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Synthesis of Hexa- and Pentasubstituted Diketopiperazines from Sterically Hindered Amino Acids

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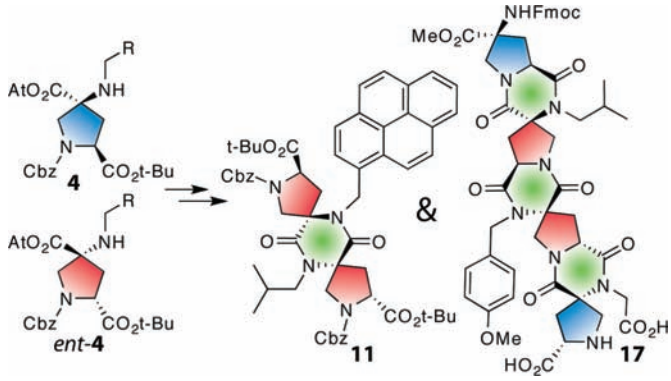
ABSTRACT



Steric hindrance assists in the formation of hindered diketopiperazines using acyl-transfer coupling. In acyl-transfer coupling, the carboxylate of an unprotected *N*-alkylamino acid attacks an active ester to form a transient anhydride that undergoes an O,N acyl transfer to form a tertiary amide. If the active ester is part of an *N*-alkylamino acid it will form a diketopiperazine. It is demonstrated here that acyl-transfer coupling can assemble highly functionalized bis-peptides bearing a functional group on every monomer.

While exploring new approaches to forming asymmetric hexa- and pentasubstituted diketopiperazines, we recently developed a new reaction for forming extremely hindered tertiary amides.[1−3] In this reaction, the carboxylic acid of an unprotected *N*-alkylamino acid acts as a nucleophile to attack an active ester to form a transient anhydride. This anhydride spontaneously undergoes an O,N acyl transfer to form a tertiary amide bond. This O,N acyl transfer is similar to the driving step of the Ugi reaction,[4] the acyl transfer of native chemical ligation,[5] and others.[6−8] We have continued to develop this reaction and demonstrate here that it can be used to synthesize asymmetric hexa- and pentasubstituted diketopiperazines under mild conditions with excellent yields. Diketopiperazines are considered privileged structures in medicinal chemistry and are valuable motifs for the discovery of new lead compounds by combinatorial chemistry and the rational development of new therapeutic agents.[9−11] We also demonstrate that this reaction can be applied sequentially to

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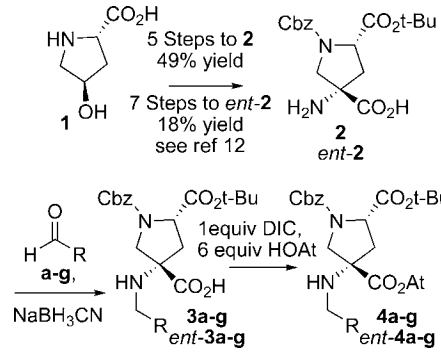
assemble bis-peptides that display a functional group on every monomer.

Toward the rational design of preorganized arrays of functional groups that can be designed to selectively bind protein surfaces we have developed bis-peptides.[12] Bis-peptides are spiro-cyclic oligomers assembled from stereochemically pure, cyclic bis-amino acids. Bis-amino acids display two α-amino acid groups mounted on a cyclic core. In the assembly of bis-peptides, diketopiperazine rings are formed sequentially between adjacent monomers to create spiro-ladder oligomers with well-defined three-dimensional structures. We have demonstrated the synthesis of many bis-amino acids and their assembly into a wide variety of oligomers with different shapes.[12] Modeling suggests that properly designed functionalized bis-peptides could mimic the display of side chains on α-helical peptides. In recent years, many groups have described non-natural oligomers that adopt helical structures.[13,14] *β*-Peptides and α/*β*-peptide hybrids adopt a variety of helical structures, some of which are able to mimic the presentation of α-helical peptides and disrupt helix−protein interactions.[15−18] Other oligomers have been demonstrated to fold into helical structures that present side chains including peptoids,[19−21] N,N′-linked oligo ureas,[22] aromatic amino acid oligomers,[23] quinoline oligoamides,[24] and pyridine dicarboxamides.[25] Another approach to mimicking α-helices is to construct scaffolds that present functional groups with spacing and orientation that is as similar to α-helical side-chain presentation as possible. This approach is demonstrated by the trisubstituted terphenyl derivatives[26] and the benzoylurea oligomers[27] of Hamilton and co-workers.

