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Synthesis of a C-2 Stapled Bis-Lexitropsin

Naim H. Al-Said[1,*] and **Sami Klaib**[2]

[1] Department of Applied Chemical Sciences, Faculty of Science, Jordan University of Science and Technology, Irbid, Jordan
[2] Department of Chemistry, Tafila Technical University, Tafila, Jordan

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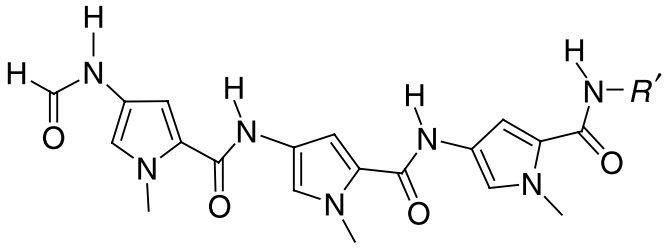
Summary. A convenient synthesis method was developed for the preparation of C-stapled homodimeric bis-lexitropsins connected through the nitrogen atoms of the central pyrrole ring with a bis-methylene linker. This lexitropsin is designed as a standard for other bis-lexitropsins with longer chains in biological evaluation and NMR studies. The key step in this method is the treatment of ethyl 4-nitropyrrole-2-carboxylate with flame-dried potassium carbonate in *DMF* followed by the addition of 1,2-dibromoethane to form the 1,2-dipyrroloethane derivative.

Keywords. Synthesis; Homodimeric; Bis-lexitropsin; Minor groove.

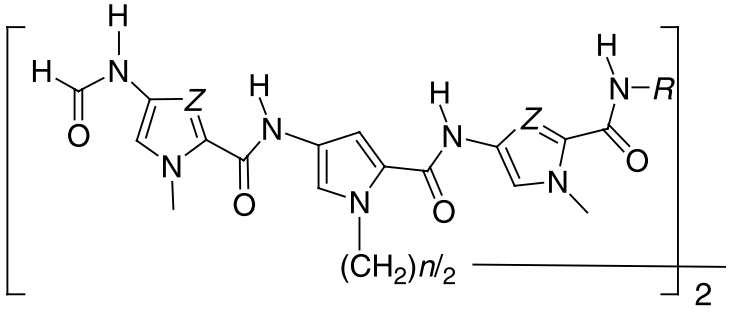
Introduction

Understanding the interactions of double helical *DNA* with small molecules at the molecular level is crucial in designing new chemotherapeutic antibiotic, antitumor, and antiviral drugs [1, 2]. Distamycin (**1**) is a well-studied member of the crescent-shaped pyrrolocarboxamides family of the naturally occurring oligopeptide antibiotics that binds reversibly in the minor groove of double-helical *B-DNA* at regions with at least four adjacent *AT* base pairs [3–5]. The high cytotoxicity of these compounds disqualifies them from being used as drugs. However, this class of pyrrole polyamides has been used as *DNA* sequence selective vehicles for the delivery of alkylating agents to *DNA* targets [6–8]. Structural modifications by replacement of pyrrole by other heterocycles resulted in designing a novel class of minor groove binding agents called lexitropsins, *i.e.* "information reading molecules" [11–14]. Recent NMR studies have confirmed that the minor groove can accommodate two peptidic lexitropsins stacked side-by-side in an antiparallel fashion filling the minor groove of the *DNA* [15–19]. As a consequence, two types of bis-lexitropsin structures have been designed: a hairpin, in which the two oligopeptides units are covalently linked in head-to-tail fashion by a linker [20–22], and cross-linked (stapled-lexitropsins), where the central rings of the two oligopeptides are linked *via* a polymethylene chain [23–26]. These bis-lexitropsins recognize longer *DNA* sequences with stronger binding affinity and higher specificity compared with the monomer when the linker has the appropriate length. Previously, we have reported the synthesis of cross-linked bis-lexitropsins **2** connected through the nitrogens of the central pyrrole ring with polymethylene chains $(CH_2)_n$ of different lengths (**2**, $n = 3$–10) [23–25]. Moreover, recently we have accomplished a practical synthesis route to a terminally linked homodimeric bis-distamycin analog [27]. However, several attempts to prepare stapled lexitropsins with a very short linker (**3**, $n = 2$) were unsuccessful so far.

