The Natural Product Avrainvillamide Binds to the Oncoprotein Nucleophosmin.

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Supporting Information

Index:

A. Chemistry

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Organic solutions were concentrated at ambient temperature (23 °C) by rotary evaporation at 40 Torr (house vacuum). Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore-size, 230-400 mesh, Merck KGA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light, then were stained with iodine or by submersion in aqueous ceric ammonium molybdate (CAM), followed by brief heating on a hot plate. Flash-column chromatography was performed as described by Still et al.,[1] employing silica gel (60 Å, 32-63 μM, standard grade, Sorbent Technologies).

Materials. Commercial solvents and reagents were used as received with the following exceptions. Dichloromethane, benzene, tetrahydrofuran, and acetonitrile were purified by the method of Pangborn et al.[2] Biotinylated alkene **7**,[3] iodoarene **12**,[3] vinyl iodide **14**,[4] nitroarene **30**,[5] iodoarene **34**,[6] and nitroaniline **36**[7] were prepared as described previously.

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400 or 500 MHz at 23 ºC. Proton chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane, and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26; C_6HD_5, δ 7.15). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sext = sextet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, and coupling constant in Hertz. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 100 or 125 MHz at 23 ºC unless otherwise noted. Carbon chemical shifts are reported in parts per million downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent ($CDCl_3$, δ 77.0; C_6D_6, δ 128.0). Infrared (IR) spectra were obtained using a Perkin-Elmer FT-IR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Low- and high-resolution mass spectra were obtained at the Harvard University Mass Spectrometry Facility.

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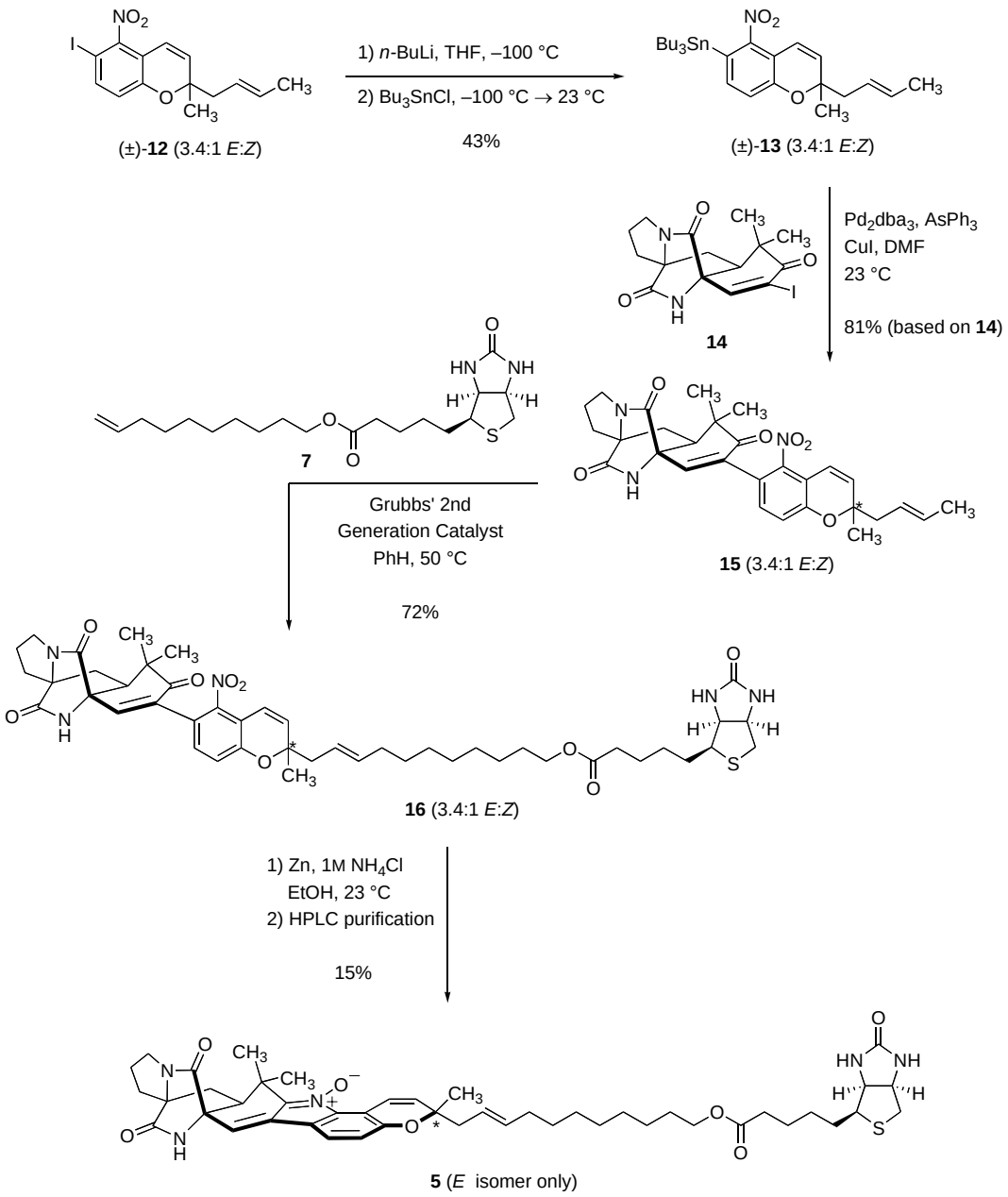
[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem*. **1978**, *43*, 2923.
[2] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.
[3] Wulff, J. E.; Herzon, S. B.; Siegrist, R.; Myers, A. G. *J. Am. Chem. Soc.* **2007**, *129*, 4898.
[4] Herzon, S. B.; Myers, A. G. *J. Am. Chem. Soc*. **2005**, *127*, 5342.
[5] Liu, L.; Zhang, Y.; Xin, B. *J. Org. Chem.* **2006**, *71*, 3994.
[6] Maya, F.; Chanteau S. H.; Cheng L.; Stewart M. P.; Tour J. M. *Chem. Mater.* **2005**, *17*, 1331.
[7] Seko, S.; Miyake, K.; Kawamura, N. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1437.

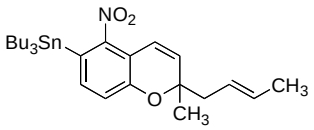
Synthetic Procedures.

For clarity, intermediates that have not been assigned numbers in the text are numbered sequentially in the supporting information, beginning with **12**.



* a 1:1 mixture of stereoisomers at this center

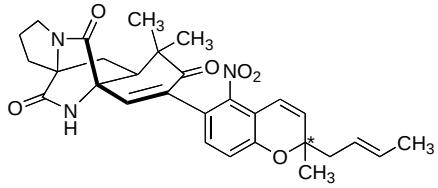
Scheme S1. Synthesis of the biological probe **5**.



(±)-**13** (3.4:1 *E*:*Z*)

Stannane **13**. *n*-Butyllithium in hexanes (2.4 M, 0.44 mL, 1.05 mmol, 1.05 equiv) and tributyltin chloride (0.28 mL, 1.05 mmol, 1.05 equiv) were added in sequence to a solution of the iodoarene **12** (371 mg, 1.0 mmol, 1.00 equiv) in tetrahydrofuran (10 mL) cooled to –100 °C. The cooling bath was removed and the dark red solution was allowed to warm to 23 °C over 45 min. The solution was diluted with hexanes-ethyl ether (2:1) and the diluted solution was washed successively with water and saturated aqueous sodium chloride solution. The washed solution was dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography on silica gel (deactivated with 20% triethylamine-ethyl acetate, eluting with hexanes-ethyl acetate, 100:1), furnishing the stannane **13** (3.4:1 mixture of *E*- and *Z*-geometrical isomers, respectively, 228 mg, 43%) as an orange oil.

R_f = 0.68 (hexanes-acetone 100:4). ^{1}H NMR (500 MHz, $CDCl_3$, signals for the major isomer), δ 7.26 (d, 1H, *J* = 7.8 Hz), 6.98 (d, 1H, *J* = 7.8 Hz), 6.68 (d, 1H, *J* = 10.3 Hz), 5.77 (d, 1H, *J* = 10.3 Hz), 5.55–5.43 (m, 2H), 2.49–2.36 (m, 2H), 1.66 (d, 3H, *J* = 5.4 Hz), 1.57–1.38 (m, 6H), 1.41 (s, 3H), 1.32 (sext, 6H, *J* = 7.3 Hz), 1.14–1.01 (m, 6H), 0.88 (t, 9H, *J* = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, signals for the major isomer), δ 154.8, 153.0, 136.9, 132.8, 129.8, 127.7, 124.9, 120.7, 118.5, 116.0, 78.4, 44.1, 29.2, 27.5, 25.9, 18.3, 13.9, 10.9. IR (NaCl, thin film), cm^{-1} 2957(m), 2921(m), 2872(m), 2854(m), 1522(s), 1279(s).



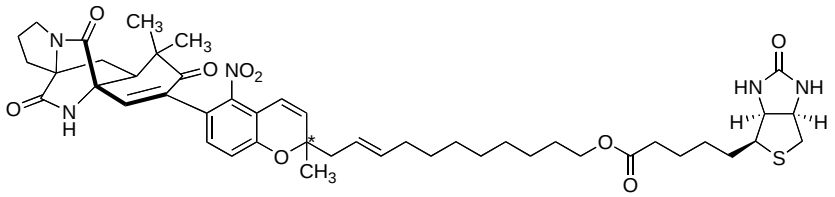
15 (3.4:1 *E*:*Z*)

Nitroarene **15**. A mixture of tris(dibenzylideneacetone)dipalladium (11.5 mg, 12.6 μmol, 25.1 μmol Pd) and triphenylarsine (15.4 mg, 50.2 μmol, 2 equiv based on Pd) in *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use) was stirred at 23 °C for 30 min. In a separate flask, a suspension of copper iodide (5 mg, 26.3 μmol) in *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use) was stirred at 23 °C for 30 min.

A third flask was charged with the vinyl iodide **14** (20 mg, 50 μmol, 1 equiv), the stannane **13** (53 mg, 100 μmol, 2 equiv), and *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the

solvent for 1 h before use). The resulting solution was treated sequentially with the tris(dibenzylideneacetone)dipalladium-triphenylarsine and copper iodide solutions prepared above (100 μL each). The reaction mixture was stirred at 23 °C for 48 h. The product solution was diluted with hexanes-ethyl ether (2:1, 100 mL). The diluted solution was washed successively with water and saturated aqueous sodium chloride solution. The combined aqueous layers were extracted with hexanes-ethyl ether (2:1). The combined organic phases were dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (dichloromethane-methanol, 100:1 to 100:2), affording the nitroarene **15** (a 1:1 mixture of diastereoisomers at C(21), and a 3.4:1 mixture of *E*- and *Z*-geometrical isomers, respectively, 21 mg, 81%) as a yellow solid.

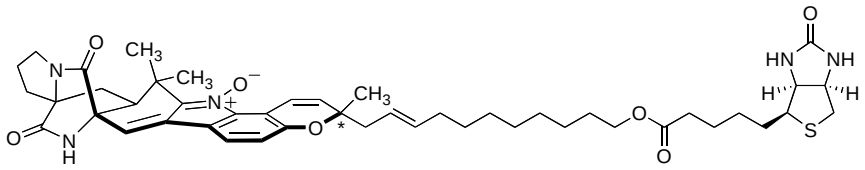
R_f = 0.50 (hexanes-ethyl acetate 1:9). ^{1}H NMR (500 MHz, $CDCl_3$, signals for the major diastereoisomers), δ 7.45–7.30 (1H, br m), 7.11 (d, 1H, *J* = 8.3 Hz), 6.95–6.92 (m, 1H), 6.88 (s, 1H), 6.51 (d, 1H, *J* = 10.3 Hz), 5.79 (d, 1H, *J* = 10.3 Hz), 5.61–5.40 (m, 2H), 3.64–3.59 (m, 1H), 3.47–3.45 (m, 1H), 2.80–2.74 (m, 2H), 2.42–2.40 (m, 2H), 2.23–2.19 (m, 1H), 2.07–1.95 (m, 2H), 1.86–1.81 (m, 3H), 1.67–1.60 (m, 2H), 1.45–1.41 (m, 3H), 1.09 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, signals for the major diastereoisomers), δ 199.2, 172.8, 167.5, 154.6, 146.7, 140.4, 137.9, 133.7, 133.7, 131.4, 130.1, 130.0, 124.7, 124.6, 122.5, 119.7, 119.7, 117.7, 117.6, 115.2, 115.2, 79.2, 67.8, 61.1, 51.0, 45.2, 44.6, 44.2, 44.1, 32.5, 29.5, 26.0, 25.9, 24.8, 23.3, 18.5, 18.3. IR (NaCl, thin film), cm^{-1} 3215(br), 2973(w), 2935(w), 2881(w), 1686 (s), 1530 (s), 1353 (m). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{29}H_{32}N_3O_6^+$, 518.2291; found, 518.2301.



16 (3.4:1 *E*:*Z*)

Biotinylated nitroarene **16**. A solution of the nitroarene **15** (19 mg, 37 μmol, 1.0 equiv), biotinylated alkene **7** (71 mg, 185 μmol, 5.0 equiv), and Grubbs' second-generation catalyst (3.1 mg, 3.7 μmol, 0.1 equiv) in benzene (20 mL) was stirred at 50 °C for 24 h. A second portion of Grubbs' second-generation catalyst (1.6 mg, 1.8 μmol, 0.05 equiv) was added and the solution was stirred at 50 °C for 18 h. The brown reaction mixture was allowed to cool to 23 °C and the cooled solution was concentrated in vacuo. The residue was purified by flash-column chromatography (dichloromethane-methanol, 25:1) to afford the biotinylated derivative **16** (a 1:1 mixture of diastereoisomers at C(21), and a 3.4:1 mixture of *E*- and *Z*-geometrical isomers, respectively, 23 mg, 72%) as a yellow film.

R_f = 0.42 (dichloromethane-methanol 9:1). ^{1}H NMR (500 MHz, $CDCl_3$, signals for the major diasteroisomers), δ 9.09 (s, 1H), 7.18–7.10 (m, 1H), 6.98–6.86 (m, 2H), 6.55–6.50 (m, 1H), 6.17 (s, 1H), 5.82–5.76 (m, 1H), 5.55–5.37 (m, 2H), 5.34 (s, 1H), 4.50–4.44 (m, 1H), 4.27–4.22 (m, 1H), 4.08–3.99 (m, 2H), 3.66–3.60 (m, 1H), 3.47 (dt, 1H, *J* = 11.7, 7.3 Hz), 3.13–3.08 (m, 1H), 2.90–2.86 (m, 1H), 2.82–2.75 (m, 2H), 2.71 (d, 1H, *J* = 12.7 Hz), 2.44–2.38 (m, 2H), 2.33–2.29 (m, 2H), 2.23–2.18 (m, 1H), 2.09–1.97 (m, 4H), 1.88–1.82 (m, 2H), 1.72–1.56 (m, 6H), 1.45–1.23 (m, 15H), 1.10–1.06 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, signals for the major diastereoisomers), 199.4, 199.3, 174.0, 173.7, 173.5, 167.8, 167.7, 164.0, 163.9, 154.6, 154.5, 146.7, 146.7, 140.3, 140.0, 138.5, 135.6, 135.4, 133.8, 131.7, 131.6, 123.7, 123.5, 122.9, 122.8, 122.7, 119.9, 119.7, 117.7, 117.6, 115.2, 115.1, 79.4, 79.1, 67.7, 64.8, 64.8, 61.9, 61.9, 61.1, 61.0, 60.6, 60.5, 60.3, 55.6, 55.6, 51.0, 50.9, 46.1, 45.2, 45.1, 44.5, 44.0, 40.8, 34.2, 34.2, 32.7, 32.6, 32.5, 29.9, 29.5, 29.4, 29.4, 29.3, 29.2, 29.1, 29.0, 28.8, 28.6, 28.4, 28.4, 27.6, 26.3, 26.1, 26.1, 25.9, 25.2, 25.1, 24.9, 23.4, 23.2, 18.6, 18.5. IR (NaCl, thin film), cm^{-1} 3258(br), 2928(m), 2855(w), 1701(s), 1684(s), 1529(m), 1458(m), 1351(m), 1267(w). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{46}H_{60}N_5O_9S^+$, 858.4106; found, 858.4124.

