An Efficient Synthesis of Pyrrolo[2,1-*c*][1,4]benzodiazepine. Synthesis of the Antibiotic DC-81

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Pyrrolo[2,1-*c*][1,4]benzodiazepines (**1**) (PBDs) are a group of potent, naturally occurring antitumor antibiotics produced by *Streptomyces* species.[1] The cytotoxic and antitumor effects of these compounds are believed to arise from modification of DNA, which leads to inhibition of nucleic acid synthesis and production of excision-dependent single- and double-strand breaks in cellular DNA.[2] These antibiotics have been proposed to covalently bond to N2 of guanine to form a neutral minor groove adduct (**2**) (Scheme 1).[3] Tomaymycin (**3**), cross-linker DSB-120 (**4**),[3d,4] and DC-81 (**5**) are the best known examples of the PBDs (Figure 1). Synthetic approaches to these compounds have been reported;[4−6] however, most of them are tedious. For instance, one of the widely used methods involves the cyclization of amino dithioacetals using mercuric chloride to give the imine products (Scheme 2).

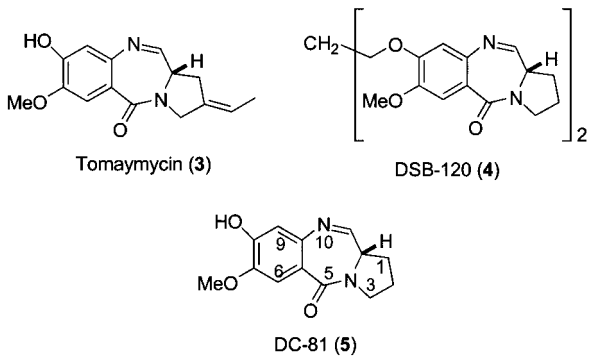

Figure 1. PBD analogues.

Nevertheless, it takes six steps to synthesize the starting material, (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**7**), from L-proline (**6**). The overall yield of this 10-step synthesis of DC-81 is about 15−20%.[4,6] More recently, Wang et al. reported the total synthesis of DC-81 over 13 steps in 4% yield.[5b]

Herein we would like to report a very short route and efficient synthesis of PBD analogue, DC-81. The synthesis started with reduction of substituted 2-nitrobenzoic acid (**8**)[6] with stannous chloride to give amine **10** in 92% yield (Scheme 3). Reaction of the resulting amine **10** with triphosgene in THF under reflux generated isatoic anhydride (**11**) in excellent yield (98%).[7] Compound **11** was coupled to L-proline in DMSO at 120 °C to produce dilactam **12**, followed by conversion of the amide to *N*-(10-methoxymethyl)-8-benzyloxy-7-methoxypyrrolo[2,1-*c*][1,4]-benzodiazepine-5,11-dione (**13**) with MOMCl in high yield.

The key step of this synthesis is hydride reduction of MOM-protected dilactam **13**. Mori reported that the imine form of PBD analogues could be prepared via reduction of MOM-protected dilactam with 10 molar equiv of $NaBH_4$ in MeOH at 0 °C, followed by silica gel chromatography.[8] Unfortunately, after the MOM-protected dilactam **13** was prepared, as Thurston reported,[9] it also failed in our hands to afford the corresponding imine **14**, using either the reported[8] condition or a number of variations. Instead, ring-opening products were obtained via 3-aza-Grob fragmentation.[10] In light of straightforward reaction sequence consideration, we explored this step with different reagents for conversion of compound **13** to its imine form. After careful studies, we found that MOM-protected dilactam **13** was successfully converted to benzyl DC-81 (**14**) in 50% yield (95% on the basis of the recovered starting material), by treating with $LiBH_4$ (1 molar equiv) in THF at −10 °C for 9 h. Further attempts to complete the reaction with longer reaction time, more reagents, or higher temperature would produce overreduction amine product. Finally, benzyl DC-81 (**14**) was converted to DC-81 (**5**) as reported.[6] The overall yield of the six-step synthesis of

