DISTILLATION №18 · ALEMBIC LABS

SELANK — N-METHYLATION OF THE BACKBONE AMIDE NITROGEN OF GLY-6 (GLY → N-METHYL-GLY / SARCOSINE SUBSTITUTION AT POSITION 6)

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PROMISING COGNITIVE N-METHYLATION OF THE BACKBONE AMIDE NITROGEN OF GLY-6 (GLY → N-METHYL-GLY / SARCOSINE SUBSTITUTION AT POSITION 6) TUFTSIN/NEUROTENSIN-LIKE RECEPTOR (PUTATIVE); GABA-A RECEPTOR ALLOSTERIC MODULATION

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
86.9%	0.078 / 0.000	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Tuftsin/neurotensin-like receptor (putative); GABA-A receptor allosteric modulation	—	—

TLDR

FOLD №18 explores N-methylation of Gly-6 in Selank (TKPRPGP) via sarcosine substitution, hypothesizing protection of the Pro-Gly-Pro motif from prolyl-oligopeptidase cleavage. Boltz-2 predicted a confidently folded structure (pLDDT 0.87) with preserved backbone geometry of the PGP turn and intact N-terminal TKPR pharmacophore arrangement. This PROMISING verdict complements Fold #8's C-terminal amidation strategy, representing an orthogonal stabilization axis targeting endopeptidase rather than exopeptidase activity. No receptor complex was modeled, so functional preservation at the GABA-A allosteric site remains structurally inferred rather than computationally demonstrated.

EXECUTIVE SUMMARY

FOLD №18: [Sar6]-Selank sarcosine substitution predicted with pLDDT 0.87 — PGP turn geometry preserved, TKPR pharmacophore intact. PROMISING structural signal for orthogonal endopeptidase resistance; POP cleavage assays needed to validate the core hypothesis.

DETAILED ANALYSIS

Selank (TKPRPGP) is a synthetic heptapeptide derived from the endogenous immunomodulatory tetrapeptide tuftsin, with a C-terminal Pro-Gly-Pro extension appended to confer metabolic resilience over its parent compound. Its pharmacological profile — anxiolytic, nootropic, stress-protective, and immunomodulatory — is well-documented in rodent models, and a human neuroimaging study (PMID:32342318) confirms detectable functional CNS effects within minutes of intranasal administration. Mechanistically, the most consistently supported hypothesis is allosteric modulation of the GABA-A receptor system, inferred from gene-expression studies in rat frontal cortex and neuroblastoma cells rather than direct binding assays. A discrete tuftsin or neurotensin-like receptor has not been pharmacologically validated; the N-terminal TKPR pharmacophore's receptor identity remains an open question in the literature.

The central modification hypothesis in this fold targets a distinct and orthogonal degradation vulnerability from the one addressed in Fold #8. Where C-terminal amidation (Fold #8, pLDDT 0.90, PROMISING) was designed to block carboxypeptidase-mediated removal of the terminal Pro-7, the present distillation addresses the interior Pro-Gly bond within the PGP tripeptide — the canonical substrate motif for prolyl-oligopeptidase (POP) and related post-proline cleaving enzymes. These serine proteases recognize and hydrolyze the amide bond following a proline residue; N-methylation of the scissile nitrogen (converting Gly-6 to sarcosine, N-methyl-glycine) sterically occludes the active-site serine from forming the requisite acyl-enzyme intermediate, abolishing substrate recognition without altering side-chain volume. Sarcosine is the minimal possible N-methylated residue: it retains the glycine Cα hydrogen, preserves backbone flexibility, and is itself an endogenous CNS-active metabolite with established favorable ADME properties.

Structural prediction via Boltz-2 yielded a pLDDT of 0.87 for [Sar6]-Selank, marginally lower than Fold #8's 0.90 but well within the high-confidence range for a heptapeptide. The predicted structure maintains the extended/turn topology around the C-terminal Pro-Sar-Pro region, and the backbone geometry of the PGP-equivalent turn appears preserved relative to the native sequence. The N-terminal TKPR pharmacophore retains its spatial arrangement, consistent with sarcosine functioning as a near-isosteric Gly replacement. The pTM score of 0.078 and absent ipTM are expected for a single-chain peptide prediction without a binding partner

modeled, and carry no negative information about receptor engagement. No Chai-1 comparative run was available for this fold, and no affinity module outputs were produced, which appropriately constrains confidence in functional predictions.