* Corresponding author. E-mail: naim@just.edu.jo



1 (R' = –$(CH_2)_2$C=NH (NH_2))



2 (Z = N or CH; n = 3–10, R = –NH–$(CH_2)_3NMe_2$) **3** (Z = CH, n = 2)

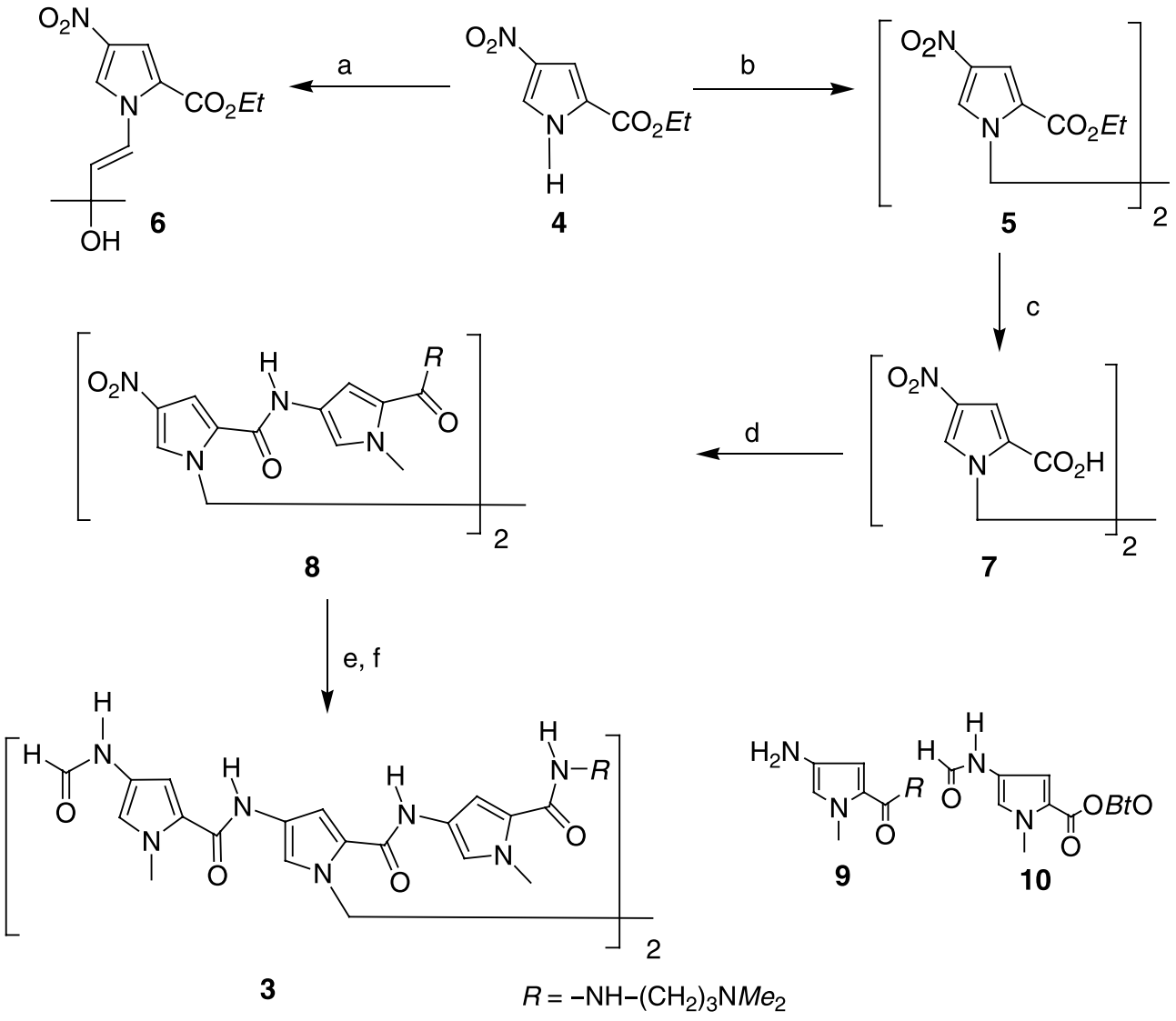
Herein, we report the synthesis of C2-stapled bis-lexitropsins **3** (Z = CH, n = 2) in which the two-tri-pyrrolocarboxamide chains are connected through the nitrogen atoms of the central pyrrole rings by a tether of two methylene units. This lexitropsin is designed as a standard for other bis-lexitropsins with longer chains in biological evaluation and NMR studies. The length of the linker in **3** allows only one strand to bind in the minor groove of the *DNA*.

