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An Efficient Synthesis of a Probe for Protein Function: 2,3-Diaminopropionic Acid with Orthogonal Protecting Groups

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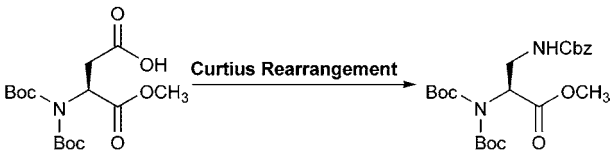
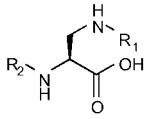
ABSTRACT



An efficient and cost-effective synthesis of *N*(α)-Boc$_2$-*N*(*β*)-Cbz-2,3-diaminopropionic acid is reported. The synthesis starts from commercially available *N*(α)-Boc-Asp(OBn)-OH and employs a Curtius rearrangement to establish the *β*-nitrogen. Proper protection of the α-nitrogen is essential for the success of the Curtius rearrangement.

Incorporation of 2,3-diaminopropionic acid (Dap, Figure 1) has frequently been used to probe many aspects of peptide and protein structure. For example, substituting Dap for lysine has been used to demonstrate that side chain length affects the stability of α helix formation in simple polypeptides.[1] Lysine-associated hydrophobic interactions, arising from interactions with the methylenes of lysine, have also been investigated using Dap, in combination with N-methylated analogues of Dap.[2] In addition, lysine/Dap substitution can also be used to probe the importance of salt bridge formation.[3]



(*S*)-2,3-diaminopropionic acid
(R_1 and R_2 are usually Boc, Fmoc, or Cbz)

Figure 1.

In our work, we are exploring the effects of Dap substitution in RNA-binding peptides. Many RNA-binding proteins and peptides rely on highly cationic amino acid sequences in order to achieve high binding affinity.[4] Therefore, such RNA-binding sequences always contain large numbers of lysine and arginine residues. To design effective RNA-binding drugs, molecules must be designed so that high binding affinity and selectivity to an RNA target can be achieved. The highly charged nature of lysine and arginine, combined with their extended side chains, can contribute to nonspecific binding, which greatly reduces selectivity. Moving the charge closer to the peptide backbone should reduce the propensity of the cationic charge to interact with random anionic species. Confining the charge associated with lysine (and arginine) to positions that are closer to the polypeptide backbone could impact the secondary structure of the peptide and the binding interaction between polypeptides and RNA. NMR studies indicate that not all charged residues in RNA-binding polypeptides are directly coordinated to the RNA fold.[5] We theorize that incorporation of Dap (and guanylated Dap) into peptides with known RNA binding targets could

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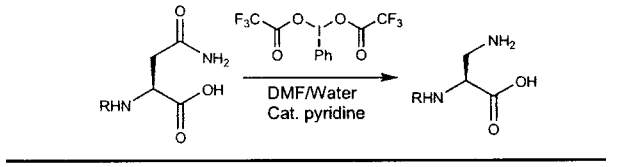
provide important benefits such as decreased nonspecific binding. Incorporation of nonnatural residues also discourages decomposition of peptides in vivo.[6] Given the high cost of Dap, and our desire to incorporate this residue into multiple positions within polypeptides, we have developed an efficient and economical route to a protected form of Dap.

Extensive studies using Dap can be limited due to the cost associated with purchasing this compound. Although commercially available, the typical price for Dap (with orthogonal protecting groups) is about $35/mmol.[7] This cost can be prohibitive when using an excess of monomer in the solid-phase synthesis of a polypeptide containing multiple Dap residues. Furthermore, only the L-enantiomer is available commercially.

The most common synthesis of Dap involves a Hoffman rearrangement of protected asparagine using a trivalent iodine reagent, most often bis-[trifluoroacetoxy]-iodo benzene (Figure 2). When this reagent is used, the trifluoroacetic acid



