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NEW APPROACHES TO PYRROLO[2,1-c][1,4]BENZODIAZEPINES: SYNTHESIS, DNA-BINDING AND CYTOTOXICITY OF DC-81

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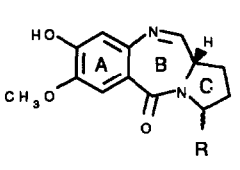
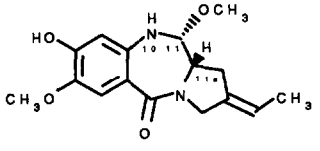
Abstract: *Two routes to the naturally occurring DNA-binding antitumour antibiotic DC-81 are described, one of which involves a novel cyclization process based on acid resin. The second route involves the synthesis of a new compound, 6-nitrovanillic acid, a key A-ring component of many naturally occurring PBDs. These routes have provided a sufficient quantity of DC-81 to allow complete characterization and evaluation in DNA-binding and in vitro cytotoxicity studies.*

The carbinolamine-containing pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are a group of potent, naturally occurring antitumour antibiotics from *Streptomyces* species, well-known members of which include anthramycin, tomaymycin and DC-81[1,2]. They exert their biological activity through sequence-selective covalent binding to the N2 of guanine in the minor groove of DNA, *via* an electrophilic imine or carbinolamine functionality at N10-C11. An (S)-stereochemistry at the C11a position provides the molecules with the appropriate three-dimensional shape to fit perfectly in the minor groove of DNA. Recent studies have shown that the PBDs recognize a three base-pair motif with a preference for purine-guanine-purine triplets[3]. There is presently considerable interest in compounds of this type because of their potential as antitumour agents, gene regulators and DNA probes[4].



DC-81: R = H (1)
NEOTHRAMYCIN: R = OH (2)

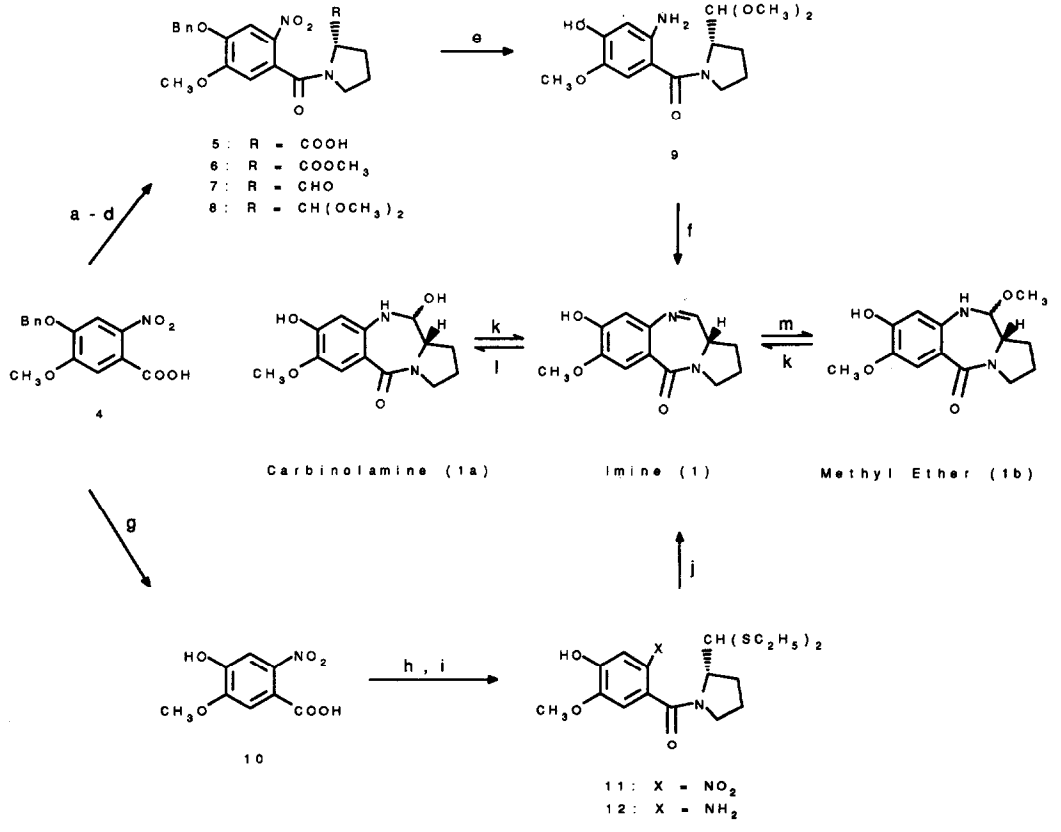


TOMAYMYCIN (3)

