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Synthesis of a Ketomethylene Isostere of the Fibrillating Peptide SNNFGAILSS

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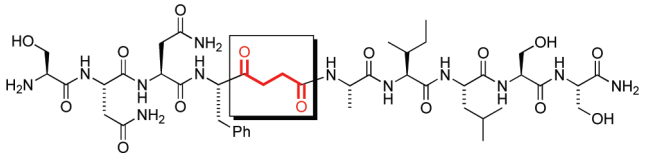

The direct synthesis of a ketomethylene isostere of the fibril-forming decapeptide SNNFGAILSS is presented with the goal of understanding how small structural changes alter the ability of such peptides to recognize each other for β-sheet formation. The key synthetic step relies on a SmI_2-mediated coupling of a *N*-tetrapeptidyl oxazolidinone with a simple acrylate followed by deprotection of the carboxylic acid and a peptide coupling step with the pentapeptide H-AILSS-NH_2.

Deposition of human islet amyloid polypeptide (hIAPP) as fibrillar amyloid in the pancreatic islets of Langerhans is a characteristic histopathological marker for type II diabetes mellitus (T2DM) and is found in more than 90% of the affected patients.[1,2] Extensive studies with fragments of hIAPP have revealed that $hIAPP_{20-29}$ (SNNFGAILSS),[3,4] $hIAPP_{22-27}$ (NFGAIL),[5] and $hIAPP_{22-29}$ (NFGAILSS)[6] all form amyloid fibrils with morphologies very similar to those of the full-length polypeptide. This region of the peptide is therefore believed to form the fibrillation core domain of fibrils in the pancreas of type II diabetes patients. We have recently determined the complete 3D β-sheet fibril structure of one of the hIAPP fragments with 0.5 Å atomic resolution using solid-state nuclear magnetic resonance spectroscopy with a fully ^{15}N,^{13}C-labeled NFGAIL fragment of the $hIAPP_{20-29}$ SNNFGAILSS-NH_2 decapeptide.[7] The 3D structure of these β-sheet forming fibrils can be used to provide atomic-resolution insight into the key interactions that hold these fibrils together.

Parallel to this work, we have set out to prepare analogues of SNNFGAILSS **1** incorporating small structural changes in order to examine their influence on fibril formation and growth (Figure 1). To this end, the ketomethylene isostere (SNNF-Ψ(CO-CH_2)-GAILSS) **2** represents an interesting target. Such a structural modification will remove a single amide bond, thereby eliminating one hydrogen bond interaction. How this variation will influence the ability of such peptides to form β-sheet fibrils will be of particular interest for understanding stability of the fibril structures formed from the parent peptide.

Synthesis of ketomethylene isosteres are generally not simple operations and usually require multiple steps for preparing these classes of dipeptide analogues.[8] Recently, we reported a novel and direct route to dipeptidyl ketomethylene isosteres through the coupling of *N*-acyl oxazolidinone derivatives of amino acids or peptides with acrylamides.[9−14] In this way, these peptide isosteres can be accessed directly from a single coupling step. Most importantly, the reaction conditions are mild and hence epimerization is avoided at the adjacent stereogenic center to the newly formed ketone. With this reaction in mind, we set out to prepare a ketomethylene dipeptide isostere of the fibrillating peptide SNNFGAILSS at the structurally simplest peptide bond position (Phe-Gly). The results of this work again illustrate the usefulness of this lanthanide reagent for performing carbon−carbon bond-forming reactions with important biomolecules.

To commence this study, we initially focused our attention on the synthesis of a ketomethylene isostere of the smaller peptide NFGAIL as a model to examine the suitability of the C−C bond-forming approach to this class of peptides. In a recent publication, we reported that the SmI_2-mediated

