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Synthesis of pyrrole urea and pyrrole carbonylurea derivatives

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ABSTRACT

A series of urea and carbonylurea distamycin analogs whereby the linker has two NH groups for hydrogen bonding with base pairs of DNA were synthesized. The urea and carbonylurea derivatives are prepared from the in situ generation of pyrrole isocyanate (prepared from compound **3**) and acyl isocyanate (compound **9**), followed by the reaction with an amine. The synthetic feasibility for the further transformations of the pyrrole urea and pyrrole carbonylurea derivatives was also addressed. The binding abilities of these molecules to calf thymus DNA were evaluated by DNA melting temperature (T_m) analysis.

1. Introduction

The rational design and synthesis of non-covalent DNA binding small molecules have become increasingly important due to their ability to sequence-selectively recognize and discriminate between DNA,[1] and as a consequence, the control of specific processes involved in gene expression implicated in a disease.[2] The most widely investigated non-covalent DNA minor groove binding small molecules are the naturally occurring antibiotics distamycin and netropsin, composing of *N*-methylpyrrole polyamides and their structural analogs.[2,3] Recent strategies in analogs design have been based on the replacement of *N*-methylpyrrole component by other heterocyclic rings such as *N*-methylimidazole, thiophene, thiazole, triazole, furan, and pyrazole to further improved discrimination of the base pairs in DNA[4–6] Furthermore, the use of 3-hydroxy-*N*-methyl pyrrole having an additional hydrogen bond has lead to specific recognition of thymine O-2 in DNA.[7,8]

The design and preparation of analogs based on the amide component in distamycin and netropsin have been relatively unexplored. In this study, we have designed the pyrrole urea and pyrrole carbonylurea derivatives (Fig. 1). The urea and carbonylurea share similar structural features to the amide, but have two NH groups available for hydrogen bonding instead of one NH, and may affect the binding of molecules to DNA minor groove. Surprisingly, the isocyante and acyl isocyanate of pyrrole for the synthesis of urea and carbonylurea have not, until, now been systematically studied. Herein, we report the synthetic access and versatility of the urea and carbonylurea pyrrole derivatives.

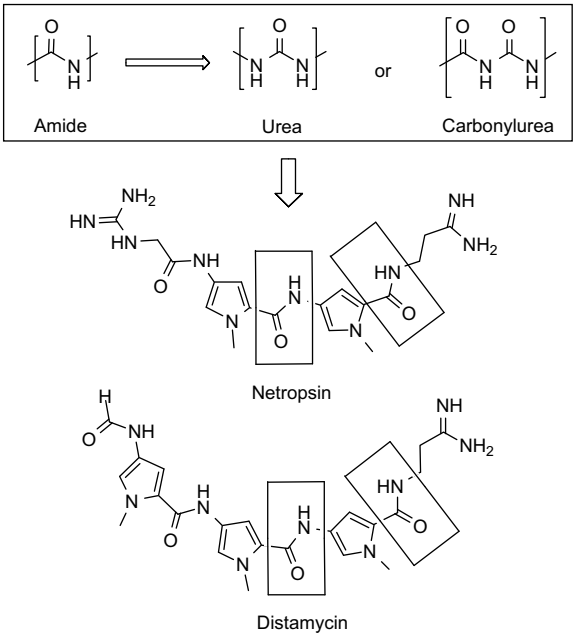

Figure 1. Design of new linking groups at various positions.

