Supporting Information

Synthesis of 3,3’-Di-O-Methyl Ardimerin and Exploration of its DNA Binding Properties

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Spectroscopic data for compounds:

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**General Methods.** Distilled water was used in all of the experiments. Organic extracts were dried over Na$_2$SO$_4$, filtered, and concentrated using a rotary evaporator at aspirator pressure (20-30 mmHg). Chromatography refers to flash chromatography and was carried out on SiO$_2$ (silica gel 60, 230-400 mesh). $^1$H and $^{13}$C NMR spectra were measured in CDCl$_3$ at 400 MHz and 100 MHz, respectively, using Me$_4$Si as internal standard. Chemical shifts are reported in ppm downfield (δ) from Me$_4$Si.
Alcohol 2a\textsuperscript{6} (5.0 g, 9.2 mmol) was dissolved in 2:1 CH\textsubscript{2}Cl\textsubscript{2}: trifluoroacetic anhydride (31 mL, 0.3 M) and stirred at room temperature for 30 minutes. The solvent was concentrated \textit{in vacuo} and azeotroped with toluene (3x15 mL). To the residue was added CH\textsubscript{2}Cl\textsubscript{2} (31 mL, 0.3M) and trimethoxybenzene (3a, 3.86 g, 23 mmol, 2.5 equivalents) and the mixture was cooled to 0\textdegree C. Boron trifluoride-diethyl etherate (1.95 g, 13.8 mmol, 1.5 equivalents) was added and the solution was stirred at 0\textdegree C for 30 minutes. A solution of saturated NaHCO\textsubscript{3} (20 mL) and ether (20 mL) was added to the reaction mixture. The phases were separated and the aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated under reduced pressure to give a crude oil. Purification of the residue by flash chromatography (8:1 hexanes: ethyl acetate) afforded 4 (3.93 g, 5.7 mmol, 62%).

\textit{See spectra on page S13}

\textsuperscript{1}H NMR: (400 MHz, CDCl\textsubscript{3})

7.39-7.10 (m, 20H); 6.92 (d, \textit{J}=7.6 Hz, 1H); 6.70 (d, \textit{J}=7.6 Hz, 1H); 4.91(m, 3H); 4.68-4.41 (m, 7H); 4.06 (d, \textit{J}=10.8 Hz, 1H); 3.88 (s, 3H); 3.86 (s, 3H); 3.93 (s, 3H); 3.85-3.70 (m, 3H); 3.60 (m, 1H).

\textsuperscript{13}C NMR: (100 MHz, CDCl\textsubscript{3})

153.6; 152.7; 142.3; 138.9; 138.5; 138.0; 128.5; 128.0; 127.9; 127.6; 125.6; 107.8; 87.1; 83.5; 79.4; 78.5; 77.4; 71.8; 69.3; 61.7; 60.8; 56.1.

HRMS (ESI): calculated for C\textsubscript{43}H\textsubscript{46}NaO\textsubscript{8} 713.3090 found 713.3114 (M+Na)\textsuperscript{+}

[\alpha]\textsuperscript{25}\text{D}: +1.6\textdegree (c = 0.005, CH\textsubscript{2}Cl\textsubscript{2})
Compound **4** (3.93 g, 5.7 mmol) was dissolved in CHCl₃ (5.7 mL, 1.0M). NBS (1.02 g, 5.77 mmol, 1.01 equiv) was added and the solution was refluxed for one hour. The solution was cooled to room temperature. The reaction was then carefully quenched with saturated NaHCO₃ (10 mL) and ether (10 mL). The phases were separated and the aqueous layer was extracted with ether (2 x 25 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude oil. Purification of the residue by flash chromatography (10:1 hexanes:ethyl acetate) afforded **5** (3.90 g, 5.13 mmol, 90%).

*See spectra on page S14*

**¹H NMR:** (400 MHz, CDCl₃)

7.40-7.28 (m, 22H); 7.08 (m, 2H); 5.14 (dd, J=17.5, 11.2 Hz, 2H); 5.07 (d, J=10.8 Hz, 1H); 4.83-4.67 (m, 5H); 4.32 (d, J=10.8 Hz, H); 4.06 (s, 3H); 4.04 (s, 3H); 4.01 (s, 3H); 3.99-3.77 (m, 6H).

