Supplemental Information

Design, Synthesis and Biophysical and Biological Evaluation of a Series of Pyrrolobenzodiazepine–Poly(*N*-Methylpyrrole) Conjugates

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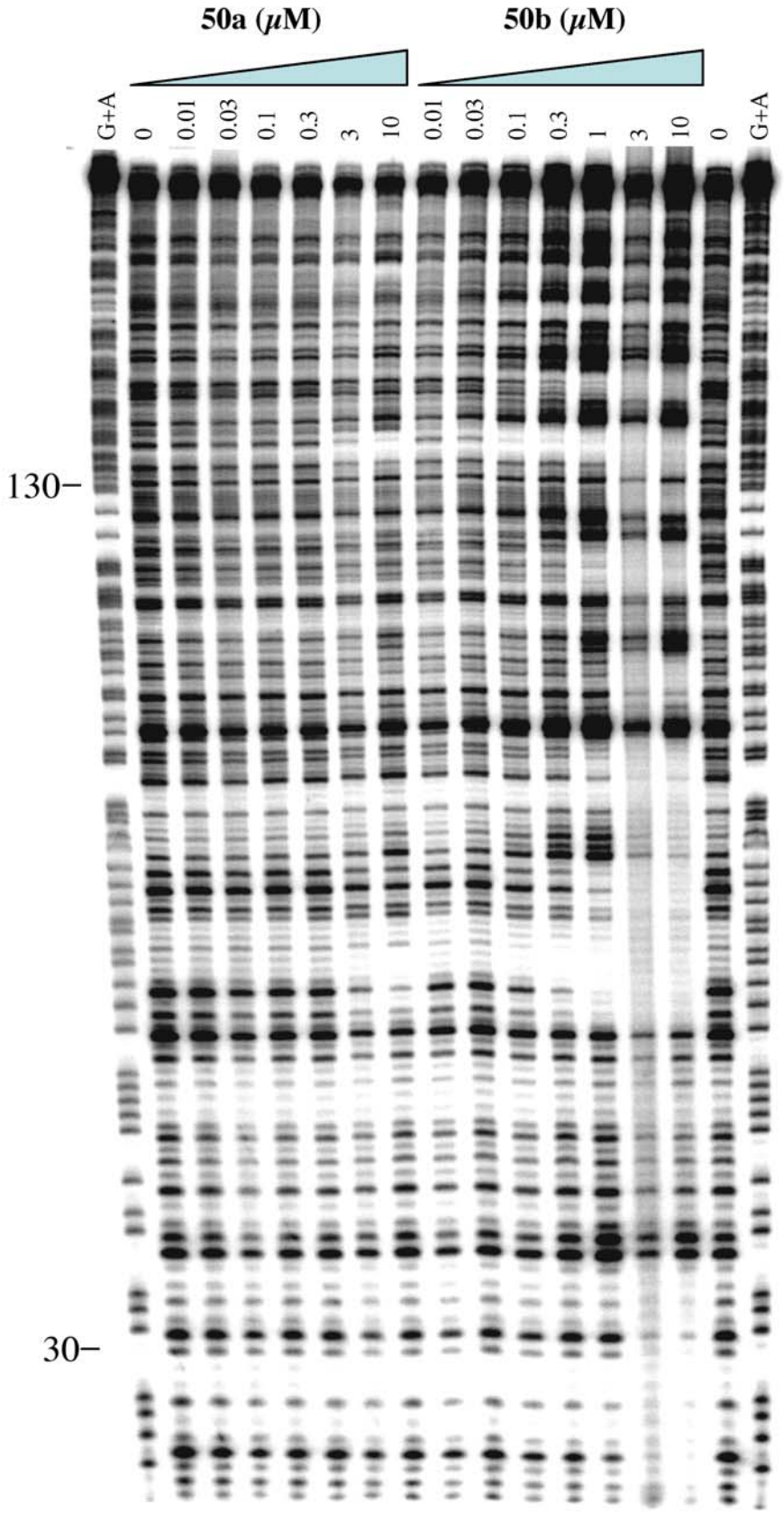
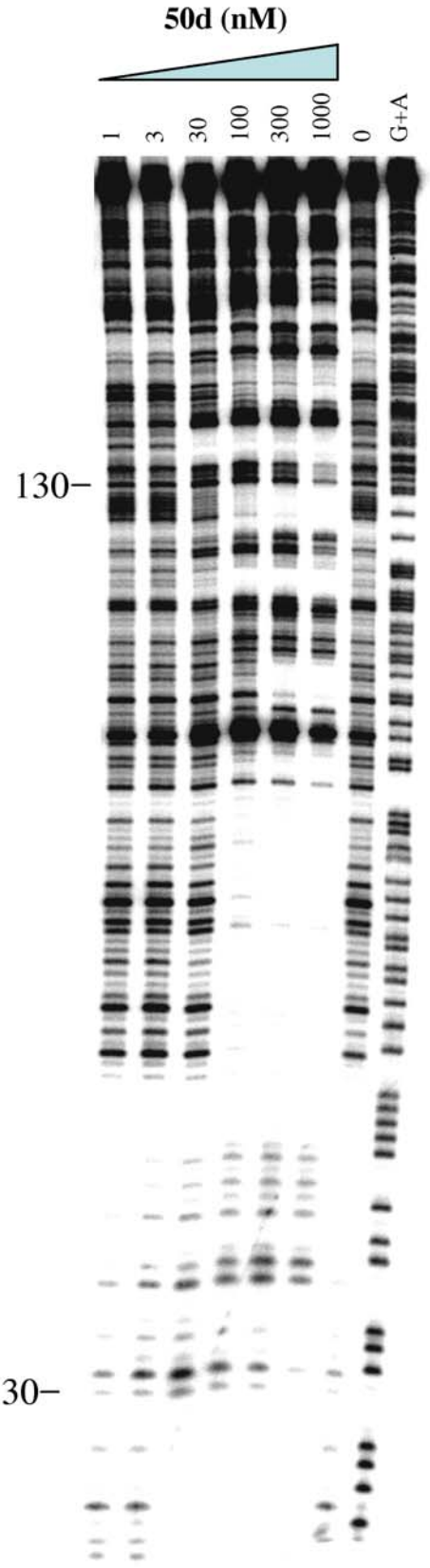
S1-A



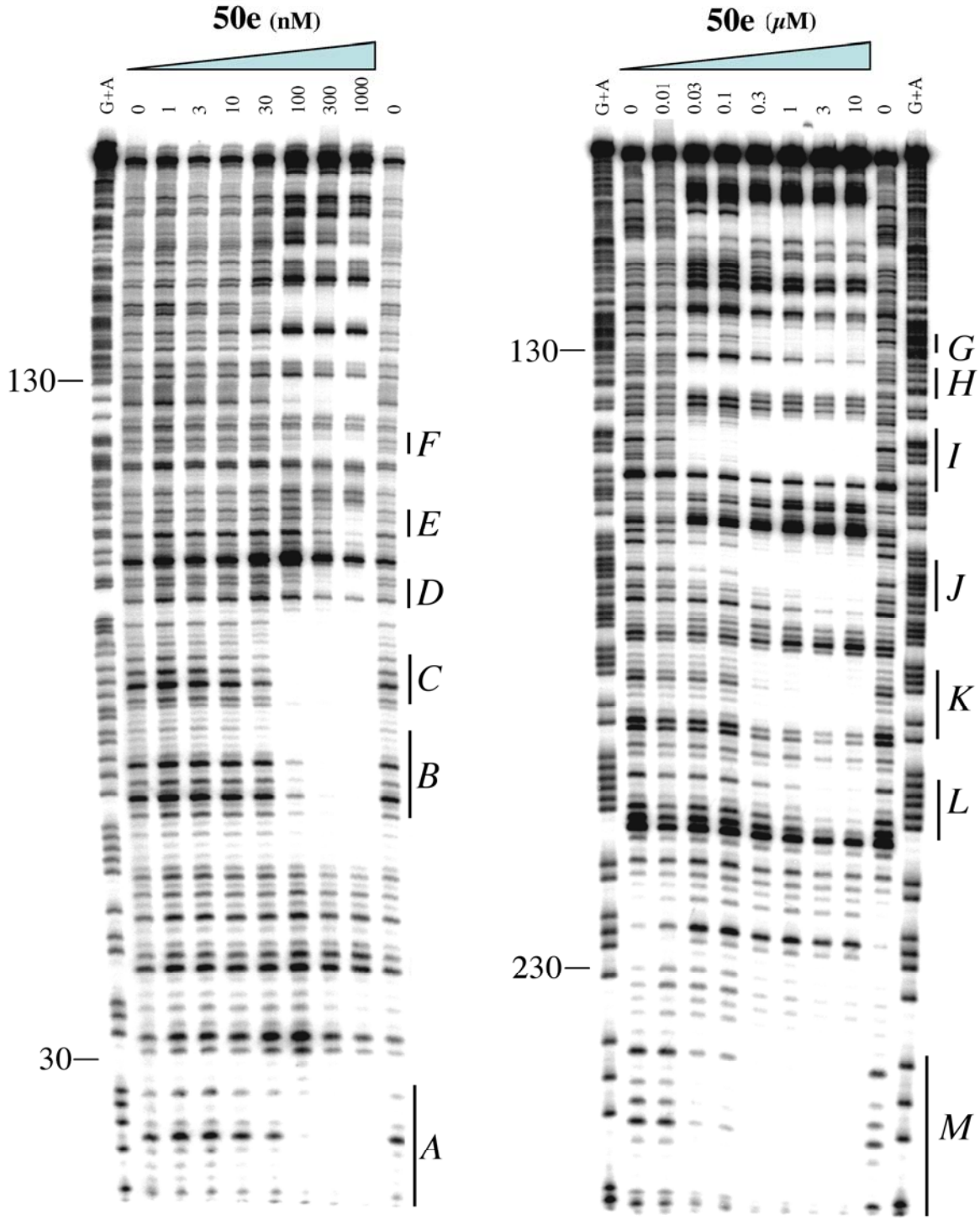
S1-A: Footprinting Gels for 50a and 50b (Forward-labelled MS2F DNA fragment).

S1B



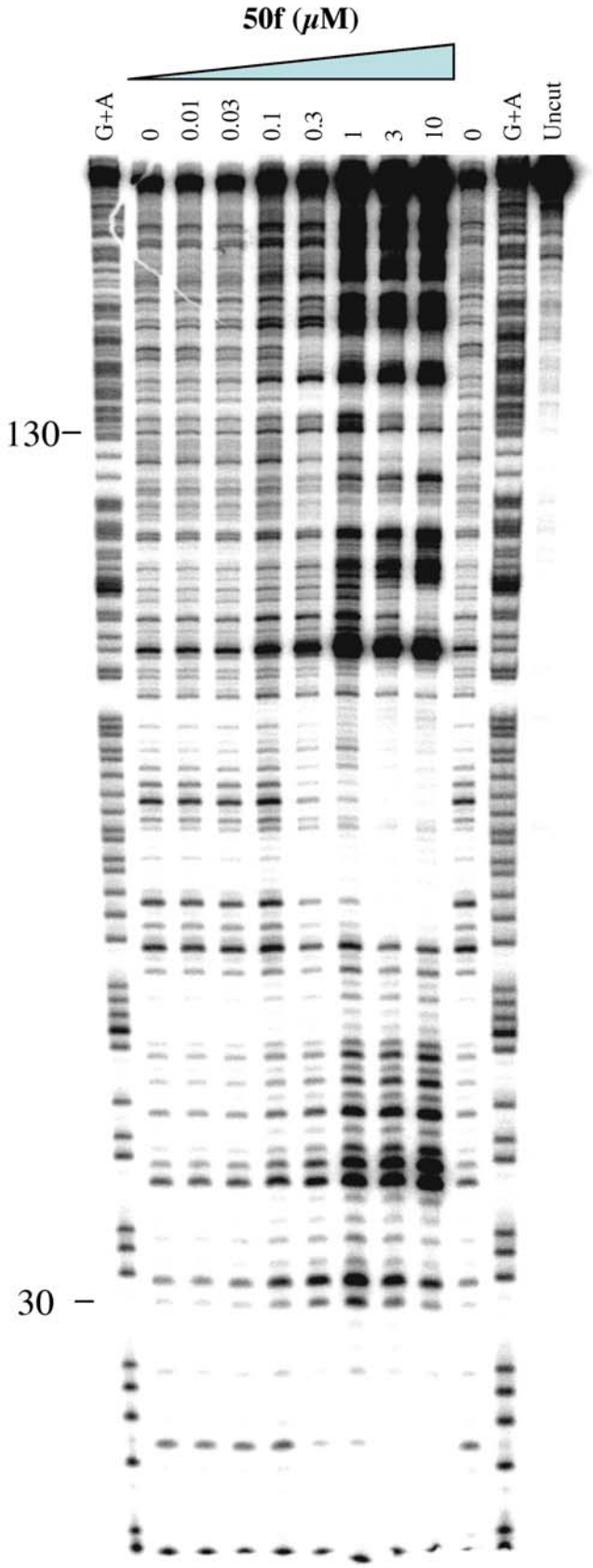
S1-B: Footprinting Gel for 50d (Forward-labelled MS2F DNA fragment).

