# SYNTHESIS OF LACTAM MODULATORS OF THE INTERLEUKIN-1 RECEPTOR FOR DELAYING LABOR AND IMPROVING NEONATAL OUTCOMES

**Charity D. Yongo-Luwawa[1], Chirstiane Quiniou[2], Sylvain Chemtob[2,3] and William D. Lubell[1]**

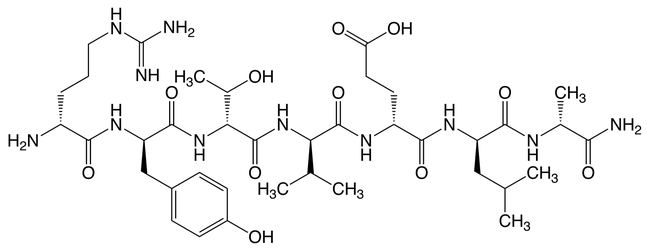
*[1] Département de Chimie, Université de Montréal, Montréal, Canada, [2] Hôpital Sainte-Justine Research Centre, Montréal, QC, Canada,*
*[3] Departments of Pediatrics, Pharmacology and Physiology, and Ophthalmology, Université de Montréal, Montréal, QC, Canada*

## Abstract

The central pro-inflammatory cytokine, interleukin-1β (IL-1β) and its receptor (IL-1R) play key roles in the induction of labor and immune vigilance against invading pathogens. Premature birth remains an unmet costly medical need. Employing lactam analogs in a peptide-based approach, modulators of the IL-1R which delay labor without effect on immune vigilance have been conceived based on the peptide **101.10** (H-D-Arg-D-Tyr-D-Thr-D-Val-D-Glu-D-Leu-D-Ala-$NH_2$).

## Premature birth

High morbidity and mortality rates are associated with premature birth (<37 weeks gestation).[1] Occuring in ≈10% of all pregnancy worldwide, prematurity is expensive and causes long-term health problems.[1,2] For example, retinopathy of prematurity due incubator care can have serious consequences on newborn vision.[3]



Peptide **1** (**101.10**)

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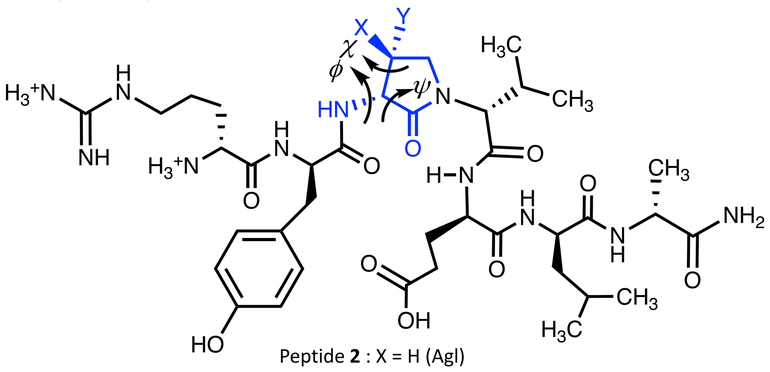
- Blocked IL-1R proinflammatory activity without inhibiting NF-kB signaling thereby maintaining immune vigilance.[4]
- Inhibited uterine inflammation and delayed birth.[4]
- Improved newborn health by blocking prenatal inflammation in mother and fetus
- After birth, blocked hyperoxia caused inflammation in the retinal vasculature in an oxygen induced retinopathy model[5]

**Goals**

- Employ lactam constraints to study structure-activity relationships of peptide **1**
- Stabilize bioactive backbone and side chain conformers
- Develop improved peptide mimic analogs

## Application of lactams to stabilize β-turns

α-Amino γ-lactam (Agl, Freidinger-Veber lactam) residues were used as covalent constraints to restrict rotation around the $\phi$- and $\psi$-dihedral angles to stabilize turns in peptide **1**.[6,7]



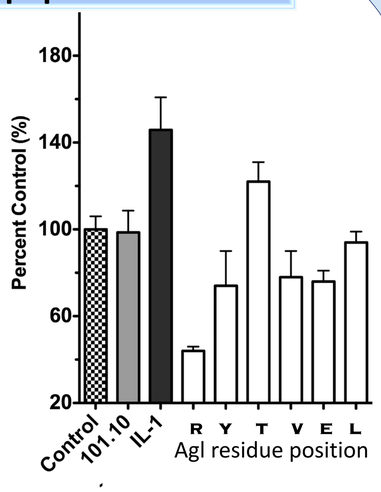
Peptide **2** : X = H (Agl)
Peptide **3** : X = OH (Hgl)
Peptide **4** : X = 4-Phenyltriazole

β-Hydroxy Agl (Hgl) residues were used to serve as rigid D-Thr analogs to add constraint on the $\chi$-dihedral angles.[6,8] Contingent on stereochemistry, Agl and Hgl residues induced β-turn conformation in peptide analogs **2** and **3**.[6]

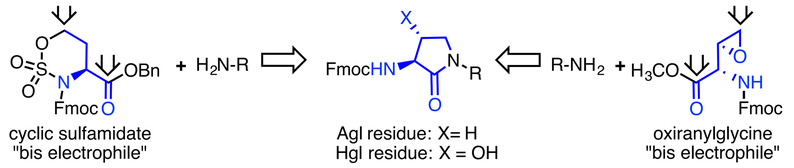
## Agl scan of peptide 1

Systematic replacement of each amino acid in peptide **1** with an Agl residue provided a set of analogs that typically inhibited IL-1β-induced proliferation of TF-1 thymocytes better than the parent peptide.[9]

