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CONFORMATIONAL CONTROL OF A PYRROLE-BASED AMIDE PENTAMER BY DIHYDROGEN PHOSPHATE ANION BINDING

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Abstract – Conformational control is of importance for compounds to implement their properties and functions. In this work, a pyrrole-based amide pentamer **1** was synthesized. NOESY NMR experiments show it adopts two main conformations, one of which can be sorted out and stabilized via binding of a dihydrogen phosphate anion.

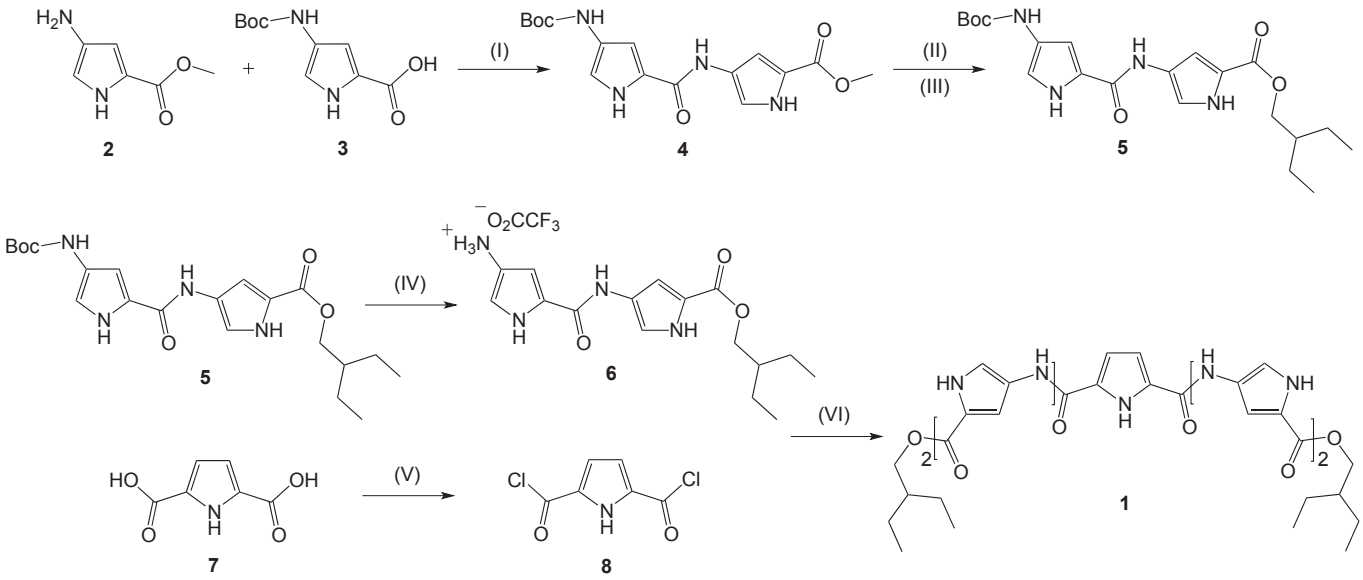
Stimuli-responsive conformational control of biomacromolecules is key to their chemical and biological functions. In supramolecular chemistry, a number of artificial oligomers has been established in recent years to mimic such responsive systems.[1-3] Most examples show the conformational control relies on the host-guest interaction, especially by hydrogen binding.[4,5] These artificial oligomers are usually made of aromatic heterocycles bearing hydrogen donor and acceptor bonding sites.

Pyrrole, a five-membered aromatic heterocyclic compound, is an important subunit for many macrocycles in living systems. Artificial pyrrole-based oligomers have been synthesized as useful agents for recognision carbohydrates,[6] anions,[7] and even DNA.[8,9] However, no report is available on the study of conformational control to this kind of oligomer. Herein, we have synthesized a series of pyrrole-based oligoamides and present their conformational control behavior. We notably show that the pentamer is able to adopt two bent conformations with different diameters, which can be controlled into one conformation via anion binding.

Preparation of the pyrrole-based oligoamides

The structures of the pyrrole-based oligoamides and their synthetic procedures were listed in Scheme 1.

The corresponding dimers **4** can be obtained with a good yield by coupling monoamine **2** and monoacid via the traditional peptide coupling method. To synthesis of pentamer, we initially chose 2,5-bis-(carboxylic acid)-1*H*-pyrrole **7** as a unit linker to connect with the dimer **4**. However, the resulting pyrrole pentamer has poor solubility and thus hampered its study in solution. For the sake of solubility improvement, we modified the methyl ester of pyrrole dimer **4** with a 2-ethylbutyl ester. Afterward, the activation of dimer **5** was carried out in the presence of TFA (trifluoroacetic acid) reagent to generate dimer amine **6**. The desired pentamer **1** was successfully achieved in moderate yield by the coupling reaction between dimer amine **6** and mono-diacid chloride **8**.



