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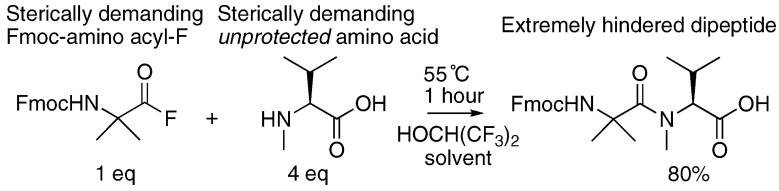
J. Am. Chem. Soc., **2008**, 130 (44), 14382-14383 • DOI: 10.1021/ja806063k • Publication Date (Web): 08 October 2008



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Published on Web 10/08/2008

Exploiting an Inherent Neighboring Group Effect of α-Amino Acids To Synthesize Extremely Hindered Dipeptides

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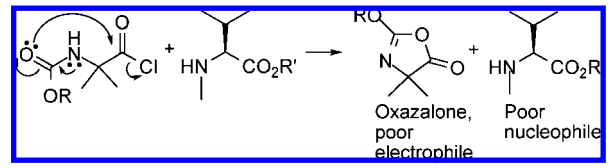
The synthetic methodology for creating peptides is among the most highly developed synthetic methodologies in chemistry.[1,2] While the creation of proteogenic peptides is largely a solved problem, the creation of highly hindered peptides that contain combinations of non-natural N-alkyl amino acids and N-alkyl-α,α-disubstituted amino acids remains a formidable challenge.[3] Hindered, non-natural amino acids are of interest because they import resistance to proteolysis and unusual conformational properties to peptides.[4] Toward a solution to this problem, we describe a new approach to creating extremely hindered dipeptides that is operationally simple and uses mild conditions and commercially available amino acids. The approach reduces the need for protecting groups and yields urethane-protected dipeptide acids that can be used as building blocks in the synthesis of larger peptides. This approach appears to take advantage of a previously unexploited intramolecular acyl transfer.

Urethane-based amine protecting groups including the carboxybenzoyl group (Cbz),[5] the *tert*-butylcarboxyl group (*t*-Boc)[2] and the 9-fluorenylmethylenecarboxyl group (Fmoc)[6] are by far the most common amine protecting groups used in peptide synthesis. Urethane-protected amino acids are commercially available for all proteogenic amino acids and many unnatural amino acids. These groups protect the amine of amino acids from unwanted reactions, they protect the amino acid from racemization during activation of the carboxyl group, and they are easily removed using mild conditions. Many approaches have been demonstrated for coupling proteogenic and hindered amino acids in which either the amino group (i.e., *N*-alkyl amino acids) or the carboxylic group (i.e., α,α-disubstituted amino acids) are relatively hindered.[7,8] On the other hand, there is as yet no efficient way to form amide bonds between hindered urethane−amino acids (such as Fmoc-Aib-OH) with carboxyl-masked hindered amino acids such as *N*-alkyl amino acids.[3,9] The challenge is that the amine of an *N*-alkyl amino acid is a very poor nucleophile, and, to compensate, strong activation of the urethane-amino acid, such as through use of a urethane-amino acid chloride, would be required to achieve coupling. However, acid chlorides are difficult to handle and strong activation of urethane-amino carboxyls leads to the rapid formation of oxazalones, which are poor electrophiles (Scheme 1) and prone to racemization. Sulfonamides have been developed as alternatives to urethane protecting groups[10] and used to couple N-methyl amino acids, but they cannot yet match the low cost, commercial availability, excellent stability, and convenience of urethane-protected amino acids.

To form a dipeptide we simply combine an Fmoc-amino acid fluoride with an excess of unprotected amino acid (Scheme 2) dissolved in hexafluoroisopropanol. We followed the literature procedure[11] with slight modifications to form the acid fluoride **1**. The Fmoc-amino acid fluoride was used without purification and added directly to a solution of 105 mg (0.8 mmol) of *N*-methylvaline **2** dissolved in 4 mL of hexafluoroisopropanol that had been

Scheme 1. The Challenge of Hindered Amide Bond Synthesis



Scheme 2. Synthesis of Fmoc-Aib-NMeVal-OH **3**

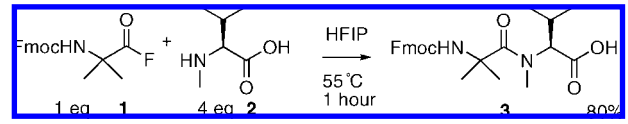


Table 1. Yields of a Variety of Hindered Dipeptides[a]

entry	dipeptide	yield (%)	time (min)
4	Fmoc-Aib-(*S*)-Tic-OH	86	60
5	Fmoc-Aib-NMeAib-OH	60	60
6	Fmoc-(*S*)-NMeVal-(*S*)-NMeVal-OH	78	5
7	Fmoc-(*S*)-NMeVal-(*S*)-Tic-OH	80	5
8	Fmoc-(*S*)-NMeVal-NMeAib-OH	68	5
9	Fmoc-(*S*)-NMeVal-Sar-OH	78	5
10	Fmoc-Ac$_5$c-NMeVal-OH	74	45
11	Fmoc-Ac$_5$c-(*S*)-Tic-OH	79	45
12	Fmoc-Ac$_5$c-NMeAib-OH	60	45
13	Fmoc-Ac$_5$c-Sar-OH	78	45