Activity of [(3*R*)-Agl[3]]-**1** (**2**) was lower than that of peptide **1**, probably due to the absence of the hydroxyl group of the D-Thr[3] residue side chain.[9]




## Agl and Hgl peptide synthesis and activity




Using cyclic sulfamidate and oxiranylglycine bis-electrophiles, the *N*-terminal amine of a peptide can be alkylated and acylated to add Agl and Hgl residues.[6,8,9]

Comparison of different stereoisomers of Agl and Hgl at position 3 of peptide **1** in *in vitro* assays of kinase phosphorylation (Western blots), cytokine expression (qPCR) and NF-kB signaling (QUANTI-blue assay) as well as in *in vivo* models of LPS-induced preterm birth (PTB) and oxygen-induced retinopathy (OIR) of prematurity, all demonstrated that [(3*R*,4*S*)-Hgl[3]]-**1** (**3**) exhibited identical activity to that of parent peptide.[10]

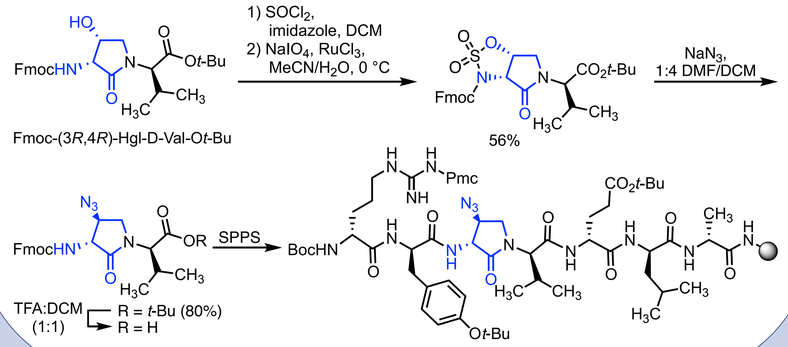
| Structure | Western Blot | | | qPCR | | | Nf-κB | *In vivo* | |
|---|---|---|---|---|---|---|---|---|---|
| | JNK | p38 | ROCK2 | COX2 | IL-1β | IL-6 | | PTB | OIR |
| [(3R)-Agl[3]]-**1** | 4 | 0 | 0 | 3 | 4 | 3 | 0 | 4 | 0 |
| [(3R,4R)-Hgl[3]]-**1** | 0 | 0 | 4 | 4 | 4 | 4 | 0 | 0 | |
| [(3R,4S)-Hgl[3]]-**1** | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 4 | 4 |
| [(3s)-Agl[3]]-**1** | 4 | 0 | 0 | 4 | 4 | 2 | 0 | 4 | 1 |
| [(3S,4R)-Hgl[3]]-**1** | 3 | 3 | 3 | 4 | 4 | 4 | 0 | 4 | 4 |
| [(3S,4S)-Hgl[3]]-**1** | 1 | 1 | 3 | 4 | 4 | 4 | 0 | 2 | 3 |
| 101.10 [H-rytvela-$NH_2$] | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 4 | 4 |
| Kineret [IL-1R-antagonist] | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 3 |

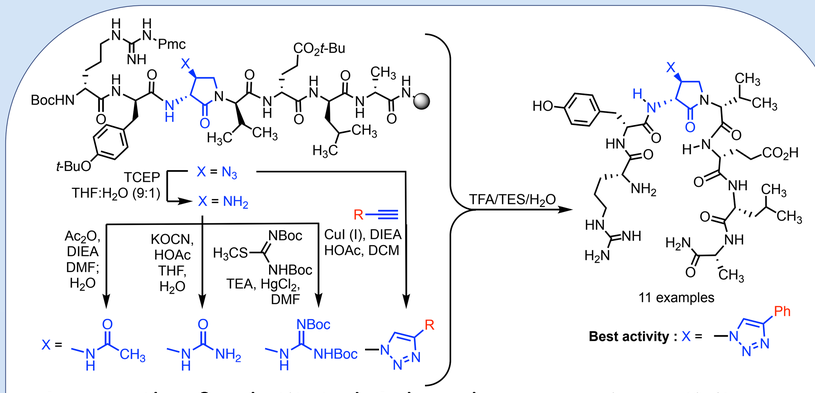
*No effect* 0 1 2 3 4 *Maximum inhibition/efficacy.* *Black = not tested*

Moreover, the circular dichroism (CD) spectrum of peptide **3** exhibited a β-turn curve shape in contrast to the random coil CD curve of parent peptide **1**.[10]

## Introduction of β-substituted Agl analogs

Methods featuring Mitsunobu and cyclic sulfamidate opening chemistry converted Hgl to β-substituted Agl residues.[6] For example, a set of eleven β-substituted Agl analogs of peptide **1** were prepared using the β-azide precursor.[11]




Among the β-substituted Agl analogs, superior activity was shown by peptide **4** [3-(4-phenyltriazolyl)-Agl[3]]-**1** on inhibiting p38 kinase phosphorylation and reducing the expression of induced cytokine genes by IL-1β. In appropriate mice models, peptide **4** delayed birth and inhibited OIR with similar or better activity than peptide **1**.[11]

## Conclusions

- Efficient syntheses in solution and on solid-phase were developed for making substituted Agl peptides analogs
- The lactam constraint on peptide **1** provided insight into the β-turn topology required to interact with IL-1R.
- Lactam peptide mimics retained peptide 1 capacity to block IL-1R proinflammatory activity without inhibiting NF-kB signaling and maintained immune vigilance.
- Lactam peptide mimics (e.g., **3**) delayed birth and improved neonatal outcomes.
- [3-(4-phenyltriazolyl)-Agl[3]]-**1** represents a new lead for treatment of preterm birth to improve the quality of life of newborns.

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