DISTILLATION №11 · ALEMBIC LABS

SS-31 — PHE-4 → 2-NAPHTHYLALANINE (2-NAL) SUBSTITUTION AT THE C-TERMINAL AROMATIC RESIDUE

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PROMISING LONGEVITY

PHE-4 → 2-NAPHTHYLALANINE (2-NAL) SUBSTITUTION AT THE C-TERMINAL AROMATIC RESIDUE

CARDIOLIPIN (MITOCHONDRIAL INNER MEMBRANE PHOSPHOLIPID)

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
85.3%	0.161 / 0.000	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Cardiolipin (mitochondrial inner membrane phospholipid)	—	—

TLDR

Fold #11 explores replacing the C-terminal phenylalanine of SS-31 (elamipretide) with 2-naphthylalanine (2-Nal) to deepen hydrophobic insertion into cardiolipin-rich mitochondrial membranes. The structure prediction returned a notably high pLDDT of 0.853 for this short tetrapeptide — well above the MOTS-c precedent in Fold #5 — with the expanded naphthyl side chain accommodated without backbone distortion, preserving the alternating cationic-aromatic pharmacophore geometry. No cardiolipin or membrane co-model was included, so the prediction captures intrinsic peptide conformation only, and functional claims remain speculative. The heuristic stability and low aggregation scores are encouraging, but the absence of any published SAR data at position 4 of SS-31 means this modification enters genuinely uncharted territory.

EXECUTIVE SUMMARY

SS-31 2-Nal4 variant: pLDDT 0.853, alternating cationic-aromatic pharmacophore preserved. Deeper cardiolipin insertion is structurally plausible — but no lipid co-model was run, and the CL-selectivity balance remains an open question.

DETAILED ANALYSIS

SS-31 (elamipretide, Bendavia) is a synthetic tetrapeptide — D-Arg-Dmt-Lys-Phe-NH2 — whose pharmacological identity is inseparable from its alternating cationic-aromatic architecture. Cationic residues (D-Arg at position 1, Lys at position 3) engage the anionic phosphodiester headgroups of cardiolipin through electrostatic attraction, while the aromatic residues (Dmt at position 2, Phe at position 4) contribute hydrophobic and van der Waals contacts with cardiolipin's acyl chain region. The Dmt residue carries an additional antioxidant burden — its dimethyltyrosine side chain can quench reactive oxygen species and engage in π-cation interactions — making it the better-characterized of the two aromatic positions. Phe-4, by contrast, is structurally underexplored: no published SAR study has systematically evaluated its contribution to insertion depth, headgroup contact, or downstream mitochondrial function.

The modification hypothesis in this DISTILLATION is conceptually straightforward: replace Phe-4 with 2-naphthylalanine (2-Nal), expanding the aromatic π-surface at the C-terminus to deepen van der Waals contact with cardiolipin acyl chains. The naphthyl ring system is a well-validated bioisostere of phenylalanine in membrane-active peptides — gramicidin and GLP-1 analogs among them — and confers approximately one additional log unit of lipophilicity without introducing chirality complications or oxidation liabilities. Because Phe-4 sits at the C-terminus in a solvent- and membrane-facing orientation rather than buried within an intramolecular fold, an increase in aromatic volume at this position is mechanistically plausible without invoking conformational disruption.

The AlphaFold structure prediction returned a pLDDT of 0.853 — surprisingly high for a four-residue peptide containing two non-canonical amino acids (D-Arg and Dmt). For context, the MOTS-c Nle substitution in Fold #5 achieved a pLDDT of 0.62, and the Epitalon D-Ala swap in Fold #6 collapsed to 0.34. The elevated confidence here likely reflects the conformational simplicity of a short extended tetrapeptide with minimal backbone ambiguity rather than any deep structural certainty — but it does confirm that the 2-Nal substitution is not introducing backbone strain or geometric incompatibility. The pTM score of 0.161 is expected and uninformative for a monomer of this length; ipTM is absent as no complex was modeled. The structural caption confirms the alternating cationic-aromatic spatial arrangement is preserved and the naphthyl side chain is accommodated without distortion.

From a heuristic profile perspective, the aggregation propensity of 0.068 is low and favorable — a meaningful consideration given that increased hydrophobicity from 2-Nal could, in principle, promote self-aggregation in aqueous solution. The stability score of 0.566 is moderate, and the half-life estimate of 30 minutes to 2 hours is consistent with the unmodified SS-31 range. BBB penetration of 0.25 is low but largely irrelevant for a mitochondrially-targeted compound designed to act in peripheral tissues, particularly cardiac and renal. The heuristic profile does not raise a red flag for solubility collapse, though this must be treated cautiously — real aqueous solubility at micromolar concentrations requires experimental measurement.

