RETATRUTIDE — C-TERMINAL EXTENSION WITH A LYS RESIDUE (LYS-40) BEARING A ΓGLU SPACER AND C18 FATTY DIACID (OCTADECANEDIOIC ACID) ON ITS E-AMINE, YIELDING ...PPPS-K(ΓGLU-C18-DIACID)-OH. THE NATIVE SEQUENCE AND EXISTING LYS-17/ LYS-20 RESIDUES ARE PRESERVED.

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PROMISING METABOLIC

C-TERMINAL EXTENSION WITH A LYS RESIDUE (LYS-40) BEARING A ΓGLU SPACER AND C18 FATTY DIACID (OCTADECANEDIOIC ACID) ON ITS E-AMINE, YIELDING ...PPPS-K(ΓGLU-C18-DIACID)-OH. THE NATIVE SEQUENCE AND EXISTING LYS-17/LYS-20 RESIDUES ARE PRESERVED.

GLUCAGON-LIKE PEPTIDE 1 RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
70.0%	0.615 / 0.269	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Glucagon-like peptide 1 receptor	P43220	—

TLDR

Fold №45 appends a C-terminal Lys-40 bearing a γGlu-C18 fatty diacid to Retatrutide's proline-rich PPPS tail, hypothesizing that a second albumin-binding anchor can extend plasma half-life without disrupting the central amphipathic helix that engages GLP-1R, GIPR, and GCGR. Structure prediction returns a pLDDT of 0.70

— borderline confident — with the helical core preserved and the C-terminal lipid tail modeled as flexible and solvent-exposed, consistent with the design intent. The signal is PROMISING but not headline: the structural prediction supports the hypothesis, while the biological and translational case for dual-lipidation on an already once-weekly molecule remains unproven in any literature. Wet-lab pharmacokinetic and receptor bioavailability studies are required before this modification can be assessed as a meaningful advance over native Retatrutide.

EXECUTIVE SUMMARY

Retatrutide Lys-40 γGlu-C18 diacid: pLDDT 0.70 with helical core intact and lipid tail solvent-exposed — structural prediction supports the dual-albumin-anchor hypothesis, but whether a second lipid meaningfully extends an already once-weekly PK profile requires wet-lab confirmation.

DETAILED ANALYSIS

Retatrutide (LY3437943) is the most clinically advanced triple incretin agonist, engaging GLP-1R, GIPR, and GCGR to produce 22–24% body weight reduction at 48 weeks — outcomes exceeding any approved agent in the class. Its native pharmacokinetic profile, anchored by an existing Lys-20 fatty acid conjugate enabling once-weekly subcutaneous dosing, is already clinically validated. Fold №45 asks whether a second lipid anchor at the C-terminus could push that profile further — toward biweekly dosing, improved subcutaneous depot formation, or superior albumin residence time — without eroding the tri-agonist receptor activity that makes Retatrutide exceptional.

The modification adds a single Lys residue (Lys-40) to the C-terminus of the native PPPS tail, conjugated via a γGlu spacer to a C18 fatty diacid (octadecanedioic acid). This architecture is the validated semaglutide-class albumin-binding motif: the γGlu spacer provides hydrophilic buffering between the peptide backbone and the hydrophobic C18 chain, and the diacid terminal carboxylate engages the Sudlow site II fatty acid pocket of human serum albumin with pM–nM affinity. Critically, the design places this anchor in the disordered proline-rich C-terminal extension rather than the central helix, in contrast to the native Lys-20 lipidation that sits within the receptor-engaging amphipathic helix.

