Supporting Information

Attaching the NorA Efflux Pump Inhibitor INF55 to Methylene Blue Enhances Antimicrobial Photodynamic Inactivation of Methicillin-Resistant *Staphylococcus aureus in vitro* and in *vivo*

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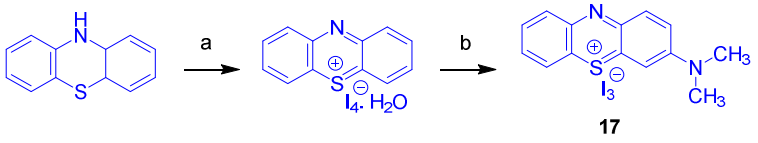
Section 1–Chemistry

Synthesis of EPI-MBs **1-16** made use of the versatile coupling reaction reported by Strekowski *et al.*,[1] where secondary amines are reacted with 3-(dimethylamino)phenothiazine-5-ium triiodide salt **17** to create asymmetric MB derivatives. Adapting this chemistry to produce the target EPI-MBs required preparation of Boc-piperazine- and Boc-*N*,*N*'-dimethylethylenediamine-based precursors **18-33** and reacting them with **17** (following Boc-deprotection) in the final step (Supplementary Figure 2).

(a) Improved synthesis of 17

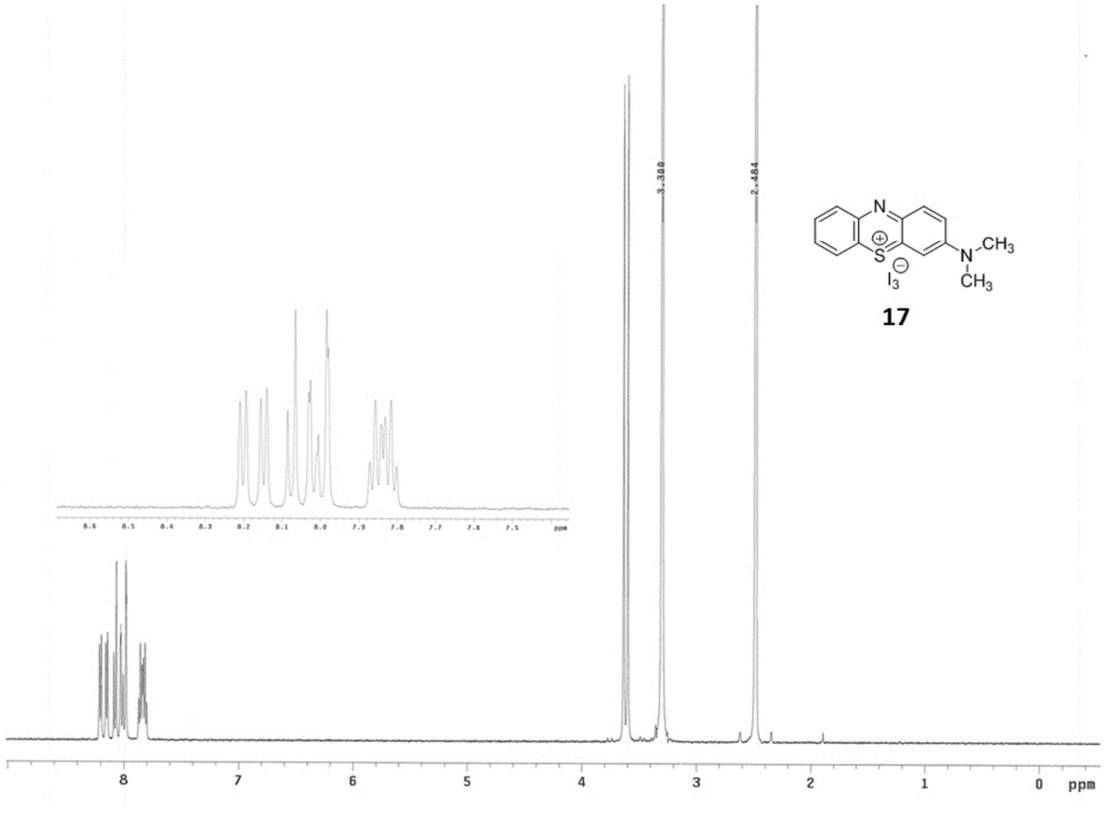
Key intermediate **17** was synthesized by adapting Strekowski's method.[1] In the first step, commercially available phenothiazine was stirred with I_2 in wet chloroform at 5 ^{o}C for 4 hr before allowing the mixture to warm to room temperature. The resulting dark precipitate was washed with copious chloroform until no further yellow washings were obtained. The resulting phenothiazinium tetraiodide salt was obtained in 83% yield after overnight air drying (Supplementary Scheme 1). Reaction of the tetraiodide salt using Strekowski's method[1] with 2 eq of dimethylamine gave **17** in 24% yield. This method involved stirring the salt for 3 hr in MeOH at room temperature. While Strekowski also reported a 24% yield for this reaction, New and Dolphin reported a 48% yield under the same conditions.[2] We found that the yield of **17** could be increased to 60% simply by changing the reaction solvent from MeOH to 15% MeOH in $CHCl_3$, yielding pure **17** after a simple filtration.



17

Supplementary Scheme 1. Improved synthesis of key phenothiazinium intermediate **17**. *Reagents and Conditions*: (a) I_2, $CHCl_3$, rt, 4 hr, 83%; (b) $(CH_3)_2NH$, 15% MeOH in $CHCl_3$, rt, 4 hr, 60%.

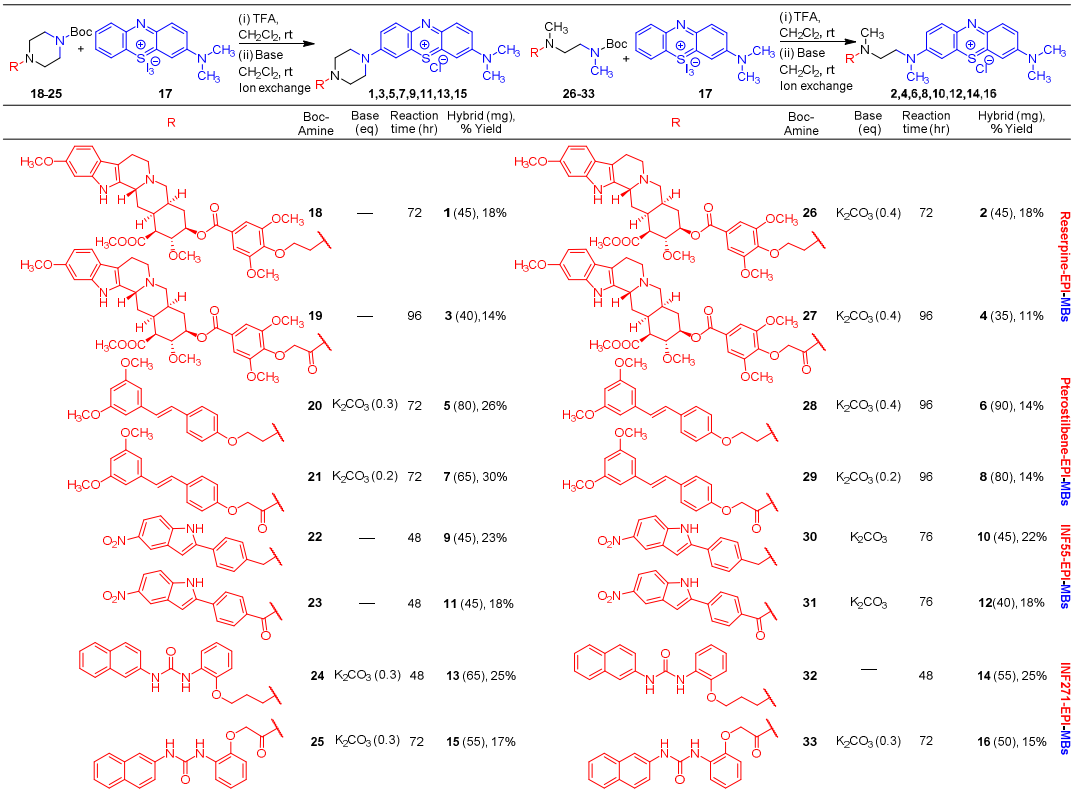
The reaction required preparation of a methanolic solution of $NH(CH_3)_2$ of known concentration. This was achieved by bubbling the gas into MeOH over several minutes and determining the exact molar ratio of $NH(CH_3)_2$:MeOH in the resulting solution by ^{1}H NMR integration. The required volume was then gradually added to a stirring solution of the tetraiodide salt in 15% MeOH/$CHCl_3$ over 4 hr at 23 ^oC. Stirring for a further 1 hr at room temperature gave the desired product as a dark solid. Reaction progress was monitored by TLC (3% $NH_4OAc_{(aq)}$ in MeOH, 15:85). Under these conditions **17** appeared as a dark spot with R_f = 0.43. A clean and sharp ^{1}H NMR spectrum was obtained for **17** in DMSO-d_6 (Supplementary Figure 1), in contrast to the poor spectra obtained in the NMR solvent reported by Strekowski (D_2O).[1]



Supplementary Figure 1. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of 3-(dimethylamino) phenothiazin-5-ium triiodide **17**.

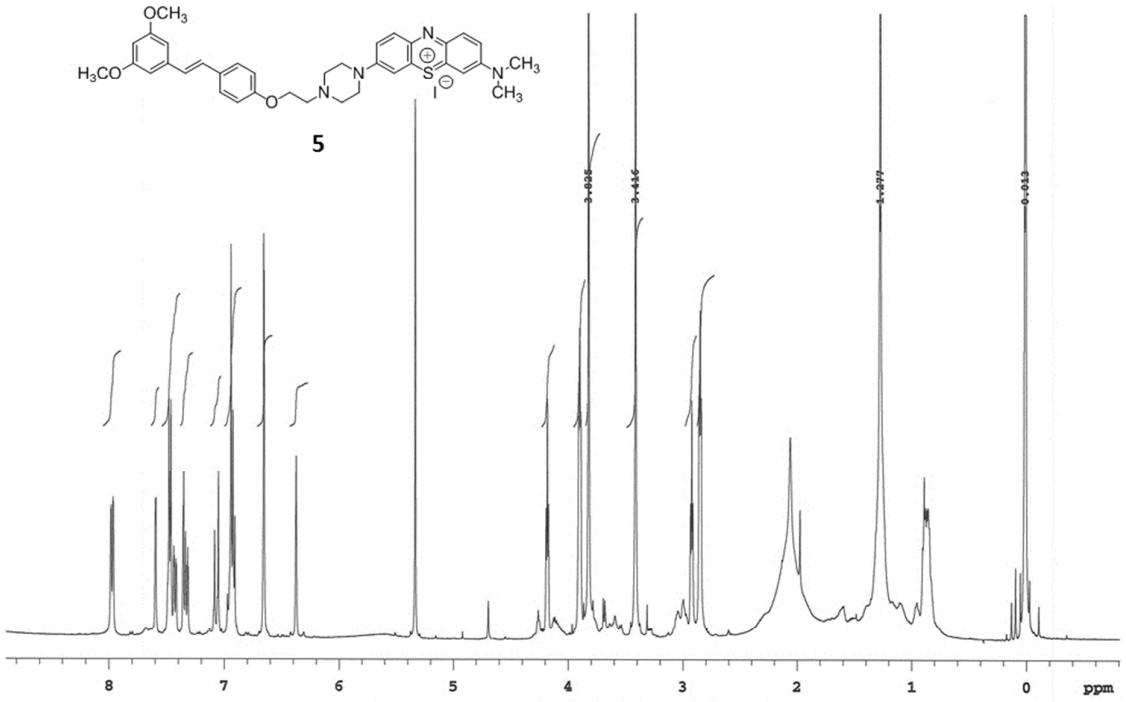
(b) Coupling reactions of Boc-piperazine (18-25)- and Boc-*N*,*N*'-dimethylethylene diamine (26-33)-based precursors with 17.

Synthesis of reserpine-EPI-MBs **1** and **3** (carrying piperazine-based linkers) was accomplished by deprotecting the Boc groups of **18** and **19** with trifluoroacetic acid (TFA) in CH_2Cl_2. Stirring the resulting TFA salts at room temperature with **17** in dry CH_2Cl_2 gave hybrids **1** and **3** as dark blue powders in 18% and 14% yields, respectively, after preparative thin-layer chromatography (TLC) and anion exchange (to chloride salts). Analogous procedures were employed for all other EPI-MBs (Supplementary Figure 2). K_2CO_3 was added where necessary to assist coupling reactions. Individual reaction yields were not optimized as sufficient quantities of all hybrids were obtained for the study using the procedures described. The chemistry used to prepare Boc-protected intermediates **18-33** is detailed below.

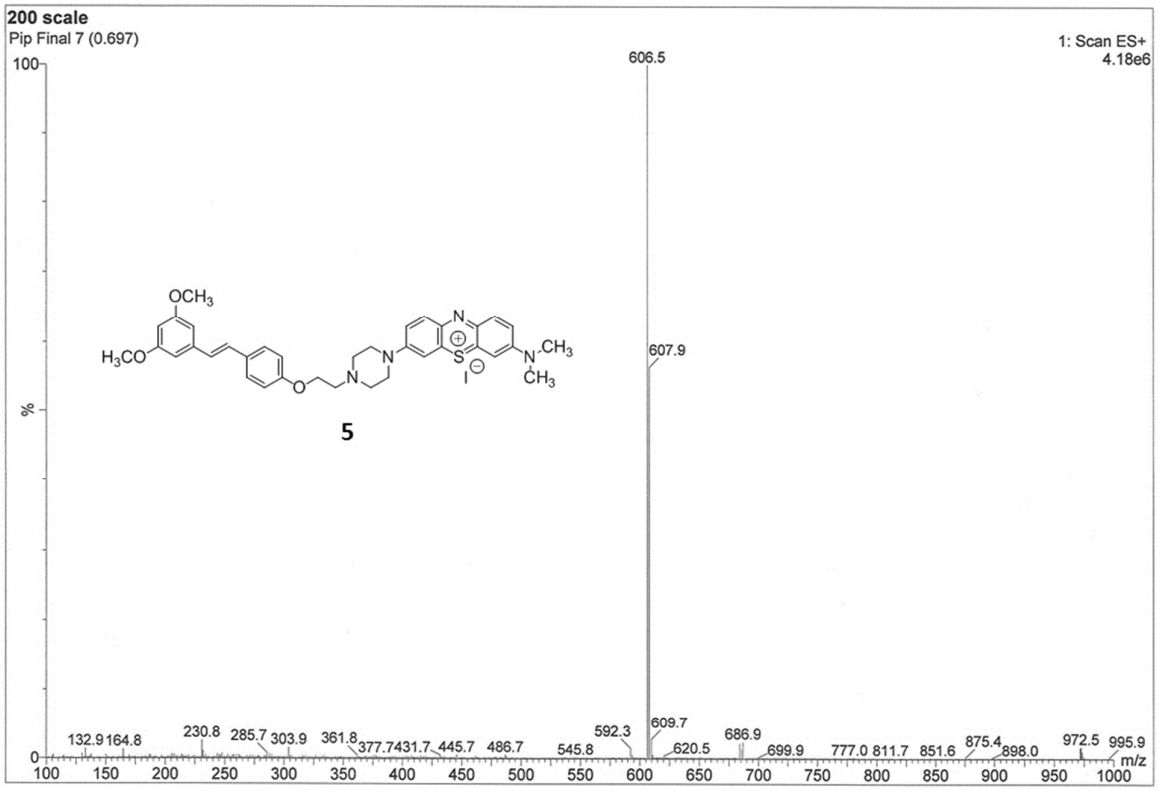


Supplementary Figure 2. Reactions of Boc-proteced intermediates **18-33** with 3-(dimethylamino) phenothiazine-5-ium triiodide salt **17** to provide EPI-MB hybrids **1-16**.