2. Results and discussion

The key step for the synthesis of the pyrrole urea unit is the reaction of pyrrole isocyanate with a primary amine. The isocyanate can be generated in situ from the well known Curtius rearrangement of an acyl azide.[9] The primary synthetic challenges thus involved finding an appropriate reaction conditions for the

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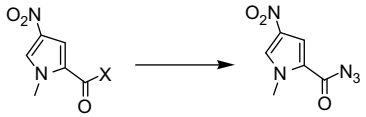
Table 1
Synthetic conditions for acyl azide

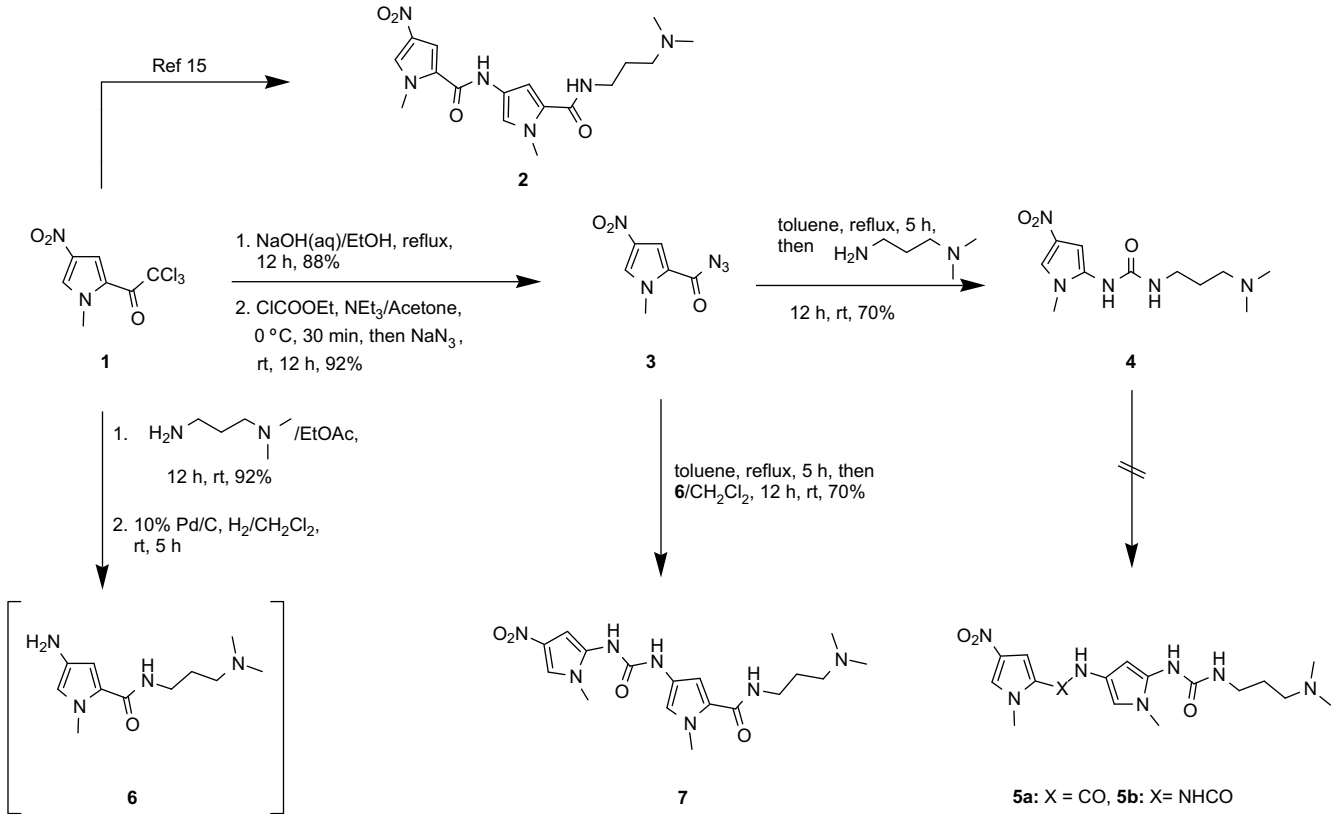


Starting material	Conditions	Yield (%)
X=CCl_3	1)NaN_3/acetone, cat DMSO; rt, 12 h.	0%
X=Cl	1)NaN_3/acetone cat DMSO; rt, 12 h.	Low yield
X=Cl	1)$TMSN_3$/toluene, reflux; 12 h 2)$NH_2CH_2CH_2CH_2C(CH_3)_2$/toluene	0%[a]
X=OCOOEt	1)NaN_3/acetone cat DMSO, rt, 12 h.	92%