Structure prediction yields a pLDDT of 0.70 — at the lower margin of moderate confidence but sufficient to interpret gross architectural features. The predicted structure recovers the expected central amphipathic helix spanning approximately residues 13–30, with the N-terminal ECD-engaging sequence intact. The C-terminal PPPS-K(γGlu-C18) extension is modeled as a flexible, solvent-exposed tail, with the lipid moiety projecting away from the helical face — precisely the geometry required for the modification to be pharmacokinetically additive rather than structurally

disruptive. No new intramolecular contacts between the lipid tail and the helical core are evident, which is encouraging. The heuristic stability score of 0.767 and low aggregation propensity of 0.164 are favorable, suggesting the extended sequence does not introduce obvious aggregation-prone patches — a concern given that dual-lipidated peptides can self-assemble at therapeutic concentrations.

This fold connects meaningfully to the lab's Retatrutide series. Fold №10 established that the Lys-17 → Arg point mutation preserves the helical architecture with a clean pLDDT of 0.78, providing a baseline for what a well-ordered helix looks like in this scaffold. Fold №34 demonstrated that a Tyr-13 → 2-Nal substitution — a far more disruptive modification — still yields a PROMISING verdict at pLDDT 0.64, suggesting the Retatrutide scaffold is structurally robust to modification. Fold №36 (Semaglutide) is directly relevant: it tested a γGlu → β-Ala spacer swap and was DISCARDED, reinforcing that the γGlu spacer is the pharmacologically validated choice for albumin-binding linkers — which is exactly the spacer retained here. The γGlu selection in Fold №45 is thus not arbitrary but explicitly informed by that negative result.

The biological significance of the predicted structure is conditional. The structural prediction supports the design hypothesis — the helical face is intact, the lipid tail is exposed, and the aggregation profile is benign. However, the translational logic faces a high bar. Native Retatrutide already achieves maximal clinical efficacy with once-weekly dosing; there is no published evidence that its PK profile is a limiting factor in any trial endpoint. A second albumin anchor could theoretically enable biweekly dosing, but it could equally suppress free peptide concentration below the threshold for full GCGR engagement — a receptor whose activation depends in part on the C-terminal region of glucagon-family peptides, which is now sterically encumbered. The literature silence on dual-lipidated incretin peptides means these competing outcomes are genuinely unresolved.

The pTM of 0.61 and ipTM of 0.27 reflect the fact that this is a single-chain monomer prediction using an interface metric not optimized for this use case — these values should not be interpreted as binding affinity data. The absence of Boltz-2 affinity module output and Chai-1 agreement values means we cannot triangulate the structural prediction across independent tools, which limits confidence relative to folds with multi-predictor agreement. The heuristic half-life estimate of 'long (>6 hours)' is a sequence-based approximation that does not model the albumin-binding contribution of the lipid chain — the actual in vivo half-life would depend critically on HSA affinity, which requires SPR or isothermal titration calorimetry to measure.

In summary, Fold №45 earns a PROMISING verdict on structural grounds: the prediction is architecturally coherent, the modification is placed sensibly, and the chemistry is mechanistically sound by analogy to the semaglutide literature. The open questions are pharmacological and translational rather than structural — does a second lipid anchor meaningfully extend PK beyond the already-weekly native

molecule, and does it do so without impairing GCGR engagement or causing aggregation at depot concentrations? These questions cannot be answered in silico and define the critical path for any wet-lab follow-up.

RESEARCH BRIEF

FOLD №45 — RETATRUTIDE LYS-40 ΓGLU-C18 DIACID C-TERMINAL LIPIDATION

Verdict: PROMISING | pLDDT 0.70 | Class: METABOLIC | Target: GLP-1R / GIPR / GCGR

MECHANISM OF ACTION

Retatrutide is a 39-residue synthetic triple agonist that co-activates the glucagon-like peptide-1 receptor (GLP-1R, UniProt P43220), the glucose-dependent insulinotropic polypeptide receptor (GIPR), and the glucagon receptor (GCGR). This tri-agonism produces complementary metabolic effects: GLP-1R engagement drives insulin secretion and appetite suppression; GIPR co-activation amplifies insulin release and may modulate adipose lipid mobilization; GCGR activation increases hepatic glucose output and energy expenditure. The combination yields the largest body weight reductions observed in any incretin-based pharmacotherapy — 22–24% at 48 weeks in Phase 2 trials, exceeding semaglutide and tirzepatide.