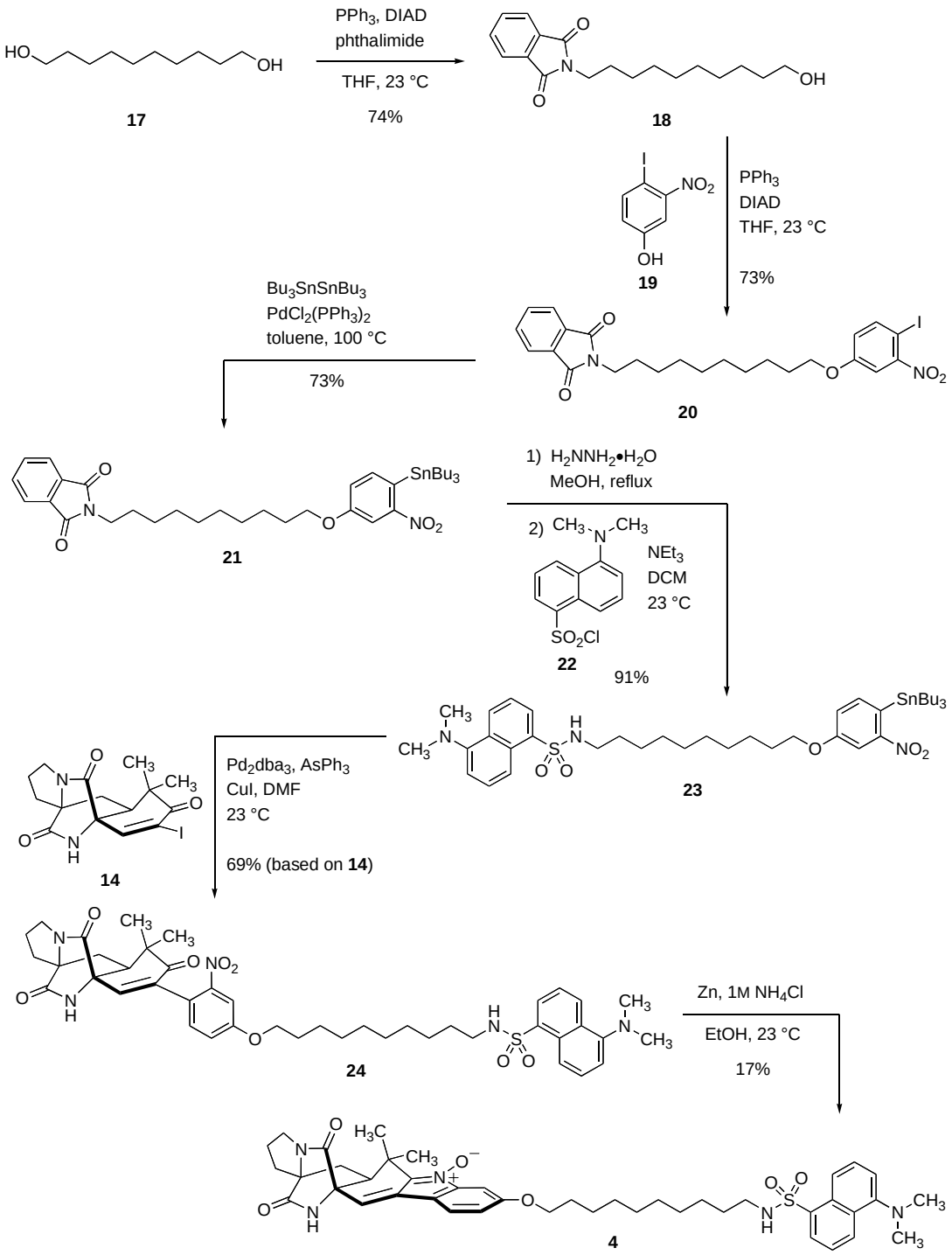


5

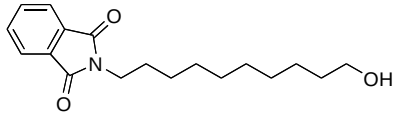
* 1:1 mixture of stereoisiomers at this center

Biotinylated nitrone **5**. Aqueous ammonium chloride solution (1 M, 22.4 μL, 22.4 μmol, 3.2 equiv) was added to a solution of the nitroarene **16** (5.6 mg, 7 μmol, 1 equiv) in ethanol (350 μL). Zinc powder (2.3 mg, 35 μmol, 5 equiv) was added and the resulting yellow suspension was stirred 23 °C for 2 h. The suspension was diluted with ethyl acetate and the diluted suspension was filtered through Celite. The filtrate was washed with saturated aqueous sodium chloride solution, the washed solution was dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (dichloromethane-methanol, 10:1) and further by HPLC (reverse phase, Beckman Coulter Ultrasphere ODS 5 μM, 30% to 100% acetonitrile in water) to afford the nitrone **5** (a 1:1 mixture of diastereoisomers at C(21), 788 μg, 15%) as a yellow solid.

R_f = 0.39 (dichloromethane-methanol 85:15). ^{1}H NMR (500 MHz, C_6D_6, signals for the major diastereoisomers), δ 9.22 (br s, 1H), 8.44–8.40 (m, 1H), 6.88–6.85 (m, 1H), 6.77–6.72 (m, 1H), 6.18 (br s, 1H), 5.86 (br s, 1H), 5.57–5.38 (m, 3H), 5.11 (br s, 1H), 4.14–3.99 (m, 3H), 3.73–3.71 (m, 1H), 3.63–3.59 (m, 1H), 3.56–3.53 (m, 1H), 3.41–3.34 (m, 1H), 3.22–3.17 (m, 1H), 2.97–2.85 (m, 1H), 2.72–2.64 (m, 1H), 2.45–1.97 (m, 8H), 1.58–1.08 (m, 31H). IR (NaCl, thin film), cm^{-1} 3140(br), 3048(w), 2931(w), 2856(w), 1701(s), 1404(m). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{46}H_{60}N_5O_7S^+$, 826.4213; found, 826.4232.



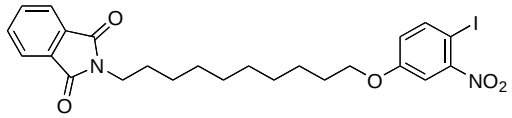
Scheme S2. Preparation of the biological probe **4**.



18

Phthalimide **18**. Diisopropyl azodicarboxylate (11.81 mL, 60 mmol, 1.2 equiv) was added slowly to an ice-cooled solution of 1,10-decanediol (**17**) (26.14 g, 150 mmol, 3.0 equiv), triphenylphosphine (15.73 g, 60 mmol, 1.2 equiv), and phthalimide (7.36 g, 50 mmol, 1.0 equiv) in tetrahydrofuran (125 mL). The resulting yellow solution was stirred at 23 °C for 20 h. The yellow product mixture was concentrated in vacuo and the residue was subjected to flash-column chromatography (hexanes-ethyl acetate, 7:3 to 1:1), affording the phthalimide **18** (11.28 g, 74%) as a white solid.

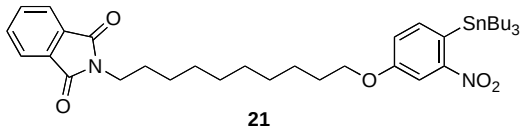
R_f = 0.30 (hexanes-ethyl acetate 3:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.84 (dd, 2H, *J* = 5.4, 2.9 Hz), 7.71 (dd, 2H, *J* = 5.4, 2.9 Hz), 3.68 (t, 2H, *J* = 7.3 Hz), 3.64 (dd, 2H, *J* = 12.2, 6.4 Hz), 1.68–1.66 (m, 2H), 1.59–1.53 (m, 2H), 1.33–1.27 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$), δ 168.7, 134.1, 132.4, 123.4, 63.3, 38.3, 33.0, 29.7, 29.5, 29.3, 28.8, 27.0, 25.9, 22.2. IR (NaCl, thin film), cm^{-1} 3410(br), 2927(m), 2854(m), 1773(m), 1705(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{18}H_{26}NO_3^+$, 304.1907; found, 304.1900.



20

Iodoarene **20**. Diisopropyl azodicarboxylate (3.25 mL, 16.5 mmol, 1.1 equiv) was added dropwise to a solution of 4-iodo-3-nitrophenol (**19**) (3.98 g, 15.0 mmol, 1.0 equiv), the alcohol **18** (5.01 g, 16.5 mmol, 1.1 equiv), and triphenylphosphine (4.33 g, 16.5 mmol, 1.1 equiv) in tetrahydrofuran (37 mL). The orange solution was stirred at 23 °C for 16 h. The product solution was concentrated in vacuo and the residue was recrystallized from chloroform, furnishing the iodoarene **20** (6.03 g, 73%) as a pale yellow solid.

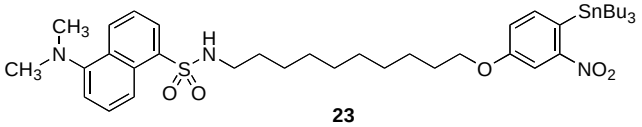
R_f = 0.64 (hexanes-ethyl acetate 3:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.86–7.83 (m, 3H), 7.71 (dd, 2H, *J* = 5.4, 2.9 Hz), 7.40 (d, 1H, *J* = 2.4 Hz), 6.85 (dd, 1H, *J* = 8.8, 2.9 Hz), 3.97 (t, 2H, *J* = 6.4 Hz), 3.68 (t, 2H, *J* = 7.3 Hz), 1.81–1.76 (m, 2H), 1.69-1.66 (m, 2H), 1.45–1.42 (m, 2H), 1.33–1.25 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 168.7, 159.9, 153.7, 142.2, 134.1, 132.4, 123.4, 121.1, 111.7, 74.3, 69.1, 38.3, 29.6, 29.5, 29.4, 29.3, 29.1, 28.8, 27.0, 26.0. IR (NaCl, thin film), cm^{-1} 2928(m), 2854(m), 1772(m), 1706(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{24}H_{28}IN_2O_5^+$, 551.1037; found, 551.1039.



21

Stannane **21**. A solution of the iodoarene **20** (1.10 g, 2.0 mmol, 1 equiv), bis(tributyltin) (1.11 mL, 2.2 mmol, 1.1 equiv), bis(triphenylphosphine)palladium(II) dichloride (14 mg, 20 μmol, 0.01 equiv), and triphenylphosphine (11 mg, 40 μmol, 0.02 equiv) in toluene (20 mL) was stirred at 100 °C for 58 h. The brown suspension was allowed to cool to 23 °C and the cooled mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified by flash-column chromatography on silica gel (deactivated with 20% triethylamine-ethyl acetate, eluting with hexanes initially, grading to 10% ethyl acetate-hexanes), furnishing the stannane **21** (1.04 g, 73%) as a yellow oil.

R_f = 0.57 (hexanes-ethyl acetate 4:1). ^{1}H NMR (500 MHz, C_6D_6), δ 7.86 (d, 1H, *J* = 2.4 Hz), 7.49 (d, 1H, *J* = 8.1 Hz), 7.46 (dd, 2H, *J* = 5.4, 2.9 Hz), 6.99 (dd, 1H, *J* = 8.1, 2.4 Hz), 6.86 (dd, 2H, *J* = 5.4, 2.9 Hz), 3.56 (t, 2H, *J* = 7.1 Hz), 3.47 (t, 2H, *J* = 6.35 Hz), 1.70–1.58 (m, 8H), 1.55–1.49 (m, 2H), 1.37 (sext, 6H, *J* = 7.3 Hz), 1.31–1.17 (m, 18H), 0.90 (t, 9H, *J* = 7.3 Hz). ^{13}C NMR (100 MHz, C_6D_6), δ 167.9, 160.5, 155.2, 138.1, 133.3, 132.6, 129.5, 122.8, 121.5, 109.3, 68.2, 37.8, 29.6, 29.6, 29.4, 29.3, 29.3, 29.2, 28.8, 27.6, 27.0, 26.1, 13.8, 11.2. IR (NaCl, thin film), cm^{-1} 2925(m), 2854(m), 1773(w), 1712(s), 1603(w), 1524(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{36}H_{55}N_2O_5Sn^+$, 715.3133; found, 715.3140.

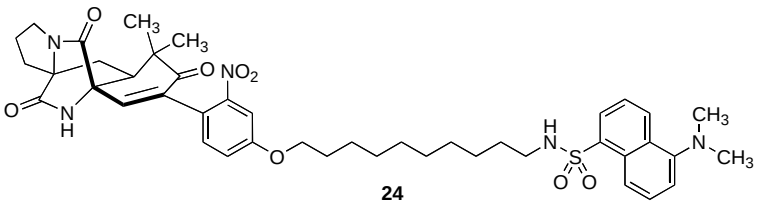


23

Stannane **23**. Hydrazine monohydrate (0.14 mL, 2.89 mmol, 2 equiv) was added to a solution of the stannane **21** (1.03 g, 1.44 mmol, 1 equiv) in methanol (15 mL). The yellow solution was heated to reflux for 2 h. The product solution was allowed to cool to 23 °C and the cooled solution was concentrated in vacuo. The residue was suspended in dichloromethane (ca. 15 mL) and the suspension was dried over anhydrous sodium sulfate. The solids were removed by filtration through Celite and the filtrate was concentrated in vacuo. The resulting yellow oil was dissolved in dichloromethane (5 mL). Dansyl chloride (**22**) (388 mg, 1.44 mmol, 1 equiv) and triethylamine (0.40 mL, 2.89 mmol, 2 equiv) were added. The yellow solution was stirred at 23 °C for 12 h. The product mixture was concentrated in vacuo and the residue was purified by flash-column chromatography (hexanes-ethyl acetate-triethylamine, 9:1:0.2 to 8:2:0.2), affording the stannane **23** (1.07 g, 91%) as a yellow oil.

R_f = 0.73 (hexanes-ethyl acetate 3:2). ^{1}H NMR (500 MHz, C_6D_6), δ 8.68 (d, 1H, *J* = 8.7 Hz), 8.40 (d, 1H, *J* = 8.7 Hz), 8.36 (dd, 1H, 7.3, 1.4 Hz), 7.87 (d, 1H, *J* = 2.3 Hz), 7.50 (d, 1H, *J* = 7.8 Hz), 7.38 (dd, 1H, *J* =

8.7, 7.3 Hz), 7.09 (dd, 1H, *J* = 8.7, 7.3 Hz), 7.00 (dd, 1H, 7.8, 2.3 Hz), 6.84 (d, 1H, *J* = 7.3 Hz), 4.21–4.18 (m, 1H), 3.49 (t, 2H, *J* = 6.4 Hz), 2.64 (q, 2H, *J* = 6.9 Hz), 2.48 (s, 6H), 1.66-1.60 (m, 6H), 1.54 (dt, 2H, *J* = 15.1, 6.4 Hz), 1.37 (sext, 6H, *J* = 7.3 Hz), 1.31–1.21 (m, 8H), 1.19–1.07 (m, 6H), 1.04–0.93 (m, 4H), 0.90 (t, 9H, *J* = 7.3 Hz), 0.87–0.82 (m, 2H). ^{13}C NMR (100 MHz, C_6D_6), δ 160.5, 155.2, 152.0, 138.2, 136.4, 130.3, 130.1, 129.7, 129.5, 128.3, 128.2, 123.3, 121.4, 119.9, 115.4, 109.4, 68.2, 45.0, 43.3, 29.7, 29.6, 29.5, 29.5, 29.5, 29.2, 29.1, 27.6, 26.5, 26.1, 13.8, 11.2. IR (NaCl, thin film), cm^{-1} 3284(br), 2953(w), 2925(m), 2854(w), 1525(s), 1330(s), 1161(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{40}H_{64}N_3O_5SSn^+$, 818.3583; found, 818.3589.

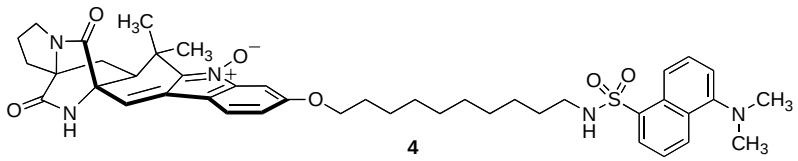


24

Nitroarene **24**. A mixture of tris(dibenzylideneacetone)dipalladium (9 mg, 9.8 μmol, 19.6 μmol Pd) and triphenylarsine (12 mg, 39.2 μmol, 2 equiv based on Pd) in *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use) was stirred at 23 °C for 30 min. In a separate flask, a suspension of copper iodide (3.8 mg, 20 μmol) in *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use) was stirred at 23 °C for 30 min. A third flask was charged with the vinyl iodide **14** (8 mg, 20 μmol, 1 equiv), the stannane **23** (33 mg, 40 μmol, 2 equiv), and *N*,*N*-dimethylformamide (150 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use). The resulting solution was treated sequentially with the tris(dibenzylideneacetone)dipalladium-triphenylarsine and copper iodide solutions prepared above (50.0 μL each). The reaction mixture was stirred at 23 °C for 65 h. The product solution was diluted with hexanes-ethyl ether (1:1, 100 mL). The diluted solution was washed with saturated aqueous sodium chloride solution. The aqueous layer was extracted with hexanes-ethyl ether. The combined organic phases were dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by radial chromatography (1-mm rotor, eluting with dichloromethane-triethylamine (100:1) initially, grading to dichloromethane-methanol-triethylamine (100:2:1), affording the nitroarene **24** (11 mg, 69%) as a yellow oil.