**¹³C NMR:** (100 MHz, CDCl₃)

152.3; 151.3; 147.5; 138.9; 138.4; 137.8; 130.1; 128.5; 128.4; 128.2; 128.0; 127.8; 127.7; 112.0; 87.2; 83.1; 79.5; 78.5; 75.6; 72.2; 75.1; 73.5; 69.2; 61.7; 61.1; 61.0

**HRMS (ESI):** calculated for C₄₃H₄₅BrNaO₈ 791.2196 found 791.2245 (M+Na)⁺

[^25]D: -9.5° (c = 0.002, CH₂Cl₂)
Isopropylmagnesium chloride (1.2 mL, 2.4 mmol, 2M in THF) was added to THF (4 mL) and the solution was cooled to 0°C. A solution of n-butyllithium (2.4 mL, 4.8 mmol, 2 M in cyclohexane) was added and the mixture was stirred at 0°C for 10 minutes. The solution was cooled to -78°C and stirred for ten minutes. Compound 5 (1.51 g, 2 mmol) was dissolved in THF (4 mL) and then added dropwise to the magnesiate solution, and the mixture was stirred at -78°C for 40 minutes. The reaction was warmed to -20°C for five minutes, and then anhydrous DMF (0.8 mL) was added rapidly to the mixture. After stirring at -20°C for 30 minutes, a saturated aqueous solution of NaHCO$_3$ (10 mL) and ether (10 mL) was added. The phases were separated and the aqueous layer was extracted with ether (2 x 25 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give a crude oil. Purification of the residue by flash chromatography (8:1 hexanes: ethyl acetate) afforded aldehyde 6a (1.01 g, 1.42 mmol, 71%).

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$^1$H NMR: (400 MHz, CDCl$_3$)
10.38 (s, 1H); 7.83 (s, 1H); 7.46-7.22 (m, 18 H); 7.00 (m, 2H); 5.00 (m, 3H); 4.79-4.60 (m, 4H); 4.23 (d, J=11.2 Hz, 1H); 4.12 (s, 3H); 3.99 (s, 3H); 3.93 (s, 3H); 3.99-3.87 (m, 6H); 3.75 (m, 1H).

$^{13}$C NMR: (100 MHz, CDCl$_3$)
188.6; 158.3; 157.1; 145.7; 138.8; 138.5; 138.3; 137.8; 129.3; 128.5; 128.4; 128.3; 128.2; 128.1; 127.8; 127.6; 125.5; 87.2; 83.2; 79.6; 78.5; 75.7; 75.5; 75.4; 75.2; 75.1; 75.0; 73.5; 73.4; 73.3; 69.3; 62.4; 61.6; 61.5; 60.8; 60.7.

HRMS (ESI): calculated for C$_{44}$H$_{46}$NaO$_7$ 741.3040 found 741.3056 (M+Na)$^+$

$[\alpha]_{25}^{25}$: -16.6° (c=0.001, CH$_2$Cl$_2$)
Aldehyde 6 (42 mg, 0.058 mmol) was dissolved in CH$_3$CN (0.23 mL, 0.25 M) and AlCl$_3$ (9 mg, 1.1 equiv) was added. Then NaI (13 mg, 1.5 equiv) was added and the solution was degassed with argon. The mixture was then heated to 80°C and stirred for one hour. The reaction mixture was then cooled to room temperature and diluted with toluene (3 mL). 1N HCl (3 mL) was added and the solution was stirred for one hour until the aqueous layer was clear and colorless. Ether (3 mL) was added and the layers were separated. The aqueous layer was then extracted with ether (2 x 15 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give a crude oil. Purification of the residue by flash chromatography (5:1 hexanes: ethyl acetate) afforded aldehyde 7 (35 mg, 0.052 mmol, 90%).

*See spectra on page S16*

$^1$H NMR: (400 MHz, CDCl$_3$)

- 11.27 (s, 1H);
- 9.65 (s, 1H);
- 7.39-7.15 (m, 20H);
- 6.94 (d, $J= 7.2$ Hz, 1H);
- 4.99-4.90 (m, 3H);
- 4.67-4.53 (m, 4H);
- 4.23 (d, $J=11.2$ Hz, 1H);
- 4.01 (s, 3H);
- 3.93 (s, 3H);
- 3.92-3.76 (m, 6H);
- 3.64 (m, 1H).

$^{13}$C NMR: (100 MHz, CDCl$_3$)

- 195.4; 158.2; 156.1; 139.8; 138.6; 138.2; 138.1; 137.5; 128.5; 128.4; 128.3; 128.1; 128.0; 127.9;
- 127.8; 127.7; 127.6; 127.5; 125.0; 117.5; 87.2; 82.6; 79.3; 78.4; 75.6; 75.1; 74.7; 73.4; 69.1;
- 61.6; 60.6; 30.3; 29.7.