The synthesis of PBDs is generally problematic due to the instability of the N10-C11 imine moiety (or the carbinolamine or methyl ether equivalent) which is usually incorporated at the final synthetic step[5]. All reported syntheses of PBD analogs with phenolic A-rings, including DC-81 (**1**), neothramycin (**2**), tomaymycin (**3**) and chicamycin, have involved initial protection of the phenolic hydroxyl followed by deprotection at the penultimate synthetic step[2]. This was thought to be necessary due to the possible formation of degradation products over the course of several synthetic steps. In particular, two syntheses of DC-81 have been reported[6], one of which provides low yields due to the necessity of cleaving an A-ring benzyl ether at the final synthetic step and in the presence of a reactive N10-C11 imine species[6b]. We have now developed two new routes that have provided a sufficient

quantity of DC-81 to allow complete characterisation, and biological and biochemical evaluation. These routes should be generally applicable to other members of the PBD family, and illustrate that protection of the phenolic A-ring hydroxyl is not required.

4-Benzyloxy-5-methoxy-2-nitrobenzoic acid **4** is an important intermediate in the synthesis of many PBD analogs and was required for the synthesis of DC-81[6b]. It was converted to the acid chloride (oxalyl chloride/THF), coupled to (S)-proline (**5**, Et_3N/H_2O), and then methylated (oxalyl chloride/CH_3OH) to give the nitro ester **6** in 95% yield. Reduction to the aldehyde (**7**) with DIBAL (toluene/-78°C) was followed by conversion to the dimethyl acetal (**8**) using Dowex 50X resin and trimethyl orthoformate. Catalytic hydrogenation (H_2/Pd-C) of **8** led to reduction of the nitro group with simultaneous deprotection of the benzyl ether to provide **9** in 52% yield. The novel cyclisation procedure involved treatment of a solution of the amino acetal **9** in CH_3CN/H_2O with Amberlite IR-120(H^+) resin. Reaction was complete within four hours, and DC-81 was obtained in the N10-C11 imine form (**1**) in 47% yield.



Reagents: a, $(COCl)_2$/THF/(S)-Proline; b, $(COCl)_2/CH_3OH$; c, DIBAL-H/Toluene/-78°C; d, $(CH_3O)_3CH$/Dowex 50X resin/CH_2Cl_2; e, 10% Pd-C/H_2/EtOH; f, Amberlite IR-120(H^+)/CH_3CN/H_2O; g, $BF_3.OEt_2$-EtSH or 30% HBr/AcOH; h, $(COCl)_2$/THF/(2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal/Et_3N/H_2O; i, $SnCl_2.2H_2O$/$CH_3OH/\Delta H$; j, $HgCl_2/CaCO_3/CH_3CN$:H_2O (4:1); k, $CHCl_3$; l, H_2O; m, CH_3OH.

An alternative approach was developed based on the well-established method[7] of $HgCl_2$-induced cyclisation of an amino thioacetal of type **12.** In one previously reported route to DC-81[6b], the phenolic hydroxyl was initially protected as a benzyl ether which was cleaved at the final step. This strategy has two disadvantages. First, the N10-C11 imine species is highly reactive and is usually only generated at the final synthetic step. Therefore, a further step such as deprotection is undesirable after the imine has been formed. Second, certain hydrogenolytic conditions used to remove the benzylic protective group can reduce the N10-C11 imine to the secondary amine (-NH-CH_2-) which is difficult to separate chromatographically leading to reduced yields[5], and is biologically inert. To overcome these problems, we have synthesised the previously unknown 6-nitrovanillic acid (**10**) which is likely to be a generally useful starting material for PBDs with the 8-hydroxy-7-methoxy substitution pattern.

