Organic LETTERS

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Fully Automated Synthesis of DNA-Binding Py-Im Polyamides Using a Triphosgene Coupling Strategy

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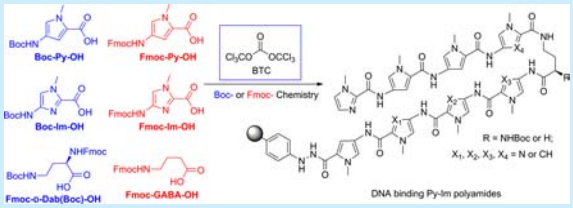
Supporting Information

ABSTRACT: The fully automated solid-phase synthetic strategy of hairpin pyrrole−imidazole polyamides is described. A key advance is the development of methodology for the application of triphosgene as a coupling agent in the automated synthesis of hairpin polyamides without racemization. This automated methodology is compatible with all the typical building blocks, enabling the facile synthesis of polyamide libraries in good yield (9−15%) and crude purity.



N-Methylpyrrole-*N*-methylimidazole (Py-Im) polyamides are cell-permeable small molecules capable of recognizing predetermined sequences of double-stranded DNA (dsDNA) by binding in the minor groove (Figure 1).[1] Sequence selectivity is achieved by precisely oriented pairs of aromatic Py and Im amino acids, such that an antiparallel Py/Py preferentially targets A·T or T·A base pairs; Im/Py targets a C·G base pair, while a Py/Im pair targets a C·G base pair.[2] As a consequence of their predictable binding and nanomolar binding affinity for target dsDNA sequences, Py-Im polymaides have considerable potential as exogenous agents that can regulate transcription by disrupting transcription factor−dsDNA binding interactions in vivo.[3] Additionally, the ability of Py-Im polyamides to discriminate between match and mismatched dsDNA sequences renders these molecules as excellent molecular probes for diagnostic applications[4] and in the design of DNA-based nanophotonic materials.[5]

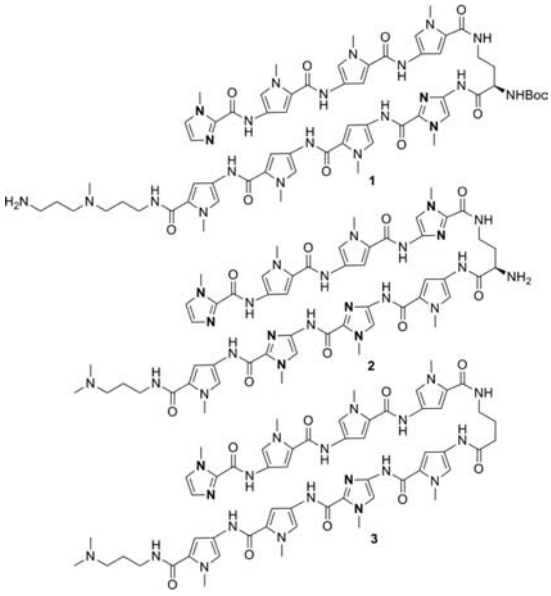


Figure 1. Structure of Py-Im polyamides.

Over the past two decades, much attention has been devoted to the total synthesis of Py-Im polyamides.[6] The syntheses of these molecules are challenging as a consequence of the low inherent nucleophilicity of the immobilized aromatic amine. The uses of conventional coupling reagents such as HATU or HOAt/DCC produce the corresponding coupled product in poor yield. This problem is exacerbated when Im building blocks are used.[6c,g] To circumvent this issue, a number of strategies have been employed such as the preparation of dimeric, trimeric, and tetrameric carboxylic acids in solution phase[6a,d,j] or using solution-phase total synthesis of the Py-Im polyamide core.[6e,f] Recently, microwave-assisted synthesis was utilized to facilitate the manual synthesis of Py-Im polyamides.[6h,i] Although this method could significantly enhance the coupling yields and reduce coupling times, the difficulty in conducting immobilized imidazole-amine couplings was noted even at elevated temperatures (60−80 °C). Machine-assisted syntheses have been employed by the Dervan and Sugiyama laboratories using Boc- and Fmoc- chemistry.[6a,b,j] In these cases, the use of Boc-Py-Im-OH/Fmoc-Py-Im-OH dimers was a necessary requirement to overcome the difficulties afore-