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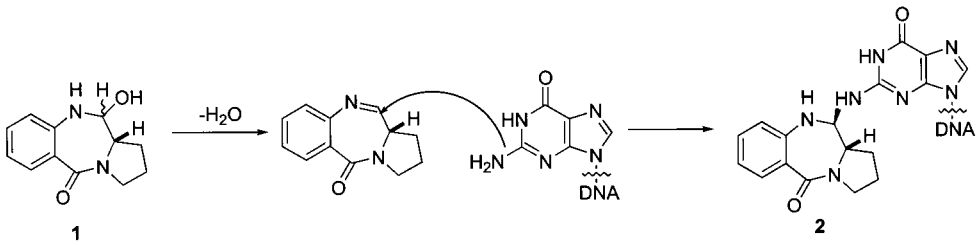
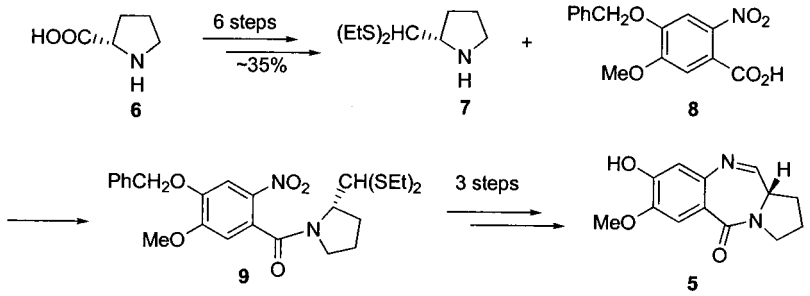
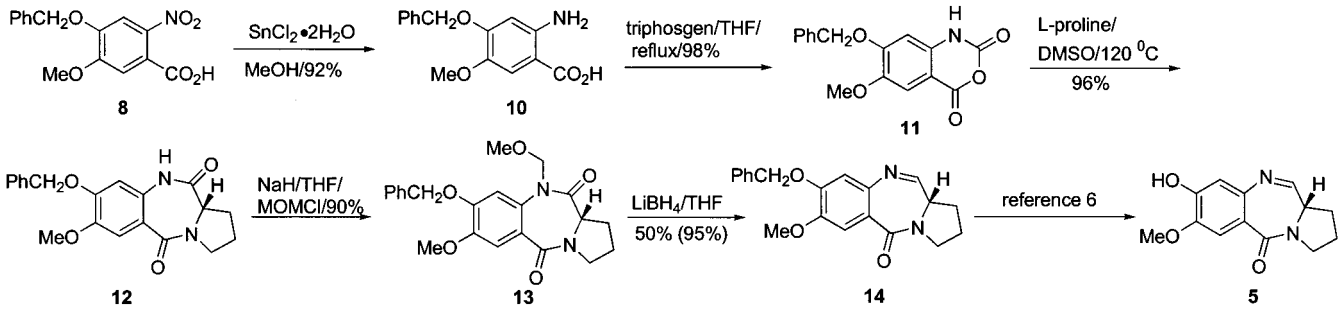
Scheme 1. Possible Mechanism of the Formation of PBD−DNA Adduct 2



Scheme 2. Synthesis of DC-81 (5) via Cyclization of Amino Dithioacetal 9



Scheme 3. Total Synthesis of DC-81 (5)[a]



[a] Yield in parentheses is based upon the recovered starting material.

DC-81 is about 35% (67% yield based upon the recovered starting material **13**, at the fifth step). The other advantage of this methodology is that the reaction can be carried out at much larger scale (10 g) than previously reported syntheses. Furthermore, in the first three steps, the products were easily recrystallized and pure enough for next subsequent reactions. The intermediate **11** can serve as a versatile leaping point for further analogue synthesis to establish the SAR of substituted prolidine C ring.

In conclusion, we have described a practical and large-scale total synthesis of antibiotic DC-81. The efficiency and adaptability of the synthetic procedure detailed above make possible the application of this methodology to the preparation of other PBD analogues, such as compound **3** and **4**.

Experimental Section

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using $CDCl_3$ as a solvent. ^{1}H NMR chemical shifts are referenced to TMS or $CDCl_3$ (7.26 ppm). ^{13}C NMR was referenced to $CDCl_3$ (77.0 ppm). Multiplicities were determined by the DEPT sequence as s, d, t, q. Mass spectra and high-resolution mass spectra (HRMS) were measured using the electron-impact (EI, 70 eV) technique by Taichung Regional Instrument Center of NSC at NCHU. Elemental analyses were performed by Tainan Regional Instrument Center of NSC at NCKU. Flash chromatography was carried out on silica gel 60 (E. Merck, 230−400 mesh).

