SS-31 — PHE-4 → L-4,4'-BIPHENYLALANINE (BIP) SUBSTITUTION AT THE C-TERMINAL AROMATIC RESIDUE, EXTENDING THE AROMATIC SURFACE TO ENGAGE THE DEEP HYDROPHOBIC S1 POCKET OF THE SEROTONIN TRANSPORTER (SERT, SLC6A4)

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DISCARDED | LONGEVITY

PHE-4 → L-4,4'-BIPHENYLALANINE (BIP) SUBSTITUTION AT THE C-TERMINAL AROMATIC RESIDUE, EXTENDING THE AROMATIC SURFACE TO ENGAGE THE DEEP HYDROPHOBIC S1 POCKET OF THE SEROTONIN TRANSPORTER (SERT, SLC6A4)

SODIUM-DEPENDENT SEROTONIN TRANSPORTER (SERT)

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
—	— / —	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Sodium-dependent serotonin transporter (SERT)	—	—

DISCARDED BY PREDICTABILITY GATE

target_not_predictable: no UniProt ID resolved — target identity unconfirmed

TLDR

FOLD №64 was DISCARDED before structural prediction could run — the orchestrator's predictability gate could not resolve a confirmed UniProt ID for SERT

(SLC6A4) from the pipeline's canonical target list, making the fold non-evaluable by current tools. The hypothesis itself — that a Phe-4 → biphenylalanine (Bip) substitution on SS-31 could engage SERT's deep hydrophobic S1 pocket via an extended π-surface — is structurally motivated and novel, as no published data exist on SS-31/SERT interactions. This is a tool-limit failure, not a biological invalidation: the question remains entirely open and scientifically interesting.

EXECUTIVE SUMMARY

FOLD №64 DISCARDED: pipeline target resolution gate failed to confirm UniProt ID for SERT (SLC6A4) — no structural prediction ran. The SS-31 Phe-4→Bip/SERT hypothesis is novel, structurally motivated, and fully open. Re-run with P31645 explicit input recommended.

DETAILED ANALYSIS

SS-31 (elamipretide; D-Arg-Dmt-Lys-Phe-NH2) is a synthetic mitochondria-targeting tetrapeptide whose alternating cationic-aromatic architecture drives its canonical mechanism: selective binding to cardiolipin in the inner mitochondrial membrane, suppression of reactive oxygen species, and restoration of mitochondrial membrane potential. Its clinical and preclinical profile spans renal, cardiac, neurological, and musculoskeletal indications — all rooted in mitochondrial biology. Yet the same cation-aromatic motif that governs cardiolipin affinity structurally resembles the basic-plus-aromatic pharmacophore of monoamine substrates and several SSRI scaffolds, raising the question of whether SS-31 variants might engage monoamine transporters as an off-target or deliberate secondary interaction.

The hypothesis tested in this fold was specific: replace the C-terminal Phe-4 of SS-31 with L-4,4'-biphenylalanine (Bip), a conformationally rigid, extended biaryl amino acid. The rationale draws on SERT's (SLC6A4) well-characterized S1 orthosteric pocket, which accommodates halogenated and extended biaryl aryl systems in citalopram, paroxetine, and sertraline. Bip extends the Phe ring by one rigid phenyl plane while preserving planarity and hydrophobicity — analogous to the medicinal chemistry strategy used to improve hydrophobic subsite engagement in SSRI development. The hypothesis predicted that the D-Arg/Lys cations would engage Asp98 at the SERT Na1 site while the biaryl system stacked against Tyr95, Phe335, and Phe341 in the deep hydrophobic subsite.

This fold is part of a productive series of C-terminal aromatic substitutions at SS-31's Phe-4 position in this lab. Fold #11 (Phe-4 → 2-Naphthylalanine) and Fold #17 (Phe-4 → 1-Naphthylalanine) both returned PROMISING verdicts with pLDDT 0.85, demonstrating that the pipeline can successfully model expanded aromatic substitutions at this position against well-resolved protein targets. Fold #56 (Phe-4 → Tyr, targeting P-glycoprotein/ABCB1) was also DISCARDED — a pattern that

suggests the pipeline's target resolution gate, rather than the chemistry, is the limiting factor when the target deviates from canonical cardiolipin biology. The Bip substitution proposed here is chemically more ambitious than either Nal regioisomer, extending the aromatic surface further, and would be the natural continuation of that series if the target resolution barrier can be cleared.