Results and Discussion

The synthesis of the C2-stapled bis-lexitropsin **3** started with the synthesis of a specifically functionalized bispyrrole unit. The coupling of ethyl 4-nitropyrrol-2-carboxylate (**4**) [28] and 1,2-dibromoethane to form the central homo-unit of the 1,2-dipyrrole derivative **5** employing the conditions (LiI, K_2CO_3, boiling acetone) that we have established with several 1,*n*-dihaloalkanes X-$(CH_2)_n$-X (X = Cl, Br) was unsuccessful [23, 24]. This reaction is particularly striking since the starting material was completely consumed. The TLC indicated the formation of at least three products. Preliminary structural analyses for the major component of the products point toward the formation of the alcohol **6** as depicted in Scheme 1. Currently, this reaction is under investigation to determine the exact structure of the major product and the mechanism of its formation under these mild conditions.

With these discouraging results in hand, we then turned to utilizing non-electrophilic solvents. When a number of other solvents (*THF*, *DME*, 1,4-dioxane) were screened, none of them furnished the required 1,2-dipyrrole **5**. The use of other weak bases (Li_2CO_3, Na_2CO_3) was found to provide similar results.

Fortunately, the required dipyrrole derivative **5** was finally obtained in moderate yield when *DMF* was used as the solvent. The highest yield (49%) of **5** was obtained when flame-dried K_2CO_3 and **4** were stirred in *DMF* at 50°C for 1 h, and then cooled to room temperature before 1,2-dibromoethane was added. The starting material **4** was consumed after stirring the reaction mixture at 50°C for 1 h. After evaporating the solvent, the product **5** was isolated from the reaction mixture by washing with CH_2Cl_2. The organic filtrate was concentrated and the residue was recrystallized from a mixture of CH_2Cl_2-*n*-hexane to afford pure **5**. It is noteworthy that the yield of **5** was lower when the reaction was conducted at higher temperature or for longer time. The ^{1}H NMR spectrum of **5** displayed the aromatic



a) $BrCH_2CH_2Br$, LiI, K_2CO_3, acetone, 60°C; b) i. K_2CO_3, *DMF*, 50°C, 1 h.

ii. Br-$(CH_2)_2$-Br, rt, then 50°C, 1 h, 49%; c) NaOH, *Me*OH; then H_3O^+;

d) **9**, *DMF*, Et_3N, 77%; e) H_2/Pd/C, *Me*OH; f) **10**, *DMF*, Et_3N, 66%.

Scheme 1

protons at $\delta = 7.46$ and 7.36 (each, 2d, 2H) ppm. The methylene protons were observed at $\delta = 4.78$ (s, $J = 4$ Hz) ppm. Furthermore, elemental analysis and high-resolution mass spectrometry confirmed the exact molecular mass of this compound.

Securing **5** allowed further progress toward the projected final product. Thus, at this stage it was decided to introduce the pyrrole units carrying the dimethylaminopropyl group. The dimethylaminopropyl group is essential for the final product. It plays an important role in the solubility of the drug in the biological system and binding in the minor groove of the *DNA*. The ester groups in **5** were hydrolyzed to the corresponding dicarboxylic acids **7** in basic methanol. The resulting dicarboxylic acid was activated for amide formation by stirring in

DMF with *DCC* in the presence of HO*Bt* and triethylamine for 0.5 h followed by the addition of freshly prepared labile intermediate **9** [23, 24] to afford the tetrapyrrole derivative **8** in good yield (77%). The ^{1}H NMR spectrum of **8** displayed the two *N*-methyl groups at $\delta = 3.81$ ppm and the methyl groups attached to the aliphatic amine at $\delta = 2.27$ ppm.

