Synthetic approach to hypoxyxylone, novel inhibitor of topoisomerase I

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SUPPORTING INFORMATION

3-Benzyloxy-1,6,8-trimethoxy-naphthalene-2-carboxylic Acid 3-Bromo-4,5,7-trimethoxy-naphthalen-2-yl Methyl Ester (4).

To a stirred solution of acid **5** (203 mg, 0.552 mmol), alcohol **6** (180 mg, 0.549 mmol), and triphenylphosphine (159 mg, 0.606 mmol) in anhydrous THF (6.8 mL) at 0 °C under argon was added dropwise diethyl azodicarboxylate (96 µL, 0.610 mmol). Stirring was continued at room temperature for 3 h, whereupon the mixture was diluted with ether and, after the usual workup, the crude product was chromatographed (silica gel, hexane-EtOAc 7:3). The residue obtained was dissolved in ether and washed several times with aqueous HCl (6 N) (to remove the byproduct of reduced DEAD) and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to afford ester **4** as a white solid (356 mg, 95%).

mp 136-138 °C (hexane-EtOAc);

IR (neat) 2926, 2850, 1728, 1619, 1580 cm⁻¹;

¹H NMR (CDCl₃, 200 MHz) δ 7.66 (s, 1H), 7.40-7.10 (m, 5H), 6.86 (s, 1H), 6.59 (d, *J* = 2.1 Hz, 2H), 6.50 (d, *J* = 2.4 Hz, 1H), 6.39 (d, *J* = 2.1 Hz, 1H), 5.58 (s, 2H), 5.20 (s, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.77 (s, 3H);

¹³C NMR (CDCl₃, 50.3 MHz) δ 166.1 (C), 159.3 (C), 158.4 (C), 157.5 (C), 156.4 (C), 155.4 (C), 153.8 (C), 153.4 (C), 139.1 (C), 136.5 (C), 134.1 (C), 128.4 (CH), 126.6 (CH), 123.2 (CH), 117.1 (C), 116.2 (C), 113.0 (C), 110.7 (C), 103.5 (CH), 99.6 (CH), 98.9 (CH), 98.4

(CH), 97.3 (CH), 69.9 (CH₂), 66.7 (CH₂), 63.9 (CH₃), 61.5 (CH₃), 56.1 (CH₃), 55.9 (CH₃), 55.2 (CH₃), 55.1 (CH₃);

MS (DCI) *m/z* 679 (MH⁺, 100), 369 (8), 351 (41), 311 (77), 233 (14);

HRMS calcd for C₃₅H₃₄BrO₉: 677.1386. Found: 677.1339 (MH⁺).

1-(3-Benzyloxy-1,6,8-trimethoxy-naphthalen-2-yl)-1-(3-hydroxymethyl-1,6,8-trimethoxy-naphthalen-2-yl)-methanone (20).

Under argon, ester **4** (269 mg, 0.397 mmol) was dissolved in THF (1.59 mL, C = 0.25 M, from which residual water had been removed with *n*-butyllithium and *o*-phenanthroline), and cooled to -45/-55 °C. *n*-Butyllithium (203 µL, 0.437 mmol) was then added dropwise. After the solution was stirred for 2 h, the reaction mixture was quenched by addition of saturated aqueous NH₄Cl, allowed to warm to ambient temperature, and diluted with ethyl acetate. After the usual workup, the crude product was purified by radial thin-layer chromatography (hexane-EtOAc 9:1, 8:2, 7:3, and then 5:5) to give a white solid **20** (130 mg, 55%).

mp 220-224°C (cyclohexane-dichloromethane);

IR (neat) 3454, 2957, 2926, 2853, 1619, 1573, 1458 cm⁻¹;

¹H NMR (CDCl₃, 200 MHz) δ 7.41 (s, 1H), 7.35-7.05 (m, 5H), 6.87 (s, 1H), 6.70 (d, *J* = 2.1 Hz, 1H), 6.60 (d, *J* = 2.1 Hz, 1H), 6.45 (d, *J* = 2.4 Hz, 1H), 6.37 (d, *J* = 2.4 Hz, 1H), 5.03 (s, 2H), 4.55 (d, *J* = 6.8 Hz, 2H), 3.90 (s, 6H), 3.87 (s, 3H), 3.83 (s, 3H), 3.57 (s, 3H), 3.41 (s, 3H);

