DSIP — HEAD-TO-TAIL (BACKBONE) CYCLIZATION VIA AMIDE BOND BETWEEN TRP-1 A-AMINE AND GLU-9 A-CARBOXYLATE, YIELDING CYCLO(WAGGDASGE)

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DISCARDED | PERFORMANCE | HEAD-TO-TAIL (BACKBONE) CYCLIZATION VIA AMIDE BOND BETWEEN TRP-1 A-AMINE AND GLU-9 A-CARBOXYLATE, YIELDING CYCLO(WAGGDASGE) | GABA-A RECEPTOR (BENZODIAZEPINE/NEUROSTEROID-MODULATED CHLORIDE CHANNEL COMPLEX IMPLICATED IN DSIP'S

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
—	— / —	DISCARDED

TARGET	UNIPROT	BINDING PROBABILITY
GABA-A receptor (benzodiazepine/ neurosteroid-modulated chloride channel complex implicated in DSIP's	—	—

DISCARDED BY PREDICTABILITY GATE

target_not_predictable: no UniProt ID resolved — target identity unconfirmed

TLDR

Fold №62 attempted head-to-tail backbone cyclization of DSIP (cyclo-WAGGDASGE) to pre-organize a β-turn conformation and simultaneously block both amino- and carboxypeptidase attack. The fold was DISCARDED before structural prediction began — the orchestrator's predictability gate could not resolve a UniProt ID for the proposed GABA-A receptor target, making a meaningful docking or binding affinity

prediction impossible. No pLDDT, pTM, or binding metrics were generated. Critically, this is a tool-limit discard, not a biological invalidation of the cyclization strategy, which retains meaningful literature support from constrained DSIP analogue studies.

EXECUTIVE SUMMARY

Fold №62 — DSIP head-to-tail cyclization — was discarded before structural prediction ran: no UniProt ID could be resolved for the GABA-A target, halting the pipeline at the orchestrator gate. The cyclization hypothesis is scientifically coherent and supported by constrained-analogue literature, but requires wet-lab synthesis or alternative computational tools to evaluate.

DETAILED ANALYSIS

DSIP (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu) is a nonapeptide first isolated in 1977 from rabbit cerebral venous blood and described as a delta-sleep-inducing agent. Despite over 45 years of intermittent research, no dedicated receptor, gene, or direct molecular binding partner has been identified for DSIP. The peptide remains one of the most biologically suggestive yet mechanistically opaque entries in the performance peptide literature — capable of modulating sleep architecture, thermoregulation, hormonal release, and pain thresholds, yet stubbornly resistant to classical receptor pharmacology. The proposed target for this fold, the GABA-A receptor complex (specifically its benzodiazepine/neurosteroid-modulated chloride channel), is a reasonable hypothesis derived from DSIP's sedative phenotype but has never been confirmed by radioligand displacement, patch-clamp electrophysiology, or any structural method. This absence of a confirmed, annotated molecular target is precisely what triggered the discard.

The modification rationale for Fold №62 is scientifically well-grounded. DSIP's two central glycines (positions 3 and 4) and overall lack of secondary structure mean the linear peptide samples an enormous conformational ensemble — a property that dilutes any productive binding pose and maximises entropy costs upon receptor engagement. Head-to-tail backbone cyclization, joining the Trp-1 α-amine to the Glu-9 α-carboxylate via a direct amide bond, represents the most radical conformational constraint achievable without introducing non-canonical residues: it forces backbone closure, dramatically restricts accessible φ/ψ space, and blocks both termini from exopeptidase recognition in a single chemical step. This strategy has precedent in the broader peptide field — somatostatin and its clinical analogues (octreotide, lanreotide) are the canonical example, where head-to-tail cyclization of a 14-mer yielded nanomolar potency and metabolic stability from a flexible, rapidly cleared linear precursor.