The PROMISING verdict rests on the structural coherence of the prediction rather than any affinity or binding-change signal. The heuristic peptide profile estimates a moderate half-life (30 minutes to 2 hours) and a low aggregation propensity of 0.0, both consistent with a small, flexible, hydrophilic peptide. The BBB penetration estimate of 0.066 is low in absolute terms but should be interpreted cautiously: this is a sequence-based heuristic, and the native Selank — with comparable physicochemical characteristics — demonstrably penetrates the CNS in humans via intranasal delivery. The heuristic model does not account for route-of-administration effects, carrier-mediated transport, or the established nasal-to-brain pathway relevant to this peptide class.

The most significant biological uncertainty in this fold is the unvalidated premise that POP-mediated cleavage of the Pro-Gly bond is a rate-limiting inactivation step for Selank in vivo. No published work maps Selank's degradation profile, identifies which peptide bonds are hydrolyzed, or quantifies the contribution of POP versus aminopeptidases or endopeptidase 24.11. If the dominant inactivation pathway targets the N-terminal Thr-Lys rather than the interior Pro-Gly, sarcosine substitution at position 6 would provide no meaningful protection. This gap is the most critical weakness of the hypothesis and represents the first priority for wet-lab follow-up. A second uncertainty concerns the impact of N-methylation on the β-turn hydrogen bond network of the PGP motif: if the Gly-6 amide NH participates in an intra-turn hydrogen bond that stabilizes the native conformation, its removal could subtly destabilize turn geometry in ways below the resolution of a single-run pLDDT score.

Within the Alembic lab narrative, Fold #18 and Fold #8 now represent complementary strategies for extending Selank's biological lifetime — one addressing exopeptidase activity at the C-terminus, the other targeting endopeptidase activity within the backbone. The logical next distillation would be a dual-modified analogue, [Sar6]-Selank-NH2, combining both modifications to probe whether their protective effects are additive. This parallels the iterative logic applied in the Semax series (Fold #1), where N-terminal acetylation established a baseline structural confidence before further elaboration. The structural predictions for both Fold #8 and Fold #18 are sufficiently high-confidence to support moving toward synthesis and in vitro stability assays, particularly POP cleavage assays comparing native Selank, [Sar6]-Selank, Selank-NH2, and the dual analogue.

All conclusions in this analysis are derived from in silico prediction and literature review only. No wet-lab synthesis, stability measurement, receptor binding assay, or in vivo pharmacokinetic study has been conducted. The structural prediction is a single Boltz-2 run without ensemble modeling; pLDDT scores for heptapeptides reflect chain-level confidence and should not be interpreted as direct surrogates for

binding affinity or biological activity. This fold is exploratory research and carries no medical or therapeutic claims.

RESEARCH BRIEF

FOLD №18 — [SAR6]-SELANK: N-METHYLATION OF GLY-6 FOR ENDOPEPTIDASE RESISTANCE

Verdict: PROMISING | pLDDT 0.87 | Peptide: TKPRP[MeG]P

MECHANISM OF ACTION

Selank (TKPRPGP) is a synthetic heptapeptide analogue of tuftsin with an appended C-terminal Pro-Gly-Pro (PGP) tripeptide. Its pharmacological effects — anxiolysis, nootropic activity, stress protection, and immunomodulation — are well-documented in rodent models and supported by a human neuroimaging study showing altered amygdala–temporal cortex functional connectivity within 5–20 minutes of intranasal administration (PMID:32342318). The most consistently supported mechanistic hypothesis is allosteric modulation of the GABA-A receptor system, inferred from gene-expression studies in rat frontal cortex (PMID:26924987) and neuroblastoma cells (PMID:28293190). Selank does not directly alter GABAergic gene expression in isolation but modulates the response to exogenous GABA, consistent with an allosteric rather than orthosteric mechanism. A discrete tuftsin receptor or neurotensin-like receptor has not been pharmacologically validated; receptor identity for the N-terminal TKPR pharmacophore remains an open question.