O-Debenzylation of **4** was initially problematic due to the presence of nitro and methoxy groups sensitive to many of the known reagents useful for this process. For example, catalytic transfer hydrogenation[8] using 1,4-cyclohexadiene/10% Pd-C proceeded to completion within 45 min although partial reduction (approx. 15%) of the nitro group occurred, necessitating chromatographic separation. TMS-I/CH_2Cl_2[9] gave approximately 65% yield of **10** but was not suitable for large scale preparations. Although 30% HBr/AcOH completely removed the benzyl group in 0.5 h (77% yield), $BF_3.OEt_2$/EtSH combination[10] was found to be the most efficient method, providing pure **10** in 90% yield after 5 h at room temperature.

Treatment of (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal[7] with the acid chloride derived from **10** afforded the nitro thioacetal (**11**) in high yield with no apparent interference by the unprotected phenolic hydroxyl. Reduction with $SnCl_2.2H_2O$ (ΔH/methanol) afforded the amino thioacetal **12** in 86% yield. Cyclization[7] of **12** with $HgCl_2$ in CH_3CN/H_20 (4:1) for 2.5 h at room temperature afforded DC-81 (**1**) directly in 80% yield. To our knowledge this represents the first example of the synthesis of a C8-hydroxyl substituted PBD without the use of a hydroxyl protective group. The synthetic DC-81 was purified by column chromatography on silica gel, and was eluted (3% $CH_3OH/CHCl_3$) in the N10-C11 imine form as a stable yellow oil. The optical rotation of this product ($[\alpha]_D23$ = +371 (c = 0.68, $CHCl_3$)) was significantly higher than that reported for synthetic DC-81[6b] ($[\alpha]_D23$ = +310 (c = 0.02, $CHCl_3$)) or for the natural product[11] ($[\alpha]_D22$ = +135 (c = 0.20, $CHCl_3$)).

The electrophilic properties of this compound were studied[12] by dissolving it in CD_3OD and monitoring nucleophilic attack at C11 by ^{1}H-NMR. The first spectrum examined (after 4.5 min) indicated that reaction with CD_3OD was already complete. Based on chemical shifts and coupling patterns for the N10, C11, C11-O$\underline{CH_3}$ and C11a protons[5], it was evident that nucleophilic attack had occurred stereospecifically from the β-face of the molecule to give an initial predominance of the C11(S) adduct (**1b**). However, slow conversion to a predominance of the C11(R) diastereomer occurred over 24 hours, suggesting that it is the thermodynamically-preferred form. Conversion of the methyl ether forms (**1b**) back to the N10-C11 imine (**1**) could be achieved by two cycles of dissolving in $CHCl_3$ followed by evaporation of the solvent *in vacuo*. Similarly, treatment of the imine with H_2O afforded the corresponding carbinolamine forms (**1a**).

In vitro cytotoxicity was examined in three different cell lines, L1210, PC6 and CH1[13]. IC_{50} values of 0.38, 0.33 and 0.1μM were observed respectively, denoting significant cytotoxicity. Footprinting studies were carried out using linearised 5'-end labelled pBR322 DNA[13]. Electrophoresis on a polyacrylamide gel resolved approxi-

mately the first 300 base-pairs. After incubation with DC-81 (synthesized by the amino thioacetal route) at 37°C for 24 hours, significant binding was observed at a number of sites. In common with other PBDs, purine-guanine-purine triplets appeared to be preferred[3]. However, one particularly strong binding site was observed at a 3'-AGA triplet flanked on both sides by two thymine bases (3'-TTAGATT). The overall pattern of sequence-selectivity was similar to that for tomaymycin under identical conditions although, in general, binding affinity was significantly less. As the A-ring fragments of both compounds are identical, this demonstrates that a C3-ethylidene moiety plays a crucial role in enhancing binding affinity. This appears to be reflected in the higher cytotoxicity of tomaymycin in the same cell lines (IC_{50} μM: L1210 = 0.0037; PC6 = 0.0018; CH1 = 0.00013).