Scheme 1. Synthesis of **1**. I) PyBOP, DIEA, DCM, rt, overnight; II) 1 M NaOH, MeOH, rt, 24 h; III) DCC, 2-ethylbutan-1-ol, DMAP, DCM, rt, overnight; IV) TFA, DCM, rt, 1 h; V) $SOCl_2$, DCM, reflux, overnight; VI) DCM, DIEA, rt, overnight.

Structural studies

Single crystal X-ray diffraction provided detailed structure information about the pyrrole-based oligoamides. Colorless crystals of dimer **4** suitable for crystallographic analysis were obtained upon diffusion of hexane into its ethyl acetate solution. The crystal structure of **4** show a planar and bent conformation with a feature that the amide proton points oppositely to the two pyrrole protons (Figure 1a). NOESY (Nuclear Overhauser Effect Spectroscopy) experiments further surpport that the only conformation of **4** in solution is in consistent with the crystal structure, that no cross peak was observed between amide proton and the two pyrrole protons (Figure S2). We attributed this conformational selectivity to the preferance of dipolar orientation.[10] The conformation of pentamer **1** was examined next. Although the single crystal of pentamer **1** was failed to obtained, its conformation was determined by NOESY experiments (Figure 1b). The appearance of strong cross peaks of H^1-H^4, H^4-H^9 indicates **1** has

conformational flexibility and thus two conformers were proposed in Figure 1c. It is worth noting that this conformational change only occurs at the connection between pyrrole diacid fragment and the adjacent amide moities, and the conformation on other parts of the molecule keeps constant, since the rest of NOE signals of **1** appear similar to that of **4**.

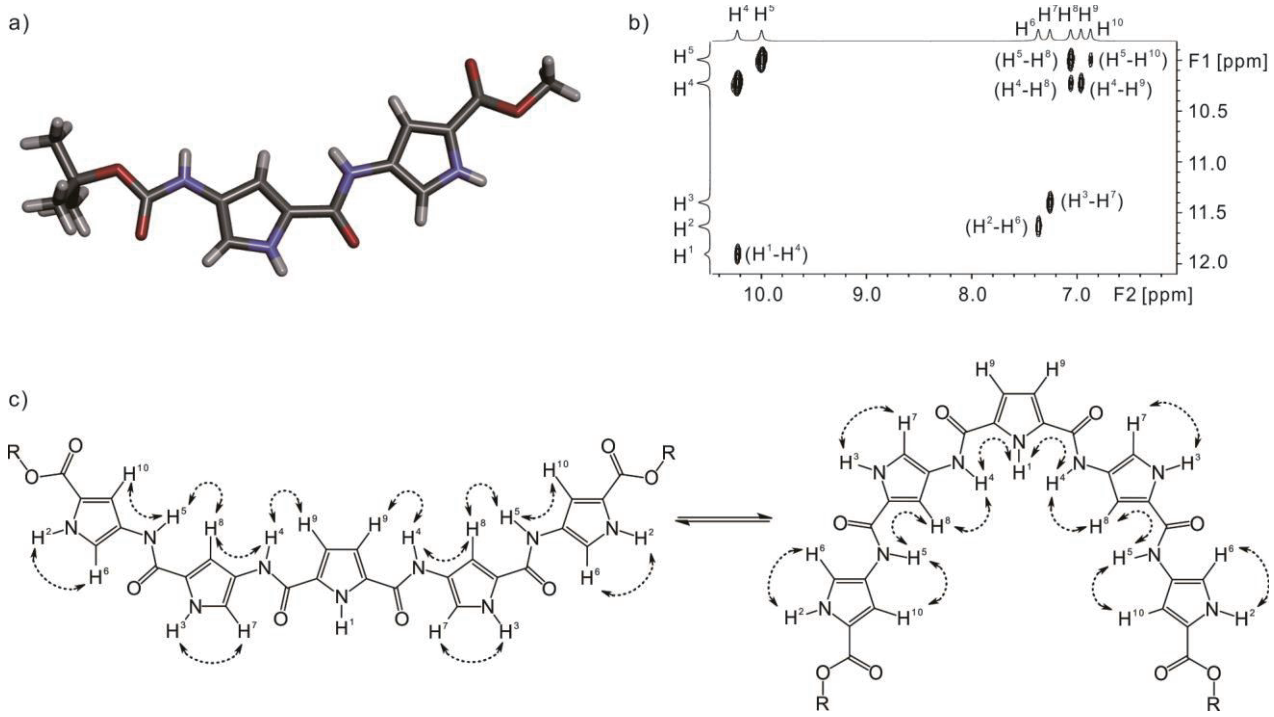


Figure 1. a) Crystal structure of **4**. b) Part of the 2D NOESY NMR spectrum (400 MHz, 298 K) of **1** (10 mM) in DMSO-d_6. c) Proposed structures of **1** in solution. Arrows represent part of the NOE correlations (R=C_6H_{13}).