The structures of all hybrids were supported by high resolution electrospray ionisation mass spectrometry (HRESI-MS) data. For example, peaks at *m/z* 945.4208 corresponding to the $C_{52}H_{61}N_6O_9S$ $[M]^+$ ion for **1** and *m/z* 959.4003, corresponding to the $C_{52}H_{59}N_6O_{10}S$ $[M]^+$ for **3**, were observed in their mass spectra. While the ^1H NMR spectrum of phenothiazinum salt **17** was as expected (DMSO-d_6, Supplementary Figure 1), the aromatic regions in the spectra of all EPI-MB hybrids (except **5**) showed substantially broadened and poorly integrating signals for the phenothiazinium aryl protons in all deuterated solvents at a variety of temperatures. The ^{13}C NMR spectra were similarly lacking in phenothiazinium carbon signals. For unknown reasons, hybrid **5** displayed the expected phenothiazinium aryl ^1H NMR signals in CD_2Cl_2 (Supplementary Figure 3). The phenomenon of broadened NMR signals with phenothiazinium salts has been noted previously in the literature.[3, 4] The EPI-MBs (except **5**) were thus unable to be comprehensively characterized by NMR spectroscopy. Nevertheless, all EPI-MBs appeared as single spots by TLC analysis and showed clean ESI-mass spectra supporting their structure and purity, as exemplified for **5** in Supplementary Figure 4.



Supplementary Figure 3. ^{1}H NMR spectrum of **5** (500 MHz, CD_2Cl_2) showing the presence of signals for the phenothiazinium protons.



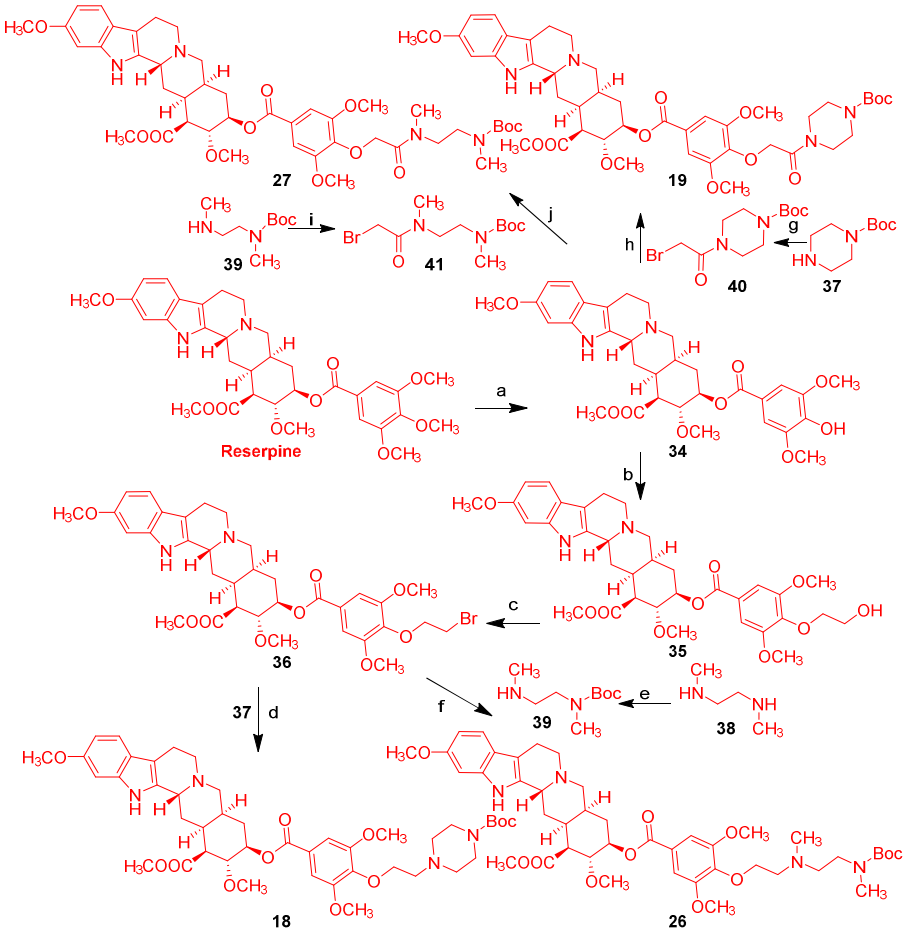
Supplementary Figure 4. Low resolution electrospray ionisation mass spectrum (ESI-MS, +ve ion) of pterostilbene-EPI-MB **5**.

(c) Synthesis of reserpine-EPI-MB intermediates 18, 19, 26 and 27

Selective demethylation of the central methyl ether in reserpine had been reported previously using Lewis acids (BBr_3, $AlCl_3$ and pyridinium chloride Py-HCl) in a variety of solvents, including petroleum ether and EtOAc/HCl.[5] Existence of the monophenolic reserpine derivative **34** suggested that reserpine EPI-MBs would be obtainable by attaching the linker moieties via *O*-alkylation. After testing several reported conditions it was found that $AlCl_3$ in CH_2Cl_2 provided the best yield of monophenolic reserpine **34**. Adding an excess (~ 50 mol eq) of finely ground $AlCl_3$ in one portion to a stirring solution of reserpine in CH_2Cl_2, followed by stirring for an additional 2 hr at room temperature, afforded **34** in 95% (lit. yield 47%)[5]. *O*-alkylation of **34** with 2-bromoethanol was carried out in the presence of K_2CO_3 in DMF with stirring at 80 ^oC overnight. The desired alcohol **35** was obtained as a yellowish solid after silica gel column chromatography in 96% yield. Formation of bromide **35** was achieved using standard Appel conditions with CBr_4/PPh_3 in dry THF, where stirring for 3 hr at room temperature gave **36** in 91% yield. *N*-Alkylation of *N*-Boc-piperazine **37** with bromide **36** in dry THF with Et_3N at reflux provided pure **18** in 80% yield after 24 hr.

Analogous chemistry was used to prepare reserpine-containing intermediate **26** bearing an *N*-alkyl-*N*,*N*'-dimethylethylenediamine linker. *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** was synthesized from the commercially available *N*,*N*'-dimethylethylenediamine **38** using the literature procedure.[6] Briefly, **38** was reacted with Boc_2O in MeOH in the presence of Et_3N and after overnight stirring (monitored by TLC; EtOAc:MeOH:Et_3N, 87:10:3; ninhydrin staining) the product **39** was isolated in 45% yield following silica gel column chromatography. Reaction of **39** with bromide **36** in dry THF in the presence of Et_3N at reflux afforded intermediate **26** in 75%. Similar strategies were applied in the synthesis of intermediates **19** and **27**, which carried amide linkages from the piperazine and *N*,*N*'-dimethylethylenediamine groups to the EPIs. Commercially available *N*-

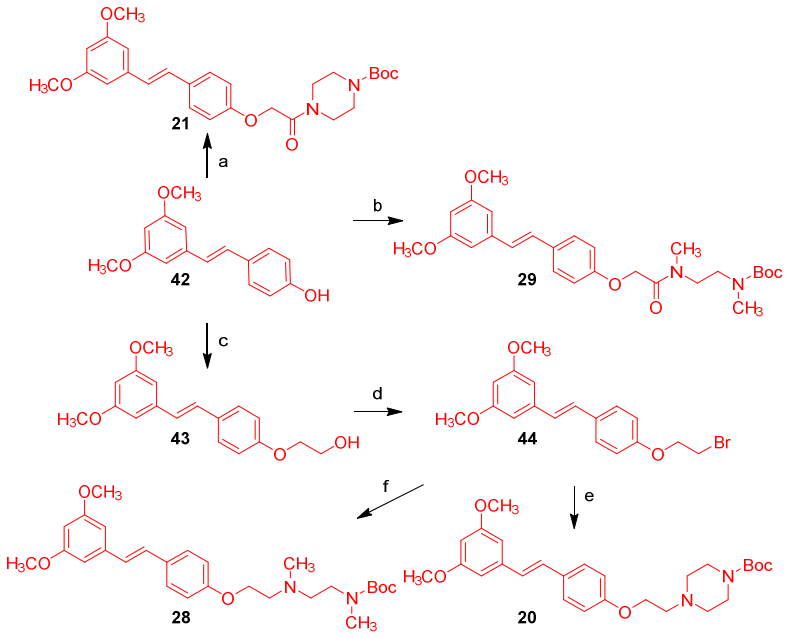
Boc-piperazine **37** and the synthesized *N*-Boc-*N,N'*-dimethylethylenediamine **39** were coupled to bromoacetyl chloride in the presence of *N,N*-diethylaniline to give **40** and **41** in 35% and 40% yields, respectively. The reactions were carried out by stirring in acetone (0 ^{o}C to room temperature) for 16 hr for **40** and 48 hr for **41**. Intermediates **19** and **27** were synthesized from phenolic reserpine derivative **34** by *O*-alkylation with **40** and **41**, respectively. The reactions were carried out using K_2CO_3 or Cs_2CO_3 in DMF at 80 ^{o}C and provided respective yields of 64% and 70% (Supplementary Scheme 2).



Supplementary Scheme 2. Synthesis of reserpine-EPI-MB intermediates **18**, **19**, **26** and **27**. *Reagents and Conditions*: (a) $AlCl_3$, CH_2Cl_2, rt, 2 hr, 95%; (b) 2-bromoethanol, K_2CO_3, DMF, 80 ^{o}C,16 hr, 96%; (c) CBr_4/PPh_3, THF, rt, 3 hr, 91%; (d) **37**, Et_3N, THF, reflux, 24 hr, 80%; (e) Boc_2O, Et_3N/MeOH, 16 hr, 45%; (f) **39**, Et_3N, THF, reflux, 48 hr, 75%; (g) Bromoacetyl chloride, *N,N*-diethylaniline, acetone, 0 ^{o}C → rt, 16 hr, 35%; (h) **40**, K_2CO_3, DMF, 80 ^{o}C, 16 hr, 64%; (i) Bromoacetyl chloride, *N,N*-diethylaniline, acetone, 0 ^{o}C → rt, 48 hr, 40%; (j) **41**, Cs_2CO_3, DMF, 80 ^{o}C, 24 hr 70%.

(d) Synthesis of pterostilbene-EPI-MB intermediates 20, 21, 28 and 29

Pterostilbene-EPI-MB intermediates **21** and **29** were synthesized from the commercially available phenolic pterostilbene derivative **42** by *O*-alkylation reactions using *N*-bromoacetyl-*N*'-Boc-piperazine **40** and *N*-bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylenediamine **41**, respectively. The reactions were carried out using K_2CO_3 in DMF at 80 ^oC and gave identical yields (77%). *O*-Alkylation of **42** with 2-bromoethanol in the presence of K_2CO_3 afforded the phenoxyethyl alcohol **43** in 90% yield after overnight stirring in DMF at 80 ^oC. The Appel reaction was used for bromination of alcohol **43**, where stirring with CBr_4/PPh_3 in THF for 3 hr at room temperature afforded bromide **44** in 94% yield. *N*-alkylation of *N*-Boc-piperazine **37** and *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** with bromide **44** afforded **20** and **28** in 87% and 81% yields, respectively (Supplementary Scheme 3).

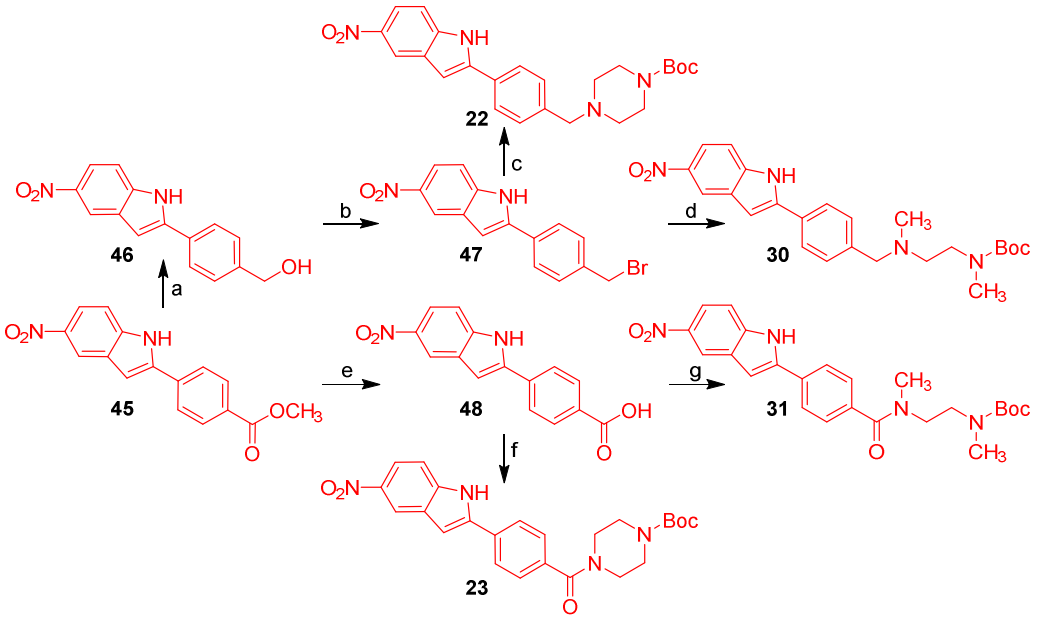


Supplementary Scheme 3. Synthesis of pterostilbene-EPI-MB intermediates **20**, **21**, **28** and **29**. *Reagents and Conditions*: (a) *N*-bromoacetyl-*N*'-Boc-piperazine **40**, K_2CO_3, DMF, 100 ^oC, 48 hr, 77%; (b) *N*-bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylenediamine **41**, K_2CO_3, DMF, 80 ^oC, 24 hr, 77%; (c) 2-bromoethanol, K_2CO_3, DMF, 80 ^oC, 16 hr, 90%; (d) CBr_4/PPh_3, THF, rt, 3 hr, 94%; (e) *N*-Boc-piperazine **37**, KI (40 mol %), THF, reflux, 48 hr, 87%; (f) *N*-Boc-*N*,*N*'-dimethylethylenediamine **39**, K_2CO_3, CH_2Cl_2, reflux, 48 hr, 81%.

(e) Synthesis of INF55-EPI-MB intermediates 22, 23, 30 and 31

INF55 methyl ester derivative **45** was synthesized using the reported method.[7] Ester **45** was quantitatively reduced to the benzylic alcohol **46** using $NaBH_4$ in THF. Bromination of **46** using CBr_4/PPh_3 in Et_2O/THF afforded **47** in 85% yield. Purification was achieved by silica gel column chromatography. Bromide **47** was reacted separately with commercial *N*-Boc-piperazine **37** and *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** in anhydrous THF in the presence of KI. After 2 days at reflux the alkylated products **22** and **30** were isolated in 96% and 78% yields, respectively, after silica gel column chromatography.

In the synthesis of INF55-EPI-MB intermediates **23** and **31** containing amide linkages from the piperazine and *N*,*N*'-dimethylethylenediamine groups to the EPI, INF55 acid derivative **48** was first obtained by hydrolysing **45** with NaOH. The crude acid **48** was coupled to *N*-Boc-piperazine **37** and *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** using HATU and diisopropylethylamine (DIPEA) as base. Stirring the reaction at room temperature in THF for 5 hr and 7 hr gave the Boc-protected intermediates **23** and **31** in 85% and 78% yields, respectively (Supplementary Scheme 4).