S1-C.



S1-C: Footprinting Gels for 50e. Left Panel – Forward-labelled MS2F DNA fragment; **Right Panel** – Reverse-labelled MS2R DNA fragment. Strong footprints are indicated (by letters) adjacent to the binding sites. These letters correspond to those used to label footprints in **Figures 4 and 5** of the main text.

S1-D



S1-D: Footprinting Gel for 50f (Forward-labelled MS2F DNA fragment).

S2-A

Molecule \ Footprint Position	*A*	*B*	*C*	*D*	*E*	*F*
50a	-	+	+	-	-	-
50b	++	++	++	+	-	++
50c	+++	+++	+++	+++	+	+++
50d	+++	++	++	++	++	+
50e	++	++	++	++	+	++
50f	++	++	++	++	+	++

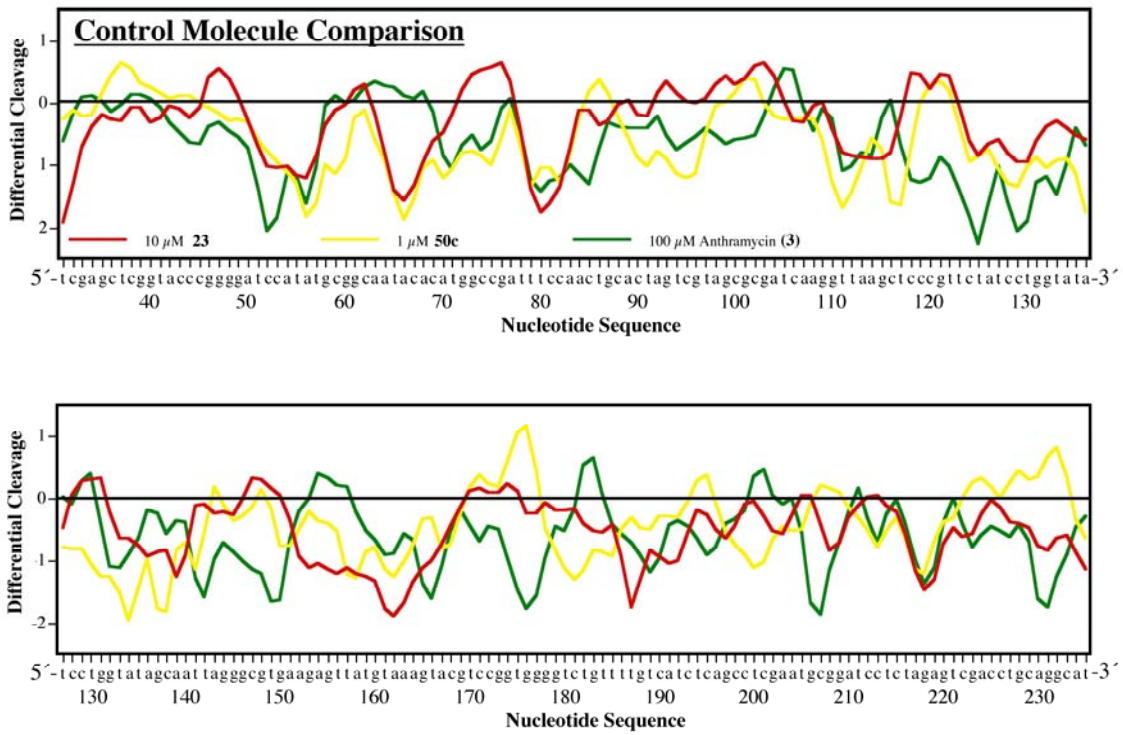
S2-A: Footprints analysed from the MS2F DNA (Forward-labelled DNA fragment). "+" indicates weak (*i.e.*, low micromolar) footprints; "++" indicates medium (*i.e.*, mid-nanomolar) footprints; "+++" indicates strong (*i.e.*, low nanomolar) footprints.

S2-B

Molecule \ Footprint Position	*G*	*H*	*I*	*J*	*K*	*L*	*M*
50a	+	+	+	+	+	+	+
50b	+	+++	++	+++	+++	++	++
50c	+++	+++	+++	++	++	+++	++
50d	++	+++	++	++	++	++	+++
50e	++	+++	+++	+++	++	++	+++
50f	++	++	++	+	-	-	+

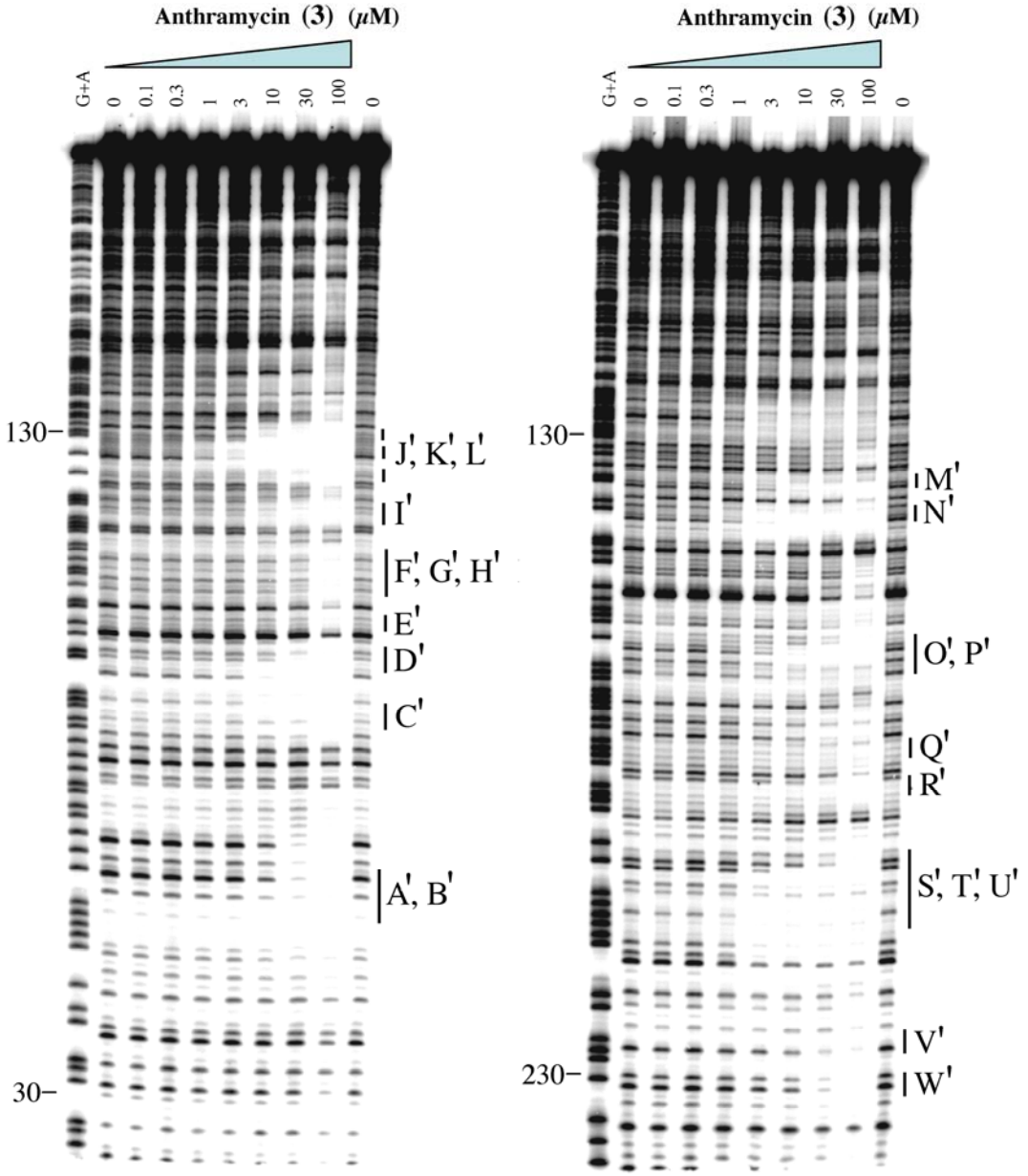
S2-B: Footprints analysed from the MS2R DNA (Reverse-labelled DNA fragment). "+" indicates weak (*i.e.*, low micromolar) footprints; "++" indicates medium (*i.e.*, mid-nanomolar) footprints; "+++" indicates strong (*i.e.*, low nanomolar) footprints.

S3



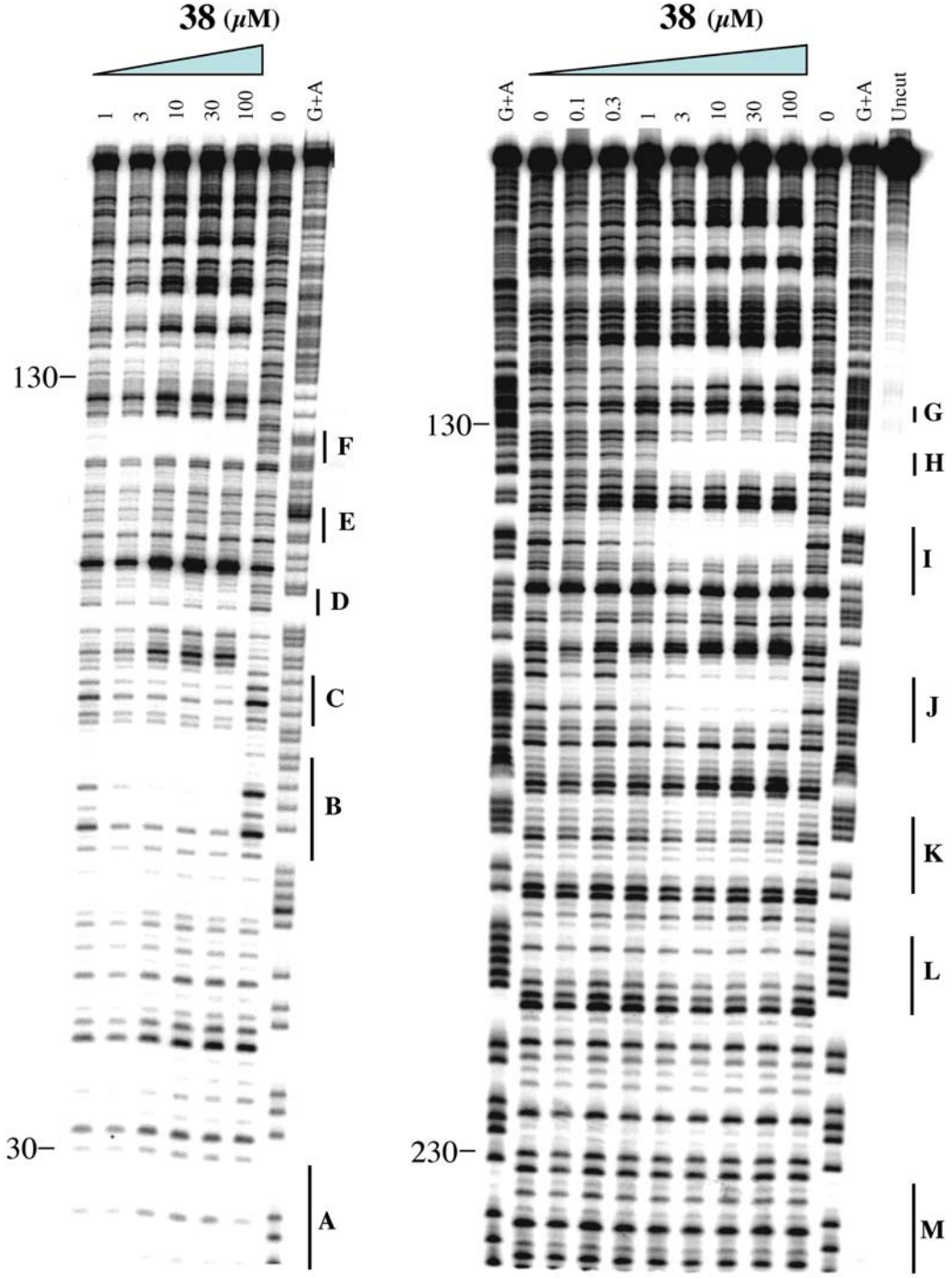
S3: Differential cleavage plots showing the relative positions of footprints produced by **50c** (yellow line), **23** (red line) and anthramycin methyl ether (**3**) (green line). There is evidence of (i) sequences bound by all three molecules, *e.g.*, 5′-^{216}TAGAG^{220}-3′, (ii) sequences bound by a combination of two molecules, *e.g.*, 5′-^{62}CAATACA^{68}-3′ (bound by **50c** and **23**) or 5′-^{69}CATGGCCG^{76}-3′ (bound by **50c** and anthramycin), and (iii) sequences bound by a single molecule, *e.g.*, 5′-^{173}GGTGGGG^{179}-3′ (bound by anthramycin) or 5′-^{178}GGTCTGTTT^{186}-3′ (bound by **50c**).