2-Amino-4-benzyloxy-5-methoxybenzoic acid (10). A solution of substituted nitrobenzoic acid **8** (18.18 g, 60 mmol) and $SnCl_2 \cdot 2\,H_2O$ (137 g, 0.6 mol) in MeOH (800 mL) was stirred at 70 °C for 5 h. The mixture was concentrated under vacuum to a thick syrup; then ethyl acetate (300 mL) was added. The organic phase was washed with water until it turned to a clear solution, and then it washed with brine and dried over $MgSO_4$. After removal of solvent, the crude material was recrystallized from ethyl acetate to afford a yellow solid. **10**: yield 15.1 g (92%); mp 159−161 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz) δ 7.43−7.29 (m, 6H), 6.22 (s, 1H), 5.12 (s, 2H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz) δ 169.6 (s), 153.5 (s), 146.9 (s), 140.3 (s), 136.1 (s), 128.2 (d), 127.6 (d), 126.8 (d), 114.0 (d), 102.4 (s), 100.8 (d), 70.0 (t), 56.3 (q); LRMS (EI, *m*/*z*) 273 (M^+); HRMS (EI, *m*/*z*) for $C_{15}H_{15}NO_4$ calcd 273.1002, found 273.0995. Anal. Calcd for $C_{15}H_{15}NO_4$: C, 65.93; H, 5.53; N, 5.13. Found: C, 65.67; H, 5.53; N, 4.89.

7-Benzyloxy-6-methoxyisatoic anhydride (11). To a solution of compound **10** (20.1 g, 73.6 mmol) in THF (300 mL) was added triphosgene (16.2 g, 51.5 mmol) in one portion. The reaction mixture was refluxed for 3 h. After being cooled to room temperature, the solution was poured into ice/water. The resulting precipitate was filtered and recrystallized from MeOH to give a white solid **11**: yield 21.5 g (98%); mp 232−235 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz) δ 11.44 (s, NH), 7.47−7.33 (m, 6H), 6.74 (s, 1H), 5.21 (s, 2H), 3.89 (s, 3H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz) δ 159.5 (s), 156.0 (s), 147.9 (s), 146.4 (s), 137.6 (s), 135.3 (s), 128.6 (d), 128.3 (d), 127.6 (d), 109.2 (d), 101.7 (s), 99.4 (d), 70.9 (t), 56.2 (q); LRMS (EI, *m*/*z*) 299 (M^+); HRMS (EI, *m*/*z*) for $C_{16}H_{13}NO_5$ calcd 299.0794, found 299.0792. Anal. Calcd for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.60. Found: C, 63.99; H, 4.43; N, 4.50.

8-Benzyloxy-7-methoxypyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (12). The mixture of anhydride **11** (15.9 g, 53.2 mmol) and L-proline (8.2 g, 68.4 mmol) in DMSO (300 mL) was heated at 120 °C for 4 h. After being cooled to room temperature, the solution was poured into water (600 mL) and kept in freezer for 6 h. The resulting precipitate was filtered and recrystallized from MeOH to give a white solid. **12**: yield 18.0 g (96%); mp 190−193 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (br s, NH), 7.43−7.33 (m, 5H), 7.27 (s, 1H), 6.45 (s, 1H), 5.17 (s, 2H), 4.30 (d, 3.2 Hz, 1H), 3.93 (s, 3H), 3.78−3.75 (m, 1H), 3.63−3.56 (m, 1H), 2.74−2.70 (m, 1H), 2.05−1.97 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8 (s), 165.2 (s), 151.4 (s), 147.0 (s), 135.9(s), 129.2 (s), 128.8 (d), 128.3 (d), 127.2 (d), 119.8 (s), 112.6 (d), 106.5 (d), 71.1 (t), 56.8 (d),. 56.2 (q), 47.3 (t), 26.2 (t) 23.5 (t); LRMS (EI, *m*/*z*) 352 (M^+); HRMS (EI, *m*/*z*) for $C_{20}H_{20}N_2O_4$ calcd 352.1424, found 352.1429. Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.01; H, 5.84; N, 7.80.

