_{NH\text{-}H\alpha}$=6.9 Hz $J_{H\alpha\text{-}H\beta pro\text{-}R}$=9.1 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.1 Hz	2.463 (*pro-R*) 2.352 (*pro-S*) $J_{H\alpha\text{-}H\beta pro\text{-}R}$=9.1 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.1 Hz $J_{H\beta pro\text{-}R\text{-}H\beta pro\text{-}S}$=15.8 Hz				
His[6]	8.528 $J_{NH\text{-}H\alpha}$=8.2 Hz	4.684 $J_{NH\text{-}H\alpha}$=8.2 Hz $J_{H\alpha\text{-}H\beta pro\text{-}R}$=8.6 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.6 Hz	3.069 (*pro-S*) 2.920 (*pro-R*) $J_{H\alpha\text{-}H\beta pro\text{-}R}$=8.6 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=5.6 Hz $J_{H\beta pro\text{-}R\text{-}H\beta pro\text{-}S}$=15.7 Hz				

Residue	NH/NMe	Hα	Hβ	Hγ	Hδ	Hε/NHε	Hζ/Others
nal[7] (D-Naphthyl Alanine)	8.170 $J_{NH\text{-}H\alpha}$=8.2 Hz	4.909 $J_{NH\text{-}H\alpha}$=8.2 Hz $J_{H\alpha\text{-}H\beta pro\text{-}R}$=5.6 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=8.7 Hz	3.046 (*pro-R*) 2.925 (*pro-S*) $J_{H\alpha\text{-}H\beta pro\text{-}R}$=5.6 Hz $J_{H\alpha\text{-}H\beta pro\text{-}S}$=8.7 Hz $J_{H\beta pro\text{-}R\text{-}H\beta pro\text{-}S}$=14.2 Hz				
NMe-Arg[8]	1.989	5.238 $J_{H\alpha\text{-}H\beta}$=7.0 Hz $J_{H\alpha\text{-}H\beta'}$=7.0 Hz	1.383 (β) 1.196 (β') $J_{H\alpha\text{-}H\beta}$=7.0 Hz $J_{H\alpha\text{-}H\beta'}$=7.0 Hz	1.083	2.933 $J_{H\delta\text{-}H\varepsilon}$=6.0 Hz	7.491 $J_{H\delta\text{-}H\varepsilon}$=6.0 Hz	
NMe-Trp[9]	2.677	5.829 $J_{H\alpha\text{-}H\beta}$=4.9 Hz $J_{H\alpha\text{-}H\beta'}$=9.8 Hz	3.139 (β) 3.005 (β') $J_{H\alpha\text{-}H\beta}$=4.9 Hz $J_{H\alpha\text{-}H\beta'}$=9.8 Hz $J_{H\beta\text{-}H\beta'}$=14.8 Hz		7.193 $J_{H\delta\text{-}H\varepsilon}$=2.5 Hz	10.766 $J_{H\delta\text{-}H\varepsilon}$=2.5 Hz	
NMe-Lys[10]	2.633	4.988 $J_{H\alpha\text{-}H\beta}$=12.3 Hz $J_{H\alpha\text{-}H\beta'}$=3.2 Hz	1.896 (β) 1.644 (β') $J_{H\alpha\text{-}H\beta}$=12.3 Hz $J_{H\alpha\text{-}H\beta'}$=3.2 Hz	1.462 1.329	1.220 1.005	3.294 (ε) 2.975 (ε') $J_{H\delta\text{-}H\varepsilon}$=7.3 Hz $J_{H\delta\text{-}H\varepsilon'}$=2.0 Hz	7.885 $J_{H\varepsilon\text{-}H\zeta}$=7.3 Hz $J_{H\varepsilon\text{-}H\zeta'}$=2.0 Hz 7.1379 & 7.3456 (protons of CONH₂ protection)

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[0007] Table 8: Temperature coefficient data of **15** and **17** in DMSO-d6. 1H-1D spectra were recorded for both the compounds at variable temperatures ranging from 295 K to 315 K in 5 K intervals and the temperature coefficients were calculated according to ($\Delta\delta$*1000)/ΔT where in $\Delta\delta$ and ΔT are changes in chemical shift of the corresponding NH and the change in sample temperature, respectively.

Name of the Compound			
15		**17**	
Name of the NH	Temperature Coefficient (ppb/K)	Name of the NH	Temperature Coefficient (ppb/K)
Nle[4]-NH	-3.50	Nle[4]-NH	-4.00
Asp[5]-NH	-6.50	Asp[5]-NH	-6.00
--	--	His[6]-NH	-4.50
Nal[7]-NH	-4.20	Nal[7]-NH	-3.00
Trp[9]-NH	-4.50	--	--
Lys[10]-NH	-3.00	--	--
Lys[10]-Hz	-5.40	Lys[10]-Hz	-3.00

[0008] Table 9: Backbone dihedral angles (in the cyclic core) measured from DG structures of 15 and 17.

Backbone Dihedral Angles in the order 15/17				
Dihedral Angle	NMe-His[6]/ His[6]	Nal[7]	NMe-Arg[8]	Trp[9]/ NMe-Trp[9]
ϕ	-119.5/-78.7	103.4/104.1	-112.8/-107.4	-90.3/-144.0
ψ	36.4/51.9	-84.9/-155.0	28.4/88.9	6.5/51.7

Dihedral Angles for the SHU portion									
SHU1/ NMe-SHU1	SHU2/ NMe-SHU2	SHU3/ NMe-SHU3	SHU4/ NMe-SHU4	SHU5/ NMe-SHU5	SHU6/ NMe-SHU6	SHU7/ NMe-SHU7	SHU8/ NMe-SHU8	SHU9/ NMe-SHU9	SHU10/ NMe-SHU10
167.9/ 63.8	-50.3/ -99.4	171.6/ 159.8	148.6/ 31.5	3.2/ -95.0	83.7/ 175.5	173.6/ 175.5	142.0/ -104.3	54.8/ 124.5	-125.2/ -69.7

[0009] Table 10: List of distance restraints that were used to derive the DG structure of 15 and the corresponding violations in rMD trajectory.

Residue	Atom	Residue	Atom	Upper Bound	Lower Bound	Mean distance (from MD)	Violation (Å)
Nle	Hα	Asp	NH	2,34	1,91	2,20	
Nle	Hα	Asp	Hα	4,82	3,94	4,27	
Asp	Hα	NMe-His	Hα	5,17	4,23	4,35	
Asp	Hα	nal	NH	4,35	3,56	3,99	
Asp	Hα	Lys	Hz	4,09	3,35	3,29	
Asp	Hβpro-R	Asp	NH	2,75	2,25	2,45	
Asp	Hβpro-R	nal	NH	4,7	3,85	4,84	+0,1
Asp	Hβpro-R	Lys	Hz	2,75	2,25	2,20	-0,05
Asp	Hβpro-S	Asp	NH	2,92	2,39	2,47	
Asp	Hβpro-S	nal	NH	4,71	3,85	3,96	
Asp	Hβpro-S	Lys	Hz	3,5	2,86	3,32	
NMe-His	Hα	NMe-His	NMe	4,85	3,64	3,72	
NMe-His	Hα	nal	NH	2,96	2,42	2,61	
NMe-His	NMe	Asp	NH	6,37	4,89	4,97	
NMe-His	NMe	Asp	Hα	3,15	2,25	2,68	
NMe-His	NMe	nal	NH	3,86	2,83	3,58	
NMe-His	NMe	Lys	Hα	5,66	4,3	3,76	-0,6
NMe-His	NMe	Lys	Hz	5,98	4,56	5,16	
nal	Hα	NMe-His	Hα	5,34	4,37	4,12	-0,2
nal	Hα	nal	NH	3,12	2,56	2,83	
nal	Hα	Lys	NH	5,23	4,28	5,25	+0,02
NMe-Arg	NMe	nal	NH	5,11	3,85	5,12	+0,01
NMe-Arg	NMe	nal	Hα	3,33	2,4	2,68	
NMe-Arg	NMe	NMe-Arg	Hα	5,13	3,87	3,68	-0,2
Lys	Hα	Asp	Hα	5,92	4,84	4,92	
Asp	Hα	Trp	he3 (NH)	5,17	4,23	6,03	+0,9
NMe-Arg	NMe	Trp	hδ1	5	3,76	4,25	
NMe-Arg	NMe	Trp	he1	6,19	4,74	5,42	

[0010] Table 11: List of distance restraints that were used to derive the DG structure of 17 and the corresponding violations in fMD trajectory.

Residue	Atom	Residue	Atom	Upper Bound	Lower Bound	Mean distance (from MD)	Violation (Å)
Nle	NH	Nle	Hα	3,16	2,33	2,89	
Nle	NH	Asp	Hα	3,79	2,8	4,87	+1,0
Asp	NH	Nle	Hα	2,38	1,76	2,50	+0,1
Asp	NH	Asp	Hα	2,99	2,21	2,89	
Asp	NH	His	Hα	4,86	3,59	5,04	+0,2
Asp	Hα	NMe-Trp	NMe	5,42	3,71	3,72	
His	NH	Asp	NH	5,23	3,88	4,14	
His	NH	Asp	Hα	2,35	1,74	2,30	
His	NH	His	Hα	2,98	2,21	2,88	
His	NH	nal	NH	3,84	2,84	3,86	+0,1
His	NH	nal	Hα	5,49	4,06	5,15	
His	NH	NMe-Trp	NMe	4,78	3,24	3,79	
nal	NH	Asp	Hα	4,44	3,28	5,03	+0,6
nal	NH	Asp	Hβpro-S	4,41	3,26	4,11	
nal	NH	His	Hα	2,43	1,79	2,22	
nal	NH	nal	Hα	3,08	2,27	2,85	
nal	NH	NMe-Arg	Hα	5,25	3,88	4,74	
nal	NH	NMe-Arg	NMe	6,77	4,71	5,17	
nal	Hα	NMe-Arg	NMe	3,1	1,99	2,74	
nal	hb2	NMe-Arg	NMe	4,18	2,79	3,47	
nal	NH	NMe-Trp	NMe	4,99	3,39	4,82	
nal	Hα	NMe-Trp	NMe	5,04	3,43	4,55	
NMe-Arg	NMe	His	NH	7,61	5,33	6,88	
NMe-Arg	Hα	nal	Hα	5,42	4,01	4,34	
NMe-Arg	Hα	NMe-Arg	NMe	4,74	3,21	3,73	
NMe-Arg	Hα	NMe-Trp	NMe	3,16	2,04	2,83	
NMe-Arg	Hα	NMe-Lys	NMe	3,44	2,25	4,38	+1,0
NMe-Trp	Hα	NMe-Arg	Hα	5,03	3,72	4,26	

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Table 11 *continued*

NMe-Trp	Hα	NMe-Arg	NMe	5.52	3.79	5.01	
NMe-Trp	NMe	NMe-Arg	NMe	5.78	3.68	4.63	
NMe-Trp	Hα	NMe-Trp	NMe	3.44	2.25	3.73	+0.3
NMe-Trp	Hα	NMe-Lys	Hα	4.72	3.49	4.29	
NMe-Trp	Hα	NMe-Lys	NMe	3.19	2.06	2.82	
NMe-Lys	Hz	Asp	Hβpro-R	2.72	2.01	2.46	
NMe-Lys	Hz	NMe-Arg	Hα	4.89	3.62	4.86	
NMe-Lys	Hα	NMe-Trp	NMe	4.46	3	4.85	+0.4
NMe-Lys	Hα	NMe-Lys	NMe	4.32	2.9	3.72	

[0011] As used herein, the term "about" refers to plus or minus 10% of the referenced number.