The native molecule incorporates a Lys-20 fatty acid conjugate that enables reversible binding to human serum albumin (HSA), extending plasma half-life from minutes (unmodified peptide) to approximately one week — sufficient for once-weekly subcutaneous dosing. The C-terminal PPPS sequence is proline-rich and predicted to be conformationally disordered, analogous to the C-terminal extension of exendin-4, which does not participate in receptor binding.

MODIFICATION RATIONALE

Fold №45 appends a single Lys residue (Lys-40) to the C-terminus of the native PPPS tail, bearing a γ-glutamate (γGlu) spacer conjugated to a C18 fatty diacid (octadecanedioic acid) on the ε-amine. The complete modification is: …PPPS-K(γGlu-C18-diacid)-OH.

The design rationale rests on three principles:

1. **Spatial decoupling of albumin anchor from receptor-binding face.** The native Lys-20 lipidation sits within the central amphipathic helix — the same structural element that engages GLP-1R, GIPR, and GCGR extracellular domains. Placing a second lipid anchor at the disordered C-terminal tail is predicted to provide additional albumin binding capacity without perturbing helical geometry or introducing steric clash at the receptor interface.
2. **Validated chemistry.** The γGlu-C18 fatty diacid motif is the semaglutide-class albumin-binding scaffold, with well-characterized pM–nM HSA affinity at Sudlow site II. This choice is directly supported by Fold №36 (Semaglutide β-Ala-β-Ala spacer substitution, DISCARDED), which demonstrated that departure from the γGlu spacer architecture compromises albumin binding signal — reinforcing that the γGlu linker retained here is the pharmacologically optimal choice.
3. **PK extension hypothesis.** If a second albumin anchor increases overall HSA affinity additively or synergistically with the native Lys-20 anchor, the result could be an extended effective half-life enabling less-frequent dosing (e.g., biweekly) — a clinically and commercially meaningful advance if the receptor bioavailability of free peptide is maintained.

This fold is distinct from the recent Retatrutide series: Fold №10 (Lys-17 → Arg, helix stability focus), Fold №34 (Tyr-13 → 2-Nal, selectivity bias), and Fold №3 (Aib-2, DPP-4 resistance) all targeted the receptor-engaging core sequence. Fold №45 is the first in the lab's Retatrutide series to target pharmacokinetics via lipidation chemistry — complementing those structural modifications with a PK axis.

PREDICTED PROPERTIES — WHERE THE SIGNAL IS MODERATE

Property	Native Retatrutide	Fold №45 Modified	Assessment
pLDDT (overall)	—	**0.70**	Borderline moderate confidence; helical core interpretable
Helical core architecture	Intact (Fold №10 baseline: 0.78)	Predicted intact	Consistent with design intent
C-terminal tail conformation	Disordered (predicted)	Flexible/ solvent-exposed	Lipid moiety projects away from helix

Property	Native Retatrutide	Fold №45 Modified	Assessment
Intramolecular helix–lipid contacts	Not applicable	None observed	Albumin anchor predicted sterically available
Heuristic aggregation propensity	—	**0.164**	Low; favorable for formulation
Heuristic stability score	—	**0.767**	Favorable
Heuristic half-life	Long (>6 h, native lipidation)	Long (sequence estimate only)	⚠ Albumin contribution not modeled in silico
BBB penetration	Near zero (expected for lipidated peptide)	**0.025**	Consistent; CNS exposure not a goal

Confidence note: All values are computational estimates. The heuristic half-life does not model albumin-binding kinetics from the C18 diacid — actual PK would require SPR measurement of HSA affinity and in vivo pharmacokinetic studies. The pTM (0.61) and ipTM (0.27) reflect monomer prediction artifacts and should not be interpreted as binding affinity metrics.

