Summer Scholar Report

Novel approach to symmetrical amino acid-derived peptide isosteres

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Abstract

Mimicry of biological peptides through isosteric replacement of the amide linkage has been demonstrated to inhibit activity of enzymes including the HIV aspartyl pro- tease, whose functions are essential for propagation of the virus. Currently reported peptide mimics lack either the restricted rotation or hydrogen bonding capability of the amide bond and thus suffer from decreased efficacy. Methodology developed within the Zercher group allows efficient access to cyclopropanol isosteres that are expected to exhibit uncompromised mimicry of natural peptides. Cyclopropanol isosteres were accessed from amino acid-derived diketones, which were prepared using a Lewis acid- assisted mixed Claisen reaction. Proline and phenylalanine-derived systems were investigated with the desired proline-derived cyclopropanol isolated in moderate yield. Investigation of phenylalanine-containing systems is currently under way, as is investigation of a novel intramol- ecular rearrangement of these cyclopropanols.

Background

Through hydrolytic cleavage of an amide linkage, the HIV aspartyl protease provides peptide fragments necessary for viral propagation.[1] Isosteric replacement of the amide functionality with a functionality resistant to hydrolysis, such as a ketomethylene or hydroxyethylene unit, has served as inspiration in the design of successful protease inhibitors.[1] Preparation of these isosteres, however, can be lengthy and without fine stereocontrol.[2,3] Furthermore, free rotation around these amide replacements decreases their inhibitory efficacy.[4] This led to development of cyclopropyl and alkenyl isosteres, which exhibited rigid structures reminiscent of the amide bond, but also sacrificed mimicry of the hydrogen bonding ability.[4] Methodology developed recently within the Zercher group provides efficient access to a unique and otherwise unreported class of isosteres replacing the amide bond with a cyclopropanol moiety.[5] This cyclo-propanol provides the desired rigidity, while also maintaining hydrogen bonding capability and mimicking the geometry of the tetrahedral intermediate formed during amide hydrolysis (**Fig. 1**).

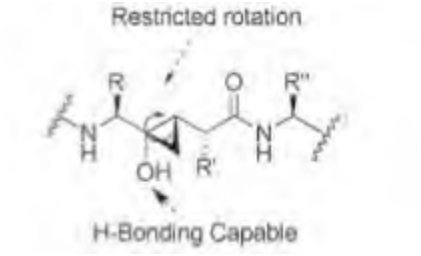
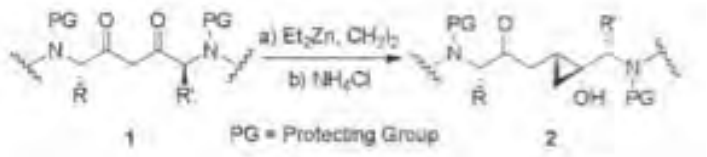


Fig. 1 – *Unique cyclopropanol isostere*

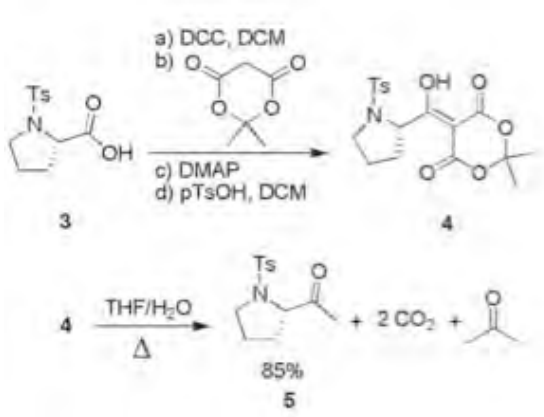
Exposure of the appropriate β-dicarbonyl-containing compound to a bis(iodomethyl)zinc carbenoid, similar to the Furukawa-modified Simmons-Smith carbenoid, results in homologation of the carbon backbone followed by cyclo-propanation to give the cyclopropanol isostere in a one vessel reaction.[5] When considering potential enzyme inhibitors to be targeted through this methodology, the C_2 symmetric nature of the HIV aspartyl protease indicates that a symmetric or psuedosymmetric substrate may have strong selectivity and potency.[6] This was confirmed by Kempf and co-workers who prepared a large library of C_2 symmetric hydroxyethylene isosteres and found many of them to have sub-nanomolar IC_{50}'s.[7] With this in mind, we are developing the homologation-cyclopropanation of a novel class of symmetrical amino acid-derived β-diketones **1** to form pseudosymmetric cyclopropanol peptide isosteres **2** (**Scheme 1**).