R_f = 0.73 (hexanes-ethyl acetate 3:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.53 (d, 1H, *J* = 8.8 Hz), 8.28 (d, 1H, *J* = 8.8 Hz), 8.24 (dd, 1H, *J* = 7.3, 1.5 Hz), 7.60–7.50 (m, 1H), 7.56 (d, 1H, *J* = 7.8 Hz), 7.52 (dd, 1H, *J* = 8.8, 7.3 Hz), 7.31 (br s, 1H), 7.18 (d, 1H, *J* = 7.3 Hz), 7.15–7.11 (m, 1H), 6.91 (br s, 1H), 6.83 (s, 1H), 4.70 (t, 1H, *J* = 5.9 Hz), 4.01 (t, 2H, *J* = 6.6 Hz), 3.66–3.62 (m, 1H), 3.51–3.46 (m, 1H), 2.97–2.78 (m, 4H), 2.89 (s, 6H), 2.24 (dd, 1H, *J* = 13.2, 10.3 Hz), 2.11–1.96 (m, 2H), 1.90–1.84 (m, 2H), 1.81–1.76 (m, 2H), 1.45–

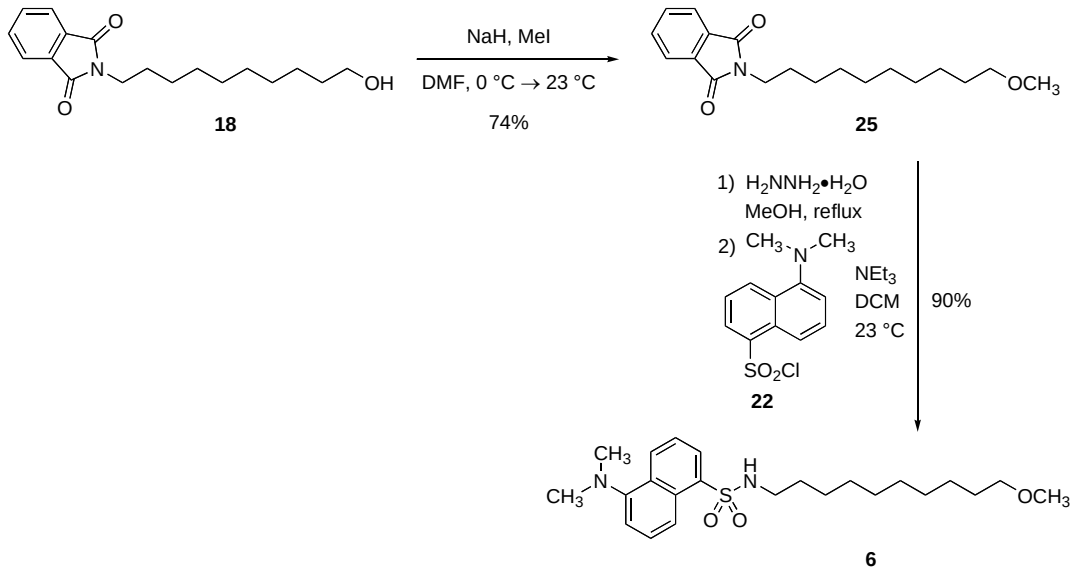
1.05 (m, 14H), 1.11 (app s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 199.2, 172.8, 171.4, 167.6, 160.1, 152.3, 149.2, 141.8, 136.1, 135.0, 133.1, 130.6, 130.1, 129.9, 128.6, 123.4, 123.2, 120.3, 118.9, 115.4, 110.2, 69.0, 67.8, 51.1, 45.6, 45.2, 44.6, 43.5, 32.5, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 26.6, 26.0, 24.9, 23.5, 18.7. IR (NaCl, thin film), cm^{-1} 3245(br), 2958(w), 2927(m), 2854(w), 1697(s), 1533(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{43}H_{54}N_5O_8S^+$, 800.3688; found, 800.3655.



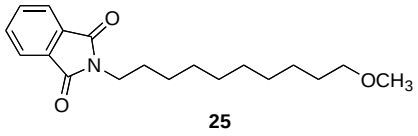
4

Dansylated nitrone **4**. Ammonium chloride solution (1 M, 22 μL, 22 μmol, 3.2 equiv) was added to a solution of the nitroarene **24** (5.6 mg, 7 μmol, 1 equiv) in ethanol (350 μL) and tetrahydrofuran (100 μL). Zinc powder (2.3 mg, 35 μmol, 5 equiv) was added. The resulting pale yellow suspension was stirred at 23 °C for 1 h. The product mixture was diluted with ethyl acetate (9 mL) and the diluted mixture was filtered through Celite. The filtrate was washed with saturated aqueous sodium chloride solution, the washed solution was dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was subjected to flash-column chromatography (ethyl acetate to ethyl acetate-methanol 20:1). The semi-purified product was purified by HPLC (reverse phase, Beckman Coulter Ultrasphere ODS 5 μM, 30% to 100% acetonitrile in water) to afford the nitrone **4** (929 μg, 17%) as a yellow solid.

R_f = 0.35 (ethyl acetate-methanol 100:4). ^{1}H NMR (500 MHz, C_6D_6), δ 8.73 (d, 1H, *J* = 8.7 Hz), 8.40 (d, 1H, *J* = 8.7 Hz), 8.38 (d, 1H, *J* = 7.3 Hz), 7.55 (d, 1H, *J* = 2.3 Hz), 7.38 (t, 1H, *J* = 8.2 Hz), 7.21–7.08 (m, 2H), 6.90 (dd, 1H, *J* = 8.2, 2.3 Hz), 6.85 (d, 1H, *J* = 7.8 Hz), 6.17 (s, 1H), 5.54 (s, 1H), 4.80 (t, 1H, *J* = 6.2 Hz), 3.66 (t, 2H, *J* = 6.2 Hz), 3.23–3.18 (m, 1H), 2.91 (dt, 1H, *J* = 11.0, 7.3 Hz), 2.72–2.62 (m, 3H), 2.49 (s, 6H), 1.99 (dd, 1H, *J* = 10.1, 6.4 Hz), 1.60 (s, 3H), 1.59–1.53 (m, 2H), 1.46–1.39 (m, 2H), 1.31–0.97 (m, 13H), 1.24 (s, 3H), 0.91–0.81 (m, 4H). IR (NaCl, thin film), cm^{-1} 3300 (br), 2926(m), 2872(w), 1697(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{43}H_{54}N_5O_6S^+$, 768.3789; found, 768.3780.



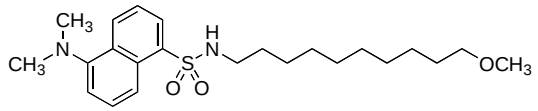
Scheme S3. Preparation of the control compound **6**.



Phthalimide **25**. 60% Sodium hydride in mineral oil (360 mg, 9 mmol, 1.5 equiv) was added in one portion to an ice-cooled solution of the alcohol **18** (1.82 g, 6 mmol, 1.0 equiv) in *N*,*N*-dimethylformamide (20 mL) (gas evolution). The mixture was stirred at 0 °C for 15 min. Methyl iodide (0.56 mL, 9 mmol, 1.5 equiv) was added dropwise. The cooling bath was removed, the reaction mixture was allowed to warm to 23 °C, and the mixture was stirred at 23 °C for 20 h. The product mixture was poured on water and ice (160 mL). The resulting mixture was extracted three times with hexane-ethyl ether (2:1). The combined organic phases were washed with saturated aqueous sodium chloride solution, the washed solution was dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (hexanes-ethyl acetate, 100:20), furnishing the phthalimide **25** (1.41 g, 74%) as a white solid.

R_f = 0.71 (hexanes-ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.84 (dd, 2H, *J* = 5.37, 2.93 Hz), 7.71 (dd, 2H, *J* = 5.37, 2.93 Hz), 3.67 (t, 2H, *J* = 7.3 Hz), 3.35 (t, 2H, *J* = 6.8 Hz), 3.32 (s, 3H), 1.66 (dt, 2H, *J* = 14.2, 7.3 Hz), 1.59–1.52 (m, 2H), 1.32–1.27 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 168.7, 134.1, 132.4, 123.4, 73.2, 58.8, 38.3, 29.9, 29.7, 29.7, 29.6, 29.4, 28.8, 27.1, 26.3. IR (NaCl, thin film), cm^{-1} 2928 (m),

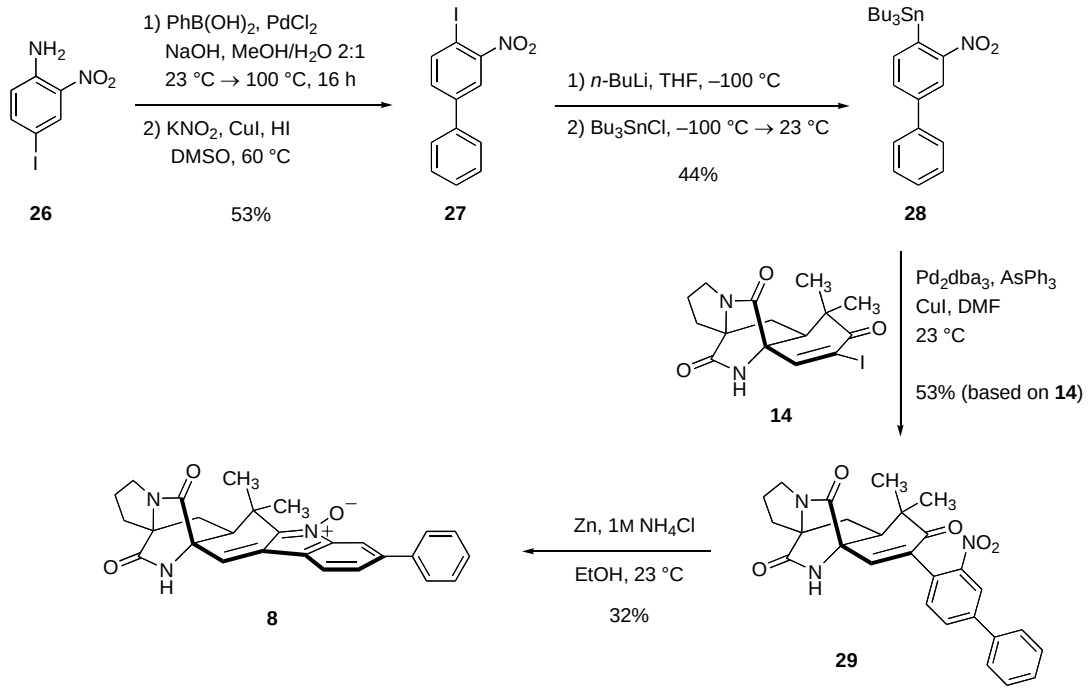
2855(m), 1773(m), 1708 (s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{28}NO_3^+$, 318.2069; found, 318.2059.



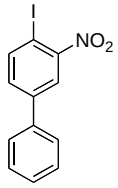
6

Dansylated derivative **6**. Hydrazine monohydrate (0.22 mL, 4.5 mmol, 2 equiv) was added to a solution of the phthalimide **25** (714 mg, 2.25 mmol, 1 equiv) in methanol (20 mL). The clear solution was heated to reflux for 2 h. The product solution was allowed to cool to 23 °C and the cooled solution was concentrated in vacuo. The residue was suspended in dichloromethane (ca. 20 mL), the suspension was dried over anhydrous sodium sulfate, the solids were removed by filtration through Celite, and the filtrate was concentrated in vacuo. The residue was dissolved in dichloromethane (10 mL). Dansyl chloride (**22**) (607 mg, 2.25 mmol, 1 equiv) and triethylamine (0.63 mL, 4.5 mmol, 2 equiv) were added. The yellow solution was stirred at 23 °C for 20 h. The product solution was concentrated in vacuo and the residue was purified by flash-column chromatography (hexanes-ethyl acetate-triethylamine, 100:10:2 to 100:20:2), affording the dansylated control **6** (852 mg, 2.03 mmol, 90 %) as a yellow oil.

R_f = 0.60 (hexanes-ethyl acetate 3:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.54 (d, 1H, *J* = 8.8 Hz), 8.28 (d, 1H, *J* = 8.8 Hz), 8.25 (dd, 1H, *J* = 7.3, 1.0 Hz), 7.57 (dd, 1H, *J* = 8.8, 7.3 Hz), 7.53 (dd, 1H, *J* = 8.8, 7.3 Hz), 7.19 (d, 1H, *J* = 7.3 Hz), 4.53 (t, 1H, *J* = 6.3 Hz), 3.35 (t, 2H, *J* = 6.8 Hz), 3.33 (s, 3H), 2.89 (s, 6H), 2.89–2.86 (m, 2H), 1.54 (dt, 2H, *J* = 14.6 6.8 Hz), 1.37–1.32 (m, 2H), 1.30–1.09 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 152.3, 134.9, 130.6, 130.1, 129.9, 129.9, 128.6, 123.4, 118.9, 115.4, 73.2, 58.8, 45.6, 43.6, 29.9, 29.7, 29.6, 29.5, 29.1, 26.6, 26.3. IR (NaCl, thin film), cm^{-1} 3301(br), 2928(m), 2854(m), 1589(w), 1576(w), 1457(m), 1321(s), 1160(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{23}H_{37}N_2O_3S^+$, 421.2525; found, 421.2538.



Scheme S4. Synthesis of analogue **8**.

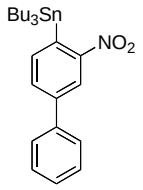


27

Iodoarene **27**. A mixture of 4-iodo-2-nitroaniline (**26**) (1.06 g, 4.0 mmol, 1 equiv), phenylboronic acid (536 mg, 4.4 mmol, 1.1 equiv), palladium chloride (35 mg, 0.2 mmol, 0.05 equiv), and sodium hydroxide (640 mg, 16 mmol, 4 equiv) in methanol-water (2:1, 15 mL) was stirred at 23 °C for 19 h and further at 100 °C for 3 h. The mixture was allowed to cool to 23 °C and the cooled mixture was concentrated in vacuo. The residue was neutralized with 5% hydrochloric acid solution. The resulting solution was extracted four times with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The resulting brown solid, potassium nitrite (857 mg, 4.0 mmol, 1 equiv), and copper iodide (762 mg, 4.0 mmol, 1 equiv) were suspended in dimethylsulfoxide and the mixture was heated to 60 °C. A solution of 55% hydroiodic acid (5 mL) in dimethylsulfoxide was added dropwise to the warmed reaction mixture. The resulting dark red solution was stirred at 60 °C for 30 min. The solution was allowed to cool to 23 °C and the cooled reaction mixture was poured onto a mixture of potassium carbonate (5 g) in ice-water (100 mL). The mixture was extracted three times with ethyl ether. The combined organic phases were washed successively with water and saturated aqueous sodium chloride solution. The washed solution was dried

over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (hexanes-dichloromethane, 9:1 to 8:2), affording the iodoarene **27** (684 mg, 53%) as a yellow solid.

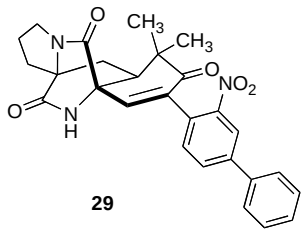
R_f = 0.32 (hexanes-acetone 100:4). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.10–8.07 (m, 2H), 7.60–7.58 (m, 2H), 7.51–7.42 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 143.0, 142.4, 137.9, 132.0, 129.5, 129.1, 127.1, 124.1, 84.6. IR (NaCl, thin film), cm^{-1} 3086(w), 3064(w), 2871(w), 1540(s), 1507(m), 1465(m), 1345(m)1025(m), 1019(m).



28

Stannane **28**. *n*-Butyllithium in hexanes (2.48 M, 0.42 mL, 1.05 mmol, 1.05 equiv) and tributyltin chloride (0.28 mL, 1.05 mmol, 1.05 equiv) were added in sequence to a solution of iodoarene **27** (325 mg, 1.0 mmol, 1 equiv) in tetrahydrofuran (10 mL) cooled to –100 °C. The cooling bath was removed and the brown solution was allowed to warm to 23 °C over 45 min. The solution was diluted with hexanes-ethyl ether (2:1) and the diluted solution was washed successively with water and saturated aqueous sodium chloride solution. The washed solution was dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography on silica gel (deactivated with 20% triethylamine-ethyl acetate, eluting with hexanes-ethyl acetate 100:2), furnishing the stannane **28** (213 mg, 44%) as a yellow oil.

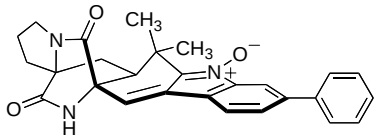
R_f = 0.75 (hexanes-acetone 100:4). ^{1}H NMR (500 MHz, C_6D_6), δ 8.49 (d, 1H, J = 1.5 Hz), 7.61 (d, 1H, J = 7.8 Hz), 7.45 (dd, 1H, J = 7.8, 1.5 Hz), 7.28–7.26 (m, 2H), 7.17–7.11 (m, 3H), 1.67–1.60 (m, 6H), 1.38 (sext, 6H, J = 7.3 Hz), 1.28–1.24 (m, 6H), 0.91 (t, 9H, J = 7.3 Hz). ^{13}C NMR (125 MHz, C_6D_6), δ 154.8, 142.8, 138.9, 138.1, 131.7, 129.1, 128.3, 128.2, 127.1, 122.5, 29.4, 27.6, 13.8, 11.3. IR (NaCl, thin film), cm^{-1} 2956 (m), 2922(m), 2852(w), 1534(s), 1343(m).