[0012] The disclosures of the following U.S. Patents are incorporated in their entirety by reference herein: U.S. Pat. No. 14/117,949 and U.S. Pat. No. 5,674,839

[0013] Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference cited in the present application is incorporated herein by reference in its entirety.

[0014] Although there has been shown and described the preferred embodiment of the present invention, it will be readily apparent to those skilled in the art that modifications may be made thereto which do not exceed the scope of the appended claims. Therefore, the scope of the invention is only to be limited by the following claims. Reference numbers recited in the claims are exemplary and for ease of review by the patent office only, and are not limiting in any way. In some embodiments, the figures presented in this patent application are drawn to scale, including the angles, ratios of dimensions, etc. In some embodiments, the figures are representative only and the claims are not limited by the dimensions of the figures. In some embodiments, descriptions of the inventions described herein using the phrase "comprising" includes embodiments that could be described as

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"consisting of", and as such the written description requirement for claiming one or more embodiments of the present invention using the phrase "consisting of" is met.

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WHAT IS CLAIMED:

1.   A modified melanocortin receptor modulator, the modulator comprising Ac-Nle-c[Asp-His-nal-Arg-Trp-Lys]-NH₂ ("SHU9119"), wherein at least one amino acid within the c[Asp-His-nal-Arg-Trp-Lys] portion of SHU9119 is N-methylated.

2.   The modified melanocortin receptor modulator of Claim 1, wherein the modulator is a member selected from a group consisting of the following N-methylated derivatives of SHU9119:

(SEQ. No. 3)

Ac-Nle-c[Asp-His-nal-Arg-(*NMe*)Trp-Lys]-NH₂;

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-Trp-(*NMe*)Lys]-NH₂;

(SEQ. No. 9)

Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH₂;

(SEQ. No. 10)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-Trp-(*NMe*)Lys]-NH₂;

(SEQ. No. 13)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-Lys]-NH₂;

(SEQ. No. 15)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-Trp-Lys]-NH₂;

(SEQ. No. 16)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH₂;

(SEQ. No. 17)

Ac-Nle-c[Asp-His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂;

(SEQ. No. 19)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂;

(SEQ. No. 28)

Ac-Nle-c[Asp-(*NMe*)His-nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂; or

(SEQ. No. 32)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-(*NMe*)Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂.

3.   The modified melanocortin receptor modulator of Claim 1, wherein a selective hMC1 receptor agonist is a member selected from a group consisting of the following N-methylated derivatives of SHU9119:

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-Trp-(NMe)Lys]-NH₂;

(SEQ. No. 10)

Ac-Nle-c[Asp-(NMe)His-nal-Arg-Trp-(NMe)Lys]-NH₂;

(SEQ. No. 15)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-Trp-Lys]-NH₂; or

(SEQ. No. 28)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂.

4.   The modified melanocortin receptor modulator of Claim 1, wherein a highly selective hMC1 receptor antagonist is:

(SEQ. No. 32)

Ac-Nle-c[Asp-(NMe)His-(NMe)nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂.

5.   The modified melanocortin receptor modulator of Claim 1, wherein a selective hMC3 receptor antagonist is a member selected from a group consisting of the following N-methylated derivatives of SHU9119:

(SEQ. No. 8)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-Trp-(NMe)Lys]-NH₂;

(SEQ. No. 15)

Ac-Nle-c[Asp-(NMe)His-nal-(NMe)Arg-Trp-Lys]-NH₂; or

(SEQ. No. 17)

Ac-Nle-c[Asp-His-nal-(NMe)Arg-(NMe)Trp-(NMe)Lys]-NH₂.

6.   The modified melanocortin receptor modulator of Claim 1, wherein a highly selective hMC5 receptor agonist is:

(SEQ. No. 3)

Ac-Nle-c[Asp-His-nal-Arg-(NMe)Trp-Lys]-NH₂.

7.   The modified melanocortin receptor modulator of Claim 1, wherein a highly selective hMC5 receptor antagonist is a member selected from a group consisting of the following N-methylated derivatives of SHU9119:

(SEQ. No. 9)

Ac-Nle-c[Asp-His-(*NMe*)nal-Arg-Trp-(*NMe*)Lys]-NH₂; or

(SEQ. No. 13)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-Lys]-NH₂.

8.   The modified melanocortin receptor modulator of Claim 1, wherein a selective universal antagonist for an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, or an hMC5 receptor is a member selected from a group consisting of the following *N*-methylated derivatives of SHU9119:

(SEQ. No. 16)

Ac-Nle-c[Asp-(*NMe*)His-(*NMe*)nal-Arg-Trp-Lys]-NH₂; or

(SEQ. No. 19)

Ac-Nle-c[Asp-(*NMe*)His-nal-Arg-(*NMe*)Trp-(*NMe*)Lys]-NH₂.

9.   A method of modulating a melanocortin receptor, said method comprising administering to a mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient for stimulating melanin synthesis in said mammal .

10.   The method of Claim 9, wherein the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, an hMC5 receptor, or a combination thereof.

11.   A method of modulating a melanocortin receptor for regulating a weight of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to bring about a determined satiation.

12.   The method of Claim 11, wherein the melanocortin receptor is an hMC3 receptor, an hMC4 receptor, or a combination thereof.

13.   A method of modulating a hMC4 receptor for stimulating sexual functions of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to bring about an enhanced and

sustained sexual arousal.

14.   A method of modulating a melanocortin receptor for regulating pain sensed by a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to bring about sufficient pain relief.

15.   The method of Claim 14, wherein the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

16.   A method of modulating a melanocortin receptor for regulating skin pigmentation of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to bring about a desired skin coloration.

17.   The method of Claim 16, wherein the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

18.   A method of modulating an hMC1 receptor for controlling an immune system of a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to bring about stimulation of an immune response .

19.   A method of modulating a melanocortin receptor for treating atherosclerosis in a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to reduce atherosclerotic plaque inflammation and to improve vascular endothelial function.

20.   The method of Claim 19, wherein the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, or a combination thereof.

21.   A method of modulating a melanocortin receptor for treating skin cancer in a

mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to prevent proliferation of cancer cells .

22.   The method of Claim 21, wherein the skin cancer is caused by over-exposure to ultraviolet radiation.

23.   The method of Claim 21, wherein the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

24.   A method of modulating a melanocortin receptor for treating a skin disease in a mammal, said method comprising administering to the mammal in need thereof at least one of the modified melanocortin receptor modulators according to Claim 1 in a therapeutically effective amount sufficient to bring about a prevention of symptoms or a cessation of the skin disease.

25.   The method of Claim 24, wherein the melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination thereof.

26.   The method of Claim 24, wherein the skin disease is a skin pigmentation disorder.

27.   The method according to Claim 9, 11, 13, 14, 16, 18, 19, 21, or 24, wherein administration is by oral, nasal, or topical means, or by injection to the mammal.

28.   The use of the modified melanocortin receptor modulator according to claim 1 for modulating a melanocortin receptor for stimulating melanin synthesis, wherein the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, an hMC4 receptor, an hMC5 receptor, or a combination thereof.

29.   The use of the modified melanocortin receptor modulator according to claim 1 for modulating a melanocortin receptor for regulating weight by bringing about a determined satiation, wherein the melanocortin receptor is an hMC3 receptor, an hMC4 receptor, or a combination thereof.

30.   The use of the modified melanocortin receptor modulator according to claim
       1 for modulating an hMC4 melanocortin receptor for stimulating sexual functions
       by bringing about an enhanced and sustained sexual arousal.

31.   The use of the modified melanocortin receptor modulator according to claim
       1 for modulating a melanocortin receptor for regulating pain by bringing about
       sufficient pain relief, wherein the melanocortin receptor is an hMC1 receptor, an
       hMC5 receptor, or a combination thereof.

32.   The use of the modified melanocortin receptor modulator according to claim
       1 for modulating a melanocortin receptor for regulating skin pigmentation by
       bringing about a desired skin coloration, wherein the melanocortin receptor is an
       hMC1 receptor, an hMC5 receptor, or a combination thereof.

33.   The use of the modified melanocortin receptor modulator according to claim
       1 for modulating an hMC1 receptor for controlling an immune system by bringing
       about a stimulation of an immune response.

34.   The use of the modified melanocortin receptor modulator according to claim
       1 for modulating a melanocortin receptor for treating atherosclerosis by reducing
       atherosclerotic plaque inflammation and improving vascular endothelial function,
       wherein the melanocortin receptor is an hMC1 receptor, an hMC3 receptor, or a
       combination thereof.

35.   The use of the modified melanocortin receptor modulator according to claim
       1 for modulating a melanocortin receptor for treating skin cancer by preventing
       proliferation of cancer cells, wherein the skin cancer is caused by over-exposure
       to ultraviolet radiation, wherein the melanocortin receptor is an hMC1 receptor,
       an hMC5 receptor, or a combination thereof.

