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Highly Efficient Synthesis of DNA-Binding Hairpin Polyamides via the Use of a New Triphosgene Coupling Strategy

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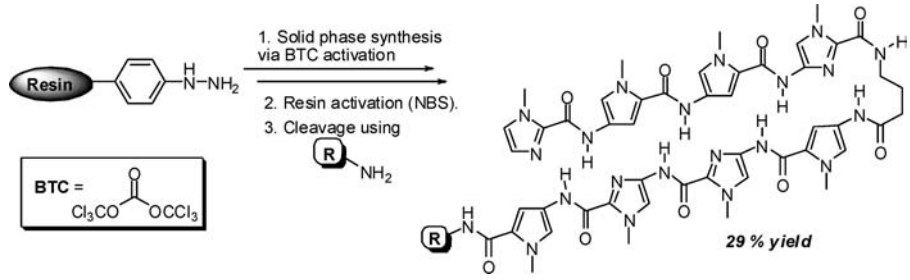
ABSTRACT



A facile and highly efficient solid phase synthesis method is reported for the preparation of hairpin DNA-binding polyamides using the cost-effective triphosgene (BTC) activating agent. Difficult polyamide sequences were prepared from *N*-methylimidazole (Im) and *N*-methylpyrrole (Py) building blocks with high stepwise yields (>98%) using Boc chemistry. The versatility of the triphosgene coupling approach was also demonstrated for the first time on aryl hydrazine resins to afford biomedically relevant tail-truncated polyamides in excellent isolated yields.

The ability to modulate the expression of any gene is one of the key goals in molecular medicine. Genetic-based methods such as RNA interference (RNAi) provide the means to silence specific gene expression; however, RNAi still poses challenges both as a research tool[1] and as a therapeutic strategy.[2,3] Pyrrole-Imidazole (Py−Im) polyamides[4−6] are cell-permeable[7−12] synthetic ligands which can be programmed to bind to predetermined sequences of DNA with nanomolar binding affinity and with specificities that equal or exceed natural eukaryotic transcriptional regulatory proteins.[4,6,13−15]

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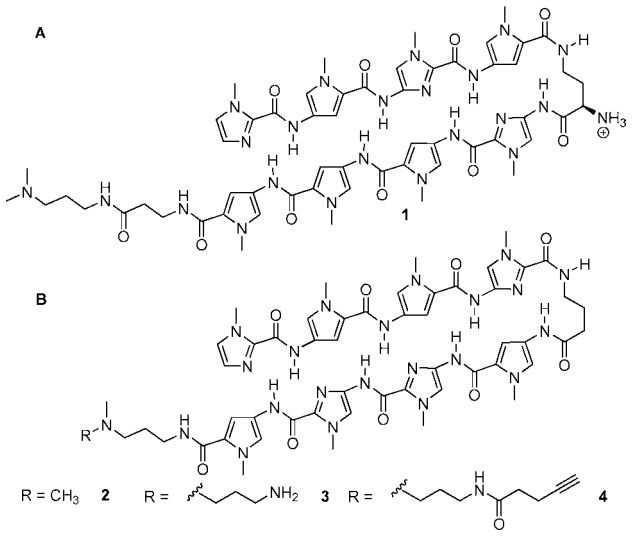


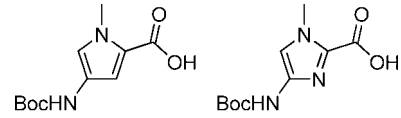
Figure 1. Hairpin polyamides **1**−**4** prepared in this study. (A) Polyamide **1** was prepared using the β-Ala-PAM resin. (B) Polyamides **2**−**4** were prepared using the aryl hydrazine resin.

DNA-binding polyamides provide an alternative small molecule strategy to the RNAi-based gene silencing approach by binding in the minor groove of DNA and blocking gene transcription.[4,6] Specificity is achieved according to a series of base pairing rules[4] where an antiparallel arrangement of Py−Py building blocks binds preferentially to A•T or T•A base pairs, whereas an Im−Py arrangement preferentially targets G•C over C•G, A•T, or T•A base pairs. Over 250 analogues of polyamides[16] have been prepared by both solid and solution phase synthesis methodologies over the past 20 years,[17,17−21] which has enabled their utilization in areas ranging from biology[4,6] through to nanotechnology,[22−24] yet despite their growing utility there is still no generally applicable method for the facile, efficient, and modular preparation of combinatorial collections of polyamides in high yield and purity, an essential requirement for their widespread utility. In this communication, we report such a method for the preparation of hairpin polyamides bearing Py, Im, and aliphatic amino acid building blocks which overcomes the low yields of existing methods.