HRMS (ESI): calculated for C$_{43}$H$_{45}$O$_7$ 705.3064 found 705.3006 (M+H)$^+$

$[\alpha]_{D}^{25}$: -29.5° (c=0.0009, CH$_2$Cl$_2$)
Aldehyde 7 (130 mg, 0.184 mmol) was dissolved in DMF (0.5 mL) and THF (0.5 mL), and benzyl chloride (0.041 mL, 0.36 mmol) was added. The solution was cooled to -78°C, and then NaHMDS (0.13 mL, 2M in THF, 1.5 equiv) was added dropwise. The mixture was allowed to warm to room temperature, and tetra-n-butylammonium iodide (10 mg) was added. The solution was allowed to stir 18 hours under an atmosphere of argon. The mixture was then quenched by the slow addition of saturated NaHCO₃ (5 mL) and ether (5 mL) and the layers were separated. The aqueous layer was then extracted with ether (2 x 15 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude oil. Purification of the residue by flash chromatography afforded the benzylated aldehyde (133 mg, 0.167 mmol, 91%).

The benzylated aldehyde (133 mg, 0.167 mmol) was dissolved in 0.5 mL acetone and cooled to 0°C. Then sulfamic acid (16 mg, 1.0 equiv) was added followed by water (0.2 mL) The mixture was stirred rapidly and then NaClO₂ (21 mg, 1.1 equiv) was added and the reaction was stirred for 20 minutes, at which time a deep yellow color was evident. The mixture was diluted with ether (10 mL) and water (5 mL) and the layers were separated. The ether layer was dried over anhydrous sodium sulfate and evaporated to give a crude oil. Purification of the residue by flash chromatography (3:1 hexanes:ethyl acetate) afforded carboxylic acid 8 (75 mg, 0.092 mmol, 50%).

See spectra on page S17

1H NMR: (400 MHz, CDCl₃)
7.98 (s, 1 H); 7.49-7.15 (m, 24H); 6.93 (m, 2H); 5.38 (d, J=6.1 Hz, 2H); 4.94-4.86 (m, 4H); 4.72-4.53 (m, 6H); 4.11 (d, J=11.0 Hz, 1H); 3.94 (s, 3H); 3.91 (s, 3H); 3.90-3.65 (m, 6H).

13C NMR: (100 MHz, CDCl₃)
164.6; 157.1; 151.4; 145.3; 138.6; 138.3; 138.2; 138.1; 137.7; 134.6; 130.0; 129.4; 129.1; 128.9; 128.7; 128.6; 128.5; 128.3; 128.2; 128.1; 127.9; 127.8; 127.7; 127.6; 127.5; 127.3; 117.8; 117.4; 98.1; 87.1; 82.8; 80.1; 79.9; 79.4; 78.4; 76.7; 75.4; 75.1; 74.9; 74.8; 73.3; 72.0; 69.2; 68.5; 67.8; 67.3; 61.5; 60.9; 31.9; 30.3; 29.6; 29.3; 22.6; 17.8; 14.1.

HRMS (ESI): calculated for C₅₀H₅₀NaO₁₀ 833.3302 found 833.3267 (M+Na)⁺

[α]D²⁵: -17.5° (c=.003, CH₂Cl₂)
Aldehyde 10a\textsuperscript{14} (130 mg, 0.714 mmol) was dissolved in DMF (0.5 mL) and THF (0.5 mL), and was cooled to -78°C. Then NaHMDS (0.45 mL, 2M in THF, 0.89 mmol, 1.2 equiv) was added dropwise. The mixture was allowed to warm to room temperature, providing a solution of sodium alkoxide 10b. Separately, acid 9a\textsuperscript{13} (100 mg, 0.35 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (1 mL) and DMF (1 drop) was added. The mixture was cooled to 0°C and oxalyl chloride (0.033 mL, 1.1 equiv) was added dropwise. The mixture was stirred for 15 minutes and concentrated \textit{in vacuo}. The residue was resuspended in THF and cooled to 0°C. Sodium alkoxide 10b was added rapidly via cannula and the mixture was allowed to warm to room temperature over one hour. The reaction mixture was then diluted with saturated NaHCO\textsubscript{3} solution (10 mL) and ether (10 mL) and the phases were separated. The aqueous layer was then extracted with ether (2 x 15 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated under reduced pressure to give a crude oil. Rapid filtration through a plug of silica gel (1:1 hexanes: ethyl acetate) to remove excess 10a afforded the coupled benzyl aldehyde intermediate.