PERFORMANCE APPLICATIONS

Based on the established Selank literature (not on any new data generated here), the parent peptide has demonstrated: - **Anxiolytic activity** comparable to diazepam in morphine withdrawal models (PMID:36322304) - **Nootropic / memory-protective effects**, including mitigation of ethanol-induced memory impairment via BDNF modulation in hippocampus and prefrontal cortex (PMID:31625062) - **Stress-protective and immunomodulatory activity**, including normalization of cytokine profiles under chronic stress (PMID:32621722) and hepatoprotection (PMID:31243679) - **Demonstrated CNS penetration in humans** via intranasal route, with fMRI-detectable effects within minutes (PMID:32342318)

The [Sar6] modification is hypothesized to extend the duration of these effects by protecting the peptide backbone from endopeptidase degradation — not to alter the pharmacodynamic profile itself. Whether prolonged exposure translates to greater efficacy is genuinely uncertain: dose-response data for native Selank suggest a ceiling effect at ~300 µg/kg, raising the possibility that receptor saturation limits the benefit of extended residence.

MODIFICATION RATIONALE

Selank's C-terminal PGP tripeptide is understood to be the primary stability-conferring element relative to native tuftsin, but the specific enzymatic vulnerabilities of the intact Selank backbone have not been mapped in published work. The Pro-Gly bond within PGP is a canonical substrate motif for **prolyl-oligopeptidase (POP)** and related post-proline endocleaving serine proteases, which recognize and hydrolyze amide bonds following proline residues. This represents a **distinct and orthogonal degradation route** from the C-terminal exopeptidase vulnerability addressed by amidation in **Fold #8**.

N-methylation of the scissile amide nitrogen — converting Gly-6 to sarcosine (N-methyl-glycine, Sar) — is a well-validated peptidomimetic strategy that abolishes POP substrate recognition by sterically occluding the active-site serine, preventing formation of the acyl-enzyme intermediate. Sarcosine is the minimal N-methylated residue: it retains the glycine Cα hydrogen, preserves backbone flexibility, maintains near-identical side-chain volume (one methyl group versus hydrogen), and is itself an endogenous CNS-active amino acid with established favorable ADME characteristics and no toxicological concern.

This fold is the second in a planned orthogonal stabilization strategy for Selank. Fold #8 addressed the C-terminus (pLDDT 0.90, PROMISING); Fold #18 addresses the interior backbone. The logical convergence is a dual-modified analogue combining both interventions — see Suggested Next Steps.

This distillation also parallels the logic applied to Semax in **Fold #1**, where N-terminal acetylation established a structural baseline for further analogue elaboration. The Selank series is building a similar iterative SAR narrative.

PREDICTED PROPERTIES (WHERE SIGNAL IS MODERATE)

Property	Observation	Confidence
pLDDT (Boltz-2)	**0.87**	High for a heptapeptide; supports confident chain folding
PGP turn geometry		Moderate — single run, no ensemble

Property	Observation	Confidence
	Preserved in predicted structure	
TKPR pharmacophore arrangement	Spatially intact	Moderate — inferred from pLDDT, no receptor complex modeled
Aggregation propensity (heuristic)	0.0	Low — consistent with small hydrophilic peptide
Stability score (heuristic)	0.5	Moderate — sequence-based estimate only
Half-life estimate (heuristic)	~30 min – 2 hours	Speculative; does not account for route of administration
BBB penetration (heuristic)	0.066	Low by sequence heuristic, but native Selank crosses BBB intranasally — interpret with caution
Binding affinity change	Not modeled	No receptor complex; cannot assess GABA-A allosteric engagement

Structural interpretation: The sarcosine substitution does not perturb the predicted backbone conformation at the resolution of this prediction, consistent with sarcosine's near-isosteric character. The N-methyl group adds steric bulk to the amide nitrogen but does not introduce a new side-chain, preserving the compact geometry of the PGP turn. The slight pLDDT reduction relative to Fold #8 (0.87 vs. 0.90) may reflect marginally increased local conformational uncertainty introduced by N-methylation, or may simply reflect run-to-run variation in single-chain heptapeptide predictions — the difference is not interpretable as a meaningful signal.

WHAT WOULD STRENGTHEN THIS SIGNAL

Computational priorities: 1. **Ensemble prediction** — run multiple Boltz-2 seeds and Chai-1 for [Sar6]-Selank to assess conformational reproducibility and establish whether the turn geometry is genuinely stable or a single-run artifact. 2. **Dual-modified analogue** — predict [Sar6]-Selank-NH2 (combining Fold #8 amidation and Fold #18 sarcosine) to assess whether dual modification preserves structural integrity and to establish the next fold in this series. 3. **Receptor complex modeling** — if a GABA-A allosteric binding site model becomes available, dock [Sar6]-Selank to assess whether backbone N-methylation at position 6 disrupts the conformational presentation required for allosteric engagement. 4. **POP cleavage site mapping** — molecular docking of native Selank and [Sar6]-Selank into the POP active site (PDB: 1QFM or similar) to directly assess whether sarcosine sterically occludes the catalytic serine.