S4



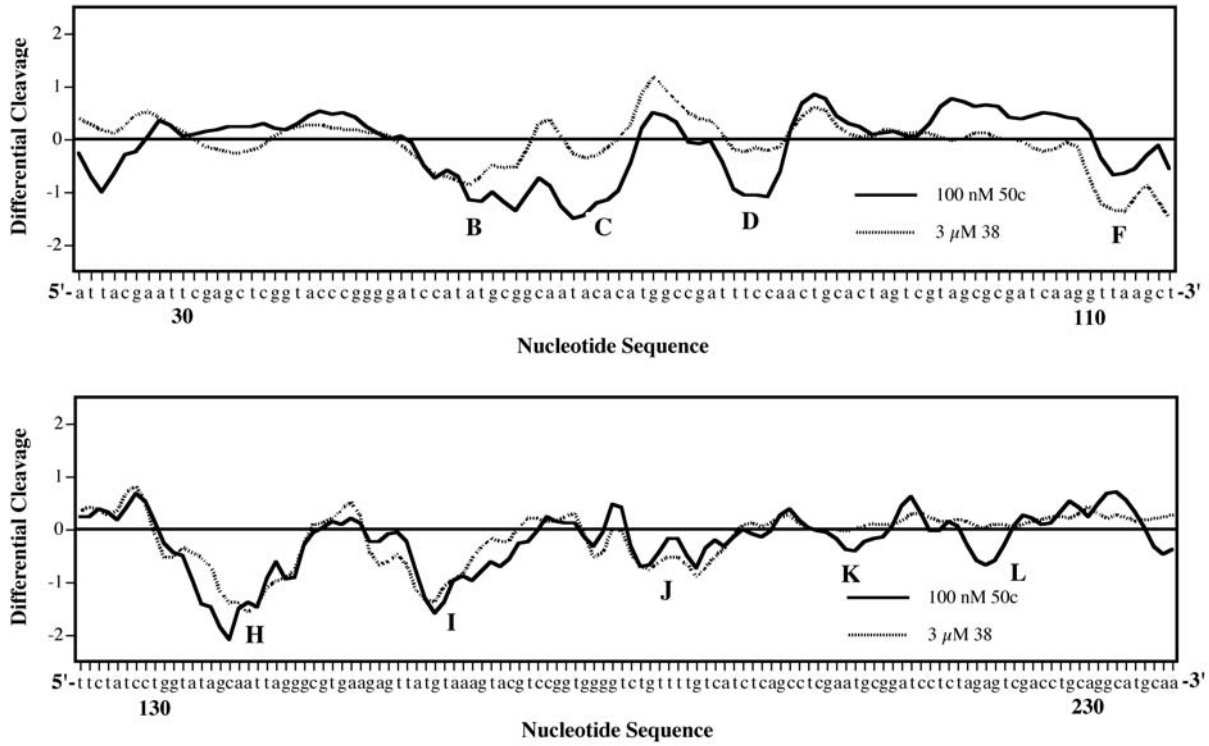
S4: DNase I footprinting gel of anthramycin methyl ether (**3**) binding to MS2 DNA. **Left Panel** – Forward-labelled MS2F DNA fragment; **Right Panel** – Reverse-labelled MS2R DNA fragment. The most probable footprints include: A' – 5′-^{48}GGA^{50}-3′, B' - 5′-^{51}TCC^{53}-3′, C' – 5′-^{71}TGG^{73}-3′, D' – 5′-^{80}TCC^{82}-3′, E' - 5′-^{86}TGC^{88}-3′, F' - 5′-^{92}AGT^{94}-3′, G' - 5′-^{95}CGT^{97}-3′, H' - 5′-^{98}AGC^{100}-3′, I' - 5′-^{108}AGG^{110}-3′, J' - 5′-^{118}CCC^{120}-3′, K' – 5′-^{123}TCT^{125}-3′, L' - 5′^{128}CCT^{130}-3′, M' – 5′-^{143}AGG^{145}-3′, N' - 5′-^{152}AGA^{154}-3′, O' – 5′-^{176}GGG^{178}-3′, P' - 5′-^{180}TCT^{182}-3′, Q' - 5′-^{192}TCT^{194}-3′, R' - 5′-^{198}CCT^{200}-3′, S' – 5′-^{208}GGA^{210}-3′, T' - 5′-^{211}TCC^{213}-3′, U' - 5′-^{214}TCT^{216}-3′, V' - 5′-^{225}CCT^{227}-3′, W' – 5′-^{230}AGG^{232}-3′.

S5



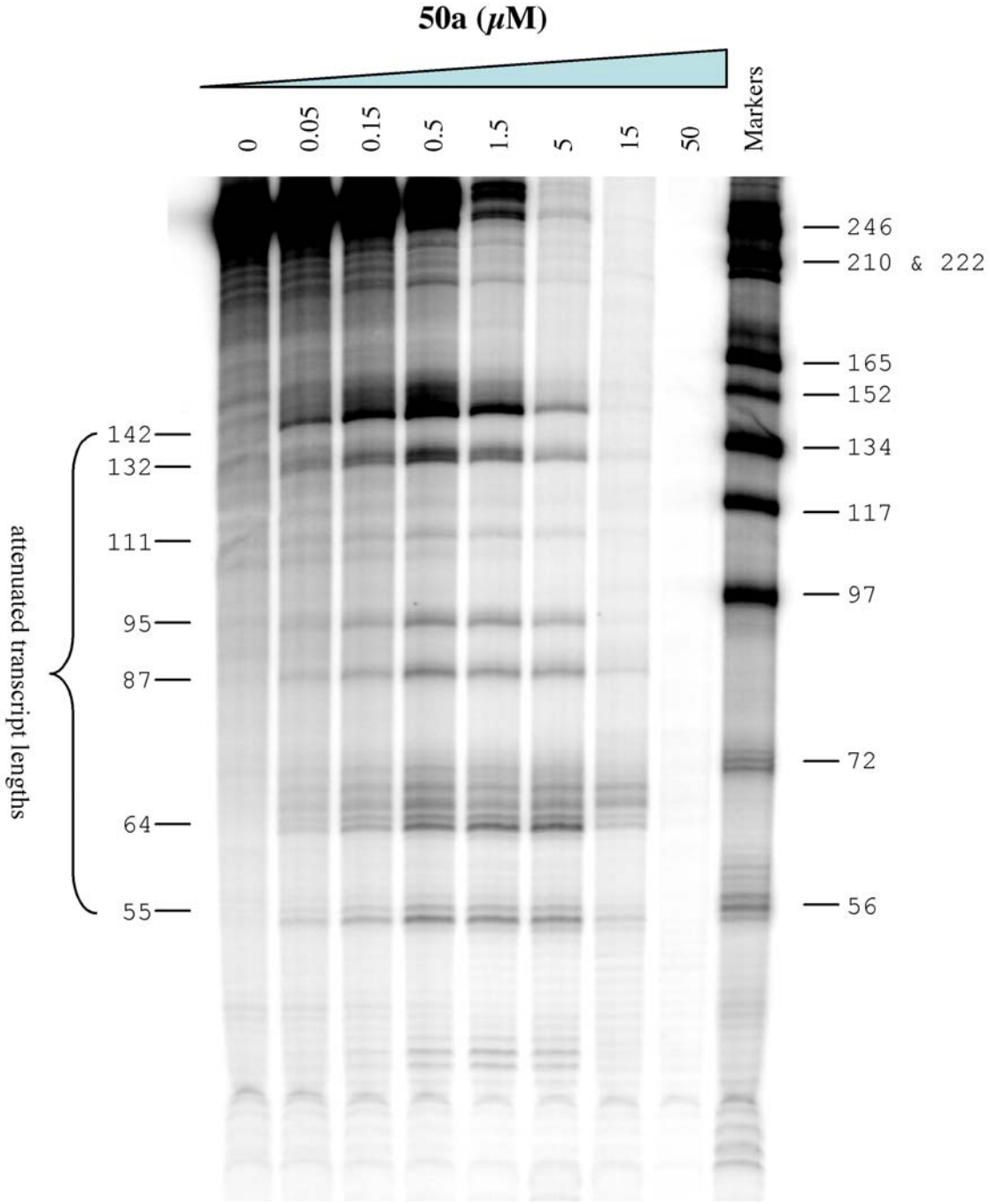
S5: DNase I footprinting gels of compound **38** binding to MS2 DNA. **Left Panel** – Forward-labelled MS2F DNA; **Right Panel** – Reverse-labelled MS2R DNA. 'G+A' indicates standard Maxam-Gilbert marker lanes. 'Uncut' represents DNA that has not been treated with DNase I. Vertical black bars and letters represent footprints corresponding to those identically labelled in **Figures 4** and **5** (main text) and **Figure S1-C (*Supplemental Information*)**.

S6



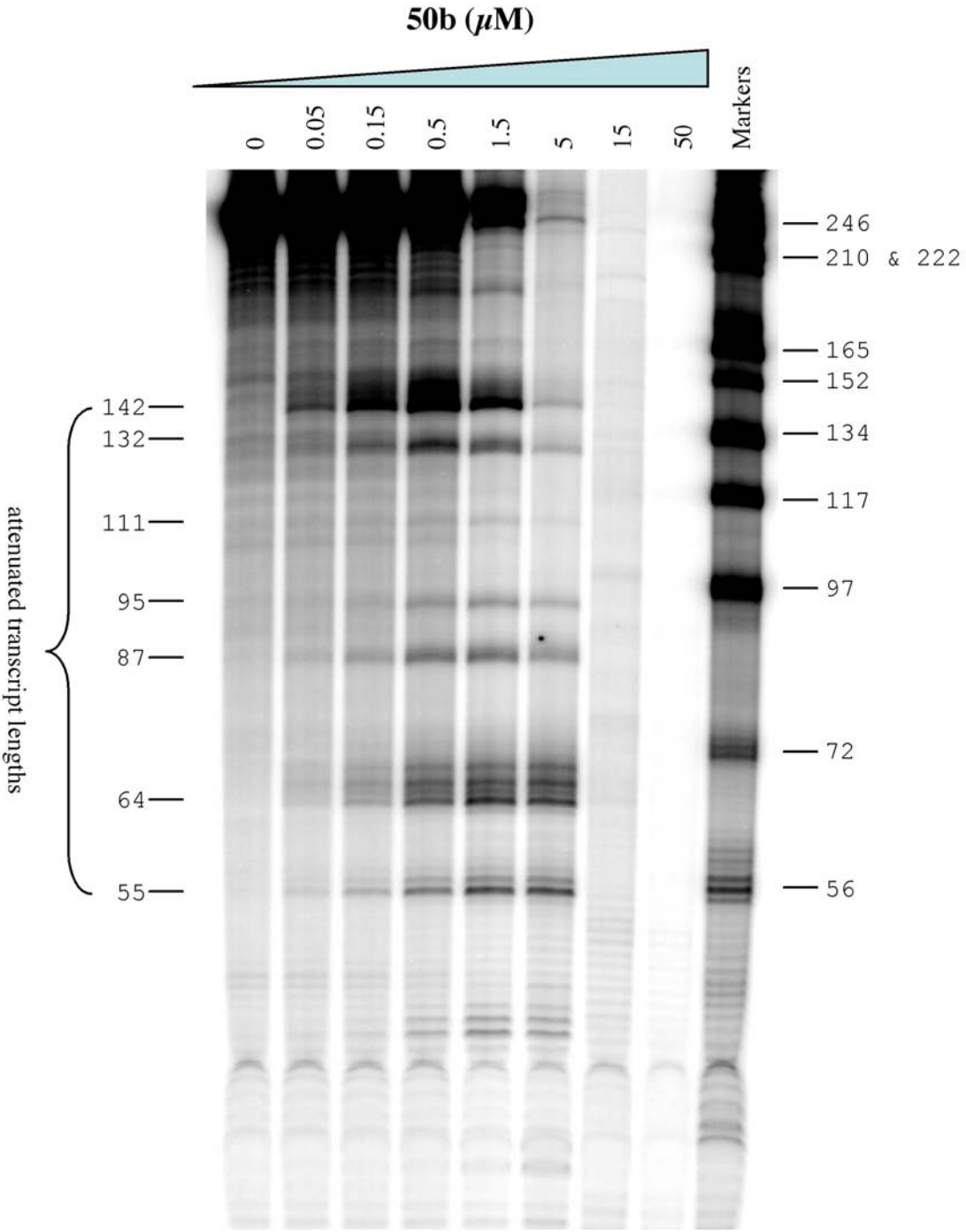
S6: Comparative differential cleavage plots for compounds **50c** and **38**. The general overlap is notable, although there are some areas of divergence (*e.g.*, regions K & L). The lettering system used to denote footprint regions is identical to that used in **Figures 4** and **5** (main text)**,** and **S1-C** and **S5** (***Supplemental Information***).