36.   The use of the modified melanocortin receptor modulator according to claim
1 for modulating a melanocortin receptor for treating a skin disease by bringing about
a prevention of symptoms or a cessation of the skin disease, wherein the
melanocortin receptor is an hMC1 receptor, an hMC5 receptor, or a combination
thereof, wherein the skin disease is a skin pigmentation disorder.
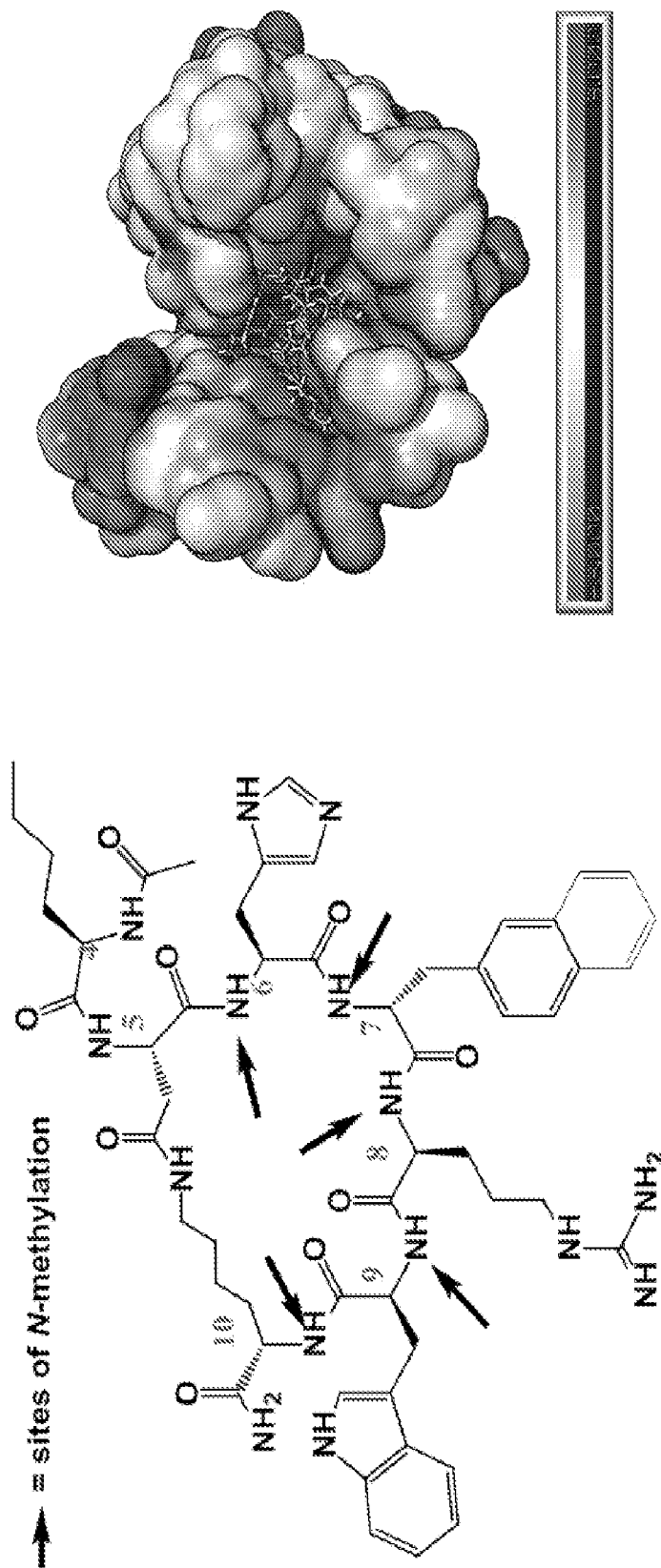
FIG. 1

Sequence

Peptide	Nle	D	H	nal	R	W	K
1	Nle	D	H	nal	R	W	K
2	Nle	D	H	nal	R	W	**K**
3	Nle	D	H	nal	R	**W**	K
4	Nle	D	H	nal	**R**	W	K
5	Nle	D	H	**nal**	R	W	K
6	Nle	D	**H**	nal	R	W	K
7	Nle	D	H	nal	R	**W**	**K**
8	Nle	D	H	nal	**R**	**W**	**K**
9	Nle	D	H	**nal**	R	W	**K**
10	Nle	D	**H**	nal	R	W	**K**
11	Nle	D	H	nal	**R**	**W**	K
12	Nle	D	H	**nal**	R	**W**	K
13	Nle	D	**H**	nal	R	**W**	K
14	Nle	D	H	**nal**	**R**	W	K
15	Nle	D	**H**	nal	**R**	W	K
16	Nle	D	**H**	**nal**	R	W	K
17	Nle	D	H	nal	**R**	**W**	**K**
18	Nle	D	H	**nal**	R	**W**	**K**
19	Nle	D	**H**	nal	R	**W**	**K**
20	Nle	D	H	**nal**	**R**	W	**K**
21	Nle	D	**H**	nal	**R**	W	**K**
22	Nle	D	**H**	**nal**	R	W	**K**
23	Nle	D	H	**nal**	**R**	**W**	K
24	Nle	D	**H**	nal	**R**	**W**	K
25	Nle	D	**H**	**nal**	R	**W**	K
26	Nle	D	**H**	**nal**	**R**	W	K
27	Nle	D	H	**nal**	**R**	**W**	**K**
28	Nle	D	**H**	nal	**R**	**W**	**K**
29	Nle	D	**H**	**nal**	R	**W**	**K**
30	Nle	D	**H**	**nal**	**R**	W	**K**
31	Nle	D	**H**	**nal**	**R**	**W**	K
32	Nle	D	**H**	**nal**	**R**	**W**	**K**

