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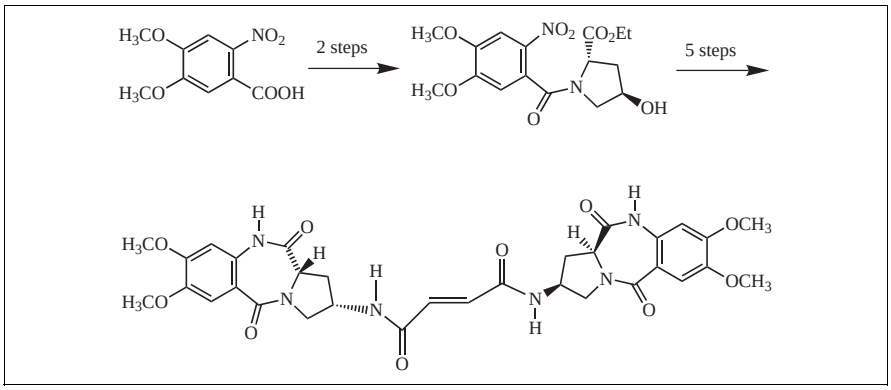

Efficient synthesis of biological important pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione dimer linked through the C-2 positions by fumarate group is described.

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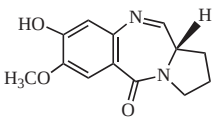
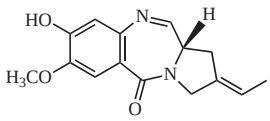
Introduction.

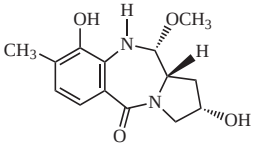
Understanding of the interactions at the molecular level between DNA and some small molecules, which are vital in antitumor, antibiotic, and antiviral chemotherapy, is crucial in facilitating the design of new drugs and probes that can recognize specific DNA sequences [1-4]. The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of naturally occurring antitumor antibiotics. DC-8 (**1**), tomamycin (**2**), and chicamycin **(3)** are examples of the PBDs family [5-7]. These small molecules recognize and bond to specific DNA sequences [8-9]. The biological activity of PBDs is associated with their ability to form covalent binding to DNA *via* nucleophilic attack of the C2-NH_2 of a guanine base at the electrophilic C11 position within the minor groove of DNA [10-11]. Dilactams of PBD such as **4**, lacking the imine moiety (or carbinolamine equivalent) at N10-C11, were shown to bind in a non-covalent fashion to DNA [12]. Recently, C-8 linked bifunctional alkylating dimers, based on PBD skeleton posses an efficient inter-strand cross-linking ability and high affinity for DNA, were described [13-16]. Since the imine moiety in such dimer has strong covalent binding ability to DNA it retards the NMR spectroscopic study of the non-covalent binding motifs.



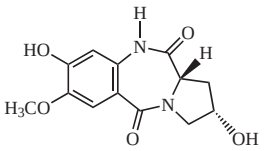
DC-8 (**1**)



Tomamycin (**2**)



Chicamycin (**3**)



Dilactam (**4**)

The current publication describes the first synthesis of PBD dilactam dimer linked through the C-2 positions. This dimer lacks the problematic unstable imine moiety design to recognize and bind to the minor groove of DNA. The synthetic pathway described here is short, amenable to large-scale preparation and generally extendable to synthesis of wide range of dimers with different linkers and functionalities.

Results and Discussion.

