DISTILLATION №74 · ALEMBIC LABS

SEMAX — GLU-2 → ASP SUBSTITUTION (SHORTENS THE ACIDIC SIDE CHAIN BY ONE METHYLENE)

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REFINED | COGNITIVE | GLU-2 → ASP SUBSTITUTION (SHORTENS THE ACIDIC SIDE CHAIN BY ONE METHYLENE) | MELANOCORTIN RECEPTOR 4

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
80.9%	0.888 / 0.867	REFINED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Melanocortin receptor 4	P32245	—

TLDR

DISTILLATION №74 tests whether replacing Glu-2 of Semax with the one-methylene-shorter Asp residue (MEHFPGP → MDHFPGP) can bias the peptide toward MC4R over MC1R by tightening a hypothesized salt-bridge interaction at the receptor's TM2/ECL1 interface. Structural prediction returned a high-confidence result: pLDDT 0.81, pTM 0.89, and a strong ipTM of 0.87, with the Asp-2 carboxylate positioned near the MC4R extracellular basic-residue rim as hypothesized. The modification earns a REFINED verdict on structural grounds, though the literature base for direct Semax–MC4R pharmacology remains thin and the Cu(II) chelation confound at Glu/Asp-2 is a meaningful biological caveat. This is the first Semax fold in the lab to target position-2, completing a growing residue-by-residue SAR map of the heptapeptide scaffold.

EXECUTIVE SUMMARY

DISTILLATION №74 — [Asp2]Semax at MC4R. pLDDT 0.81, ipTM 0.87: high-confidence interface with Asp-2 positioned for a tighter electrostatic contact at the

MC4R vestibule. First position-2 probe in the lab's Semax series. Cu(II) chelation confound requires controlled wet-lab validation.

DETAILED ANALYSIS

Semax (MEHFPGP) is a synthetic heptapeptide derived from ACTH(4-10) developed in Russia in the 1980s and studied primarily as a neuroprotective and nootropic agent. Its pharmacology in the published literature is dominated by BDNF/NGF upregulation, monoaminergic modulation, anti-inflammatory cytokine regulation, and — more recently recognized — copper chelation via the N-terminal Met-Glu-His triad. Despite its structural ancestry as an ACTH fragment, no published study has quantified Semax's binding affinity or functional potency at individual melanocortin receptor subtypes (MC1R, MC3R, MC4R, MC5R), leaving the receptor-level mechanism of action inferred rather than directly measured. This fold engages that gap from a structural-predictive angle.

The modification hypothesis is chemically conservative: Glu-2 is replaced by Asp, preserving the acidic side-chain charge while shortening the carbon backbone by one methylene group (~1.5 Å reduction in side-chain reach). The rationale draws on well-precedented melanocortin SAR logic — the depth and geometry of the electrostatic pocket presented by the extracellular vestibule differs across MCR subtypes, and small changes in side-chain length in the ACTH pharmacophore region have been shown to shift subtype selectivity substantially in other peptide series (e.g., MT-II, MS05 analogues). The specific structural premise — that a conserved Arg/Lys at the MC4R TM2/ECL1 rim sits one helical turn shallower than the corresponding residue in MC1R, making it more accessible to a shorter Asp carboxylate — is theoretically grounded but experimentally unvalidated for this scaffold.

Structural prediction with Boltz-2 produced a confidently resolved complex. The pLDDT of 0.81 is essentially identical to the target established from native Semax (~0.80) and slightly below the Hyp-5 analogue from Fold #61 (0.83), indicating that the Glu→Asp change does not destabilize the peptide's local fold. More importantly, the interface metrics are strong: pTM 0.89 and ipTM 0.87 indicate a well-defined and geometrically coherent peptide–receptor interface. The predicted binding pose preserves the His-Phe β-turn pharmacophore — the core recognition element shared across ACTH-derived melanocortin agonists — while positioning the shortened Asp-2 carboxylate near the MC4R extracellular basic-residue cluster, consistent with the salt-bridge tightening hypothesis.

Heuristic sequence-based profiling suggests a favorable biophysical profile: near-zero aggregation propensity (0.0), a reasonable stability score (0.80), and modest predicted BBB penetration (0.41). The short estimated half-life (15–45 minutes) is expected for a linear heptapeptide and is consistent with the pharmacokinetic

profiles observed for other Semax variants in the lab. No improvement in metabolic stability was targeted here — this fold is purely a selectivity probe.

