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The Synthesis of a Terminally Linked Homodimeric Bisdistamycin Analog

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Summary. A practical synthesis route to a terminally linked homodimeric bisdistamycin analog is described. In this analog the two strands of tricarboxamides of the pyrrole-pyrrole-pyrrole array are tethered from the nitrogen atom of the terminal pyrrole by a bisethoxyethane chain.

Keywords. Natural product; Stacking; *DNA*; Heterocyclic.

Introduction

The naturally occurring oligopeptide antibiotics distamycin (**1**) [1, 2] and netropsin (**2**) (Formulae 1) [3, 4] bind reversibly to specific nucleotide sequences consisting of 4–5 adjacent *AT* base pairs in the minor groove of double-helical *DNA* blocking its template function [5, 6]. 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'' [7–10]. The polypyrrolocarboxamides skeletons of these oligopeptides have been used as *DNA* sequence selective vehicles for the delivery of alkylating agents to *DNA* targets [11–15].

Increasing the number of heterocyclic carboxamide units beyond five has resulted in compounds with lower binding affinity to *DNA*. This is due to the incompatible phasing of the crescent-shaped curvature, which prevents maximum hydrogen bonding and *Van der Waals* forces between the ligand and *DNA* [16–18]. Moreover, NMR studies have confirmed that two lexitropsin residues can be accommodated, stacked side-by-side in an antiparallel fashion, filling the minor groove of the *DNA* [19–22]. As a consequence, two types of bislexitropsin structures have been designed: a hairpin **3**, in which the two units of oligopeptides are covalently linked in head-to-tail fashion by a linker [23–25], and cross-linked **4**, where the central rings of the two oligopeptides are linked *via* a polymethylene chain (Formulae 2) [26–30]. These bislexitropsins recognize longer *DNA* sequences with

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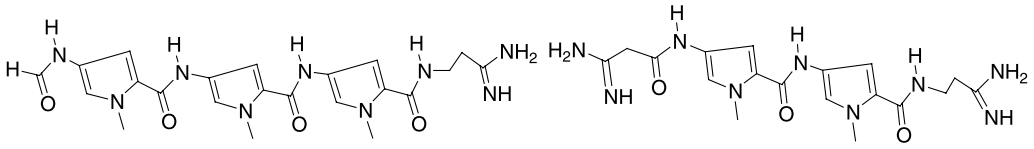
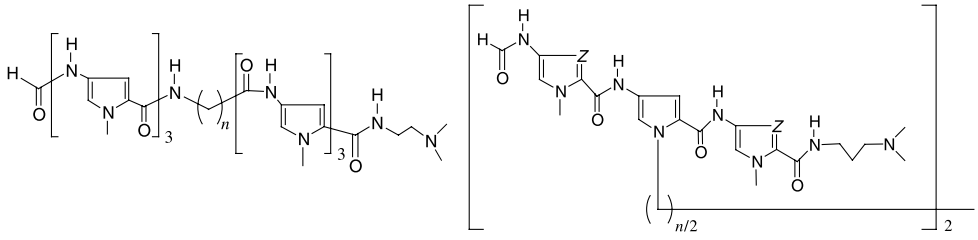
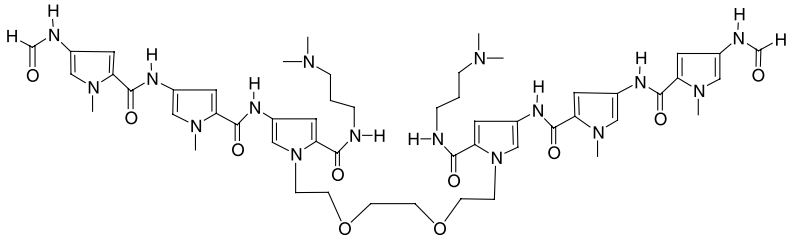


1 **2**

Formulae 1



3 **4** (Z = N or CH; n = 5–10)