S7-A



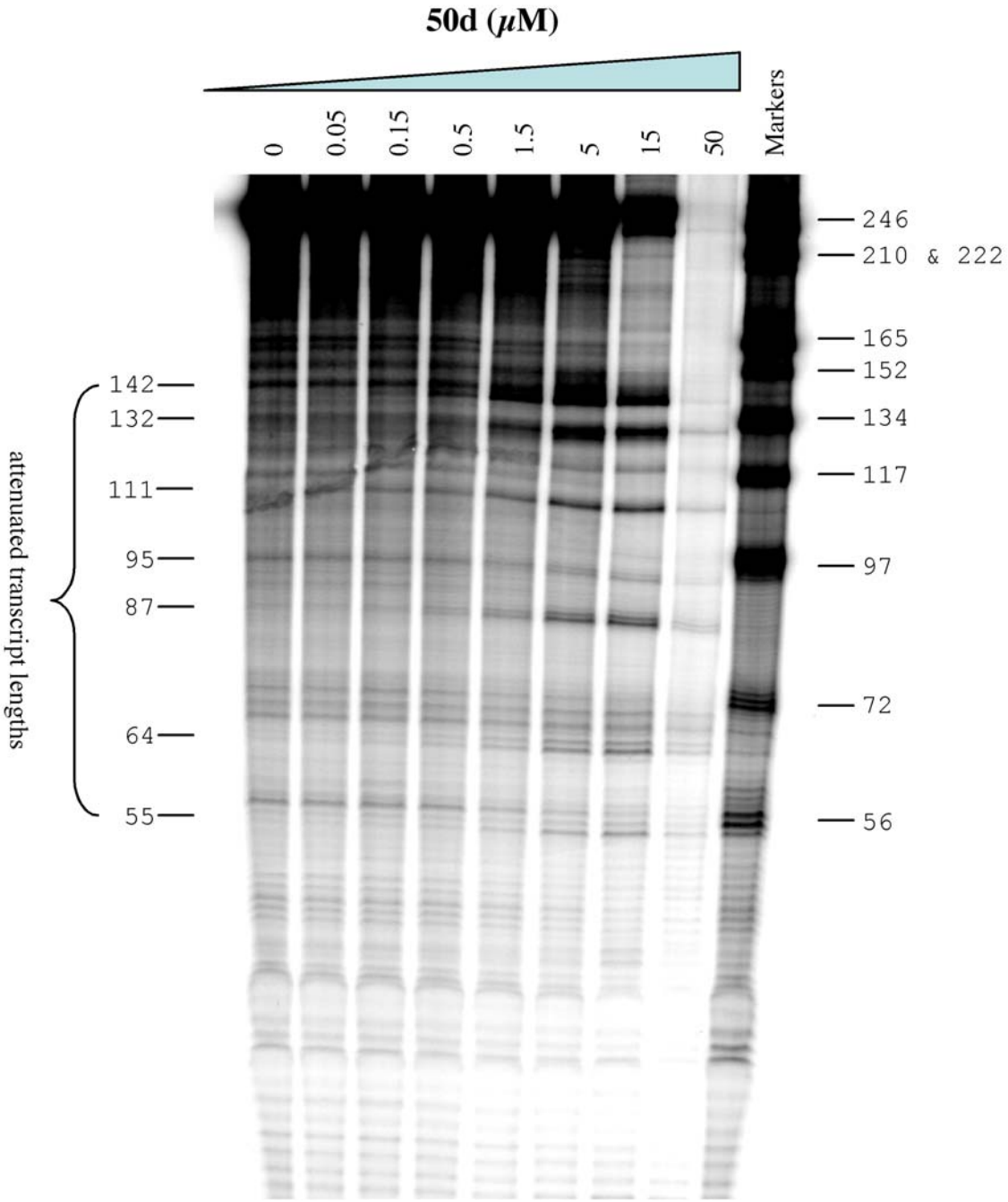
S7-A: *In vitro* transcription gel showing the T-stops produced by **50a** on the MS2 DNA fragment. Marker lengths (in nucleotides) are indicated on the far right, and the lengths of the attenuated transcripts (in nucleotides) are displayed on the left side.

S7-B



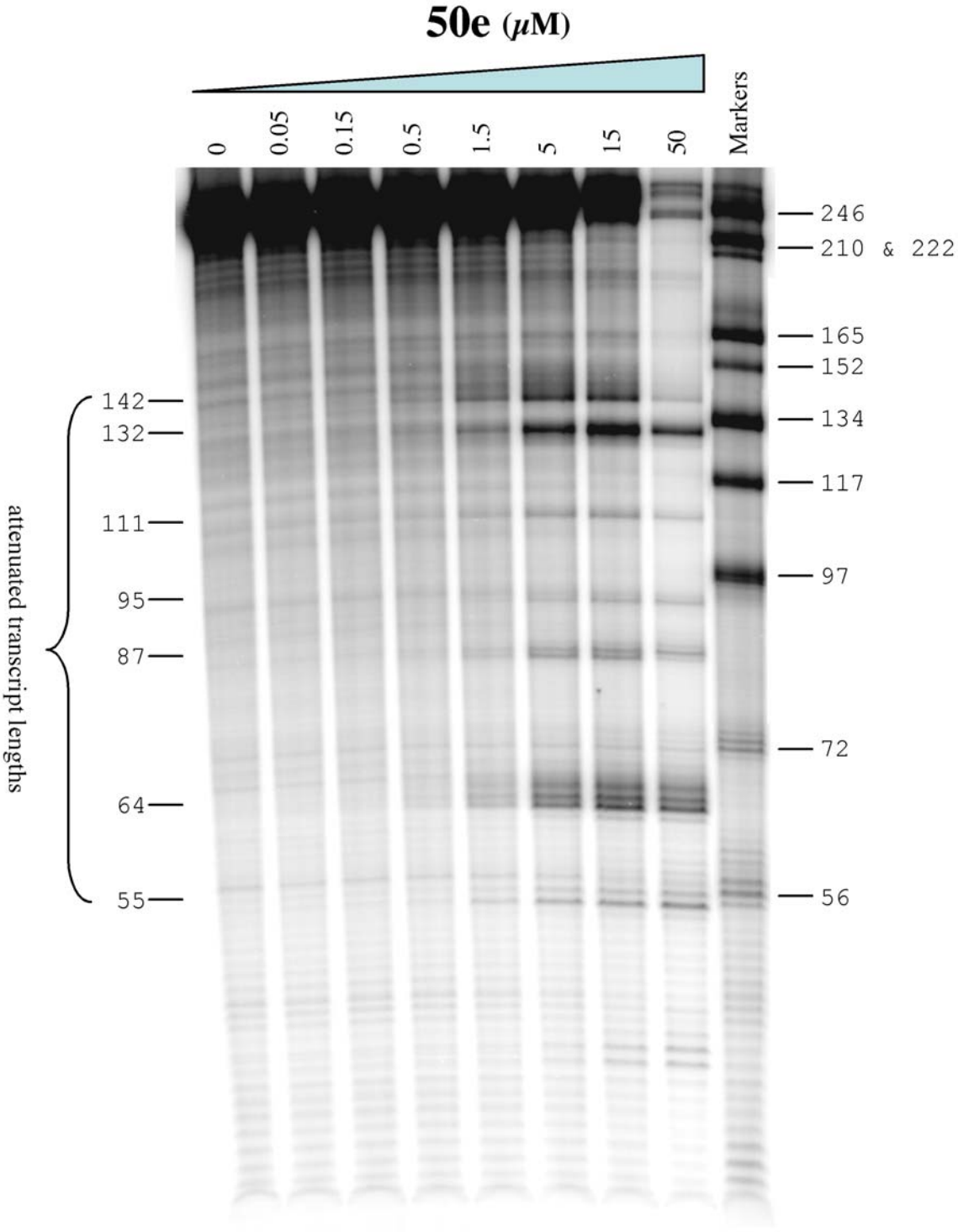
S7-B: *In vitro* transcription gel showing the T-stops produced by **50b** on the MS2 DNA fragment. Marker lengths (in nucleotides) are indicated on the far right, and the lengths of the attenuated transcripts (in nucleotides) are displayed on the left side.

S7-C



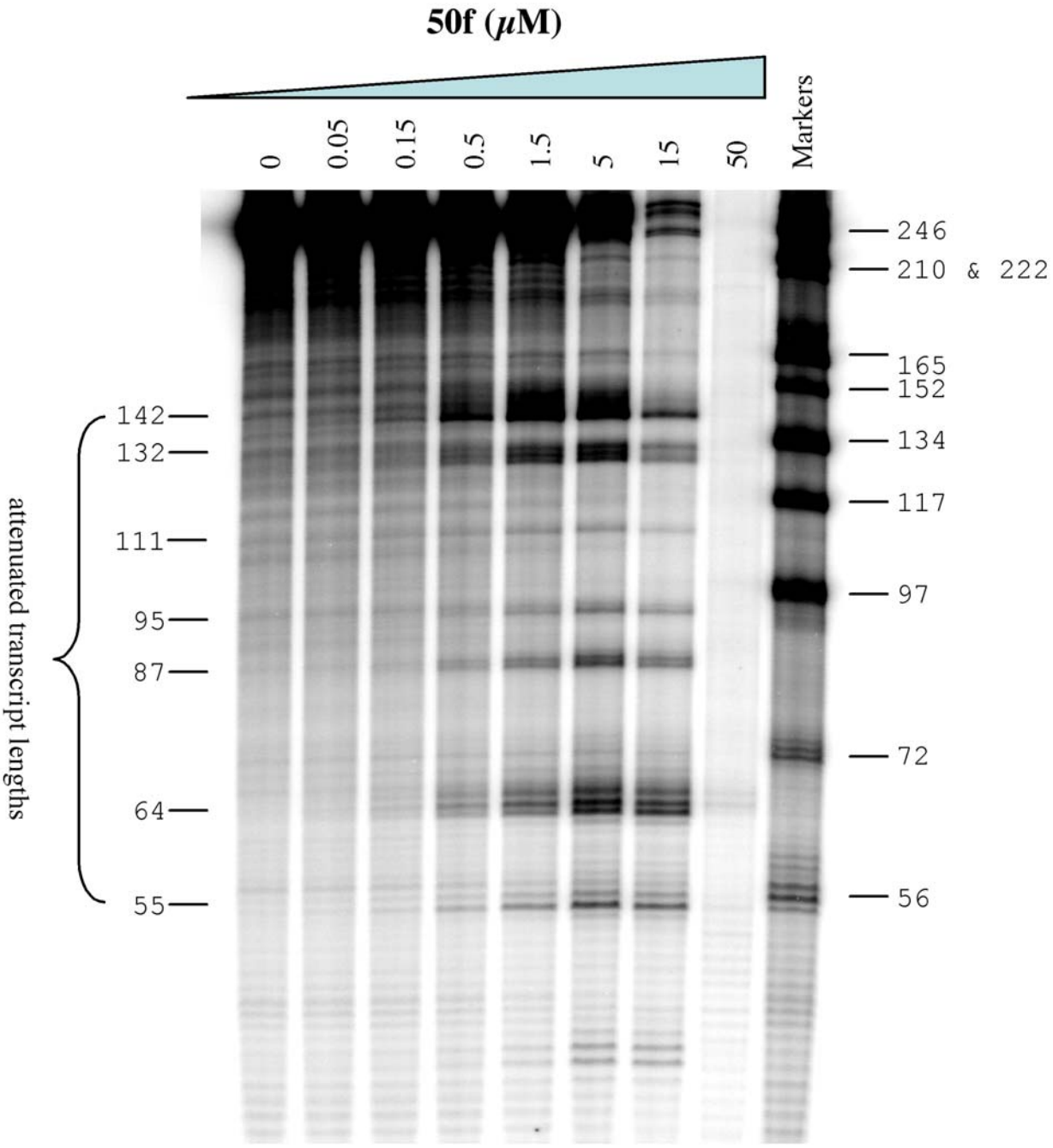
S7-C: *In vitro* transcription gel showing the T-stops produced by **50d** on the MS2 DNA fragment. Marker lengths (in nucleotides) are indicated on the far right, and the lengths of the attenuated transcripts (in nucleotides) are displayed on the left side.