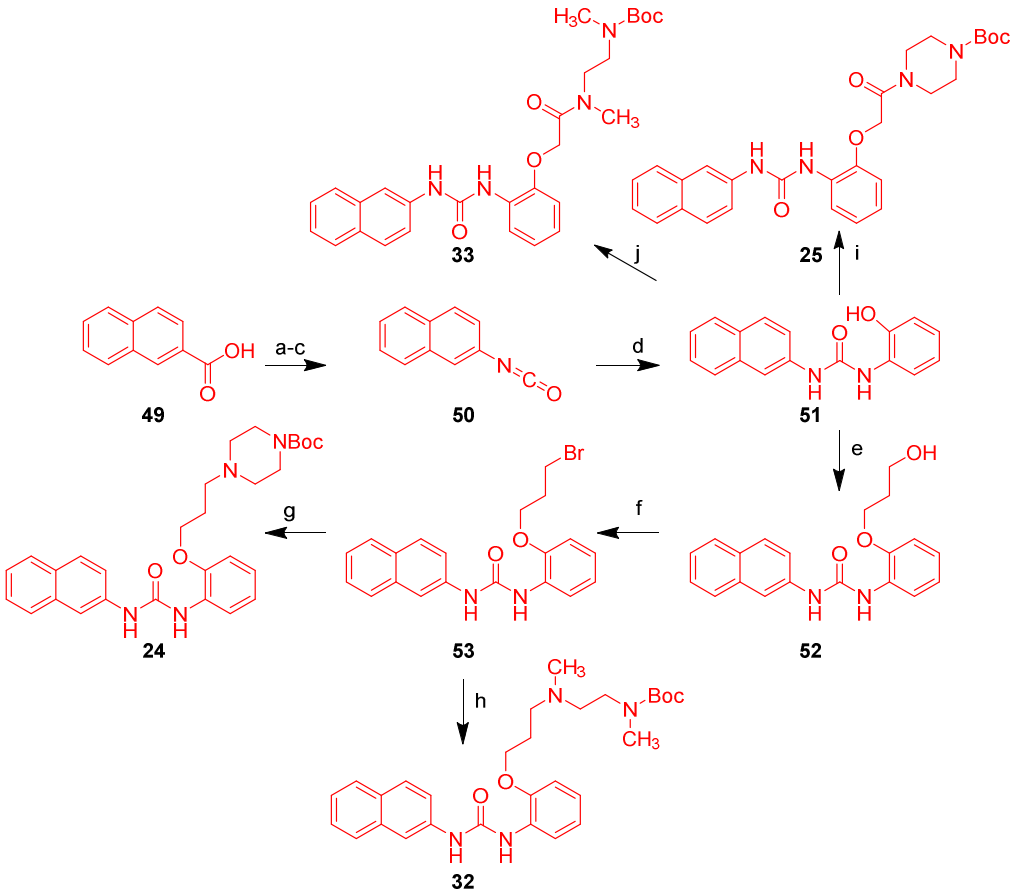
Supplementary Scheme 4. Synthesis of INF55-EPI-MB intermediates **22**, **23**, **30** and **31**. *Reagents and Conditions:* (a) $NaBH_4$, THF, rt, 24 hr, quant.; (b) CBr_4/PPh_3, Et_2O/THF, rt, 3 hr, 85%; (c) *N*-Boc-piperazine **37**, KI (45 mol%), THF, reflux, 48 hr, 96%; (d) *N*-Boc-*N*,*N*'-dimethylethylenediamine **39**, KI (50 mol%), THF, reflux, 48 hr, 78%; (e) NaOH, $MeOH/CH_2Cl_2$, rt, 30 min, then reflux, overnight, 96% (f) *N*-Boc-piperazine **37**, HATU, DIPEA, THF, rt, 5 hr, 85%; (g) *N*-Boc-*N*,*N*'-dimethylethylenediamine **39**, HATU, DIPEA, THF, rt, 7 hr, 78%.

(f) Synthesis of INF271-EPI-MB intermediates 24, 25, 32 and 33

Synthesis of the INF271-EPI-MB intermediates commenced with the chlorination of 2-naphthoic acid **49** using thionyl chloride. Thionyl chloride was gradually added to the solution of 2-naphthoic acid in benzene before stirring the reaction at reflux overnight. Upon consumption of the starting material (TLC; Pet. spirit:EtOAc, 50:50) the solvent was evaporated and the residue used without further purification. The crude acyl chloride was reacted with NaN_3/HCl in a two phase solvent system (C_6H_6/H_2O) using the phase transfer catalyst *N*,*N*'-diethylaniline. After stirring overnight at room temperature the desired acyl azide was obtained in 76% yield. Quantitative conversion to isocyanate **50** was then achieved by heating the acyl azide at reflux in benzene for 4 hr. These procedures were based on the method reported by Shafiee *et al.*[8] Reaction of 2-naphthylisocyanate **50** with *O*-aminophenol

in anhydrous CH_2Cl_2 at room temperature for 48 hr afforded the substituted urea **51** in 80% yield. The product **51** formed in the reaction as a white precipitate, which was simply filtered, dried under reduced pressure and used without further purification. *O*-Alkylation of **51** with 3-bromopropanol was achieved by stirring overnight at room temperature with K_2CO_3 in DMF, giving **52** in 75% yield. Bromination of **52** under Appel conditions using CBr_4/PPh_3 in dry THF at 40 ^oC gave the desired halide **53** in 85% yield after silica gel column chromatography.

Reaction of *N*-Boc-piperazine **37** with bromide **53** in the presence of K_2CO_3 and KI (25 mol%) in anhydrous THF afforded **24** in 85% yield after overnight heating at reflux. Intermediate **32** was synthesized by reacting *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** with bromide **53** in the presence of K_2CO_3 and KI (30 mol%) in dry THF. A yield of 85% was obtained after heating overnight at reflux. Alkylation of *N*-bromoacetyl-*N*'-Boc-piperazine **40** and *N*-bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylenediamine **41** with the phenolic urea derivative **51** in DMF at 80 ^oC in the presence of Cs_2CO_3 gave **25** and **33** in 66% and 80% yields, respectively, after silica gel column chromatography (**Supplementary Scheme 5**).



Supplementary Scheme 5. Synthesis of INF271-EPI-MB intermediates **24**, **25**, **32** and **33**. *Reagents and Conditions*: (a) $SOCl_2$, C_6H_6, reflux, 16 hr, 95%; (b) NaN_3, 5% HCl, *N*,*N*-diethylaniline, C_6H_6/H_2O, rt, 16 hr, 76%; (c) C_6H_6, reflux, 4 hr, quant.; (d) *O*-aminophenol, CH_2Cl_2, rt, 48 hr, 80%; (e) 3-bromopropanol, K_2CO_3, DMF, rt, 16 hr, 75%; (f) CBr_4/PPh_3, THF, 40 ^oC, 16 hr, 85%; (g) *N*-Boc-piperazine **37**, K_2CO_3, KI (25 mol%), THF, reflux, 16 hr, 85%; (h) *N*-Boc-*N*,*N*'-dimethylethylenediamine **39**, K_2CO_3 KI (30 mol%), THF, 80 ^oC, 48 hr, 85%; (i) *N*-bromoacetyl-*N*'-Boc-piperazine **40**, K_2CO_3, DMF, 80 ^oC, overnight, 66%; (j) *N*-bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylenediamine **41**, K_2CO_3, DMF, 80 ^oC, overnight, 80%.

(g) Synthetic procedures and compound characterisation

All reactions were performed under an argon atmosphere in flame or oven-dried glassware with magnetic stirring, unless otherwise noted. Pterostilbene was purchased from ChromaDex (NV, USA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Anhydrous acetonitrile and toluene were obtained from a PureSolv solvent purification system. *N*,*N*-dimethylformamide (DMF) was dried over

barium oxide and distilled under reduced pressure onto activated 4Å molecular sieves. Anhydrous tetrahydrofuran (THF) and diethyl ether (Et_2O) were freshly distilled from sodium benzophenone ketyl. All other solvents were of analytical reagent (AR) grade and used as received. The term petroleum spirit refers to petroleum spirit within the boiling range 40-60 ^oC.

Reaction monitoring was performed using thin layer chromatography (TLC) analysis on Merck Silica Gel 60 F_{254} (0.2 mm) aluminium-backed plates. Purification by flash column chromatography used silica gel 60 (230-400 mesh, Merck) with the indicated eluents. Purification of hybrids **1-16** was performed using Merck 60 F_{254} preparative TLC plates (1000 μm).

Melting points were determined on a Buchi digital M-560 melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Varian Unity-300 MHz or Varian-Inova-500 MHz spectrometers in deuterated solvents. Spectra were referenced using the chemical shifts of solvent resonances. UV/Vis spectra were recorded on a Shimadzu UV-1700 PharmaSpec UV/Vis spectrophotometer. Infrared spectra were obtained from neat samples using an Avator ESP spectrometer. Low resolution electrospray ionisation mass spectra (ESI-MS) were measured using a Shimadzu LC-MS (2010) spectrophotometer. High resolution electrospray ionisation mass spectra (HRESI-MS) were recorded using a factory modified Waters QToF Ultima^{TM} Spectrometer (Wyntheshawe, UK).

General procedure A (below) was used for coupling all Boc-protected intermediates **18-33** to the common intermediate **17** to form the respective EPI-MBs **1-16**. The procedure is exemplified with the synthesis of reserpine EPI-MB **1**.

3-(4-(2-(4-((((1S,2R,3R,4aS,13bR,14aS)-2,11-Dimethoxy-1-(methoxycarbonyl)-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo[2',3':3,4]pyrido[1,2-b]isoquinolin-3-yl)oxy)carbonyl)-2,6-dimethoxyphenoxy)ethyl)-piperazin-1-yl)-7-(dimethylamino) phenothiazin-5-ium chloride (1)

Boc-protected intermediate **18** (80 mg, 0.07 mmol) was dissolved in CH_2Cl_2 (25 mL) in a 50 mL round bottom flask under Ar and cooled to 0 ^oC. TFA (5 mL) was added in one portion and the reaction allowed to warm to room temperature with stirring while monitoring by TLC (Pet. spirit:EtOAc, 40:60). Upon consumption of the starting material the reaction was quenched with water and extracted with CH_2Cl_2 (3 x 30 mL). The pooled organic fractions were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude amine.TFA salt was redissolved in dry CH_2Cl_2 (15 mL) in a 25 mL oven-dried round bottom flask under Ar and 3-dimethylaminophenothiazin-5-ium triiodide **17** (120 mg, 0.19 mmol, 0.9 eq) was added in one portion. The reaction was stirred at room temperature for 72 hr while monitoring by TLC (3% $NH_4OAc_{(aq)}$:MeOH, 15:85). K_2CO_3 was added in cases where the reaction showed little or no progress after overnight stirring. The completed reaction was concentrated and the residue purified by preparative TLC using 3% $NH_4OAc_{(aq)}$/MeOH 15:85. The dark blue product obtained was dissolved in dry MeOH (25 mL) and stirred at room temperature with quaternary ammonium chloride-anion exchange resin (350 mg, 10% w/w). After 1 hr the mixture was filtered through a plug of cotton and the filtrate concentrated to give **1** (15 mg, 18%) as a dark blue powder. The light-sensitive compound was stored at -20 ^oC in a vial under Ar and wrapped in aluminium foil. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.03; Mp > 250 ºC; UV (MeOH): 292 (log ε 5.06, λ_{max}), 3.29 (log ε 4.59), 386 (log ε 4,45), 510 (log ε 4.00, λ_{min}), 610 (log ε 4.80), 651 (log ε 5.04, λ_{max}) nm; IR (neat) ν 3419, 2926, 2843, 1708, 1591, 1459, 1387, 1330, 1222, 1126 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{52}H_{61}N_6O_9S]$ 945.4215, found 945.4208.

3-((2-((2-(4-((((1S,2R,3R,4aS,13bR,14aS)-2,11-Dimethoxy-1-(methoxycarbonyl)-1,2, 3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo[2',3':3,4]pyrido[1,2-b]isoquinolin-3-yl)oxy)carbonyl)-2,6-dimethoxyphenoxy)ethyl)(methyl)amino)ethyl)(methyl) amin o)-7-(dimethylamino)phenothiazin-5-ium chloride (2)

General procedure A was used with Boc-protected intermediate **26** (100 mg, 0.12 mmol). Stirring for 3 days at room temperature in the presence of K_2CO_3 (0.4 eq) afforded **48** (22 mg, 18%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.03; Mp = 212-214 ºC; UV (MeOH): 292 (log ε 4.86, λ_{max}), 327 (log ε 4.31), 392 (log ε 4.17), 512 (log ε 3.75, λ_{min}), 609 (log ε 4.70), 656 (log ε 5.02, λ_{max}) nm; IR (neat) ν 3416, 2931, 2870, 2360, 1709, 1594, 1454, 1383, 1327, 1229, 1216 1123 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{52}H_{63}N_6O_9S$] 947.4372, found 947.4388.

3-(4-(2-(4-((((1S,2R,3R,4aS,13bR,14aS)-2,11-Dimethoxy-1-(methoxycarbonyl)-1,2,3,4,4a, 5,7,8,13,13b,14,14a-dodecahydroindolo[2',3':3,4]pyrido[1,2-b]iso quinolin-3-yl)oxy) carbonyl)-2,6-dimethoxyphenoxy)acetyl)piperazin-1-yl)-7-(dimethylamino) phenothiazin-5-ium chloride (3)

General procedure A was used with Boc-protected intermediate **19** (80 mg, 0.01 mmol). Stirring at room temperature for 4 days yielded **3** (13.6 mg, 14%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp > 250 ºC; UV (MeOH): 292 (log ε 4.88, λ_{max}), 3.27 (log ε 4.46), 392 (log ε 4,45), 512 (log ε 3.79, λ_{min}), 609 (log ε 4.67), 653 (log ε 4.97, λ_{max}) nm; IR (neat): 3744, 2933, 2845, 2361, 1714, 1700, 1592, 1458, 1384, 1329, 1209, 1179, 1152 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{52}H_{59}N_6O_{10}S$] 959.4008, found 959.4003.

3-((2-(2-(4-((((1S,2R,3R,4aS,13bR,14aS)-2,11-Dimethoxy-1-(methoxycarbonyl)-1,2, 3,4,4a,5,7,8,13, 13b,14,14a-dodecahydroindolo[2',3':3,4]pyrido[1,2-b]isoquinolin-3-yl)oxy)carbonyl)-2,6-dimethoxyphenoxy)-*N*-methylacetamido)ethyl)(methyl)amino)-7-(dimethylamino)phenothiazin-5-ium chloride (4)

General procedure A was used with Boc-protected intermediate **27** (90 mg, 0.11 mmol). Stirring for 4 days at room temperature in the presence of K_2CO_3 (0.4 eq) afforded **4** (12 mg, 11%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp > 250 ºC; UV (MeOH): 292 (log ε 4.85, λ_{max}), 329 (log ε 4.29), 392 (log ε 4.23), 512 (log ε 3.77, λ_{min}), 609 (log ε 4.70), 656 (log ε 5.00, λ_{max}) nm; IR (neat) ν 3363, 2926, 2848, 2363, 1716, 1710, 1594, 1462, 1387, 1330, 1217, 1183, 1124 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{52}H_{61}N_6O_{10}S$] 961.4164, found 961.4179.

(*E*)-3-(4-(2-(4-(3,5-Dimethoxystyryl)phenoxy)ethyl)piperazin-1-yl)-7-(dimethylamino) phenothiazin-5-ium chloride (5)

General procedure A was used with Boc-protected intermediate **20** (100 mg, 0.21 mmol). Stirring at room temperature for 72 hr in the presence of K_2CO_3 (0.3 eq) afforded **5** (35 mg, 26%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.03; Mp = 162-163 ºC; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.98 (dd, *J* = 8.0, 1.6 Hz, 1H, H36), 7.44 (d, *J* = 10.0 Hz, 2H, H6, H2), 7.42 (dd, *J* = 8.0, 1.6 Hz, 1H, H33), 7.37 (d, *J* = 10.0 Hz, 1H, H26), 7.35 (dd, *J* = 10.0, 2.4 Hz, 1H, H27), 7.08 (d, *J* = 2.4 Hz, 1H, H29), 6.86 (m, 5H, H35, H9, H3, H5, H8), 6.67 (s, 2H, H12, H14), 6.38 (s, 1H, H16), 4.31 (t, *J* = 12.0 Hz, 2H, H18), 3.75 (s, 6H, H10, H11), 3.64 and 3.60 (2s, 6H, $N(CH_3)_2$), 2.95 (t, *J* = 14.0 Hz, 2H, H19), 2.93 (dd, *J* = 7.5 Hz, 4H, H22, H24), 2.08 (dd, *J* = 7.5 Hz, 4H, H21, H25); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ ^{13}C NMR (125 MHz, CD_2Cl_2) δ 161.4, 158.8, 139.9, 139.1, 130.5, 128.8, 128.1, 126.9, 122.2, 119.8, 115.10, 106.7, 106.4,

104.5, 99.9, 66.3, 60.9, 57.0, 55.6, 54.2, 54.0, 48.4, 30.0, 28.9; UV (MeOH): 294 (log ε 4.86, λ_{max}), 323 (log ε 4.70), 415 (log ε 4.10, λ_{min}), 612 (log ε 4.60), 655 (log ε 4.84, λ_{max}) nm; IR (neat) ν 2921, 2363, 1589, 1386, 1146, 1129, 1110 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{36}H_{39}N_4O_3S]$ 607.2737, found 607.2743.

