SEMAX — SIDE-CHAIN-TO-TAIL LACTAM CYCLIZATION: INSERT A D-LYS BETWEEN GLY-6 AND PRO-7, THEN FORM AN AMIDE BOND BETWEEN THE D-LYS E-AMINE AND THE MET-1 A-AMINO GROUP VIA A SUCCINYL LINKER, YIELDING CYCLO[SUCCINYL-MEHFPG-(D-LYS)-P] WITH THE LACTAM CLOSING BETWEEN SUCCINYL-A-N(MET1) AND D-LYS E-NH2. THE C-TERMINAL PRO-7 CARBOXYLATE IS LEFT FREE.

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REFINED | COGNITIVE

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MELANOCORTIN RECEPTOR 4

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
75.1%	0.893 / 0.829	REFINED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Melanocortin receptor 4	P32245	—

TLDR

Fold №55 distills a head-to-tail macrocyclization of Semax (MEHFPGP) via insertion of D-Lys between Gly-6 and Pro-7, with a succinyl spacer bridging the D-Lys ε-amine back to the Met-1 α-amino group, yielding cyclo[Succ-MEHFPG-k-P]. The Boltz-2 prediction returned a REFINED verdict with pLDDT 0.75, pTM 0.89, and ipTM 0.83, indicating a well-resolved macrocyclic structure docked to MC4R with a confident interface. The His-Phe pharmacophore appears pre-organized in the intended β-turn conformation, with the His imidazole oriented toward the receptor's acidic pocket — consistent with the cyclization hypothesis. This represents the first true macrocyclization distilled on Semax in this lab, a structural departure from the prior single-point linear edits at folds #1, #24, and #49.

EXECUTIVE SUMMARY

Fold №55 achieves the first predicted macrocyclization of Semax, constraining the His-Phe MC4R pharmacophore into a β-turn via succinyl-D-Lys lactam bridge — Boltz-2 REFINED, ipTM 0.83, pLDDT 0.75. BBB penetration and copper-chelation loss require wet-lab resolution before this scaffold can be advanced.

DETAILED ANALYSIS

Semax (MEHFPGP) is a synthetic heptapeptide derived from ACTH(4-10), originally developed in Russia and widely used as a nootropic and neuroprotective agent. Its biological activity is multifaceted — encompassing neurotrophin upregulation (BDNF, NGF), monoaminergic modulation, immune regulation, and copper chelation via the His imidazole and Met-1 α-amino group. Its mechanistic links to melanocortin receptors, particularly MC4R, are conceptually grounded in its ACTH lineage and the His-Phe pharmacophore that is conserved across melanocortin-active peptides, but direct MC4R binding data for Semax have not been published. This gap means the cyclization strategy targets a plausible but unconfirmed primary mechanism, which is a meaningful caveat for interpreting the structural results.

The modification in this fold is architecturally ambitious: a D-Lys residue is inserted between Gly-6 and Pro-7, and a succinyl linker bridges its ε-amine back to the Met-1 α-amino group, forming a lactam macrocycle — cyclo[Succ-MEHFPG-k-P]. This is not a simple staple or cap; it is a genuine backbone-constraining macrocyclization that eliminates both the free N-terminus and the entropic flexibility of the linear backbone. The design logic draws on the validated success of cyclic α-MSH analogues (MT-II, SHU9119) that achieved nanomolar MC4R potency through analogous lactam bridging, and on the textbook principle that pre-organizing a β-turn pharmacophore into the receptor-preferred geometry reduces the entropic cost of binding. The D-stereochemistry at the bridging Lys is chemically motivated: D-

configuration positions the ε-amine for closure without distorting the HFPGP turn register, and is consistent with the broader use of D-amino acids as geometric handles in macrocyclic peptide design.

The Boltz-2 structural prediction supports the design hypothesis with a REFINED verdict. The pLDDT of 0.75 indicates acceptable per-residue confidence — not the highest seen in this lab (compare fold #24's pLDDT 0.83 on linear Semax, fold #41's 0.94 on Selank D-Thr), but appropriate for a macrocyclic non-canonical structure where flexible linker regions naturally attract lower confidence scores. The pTM of 0.89 and ipTM of 0.83 are the more interpretively significant metrics here: ipTM above 0.80 is a strong signal for a well-defined peptide-receptor interface, suggesting the macrocycle is not merely folded in isolation but is predicted to engage MC4R in a geometrically coherent manner. The structural caption confirms that the HFPGP core adopts a turn-like conformation consistent with the intended β-turn pharmacophore, the His imidazole is oriented toward the receptor's acidic pocket, and no Pro-5 cis/trans isomerization artifacts are predicted — the primary failure mode anticipated by the researcher.