The approach we chose toward functionalizing bis-peptides is to combine *N*-alkyl-α,α-disubstituted bis-amino acids such as **3a**−**g** through diketopiperazine linkages (Scheme 1).

Scheme 1. Synthesis of N-Functionalized, Activated Amino Esters



These amino acids are very sterically hindered and have a history as poor substrates for amide bond formation using traditional coupling agents.[28] In terms of forming diketopiperazines, even tetrasubstituted diketopiperazines are difficult to form from linear dipeptide precursors and traditionally require forcing conditions.[11,29,30] While steric hindrance has traditionally been regarded as an obstacle to the coupling of hindered amino acids, we demonstrate here that it can actually assist in the formation of tertiary amides and hexa- and pentasubstituted diketopiperazines.

Compounds **2** and *ent*-**2** are synthesized in five and seven steps, respectively, from *trans*-L-4-hydroxyproline **1** in 49% and 18% overall yield using previously described procedures.[12] The synthesis of each amino acid involves only one chromatographic step. The amino acids **2** and *ent*-**2** were functionalized on the amine using reductive alkylation with the aldehydes **a**−**g** shown in Table 1 to form **3a**−**g** with quantitative yield. The aldehydes **a**−**g** were chosen because they demonstrate that a variety of proteogenic and nonproteogenic functional groups can be incorporated using acyl-transfer coupling. The unprotected amino acids **3a**−**g** were then combined with 6 equiv of 1-hydroxy-7-azabenzotriazole (HOAt) followed by 1 equiv of diisopropylcarbodiimide (DIC). The excess HOAt is used to trap the *O*-acylisourea to form **4a**−**g** in near quantitative yield. When less than 6 equiv of HOAt was used, we observed the formation of symmetric hexasubstituted diketopiperazines formed from two molecules of **3**. The activated esters **4a**−**g** do not spontaneously self-react in DMF/CH_2Cl_2 solutions for several

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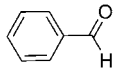
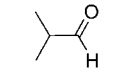
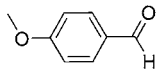
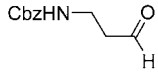
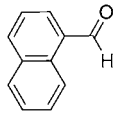
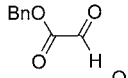
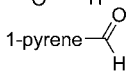
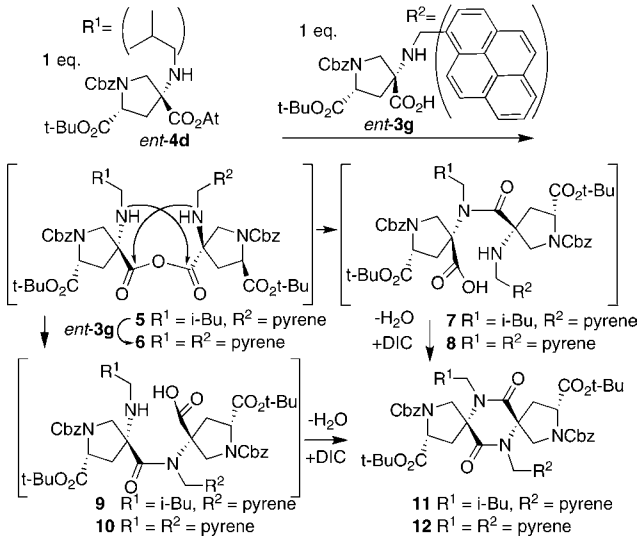
Table 1. Variety of Aldehydes Used to Functionalize Bis-amino Acids

entry	aldehyde	product	entry	aldehyde	product
a		3a	d		*ent*-3d
b		*ent*-3b	e		3e
c		3c	f		3f
			g		*ent*-3g

hours at room temperature presumably because of the extremely hindered nature of the otherwise unprotected secondary amine.

