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4-Methyltrityl-Protected Pyrrole and Imidazole Building Blocks for Solid Phase Synthesis of DNA-Binding Polyamides

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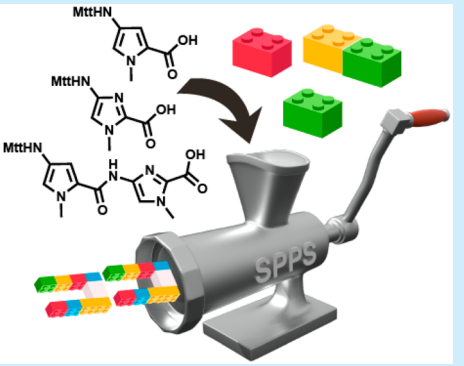
Supporting Information

ABSTRACT: DNA-binding polyamides are synthetic oligomers of pyrrole/imidazole units with high specificity and affinity for double-stranded DNA. To increase their synthetic diversity, we report a mild methodology based on 4-methyltrityl (Mtt) solid phase peptide synthesis (SPPS), whose building blocks are more accessible than the standard Fmoc and Boc SPPS ones. We demonstrate the robustness of the approach by preparing and studying a hairpin with all precursors. Importantly, our strategy is orthogonal and compatible with sensitive molecules and could be readily automated.



The discovery of the dimeric interaction of distamycin A in the DNA minor groove[1] paved Dervan's way for the development of programmable hairpin pyrrole-imidazole (Py-Im) polyamides capable of specifically targeting double-stranded (ds) DNA sequences.[2−7] During the past two decades, the vast repertoire of synthesized polyamides has demonstrated their implementation as a powerful tool to interfere with DNA-dependent processes and their potential in medicine as therapeutics. Thus, these modular probes have been involved in gene expression,[8−12] epigenetic control,[13−18] DNA replication,[19] DNA overwinding/underwinding,[20,21] and DNA cleavage.[22] Furthermore, polyamides have been conjugated to fluorophores for screenings and diagnoses.[23−27] Currently, more complex functionalization has been reported to improve or diversify the use of these molecules.[10,16,28,29]

Today, solid phase peptide synthesis (SPPS) is the method of choice for the preparation of Py-Im polyamides. To date, despite some innovations such as microwave-assisted synthesis, which shortened the time over which the yields were retained,[30] the strategies have been restricted to the use of *tert*-butyloxycarbonyl (Boc)[31−33] and fluorenylmethyloxycarbonyl (Fmoc)[32,34] protecting groups for the *N*-methyl pyrrole and *N*-methyl imidazole amino acids. However, the increasing complexity of the polyamide conjugates demands alternative approaches to expand the scope of the reactions as well as the functionalities used in the synthesis of hairpin batteries. For this purpose, we envisaged using the 4-methyltrityl (Mtt) group as the amino protecting group of the pyrrole/imidazole building blocks (Figure 1). Indeed, the Mtt protecting group not only allows deprotection procedures milder than those involved in Boc chemistry (2% TFA/DCM for Mtt vs 80% TFA/DCM/0.5 M PhSH for Boc) but also is orthogonal to the Fmoc-based synthesis as well as compatible with a broad palette of building reactions.[35] All common resins for Fmoc and Boc SPPS are compatible with our methodology except those sensitive to low acid percentages.

Herein, we describe the preparation of Mtt-protected pyrrole (**2**) and imidazole (**3**) monomers for Py-Im polyamide SPPS. Our straightforward synthesis results in yields that are higher than those used for the Fmoc and Boc analogues. In addition, we report the procedure to obtain the dimeric Mtt-protected Py-Im building block (**4**) to counter the synthetic difficulty from the electron-deficient nucleophilic imidazole amine.[36] The Mtt-protected γ-aminobutyric acid (**5**) as a turn unit of the hairpin was synthesized, too. We exemplified our approach with the synthesis of a Py-Im polyamide containing all of our new Mtt-protected compounds, ImPyPyIm-γ-PyImImPy-β-Dp (**1**), and compared the synthetic efficiency of this methodology to that of the "conventional" Fmoc SPPS due to the potential of both automation and full orthogonality.