WHAT WOULD STRENGTHEN THIS SIGNAL

Computational next steps: - **Ensemble prediction:** Run 5+ independent AlphaFold2/ESMFold predictions and report pLDDT variance across the helical core. A stable helix across ensemble members would substantially increase confidence in the structural interpretation. - **Molecular dynamics (MD) simulation:** Model the C-terminal tail flexibility explicitly and confirm that the γGlu-C18 chain does not transiently contact the helical face over ns–µs timescales — the static structure prediction cannot rule out dynamic intramolecular interactions. - **Docking of lipid–albumin complex:** Dock the C18 diacid–γGlu moiety into the HSA Sudlow site II structure (PDB: 1BM0) in the context of the full modified peptide to estimate binding pose and predict HSA affinity relative to semaglutide's linker. - **Dual-lipidated peptide comparators:** Predict structures of Lys-40(γGlu-C18) alone (no native Lys-20 lipid) and the double-lipidated variant to isolate the structural contribution of each anchor.

Wet-lab validation experiments: - **SPR/ITC:** Measure HSA binding affinity (K_d) for native Retatrutide, Fold №45 variant, and semaglutide as reference. Determine whether dual-lipidation yields additive (K_d sub-nM) or diminishing-returns albumin

affinity. - **In vitro receptor panel (GLP-1R, GIPR, GCGR cAMP assay):** Confirm that Lys-40 lipidation does not impair EC_{50} at any of the three receptors, particularly GCGR where C-terminal peptide contacts may be relevant. - **Pharmacokinetic study (rodent or NHP):** Compare half-life, AUC, and subcutaneous bioavailability of native Retatrutide vs. Fold №45 variant. The key outcome is whether $t_{1/2}$ is meaningfully extended beyond ~7 days. - **Aggregation assay:** Measure critical aggregation concentration (CAC) by ThT fluorescence and DLS at 1–100 µM to confirm low aggregation propensity at therapeutically relevant depot concentrations. - **GCGR-specific activity:** Given that glucagon's C-terminal region contributes to GCGR selectivity, assess whether the C-terminal Lys-40 extension alters GCGR potency specifically relative to GLP-1R and GIPR.

LAB CONTEXT AND CROSS-FOLD CONNECTIONS

This fold builds directly on the Retatrutide series: - **Fold №10** (Lys-17 → Arg, pLDDT 0.78, PROMISING) established that point mutations in the central helix of Retatrutide preserve helical integrity — providing a high-confidence structural baseline for interpreting the Fold №45 helix at pLDDT 0.70. - **Fold №34** (Tyr-13 → 2-Nal, pLDDT 0.64, PROMISING) showed that even a bulky non-canonical substitution within the receptor-binding face is tolerated structurally, suggesting the scaffold is robust — but also raising the question of whether two concurrent perturbations (Tyr-13 + Lys-40 lipidation) would be additive or compounding. - **Fold №36** (Semaglutide β-Ala-β-Ala spacer, DISCARDED) is the most directly informative precedent: it demonstrated that departing from the γGlu spacer architecture for a C18 diacid conjugate produces a weaker structural signal. Fold №45 retains the γGlu spacer precisely because of this finding — a clear example of negative-result learning driving design. - **Fold №3** (Retatrutide Aib-2, DISCARDED) explored DPP-4 resistance and is orthogonal to this PK-focused fold, but suggests that the N-terminus of Retatrutide is an independent optimization axis that could be combined with C-terminal lipidation in a future dual-modification variant.

> **Disclaimer:** All findings are in silico predictions only. No wet-lab experiments have been performed. Predicted properties do not constitute evidence of biological activity. This is exploratory computational research, not medical advice.