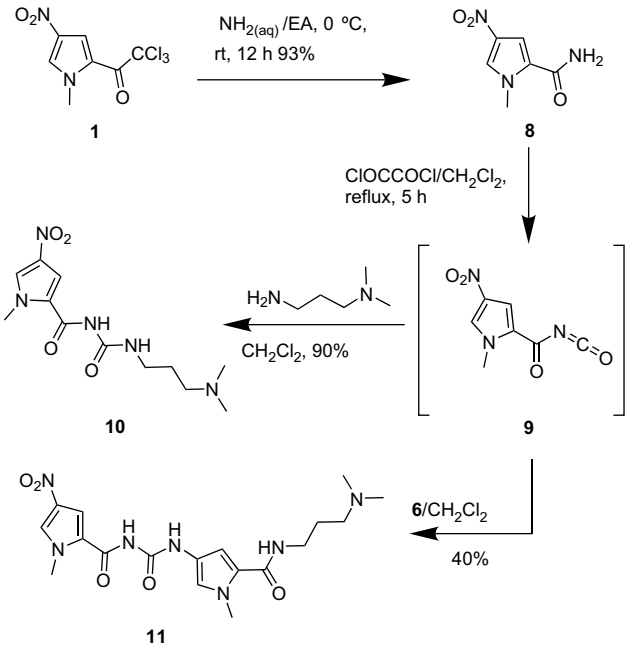
[a] We obtained in this case compound **2**, indicating the lack of reaction with TMS-CN.

conversion of the acid derivative of pyrrole to the acyl azide in high yield (Table 1). The starting material, 2-trichloroacetyl-4-nitro pyrrole **1**, was prepared as previously described by the trichloroacylation of *N*-methylpyrrole, followed by nitration.[10] The reaction of **1** with sodium azide did not give the required acyl azide. Next, compound **1** was hydrolyzed to the acid and activated as acid chloride, and this unfortunately gave low yield of the acyl azide. A one pot procedure for the preparation of urea from acid chloride using trimethylsilyl azide and an amine was also unsuccessful. Instead, the acid was converted to the mixed anhydride with ethyl chloroformate,[11] and treated with sodium azide to give the acyl azide **3** in excellent yields (92%). With the pyrrole acyl azide building block in hand, we first chose to synthesize the urea functionality between a pyrrole and the electrostatic binding group. Rearrangement to the isocyanate was effected by heating the acyl azide **3** in toluene. Then, *N*,*N*-dimethyl-1,3-propanediamine was added to give the urea product **4** in 70% yield (Scheme 1). The synthetic approach was designed to use the urea product **4** for the installation of the remaining pyrroles. The use of standard reaction conditions, hydrogenation of the nitro group to amine in **4**, followed by treatment with **1** did not give the requisite product **5a**, and TLC showed a complex mixture of products. Similar attempt to obtain the bis-urea **5b** also failed when the hydrogenated product of **4** was treated with the in situ prepared isocyanate of **3**. The implication was that the urea **4** was not suitable for the preparation of our target compounds such as **5a** and **5b**. The next step was to introduce the urea functional group between two pyrrole rings. This modification will allow the formation of the urea bond at the late stage of the synthesis. Rearrangement of acyl azide **3** to the isocyanate, and reaction with the previously reported amino-pyrrole **6** gave, after chromatographic purification, the bis-pyrrole amide-urea product **7** in good isolated yield (52%) (Scheme 1). In general, this method can be adapted for the synthesis of distamycin analogs with a urea group between the heterocyclic rings.