The discard was triggered not by structural prediction failure but by the orchestrator's predictability gate: no UniProt ID for SERT (SLC6A4; P31645) was resolved from the pipeline's canonical target list. This is a pure tool-limit event. SERT is among the most structurally characterized human membrane transporters — multiple cryo-EM and X-ray structures exist in complex with SSRIs and substrate analogs (PDB: 5I6X, 6AWO, 7LIA among others) — making it entirely tractable in principle for Boltz-2/Chai-1 complex prediction. The failure was administrative/ database resolution, not structural unpredictability.

The literature base for the specific SS-31/SERT hypothesis is essentially absent. No published experimental or computational study has examined SS-31 or any close analog at SERT. The supporting evidence is entirely analogical: structural resemblance of SS-31's pharmacophore to monoamine ligands, a 2024 Stefaniak et al. preprint showing SS-31's cation-aromatic surface can engage non-cardiolipin targets (alpha-synuclein/membrane displacement), and Bip's established use in medicinal chemistry for hydrophobic subsite extension. Complicating factors include SS-31's tetrapeptide size (~640 Da, net +3 charge) versus small-molecule SSRIs, the presence of D-amino acids at positions 1 and 2 (potential steric conflict with SERT's stereospecific elements), and no published evidence of serotonergic behavioral phenotypes in SS-31-treated animals.

From a cross-fold perspective, this work sits at an interesting junction in the lab's narrative. The 2-Nal and 1-Nal series (Folds #11 and #17) established that expanded C-terminal aromatics on SS-31 produce promising structural signals against protein targets. The Bip modification is the logical extension — a longer, more rigid biaryl system — but directed at a genuinely novel target (SERT) rather than the canonical cardiolipin interface. If the target resolution barrier is cleared in a future fold, the pipeline already has precedent for modeling this position successfully.

In silico limitations are significant here beyond the gate failure. Even if successfully modeled, a tetrapeptide's interaction with a transporter's orthosteric pocket raises questions that structure prediction alone cannot resolve: functional transport inhibition versus mere binding, pharmacokinetic access to neuronal SERT in vivo, and whether D-amino acid chirality at positions 1-2 is compatible with the binding geometry predicted. These questions require wet-lab adjudication.

RESEARCH BRIEF

FOLD №64 — SS-31 PHE-4 → BIPHENYLALANINE (BIP): SERT S1 POCKET PROBE

Verdict: DISCARDED | Class: LONGEVITY | Focus: AFFINITY

TLDR

This fold was **DISCARDED** due to a tool-limit failure: the orchestrator's predictability gate could not resolve a confirmed UniProt ID for SERT (SLC6A4) from the pipeline's canonical target list, and structural prediction was never attempted. This is a **database/pipeline resolution failure — not a biological invalidation** of the hypothesis. The chemistry is novel, the target is tractable in principle, and the question remains entirely open.

WHAT WE TRIED

SS-31 (elamipretide; D-Arg-Dmt-Lys-Phe-NH2) carries an alternating cationic-aromatic tetrapeptide motif that mirrors the basic-plus-aromatic pharmacophore of monoamine substrates and several SSRI scaffolds. This fold asked: can replacing the C-terminal Phe-4 with L-4,4'-biphenylalanine (Bip) — a rigid, extended biaryl amino acid — create a measurable, structure-prediction-tractable interface with the serotonin transporter (SERT/SLC6A4) S1 orthosteric pocket?

The hypothesis drew on SERT's well-characterized deep hydrophobic subsite, which accommodates the halogenated/extended aryl moieties of citalopram, paroxetine, and sertraline. Bip extends Phe-4's single ring by one rigid phenyl plane without sacrificing planarity or hydrophobicity — an analogue of the medicinal chemistry strategy used in SSRI hydrophobic subsite optimization. The predicted binding geometry placed D-Arg and Lys cations engaging SERT's Asp98 (Na1 coordination site) while the biaryl system stacked against Tyr95, Phe335, and Phe341 in the deep hydrophobic subsite. A native Phe-4 SS-31 parallel model was intended as an internal comparator.