Interestingly, DC-81 obtained by the amino acetal/acid resin route appeared to be racemic. This was supported by lower cytotoxicity in the same cell lines under identical conditions (IC_{50} μM: L1210 = 5.3; PC6 = 2.7; CH1 = 0.3). Acid resin was chosen as a mild reagent for the cyclization process, as there was some evidence from the literature that strong acid conditions may cause racemization at the acetal-bearing carbon in amino acetals of type **9**, adversely affecting the DNA-binding potential of final products[7]. Although racemization of DC-81 synthesized by this route could have occurred during either work-up or storage, this result suggests that the thioacetal/$HgCl_2$ route should be utilized if optical activity in the final product is crucial.

EXPERIMENTAL

All reagents and reaction solvents were purchased from Aldrich Chemical Co. Solvents were dried and distilled prior to use. Petroleum ether refers to the fraction boiling between 60-80°C. Analytical silica gel 60 GF_{254} TLC plates with fluorescent indicator were used. Flash chromatography was carried out with silica gel grade 60 (230-400 mesh) purchased from the Aldrich Chemical Co. Melting points (m.p.) were determined on a Gallenkamp P1384 digital melting point apparatus and are uncorrected. Infrared spectra were recorded using a Perkin-Elmer 297 spectrophotometer. ^{1}H- and ^{13}C-NMR were recorded on a JEOL GSX 270MHz FT-NMR spectrometer operating at 293K ± 1K. Mass spectra (MS) were recorded using a JEOL JMS-DX 303 GC-MS mass spectrometer (EI mode: 70 eV, source 390-420K; CI gas: isobutane). Accurate molecular masses (HRMS) were determined by peak matching using perfluorokerosene (PFK) as an internal mass marker. Optical rotations at the Na-D line were obtained at ambient temperature using a Perkin-Elmer 141 Polarimeter.

(2S)-N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2- carboxylic acid (5):

4-Benzyloxy-5-methoxy-2-nitrobenzoic acid **4** (5.0g, 16.5mmol) and oxalyl chloride (2.51g, 19.8mmol) were dissolved in anhydrous toluene (50ml) and DMF (4 drops) added. Stirring at RT was continued until all solid material had dissolved and the liberation of CO_2 had ceased. The toluene was evaporated *in vacuo*, the residue dissolved in anhydrous THF (20ml), and added dropwise over 20 min to a stirred mixture of L-proline (1.91g, 16.6mmol) and triethylamine (3.53g, 34.9mmol) in water (25ml) at 0°C. The mixture was then allowed to reach RT and stirred for a further 30 min, after which time the THF was evaporated *in vacuo*. The resultant aqueous residue was acidified to pH 1.0 with HCl (11.5M) and extracted with EtOAc (3 x 25ml). The combined organic phase was washed with water (2 x 50ml), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a white sticky solid. Recrystallisation from ethanol/water afforded 5.35g (81%) of **5** as a yellow amorphous solid: m.p. 171-173°C; ^{1}H-NMR ($CDCl_3$): δ 1.89-2.06 (m, 2H), 2.25-2.34 (m, 2H), 3.13-3.22 (m, 1H), 3.26-3.34 (m, 1H),

4.00 (s, 3H), 4.79 (dd, 1H, J_1 = 7.4Hz, J_2 = 5.4Hz), 5.22 (s, 2H), 6.89 (s, 1H), 7.26-7.48 (m, 5H), 7.77 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 24.6, 28.8, 48.9, 56.9, 59.4, 71.4, 108.9, 109.5, 126.7, 127.6, 128.5, 128.6, 128.8, 128.9, 135.2, 137.0, 148.4, 155.0, 168.2, 173.9; IR (KBr): ν 3650-3300, 3200-2700, 1725, 1630, 1580, 1520, 1445, 1335, 1285, 1220, 1067, 761, 702 cm^{-1}; MS (EI) m/z (relative intensity): 400 ($M^{+\cdot}$, 2%), 355, 322, 303, 286, 270, 241, 231, 196, 121, 105, 91 (100%), 70; HRMS: Calc. for 400.1271 ($C_{20}H_{20}N_2O_7$), found 400.1396.