29

Nitroarene **29**. A mixture of tris(dibenzylideneacetone)dipalladium (9 mg, 9.8 μmol, 19.6 μmol Pd) and triphenylarsine (12 mg, 39.2 μmol, 2 equiv based on Pd) in *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use) was stirred at 23 °C for 30 min. In a separate flask, a suspension of copper iodide (3.8 mg, 20 μmol) in *N*,*N*-dimethylformamide (500 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use) was stirred at 23 °C for 30 min. A third flask was charged with vinyl iodide **14** (8 mg, 20 μmol, 1 equiv), stannane **28** (20 mg, 40 μmol, 2 equiv), and *N*,*N*-dimethylformamide (150 μL, deoxygenated by bubbling argon gas through the solvent for 1 h before use). The resulting solution was treated sequentially with the tris(dibenzylideneacetone)dipalladium-triphenylarsine and copper iodide solutions prepared above (50.0 μL each). The reaction mixture was stirred at 23 °C for 61 h. The product solution was diluted with hexanes-ethyl ether (2:1, 100 mL). The diluted solution was washed with saturated aqueous sodium chloride solution. The aqueous layer was extracted with hexanes-ethyl ether (2:1). The combined organic phases were dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by radial chromatography (1-mm rotor, eluting with dichloromethane-methanol, 100:1), affording the nitroarene **29** (5 mg, 53%) as a pale yellow solid.

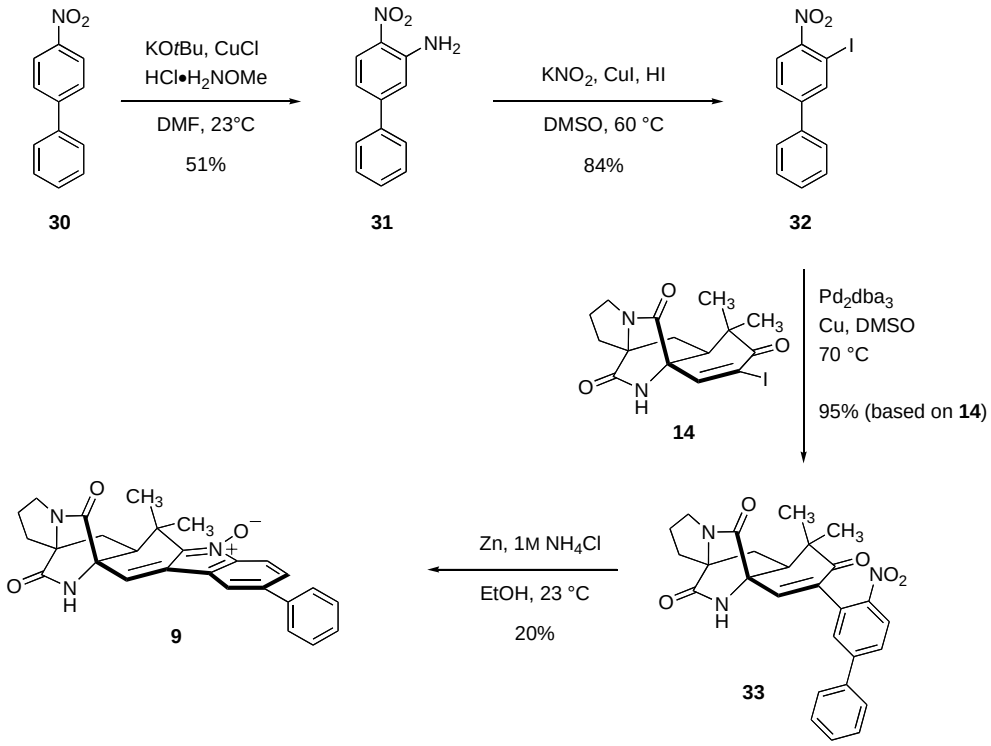
R_f = 0.35 (hexanes-ethyl acetate 1:9). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.31 (s, 1H), 7.86 (d, 1H, *J* = 7.8 Hz), 7.62 (d, 2H, *J* = 7.3 Hz), 7.52–7.43 (m, 4H), 6.92 (s, 1H), 6.77 (br s, 1H), 3.69–3.64 (m, 1H), 3.50 (dt, 1H, *J* = 11.2, 7.6 Hz), 2.96–2.80 (m, 2H), 2.29 (dd, 1H, *J* = 13.2, 10.3 Hz), 2.14–1.99 (m, 2H), 1.93–1.86 (m, 2H), 1.16 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 199.0, 172.7, 167.5, 149.0, 143.4, 142.0, 138.3, 136.5, 132.7, 132.1, 129.9, 129.4, 129.0, 127.3, 123.1, 67.8, 61.1, 51.1, 45.2, 44.6, 32.5, 29.6, 24.9, 23.5, 18.8. IR (NaCl, thin film), cm^{-1} 2921(w), 1686(s), 1532(m), 1352(w). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}N_3O_5^+$, 472.1867; found, 472.1850.



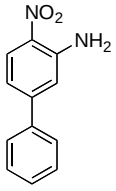
8

Nitrone **8**. Ammonium chloride solution (1 M, 15 μL, 15 μmol, 2.2 equiv) was added to a solution of nitroarene **29** (3.3 mg, 7 μmol, 1 equiv) in ethanol (350 μL). Zinc powder (2.3 mg, 35 μmol, 5 equiv) was added. The resulting pale yellow suspension was stirred at 23 °C for 15 min. The product mixture was diluted with ethyl acetate (9 mL) and the diluted mixture was filtered through Celite. The filtrate was washed with saturated aqueous sodium chloride solution, the washed solution was dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was filtered through a plug of silica gel, eluting with dichloromethane-acetone (2:1). The filtrate was concentrated in vacuo and the residue was purified by radial chromatography (1-mm rotor, eluting with dichloromethane-methanol 100:1 initially, grading to dichloromethane-methanol 100:3), affording the nitrone **8** (970 μg, 32%), as a yellow solid.

R_f = 0.40 (dichloromethane-methanol 100:6). ^{1}H NMR (500 MHz, C_6D_6), δ 8.17 (s, 1H), 7.40–7.36 (m, 4H), 7.21–7.03 (m, 3H), 6.17 (s, 1H), 5.36 (br s, 1H), 3.19–3.15 (m, 1H), 2.89 (dt, 1H, *J* = 11.2, 7.3 Hz), 2.65–2.60 (m, 1H), 1.91 (dd, 1H, *J* = 9.8, 6.8 Hz), 1.57 (s, 3H), 1.43–1.08 (m, 5H), 1.23 (s, 3H). IR (NaCl, thin film), cm^{-1} 3215(w), 2925(w), 1702(s), 1686(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}N_3O_3^+$, 440.1969; found, 440.1962.



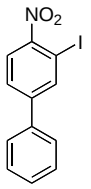
Scheme S5. Synthesis of analogue **9**.



31

Nitroaniline **31**. A solution of the nitroarene **30** (1.54 g, 7.73 mmol, 1.0 equiv) and methoxylamine hydrochloride (807 mg, 9.66 mmol, 1.25 equiv) in dimethylformamide (12 mL) was added over 5 min to a solution of potassium *tert*-butoxide (3.69 g, 32.85 mmol, 4.25 equiv) and copper chloride (77 mg, 0.1 mmol, 0.1 equiv) in dimethylformamide (27 mL). The resulting dark red solution was stirred at 23 °C for 1.5 h. The product solution was diluted with saturated ammonium chloride solution and the diluted solution was extracted three times with dichloromethane. The combined organic phases were dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by recrystallization (hexanes-ethyl acetate), affording the nitroaniline **31** (853 mg, 51%) as a yellow solid.

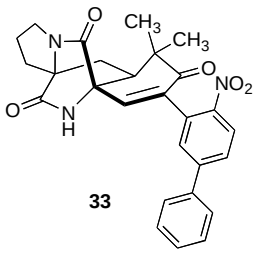
R_f = 0.35 (hexanes-ethyl acetate 8:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.19 (d, 1H, *J* = 8.8 Hz), 7.59–7.57 (m, 2H), 7.49–7.41 (m, 3H), 6.99 (d, 1H, *J* = 2.0 Hz), 6.94 (dd, 1H, *J* = 8.8, 2.0 Hz), 6.15 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 148.8, 145.1, 139.2, 131.7, 129.2, 129.1, 127.4, 127.1, 116.8, 116.7. IR (NaCl, thin film), cm^{-1} 3487(m), 3369(m), 3179(w), 3066(w), 1620(s), 1572(s), 1483(s), 1444(s), 1416(m), 1331(s), 1282(s), 1231(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{11}N_2O_2^+$, 215.0815; found, 215.0811.



32

Iodoarene **32**. A solution of 55% hydroiodic acid (4.93 mL) in dimethylsulfoxide (16 mL) was added dropwise to a mixture of the nitroaniline **31** (840 mg, 3.92 mmol, 1 equiv), potassium nitrite (734 mg, 8.62 mmol, 2.2 equiv), and copper iodide (747 mg, 3.92 mmol, 1 equiv) in dimethylsulfoxide (20 mL) at 60 °C. The dark red mixture was stirred at 60 °C for 30 min. The mixture was allowed to cool to 23 °C and the cooled mixture was poured onto potassium carbonate (5 g) in ice-water (100 mL). The mixture was extracted three times with ethyl ether. The combined organic phases were washed successively with water and saturated aqueous sodium chloride solution. The washed solution was dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (hexanes-dichloromethane, 9:1 to 8:2), affording the iodoarene **32** (1.07 g, 84%) as a pale yellow solid.

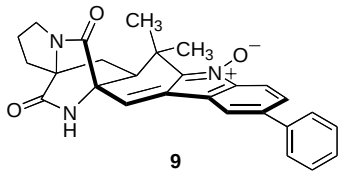
R_f = 0.68 (hexanes-ethyl acetate 8:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.26 (d, 1H, *J* = 1.8 Hz), 7.98 (d, 1H, *J* = 8.5 Hz), 7.68 (dd, 1H, *J* = 8.5, 1.8 Hz), 7.59–7.57 (m, 2H), 7.52–7.44 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 146.9, 140.7, 137.6, 129.5, 129.4, 127.7, 127.6, 126.2, 87.3. IR (NaCl, thin film), cm^{-1} 3061(w), 3031(w), 1583(m), 1566(m), 1522(s), 1345(m). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{23}H_{37}N_2O_3S^+$, ; found, .



33

Nitroarene **33**. A mixture of the vinyl iodide **14** (8 mg, 20 mmol, 1.0 equiv), the aryl iodide **32** (16.3 mg, 50 mmol, 2.5 equiv), tris(dibenzylideneacetone)dipalladium (1.8 mg, 2 mmol, 0.1 equiv), and copper (6.4 mg, 100 mmol, 5.0 equiv) in dimethylsulfoxide (200 mL) was stirred at 70 °C for 4 h. The brown product mixture was allowed to cool to 23 °C and the cooled mixture was diluted with dichloromethane. The diluted mixture was washed with saturated aqueous ammonium solution-water-ammonium hydroxide (4:1:0.5). The layers were separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (dichloromethane-methanol, 100:1), furnishing the nitroarene **33** (9 mg, 95%) as a pale yellow solid.

R_f = 0.40 (hexanes-ethyl acetate 1:9). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.20 (d, 1H, J = 8.8 Hz), 7.73–7.62 (m, 3H), 7.50–7.44 (m, 4H), 6.91 (s, 1H), 6.90 (br s, 1H), 3.66–3.61 (m, 1H), 3.52–3.47 (m, 1H), 2.95–2.77 (m, 2H), 2.29–2.25 (m, 1H), 2.10–1.99 (m, 2H), 1.93–1.84 (m, 2H), 1.15 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 199.0, 172.9, 167.4, 147.3, 147.2, 142.5, 138.5, 136.5, 132.2, 130.8, 129.4, 129.2, 128.2, 127.7, 125.4, 67.8, 61.2, 50.8, 45.2, 44.6, 32.5, 29.6, 24.8, 23.9, 18.9. IR (NaCl, thin film), cm^{-1} 2968(w), 1688(s), 1520(m), 1350(w). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}N_3O_5^+$, 472.1867; found, 472.1865.

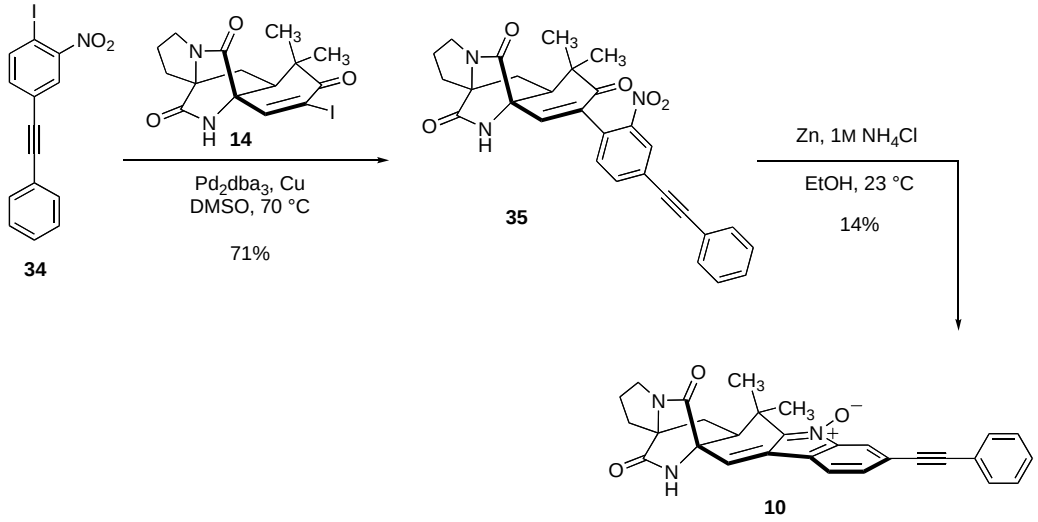


9

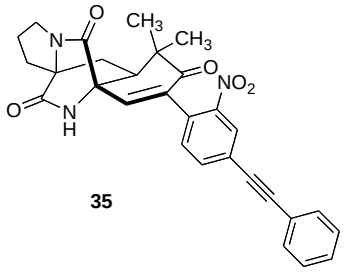
Nitrone **9**. Ammonium chloride solution (1 M, 18 μL, 18 μmol, 2.2 equiv) was added to a solution of the nitroarene **33** (3.8 mg, 8 μmol, 1 equiv) in ethanol (400 μL). Zinc powder (2.6 mg, 40 μmol, 5 equiv) was added. The resulting pale yellow suspension was stirred at 23 °C for 30 min. The product mixture was diluted with ethyl acetate (9 mL) and the diluted mixture was filtered through Celite. The filtrate was washed with saturated aqueous sodium chloride solution, the washed solution was dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was filtered through a plug of silica gel, eluting with dichloromethane-acetone (2:1). The filtrate was

concentrated in vacuo and the residue was purified by radial chromatography (1-mm rotor, eluting with dichloromethane-methanol 100:1 initially, grading to dichloromethane-methanol 100:3), affording the nitrone **9** (702 μg, 20%) as a yellow solid.

R_f = 0.35 (dichloromethane-methanol 100:6). ^{1}H NMR (500 MHz, C_6D_6), δ 7.81 (d, 1H, *J* = 8.3 Hz), 7.49 (d, 1H, *J* = 1.5 Hz), 7.34 (d, 2H, *J* = 7.3 Hz), 7.31–7.17 (m, 2H), 7.23 (d, 2H, *J* = 7.3 Hz), 6.10 (s, 1H), 5.49 (1H, br s), 3.21–3.16 (m, 1H), 2.89 (dt, 1H, *J* = 11.2, 7.3 Hz), 2.66–2.61 (m, 1H), 1.97 (dd, 1H, *J* = 10.3, 6.8 Hz), 1.58 (s, 3H), 1.45–1.13 (m, 5H), 1.23 (s, 3H). IR (NaCl, thin film), cm^{-1} 3226(w), 2961(w), 2928(w), 1701(s), 1689(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}N_3O_3^+$, 440.1969; found, 440.1969.



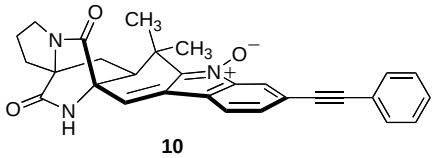
Scheme S6. Synthesis of analogue **10**.



Nitroarene **35**. A mixture of the vinyl iodide **14** (8 mg, 20 mmol, 1.0 equiv), the iodoarene **34** (17.5 mg, 50 mmol, 2.5 equiv), tris(dibenzylideneacetone)dipalladium (1.8 mg, 2 mmol, 0.1 equiv), and copper (6.4 mg, 100 mmol, 5.0 equiv) in dimethylsulfoxide (200 mL) was stirred at 70 °C for 5 h. The brown product mixture was allowed to cool to 23 °C and the cooled mixture was diluted with dichloromethane. The diluted mixture was washed with saturated aqueous ammonium solution-water-

ammonium hydroxide (4:1:0.5). The layers were separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (dichloromethane-methanol, 100:1), furnishing the nitroarene **35** (7 mg, 71%) as a pale yellow solid.