[a] All reactions were carried out as described in Scheme 2 varying only the duration of the reaction.

prewarmed to 55 °C (Scheme 2). The solution was allowed to react at 55 °C for one hour and then subjected to reverse-phase high performance liquid chromatography with mass spectrometry (HPLC−MS). The same approach was used to synthesize a variety of other dipeptides with good yields (Table 1). Fmoc-amino acids activated as 1-oxy-7-azabenzotriazole (OAt)) esters using diisopropylcarbodiimide and HOAt produced dipeptide, but the reactions were ∼50% slower than those using acid fluorides.[7]

Reverse phase chromatography of the product of Scheme 2 provides the dipeptide Fmoc-Aib-NMeVal-OH (80%), the recovered starting material Fmoc-Aib-OH (11%), and the ester Fmoc-Aib-OCH(CF$_3$)$_2$ (9%) (Figure 1). Hexafluoroisopropanol is used as the solvent for the dipeptide coupling because it is the only solvent that we have as yet identified that consistently maintains solubility of both the amino acids and the Fmoc-amino acid fluoride. The drawback of using this solvent is that it reacts with the acid fluoride to produce small amounts of hexafluoroisopropyl ester. It is important to completely avoid base in these reactions because base leads to quantitative esterification with solvent. The byproducts and unreacted amino acid starting material can be removed by extraction and chromatography. For precious Fmoc-amino acids the ester can

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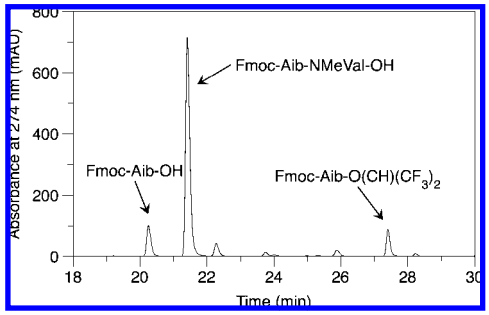


Figure 1. HPLC chromatogram of the reaction mixture of Fmoc-Aib-NMeVal-OH **3**. The unlabeled peaks did not have identifiable masses.

be recovered and recycled using acid or base mediated hydrolysis depending on whether N-Fmoc or N-Boc urethane protection is used.

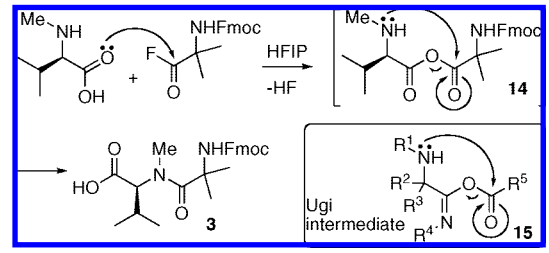
Low resolution HPLC−MS gave *m*/*z* values that were consistent with the structure of each dipeptide **3** to **13**. High resolution mass spectrometry of **3**, **4**, and **10** were consistent with the predicted masses of these dipeptides. The structures of **3**, **4**, and **10** were confirmed using HMQC and HMBC two-dimensional NMR experiments, which in the case of **3** demonstrated correlations between the hydrogens of the N-Me group and the carbonyl carbon of the Aib residue as well as to the α carbon of the NMeVal residue. In addition, the methyl groups of the Aib residue of **3** have different chemical shifts, indicating that they are diastereotopic. We collected proton spectra of **3** at a variety of temperatures and the peaks are broad at room temperature and become sharp at 360 K. This is consistent with two amide rotamers interconverting slowly at room temperature and interconverting rapidly at high temperature. Other examples of dipeptides that we synthesized demonstrated proton NMR spectra that were consistent with mixtures of slowly interconverting amide rotamers at room temperature (e.g., dipeptide **6**).

We carried out the dipeptide formation reaction using (*S*)-Fmoc-NMeVal-F and racemic NMeVal-OH, and by reverse-phase HPLC we saw two peaks consistent with two diastereomeric dipeptides. The reverse-phase HPLC of **6** displayed only one dipeptide peak, which demonstrates that the dipeptide formation reaction maintains the stereochemical integrity of both amino acid stereocenters.

