

## Research Article

# Convenient and Scalable Synthesis of Fmoc-Protected Peptide Nucleic Acid Backbone

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The peptide nucleic acid backbone Fmoc-AEG-OBn has been synthesized via a scalable and cost-effective route. Ethylenediamine is mono-Boc protected, then alkylated with benzyl bromoacetate. The Boc group is removed and replaced with an Fmoc group. The synthesis was performed starting with 50 g of Boc anhydride to give 31 g of product in 32% overall yield. The Fmoc-protected PNA backbone is a key intermediate in the synthesis of nucleobase-modified PNA monomers. Thus, improved access to this molecule is anticipated to facilitate future investigations into the chemical properties and applications of nucleobase-modified PNA.

## 1. Introduction

Peptide nucleic acid (PNA) [1] has recently emerged as a promising alternative to the native nucleic acids DNA and RNA (Figure 1) for a wide variety of applications including antisense therapy [2] and gene diagnostics [3]. The key advantages of PNA over DNA and RNA are its resistance to degradation by cellular nucleases [4] and its relatively higher binding affinity and mismatch selectivity in duplex formation [5]. PNA can be generated by Fmoc- or Boc-solid phase peptide synthesis [6], and Fmoc-protected monomers bearing each of the four canonical nucleobases are commercially available. Recently, the incorporation of modified nucleobases into PNA has been shown to enable synthesis of nucleic acids having unique physicochemical properties [7]. However, PNA monomers bearing modified nucleobases are not commercially available, and must instead be synthesized in the laboratory. Suitable reactions have been reported for preparation of modified nucleobases and coupling of these nucleobase acetic acids to the PNA backbone (Figure 2) [7–9]. However, to our knowledge, a scalable and cost-effective synthesis for the protected *N*-[2-(Fmoc)aminoethyl]glycine benzyl ester (Fmoc-AEG-OBn) backbone **1** has yet to be reported. Synthesis of the Fmoc-protected carboxylic acid backbone Fmoc-AEG-OH has been reported [10], and coupling of nucleobase acetic acids with

Fmoc-AEG-OH has been described in the patent literature [11]. However, this coupling reaction provides moderate-to-low yields of PNA monomer [12, 13]. Here, we describe a synthesis of **1** that proceeds in four steps with an overall yield of 32%, utilizes inexpensive reagents, and can be scaled to produce large quantities of final product in a single batch with only minimal purification.

## 2. Materials and Methods

**2.1. General Methods.** Unless otherwise noted, all starting materials were obtained from commercial suppliers and were used without further purification. Flash column chromatography was carried out using silica gel 60 (230–400 mesh).  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts are expressed in parts per million ( $\delta$ ) using residual solvent protons as internal standard ( $\delta$  7.26 ppm ( $^1\text{H}$ ) and 77.16 ppm ( $^{13}\text{C}$ ) for  $\text{CHCl}_3$ ). Coupling constants,  $J$ , are reported in Hertz (Hz), and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and app (apparent). Mass spectra were obtained through the Mass Spectrometry Facility, University of Utah.

**2.2. *tert*-Butyl(2-aminoethyl)carbamate (6).** A 2 L round bottom flask was charged with ethylenediamine (306.5 mL,



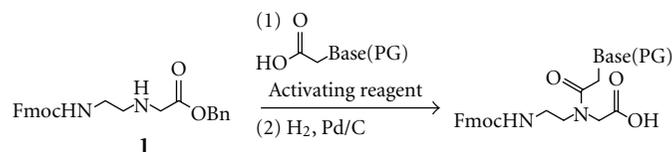


FIGURE 2: Synthesis of Fmoc-protected PNA monomers.