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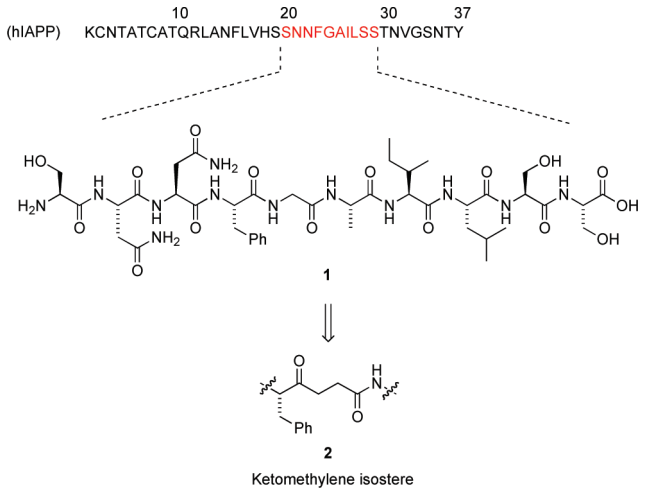
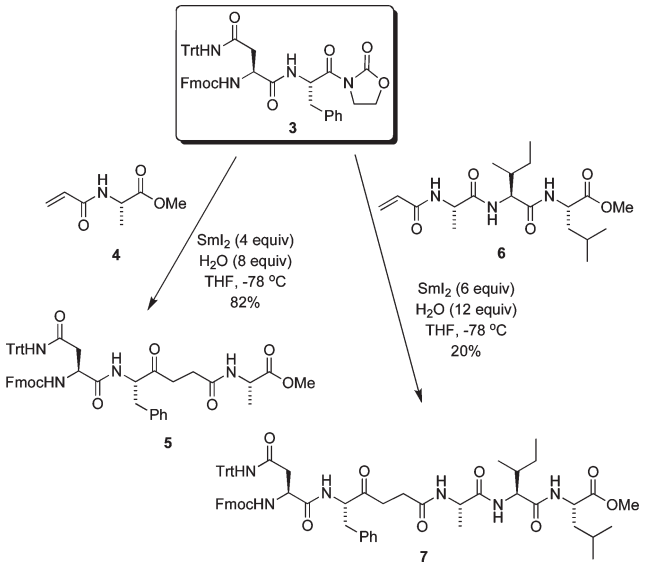


FIGURE 1. SNNFGAILSS decapeptide and the corresponding ketomethylene isostere.

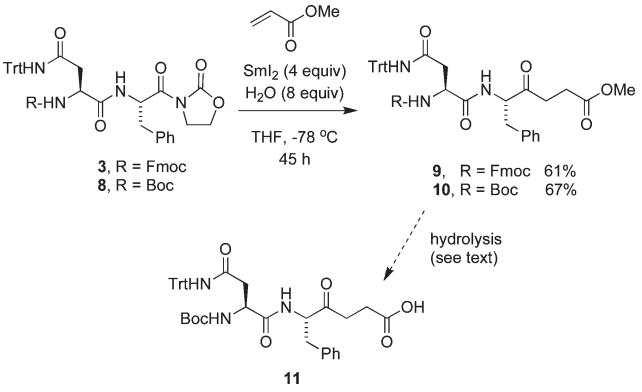
SCHEME 1. Previously Published SmI_2-Promoted Reactions with Peptide-Derived Acrylamides



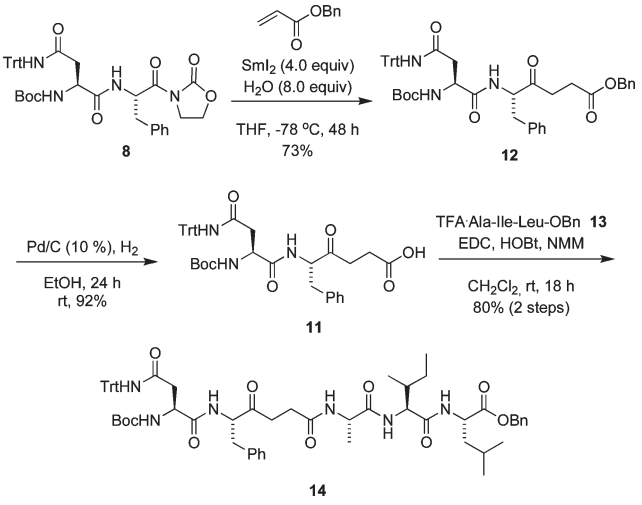
coupling of the *N*-dipeptidyl oxazolidinone **3** and *N*-acryloyl-L-alanine methyl ester **4** provided the fully protected ketomethylene isostere **5** of the tetrapeptide NFGA in a good 82% yield (Scheme 1).[13] However, upon extrapolation to the hexapeptide analogue, the reaction between **3** and the larger acrylamide, *N*-acryloyl-L-alanyl-L-isoleucyl-L-leucine methyl ester **6** was impeded by the low solubility of the electron-poor alkene under the reaction conditions leading to the formation of the coupling product **7** in only a 20% yield.