Received: November 21, 2014
Published: December 12, 2014

mentioned.[6a,b,i,j] We previously reported a facile and highly efficient solid-phase synthesis of hairpin DNA-binding polyamides using the cost-effective BTC [bis(trichloromethyl) carbonate or triphosgene] activating agent.[6g] This strategy was employed with great effect to produce Py-Im polyamides in 8−33% yield by manual solid-phase synthesis. At present, a flexible, modular, and facile protocol for the fully automated synthesis of Py-Im polyamides using monomeric building blocks much akin to solid-phase peptide synthesis is not available. In this paper, we report such a strategy to produce a palette of hairpin Py-Im polyamides using BTC as the condensing agent for all coupling steps.

BTC putatively forms highly electrophilic acid chlorides in the presence of carboxylic acids in situ, which undergo facile amidation of sterically hindered and electronically less reactive amines.[7] One limitation with current BTC-mediated coupling protocols is the strong exothermic nature of the reaction, resulting in the emission of CO_2 accompanied by large amount of collidine hydrochloride precipitate (Figure 2). In order to

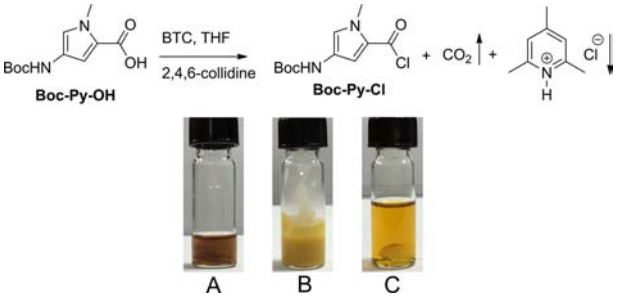


Figure 2. Activation of Boc-Py-OH with BTC: (A) solution of Boc-Py-OH and BTC in THF; (B) slurry generated by addition of 2,4,6-collidine to A; (C) clear solution obtained by addition of a 10% DIEA/DMF solution to B.

render BTC couplings amenable to automation, methods were therefore required to mitigate the exotherm and to prevent clogging of the lines of the automated synthesizer by the precipitate. Preactivation of the amino acids with BTC outside the peptide synthesizer was employed by Konig et al. in the machine-assisted synthesis of aromatic oligoamides as the solution could be freed of insoluble particles by syringe filtration.[8] However, the preactivated amino acid chloride has limited stability, which is not feasible for use in the automated synthesis of Py-Im polyamides. Therefore, development of a milder and solid-free activation method is crucial for the automation of the BTC strategy.

In a primary study, the reaction conditions of BTC-mediated in situ activation were investigated in order to meet the requirements of commercially available peptide synthesizers. As observed by Fuse et al. in the formation of acid chloride in a microflow system,[9] the combinations of BTC, organic bases (Et_3N, DIEA, 2,4,6-collidine, pyridine), and solvents (THF, 1,4-dioxane, DMF) generated precipitations. The only combination of BTC, DIEA, and DCM generating no precipitation resulted in low coupling yields as well as racemization of the chiral amino acids. Because Py-Im polyamides syntheses usually involved the coupling of a chiral amino acid as γ-turn unit, the retention of the chirality during the incorporation of this amino acid had to be addressed (Figure 3). Recently, we developed a reproducible, highly efficient, and racemization free protocol for triphosgene-

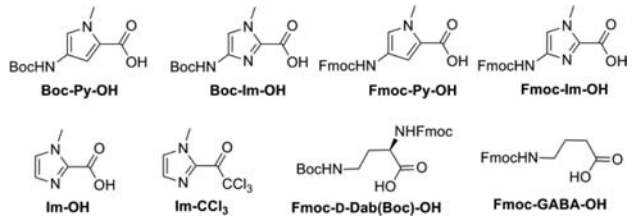


Figure 3. Building blocks for the synthesis of Py-Im polyamides.

