Synthesis of polyamide containing pyrrole and imidazole amino acids

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A polyamide containing *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) amino acids was synthesized by coupling two of the four-ring oligopeptide chains using DCC/HOBT as promoting additives. The structure of the polyamide was confirmed by IR, NMR and MS spectra.

Keywords DNA recognizing molecule, polyamides, synthesis

Introduction

Polyamides containing aromatic amino acids, *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) have attracted considerable attention recently because they can recognize and bind to predetermined sequence in the minor groove of DNA with high specificity, and have been shown to permeate living cell[1-3] and inhibit specific gene expression.[4] Dervan *et al*. has established that the sequence-specificity of the binding depends on the side-by-side amino acid pairings in the minor groove of DNA.[5-7] Antiparallel pairing of Py/Im distinguishes C·G from G·C, A·T and T·A base pairs; while an Im/Py pair targets a G·C base pair. Py/Py recognizes both A·T and T·A base pairs.[5-8]

In this article, the binding sequence (5′-TCCT-3′) of a natural antitumor antibiotic-Calicheamicin[9,10] was chosen as the target site, and a new polyamide according to the pairing rule established by Dervan *et al*. has been designed as shown in Fig. 1.

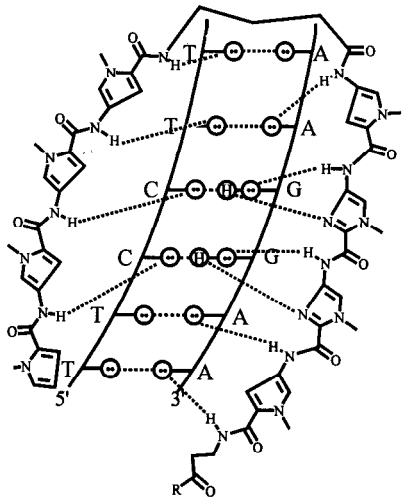

Fig. 1 Binding model of the polyamide designed with 5′-TCCT-3′.

The rationale of the design of this polyamide through the covalent coupling of two four-ring oligopeptides *via* γ-aminobutyric acid to form the eight-ring polyamide is as follows: (1) the γ-aminobutyric acid (γ) facilitates the formation of γ-turn; (2) the β-alanine (β) increases polyamide-DNA binding affinity.

Results and discussion

The polyamide in Fig. 1 was made up of two an-

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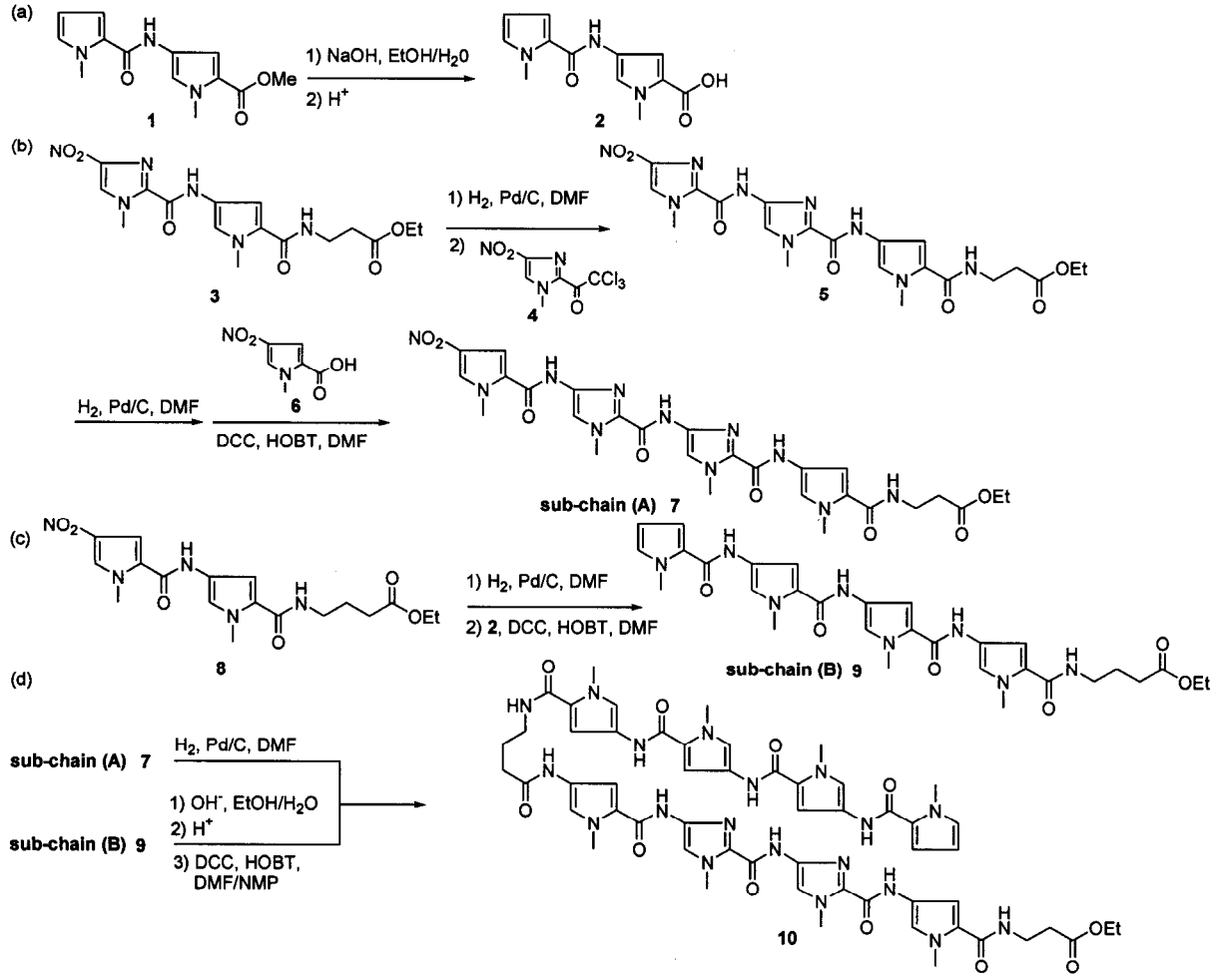
tiparallel sub-chains. According to a retrosynthetic analysis, disconnecting at the joining point of the two sub-chains will dramatically reduce the steps for the total synthesis of the polyamide. In the synthesis of this polyamide, key reactants[11,12] were $NO_2PyCOOH$, $NO_2ImCOCCl_3$, PyPyCOOMe, $NO_2ImPy\beta OEt$, NO_2Py-$Py\gamma OEt$, and the synthetic route is shown in Scheme 1.