¹³C NMR (CDCl₃, 75.4 MHz) δ 198.6 (C), 159.6 (C), 159.4 (C), 157.8 (C), 157.6 (C), 156.0 (C), 155.1 (C), 139.5 (C), 139.3 (C), 136.3 (C), 131.3 (C), 128.3 (CH), 127.8 (CH), 127.4 (CH), 125.4 (C), 124.4 (CH), 114.7 (C), 111.1 (C), 103.2 (CH), 99.3 (CH), 98.7 (CH), 97.4 (CH), 70.2 (CH₂), 64.7 (CH₂), 64.1 (CH₃), 64.0 (CH₃), 63.9 (CH₃), 56.0 (CH₃), 55.4 (CH₃), 55.3 (CH₃);

MS (DCI, NH₃+isobutane) *m/z* 599 (MH⁺, 50), 279 (100);

HRMS calcd for C₃₅H₃₄O₉: 598.2203. Found: 598.2211 (MH⁺).

1-(3-Benzyloxy-1,6,8-trimethoxy-naphthalen-2-yl)-1-(1,6,8-trimethoxy-3-methoxy methyl-naphthalen-2-yl)-methanone (20c).

To a solution of alcohol **20** (180 mg, 0.301 mmol) in dichloromethane (3.2 mL) at room temperature was added silver (I) oxide (280 mg, 1.25 mmol) and iodomethane (150 μ L, 2.41 mmol). The reaction mixture was stirred in the dark. After 48 h, 72 h, 96 h, and 144 h, additional silver (I) oxide (145 mg, 0.626 mmol) and iodomethane (70 μ L, 1.12 mmol) were added (until disappearance of the starting material in thin-layer chromatography). The slurry was then filtered and the solvent was removed in vacuo to give the product **20c** (190 mg).

mp 175-178 $^{\circ}$ C (pentane-dichloromethane, white solid);

IR (neat) 2935, 2845, 1662, 1620, 1578 cm^{-1} ;

^1H NMR (CDCl_3 , 200 MHz) δ 7.65 (s, 1H), 7.20 (s, 5H), 6.86 (s, 1H), 6.74 (d, J = 2.1 Hz, 1H), 6.59 (d, J = 2.1 Hz, 1H), 6.43 (d, J = 2.1 Hz, 1H), 6.35 (d, J = 2.4 Hz, 1H), 5.08 (s, 2H), 4.73 (s, 2H), 3.88 (s, 6H), 3.85 (s, 3H), 3.83 (s, 3H), 3.47 (s, 3H), 3.43 (s, 3H);

^{13}C NMR (CDCl_3 , 75.4 MHz) δ 196.6 (C), 159.2 (2C), 157.7 (C), 157.6 (C), 156.6 (C), 156.0 (C), 155.1 (C), 139.2 (C), 138.9 (C), 137.1 (C), 136.4 (C), 130.2 (C), 128.3 (CH), 127.6 (CH), 127.1 (CH), 125.3 (C), 120.7 (CH), 114.0 (C), 110.8 (C), 103.3 (CH), 99.1 (CH), 98.7 (CH), 98.5 (CH), 97.1 (CH), 71.8 (CH_2), 70.0 (CH_2), 64.0 (CH_3), 63.6 (CH_3), 58.6 (CH_3), 55.9 (2CH_3), 55.3 (CH_3), 55.2 (CH_3);

MS (EI) m/z 612 (M^+ , 43), 581 (100), 459 (41);

HRMS calcd for $\text{C}_{36}\text{H}_{36}\text{LiO}_9$: 619.2519. Found: 619.2536 (MLi^+).

1-(3-Hydroxy-1,6,8-trimethoxy-naphthalen-2-yl)-1-(1,6,8-trimethoxy-3-methoxymethyl-naphthalen-2-yl)-methanone (20d).