This fold is the third C-terminal aromatic substitution at SS-31's Phe-4 position explored in this lab. **Fold #11** (Phe-4 → 2-Naphthylalanine, pLDDT 0.85, PROMISING) and **Fold #17** (Phe-4 → 1-Naphthylalanine, pLDDT 0.85, PROMISING) demonstrated that the pipeline can successfully model expanded C-terminal aromatics at this position. Bip is the next logical step: longer rigid biaryl extension,

directed at a novel protein target rather than the cardiolipin interface. **Fold #56** (Phe-4 → Tyr, targeting P-glycoprotein) was also DISCARDED — a recurrent pattern suggesting the gate is the constraint when targets deviate from the canonical SS-31 biology.

WHY IT WAS DISCARDED

Primary reason (orchestrator gate): `target_not_predictable: no UniProt ID resolved — target identity unconfirmed`

The pipeline's canonical target resolution step could not confirm a UniProt entry for SERT (SLC6A4; canonical ID P31645; ChEMBL CHEMBL228) from its internal list. Structural prediction (Boltz-2/Chai-1) was therefore never run. This is a **pipeline database gap**, not a reflection of SERT's structural tractability. SERT is among the best-characterized human membrane transporters: high-resolution cryo-EM and X-ray structures exist in complex with SSRIs and substrate analogs (e.g., PDB 5I6X — LeuT-SERT chimera with S-citalopram; PDB 6AWO — hSERT + ibogaine; PDB 7LIA — hSERT cryo-EM). In principle, Boltz-2/Chai-1 should be able to model a peptide–SERT complex if the target is correctly specified.

No structural prediction metrics (pLDDT, pTM, ipTM, binder probability, Chai-1 agreement) were generated for this fold.

WHAT THIS DOESN'T MEAN

DISCARDED is not "disproved." The discard here reflects a pipeline database resolution failure — the question of whether SS-31 Phe-4→Bip engages SERT's S1 pocket was never evaluated, computationally or experimentally. The hypothesis remains scientifically novel and structurally motivated: no published study (experimental or computational) has examined SS-31 or any modified analog at SERT or any SLC6-family transporter. The literature provides no empirical precedent — positive or negative — for this interaction. The alternating cation-aromatic motif of SS-31 structurally resembles monoamine pharmacophores, Bip is an established medicinal chemistry tool for SSRI hydrophobic subsite extension, and the 2024 Stefaniak et al. preprint demonstrates SS-31's cation-aromatic surface can engage non-cardiolipin molecular targets. This is an open, unexplored question that deserves proper evaluation.

WHAT WOULD ANSWER THE QUESTION

- **Re-run with explicit UniProt ID:** Specify SERT as P31645 (human SLC6A4) directly to the Boltz-2/Chai-1 pipeline, using PDB 7LIA or 6AWO as template

structure. The Bip modification requires a SMILES/CIF input for the non-canonical residue — this is the most immediate path to a structural verdict within this lab.

- **Radioligand displacement assay (SPQ or [³H]-imipramine):** Quantitative binding assay at recombinant hSERT expressed in HEK293 cells would give a direct K_i for SS-31-Bip vs. native SS-31 vs. a known SSRI comparator (citalopram IC_{50} ~1 nM as anchor). Even a weak K_i (>10 µM) would be informative as a starting SAR point.
- **Electrophysiology / uptake inhibition (ASP⁺ or [³H]-5-HT assay):** Functional serotonin transport inhibition assay (e.g., fluorescent ASP^+ uptake in SERT-HEK293) would confirm whether any binding translates to transporter blockade — relevant because tight binding without functional inhibition is possible for large ligands at allosteric sites.
- **Free energy perturbation (FEP) or Glide SP docking:** If a SERT crystal structure is used as rigid receptor input (PDB 7LIA recommended for open-outward hSERT), standard small-molecule docking tools (Glide, AutoDock-GPU) could accommodate a tetrapeptide with non-canonical Bip residue and provide a ranked binding pose and estimated ΔG — circumventing the AlphaFold-based pipeline's target resolution gate entirely.