Methyl (2S)-N-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxylate (6):

DMF (2 drops) was added to a suspension of the nitro acid **5** (3.0g, 7.5mmol) and oxalyl chloride (1.50g, 11.8mmol) in anhydrous toluene (30ml), and the mixture stirred at RT for 4.5 h until all solid material had dissolved and evolution of CO_2 had ceased. Methanol (20ml) was then added and the mixture stirred for a further 1h, after which time the solvent was evaporated *in vacuo* and the residue dissolved in EtOAc (50ml). This solution was washed with aq. $NaHCO_3$ (3 x 20ml), water (2 x 20ml), and then dried ($MgSO_4$) prior to evaporation *in vacuo* to afford 2.95g (95%) of pure **6** as a yellow oil: ^{1}H-NMR ($CDCl_3$): δ 1.94-2.10 (m, 3H), 2.12-2.31 (m, 1H), 3.18-3.20 (m, 1H), 3.30-3.42 (m, 1H), 3.81 (s, 3H), 3.92 (s, 3H), 4.73-4.76 (m, 1H), 5.21 (s, 2H), 6.87 (s, 1H), 7.35-7.52 (m, 5H), 7.76 (s, 1H); IR (neat): ν 3100-2800, 2940, 1730, 1635, 1510, 1418, 1330, 1270, 1205, 1150, 1057, 750, 695 cm^{-1}; MS (EI) m/z (relative intensity): 414 ($M^{+\cdot}$, 14%), 355, 323, 286, 270, 241, 196, 121, 105, 91 (100%), 65.

(2S)-N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde (7):

The amide **6** (2.0g, 4.8mmol) was dissolved in anhydrous toluene (75ml) and diisobutylaluminium hydride solution (9.6mmol; 6.4ml of a 1.5M solution in toluene) added dropwise over 30 min at -78°C (dry ice/acetone) under a N_2 atmosphere. The mixture was maintained at -78°C for a further 45 min until TLC (EtOAc/petroleum ether, 1:1) indicated complete reaction. The mixture was then quenched with methanol (10ml) and then HCl (1M, 30ml) and allowed to warm to RT. The organic layer was separated and the aqueous phase extracted with EtOAc (4 x 25ml). The combined organic phase was backwashed with water (2 x 100ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford a yellow oil. Purification by flash chromatography ($CHCl_3$/CH_3OH, 95:5) afforded 0.80g (43%) of **7** as an amorphous yellow solid: m.p. 164-166°C; ^{1}H-NMR ($CDCl_3$): δ 1.88-1.97 (m, 2H), 2.10-2.24 (m, 2H), 3.18-3.33 (m, 2H), 4.00 (s, 3H), 4.69 (t, 1H, J = 6.3Hz), 5.22 (s, 2H), 6.87 (s, 1H), 7.32-7.48 (m, 5H), 7.78 (s, 1H), 9.79 (d, 1H, J = 1.8Hz); ^{13}C-NMR ($CDCl_3$): δ 24.7, 26.3, 48.5, 56.7, 64.8, 71.4, 109.0, 109.1, 127.4, 127.6, 128.6, 128.8, 135.2, 137.3, 148.3, 155.0, 167.1, 199.1; IR (KBr): ν 1725, 1630, 1568, 1510, 1440, 1420, 1325, 1273, 1210, 1050, 985, 860, 750 cm^{-1}; MS (EI) m/z (relative intensity): 355 ($M^{+\cdot}$-29, 5%), 286, 121, 91 (100%), 84, 65; MS (CI) m/z (relative intensity): 385 ($MH^{+\cdot}$, 32%), 323, 295, 171, 107, 86 (100%).

(2S)-N-(4-Benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde dimethyl acetal (8):

Trimethyl orthoformate (0.314g, 3.0mmol) and Dowex 50X acid resin (0.2g, washed in water, dilute HCl and finally water) were added to a solution of the nitro aldehyde **7** (0.5g, 1.3mmol) in anhydrous CH_2Cl_2 (5ml). The mixture was stirred at RT for 16 h until TLC ($CHCl_3$/CH_3OH, 95:5) indicated that reaction was complete. The resin was removed by filtration and the solvent evaporated *in vacuo* to afford an oil, which was purified by flash chromatography ($CHCl_3$/CH_3OH, 99:1) to afford 0.52g (93%) of **8** as an orange gum. This was used in the next step without purification: ^{1}H-NMR ($CDCl_3$): δ 1.67-1.79 (m, 1H), 1.92-2.10 (m, 2H), 2.17-2.28 (m, 1H), 3.06-3.21 (m, 2H), 3.57 (s, 6H), 3.97 (s, 3H), 4.42 (bs, 1H), 4.94 (d, 1H, J = 2.6Hz), 5.21 (s, 2H), 6.76 (s, 1H),