FIG. 2a

FIG. 2b

FIG. 2c

FIG. 2d

FIG. 2e
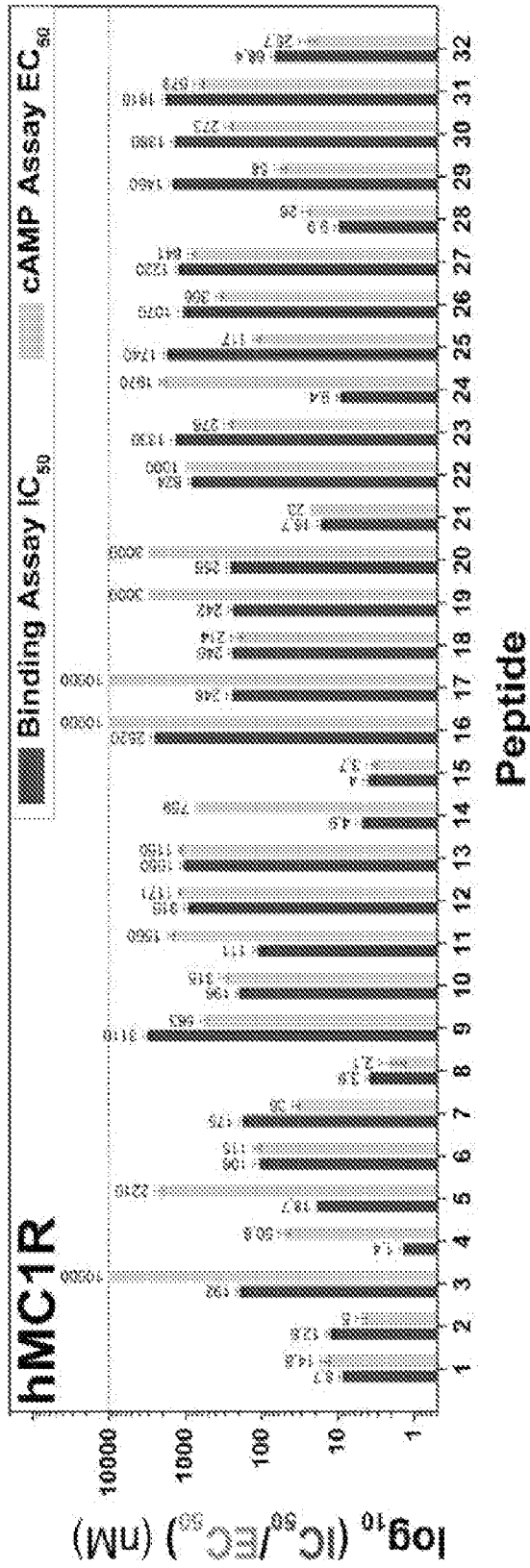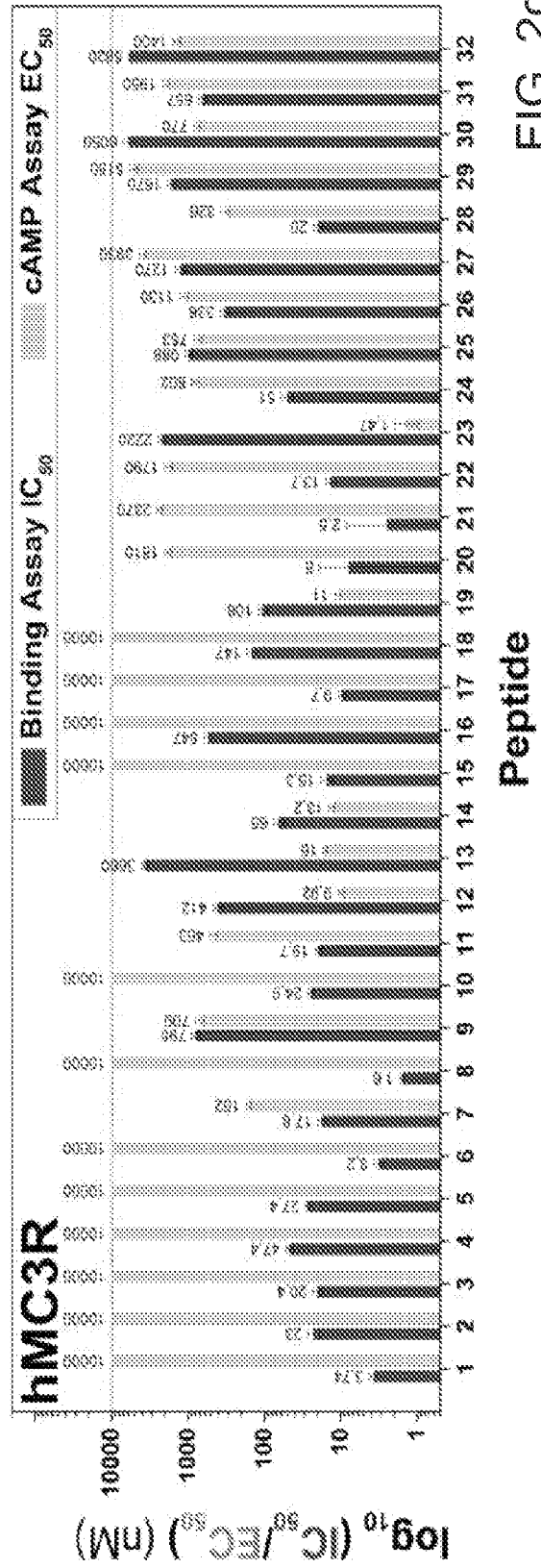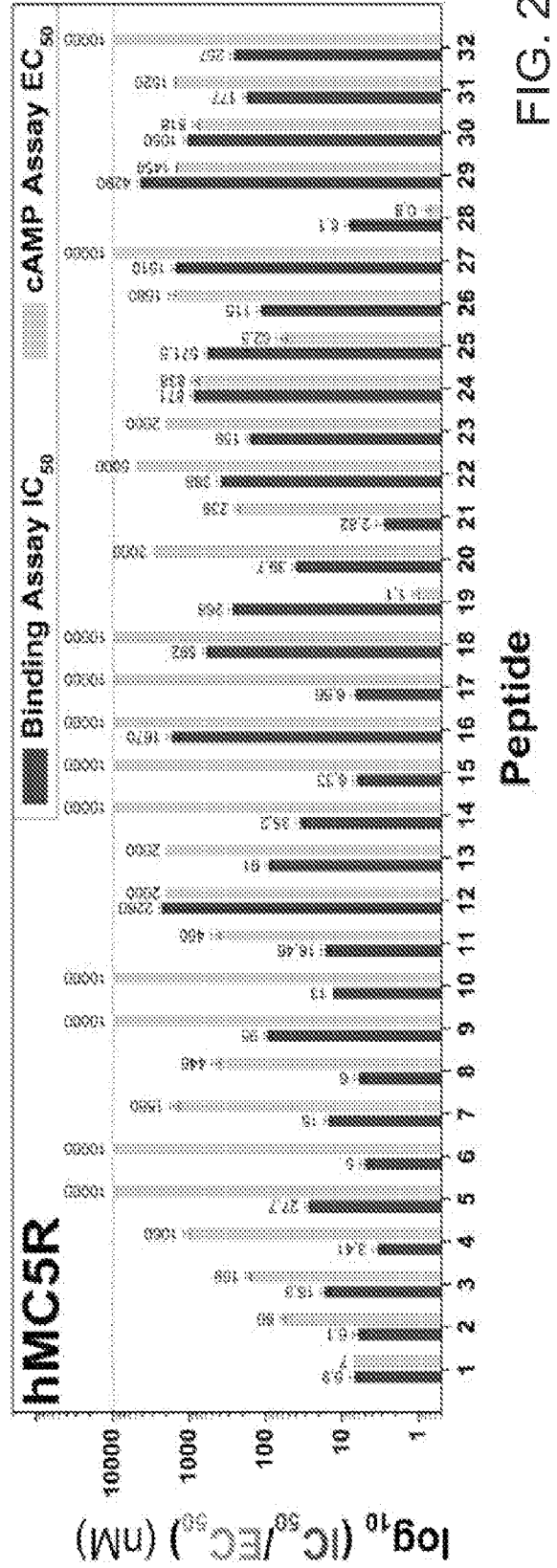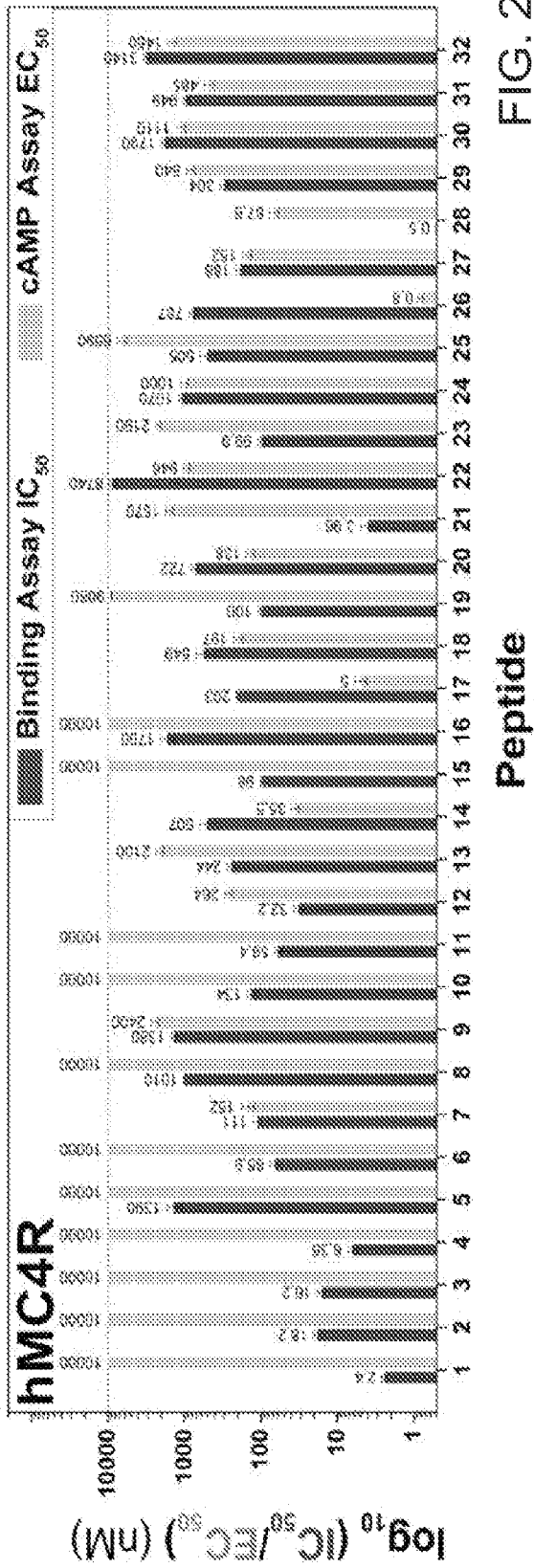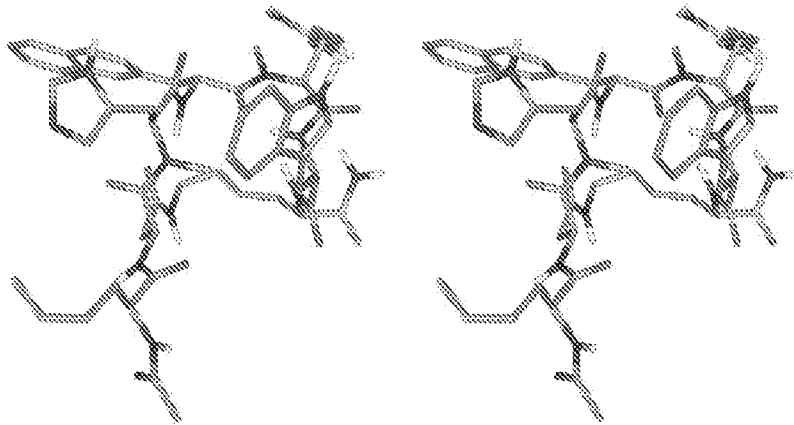
FIG. 3a