Finally, we explored the introduction of a carbonylurea functionality in the pyrrole ring. Weikert et al.[12] has reported the conversion of an acid to the acyl isocyanate intermediate via the treatment of an amide with oxalyl chloride, and the subsequent reaction with amines then gave the carbonylurea product. The synthesis of a suitable pyrrole carboxamide synthon for the synthesis of carbonylurea has not been reported. Interestingly, the reaction of 2-trichloriacetyl-4-nitro pyrrole **1** with ammonium hydroxide in ethyl acetate at 0 °C gave the pyrrole carboxamide **8** (93%). This was converted in situ to the acyl isocyanate **9** by reacting with oxalyl chloride.[13] Condensation of the acyl isocyanate **9** with *N*,*N*-dimethyl-1,3-propanediamine in 1,2-dichloroethane gave the carbonylurea **10**. We were surprised to see that hydrogenation of the nitro group to amine in **10**, followed by treatment with **1** also failed to give the coupled amide product. Once again, we have to change our synthetic strategy. Next, the acyl isocyanate **9** was reacted with amino-pyrrole **6** to give, after chromatographic purification, **11** in moderate yield (45%) (Scheme 2).



Scheme 1. Synthesis of pyrrole urea derivatives.



Scheme 2. Synthesis of pyrrole carbonylurea derivatives.

With the newly designed urea **4**, **7** and carbonylurea **10**, **11** compounds in hand, we next investigated their DNA binding abilities. The polyamide–oligonucleotide interaction has been reported to increases the thermal stability of the double helix,[14] and we have performed preliminary UV melting experiments of calf thymus (CT) DNA in the absence and in the presence of the synthesized urea and carbonylureas. A shift to higher melting temperature (T_m) in the presence of the compounds is indicative of the strength of their interaction with DNA. As shown in Table 2, all the synthesized urea and carbonylureas showed little increase in the melting temperature of CT DNA (ΔT_m<1 °C). Qualitatively, their binding ability can be compared using the same drug/DNA ratio. With distamycin a much higher melting temperature (ΔT_m=14 °C) was observed. Furthermore, when we increase the drug/DNA ratio to 1 for compound **7**, weak binding was observed. For a clearer comparison and interpretation, we have measured the melting temperature for compound **2**,[15] which showed a ΔT_m of 2.5 °C. Thus in this case, when comparing the results for compound **7**, **10**, and **2**, we found that they have a much lower melting temperature than distamycin. It has been reported that the presence of a minimum of three pyrrole rings without the leading amide at the terminus is necessary to provide at least a minimal binding with DNA.[16] Furthermore, the urea **7** and carbonylurea **8** bind less well than the nitro **2**. Since CT DNA is known to be richer in G–C sequence, this result may support that, in this case, urea and carbonylurea bind more favorably to T-site of the oligonucleotide, explaining the smaller ΔT_m observed.

Table 2
Melting temperature with calf thymus DNA (27.7 μM)

Compound	Concentration (μM)	ΔT_m (°C)
2	4.0	2.5
4	4.0	−0.1
7	4.0	0.1
7	13.8	0.7
7	27.7	1.1
10	4.0	0.3
11	4.0	0.2
Distamycin	4.0	14.1

Although the data presented here do not allow definitive conclusions, the current work reports the attempt to replace the amide of polypyrrole with the new urea and carbonylurea functionality. However, more synthetic and analytical work has to be carried out to optimize the strength of binding to DNA and to verify the proposed model for their interaction.

3. Conclusions

We describe in this paper the successful synthetic protocol for the efficient conversion of the acid derivative of pyrrole acid to the isocyanate and acyl isocyanate intermediates, respectively. Importantly, this opens up possibilities for access to polypyrrole bearing urea and carbonylurea in their structures, which could influence the selectivity of the interaction with DNA minor groove. Preliminary DNA binding studies showed weak interaction of the urea and carbonylurea derivatives. However, more synthetic and analytical work has to be carried out to optimize the strength of binding to DNA.