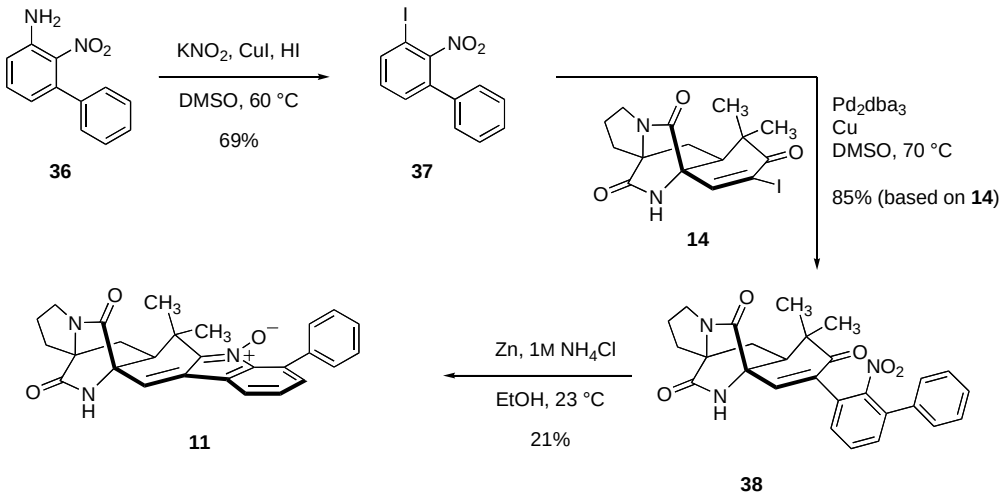
R_f = 0.45 (hexanes-ethyl acetate 1:9). ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.24 (d, 1H, *J* = 1.4 Hz), 7.77 (dd, 1H, *J* = 7.8, 1.4 Hz), 7.57–7.55 (m, 2H), 7.44–7.37 (m, 4H), 6.90 (s, 1H), 6.81 (br s, 1H), 3.68–3.63 (m, 1H), 3.50 (dt, 1H, *J* = 11.4, 7.6 Hz), 2.85–2.80 (m, 2H), 2.27 (dd, 1H, *J* = 13.3, 10.0 Hz), 2.13–1.99 (m, 2H), 1.92–1.86 (m, 2H), 1.14 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 198.7, 172.7, 167.4, 148.6, 141.7, 136.9, 136.3, 132.4, 132.1, 130.8, 129.4, 128.7, 127.4, 125.8, 122.3, 93.0, 86.8, 67.8, 61.1 51.0, 45.2, 44.6, 32.4, 29.6, 24.9, 23.5, 18.8. IR (NaCl, thin film), cm^{-1} 2924(w), 1688(s), 1531(m), 1353(m). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{29}H_{26}N_3O_5^+$, 496.1867; found, 496.1872.



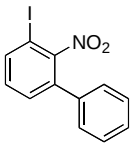
10

Nitrone **10**. Ammonium chloride solution (1 M, 18 μL, 18 μmol, 2.2 equiv) was added to a solution of nitroarene **35** (4.0 mg, 8 μmol, 1 equiv) in ethanol (400 μL). Zinc powder (2.6 mg, 40 μmol, 5 equiv) was added. The resulting pale yellow suspension was stirred at 23 °C for 1 h. The product mixture was diluted with ethyl acetate (9 mL) and the diluted mixture was filtered through Celite. The filtrate was washed with saturated aqueous sodium chloride solution, the washed solution was dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was filtered through a plug of silica gel, eluting with dichloromethane-acetone (2:1). The filtrate was concentrated in vacuo and the residue was purified by radial chromatography (1-mm rotor, eluting with dichloromethane-methanol 100:1 initially, grading to dichloromethane-methanol 100:3), affording the nitrone **10** (489 μg, 14%) as a yellow solid.

R_f = 0.31 (dichloromethane-methanol 100:6). ^{1}H NMR (500 MHz, C_6D_6), δ 8.20 (s, 1H), 7.50–7.49 (m, 2H), 7.41–7.40 (m, 1H), 7.22–7.00 (m, 4H), 6.08 (s, 1H), 5.24 (br s, 1H), 3.17–3.12 (m, 1H), 2.87 (dt, 1H, *J* = 11.2, 7.3 Hz), 2.63–2.56 (m, 1H), 1.84 (dd, 1H, *J* = 10.3, 6.3 Hz), 1.55 (s, 3H), 1.42–1.11 (m, 5H), 1.16 (s, 3H). IR (NaCl, thin film), cm^{-1} 2954(w), 2913(w), 2851(w), 1692(s), 1260(m). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{29}H_{26}N_3O_3^+$, 464.1969; found, 464.1992.



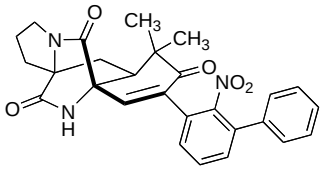
Scheme S7. Synthesis of analogue **11**.



37

Iodoarene **37**. A solution of 55% hydroiodic acid (3.89 mL) in dimethylsulfoxide (12 mL) was added dropwise to a mixture of the nitroaniline **36** (666 mg, 3.11 mmol, 1 equiv), potassium nitrite (582 mg, 6.84 mmol, 2.2 equiv), and copper iodide (592 mg, 3.11 mmol, 1 equiv) in dimethylsulfoxide (15 mL) at 60 °C. The dark red mixture was stirred at 60 °C for 30 min. The mixture was allowed to cool to 23 °C and the cooled mixture was poured onto potassium carbonate (5 g) in ice-water (100 mL). The mixture was extracted three times with ethyl ether. The combined organic phases were washed successively with water and saturated aqueous sodium chloride solution. The washed solution was dried over anhydrous sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (hexanes-dichloromethane, 9:1 to 8:2), affording the iodoarene **37** (693 mg, 69%) as a white solid.

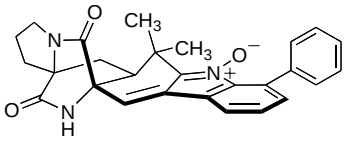
R_f = 0.46 (hexanes-ethyl acetate 8:2). ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.90–7.88 (m, 1H), 7.44–7.40 (m, 3H), 7.35–7.32 (m, 2H), 7.24 (t, 2H, J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ 139.3, 136.1, 136.0, 131.4, 131.3, 129.2, 129.1, 128.2, 85.8. IR (NaCl, thin film), cm^{-1} 3084(w), 3070(m), 3032(w), 1522(s), 1367(s). HRMS-ESI (*m/z*): $[M + K]^+$ calcd for $C_{12}H_8IKNO_2^+$, 363.9231; found, 363.9229.



38

Nitroarene **38**. A mixture of the vinyl iodide **14** (8 mg, 20 μmol, 1.0 equiv), the iodoarene **37** (16.3 mg, 50 μmol, 2.5 equiv), tris(dibenzylideneacetone)dipalladium (1.8 mg, 2 μmol, 0.1 equiv), and copper (6.4 mg, 100 μmol, 5.0 equiv) in dimethylsulfoxide (200 μL) was stirred at 70 °C for 5 h. The brown product mixture was allowed to cool to 23 °C and the cooled mixture was diluted with dichloromethane. The diluted mixture was washed with saturated aqueous ammonium solution-water-ammonium hydroxide (4:1:0.5). The layers were separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by flash-column chromatography (dichloromethane-methanol, 100:1), furnishing the nitroarene **38** (8 mg, 85%) as a pale yellow solid.

R_f = 0.35 (hexanes-ethyl acetate 1:9). ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.59–7.55 (m, 1H), 7.45 (dd, 1H, *J* = 7.8, 1.4 Hz), 7.43–7.35 (m, 4H), 7.34–7.32 (m, 2H), 6.90 (s, 1H), 6.67–6.56 (m, 1H), 3.67–3.62 (m, 1H), 3.49 (dt, 1H, *J* = 11.4, 7.3 Hz), 2.85–2.78 (m, 2H), 2.25 (dd, 1H, *J* = 13.3, 10.5 Hz), 2.12–1.98 (m, 2H), 1.90–1.84 (m, 2H), 1.14 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ 199.1, 172.6, 167.1, 149.5, 139.9, 139.0, 137.0, 135.7, 132.1, 130.8, 130.6, 129.8, 128.9, 128.7, 128.2, 67.8, 61.1, 51.1, 45.3, 44.6, 32.5, 29.6, 24.9, 23.3, 18.7. IR (NaCl, thin film), cm^{-1} 2925(m), 1686(s), 1533(m), 1358(m). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}N_3O_5^+$, 472.1867; found, 472.1861.



11

Nitrone **11**. Ammonium chloride solution (1 M, 18 μL, 18 μmol, 2.2 equiv) was added to a solution of nitroarene **38** (3.8 mg, 8 μmol, 1 equiv) in ethanol (400 μL). Zinc powder (2.6 mg, 40 μmol, 5 equiv) was added. The resulting pale yellow suspension was stirred at 23 °C for 2 h. The product mixture was diluted with ethyl acetate (9 mL) and the diluted mixture was filtered through Celite. The filtrate was washed with saturated aqueous sodium chloride solution, the washed solution was dried over sodium sulfate, the solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was subjected to flash-column chromatography (dichloromethane-ethyl acetate, 4:1 to 5:3), giving the nitrone **11** (731 μg, 21%) as a yellow solid.

R_f = 0.39 (dichloromethane-methanol 100:6). ^{1}H NMR (500 MHz, C_6D_6), δ 7.56 (d, 2H, *J* = 6.8 Hz), 7.28–7.00 (m, 6H), 6.08 (s, 1H), 5.37 (br s, 1H), 3.21–3.17 (m, 1H), 2.89 (dt, 1H, *J* = 11.2, 7.3 Hz), 2.64–2.59 (m, 1H), 1.96 (dd, 1H, *J* = 10.3, 6.3 Hz), 1.45–1.12 (m, 5H), 1.42 (s, 3H), 1.14 (s, 3H). IR (NaCl, thin film), cm^{-1} 3222(w), 2961(w), 2927(w), 1701(s), 1684(s). HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}N_3O_3^+$, 440.1969; found, 440.1986.

B. Biology

General Experimental Procedures. All cell-culture work was conducted in a class II biological safety cabinet. Buffers were filter-sterilized (0.2 μm) prior to use. Antiproliferative assays and other operations requiring the handling of nitrone species were carried out in the dark to prevent the occurrence of photochemical rearrangement reactions. Compounds **1-7** were typically stored in the dark as 5 mM stock solutions in DMSO, and were kept at –80 ºC. Compounds **8-11** were stored at –80 °C as dry solids (100-μg portions). Stock solutions (5 mM in DMSO) were prepared immediately prior to use.

Materials. LNCaP, T-47D, and HeLa-S3 cells were purchased from ATCC. COS-7 cells were kindly provided by Professor Alan Saghatelian. All cells were cultured in RPMI 1640 (Mediatech) containing 10% fetal bovine serum (Hyclone), 10 mM HEPES, and 2 mM L-glutamine. Cells were grown in BD Falcon tissue culture flasks with vented caps. Bradford reagent and Laemmli loading buffer (2X concentration) were purchased from Sigma Aldrich. Antiproliferative assays were conducted in pre-sterilized 96-well flat-bottomed plates from BD Falcon. Solutions of resazurin were purchased from Promega as part of the CellTiter-Blue Cell Viability Assay kit, and were used according to the manufacturer's instructions. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using precast Novex tris-glycine mini gels (10%, 12% or 4-20% gradient, Invitrogen). Electrophoresis and semi-dry electroblotting equipment was purchased from Owl Separation Systems. Nitrocellulose membranes were purchased from Amersham Biosciences. A mouse monoclonal antibody to nucleophosmin (B23) was purchased from Santa Cruz Biotechnology (sc-32256). A rabbit polyclonal antibody to peroxiredoxin 1 was purchased from GeneTex (GTX15571). Rabbit polyclonal antibodies to exportin 1 and p53 were purchased from Santa Cruz Biotechnology (XPO1: sc-5595; p53: sc-6243). An Alexafluor 647 goat anti-mouse secondary antibody, together with Image-iT FX Signal Enhancer blocking solution, was purchased from Invitrogen (A31625). Western-blot detection was performed using the SuperSignal West Pico Chemiluminscence kit (including a goat anti-rabbit-HRP or goat anti-mouse-HRP conjugate) from Pierce. Western blots were visualized using CL-XPosure X-ray film from Pierce, or were imaged on an AlphaImager. Streptavidin-agarose was purchased from Sigma Aldrich. Protein bands were visualized using the Novex Colloidal Blue staining kit from Invitrogen, and were analyzed at the Taplin Biological Mass Spectrometry Facility (Harvard University). Yo-Pro iodide was purchased from Invitrogen.

Instrumentation. Absorbance and fluorescence measurements were made using Molecular Dynamics multiwell plate readers (absorbance: SPECTRAmax PLUS 384, fluorescence: SPECTRAmax GEMINI XS). Data was collected using SOFTmax PRO v. 4.3 (Molecular Dynamics), and was manipulated in Excel (Microsoft). The XLfit4 plugin (IDBS software) running in Excel was used for curve fitting. Analytical HPLC measurements were made on a Beckman Coulter System Gold HPLC, equipped with a reverse phase Beckman Coulter Ultrasphere ODS column (5 μM, 4.6 mm x 25 cm). Fluorescence microscopy

experiments were performed using a Zeiss upright microscope, equipped with 355 nm, 488 nm, 543 nm and 633 nm lasers. Flow cytometry experiments were performed on an LSR II flow cytometer (BD Biosciences).

Preparation of Solutions.

RIPA buffer:
50 mM Tris·HCl, pH 7.35
150 mM NaCl
1 mM EDTA
1% Triton X-100
1% Sodium deoxycholate
0.1% SDS
1 mM PMSF
5 μg/mL aprotinin
5 μg/mL leupeptin
200 μM Na_3VO_4
50 mM NaF

Wash buffer:
50 mM Tris·HCl, pH 7.6
75 mM NaCl
0.5 mM EDTA
0.5% Triton X-100
0.5% Sodium deoxycholate
0.05% SDS

Tris Buffer:
50 mM Tris·HCl, pH 7.8

Sucrose-Hypotonic Buffer:
25 mM Tris·HCl, pH 6.8
250 mM Sucrose
0.05% digitonin
1 mM DTT
1 mM PMSF
5 μg/mL leupeptin
200 μM Na_3VO_4
50 mM NaF

Apoptosis Detection Buffer
100 nM Yo-Pro iodide
1.5 μM Propidium iodide
1 mM EDTA
1% BSA
in PBS

Preparation of Resins.

A 400-μL aliquot of Sepharose 6B suspension (Sigma) was transferred to a 1.5-mL centrifuge tube. Wash buffer (1.0 mL, see above for formulation) was added, and the resulting slurry was mixed for 5 min at 4 ºC. The resin was centrifuged (12000 x g, 2 min, 4 ºC), and the supernatant was discarded. The resin was washed twice with 1.0 mL wash buffer (each wash: 5 min mixing at 4 ºC, followed by 2 min centrifugation at 12000 x g, 4 ºC), then was suspended in 800 μL wash buffer and mixed thoroughly prior to use.

A 400-μL aliquot of streptavidin-agarose suspension (Sigma) was transferred to a 1.5-mL centrifuge tube. Wash buffer (1.0 mL, see above for formulation) was added, and the resulting slurry was mixed for 5 min at 4 ºC. The resin was centrifuged (12000 x g, 2 min, 4 ºC), and the supernatant was

discarded. The resin was washed twice with 1.0 mL wash buffer (each wash: 5 min mixing at 4 ºC, followed by 2 min centrifugation at 12000 x g, 4 ºC), then was suspended in 800 µL wash buffer and mixed thoroughly prior to use.

Abbreviations and Acronyms.

Tris = trishydroxymethylaminomethane (2-Amino-2-(hydroxymethyl)propane-1,3-diol)

SDS = sodium dodecyl sulfate

EDTA = ethylenediamine tetraacetic acid

PMSF = phenylmethanesulfonylfluoride

PBS = phosphate buffered saline

TBS = tris-buffered saline

DTT = dithiothreitol

BSA = bovine serum albumin

Antiproliferative Assays.

LNCaP and T-47D cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellet was resuspended in fresh medium to achieve a concentration of approximately 1.0 to 1.5 x 10^6 cells/mL. A sample was diluted 10-fold in fresh medium, and the concentration of cells was determined using a hemacytometer.

The cell suspension was diluted to 1.0 x 10^5 cells/mL. A multichannel pipette was used to charge the wells of a 96-well plate with 100 μL per well of the diluted cell suspension. The plates were incubated for 24 h at 37 ^oC under an atmosphere of 5% CO_2.