To test if the *N*-alkylamino-OAt esters **4a**−**g** could couple with hindered *N*-alkylamino acids through an acyl-transfer mechanism we combined 110 μmol of *ent*-**4d** with 110 μmol of *ent*-**3g** in 2 mL of 1:2 DMF/CH_2Cl_2 and stirred at room temperature for 1.5 h (Scheme 2). High-performance liquid

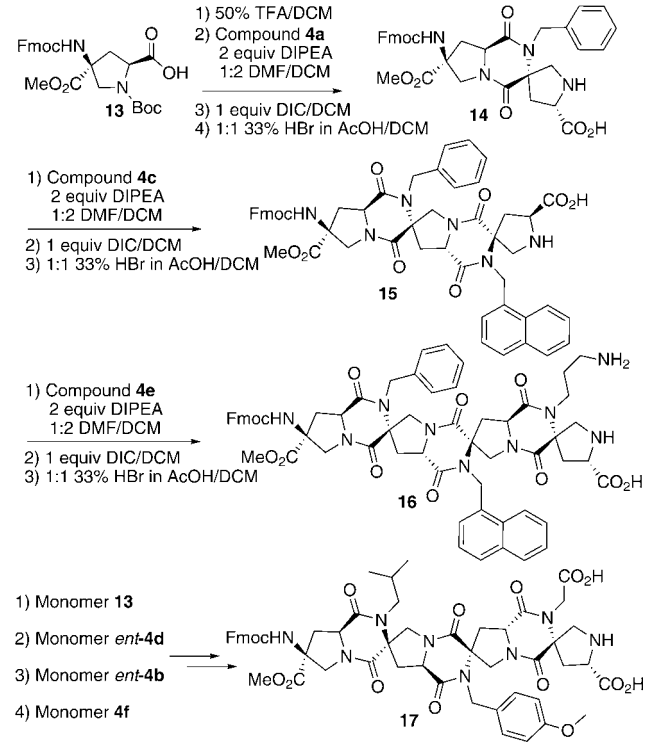
Scheme 2. Proposed Mechanism for the Synthesis of Hexasubstituted Diketopiperazines



chromatography with mass spectrometry (RP-HPLC−MS) analysis of the reaction mixture showed a ratio of *ent*-**3g**:**7**/**9**:**11**:**8**/**10**:**12** of 20:35:35:8:2. We then added 1 equiv of DIC to the reaction mixture, allowed it to stir for 1 h, and observed the ratio of *ent*-**3g**:**11**:**12** of 4:81:15. Thus, the yield of **11** was 81% relative to *ent*-**3g**. In the initial reaction of *ent*-**3g** with *ent*-**4d**, the only competent nucleophile is the carboxylic acid of *ent*-**3g** which we propose attacks the −OAt ester of *ent*-**4d** to form the transient anhydride **5** which spontaneously rearranges one of two ways to form **7** and/or **9**. We propose that the traces of **8**, **10**, and **12** that are observed come about from the reaction of *ent*-**3g** with **5** to form the anhydride **6** followed by acyl transfer to form **12**. Each of the amide products **7** and **9** proceed to the same diketopiperazine product **11** by spontaneous dehydration or assisted by the addition of a dehydrating agent such as DIC. Diketopiperazine closure requires the first amide **7** and/or **9** to adopt a *cis* conformation; this is greatly assisted in these cases by the sterically crowded nature of the tertiary amides.