***N*-(10-Methoxymethyl)-8-benzyloxy-7-methoxypyrrolo-[2,1-*c*][1,4]benzodiazepine-5,11-dione (13).** To a stirred solution of aromatic lactam **12** (12 g, 34.1 mmol) in THF (150 mL) was added NaH (3.4 g, 85.3 mmol) under nitrogen at 0 °C, and the reaction mixture was stirred at the same temperature for 30 min. MOMCl (6.4 mL, 77.6 mmol) was added dropwise into the reaction mixture. The resulting solution was stirred at room temperature for 24 h. The reaction mixture was poured into ice/water (150 mL) and extracted four times with ethyl acetate. The combined organic phases were washed with saturated $NaHCO_3$, H_2O, and brine, and dried over $MgSO_4$. After removal of solvent, the residue was purified by flash chromatography (CH_2Cl_2/MeOH = 40:1) to give a yellow oil product. **13**: yield 12.2 g (90%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45−7.29 (m, 6H), 7.18 (s, 1H), 5.37 (d, J = 10 Hz, 1H), 5.21 (s, 2H), 4.44 (d, J = 10 Hz, 1H), 4.09 (dd, J = 8, 2.4 Hz, 1H), 3.95 (s, 3H), 3.77−3.72 (m, 1H), 3.59∼3.52 (m, 1H), 3.40 (s, 3H), 2.72−2.68 (m, 1H), 2.11−1.94 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3 (s), 165.1 (s), 150.7 (s), 147.5 (s), 136.0 (s), 133.7 (s), 128.6 (d), 128.1 (d), 127.4 (d), 122.3 (s), 111.5 (d), 107.1 (d), 79.7 (t), 71.0 (t), 57.5 (d), 56.8 (q), 56.2 (q), 46.7 (t), 26.5 (t) 23.7 (t); LRMS (EI, *m*/*z*) 396 (M^+); HRMS (EI, *m*/*z*) for $C_{22}H_{24}N_2O_5$ calcd 396.1686, found 396.1688. Anal. Calcd for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.42; H, 6.20; N, 6.85.

8-Benzyloxy-7-methoxypyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (14, 8-Benzyl DC-81). To a solution of MOM-protected aromatic lactam **13** (9.6 g, 23.2 mmol) in THF (50 mL) was added lithium borohydride (516 mg, 23.2 mmol) in one portion at −10 °C, and the reaction mixture was stirred at same temperature for 8 h. The reaction mixture was poured into ice/water (100 mL) and extracted four times with ethyl acetate. The combined organic phases were washed with saturated $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After removal of solvent, the residue was purified by flash chromatography (CH_2Cl_2/MeOH = 70:1) to give a light yellow solid product **14** (yield 3.89 g, 50%) and compound **13** (4.77 g, 95% yield based upon the recovered starting material): mp 58−61 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47−7.29 (m, 6H), 7.18 (s, 1H), 5.38 (d, J = 9.8 Hz, 1H), 5.21 (s, 2H), 4.45 (d, J = 9.8 Hz, 1H), 4.12−4.07 (m, 1H), 3.95 (s, 3H), 3.75−3.54 (m, 2H), 3.40 (m, 3H), 2.70 (t, J = 2.0 Hz, 1H), 2.06−1.95 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.6 (s), 162.4 (d), 150.4 (s), 148.0 (s), 140.5 (s), 136.2 (s), 128.6 (d), 128.0 (d), 127.3 (d), 120.5 (s), 111.7 (d), 111.3 (d), 70.8 (t), 56.1 (q), 53.6-(d), 46.6 (t), 29.6 (t) 24.1 (t); LRMS (EI, *m*/*z*) 336 (M^+); HRMS (EI, *m*/*z*) for $C_{20}H_{20}N_2O_3$ calcd 336.1475, found 336.1473.

8-Hydroxy-7-methoxypyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5, DC-81). To a solution of compound **14** (5.93 g, 17.64 mmol) in absolute EtOH (130 mL) was added 10% Pd/C (8.8 g) under nitrogen.[6] 1,4-Cyclohexadiene (17 mL, 176 mmol) was added to the solution dropwise. The resulting solution was stirred at room temperature for 2.5 h until TLC (reversed-phase C_{18}: H_2O/MeOH = 1:3) indicated that the reaction was complete. The reaction mixture was filtered through Celite. Purification of the residue by flash chromatography (CH_2Cl_2/MeOH = 40:1) gave a colorless solid product. **5**: yield 3.91 g (90%); mp 135−138 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (d, J = 4.4 Hz, 1H), 7.52 (s, 1H), 6.89 (s, 1H), 6.41 (br s, −OH), 3.96 (s, 3H), 3.85−3.79 (m, 1H), 3.74−3.70 (m, 1H), 3.61−3.54 (m, 1H), 2.35−2.29 (m, 2H), 2.09−2.01 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ164.8 (s), 162.6 (d), 148.5 (s), 145.5 (s), 141.2 (s), 120.0 (s), 112.7 (d), 111.2 (d), 56.3 (q), 53.7 (d), 26.6 (t) 24.2 (t); LRMS (EI, *m*/*z*) 246 (M^+); HRMS (EI, *m*/*z*) for $C_{13}H_{14}N_2O_3$ calcd 246.1005, found 246.0996.

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Supporting Information Available: Proton and carbon spectra for compounds **10**−**14** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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