Scheme 1



The transformation of the ester to carboxylic acid was necessary for the coupling reaction using DCC/HOBT as promoting additives. In the mixed solution of basic alcohol and water, the ester was converted into a carboxylic acid salt. After saponification, a solution of 6 mol/L HCl was used to neutralize the basic solution, and then acid was isolated from the mixed solution in excellent yield.

There were two crucial elements in the synthesis of four-ring oligopeptide and the polyamide by the coupling reaction using DCC/HOBT. One was the quantitative reduction of the nitro group to an amino group, and another was the preparation of an active ester of the carboxyl component.

In the reduction reaction, the termination of hydrogenation in time is very important. Although the hydrogenation was fulfilled almost quantitatively in most cases, the amino products were rather unstable. Under the reductive hydrogen atmosphere, the amino compounds thus obtained gradually decomposed on long standing at room temperature. To achieve the optimum result, TLC was employed to monitor the progress of the hydrogena-

tion. When the hydrogenation was finished, the reaction was terminated. The temperature of the reaction was also critical. Elevated temperature was needed in some cases. For example, the hydrogenation of **5** containing *N*-methylimidazole unit at 55℃ dramatically reduced the reaction time from days at ambient temperature to only several hours.

In the preparation of the active ester of PyPyPyPyγCOOH, large amount of *N*, *N*-dimethylformamide (DMF) was needed to dissolve this acid of low solubility. The low concentration of the reactant resulted in small amount of active ester. Consequently, only 16% yield of PyPyPyPyγPyImImPyβOEt (**10**) from the coupling reaction was achieved. In contrast, the acid was quite soluble in a mixed solvent of DMF and *N*-methylpyrrolidone (NMP) (6∶1) and the yield of the coupling reaction was found to increase to 32%. Further experiment indicated that adding excess of DCC facilitated the formation of the active ester. When three equivalents of DCC were added to the reaction solution, a significantly higher yield of **10** (74%) was achieved.

The polyamide ester **10** is a very useful intermediate, which can be conjugated with other functional groups (such as enediyne antibiotics, photochemical DNA cleavers, DNA alkylating molecules, *etc*.) to form sequence-specific DNA-cleaving agents for the design of new gene-selective drugs.

The significant feature of this procedure was that there was no need of protecting and deprotecting amino group in the synthetic route and thus dramatically reduced the number of the synthetic steps. This work provided a very important strategy for the design and synthesis of new DNA recognizing molecules-polyamides.

Experimental

IR spectra were obtained on a Bruker VECTOR22 FT-IR spectrometer. NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker ARX 400 and a Varian 200 nuclear magnetic resonance spectrometers. High-resolution mass spectra (HRMS) were recorded on a Bruker APEXII-FTICR mass spectrometer.