The following day, a 6.5-μL aliquot of nitrone solution, at 5 mM in DMSO, was diluted in 643.5 μL of medium to achieve a working concentration of 50 μM. Serial dilutions were employed to generate a range of different concentrations for analysis. Finally, 100-μL aliquots of the diluted nitrone solutions were added to the wells containing adhered cells, resulting in final assay concentrations of up to 25 μM.

The treated cells were incubated for 72 h at 37 ^oC (5% CO_2). To each well was added 20 μL of CellTiter-Blue reagent, and the samples were returned to the incubator. Fluorescence (560 nm excitation / 590 nm emission) was recorded on a 96-well plate reader following a 4.0 h incubation period (37 ^oC, 5% CO_2).

Percent growth inhibition was calculated for each well, based upon the following formula:

$$\text{Percent growth inhibition} = 100 \times (S - B_0) / (B_t - B_0)$$

where S is the sample reading, B_t is the average reading for a vehicle-treated population of cells at the completion of the assay, and B_0 is the average reading for an untreated population of cells at the beginning of the assay.

Each analogue was run a minimum of eight times, over a period of at least two weeks. For each compound, 14 separate concentrations were used in the assay, ranging from 25 μM to 8 nM. The average inhibition at each concentration was plotted against concentration, and a curve fit was generated. To eliminate positional effects (e.g., cell samples in the center of the plate routinely grew more slowly than those near the edge), the data was automatically scaled to ensure that the curves showed no inhibition at negligible concentrations of added compound. Such a precaution was found to generate more consistent data from week to week, without affecting the final results. Final GI_{50} values reflect the concentrations at which the resulting curves pass through 50 percent inhibition.

Fluorescence Microscopy Experiments.

HeLa-S3 cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded and the cell pellet was resuspended in fresh medium to achieve a concentration of approximately 1.0 to 1.5 x 10^6 cells/mL. A sample was diluted 10-fold in fresh medium, and the concentration of cells was determined using a hemacytometer.

The cell suspension was diluted to 2.0 x 10^4 cells/mL. A 6-well plate was charged with one 22 mm x 22 mm number 1.5 glass coverslip per well, followed by 4 mL/well of cell suspension. The plate was incubated for 24 h at 37 ºC under an atmosphere of 5% CO_2.

The following day, 5.94 μL of a 5 mM stock solution of probe **4** in DMSO was added to 1094 μL of cell-culture medium. From the resulting 27 μM solution, 500 μL was added to one well of the 6-well plate, resulting in a final concentration of 3 μM probe **4**. Other samples were prepared in a similar manner, but with final concentrations of 1 μM or 0 μM (vehicle control) probe **4**. All samples contained 0.06% DMSO.

The plate was returned to the incubator for 2 h, then the coverslips were carefully removed. Each coverslip was immersed in 5 mL methanol at –20 ºC for 3 min to fix the cells, then was washed three times (5 min per wash) in 5 mL PBS. The cells were permeablized by immersing the coverslips in 5 mL of 0.1% Triton X-100 in PBS for 5 min at 23 ºC, followed by three washes (5 min in 5 mL PBS). The coverslips were coated with a film of Image-iT FX Signal Enhancer and incubated at 23 ºC for 30 min, then were washed three times (5 min in 5 mL PBS).

The 3 μM and vehicle control samples were rinsed briefly in water, then mounted on slides with 20 μL Mowiol mounting mixture (containing 0.1% *p*-phenylene diamine).

The 1 μM sample was treated with 150 μL of primary antibody solution (0.5 μL of mouse anti-B23, Santa Cruz Biotechnology (sc-32256) in 499.5 μL PBS) for 30 min, then washed three times (5 min in 5 mL PBS) and treated with 150 μL of secondary antibody solution (0.5 μL of Alexafluor 647 goat anti-mouse, Invitrogen (A31625) in 499.5 μL PBS) for 30 min. The coverslip was washed three more times (5 min in 5 mL PBS), rinsed briefly in water, and mounted onto a slide with 20 μL Mowiol mounting mixture (containing 0.1% *p*-phenylene diamine).

Fluorescence microscopy experiments (λ_{ex} = 355 nm) showed that the dansyl group of the activity-based probe **4** was detectable above the background; e.g., cells treated with 3 μM of probe **4** (Figure S1A) showed a higher fluorescence output than cells treated with vehicle control (Figure S1B).

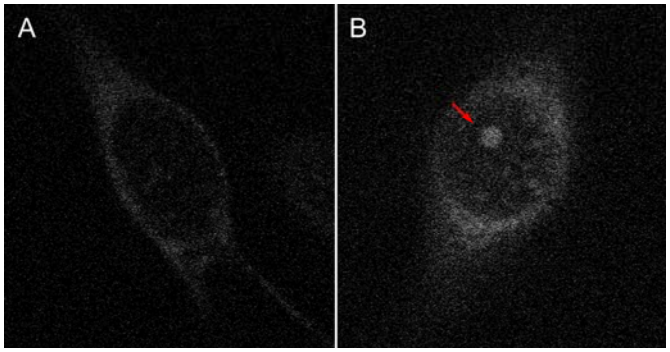


Figure S1. Fluorescence microscopy experiments with activity-based probe **4** in HeLa S3 cells. (A) Vehicle control reveals background fluorescence. (B) Treatment with 3 μM probe **4** shows both extra- and intranuclear localization. Red arrow indicates a localized concentration of **4** observed inside the nucleus. Data is representative of several cells analyzed.

Probe **4** was observed in both the cytosol and nucleus of HeLa S3 cells at concentrations of both 1 μM and 3 μM. Within the nucleus, the probe appeared to be concentrated within a smaller intranuclear region, identified as the nucleolus by immunofluorescence experiments using nucleophosmin as a nucleolar marker (Figure S1B, and text Figure 2).

Data from similar experiments in T-47D cells are shown below in Figure S2.

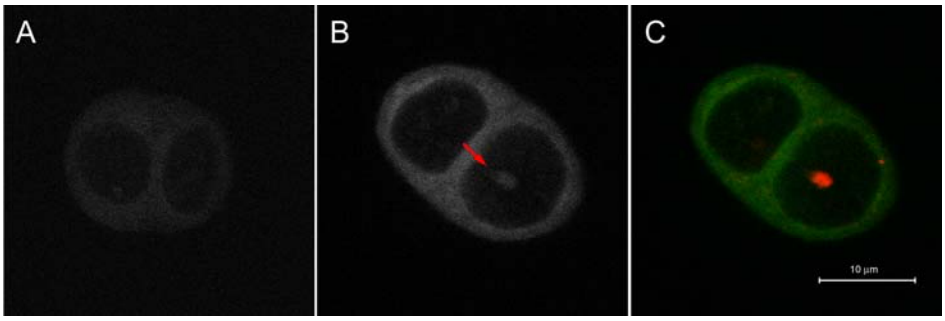


Figure S2. Fluorescence microscopy experiments with activity-based probe **4** in T-47D cells. (A) Vehicle control reveals background fluorescence. (B) Treatment with 1 μM probe **4** shows both extra- and intranuclear localization. Red arrow indicates a localized concentration of **4** observed inside the nucleus. (C) Direct fluorescence from **4** (green) overlaid with immunofluorescent localization of nucleophosmin (red) as a nucleolar marker.

Identification of Nucleophosmin by LC-MS/MS.

T-47D cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellet was resuspended in fresh medium to achieve a concentration of approximately 1.0 to 1.5 x 10^6 cells/mL. A sample was diluted 10-fold in fresh medium, and the concentration of cells was determined using a hemacytometer.

The cell suspension was diluted to 4.5 x 10^5 cells/mL. Cell culture flasks (75 cm^2) were charged with 12 mL of the suspension, and were then incubated for 2 d at 37 ºC under an atmosphere of 5% CO_2.

The medium was removed from the growing cells, and replaced with 12 mL of medium containing either 8 μM of the biotinylated probe **3** or (as a control) 8 μM of compound **7**. Incubation (at 37 ºC and 5% CO_2) was continued for 1 d, after which the medium (including any detached cells) from each sample was transferred to a 50-mL centrifuge tube. The cells were rinsed with 10 mL PBS, which was added to centrifuge tubes. Adhered cells were detached from the culture flask by trypsinization (10 min, 37 ºC, 3 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh medium (6 mL) was added, and the resulting suspension was added to the centrifuge tubes, along with a 10 mL PBS rinse.

The samples were centrifuged (10 min at 183 x g), and the supernatant was discarded. The cells were resuspended in 1 mL of PBS, transferred to a 1.5-mL centrifuge tube, and centrifuged again (5 min at 500 x g). The supernatant was discarded, and the cells were washed with 1 mL of PBS.

The washed cells were cooled on ice, then lysed by addition of 500 μL per sample ice-cold RIPA buffer (see above for formulation). The samples were mixed end-over-end for 1 h at 4 ºC with occasional vortexing, then 500 μL per sample Tris buffer was added. The samples were centrifuged (12000 x g, 10 min, 4 ºC), and insoluble material was removed with a pipette tip. The lysates were transferred to fresh 1.5-mL centrifuge tubes.

The protein concentration in each lysate was determined (Bradford method[8]), and the samples were diluted with wash buffer to a final concentration of 3500 μg protein in 1100 μL. Each sample was treated with a 50-μL aliquot of washed, twice-diluted sepharose (see above for resin preparation) and the resulting slurry was mixed end-over-end for 1 h at 4 ºC. The samples were centrifuged (12000 x g, 10 min, 4 ºC), and 1 mL of supernatant from each sample was transferred to a clean 1.5-mL centrifuge tube. This was treated with two 30-μL aliquots of washed, well-suspended, two-fold diluted streptavidin-agarose resin (see above for resin preparation). The resulting slurry was mixed for 15 h at 4 ºC, then was centrifuged (12000 x g, 10 min, 4 ºC). The supernatant was discarded.

The collected resins were washed with wash buffer at 4 ºC, then with tris buffer at 4 ºC, then twice with tris buffer at 23 ºC. Each wash consisted of 10 min mixing, followed by 10 min centrifugation (either 12000 x g at 4 ºC, or 10000 x g at 23 ºC). See above for solution preparation.

[8] Bradford, M. M. *Anal. Biochem*. **1976**, *72*, 248.

The washed resin was suspended in Laemmli loading buffer (Sigma, 2X concentration, 50 μL per sample), and the samples were heated to 95 ºC for 6 min. A tris-glycine mini gel (10%, 12-well) was loaded with 20 μL per lane of the denatured protein mixture. The protein samples were electroeluted (20 min, 23 ºC, 150 V) until all of the loaded protein had migrated into the gel.

The resulting gel was stained with Colloidal Blue. The entire lanes (approximately 1 cm) corresponding to the protein from the two samples were submitted for protein sequencing by LC–MS/MS. Results are shown in Table S1.

Table S1: LC–MS/MS Analysis of Proteins Identified Following Affinity-Isolation with Probe **3**

protein	MW (kDa)	percent coverage (by mass)		assignment
		8 μM **3**	8 μM **7**	
cellular myosin heavy chain, type a	226	40%	41%	nonspecific binder: myosin
actin-like protein Q562X8	12	28%	28%	nonspecific binder: actin
actin-like protein actg1	29	18%	18%	nonspecific binder: actin
actin-like protein Q562P9	11	17%	17%	nonspecific binder: actin
60s ribosomal protein l7	**29**	**10%**	**-**	**possible selective binding protein**
cellular myosin heavy chain, type b	229	8%	12%	nonspecific binder: myosin
tubulin alpha-2 chain	50	8%	4%	nonspecific binder: tubulin
nucleophosmin	**33**	**7%**	**-**	**possible selective binding protein**
actin, alpha 1, skeletal muscle	32	7%	7%	nonspecific binder: actin
actin-like protein Q6ZSQ4	24	5%	5%	nonspecific binder: actin
actin-like protein Q9BYX7	42	4%	4%	nonspecific binder: actin
glyceraldehyde-3-phosphate dehydrogenase	36	4%	10%	nonspecific binder: abundant protein
pyruvate kinase muscle isozyme	58	4%	-	observed in other experiments as a nonspecific binding protein
pyruvate carboxylase	130	3%	14%	biotinylated protein
tubulin alpha-6 chain	50	3%	3%	nonspecific binder: tubulin
myosin heavy chain, smooth muscle isoform	227	1%	1%	nonspecific binder: myosin
myosin heavy chain, nonmuscle iic	228	1%	1%	nonspecific binder: myosin
methylcrotonoyl-coa carboxylase subunit alpha	80	-	13%	biotinylated protein
propionyl-coa carboxylase alpha chain	77	-	4%	biotinylated protein
propionyl-coa carboxylase beta chain	58	-	3%	biotinylated protein
methylcrotonoyl-coa carboxylase beta chain	61	-	3%	biotinylated protein
heat-shock protein beta-1	23	-	8%	known to associate with tubulin

Among several proteins common to both the sample and control lanes (in particular structural proteins such as myosin, actin, and tubulin, as well as biotinylated proteins), we observed only three proteins which were present in the sample originating from treatment with probe **3**, but not in the control sample originating from treatment with compound **7**. Of these, pyruvate kinase muscle isozyme was considered not to be a selective binding protein, since it had previously been detected in both sample and control lanes from other experiments.

In subsequent Western-blotting experiments, the 60s ribosomal protein was likewise revealed to be a nonselective binding protein (data not shown), while nucleophosmin was found to selectively bind to the biotinylated probes **3** and **5** (see below).

Attempts to directly identify nucleophosmin in a similar full-gel analysis by LC–MS/MS with the natural product-like probe **5** were unsuccessful (despite the fact that **5** binds more efficiently than **3** to nucleophosmin, as discussed below), as these analyses were invariably complicated by an overabundance of the nonspecific binding proteins discussed above. However, when a narrower region of the gel was submitted for analysis following affinity isolation with probe **5** and electroelution, nucleophosmin was detected by LC–MS/MS analysis. Nucleophosmin was not detected by LC–MS/MS analysis in control experiments using (+)-avrainvillamide (**1**) or **7** in lieu of probe **5** (equal concentrations).

Affinity-Isolation Experiments from Incubations with Live Cells, then Lysis.

1. Preparation of Cellular Lysates from Treated Cells.

T-47D cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellet was resuspended in fresh medium to achieve a concentration of approximately 1.0 to 1.5 x 10^6 cells/mL. A sample was diluted 10-fold in fresh medium, and the concentration of cells was determined using a hemacytometer.

The cell suspension was diluted to 3.0 x 10^5 cells/mL. Cell culture flasks (75 cm^2) were charged with 12 mL of the suspension, and were then incubated for 2 d at 37 ºC under an atmosphere of 5% CO_2. The medium was removed, and 12 mL fresh cell culture medium was added. Incubation was continued for 24 h. The cells were ~65% confluent.

The medium was removed from the growing cells, and replaced with 12 mL of medium containing the following activity-based probes and control compounds (from 5 mM stocks in DMSO):

sample:	volume medium	volume DMSO	volume **5** 5000 μM	volume **3** 5000 μM	volume (+)-**1** 5000 μM	volume **2** 5000 μM	volume **7** 5000 μM	% DMSO
1	12.5 mL	45.0 μL	x	x	x	x	x	0.36%
2	12.5 mL	37.5 μL	7.5 μL (3 μM)	x	x	x	x	0.36%
3	12.5 mL	30.0 μL	x	x	7.5 μL (3 μM)	x	7.5 μL (3 μM)	0.36%
4	12.5 mL	22.5 μL	x	22.5 μL (9 μM)	x	x	x	0.36%
5	12.5 mL	x	x	x	x	22.5 μL (9 μM)	22.5 μL (9 μM)	0.36%

The cells were incubated for 90 min at 37 ºC under an atmosphere of 5% CO_2. The medium (including any detached cells) from each sample was transferred to a 50-mL centrifuge tube. The cells were rinsed with 10 mL PBS, which was added to the centrifuge tubes. Adhered cells were detached from the culture flask by trypsinization (10 min, 37 ºC, 5 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh medium (10 mL) was added and the resulting suspension was added to the centrifuge tubes, along with a 5-mL PBS rinse.

The samples were centrifuged (10 min at 183 x g), and the supernatant was discarded. The cells were resuspended in 1 mL of PBS, the suspension was transferred to a 1.5-mL centrifuge tube, and the cells were again pelleted by centrifugation (5 min at 500 x g). The supernatant was discarded, and the cells were washed twice with 1 mL of PBS.

The washed cells were cooled on ice, then were lysed by addition of 500 μL per sample ice-cold RIPA buffer (see above for formulation). The samples were mixed end-over-end for 1 h at 4 ºC with occasional vortexing, then 500 μL per sample Tris buffer was added. The samples were centrifuged (12000 x g, 10 min, 4 ºC), and insoluble material was removed with a pipette tip. The lysates were transferred to fresh 1.5-mL centrifuge tubes.

2. Affinity-Isolation of Bound Proteins.

Each individual sample lysate from section 1 was treated with 50 μL of washed, well-suspended, two-fold diluted Sepharose resin (see above for resin preparation). The resulting slurry was mixed for 6 h at 4 ºC, then was centrifuged (12000 x g, 2 min, 4 ºC). The supernatant was transferred to a clean 1.5-mL centrifuge tube. The protein concentration in each lysate was analyzed by the Bradford method,[8] and found to be consistent across all samples, within experimental error.