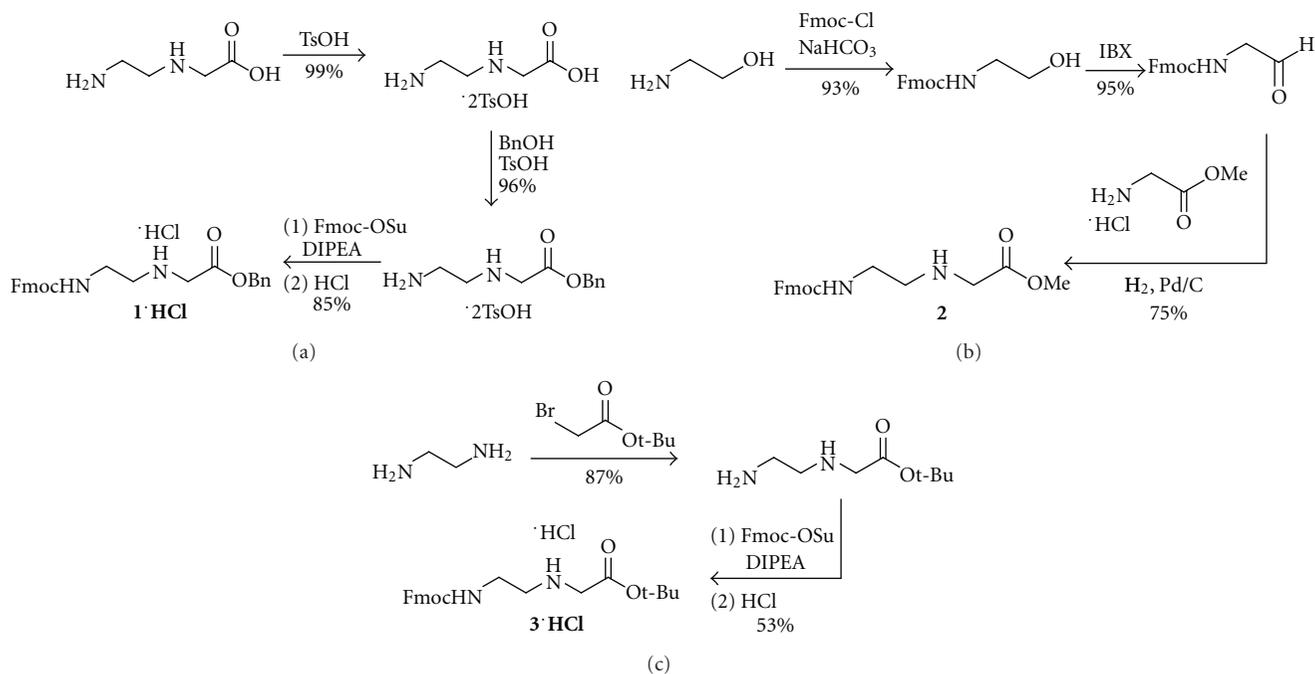


FIGURE 3: Reported synthetic routes to the Fmoc-AEG-OR backbone.

would first be monoprotected with Fmoc-OSu, then alkylated with benzyl bromoacetate to give **1**. However, Fmoc-ethylenediamine cannot be directly prepared by the reaction of ethylenediamine with Fmoc-Cl or Fmoc-OSu. Rather, a three-step process is required in which ethylenediamine is mono-Boc protected (**6**), then Fmoc protected (**7**), and finally the Boc group is removed under acidic conditions to give **8** as the TFA salt [16]. Unfortunately, our attempts to alkylate **8**·TFA with benzyl bromoacetate failed to yield the desired product **1**, likely due to the instability of the free base of **8** (Figure 4(b)).

Fortunately, we were able to obtain Boc-ethylenediamine **6** in 80% yield from ethylenediamine and Boc anhydride using a modified version of a reported procedure [17], and this was successfully alkylated with benzyl bromoacetate to give **9** in 72% yield. We then deprotected the Boc group using trifluoroacetic acid (TFA) to give a quantitative yield of free amine, which was importantly found to be stable to cyclization when isolated as the TFA salt. In the final step, we combined the amine TFA salt with Fmoc-OSu prior to adding base, so that protection of the primary amine could compete with cyclization to give the desired product **1** in 55%

yield. Starting with 50 g of Boc anhydride, we were able to generate 31 g of analytically pure **1** in a single batch using inexpensive reagents (Figure 4(c)) [18].

A key to the scalability of our synthetic route is the relatively facile purification of the synthetic intermediates and final product. The Boc protection step to give **6** requires only aqueous workup, and the deprotection step requires simple concentration and removal of TFA via formation of an azeotrope with toluene. The alkylation to produce **9** and the Fmoc protection to give **1** require flash column chromatography, but a large difference in  $R_f$  between the products and impurities makes purification possible using only a silica plug.