Having completed the assembly of the four-ring intermediate **8**, it remained to introduce the pyrrole ring carrying the formyl group. The methodology chosen for this task was designed to minimize the number of reaction steps required from **8** to the final product **3** due to the high polarity of this intermediate, which causes difficulties in the purification process. This problem was solved by implementation of the activated ester **10** [29]. The final incorporation of the third pyrrole moieties was accomplished by first catalytic transfer hydrogenation of the nitro groups in **8** using Pd/C in methanol to give the corresponding unstable diamine. The resulting diamine was not isolated, but immediately allowed to react with excess activated ester **10** in *DMF* at 50°C in the presence of triethylamine to generate the final product in reasonable yield (66%). The target compound was purified on silica gel and eluted from the column using 5% NH_4OH in methanol:chloroform (1:1). It was fully characterized using FABMS and NMR. Its FABMS showed the molecular ion at m/z 991.50 (MH+), in agreement with the molecular formula $C_{48}H_{63}N_{16}O_8$. The ^{1}H NMR provided conclusive evidence for the formation of the required C-2 stapled bis-lexitropsin **3**.

In conclusion, we have established an efficient and short methodology for preparing the C-2 stapled bis-distamycin analog **3** characterized by a very short linker. With this selected cross-linked lexitropsin now available, biochemical and biophysical analysis of its interactions with *DNA* is possible. These interactions could be used as a standard for the study of interactions of bis-lexitropsins having longer linker with *DNA*.

Experimental

Melting points were determined on an Electrothermal melting point apparatus. Infrared (IR) spectra were recorded on a Nicolet 7199 FTIR spectrometer either in $CHC1_3$ or as KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AM-300 spectrometers using $CDC1_3$ and *DMSO*-d_6 as solvent and internal standard. Chemical shifts are reported in ppm relative to the residual solvent peak of $CDC1_3$ or *DMSO*-d_6 defined to be $\delta = 7.26$ and 2.49 ppm. For carbon spectra (APT), (a) and (p) are used to denote the signals which are antiphase (methyl, methine groups) and in phase (methylene, and quaternary), relative to the solvent peaks. High-resolution mass spectra (HRMS) and FABMS were obtained using a Kratos AEI MS-9 and MS-50 mass spectrometer. Elemental analysis were performed with a Perkin Elmer analyzer; their results agreed favorably with the calculated values.

1,1′-(1,2-Ethanediyl)bis[N-(2-carboethoxy)-4-nitropyrrole] (**5**, $C_{16}H_{18}O_8N_4$)

A solution of 2.21 nitro ester **4** (12.0 mmol) and 2.66 g K_2CO_3 (20.0 mmol) in 20 cm^3 *DMF* were stirred at 50°C for 1 h and then cooled to room temperature. To this mixture was added 0.935 g 1,2-dibromoethane (5.0 mmol). The resulting mixture was stirred at 50°C for 1 h and then the solvent was evaporated *in vacuo*. The residue was washed with boiling CH_2C1_2 (6×50 cm^3) and the washings were filtered through a short pad of silica gel. The filtrate was concentrated and the residual solid was recrystallized from 30% CH_2C1_2 in *n*-hexane to give 0.90 g (49%) pure dipyrrole **5**. Mp 221°C; ^{1}H NMR (CD_2C1_2): $\delta = 7.46$ and 7.36 (2d, $J = 2$ Hz, 2×2pyrr-H), 4.78 (s, $2CH_2$), 4.32 (q, $J = 7$ Hz, $2OCH_2$), 1.38 (t, $J = 7$Hz, $2CH_3$) ppm; ^{13}C NMR (CD_2C1_2): $\delta = 160.42$ (p), 136.28 (p), 127.36 (a), 123.09 (p), 113.41 (a), 61.77 (p), 50.75 (p), 14.37 (a) ppm; IR (KBr): $\bar{\nu} = 3148$, 1718, 1505, 1317, 1265, 1087 cm^{-1}; HRMS: $m/z = 394.1120$ (M$^+$, 39%).