5

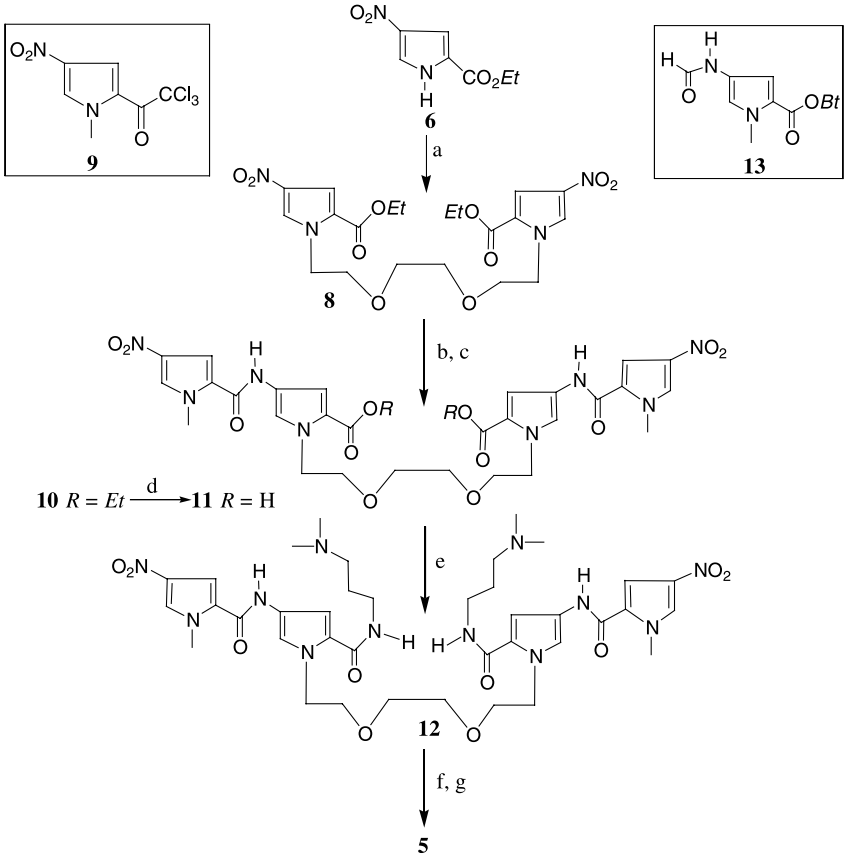
Formulae 2

stronger binding affinity and higher specificity compared with the monomer when the linker has the appropriate length. Additionally, these molecules also exhibit activity against retroviruses including HIV-1 [23–32]. Previously, we reported the synthesis of homodimeric cross-linked bis-lexitropsins **4** connected through the nitrogens of the two central pyrrole rings with polymethylene linkers [26, 27].

Herein, the synthesis of a third generation of bisdistamycin analog **5** is described. In this generation the two pyrrolocarboxamide chains are connected through the nitrogen atoms of the two terminal pyrrole rings by a tether of a bis-ethoxyethane chain. The oxygen atoms are introduced in the chain to increase the solubility of the dimer in aqueous solution.

Results and Discussion

The key to the synthesis of terminally linked homodimeric bislexitropsins was the coupling of easily accessible ethyl 4-nitropyrrol-2-carboxylate (**6**) [31] and the commercially available bis(2-chloroethoxy)ethane (**7**) to form the central homo-



a) $Cl(CH_2)_2O(CH_2)_2O(CH_2)_2Cl$, LiI, *DMF*, 80°C, 2 h, 62%; b) H_2, 5% Pd/C, 18 h, *Me*OH, *EtOAc*; c) **9**, *DMF*, Et_3N, 50°C, 3 h, 77% (2 steps); d) 2 *N* NaOH, *Me*OH, 50°C, 4 h, 71%; e) *EDCI*, *DMF*, $H_2N(CH_2)_3NMe_2$, Et_3N, HO*Bt*, rt, 3 h; f) H_2, 5% Pd/C, *Me*OH, rt, 6 h; g) **13**, Et_3N, *DMF*, 50°C, 68%

Scheme 1

unit of the 1,8-dipyrrole derivative **8**. Initially, this compound was prepared in low yield by SN2 reaction between **6** and **7** in *DMF* at ambient temperature using K_2CO_3 as a base, but a reasonable yield (>60%) was realized using bis(2-iodoethoxy)ethane. Bis(2-iodoethoxy)ethane was generated *in situ* by treatment of **7** with excess lithium iodide at 80°C in *DMF* for 2 h. After cooling to ambient temperature, flame-dried K_2CO_3 and **6** were added (Scheme 1) and the reaction was stirred for 18 h. The product of the coupling was isolated from the reaction mixture by recrystallization of the solid residue obtained after concentrating the organic extracts. The ^1H NMR spectrum of **8** displayed the methylene units at $\delta = 4.54$ (t, $J = 5$ Hz), 4.31 (q, $J = 7$ Hz), 3.78 (t, $J = 5$ Hz) and 3.51 (s) ppm and the methyl groups at $\delta = 1.35$ (t, $J = 7$ Hz) ppm. Furthermore, its HRMS showed the molecular ion at $m/z = 482.1649$ (M^+) in agreement with the molecular formula $C_{20}H_{26}N_4O_{10}$.