(*E*)-3-((2-((2-(4-(3,5-Dimethoxystyryl)phenoxy)ethyl)(methyl)amino)ethyl)(methyl) amino)-7-(dimethylamino) phenothiazin-5-ium chloride (6)

General procedure A was used with Boc-protected intermediate **28** (70 mg, 0.15 mmol). Stirring for 3 days at room temperature in the presence of K_2CO_3 (0.4 eq) provided **6** (27 mg, 28%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp = 178-180 ºC; UV (MeOH): 293 (log ε 5.10, λ_{max}), 324 (log ε 4.95), 413 (log ε 3.26, λ_{min}), 611 (log ε 4.63), 661 (log ε 4.92, λ_{max}) nm; IR (neat) ν 2919, 2848, 2361, 1589, 1455, 1386, 1174, 1145, 1137 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{36}H_{41}N_4O_3S]$ 609.2894, found 609.2901.

(*E*)-3-(4-(2-(4-(3,5-Dimethoxystyryl)phenoxy)acetyl)piperazin-1-yl)-7-(dimethyl amino)phenothiazin-5-ium chloride (7)

General procedure A was used with Boc-protected intermediate **21** (60 mg, 0.12 mmol). Stirring at room temperature for 3 days in the presence of K_2CO_3 (0.2 eq) yielded **7** (25 mg, 30%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.04; Mp = 185-187 ºC; UV (MeOH): 292 (log ε 5.10, λ_{max}), 320 (log ε 4.91), 412 (log ε 3.37, λ_{min}), 615 (log ε 4.64), 654 (log ε 4.87, λ_{max}) nm; IR (neat) ν 3261, 2921, 2363, 1589, 1386, 1146, 1129, 1110 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{36}H_{37}N_4O_4S]$ 621.2530, found 621.2546.

(*E*)-3-((2-(2-(4-(3,5-Dimethoxystyryl)phenoxy)-*N*-methylacetamido)ethyl)(methyl) amino) -7-(dimethylamino)phenothiazin-5-ium chloride (8)

General procedure A was used with Boc-protected intermediate **29** (80 mg, 0.16 mmol). Stirring for 2 days at room temperature in the presence of K_2CO_3 (0.2 eq) afforded **8** (28 mg, 26%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp = 194-196 ºC; UV (MeOH): 293 (log ε 5.17, λ_{max}), 324 (log ε 4.99), 415 (log ε 3.29, λ_{min}), 613 (log ε 4.67), 661 (log ε 4.95, λ_{max}) nm; IR (neat) ν 2938, 2842, 2360, 1664, 1589, 1456, 1203, 1149, 1065, 1055 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{36}H_{39}N_4O_4S]$ 623.2687, found 623.2692.

3-(Dimethylamino)-7-(4-(4-(5-nitro-1*H*-indol-2-yl)benzyl)piperazin-1-yl)phenothiazine-5-ium chloride (9)

General procedure A was used with Boc-protected intermediate **22** (90 mg, 0.20 mmol). Stirring at room temperature for 48 hr gave **9** (29 mg, 23 %) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.04; Mp > 250 ºC; UV (MeOH): 295 (log ε 4.99, λ_{max}), 415 (log ε 3.43, λ_{min}), 610 (log ε 4.62), 656 (log ε 4.91, λ_{max}) nm; IR (neat) ν 3205, 2924, 2361, 1683, 1591, 1517, 1472, 1387, 1329, 1220, 1134, 1045; HRESI-MS: *m/z* calculated for $[C_{33}H_{31}N_6O_2S]$ 575.2224, found 575.2236.

3-(Dimethylamino)-7-(methyl(2-(methyl(4-(5-nitro-1*H*-indol-2-yl)benzyl)amino)ethyl) amino)phenothiazin-5-ium chloride (10)

General procedure A was used with Boc-protected intermediate **30** (80 mg, 0.18 mmol). Stirring at room temperature for 48 hr yielded **10** (25 mg, 22%) as dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp > 250 ºC; UV (MeOH): 295 (log ε 5.03, λ_{max}), 415 (log ε 3.65, λ_{min}), 609 (log ε 4.66), 650

(log ε 4.86, λ_{max}) nm; IR (neat) ν 3245, 2922, 2850, 2362, 1663, 1591, 1519, 1458, 1386, 1329, 1230, 1219, 1135 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{33}H_{33}N_6O_2S$] 577.2380, found 577.2386.

3-(Dimethylamino)-7-(4-(4-(5-nitro-1*H*-indol-2-yl)benzoyl)piperazin-1-yl)pheno-thiazin-5-ium chloride (11)

General procedure A was used with Boc-protected intermediate **23** (100 mg, 0.23 mmol). Stirring at room temperature for 76 hr in the presence of K_2CO_3 (0.3 eq) provided **11** (25 mg, 18%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.03; Mp > 250 ºC; UV (MeOH): 297 (log ε 5.06, λ_{max}), 419 (log ε 3.49, λ_{min}), 609 (log ε 4.68), 651 (log ε 4.91, λ_{max}) nm; IR (neat) ν 3305, 2975, 2922, 2360, 1695, 1558, 1553, 1549, 1540, 1472, 1409, 1400, 1331, 1220, 1134 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{33}H_{29}N_6O_3S$] 589.2016, found 589.2022

3-(Dimethylamino)-7-(methyl(2-(*N*-methyl-4-(5-nitro-1*H*-indol-2-yl)benzamido) ethyl)amino)phenothiazin-5-ium chloride (12)

General procedure A was used with Boc-protected intermediate **31** (120 mg, 0.26 mmol). Stirring at room temperature for 76 hr in the presence of K_2CO_3 (0.4 eq) afforded **12** (30 mg, 18%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp > 250 ºC; UV (MeOH): 294 (log ε 5.02, λ_{max}), 413 (log ε 3.39, λ_{min}), 610 (log ε 4.46), 659 (log ε 4.82, λ_{max}) nm; IR (neat) ν 3240, 2921, 2853, 2361, 1665, 1591, 1516, 1456, 1387, 1328, 1226, 1218, 1130 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{33}H_{31}N_6O_3S$] 591.2173, found 591.2181.

3-(Dimethylamino)-7-(4-(3-(2-(3-(naphthalen-2-yl)ureido)phenoxy)propyl)piperazin-1-yl)phenothiazin-5-ium chloride (13)

General procedure A was used with Boc-protected intermediate **24** (100 mg, 0.19 mmol). Stirring at room temperature for 2 days afforded **13** (34 mg, 25 %) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH, 15:85) = 0.04; Mp > 250 ºC; UV (MeOH): 287 (log ε 4.86), 295 (log ε 4.88, λ_{max}), 323 (log ε 4.14), 368 (log ε 3.37, λ_{min}), 608 (log ε 4.61), 655 (log ε 4.88, λ_{max}) nm; IR (neat) ν 3355, 2972, 2921, 2740, 2360, 1704, 1595, 1458, 1353, 1136, 1127, 1118 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{38}H_{39}N_6O_2S]$ 643.2850, found 643.2867.

3-(Dimethylamino)-7-(methyl(2-(methyl(3-(2-(3-(naphthalen-2-yl)ureido)phenoxy) propyl) amino)ethyl)amino)phenothiazin-5-ium chloride (14)

General procedure A was used with Boc-protected intermediate **32** (100 mg, 0.19 mmol). Stirring at room temperature for 48 hr provided **14** (34 mg, 25%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.03; Mp = 239-241 ºC; UV (MeOH): 290 (log ε 4.86), 327 (log ε 4.06), 415 (log ε 3.03, λ_{min}), 610 (log ε 4.60), 659 (log ε 4.97, λ_{max}) nm; IR (neat) ν 3299, 2968, 2925, 2710, 2362, 1700, 1593, 1450, 1386, 1327, 1231, 1195, 1136, 1126, 1117 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{38}H_{41}N_6O_2S]$ 645.3006, found 645.2993.

3-(Dimethylamino)-7-(4-(2-(2-(3-(naphthalen-2-yl)ureido)phenoxy)acetyl) piperazin-1-yl)phenothiazin-5-ium chloride (15)

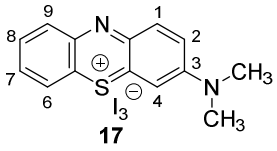
General procedure A was used with Boc-protected intermediate **25** (90 mg, 0.18 mmol). Stirring at room temperature for 3 days with K_2CO_3 (0.3 eq) yielded **15** (21 mg, 17%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3%

$NH_4OAc_{(aq)}$:MeOH 15:85) = 0.03; Mp 228-231 ºC; UV (MeOH): 289 (log ε 4.93), 293 (log ε 4.94, λ_{max}), 324 (log ε 4.10), 410 (log ε 2.04, λ_{min}), 608 (log ε 4.67), 652 (log ε 4.96, λ_{max}) nm; IR (neat) ν 3324, 2989, 2923, 2738, 2366, 1700, 1653, 1593, 1458, 1386, 1215, 1196, 1136, 1127 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{37}H_{35}N_6O_3S]$ 643.2486, found 643.2477.

3-(Dimethylamino)-7-(methyl(2-(*N*-methyl-2-(2-(3-(naphthalen-2-yl)ureido)phenoxy) acetamido)ethyl)amino)phenothiazin-5-ium chloride (16)

General procedure A was used with Boc-protected intermediate **33** (80.0 mg, 0.16 mmol). Stirring at room temperature for 3 days with K_2CO_3 (0.3 eq) afforded hybrid **16** (15 mg, 15%) as a dark blue powder after preparative TLC and anion exchange. TLC R_f (3% $NH_4OAc_{(aq)}$:MeOH 15:85) = 0.02; Mp = 246-248 ºC; UV (MeOH): 293 (log ε 4.93, λ_{max}), 325 (log ε 4.16), 413 (log ε 3.09, λ_{min}), 610 (log ε 4.73), 656 (log ε 5.05, λ_{max}) nm; IR (neat) ν 3311, 2968, 2923, 2851, 2736, 2365, 1706, 1654, 1593, 1457, 1387, 1327, 1231, 1192, 1136, 1126, 1119 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{37}H_{37}N_6O_3S]$ 645.2642, found 645.2663.

3-(Dimethylamino)phenothiazin-5-ium triiodide (17)

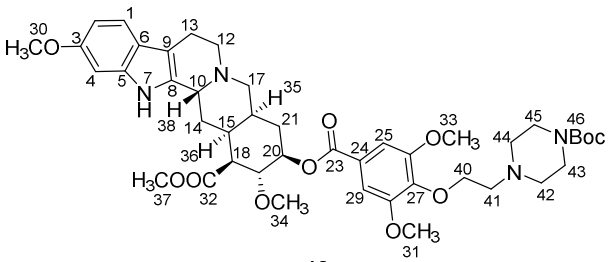


17

Dimethylamine in methanol (6.2 g, 11.9 mmol) was added dropwise to a solution of phenothiazin-5-ium tetraiodide hydrate (5.0 g, 6.90 mmol) in 80 mL chloroform at room temperature. The reaction was stirred for 5 h while monitoring by TLC (3% $NH_4OAc_{(aq)}$/MeOH, 15:85). The resulting precipitate was filtered, washed with chloroform and air dried to give **17** (205 mg, 60%) as a dark blue solid. TLC R_f (3% $NH_4OAc_{(aq)}$/MeOH, 15:85) = 0.28; Mp 144 -145 ºC. ^1H NMR (500 MHz, $(CD_3)_2SO$) δ 8.18 (dd, J = 8.0, 1.6 Hz, 1H, H9), 8.13 (dd, J = 8.0, 1.6 Hz, 1H, H6), 8.06 (d, J = 10.0 Hz, 1H, H1), 7.98 (dd, J = 10.0, 2.4 Hz, 1H, H2), 7.94 (d, J = 2.4 Hz, 1H, H4), 7.82 (m, 2H, H7, H8), 3.64 and 3.60 (2s, 6H, $N(CH_3)_2$); ^{13}C NMR (125

MHz, $(CD_3)_2SO$) δ 156.1, 144.1, 139.8, 139.6, 138.0, 134.6, 133.2, 129.8, 126.3, 126.1, 125.8, 109.7, 43.4, 42.9; IR (neat) ν 2800, 1617, 1559, 1489, 1429, 1411, 1252, 1118, 1078, 887, 835, 772 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{14}H_{13}N_2S]$ 241.0794, found 241.0767.

Methyl (1S,2R,3R,4aS,13bR,14aS)-3-((4-(2-(4-(*tert*-butoxy carbonyl) piperazin-1-yl)ethoxy)-3,5-dimethoxybenzoyl)oxy)-2,11-dimethoxy-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo [2',3':3,4]pyrido[1,2-b]isoquinoline-1-carboxylate (18)

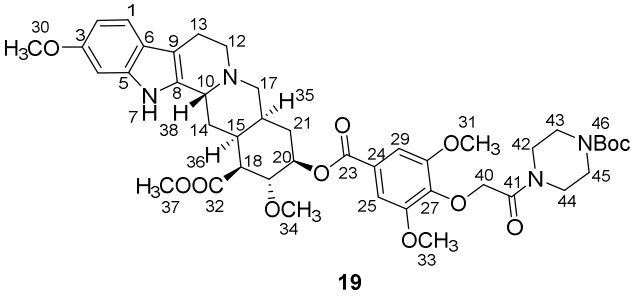


18

Bromide **36** (200 mg, 0.28 mmol) was dissolved in dry THF (25 mL) in a 50 mL flame-dried round bottom flask under Ar and *N*-Boc-piperazine (150 mg, 0.78 mmol) and triethylamine (500 mg, 5.0 mmol) were successively added. A reflux condenser was attached and the mixture heated at 100 ^oC with stirring 16 hr while monitoring by TLC (60% EtOAc/Pet. spirit). The solvent was evaporated and the residue purified by silica gel column chromatography (Pet. spirit:EtOAc, 50:50) to provide **18** (184 mg, 80%) as a yellow-brown solid. TLC R_f (Pet. spirit:EtOAc, 60:40) = 0.25; Mp 170-172 ºC; ^1H NMR (500 MHz, $CDCl_3$) δ 7.55 (br s, 1H, H7), 7.31 (m, 3H, H25, H29, H1), 6.78 (app d, J = 2.0 Hz, 1H, H4), 6.69 (d, J = 10.0 Hz, 2.0 Hz, 1H, H2), 5.03 (sextet, J = 4.5 Hz, 1H, H20), 4.46 (app s, 1H, H19), 4.27 (t, J = 10.0, 2H, H40), 3.90 (s, 6H, H31, H33), 3.87 (t, J = 10.0 Hz, 1H, H38), 3.83 (s, 3H, H30), 3.80 (s, 3H, H37), 3.71 (t, J = 10.0 Hz, 2H, H41), 3.50 (s, 3H, H34), 3.41 (app dd, J = 8.5 Hz, 4H, H43, H45), 3.18 (d, J = 7.1 Hz, 2H, H17), 3.08 (d, J = 7.1 Hz, 1H, H18), 2.94 (sextet, J = 7.1 Hz, 1H, H36) 2.68 (dd, J = 10.0 Hz, 5.0 Hz, 2H, H14), 2.56 (app dd, J = 8.5 Hz, 4H, H42, H44), 2.50-2.24 (m, 3H, H21, H35), 2.18-1.92 (m, 4H, H12, H13), 1.45 (s, 9H, H46); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1 (C32), 166.0 (C23), 158.8 (C46), 156.2 (C3), 148.9 (C26, C28),

138.8 (C5, C8), 137.3 (C27), 121.2 (C24), 120.82 (C6, C9), 118.1 (C1), 109.0 (C2), 106.3 (C25, C29), 94.5 (C4), 79.4 (C46), 69.8 (C40), 68.5 (C10), 60.6 (C19, C20), 59.1 (C41), 57.2 (C30, C31, C33), 56.1(C42, C44), 56.1 (C34), 52.0 (C37), 51.6 (C18), 51.1 (C17), 48.8 (C12), 45.8 (C43, C45), 33.0 (C15), 32.0 (C14), 31.5 (C14), 29.2 (C36), 27.9 (C46), 23.7 (C21), 16.3 (C13); IR (neat) ν 3741, 2935, 2794, 1700, 1695, 1457, 1418, 1248, 1152 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{43}H_{58}N_4O_{11}$] 806.4102, found 806.4108.