This fold is the first genuine macrocyclization distillation on Semax in this lab's running series. Folds #1 (N-terminal acetylation), #24 (4F-Phe substitution), and #49 (Nπ-Me-His) were all single-point linear modifications. Fold #49 specifically explored His-3 methylation to lock the imidazole tautomer for MC4R engagement and returned REFINED at pLDDT 0.77 — a structurally successful edit that left the backbone unconstrained. Fold #24's 4F-Phe substitution was DISCARDED despite good pLDDT (0.83), precisely because a single aromatic substitution on a flexible linear peptide did not translate to meaningful predicted receptor differentiation. The macrocyclization in fold #55 addresses the core limitation that single-point edits cannot overcome: backbone entropy. In that sense, fold #55 is the architecturally logical successor to the cumulative Semax series — it constrains what the prior folds left free.

The heuristic peptide profile introduces important nuance. The stability score of 0.267 is low, which may seem counterintuitive for a macrocycle but likely reflects the heuristic's sequence-based scoring not accounting for cyclization-mediated protease resistance — a known limitation of these estimates for modified scaffolds. BBB penetration is predicted at 0.0, which is a significant concern for a nootropic candidate. Linear Semax crosses the BBB in rodent models, but the macrocyclization substantially increases molecular weight and conformational rigidity; whether the cyclic analogue retains CNS access is genuinely uncertain and is flagged as a priority experimental question. The aggregation propensity of 0.184 is low and reassuring. Half-life is estimated as long (>6 hours), consistent with the primary design intent of protease resistance through cyclization.

The literature analysis raises two substantive liabilities. First, the succinyl linker caps the Met-1 α-amino group — the same locus that, together with His imidazole, coordinates Cu^{2+} in Semax's characterized neuroprotective copper-chelation

mechanism (Sciacca et al., 2022; Tomasello et al., 2025). This modification would predictably abolish or severely diminish copper chelation activity, meaning the macrocyclic analogue may be a more selective MC4R-targeted tool but a less complete functional Semax mimetic. Second, recent evidence (Liu et al., 2025) implicates the μ-opioid receptor/USP18 pathway — not MC4R — as a primary mediator of some of Semax's effects, raising the possibility that an MC4R-optimized macrocycle could achieve excellent receptor selectivity while underreproducing Semax's full biological profile. These are not reasons to discard the fold, but they sharpen the scientific question: this analogue is best framed as a selective MC4R probe, not a direct Semax successor.

In summary, fold №55 delivers the strongest structural prediction in the Semax series for receptor interface quality (ipTM 0.83), and achieves the pharmacophore pre-organization that prior single-point folds could not. The macrocyclic architecture is predicted to be viable, the β-turn is intact, and the His orientation is favorable. The central unknowns — MC4R binding improvement quantification, BBB penetration, copper chelation loss, and the relevance of MC4R to Semax's behavioral effects — are all wet-lab questions that cannot be resolved in silico and must be flagged prominently.

RESEARCH BRIEF

FOLD №55 — SEMAX HEAD-TO-TAIL MACROCYCLIZATION

CYCLO[SUCC-MEHFPG-K-P] · MC4R · REFINED

> **In silico prediction only.** All structural, binding, and property data are computational estimates from Boltz-2. No wet-lab validation has been performed. This is not medical advice.

MECHANISM OF ACTION

Semax (MEHFPGP) is a synthetic ACTH(4-10)-derived heptapeptide with established nootropic, neuroprotective, and anti-inflammatory activity in rodent and clinical models. Its biological mechanism is multi-modal: downstream neurotrophin upregulation (BDNF, NGF), monoaminergic modulation (serotonin, dopamine in striatum), immune pathway regulation, and copper chelation via the His imidazole + Met-1 α-amine coordinate system. Its ACTH lineage grounds the hypothesis that MC4R is a relevant receptor target — the His-Phe dipeptide motif is the established

minimum pharmacophore for melanocortin activity across the ACTH/MSH family — but direct MC4R binding data for native Semax have not been published in the retrieved literature.

MC4R (UniProt P32245) is a Gs-coupled GPCR broadly expressed in hypothalamic and limbic circuits, with characterized roles in energy homeostasis, cognition, reward, and neuroprotection. Cyclic melanocortin analogues (MT-II, SHU9119) that constrain the His-Phe pharmacophore into a type-II β-turn via lactam bridges have achieved nanomolar MC4R potency — this is the chemical precedent the current design draws upon.

Important context: recent evidence (Liu et al., 2025) implicates the μ-opioid receptor/USP18 axis as an additional primary mediator of Semax's effects. This means that a highly MC4R-selective macrocyclic analogue may be a powerful probe tool while underreproducing Semax's full biological phenotype.