Each sample was treated with two 30-μL aliquots of washed, well-suspended, two-fold diluted streptavidin-agarose resin (see above for resin preparation). The resulting slurry was mixed for 15 h at 4 ºC, then was centrifuged (12000 x g, 10 min, 4 ºC). The supernatant was discarded.

The collected resins were washed with wash buffer at 4 ºC, then with tris buffer at 4 ºC, then twice with tris buffer at 23 ºC. Each wash consisted of 10 min mixing, followed by 10 min centrifugation (either 12000 x g at 4 ºC, or 10000 x g at 23 ºC). See above for solution preparation.

The washed resin was suspended in Laemmli loading buffer (Sigma, 2X concentration, 70 μL per sample), and the samples were heated to 95 ºC for 6 min.

3. Western-Blot Detection of Nucleophosmin.

A tris-glycine mini gel (4 – 20%, 12-well) was loaded with 15 μL per lane of the denatured protein mixture from section 2. One lane was loaded with 8 μL of Benchmark prestained protein ladder

(Invitrogen). The protein samples were electroeluted (1 h, 23 ºC, 150 V), then transferred under semi-dry conditions to a nitrocellulose membrane (100 mA, 23 ºC, 12 h).

The membrane was blocked for 1 h (40 mL 3% low-fat milk in TBS buffer with 0.1% tween-20), then rinsed (two ten min washes with TBS buffer containing 0.1% tween-20), and treated 1 h with primary antibody solution (20 mL of 1% low-fat milk in TBS buffer with 0.1% tween-20, containing 10 μg of mouse anti-B23 antibody). The membrane was rinsed again (two 10-min washes with 40 mL TBS buffer containing 0.1% tween-20) and treated with secondary antibody solution (20 mL of 1% low-fat milk in TBS buffer with 0.1% tween-20, containing 20 μg of goat anti-mouse-HRP conjugate). The membrane was rinsed once more (three 10-min washes with 40 mL TBS buffer containing 0.1% tween-20) and treated with 6 mL of a 1:1 mixture of stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western-blot of Figure 3A.

Affinity-Isolation Experiments from Incubations with Cell Lysates.

1. Preparation of Whole Cell Lysate.

T-47D cells were grown to approximately 90% confluence in 9 T-150 tissue culture flasks. The medium was discarded, and the cells were washed with PBS (10 mL per flask). The cells were harvested by trypsinization (10 min, 37 ºC, 8 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh cell-culture medium (16 mL) was added to each flask, and the suspension was transferred to 50-mL centrifuge tubes. The cells were pelletted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellets were resuspended in PBS (10 mL) and transferred to 15-mL centrifuge tubes. The cells were pelletted once again by centrifugation (10 min at 183 x g), then were washed twice with 5 mL PBS.

Packed cells (1.5 mL) were cooled on ice. Ice-cold RIPA buffer (5 mL, see above for formulation) was added, and the mixture was rotated end-over-end for 1 h at 4 ºC. Tris buffer (5 mL) was added, and the lysate was centrifuged (12000 x g, 10 min, 4 ºC). Insoluble material was removed with a pipette tip, and the remaining lysate was transferred to a clean 15-mL centrifuge tube. A 750-μL aliquot of washed, well-suspended, two-fold diluted streptavidin-agarose resin (see above for resin preparation) was added, and the resulting slurry was mixed for 5 h at 4 ºC, then was centrifuged (12000 x g, 10 min, 4 ºC). The supernatant lysate was carefully removed, briefly mixed, and partitioned into ten 1-mL aliquots, which were flash-frozen in liquid N_2 and stored at –80 ºC prior to use. The lysate contained 7.6 mg/mL total protein (Bradford method[8]).

2. Preparation of Nuclear-Enriched Lysate.

T-47D cells were grown to approximately 90% confluence in 11 T-150 tissue culture flasks. The medium was discarded, and the cells were washed with PBS (10 mL per flask), then harvested by trypsinization (10 min, 37 ºC, 8 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh cell-culture medium (16 mL) was added to each flask, and the resulting suspension was transferred to 50-mL centrifuge tubes. The cells were pelletted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellets were resuspended in PBS (10 mL) and transferred to a 15-mL centrifuge tubes. The cells were pelletted once again by centrifugation (10 min at 183 x g), then were washed twice with 5 mL PBS.

Packed cells (2.1 mL) were cooled on ice. Ice-cold sucrose-hypotonic buffer (5 mL, see above for formulation) was added. The suspension was mixed for 1 min on ice, then was centrifuged (6800 x g, 3 min, 4 ºC). The supernatant (cytosolic lysate) was removed, and the remaining pellet was washed twice with 4 mL PBS, then was lysed by the addition of 6 mL RIPA buffer (see above for formulation). The suspension was mixed end-over-end for 1 h at 4 ºC, then was diluted with 6 mL tris buffer and centrifuged (12000 x g, 10 min, 4 ºC). Insoluble material was removed using a pipette tip, and the remaining nuclear-enriched lysate was carefully removed, briefly mixed, and partitioned into ten 1-mL aliquots, which were flash-frozen in liquid N_2 and stored at –80 ºC prior to use. The lysate contained 6.2 mg/mL total protein (Bradford method[8]).

3. Titration of Probe **5**–Nucleophosmin Binding.

A 1-mL aliquot of T-47D whole cell lysate was thawed at 4 ºC and diluted with 4 mL wash buffer, to afford a working lysate of 1.5 mg/mL total protein. This was partitioned into 1.5-mL centrifuge tubes, and treated (on ice, in the dark) with DMSO and solutions of **5** (prepared by serial dilution from an initial 5 mM stock in DMSO) as indicated:

sample:	volume lysate	volume DMSO	volume **5** 5 μM	volume **5** 50 μM	volume **5** 500 μM	final volume	% DMSO
1	384 μL	16 μL	x	x	x	400 μL	4%
2	384 μL	8 μL	8 μL (100 nM)	x	x	400 μL	4%
3	384 μL	12 μL	x	4 μL (500 nM)	x	400 μL	4%
4	384 μL	8 μL	x	8 μL (1 μM)	x	400 μL	4%
5	384 μL	8 μL	x	x	8 μL (10 μM)	400 μL	4%

The samples were mixed end-over-end in the dark for 4 h at 4 ºC. Each sample was treated with two 30-μL aliquots of washed, well-suspended, two-fold diluted streptavidin-agarose resin (see above for resin preparation). The resulting slurry was mixed for 15 h at 4 ºC, then was centrifuged (12000 x g, 10 min, 4 ºC). The supernatant was discarded.

The collected resins were washed with wash buffer at 4 ºC, then with tris buffer at 4 ºC, then twice with tris buffer at 23 ºC. Each wash consisted of 10 min mixing, followed by 10 min centrifugation (either 12000 x g at 4 ºC, or 10000 x g at 23 ºC). See above for solution preparation.

The washed resin was suspended in Laemmli loading buffer (Sigma, 2X concentration, 90 μL per sample), and the samples were heated to 95 ºC for 6 min.

A tris-glycine mini gel (4 – 20%, 12-well) was loaded with 15 μL per lane of the denatured protein mixture. One lane was loaded with 8 μL of Benchmark prestained protein ladder (Invitrogen). The protein samples were electroeluted (1 h, 23 ºC, 150 V), then transferred under semi-dry conditions to a nitrocellulose membrane (100 mA, 23 ºC, 12 h).

The membrane was blocked for 1 h (40 mL 3% low-fat milk in TBS buffer with 0.1% tween-20), then rinsed (two 10-min washes with TBS buffer containing 0.1% tween-20), and treated 1 h with primary antibody solution (20 mL of 1% low-fat milk in TBS buffer with 0.1% tween-20, containing 10 μg of mouse anti-B23 antibody). The membrane was rinsed again (two 10-min washes with 40 mL TBS buffer containing 0.1% tween-20) and treated with secondary antibody solution (20 mL of 1% low-fat milk in TBS buffer with 0.1% tween-20, containing 20 μg of goat anti-mouse-HRP conjugate). The membrane was rinsed once more (three 10-min washes with 40 mL TBS buffer containing 0.1% tween-20) and treated with 6 mL of a 1:1 mixture of stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western-blot of Figure 3B.

4. Competitive Binding Affinity-Isolation Experiments.

Aliquots of T-47D whole cell and nuclear-enriched lysates were thawed at 4 ºC and diluted with wash buffer to provide working lysates of 1.5 mg/mL total protein. These were partitioned into 1.5-mL centrifuge tubes, and treated (on ice, in the dark) with DMSO and solutions of **5**, **1**, *ent*-**1** and **2**, as indicated:

sample:	volume lysate	volume DMSO	volume **5** 500 μM	volume **1** 5 mM	volume *ent*-**1** 5 mM	volume **2** 5 mM	final volume	% DMSO
1 A	nuclear 384 μL	8 μL	8 μL (10 μM)	x	x	x	400 μL	4 %
2 A	nuclear 384 μL	0 μL	8 μL (10 μM)	8 μL (100 μM)	x	x	400 μL	4 %
3 A	nuclear 384 μL	0 μL	8 μL (10 μM)	x	8 μL (100 μM)	x	400 μL	4 %
4 A	nuclear 384 μL	0 μL	8 μL (10 μM)	x	x	8 μL (100 μM)	400 μL	4 %
1 B	whole cell 384 μL	8 μL	8 μL (10 μM)	x	x	x	400 μL	4 %
2 B	whole cell 384 μL	0 μL	8 μL (10 μM)	8 μL (100 μM)	x	x	400 μL	4 %
3 B	whole cell 384 μL	0 μL	8 μL (10 μM)	x	8 μL (100 μM)	x	400 μL	4 %
4 B	whole cell 384 μL	0 μL	8 μL (10 μM)	x	x	8 μL (100 μM)	400 μL	4 %

The samples were mixed end-over-end in the dark for 4 h at 4 ºC. Each sample was treated with two 30-μL aliquots of washed, well-suspended, two-fold diluted streptavidin-agarose resin (see above). The resulting slurry was mixed for 15 h at 4 ºC, then was centrifuged (12000 x g, 10 min, 4 ºC). The supernatant was discarded.

The collected resins were washed with wash buffer at 4 ºC, then with tris buffer at 4 ºC, then twice with tris buffer at 23 ºC. Each wash consisted of 10 min mixing, followed by 10 min centrifugation (either 12000 x g at 4 ºC, or 10000 x g at 23 ºC). See above for solution preparation.

The washed resin was suspended in Laemmli loading buffer (Sigma, 2X concentration, 90 μL per sample), and the samples were heated to 95 ºC for 6 min.

A tris-glycine mini gel (4 – 20%, 12-well) was loaded with 15 μL per lane of the denatured protein mixture. One lane was loaded with 8 μL of Benchmark prestained protein ladder (Invitrogen). The protein samples were electroeluted (1 h, 23 ºC, 150 V), then transferred under semi-dry conditions to a nitrocellulose membrane (100 mA, 23 ºC, 12 h).

The membrane was blocked for 1 h (40 mL 3% low-fat milk in TBS buffer with 0.1% tween-20), then rinsed (two 10-min washes with TBS buffer containing 0.1% tween-20), and treated 1 h with primary antibody solution (20 mL of 1% low-fat milk in TBS buffer with 0.1% tween-20, containing 10 μg of

mouse anti-B23 antibody). The membrane was rinsed again (two 10-min washes with 40 mL TBS buffer containing 0.1% tween-20) and treated with secondary antibody solution (20 mL of 1% low-fat milk in TBS buffer with 0.1% tween-20, containing 20 μg of goat anti-mouse-HRP conjugate). The membrane was rinsed once more (three 10-min washes with 40 mL TBS buffer containing 0.1% tween-20) and treated with 6 mL of a 1:1 mixture of stabilized peroxide solution:enhanced luminol solution (Pierce; WestPico Chemiluminescent Substrate kit) for 3 min. Finally, the membrane was sealed in plastic wrap and exposed to X-ray film to provide the Western-blot of Figure 3C.

Western-blot detection of exportin-1 (XPO1) and peroxiredoxin 1 (PRX1) showed that all three inhibitors (**1**, *ent*-**1** and **2**) were capable of blocking the binding of probe **5** to these proteins whereas the three inhibitors exhibited differential blocking of the binding of probe **5** to nucleophosmin, with the natural product **1** being most effective (Figure S3).

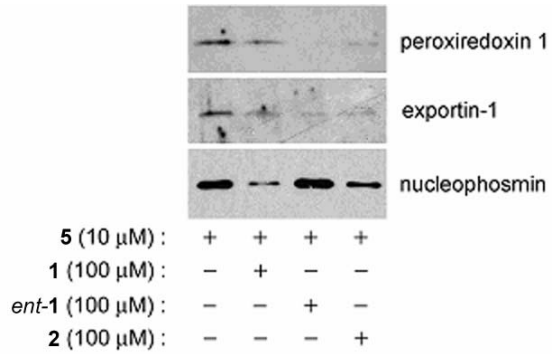


Figure S3. Western-blot detection of peroxiredoxin 1, exportin-1 and nucleophosmin following affinity-isolation experiments in whole-cell lysate.

5. Affinity-Isolation Experiments following Co-Incubation with Iodoacetamide.

Identical affinity-isolation experiments to those described in the previous section were performed, except that iodoacetamide (8 μL of a freshly prepared 500 mM solution in DMSO) was added to one sample:

sample:	volume lysate	volume DMSO	volume **5** 500 μM		volume iodoacetamide 500 mM		final volume	% DMSO
1	whole cell 384 μL	8 μL	8 μL	(10 μM)	x		400 μL	4 %
2	whole cell 384 μL	0 μL	8 μL	(10 μM)	8 μL	(10 mM)	400 μL	4 %

Western-blot detection (as described above) revealed a reduction in affinity-isolated nucleophosmin for the sample treated with iodoacetamide.

Site-Directed Mutagenesis Experiments.

1. Preparation of Mutant Sequences.

An *E. coli* DH10B clone carrying a pCMV-SPORT6 vector (including an ampicillin resistance gene) containing a *c*DNA that encodes for NPM1.3 was purchased from Open Biosystems (clone 3877633, catalogue number MHS1010-73718). A clone was streaked onto ampicillin-treated agar plates and incubated overnight at 37 ºC. The following day, individual colonies were selected and amplified overnight in 5 mL of ampicillin-containing broth. Plasmid DNA was isolated from individual colonies using the QIAGEN miniprep kit.

Cysteine → alanine mutations were carried out using the QuikChange Site-Directed Mutagenesis Kit (Invitrogen), following the manufacturer's directions. The following primers were used to effect the desired mutations:

$Cys^{21} \rightarrow Ala^{21}$: Forward primer:

5'-GCCCCAGAACTATCTTTTCGGTGCTGAACTAAAGGCCGAC-3'

Reverse primer:

5'-GTCGGCCTTTAGTTCAGCACCGAAAAGATAGTTCTGGGGC-3'

$Cys^{104} \rightarrow Ala^{104}$: Forward primer:

5'-TGGTCTTAAGGTTGAAGGCTGGTTCAGGGCCAGTGC-3'

Reverse primer:

5'-GCACTGGCCCTGAACCAGCCTTCAACCTTAAGACCA-3'

$Cys^{275} \rightarrow Ala^{275}$: Forward primer:

5'-AAGCCAAATTCATCAATTATGTGAAGAATGCCTTCCGGATGACTGAC-3'

Reverse primer:

5'-GTCAGTCATCCGGAAGGCATTCTTCACATAATTGATGAATTTGGCTT-3'

After codon exchange, the modified DNA was used to transform TOP10 chemically competent *E. coli* (Invitrogen) following the manufacturer's directions. The cells were plated on an ampicillin-treated agar plate and incubated overnight at 37 ºC. The following day, individual colonies were collected and amplified overnight in 5 mL of ampicillin-containing broth. Plasmid DNA was isolated (using the QIAGEN miniprep kit) and submitted for sequencing (Genewiz; forward primer = CACCATGGAAGATTCGATGGACATGG, reverse primer = TTAAAGAGACTTCCTCCACTGCC).

Colonies expressing the desired plasmids were grown for 20 h at 37 ºC, in 50 mL of broth containing 100 μg/mL ampicillin. The following day, plasmid DNA was isolated (using the QIAGEN midiprep kit), quantified and sequenced (Genewiz).

2. Transformation of COS-7 Cells.

COS-7 cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded, the cell pellet was resuspended in fresh medium, and the concentration of the resulting suspension was determined using a hemacytometer.

Cell culture flasks (75 cm^2) were charged with 12 mL of a 3 x 10^5 cells/mL suspension, and incubated overnight at 37 ºC under an atmosphere of 5% CO_2.

The following day, Lipofectamine 2000 (480 μL) was added to Opti-MEM reduced serum medium (3520 μL). Plasmid DNA (15 μg in QIAGEN extraction buffer) was added to Opti-MEM (to a final volume of 500 μL) for each sample (A: no DNA; B: NPM1.3; C: NPM1.3c^{21}-a; D: NPM1.3c^{104}-a; E: NPM1.3c^{275}-a). A 500-μL aliquot of the diluted Lipofectamine solution was added to each sample, and the resulting transfection complex solutions were incubated for 10 min at 23 ºC, then were diluted with 5 mL of Opti-MEM.

