Synthesis and application of β-amino γ-lactams in the study of interleukin-1 and cluster of differentiation-36 receptors modulating peptides

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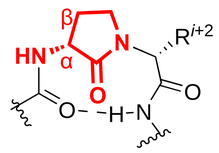
Abstract

Freidinger-Veber lactam (e.g., α-amino γ-lactam, Agl) residues are a mainstay in the study of peptide structure-activity relationships. Although less well studied, the β-amino γ-lactam (Bgl) counterparts can also favour β-turn conformers important for molecular recognition. For example, Bgl analogs of the peptide **101.10** (H-D-Arg-D-Tyr-D-Thr-D-Val-D-Glu-D-Leu-D-Ala-NH_2) have revealed active conformers of this allosteric modulator of the interleukin-1 receptor (IL-1R). Similarly, Bgl analogs of **GHRP-6** (H-His-D-Tyr-Ala-Trp-D-Phe-Lys-NH_2) exhibit promise as cluster-of-differentiation-36 receptor (CD36) modulators. New methods are presented for the synthesis and incorporation of Bgl residues into **101.10** and **GHRP-6**.

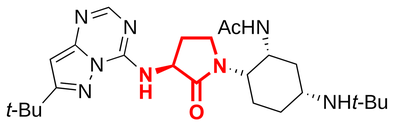
Application of Freidinger-Veber lactams



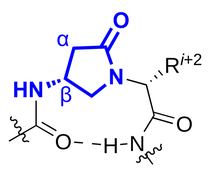
α-amino γ-lactam [(*R*)-Agl] residue in turn conformation

Agl residues add covalent constraints that restrict backbone ψ- and ω-dihedral angles stabilizing turn geometry.[1,2]

An Agl residue is used in the clinical candidate **BMS-813160** under investigation for treating pancreatic and colorectal cancer.[3]



BMS-813160
Selective CCR2/5 dual antagonist

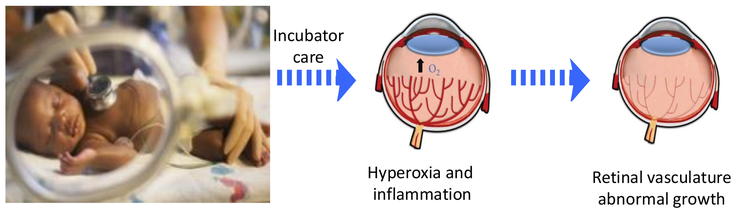


β-amino γ-lactam [(*R*)-Bgl] residue in turn conformation

In spite potential for peptide and medicinal chemistry Bgl residues are less well studied due in part to limited synthetic access.[4]

Premature birth and interleukin 1 receptor (IL-1R)

A major costly healthcare challenge, PTB (<37 weeks of gestation) occurs in about 10% of all pregnancies globally causing high rates of morbidity, mortality and later in life health problems.[5,6] Notably, incubator care of preterm newborns under high oxygen atmosphere can lead to retinopathy of prematurity (ROP) the leading cause of infant vision loss.[7]



Peptide 1 (H-D-Arg-D-Tyr-D-Thr-D-Val-D-Glu-D-Leu-D-Ala-NH_2) **(101.10)**

- Blocks IL-1R proinflammatory activity without inhibiting NF-κB signaling, thereby maintaining immune vigilance. [8]
- Inhibits uterine inflammation and delays birth.[8]
- Improves newborn health by blocking prenatal inflammation in mother and fetus.[8]
- After birth, blocks hyperoxia caused inflammation in the retinal vasculature in an oxygen induced retinopathy model.[9]

Goals

- Employ Bgl constraints to study the structure-activity relationships of peptide **1**, stabilize bioactive conformer and improve activity.