RAW METRICS

Metric	Value
pLDDT	Not generated (gate failure)
pTM	Not generated
ipTM	Not generated
Binder probability (Boltz-2)	Not generated
Chai-1 agreement	Not generated
Heuristic stability/BBB/aggregation	Not generated

No structural prediction was attempted. All metrics above reflect pipeline gate failure, not structural prediction output.

This fold is part of the lab's ongoing SS-31 C-terminal aromatic substitution series. See Fold #11 (2-Nal, PROMISING), Fold #17 (1-Nal, PROMISING), and Fold #56 (Tyr → ABCB1, DISCARDED) for prior entries. The Bip extension is the most chemically ambitious variant in this series and the first directed at a monoamine transporter target.

SEQUENCES

NATIVE

```
DArgDmtLysPhe
```

MODIFIED

```
DArg-Dmt-Lys-Bip
```

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
- fold was DISCARDED at the orchestrator gate — no structural prediction metrics (pLDDT, pTM, ipTM, binder probability) were generated; this is a tool-limit/ database failure, not a biological invalidation
- SERT (SLC6A4) is a well-characterized target in principle, but pipeline target resolution failed; manually specifying UniProt P31645 is required for a future structural attempt
- biphenylalanine (Bip) is a non-canonical amino acid — Boltz-2/Chai-1 require explicit SMILES or CIF parameterization for non-canonical residues; this is an additional technical hurdle beyond target resolution
- SS-31 is a tetrapeptide (~640 Da, net +3 charge) substantially different in size and charge from small-molecule SSRIs; whether a tetrapeptide can adopt a productive binding geometry in SERT's S1 pocket is unresolved
- D-amino acids at positions 1 (D-Arg) and 2 (Dmt) may introduce steric conflicts with SERT's stereospecific recognition elements — this cannot be assessed without structural modeling
- no heuristic property estimates (aggregation, stability, BBB penetration, half-life) were generated due to gate failure

CITATIONS

1. **PMID —** (2020) — — Mitochondrial protein interaction landscape of SS-31
2. **PMID —** (2024) — — Therapeutic Peptide SS-31 Modulates Membrane Binding and Aggregation of Alpha-Synuclein and Restores Impaired Mitochondrial Function

3. **PMID —** (2022) — — SS-31, a Mitochondria-Targeting Peptide, Ameliorates Kidney Disease
4. **PMID —** (2019) — — Elamipretide (SS-31) improves mitochondrial dysfunction, synaptic and memory impairment induced by lipopolysaccharide in mice
5. **PMID —** (2023) — — SS-31 Improves Cognitive Function in Sepsis-Associated Encephalopathy by Inhibiting the Drp1-NLRP3 Inflammasome Activation
6. **PMID —** (2020) — — A Comprehensive Drug Repurposing Study for Covid19 Treatment: Novel Putative DHODH Inhibitors Show Association to Serotonin-Dopamine Receptors
7. **PMID —** (2025) — — SS-31@Fer-1 Alleviates ferroptosis in hypoxia/reoxygenation cardiomyocytes via mitochondrial targeting
8. **PMID —** (2024) — — New insight for SS-31 in treating diabetic cardiomyopathy: Activation of mitoGPX4 and alleviation of mitochondria-dependent ferroptosis
9. **PMID —** (2025) — — SS-31 Targets NOS2 to Enhance Osteogenic Differentiation in Aged BMSCs by Restoring Mitochondrial Function
10. **PMID —** (2024) — — SS-31 protects against bleomycin-induced lung injury and fibrosis

SOLANA SIGNATURE ckk5sJhA7bEtHPr37hbqL5ijHRbQHa7DZ8uMptazxr89uaGrfRzVwHx8KMmcGZm2sXRhLMEoGWgyjorKvZyuMiN
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