The benzylated aldehyde was dissolved in 1.0 mL acetone and cooled to 0°C. Then sulfamic acid (34 mg, 1.0 equiv) was added followed by water (0.4 mL) The mixture was stirred rapidly and then NaClO\textsubscript{2} (35 mg, 1.1 equiv) was added and the reaction was stirred for 20 minutes, at which time a deep yellow color was evident. The mixture was diluted with ether (10 mL) and water (10 mL) and the layers were separated. The ether layer was dried over anhydrous sodium sulfate and evaporated to give a crude oil. Purification of the residue by flash chromatography (1.5:1 hexanes: ethyl acetate) afforded carboxylic acid 11 (129 mg, 0.276 mmol, 79%).

\textit{See spectra on page S18}

\textsuperscript{1}H NMR: (400 MHz, CDCl\textsubscript{3})

7.96 (d, J=8.92 Hz, 1H); 7.86 (d, J=8.96 Hz, 1H); 7.54 (d, J=8.24 Hz, 1H); 7.29 (m, 4H); 6.88 (d, J=9.0 Hz, 1H); 6.79 (d, J=8.96 Hz, 1H); 5.14 (s, 2H); 3.96 (s, 3H); 3.95 (s, 3H); 3.89 (s, 3H); 3.80 (s, 3H).

\textsuperscript{13}C NMR: (100 MHz, CDCl\textsubscript{3})

168.7; 162.7; 158.0; 157.7; 154.2; 145.6; 143.2; 142.1; 137.5; 128.4; 128.2; 128.0; 127.9; 127.7; 117.1; 115.9; 108.9; 107.2; 61.1; 61.0; 56.2; 56.1.

HRMS (ESI): calculated for C\textsubscript{25}H\textsubscript{24}NaO\textsubscript{9} 491.1318 found 491.1347 (M+Na)+
Benzylxoy acid 11 (50 mg, 0.106 mmol) was dissolved in SOCl\(_2\) (1 mL) and the mixture was stirred at 80°C for three hours under argon. After cooling to room temperature, the mixture was diluted with benzene (5 mL) and was concentrated in vacuo until there was approximately 1 mL of solvent remaining. This process was repeated again, and then the residue was diluted to a volume of 20 mL with benzene. 4-Dimethylaminopyridine (39 mg, 0.318 mmol, 3 equiv) was added, along with sodium sulfate (200 mg) and the mixture was allowed to stir at room temperature for 12 hours. The mixture was diluted with ether (10 mL) and saturated NaHCO\(_3\) solution (10 mL) and the layers were separated. The organic layer was further washed with saturated NaHCO\(_3\) (10 mL), 1N HCl (10 mL), and saturated NaHCO\(_3\) (10 mL). The organic extracts were dried over anhydrous sodium sulfate and evaporated to give a crude oil. Purification of the residue by flash chromatography (1.5:1 hexanes:ethyl acetate) afforded diolide 12 (20 mg, 0.058 mmol, 55%).

*See spectra on page S19*

\(^1\)H NMR: (400 MHz, CDCl\(_3\))

7.65 (d, \(J=8.76\) Hz, 2H); 6.97 (d, \(J=8.76\) Hz, 2H); 3.98 (s, 3H); 3.92 (s, 3H).

\(^13\)C NMR: (100 MHz, CDCl\(_3\))

163.3; 156.9; 142.1; 141.9; 126.2; 117.9; 109.8; 60.7; 56.1.

HRMS (ESI): calculated for C\(_{18}\)H\(_{17}\)O\(_8\) 361.0923 found 361.0960 (M+H)^+
Aldehyde 7a (74 mg, 0.1 mmol) was dissolved in THF (1.0 mL) and was cooled to 0°C. Then KOr-Bu (0.1 mL, 1M in THF, 0.1 mmol) was added dropwise. The mixture was allowed to stir for 10 minutes, providing a solution of potassium alkoxide 7b. Separately, acid 8a (55 mg, 0.07 mmol) was dissolved in CH₂Cl₂ (1.0 mL) and one drop of DMF was added. Oxalyl chloride (0.01 mL) was added and a vigorous evolution of gas from the reaction mixture was noted. After five minutes the mixture was diluted with 1:1 benzene:hexanes (3 mL) and stirred for 2 minutes. The mixture was filtered through a cotton plug and concentrated in vacuo. The oil was taken up in THF (0.5 mL) and was added dropwise to the solution of potassium alkoxide. The reaction was allowed to stir overnight at room temperature. The reaction mixture was then diluted with saturated NaHCO₃ solution (10 mL) and ether (10 mL) and the phases were separated. The aqueous layer was then extracted with ether (2 x 15 mL). The combined organic extracts were once washed with 20 mL sat. aq. NaCl, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude oil. Rapid filtration through a plug of silica gel (4:1 → 2:1 hexanes: ethyl acetate) to remove excess 7a afforded the coupled aldehyde intermediate (92.7 mg, 0.062 mmol, 89%).