7.35-7.47 (m, 5H), 7.77 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 48.9, 56.6, 57.8, 59.0, 71.4, 104.8, 109.2, 127.6, 128.5, 128.8, 135.3, 137.3, 148.0, 154.8, 166.5; IR (neat): ν 3100-2880, 2940, 2830, 1635, 1576, 1515, 1420, 1331, 1275, 1210, 1060, 980, 750, 698 cm^{-1}; MS (EI) m/z (relative intensity): 430 ($M^{+\cdot}$, 1%), 414, 399, 355, 301, 286, 167, 149, 121, 91, 84 (100%), 79; MS (CI) m/z (relative intensity): 431 ($MH^{+\cdot}$, 58%), 415, 399, 385, 341, 309, 284, 146, 107, 86 (100%), 75.

(2S)-N-(2-Amino-4-hydroxy-5-methoxybenzoyl)pyrrolidine-2-carboxaldehyde dimethyl acetal (9):

The nitro acetal **8** (0.75g, 1.7mmol) was dissolved in methanol (5ml, HPLC-grade) and 10% Pd-C (0.075g) added. The mixture was hydrogenated at RT and atmospheric pressure for 20 min until TLC ($CHCl_3/CH_3OH$, 97:3) indicated that reaction was complete. The catalyst was removed by filtration through celite, and the solvent evaporated *in vacuo* to afford a residue which was further purified by column chromatography (EtOAc/petroleum ether, 95:5, silica gel) to afford 0.28g (52%) of **9** as a pale orange oil: ^{1}H-NMR ($CDCl_3$): δ 1.65-1.79 (m, 1H), 1.91-2.18 (m, 3H), 3.47 (s, 3H), 3.50 (s, 3H), 3.53-3.56 (m, 2H), 3.80 (s, 3H), 4.44 (bs, 2H), 4.74 (bs, 1H), 6.30 (s, 1H), 6.74 (s, 1H); IR (neat): ν 3600-2700, 3320, 2910, 1570, 1500, 1400, 1290, 1250, 1170, 1115, 1050, 740 cm^{-1}; MS (EI) m/z (relative intensity): 310 ($M^{+\cdot}$, 16%), 278, 235, 209, 193, 166 (100%), 138, 123, 95, 75, 70; MS (CI) m/z (relative intensity): 311 ($MH^{+\cdot}$, 100%), 279, 166.

4-Hydroxy-5-methoxy-2-nitrobenzoic acid (10):

Method A. 30% HBr in AcOH (5ml) was added slowly to a stirred solution of **4** (0.5g, 1.7mmol) in CH_2Cl_2 at 0°C for 10 min. Stirring was continued for a further 0.5 h until TLC ($CH_3OH/CHCl_3$, 1:9) indicated a complete loss of starting material. The reaction mixture was quenched with water (2 x 10ml), extracted with EtOAc (2 x 50ml), and the combined organic phase washed with water (2 x 20ml) and brine (2 x 15ml). After drying (Na_2SO_4), the solvent was removed by evaporation *in vacuo* to afford a brown gum which was crystallized from Et_2O/hexane to give 0.27g (77%) of **10** as brown needles.

Method B. EtSH (11.5g, 185.0mmol) and $BF_3.OEt_2$ (9.4g, 66.0mmol) were added dropwise to a stirred solution of **4** (2.0g, 6.6mmol) in CH_2Cl_2 (25ml) at 0°C. The mixture was stirred at RT for 5 h until TLC ($CH_3OH/CHCl_3$, 1:9) indicated complete loss of starting material. The reaction was quenched with water, extracted with EtOAc (3 x 100ml), and the combined organic phase washed with brine (2 x 50ml). After drying (Na_2SO_4), the solvent was removed by evaporation *in vacuo* to afford a crude brown solid which was crystallized from Et_2O/hexane to give 1.26g (90%) of **10** as yellow prisms: m.p. 174-176°C; ^{1}H-NMR ($CDCl_3$): δ 3.90 (s, 3H), 7.26 (s, 1H), 7.32 (s, 1H), 10.61 (bs, 1H), 13.4 (bs, 1H); ^{13}C-NMR ($CDCl_3$): δ 56.2, 110.3, 111.8, 119.4, 141.5, 148.6, 150.7, 166.0; IR (KBr): ν 3430, 3300-2380, 1700, 1600, 1520, 1460, 1420, 1335, 1270, 1225, 1210, 1045, 995, 875, 865, 805 cm^{-1}; MS (EI) m/z (relative intensity): 213 ($M^{+\cdot}$, 100), 183, 167, 152, 139, 124, 111, 107, 96, 91, 79, 69, 51; HRMS: Calc. for 213.0273 ($C_8H_7NO_6$), found 213.0294.