4. Experimental

4.1. General

All reactions were conducted under an atmosphere of nitrogen in oven-dried glassware. Dichloromethane was distilled over calcium hydride. 3-[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carbonylamino) pyrrole-2-carbonylamino] **2**,[15] 1-methyl-4-nitro-2-trichloroacetylpyrrole **1**,[10] and 4-amino-1-methyl-1*H*-pyrrole-2-carboxylic acid (3-dimethylamino-propyl)amide **6**[17] were prepared according to the published procedures. Nuclear magnetic resonance spectra were recorded on a Varian Unity-INOVA-500 MHz spectrometer. Mass spectra were obtained on JEOL JMS-HX110 or ESI on Bruker APE (II) FT-MS.

4.2. DNA melting temperature (T_m) measurement

The experiments were conducted with a Pharmacia Biotect Ultrospec 4000 UV/Visible Spectrophotometer with a temperature controller in 3 mL quartz cuvettes with a Teflon cap. The absorbance of the DNA–compound complex was monitored at 260 nm as a function of temperature and DNA without compound was used as a control. Cuvettes were mounted in a thermal block and the solution temperatures were monitored with a heating rate of 0.5 °C/min. The concentration of DNA was determined by measuring the absorbance at 260 nm. A ratio of approximately 1:7 compound/DNA (4 μM: 27.7 μM) to a ratio of 1:1 compound/DNA was used in the studies. The compounds were dissolved in DMSO and kept below 1% at the final concentration in the cuvettes. All the binding experiments were carried out in an aqueous solution of 10% 1X PBS buffer (pH: 6.8) at 25 °C.

4.3. Synthesis

*4.3.1. 1-Methyl-4-nitro-2-azido-acetylpyrrole (**3**).* To a solution of compound **1** (5 g, 18 mmol) in EtOH (50 mL) was added 5% aqueous NaOH (20 mL) and refluxed for 12 h. After cooling to room temperature, the reaction mixture was neutralized with 10% aqueous HCl, and on further cooling in an ice bath gave precipitate of the 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid. The precipitate was collected, washed with additional cold water, and dried to give the product in 88% yield.

Ethylchloroformate (0.68 mL, 7.1 mmol) was added slowly to the acid above (1 g, 5.89 mmol) in acetone (30 mL) and NEt_3 (0.9 mL, 6.05 mmol) at 0 °C and stirred for 1 h to form the active anhydride. NaN_3 (0.8 g, 12.30 mmol) was added in portions to the reaction

mixture and stirred overnight at room temperature. The solvent was evaporated and the residue was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and concentrated to give the crude product, and purified by column chromatography using EtOAc/Hexane (1:3). The desired product **3** was obtained (1.057 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.68 (d, J=2 Hz, 1H), 7.47 (d, J=2 Hz, 1H), 4.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.73, 135.47, 129.33, 123.56, 114.27, 38.19; LRMS (FAB): 154 ($M+H^+-N_3$).

*4.3.2. 1-(3-Dimethylamino-propyl)-3-(1-methyl-4-nitro-1H-pyrrol-2-yl)-urea (**4**).* A solution of **3** (1 g, 5.13 mmol) in toluene (30 mL) was refluxed for 5 h to induce rearrangement into the isocyanate. After cooling to room temperature, *N*,*N*-dimethyl-1,3-propanediamine (0.9 mL, 7.10 mmol) was added and left to react overnight. The reaction mixture was first evaporated, then dissolved in MeOH, and directly subjected to column chromatography on silica gel with MeOH as eluent to give the desire urea **4** (0.97 g, 70%). Mp 141–143 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 (br s, 1H), 6.55 (br s, 1H), 3.57 (s, 3H), 3.12 (q, J=6 Hz, 2H), 2.41 (br s, 2H), 2.11 (s, 6H), 1.64(br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 157.23, 134.53, 127.13, 120.61, 101.95, 59.03, 44.88, 40.89, 33.50, 25.29; HRMS (ESI): calcd for $C_{11}H_{19}N_5O_3$ 270.1566, found 270.1565.