S7-D

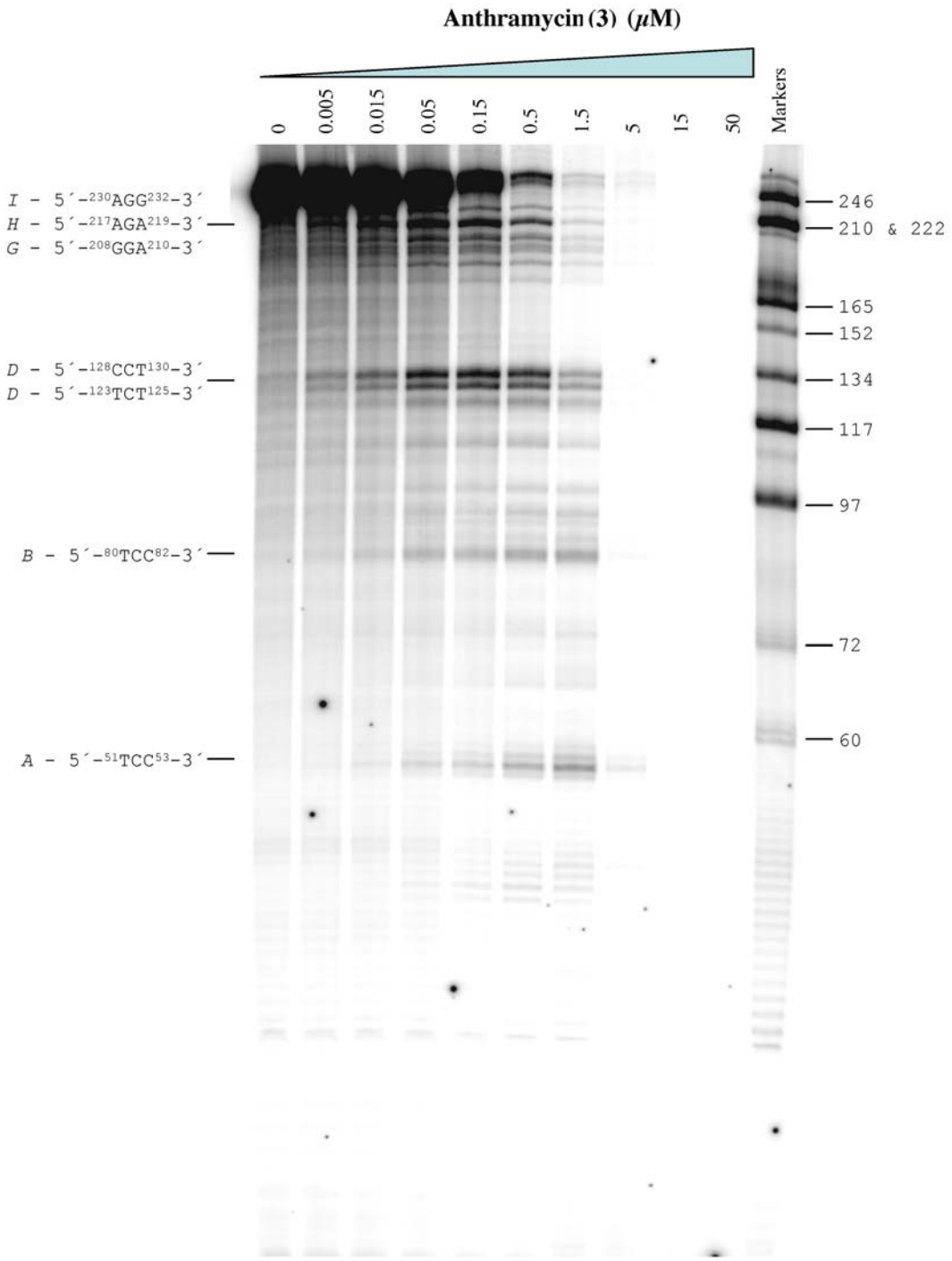


S7-D: *In vitro* transcription gel showing the T-stops produced by **50e** on the MS2 DNA fragment. Marker lengths (in nucleotides) are indicated on the far right, and the lengths of the attenuated transcripts (in nucleotides) are displayed on the left side.

S7-E



S7-E: *In vitro* transcription gel showing the T-stops produced by **50f** on the MS2 DNA fragment. Marker lengths (in nucleotides) are indicated on the far right, and the lengths of the attenuated transcripts (in nucleotides) are displayed on the left side.



S8: *In vitro* transcription gel showing the T-stops produced by anthramycin methyl ether (**3**) on the MS2 DNA fragment. Marker lengths (in nucleotides) are indicated on the far right. The presence of a 60 nucleotide marker and the absence of a 56 nucleotide marker (as present in **Figures S7A-E**) is due to the use of an alternative enzyme (see *Experimental Section* of main text). The positions of attenuated transcripts and their related PBD-binding sites are shown on the left side of the gel.

S9

Experimental Details (and references) for synthesis of compounds 16, 17, 19, 20, 24, 26, 28, 31, 32, 40-45 and 52-54.

Synthesis of *N*-Methylpyrrole Oligomers

Methyl 4-[(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino}-1-methyl-1*H*-pyrrole-2-carboxylate (16)[1, 2]

The Boc-protected pyrrole acid **15** (0.25 g, 1.05 mmol) and the methyl pyrrolecarboxylate **14** (0.20 g, 1.05 mmol, 1 eq) were dissolved in anhydrous DMF (5 mL) with stirring. This solution was treated with EDCI (0.403 g, 2.1 mmol, 2 eq) and DMAP (0.320 g, 2.6 mmol, 2.5eq) then stirred for 18 h at room temperature. The reaction mixture was diluted with EtOAc (50 mL) and washed with 10 % HCl solution (3 × 50 mL) and saturated $NaHCO_3$ solution (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give an off white foam, **16** (0.368 g, 94%). mp 78 °C (lit 78-79 °C[1]); ^{1}H-NMR (d_6-DMSO) δ 9.85 (1H, s, NH), 9.09 (1H, s, Boc-NH), 7.46 (1H, s, Py-H), 6.92 (1H, s, Py-H), 6.91 (1H, s, Py-H), 6.85 (1H, s, Py-H), 3.85 (3H, s, OCH_3), 3.82 (3H, s, NCH_3), 3.75 (3H, s, NCH_3), 1.48 (9H, s, $C[CH_3]_3$). **Note**: The NMR spectrum for **16** differed (>0.1 ppm) from that recorded in the literature for one out of four signals in the aromatic region (δ 7.46, 7.11, 6.96, 6.90 lit[1].; 7.46, 6.92, 6.91, 6.85 observed). The spectra we recorded were consistent for several samples.

4-[(4-*tert*-Butyloxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carboxylic acid (17)[2]

A stirred solution of Boc-pyrrole dimer **16** (0.805 g, 2.1 mmol) in MeOH (40 mL) was treated with 1M NaOH solution (25 mL). The reaction mixture was stirred at room temperature for 18 h. The volume was reduced *in vacuo* and the aqueous solution extracted with EtOAc (50 mL). The solvent was removed from the EtOAc fraction and the residue was treated with 1M NaOH solution (10 mL) for a further 3 h. This was combined with the previous aqueous fraction and acidified to pH 2-3 with 1M HCl solution and the resulting suspension extracted with EtOAc (3 × 75 mL). The organic fractions were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow foam, **17** (0.781 g, 100%). ^{1}H-NMR (d_6-DMSO) δ 12.07 (1H, bs, OH), 9.81 (1H, s, NH), 9.08 (1H, s, NH), 7.40 (1H, d, J = 1.9 Hz, Py-H), 6.88 (1H, s, Py-H), 6.84 (1H, s, Py-H), 6.83 (1H, s, Py-H), 3.81 (3H, s, NCH_3), 3.80 (3H, s, NCH_3), 1.45 (9H, s, $C[CH_3]_3$); ^{13}C-NMR (d_6-DMSO) δ 171.9, 161.9, 158.3, 152.8, 122.6, 122.3, 120.2, 119.4, 117.0, 108.3, 103.7, 78.3, 36.1, 36.1, 28.1.

Methyl 4-({4-[(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carboxylate (19)[1, 2]

The Boc-protected pyrrole dimer **16** (0.25 g, 0.66 mmol) was placed in a dry round-bottomed flask and treated with 4M HCl in dioxane (5 mL). The resulting solution became cloudy over a period of 30 min. The solvent was removed *in vacuo* to give a yellow solid which was then dried *in vacuo*. The residue was dissolved in anhydrous DMF (9 mL) and the Boc-pyrrole acid **15** (0.176 g, 0.726 mmol, 1.1 eq) was added followed by EDCI (0.191 g, 0.99 mmol, 1.5 eq) and DMAP (0.097 g, 0.79 mmol, 1.2 eq). The reaction mixture was stirred at room temperature for 18 h then diluted with EtOAc (50 mL) and washed with 1M HCl solution (3 × 50 mL), then saturated

$NaHCO_3$ solution (3 × 50 mL), dried ($MgSO_4$) then concentrated *in vacuo* to give a tan foam. This solid was suspended in a 1:1 mixture of MeOH and 1M NaOH solution (40 mL) and stirred at room temp for 30 min. EtOAc was added and the organic layer washed with saturated $NaHCO_3$ solution (3 × 50 mL) and dried ($MgSO_4$). Concentration *in vacuo* gave an off white foam, **19** (0.160 g, 48%). mp 134 °C (lit 131-133 °C[1]); ^{1}H-NMR (d_6-DMSO) δ 9.90 (1H, s, NH), 9.86 (1H, s, NH), 9.13 (1H, s, Boc-NH), 7.46 (1H, d, *J* = 1.9 Hz, Py-H), 7.21 (1H, d, *J* = 1.7Hz, Py-H), 7.06 (1H, d, *J* = 1.7 Hz, Py-H), 6.91 (1H, s, Py-H), 6.90 (1H, s, Py-H), 6.85 (1H, s, Py-H), 3.84 (6H, s, NCH_3), 3.81 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 1.46 (9H, s, $C[CH_3]_3$). **Note**: The NMR spectrum for **19** differed (>0.1 ppm) from that recorded in the literature for the N-H signals (δ 9.26, 9.23, 8.13 lit[1].; 9.90, 9.86, 9.13 observed). The spectra we recorded were consistent for several samples.

4-({4-[(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carboxylic acid (20)[2]

The Boc-pyrrole trimer **19** (0.60 g, 1.2 mmol) was dissolved in MeOH (5 mL) and treated with NaOH solution (0.1 g in 5 mL H_2O). The reaction mixture was stirred overnight then heated at 60°C for 2 h. The MeOH was removed *in vacuo* and the aqueous fraction extracted with EtOAc (25 mL). The aqueous layer was adjusted to pH 2-3 with 1M HCl solution then extracted with EtOAc (3 × 30 mL). The combined organic layers were dried ($MgSO_4$) then concentrated *in vacuo* to give an orange solid. The solid was suspended in Et_2O (10 mL) and collected on a filter then dried *in vacuo* to give an orange solid, **20** (0.431 g, 74%). ^{1}H-NMR (d_6-DMSO) δ 12.11 (1H, s, OH), 9.89 (1H, s, NH), 9.86 (1H, s, NH), 9.09 (1H, s, Boc-NH), 7.43 (1H, d, *J* = 1.9 Hz, Py-H), 7.22 (1H, d, *J* = 1.7 Hz, Py-H), 7.06 (1H, d, *J* = 1.7 Hz, Py-H), 6.90 (1H, s, Py-H), 6.86 (1H, d, *J* = 1.9 Hz, Py-H), 6.84 (1H, s, Py-H), 3.85 (3H, s, NCH_3), 3.83 (3H, s, NCH_3), 3.82 (3H, s, NCH_3), 1.46 (9H, s, $C[CH_3]_3$); ^{13}C-NMR (d_6-DMSO) δ 161.9, 158.4, 158.4, 152.8, 122.8, 122.7, 122.5, 122.4, 122.3, 120.2, 119.5, 118.4, 117.0, 108.4, 104.7, 103.8, 78.2, 36.1, 36.0, 28.1.