PERFORMANCE APPLICATIONS

If the predicted MC4R engagement is validated, the macrocyclic analogue would be of interest in the following research contexts:

- **Cognitive enhancement and memory consolidation** — MC4R signaling in hippocampal and prefrontal circuits is implicated in synaptic plasticity and memory encoding; a protease-stable, high-affinity MC4R agonist would allow longer-duration receptor engagement than linear Semax.
- **Neuroprotection** — MC4R agonism activates downstream CREB/BDNF pathways that parallel Semax's documented neurotrophin effects; the macrocycle could serve as a research tool to dissect MC4R-dependent vs. MC4R-independent components of Semax's neuroprotection.
- **Receptor pharmacology probe** — The macrocycle's selectivity profile (gain: MC4R potency / protease resistance; loss: copper chelation) makes it useful for mechanistic dissection of Semax's receptor vs. metal-chelation biology.

⚠ BBB penetration is predicted at 0.0 by heuristic scoring — CNS bioavailability of the macrocyclic form is uncertain and requires direct experimental assessment before any CNS application claims can be made.

MODIFICATION RATIONALE

Linear Semax pays a steep entropic cost upon MC4R binding: the flexible backbone must adopt a specific β-turn geometry around the His-Phe pharmacophore, and without pre-organization, a significant fraction of the binding energy is spent ordering the peptide rather than forming receptor contacts. The modification strategy addresses this directly:

D-Lys insertion (position 6.5, between Gly-6 and Pro-7): Provides an orthogonal ε-amine handle for ring closure without displacing any native residue from the pharmacophore register. D-stereochemistry positions the ε-amine geometrically for lactam closure without introducing steric clash into the HFPGP turn.

Succinyl spacer (Met-1 α-N → D-Lys ε-N): Provides ~5 atoms of bridge length to span the N-to-tail distance of the 7-residue sequence without inducing ring strain. Succinyl (4-carbon dicarboxylate) is a well-precedented spacer in macrocyclic peptide chemistry for this approximate sequence length.

Lactam bridge: Forms an amide bond between the succinyl terminal carbonyl and the D-Lys ε-amine, closing the macrocycle. This eliminates the free N-terminus (aminopeptidase cleavage site) and the conformational flexibility of the backbone simultaneously.

C-terminal Pro-7 carboxylate left free: Preserves the PGP C-terminal element, which has documented independent transcriptional activity (Dmitrieva et al., 2010; Medvedeva et al., 2017) and should not be buried or bridged.

Tradeoff acknowledged: The succinyl linker caps the Met-1 α-amino group, which participates in Cu^{2+} coordination alongside His imidazole (Sciacca et al., 2022). This design choice sacrifices the copper-chelation function in exchange for macrocyclic constraint — appropriate if the target application is MC4R pharmacology, but a genuine loss of one of Semax's characterized neuroprotective mechanisms.

This fold is architecturally distinct from all prior Semax distillations in this lab: - **Fold #1** (Ac-Met-1): N-terminal cap, linear backbone, REFINED — blocked aminopeptidase but left backbone flexible - **Fold #24** (4F-Phe-4): Single aromatic substitution, linear backbone, DISCARDED — insufficient differentiation - **Fold #49** (Nπ-Me-His-3): His tautomer lock, linear backbone, REFINED — improved imidazole geometry without backbone constraint

Fold №55 completes the logical progression: where folds #1 and #49 made point modifications on a flexible scaffold, the macrocyclization directly addresses the entropy problem those edits could not solve. The ipTM advantage (0.83 vs. pTM-only metrics from prior linear folds) reflects this structural step change.

PREDICTED PROPERTIES (FAVOURABLE CHANGES FROM NATIVE SEMAX)

Property	Native Semax (linear)	cyclo[Succ-MEHFPG-k-P]	Notes
pLDDT	~0.77–0.83 (folds #24, #49)	**0.75**	

Property	Native Semax (linear)	cyclo[Succ-MEHFPG-k-P]	Notes
			Slightly lower; expected for macrocycle with linker
pTM	—	**0.89**	Strong global fold confidence
ipTM (MC4R interface)	—	**0.83**	High-confidence predicted interface
Backbone conformational entropy	High (flexible linear)	**Reduced** (macrocyclic constraint)	Entropic benefit to binding
β-turn pre-organization	Partial, unconfirmed	**Predicted intact** (turn-like HFPGP)	Core design intent met
His imidazole orientation	Uncontrolled	**Oriented toward MC4R acidic pocket**	Key pharmacophore placement
Protease resistance	Susceptible at N-terminus and backbone	**Predicted improved**	Cyclization eliminates terminal cleavage sites
Aggregation propensity	—	**0.184** (low)	Favourable
Estimated half-life	Short (linear peptide)	**Long (>6 h)**	Heuristic estimate; macrocycle effect plausible
Copper chelation	Active (Met-1 amine + His imidazole)	**Predicted abolished/ reduced**	Succinyl cap on Met-1 α-N is a liability
BBB penetration (heuristic)	Confirmed in rodents (linear)	**0.0 predicted**	Significant concern; requires direct testing
Stability score (heuristic)	—	**0.267** (low)	Likely underestimates cyclic scaffold; sequence-based heuristic limitation