The cross-fold narrative is important context. The Alembic Semax series has now explored N-terminal acetylation (Fold #1, REFINED), Phe-4 fluorination (Fold #24, DISCARDED on biological grounds), His-3 methylation (Fold #49, REFINED), cyclization (Fold #55, REFINED), and Pro-5 hydroxylation (Fold #61, REFINED). Each of these touched a different residue or terminus. Fold #74 is the first to interrogate position 2 — the Glu residue that anchors the Met-Glu-His N-terminal triad. This position is doubly interesting: it is simultaneously a putative receptor contact point and a copper-chelation participant, making it one of the most pharmacologically loaded residues in the sequence.

The copper-chelation confound is the most significant biological caveat raised by the literature agent. The Sciacca (2022) and Tomasello (2025) studies establish that the Met-Glu-His triad forms a high-affinity Cu(II) complex, and Glu-2 coordinates directly to the metal. A Glu→Asp substitution alters the coordination geometry of this complex — potentially reducing Cu(II) affinity or changing the complex's redox properties — which in CNS tissue (copper-rich environment) could confound any phenotypic selectivity readout. Disentangling altered metal chelation from altered receptor selectivity in cell-based assays would require careful experimental design, ideally including copper-chelated and copper-free conditions in parallel.

Limitations are multi-layered: (1) This is a single-run in silico prediction with no ensemble averaging; (2) the selectivity inference (MC4R over MC1R) is based on a single modelled complex — no MC1R counter-screen was run computationally; (3) the structural prediction cannot model the dynamic conformational ensemble of the ECL regions, which are notoriously flexible in GPCRs; (4) Boltz-2's affinity module did not return quantitative ΔΔG values, so the predicted tighter salt bridge is a geometric inference rather than an energy-quantified claim; (5) the literature base for Semax as a direct MCR ligand is absent, meaning the entire selectivity engineering exercise is predicated on a plausible but unconfirmed pharmacological premise. These caveats do not undermine the REFINED verdict — which reflects structural prediction confidence — but they substantially temper the biological conclusions that can be drawn.

RESEARCH BRIEF

DISTILLATION №74 — REFINED

[ASP2]SEMAX · MC4R SELECTIVITY PROBE

Sequence: MDHFPGP (Glu-2 → Asp) | **Target:** MC4R (UniProt P32245) | **Class:** Cognitive

MECHANISM OF ACTION

Semax is structurally derived from ACTH(4-10), and ACTH signals through all five melanocortin receptors (MC1R–MC5R). The His-Phe dipeptide at positions 3-4 constitutes the minimal pharmacophore for melanocortin receptor engagement — a β-turn motif that inserts into the extracellular vestibule and makes hydrophobic and electrostatic contacts with conserved receptor residues. The N-terminal Met-Glu segment of Semax is believed to contribute an electrostatic anchoring interaction with a basic residue (Arg or Lys) presented at the TM2/ECL1 interface, a contact geometry that differs subtly across receptor subtypes due to differences in the depth and identity of the partnering residue.

Importantly, the CNS effects of Semax documented in the literature — BDNF/NGF upregulation, monoaminergic modulation, anti-inflammatory cytokine regulation, and copper chelation — are likely pleiotropic and not exclusively MCR-mediated. Liu et al. (2025) identify opioid/USP18 pathways in spinal cord injury contexts; Medvedeva et al. (2017) document immune/interferon signaling; and the Eremin et al. (2005) paper explicitly links melanocortinergic and monoaminergic systems in the context of Semax's striatal serotonergic effects. Any selectivity engineering at the receptor level must be interpreted against this multi-mechanistic backdrop.

PERFORMANCE APPLICATIONS

MC4R is a therapeutically validated target for metabolic regulation (appetite suppression, energy expenditure) and has been implicated in cognitive and neuroprotective signaling, mood regulation, and erectile function. Enhanced MC4R selectivity over MC1R is desirable for two reasons: (1) MC1R activation drives melanogenesis and pigmentation effects — an unwanted side effect for a cognitive/neuroprotective agent; (2) MC5R activation is associated with sebaceous gland stimulation. A Semax analogue with improved MC4R/MC1R selectivity ratio could, in principle, preserve or enhance the nootropic and neuroprotective profile while reducing dermatological off-target activity.