During the course of our research program into the utilization of polyamide ligands, we were unable to obtain suitable quantities of polyamides such as **1**, using conventional methods (Figure 1).[18−20] These synthetic protocols utilize activated benzotriazole esters as a means to couple the hetereocyclic Boc/Fmoc-protected amino acids.[18,20−23] These methods proceed well for resin-bound aliphatic and Py amine couplings with 4-[(*tert*-butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylic acid [BocPyOH]; however, as a consequence of its lower inherent nucleophilicity, the coupling efficiencies of resin bound Im amines [4-[(*tert*-butoxycarbonyl)amino]-1-methylimidazole-2-carboxylic acid (BocImOH)] are rather poor.[20] As a consequence, the preparation of polyamides in high yield and purity, which contain Im building blocks located within the core of the polyamide such as in the case of polyamide **1**, has proved challenging.

The most common reagents used for the formation of activated benzotriazole esters for polyamide synthesis have been uronium salts (HBTU/HATU) or DCC/HOAt. Both reagent sets form the activated benzotriazole ester which can be either isolated or utilized directly in situ. Quantification of these coupling efficiencies by HPLC analysis revealed that the HATU coupling agent was only efficient in effecting Py−Py couplings under the typical HATU activation conditions, whereas the DCC/HOAt method is efficient in effecting amide bond formation for both Py−Py and Im−Im couplings (Table 1). When these reagent sets were utilized for the formation of the difficult Resin-Im-NH_2/BocPyOH coupling, both HATU and DCC/HOAt methods afforded only 5−8% of the coupled product after 20 min (Table 1).

Table 1. Comparative Coupling Yields of Polyamide Building Blocks[a]



amide bond[b]	HATU (%)[c]	DCC/HOAt (%)[d]	BTC (%)[e]
BocPyOH → H_2NIm-Resin	5	8	>98
BocPyOH → H_2NPy-Resin	95	95	>98
BocImOH → H_2NIm-Resin	12	>98	>98

[a] Yields were based on HPLC peak integration. [b] Resin = β-Ala PAM. [c] Activation in 1:1 DMF/NMP, Boc-monomer/HATU/DIEA, 3−5 min; coupling for 20 min. [d] Activation in 1:1 DMF/NMP, Boc-monomer/DCC/HOAt, 2 h, DIEA; coupling for 20 min. [e] Activation in THF, Boc-monomer/BTC/Collidine, 1 min, DIEA; coupling for 20 min.

Although the solution-based preparation of BocPy−ImOH dimers provides a potential route to circumvent this problematic Im−Py coupling,[18] we found their utility in polyamide synthesis compromised by their inherent insolubility in typical coupling solvents [e.g., DMF, NMP, DMSO], resulting in the formation of polyamide products in low yield

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and purity. In light of these limitations, we surmised that if one could increase the electrophilicity of the activated carboxylic acid, one could subsequently increase the efficiency of these problematic Im-NH_2/BocPyOH couplings.

In 2002, Jung et al. reported an Fmoc-based synthesis of cyclic peptides containing sterically hindered secondary amines on a solid support in which BTC [bis(trichloromethyl)carbonate or triphosgene] was used as the coupling agent.[25,26] The BTC reagent putatively forms acid chlorides in situ in high yield and has shown application as a highly efficient coupling agent for the Fmoc-mediated synthesis of aromatic oligoamides using automated peptide synthesizers.[27] It has also been shown that BTC performs better than TFFH[28] and $POCl_3$[29] in the solid phase synthesis of difficult sequences.

The in situ formation of the putative acid chloride also enables the utilization of acid-labile protecting groups such as *tert*-butyl esters, which are reported to have a limited shelf life in the presence of acid chlorides.[30] Although there have been no reports of its use in Boc-based solid phase synthesis, we reasoned that the increased electrophilicity of the acid chloride generated in situ by the reaction of BTC with the appropriate aromatic carboxylic acid would considerably enhance coupling efficiencies of polyamides and reduce reaction times. Indeed the BTC method was found to be far superior to current benzotriazole-based protocols in all couplings tested (Table 1). Activation times of both the BocPyOH and BocImOH only required 1 min when 0.33 equiv of BTC was used, compared to 2 h for DCC/HOAt-mediated activations.[20] Coupling times of 20 min were typically required for quantitative conversion to coupled products using BTC according to HPLC analysis, which enabled each deprotection−coupling−wash cycle to be effected well within 1 h. To the best of our knowledge, this is the first demonstration of a BTC-mediated solid phase synthesis protocol using Boc chemistry.