*4.3.3. 1-[5-(4-Dimethylamino-butyryl)-1-methyl-1H-pyrrol-3-yl]-3-(1-methyl-4-nitro-1H-pyrrol-2-yl)-urea (**7**).* Compound **7** was similarly prepared as above with the use of **6**. Yield: **7**, 1.04 g, 52%. Mp 154–156 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 7.65 (d, J=2.0 Hz, 1H), 6.93 (d, J=1.8 Hz, 1H), 6.69 (d, J=2.0 Hz, 1H), 6.52 (d, J=1.8 Hz, 1H), 3.85 (s, 3H), 3.58 (s, 3H), 3.36 (t, J=6.0 Hz, 2H), 2.93 (t, J=6.0 Hz, 2H), 2.71 (s, 6H), 1.91 (t, J=6.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.67, 135.55, 128.97, 122.21, 101.36, 57.08, 44.02, 37.08, 36.85, 33.74, 27.02; HRMS (ESI): calcd for $C_{17}H_{24}N_6O_4$ 392.2044, found 392.2046.

*4.3.4. 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid amide (**8**).* The excess aqueous NH_3 (28%) and EtOAc (15 mL) in a round-bottom flask was cooled to 0 °C and to this was added slowly a solution of trichloroacetylpyrrole **1** (1 g, 3.6 mmol) in EtOAc (20 mL) and stirred for 12 h. Water and additional EtOAc was added to extract the product. After work up, the desired acid amide **8** (0.56 g, 93%) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.59 (d, J=1.5 Hz, 1H), 7.14 (d, J=1.5 Hz, 1H), 4.00 (s, 3H); HRMS (ESI): calcd for $C_6H_7N_3O_3$ 170.0565, found 170.0566.

*4.3.5. 1-(3-Dimethylamino-propyl)-3-(1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-urea (**10**).* To a solution of **8** (1 g, 5.92 mmol) in 1,2-dichloroethane (30 mL) at room temperature was gradually added oxalyl chloride (3 mL, 23.3 mmol) and stirred until all the suspension dissolved. The mixture was then further refluxed for 5 h. After cooling, the reaction mixture was concentrated to remove excess oxalyl chloride. To the residue was added CH_2Cl_2 (20 mL), followed by *N*,*N*-dimethyl-1,3-propanediamine (0.9 mL, 7.10 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was stirred at room temperature overnight. The product precipitated out of the solution after the reaction and was collected by filtration. Purification by chromatography on silica gel with MeOH as eluent gave the pure urea **10** (1.58 g, 90%). Mp 233–235 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.71 (br s, 1H), 8.79 (t, 1H), 8.12 (d, J=1.5 Hz, 1H), 7.62 (d, J=1.5 Hz, 1H), 4.01 (s, 3H), 3.52 (q, J=7 Hz, 2H), 2.40 (t, J=7.5 Hz, 2H), 2.24 (s, 6H), 1.81(t, J=7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.09, 154.39, 135.34, 128.22, 124.09, 111.58, 57.11, 45.39, 38.44, 27.30; HRMS (ESI): calcd for $C_{12}H_{19}N_5O_4$, 298.1514, found, 298.1515.

*4.3.6. 1-Methyl-4-[3-(1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-ureido]-1H-pyrrole-2-carboxylic acid (3-dimethylamino-propyl)-amide(**11**).* Compound **11** was similarly prepared as above with the use of **6**. Yield: 0.496 g, 40%. Mp 213–215 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.04 (br s, 1H,), 8.89 (br s, 1H), 8.78 (t, 1H), 7.56 (br s, 1H), 7.48 (br s, 1H), 7.36 (d, J=4.5 Hz, 1H), 7.01 (br s, 1H), 4.01 (s, 3H), 3.90 (s, 3H), 3.43 (q, 2H), 2.36 (t, J=18 Hz, 2H), 2.19 (s, 6H), 1.78 (t, J=2.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.01, 161.99, 150.29, 134.07, 130.15, 123.95, 123.54, 120.14, 117.72, 111.23, 103.98, 57.00, 45.17, 38.12, 37.05, 36.06, 27.16; HRMS (ESI): calcd for for $C_{18}H_{25}N_7O_5$ 420.1995, found, 420.1996.

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