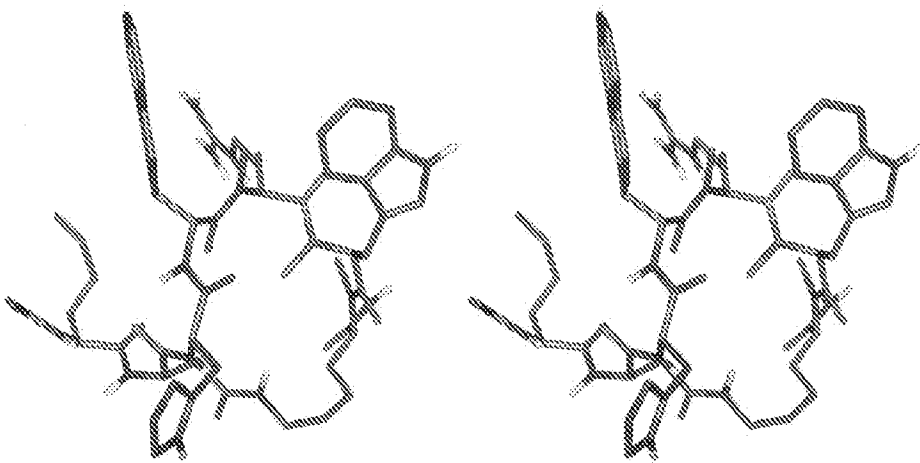
FIG. 3b
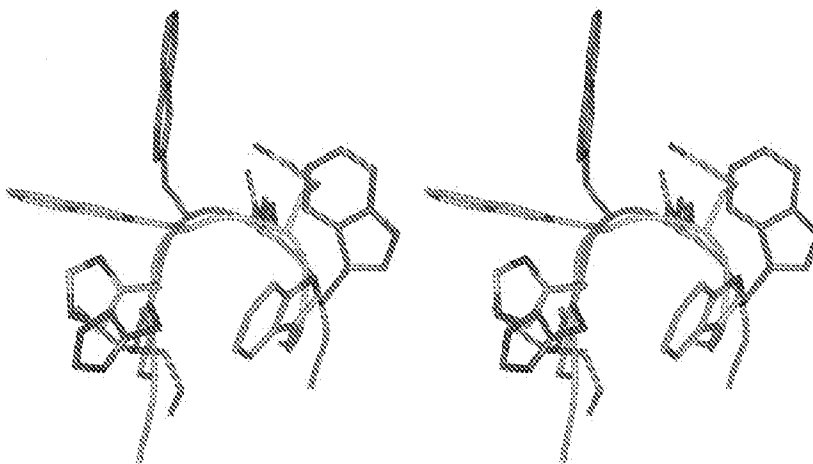
FIG. 3c
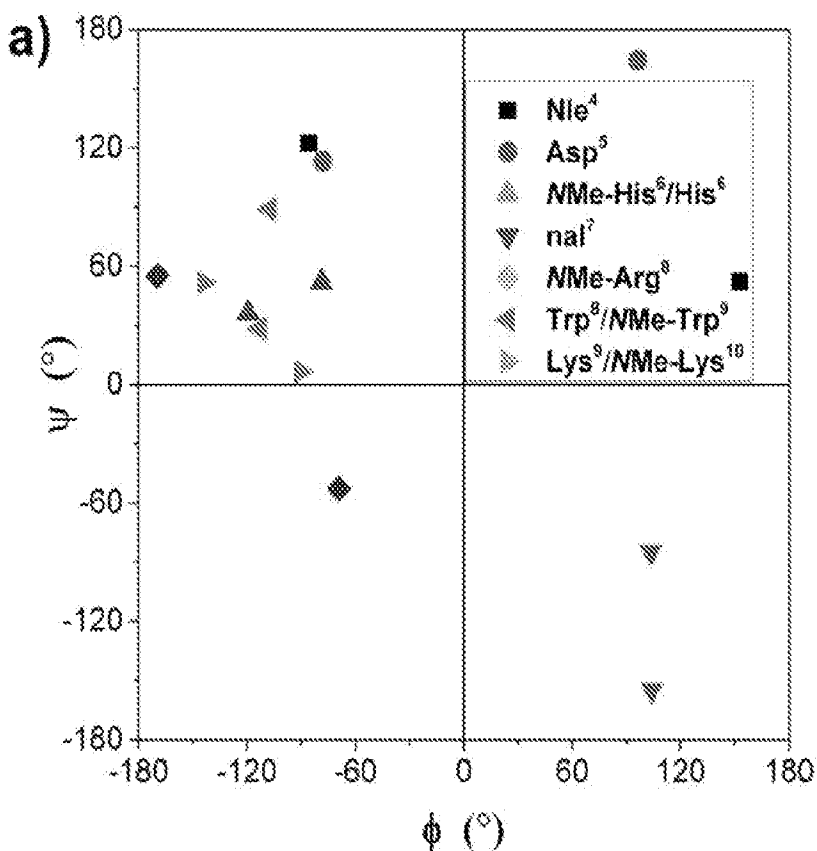
FIG. 4a
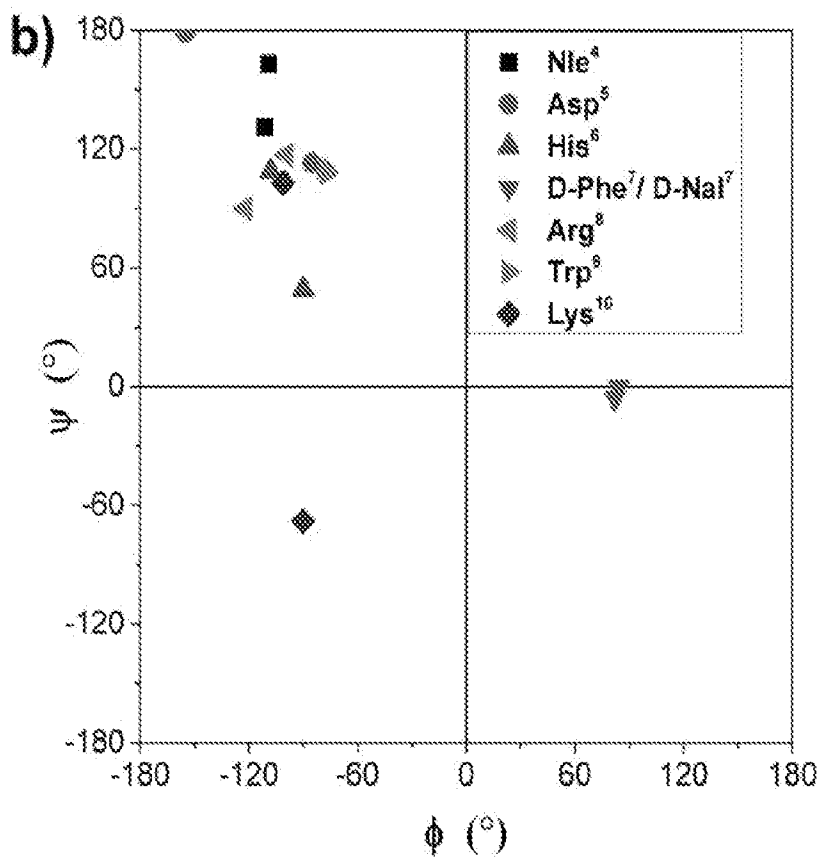
FIG. 4b
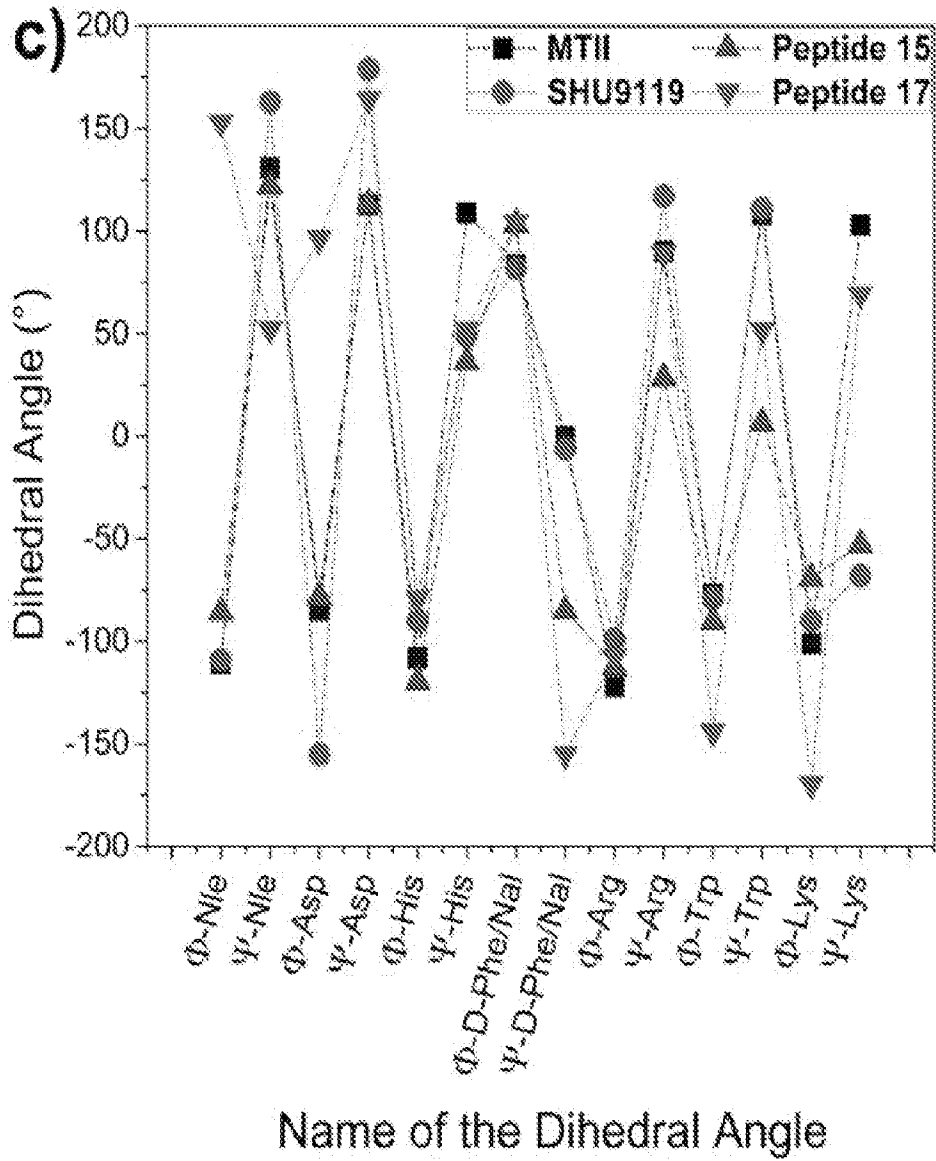
FIG. 4c