The literature context is both supportive and cautionary. Birk et al. (2013) established that aromatic residues engage cardiolipin acyl chains, providing the direct rationale for expanded aromatic surface area. Romanova et al. (2025) demonstrated that SS-31's membrane insertion geometry is functionally relevant for oxidative phosphorylation supercomplex stabilization — implying that modifications altering insertion depth are biologically meaningful, not merely physicochemical curiosities. The Stefaniak preprint (2024) suggests membrane affinity is tunable, with competitive binding to negatively charged membranes being dose-dependent. Together, these support the hypothesis that deeper acyl chain insertion could enhance cardiolipin engagement. However, a critical countervailing concern is selectivity: SS-31's functional specificity for cardiolipin-rich mitochondrial inner membranes depends on a finely tuned balance of electrostatic and hydrophobic contributions. A variant that tips this balance toward excess hydrophobicity could reduce selectivity for CL over other anionic phospholipids (phosphatidylserine, phosphatidylglycerol), potentially diluting mitochondrial targeting.

No co-modeling of cardiolipin, a lipid bilayer, or any protein partner was performed in this DISTILLATION. This is a fundamental limitation: the prediction captures intrinsic peptide conformation only, and all inferences about insertion depth, headgroup contact geometry, and supercomplex interactions are extrapolated from sequence-based heuristics and literature analogy. Molecular dynamics simulation of the 2-Nal variant in a cardiolipin-containing bilayer would be the natural computational next step. The Boltz-2 affinity module returned no values, and Chai-1 agreement is absent, meaning the promising verdict rests on structural plausibility and heuristic properties rather than any binding affinity prediction.

In the context of the lab's running narrative, this fold represents a continuation of the aromatic residue expansion strategy that was implicitly introduced with the MOTS-c Nle substitution in Fold #5 — though the mechanistic logic differs. Fold #5 was an isosteric oxidation-protection strategy; Fold #11 is an explicit membrane-pharmacophore enhancement strategy. Both share a common thread: non-canonical amino acid substitutions that are structurally conservative at the backbone level but functionally hypothesis-generating at the side chain level. The verdict of PROMISING is appropriate — the structural prediction does not contradict the modification hypothesis, the heuristic profile is not alarming, and the literature provides a

coherent rationale — but wet lab validation is required before any functional claim can be made.

RESEARCH BRIEF

DISTILLATION №11 — PROMISING

SS-31 PHE-4 → 2-NAPHTHYLALANINE: DEEPENING CARDIOLIPIN MEMBRANE ANCHORING

Peptide: SS-31 (D-Arg-Dmt-Lys-Phe-NH_2) → Modified: D-Arg-Dmt-Lys-**2Nal**-NH_2
Class: Longevity
Target: Cardiolipin (mitochondrial inner membrane)
Fold verdict: PROMISING
pLDDT: 0.853 | **pTM:** 0.161

MECHANISM OF ACTION

SS-31 (elamipretide/Bendavia) is a synthetic tetrapeptide whose mechanism is defined by its alternating cationic-aromatic pharmacophore. The two cationic residues — D-Arg (position 1) and Lys (position 3) — provide electrostatic attraction to cardiolipin's anionic phosphodiester headgroups, while the two aromatic residues — Dmt (position 2) and Phe (position 4) — engage the hydrophobic acyl chain region of the cardiolipin bilayer through van der Waals and π-stacking interactions.

The foundational Birk et al. (2013, PMID:23813215) study established that this cardiolipin binding inhibits cytochrome c peroxidase activity, protecting cardiolipin from oxidative peroxidation. Downstream consequences include stabilization of mitochondrial cristae ultrastructure, support of respiratory supercomplex (OxPhos complex I–IV) assembly, reduction in mitochondrial ROS generation, and preservation of membrane potential. Romanova et al. (2025, PMID:39880166) demonstrated these effects in biomimetic CL-containing nanoliposomes, confirming that membrane insertion geometry is sufficient to restore supercomplex assembly — establishing that the physical interaction with the bilayer, not secondary protein signaling, is the operative mechanism.

The Dmt residue at position 2 has additional pharmacological significance: its dimethyltyrosine side chain confers free radical scavenging capacity and engages in π-cation interactions with the cardiolipin headgroup. Phe-4, by contrast, is the less characterized of the two aromatic residues — its specific contribution to insertion depth versus pharmacophore structural integrity remains experimentally unresolved.

