Synthesis of DNA-recognizing polyamides containing N-methylpyrrole and N-methylimidazole

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Dedicated with best wishes to Professor Zhi-Tang Huang on the occasion of his 75th birthday
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Abstract
Two novel polyamides containing N-methylpyrrole (Py) and N-methylimidazole (Im) were designed and synthesized by a trichloroacetyl condensation reaction and DCC/HOBT coupling reaction without amino protection and deprotection in solution.

Keywords: Polyamide, N-methylpyrrole, N-methylimidazole

Introduction

The design of synthetic ligands that read the information in the DNA double helix has been a central goal at the interface of chemistry and biology. Syntheses of DNA-binding molecules, such as triplex-forming oligonucleotides, peptide nucleic acids, oligosaccharide and oligopeptides have been exploited. Recently, polyamides containing N-methylpyrrole and N-methylimidazole amino acids have attracted considerable attention on the part of synthetic and biological chemists because they recognize and bind in the minor groove of predetermined DNA sequences with high specificity and affinity. Since these polyamides can permeate living cell membranes, they have the potential to control specific gene expression. Here we report one simple procedure for the synthesis of two novel polyamides (PyPyγImImImβDp(1) and PyPyβImImImβDp(2), where Py = N-methylpyrrole, Im= N-methylimidazole, γ = γ-aminobutyric acid, β = β-alanine, Dp=N, N-dimethylpropyldiamine.)
Results and Discussion

The polyamide 1 and 2 are made up of two subchains, NO$_2$ImImImβCOOEt and PyPyPyγCOOH or PyPyPyβCOOH, by retrosynthetic analysis. In the synthesis of NO$_2$ImImImβCOOEt (6), we choose a trichloroacetyl condensation reaction and take the step-by-step synthetic strategy to introduce imidazole ring from C-terminus sequentially. For the trichloroacetyl condensation reaction, the key starting material is 4-nitro-N-methyl-2-trichloroacetylimidazole (3). This compound was easily prepared from commercially available N-methylimidazole through trichloroacetylation and nitration. NO$_2$ImImImβCOOEt (6) was obtained smoothly by using the trichloroacetyl condensation reaction with good yield without resorting to column chromatography.

Scheme 1. Synthesis of compound 6 by the trichloroacetyl condensation reaction

The main difficulty in the synthesis of PyPyPyγCOOEt and PyPyPyβCOOEt is that the trichloroacetyl condensation reaction between N-methyl-2-trichloroacetylpyrrole and the amine component containing N-methylpyrrole are not satisfactory even in the presence of a catalyst. In this case, the DCC/HOBT coupling reaction is an alternative method for the synthesis of compounds (8, 9). PyPyPyγCOOEt (8) and PyPyPyβCOOEt (9) were synthesized by the coupling of PyPyCOOH (7) with NH$_2$PyβCOOEt and NH$_2$PyγCOOEt using the DCC/HOBt coupling reaction in higher yield, respectively.
Scheme 2. Synthesis of compound 8 and 9

Scheme 3. Synthetic routes of compound 1 and 2

In synthesis of the compound 1, first the reduction of the nitro compound 6 to an amine 6a and the saponification of the ester 8 to carboxylic acid 8a for the construction of polyamide 10 by the DCC/HOBt coupling reaction. In the DCC/HOBt coupling reaction, the carboxyl component must be transformed into an active ester; this is a key step in synthesis of 10. Then 10 was saponified with NaOH and neutralized with hydrochloric acid, the acid 12 was isolated from the solvent in good yield. It was worth noting that the control of pH after saponification was very
important for the yield. In the last, ⁴N, ⁴N-dimethylpropyldiamine was introduced to the polyamide ¹², and ¹ was produced in a good yield. The synthetic procedure similar to that for ¹ was followed for the preparation of ².

Polyamide ¹ and ² containing ⁴N-methylpyrrole and ⁴N-methylimidazole have been synthesized by the trichloroacetyl condensation reaction and DCC/HOBT coupling reaction without amino protection and deportation. Compared with solid-phase synthesis for polyamides, this method will save reactants. Because the acid was converted to the active ester first and the use of an excess of acid was not necessary, so the equimolar amount of acid and amine component was employed in the coupling reaction using DCC/HOBT. This simple procedure will be used in the synthesis of Py/Im hairpin polyamides by connection of two subchains in a single step.

**Experimental Section**

**General Procedures.** Thin-layer chromatography (TLC) was performed on a silica gel 60 GF254 (240-400 mesh) plates and column chromatography was performed with silica gel 60G. Visualization was accomplished by UV light. Infrared spectra were recorded in the FT-IR mode. ¹H NMR spectra were recorded by a Varian HY 200 NMR spectrometer. Fast-atom bombardment (FAB) mass spectra were obtained using thioglycerol as a matrix with a VG-ZAB-HS mass spectrometer. ⁴N, ⁴N-dimethylformamide, ethanol, methanol, ethyl acetate, and tetrahydrofuran were dried and purified according to standard procedures.