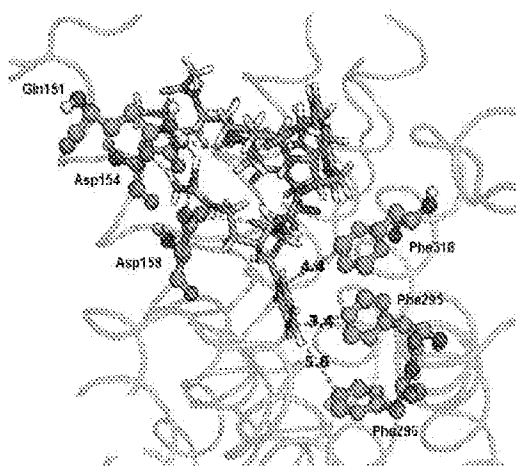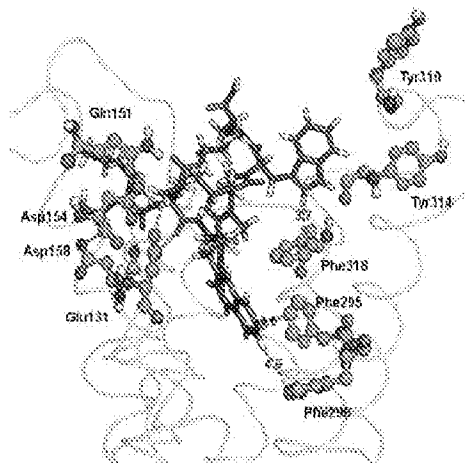Ligands in contact with residues mutated in Yang 2005
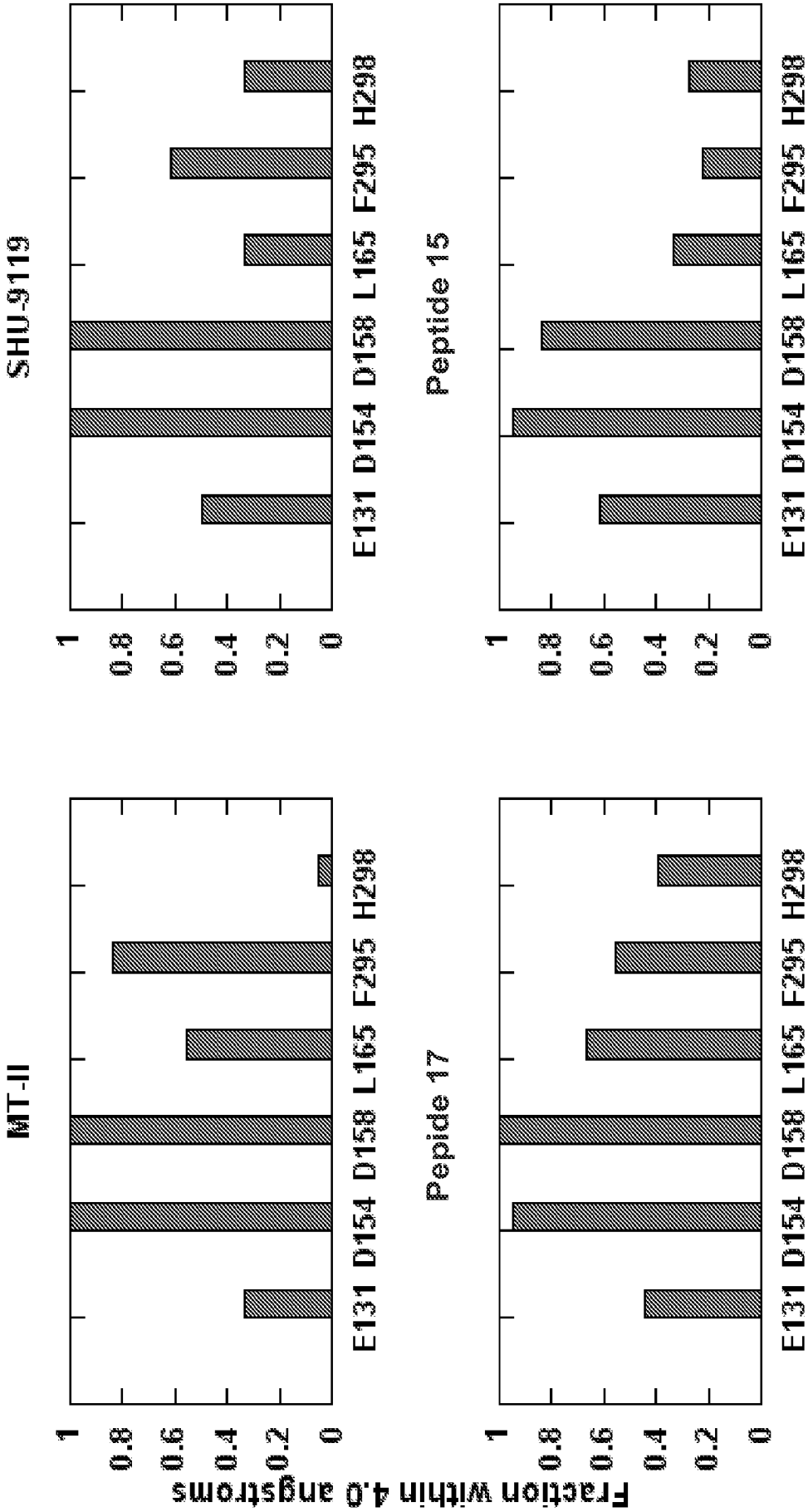
FIG. 5a
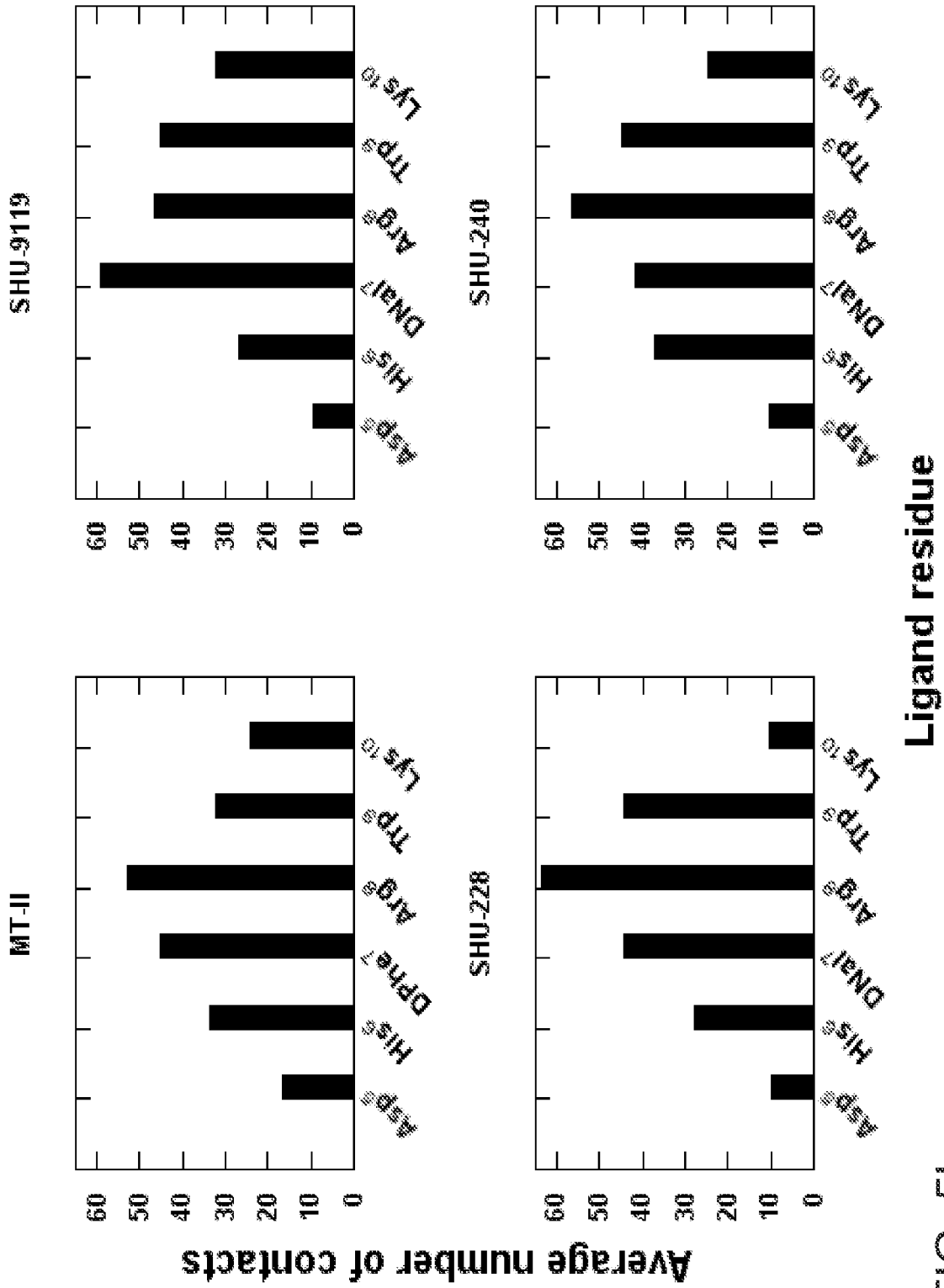
FIG. 5b