The aldehyde 13a (45 mg, 0.03 mmol) was dissolved in 0.6 mL tBuOH and 2-methyl-2-butene (0.126 mL) was added. An additional 0.1 mL acetone was then added to achieve solubility. An aqueous solution (0.21 mL) of NaClO₂ and NaH₂PO₄ (100 mg NaClO₂ and 112 mg NaH₂PO₄ per mL H₂O) was added and the solution was allowed to stir for one hour at room temperature, at which point complete conversion of the starting material had been achieved. The mixture was diluted with CH₂Cl₂ (10 mL) and water (10 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulfate and evaporated to give a crude oil. Purification of the residue by flash chromatography (2:1 hexanes: ethyl acetate) afforded carboxylic acid 13b (29.4 mg, 0.0195 mmol, 65%).

See spectra on page S20

^1^H NMR: (400 MHz, CDCl₃)

8.30 (s, 1H); 8.00 (s, 1H); 7.59 (d, J=6.6 Hz, 2H); 7.38-7.13 (m, 39H); 6.98 (m, 3H); 6.95 (d, J=8.0 Hz, 2H); 5.23 (s, 2H); 5.03-4.94 (m, 5H); 4.90-4.85 (m, 3H); 4.66 (m, 3H); 4.61 (m, 6H); 4.59 (m, 1H); 4.16 (d, J=10.4 Hz, 1H); 4.11 (d, J=10.6 Hz, 1H); 3.94 (s, 3H); 3.88 (s, 3H); 3.81-3.60 (m, 12H); 3.82 (s, 3H); 3.79 (s, 3H).

^1^C NMR: (100 MHz, CDCl₃)

161.9; 156.4; 138.7; 138.6; 138.3; 138.2; 137.8; 137.7; 137.4; 137.2; 131.1; 128.5; 128.4; 128.3; 128.2; 128.0; 127.8; 127.7; 127.6; 127.5; 125.5; 120.0; 87.0; 86.9; 83.2; 79.5; 78.9; 78.5; 78.1; 77.2; 76.3; 75.5; 75.4; 75.3; 75.1; 75.0; 73.4; 69.2; 68.7; 65.8; 61.6; 61.1; 61.0; 60.6; 60.3; 55.9.

HRMS (ESI): calculated for C₉₃H₉₂NaO₁₉ 1535.6131 found 1535.6153 (M+Na)⁺

[α]D^25_(13c): -19.5° (c=0.002, CH₂Cl₂)
Benzyloxy acid 13b (21 mg, 0.014 mmol) was dissolved in toluene (1 mL) and dimethyl sulfide (0.4 mL) was added. Then TFA (1 mL) was added and the mixture was stirred for 5 minutes at room temperature. The solution was concentrated in vacuo and the residue was dissolved in a minimal volume of CH₂Cl₂ and rapidly filtered through a plug of silica gel with 1.5:1 hexanes:ethyl acetate. Concentration of the eluted fractions in vacuo gave seco acid 13c ([α]²⁵_D =-19.5°, CH₂Cl₂).

Seco acid 13c (~0.014 mmol) was azeotroped with benzene (3x3 mL) and then dissolved in benzene (15 mL) and 4Å molecular sieves (100 mg) was added. Triethylamine (116 µL, 0.81 mmol) and 2,4,6-trichlorobenzoyl chloride (84 µL, 0.53 mmol) were added and the mixture was stirred at room temperature for five minutes. DMAP (17 mg, 0.139 mmol) was added and the mixture was allowed to stir at room temperature under argon overnight. The mixture was then diluted with ether (20 mL) and saturated NaHCO₃ solution (10 mL) and the phases were separated. The aqueous layer was then extracted with ether (2 x 15 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude oil. Purification of the residue by flash chromatography (4:1 3:1 hexanes: ethyl acetate) afforded 14 (9.8 mg, 0.007 mmol, 50%).

See spectra on page S21

¹H NMR: (400 MHz, CDCl₃)
7.34-7.29 (m, 30 H); 7.20-7.15 (10 H); 6.92 (d, J=6.5 Hz, 2H); 4.93 (m, 4H); 4.97 (d, J=10.8 Hz, 2H); 4.60 (m, 6H); 4.54 (d, J=12.3 Hz, 3H); 4.45 (d, J=12.1 Hz, 3H); 4.12 (d, J=11.0 Hz, 2H); 3.87-6.61 (m, 8H); 3.74 (s, 3H); 3.71 (s, 3H); 3.53 (m, 4H).