To avoid this potential reactant solubility issue for this key coupling step, which undoubtedly would be more prominent if this synthetic approach were to be extended to the larger decapeptide, we decided to pursue a slightly modified synthetic route to the ketomethylene isostere. In this alternative approach, the *N*-acyl oxazolidinone would initially be coupled to a simple acrylate followed by liberation of the carboxylic acid and a standard peptide coupling step with a *C*-terminally protected peptide. The solubility issue in the C−C bond-forming step is in this way resolved by utilizing a

SCHEME 2. Synthesis of the Tripeptide NF-Ψ(CO-CH_2)-G Isostere



SCHEME 3. Synthesis of the Fully Protected NF-Ψ(CO-CH_2)-GAIL



smaller and more soluble electron-deficient alkene as the coupling partner.

Preliminary efforts were focused on the coupling of the *N*-dipeptidyl oxazolidinones **3** and **8** with methyl acrylate, as shown in Scheme 2. The coupling products **9** and **10** were obtained by dropwise addition of SmI_2 (0.1 M in THF) over 30 min to a cold solution of either peptidyl oxazolidinone **3** or **8** and methyl acrylate in the presence of water followed by stirring for 45 h. Both **9** and **10** could be isolated in good yields, and as expected from earlier work, the Fmoc and Boc protecting groups on the *N*-terminal amino acid were well tolerated.[13]

To obtain the free carboxylic acid for further peptide coupling, hydrolysis of the methyl ester was examined using several protocols with the goal of avoiding epimerization of the chiral carbon center adjacent to the ketone functionality. Whereas LiOH in THF/water (4:1)[16] and potassium trimethylsilanolate in THF[17] proved effective for the hydrolysis of the methyl ester **10**, considerable epimerization was observed in the product in both

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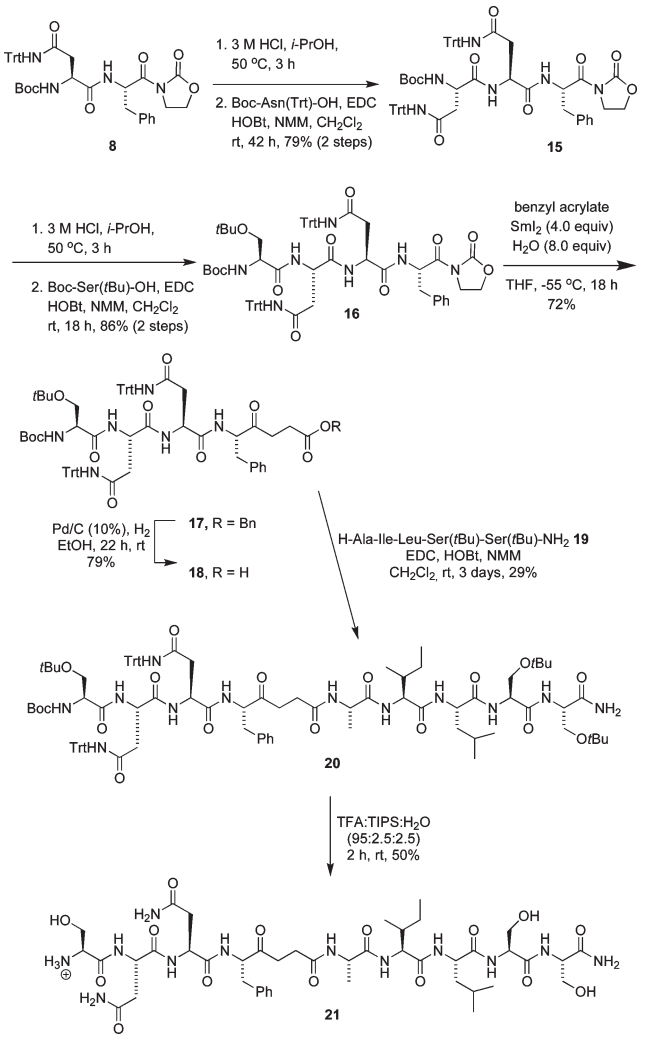
SCHEME 4. Synthesis of SNNF-Ψ(CHOH-CH_2)-GAILSS-NH_2