The product **20c** (190 mg) and Pd(OH)₂/C (68 mg) were stirred in dichloromethane (5.2 mL) and ethanol (13 mL) under a hydrogen atmosphere for 16 h. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to give a yellow solid (170 mg).

mp 115-116 °C;

IR (KBr) 3452, 2935, 2849, 1640, 1621, 1560 cm⁻¹;

¹H NMR (CDCl₃, 200 MHz) δ 11.97 (s, 1H), 7.47 (s, 1H), 6.92 (s, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.19 (d, *J* = 2.1 Hz, 1H), 4.41 (s, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.24 (s, 3H), 3.17 (s, 3H);

¹³C NMR (CDCl₃, 75.4 MHz) δ 201.5 (C), 162.8 (2C), 161.3 (C), 158.8 (C), 158.6 (C), 158.3 (C), 157.4 (C), 152.9 (C), 142.6 (C), 137.6 (C), 134.5 (C), 132.1 (C), 121.4 (CH), 115.9 (C), 114.8 (C), 110.1 (C), 107.1 (CH), 99.1 (2CH), 97.4 (CH), 96.7 (CH), 72.4 (CH₂), 64.2 (CH₃), 63.4 (CH₃), 58.4 (CH₃), 56.0 (CH₃), 55.7 (CH₃), 55.3 (CH₃), 55.2 (CH₃);

MS (DCI, NH₃+isobutane) *m/z* 523(MH⁺, 100), 493 (19), 289 (15), 261 (64), 233 (22);

HRMS calcd for C₂₉H₃₀LiO₉: 529.2050. Found: 529.2071 (MLi⁺).

1,3,8,9,11-Pentamethoxy-6-methoxymethyl-14-oxa-benzo[α]naphthacen-7-one (2).

A solution of the product **20d** (170 mg) and potassium hydroxide (46 mg, 0.829 mmol) in methanol (10.9 mL) was refluxed overnight. After being allowed to cool to room temperature, the reaction was quenched by addition of dilute aqueous HCl (0.1 N) and the solvents were removed under reduced pressure. The residue was treated with aqueous HCl (0.1 N) and then extracted with dichloromethane. After the usual workup, the resultant crude product was triturated with ether to leave xanthone **2** as a yellow solid (155 mg, 91% overall yield for three steps).

mp 238-240 °C;

IR (KBr) 2936, 2850, 1647, 1617, 1558 cm⁻¹;

UV λ_{max} (CHCl₃)/ nm 242 (log ε, 4.63), 297 (4.83), 412 (4.07);

¹H NMR (CDCl₃, 500 MHz) δ 7.74 (s, 1H, H_a), 7.48 (s, 1H, H_d), 6.76 (d, *J* = 2.4 Hz, 1H, H_b), 6.67 (d, *J* = 2.1 Hz, 1H, H_e), 6.56 (d, *J* = 2.4 Hz, 1H, H_c), 6.40 (d, *J* = 2.1 Hz, 1H, H_f), 5.28 (s, 2H), 4.06 (s, 3H, H₃ or H₆), 4.04 (s, 3H, H₃ or H₆), 3.97 (s, 3H, H₅), 3.92 (s, 3H, H₄ ou H₂), 3.91 (s, 3H, H₄ ou H₂), 3.62 (s, 3H, H₁);

¹³C NMR (CDCl₃, 125.7 MHz) δ 178.2 (C), 161.1 (C), 160.6 (C), 160.0 (C), 159.6 (C), 159.4 (C), 155.6 (C), 153.4 (C), 140.3 (C), 139.8 (C), 138.1 (C), 119.1 (CH, Ca), 115.4 (C), 114.0 (2C), 109.5 (C), 108.3 (CH, Cd), 99.6 (CH, Cb or Cc), 99.3 (CH, Cb or Cc), 98.5 (CH, Cf), 97.3 (CH, Ce), 73.8 (CH₂), 63.4 (CH₃, C3 or C6), 59.0 (CH₃, C1), 56.3 (2CH₃, C5 and C3 or C6), 55.5 (2CH₃, C2 and C4);

MS (EI) 490 (M⁺, 5), 475 (50), 232 (20);

HRMS calcd for C₂₈H₂₇O₈: 491.1706. Found: 491.1705 (MH⁺).