¹³C NMR: (150 MHz, CDCl₃)
163.6; 155.6; 144.6; 143.8; 138.4; 137.9; 137.2; 133.0; 128.3; 128.2; 127.9; 127.7; 127.5; 123.2; 118.9; 86.9; 83.1; 79.4; 78.2; 75.5; 75.1; 75.0; 73.7; 73.4; 68.9; 61.3; 60.7.

HRMS (ESI): calculated for C₈₆H₆₄NaO₁₈ 1427.5555 found 1427.5548 (M+Na)⁺

[α]²⁵_D: -8.8° (c=.008, CH₂Cl₂)
Compound 14 (22 mg, 0.016 mmol) was dissolved in THF (1 mL) and EtOH (1 mL). Pearlman’s catalyst (13 mg, 20% Pd(OH)$_2$ on C) was added and the mixture stirred under a hydrogen atmosphere for 18 hours. The mixture was then filtered through a bed of celite with 20% CH$_3$OH in EtOAc, and the solvent was concentrated in vacuo to give an oil (~10 mg, 0.014 mmol, 90%).

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$^1$H NMR: (400 MHz, CD$_3$OD)

7.42 (s, 2H); 4.94 (br s, 10H); 4.37 (t, $J=7.0$ Hz, 2H); 4.38 (t, $J=6.36$ Hz, 4H); 3.90 (s, 12H); 3.47-3.36 (m, 5H); 3.32 (s, 1H).

$^{13}$C NMR: (100 MHz, CDCl$_3$)

179.4; 161.5; 156.3; 144.9; 133.5; 123.0; 119.0; 81.1; 78.4; 74.8; 73.9; 70.4; 68.9; 61.6; 58.6; 57.7.

HRMS (ESI): calculated for C$_{30}$H$_{36}$NaO$_{18}$ 707.1799 found 707.1782 (M$+$Na)$^+$

$[\alpha]_{D}^{25}$: +15.5° (c=0.0009, CH$_3$OH)

UV: $\lambda_{max}$(log $\varepsilon$)=214 nm (5.14)
Compound 15 (2.0 mg, 0.003 mmol) was dissolved in acetyl chloride (1.2 mL) and was stirred under argon at room temperature overnight. The mixture was concentrated in vacuo and purified by flash chromatography (1:1 → 3:1 hexanes: ethyl acetate) to afford 16 (~2.6 mg, 0.0025 mmol, 85%)

See spectra on page S23

$^1$H NMR: (400 MHz, CDCl$_3$)

7.25 (s, 2H); 5.36-5.25 (m, 4H); 5.17 (t, J=9.4 Hz, 2H); 4.52 (d, J=8.5 Hz, 2H); 4.21 (d, J=10.0 Hz, 2H) 4.11 (d, J=12.2 Hz, 2H); 3.98 (br s, 12H); 3.89 (br s, 2H); 2.07 (br s, 9H); 2.06 (br s, 15 H).

$^{13}$C NMR: (100 MHz, CDCl$_3$)

170.5; 170.2; 169.4; 168.4; 162.9; 145.4; 145.1; 144.7; 129.0; 124.4; 118.7; 73.9; 71.0; 68.5; 68.1; 62.0; 61.7; 61.2; 61.0; 20.7; 20.6; 20.5; 20.3.

HRMS (ESI): calculated for C$_{46}$H$_{52}$NaO$_{26}$ 1043.2645 found 1043.2615 (M+Na)$^+$

$[\alpha]^{25}_D$: -49.3 (c=0.003, CH$_2$Cl$_2$)
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^{1}$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^{1}H\ (400\ MHz)\ and\ ^{13}C\ NMR\ (100\ MHz)\ spectra$
$^1$H (400 MHz) and $^{13}$C NMR (150 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra
$^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra

![Chemical structure and spectra](image-url)
$^1$H-$^1$H COSY 16 (CDCl$_3$, 400MHz)
$^1$H-$^1$H NOESY 16 (CDCl$_3$, 400MHz)

$n$Oe's observed

- $H_a \leftrightarrow H_1$
- $H_a \leftrightarrow H_2$
- $OCH_3 \leftrightarrow H_1$
- $H_5 \leftrightarrow H_1$
- $H_5 \leftrightarrow H_3$
- $H_5 \leftrightarrow H_6$
- $H_4 \leftrightarrow H_6$
**Procedure for UV absorption measurements**

A stock solution of compound 15 (11 mg in 2.67 mL of 2:1 MeOH:DMSO, 6.02 mM) was serially diluted with 10mM Tris-EDTA buffer solution (prepared according to the procedure of Jenkins: Jenkins, T.C. Optical Absorbance and Fluorescence Techniques for Measuring DNA-Drug Interactions. In *Methods in Molecular Biology, Drug-DNA Interaction Protocols*; Fox, K.R., Ed. Humana: Totowa, 1997; Vol 90, pp.195-221) to give eight separate samples of final concentrations 0.79 µM, 1.18 µM, 1.95 µM, 2.70 µM, 3.80 µM, 5.56 µM, 8.81 µM, and 11.76 µM, respectively.