Methyl 4-{[4-({4-[(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-*1H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carboxylate (24)

The Boc-pyrrole dimer **16** (0.207 g, 0.54 mmol) in a dry round-bottomed flask was treated with 4M HCl in dioxane (5 mL) with stirring. The reaction mixture was stirred for 30 min during which time a precipitate formed. The solvent was removed and the residue dried *in vacuo*. The residue was dissolved in anhydrous DMF (5 mL) and the Boc-pyrrole dimer acid **17** (0.2 g, 0.55 mmol) was added followed by EDCI (0.159 g, 0.83 mmol, 1.5 eq) and DMAP (0.081 g, 0.66 mmol, 1.2 eq). The reaction mixture was stirred for 48 hours then diluted with EtOAc (50 mL) and washed with 10% HCl solution (3 × 30 mL) then saturated $NaHCO_3$ solution (3 × 30 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo* to give an orange solid, **24** (0.310 g, 90%). ^{1}H-NMR (d_6-DMSO) δ 9.93 (2H, s, NH), 9.86 (1H, s, NH), 9.08 (1H, s, Boc-NH), 7.47 (1H, d, *J* = 1.9 Hz, Py-H), 7.23 (1H, d, *J* = 1.8 Hz, Py-H), 7.22 (1H, d, *J* = 1.7 Hz, Py-H), 7.07 (1H, d, *J* = 1.8 Hz, Py-H), 7.05 (1H, d, *J* = 1.8Hz, Py-H), 6.91 (1H, d, *J* = 1.9 Hz, Py-H), 6.89 (1H, d, *J* = 1.9 Hz, Py-H), 6.84 (1H, d, *J* = 1.7 Hz, Py-H), 3.85 (3H, s, NCH_3), 3.84 (6H, s, NCH_3), 3.81 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 1.46 (9H, s, $C[CH_3]_3$).

Methyl 4-[(4-{[4-({4-[(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carboxylate (26)

The Boc-pyrrole trimer **19** (0.2 g, 0.40 mmol) in a dry round-bottomed flask was treated with 4M HCl in dioxane (5 mL). The solution was stirred for 30 min during which time a precipitate formed. The solvent was removed and the residue dried *in vacuo*. The residue was dissolved in anhydrous DMF (2.5 mL) and the Boc-pyrrole dimer acid **17** (0.144 g, 0.40 mmol, 1 eq) was added followed by EDCI (0.115 g, 0.60 mmol, 1.5 eq) and DMAP (0.058 g, 0.47 mmol, 1.2 eq). The reaction mixture was stirred for 48 h then diluted with EtOAc (50 mL) and washed with 10 % HCl solution (3 × 30 mL) then saturated $NaHCO_3$ (3 × 30 mL). The organic layer was dried ($MgSO_4$) then concentrated *in vacuo* to give an orange solid, **26** (0.253 g, 85%). ^{1}H-NMR (d_6-DMSO) δ 9.95 (1H, s, NH), 9.93 (2H, s, NH), 9.86 (1H, s, NH), 9.08 (1H, s, Boc-NH), 7.47 (1H, d, *J* = 1.9 Hz, Py-H), 7.25 (1H, d, *J* = 2.1 Hz, Py-H), 7.24 (1H, d, *J* = 2.4Hz, Py-H), 7.23 (1H, d, *J* = 1.7 Hz, Py-H), 7.08 (1H, d, *J* = 1.9 Hz, Py-H), 7.07 (1H, d, *J* = 1.9 Hz, Py-H), 7.07 (1H, d, *J* = 1.9 Hz, Py-H), 6.91 (2H, d, *J* = 2.0 Hz, Py-H), 6.85 (1H, s, Py-H), 3.86 (3H, s, NCH_3), 3.85 (6H, s, NCH_3), 3.84 (3H, s, NCH_3), 3.81 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 1.46 (9H, s, $C[CH_3]_3$).

Methyl 4-({4-[(4-{[4-({4-[(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carboxylate (28)

The Boc-pyrrole trimer **19** (0.2 g, 0.40 mmol) in a dry round-bottomed flask was treated with 4M HCl in dioxane (2.5 mL). The reaction mixture was stirred at room temperature for 30 min during which time a precipitate formed. The solvent was removed and the residue dried *in vacuo*. The residue was dissolved in anhydrous DMF (2.5 mL) and the Boc-pyrrole trimer acid **20** (0.194 g, 0.40 mmol, 1 eq) was added followed by EDCI (0.115 g, 0.6 mmol, 1.5 eq) and DMAP (0.058 g, 0.47 mmol, 1.2 eq). The reaction mixture was stirred for 48 h then diluted with EtOAc (50 mL) and washed with 10% HCl solution (3 × 30 mL) and saturated $NaHCO_3$ solution (3 × 30 mL). The organic layer was dried ($MgSO_4$) then concentrated *in vacuo* to give an orange solid, **28** (0.185 g, 54%). ^{1}H-NMR (d_6-DMSO) δ 9.95 (2H, s, NH), 9.93 (2H, s, NH), 9.86 (1H, s, NH), 9.08 (1H, s, Boc-NH), 7.47 (1H, d, *J* = 1.8 Hz, Py-H), 7.25 (1H, d, *J* = 2.2 Hz, Py-H), 7.24 (2H, d, *J* = 2.0 Hz, Py-H), 7.22 (1H, d, *J* = 1.6Hz, Py-H), 7.07 (2H, d, *J* = 1.6 Hz, Py-H), 7.07 (1H, d, *J* = 2.0 Hz, Py-H), 6.91 (2H, d, *J* = 1.9 Hz, Py-H), 6.89 (1H, s, Py-H), 6.84 (1H, s, Py-H), 3.86 (3H, s, NCH_3), 3.86 (6H, s, NCH_3), 3.85 (3H, s, NCH_3), 3.84 (3H, s, NCH_3), 3.81 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 1.46 (9H, s, $C[CH_3]_3$).

Synthesis of C_3 Boc Protected PBD Acid

4-(2-Benzyloxycarbonylethoxy)-5-methoxy-2-nitrobenzoic acid (31)

4-Toluenesulfonic acid (8.1 g, 0.16 eq) and benzyl alcohol (176.6 g, 169 mL, 6.14 eq) were added to a suspension of diacid **30**[3] (76 g, 0.266 mol) in toluene (760 mL). The mixture was heated at reflux for 3.5 h then allowed to cool to room temperature. The reaction mixture was extracted with a saturated solution of aqueous $NaHCO_3$ and combined aqueous extracts were acidified to pH 2 with 1M HCl. The mixture was extracted with EtOAc (3 × 300 mL) and the precipitated material dissolved in EtOAc (600 mL). The combined EtOAc solutions were dried ($MgSO_4$) and concentrated *in*

vacuo to give a yellow solid. Recrystallisation from EtOAc-hexane gave **31**, (55.9 g, 56%). ^{1}H-NMR (d_6-DMSO) δ 7.63 (1H, s, phenyl H-3), 7.37-7.30 (6H, m, phenyl H-6, benzyl), 5.15 (2H, s, benzyl CH_2), 4.36 (2H, t, J = 5.8 Hz, sidechain H-1), 3.90 (3H, s, OCH_3), 2.91 (2H, t, J = 5.9 Hz, sidechain H-2); ^{13}C-NMR (d_6-DMSO) δ 170.4, 166.0, 151.7, 148.9, 141.3, 136.0, 128.3, 127.9, 127.7, 121.4, 111.4, 108.2, 65.6, 65.0, 56.4, 33.7; IR ($CHCl_3$) 1704, 1603, 1537, 1424, 1395, 1349, 1278, 1213, 1181, 1050, 1022, 873, 753 cm^{-1}; MS (ES^-) *m/z* (relative intensity) 374 ([M - H]$^{-\cdot}$, 100%).

3-[4-((2*S*)-2-Hydroxymethylpyrrolidine-1-carbonyl)-2-methoxy-5-nitro-phenoxy]propionic acid benzyl ester (32)

Benzyl ester **31** (30 g, 79.9 mmol) was suspended in anhydrous CH_2Cl_2 (300 mL) with stirring in a round-bottomed flask equipped with a drying tube. Oxalyl chloride (11.16 g, 7.66 mL, 1.1 eq) was added followed by a few drops of DMF. The mixture was stirred for 18 h at room temperature. Triethylamine (17.75 g, 24.45 mL, 2.2 eq) and (2*S*)-(+)-pyrrolidinemethanol (8.88 g, 8.66 mL, 1.1 eq) were dissolved in anhydrous CH_2Cl_2 (150 mL) under N_2. The solution was cooled to below –30 °C. The acid chloride solution was added dropwise over 6 h maintaining the temperature below -30°C. It was then left to stir overnight at room temperature. The resulting solution was extracted with 1M HCl solution (2 × 200 mL), then washed twice with water and once with brine. After drying ($MgSO_4$) and concentration *in vacuo* to gave **32** as a yellow-brown oil which solidified on standing (quantitative yield). This material was used in the next step without further purification. $[\alpha]^{25}_D$ = -532° (*c* = 0.12, $CHCl_3$); ^{1}H-NMR (d_6-DMSO) δ 7.75 (1H, m, phenyl H-3), 7.34 (5H, m, benzyl), 6.82 (1H, s, phenyl H-6), 5.20 (2H, s, benzyl CH_2), 4.37-4.34 (3H, m, sidechain H-1, pyrrolidine H-2), 3.94-3.80 (5H, m, OCH_3, pyrrolidine H-5), 3.16-3.08 (2H, m, pyrrolidine CH2-OH), 2.96 (2H, t, J = 6.2 Hz, sidechain H-2), 2.23-2.13 (1H, m, pyrrolidine H-3), 1.92-1.69 (3H, m, pyrrolidine H-3,4); ^{13}C-NMR (d_6-DMSO) δ 170.4, 165.5, 154.0, 147.2, 136.9, 136.0, 128.6, 128.3, 128.2, 128.1, 127.9, 127.7, 109.9, 108.3, 79.1, 65.6, 64.8, 64.7, 61.7, 60.6, 59.9, 58.6, 56.6, 48.4, 45.7, 33.7, 27.4, 27.0, 23.4, 21.6; IR ($CHCl_3$) 1735, 1618, 1577, 1519, 1453, 1427, 1383, 1332, 1275, 1218, 1171, 1058, 871, 750, 698, 649 cm^{-1}; MS (ES^+) *m/z* (relative intensity) 459 ([M + H]$^{+\cdot}$, 100%).

Synthesis of C_4 Alloc/THP PBD acid

4-(4-Formyl-2-methoxyphenoxy)butanoic acid methyl ester (40)[4]

A slurry of vanillin **39** (40 g, 0.262 mol), methyl-4-bromobutyrate (50 g, 34.2 mL, 1.05 eq) and K_2CO_3 (54 g, 1.5 eq) in DMF (200 mL) was stirred at room for 16 h. Water (1 L) was then added whilst stirring and the white precipitate was collected on a filter, washed with water and dried to yield **40**, (60 g, 85%). mp 73 °C; ^{1}H-NMR ($CDCl_3$) δ 9.80 (1H, s, formyl-H), 7.46-7.40 (2H, m, H-3,5), 6.97 (1H, d, J = 8.1 Hz, H-6), 4.16 (2H, t, J = 6.3 Hz, sidechain H-1), 3.92 (3H, s, OCH_3), 3.70 (3H, s, sidechain CO_2CH_3), 2.57 (2H, t, J = 7.2 Hz, sidechain H-3), 2.20 (2H, p, J = 6.7 Hz, sidechain H-2); ^{13}C-NMR ($CDCl_3$) δ 190.9, 173.4, 153.8, 149.9, 130.1, 126.8, 111.5, 109.2, 67.8, 56.0, 51.7, 30.3, 24.2; IR (solid) ν_{max} 1728, 1678, 1582, 1508, 1469, 1426, 1398, 1262, 1174, 1133, 1015, 880, 809, 730 cm^{-1}; MS (ES^+) *m/z* (relative intensity) 253 ([M + H]$^{+\cdot}$, 100%).