PERFORMANCE APPLICATIONS

SS-31 is under active clinical investigation across multiple mitochondrial dysfunction indications: heart failure with preserved ejection fraction (PROGRESS-HF), primary mitochondrial myopathy (MMPOWER-3, TAZPOWER), and Leber's hereditary optic neuropathy (ReCLAIM). Preclinical data support utility in renal ischemia-reperfusion injury, age-related cardiac dysfunction, neurodegenerative models, and Barth syndrome (TAZ deficiency with defective CL remodeling).

In the longevity and performance context, SS-31 is of interest for its capacity to enhance mitochondrial bioenergetics in aging tissue — improving ATP production efficiency and reducing mitochondrial ROS — without requiring mitochondrial membrane potential-dependent uptake (unlike TPP-conjugated compounds). The 2-Nal modification is hypothesized to extend this profile by strengthening the primary pharmacological anchor (CL binding), potentially enhancing efficacy in contexts where cardiolipin is oxidized or reduced in abundance, such as aged or ischemic mitochondria.

MODIFICATION RATIONALE

2-Naphthylalanine (2-Nal) is a well-validated aromatic amino acid bioisostere with a fused bicyclic ring system that expands the π-surface relative to phenylalanine's monocyclic ring. Key rationale points:

1. Aromatic volume expansion without backbone disruption. Because Phe-4 sits at the solvent- and membrane-facing C-terminus of a short linear peptide rather than within a compact intramolecular fold, increased aromatic volume at this position is unlikely to generate steric clashes with adjacent backbone atoms. The structure prediction confirms this: the naphthyl side chain is accommodated at position 4 without backbone distortion (pLDDT 0.853), and the alternating cationic-aromatic spatial arrangement is preserved. This is analogous to the logic behind Fold #5 (MOTS-c Nle substitution, PROMISING) — structurally conservative at the backbone, functionally targeted at the side chain — though the mechanistic intent differs: Fold #5 was oxidation protection; Fold #11 is membrane-pharmacophore enhancement.

2. Increased logP supports deeper bilayer insertion. 2-Nal adds approximately +1 log unit of lipophilicity relative to Phe. In the cardiolipin acyl chain context, this should increase van der Waals contact surface with the hydrophobic interior of the bilayer, potentially deepening the insertion angle of the C-terminus.

3. Pharmacophore preservation. The alternating cationic-aromatic pattern (position 1 cationic, position 2 aromatic, position 3 cationic, position 4 aromatic) is strictly maintained. The Stefaniak preprint (2024) emphasizes that this alternating

architecture — not any single residue — is the structural requirement for membrane electrostatic activity. Substituting Phe with 2-Nal extends aromatic character without disrupting the pattern.

4. No oxidation liability, no new chirality issues. Unlike modifications to Dmt (which could disrupt the antioxidant function), 2-Nal at position 4 adds no oxidizable phenolic group and introduces no new stereocenter when used as the L-isomer. A D-2-Nal variant could be explored in a future fold for further proteolytic resistance.

PREDICTED PROPERTIES (FAVOURABLE CHANGES FROM NATIVE SS-31)

> ⚠ All values below are in silico predictions and heuristic estimates. They do not constitute experimental measurements and should not be treated as validated properties.

Property	Native SS-31 (estimated)	2-Nal Variant (predicted)	Direction
pLDDT (backbone confidence)	~0.75–0.85 (est.)	**0.853**	✅ Preserved/high
Aggregation propensity	Low	**0.068** (low)	✅ Favorable
Stability score	Moderate	**0.566**	➡ Comparable
Half-life estimate	~30 min – 2 hr	**~30 min – 2 hr**	➡ Comparable
Predicted logP	Moderate	+~1 unit vs. native	⚠ Increased (watch solubility)
BBB penetration	Low	**0.25** (low)	➡ Comparable (not a target tissue)
Aromatic π-surface at C-term	Phenyl (monocyclic)	**Naphthyl (bicyclic)**	✅ Expanded
Pharmacophore integrity	Alternating cationic-aromatic	Preserved	✅ Preserved

The most notable predicted change is the expanded naphthyl aromatic surface at position 4 with preservation of low aggregation propensity — a favorable combination that suggests the added hydrophobicity is not driving self-association at the sequence level. The stability score of 0.566 is moderate and comparable to native expectations. The absence of a predicted binding affinity value (Boltz-2 affinity module returned no output) means we cannot quantify the hypothesized improvement in cardiolipin binding affinity computationally at this time.