1,1′-(1,2-Ethanediyl)bis[N-4-nitropyrrole-2-carboxylic acid] (**7**, $C_{12}H_{10}O_8N_4$)

To a solution of 291 mg **5** (0.74 mmol) in 20 cm^3 methanol was added 2 cm^3 3 *N* NaOH and the resulting mixture was stirred in a water bath (60°C) for 2 h. The methanol was evaporated and the residue was cooled to 0°C and the *pH* was adjusted to 4 with 2 *N* HC1. The precipitate was filtered off, washed with cold methanol and dried to give 250 mg acid **7** (100%). Mp 290°C (dec.); ^{1}H NMR (*DMF*-d_7): $\delta = 8.05$ (2H obscured by the solvent peaks), 7.30 (s, 2pyrr-H), 5.00 (s, $2CH_2$) ppm; ^{13}C NMR (*DMF*-d_7): $\delta = 156.90$ (p), 130.95 (p), 124.47 (a), 119.91 (p), 107.65 (a), 45.97 (p) ppm; IR (KBr): $\bar{\nu} = 3600$–2200, 3122, 1687, 1524, 1313, 1271, 1167 cm^{-1}; HRMS: $m/z = 338.0493$ (M$^+$, 12%).

1,1′-(1,2-Ethanediyl)bis[N-5-[[[(3-dimethylamino) propylamino]carbonyl]-1-methylpyrrol-3-yl]-4-nitropyrrole-2-carboxamide] (**8**, $C_{34}H_{46}O_8N_{12}$)

A mixture of 122 mg **7** (0.36 mmol), 128 mg *EDCI* (0.73 mmol), and 103 mg HO*Bt* (0.76 mmol) in 10 cm^3 *DMF* was stirred at room temperature for 1 h. Then freshly prepared **9** (0.8 mmol) was added and the reaction mixture was stirred for 2 h. The solvent was removed under reduced pressure at 50°C and then 5% aqueous K_2CO_3 (5 cm^3) was added to the residue. The precipitate was collected, washed with water and cold methanol to give 210 mg (77%) pure yellow solid of **8**. Mp 249°C (dec.); ^{1}H NMR (*Me*OD-d_4): $\delta = 7.63$, 7.34, 7.14, 6.75 (4d, $J = 2$ Hz, 4×2pyrr-H), 4.96 (s, CH_2), 3.81 (s, $2NCH_3$), 3.33 (4H obscured by the solvent peaks, $2CH_2$), 2.41 (t, $J = 8$ Hz, $2CH_2$), 2.27 (s, $2N(CH_3)_2$), 1.77 (quin., $J = 8$ Hz, $2CH_2$) ppm;

IR (KBr): $\bar{\nu} = 3416$, 1647, 1636, 1539, 1310 cm^{-1}; FABMS: $m/z = 751.40$ (MH^+, 4%).

1,1′-(1,2-Ethanediyl)bis[N-5-[[[(3-dimethylaminopropyl) amino]carbonyl]-1-methylpyrrol-3-yl]-4-[[[4-formylamino-1-methylpyrrol-2-yl]carbonyl]amino]-pyrrole-2-carboxamide] (**3**, $C_{48}H_{63}O_8N_{16}$)

A solution of 100 mg **8** (0.13 mmol) in 15 cm^3 methanol was hydrogenated over 15 mg Pd/C for 2 h. The catalyst was removed by filtration through celite. The filtrate was concentrated and the residue was lyophilized from $CHC1_3$. To the solution of the above amine in *DMF* (10 cm^3) was added the activated acid **10**. The reaction mixture was stirred at room temperature for 1 h and at 40°C for 1 h before the solvent was removed *in vacuo*. The residue was purified on silica gel (flash chromatography, 1% of NH_4OH in methanol and $CHC1_3$ 1:1) to give 80 mg (62%) of the C-2 stapled bis-lexitropsin **3**. Mp 185°C; ^1H NMR (*Me*OD-d_4): $\delta = 8.12$ (s, 2H, HCO), 7.15, 7.13, 6.86, 6.82, 6.76. 6.71 (6d, $J = 2$ Hz, 6×2pyrr-H), 4.67 (s, CH_2), 3.85 and 3.75 (2s, 6H, $2NCH_3$), 3.34 (4H obscured by the solvent peaks, $2CH_2$), 2.44 (t, $J = 8$ Hz, $2CH_2$), 2.28 (s, $2N(CH_3)_2$), 1.77 (quint., $J = 8$ Hz, $2CH_2$) ppm; IR (KBr): $\bar{\nu} = 3426$, 2857, 1640, 1539, 1403, 1098 cm^{-1}; FABMS: $m/z = 991.75$ (MH^+, 3.2%).

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