Next, we proceeded to examine the reduction of the nitro groups in **8**. This was achieved employing Pd/C as a reduction system to furnish the corresponding unstable diamine that was coupled directly after concentration with 4-nitro-2-trichloroacetyl-1-methylpyrrole (**9**) [31] in the presence of triethylamine to afford the dinitro derivative **10** in 77% overall yield. This yield was obtained only when the solvents were degassed with N_2 prior to use. The product **10** was isolated by crystallization from ethanol. The structure was confirmed by spectral data and elemental analysis. The proton NMR spectrum displayed four signals with small coupling constant (2 Hz) representing the aromatic protons at $\delta = 8.18$, 7.53, 7.44, and 6.91 ppm.

Securing **10** allowed further progress toward the projected final product. Thus, at this stage it was decided to introduce the dimethylaminopropyl group. This group is essential, because it plays an important role in the solubility of the molecule and its binding in the minor groove of the *DNA*. It was introduced by a two steps sequence. First, the ester groups were hydrolyzed to the corresponding dicarboxylic acid **11** in basic methanol. Second, the resulting dicarboxylic acid was further transformed into the corresponding diamide **12** by standard methodology implementing *DCC* in the presence of 3-hydroxy-1,2,3-benzotriazin-4(*3H*)-one (HO*Bt*) and triethylamine.

Having completed the assembly of intermediate **12**, it remained to introduce the two pyrrole rings each having a formyl group at C-4. The methodology chosen for this task was designed to minimize the number of reaction steps required to transform **12** to the final product **5** due to the high polarity of this intermediate which caused difficulties in purification. This problem was solved by implementing the activated ester **13** [32]. Thus, the nitro groups in **12** were reduced to the corresponding unstable diamines using PtO_2 in methanol. This diamine was not isolated, but immediately allowed to react with excess activated ester **13** in *DMF* at 50°C in the presence of triethylamine to give the final product in reasonable yield (>65%). 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 $m/z = 1079.8$ (MH^+), in agreement with the molecular formula $C_{52}H_{70}N_{16}O_{10}$. The ^1H and ^{13}C NMR spectrum of **5** provided conclusive evidence for the formation of the required target of the bisdistamycin analog **5**.

In conclusion, we have established a general synthetic route to a terminally linked bisdistamycin analog **5** starting from easily accessible starting materials. Biochemical analysis of interactions between **5** and *DNA* will be reported in due course.

Experimental

Melting points were determined on an Electrothermal melting point apparatus. Infrared (IR) spectra were recorded on a Nicolet 7199 FTIR spectrometer either in $CHCl_3$ or as KBr pellets. ^1H and ^{13}C NMR spectra were recorded on Bruker AM-300 spectrometers using $CDCl_3$ and *DMSO*-d_6 as solvent and internal standard. Chemical shifts are reported in ppm relative to the residual solvent peak of $CDCl_3$ or *DMSO*-d_6, defined to be $\delta = 7.26$ and 2.49 ppm. For ^{13}C NMR spectra (APT), (a) and (p) are used to denote the signals which are antiphase (methyl and methine groups) and in phase (methylene groups and quaternary carbons), respectively, relative to the solvent peaks. High-resolution mass

spectra (HRMS) and FAB mass spectra were obtained using a Kratos AEI MS-9 and MS-50 mass spectrometer. Elemental analyses were found to be in accord with the calculated values.