4-(4-Formyl-2-methoxy-5-nitrophenoxy)butanoic acid methyl ester (41)[4]

A solution of the aldehyde **40** (50 g, 0.197 mol) in acetic anhydride (150 mL) was slowly added to a mixture of 70% HNO_3 (900 mL) and acetic anhydride (200 mL) at 0 °C. The mixture was then left to stir at 0 °C for 2.5 h. The solution was then poured on to ice in a 5 L flask and the volume adjusted to 5 L with ice and water. The resulting light-sensitive pale-yellow precipitate was immediately filtered and washed with cold water. The product **41** was used directly in the next step. ^{1}H-NMR ($CDCl_3$) δ 10.40 (1H, s, formyl-H), 7.61 (1H, s, H-6), 7.4 (1H, s, H-3), 4.21 (2H, t, J = 6.2 Hz, sidechain H-1), 4.00 (3H, s, OCH_3), 3.71 (3H, s, sidechain CO_2CH_3), 2.58 (2H, t, J = 7.1 Hz, sidechain H-3), 2.23 (2H, p, J = 6.3 Hz, sidechain H-2); ^{13}C-NMR ($CDCl_3$) δ 188.5, 172.8, 152.7, 151.0, 143.5, 124.7, 110.1, 108.2, 68.4, 56.4, 51.3, 29.7, 23.8; MS (ES^+) *m/z* (relative intensity) 298 ($[M + H]^{+\cdot}$, 100%).

5-Methoxy-4-(3-methoxycarbonylpropoxy)-2-nitrobenzoic acid (42)[5]

Nitroaldehyde **41** (80 g, wet) was dissolved in acetone (500 mL) in a 2 L flask fitted with a condenser and a mechanical stirrer. A hot solution of 10 % $KMnO_4$ (50 g in 500 mL of water) was added quickly using a dropping funnel (10 min). CAUTION!: exothermic reaction. The solution was stirred and allowed to cool for 1 h before filtration through Celite. The brown residue was washed with hot water (1 L). The filtrate was transferred in a large flask and a solution of sodium bisulfite (80 g in 500 mL 1M HCl) was added. The final volume was adjusted to 3 L by addition of water, and adjusted to pH 1 with concentrated HCl. The product acid **42** precipitated and was recovered by filtration and dried (31 g, 50% over 2 steps). ^{1}H-NMR ($CDCl_3$) δ 7.33 (1H, s, H-3), 7.19 (1H, s, H-6), 4.09 (2H, t, J = 5.7 Hz, sidechain H-1), 3.91 (3H, s, OCH_3), 3.64 (3H, s, sidechain OCH_3), 2.50 (2H, t, J = 7.0 Hz, sidechain H-3), 2.14 (2H, p, J = 6.3 Hz, sidechain H-2); ^{13}C-NMR (DMSO-d_6) δ 172.8, 166.0, 151.8, 149.1, 141.3, 121.2, 111.3, 107.8, 68.1, 56.4, 51.3, 29.7, 23.8; IR (solid) ν_{max} 1736, 1701, 1602, 1535, 1415, 1275, 1220, 1054, 936, 879, 820, 655 cm^{-1}; MS (ES^-) *m/z* (relative intensity) 312 ($[M - H]^{-\cdot}$, 100%).

4-[4-((2*S*)-2-Hydroxymethylpyrrolidine-1-carbonyl)-2-methoxy-5-nitrophenoxy]butanoic acid methyl ester (43)

Methyl ester **42** (30 g, 95.8 mmol) was suspended in anhydrous CH_2Cl_2 (300 mL) with stirring in a round-bottomed flask equipped with a drying tube. Oxalyl chloride (13.4 g, 9.20 mL, 1.1 eq) was added followed by a few drops of DMF. The mixture was stirred for 18 h at room temperature. Triethylamine (21.3 g, 29.3 mL, 2.2 eq) and (2*S*)-(+)-pyrrolidinemethanol (9.68 g, 9.44 mL, 1.1 eq) were dissolved in anhydrous CH_2Cl_2 (150 mL) under nitrogen. The solution was cooled to below –30°C and the acid chloride solution was added dropwise over 6 h, maintaining the temperature below -30°C. After stirring overnight at room temperature the resulting solution was washed with 1M HCl (2 × 200 mL), twice with water, then once with brine. After drying ($MgSO_4$), concentration *in vacuo* gave **43** as a yellow–brown oil (quantitative yield) that solidified on standing. This material was used in the next step without further purification. $[\alpha]^{24}_D$ = -84° (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.70 (1H, s, H-6), 6.80 (1H, s, H-3), 4.45-4.35 (1H, m, pyrrolidine H-2), 4.16 (2H, t, J = 6.2 Hz, sidechain H-1), 3.97 (3H, s, OCH_3), 3.97-3.70 (2H, m, pyrrolidine H-5), 3.71 (3H, s, sidechain OCH_3), 3.17 (2H, t, J = 6.7 Hz, pyrrolidine CH_2-OH), 2.57 (2H, t, J = 7.1 Hz, sidechain H-3), 2.26-2.14 (3H, m, sidechain H-2, pyrrolidine H-3), 1.90-1.70 (3H, m, pyrrolidine H-3,4); ^{13}C-NMR ($CDCl_3$) δ 173.2, 154.8, 148.4, 109.2, 108.4, 68.4, 66.1, 61.5, 56.7, 51.7, 49.5, 30.3, 28.4, 24.4, 24.2; IR (solid) ν_{max} 3400, 2953, 1734,

1618, 1517, 1432, 1327, 1271, 1219, 1170, 1051, 995, 647 cm^{-1}; MS (ES^+) *m/z* (relative intensity) 397 ($[M + H]^{+\cdot}$, 100%).

4-[5-Amino-4-((2*S*)-2-hydroxymethyl-pyrrolidine-1-carbonyl)-2-methoxy-phenoxy]-butyric acid methyl ester (44)

Nitro ester **43** (38.4 g, 97 mmol) was dissolved in EtOH (400 mL)). A slurry of 10% Pd/C (2 g) was added as a slurry in EtOH and the mixture was hydrogenated in a Parr apparatus at 40 psi until no further H_2 uptake was observed. The mixture was filtered through Celite and the solvent removed *in vacuo*. The resulting amine **44** (35.4 g, 100%) was used directly in the next step.

4-[5-Allyloxycarbonylamino-4-((2*S*)-2-hydroxymethylpyrrolidine-1-carbonyl)-2-methoxyphenoxy]butanoic acid methyl ester (45)

A batch of the amine **44** (22.5 g, 61.5 mmol) was dissolved in anhydrous CH_2Cl_2 (300 mL) containing anhydrous pyridine (10.9 mL, 134 mmol) at 0 °C. A solution of allyl chloroformate (7.17 mL, 67.5 mmol) in anhydrous CH_2Cl_2 (200 mL) was added dropwise at 0 °C. The resulting solution was allowed to stir for 18 h at room temperature. The mixture was then washed with cold 1M HCl (200 ml), water (200 mL), saturated aqueous $NaHCO_3$ (200 mL), and then brine (200 mL). The organic solution was then dried ($MgSO_4$), and the solvent was removed *in vacuo* to provide **45**, slightly contaminated by the product of diacylation (27 g, 100%). Column chromatography (EtOAc-hexane) provided an analytical sample. $[\alpha]^{26}_D$ = -67° (*c* = 0.45, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 8.78 (1H, bs, NH), 7.75 (1H, s, H-6), 6.82 (1H, s, H-3), 5.97 (1H, m, allyl H-2), 5.38-5.34 (1H, dd, *J* = 1.5, 17.2 Hz, allyl H-3), 5.27-5.24 (1H, dd, *J* = 1.3, 10.4 Hz, allyl H-3), 4.63 (2H, m, allyl H-1), 4.40 (2H, bs, C*H*$_2$-OH), 4.11 (2H, t, *J* = 6.3 Hz, sidechain H-1), 3.82 (4H, s, OCH_3, OH), 3.68-3.90 (4H, m, OCH_3, pyrrolidine H-2), 3.61-3.49 (2H, m, pyrrolidine H-5), 2.54 (2H, t, *J* = 7.4 Hz, sidechain H-3), 2.18 (2H, p, *J* = 6.7 Hz, sidechain H-2), 1.92-1.70 (4H, m, pyrrolidine H-3,4); ^{13}C-NMR ($CDCl_3$) δ 173.4,170.9, 153.6, 150.5, 144.0, 132.5, 132.0, 118.1, 115.4, 111.6, 105.6, 67.7, 66.6, 65.8, 61.1, 60.4, 56.6, 51.7, 30.5, 28.3, 25.1, 24.3; IR (solid) ν_{max} 2949, 2359, 1728, 1596, 1521, 1433, 1202, 1173, 1119, 998, 844, 652 cm^{-1}; MS (FAB^+) *m/z* 451 (M^++H 50%).

Synthesis of Dilactam Building Block

Methyl (2*S*)-1-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-pyrrolidine-2-caboxylate (52)[6]

5-Benzyloxy-4-methoxy-2-nitrobenzoic acid **51**[7] (1.0 g, 3.3 mmol) was suspended in anhydrous CH_2Cl_2 under a N_2 atmosphere. Oxalyl chloride (0.46 g, 0.316 mL, 3.63 mmol, 1.1 eq) was added, followed by DMF (2 drops). Effervescence was observed and a red solution formed. The reaction mixture was stirred over night then added to a cooled (-30 ºC) solution of L-proline methyl ester (0.593 g, 3.63 mmol, 1.1 eq), followed by a solution of Et_3N (1.668 g, 2.3 mL, 16.5 mmol, 5.0 eq) in anhydrous CH_2Cl_2 (50 mL) cooled to -30°C, over 5 min. This temperature was maintained for 1 h during which time a precipitate formed. The reaction mixture was allowed to rise to room temperature over 3 h. The organic layer was washed with 1M HCl solution (2 × 30 mL), then water (2 × 30 mL) and brine (2 × 30 mL). The organic extracts were dried ($MgSO_4$) then concentrated *in vacuo* and dried *in vacuo* to give an orange foam, **52** (1.318 g, 97%). ^{1}H-NMR ($CDCl_3$) mixture of rotamers δ 7.76 (1H, s, phenyl H-6), 7.74 (1H, s, phenyl H-6), 7.47-7.33 (5H, m, benzyl Ph-H), 6.87 (1H, s, phenyl H-3), 6.82 (1H, s, phenyl H-3), 5.21 (2H, s, benzyl CH_2), 5.20 (1H, s, benzyl CH_2), 4.75 (1H, dd, *J* = 4.5, 8.7 Hz, H-2), 4.05-4.01 (1H, m, H-2), 3.99 (3H, s, OCH_3), 3.93 (3H,

s, OCH_3), 3.81 (3H, s, OCH_3), 3.36-3.30 (1H, m, H-5), 3.22-3.16 (1H, m, H-5), 2.39-2.22 (1H, m, H-3), 2.12-1.89 (3H, m, H-3,4).

8-Hydroxy-7-methoxy-1,2,3,11a-tetrahydro-10*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (53)[6]

A solution of the nitro compound **52** (6.0 g) in EtOH (90 mL) was treated with a suspension of 10 % palladium on charcoal (0.6 g) in ethanol (10 mL). The mixture was agitated under a H_2 atmosphere (50 psi) for 3 h then filtered through a pad of Celite. The bed was washed with EtOAc and the combined filtrates concentrated *in vacuo*. The residue was further dried *in vacuo* then triturated with EtOAc to afford a grey solid which was collected by filtration and dried *in vacuo* to give the product **53**, (3.35 g, 88%) as a solid. ^{1}H-NMR (d_6-DMSO) δ 10.21 (1H, s, NH), 9.93 (1H, s, OH), 7.21 (1H, s, H-9), 6.55 (1H, s, H-6), 4.06-4.02 (1H, m, H-11a), 3.77 (3H, s, OCH_3), 3.56-3.50 (1H, m, H-3), 3.46-3.40 (1H, m, H-3), 2.47-2.44 (1H, m, H-1), 1-98-1.73 (3H, m, H-1,2).