#### 4. Discussion

Fmoc-protected PNA backbone **1** is a key intermediate in the synthesis of Fmoc-protected PNA monomers having modified nucleobases. However, to date, a scalable and cost-effective synthetic route to this molecule has yet to be reported in the literature. An efficient synthesis of the Boc-protected backbone has been reported, but our attempts to

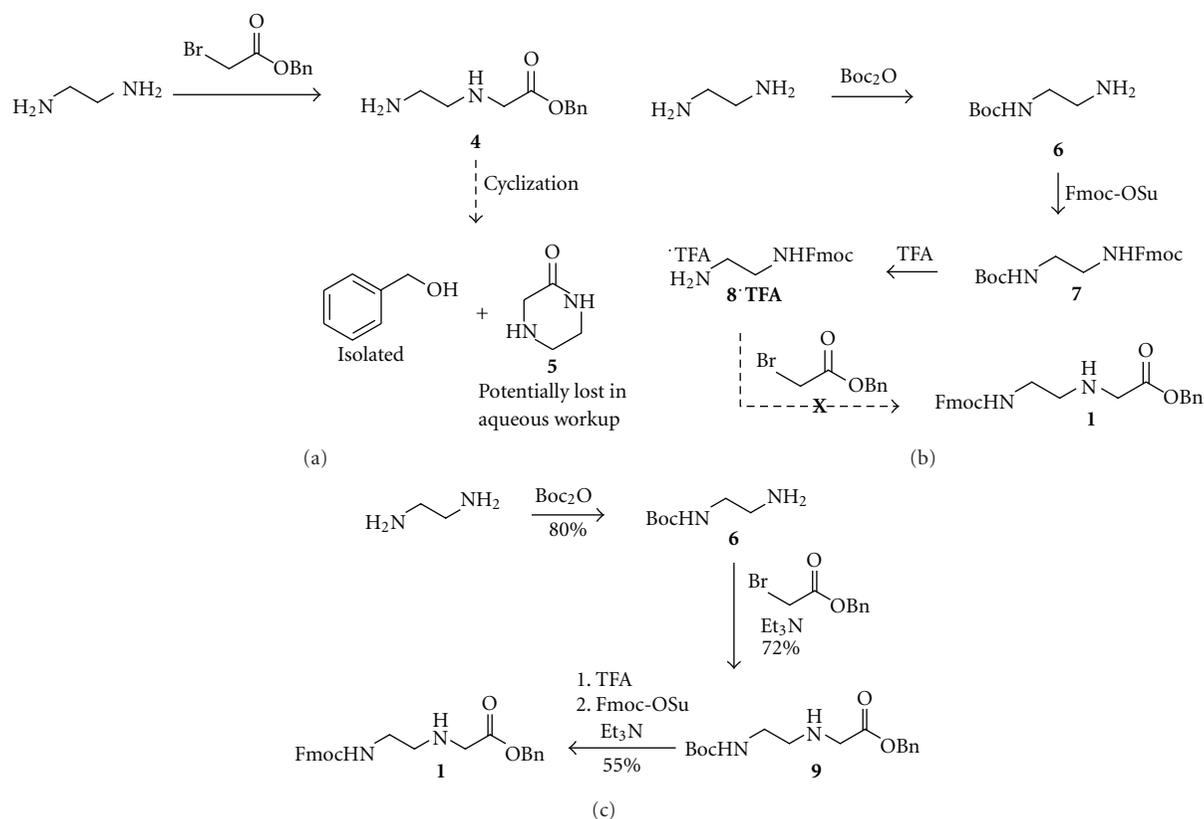


FIGURE 4: Synthetic route to Fmoc-AEG-OBn 1.

utilize this synthetic route with Fmoc in place of Boc failed to give product, likely due to the instability of synthetic intermediate **8**. Rather, synthesis of **1** can be initiated using a Boc protecting group, followed by a protecting group swap to provide the Fmoc-protected product. The first two steps of our synthetic route mirror those of the published synthesis for the Boc-protected monomer [19]. However, replacement of the Boc group with Fmoc poses a significant challenge, as this step proceeds through unstable intermediate **4**. We were able to perform this transformation by generating the free base of **4** at reduced temperature and in the presence of Fmoc-OSu, enabling Fmoc protection to effectively compete with cyclization, providing **1** in moderate yield.

In summary, we describe here a novel route to the PNA backbone Fmoc-AEG-OBn **1**. Using this route, we have rapidly synthesized 31 g of **1** using inexpensive starting materials and only minimal purification. The overall yield for our synthetic route is modest at 32%; however, the low cost of starting materials and ease of purification enable this synthesis to be tractable on a large scale. Having a convenient route to access **1** is anticipated to ease the synthesis of new Fmoc-protected PNA monomers, presumably furthering the exploration of PNA having unique modified nucleobases.

## Acknowledgment

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