The fold sits within a coherent DSIP experimental narrative at this lab. Fold №46 (Ac-WAGGDASGE-NH_2, pLDDT 0.65, PROMISING) established that N-terminal acetylation

combined with C-terminal amidation provides partial terminus protection and marginally improved predicted structure confidence — but the capping strategy is inherently incomplete because it does not constrain the backbone or close the conformational ensemble. Fold №57 attempted a more local geometric fix — Gly-3/Gly-4 → D-Ala double substitution — but was discarded, likely because point mutations at the Gly-Gly hinge are too local to globally constrain a 9-mer. Head-to-tail cyclization is the logical escalation: it addresses the global conformational problem that neither terminus capping nor D-Ala substitution could solve.

The literature base provides meaningful, if indirect, support. Kovalzon & Strekalova (2006) reported that artificial structural analogues of DSIP — not native DSIP itself — produced significant slow-wave sleep promotion in rabbits and rats. This is a critical observation: it implies the native linear, floppy conformation is suboptimal and that geometric constraint can unlock latent activity from this sequence. The clinical data of Schneider-Helmert (1984) showing dose-dependency and buildup effects are consistent with a peptide of marginal affinity limited by conformational entropy. Mu et al. (2024) demonstrated that dramatically improved CNS delivery (via a BBB-crossing fusion) substantially enhances DSIP efficacy in a validated insomnia model — suggesting intrinsic potency is a real bottleneck, which conformational preorganization is designed to address.

The structural prediction pipeline was never executed. The orchestrator's predictability gate requires a resolvable UniProt ID for the target before allocating compute to Boltz-2/Chai-1 complex modelling. DSIP's candidate target — the GABA-A receptor chloride channel complex — lacks a single canonical UniProt entry that maps cleanly to the benzodiazepine/neurosteroid modulatory site in the context of this peptide's hypothesized interaction mode. The gate correctly flagged this as target_not_predictable and halted the fold. No pLDDT, pTM, ipTM, or binding probability data were produced. Heuristic peptide property estimates were also not generated because the structural agent did not process the sequence.

It is important to emphasise what this discard does not mean. The cyclization chemistry is feasible — head-to-tail macrolactamisation of a 9-mer via standard solid-phase peptide synthesis with on-resin or solution-phase cyclization is well-established. The biological hypothesis — that pre-organizing a β-turn around the Gly-Gly hinge will improve target engagement — is not disproved. The DSIP literature's consistent finding that constrained analogues outperform native DSIP is, if anything, supportive. The discard reflects a limitation of our current in silico pipeline: it cannot evaluate peptide-target interactions when the target lacks a resolvable structural identity in the databases the tool queries. This is a tool boundary, not a verdict on the science.

Several challenges remain beyond the tool limitation. The breadth of DSIP's reported effects (sleep, thermoregulation, heart rate, pain, lymphokines, hormonal levels) suggests either extreme promiscuity or an indirect mechanism — neither is easily addressed by optimising binding to a single receptor. Head-to-tail cyclization

imposes a specific macrocyclic geometry that may not match the true bioactive conformation, particularly if DSIP acts through a mode distinct from a single compact β-turn. The DSIP literature is also dominated by pre-genomics, pre-structural-biology studies from 1984–1988 whose basic pharmacological claims have not been systematically reproduced by contemporary methods. These are biological uncertainties that persist regardless of what any in silico tool returns.

The path forward for this hypothesis likely runs through two parallel tracks: synthetic and biophysical. On the synthetic side, the cyclic peptide is accessible and could be tested in a competitive radioligand displacement assay at the GABA-A benzodiazepine site, or in primary hippocampal or cortical neurons using whole-cell patch-clamp, before any further computational investment. On the computational side, if a cryo-EM structure of the GABA-A complex bound to a neurosteroid or small-molecule modulator is used as the target scaffold — bypassing the UniProt gate — a future fold could attempt to model the cyclic peptide interaction directly. Alternatively, a molecular dynamics simulation of the free macrocycle in explicit solvent would reveal whether the predicted β-turn is genuinely the dominant low-energy conformer, independent of any target.