mediated solid-phase coupling of proteinogenic amino acids.[10] The key features of the procedure include the following: (1) the preactivation of the carboxyl acid is carried out with collidine in THF, racemization-free conditions validated by the research of Jung and Falb;[7] (2) the resulting precipitation is converted to the soluble DIEA hydrochloride salt by the addition of a DIEA/DMF solution. According to this procedure, Boc-Py-OH (1.0 equiv) and BTC (0.33 equiv) were dissolved in dry THF (1 mL) (Figure 2, A), collidine was added slowly to form a slurry (Figure 2, B).[11] Upon addition of a 10% DIEA/DMF mixture (1.5 mL), the insoluble collidine hydrochloride disappeared very quickly and a clear solution was generated (Figure 2, C). To avoid the potential clogging of the peptide synthesizer pipelines, this special combination of bases for the activation of amino acids could be utilized in the automated synthesis of Py-Im polyamides.

We then investigated whether the optimized BTC activation procedure could be conducted on a CS336X peptide synthesizer with a computer-controlled operation system. The synthesizer was programmed in the standard hardware configuration for DIC/HOBt (or HBTU/DIEA) protocols. To evaluate the automated activation efficiency of the BTC method, Boc-Py-OH (1.0 equiv) and BTC (0.33 equiv) were loaded as solids into an amino acid reservoir, which was set up to be dissolved by dry THF (1.25 mL) and activated by 2,4,6-collidine/dry THF (15%, 1.25 mL), followed by DIEA/dry DMF (10%, 2.5 mL). During each step, nitrogen was bubbled into the amino acid reservoir to blend the reaction mixture and facilitate the emission of CO_2 and heat. As a model study for the HPLC analysis, excess 2-(4-methoxyphenyl)ethylamine was added to generate the corresponding amide. As shown in Figure 4, we found that 0.33 equiv of BTC, typically used in the activation step to release equal molar of phosgene, was not sufficient for full conversion of the acid chloride (trace a). We assume that this might be due to the emission of phosgene in

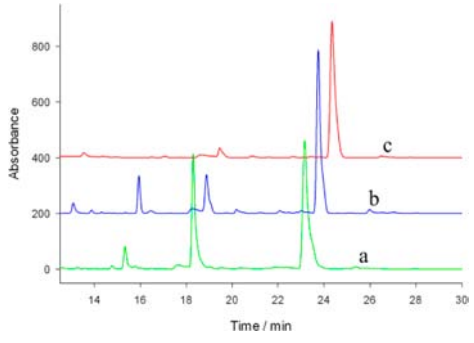


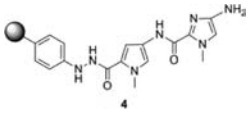
Figure 4. HPLC analysis of automated activation of Boc-Py-OH with BTC (285 nm): (a) 0.33 equiv of BTC; (b) 0.41 equiv of BTC; (c) 0.50 equiv of BTC. t_r = 18.29 (Boc-Py-OH), t_r = 23.16 (the resulting amide).

dx.doi.org/10.1021/ol503388a | Org. Lett. 2015, 17, 158−161

the nitrogen bubbling step. The activation efficiency was significantly improved when 0.50 equiv BTC was used (trace c). Similar results were achieved when Boc-Im-OH or Fmoc-D-Dab(Boc)-OH was used, except for Im-OH which has poor solubility in THF. Therefore, 0.50 equiv of BTC is an optimal ratio for the efficient activation of both aromatic amino acids and aliphatic amino acids.

In regard to the poor nucleophilicity of the resin-bound Im amines (resin-Im-NH_2), we anticipated that the successful synthesis of Py-Im polyamides on a peptide synthesizer relied on the efficient coupling of Boc-Py-OH, Boc-Im-OH, or Fmoc-D-Dab(Boc)-OH (γ-turn) to Im amines. An oligomer core (**4**) terminating with an Im nucleophile was used as a model study on the aryl hydrazine resin. We found that 40 min coupling time was optimal for complete coupling of the Boc-Im-OH monomer to amine **4**. To ensure complete coupling of Boc-Py-OH to **4**, two successive rounds of coupling were conducted (25 min each). The coupling of Fmoc-D-Dab(Boc)-OH to the Im nucleophile was surprisingly slow and required an extended coupling step (200 min) to achieve acceptable yields. In optimizing the γ-Im coupling conditions, we found that neither the reagent set DIC/HOAt/DIEA nor PyBOP/DIEA, which could be applied in the peptide synthesizer, were efficient in this amide bond formation. Further research revealed that the addition of one equivalent of HOAt to the BTC activated intermediate dramatically accelerated the rate of coupling with complete conversion observed by HPLC within 20 min. Using the combination of BTC/HOAt, the Im-Im coupling was also complete in 20 min (Table 1).