In order to verify whether the conformation of pyrrole pentamer **1** could be regulated by guest recognition, and inspired by the binding behavior for diaamidopyrroles from Gale group,[11,12] we investigated **1** for anion binding abilities by the ^{1}H NMR titration technique. In general, downfield chemical shifts of the amide protons and pyrrole N-H protons were observed when anion recognition began to work by addition of tetrabutylammonium salts of anions into the solution of **1** in DMSO (Figures S4-S6). The binding constants K_a can be determined by applying non-linear curve fitting using the 1:1 host–guest binding model.[13] And the data are summarized in Table 1. It reveals that binding of pentamer **1** prefers to dihydrogen phosphate anion, followed by chloride, but rather than bisulfate, nitrate, bromide or iodide anion.

Table 1. Binding constants (M^{-1}) for the formation of 1:1 complexes of **1** with various anions in DMSO-d_6 at 298 K, determined by ^{1}H NMR titration technique[a]

Anions	$H_2PO_4^-$	HSO_4^-	NO_3^-	Cl^-	Br^-	I^-
K_a	387	n.d.[b]	n.d.[b]	15	n.d.[b]	n.d.[b]

[a] Errors estimated to be no more than ±10%. Tetrabutylammonium salts were used as anion sources.
[b] n.d. = not determined; Δδ were too low to make an accurate estimation of the constant.

After sceening of different anions, we chose $H_2PO_4^-$, the best guest to study whether the conformation of **1** can be controlled by guest binding induction. The NOESY experiments of **1** with $H_2PO_4^-$ anion (3 eq.) show that the cross peak of H^4-H^9 disappears, but H^1-H^4 signal remains, which surpports that the structure of **1** is controlled as a more bent conformation via guest binding (Figures S7).

EXPERIMENTAL

General methods

All chemicals and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Tetrahydrofuran (THF) was distilled over sodium/benzophenone and dichloromethane (DCM) and *N*,*N*-diisopropylethylamine (DIEA) was distilled over CaH_2 prior to use. Column chromatography was carried out on Merck GEDURAN Si60 (40-63 μm). High-resolution electrospray ionization mass spectrometry (ESI-MS) was performed on a micro TOF II instrument featuring a Z spray source with electrospray ionization and modular LockSpray interface.

NMR spectra were recorded on Bruker AVANCE 600 (600 MHz), and Bruker AVANCE 400 (400 MHz) spectrometers. Chemical shifts were calibrated by DMSO-d_6 (2.50 ppm for ^{1}H NMR, 39.52 ppm for ^{13}C NMR). All chemical shifts (δ) are quoted in ppm and coupling constants (*J*) are expressed in Hertz (Hz). The following abbreviations are used for convenience in reporting the multiplicity for NMR resonances: s = singlet, d = doublet, t = triplet, and m = multiplet. Data processing was performed with Topspin 2.0 software. Assignment of all ^{1}H and ^{13}C resonances was achieved using standard 2D NMR techniques: ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY.

Starting Materials. Compounds **2**,[14] **3**[14] and **8**[15] were prepared according to the appropriate reported procedures. All other chemicals used in this study were commercially available.

***tert*-Butyl [2-((methoxycarbonyl-1*H*-pyrrol)-4-ylcarbamoyl)]-1*H*-pyrrol-4-ylcarbamate 4.** To a suspension of **2** (59 mg, 0.42 mmol), and **3** (95 mg, 0.42 mmol) in CH_2Cl_2 (5 mL), benzotriazol-1-

yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 218 mg, 0.42 mmol) and DIEA (0.07 mL, 0.42 mmol) were added, and the mixture was stirred overnight at room temperature under N_2. Then the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel (petroleum ether:EtOAc = 2:1) to give 121 mg (83%) of **4** as a brown solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.65 (s, 1H), 11.18 (s, 1H), 9.88 (s, 1H), 9.09 (s, 1H), 7.31 (s, 1H), 6.89 (s, 1H), 6.86 (s, 1H), 6.81 (s, 1H), 3.76 (s, 3H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.79, 157.84, 152.85, 124.87, 124.38, 123.36, 118.86, 114.18, 110.61, 106.29, 101.76, 78.21, 51.05, 28.23. ESI-HRMS: *m/z* calcd for $[M+H]^+$ 349.1497, found 349.1565. Elemental analysis (%): calculated for $C_{16}H_{20}N_4O_5$, C 55.17, H 5.79, N 16.08, found, C 54.96, H 5.93, N 16.51.