RESEARCH BRIEF

FOLD №62 — DSIP HEAD-TO-TAIL CYCLIZATION

cyclo(WAGGDASGE) | Verdict: DISCARDED

TLDR

Fold №62 was **DISCARDED before structural prediction began** due to a tool-limit failure: the orchestrator's predictability gate could not resolve a UniProt ID for the proposed GABA-A receptor target, making complex modelling impossible. This is a **pipeline boundary issue**, not a biological invalidation of the cyclization strategy. The hypothesis that head-to-tail backbone cyclization will preorganize DSIP's bioactive conformation and resist exopeptidase clearance remains scientifically coherent and untested by any in silico or wet-lab method.

WHAT WE TRIED

This fold proposed joining the Trp-1 α-amine of DSIP to the Glu-9 α-carboxylate via a direct amide bond, yielding the macrocyclic nonapeptide **cyclo(WAGGDASGE)**. The

hypothesis was that backbone cyclization — a global constraint strategy — would accomplish what two prior DSIP folds could not:

- **Fold №46** (Ac-WAGGDASGE-NH_2, PROMISING, pLDDT 0.65): N-terminal acetylation + C-terminal amidation provided partial terminus protection and marginally improved structural confidence, but left the backbone conformationally unconstrained and the central Gly-Gly hinge free to roam.
- **Fold №57** (Gly-3/Gly-4 → D-Ala, DISCARDED): Local D-Ala substitution at the hinge was hypothesized to bias the backbone toward a β-turn, but point mutations at two residues are insufficient to globally collapse a floppy 9-mer.

Head-to-tail cyclization is the logical escalation: it imposes a **topological constraint** across the entire backbone, forces closure of φ/ψ space across all nine residues simultaneously, and eliminates both N- and C-terminal exopeptidase recognition sites in a single chemical step. The target was the GABA-A receptor complex (benzodiazepine/neurosteroid-modulated chloride channel), hypothesized on the basis of DSIP's sedative and sleep-promoting phenotype.

The Gly-Gly hinge (positions 3–4) was expected to serve as the flexible turn point in the macrocycle, with Asp-5/Ser-6 side chains presented on one face and Trp-1 contributing aromatic character to a putative binding surface. The structural prediction was expected to yield a compact ring of approximately 10–12 Å diameter with a single dominant low-energy conformer — higher predicted confidence than the linear form due to reduced ensemble heterogeneity.

WHY IT WAS DISCARDED

Primary reason: `target_not_predictable` — no UniProt ID resolved for the GABA-A receptor complex in the context of this peptide's hypothesized interaction.

The Alembic Labs orchestrator requires a resolvable UniProt accession for the target before allocating compute to Boltz-2/Chai-1 complex modelling. The GABA-A receptor is a heteropentameric chloride channel encoded by multiple subunit genes (GABRA1–6, GABRB1–3, GABRG1–3, etc.); there is no single canonical UniProt ID that maps cleanly to the benzodiazepine/neurosteroid modulatory site relevant to DSIP's hypothesized mechanism. The pipeline could not select a definitive structural target, and the fold was halted before any prediction was run.

This is compounded by a deeper biological gap: **DSIP has no confirmed molecular target**. No dedicated DSIP receptor has been cloned, no gene has been identified, and no direct GABA-A binding study (radioligand displacement, patch-clamp, SPR, ITC) has ever been published for DSIP or any analogue. The GABA-A hypothesis is inferred from DSIP's sedative phenotype — it is a pharmacologically plausible guess, not an established mechanistic fact. The predictability gate

correctly identified that a meaningful docking calculation cannot be built on an unconfirmed target.

WHAT THIS DOESN'T MEAN

DISCARDED is not "disproved." The cyclization chemistry is entirely feasible: head-to-tail macrolactamisation of a 9-mer via solid-phase synthesis with on-resin or solution-phase cyclization is well-established and routinely executed. The biological hypothesis has genuine literature support — Kovalzon & Strekalova (2006) reported that constrained DSIP structural analogues, not native linear DSIP, produced significant slow-wave sleep promotion in animal models, directly implying that conformational preorganisation unlocks latent activity from this sequence. The failure of this fold is a **boundary of current in silico tooling**, not a statement about whether cyclo(WAGGDASGE) would bind GABA-A or promote sleep in a biological system. The question this fold asked remains open and, by the literature's account, genuinely interesting.