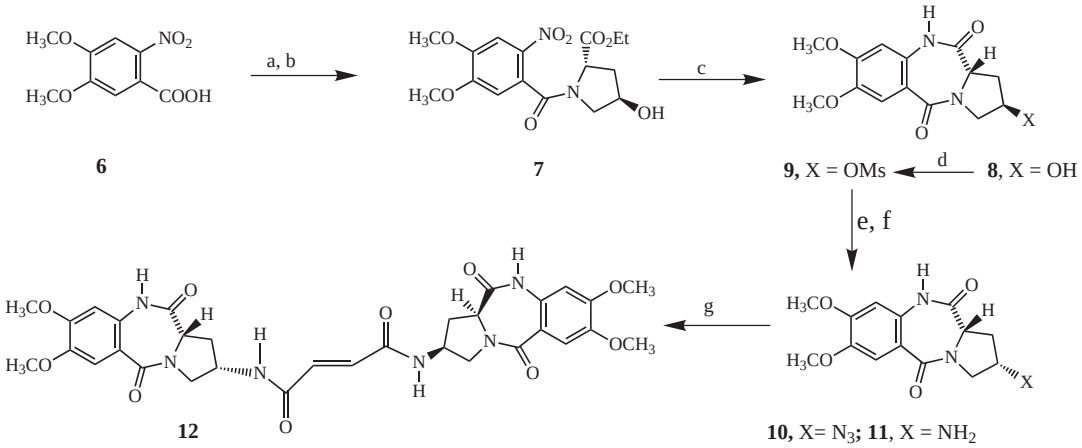
The synthesis of PBD dilactam dimer **12** started with the synthesis of a specifically functionalized dilactam unit. In the first step, commercially available *trans*-hydroxy proline ethyl ester hydrochloride (**5**) and 6-nitroveratric acid (**6**) were coupled. Following the conversion of **6** to its acid chloride using thionyl chloride, the resulting acid chloride was added to a solution of **5** in dichloromethane at 0 °C to afford the precursor **7** in excellent yield (Scheme 1). The ^{1}H-NMR ($CDCl_3$) of this pure intermediate indicated the presence of two related species in a ratio of 7:3 probably due to the restricted rotation of the amide bond. The methoxy protons were displayed as two singlets (δ 3.93 and 3.90) and the methyl protons as two triplets (δ 1.28 and 1.03).

Reduction of the nitro group in **7** in methanol over Pd/C was very slow and required addition of

catalytic amount of hydrochloric acid to accomplish the conversion of the starting material. Fortunately, this process was accompanied by a simultaneous cyclization of the resulting amine to give the required dilactam **8**.

In summary, the first pyrrolo[2,1-c][1,4]-benzodiazepine-5,11-dione dimer has been synthesized and characterized. The approach to this dimer is extendable to synthesis of a wide range of dimers with different linkers and functionalities.

Scheme 1



Reagents and conditions a) $SOCl_2$, reflux; b) *trans*-4-Hydroxyproline ethyl ester hydrochloride (**5**), CH_2Cl_2, 87%; c) H_2/Pd(C), MeOH/$HCl_{(cat)}$, 93%; d) $MeSO_2Cl$, Pyr., 87%; e) NaN_3, EtOH-DMF, 80° C, 95%; f) H_2/Pd/C, MeOH, then Fumaric acid, EDCI, HOBtO, DMF, 63%.

Forming a dimer of dilactam **8** employing ester linkages was abandoned because several attempts to couple the hydroxyl group of the dilactam **8** with fumaric acid and succinic acid or their acid chlorides under different conditions were unsuccessful.

Therefore, we decided to use the more reactive amine at C-2 position. The conversion of the hydroxyl group to amine was smoothly achieved in two steps. First, the hydroxyl group at C-2 position was converted to mesylate **9** in pyridine at 0 °C. Then, the resulting mesylate was treated with sodium azide in ethanol-dimethylformamide solution at 80 °C to afford the azido derivative **10**. The remaining problem in our synthesis consisted formally of the task of achieving reduction of the azido group to the corresponding amine **11** and coupling of the resulting amine with suitable linker. Thus, conversion of azido group to the required amine **11** was effected by hydrogenolysis method in methanol. The resulting amine **11** was used in the next step without purification. Coupling of the amine **11** with fumaric acid was accomplished by a conventional method used for amide formation. The fumaric acid was first activated by employing 1-(3-dimethylaminopropyl)-3–ethylcarbodi-imide hydrochloride (EDCI) and 3-hydroxy-1,2,3-benzo-triazin-4(3H)-one (HOBtO) followed by addition of amine **11** to furnish the first PBDs dimer **12** in reasonable overall yield.

EXPERIMENTAL

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. The spectra were recorded using Nicolet 7199 FT-IR. ^{1}H-NMR spectra were recorded on Bruker AM-300 spectrometer. High-resolution mass spectra (HRMS) and FAB mass spectra were obtained using a Kratos AEI MS-9 and MS-50 mass spectrometer and reported as m/z.