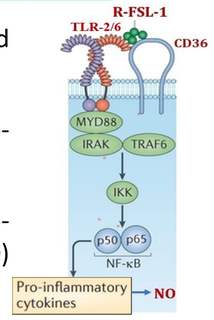
Growth Hormone Releasing Peptide-6 (GHRP-6)

- **Peptide 2** (H-His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, GHRP-6) offers promising pharmacological activity on the cardiovascular system and anti-atherosclerotic effects in apolipoprotein deficient murine models.[4,10]

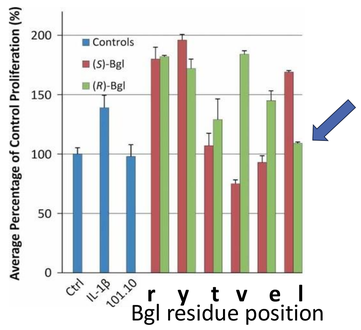
- **Peptide 2** binds both cluster of differentiation-36 and growth hormone secretagogue receptor-1a receptors (CD-36 and GHS-R1a) yielding unselective biological activity. [10]

Selective CD36 modulation is desired

- A scavenger receptor, CD36 is co-expressed and modulates the TLR-2/6 complex.[11]
- TLRs recognize pathogen- and damage-associated molecular patterns.[11]
- TLR-2/6 agonists (e.g., R-FSL-1), trigger pro-inflammatory responses [e.g., nitric oxide (NO) production].[11]



Earlier Bgl scans of 101.10 and GHRP-6

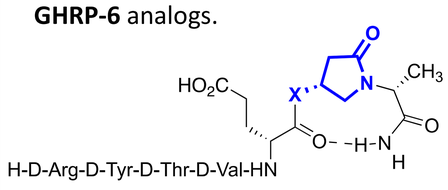


A Bgl scan of **101.10** drew attention to the D-Leu residue in a TF-1 thymocyte proliferation induced by IL-1 assay.[12] [(*R*)-Bgl[6]]-**1** exhibited similar activity as peptide **1.**

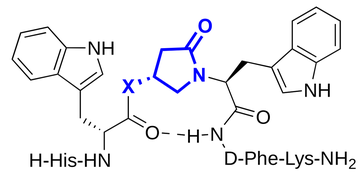
A Bgl scan of **GHRP-6** revealed the importance [Bgl[3]]-**2** analogs for inducing turn geometry for CD36 binding selectivity.[4]

Project goals

- Innovate methods for Bgl and β-hydroxy-γ-lactam (Bhl) residue introduction into peptides.
- Demonstrate proof-of-concept by synthesis of Bgl and Bhl **101.10** and **GHRP-6** analogs.

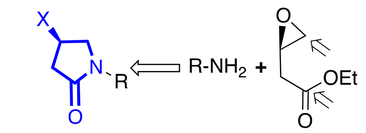


[3*R*-Bgl[6]]-**1** (**3**): **X** = **NH**
[3*R*-depsi-Bgl[6]]-**1** (**4**): **X** = **O**



[3*R*-Bgl[3]]-**1** (**5**): **X** = **NH**
[3*R*-depsi-Bgl[3]]-**1** (**6**): **X** = **O**