Methyl (1S,2R,3R,4aS,13bR,14aS)-3-((4-(2-(4-(*tert*-butoxy carbonyl)piperazin-1-yl)-2-oxoethoxy)-3,5-dimethoxybenzoyl)oxy)-2,11-dimethoxy-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo [2',3':3,4]pyrido[1,2-b]isoquinoline-1-carboxylate (19)

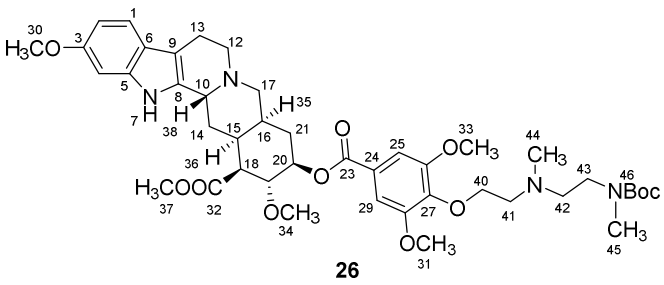


19

Phenolic reserpine **34** (50 mg, 0.08 mmol) was dissolved in DMF (7 mL) in a 25 mL flame-dried round bottom flask under Ar and K_2CO_3 (250 mg, 1.79 mmol) was added. The mixture was stirred for 20 min before *N*-bromoacetyl-*N*'-Boc-piperazine **40** (40 mg, 0.13 mmol) was added in one portion. The mixture was stirred at 80 ^oC for 48 hr while monitoring by TLC (EtOAc:MeOH, 95:5). The solvent was evaporated and the residue purified by silica gel column chromatography to yield **19** (43 mg, 64%) as a brown solid. TLC R_f (EtOAc:MeOH, 95:5) = 0.25; Mp 138-141 ºC; ^1H NMR (500 MHz, $CDCl_3$) δ 7.64 (br s, 1H, H7), 7.35 (m, 3H, H25, H29, H1), 6.82 (s, 1H, H4), 6.77 (d, J = 15.0 Hz, 1H, H2), 5.05 (sextet, J = 4.5 Hz, 1H, H20), 4.68 (s, 2H, H40), 4.44 (app s, 1H, H19), 3.88 (s, 6H, H31, H33), 3.86 (t, J = 10.0 Hz, 1H, H38), 3.85 (s, 3H, H30), 3.81 (s, 3H, H37), 3.76 (app dd, J = 8.5 Hz, 4H, H42, H44), 3.63 (app dd, J = 8.5 Hz, 4H, H43, H45), 3.48 (s, 3H, H34), 3.19 (d, J = 7.1 Hz, 2H, H17), 3.07 (d, J = 7.1 Hz, 1H,

H18), 2.94 (sextet, J = 7.1 Hz, 1H, H36) 2.69 (dd, J = 10.0 Hz, 5.0 Hz, 2H, H14), 2.56-2.24 (m, 3H, H21, H35), 2.13-1.85 (m, 4H, H12, H13), 1.48 (s, 9H, H46); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0 (C32), 168.2 (41), 165.6 (C23), 158.5 (C46), 156.0 (C3), 148.8 (C26, C28), 138.9 (C5, C8), 137.2 (C27), 121.1 (C24), 120.8 (C6, C9), 118.2 (C1), 109.1 (C2), 106.1 (C25, C29), 94.3 (C4), 79.2 (C46), 68.4 (C10), 65.7 (C40), 60.5 (C19, C20), 57.1 (C30, C31, C33), 56.0 (C34), 52.4 (C37), 51.9 (C43, C45), 51.5 (C18), 51.0 (C17), 50.6 (C42, C44), 48.6 (C12), 33.1 (C15), 31.8 (C14), 31.4 (C14), 29.1 (C36), 27.8 (C46), 23.8 (C21), 16.1 (C13); IR (neat) ν 3340, 2931, 2844, 2365, 1707, 1694, 1458, 1415, 1211, 1125 cm^{-1}; HRESI-MS: m/z calcd for [$C_{43}H_{56}N_4O_{12}$] 820.3895, found 820.3875.

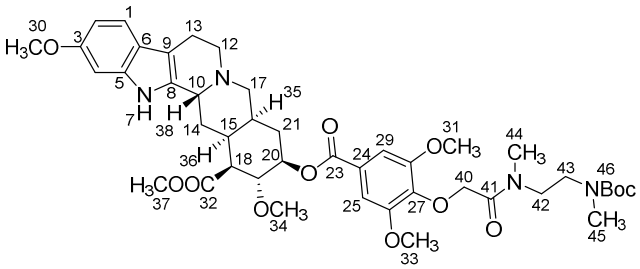
Methyl (1S,2R,3R,4aS,13bR,14aS)-3-((4-(2-((2-((*tert*-butoxy carbonyl) (methyl) amino) ethyl) (methyl)amino)ethoxy)-3,5-dimethoxybenzoyl) oxy)-2,11-dimethoxy-1,2,3,4,4a, 5,7,8,13, 13b,14,14a-dodecahydroindolo[2',3':3,4] pyrido[1,2-b]isoquinoline-1-carboxylate (26)



Bromide **36** (200 mg, 0.28 mmol) was dissolved in dry THF (20 mL) in a 50 mL flame-dried round bottom flask under Ar and *N*-Boc-*N*,*N*'-dimethylethylenedi amine **39** (150 mg, 0.78 mmol) was added followed by triethylamine (500 mg, 5.0 mmol). A reflux condenser was attached and the mixture warmed to 100 ^{o}C and stirred for 16 hr while monitoring by TLC (Pet. spirit:EtOAc 60:40). The mixture was concentrated and the residue purified by a silica gel column chromatography using 30% EtOAc/Pet. spirit to provide **26** (172 mg, 75%) as a brown solid. TLC R_f (Pet. spirit:EtOAc 60:40) = 0.23; Mp 188-191 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (br s, 1H, H7), 7.24 (m, 3H, H25, H29, H1), 6.91 (app d,

J = 2.0 Hz, 1H, H4), 6.78 (d, *J* = 10.0 Hz, 2.0 Hz, 1H, H2), 5.04 (sextet, *J* = 4.5 Hz, 1H, H20), 4.54 (app s, 1H, H19), 4.21 (t, *J* = 10.0, 2H, H40), 3.89 (s, 6H, H31, H33), 3.86 (t, *J* = 10.0 Hz, 1H, H38), 3.81 (s, 3H, H30), 3.79 (s, 3H, H37), 3.71 (t, *J* = 10.0 Hz, 2H, H41), 3.48 (s, 3H, H34), 3.22 (t, *J* = 10.0 Hz, 2H, H43), 3.16 (d, *J* = 7.1 Hz, 2H, H17), 3.03 (d, *J* = 7.1, 1H, H18), 2.96 (sextet, *J* = 7.1 Hz, 1H, H36) 2.94 (s, 3H, H45), 2.92 (S, 3H, H44), 2.68 (dd, *J* = 10.0 Hz, 5.0 Hz, 2H, H14), 2.55 (t, *J* = 10.0 Hz, 2H, H42), 2.49-2.22 (m, 3H, H21, H35), 2.16-1.90 (m, 4H, H12, H13), 1.44 (s, 9H, H46); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6 (C32), 165.8 (C23), 158.5 (C46), 156.0 (C3), 149.1 (C26, C28), 139.0 (C5, C8), 137.1 (C27), 121.0 (C24), 120.4 (C6, C9), 117.6 (C1), 108.7 (C2), 105.9 (C25, C29), 94.1 (C4), 79.2 (C46), 69.5 (C40), 68.1 (C10), 60.4 (C19, C20), 59.0 (C41), 57.0 (C30, C31, C33), 56.1 (C34), 55.3 (C42), 52.6(C43), 51.8 (C37), 51.4 (C18), 50.8 (C17), 48.6 (C12), 46.7 (C44), 36.8 (C45), 32.6 (C15), 31.6 (C14), 29.1 (C36), 28.4 (C46), 23.6 (C21), 16.1 (C13); IR (neat) ν 3419, 2941, 1709, 1684, 1458, 1330, 1216, 1158 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{43}H_{60}N_4O_{11}$] 808.4259, found 808.4969.

Methyl(1S,2R,3R,4aS,13bR,14aS)-3-((4-(2-((2-((*tert*-butoxycarbonyl)(methyl)amino) ethyl)(methyl)amino)-2-oxoethoxy)-3,5-dimethoxyben-zoyl)oxy)-2,11-dimethoxy-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo[2',3':3,4] pyrido[1,2-b]isoquinoline-1-carboxylate (27)

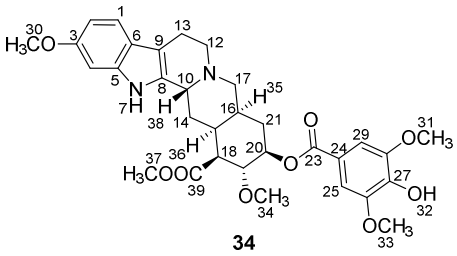


27

The phenolic reserpine derivative **34** (50 mg, 0.08 mmol) was dissolved in dry DMF (7 mL) in a 50 mL oven-dried round bottom flask under Ar and K_2CO_3 (250 mg, 1.79 mmol) was added. The mixture was stirred at room temperature for 30 min before adding *N*-

bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylenediamine **41** (40 mg, 0.13 mmol) in one portion. The reaction mixture was heated to 80 °C and stirred for 48 hr while monitoring by TLC (EtOAc:MeOH, 95:5). The solvent was evaporated and the residue purified by silica gel column chromatography using a gradient (100% EtOAc → 5% MeOH/EtOAc) to give **27** (47 mg, 70%) as a brown solid. TLC R_f (EtOAc:MeOH, 95:5) = 0.20; Mp 103-105 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (br s, 1H, H7), 7.36-7.28 (m, 3H, H25, H29, H1), 6.91 (app d, J = 1.0 Hz, 1H, H4), 6.78 (d, J = 10.0 Hz, 1.0 Hz, 1H, H2), 5.04 (sextet, J = 4.5 Hz, 1H, H20), 4.68 (s, 2H, H40), 4.51 (app s, 1H, H19), 3.89 (s, 6H, H31, H33), 3.84 (s, 3H, H30), 3.81 (s, 3H, H37), 3. 68 (app s, 1H, H38), 3.49 (s, 3H, H34), 3.40 (t, J = 10.0 Hz, 2H, H43), 3.17 (d, J = 7.1 Hz, 2H, H17), 3.03 (d, J = 7.1 Hz, 1H, H18), 2.94 (s, 3H, H45), 2.92 (S, 3H, H44), 2.88 (sextet, J = 7.1 Hz, 1H, H36), 2.70 (dd, J = 10.0 Hz, 5.0 Hz, 2H, H14), 2.55 (t, J = 10.0 Hz, 2H, H42), 2.49-2.22 (m, 3H, H21, H35), 2.16-1.90 (m, 4H, H12, H13), 1.44 (s, 9H, H46); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4 (C32), 166.4 (C41), 165.3 (C23), 158.2 (C46), 156.1 (C3), 149.3 (C26, C28), 139.0 (C5, C8), 136.0 (C27), 121.5 (C24), 119.3 (C6, C9), 117.4 (C1), 108.4 (C2), 104.7 (C25, C29), 93.0 (C4), 79.5 (C46), 69.0 (C40), 67.4 (C10), 60.2 (C19, C20), 56.8 (C30, C31, C33), 56.0 (C34), 53.3 (C43), 52.4 (C42), 51.6 (C37), 51.3 (C18), 50.7 (C17), 48.5 (C12), 43.7 (C45), 36.6 (C44), 32.5 (C15), 31.8 (C14), 29.1 (C36), 28.3 (C46), 23.3 (C21), 16.3 (C13); IR (neat) ν 3291, 2935, 2828, 2360, 2162, 1704, 1696, 1458, 1415, 1213, 1126 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{43}H_{58}N_4O_{12}$] 822.4051, found 822.4035.

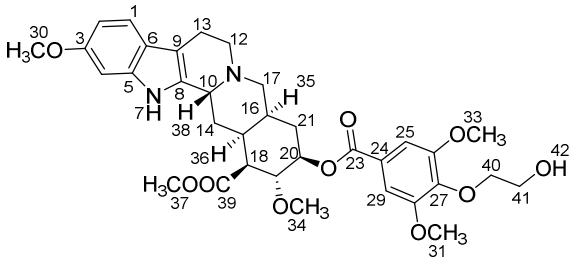
Methyl (1S,2R,3R,4aS,13bR,14aS)-3-((4-hydroxy-3,5-dimethoxy benzoyl)oxy)-2,11-dimethoxy-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo [2',3':3,4]pyrido[1,2-b]isoquinoline-1-carboxylate (34)[5]



34

Finely ground aluminium chloride (1.0 g, 7.63 mmol) was added in one portion to a stirring solution of reserpine (100 mg, 0.16 mmol) in dry CH_2Cl_2 (20 mL) at room temperature. The reaction was stirred for 2 hr while monitoring by TLC (EtOAc). The reaction was quenched by addition of 5% aqueous HCl (15 mL) and the mixture diluted with CH_2Cl_2 (30 mL) and the layers separated. The aqueous layer was extracted twice more with CH_2Cl_2 and the combined organic fractions were washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated to yield **34** (90 mg, 95%) as a yellowish solid. TLC R_f (EtOAc) = 0.45; Mp 154-156 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (br s, 1H, H7), 7.33 (s, 2H, H25, H29),7.31 (d, J = 10.0 Hz, 1H, H1), 6.88 (s, 1H, H4), 6.78 (d, J = 10.0 Hz, 1H, H2), 4.97 (sextet, J = 4.5 Hz, 1H, H20), 4.74 (app s, 1H, H19), 3.98 (t, J = 10.0 Hz, 1H, H38), 3.92 (s, 6H, H31, H33), 3.86 (s, 3H, H30), 3.81 (s, 3H, H37), 3.50 (s, 3H, H34), 3.28 (d, J = 7.1 Hz, 2H, H17), 3.21 (d, J = 7.1 Hz, 1H, H18), 2.95 (sextet, J = 7.1 Hz, 1H, H36) 2.77-2.45 (m, 5H, H14, H21, H35), 2.15-1.95 (m, 4H, H12, H13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4 (C39), 165.6 (C23), 156.7 (C3), 146.8 (C26, C28), 139.7 (C5, C8), 137.1 (C27), 121.2 (C24), 120.87 (C6, C9), 118.6 (C1), 109.7 (C2), 106.8 (C25, C29), 95.3 (C4), 60.7 (C19, C20), 56.9 (C30, C31, C33), 55.9 (C34), 54.5 (C0), 52.2 (C37), 51.4 (C18), 50.9 (C17), 48.5 (C12), 33.2 (C36), 31.3 (C35), 29.4 (C36), 23.7 (C21), 16.3 (C13); IR (neat) ν 3736, 2921, 1732, 1691, 1550, 1436, 1181, 1118 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{32}H_{38}N_2O_9$] 594.2577, found 594.2571.