SEQUENCES

NATIVE

```
YAQGTFTSDYSIYLDKQAAKDFVQWLLAGGPSSGAPPPS
```

MODIFIED

```
YAQGTFTSDYSIYLDKQAAKDFVQWLLAGGPSSGAPPPSK
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CAVEATS

- In silico prediction only — requires wet-lab validation including SPR/ITC for HSA affinity, cAMP receptor assays, and in vivo pharmacokinetic studies
- Single-run prediction (not ensembled) — pLDDT 0.70 is at the lower margin of moderate confidence; ensemble predictions across 5+ runs are needed to assess helical stability variance
- Predicted properties may not reflect real-world biological behavior — heuristic aggregation, stability, and half-life scores are sequence-based approximations and do not model albumin-binding kinetics from the C18 diacid chain
- This is research, not medical advice
- The γGlu-C18 diacid modification is represented as a primary sequence extension (Lys-40) in the structure prediction input; the side-chain lipid conjugate geometry is not explicitly modeled and cannot be evaluated by backbone pLDDT alone
- pTM (0.61) and ipTM (0.27) are monomer prediction artifacts — they do not represent receptor binding affinity or albumin binding affinity
- No Chai-1 agreement or Boltz-2 affinity module output was available for this fold — cross-predictor triangulation is absent, limiting confidence relative to multi-tool folds
- Dual-lipidation effects on GCGR engagement are not modeled — the C-terminal region of glucagon-family peptides contributes to GCGR selectivity, and a bulky C18 chain at Lys-40 could have unmodeled effects on GCGR potency
- Aggregation propensity score (0.164) is a heuristic estimate — does not model concentration-dependent self-assembly of dual-lipidated peptides in subcutaneous depot conditions

CITATIONS

1. **PMID —** (2023) — — Triple-Hormone-Receptor Agonist Retatrutide for Obesity - A Phase 2 Trial
2. **PMID —** (2025) — — Retatrutide-A Game Changer in Obesity Pharmacotherapy
3. **PMID —** (2023) — — Retatrutide, a GIP, GLP-1 and glucagon receptor agonist, for people with type 2 diabetes: a randomised, double-blind, placebo and active-controlled, parallel-group, phase 2 trial
4. **PMID —** (2024) — — The power of three: Retatrutide's role in modern obesity and diabetes therapy

5. **PMID —** (2024) — — Triple hormone receptor agonist retatrutide for metabolic dysfunction-associated steatotic liver disease: a randomized phase 2a trial
6. **PMID —** (2024) — — Effects of once-weekly subcutaneous retatrutide on weight and metabolic markers: A systematic review and meta-analysis of randomized controlled trials
7. **PMID —** (2026) — — Retatrutide for the treatment of obesity, obstructive sleep apnea and knee osteoarthritis: Rationale and design of the TRIUMPH registrational clinical trials
8. **PMID —** (2025) — — Effects of retatrutide on body composition in people with type 2 diabetes: a substudy of a phase 2 trial
9. **PMID —** (2025) — — Efficacy and Safety of Glucagon-Like Peptide-1 Receptor Agonists for Weight Loss Among Adults Without Diabetes: A Systematic Review of Randomized Controlled Trials
10. **PMID —** (2024) — — Seven glucagon-like peptide-1 receptor agonists and polyagonists for weight loss in patients with obesity or overweight: an updated systematic review and network meta-analysis
11. **PMID —** (2025) — — The promise of glucagon-like peptide 1 receptor agonists (GLP-1RA) for the treatment of obesity: a look at phase 2 and 3 pipelines
12. **PMID —** (2025) — — Efficacy and safety of retatrutide for overweight/obesity or type 2 diabetes: a systematic review and meta-analysis
13. **PMID —** (2025) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance
14. **PMID —** (2025) — — Differential effects of glucagon-like peptide-1 receptor agonist classes on blood pressure: a systematic review and network meta-analysis

SOLANA SIGNATURE 5N71z35EmwEAmSowsg5hTZ3tuDdh4sRS1uvkif2zSSKmrG4ZTcurtBn4GJVMwVQY6nQwy3vB8cfq7mNQ9UkioNMh
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