A: MT-II best dock

B: SHU9119 best dock

Notes:1)D154/D158 provide polar contracts with side chains of Arg[8], Lys[10]; 2) Q151 also stabilized Arg[8]; 3) Q158 also stablizes backbone of D-Phe[7]; 4) D-Phe[7] has T-shaped π-π staking with F295.

Notes: 1) D154/D158 provide polar contact with side chains of Arg[8]; 2) Ile 217 backbone carbonyl group stabilizes terminal NH2 group; 3) Possible T-shaped stack between DNal(2')[7] and Y310/Y314; 4) Possible T-shaped stack with Phe318 and DNal(2')[7] and/or Trp[9].

C: Peptide 15 best dock

D: Peptide 17 best dock
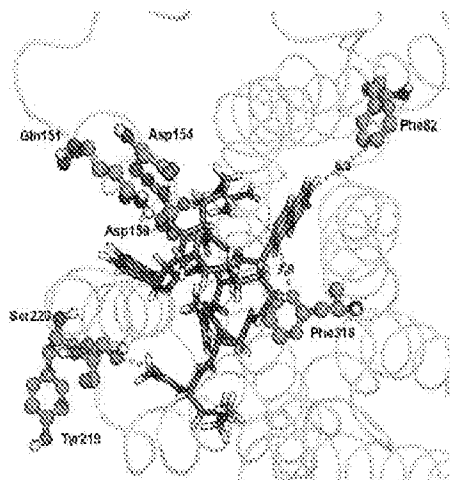
Notes: 1) Q151 and D154 polar contact with Arg[8]; 2) D158 polar contacts with side chain of Arg[8]; 3) S220 polar contact with N-terminal carbonyl group; 4) Y219 in EL loop rotated away from Trp[9]; 5) F318 T-shaped stacking with DNal (2')[7].
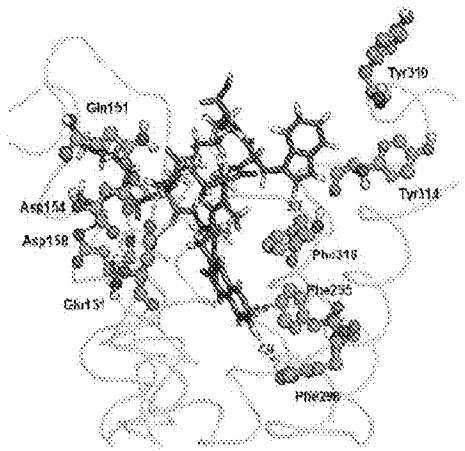
Notes: 1) Arg[8] side chain polar contact with D151, D154, and D158; 2) Polar contact between N-terminal carbonyl group and E131; 3) Y310/Y314 potential π-π stacking with Trp[9] but rotated away; 4) F31 T-shaped stacking with Trp[9]; F295/296 both T-shaped stacking with DNal(2')[7].
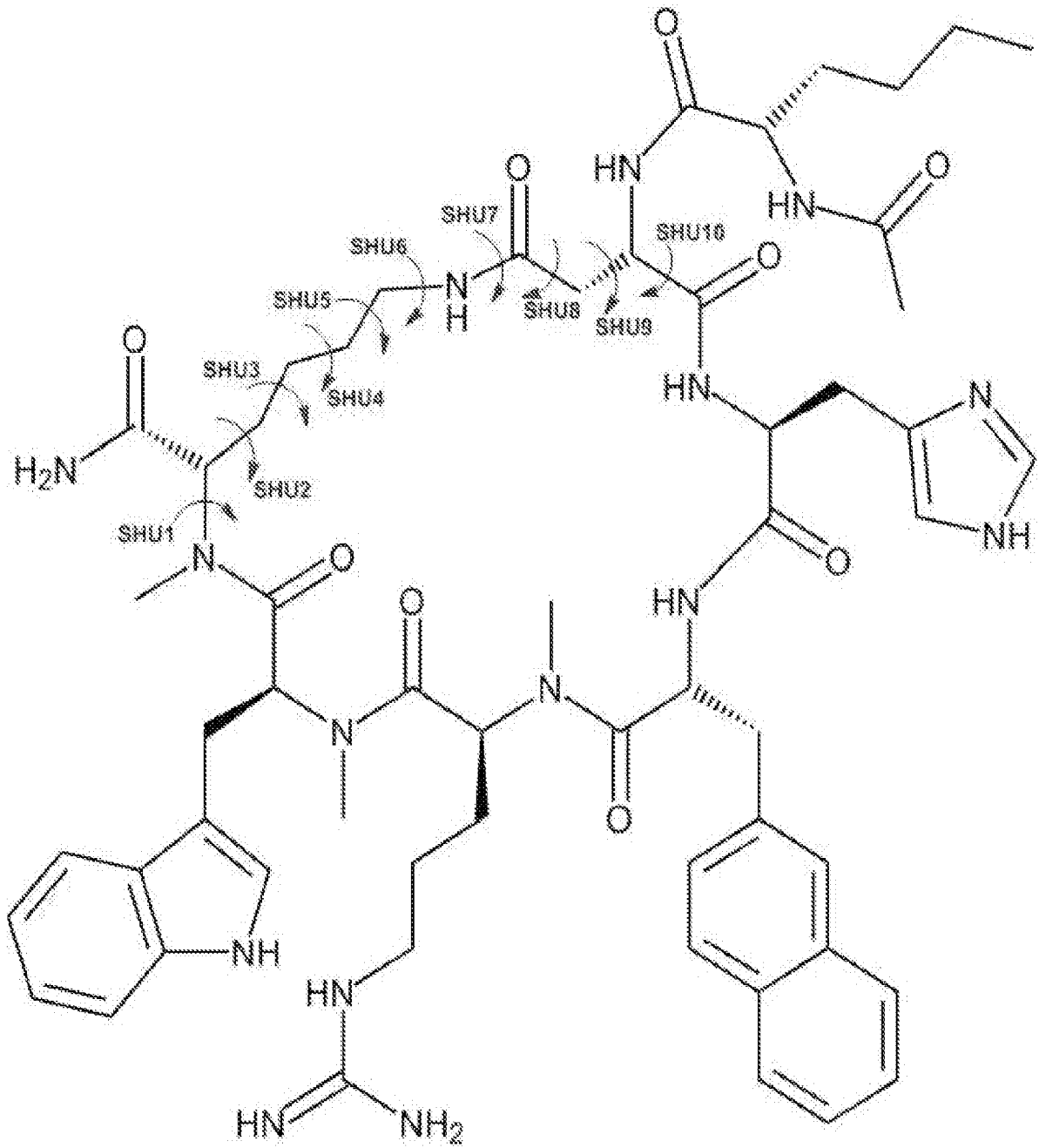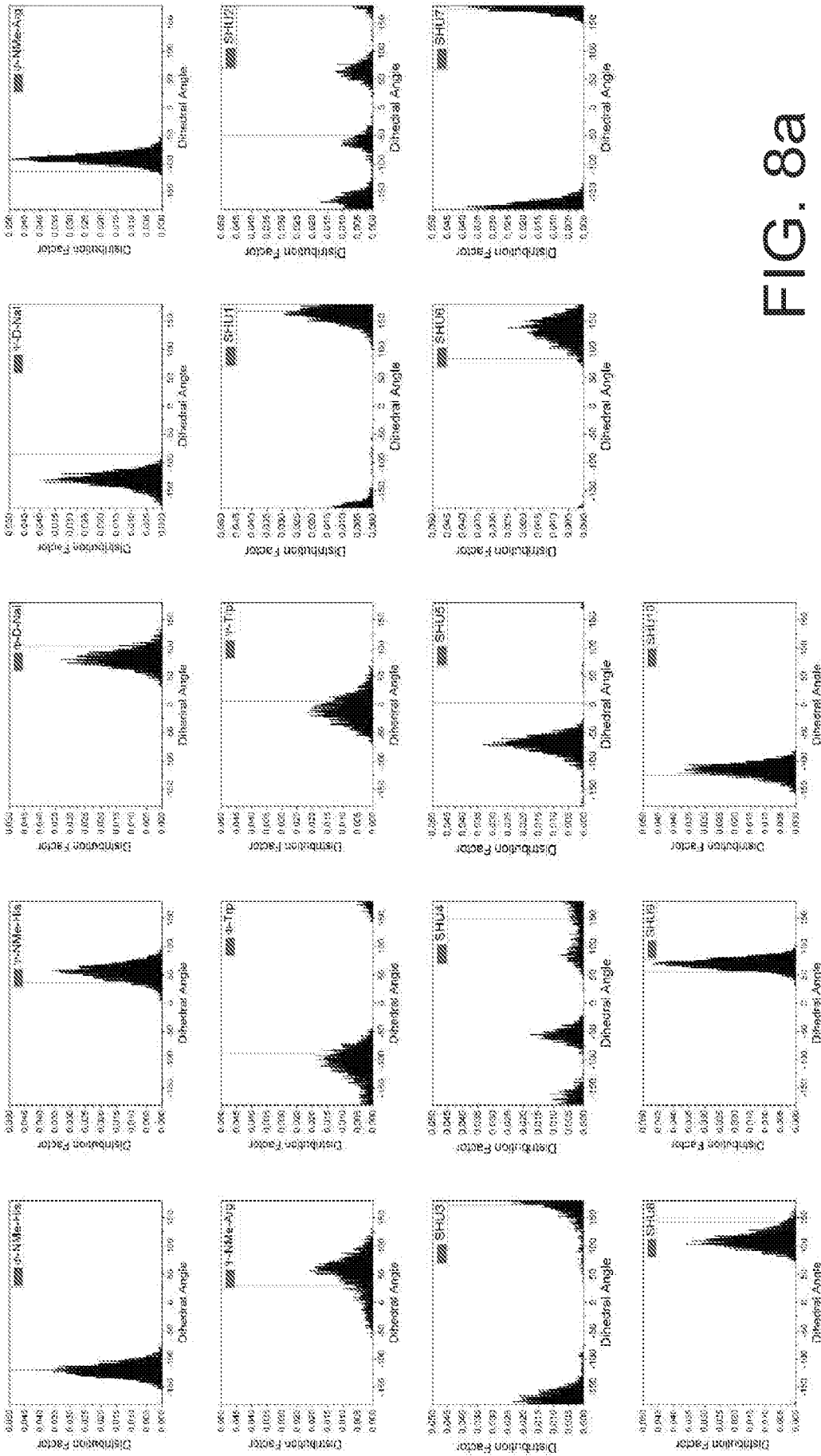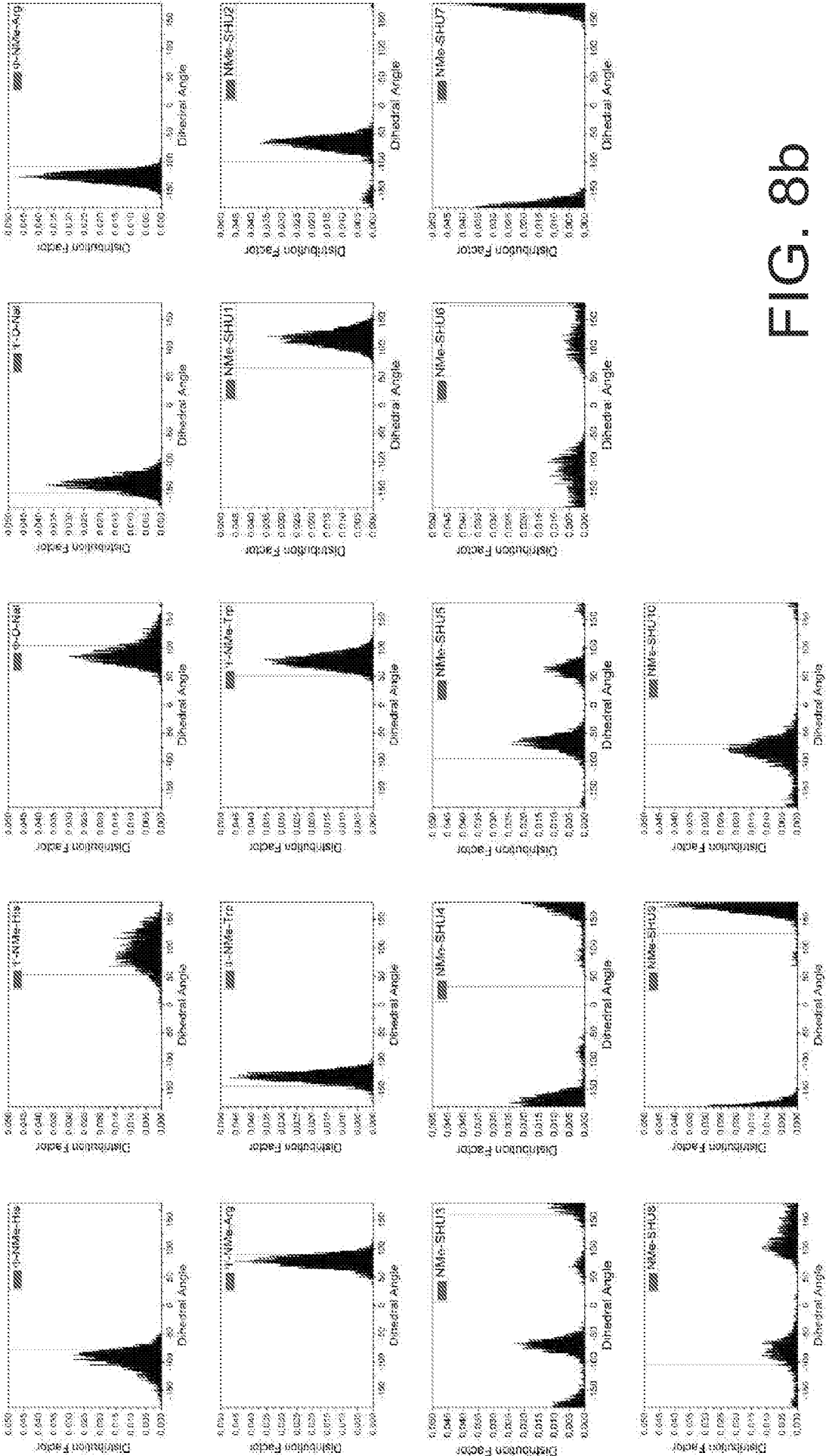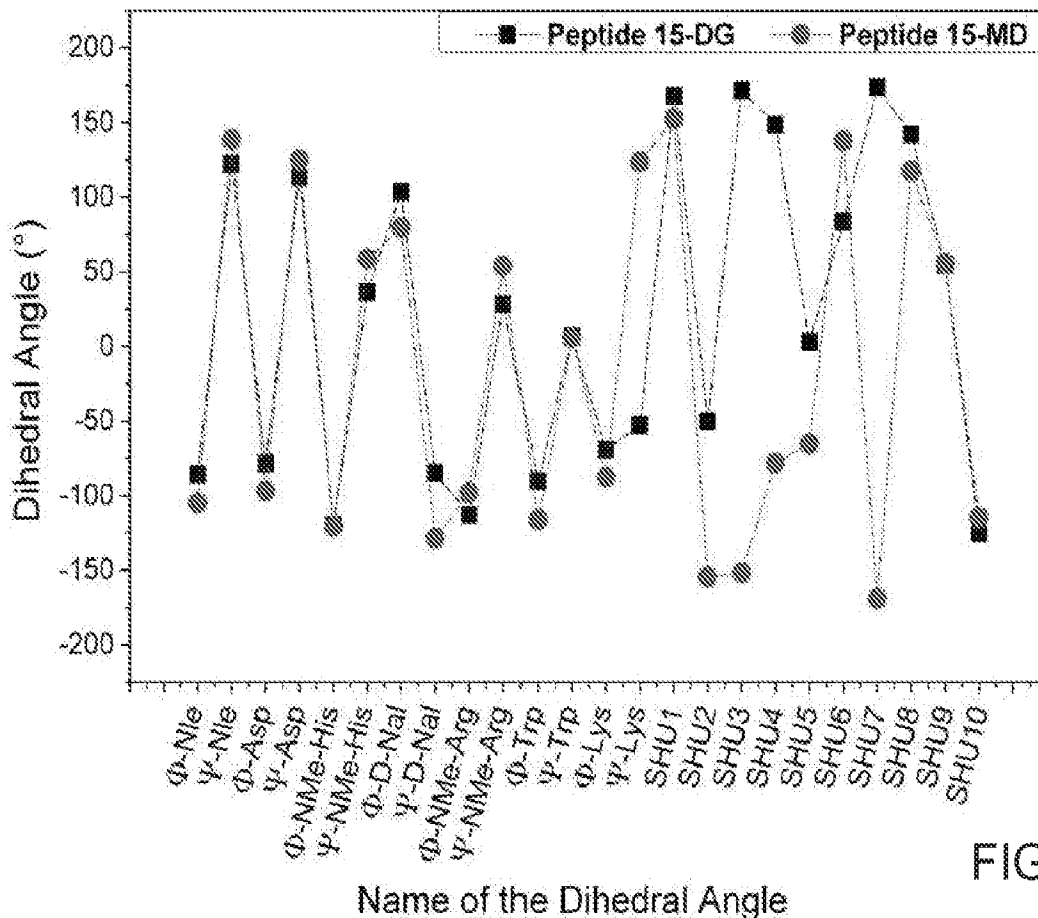
FIG. 6