Table 1. Optimized Conditions for the Coupling of Monomers to Im Amine 4

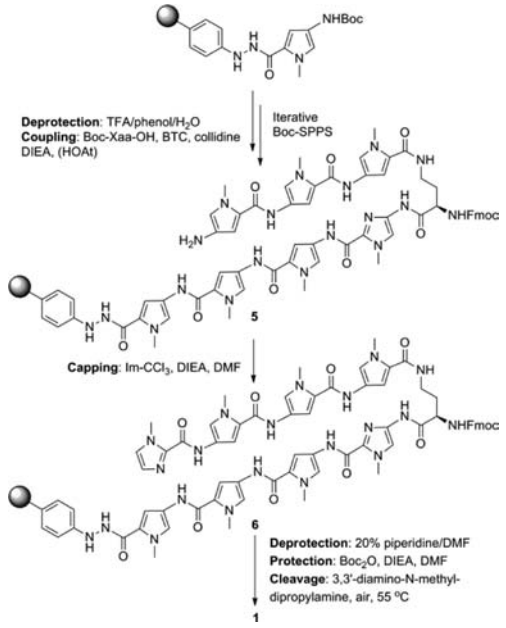


entry	monomer	monomer/BTC equiv (equiv)	HOAt[a] (equiv)	coupling time[b] (min)
1	Boc-Py-OH	4/2		50[c]
2	Boc-Im-OH	4/2		40
3	Fmoc-D-Dab(Boc)-OH	4/2		200
4	Fmoc-D-Dab(Boc)-OH	4/2	4	20
5	Boc-Im-OH	4/2	4	20

[a]After Fmoc-D-Dab(Boc)-OH was activated using a standard BTC procedure, a solution of HOAt/DMF/DIEA was added and the resulting mixture was transferred to the pretreated resin for the coupling reaction. [b]The optimal coupling times were determined by the chloranil test for free Im amines of **4**. [c]Two cycles, 25 min each.

Encouraged by this result, we then investigated the automated preparation of a biologically relevant polyamide **1**, targeting the 5′-WGWWCW-3′ (where W = A/T) subset of the consensus androgen and glucocorticoid response element.[12] The synthesis program on the CS336X peptide synthesizer was divided into three modules: (1) deprotection of the resin-bound polyamide, including removal of the Boc group with TFA/Phenol/H_2O (92.5/5/2.5) cocktail, DCM washes, DIEA/DMF wash, and DMF washes; (2) monomer coupling to the resin-bound polyamide, including BTC activation (5 min), delivery of the activated monomer, coupling (25 min), and DMF washes; (3) the terminal Im capping, including Im-CCl_3[6h] coupling and DMF washes. For a typical synthesis on resin (400 mg, 0.15 mmol/g), Boc-amino acid (4 equiv) and BTC (2 equiv) were activated just prior to the coupling step and transferred to the pretreated resin to generate the corresponding polyamide. The automated solid-phase synthesis of polyamide **1** involved eight coupling cycles plus a protecting group exchange step. The program proceeded smoothly using the optimized BTC protocol, requiring no special precautions beyond those used for general solid-phase peptide synthesis.[13] Polyamide **1** was then cleaved from the resin using 3,3′-diamino-*N*-methyldipropylamine after oxidation of the hydrazide by *N*-bromosuccinimide or atmospheric O_2 in the presence of Cu(II) salts to form a transient diazene species.[6g,14] However, better yields were obtained by direct aminolysis of resin-bound polyamides under air at 55 °C for 16 h.[6k] After semipreparative HPLC purification, polyamide **1** was obtained in 15% yield with a purity of 95% (Scheme 1). Using the

Scheme 1. Automated Synthesis of Py-Im Polyamide 1



modular program described above, polyamide **2** comprising a diverse range of couplings encountered in polyamide synthesis was attempted. These included Py-Py, Im-Py, Im-Im, γ-Py, and Im-γ couplings in addition to the known challenging Py-Im (Boc-Py-OH/Resin-Im-NH_2) coupling in the synthesis. After cleavage from the resin using dimethylamino-propylamine (Dp) and purification by semipreparative HPLC, polyamide **2**, which was one of the most difficult sequences in polyamides synthesis, was obtained in 9% yield.