N-[2-Nitro-4,5-dimethoxybezoyl]-L-hydroxyproline ethyl ester (**7**).

A solution of nitroveratric acid (**6**) (2.27 g, 10 mmol) was heated with excess thionyl chloride (6 mL) for 2 h. The solution was then concentrated and the residue was dissolved in dichloromethane (200 mL). Then, *trans*-hydroxy-L-proline ethyl ester hydrochloride (**5**) (2.93, 15 mmol) and triethylamine (4 mL) were added to the acid chloride at 0 °C. The reaction mixture was stirred for 3 h, washed with 5% HCl (100 mL), and the organic layer was concentrated. The residue was purified by column chromatography on silica gel (5% MeOH in ethyl acetate) to give amide **7** (3.21 g, 87% yield); [ ]$_D$ –72 (c 5.75, $CHC1_3$); FT-IR ($CHCI_3$) 3438, 1740 and 1647 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.65 and 6.83 (2s, 1H each, aromatic-*H*), 4.75 (t, J=8 Hz, 1H, C*H*OH), 4.20 (q, J=7 Hz, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 3.45 (dd, J=11 Hz, J'=4 Hz, 2H, CH_2), 1.28 (t, J=8 Hz, 3H, CH_3) for the major species. ^{1}H-NMR ($CDCl_3$): δ 7.55 and 6.73 (2s, 1H each, aromatic-*H*), 4.50 (t, J = 8 Hz, 1H, C*H*OH), 4.10 (q, J = 7 Hz, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 1.03 (t, J = 8 Hz, 3H, CH_3) for the minor species; HRMS calcd. for $C_{16}H_{20}N_2O_8$ 368.1220, found 368.1226.

Anal. Calcd. for $C_{16}H_{20}N_2O_8$: C, 52.17; H, 5.47; N, 7.61. Found: C, 52.20; H, 5.40; N, 7.55

(1la*S*)-2(*R*)-Hydroxy-7,8-dimethoxy-2,3,5,10,11,1la-hexahydro-5,11-dioxo-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine (**8**).

Amide **7** (1.84 g, 5 mmol) in methanol (150 mL) containing few drops of conc. HCl was hydrogenated (50 psi, 345 KPa) at 40 °C for 18 h over Pd/C. The reaction mixture was filtered and the filtrate was concentrated. The solid residue was washed with cold methanol to furnish **8** (1.38 g, 93% yield) as white solid; m.p 254 °C; FT-IR ($CHC1_3$) 3422, 1674, 1649 and 1519 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.30 (s, 1H, N*H*), 7.25 and 6.68 (2s, 1H each, aromatic-*H*), 5.13 (d, J = 4 Hz, 1H, C*H*OH), 4.28 **(**q, J = 4 Hz, 1H, NC*H*H), 4.10 (dd, J = 8 and 6 Hz, 1H, C*H*), 3.78 (s, 3H, OCH_3), 3.60 (dd, J = 12 and 4 Hz, 2H, NCHH), 3.40 (dd, J = 12 and 5 Hz, 1H, NCHH); HRMS calcd for $C_{14}H_{16}N_2O_5$ 292.1060, found 292.1061.

Anal. Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.51. Found: C, 57.28; H, 5.47; N, 9.63.

(11a*S*)-2(*R*)-Mesy1-7,8-dimethoxy-2,3,5,10,11,1la-hexahydro-5,11-dioxo-l*H*-pyrrolol[2,1-*c*][l,4]benzodiazepine (**9**).

Methanesulfonyl chloride (0.7 g, 6 mmol) was added to a solution of **8** (1.17 g, 4 mmol) in dichloromethane (50 mL) containing pyridine (3 mL). The reaction mixture was stirred at room temperature for 3 h followed by addition of 10% HCl (100 mL). The organic layer was separated, dried and concentrated. The residue was purified by flash chromatography (ethyl acetate) to afford mesylate **9** (1.2 g, 87% yield) as a white solid; mp. 185° C; $[\alpha]_D$ 299 (c 6.94, MeOH); FT-IR ($CHC1_3$) 1693,1674, 1629 and 1609 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 8.30 (s, 1H, N*H*), 7.45 and 6.45 (2s, 1H each, aromatic-*H*), 5.13 (m, 1H, C*H*OMs), 4.35 (m, 2H, NCHH), 3.10 and 2.45 (2m, 1H each, CHH), 3.05 (s, 3H, CH_3SO_2); HRMS calcd for $C_{17}H_{18}N_2O_7S$ 370.0835, found 370.0838.