(2S)-N-(4-Hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carbaldehyde diethyl thioacetal (11):

DMF (2 drops) was added to a stirred suspension of 6-nitrovanillic acid **10** (0.7g, 3.3mmol) and oxalyl chloride (0.5g, 4.0mmol) in dry THF (15ml), and stirring continued for 5 h. The THF was removed by evaporation *in vacuo* and the resultant yellow residue dissolved in dry THF (10ml) and added dropwise over a period of 30 min to a vigorously stirred suspension of (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal[7] (0.81g, 3.9mmol),

Et_3N (0.74g, 7.3mmol) and ice/water (0.8ml) cooled in an ice bath. After addition was complete, the reaction mixture was warmed to RT and stirred for a further 1.5 h. After removal of the THF by evaporation *in vacuo*, the residue was diluted with water (2 x 25ml) and extracted with EtOAc (3 x 25ml). The aqueous phase was adjusted to pH 3 with conc. HCl and extracted with EtOAc (2 x 25ml). The combined organic phase was washed with water (3 x 20ml) and brine (2 x 30ml), dried ($MgSO_4$) and then concentrated *in vacuo* to afford a dark red oil which was purified by flash chromatography (EtOAc/hexane, 1:1; TLC : EtOAc/hexane, 3:2) to give the amide **11** (0.93g, 71%) as a pale yellow oil: ^{1}H-NMR ($CDCl_3$): δ 1.20-1.38 (t, 6H, J = 8Hz), 1.77-2.38 (m, 4H), 2.65-2.87 (m, 4H), 3.19-3.35 (m, 2H), 3.98 (s, 3H), 4.67-4.75 (m, 1H), 4.88 (d, 1H, J = 3.84Hz), 6.39 (bs, 1H), 6.81 (s, 1H), 7.70 (s, 1H); IR (neat): ν 3430, 1635, 1330 cm^{-1}

(2S)-N-(2-Amino-4-hydroxy-5-methoxybenzoyl)pyrrolidine-2-carbaldehyde diethyl thioacetal (12):

A solution of the nitro thioacetal **11** (0.25g, 0.63mmol) and $SnCl_2.2H_2O$ (0.57g, 2.5mmol) in methanol (20ml) was refluxed for 45 min until TLC (EtOAc/hexane, 3:1) indicated that reaction was complete. The solvent was removed by evaporation *in vacuo*, the residue cooled to 0°C and then quenched with water (2 x 20ml). The resulting viscous yellow liquid was triturated with EtOAc (2 x 40ml) and the mixture allowed to stir at RT for 1h. The resultant suspension was filtered through a short bed of Celite which was rinsed with more EtOAc (2 x 25ml). The combined organic phase was washed with water (2 x 25ml) and brine (2 x 30ml), then dried ($MgSO_4$) and concentrated under reduced pressure to afford a yellow foam which was further purified by flash chromatography (EtOAc/hexane, 4:1) to give **12** (0.20g, 86%) as a yellow oil: ^{1}H-NMR ($CDCl_3$): δ 1.21-1.36 (m, 6H), 1.63-2.31 (m, 4H), 2.65-2.74 (m, 4H), 3.54-3.70 (m, 2H), 3.78 (s, 3H), 4.66-4.70 (m, 4H), 6.27 (s, 1H), 6.79 (s, 1H), 7.28 (s, 2H, exch. with D_2O); ^{13}C-NMR ($CDCl_3$): δ 14.9, 15.1, 25.2, 26.5, 27.3, 29.7, 51.4, 53.3, 56.7, 60.9, 103.2, 111.3, 111.7, 138.8, 141.8, 148.8, 169.9; IR (neat): ν 3439, 3425, 3340, 1625cm^{-1}.