Bgl and Bhl dipeptide synthesis

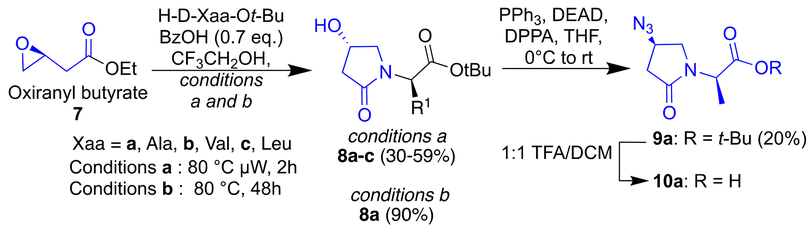


Bgl: X = NH
depsi-Bgl: X = OH

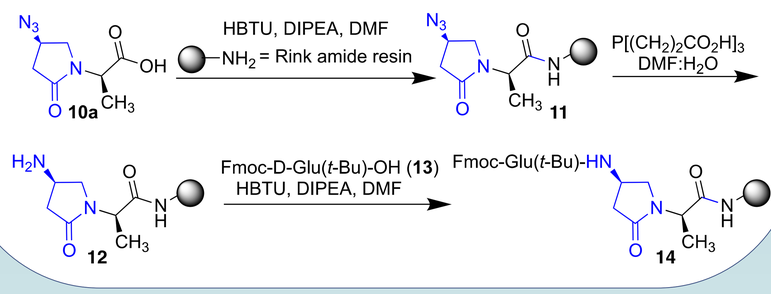
Oxiranyl butyrate "bis-electrophile"

3-Oxiranyl butyrate was designed to act as "bis-electrophile" for amine alkylation and acylation to synthesize Bgl and Bhl residues.

Oxirane **7** was synthesized in three steps (43% yield) from commercial (*S*)-(–)-β-hydroxy-γ-butyrolactone,[15] and used to alkylate and acylation of different amino esters to provides Bhl analogs **8a-c**. Alcohol displacement with azide under modified Mitsunobu conditions converted Bhl **8a** into Bgl dipeptide **9a**.[16]

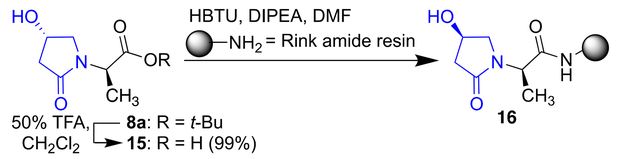


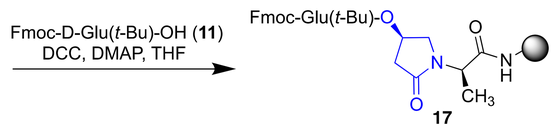
The synthesis of [*R*-Bgl[6]]-**1** (**3**) is being pursued from Bgl dipeptide **10a** by coupling to Rink amide resin, reduction with TCEP and acylation with Fmoc-Glu(*t*-Bu)-OH (**13**).



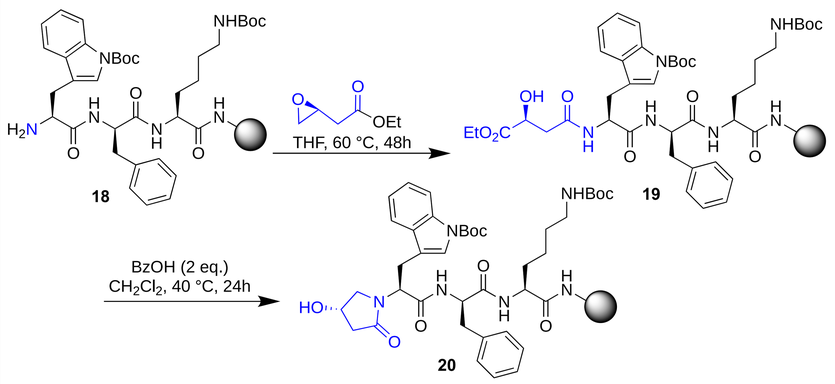
Bhl peptide synthesis

Peptide synthesis with Bhl **8a** entails ester solvolysis, coupling onto Rink amide resin and acylation with Fmoc-D-Glu(*t*-Bu)-OH to give depsi-peptide **17** with conversion over 90%.





Solid-phase Bhl peptide synthesis was pursued by alkylation and acylation of tripeptide **18** which yielded tetrapeptide **20** of 60% purity by HPLC-MS analysis.[17]



Conclusions and perspectives

- Oxiranyl butyrate is a novel "bis-electrophile" for Bhl and Bgl peptide synthesis by solution and solid-phase methods.
- Application of Bhl and Bgl analogs in peptides **1** and **2** is under study to examine effects on IL-1R and CD36 mediated inflammatory pathways.

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