In the biohacker and research-use context, this makes [Asp2]Semax an interesting selectivity probe — not a dose-escalation or stability play, but a subtype-discrimination experiment. If wet-lab validation confirmed improved MC4R/MC1R selectivity, it would also open the door to metabolic and appetite-regulatory applications that are pharmacologically distinct from native Semax's established neuroprotective profile.

MODIFICATION RATIONALE

The Glu-2 → Asp substitution is the simplest possible acidic-side-chain truncation: it removes one methylene group (~1.5 Å), preserving the negative charge and hydrogen-bonding donor/acceptor profile while reducing side-chain reach. The logic is geometric: if the basic residue at the MC4R TM2/ECL1 rim sits shallower in the binding vestibule than the corresponding residue in MC1R (as suggested by comparative receptor homology analysis), then a shorter carboxylate would achieve optimal salt-bridge geometry at MC4R while losing contact efficiency at MC1R. This is a well-established medicinal chemistry strategy in GPCR peptide SAR — the MT-II/MS05 melanocortin analogue series, for example, demonstrates that single-atom changes in the acidic recognition element can shift subtype selectivity by orders of magnitude.

This modification is meaningfully distinct from all prior Semax folds in this lab. Fold #1 (N-terminal acetylation) modified the Met-1 α-amino group — abolishing the positive charge at the terminus, a change orthogonal to the Glu-2 carboxylate geometry. Fold #49 (His-3 methylation) targeted the imidazole tautomer to optimize aromatic stacking at the receptor. Fold #61 (Pro-5 → Hyp) rigidified the central β-turn via stereoelectronic effects. Fold #55 (cyclization) constrained the global topology. None of these touched Glu-2, leaving the position-2 SAR point entirely unexplored until now.

The Glu-2 residue is also a participant in the Met-Glu-His Cu(II) chelation complex characterized by Sciacca et al. (2022) and Tomasello et al. (2025). Shortening this residue will alter the chelation geometry — a meaningful confound for in vivo or cell-based selectivity assays (discussed under limitations).

PREDICTED PROPERTIES (FAVOURABLE CHANGES FROM NATIVE)

Property	Native Semax (MEHFPGP)	[Asp2]Semax (MDHFPGP)	Direction
pLDDT (MC4R complex)	~0.80 (reference)	0.81	→ Maintained
pTM	reference	0.89	↑ Strong
ipTM (interface)	reference	0.87	↑ Strong
Aggregation propensity	reference	0.0	→ Favorable
Stability score	reference	0.80	→ Comparable
	reference	0.41	→ Moderate

Property	Native Semax (MEHFPGP)	[Asp2]Semax (MDHFPGP)	Direction
BBB penetration (heuristic)			
Half-life (heuristic)	short	short (~15–45 min)	→ Unchanged
MC4R/MC1R selectivity (predicted)	baseline	improved (geometric inference)	↑ Hypothesized
Cu(II) chelation geometry	Met-Glu-His triad	Altered (Asp-2 shorter)	⚠ Confound

All values are in silico predictions or heuristic estimates. Selectivity improvement is a geometric inference from the predicted binding pose — no quantitative ΔΔG or MC1R counter-screen was computed.

The structural prediction places the Asp-2 carboxylate in proximity to a basic residue at the MC4R TM2/ECL2 rim, consistent with the hypothesized tighter salt-bridge. The His-Phe pharmacophore core is fully preserved, suggesting the substitution does not disrupt the primary receptor-recognition element. The near-zero aggregation propensity is an improvement over many modified peptides and suggests the variant should be synthetically tractable and solution-stable.

SUGGESTED NEXT STEPS

Further variants to consider: - **[β-Asp2]Semax** — replace Glu-2 with beta-aspartic acid (an isomer with an extended backbone connectivity) to probe whether the backbone geometry matters as much as the side-chain length at position 2. - **[Gln2]Semax** — neutral amide isostere of Glu-2 as a negative control; if selectivity shifts disappear, this confirms the electrostatic mechanism. - **[D-Asp2]Semax** — D-amino acid at position 2 would also disrupt the Cu(II) chelation triad and provide a stereochemical probe of the salt-bridge geometry; compare with Fold #41 (D-Thr-1 Selank), which earned a PROMISING verdict on a related design logic in the same lab series. - **Counter-screen fold: [Asp2]Semax vs. MC1R** — a computational fold docking the same MDHFPGP sequence against MC1R (UniProt Q01726) would provide the selectivity ratio inference that this fold alone cannot deliver. This is the highest-priority next computational step.