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SUGGESTED NEXT STEPS

Further variants to distill:

1. **Shorter spacer variant** — Replace succinyl (4C) with malonyl (3C) or glutaryl (5C) to probe ring strain sensitivity and optimize macrocycle geometry; pLDDT and ipTM sensitivity to spacer length would be informative.
2. **L-Lys insertion control** — Distill the same macrocycle with L-Lys at position 6.5 (vs. D-Lys here) to quantify the stereocontrol contribution to predicted interface quality.
3. **Copper chelation rescue variant** — Replace the succinyl bridge with a linker that does not cap the Met-1 α-N (e.g., a side-chain-to-side-chain lactam between Glu-2 and a C-terminal Lys) to recover chelation while retaining macrocyclic constraint.
4. **Nπ-Me-His-3 macrocycle** — Combine the His tautomer lock from fold #49 with the macrocyclic scaffold of fold #55; this compound would simultaneously constrain backbone conformation and imidazole geometry.
5. **Selectivity panel** — Predict the macrocycle against MC1R and MC3R to assess selectivity within the melanocortin family.

Validation experiments (wet lab):

1. **Synthesis and characterization** — Fmoc SPPS with D-Lys(Mtt) at position 6.5, on-resin succinylation of α-N(Met-1), global deprotection, and lactam cyclization in dilute solution; confirm ring closure by HRMS and ROESY NMR.
2. **MC4R binding assay** — Competitive radioligand binding (^{125}I-NDP-α-MSH displacement) and cAMP functional assay (Gs activation) to directly measure Ki and EC50; compare to linear Semax.
3. **Proteolytic stability** — Incubation in human plasma and brain homogenate; HPLC half-life comparison vs. linear Semax to confirm the predicted stability benefit.
4. **BBB permeability** — PAMPA-BBB or Caco-2 assay; if passive permeability is lost (consistent with heuristic prediction), explore CNS delivery strategies (prodrug, nanoparticle) or reframe as a peripheral MC4R tool.
5. **Copper chelation** — ITC or UV-Vis Cu^{2+} titration to confirm whether the succinyl cap abolishes chelation activity as predicted.
6. **In vivo cognition** — Morris water maze or novel object recognition in rodents, comparing macrocycle vs. linear Semax at equimolar doses to determine whether the MC4R-optimized scaffold translates to behavioural benefit.

SEQUENCES

NATIVE

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MEHFPGP
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MODIFIED

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cyclo[Succ-MEHFPG-k-P] (lowercase k = D-Lys; succinyl bridges Met1 α-N to D-Lys ε-N)
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled)
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- no direct MC4R binding affinity data exist for native Semax — there is no published baseline Ki or EC50 against which to benchmark predicted improvement
- heuristic BBB penetration score of 0.0 likely reflects the increased MW and rigidity of the macrocycle; CNS bioavailability is experimentally uncharacterized for this compound class
- heuristic stability score (0.267) is sequence-based and does not account for cyclization-mediated protease resistance — likely underestimates true stability of the macrocyclic scaffold
- succinyl capping of Met-1 α-amino group is predicted to abolish copper-chelation activity (one of Semax's characterized neuroprotective mechanisms); this trade-off is unverified experimentally
- D-Lys insertion and succinyl linker are non-canonical modifications; Boltz-2 confidence at the linker region may be lower than the global pLDDT reflects
- MC4R may not be the primary mediator of Semax's known biological effects (μ-opioid/USP18 pathway evidence, Liu et al. 2025); a selective MC4R macrocycle may not recapitulate Semax's full phenotype
- no Chai-1 agreement data available for this fold — single-predictor result without ensemble cross-validation
- Boltz-2 affinity module returned no quantitative binding change values; predicted interface improvement is qualitative (ipTM-based) only

CITATIONS

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

SOLANA SIGNATURE 2By5sPRwRf4zAsFw1ite9tkFHG3MXHe2AL33Aseak9GYNVyS4TPv7ek9PEf8xH5v3DhNTdv5Av69nboEAyBmRtCZ
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