To determine if we could use this approach to sequentially form pentasubstituted diketopiperazines and assemble functionalized bis-peptides we synthesized compounds **16** and **17** (Scheme 3). To synthesize the functionalized bis-peptide

Scheme 3. Synthesis of Oligomers **16** and **17**



16, we started from compound **13**, a protected bis-amino acid that we have previously described.[12] The Boc group was removed with 50% trifluoroacetic acid (TFA/CH_2Cl_2) and the solution was concentrated under reduced pressure. The resulting amino acid was combined with active ester **4a** and allowed to stir at room temperature overnight. By C_{18} RP-HPLC−MS we observed two products, one had a mass consistent with the formation of a single amide bond between the two amino acids and the other had a mass consistent with the desired diketopiperazine product in a ratio of 27:73. We then added 1 equiv of DIC directly to the reaction mixture and stirred for 2 h at room temperature. At this point, the diketopiperazine was the only product observed by RP-HPLC−MS. The diketopiperazine was isolated and treated with HBr in acetic acid to form the amino acid **14**. We isolated compound **14** by C_{18} RP-HPLC (68% yield relative to **13**). We then repeated the process with a new monomer,

combining **14** and the activated, 2-methylenenaphthylene-functionalized bis-amino acid **4c** which again formed a mixture of amide and diketopiperazine products to which we added 1 equiv of DIC and then treated with HBr/AcOH to form **15** (77% yield from **14**). The process was repeated a third time, combining **15** with the activated, carboxybenzoyl-aminopropyl-functionalized bis-amino acid **4e**. The single amide and diketopiperazine products of this were treated with DIC and then HBr/AcOH to form **16** (59% yield from **15**). Oligomer **17** was synthesized using a different sequence of functionalized monomers with the indicated stereochemistry with similar yields.

The composition of **16** and **17** was verified by high-resolution mass spectrometry, and the structures were validated using two-dimensional nuclear magnetic resonance experiments. ROESY correlations were consistent with minimum energy conformations of **16** and **17** identified using the Amber94 force field.[31] In the structure of **16** the stereochemistry of the component monomers arranges the three functional groups in a left-handed helical arrangement, and they superimpose well on the side chains of residues *i*, $i + 3$, and $i + 6$ of a model α-helical peptide (Figure 1). The distance between each successive pairs of functionalized amide nitrogens is 5.6 Å. In the structure of **17** the functional groups are in a right-handed helical arrangement that superimpose well on the side-chains of residues *i*, $i + 4$, and $i + 8$ of a model α-helix with a distance of 5 Å between each successive pair of functionalized amide nitrogens.

We have demonstrated that acyl-transfer coupling allows the synthesis of hexa- and pentasubstituted diketopiperazines and the sequential solution phase synthesis of short bis-peptide oligomers that carry a different functional group on each monomer. The three-dimensional display of the functional groups is controlled by the sequence and stereochemistry of the monomers and a wide assortment of proteogenic and nonproteogenic functional groups can be incorporated.

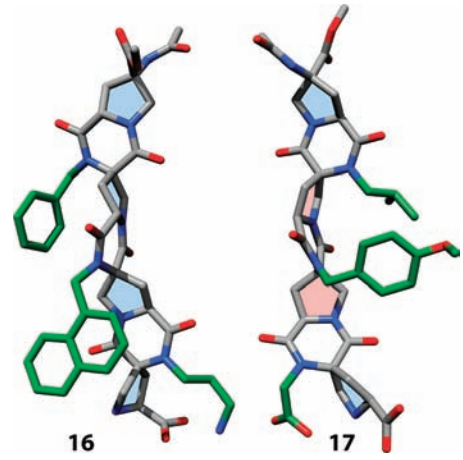


Figure 1. Models of **16** and **17**. Pyrrolidine rings with (*S*,*S*) stereochemistry are shaded blue and those with (*R*,*R*) stereochemistry are shaded red. In **16**, the side chains project in a left-handed helical sense, and in **17**, they project in a right-handed helical sense.

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Supporting Information Available: Synthesis and characterization of the functionalized bis peptides and coupling experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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