To further confirm whether the stereochemical information encoded in the turn unit would survive several additional coupling steps in the automated synthesis, we synthesized the two enantiomers of polyamide **6**[6h] from Fmoc-L-Dab(Boc)-OH and Fmoc-D-Dab(Boc)-OH, respectively. Mosher amide derivatives 7-(***R,S***) and 7-(***S,S***) were formed by the reaction of both enantiomers of **6** with (*S*)-Mosher's acid chloride. 7-(***R,S***), 7-(***S,S***), and a mixture of them were subjected to HPLC analysis using a CHIRALPAK ID column to separate the two diastereomers. As shown in Figure 5, less than 1% epimer (estimated by integration) could be detected for each diastereomer (trace b and c), indicating the chirality of the γ-

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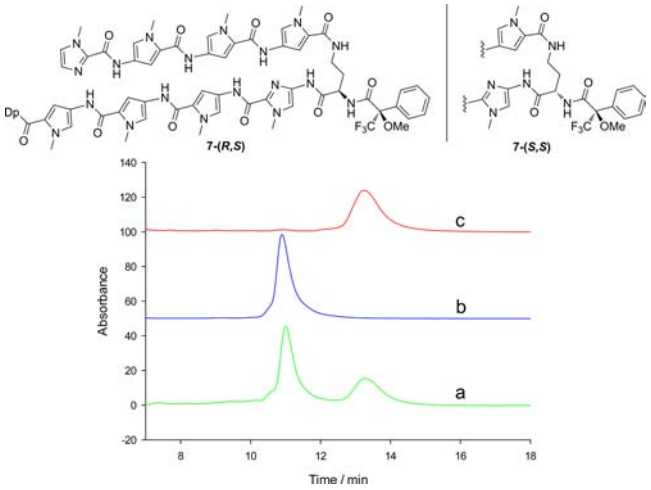

Figure 5. HPLC analysis of polyamide diastereomers (310 nm): (a) a mixture of 7-(*R*,*S*) and 7-(*S*,*S*); (b) 7-(*R*,*S*), t_r = 10.90 min; (c) 7-(*S*,*S*), t_r = 13.25 min.

turn unit was retained during the automated Py-Im polyamides synthesis.

Finally, a further application of the optimized BTC protocol was explored via the preparation of polyamide **3** using an Fmoc- strategy. The deprotection step was conducted by twice deblocking (10 min) with 20% piperidine/DMF to remove Fmoc group from the resin-bound polyamide. Fmoc-Py-OH, Fmoc-Im-OH, and Fmoc-GABA-OH were activated by the same BTC activation procedure. It should be noted that two coupling cycles were needed to drive the reaction of Fmoc-Py-OH/Resin-Im-NH_2 to completion (60 min each). All other couplings were carried out with a single-coupling cycle with extended reaction time (40 min). After capping with Im-CCl_3, cleavage from the resin and semipreparative HPLC purification, polyamide **3** was obtained in 12% yield.

In summary, an automated protocol to solid phase Py-Im polyamide synthesis using BTC as the activating agent has been developed. This protocol was facile and fully amenable for automation on a CS336X peptide synthesizer using monomeric *N*-protected carboxylic acids regardless of the required sequence or *N*-protection strategy. The automation of the BTC strategy represented a significant step forward for the multiple, parallel synthesis of structurally diverse peptoids for biomedical studies and also for the large-scale production of this family of molecules for further exploration as therapeutic scaffolds.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the "Hundred Talents Program" of the Chinese Academy of Sciences, Shenzhen Sciences & Technology Innovation Council (CXZZ20120831175831692, KQCX20130628112914285), and the National Natural Science Foundation of China (Grant No. 21432003 and 21402232). We are grateful for assistance at the mass facility at Peking University Shenzhen Graduate School.

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