1-Methyl-4-(1-methylpyrrole-2-carboxamido)pyrrole-2-carboxylic acid (PyPyCOOH) (**2**)

To a solution of PyPyCOOEt (2.75 g, 10.0 mmol) in 70 mL of ethanol was added NaOH (1.20 g in 50 mL of water), and the solution was stirred at room temperature for 12 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. A solution of 6 mol/L HCl was used to adjust the acidity of the aqueous solution to pH = 1. The acid was collected by filtration, and then washed with water and dried under IR lamp to offer 2.34 g of **2** (95% yield). ν_{max}(film): 3463, 3326, 2952, 2624, 1665, 1581, 1459, 1418, 1320, 1289, 1260, 1199, 1123, 1061, 728, 657, 607 cm^{-1}. δ_H(DMSO-d_6): 14.13(b, 1H), 11.99(s, 1H), 9.60(s, 1H), 9.12(s, 1H), 9.07(s, 1H), 9.00(s, 1H), 8.23(s, 1H), 4.04(s, 3H), 4.00(s, 3H). HRMS *m/z*: 248.1036[M + H]$^+$ (248.1035 calcd for $C_{12}H_{14}N_3O_3$).

Ethyl β-[1-methyl-4-[1-methyl-4-(1-methyl-4-nitroimidazole-2-carboxamido)imidazole-2-carboxamido]pyrrole-2-carboxamido]alaninate (NO_2ImImPyβOEt) (**5**)

To a solution of NO_2ImPyβOEt (3.70 g, 9.4 mmol) in 25 mL of DMF was added 0.70 g of Pd/C catalyst (10%), and the mixture was stirred under a slight positive pressure of H_2 at 55℃ for 12 h. The catalyst was removed by filtration through Celite. To the filtrate was added 1-methyl-4-nitro-2-trichloroacetylimidazole (2.58 g, 9.5 mmol) immediately. The reaction mixture was stirred for 12 h. After filtration, yellow solid was collected, washed with ethanol and ethyl acetate, and dried to give 3.23 g of **5** in 66.5% yield. ν_{max}(film): 3373, 3250, 3125, 2970, 1727, 1642, 1545, 1478, 1436, 1378, 1309, 1262, 1179, 1119, 1050, 749, 623 cm^{-1}. δ_H($CDCl_3$, 200 MHz): 10.34(s, 1H), 10.17(s, 1H), 8.66(s, 1H), 8.10(t, *J* = 5.4 Hz, 1H), 7.58(s, 1H), 7.24(s, 1H), 6.97(s, 1H), 4.08(q, *J* = 7.2 Hz, 2H), 4.07(s, 3H), 4.02(s, 3H), 3.80(s, 3H), 3.41(q, *J* = 6.4 Hz, 2H), 2.54(t, *J* = 7.2 Hz, 2H), 1.20(t, *J* = 7.2 Hz, 3H). HRMS *m/z*: 516.1954 [M + H]$^+$ (516.1955 calcd for $C_{21}H_{26}N_9O_7$).

Ethyl β-[1-methyl-4-[1-methyl-4-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-imidazole-2-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]alaninate (NO_2PyImImPyβOEt) (**7**)

To a solution of 1-methyl-4-nitropyrrole-2-car-

boxylic acid (0.77 g, 4.50 mmol) in 10 mL of DMF were added HOBT (0.62 g, 4.60 mmol) and DCC (0.93 g, 4.50 mmol), and the solution was allowed to stir for 18 h at room temperature. Separately, a solution of NO_2ImImPyβOEt (2.33 g, 4.52 mmol) in 25 mL of DMF was mixed with 0.50 g of Pd/C catalyst (10%), and the mixture was stirred under a slight positive pressure of H_2 at 55℃ for 12 h. The catalyst was removed by filtration through Celite, and the filtrate was added to the active ester, followed by stirring for another 12 h. After filtration, 100 mL of $CHCl_3$ was added to the filtrate and the organic layer was washed with brine and dried over anhydrous $MgSO_4$. The desiccator was removed by filtration, and the filtrate was concentrated *in vacuo*. After purification by column chromatography with a mixed solvent of $CHCl_3$ and CH_3OH as eluant, 2.13 g of **7** was obtained as a light yellow solid in 74% yield. ν_{max} (film): 3371, 3132, 2950, 1726, 1650, 1543, 1474, 1381, 1309, 1261, 1192, 1121, 1067, 1022, 786, 623 cm^{-1}. δ_H (DMSO-d_6): 10.90 (s, 1H), 10.26(s, 1H), 9.43(s, 1H), 8.23(s, 1H), 8.11 (t, J = 5.4 Hz, 1H), 7.77(s, 1H), 7.67(s, 1H), 7.59(s, 1H), 7.24(s, 1H), 6.69(s, 1H), 4.07(q, J = 7.0 Hz, 2H), 4.02(s, 3H), 4.01(s, 3H), 3.97 (s, 3H), 3.81(s, 3H), 3.42(q, J = 6.4 Hz, 2H), 2.54(t, J = 7.0 Hz, 2H), 1.19(t, J = 7.2 Hz, 3H). HRMS m/z: 638.2433 [M + H]$^+$ (638.2435 calcd for $C_{27}H_{32}N_{11}O_8$).