Absorbance spectra were recorded in a quartz cuvette on an HP 8452A Diode Array Spectrophotometer connected to a 89090A Peltier Temperature Controller with the cell holder thermostatted at 25°C.

![UV absorption spectra of 15](image)

**Figure 3.** UV absorption spectra (10mM Tris-EDTA buffer) of 15 at varying concentrations, \([15] = 0.79, 1.18, 1.95, 2.70, 3.80, 5.56, 8.81, \) and 11.76 x 10^{-6} mol L^{-1} for curves 1-8, respectively.

**Procedures for DNA binding studies**

**UV protocols**

CT-DNA and Salmon Testes DNA were purchased from CALBIOCHEM. Solutions of CT-DNA and Salmon Testes DNA were prepared in 10mM Tris-EDTA buffer at pH 7.1 (as described in Jenkins, *vide supra*) and gave a 1.80:1 absorbance ratio at 260 nm and 280 nm. The concentration of the DNA was determined using 8452A HP Diode Array Spectrophotometer (ε_{260} = 6600 M^{-1} cm). The solution pH was measured using Beckman 350 pH meter.
A 7.94 x10^{-7} M solution of 15 in 10mM Tris-EDTA buffer was placed in a quartz cuvette (2 mL) and the UV spectrum was recorded ($\lambda_{\text{max}}$ = 214 nm). Aliquots of CT-DNA stock solution (9.84 mM) were then added and the UV spectrum was again recorded after each addition. Each addition produced final CT-DNA concentrations of 0.56, 1.68, 3.91, 8.30, 16.33, 41.35, and 84.50 x 10^{-7} M.

Fig. 4 UV absorption spectra of compound 15 in the presence of varying concentrations of CT-DNA, $C_{15} = 7.94 \times 10^{-7}$ mol L^{-1}, $C_{\text{DNA}} = 0.00, 0.56, 1.68, 3.91, 8.30, 16.33, 41.35, \text{and } 84.50 \times 10^{-7}$ mol L^{-1} for curves 1-8, respectively.
A 7.94 x10^{-7} M solution of daunorubicin in 10mM Tris-EDTA buffer was placed in a quartz cuvette (2 mL) and the UV spectrum was recorded ($\lambda_{\text{max}}=486 \text{ nm}$). Aliquots of CT-DNA stock solution (9.84 mM) were then added and the UV spectrum was again recorded after each addition. Each addition produced final CT-DNA concentrations of 1.68, 3.91, 8.30, 16.33, and 45.00 x 10^{-7} M.

Fig. 5  UV absorption spectra of Daunorubicin in the presence of varying concentrations of CT-DNA; [DNR] = 7.93 x 10^{-7} \text{ mol L}^{-1}, [\text{CT-DNA}] = 0.00 – 4.50 x 10^{-6} \text{ mol L}^{-1}.
DNA thermal denaturation experiments

DNA thermal denaturation experiments were conducted using 8452A HP Diode Array Spectrophotometer connected to 89090A Peltier Temperature Controller.

a. A stock solution of Salmon Testes DNA (9.84 mM) was diluted with 10mM Tris-EDTA buffer to give a working concentration of 5.7 µM. This solution (2 mL) was placed in a quartz cuvette and absorbance readings were taken in 1°C increments for temperatures ranging from 25 ºC to 84 ºC with heating at a rate of 1°C/min and absorbance monitoring at 260 nm.

b. 2mL of a solution containing Salmon Testes DNA (5.7 µM) and 15 (6.21 x10^8M) prepared from the corresponding stock solutions noted above was placed in a quartz cuvette and absorbance readings were taken in 1°C increments for temperatures ranging from 25 ºC to 84 ºC with heating at a rate of 1°C/min and absorbance monitoring at 260 nm.