Methyl (1S,2R,3R,4aS,13bR,14aS)-3-((4-(2-hydroxyethoxy)-3,5-dimethoxy benzoyl) oxy)-2,11-dimethoxy-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydroindolo[2',3':3,4] pyrido[1,2-b] isoquinoline-1-carboxylate 35

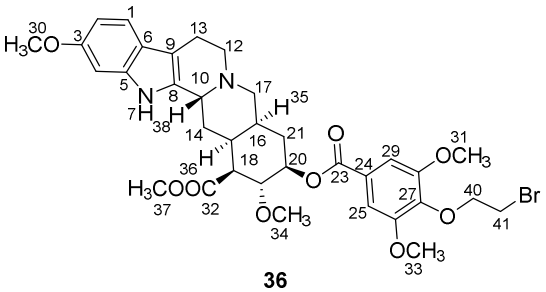


35

The phenolic reserpine **34** (100 mg, 17 mmol) was dissolved in dry DMF (5 mL) in a 25 mL flame-dried round bottom flask under Ar and K_2CO_3 (1.00 g, 17.8 mmol) was added. The mixture was stirred at room temperature for 30 min before adding 2-bromoethanol (50 mg, 40 mmol) and warming to 80 ^oC. After overnight stirring and monitoring by TLC (EtOAc) the reaction mixture was concentrated and the residue re-dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography (60% EtOAc/Pet, Spirit) to afford **35** (100 mg, 96%) as a yellow solid. TLC R_f (EtOAc) = 0.38; Mp 121-123 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (br s, 1H, H7), 7.33 (m, 3H, H25, H29, H1), 6.84 (app d, J = 2 Hz, 1H, H4), 6.78 (d, J = 10.0 Hz, 2.0 Hz, 1H, H2), 5.05 (sextet, J = 4.5 Hz, 1H, H20), 4.47 (app s, 1H, H19), 4.25 (t, J = 10.0 Hz, 2H, H40), 3.92 (s, 6H, H31, H33), 3.89 (t, J = 10.0 Hz, 1H, H38), 3.84 (s, 3H, H30), 3.82 (s, 3H, H37), 3.73 (t, J = 10.0 Hz, 2H, H41), 3.50 (s, 3H, H34), 3.18 (d, J = 7.1 Hz, 2H, H17), 3.05 (d, J = 7.1 Hz, 1H, H18), 2.96 (sextet, J = 7.1 Hz, 1H, H36) 2.70 (dd, J = 10.0 Hz, 5.0 Hz, 2H, H14), 2.52-2.27 (m, 3H, H21, H35), 2.15-1.95 (m, 4H, H12, H13) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8 (C39), 165.8 (C23), 156.4 (C3), 149.2 (C26, C28), 140.1 (C5, C8), 137.5 (C27), 121.5 (C24), 120.84 (C6, C9), 118.3 (C1), 109.2 (C2), 106.5 (C25, C29), 94.9 (C4), 69.8 (C40), 60.7 (C19, C20), 59.5 (C41), 56.9 (C30, C31, C33), 55.9 (C34), 54.5 (C0), 52.2 (C37), 51.4 (C18), 50.9 (C17), 48.5 (C12), 33.2 (C36), 31.3

(C35), 29.4 (C36), 23.7 (C21), 16.3 (C13) ; IR (neat) ν 3741, 2918, 1731, 1688, 1553, 1437, 1182, 1121 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{34}H_{42}N_2O_{10}$] 638.2839, found 638.2844.

Methyl (1S,2R,3R,4aS,13bR,14aS)-3-((4-(2-bromoethoxy)-3,5-dimethoxybenzoyl) oxy)-2,11-dimethoxy-1,2,3,4,4a,5,7,8,13,13b,14,14a-dodeca-hydroindolo[2',3': 3,4] pyrido[1,2-b] isoquinoline-1-carboxylate (36)

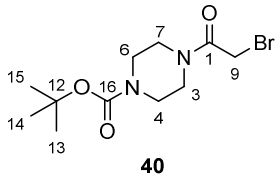


36

O-alkylated reserpine alcohol **35** (100 mg, 0.16 mmol) was dissolved in dry THF (20 mL) in a 50 mL flame-dried round bottom flask under Ar. CBr_4 (200 mg, 0.60 mmol) was added and the mixture stirred at room temperature for 10 min before adding PPh_3 (100 mg, 0.38 mmol) in one portion. Stirring was continued at room temperature for a further 3 hr while monitoring by TLC (Pet. spirit:EtOAc, 70:30). The solvent was evaporated and the residue purified by silica gel column chromatography using a gradient (Pet. spirit:EtOAc, 60:40 → 100% EtOAc) to give **36** (98 mg, 91%) as a yellow-brown solid. TLC R_f (Pet. spirit:EtOAc, 60:40) = 0.55; Mp 140-143 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (br s, 1H, H7), 7.32 (m, 3H, H25, H29, H1), 6.85 (app d, J = 2.0 Hz, 1H, H4), 6.78 (d, J = 10.0 Hz , 2.0 Hz, 1H, H2), 5.10 (sextet, J = 4.5 Hz, 1H, H20), 4.75 (t, J = 10.0 Hz, 2H, H40), 3.91 (s, 7H, H31, H33, H19), 3.77 (app t, 7H, H30, H37, H38), 3.63 (t, J = 10.0 Hz, 2H, H41), 3.52 (s, 3H, H34), 3.18 (d, J = 7.1 Hz, 2H, H17), 3.05 (d, J = 7.1 Hz, 1H, H18), 2.97 (sextet, J = 7.1 Hz, 1H, H36) 2.73 (dd, J = 10.0 Hz, 5.0 Hz, 2H, H14), 2.54-2.22 (m, 3H, H21, H35), 2.12-1.88 (m, 4H, H12, H13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1 (C39), 165.4 (C23), 156.6 (C3), 149.0 (C26, C28), 140.5 (C5, C8), 137.0 (C27), 122.0 (C24), 120.2 (C6, C9), 118.8 (C1), 109.0 (C2), 106.0 (C25, C29), 94.7 (C4), 69.3 (C40), 60.2 (C19, C20), 57.2 (C30, C31, C33), 56.1 (C34), 54.5 (C0), 52.0 (C37),

51.1 (C18), 51.2 (C17), 48.2 (C12), 33.0 (C36), 31.6 (C41), 31.1 (C35), 29.6 (C36), 23.9 (C21), 18.1 (C13); IR (neat) ν 3744, 2916, 1734, 1683, 1558, 1437, 1183, 1119 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{34}H_{41}{}^{79}BrN_2O_9$] 701.1995, found 701.1986.

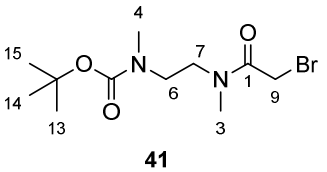
***Tert*-butyl 4-(2-bromoacetyl)piperazine-1-carboxylate (40)**



40

Bromoacetylchloride (500 g, 3.2 mmol) was added dropwise during 30 min to a solution of *tert*-butyl piperazine-1-carboxylate **37** (500 mg, 2.7 mmol) and K_2CO_3 (2.0 g, 14.6 mmol) in 50 mL anhydrous THF. The reaction was stirred for 12 h while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The reaction mixture was concentrated and the residue re-dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography (30% EtOAc/Pet, Spirit) to afford **40** (250 mg, 35%) as an off-white solid. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.48; Mp 114 -115 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 2H, H9), 3.62-3.42 (m, 8H, H3, H4, H6, H7), 1.45 (s, 9H, H13, H14, H15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1, 154.3, 80.4, 46.5 (2C), 41.9 (2C), 28.3 (3C), 25.6; IR (neat) ν 1762, 1723, 1560, 1498, 1429, 1413, 1250, 1120 cm^{-1}; HRESI-MS: *m/z* calcd for $C_{11}H_{19}{}^{79}BrN_2O_3$) 307.0579, found 307.0590.

***Tert*-butyl (2-(2-bromo-*N*-methylacetamido)ethyl)(methyl) carbamate (41)**



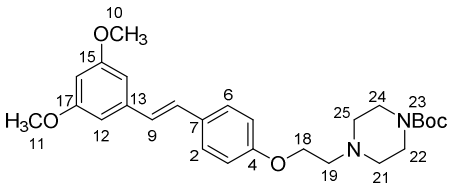
41

Bromoacetylchloride (500 g, 3.2 mmol) was added dropwise over 30 min to a solution of *tert*-butyl methyl(2-(methylamino)ethyl)carbamate **39** (500 mg, 2.7 mmol) and K_2CO_3 (1.2 g, 8.7 mmol) in 30 mL anhydrous CH_2Cl_2. The reaction was stirred for 16 h while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The

reaction mixture was concentrated and the residue re-dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography (30% EtOAc/Pet, Spirit) to afford **41** (330 mg, 40%) as an off-white solid. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.46; Mp 129 - 131 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.26 (s, 2H, H9), 3.46 (t, 2H, H7), 3.28 (t, 2H, H6), 3.25 (s, 3H, H3), 2.89 (s, 3H, H4), 1.45 (s, 9H, H13, H14, H15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 153.9, 79.6, 51.8, 47.5, 35.3, 34.9, 28.2 (3C), 27.6; IR (neat) ν 1752, 1717, 1559, 1489, 1429, 1411, 1252, 1118 cm^{-1}; HRESI-MS: *m/z* calcd for $C_{11}H_{21}$ $^{79}BrN_2O_3$) 309.0735, found 309.0752.

***Tert*-butyl (*E*)-4-(2-(4-(3,5-dimethoxystyryl)phenoxy)ethyl)piperazine-1-carboxylate (20)**

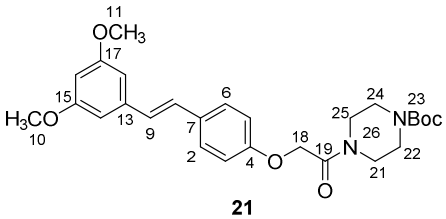
To a solution of bromide **44** (150 mg, 0.41 mmol) in anhydrous THF (30 mL) in a 50 mL oven-dried round bottom flask under Ar was added KI (300 mg, 1.80 mmol). *N*-Boc-piperazine **37** (120 mg, 0.63 mol) was then added in one portion, a reflux condenser attached and the reaction heated at 100 ^{o}C with stirring for 48 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was evaporated and the residue re-dissolved in EtOAc (50 mL). The organic layer was washed successively with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using a gradient (10 → 15% EtOAc/Pet. Spirit) to give **20** (166 mg, 87%) as an off-white solid. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.30; Mp 103-105 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, J = 10.0 Hz, 2H, H6, H2), 7.12 (d, J = 15.8 Hz, 1H, H9), 6.90 (m, 3H, H3, H5, H8), 6.70 (s, 2H, H12, H14), 6.38 (s, 1H, H16), 4.31 (t, J = 12.0 Hz, 2H, H18), 3.75 (s, 6H, H10, H11), 3.43 (dd, J = 7.5 Hz, 4H, H22, H24), 3.65 (t, J = 14.0 Hz, 2H, H19), 2.65 (dd, J = 7.5 Hz, 4H,



20

H21, H25), 1.46 (s, 9H, H23) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.4 (C23), 160.8 (C15, C17), 159.9 (C4), 141.5 (C13), 131.6 (C2, C6), 128.1 (C9), 127.6 (C8), 115.0 (C3, C5), 106.0 (C12, C14), 100.4 (C16), 76.8 (C23), 69.4 (C18), 58.9 (C19), 57.6 (C21, C25), 54.5 (C10, C11), 47.4 (C22, C24), 28.9 (C23); IR (neat) ν 2934, 2836, 1685, 1584, 1513, 1425, 1248, 1145 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{27}H_{36}N_2O_5$] 468.2624, found 468.2637.

***Tert*-butyl(*E*)-4-(2-(4-(3,5-dimethoxystyryl)phenoxy)acetyl)piperazine-1-carboxylate(21)**

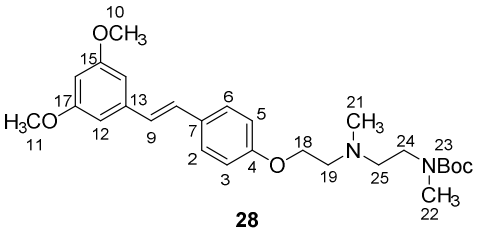


21

To a solution of phenolic pterostilbene **42** (256 mg, 1.0 mmol) in anhydrous DMF (10 mL) in a 25 mL oven-dried round bottom flask under Ar was added K_2CO_3 (552 mg, 4.0 mmol). *N*-bromoacetyl-*N*'-Boc-piperazine **40** (460 mg, 1.5 mmol) was then added in one portion and a reflux condenser attached and the reaction stirred at 100 ^{o}C for 48 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was evaporated and the residue redissolved in CH_2Cl_2 (50 mL). The organic layer was washed successively with water and brine, dried over anhydrous $NaSO_4$ and concentrated. The crude residue was purified by silica gel column chromatography using 15% EtOAc/Pet. spirit to afford **21** (371 mg, 77%) as an off-white solid. TLC R_f (Pet. spirit:EtOAc, 55:45) = 0.42; Mp 52-53 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 10.0 Hz, 2H, H6, H2), 7.11 (d, J = 15.8 Hz, 1H, H9), 7.05-6.86 (m, 3H, H3, H5, H8), 6.63 (app d, J = 1.5 Hz, 2H, H12, H14), 6.37 (s, 1H, H16), 4.71 (s, 2H, H18), 3.81 (s, 6H, H10, H11), 3.57 (dd, J = 7.5 Hz, 4H, H21, H25), 3.42 (dd, J = 7.5 Hz, 4H, H22, H24), 1.46 (s, 9H, H23); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8 (C19), 161.2 (C15, C17), 157.6 (C4), 154.7 (C23), 139.7 (C13), 131.1 (C7), 128.6 (C9), 128.1 (C2, C6), 127.5 (C8), 115.0 (C3, C5), 104.5 (C12, C14), 100.0 (C16), 80.6 (C23), 68.2 (C18), 55.6 (C10, C11), 45.6

(C22, C24), 42.2 (C21, C25), 28.6 (C23); IR (neat) ν 2930, 2886, 1684, 1651, 1589, 1510, 1436, 1232, 1219 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{27}H_{34}N_2O_6$] 482.2417, found 482.2423.

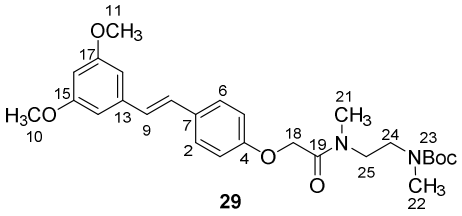
***Tert*-butyl(*E*)-(2-((2-(4-(3,5-dimethoxystyryl)phenoxy)ethyl)(methyl)amino)ethyl) (methyl) carbamate (28)**



28

To a stirring solution of pterostilbene alkyl bromide **44** (150 mg, 0.41 mol) in anhydrous CH_2Cl_2 (30 mL) in a 50 mL oven-dried round bottom flask under Ar was added K_2CO_3 (250 mg, 1.80 mmol). *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** (120 mg, 0.63 mol) was then added in one portion, a reflux condenser attached and the reaction heated at 60 ^oC for 48 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The cooled reaction mixture was diluted with CH_2Cl_2 (30 mL) and the organic layer washed successively with water and brine, dried over anhydrous Na_2CO_3 and concentrated. The crude residue was purified by silica gel column chromatography using a gradient (15% EtOAc/Pet. spirit to 100% EtOAc) to provide **28** (154 mg, 81%) as a pale yellow solid. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.27; Mp 131-135 ºC; ^1H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 10.0 Hz, 2H, H6, H2), 7.14 (d, J = 15.8 Hz, 1H, H9), 7.06-6.90 (m, 3H, H3, H5, H8), 6.65 (s, 2H, H12, H14), 6.37 (s, 1H, H16), 4.29 (t, J = 12.0 Hz, 2H, H18), 3.75 (s, 6H, H10, H11), 3.13 (t, J = 7.5 Hz, 2H, H24), 2.75 (t, J = 7.1 Hz, 2H, H19), 2.57 (s, 3H, H22), 2.43 (3, J = 7.1 Hz, 2H, H25), 2.19 (s, 3H, H21), 1.45 (s, 9H, H23); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2 (C23), 160.7 (C15, C17), 159.6 (C4), 141.0 (C13), 131.3 (C2, C6), 128.0 (C9), 127.2 (C8), 114.7 (C3, C5), 105.5 (C12, C14), 99.8 (C16), 78.1 (C23), 67.9 (C18), 58.9 (C19), 56.2 (C25), 54.4 (C10, C11), 53.1 (C24), 46.4 (C22), 37.5 (C21), 28.7 (C23); IR (neat) ν 2944, 2861, 1687, 1585, 1511, 1422, 1247, 1125 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{27}H_{38}N_2O_5$] 470.2871, found 470.2864.