Ethyl γ-[1-*methyl*-4-[1-*methyl*-4-[1-*methyl*-4-(1-*methylpyrrole*)-2-*carboxamido*]*pyrrole*-2-*carboxamido*]-*pyrrole*-2-*carboxamido*]*pyrrole*-2-*carboxamido*] *butyricate* (PyPyPyPyγOEt) (**9**)

A similar synthetic procedure as that for the preparation of NO_2PyImImPyβOEt (**7**) was followed for the preparation of **9** and 2.13 g of **9** was obtained in 78% yield. ν_{max} (film): 3306, 3126, 2939, 1721, 1641, 1586, 1538, 1467, 1405, 1316, 1253, 1204, 1109, 1061, 737, 670, 608 cm^{-1}. δ_H(DMSO-d_6): 8.19(s, 1H), 8.05(s, 1H), 7.79(s, 1H), 7.17(s, 1H), 7.13(s, 2H), 6.77(s, 1H), 6.75(s, 1H), 6.71(s, 1H), 6.57(s, 1H), 6.55(s, 1H), 6.43(t, J = 6.0 Hz, 1H), 6.10(t, J = 3.2 Hz, 1H),4.10(q, J = 7.2 Hz, 2H), 3.95(s, 3H), 3.86(s, 3H), 3.82(s, 6H), 3.39(q, J = 6.2 Hz, 2H), 2.38(t, J = 7.0 Hz, 2H), 1.82—1.94(m, J = 6.8 Hz, 2H), 1.21 (t, J = 7.2 Hz, 3H). HRMS m/z: 605.2833 [M + H]$^+$ (605.2836 calcd for $C_{30}H_{37}O_6N_8$).

PyPyPyPyγPyImImPyβOEt (**10**)

To a solution of the acid of **9** (0.40 g, 0.70 mmol) in 4 mL of DMF and 0.7 mL of *N*-methylpyrrolidone (NMP) was added HOBT (0.29 g, 2.2 mmol) and DCC (0.42 mg, 2.0 mmol), and the solution was stirred overnight. Separately, to a solution of **7** (0.44 g, 0.70 mmol) in 9 mL of DMF was added 0.13 g of Pd/C catalyst (10%) and the mixture was stirred under a slight positive pressure of H_2 at 70℃ for 6 h. The catalyst was removed by filtration through Celite. The filtrate was directed into the active ester solution and the solution was stirred overnight. After filtration, the filtrate was concentrated *in vacuo*. Column chromatography of the residue (gradient eluant 0—5% CH_3OH in $CHCl_3$) provided 0.60 g of **29** in 74% yield. ν_{max} (film): 3750—2500, 3305, 3125, 2943, 1710, 1647, 1533, 1468, 1437, 1400, 1252, 1202, 1110, 1061, 900, 751, 614 cm^{-1}. δ_H(DMSO-d_6): 10.19(s, 1H), 10.04(s, 1H), 9.77(s, 1H), 9.73(s, 1H), 9.71(s, 1H), 9.67(s, 1H), 9.36(s, 1H), 7.92(t, J = 5.6 Hz, 1H), 7.90(t, J = 5.6 Hz, 1H), 7.58 (s, 1H), 7.53(s, 1H), 7.25(d, J = 1.6 Hz, 1H), 7.20(s, 3H), 7.15(d, J = 1.8 Hz, 1H), 7.05(d, J = 1.8 Hz, 1H), 7.04(d, J = 1.8 Hz, 1H), 6.95(t, J = 1.7 Hz, 2H), 6.90(d, J = 1.8 Hz, 3H), 6.05 (t, J = 2.6 Hz, 1H), 4.08(q, J = 7.1 Hz, 2H), 4.02(s, 3H), 4.01(s, 3H), 3.89(s, 3H), 3.86(s, 3H), 3.85(s, 3H), 3.84(s, 3H), 3.82(s, 3H), 3.81(s, 3H), 3.42(q, J = 6.5 Hz, 2H), 3.25(q, J = 6.5 Hz, 2H), 2.53(t, J = 7.0 Hz, 2H), 2.31(t, J = 7.3 Hz, 2H), 1.78—1.86(m, J = 7.2 Hz, 2H), 1.19(t, J = 7.1 Hz, 3H). HRMS m/z: 1166.5026 [M + H]$^+$ (1166.5032 calcd for $C_{55}H_{64}N_{19}O_{11}$).

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