***tert*-Butyl [2-(2-ethylbutoxycarbonyl)-1*H*-pyrrol-4-ylcarbomoyl]-1*H*-pyrrol-4-ylcarbamate 5.** Compound **4** (100 mg, 0.26 mmol) in 1 M NaOH (10 mL) and MeOH (10 mL) was stirred at room temperature for 24 h. MeOH was then evaporated, the pH of the remaining aqueous solution was adjusted to 2 pH by adding 2 M HCl. The precipitate was collected by filtration and washed by proper amount of Et_2O to give a brown solid. To a suspension of the brown solid in DCM (3.0 mL), dicyclohexylcarbodiimide (DCC, 64 mg, 0.31 mmol), 4-dimethylaminopyridine (DMAP, 1.6 mg, 0.013 mmol) and 2-ethylbutan-1-ol (53 mg, 0.52 mmol) were added, the mixture was stirred overnight at room temperature. The solvent was then evaporated under reduced pressure and the crude product was purified by flash column chromatography on silica gel (petroleum ether:EtOAc = 1:1) to give 79 mg (73%) of **5** as a light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.58 (s, 1H), 11.16 (s, 1H), 9.87 (s, 1H), 9.08 (s, 1H), 7.33 (s, 1H), 6.88 (s, 1H), 6.85 (s, 1H), 6.81 (s, 1H), 4.12 (d, J = 5.6 Hz, 2H), 1.61 – 1.52 (m, 1H), 1.46 (s, J = 13.0 Hz, 9H), 1.43 – 1.34 (m, 4H), 0.90 (t, J = 7.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.50, 157.80, 152.85, 124.82, 124.40, 123.35, 119.07, 114.15, 110.59, 106.18, 101.69, 78.19, 65.14, 40.03, 28.23, 22.93, 10.96. ESI-HRMS: *m/z* calcd for $[M+H]^+$ 419.2296, found 419.2299. Elemental analysis (%): calculated for $C_{21}H_{30}N_4O_5$, C 60.27, H 7.23, N 13.39, found, C 60.26, H 7.24, N 13.39.

2,5-Bis(2-ethylbutoxycarbonyl-1*H*-pyrrol)-4-ylcarbamoyl]]-1*H*-pyrrol-4-ylcarbamoyl]-1*H*-pyrrole 1. To a suspension of **7** (65 mg, 0.42 mmol) in dry DCM (25 mL), thionyl chloride (0.31 mL, 4.3 mmol) was added, followed by two drops of DMF (*N*,*N*-dimethylformamide). The mixture was refluxed overnight to become a clear, yellow solution. The solvent and the excess of thionyl chloride were removed in vacuo. The crude product **8** was used in the following steps without further purification. On the other hand, to a suspension of **5** (200 mg, 0.48 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA, 0.5 mL) under N_2. After being stirred at rt for 1 h, the solvent was evaporated to give white solid compound **6**, Then, dissolve above compound **6** and DIEA (0.07 mL, 0.42 mmol) into DCM (3 mL), and

the mixture was stirred under N_2, and was cooled to 0 °C. A solution of **8** prepared before in 5 mL DCM was slowly drop into the mixture. The reaction was stirred overnight, and then the solvent was removed in vacuo. The solid was washed with 2 M HCl, water and ether. The resulting crude product was recrystallized with methanol and petroleum ether to give 112 mg (62%) of **1** as a light brown solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1H), 11.64 (s, 2H), 11.40 (s, 2H), 10.22 (s, 2H), 9.98 (s, 2H), 7.36 (s, 2H), 7.25 (s, 2H), 7.06 (s, 2H), 6.96 (s, 2H), 6.87 (s, 2H), 4.13 (d, J = 5.4 Hz, 4H), 1.62 – 1.54 (m, 2H), 1.43 – 1.36 (m, 8H), 0.91 (t, J = 7.4 Hz, 12H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.52, 157.73, 156.91, 129.05, 124.75, 123.74, 123.57, 119.09, 114.30, 112.25, 106.28, 102.67, 65.18, 40.02, 22.91, 10.96. ESI-HRMS: *m/z* calcd for $[M+H]^+$ 756.3481, found 756.3185. Elemental analysis (%): calculated for $C_{38}H_{45}N_9O_8$, C 60.39, H 6.00, N 16.68, found, C 60.67, H 6.35, N 16.39.

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