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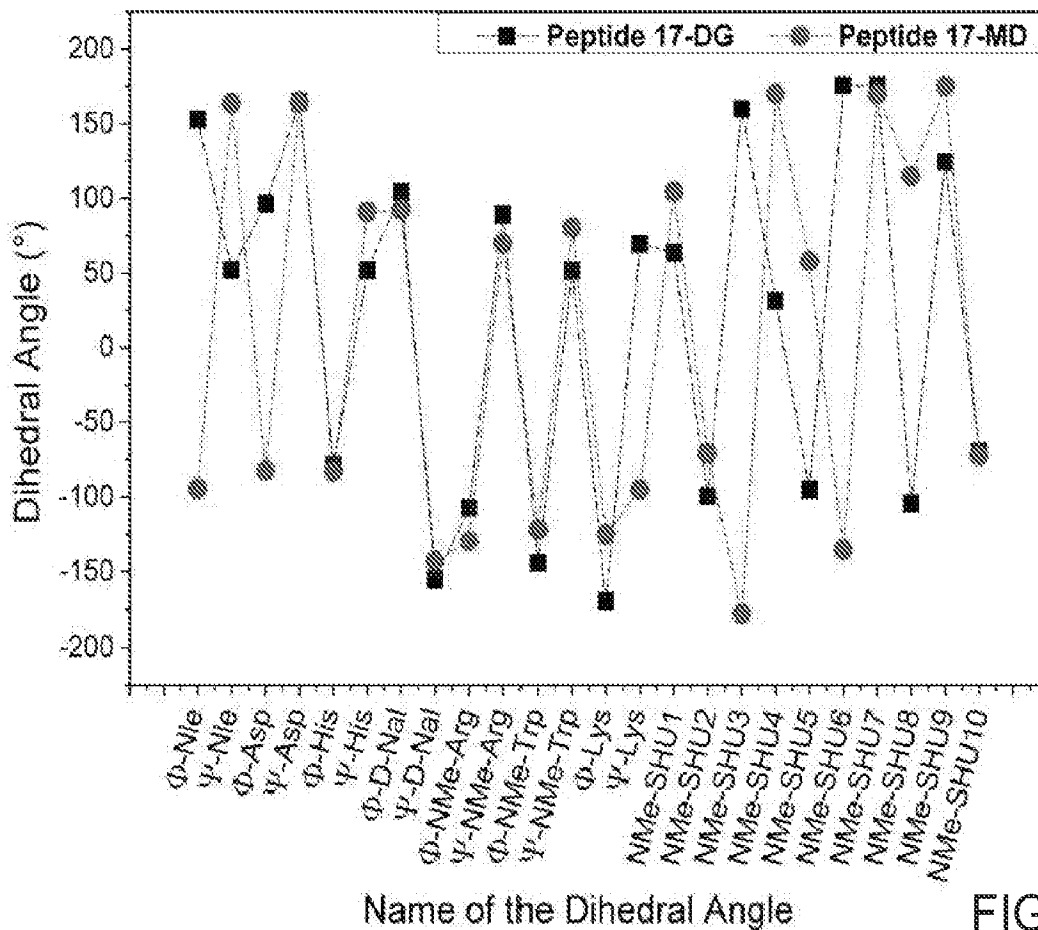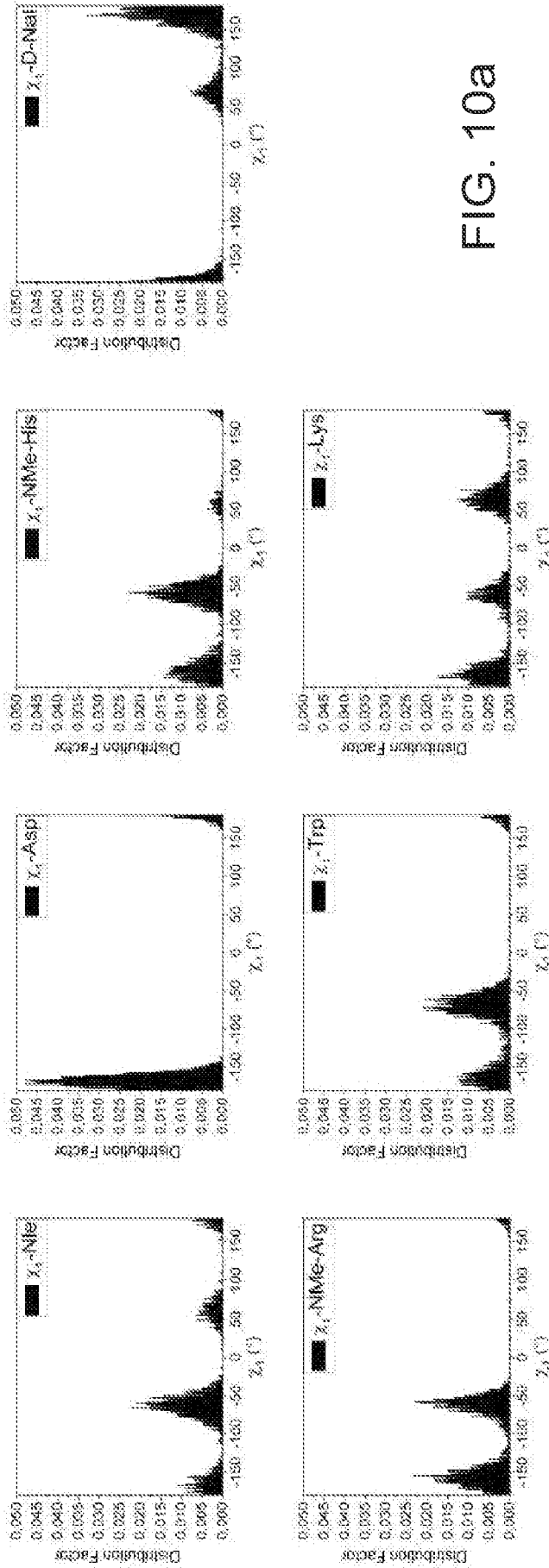## FIG. 7

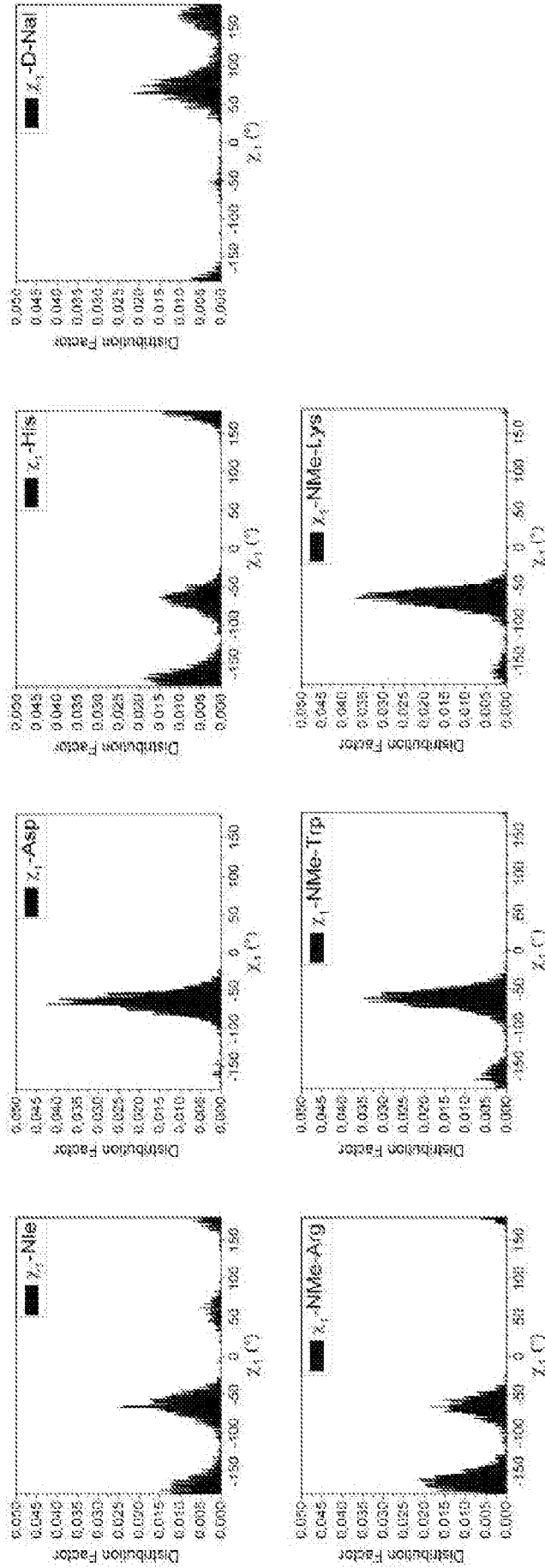FIG. 8a

FIG. 8b

FIG. 9a

FIG. 9b

FIG. 10a

FIG. 10b

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter*.1(c) and (d) and 39)

Applicant's or agent's file reference UNIA 14.05 PCT	IMPORTANT DECLARATION	Date of mailing *(day/month/year)* **19 AUG 2015**
International application No. PCT/US15/35180	International filing date *(day/month/year)* 10 June 2015	(Earliest) Priority Date *(day/month/year)* 10 June 2014

International Patent Classification (IPC) or both national classification and IPC

IPC: C12Q 1/68; CPC: C12Q 2600/158

Applicant THE ARIZONA BOARD OF REGENTS ON BEHALF OF THE UNIVERSITY OF ARIZONA

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:

    a. ☐ scientific theories

    b. ☐ mathematical theories

    c. ☐ plant varieties

    d. ☐ animal varieties

    e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes

    f. ☐ schemes, rules or methods of doing business

    g. ☐ schemes, rules or methods of performing purely mental acts

    h. ☐ schemes, rules or methods of playing games

    i. ☐ methods for treatment of the human body by surgery or therapy

    j. ☐ methods for treatment of the animal body by surgery or therapy

    k. ☐ diagnostic methods practised on the human or animal body

    l. ☐ mere presentations of information

    m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art .

2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

    ☐ the description   ☐ the claims   ☐ the drawings

3. ☒ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:

    ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

    ☒ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

    ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

4. Further comments:
    Applicant failed to submit a valid electronic seq. listing in respone to the ISA/225. .

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Authorized officer Blaine Copenheaver
Facsimile No. 571-273-8300	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/203 (January 2015)