At 55 °C the reactions with Fmoc-NMeVal-F (**6** to **9**) are over in 5 min. When the Fmoc-amino acid fluoride is more hindered (such as Fmoc-Aib-F), the dipeptide formation is slower but the reaction with solvent also slows down so the yields remain high. The time-course of the formation of **3** is shown in the Supporting Information. We observed no premature deprotection of the Fmoc group in any of these reactions. Our attempts to couple Fmoc-Aib-OH with NMeVal-OMe in dimethylformamide using a variety of activated species, including acid fluorides, yielded no significant dipeptide and extensive Fmoc deprotection as seen by others.[12] We also carried out a competition experiment in which we added Fmoc-Aib-F to 4 equiv of NMeVal-OH and 4 equiv of NMeVal-OMe.HCl in HFIP in one pot. By RP-HPLC we observed a ratio of Fmoc-Aib-NMeVal-OH/Fmoc-Aib-NMeVal-OMe of 97:3. The free carboxylic acid of the unprotected amino acid is necessary to achieve efficient acylation.

The observation that the degree of steric crowding of the amine of the amino acid has little effect on the dipeptide yield led us to propose the mechanism shown in Scheme 3. We propose that the carboxylic acid of the amino acid attacks the acid-fluoride to form a transient anhydride that spontaneously rearranges through a five-membered ring transition state to form the hindered amide bond in an intramolecular O,N-acyl transfer. To test this hypothesis we

Scheme 3. Proposed Mechanism for Dipeptide Formation



combined **1** with 4 equiv *N*-(2-methylnaphthylene)-sarcosine in HFIP and observed that almost 8% of it was converted to the HFIP ester, consistent with the formation of an anhydride that undergoes solvolysis because it can not undergo O,N-acyl transfer. The proposed amino-anhydride intermediate and five-member ring transition state is similar to that of the Ugi reaction,[13] which has been described as a "remote Mumm rearrangement".[14] It is reminiscent of the S,N-acyl shift invoked in native chemical ligation[15] and the O,N-acyl shift[16] invoked in the "depsipeptide technique"[17] and "switch peptides".[18] It is also reminiscent of the pioneering work of Kemp and co-workers in their development of O,N-acyl transfer groups.[19]

In conclusion, we have demonstrated an operationally simple approach to synthesizing extremely hindered dipeptides using commercially available amino acids and mild reaction conditions that provides high yields of dipeptide and maintains stereochemical integrity.

Acknowledgment. The authors would like to thank Dr. Jason Moss for valuable conversations. This work was supported by the NIH/NIGMS (GM067866).

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

References

(1) Merrifield, B. *Methods Enzymol.* **1997**, *289*, 3–13. Albericio, F. *Curr. Opin. Chem. Biol* **2004**, *8*, 211–221.
(2) Bodanszky, M. *Principles of Peptide Chemistry*; Springer-Verlag: New York, 1984.
(3) Carpino, L. A.; Ionescu, D.; El-Faham, A.; Henklein, P.; Wenschuh, H.; Bienert, M.; Beyermann, M. *Tetrahedron Lett.* **1998**, *39*, 241–244.
(4) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243–2266.
(5) Bergmann, M.; Zervas, L. *Chem. Ber.* **1932**, *65*, 1192–1201.
(6) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401–407.
(7) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
(8) Wenschuh, H.; Beyermann, M.; Krause, E.; Brudel, M.; Winter, R.; Schumann, M.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1994**, *59*, 3275–3280. Thern, B.; Rudolph, J.; Jung, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 2307–2309. Katritzky, A. R.; Todadze, E.; Angrish, P.; Draghici, B. *J. Org. Chem.* **2007**, *72*, 5794–5801. Cianci, J.; Baell, J. B.; Harvey, A. J. *Tetrahedron Lett.* **2007**, *48*, 5973–5975.
(9) Moretto, V.; Valle, G.; Crisma, M.; Bonora, G. M.; Toniolo, C. *Int. J. Biol. Macromol.* **1992**, *14*, 178–184.
(10) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1998**, *120*, 2690–2691.
(11) Kaduk, C.; Wenschuh, H.; Beyermann, M.; Forner, K.; Carpino, L.; Bienert, M. *Lett. Pept. Sci.* **1996**, *2*, 285–288.
(12) Wenschuh, H.; Beyermann, M.; Winter, R.; Bienert, M.; Ionescu, D.; Carpino, L. A. *Tetrahedron Lett.* **1996**, *37*, 5483–5486.
(13) Ugi, I. *Angew. Chem., Int. Ed.* **1962**, *1*, 8–21. Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168–3210.
(14) Mumm, O. *Chem. Ber.* **1910**, *43*, 886–893.
(15) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–779.
(16) Bergmann, M.; Brand, E.; Weinmann, F. *Hoppe-Seyler's Z. Physiol. Chem.* **1923**, *131*, 1–17.
(17) Coin, I.; Dolling, R.; Krause, E.; Bienert, M.; Beyermann, M.; Sferdean, C.; Carpino, L. *J. Org. Chem.* **2006**, *71*, 6171–6177.
(18) Dos Santos, S.; Chandravarkar, A.; Mandal, B.; Mimna, R.; Murat, K.; Saucede, L.; Tella, P.; Tuchscherer, G.; Mutter, M. *J. Am. Chem. Soc.* **2005**, *127*, 11888–11889.
(19) Kemp, D. S.; Carey, R. I. *J. Org. Chem.* **1993**, *58*, 2216–2222.

JA806063K