A synthetic route to N-methylpyrrole containing polyamide/peptide conjugate

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A N-methylpyrrole containing polyamide/peptide conjugates which are synthetic analogues of natural products with DNA affinity was synthesised. It can efficiently hydrolyse PUC19 plasmid DNA. The structure of these compound was confirmed by ^{1}H NMR, MS and IR.

Keywords: DNA, polyamide/peptide conjugates, cleavage agents

Recently, polyamides containing the N-methylpyrrole and N-methylimidazole amino acids have attracted considerable attention on the part of synthetic and biological chemists because they recognise and bind in the minor groove of predetermined DNA sequences with high affinity and specificity.[1-3] Since these polyamides can permeate living cell membranes, they have the potential to control specific gene expression.[4-7] 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.[8-10] Antiparallel pairings 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 recognises both A•T and T•A base pairs.[8-11]

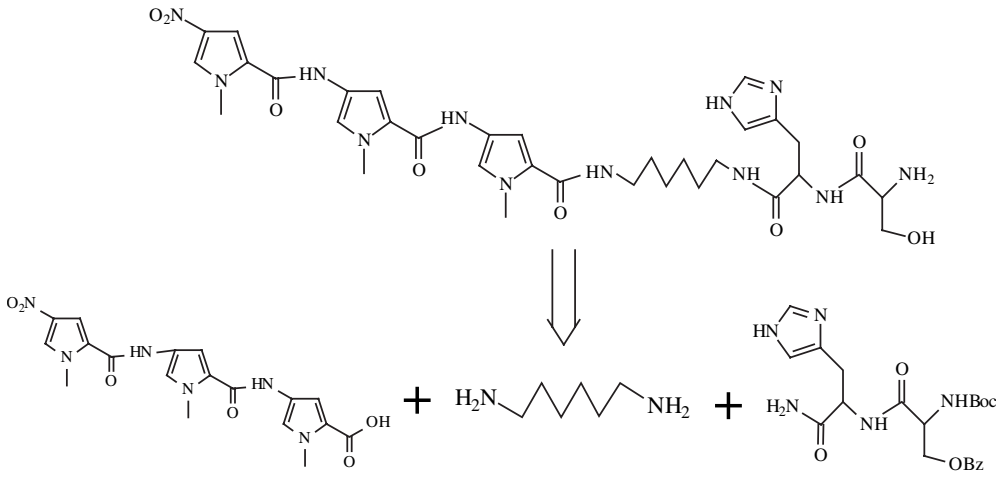
Our laboratory carried out many investigations on small peptides and found that seryl-histidine dipeptide and related oligopeptides can cleave DNA, protein and carboxyl ester,[12,13] which is the shortest peptide ever reported in cleavage agents.

In this article, we report a synthetic route to N-methyl-pyrrole containing polyamide/peptide conjugates, and then the study of their interaction with DNA. The title compound (shown in Scheme 1) is made up of three parts. According to retrosynthetic analysis, the coupling of the three parts will results in a convergent synthesis with fewer linear steps. The synthetic procedure can be performed in two ways: (a) tripolypyrrole acid reacts with hepanamine firstly, then reacts with His-Ser dipeptide containing protected group. (b) His-Ser dipeptide containing protected group reacts with hepanamine firstly, then reacts with tripolypyrrole. Because of the birge block of the His-Ser dipeptide containing protected group, the economy; of the two routes is very poor. So we adopted another synthetic route. The synthetic route is shown in Scheme 2. The compound **9** is our targeted molecule, which consists of polyamides and a seryl-histidine dipeptide. The polyamide is believed to specifically bind DNA, and the dipeptide has been demonstrated to induce DNA cleavage. The structure of these compounds was confirmed by ^{1}H NMR, MS and IR.

Reaction conditions (**1**) NaOMe, MeOH; (**2**) $NH_2(CH_2)_6NH_2$, $CHCl_3$; (**3**) i. Pd-C, H_2; ii. $NO_2PyCOCCl_3$; (**4**) Boc-His, DCC, HOBt; (**5**) NaOH, H_2O, EtOH; (**6**) i. Pd-C, H_2; ii. DCC, HOBt; (**7**) i. TFA/H_2O; ii.NH_4OH, iii.Boc-Ser-Bzl, DCC, HOBt; (**8**) i. TFMSA, Thioanisol, TFA; ii.NH_4OH

The transformation of dipolyamide ester **4** to carboxylic acid **6** was essential to the coupling reaction. In the mixed heated solution of basic alcohol and water, the ester **4** was converted into a carboxylic acid salt. After a solution of 6N HCl was used to neutralise the basic solution, the acid **6** was isolated from the mixed solution in excellent yield.