R	Yield
Boc	42%
Cbz	63%
Fmoc	0%

Figure 2. Hoffman rearrangement with bis-[trifluoroacetoxy]-iodo benzene.[12]

formed in solution[8] is believed to catalyze the hydrolysis of the isocyanate intermediate to an amine, reducing urea formation from reaction of amine with remaining isocyanate.[9] However, the acid generated can also remove a Boc protecting group. As shown in Figure 2, Boc-protected asparagine produced significantly lower yields than Cbz-protected asparagine when subjected to the same conditions for the Hoffman rearrangement. Fmoc-protected asparagine had poor solubility under the same reaction conditions, which accounts for the failure of the Hoffman rearrangement when using this form of protected asparagine. If Cbz protection is used, which is optimal, then an α-amine protecting group must be converted to a Boc or Fmoc group in order to be compatible with solid-phase peptide synthesis.[10] Because we required access to large amounts of Boc-protected Dap, the Hoffman-based synthesis of Dap quickly became prohibitive to the progress of our research. The main problems we encountered were the constant need to exchange protecting groups, the high cost of bis-[trifluoroacetoxy]-iodo benzene (~$0.95/mmol[11]), and the requirement to use an excess (at least 2 equiv) of this reagent. Therefore, we began to develop a more direct and less costly route to Dap.

We felt that a Curtius rearrangement of an aspartic acid derivative would afford an isocyanate that could be trapped with benzyl alcohol to directly afford a derivative of Dap with orthogonal protecting groups suitable for solid-phase peptide synthesis. Starting from Boc-Asp-OBn (**1**), a Curtius rearrangement proceeded to give a product with ^{1}H NMR consistent with the expected isocyanate intermediate **2** (Figure 3). To our surprise, multiple efforts to trap the

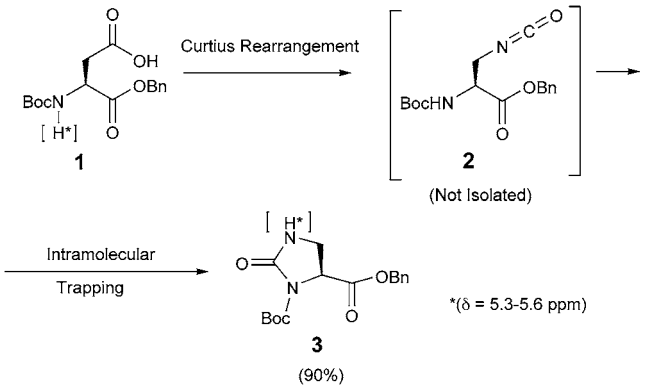


Figure 3. Cyclization with a single Boc-protecting group during Curtius rearrangement.

supposed isocyante failed. Additional ^{13}C NMR, IR, and mass spectrometry data confirmed that cyclic urea **3** was present instead. In the ^{1}H NMR of **3**, the urea proton (H*) appeared in the same region (5.3−5.6 ppm in $CDCl_3$) as most carbamate protons, which was initially deceptive. Also, there was no discernible change in the couplings or chemical shifts of the α or β protons to indicate urea versus isocyanate formation. Deprotection of the Boc group from **3** provided the unprotected urea, which was also fully characterized. A number of studies have shown that carbamate-protected amines can trap isocyanates via intramolecular cyclization.[13] In our case, the cyclization to form a five-membered ring urea was especially efficient. We were unable to intercept the isocyanate with other nucleophiles.

We were able to prevent the intramolecular trapping of the isocyanate after the Curtius rearrangement by dually protecting the reactive nitrogen.[14] Starting from protected aspartic acid **4**, which is commercially available, the carboxylic acid was first converted to methyl ester **5**, followed by introduction of a second Boc group[13a] to give **6** (Scheme