Anal. Calcd. for $C_{17}H_{18}N_2O_7S$: C, 48.64; H, 4.90; N, 5.56. Found: C, 48.72; H, 4.82; N, 5.63.

(1la*S*)-2(*S*)-azido-7,8-dimethoxy-2,3,5,10,11,11a-hexahydro-5,l1-dioxo-l*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine (**10**).

Mesylate **9** (1.85 g, 5 mmol) and sodium azide (0.98 g 15 mmol) in ethanol-dimethylformamide solution (1:1, 100 mL) was stirred at 80 °C for 18 h. The solution was concentrated and the residue was dissolved in $CHCl_3$ (100 mL). The organic layer was washed with water, dried and concentrated. The solid residue was recrystallized (1:1, $CHCl_3$-hexane) to afford **10** (1.52 g, 95% yield); mp. 126 °C; FT-IR ($CHCl_3$) 2102, 1696,1627, 1609 and 1431 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 9.20 (s, 1H, N*H*), 7.45 and 6.55 (2s, 1H each, aromatic-*H*), 4.35 (m, 1H,C*H*N_3), 4.16 (dd, J = 9 and 3 Hz, 1H, NCHH), 3.80 (dd, J = 5 and13 Hz, 1H, NCH*H*), 3.90 (s, 6H, OCH_3); FIRMS calcd. for $C_{14}H_{15}N_50_4$ 317.1124, found 317.1125.

Anal. Calcd. for $C_{14}H_{15}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 53.24; H, 4.62; N, 22.21.

Dimer **12**.

Azide **10** (735 mg, 2.3 mmol) in methanol (100 mL) was hydrogenated (30 psi, 207 KPa) for 2 h over Pd/C. The mixture was filtered and concentrated. The residue was collected and washed with 10% ethyl acetate in hexane to give pure amine **11**. The resulting amine was dissolved in DMF (10 mL) containing triethylamine (1 mL). The resulting mixture was added to a DMF (10 mL) solution of fumaric acid (116 mg, 1 mmol), EDCI (2.2 mmol) and HOBtO (2 mmol). The reaction mixture was stirred for 24 h. The mixture was concentrated under reduced pressure. The residue was dissolved in chloroform and the resulting solution was washed with water, dried and concentrated. The solid residue was collected and washed with cold methanol to give pure dimmer **12** (920 mg, 63% yield) as a white powder; mp. 310 °C (dec); $[\alpha]_D$ +399 (c 3.95, DMSO); FT-IR (CH_2Cl_2) 1677, 1654, 1620, 1606 and 1432 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.32 (s, 2H, NH), 8.53 and 8.52 (2s, 1H each NH), 7.25 and 6.76 (2s, 2H each, aromatic-*H*), 6.68 (s, 2H, *H*C=C*H*), 4.28 (dd, J = 12 and 5 Hz, 2H), 4.15 (dd, J = 8 and 5 Hz, 2H), 3.98 (dd, J = 12 and 8 Hz, 2H), 3.76 and 3.7 (2s, 3H each, OCH_3), 3.28 (m, 2H), 2.65 (m, 2H), and 2.27 (m, 2H); ^{13}C-NMR (DMSO-d_6) δ 170.0, 164.9, 163.7, 151.8, 145.3, 132.5, 130.8, 117.6, 111.9, 104.4, 55.6, 51.6, 47.4, 30.9; FABMS calcd. for $C_{32}H_{35}N_6O_{10}$ 662.2, found 663.1 (MH^+, 5%).

Anal. Calcd. for $C_{32}H_{34}N_6O_{10}$: C, 58.00; H, 5.17; N, 12.68. Found: C, 58.32; H, 5.32; N, 12.51.

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