8-Hydroxy-1,3,9,11-tetramethoxy-6-methoxymethyl-14-oxa-benzo[α]naphthacene-7-one (21).

A solution of xanthone **2** (95 mg, 0.194 mmol) and lithium chloride (40 mg, 0.944 mmol) in DMF (2.8 mL) was stirred at 110 °C for 13 h. After being allowed to cool to room temperature, the mixture was diluted with water and then extracted with chloroform. The organic layer was washed several times with water and then brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo to give xanthone **21** as a red-brown solid (82 mg, 89%).

mp 268-269°C;

IR (KBr) 3411, 2925, 1650, 1620, 1602, 1573 cm⁻¹;

¹H NMR (CDCl₃, 500 MHz) δ 7.74 (s, 1H, Hc), 7.10 (s, 1H, Hf), 6.80 (d, *J* = 2 Hz, 1H, Hd), 6.66 (d, *J* = 2.5 Hz, 1H, Ha), 6.61 (d, *J* = 2 Hz, 1H, He), 6.39 (d, *J* = 2 Hz, 1H, Hb), 5.26 (d, *J* = 1 Hz, 2H), 4.10 (s, 3H, C3), 4.03 (s, 3H, C5), 3.95 (s, 3H, C4), 3.95 (s, 3H, C2), 3.66 (s, 3H, C1);

¹³C NMR (CDCl₃, 75.4 MHz) δ 183.8 (C), 163.9 (C), 161.8 (C), 161.7 (C), 161.0 (C), 159.8 (C), 157.2 (C), 152.0 (C), 141.9 (C), 140.5 (C), 137.5 (C), 119.7 (CH), 112.4 (C), 109.7 (C), 108.2 (C), 104.4 (C), 100.8 (CH), 99.8 (CH), 99.6 (CH), 97.9 (CH), 96.9 (CH), 73.6 (CH₂), 59.1 (CH₃), 56.4 (CH₃), 56.3 (CH₃), 55.6 (CH₃), 55.5 (CH₃);

MS (DCI, NH₃ + isobutane) *m/z* 477 (MH⁺, 16), 102 (100).

1,3,9,11-Tetramethoxy-6-methoxymethyl-7H-14-oxa-benzo[α]naphthacen-8-ol (22).

To a solution of xanthone **21** (37.5 mg, 0.079 mmol) in dry dichloromethane (7.9 mL) was added dropwise borane-methyl sulfide complex (75 μl, 0.791 mmol). The mixture was refluxed for 48 h, and then allowed to cool to room temperature, diluted with an aqueous solution of HCl (0.5N), and extracted with dichloromethane. After the usual workup, xanthene **22** (37.1 mg) was obtained, which was used without further purification.

¹H NMR (CDCl₃, 300 MHz) δ 7.39 (s, 1H), 6.94 (s, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 6.63 (d, *J* = 2.1 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 6.32 (d, *J* = 2 Hz, 1H), 4.63 (s, 2H), 4.07 (s, 2H), 4.02 (s, 3H), 4.01 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.48 (s, 3H);

MS (DCI, NH₃ + isobutane) *m/z* 461 (18), 197 (100).

Trimethyl-(1,3,6,9,11-tetramethoxy-6-methoxymethyl-7*H*-14-oxa-benzo[α]naphthacen-8-yloxy)-silane (22c**).**

To a stirred solution of crude product **22** (37.1 mg) in anhydrous dichloromethane (0.85 mL) at 0 °C were added dropwise 2,6-lutidine (64 μ L, 0.552 mmol) and then trimethylsilyl trifluoromethanesulfonate (71 μ L, 0.394 mmol). After 2.5 h, the reaction mixture was quenched by addition of saturated aqueous NH_4Cl and then extracted with dichloromethane. The organic layer was washed with water, saturated aqueous CuSO_4 , and brine, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to afford xanthene **22c** (42.3 mg) as a green substance, which was used without further purification.