Encouraged by our model studies, we then investigated whether a BTC-based coupling methodology could be applied to the preparation of challenging hairpin polyamide sequences. We chose to investigate the synthesis of polyamide **1** which targets the DNA sequence 5′-WWGWGCW-3′ (where W is either A or T) with nanomolar affinity.[31] Compound **1** was prepared in only 0.1% reported yield using the β-Ala PAM resin via a traditional Boc-chemistry/benzotriazole-based ester protocol.[30] The low yield is most likely attributed to a challenging Resin-ImNH_2/BocPyOH coupling late in the synthesis sequence, which was confirmed in our laboratory using the conventional coupling methodology. This polyamide sequence also comprises other typical coupling sequences such as Py−Py, Py−Im, and Im−aliphatic couplings which enable us to ascertain the scope of the BTC coupling methodology. Using Boc-based chemistry and exclusively our BTC protocol, we were pleased to prepare polyamide **1** in 33% yield after CBz deprotection of the γ-turn motif; i.e. *this is a 330-fold increase in isolated yield for* ***1*** *using the BTC coupling protocol*.

Encouraged by this result, we then investigated the preparation of polyamides using solid supports that do not install an A•T/T•A encoding β-Ala tail on the C-termini of polyamides. This is an important requisite for biological applications as the presence of β-Ala tails is known to correlate with generally poor cellular uptake as well as enforcing an A•T/T•A encoding end sequence.[4,10,17] The Dervan group recognized this limitation and developed a polyamide synthetic protocol which utilized the Kaiser resin to delete the β-Ala tail.[32] As a consequence of their increased stability in strongly acidic and basic conditions coupled with a mild oxidative resin release protocol that enables the installation of various tail functionalities, aryl hydrazide resins offer potential advantages over Kaiser oxime resins currently used for truncated polyamide synthesis.[31] We evaluated the compatibility of the BTC coupling strategy with aryl hydrazide resins via the preparation of polyamide **2**.

Polyamide **2** was chosen as it comprises a diverse range of couplings not encountered in the synthesis of **1** such as an Im−Im coupling, in addition to the known challenging Resin-ImNH_2−BocPyOH coupling late in the synthesis. The solid phase synthesis of polyamide **2** proceeded smoothly using the BTC protocol. Polyamide release from the hydrazine resin was then achieved by oxidative activation of the resin in the presence of 2.0 equiv of NBS in pyridine for 10 min at room temperature. Cleavage of the polyamide from the activated diazene solid support with dimethylamino propylamine (Dp) at 40 °C for 5 h afforded the crude polyamide product **2** in 73% purity. After semipreparative HPLC purification, the isolated yield of **2** was 29% yield and with a purity of 98% (Figure 2). The versatility

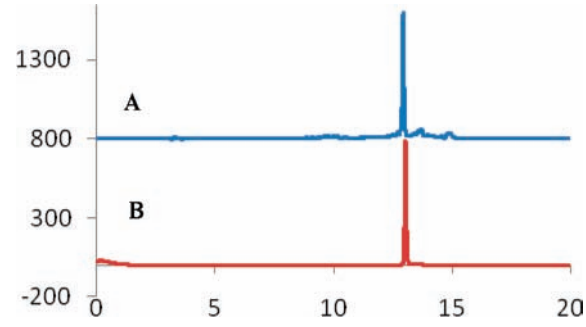

Figure 2. HPL chromatogram of (A) crude and (B) purified polyamide **2**. The retention time of **2** is 13.02 min.[33]

of the aryl hydrazine method was also demonstrated by the NBS-mediated oxidative release of polyamides outfitted with amino- (**3**) and alkyne-modified (**4**) tail structures in 22% and 12% yields, respectively.

According to the high purities of crude **3** and **4** by HPLC analysis, the lower isolated yield of these polyamides compared to polyamide **1** most likely correlates to their

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inherent resistance to precipitation in diethyl ether rather than suboptimal coupling. Polyamides **3** and **4** comprise useful conjugating amine (**3**) and alkyne (**4**) functionalities which highlight the flexibility of the aryl hydrazine resin protocol coupled with the high yielding BTC coupling methodology.

In summary, we have described a new and highly effective approach to solid phase polyamide synthesis using BTC as the coupling agent. The BTC protocol was found to be experimentally simple, fast, and highly- atom and step-efficient for their preparation of polyamides on both known (PAM resins) and novel (aryl hydrazine) solid supports regardless of their sequence. Efforts are now underway to investigate the broader application of the BTC coupling protocol for the preparation of polyamides comprising diverse building blocks both in solution and by automated solid phase methods.

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Supporting Information Available: Information concerning the comparative coupling efficiencies of HATU, DCC/HOAt, and BTC, the preparative procedures and MALDI-ToF data of polyamides **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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