The synthesis of the Mtt-protected precursors is shown in Scheme 1. The transformation of the corresponding commercially available NO_2-Py-OMe and NO_2-Im-OMe compounds (**10** and **12**, respectively) into the desired Mtt-protected Py and Im amino acids (**2** and **3**, respectively) was carried out in a two-step procedure. Thus, palladium-catalyzed reductive chemistry followed by the previously reported conditions used for the Mtt protection of ethylene diamine[35,37] yielded the in situ protection of the resulting amine. Subsequent ester hydrolysis afforded the Mtt-protected monomers with excellent yields of 84% for Mtt-Py-OH (**2**) and 68% for Mtt-Im-OH (**3**). Comparing the synthetic accessibility of our Mtt-protected Py and Im monomers to

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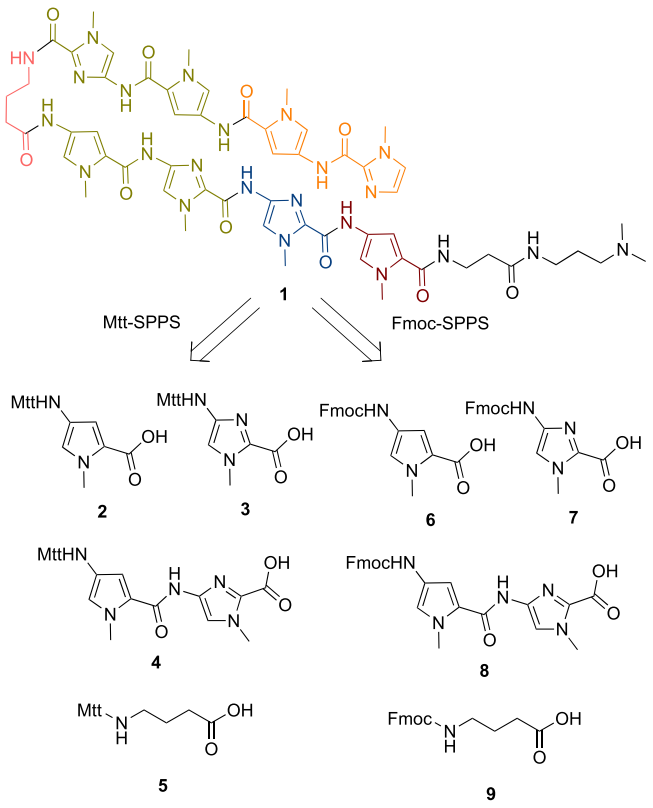
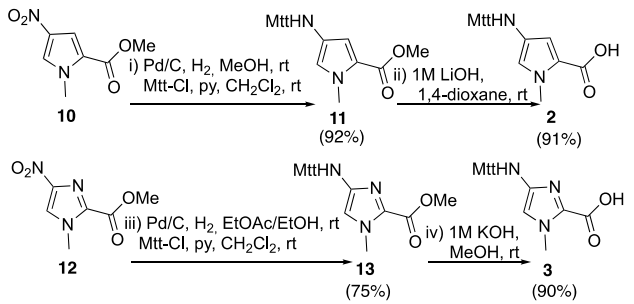


Figure 1. ImPyPyIm-γ-PyImImPy-β-Dp (**1**) prepared from Mtt SPPS and Fmoc SPPS. The colors highlight the different building blocks incorporated during the synthesis.

Scheme 1. Synthesis of the Mtt-Protected Pyrrole and Imidazole Monomers



that of the previously reported compounds, ours have yields higher than those of the Boc- and Fmoc-protected analogues[31,34] (64% for Boc-Py-OH and 42% for Boc-Im-OH; 58% for Fmoc-Py-OH and 30% for Fmoc-Im-OH). Mtt-Im-OH synthesis reduced by half the patented procedure for Fmoc-Im-OH preparation.[38] Furthermore, both the Mtt-PyIm-OH dimer (**4**) and the Mtt-γ-OH turn motif (**5**) were prepared following the same reaction sequence from the previously reported NO_2-PyIm-OMe[31] and the commercially available methyl γ-aminobutyrate hydrochloride (Scheme S4).

The different π-electron density of the N-heterocycles entails a distinct reactivity toward the Mtt deprotection by using TFA. As expected, in the case of the electron-rich pyrrole, the Mtt group was more acid-labile. Thus, in the presence of 0.1% TFA for 3 min, the removal of the protecting group of Mtt-Py-OMe was almost complete while the electron-deficient imidazole analogue required 2.0% TFA for 3 min for efficient cleavage. In Figure 2, a close-up of the ^{1}H NMR spectra of the Mtt-methyl group in its bound and free form is shown at different TFA