WHAT WOULD STRENGTHEN THIS SIGNAL

Computational experiments (near-term): - **Molecular dynamics (MD) simulation** of both native SS-31 and the 2-Nal variant in a cardiolipin-containing bilayer (e.g., POPC/TOCL at 80:20 mol%). This is the most direct way to test the insertion depth hypothesis and would provide quantitative membrane penetration depth, orientation angle, and acyl chain contact surface data. - **Ensemble structure prediction** (multiple seeds, Chai-1 agreement) to verify that the high pLDDT reflects genuine backbone preference rather than a single-run artifact. The current single-run pLDDT of 0.853 is encouraging but not ensembled. - **Binding affinity prediction** with an explicit cardiolipin model once tools capable of peptide-lipid affinity scoring are available in this pipeline. The current Boltz-2 module returned no values, likely due to the lipid (non-protein) target. - **D-2-Nal variant fold** (Fold #N+1 candidate): exploring the D-configuration of 2-Nal at position 4 to combine deeper insertion with potential proteolytic resistance at the C-terminal residue.

Wet lab validation experiments (recommended priority order): 1. **Fluorescence lipid bilayer binding assay** (polarity-sensitive dye, as used in Birk 2013): compare CL-binding affinity of 2-Nal variant vs. native SS-31 in biomimetic liposomes (20 mol% CL). This is the direct functional test of the insertion hypothesis. 2. **Zeta potential and membrane rigidity measurement** (as in Romanova 2025): test whether 2-Nal variant more potently decreases zeta potential of CL-containing nanoliposomes — a proxy for deeper/stronger membrane insertion. 3. **Cytochrome c peroxidase inhibition assay**: quantify whether the 2-Nal variant maintains or enhances inhibition of CL-bound cytochrome c peroxidase activity, the canonical functional readout of SS-31 pharmacology. 4. **Aqueous solubility measurement**: directly test whether +1 logP from 2-Nal causes solubility collapse at physiologically relevant concentrations (target: >1 mg/mL). 5. **Cell-free ROS scavenging assay**: confirm that removing the Phe-4 phenyl ring (replacing with naphthyl) does not impair free radical quenching capacity — primarily a Dmt function, but the 2-Nal's extended conjugation could in theory contribute or interfere.

What this fold cannot yet tell us: - Whether deeper acyl chain insertion enhances or disrupts the electrostatic headgroup contact geometry (the cationic-aromatic balance concern remains unresolved). - Whether CL selectivity is maintained vs. other anionic phospholipids (PS, PG) — a critical specificity question with no current computational answer. - Mitochondrial uptake kinetics and in vivo pharmacokinetics.

SEQUENCES

NATIVE

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DArgDmtLysPhe
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MODIFIED

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DArg-Dmt-Lys-2Nal
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled) — pLDDT 0.853 should be confirmed across multiple seeds
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- cardiolipin is a lipid target — no protein structure co-modeling was performed; all binding and insertion claims are hypothetical extrapolations
- Boltz-2 affinity module returned no values; no quantitative binding affinity prediction is available for this fold
- heuristic logP increase (+~1 unit for 2-Nal vs. Phe) is a sequence-based estimate; real aqueous solubility requires experimental measurement
- CL selectivity vs. other anionic phospholipids (phosphatidylserine, phosphatidylglycerol) cannot be assessed computationally at this time
- no published SAR data exists for SS-31 position-4 aromatic modifications — all functional inferences are by analogy

CITATIONS

1. **PMID —** (2013) — — The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin
2. **PMID —** (2020) — — Mitochondrial protein interaction landscape of SS-31
3. **PMID —** (2025) — — Elamipretide: A Review of Its Structure, Mechanism of Action, and Therapeutic Potential
4. **PMID —** (2025) — — Cadmium-cardiolipin disruption of respirasome assembly and redox balance through mitochondrial membrane rigidification
5. **PMID —** (2019) — — Mitochondrial protein interaction landscape of SS-31 (preprint)

6. **PMID —** (2024) — — Therapeutic Peptide SS-31 Modulates Membrane Binding and Aggregation of Alpha-Synuclein and Restores Impaired Mitochondrial Function
7. **PMID —** (2021) — — Barth syndrome cellular models have dysregulated respiratory chain complex I and mitochondrial quality control due to abnormal cardiolipin
8. **PMID —** (2024) — — New insight for SS-31 in treating diabetic cardiomyopathy: Activation of mitoGPX4 and alleviation of mitochondria-dependent ferroptosis
9. **PMID —** (2022) — — SS-31, a Mitochondria-Targeting Peptide, Ameliorates Kidney Disease
10. **PMID —** (2025) — — SS-31@Fer-1 Alleviates ferroptosis in hypoxia/reoxygenation cardiomyocytes via mitochondrial targeting

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