Validation experiments: - **Radioligand displacement assay** — competitive binding of [Asp2]Semax vs. [125I]-NDP-α-MSH at hMC4R and hMC1R expressed in HEK293 cells; this is the definitive selectivity readout and would establish Ki values at both subtypes. - **cAMP functional assay (Gs)** — measure EC50 and Emax at MC4R and MC1R in parallel to capture any functional selectivity (biased agonism) not captured by binding alone. - **Cu(II) chelation titration** — ITC or UV-vis spectroscopic titration of [Asp2]Semax with Cu(II) vs. native Semax to quantify the

Kd shift introduced by the Glu→Asp change; this would allow the copper confound to be characterized and controlled for in cellular assays. - **SPR binding kinetics** — surface plasmon resonance on immobilized MC4R ECD (or nanodisc-reconstituted receptor) to compare kon/koff for native vs. [Asp2]Semax and estimate whether the interface improvement predicted by ipTM translates to slower off-rate. - **NMR structural characterization** — 2D NOESY in aqueous solution to confirm that the His-Phe β-turn geometry is maintained in [Asp2]Semax and that the Asp-2 side chain adopts a conformation compatible with the predicted salt-bridge geometry.

Cross-fold integration: This fold complements the emerging Semax SAR map in this lab. The combination of Fold #49 (His-3 methylation, optimizing the aromatic stacking interaction) with Fold #74's Asp-2 substitution (optimizing the electrostatic anchor) represents a logical double-substitution candidate — [D-mHis3, Asp2]Semax — that could be explored in a future fold to assess whether the two modifications are additive or interfering at the receptor interface.

SEQUENCES

NATIVE

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MEHFPGP
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MODIFIED

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MDHFPGP
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled); no Chai-1 agreement score available for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- MC4R/MC1R selectivity improvement is a geometric inference from a single docked pose — no MC1R counter-screen was computed and no quantitative ΔΔG values were returned by Boltz-2's affinity module
- Glu-2 participates in the Met-Glu-His Cu(II) chelation complex; Glu→Asp substitution alters chelation geometry and this confound is not resolved by structural prediction alone

- no published wet-lab data quantifies Semax's binding affinity at any MCR subtype — the entire selectivity hypothesis is built on a plausible but unvalidated pharmacological premise
- heuristic BBB penetration, stability, and half-life estimates are sequence-based approximations, not experimental measurements
- GPCR extracellular loop regions are conformationally flexible; static structural predictions may not capture the dynamic ensemble relevant to binding

CITATIONS

1. **PMID —** (2005) — — Semax, an ACTH(4-10) analogue with nootropic properties, activates dopaminergic and serotoninergic brain systems in rodents.
2. **PMID —** (2022) — — Semax, a Synthetic Regulatory Peptide, Affects Copper-Induced Abeta Aggregation and Amyloid Formation in Artificial Membrane Models.
3. **PMID —** (2025) — — Semax, a Copper Chelator Peptide, Decreases the Cu(II)-Catalyzed ROS Production and Cytotoxicity of aβ by Metal Ion Stripping and Redox Silencing.
4. **PMID —** (2021) — — Semax, synthetic ACTH(4-10) analogue, attenuates behavioural and neurochemical alterations following early-life fluvoxamine exposure in white rats.
5. **PMID —** (2017) — — Semax, an analog of ACTH
6. **PMID —** (2025) — — Semax peptide targets the μ opioid receptor gene Oprm1 to promote deubiquitination and functional recovery after spinal cord injury in female mice.
7. **PMID —** (2010) — — Semax and Pro-Gly-Pro activate the transcription of neurotrophins and their receptor genes after cerebral ischemia.
8. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions.

SOLANA SIGNATURE 3qCN1JE66XHvLqukwDgYZB9y8QPahnUGzq8QT3Z9u1ikYwWCN2WL2FpJGGoFVTaT3L4XwR9ddpG7R5CmcCJuYufX

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