cases by ^{1}H NMR spectroscopy. Trimethyltin hydroxide has recently been reported as a mild and selective method for the hydrolysis of epimerization-prone methyl esters.[18] Treatment of **9** with Me_3SnOH (3.0 equiv) in dichloroethane at 70 °C for 4.5 h provided the desired carboxylic acid **11** with no epimerization, but only a low yield of the product was obtained. Extended reaction times afforded higher yields of **11**, though with signs of epimerization according to ^{1}H NMR and HPLC analysis.

To circumvent these problems, the C−C bond-forming reaction was carried out with benzyl acrylate and the *N*-peptidyl oxazolidinone **8** leading to the formation of the ketone **12** in a good yield (Scheme 3). The following hydrogenolysis was accomplished using palladium on charcoal under a hydrogen atmosphere and afforded the free carboxylic acid **11** in a 92% yield. ^{1}H NMR analysis of tripeptide NFG analogue revealed that no detectable epimerization had occurred during either the coupling or the hydrogenolysis step.

The tripeptide **13** required for the subsequent peptide coupling was synthesized from the *p*-toluenesulfonate salt of L-leucine benzyl ester and *N*-Boc-L-isoleucine using EDC as the coupling reagent. Removal of the Boc-protecting group of the resulting *N*-Boc-L-isoleucyl-L-leucine benzyl ester with trifluoroacetic acid in dichloromethane followed by further extension of the peptide chain with alanine and deprotection yielded the tripeptide **13** ready for peptide coupling with **11**. Applying standard EDC peptide coupling conditions afforded the desired, fully protected NFGAIL isostere **14** in an 80% overall yield for the last two steps.

With the successful synthesis of the hexapeptide analogue as a model, we immediately proceeded to apply this synthetic approach for accessing the ketomethylene isostere of the decapeptide as illustrated in Scheme 4. Synthesis of the required tetrapeptidyl oxazolidinone **16** was performed by two consecutive cycles of *N*-terminal protecting group removal with 3 M HCl in *i*-PrOH at 50 °C followed by an EDC-mediated peptide coupling via the tripeptide **15**. The key C−C bond-forming step was initially performed under standard conditions for peptidyl oxazolidinones and acrylate couplings at −78 °C and provided the ketomethylene isostere **17** of the pentapeptide SNNFG in a 54% yield, which is consistent with the yields previously published for this type of reaction.[13] However, increasing the reaction temperature to −55 °C from −78 °C and doubling the amount of benzyl acrylate had a beneficial effect on the yield and **17** was isolated in a good 72% yield. Hydrogenolysis of the coupling product afforded the partially deprotected isostere **18**.

Finally, the peptide chain was elongated to the corresponding decapeptide isostere **20** by liquid-phase peptide coupling in DMF between the protected peptide amide fragment **19**, which was prepared by automated solid-phase peptide synthesis, and the carboxylic acid **18**. Treatment of the protected decapeptide ketomethylene isostere **20** with 95% trifluoroacetic acid afforded the desired peptide isostere **21** in a 50% yield after HPLC purification. The decapeptide analogue was prepared as its C-terminal amide, as we have earlier shown that the same derivative of the parent peptide can easily be fibrillated,[7] thus facilitating the subsequent fibrillation studies of this new ketomethylene isostere.

In summary, we have prepared a ketomethylene isostere of the fibrillating SNNFGAILSS fragment of the human islet amyloid polypeptide with the purpose of exploiting such analogues for understanding how small structural alterations influence the ability of such peptides to aggregate. The isostere was prepared exploiting an efficient SmI_2-mediated C−C bond forming reaction as the key step for attaining a peptidyl ketone intermediate. Work is now underway to investigate whether this decapeptide analogue can fibrillate, as well as examining its influence on the fibrillation of the parent peptide. These studies will be reported in due course.

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Supporting Information Available: Experimental details for all compounds including copies of ^{1}H NMR, ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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