**PyPyPyγImImImβCO₂Et (10).** To PyPyPyγCO₂H (8a) (0.80 g, 1.76mmol) in 7 mL of DMF was added DCC (0.36 g, 1.76 mmol), followed by HOBT (0.24 g, 1.76 mmol). The reaction solution was stirred overnight. Separately, to a solution of NO₂ImImImβCO₂Et (6) (0.91 g, 1.76 mmol) in 12 mL of DMF was added Pd/C catalyst (10%,100 mg), and the mixture was stirred under a slight positive pressure of H₂ overnight. The catalyst was removed by filtration through Celite, and the filtrate was directed into the active ester solution and the mixture was stirred for 20 h. Then filtration, the filtrate was concentrated in vacuo. After purification by column chromatography with a mixture of methanol and chloroform as eluent (gradient eluate), 1.37g of compound ¹⁰ was obtained (84% yield). IR (KBr) 3446, 1652, 1541, 1473, 1436, 1418, 1313, 1253, 1190, 1114, 1019, 733, 626 cm⁻¹. ¹H NMR(200MHz, DMSO-d₆, δppm): 1.18 (t, J=7.2Hz, 3H), 1.81 (t, J=6.4Hz, 2H), 2.38(t, J= 7.2Hz, 2H), 2.57 (br, 2H), 3.24(m, 2H), 3.45(m, 2H), 3.81(s, 3H), 3.84(s, 3H), 3.88(s, 3H), 3.96(s, 3H), 4.01(s, 3H), 4.05(s, 3H), 6.06(m, 1H), 6.91(m, 3H), 7.04(m, 1H), 7.19 (m, 1H), 7.24(m, 1H), 7.54 (s, 2H), 7.65(s, 1H), 8.06(m, 1H), 8.29(m, 1H), 8.32(s, 1H), 9.74 (m, 1H), 9.84(s, 1H), 9.91 (s, 1H), 10.44(s, 1H). FAB-MS calcd for C₄₂H₅₁N₁₆O₉ (M+H⁺) m/z 923, found m/z 923.

**PyPyPyβImImImβCO₂Et (11).** A synthetic procedure similar to that for PyPyPyγImImImβCO₂Et (10)
was followed for the preparation of PyPyPyβImImImβCO₂Et (11) (yield 79%). IR (KBr) 3446, 1647, 1541, 1473, 1312, 1253, 1192, 1114, 740, 626 cm⁻¹. ¹H NMR (200MHz, DMSO-d₆, δ ppm): 1.18 (t, J=7.2Hz, 3H), 2.60 (m, 4H), 3.45(m, 4H), 3.82(s, 3H), 3.85(s, 3H), 3.89(s, 3H), 3.96(s, 3H), 3.98(s, 3H), 4.01(s, 3H), 4.09(q, J=7.2Hz, 2H), 6.06(m, 1H), 6.91(m, 3H), 7.06(m, 1H), 7.24(m, 2H), 7.54 (d, 2H), 7.65(s, 1H), 8.09(m, 2H), 8.29(m, 2H), 9.75 (m,1H), 9.84(s, 1H), 9.91 (s, 1H), 10.48(s, 1H). FAB-MS calcd for C₄₁H₄₉N₁₆O₉ (M+H⁺) m/z 909, found m/z 909.

**PyPyPyγImImImβDp (1).** To a solution of PyPyPyγImImImβCOOH (12) (200 mg, 0.22 mmol) in 3 mL of DMF was added HOBT (60 mg, 0.44 mmol), followed by DCC (90 mg, 0.44 mmol). The reaction solution was stirred overnight. N,N-dimethylpropyldiamine (23 µL) was added to the reaction solution, and the stirring was continued for another 12 h. DCU was removed by filtration, and the filtrate was concentrated in vacuo. Column chromatography of the residue (CH₃OH/CHCl₃=2:1 and CH₃OH/CHCl₃=1:2) afforded 150 mg of the polyamide 1 (69% yield). IR (KBr) 3446, 1647, 1558, 1538, 1311, 626 cm⁻¹. ¹H NMR(200MHz, DMSO-d₆, δ ppm):1.51 ( m, 2H), 1.81 (m, 2H), 2.09(m, 8H), 2.19 (m, 2H), 2.37(m, 4H), 3.06(m, 2H), 3.22(m, 2H), 3.81(s, 3H), 3.85(s, 3H), 3.88(s, 3H), 3.96(s, 3H), 3.98(s, 3H), 4.01(s, 3H), 6.06(m, 1H), 6.91(m, 4H), 7.04(m, 1H), 7.19 (m, 2H), 7.53(s, 2H), 7.64 (s, 1H), 8.06(m, 3H), 8.31(s, 1H), 9.90(m, 2H), 10.45 (s,1H). FAB-MS calcd for C₄₅H₅₉N₁₈O₈ (M+H⁺) m/z 979, found m/z 979.

**PyPyPyβImImImβDp(2).** A synthetic procedure similar to that for PyPyPyγImImImβDp (1) was followed for the preparation of PyPyPyβImImImβDp(2) (yield 59%). IR (KBr) 3446, 1647, 1558, 1541, 1473, 1312, 627 cm⁻¹. ¹H NMR(200MHz, DMSO-d₆, δ ppm): 1.51 (m, 2H), 2.08(s, 6H), 2.19 (m, 2H), 2.38(m, 2H), 2.56 (m, 2H), 3.06 (m, 4H), 3.43(m, 2H), 3.82(s, 3H), 3.84(s, 3H), 3.88(s, 3H), 3.96(s, 3H), 3.98(s, 3H), 4.01(s, 3H), 6.06(m, 1H), 6.86(m, 1H), 6.95(m, 3H), 7.03(m, 1H), 7.23 (m, 2H), 7.54(m, 2H), 7.65 (s, 1H), 7.93(m, 1H), 8.09(m, 1H), 8.21(m, 1H), 8.32(s, 1H), 9.84(s, 1H), 9.91(s, 1H), 10.45 (s,1H). FAB-MS calcd for C₄₄H₅₇N₁₈O₈(M+H⁺) m/z 965, found m/z 965.

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