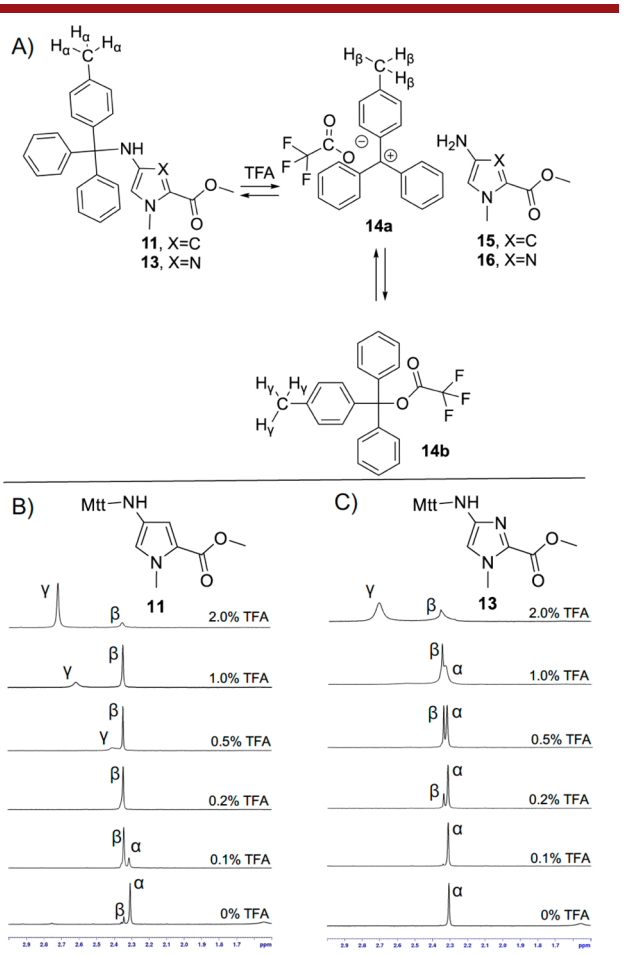


Figure 2. (A) Reaction scheme of Mtt deprotection. Close-ups of the aliphatic regions of the ^{1}H NMR spectra of (B) Mtt-Py-OMe (**11**) and (C) Mtt-Im-OMe (**13**) in $CDCl_3$ at 25 °C after addition of different amounts of TFA and incubation for 3 min. The signals of the methyl protons of the Mtt protecting group are marked in the scheme and in the corresponding NMR spectra as α, β, and γ. The small signal of the protons of the Mtt free (β) in panel B at 0% TFA is due to acid traces in $CDCl_3$.

concentrations in $CDCl_3$. At higher TFA concentrations, the signals are low-field-shifted, which is in agreement with our observations of the ^{1}H NMR spectra of Mtt-Cl under the same conditions (Figure S68) as well as reported trityl derivatives.[39,40] The two different peaks for the free Mtt-methyl group can be explained by an equilibrium with TFA (Figure 2A), which was described previously.[41,42]

To test the applicability of our Mtt-protected compounds for Py-Im SPPS, we next synthesized a hairpin polyamide [ImPyPyIm-γ-PyImImPy-β-Dp (**1**)] containing all of them (Figure 1). For comparison, we prepared the same sequence using the Fmoc-protected compounds, too. Our strategy offers a mild acid–based alternative to the current protocols for the cleavage of the temporary protecting groups, which must be particularly interesting for basic-sensitive conjugates and those susceptible to either strong acidic conditions or nucleophilic attack. Along these lines, the conventional *N,N*-dimethylaminopropylamine (Dp) aminolysis used as the final cleavage[34] (68 equiv, 55 °C, 18 h) was exchange for a controlled amide

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bond formation in solution once the acid hairpin polyamides were obtained from both methodologies. The implementation of Mtt SPPS was straightforward using the same coupling and capping conditions as those described in Fmoc SPPS[9] except the deprotection step. Thus, 2% TFA in CH_2Cl_2 for 20 min served as a base-free alternative to release the reactive amine, instead of the 20% piperidine in DMF used in Fmoc SPPS. The aromatic amine and the color of the pyrrole and imidazole prevent the use of both the ninhydrin[43] and the TNBS test[44] to monitoring the progress of the coupling reactions. However, stepwise cleavage of a sample of resin and reverse phase (RP) HPLC analysis verified high yields until the incorporation of the turn amino acid (>95%) (Figure S82). We observed that, unlike the Fmoc-γ-OH, the coupling of the Mtt-γ-OH did not occur under the standard conditions: 4 equiv of Mtt-γ-OH, 4 equiv of HATU, and 12 equiv of DIPEA. NMR experiments revealed that the electron-rich protecting group Mtt increased the nucleophilicity of the secondary amine, yielding the corresponding deactivated Mtt-butyrolactam (Scheme S6 and Figures S76−S81) in the presence of HATU and DIPEA (pK_a = 10.75).[45] Indeed, the acetylated polyamide starting material was the only product detected in the HPLC chromatogram (Figure S83a). However, the use of weaker bases such as NMM (pK_a = 7.38)[46] and pyridine (pK_a = 5.21)[46] decreased the level of intramolecular deactivation and allowed the successful coupling, yielding the desired product with 10% and 65% yields, respectively (Table 1, entries B and C, respectively). Coupling with PyBOP increased the amount of desired product to 75% (Table 1, entry G) and corroborated the general tendency observed with the different bases. Importantly, due to the Mtt orthogonality to Fmoc synthesis, the co-synthesis with Fmoc-γ-OH was possible, too.