There were two elements in the synthesis of compound **7**. One is the qualitative reduction of the nitro group to an amino group, and another was the preparation of an active ester of the carboxyl component. To achieve the optimum result, TLC was used to monitor the progress of the hydrogenation. Due to the low solubility of this acid, a large amount of N, N-dimethylformamide was needed in the preparation of the active ester. Adding excess DCC facilitated the formation of the active ester, when three equivalents of DCC were added to the reaction solution, a significantly higher yield of **7** was achieved.



Scheme 1

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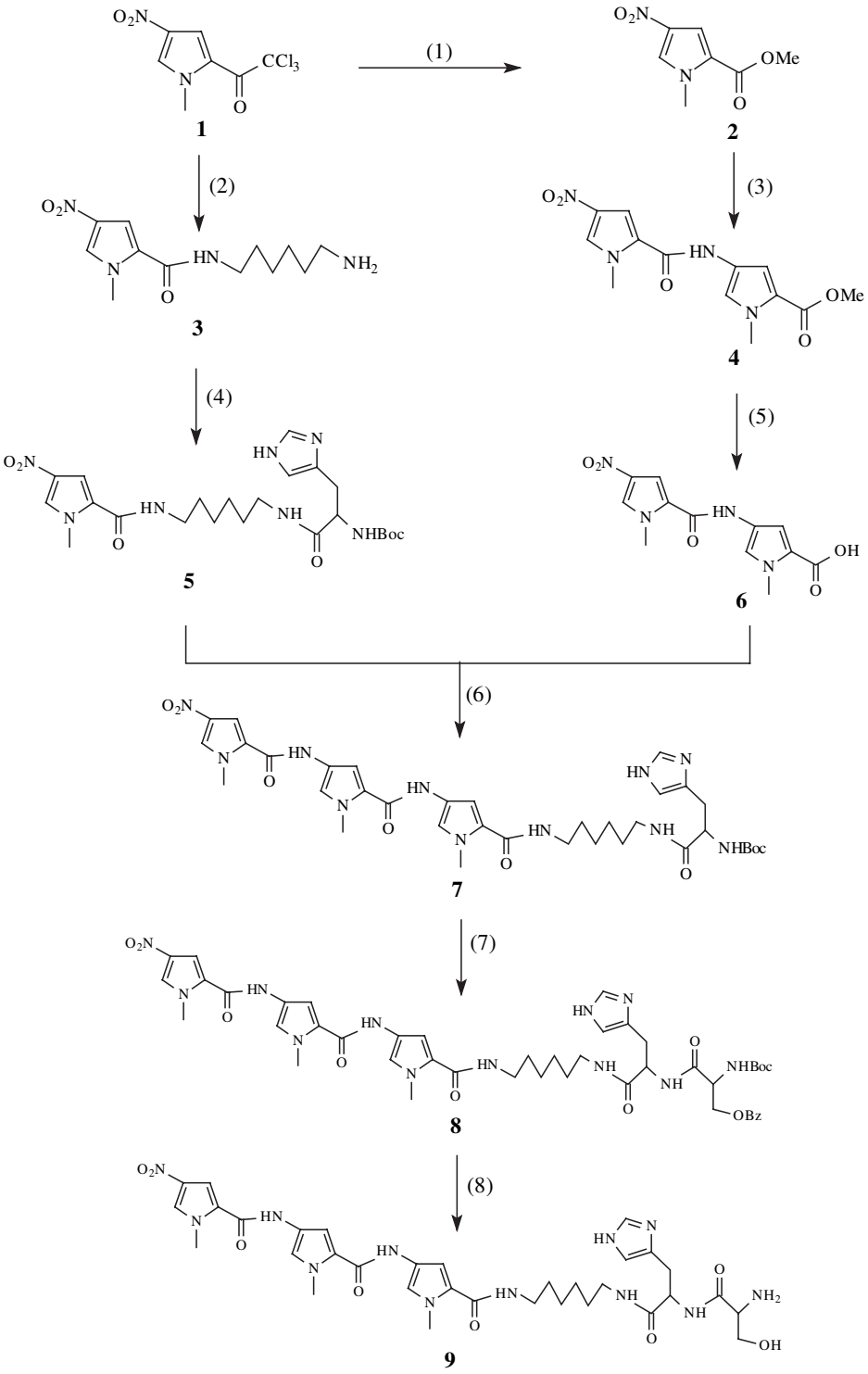