Scheme 1 – *Amino acid-derived cyclopropanol isosteres from novel β-diketones*

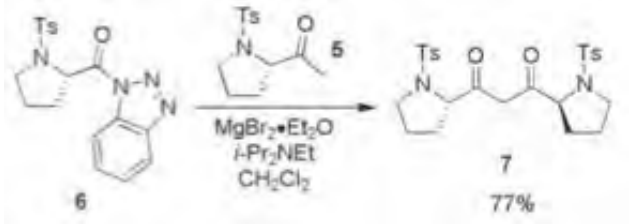
Preparation of β-Diketone Precursors

Preparation of amino acid-derived β-diketones was envisioned through a mixed Claisen condensation of a ketone with some form of activated ester. Many different activated esters and enolate forming conditions were attempted before two successful methods were identified. The first step in preparation of the ketone and activated ester was *N*-protection of the amino acid to prevent quenching of an intermediate enolate formed during the homologation- cyclopropanation reaction. Due to its ease of handling and protection, L-proline was chosen as the model system with which to optimize β-diketone and cyclopropanol forming conditions. L-Proline was protected as the sulfonamide **3**, then subjected to a DCC-facilitated coupling reaction with Meldrum's acid to form the adduct **4**. Hydrolysis of this adduct and flash chromatography of the crude mixture gave the ketone **5** (**Scheme 2**).



Scheme 2 – *Preparation of amino acid-derived ketone **5***

Tosyl protected proline (**3**) was also reacted with thionyl chloride and 1H-benzotriazole to form the activated species **6**, which was condensed with ketone **5** in a Lewis acid-assisted mixed Claisen reaction to form the desired β- diketone **7** (**Scheme 3**). Recrystallization of the crude mixture from ethanol provided analytical grade β-diketone.



Scheme 3 – *Mixed Claisen to form β-diketone 7*

β-Diketone **7** was also prepared through a more traditional mixed Claisen reaction, using lithium diisopropy-lamide at -78 °C to form the enolate of **5**, which was then reacted with active species **6**. This procedure resulted in poorer yields of the desired product and a crude mixture that was not amenable to

recrystallization. Through ^{1}H NMR analysis, it was observed that β-diketone **7** exists predominantly in its enol form, with the exact ratio of enol to keto species differing slightly with concentration. The presence of a single resonance for the enol form's sp^2 CH moiety suggests that this material was prepared with negligible epimerization, barring isochronicity of the two diastereomers.

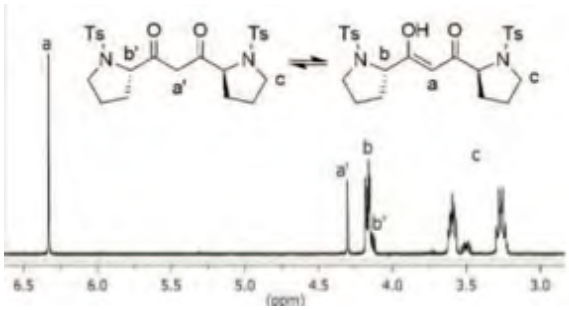
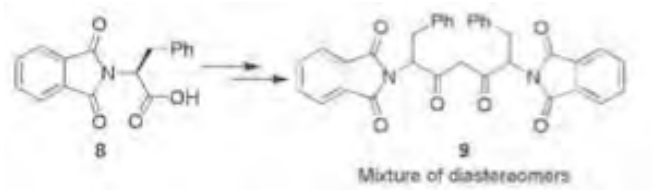


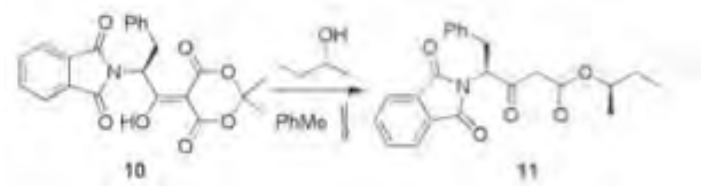
Figure 2 – *^{1}H NMR expansion of enol and keto forms of β- diketone 7*

The phenylalanine-derived analog of β-diketone **7** was also prepared using similar methodology. Protection as the phthalimide **8** followed by Meldrum's acid coupling and exposure to modified mixed Claisen conditions gave the β- diketone **9** (**Scheme 4**), though in poorer yield than the pro- line-derived system, and in a crude mixture which required flash chromatography to separate. Unfortunately, ^{1}H NMR analysis of the purified material revealed two different resonances from the enol form of **9**, indicating that epimerization of the phenylalanine residue had occurred at some point during the preparation.



Scheme 4 – *Preparation of phenylalanine derived β-dike- tone **9***

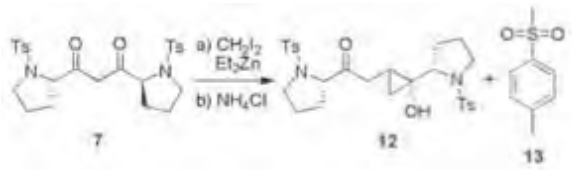
To investigate this epimerization, the Meldrum's acid adduct **10** was opened with the chiral alcohol (*R*)-2-butanol, forming β-keto ester **11** (**Scheme 5**) in what an initial analysis indicates to be a mixture of diastereomers. This suggests that the material leading into the mixed Claisen step has already been epimerized, and appropriate steps are currently being taken to determine exactly when the epimerization occurred.