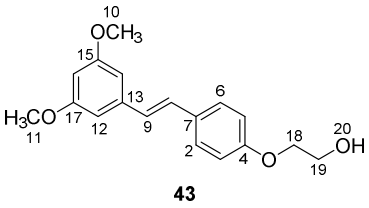
***Tert*-butyl (*E*)-(2-(2-(4-(3,5-dimethoxystyryl)phenoxy)-N-methylacetamido)ethyl) (methyl) carbamate (29)**



29

To a stirring solution of phenolic pterostilbene derivative **42** (256 mg, 1.0 mmol) in anhydrous DMF (10 mL) in a 25 mL oven-dried round bottom flask under Ar was added K_2CO_3 (552 mg, 4.0 mmol). *N*-bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylenediamine **41** (460 mg, 1.5 mmol) was added in one portion, a reflux condenser attached and the reaction heated at 80 ^oC for 24 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The reaction mixture was concentrated and the residue redissolved in CH_2Cl_2 (30 mL). The organic layer was washed successively with water and brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography using a gradient (Pet. spirit:EtOAc, 85:15 to 100% EtOAc) to afford **29** (255 mg, 77%) as a pale yellow solid. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.27; Mp 118-120 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, J = 18.0 Hz, 2H, H6, H2), 7.07-6.86 (m, 4H, H3, H5, H8, H9), 6.64 (s, 2H, H12, H14), 6.38 (s, 1H, H16), 4.69 (d, J = 18.0 Hz, 2H, H18), 3.82 (s, 6H, H10, H11), 3.54 (t, J = 8.5 Hz, 2H, H25), 3.39 (t, J = 8.5 Hz, 2H, H24), 3.10 (s, 3H, H21), 2.86 (s, 3H, H22), 1.45 (s, 9H, H23); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8 (C19), 160.9 (C15, C17), 157.7 (C4), 155.9 (C23), 139.6 (C13), 130.8 (C7), 128.5 (C9), 127.8 (C2, C6), 127.0 (C8), 114.9 (C3, C5), 104.4 (C12, C14), 97.7 (C16), 79.8 (C23), 67.1 (C18), 55.3 (C10, C11), 47.4 (C24), 45.5 (C25), 34.8 (C21, C22), 28.4 (C23); IR (neat) ν 2935, 2831, 1684, 1659, 1590, 1509, 1457, 1204, 1173, 1148 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{27}H_{36}N_2O_6$] 484.2573, found 484.2586.

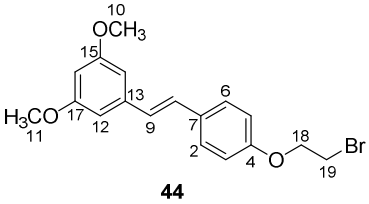
(*E*)-2-(4-(3,5-Dimethoxystyryl)phenoxy)ethan-1-ol (43)



43

To a solution of phenolic pterostilbene derivative **42** (300 mg, 1.1 mmol) in anhydrous DMF (10 mL) in a 25 mL flame-dried round bottom flask under Ar was added K_2CO_3 (260 mg, 1.1 mmol) and the mixture was stirred at room temperature for 20 min. 2-Bromoethanol (300 mg, 2.5 mmol) was added dropwise over 10 min and the reaction mixture warmed to 80 ^oC and stirred for 16 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was evaporated under reduce pressure and the residue re-dissolved in CH_2Cl_2. The organic layer was washed successively with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using 10% EtOAc/Pet. spirit to afford **43** (280 mg, 90%) as an off-white powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.45; Mp 83-84 ºC; ^{1}H NMR (500 MHz, CD_3OD) δ 7.46 (d, J = 10.0 Hz, 2H, H6, H2), 7.05 (d, J = 15.8 Hz, 1H, H9), 6.93 (m, 3H, H3, H5, H8), 6.67 (s, 2H, H12, H14), 6.38 (s, 1H, H16), 4.31 (t, J = 12.0 Hz, 2H, H18), 3.78 (s, 6H, H10, H11), 3.65 (t, J = 14.0 Hz, 2H, H19); ^{13}C NMR (125 MHz, CD_3OD) δ 162.6 (C4), 161.8 (C15, C17), 141.5 (C13), 130.8 (C2, C6), 128.3 (C9), 127.9 (C8), 114.9 (C3, C5), 105.0 (C12, C14), 100.4 (C16), 69.7 (C18), 60.9 (C19), 54.8 (C10, C11); IR (neat) ν 3671, 2927, 2348, 1586, 1146, 1129, 1110 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{18}H_{19}NaO_4$] 300.1362, found 300.1389.

(*E*)-1-(4-(2-Bromoethoxy)styryl)-3,5-dimethoxybenzene (44)



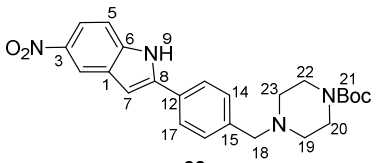
44

To a solution of alcohol **43** (200 mg, 0.67 mmol) in anhydrous THF (25 mL) in a 50 mL flame-dried round bottom flask under Ar was added CBr_4 (850 mg, 2.5 mmol). PPh_3

(262 mg, 1.0 mmol) was then added in one portion and the reaction stirred for 3 hr at room temperature while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was evaporated and the residue re-dissolved in EtOAc. The organic layer was washed successively with water and brine, dried over anhydrous $MgSO_4$ and concentrated. The crude residue was purified by silica gel column chromatography to afford **44** (225 mg, 94%) as an off-white foam. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.69; Mp 76-77 ºC; ^{1}H NMR (500 MHz, CD_3OD) δ 7.45 (d, J = 10.0 Hz, 2H, H6, H2), 7.08 (d, J = 15.8 Hz, 1H, H9), 6.95 (m, 3H, H3, H5, H8), 6.66 (s, 2H, H12, H14), 6.35 (s, 1H, H16), 4.30 (t, J = 12.0 Hz, 2H, H18), 3.76 (s, 6H, H10, H11), 3.63 (t, J = 14.0 Hz, 2H, H19); ^{13}C NMR (125 MHz, CD_3OD) δ 161.9 (C4), 161.5 (C15, C17), 141.0 (C13), 130.8 (C2, C6), 127.8 (C9), 127.9 (C8), 115.2 (C3, C5), 106.1 (C12, C14), 99.8 (C16), 69.5 (C18), 60.8 (C19), 55.01 (C10, C11) ; IR (neat) ν 2928, 2346, 1583, 1145, 1122, 1115 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{18}H_{19}BrO_3$] 362.0518, found 362.0524.

***Tert*-butyl 4-(4-(5-nitro-1*H*-indol-2-yl)benzyl)piperazine-1-carboxylate (22)**

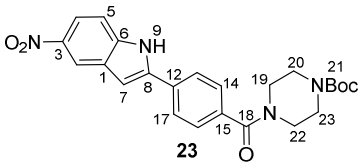
Bromide **47** (70 mg, 0.21 mmol) was dissolved in anhydrous THF (25 mL) under Ar and KI (175 mg, 1.1 mmol) added. The mixture was stirred at room temperature for 30 min before adding *N*-Boc-piperazine **37** (100 mg, 0.53 mmol) in one portion. The reaction was then heated at reflux for 48 hr while monitoring by TLC (Pet. spirit:EtOAc, 70:30). The mixture was concentrated and the residue purified by silica gel column chromatography using Pet. spirit:EtOAc 85:15 to afford **22** (89 mg, 96%) as a yellow solid. TLC R_f (Pet. spirit:EtOAc, 70:30) = 0.43; Mp 208-210 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.25 (br.s, 1H, H9), 8.53 (s, 1H, H2), 7.89 (d, J = 7.5 Hz, 2H, H5), 7.84 (d, J = 8.5 Hz, 2H, H13, H17), 7.53 (d, J = 7.5 Hz,1H, H4), 7.42 (d, J = 8.5, 2H, H14, H16), 7.15 (s, 1H, H7), 3.51 (s, 2H, H18), 3.30 (m, 4H, H20, H22), 2.33 (m, 4H, H19, H23), 1.37 (s, 9H, H21) ; ^{13}C



22

NMR (125 MHz, $(CD_3)_2SO$) δ 155.0 (C21), 142.4 (C6), 141.2 (C15), 139.9 (C8), 139.0 (C12), 130.2 (C3), 130.0 (C14, C16), 128.7 (C1), 125.5 (C13, C17), 118.0 (C4), 117.7 (C5), 110.9 (C2), 101.6 (C7), 70.9 (C21), 62.7 (C18), 53.0 (C19, C23, C20, C22), 28.6 (C21) ; IR (neat) ν 3272, 2969, 2811, 2769, 1663, 1520, 1476, 1330, 1318, 1216, 1158 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{24}H_{28}N_4O_4]$ 436.2111, found 436.2113.

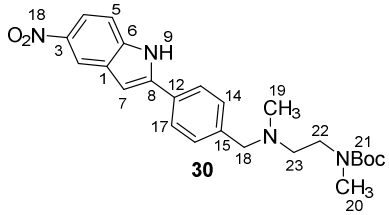
***Tert*-butyl 4-(4-(5-nitro-1*H*-indol-2-yl)benzoyl)piperazine-1-carboxylate (23)**



23

The acid **48** (20 mg, 0.07 mmol) was dissolved in anhydrous THF (10 mL) under Ar and DIPEA (9.0 mg, 0.07 mmol) added. *N*-Boc-piperazine **37** (16 mg, 0.08 mmol) and HATU (35 mg, 0.09 mmol) were then successively added and the reaction stirred at room temperature for 5 hr while monitoring by TLC (Pet. spirit:EtOAc, 30:70). The solvent was removed under reduced pressure and the residue redissolved in CH_2Cl_2. The organic layer was washed with 5% aqueous HCl, water and brine and then dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using Pet. spirit:EtOAc 60:40 to afford **23** (26 mg, 85%) as a yellow solid. TLC R_f (Pet. spirit:EtOAc, 30:70) = 0.50; Mp 210-12 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 11. 05 (br.s, 1H, H9), 8.58 (s, 1H, H2), 8.06 (d, J = 7.5 Hz, 1H, H5), 7.99 (d, J = 8.5 Hz, 2H, H13, H17), 7.74 (d, J = 8.5, 2H, H14, H16), 7.60 (d, J = 8.5 Hz,1H, H4), 7.27 (s, 1H, H7), 3.35 (m, 4H, H19, H22), 2.13 (m, 4H, H20, H23), 1.43 (s, 9H, H21); ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ 171.1 (C18), 151.8 (C21), 147.5 (C6), 142.5 (C15), 140.6 (C8), 139.0 (C12), 131.4 (C3), 130.8 (C14, C16), 129.5 (C1), 125.9 (C13, C17), 119.1 (C4), 118.3 (C5), 110.5 (C2), 101.8 (C7), 68.2 (C21), 46.0 (C20, C23), 40.4 (C19, C22), 29.3 (C21); IR (neat) ν 3267, 2957, 2795, 2768, 1701, 1663, 1521, 1475, 1322, 1312, 1215, 1119 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{24}H_{26}N_4O_5]$ 450.1903, found 450.1916.

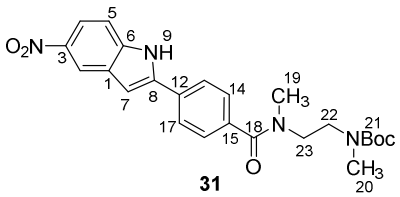
***Tert*-butyl methyl(2-(methyl(4-(5-nitro-1*H*-indol-2-yl)benzyl)amino)ethyl)carbamate(30)**



30

Bromide **47** (80 mg, 0.24 mmol) was dissolved in anhydrous THF (25 mL) under Ar in a 50 mL oven-dried round bottom flask. KI (200 mg, 1.12 mmol) was added and the mixture stirred at room temperature for 30 min before adding *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** (100 mg, 0.53 mmol) in one portion. A reflux condenser was attached and the reaction heated to reflux for 48 hr while monitoring by TLC (Pet. spirit:EtOAc, 70:30). The solvent was evaporated and the residue purified by silica gel column chromatography using a gradient from Pet. spirit:EtOAc 60:40 to 100% EtOAc to yield **30** (63 mg, 78%) as a yellow solid. TLC R_f (Pet. spirit:EtOAc, 70:30) = 0.23; Mp 118-120 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.26 (br.s, 1H, H9), 8.55 (s, 1H, H2), 8.00-7.82 (m, 3H, H5, H13, H17), 7.60-7.38 (m, 3H, H4, H14, H16), 7.21 (s, 1H, H7), 4.00 (s, 2H, H18), 3.37 (m, 4H, H23, H22), 2.66 (s, 3H, H20), 1.96 (s, 3H, H19), 1.36 (s, 9H, H21); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 158.8 (C21), 142.1 (C6), 141.2 (C15), 140.0 (C8), 139.7 (C12), 129.9 (C3), 129.6 (C14, C16), 128.6 (C1), 125.3 (C13, C17), 117.8 (C4), 117.5 (C5), 110.7 (C2), 101.2 (C7), 79.4 (C21), 62.0 (C23), 47.1 (C22), 42.7 (C19), 35.0 (C20), 28.4 (C21); IR (neat) ν 3268, 2961, 2810, 2776, 1668, 1523, 1475, 1396, 1330, 1317, 1211, 1153 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{24}H_{30}N_4O_4$] 438.2267, found 438.2280.

***Tert*-butyl methyl(2-(*N*-methyl-4-(5-nitro-1H-indol-2-yl)benzamido)ethyl)carbamate(31)**

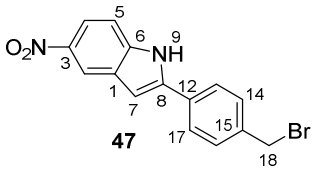


31

Acid **48** (20 mg, 0.07 mmol) was dissolved in anhydrous THF (9 mL) under Ar in a 25 mL flame-dried round bottom flask. DIPEA (9.0 mg, 0.07 mmol), *N*-Boc-*N*,*N*'-dimethylethylenediamine **39** (16 mg, 0.088 mmol) and HATU (35 mg, 0.09 mmol) were successively added and the reaction stirred at room

temperature for 7 hr while monitoring by TLC (Pet. spirit:EtOAc, 70:30). The solvent was evaporated and the residue redissolved in CH_2Cl_2 (30 mL). The organic layer was washed with 5% aqueous HCl, water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using a gradient from Pet. spirit:EtOAc 60:40 to 100% EtOAc to provide **31** (23 mg, 78%) as a yellow solid. TLC R_f (Pet. spirit:EtOAc, 70:30) = 0.45; Mp 162-164 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.20 (br.s, 1H, H9), 8.57 (s, 1H, H2), 7.61 (d, J = 11.5 Hz, 2H, H13, H17), 7.49 (d, 1H, H5), 7.43-7.27 (m, 3H, H4, H14, H16), 6.91 (d, J = 11.4 Hz,1H, H7), 3.76-3.32 (m, 4H, H23, H22), 2.76 (s, 3H, H20), 1.68 (s, 3H, H19), 1.46 (s, 9H, H21); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0 (C18), 157.0 (C21), 142.0 (C6), 140.4 (C15), 139.8 (C8), 139.1 (C12), 129.1 (C3), 128.1 (C14, C16), 127.5 (C1), 125.2 (C13, C17), 116.9 (C4), 116.3 (C5), 110.3 (C2), 101.2 (C7), 79.8 (C21), 61.9 (C23), 47.1 (C22), 43.1 (C19), 36.8 (C20), 28.5 (C21); IR (neat) ν 3233, 2981, 2790, 1697, 1675, 1598, 1472, 1398, 1332, 1317, 1166, 1028 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{24}H_{28}N_4O_5$] 452.2060, found 452.2088.