Fig.6 Salmon Testes DNA denaturation; \( C_{\text{DNA}} = 5.70 \times 10^{-6} \text{ mol L}^{-1} \)
Fig. 7 Salmon Testes DNA denaturation in the presence of 15; $C_{15} = 6.21 \times 10^{-8} \text{ mol L}^{-1}$, $C_{\text{DNA}} = 5.70 \times 10^{-6} \text{ mol L}^{-1}$; $C_{15}:C_{\text{DNA}} = 0.01$

Fig 8. Melting Curves of Salmon Testes DNA in the absence and presence of Compound 15; $C_{15} = 6.21 \times 10^{-8} \text{ mol L}^{-1}$ and $C_{\text{DNA}} = 5.70 \times 10^{-6} \text{ mol L}^{-1}$.
b. 2mL of a solution containing Salmon Testes DNA (16.88 µM) and 15 (0.98 µM) prepared from the corresponding stock solutions noted above was placed in a quartz cuvette and absorbance readings were taken in 1°C increments for temperatures ranging from 25 °C to 84 °C with heating at a rate of 1°C/min and absorbance monitoring at 260 nm.

Fig 9. Salmon Testes DNA denaturation in the absence of 15; $C_{\text{DNA}} = 16.88 \times 10^{-6}$ mol L$^{-1}$
Fig 10. Salmon Testes DNA denaturation in the presence of 15; $C_{15} = 0.98 \times 10^{-6} \text{ mol L}^{-1}$, $C_{DNA} = 16.88 \times 10^{-6} \text{ mol L}^{-1}$; $C_{15}:C_{DNA} = 0.058$

Fig 11. Melting curves of salmon testes DNA in the absence and presence of 15; $[15] = 0.98 \times 10^{-6} \text{ mol L}^{-1}$ and $[DNA] = 16.88 \times 10^{-6} \text{ mol L}^{-1}$. $f_{ss} = (A - A_0)/(A_f - A_0)$, where $A_0$ is the initial absorbance intensity, $A$ is the absorbance intensity corresponding to its temperature, and $A_f$ is the final absorbance intensity.
Fig. 12 Salmon Testes DNA denaturation first derivative; $C_{15} = 0.98 \times 10^{-6}$ mol L$^{-1}$; $C_{DNA} = 16.88 \times 10^{-6}$ mol L$^{-1}$
**Fluorescence studies (Perkin Elmer Luminescence Spectrometer (LS 50B))**:

Ethidium bromide displacement studies were performed on a Perkin Elmer Luminescence Spectrometer (LS 50B) with $\lambda_{\text{ex}} = 525.0$ nm, $\lambda_{\text{em}} = 586$ nm (10 nm slits).

8 Eppendorf tubes were prepared with 2 mL total volume of EB-DNA complex (1.08 x $10^{-6}$ mol L$^{-1}$ CT-DNA and 1.12 x $10^{-6}$ mol L$^{-1}$ EB) and concentrations of 15 of 0.00, 0.08, 0.24, and 0.40, 0.71, 0.87, 1.03, or 1.19 x $10^{-6}$ mol L$^{-1}$. Fluorescence spectra were recorded from 525 nm to 670 nm after an equilibration period of 5 min.

![Emission Spectra of EB-DNA System](image)

Fig. 8 Emission Spectra of EB-DNA System (1.08 x $10^{-6}$ mol L$^{-1}$ DNA and 1.12 x $10^{-6}$ mol L$^{-1}$ EB) [Compound 15] = 0.00, 0.08, 0.24, and 0.40, 0.71, 0.87, 1.03, and 1.19 x $10^{-6}$ mol L$^{-1}$; $\lambda_{\text{ex}} = 525.0$ nm, $\lambda_{\text{em}} = 525.0 - 670.0$ nm
8 Eppendorf tubes were prepared with 2 mL total volume of EB-DNA complex (1.08 x 10^{-6} mol L^{-1} CT-DNA and 1.12 x 10^{-6} mol L^{-1} EB) and concentrations of daunorubicin of 0.00, 0.08, 0.24, or 0.39 x 10^{-6} mol L^{-1}. Fluorescence spectra were recorded from 525 nm to 670 nm after an equilibration period of 5 min. Due to the overlapping fluorescence emission from daunorubicin at 555 nm, the higher concentrations of daunorubicin (0.40, 0.71, 0.87, 1.03, or 1.19 x 10^{-6} mol L^{-1}) showed apparent fluorescence enhancement at 586 nm instead of quenching.

Fig.9 Emission Spectra of EB-DNA System (1.08 x 10^{-6} mol L^{-1} DNA and 1.12 x 10^{-6} mol L^{-1} EB); [DNR] = 0.00, 0.08, 0.24, and 0.39 x 10^{-6} mol L^{-1}; λ_{ex} = 525 nm, λ_{em} = 586 nm