Scheme 5 – *Opening of phenylalanine Meldrum's acid adduct with chiral alcohol*

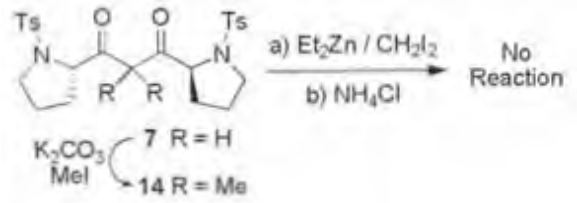
Preparation of Cyclopropanol Isosteres

L-Proline-derived β-diketone **7** was exposed to the bis(iodomethyl)zinc carbenoid at 0 °C and a crude mixture containing many different products was obtained. After chromatographic separation of the crude mixture, minor amounts of a diastereomeric mixture of desired cyclo-propanol **12** were obtained, as well as methyl sulfone **13** and other unidentified products (**Scheme 6**). Sulfone **13** is believed to be formed through elimination of *p*-toluenesulfinate from one of the proline residues, followed by methylation of this species by the zinc carbenoid.



Scheme 6 – *Initial homologation-cyclopropanation of β- diketone 7*

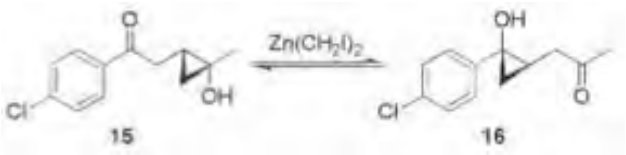
In order to limit the formation of sulfone **13**, it was necessary to know whether the sulfone was formed as a decomposition product of some intermediate species, or through an undesired reaction of the starting material itself with zinc carbenoid. The first step of homologation-cyclopropanation involves deprotonation of the β-diketone and formation of a zinc stabilized enolate. If a species analogous to β-diketone **7** but incapable of forming an enolate could be prepared, then reaction of this species with zinc carbenoid would reveal whether loss of the tosyl protecting group occurred before homologation-cyclopropanation or sometime after this process began. Thus, β-diketone **7** was modified as the *gem*-dimethyl compound **14** and subjected to homologation- cyclopropanation conditions, resulting in no reaction (**Scheme 7**).



Scheme 7 – *Preparation and exposure of dimethyl β-dike- tone **14** to zinc carbenoid*

This indicated to us that loss of the tosyl protecting group was likely due to the high reactivity of one or more reaction intermediates. Strong evidence exists to suggest that the homologation-cyclopropanation reaction proceeds through a series of zinc stabilized enolates and homoenolates.[8,9] It was hypothesized that introduction of a reagent to trap these species may diminish formation of unwanted byproducts during the homologation-cyclopropanation reaction. Exposure of β-diketone **7** to homologation-cyclopropa- nation conditions in the presence of trimethylsilylchloride (TMS-Cl) followed by selective removal of the silyl group resulted in a much cleaner crude reaction mixture with formation of cyclopropanol **12** in >50% yields. Sulfone **13** and at least one other byproduct were still present, though in decreased amounts.

An interesting consequence of introducing TMS-Cl to the reaction mixture was an increase of the diastereomeric ratio in which cyclopropanol **12** was observed. When standard homologation-cyclopropanation conditions were used, a roughly one to one mixture of diastereomers was observed. When the zinc carbenoid reaction is performed in the presence of TMS-Cl, however, diastereomeric ratios of approximately 1:4 were observed. We believe this is due to TMS protection of the cyclopropanol, which minimizes the possibility of interconversion between diastereomers. Investigation of the formation and reactivity of β-diketone derived cyclopropanols **15** and **16** revealed a unique isomeric interconversion when exposed to bis(iodomethyl)zinc carbenoid (**Scheme 8**).



Scheme 8 – *Interconversion of cyclopropanol isomers*

This rearrangement is hypothesized to proceed through fragmentation of the cyclopropane with concurrent

nucleophilic attack of the ketone functionality. If zinc were to be complexed between the Lewis basic oxygens, rearrangement through this conformation would be predicted to result in the interconversion of diastereomers. Therefore, by protecting the cyclopropanol moiety as it is formed with TMS, this mechanistic pathway is shut down, no rearrangement is allowed to occur, and the cyclopropanol product is formed with greater diastereoselectivity. To further investigate this theory, the TMS-protected analog of cyclopropanol **12** was isolated and heated at 200 °C in a closed vessel under inert atmosphere for one hour, resulting in no change of the diastereomeric ratio as observed via ^{1}H NMR.

Phenylalanine derived β-diketone **9** has also been exposed to homologation-cyclopropanation conditions, both in the presence and absence of TMS-Cl, and initial NMR analysis of the crude mixtures indicates formation of potential product material.

Future Work

Epimerization during the formation of phenylalanine derived β-diketone **9** is an issue needing a solution. Recent results have suggested where stereochemical integrity is lost, and optimization of the synthetic route will be under- taken to minimize epimerization.

Optimization studies of cyclopropanol- forming conditions are also underway. Recent results indicate that cooling the reaction vessel to 0 °C for the entirety of the reaction may further decrease formation of sulfone **13** and other byproducts, as well as increase the diastereoselectivity of the reaction. Controlled rearrangement of the cyclopropanol isomers is also being attempted in an effort to identify the thermodynamic characteristics of the rearrangement.

Acknowledgements

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