WHAT WOULD ANSWER THE QUESTION

- **Radioligand displacement at GABA-A**: Synthesize cyclo(WAGGDASGE) and measure displacement of [³H]-flunitrazepam (benzodiazepine site) or [³H]-TBPS (chloride channel blocker) in rat cortical membrane preparations. This is the most direct test of the GABA-A hypothesis and requires no prior knowledge of which subunit is relevant.
- **Whole-cell patch-clamp electrophysiology**: Apply cyclo(WAGGDASGE) to primary hippocampal or cortical neurons and measure GABA-evoked chloride currents with and without the peptide. A potentiating or inhibiting effect would confirm functional GABAergic engagement.
- **Molecular dynamics simulation of the free macrocycle**: An explicit-solvent MD run (e.g., using GROMACS or OpenMM with CHARMM36m) of the cyclic nonapeptide would reveal whether the predicted β-turn is genuinely the dominant low-energy conformer and define the accessible conformational ensemble — independent of any target assumption.
- **Future fold using a cryo-EM-derived GABA-A structure as scaffold**: Several high-resolution cryo-EM structures of GABA-A complexes with modulators (e.g., PDB: 6HUG, 6X3X) are available. A future Alembic fold could attempt to model cyclo(WAGGDASGE) against one of these structures directly, bypassing the UniProt gate by specifying the PDB ID rather than a UniProt accession.

RAW METRICS

Metric	Value
pLDDT	Not generated (prediction not run)
pTM	Not generated
ipTM	Not generated
Binder probability	Not generated
Chai-1 agreement	Not generated
Boltz-2 affinity	Not generated
Heuristic peptide profile	Not generated

No computational output was produced for this fold. All metrics above reflect pipeline halt at the orchestrator gate stage.

SEQUENCES

NATIVE

```
WAGGDASGE
```

MODIFIED

```
cyclo(WAGGDASGE)
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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
- DISCARDED due to target_not_predictable: no UniProt ID resolved for the GABA-A receptor complex — this is a tool-limit failure, not biological invalidation
- no structural metrics (pLDDT, pTM, ipTM, binder probability) were generated — the pipeline halted before prediction was run
- DSIP has no confirmed molecular target after 45+ years of research — the GABA-A hypothesis is inferred from phenotype, not direct biochemical evidence

- heuristic peptide property estimates (aggregation, stability, BBB penetration, half-life) were not generated for this fold
- head-to-tail cyclization geometry may or may not be compatible with an unstrained 9-residue macrocycle — ring strain feasibility has not been computationally evaluated
- the DSIP literature base is sparse and largely pre-genomics (1984–1988); basic pharmacological claims have not been systematically reproduced by contemporary methods

CITATIONS

1. **PMID —** (2006) — — Delta sleep-inducing peptide (DSIP): a still unresolved riddle.
2. **PMID —** (1984) — — Delta-sleep-inducing peptide (DSIP): a review.
3. **PMID —** (1986) — — Delta-sleep-inducing peptide (DSIP): an update.
4. **PMID —** (1984) — — DSIP in insomnia.
5. **PMID —** (1988) — — DSIP--a tool for investigating the sleep onset mechanism: a review.
6. **PMID —** (2024) — — Pichia pastoris-secreted DSIP-CBBBP fusion peptides: sleep-enhancing effects in PCPA-induced insomnia model.
7. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions.
8. **PMID —** (2023) — — Protecting Group-Minimum, Practical N-to-C Peptide Synthesis.

SOLANA SIGNATURE 43XtXuvfWHH3VduwnbzrS4QkEzQ9RTR9FDZPpVk5vB37H9aXzmSGtdyNWaj7CMKC5SwdhJTPf17e6B6g4u7FY4jf

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