The medium was removed from the growing cells and replaced with the prepared transfection complex solutions. The samples were incubated at 37 ºC, under an atmosphere of 5% CO_2, for 5 h. The supernatant was removed from the adhered cells, and replaced with 12 mL of fresh serum-containing media. The samples were returned to incubation (37 ºC, 5% CO_2) for 60 h. The medium (including any detached cells) from each sample was transferred to a 50-mL centrifuge tube. The cells were rinsed with 10 mL PBS, which was added to centrifuge tubes. Adhered cells were detached from the culture flask by trypsinization (10 min, 37 ºC, 5 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh medium (10 mL) was added and the resulting suspension was added to the centrifuge tubes, along with a 5-mL PBS rinse.

The samples were centrifuged (10 min at 183 x g), and the supernatant was discarded. The cells were resuspended in 1 mL of PBS, transferred to a 1.5-mL centrifuge tube, and centrifuged again (5 min at 500 x g). The supernatant was discarded, and the cells were washed twice with 1 mL of PBS.

The washed cells were cooled on ice, then lysed by addition of 500 μL per sample ice-cold RIPA buffer (see above for formulation). The samples were mixed end-over-end for 1 h at 4 ºC with occasional vortexing, then 500 μL per sample Tris buffer was added. The samples were centrifuged (12000 x g, 10 min, 4 ºC), and insoluble material was removed with a pipette tip. The lysates were transferred to fresh 1.5-mL centrifuge tubes. A 50-μL aliquot of washed, twice-diluted streptavidin-agarose resin (see above for wash conditions) was added to each sample, and the resulting slurry was rotated end-over-end for 15 h at 4 ºC. The samples were centrifuged (12000 x g, 10 min, 4 ºC), and the protein concentration in the supernatants was measured (Bradford method[8]).

An aliquot from each supernatant was diluted with wash buffer to provide individual 397-µl samples, each containing 2 mg/mL total protein. These were mixed, then 5 µL was removed from each sample and added to Laemmli loading buffer (Sigma, 2X concentration, 45 µL per sample). The resulting solutions were heated to 95 ºC for 6 min, then were further diluted 5-fold with Laemmli loading buffer. A tris-glycine mini gel (12%, 12-well) was loaded with 15 µL per well of the diluted denatured protein mixture. The protein samples were electroeluted (150 V, 23 ºC, 90 min) and transferred to a nitrocellulose membrane (100 mA, 23 ºC, 12 h). Nucleophosmin (both native NPM1.1 and expressed NPM1.3) was detected by Western-blot using the procedure outlined above.

To the remaining 392-µL lysates, 8-µL aliquots of a 50 µM solution of probe **5** in DMSO were added (on ice, in the dark), to afford a final concentration of 1 µM probe **5**, in each of the five 400-µL samples. The samples were mixed end-over-end at 4 ºC for 4 h. Two 30-µL aliquot of washed, twice-diluted streptavidin-agarose resin (see above for wash conditions) was added to each sample, and the resulting slurry was rotated end-over-end for 15 h at 4 ºC.

The collected resins were washed with wash buffer at 4 ºC, then with tris buffer at 4 ºC, then twice with tris buffer at 23 ºC. Each wash consisted of 10 min mixing, followed by 10 min centrifugation (either 12000 x g at 4 ºC, or 10000 x g at 23 ºC). See above for solution preparation.

The washed resin was suspended in Laemmli loading buffer (Sigma, 2X concentration, 50 µL per sample), and the samples were heated to 95 ºC for 6 min. A tris-glycine mini gel (12%, 12-well) was loaded with 15 µL per well of the liberated protein mixture. The protein samples were electroeluted (150 V, 23 ºC, 90 min) and transferred to a nitrocellulose membrane (100 mA, 23 ºC, 12 h). Nucleophosmin (both native NPM1.1 and expressed NPM1.3) was detected by Western-blot using the procedure outlined above.

The results of the Western-blotting experiments (Figure 5, text) suggest that cysteine-275 of nucleophosmin is required for binding to probe **5**.

Transfection / Apoptosis Experiments

HeLa S3 cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellet was resuspended in fresh medium. The concentration of the cell suspension was determined using a hemacytometer, and a suspension of 1 x 10^5 cells/mL was prepared.

siPORT NeoFX (100 µL) was added to Opti-MEM reduced serum medium (1900 µL). A siRNA targeting NPM1.1 (Applied Biosystems, Cat. No. AM16708; ID 143640; 11.4 µL from a 50 µM stock solution) was added to Opti-MEM (938.6 µL). At the same time, a control siRNA (Applied Biosystems, Cat. No. AM4611; 11.4 µL from a 50 µM stock) was similarly added to Opti-MEM (938.6 µL). A 950-µL aliquot of the diluted NeoFX solution was added to each sample, and the resulting transfection complex solutions were incubated for 10 min at 23 ºC.

Cell culture flasks (75 cm^2) were charged with 1.8 mL of the prepared transfection complex solution, followed by 16.2 mL of the HeLa S3 cell suspension (at 1 x 10^5 cells/mL). The samples were incubated for 2 d at 37 ºC, under an atmosphere of 5% CO_2. At the end of this period, the cells (which had reached ~90% confluence) were stripped of media, rinsed with trypsin buffer, then detached from the culture flasks by trypsinization (5 min, 37 ºC, 5 mL per flask, 0.05% trypsin, 0.53 mM EDTA). Fresh medium (10 mL) was added and the resulting suspensions were transferred quantitatively to 50-mL centrifuge tubes. The culture flasks were rinsed with an additional 5 mL medium, which was likewise added to the centrifuge tubes.

The samples were centrifuged (10 min at 183 x g). The supernatant was discarded, and the cells were resuspended in 30 mL per sample of fresh medium. The concentration of the cell suspensions was determined using a hemacytometer. Over the course of the 2 d transfection period, both the transfected and mock-transfected cells grew ~4-fold. No statistically significant difference in growth rate was observed for the two populations of cells in this experiment, or in several related experiments, using various means of measurement (counting by hemacytometer, assaying cell viability with CellTiter-Blue, and quantifying total protein in lysed cells).

12-well plates were charged with 3 mL per well of suspensions of the transfected or mock-transfected cells, at 2.5 x 10^4 cells per mL. The samples were incubated overnight at 37 ºC, under an atmosphere of 5% CO_2. The following day, solutions of cell culture medium containing (+)-avrainvillamide or vehicle control were prepared. 500-μL aliquots of these solutions were added to the 3-mL samples. The treated samples were returned to the incubator (37 ºC, 5% CO_2) for 24 h.

The medium (containing any detached cells) from each sample was transferred to a 15-mL centrifuge tube. The cells were rinsed with 1 mL PBS, which was added to the centrifuge tubes. Adhered cells were detached from the 12-well plates by trypsinization (5 min, 37 ºC, 300 μL per sample, 0.05% trypsin, 0.53 mM EDTA). The trypsin was quenched by the addition of 1 mL fresh medium, and the resulting suspension was added to the centrifuge tubes, along with a 1 mL rinse (PBS, with 1 mM EDTA and 1% BSA).

The samples were centrifuged (10 min at 183 x g), and the supernatant was discarded. The cells from each sample were resuspended in 1 mL PBS (containing 1 mM EDTA and 1% BSA), transferred to a 1.5-mL centrifuge tube, and centrifuged again (5 min at 500 x g). The supernatant was discarded, and the samples were cooled on ice. Apoptosis detection buffer (500 μL; see above for preparation) was added to each sample. The resulting suspensions were mixed and incubated on ice for 1 h, prior to analysis.

Each sample was analyzed on an LSRII flow cytometer, with 20,000 events recorded per sample. Apoptotic cells were defined as those permeable to Yo-Pro iodide, but not to propidium iodide (PI). Viable cells were defined as those permeable to neither die. Compensation controls were set manually, to achieve the greatest distinction between viable and apoptotic cell populations (PI vs. Yo-Pro: 30%; Yo-Pro vs. PI: 2%). The results (Figure 6A, text) indicate that the transfected cells were more susceptible to avrainvillamide-induced apoptosis.

The experiment was carried out three times, with qualitatively similar results each time. Attempts to replicate these results with a second siRNA (Applied Biosystems, Cat. No. AM16708; ID 284660) were unsuccessful; Western-blotting experiments suggest that this siRNA afforded less complete suppression of nucleophosmin (Figure S4).

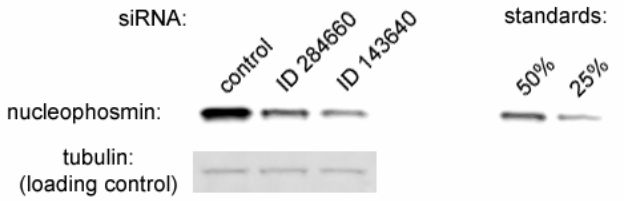


Figure S4. Western-blot detection of nucleophosmin (and tubulin, as a loading control), 2 d after transfection with two commercially available siRNA reagents (Applied Biosystems, Cat. No. AM16708) or a control siRNA (Applied Biosystems, Cat. No. AM4611). Knockdown was estimated at ~50% for ID 284660 and ~75% for ID 143640.

p53 / Nucleophosmin Changes with (+)-Avrainvillamide Incubation.

1. Treatment of cells with (+)-Avrainvillamide.

LNCaP and T-47D cells were grown to approximately 80% confluence, then were trypsinized, collected, and pelleted by centrifugation (10 min at 183 x g). The supernatant was discarded, and the cell pellets were resuspended in fresh medium. The cell concentration in the resulting suspension was determined using a hemacytometer.

Four 6-well plates (two for each cell line) were charged with 6 mL per well of cell suspension at 2 x 10^5 cells/mL. The cells were incubated overnight at 37 ºC, under an atmosphere of 5% CO_2. The following day, stock solutions of (+)-avrainvillamide (**1**) in fresh cell culture medium were prepared as indicated below:

sample :	1	2	3	4	5
DMSO :	22.32 µL	19.53 µL	16.74 µL	11.16 µL	x
volume (+)-avrainvillamide (**1**) : (5 mM in DMSO)	x	2.79 µL	5.58 µL	11.16 µL	22.32 µL
Medium :	877.68 µL	877.68 µL	877.68 µL	877.68 µL	877.68 µL
[**1**] x $^{200}/_{6200}$:	x	0.5 µM	1 µM	2 µM	4 µM
[DMSO] x $^{200}/_{6200}$:	0.08%	0.08%	0.08%	0.08%	0.08%

To each 6-mL sample, a 200-μL aliquot of the appropriate stock solution was added, resulting in a final concentration of 0 – 4 μM (+)-avrainvillamide (**1**). The samples were returned to the incubator (37 ºC, 5% CO_2) for 24 h.

The following day, the medium (containing any detached cells) from each sample was transferred to a 15-mL centrifuge tube. The cells were rinsed with 1 mL PBS, which was added to the centrifuge tubes. Adhered cells were detached from the 12-well plates by trypsinization (5 min, 37 ºC, 500 μL per sample, 0.05% trypsin, 0.53 mM EDTA). Fresh medium (1 mL) was added and the resulting suspension was added to the centrifuge tubes, along with a 2-mL rinse with PBS.

The samples were centrifuged (10 min at 183 x g), and the supernatant was discarded. The cells from each duplicate sample were combined (such that each sample contained the cells from two wells of a 6-well plate), then were resuspended in 1 mL PBS and transferred to a 1.5-mL centrifuge tube and centrifuged again (5 min at 500 x g). The supernatant was discarded, and the cells were washed with 1 mL PBS. The cells were resuspended in 1 mL PBS and mixed thoroughly. A 500-μL aliquot from each sample was transferred to a fresh 1.5-mL centrifuge tube. All the samples were centrifuged (5 min at 500 x g) and the supernatant was discarded. The resulting 20 samples (10 samples of T-47D cells, treated with 0 – 4 μM (+)-avrainvillamide, and 10 samples of LNCaP cells, treated with 0 – 4 μM (+)-avrainvillamide (**1**), where each sample contained the number of cells from 1 well of a 6-well plate) were separated into two groups. One group of samples was lysed in RIPA buffer (see below) to prepare a series of whole-cell lysates. The other group of samples was first treated with sucrose-hypotonic buffer to prepare a series of cytosolic lysates. The remaining pellets were washed and treated with RIPA buffer to prepare a series of nuclear-enriched lysates (see below).

2. Preparation and Analysis of Whole-Cell Lysates.

From the samples prepared in section 1, five samples of T-47D cells and five samples of LNCaP cells (each treated with 0 – 4 μM (+)-avrainvillamide) were cooled on ice, treated for 1 h with ice-cold RIPA buffer (100 μL, see above for formulation), then centrifuged (12000 x g, 10 min, 4 ºC). The protein concentration in each lysate was quantified (Bradford method;[8] samples and standards were measured in triplicate), and the lysates were mixed 1:1 with Laemmli loading buffer (Sigma, 2X concentration). The resulting samples were heated to 95 ºC for 6 min, then were cooled and loaded onto tris-glycine mini gels (4 – 20%, 12-well) at 16 μg per well. The protein samples were electroeluted (1 h, 23 ºC, 150 V), then transferred under semi-dry conditions to nitrocellulose membranes (100 mA, 23 ºC, 12 h). The membranes were subjected to Western-blotting conditions for the detection of nucleophosmin, p53 and 14-3-3b (as a loading control), using an identical procedure to that described above.

3. Preparation and Analysis of Cytosolic and Nuclear-Enriched Lysates.

From the samples prepared in section 1, five samples of T-47D cells and five samples of LNCaP cells (each treated with 0 – 4 μM (+)-avrainvillamide) were cooled on ice, and treated for 1 min with ice-cold sucrose-hypotonic buffer (50 μL, see above for formulation). The samples were vortexed and centrifuged (6800 x g, 3 min, 4 ºC). The supernatants (cytosolic lysates) were carefully transferred to fresh 1.5-mL centrifuge tubes. The remaining pellets were washed twice (on ice) twice with 500 μL PBS. The washed pellets were lysed by addition of ice-cold RIPA buffer (50 μL, see above for formulation). The resulting nuclear-enriched lysates were incubated 1 h at 4 ºC, then centrifuged (12000 x g, 10 min, 4 ºC).

The protein concentration in each lysate (both cytosolic and nuclear-enriched) was quantified (Bradford method;[8] samples and standards were measured in triplicate), and the lysates were mixed 1:1 with Laemmli loading buffer (Sigma, 2X concentration). The resulting samples were heated to 95 ºC for 6 min, then were cooled and loaded onto tris-glycine mini gels (4 – 20%, 12-well) at 16 μg per well. The protein samples were electroeluted (1 h, 23 ºC, 150 V), then transferred under semi-dry conditions to nitrocellulose membranes (100 mA, 23 ºC, 12 h). The membranes were subjected to Western-blotting conditions for the detection of nucleophosmin, p53 and 14-3-3β (as a loading control), using an identical procedure to that described above.

The results from these experiments (Figures 6B, text, and S5, below) revealed an increasing concentration of p53 with increasing concentrations of (+)-avrainvillamide (**1**). The increase was observed in both T-47D cells (which have a relatively high concentration of p53 in unmodified cells) and LNCaP cells (which have a lower starting concentration of p53). Following incubation at the highest concentration of (+)-avrainvillamide (**1**), 4 μM, the T-47D cells experienced a reduction in cellular p53, presumably indicating proteasomal destruction of this protein as part of an apoptosis-related mechanism. The total concentration of nucleophosmin did not change, but translocation of nucleophosmin to the cytosol was observed following incubation with 4 μM (+)-avrainvillamide (**1**).

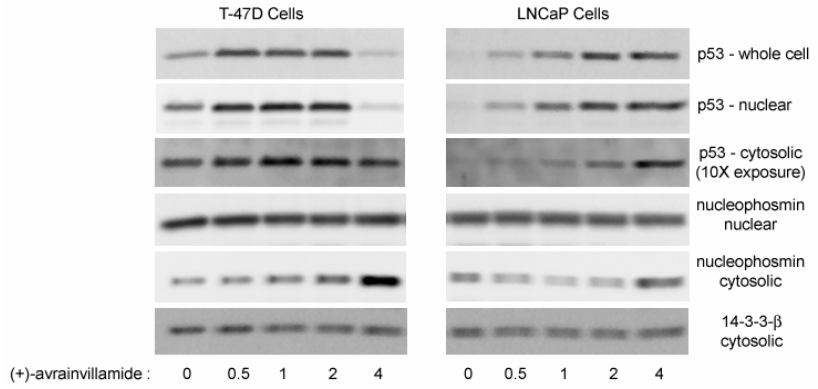


Figure S5. Western-blot detection of p53, nucleophosmin and 14-3-3β (as a loading control) following lysis of cells treated with increasing concentrations of (+)-avrainvillamide (**1**).

C. Complete references

Complete reference 7a

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