Scheme 2

Because of the high polarity of the carboxyl component, only highly polar solvents (*e.g.* DMF) can be used as reaction solvents in the synthesis of title compound **9**. Because most of the acid was converted to the active ester first, the use of an excess of acid was not necessary. The equimolar amount of acid and amine component was employed in the coupling reaction using DCC/HOBt. The deprotecting amino and hydroxyl group in the synthesis of the title compound was convenient and fast and proceeded in high yield.

Hydrolysis of PUC19 plasmid DNA by the title compound was studied at different pHs from 5.5 to 9.0 in BR buffer. We found it can be efficiently hydrolyse by compound **9**.

In conclusion, we have developed a convenient and efficient route for the synthesis of the new cleavage agents which are synthetic analogues of natural products with DNA affinity. The interaction of compound **9** with DNA is being examined.

Experimental

^{1}H NMR spectra were measured by using a Bruker AC-P400 spectrometer with TMS as the internal and with $CDCl_3$, DMSO or CD_3OD as the solvent. IR spectra were recorded as KBr pellets on a BRUCK spectrometer. Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE-LCTM ion trap spectrometer equipped with a gas nebuliser probe, capable of analysing ions up to

m/z 20000. Solvents were purified and dried by standard procedures. Compounds 1–4 and **6** were synthesised according to refs 14 and 15 respectively.

The synthesis of compound **5**: To a solution of Boc-L-His (0.529 g, 2.3 mmol) and HOBt (0.309 g, 2.3 mmol) in 15 ml DMSO was added a solution of DCC (0.527 g, 2.6 mmol) in 10 ml CH_2Cl_2, and the mixture was stirred for 12 h. The DCU was removed by filtration. To the filtrate was added compound **3** (0.605 g, 2.3 mmol). The reaction mixture was stirred for 24h and then was concentrated *in vacuo*. After purification by column chromatography with a mixed solvent of $CHCl_3$, MeOH and $NH_3.H_2O$ as eluant, 0.855 g of compound **5** was obtained as a light yellow solid in 74% yield.

^{1}H NMR (400MHz, $CDCl_3$): 7.60 (s, 1H), 7.55 (d, 1H, *J* = 1.6Hz), 7.33 (s, 1H) 6.99 (s, 1H), 6.87 (s, 1H), 6.68 (s, 1H), 6.05 (s, 1H), 4.42 (s, 1H), 3.99 (s, 3H), 3.32 (m, 2H), 3.20 (m, 2H), 3.16 (dd, 1H, *J* = 16Hz, *J* = 8.0 Hz), 3.00 (dd, 1H, *J* = 16Hz, *J* = 8.0 Hz), 1.55 (m, 2H), 1.44 (s, 9H), 1.38 (m, 2H), 1.30 (m, 2H), 1.16 (m, 2H). ESI-MS: Positive ion *m/z* 505.8 $[M+H]^+$. IR,ν/cm^{-1}:3302, 3130, 2934, 1699, 1654, 1530, 1312, 1167.

The synthesis of compound **7**: To a solution of compound 6 (0.865 g, 2.9 mmol) in 20 ml of DMF was added HOBt (0.396 g, 2.9 mmol) and DCC (0.722 g, 3.5 mmol) and the solution was allowed to stirred for 8 h at room temperature. After filtration, the active ester solution was obtained.

Separately, a solution of compound **5** (1.45 g, 2.9 mmol) in 20 ml of MeOH was mixed with 0.3 g of Pd/C catalyst (10%), and the mixture was stirred under a slight positive pressure of H_2 at room temperature for 12 h. The catalyst was removed by filtration through Celite and the filtrate was added to the active ester, followed by stirring another 12 h. Then 25 ml $CHCl_3$ was added to the mixture solution 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*. Column chromatography of the residue (eluant with $CHCl_3$ and MeOH) provided 1.565 g light yellow power of compound 7 in 73% yield.