Wet-lab priorities (in order of mechanistic necessity): 1. **Synthesize [Sar6]-Selank** and confirm identity/purity by HPLC-MS. 2. **POP cleavage assay** — incubate native Selank and [Sar6]-Selank with purified porcine or recombinant human POP; quantify cleavage products by LC-MS over time. This directly tests the core hypothesis and fills the most critical literature gap. 3. **Plasma stability assay** — incubate both peptides in rat or human plasma; compare half-lives by LC-MS. Compare with Fold #8 Selank-NH2 to map orthogonal contributions. 4. **GABA-A allosteric assay** — replicate the gene-expression paradigm from PMID:28293190 (IMR-32 cells, GABA co-treatment) with [Sar6]-Selank to verify that allosteric modulation is preserved. 5. **Behavioral pharmacology** — if in vitro data are supportive, test [Sar6]-Selank in the elevated plus maze anxiolytic paradigm used for native Selank validation.

> **Mandatory disclaimer:** All structural, stability, and property predictions in this report are in silico estimates from a single Boltz-2 prediction run. No synthesis, stability measurement, receptor binding assay, or in vivo experiment has been conducted. Predicted properties are computational approximations and may not reflect real-world biological behavior. This report is exploratory research only and constitutes no medical advice, therapeutic claim, or product endorsement.

SEQUENCES

NATIVE

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TKPRPGP
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MODIFIED

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TKPRP-Sar-P (TKPRP[MeG]P)
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CAVEATS

- In silico prediction only — requires wet-lab synthesis and validation before any biological conclusions can be drawn
- Single-run Boltz-2 prediction (not ensembled) — pLDDT scores for heptapeptides reflect chain-level confidence and should not be interpreted as surrogates for binding affinity or biological activity

- Predicted properties may not reflect real-world biological behavior — heuristic stability, BBB, and half-life estimates are sequence-based approximations, not measured values
- This is research, not medical advice — no therapeutic claims are made or implied
- No receptor complex was modeled — structural preservation of the GABA-A allosteric pharmacophore is inferred from backbone geometry, not from a docked complex
- The premise that POP-mediated Pro-Gly cleavage is the rate-limiting inactivation step for Selank is an untested assumption; no published data map Selank's degradation profile
- Heuristic BBB penetration score (0.066) is likely an underestimate for intranasal delivery routes — this metric should not be used to assess CNS access for intranasally administered peptides
- N-methylation eliminates the Gly-6 amide NH hydrogen bond donor; if this NH stabilizes the native PGP β-turn through an intra-turn hydrogen bond, sarcosine substitution could introduce conformational perturbation below the resolution of this single-run prediction
- Chai-1 comparative agreement data were unavailable for this fold; the absence of cross-predictor validation reduces confidence in structural conclusions relative to folds with multi-predictor agreement

CITATIONS

1. **PMID —** (2022) — — Selank, a Peptide Analog of Tuftsin, Attenuates Aversive Signs of Morphine Withdrawal in Rats
2. **PMID —** (2016) — — Selank Administration Affects the Expression of Some Genes Involved in GABAergic Neurotransmission
3. **PMID —** (2017) — — GABA, Selank, and Olanzapine Affect the Expression of Genes Involved in GABAergic Neurotransmission in IMR-32 Cells
4. **PMID —** (2019) — — Selank, Peptide Analogue of Tuftsin, Protects Against Ethanol-Induced Memory Impairment by Regulating of BDNF Content in the Hippocampus and Prefrontal Cortex in Rats
5. **PMID —** (2020) — — Functional Connectomic Approach to Studying Selank and Semax Effects
6. **PMID —** (2021) — — The Influence of Selank on the Level of Cytokines Under the Conditions of 'Social' Stress
7. **PMID —** (2019) — — Effect of Selank on Morphological Parameters of Rat Liver in Chronic Foot-Shock Stress
8. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions

SOLANA SIGNATURE 32yW8KSmYKKkccYheovLAzX5dyCTmzpg5s8LJTcxB5vR8bUQEu4LMaHc6pe23EEGEqACfuWtGppDKhp8HFdksjxr
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