^1H NMR (CDCl_3 , 300 MHz) δ 7.36 (s, 1H), 7.10 (s, 1H), 6.68 (d, $J = 2.3$ Hz, 1H), 6.62 (d, $J = 2.3$ Hz, 1H), 6.54 (d, $J = 2.3$ Hz, 1H), 6.32 (d, $J = 2.3$ Hz, 1H), 4.59 (s, 2H), 4.09 (s, 2H), 4.03 (s, 3H), 3.88 (s, 9H), 3.45 (s, 3H), 0.28 (s, 9H).

1,3,9,11-Tetramethoxy-6-methoxymethyl-14-oxa-benzo[α]naphthacen-8-one (23**).**

A solution of the above crude product **22c** (42.3 mg) and palladium (II) acetate (17.5 mg, 0.078 mmol) in anhydrous acetonitrile (1.32 mL) was refluxed for 12 h. After being allowed to cool to room temperature, the reaction mixture was concentrated in vacuo and the residue was immediately purified by flash column chromatography (silica gel treated with triethylamine 2.5% w/v, dichloromethane and then dichloromethane-methanol 97:3) to give **23** as a blue substance (20.4 mg, 56% for three steps).

IR (neat) 2926, 2855, 1659, 1613, 1590, 1536 cm^{-1} ;

UV λ_{max} (CH_3CN)/ nm 208 (log ϵ , 5.24), 234 (5.27), 323 (4.91), 610 (4.45);

^1H NMR (CDCl_3 , 300 MHz) δ 8.43 (s, 1H), 7.32 (s, 1H), 6.64 (d, $J = 1.9$ Hz, 1H), 6.50 (d, $J = 2$ Hz, 1H), 6.40 (d, $J = 2.3$ Hz, 1H), 6.26 (d, $J = 2.2$ Hz, 1H), 6.21 (s, 1H), 4.70 (s, 2H), 4.00 (s, 3H), 3.94 (s, 3H), 3.88 (s, 3H), 3.88 (s, 3H), 3.49 (s, 3H);

^{13}C NMR (CDCl_3 , 75.4 MHz) δ 178.6 (C), 164.3 (C), 163.9 (C), 161.1 (C), 159.3 (C), 153.5 (C), 150.7 (C), 143.9 (C), 139.3 (C), 133.8 (C), 133.2 (CH), 123.7 (C), 122.2 (CH), 113.4 (C), 112.2 (C), 109.9 (C), 101.9 (CH), 99.8 (CH), 99.5 (CH), 96.8 (CH), 95.1 (CH), 72.1 (CH_2), 58.7 (CH_3), 56.1 (CH_3), 55.9 (CH_3), 55.4 (CH_3), 55.4 (CH_3);

MS (DCI, NH_3 + isobutane) m/z 499 (7), 461 (MH^+ , 100);

HRMS calcd for $\text{C}_{27}\text{H}_{25}\text{O}_7$: 461.1600. Found : 461.1605 (MH^+).

Biological Test Protocol

Supercoiled pKMp27 DNA (0.5 μg) was incubated with 6 units of topo I at 37°C for 45 minutes in relaxation buffer (50 mM TRIS, pH 7.8, 50 mM KCL, 10 mM MgCl_2 , 1 mM Dithiothreitol, 1mM EDTA) in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteins K to 250 $\mu\text{g/mL}$. DNA samples were then added to the electrophoresis dye mixture (3 μL) and electrophoresed in a 1% agarose gel at room temperature for 3 hrs. Gel were stained with ethidium bromide (1mg/mL), washed and photographed under UV light. Similar experiments were performed using ethidium-containing agarose gels.

In the presence of topo I inhibitor, the nicked DNA fraction is significantly increased in a dose-dependent manner, reflecting the stabilization of TopoI-DNA complexes. The product **23** was found active at a dosage of 0.5 μg .