(11aS)-8-Hydroxy-7-methoxy-1,2,3,11a-tetrahydro-*5H*-pyrrolo[2,1-c][1,4]benzodiazepin-5-one (1):

Method A. Amberlite IR-120(H^+) resin (0.05g) was added to a solution of the amino acetal **9** (0.1g, 0.32mmol) in acetonitrile/water (8:2, 5ml). The mixture was stirred at RT for 4 h until TLC (EtOAc/petroleum ether, 9:1) indicated that reaction was complete. The resin was removed by filtration and the solvent evaporated *in vacuo* to afford a yellow gum. Purification by column chromatography (EtOAc/petroleum ether, 9:1, silica gel) afforded 0.037g (47%) of **1** as a yellow oil. NMR and MS data were identical to those for DC-81 obtained by Method B.

Method B. A solution of the amino thioacetal **12** (0.14g, 0.38mmol), $HgCl_2$ (0.25g, 0.92mmol) and $CaCO_3$ (0.094g, 0.94mmol) in CH_3CN/H_2O (4:1, 15ml) was stirred slowly at RT for 2.5 h until TLC (EtOAc/hexane, 4:1) indicated complete loss of starting material. After evaporation of the CH_3CN *in vacuo*, the residue was diluted with EtOAc (2 x 50ml), washed with aq. $NaHCO_3$ (2 x 30ml) and brine (2 x 25ml), and the combined aq. layer back-extracted with EtOAc (1 x 25ml). The combined organic phase was concentrated *in vacuo* to afford a residue, which was purified by flash chromatography ($CH_3OH/CHCl_3$, 1:4) to afford **1** (0.077g, 83%) as a yellow oil: ^{1}H-NMR ($CDCl_3$): δ 1.95-2.05 (m, 2H, 2 x H-1), 2.27-2.35 (m, 2H, 2 x H-2), 3.45-3.85 (m, 3H, 2 x H-3; H-11a), 3.92 (s, 3H, OCH_3), 6.89 (s, 1H, H-6 or H-9), 6.97 (bs, 1H, 8-OH, exch. with D_2O), 7.51 (s, 1H, H6 or H9), 7.66 (d, 1H, J = 4.6Hz, H-11); ^{13}C-NMR ($CDCl_3$): δ 24.2, 29.6, 46.7, 53.7, 56.2, 111.2, 112.8, 119.8, 141.0, 145.7,

148.7, 162.6, 164.8; MS (EI) m/z (relative intensity): 246 ($M^{+\cdot}$, 100%), 231 (23), 217 (10), 203 (5), 150 (10), 122 (11), 70 (48); HRMS: Calc. for 246.1004 ($C_{13}H_{14}N_2O_3$), found 246.1056; $[\alpha]_D23$ = +371 (c = 0.68, $CHCl_3$).

Formation of C11(R) and C11(S) Carbinolamine Methyl Ethers of DC-81 (1b):

The imine form of DC-81 (**1**) was dissolved in CD_3OD, and the NMR spectrum measured immediately (approximately 4 min) and again after 0.5, 1.0, 5 and 20 h. The imine signal (δ 7.66) had completely disappeared by 4.5 min and a mixture of the C11(R) and C11(S) methyl ethers (**1b**) had formed, with the C11(S) diastereomer predominating (60%). Racemization slowly occurred, so that after 20 h there was a predominance of the C11(R) diastereomer (60%). ^{1}H-NMR (CD_3OD): C11(R) Diastereomer, δ 1.81-2.27 (m, 4H), 3.29 (s, 3H), 3.50-3.96 (m, 3H), 3.80 (s, 3H), 4.55 (s, 1H), 6.21 (s, 1H), 7.36 (s, 1H), 7.90 (s, 1H); C11(S) Diastereomer, δ 1.81-2.27 (m, 4H), 3.32 (s, 3H), 3.50-3.96 (m, 3H), 3.83 (s, 3H), 4.38 (d, 1H, J = 9.0Hz), 6.45 (s, 1H), 7.2 (s, 1H), 7.90 (s, 1H).

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