Bis[2-[(2-trichloroacetyl)-4-nitropyrrol-1-yl]ethoxy]ethane (**8**, $C_{20}H_{26}N_4O_{10}$)
A solution of 3.74 g bis(2-chloroethoxy)ethane (20.0 mmol) and 7.98 g LiI (60.0 mmol) in 150 cm^3 *DMF* was stirred for 2 h at 80°C. After the solution was cooled to room temperature, 8.28 g K_2CO_3 (60.0 mmol) and 4.60 g of **6** (25.0 mmol) were added and the resulting mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure at 50°C and the residual cake was washed with 3×300 cm^3 CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried, and concentrated. The residue was recrystallized (20% *n*-hexane in CH_2Cl_2) to give 6.28 g (62%) pure **8**. Mp 102°C; ^{1}H NMR ($CDCl_3$): $\delta = 7.75$ and 7.42 (2d, $J = 2$ Hz, 2×2pyrr-H), 4.54 (t, $J = 5$ Hz, 4H, $2NCH_2$), 4.31 (q, $J = 7$ Hz, 4H, $2OCH_2$), 3.78 (t, $J = 5$ Hz, 4H, $2CH_2$), 3.51 (s, 4H, $2OCH_2$), 1.35 (t, $J = 7$ Hz, 6H, $2CH_3$) ppm; IR ($CHCl_3$ cast): $\bar{\nu} = 1715$, 1539, 1508, 1317 cm^{-1}; HRMS: $m/z = 482.1649$ (M^+).

Bis[2-[(2-carboethoxy)-4-[1-methyl-4-nitropyrrole-2-carboxamido]pyrrol-1-yl]ethoxy]ethane (**10**, $C_{32}H_{38}N_8O_{12}$)
A solution of 2.41 g **8** (5.0 mmol) and 30 mg 5% Pd/C in 300 cm^3 25% methanol in ethyl acetate was stirred under a hydrogen atmosphere (50 psi) until the starting material was consumed (18 h). After the catalyst was removed by filtration through celite, the filtrate was concentrated to dryness and the residual amine was dissolved in 50 cm^3 *DMF*. Then 3.86 g **9** (15.0 mmol) and 3 cm^3 triethylamine were added. The resultant mixture was stirred at 50°C for 3 h and then concentrated to dryness under reduced pressure at 50°C. The residue was recrystallized (ethanol) to give 2.82 g (77%) pure **10**. Mp 130°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 10.21$ (s, 2H, 2NH), 8.18, 7.53, 7.44, and 6.91 (4d, $J = 2$ Hz, 4×2pyrr-H), 4.40 (t, $J = 5$ Hz, 4H, $2NCH_2$), 4.17 (q, $J = 7$ Hz, 4H, $2OCH_2$), 3.92 (s, 6H, $2NCH_3$), 3.63 (t, $J = 5$ Hz, 4H, $2OCH_2$), 3.50 (s, 4H, $2OCH_2$), 1.25 (t, $J = 7$ Hz, 6H, $2CH_3$) ppm; IR ($CHCl_3$ cast): $\bar{\nu} = 1689$, 1660, 1565, 1379 cm^{-1}; HRMS: $m/z = 726.2631$ (M^+).

Bis[2-[(2-carboxy)-4-[1-methyl-4-nitropyrrole-2-carboxamido]pyrrol-1-yl]ethoxy]ethane (**11**, $C_{28}H_{30}N_8O_{12}$)
20 cm^3 2 *N* NaOH was added to a solution of 3.63 g **10** (5.0 mmol) in 50 cm^3 methanol and the resultant mixture was stirred at 50°C for 4 h. The reaction mixture was cooled to 0°C and the *pH* was adjusted to 2 with conc HCl. The precipitate was collected and dried to furnish 2.4 g (71%) pure **11**. Mp 157°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.50$ (s, 2H, 2COOH), 10.20 (s, 2H, 2NH), 8.15, 7.50, 7.45, and 6.84 (4d, $J = 2$ Hz, 4×2pyrr-H), 4.43 (t, $J = 5$ Hz, 4H, $2NCH_2$), 3.93 (s, 6H, $2NCH_3$), 3.64 (t, $J = 5$ Hz, 4H, $2OCH_2$), 3.48 (s, 4H, $2OCH_2$) ppm; IR (KBr): $\bar{\nu} = 1658$, 1565, 1455 cm^{-1}; FABMS: $m/z = 670.8$ (M^+).