Benzyl (11a*S*)-4-(7-methoxy-5,11-dioxo-2,3,5,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butanoate (54)

A mixture of dilactam **53** (0.100 g, 0.38 mmol), benzyl bromobutyrate (0.117 g, 0.46 mmol, 1.2 eq) and K_2CO_3 (0.079 g, 0.57 mmol, 1.5 eq) in DMF (6 mL) was stirred overnight at room temperature. The solvent was then removed *in vacuo* and the residue was digested in water (40 mL) and then extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by column chromatography (eluted with EtOAc-hexane gradient 3:7 – 5:5) to give **54** (0.103 g, 62%) as an almost colorless oil. ^{1}H-NMR ($CDCl_3$) δ 10.23 (1H, s, NH), 7.38-7.35 (5H, m, benzyl Ph-H), 7.23 (1H, s, H-9), 6.67 (1H, s, H-6), 5.10 (2H, s, benzyl CH_2), 4.07-3.95 (3H, m, H-11a, sidechain H-1), 3.76 (3H, s, OCH_3), 3.58-3.51 (1H, m, H-3), 3.47-3.42 (1H, m, H-3), 2.57-2.44 (3H, m, H-1, sidechain H-3), 2.07-2.01 (2H, m, sidechain H-2), 1.94-1.88 (3H, m, H-1,2).

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^{13}C-NMR and IR Data for Compounds 6, 22, 35, 38, 46-48, 50a-f.

(11a*S*) Methyl 4-(7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butanoate (6)
^{13}C-NMR ($CDCl_3$) δ 173.4, 164.5, 162.3, 150.5, 147.8, 140.6, 120.3, 111.6, 110.6, 67.7, 56.1, 53.6, 51.6, 46.6, 30.4, 29.6, 24.2, 24.1; IR (solid) ν_{max} 3323, 2952, 1734, 1625, 1601, 1506, 1433, 1372, 1262, 1217, 1173, 1127, 1092, 1021, 951, 875, 765, 729.

Methyl 4-({4-[(4-formamino-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carboxylate (22)
^{13}C-NMR (d_6-DMSO) δ 160.8, 158.4, 158.3, 157.9, 122.9, 122.5, 122.4, 122.3, 122.1, 120.7, 118.5, 118.5, 108.3, 104.8, 104.7, 103.9, 59.7, 50.9, 36.1, 36.0; IR (solid) ν_{max} 3285, 2957, 1688, 1581, 1433, 1401, 1248, 1102, 778 cm^{-1}.

(11*S*,11a*S*)-8-(2-Benzyloxycarbonylethoxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10-carboxylic acid *tert*-butyl ester (35)
^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5, 167.0, 149.5, 148.5, 135.6, 129.2, 128.6, 128.5, 128.3, 128.2, 126.2, 114.8, 110.8, 85.7, 81.8, 66.6, 64.7, 59.7, 56.1, 34.4, 28.8, 28.4, 23.0; IR ($CHCl_3$) 2975, 2362, 1698, 1603, 1514, 1455, 1433, 1394, 1322, 1164, 1042, 852, 731 cm^{-1}.

(11a*S*) Methyl 4-{[4-({4-[4-(7-Methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)propionylamino]-1-methyl-1*H*-pyrrole-2-carbonyl]amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carboxylate (38)
^{13}C-NMR (d_6-DMSO) δ 168.8, 164.2, 160.8, 158.4, 158.3, 122.9, 122.7, 122.4, 122.1, 120.7, 118.6, 118.4, 108.3, 104.7, 103.9, 67.4, 55.6, 50.9, 36.1, 36.1, 35.3, 29.8, 29.3, 23.2, 22.4; IR (solid) ν_{max} 3298, 2940, 1702, 1642, 1582, 1434, 1246, 1196, 1109, 750 cm^{-1}.

(11*S*,11a*S*) 11-Hydroxy-7-methoxy-8-(3-methoxycarbonylpropoxy)-5-oxo-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10-carboxylic acid allyl ester (46)
^{13}C-NMR ($CDCl_3$) δ 173.4, 167.0, 156.0, 149.9, 148.7, 131.8, 128.3, 125.9, 118.1, 113.9, 110.7, 86.0, 67.9, 66.8, 60.4, 59.9, 56.1, 51.7, 46.4, 30.3, 28.7, 24.2, 23.1, 21.1; IR (solid) ν_{max} 2951, 1704, 1604, 1516, 1458, 1434, 1313, 1272, 1202, 1134, 1103, 1041, 1013, 647 cm^{-1}.

(11*S*,11a*S*)-7-Methoxy-8-(3-methoxycarbonylpropoxy)-5-oxo-11-(tetrahydropyran-2-yloxy)-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10-carboxylic acid allyl ester (47)
^{13}C-NMR ($CDCl_3$) δ 173.4, 167.2, 149.1, 132.0, 114.5, 100.0, 98.4, 94.6, 91.7, 68.0, 67.7, 66.3, 63.9, 63.6, 63.3, 62.9, 56.1, 51.6, 51.5, 46.3, 46.3, 31.1, 30.9, 30.7, 30.4, 30.2, 29.0, 25.4, 25.3, 25.2, 24.2, 20.0, 19.8, 19.7.

(11*S*,11a*S*)-8-(3-Carboxypropoxy)-7-methoxy-5-oxo-11-(tetrahydropyran-2-yloxy)-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10-carboxylic acid allyl ester (48)

^{13}C-NMR (d_6-DMSO) δ 173.9, 173.9, 171.9, 166.1, 166.0, 149.6, 148.4, 148.3, 132.6, 116.5, 114.4, 110.5, 110.3, 99.2, 67.5, 67.4, 65.6, 65.5, 62.8, 59.4, 55.7, 45.9, 30.5, 30.2, 29.8, 29.7, 28.4, 28.3, 24.9, 24.8, 23.9, 23.8, 22.9, 22.7.

(11a*S*) Methyl 4-[4-(7-methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butyrylamino]-1-methyl-1*H*-pyrrole-2-carboxylate (50a)

^{13}C-NMR (d_6-DMSO) δ 168.8, 164.2, 163.3, 160.7, 150.2, 146.9, 122.7, 120.4, 119.8, 118.5, 111.2, 110.1, 107.6, 67.7, 55.6, 53.4, 50.9, 46.3, 36.1, 31.9, 28.8, 24.6, 23.6; IR (solid) ν_{max} 3296, 2937, 1702, 1596, 1580, 1451, 1255, 1196, 1097, 782 cm^{-1}.

(11a*S*) Methyl 4-({4-[4-(7-Methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butyrylamino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carbxylate (50b)

^{13}C-NMR (d_6-DMSO) δ 168.8, 164.2, 163.3, 160.8, 158.4, 150.2, 146.9, 140.6, 122.9, 122.5, 122.1, 120.7, 119.8, 118.5, 118.3, 111.3 , 110.1, 108.3, 104.0, 67.8, 55.6, 53.4, 50.9, 46.4, 36.1, 36.0, 31.9, 28.8, 24.7, 23.6; IR (solid) ν_{max} 3300, 2947, 1703, 1596, 1582, 1448, 1435, 1252, 1197, 1100, 781 cm^{-1}.

(11a*S*) Methyl 4-{[4-({4-[4-(7-Methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butyrylamino]-1-methyl-1*H*-pyrrole-2-carbonyl]amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carboxylate (50c)

^{13}C-NMR (d_6-DMSO) δ 168.8, 164.2, 163.3, 160.8, 158.5, 158.1, 150.2, 146.9, 140.6, 123.0, 122.7, 122.5, 122.2, 122.0, 120.7, 119.8, 118.6, 118.5, 118.2, 111.3, 110.1, 108.3, 104.0, 104.0, 55.6, 53.4, 50.9, 46.4, 36.2, 36.1, 36.0, 31.9, 28.8, 24.8, 23.7; IR (solid) ν_{max} 3300, 2946, 1702, 1594, 1579, 1433, 1249, 1199, 1104, 774 cm^{-1}.

(11a*S*) Methyl 4-[(4-{[4-({4-[4-(7-Methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butyrylamino]-1-methyl-*1H*-pyrrole-2-carbonyl]amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carboxylate (50d)

^{13}C-NMR (d_6-DMSO) δ 168.8, 164.2, 163.3, 160.8, 158.5, 158.4, 150.2, 146.9, 140.6, 123.0, 122.7, 122.5, 122.3, 122.1, 122.0, 120.7, 119.8, 118.6, 118.5, 118.1, 111.3, 110.1, 108.4, 104.8, 104.7, 104.0, 55.6, 53.4, 50.9, 46.4, 36.1, 36.1, 31.9, 28.8, 24.8, 23.7; IR (solid) ν_{max} 3289, 2947, 1706, 1632, 1580, 1433, 1250, 1199, 1106, 772 cm^{-1}.

(11a*S*) Methyl 4-({4-[(4-{[4-({4-[4-(7-Methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butyrylamino]-1-methyl-1*H*-pyrrole-2-carbonyl]amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carboxylate (50e)

^{13}C-NMR (d_6-DMSO) δ 168.8, 164.2, 163.3, 160.8, 158.5, 158.4, 150.2, 146.9, 140.6, 123.0, 122.7, 122.5, 122.3, 122.2, 122.1, 122.0, 120.7, 118.6, 118.5, 118.2, 111.3, 110.1, 108.4, 104.8, 104.8, 102.0, 67.8, 55.6, 53.4, 50.9, 46.4, 36.2, 36.1, 31.9, 28.8, 24.8, 23.7; IR (solid) ν_{max} 3297, 2945, 1701, 1631, 1579, 1434, 1251, 1199, 1106, 774 cm^{-1}.

(11a*S*) Methyl 4-{[4-({4-[(4-{[4-({4-[4-(7-Methoxy-5-oxo-2,3,5,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-8-yloxy)butyrylamino]-1-methyl-1*H*-pyrrole-2-carbonyl]amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carbonyl)amino]-1-methyl-1*H*-pyrrole-2-carbonyl}amino)-1-methyl-1*H*-pyrrole-2-carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carboxylate (50f)

^{13}C-NMR (*d*$_6$-DMSO) δ 168.8, 164.3, 163.3, 160.8, 158.5, 158.4, 150.2, 146.9, 140.6, 123.0, 122.8, 122.7, 122.5, 122.3, 122.2, 122.1, 122.0, 120.7, 119.8, 118.5, 118.5, 118.1, 111.3, 110.1, 108.4, 104.8, 104.8, 104.8, 104.7, 104.7, 67.8, 55.6, 53.4, 50.9, 46.4, 36.2, 36.2, 36.1, 36.0, 35.9, 31.9, 28.8, 24.8, 23.7; IR (solid) ν_{max} 3300, 2945, 1701, 1634, 1581, 1433, 1250, 1200, 1106, 772 cm^{-1}.

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HPLC Data for Key Compounds 6, 22, 38, 50a-f and 56.

Compound	HPLC System	Retention Time
6	LCMS[a]	2.25 min
22	LCMS[a]	2.67 min
38	LCMS[a]	2.63 min
50a	LCMS[a]	2.38 min
50a	HPLC[b]	13.74 min
50b	LCMS[a]	2.50 min
50b	HPLC[b]	14.51 min
50c	LCMS[a]	2.73 min
50d	LCMS[a]	2.85 min
50d	HPLC[c]	3.23min
50e	LCMS[a]	2.90 min
50e	HPLC[c]	3.36 min
50f	LCMS[a]	2.98 min
56	LCMS[d]	1.43 min

Notes: Purity was judged to be >95% based on area under the peak. Detection of peaks was by UV at 254 nm (diode array detector). LCMS analyses gave mass ions correct for (M^++1) for the eluted peaks. *a*. LC-MS analysis used a Luna 3µ C8(2) 50 x 4.6 mm column, flow rate 1.5 mL/min and a linear gradient from 95:5 solvent A:B at time 0 to 5:95 A:B at 4 min after sample injection then maintained at 5:95 until 7 min. Solvent A is 0.1% formic acid in water, solvent B is 0.1% formic acid in acetonitrile; *b*. HPLC analysis used a Luna 5µ C18(2) 250 x 4.6 mm column with a flow rate of 1.0 mL/min and a linear gradient solvent system going from 0:100 solvent A:B at time 1 min to 100:0 A:B at 21 min after sample injection then maintained at 100:0 until 24 min. Solvent A is 0.1% TFA in water, solvent B is 0.1% TFA in acetonitrile; *c*. HPLC analysis used a Luna 5µ C18(2) 250 x 4.6 mm column with a flow rate of 1.0 mL/min and a solvent system of 0.1% TFA in water (40%) and acetonitrile (60%); *d*. LC-MS analysis used a Luna 3µ C8(2) 50 x 4.6 mm column with a flow rate of 3 mL/min and a linear gradient solvent system going from 95:5 solvent A:B at time 0 to 5:95 A:B at 3 min after sample injection then maintained at 5:95 until 4 min. Solvent A is 0.1% formic acid in water, solvent B is 0.1% formic acid in acetonitrile.