^{1}H NMR (400MHz, CD_3OD): 7.88 (d, 1H, *J* = 1.6Hz), 7.58 (s, 1H), 7.42 (d, 1H, *J* = 2.0Hz), 7.23 (d, 1H, *J* = 1.6Hz), 7.16 (d, 1H, *J* = 1.6 Hz), 6.94 (d, 1H, *J* = 1.6 Hz), 6.83 (s, 1H), 6.78 (d, 1H, *J* = 1.6Hz), 4.33 (s, 1H), 3.99 (s, 3H), 3.90 (d, 3H), 3.86 (s, 3H), 3.29 (m, 2H), 3.14 (m, 2H), 2.98 (dd, 1H, *J* = 16Hz, *J* = 8.0 Hz), 2.85 (dd, 1H, *J* = 16Hz, *J* = 8.0 Hz), 1.56 (m, 2H), 1.45 (m, 2H), 1.38 (s, 9H), 1.31 (m, 4H). ESI-MS: Positive ion *m/z* 505.8 $[M+H]^+$. IR, ν/cm^{-1}: 3132, 2933, 1654, 1534, 1400, 1311, 753.

The synthesis of compound **8**:To a solution of Boc-*O*-benzyl-L-Ser (0.314 g, 1.1 mmol) in 20 ml of CH_2Cl_2 was added HOBt (0.18 g, 1.3 mmol) and DCC (0.4 g, 1.9 mmol) and the solution was stirred for 12 h at room temperature. After filtration, the active ester solution was obtained.

Separately, a solution of compound **7** (0.82 g, 1.1 mmol) in 10 ml of 90% CF_3COOH was stirred for 8h at room temperature. The CF_3COOH was removed *in vacuo*, then 10 ml of Et_3N and 5 ml of DMF was added to the residue. The mixture solution was added to the active ester solution, followed by stirring for 24 h, then was concentrated *in vacuo*. After purification by column chromatography, 0.81 g of compound **8** was obtained in 80% yield.

^{1}H NMR (400MHz, $CDCl_3$): 9.76 (bs, 1H), 9.13 (bs, 1H), 8.01 (s, 1H), 7.69 (s, 1H), 7.53 (s, 2H), 7.42 (s, 1H), 7.34 (s, 1H), 7.27 (m, 5H), 7.00 (bs, 1H), 6.77 (bs, 1H), 6.74 (s,1H), 6.67 (s, 1H), 6.61 (s, 1H), 5.64 (bs, 1H), 4.74 (bs, 1H), 4.49 (s, 2H), 4.29 (d, 1H), 3.99 (s, 3H), 3.92 (s, 3H), 3.83 (s, 3H), 3.67 (bs, 2H), 3.28 (m, 3H), 3.24 (m, 1H), 2.88 (m, 2H), 1.39 (m, 11H), 1.17 (m, 4H), 0.90 (m, 2H). ESI-MS: Positive ion *m/z* 927.3 $[M+H]^+$.

The synthesis of compound **9**: To a solution of compound **8** (0.926 g, 1.0 mmol) in 3.5 ml of thioanisol was added 3.5 ml of CF_3COOH and 3.5 ml of CF_3SO_3H. The solution was stirred for 8 h at 0 °C and precipitating with ether. A solution of $NH_3.H_2O$ was used to adjust the solution to ph = 7. Then concentrated *in vacuo*. After purification by column chromatography, 0.44 g of compound 9 was obtained in 60% yield.

^{1}H NMR(400MHz, DMSO): 10.32 (s, 1H), 9.96 (s, 1H), 8.19 (d, 1H), 8.44 (s, 1H), 8.02 (s, 1H), 7.88 (s, 1H), 7.61 (d, 1H), 7.53 (s, 1H), 7.28 (d, 1H), 7.19 (d, 1H), 7.04 (d, 1H), 6.86 (d, 1H), 6.78 (s, 1H), 4.43 (t, 1H), 3.97 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.54 (m, 2H), 3.50 (m, 2H), 3.14 (m, 2H), 3.00 (m, 2H), 2.91 (dd, 1H), 2.82 (dd, 1H), 1.45 (m, 2H), 1.33 (m, 2H), 1.21 (m, 4H). HRMS *m/z*: 369.1781 $[MH_2]^{2+}$ (369.1791 calcd for $C_{33}H_{44}O_8N_{12}$). IR,ν/cm^{-1}: 3281, 3130, 2935, 2846, 1662,1642, 1580, 1547, 1535, 1502, 1471, 1433, 1398, 1308, 1258, 1203, 1114, 1064, 986, 889, 815, 753;

The authors would like to thank the financial support from the National Natural Science Foundation of China (No.20132020).

Received 4 December 2004; accepted 18 February 2005
Paper 04/2929

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