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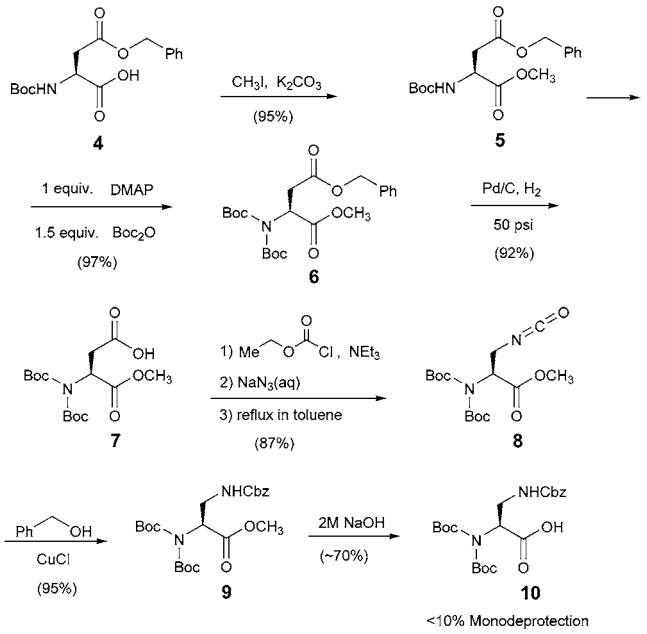
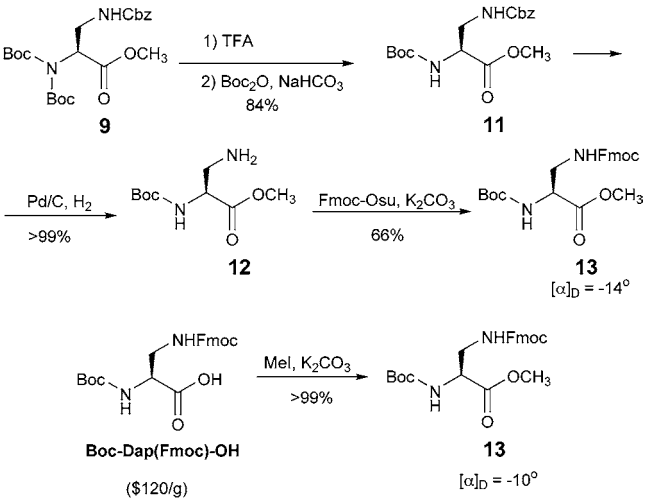
Scheme 1



Scheme 2



1). The benzyl ester was then removed via hydrogenolysis under pressure (50 psig). Next, the Curtius rearrangement was performed by first converting the acid **7** to a mixed anhydride, followed by conversion to the corresponding acyl azide, and then refluxing in toluene for 2 h. These conditions clearly formed isocyanate **8**, which has been characterized by ^{1}H NMR, ^{13}C NMR, and IR. Once isolated, the isocyanate was trapped with benzyl alcohol, using CuCl as a Lewis acid, to form the Cbz-protected amine **9**.[15] Hydrolysis of the methyl ester then gave protected Dap **10**.

A minor side product that we noticed in this synthetic route results from loss of one Boc group of the diprotected nitrogen. Partial monodeprotection most likely occurs during acidic workup after the ester hydrolysis. This byproduct is easily identified in the ^{1}H NMR spectra (see Supporting Information for data) and present in quantities of less than 10% relative to the main product (estimated by ^{1}H NMR). We have used **10** directly in solid-phase peptide synthesis without complications in the deprotection or coupling steps associated with peptide synthesis. The partially deprotected byproduct is not detrimental to the conditions of solid-phase peptide synthesis.

To confirm the enantiomeric purity of **9**, we prepared **13** and compared the optical rotation to the identical compound prepared from commercial Dap. Conversion of **9** to **13** is shown in Scheme 2. Boc-Dap(Fmoc)-OH (from Advanced Chemtech) was methylated to give **13**. The optical rotations of both compounds were within experimental error of each other, confirming that racemization does not occur in our synthesis of Dap.

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In conclusion, we have developed a convenient synthesis of orthogonally protected Dap. On the basis of the commercial prices of the reagents and the yields of this synthesis, using the Curtius rearrangement to access orthogonally protected Dap proves to be half as costly as using the Hoffman-based procedures and less time-consuming. It should be noted that this synthesis is optimal for accessing Dap for use in solid-phase peptide synthesis using the Boc group to protect the α nitrogen. For solid-phase peptide synthesis using the Fmoc strategy, the Dap monomer requires an Fmoc group on the α nitrogen and a Boc group on the *β* nitrogen. This arrangement of protecting groups could certainly be made via our synthetic route using the following series of protecting group manipulations on **10**: deprotection of the Boc groups followed by Fmoc protection and then removal of the Cbz protecting group followed by Boc protection. However, the Hoffman-based approach would provide a shorter route to α-Fmoc, *β*-Boc Dap since less protecting group manipulation would be necessary. Further studies in our group will be directed toward incorporating this amino acid into RNA binding peptides in order to probe the effects on binding affinities.

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Supporting Information Available: Experimental procedures and ^{1}H NMR characterization for compounds **4**−**10** and **13**, ^{13}C NMR characterization available for compounds **4**−**9**, and IR for **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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