Bis[2-[2-(carboxamidopropyl-3-dimethylamino)-4-[1-methyl-4-nitropyrrole-2-carboxamido] pyrrol-yl]ethoxy]ethane (**12**, $C_{38}H_{55}N_{12}O_{10}$)
A solution of 1.67 g **11** (2.5 mmol), 1.43 g *EDCI* (7.5 mmol), and 1.22 g 3-hydroxy-1,2,3-benzotriazin-4(*3H*)-one (HO*Bt*) (7.5 mmol) in 50 cm^3 *DMF* was stirred at room temperature for 1 h. Then 3 cm^3 3-dimethylaminopropylamine and 3 cm^3 triethylamine were added and the resulting mixture was stirred for 3 h. The solvent was removed under reduce pressure at 50°C and the residue was dissolved in 300 cm^3 $CHCI_3$. The solution was washed with 20 cm^3 5% K_2CO_3 and the organic layer was separated, dried, and concentrated. The crude product was purified on silica gel (flash chromatography, 5% NH_4OH in methanol:$CHCl_3$ = 1:1) to furnish 1.10 g (52%) **12**. Mp 179°C; ^{1}H NMR ($CDCl_3$): $\delta = 8.90$ (s, 2H, 2NH), 7.95 (bs, 2H, 2NH), 7.55, 7.34, 7.18, and 6.75 (4d, $J = 2$ Hz, 4×2pyrr-H), 4.43 (t, $J = 5$ Hz, 4H, $2NCH_2$), 4.02 (s, 6H, $2NCH_3$), 3.69 (t, $J = 5$ Hz, 4H, $2OCH_2$), 3.49 (s, 4H, $2OCH_2$), 3.45 (q, $J = 6$ Hz, 4H, $2NCH_2$), 2.50 (t, $J = 6$ Hz, 4H, $2NCH_2$), 2.32 (s, 12H, $2N(CH_3)_2$), 1.73 (m, 4H, $2CH_2$) ppm; IR ($CHCl_3$ cast): $\bar{\nu} = 1639$, 1592, 1573, 1504 cm^{-1}; FABMS: $m/z = 839.1$ (MH^+).

Bis[2-[2-(carboxamidopropyl-3-dimethylamino)[1-methyl-4-[1-methyl-4-formamidopyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrol-yl]ethoxy]ethane (**5**, $C_{52}H_{71}N_{16}O_{10}$)
A solution of 209 mg **12** (0.25 mmol) and 30 mg PtO_2 in 100 cm^3 methanol was stirred under hydrogen (50 psi) for 3 h. Then, the catalyst was removed by filtration through celite. The filtrate was concentrated and the residue was dissolved in $CHCl_3$. The residue left after concentration was dissolved in 20 cm^3 *DMF*. The resulting solution was added to 314 mg **13** (0.10 mmol) followed by 1 cm^3 triethylamine. The resulting mixture was stirred for 6 h and then the solvent was removed under reduced pressure at 50°C. The residue was purified on silica gel (flash chromatography, 5% NH_4OH in methanol:$CHCl_3$ = 1:1) to give 185 mg (68%) pure **5**. Mp 184°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 10.05$, 9.93, and 9.90 (3 s, 3 × 2NH), 8.15 (d, $J = 2$ Hz, 2H, 2HCO), 8.10 (t, $J = 5$ Hz, 2H, 2NH), 7.24, 7.23, 7.18, 7.03, 6.92, and 6.85 (6d, $J = 2$ Hz, 6 × 2pyrr-H), 4.40 (t, $J = 5$ Hz, 4H, $2NCH_2$), 3.84 (s, 12H, $4NCH_3$), 3.61 (t, $J = 5$ Hz, 4H, $2NCH_2$), 3.45 (s, 4H, $2OCH_2$), 3.18 (q, $J = 7$ Hz, 4H, $2CH_2$), 2.24 (t, $J = 7$ Hz, 4H, $2NCH_2$), 2.13 (s, 12H, $2N(CH_3)_2$), 1.60 (quin, $J = 7$ Hz, 4H, $2CH_2$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 161.2$ (p), 158.4 (p), 158.3 (p), 157.8 (a), 122.9 (p), 122.8 (p), 122.6 (p), 122.5 (p), 122.2 (p), 122.0 (p), 120.7 (a), 118.4 (a), 117.4 (a), 104.6 (a), 104.5 (a), 103.9 (a), 70.7 (p), 69.5 (p), 56.9 (p), 47.3 (p), 45.0 (a), 37.0 (p), 36.3 (a), 36.0 (a), 27.0 (p) ppm; IR ($CHCl_3$ cast): $\bar{\nu} = 3285$, 1641, 1580, 1527, 1435 cm^{-1}; FABMS: $m/z = 1079.8$ (MH^+).

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