Table 1. Tested Conditions for Mtt-γ-OH (5) Incorporation[a]

	coupling reagent	base	yield (%)
A	4 equiv of HATU	12 equiv of DIPEA	<5
B	4 equiv of HATU	12 equiv of NMM	10
C	4 equiv of HATU	12 equiv of pyridine	65
D	4 equiv of HATU	8 equiv of pyridine	63
E	4 equiv of PyBOP	12 equiv of NMM	42
F	4 equiv of PyBOP	8 equiv of NMM	52
G	4 equiv of PyBOP	8 equiv of pyridine	75

[a]For the reaction, 4 equiv of Mtt-γ-OH (0.3 M in NMP), 4 equiv of coupling reagent, and 8 or 12 equiv of base were preincubated for 3 min and coupled for 1 h. After capping, cleavage, and HPLC analysis, yields were calculated by integration of the peak areas with an intensity of >10% related to the highest peak in the chromatogram.

After the incorporation of the turn motif, the rest of the couplings proceeded as expected for both protocols. Once all the synthesis steps were completed, the Py-Im polyamide was cleaved from the resin, which resulted in a single peak in the RP-HPLC chromatogram (Figures S61 and S62). The retention time as well as HRMS-ESI unequivocally verified the same identity of the compounds obtained by the different methodologies, ImPyPyIm-γ-PyImImPy-β-COOH (**35**), and the yields were comparable, too. Dp was placed in solution at the C-terminus of the polyamide to increase the affinity (Scheme S5).[33]

Finally, we tested the affinity and specificity of ImPyPyIm-γ-PyImImPy-β-Dp (**1**) by thermal denaturation analysis. These spectroscopic measurements were performed on 12-mer dsDNA duplexes with the sequences 5′-GGTAGCCGTACC-3′ and 5′-GGTAGCTGTACC-3′, which contain the target and a single mismatch binding site, respectively. Satisfactorily, our Py-Im polyamide (**1**) has a higher thermal stabilization value in the presence of the target dsDNA than the mismatched one (ΔT_m of 6.6 °C versus ΔT_m of 1 °C) (Figure 3).

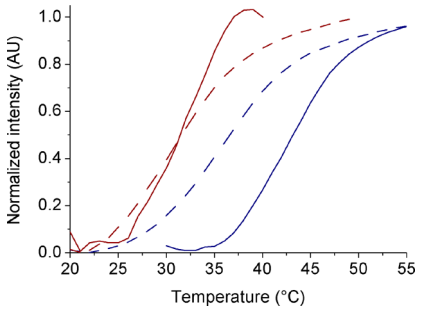

Figure 3. Melting curves of a 1 μM duplex dsDNA solution in 10 mM NaH_2PO_4 (pH 7.4): 5′-GGTAGCCGTACC-3′ (blue) or 5′-GGTAGCTGTACC-3′ (red) in the presence (straight) and absence (dotted) of 1.2 μM ImPyPyIm-γ-PyImImPy-β-Dp (**1**).

In conclusion, this work represents the first report of Mtt-protected pyrrole and imidazole building blocks for DNA-binding polyamides. Our monomers are accessible with yields higher than those use in the conventional Boc and Fmoc SPPS. We demonstrate the utility of this methodology by the synthesis and study of a DNA-binding hairpin bearing all types of Mtt-protected compounds needed. This approach is both orthogonal to Fmoc SPPS and compatible with basic-sensitive functionalities and those susceptible to either strong acidic conditions or nucleophilic attack. Therefore, we foresee that our Mtt SPPS will increase the synthetic diversity in Py-Im polyamide chemistry affording novel bifunctional polyamide conjugates. In addition, it could be readily adapted to automated synthesis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.9b04288.

Experimental procedures, spectroscopic and analytical data of all new compounds, and additional experiments (PDF)

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Notes

The authors declare no competing financial interest.

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