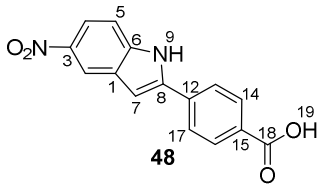
2-(4-(Bromomethyl)phenyl)-5-nitro-1*H*-indole (47)



To a solution of the methyl ester **45** (80 mg, 0.27 mmol) in dry THF (5.0 mL) under Ar was added $NaBH_4$ (50 mg, 1.3 mmol) and the reaction stirred at room temperature while monitoring by TLC (pet. Spirit:EtOAc, 50:50). After 24 hr the reaction was quenched by slow addition of sat. NH_4Cl (aq) until bubbling ceased. The mixture was diluted with water (50 mL) and extracted with EtOAc (4 x 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated to give benzylic alcohol **46** (85%) as a bright yellow powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.42; Mp 215-218 ^{o}C; ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 11.37 (br.s, 1H, H9), 8.53 (s, 1H, H2), 8.04 (d, J = 8.5 Hz, 1H, H5), 7.89

(d, J = 8.5 Hz, 2H, H13, H17), 7.59 (d, J = 8.5 Hz, 1H, H4), 7.51 (d, 2H, J = 8.5 Hz, H14, H16), 7.17 (s, 1H, H7), 4.72 (s, 2H, H18); ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ 146.3 (C6), 143.1 (C15), 141.2 (C8), 140.1 (C12), 131.5 (C3), 130.9 (C14, C16), 130.0 (C1), 125.8 (C13, C17), 119.1 (C4), 118.4 (C5), 110.6 (C2), 101.7 (C7), 64.3 (C18). The alcohol **46** (30 mg, 0.10 mmol) and CBr_4 (100 mg, 0.30 mmol) were added to a flame-dried 25 mL round bottom flask followed by anhydrous Et_2O/THF (1:1, 5 mL). The mixture was stirred at room temperature for 30 min before PPh_3 (80 mg, 0.31 mmol) was added in one portion. The reaction was stirred at room temperature for 3 hr while monitoring by TLC (Pet. spirit:EtOAc, 70:30). The solvent was evaporated and the residue purified by silica gel column chromatography using Pet. spirit:EtOAc 90:10 to yield **47** (30 mg, 94%) as a yellow powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.91; Mp 198-199 ^{o}C; ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 10.14 (br.s, 1H, H9), 9.27 (s, 1H, H2), 8.05 (d, 1H, J = 8.5 Hz, H5), 7.91 (d, 2H, J = 7.5 Hz, H4), 7.61-7.57 (m, 4H, H13, H14, H16, H17), 6.92 (s, 1H, H7), 4.51 (s, 2H, H18); ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ 143.0 (C6), 142.1 (C8), 138.3 (C15), 137.6 (C10), 135.8 (C12), 132.5 (C3), 129.5 (C14, C16), 129.2 (C7), 125.8 (C13, C17), 113.7 (C4), 112.2 (C5), 98.6 (C11), 33.3 (C2).

4-(5-Nitro-1*H*-indol-2-yl)benzoic acid (48)



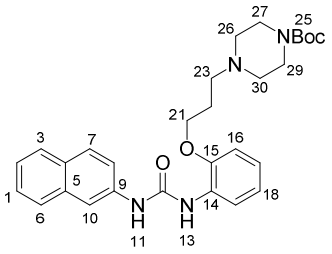
48

To a solution of methyl ester **45** (250 mg, 0.84 mmol) in MeOH/CH_2Cl_2 (10:90 v/v, 40 mL) was added methanolic NaOH (3 N, 2.5 mL). The mixture was stirred at room temperature for 30 min before attaching a reflux condenser and heating the reaction overnight at reflux while monitoring by TLC (CH_2Cl_2:MeOH, 90:10). The solvent was removed under reduced pressure and the residue redissolved in water (100 mL). The aqueous layer was washed with CH_2Cl_2 (2 x 60 mL) and the *p*H reduced to 2-3 by dropwise addition of 5% HCl. The aqueous

layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated to give **48** (230 mg, 96%) as a yellow powder. TLC R_f (CH_2Cl_2:MeOH, 90:10) = 0.21; Mp 138-139 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.41 (s, 1H, H9), 8.56 (s, 1H, H2), 8.02 (m, 5H, H5, H13, H14, H16, H17), 7.57 (d, J = 10.0 Hz, 1H, H4),7.31 (s, 1H, H7); ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ 174.2 (C18), 148.3 (C6), 143.1 (C15), 141.2 (C8), 140.1 (C12), 131.5 (C3), 130.9 (C14, C16), 130.0 (C1), 125.8 (C13, C17), 119.1 (C4), 118.4 (C5), 110.6 (C2), 101.7 (C7); IR (neat) ν 3420, 3233, 2891, 2787, 2771, 1793, 1518, 1473, 1321, 1317, 1210, 1151 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{15}H_{10}N_2O_4$] 282.0641, found 282.0660.

***Tert*-butyl4-(3-(2-(3-(naphthalen-2-yl)ureido)phenoxy)propyl)piperazine-1-carboxylate (24)**

Bromide **53** (200 mg, 0.5 mmol), *N*-Boc-piperazine **37** (200 mg, 1.1 mmol), K_2CO_3 (800 mg, 5.75 mmol) and KI (400 mg, 2.39 mol) were successively added to a 50 mL oven-dried round bottom flask under Ar. Dry THF (30 mL) was then added, a reflux condenser attached and the mixture stirred at 80 ^{o}C for 48 hr. The reaction mixture was concentrated *in vacuo* and the residue redissolved in EtOAc (200 mL). The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using a gradient (Pet. spirit:EtOAc, 50:50 to 100% EtOAc) to afford **24** (214 mg, 85%) as a brown powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.28; Mp 98-100 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.90 (s, 1H, H11), 8.28 (d, J = 7.5, 1H, H19), 8.09 (s, 1H, H13), 7.76 (d, J = 7.5 Hz, 3H, H3, H6, H8), 7.11 (s, 1H, H10), 7.46 (d, J = 7.6 Hz, 1H, H7), 7.43 (d, J = 7.5 Hz, 2H, H1), 7.36 (d, J = 7.5 Hz, 1H, H2), 7.08 (d, J = 7.4 Hz, 1H, H16), 6.9-6.10 (d, J = 7.6 Hz, 1H, H18), 6.94 (d, J = 7.5 Hz, 1H, H17), 4.73 (t, J = 7.1 Hz, 2H, H21), 3.63 (t, J = 7.1 Hz,

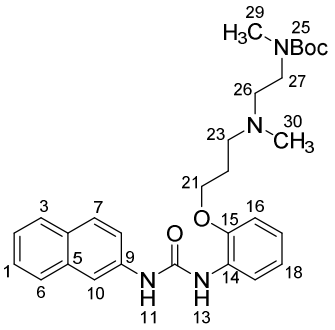


24

2H, H27), 3.44 (dd, J = 7.1 Hz, 4H, H26, H29), 3.65 (t, *J* = 7.1, 2H, H30), 2.17 (t, *J* = 7.1 Hz, 2H, H23), 1.83 (q, *J* = 7.1 Hz, 2H, H22), 1.47 (s, 9H, H25); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 155.6 (C15), 154.7 (C40), 152.9 (C12), 135.4 (C9), 133.7 (C5), 129.9 (C7), 127.8 (C17), 126.8 (C4), 126.5 (C3), 125.3 (C1), 124.6 (C6), 124.3 (C14), 121.4 (C2), 120.5 (C18), 119.9 (C8, C19), 116.7 (C10), 112.9 (C16), 79.8 (C36), 73.1 (C21), 58.2 (C23), 56.7 (C26, C30), 46.2 (C27, C29), 28.4 (C37, C38, C39), 27.7 (C25); IR (neat) ν 3327, 2979, 2930, 2741, 2365, 1690, 1601, 1528, 1449, 1248, 1233, 1193, 1164, 1117 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{29}H_{36}N_4O_4]$ 504.2737, found 504.2734.

***Tert*-butyl methyl(2-(methyl(3-(2-(3-(naphthalen-2-yl)ureido)phenoxy)propyl)amino) ethyl) carbamate (32)**

Bromide **53** (200 mg, 0.50 mmol), *N*-Boc-*N,N'*-dimethylethylenediamine **39** (200 mg, 1.0 mmol), K_2CO_3 (850 mg, 6.11 mmol) and KI (450 mg, 2.69 mmol) were added to a 50 mL flame-dried round bottom flask under Ar followed by dry THF (40 mL). The reaction was stirred for 48 hr at 80 ^{o}C while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was evaporated and the residue redissolved in EtOAc (50 mL). The organic layer was washed with 5% aqueous HCl, water and brine before being dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using a gradient (Pet. spirit:EtOAc, 70:30 to 100% EtoAc followed by EtOAc:MeOH, 90:10) to yield **32** (215 mg, 85%) as a pale yellow powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.28; Mp 94-97 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 9.27 (s, 1H, H11), 8.35 (s, 1H, H13), 7.85 (d, *J* = 7.5 Hz, 1H, H19), 7.78 (d, *J* = 7.5 Hz, 1H, H3), 7.71 (d, *J* = 7.5 Hz, 1H, H6), 7.54 (d, *J*= 7.5 Hz, 1H, H8), 7.45 (d, *J* = 7.5 Hz, 2H, H1), 7.42 (d, *J* = 7.5 Hz, 1H, H7), 7.40 (d, *J* = 7.5

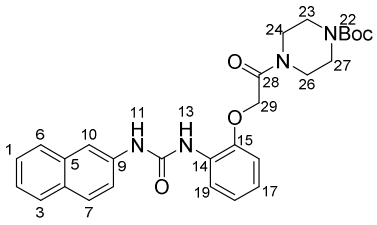


32

Hz, 1H, H2), 7.20 (d, *J* = 7.5 Hz, 1H, H16), 7.16 (s, 1H, H10), 7.10 (d, *J* = 7.5 Hz, 1H, H18), 7.08 (d, *J* = 7.5 Hz, 1H, H17), 4.04 (t, *J* = 7.1 Hz, 2H, H21), 3.27 (s, 4H, H29), 3.06 (t, *J* = 7.1 Hz, 3H, H27), 2.53 (t, *J* = 7.1 Hz, 2H, H26), 2.46 (t, *J* = 7.1 Hz, 2H, H23), 2.18 (s, 3H, H30), 1.82 (q, *J* = 7.1 Hz, 2H, H22), 1.42 (s, 9H, H37, H38, H39); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 155.6 (C15), 154.7 (C40), 152.9 (C12), 135.4 (C9), 133.7 (C5), 129.9 (C7), 127.8 (C17), 126.8 (C4), 126.5 (C3), 125.3 (C1), 124.6 (C6), 124.3 (C14), 121.4 (C2), 120.5 (C18), 119.9 (C8, C19), 116.7 (C10), 112.9 (C16), 79.8 (C36), 73.1 (C21), 55.6 (C26), 55.4 (C23), 52.4 (27), 46.9 (C30), 36 (C29), 28.4 (C37, C38, C39), 27.4 (C22); IR (neat) ν 3360, 2974, 2931, 2802, 2360, 1689, 1654, 1600, 1533, 1449, 1249, 1231, 1193, 1159, 1117 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{29}H_{38}N_4O_4]$ 506.2893, found 506.2904.

***Tert*-butyl 4-(2-(2-(3-(naphthalen-2-yl)ureido)phenoxy)acetyl)piperazine-1-carboxylate (25)**

To a solution of the phenolic urea **51** (200 mg, 0.72 mmol) in dry DMF (5 mL) in a 50 mL oven-dried round bottom flask under Ar was added Cs_2CO_3 (800 mg, 2.5 mmol). The mixture was stirred at 40 ^{o}C for 30 min before *N*-bromoacetyl-*N'*-Boc-piperazine **40** (200 mg, 0.65 mmol) in dry DMF (3 mL) was added dropwise over 5 min. The reaction was stirred at 80 ^{o}C for 16 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was removed *in vacuo* and the residue redissolved in EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography using a gradient (Pet. spirit:EtOAc, 50:50 to 100% EtOAc followed by EtOAc:MeOH, 95:5) to yield **25** (237 mg, 66%) as an off-white powder. R_f (pet. Spirit:EtOAc, 50:50) = 0.48; Mp 243-244 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 9.57 (s, 1H, H11), 8.36 (s, 1H, H13), 8.15 (d, *J* = 7.5 Hz, 1H, H19), 7.78 (d, 1H, *J* = 7.5 Hz, H3), 7.76 (d,

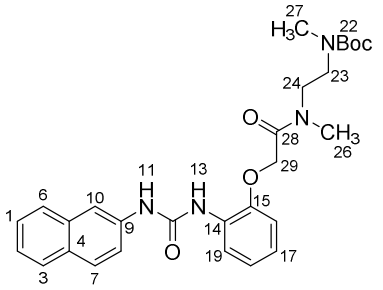


25

J = 7.5 Hz, 1H, H6), 7.72 (dd, *J* = 7.5 Hz, 2H, H7, H8), 7.38, 7.43 (dt, *J* = 7.4 Hz, 2H, H1,H2), 7.32 (d, *J* = 7.5 Hz, 1H, H17), 6.94-6.98 (m, *J* = 7.5 Hz, 3H, H10, H16, H18), 4.51 (s, 2H, H29), 3.68-3.31 (m, J = 7.11, 4H, H23, H24), 3.01 (s, 1H, H27), 2.93 (s, 1H, H26), 1.41 (s, 9H, H22); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 166.0 (C28), 153.7 (C22, C=O), 152.5 (C12), 146.8 (C15), 138.4 (C9), 137.5 (C5), 133.8 (C4), 129.0 (C14), 128.4 (C7), 127.5 (C17), 127.3 (C3), 126.8 (C1), 126.3 (C6), 123.8 (C2), 121.7 (C18), 121.1 (C19), 119.4 (C8),114.8 (C10), 113.2 (C16), 79.1 (C22), 66.5 (C27), 54.8 (C23), 43.8 (C26), 41.1 (24), 36.2 (C29), 27.9 (C22); IR (neat) ν 3363, 2984, 2925, 2360, 1707, 164, 1629, 1601, 1540, 1507, 1430, 1241, 1232, 1193, 1158, 1128, 1119 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{28}H_{32}N_4O_5]$ 504.2373, found 504.2377.

***Tert*-butyl methyl(2-(N-methyl-2-(2-(3-(naphthalen-2-yl)ureido)phenoxy)acetamido) ethyl) carbamate (33)**

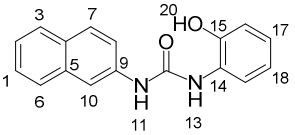
To a stirring solution of the phenolic urea **51** (200 mg, 0.72 mmol) in dry DMF (5 mL) in a 50 mL oven-dried round bottom flask under Ar was added Cs_2CO_3 (1.0 g, 3.06 mmol). The mixture was stirred at 40 ^oC for 30 min before adding *N*-bromoacetyl-*N*'-Boc-*N*,*N*'-dimethylethylene diamine **41** (200 mg, 0.64 mmol) in DMF (3 mL) was added dropwise over 5 min. The reaction was heated to 80 ^oC and stirred while monitoring by TLC (Pet. spirit:EtOAc, 50:50). The solvent was removed and the residue redissolved in EtOAc. The organic layer was washed with water and brine and the aqueous layer back-extracted with EtOAc (50 mL). The pooled organic fractions were dried over anhydrous $MgSO_4$ and concentrated. The crude residue was purified by silica gel column chromatography using a gradient (Pet. spirit:EtOAc, 70:30 to 100% EtoAc followed by EtOAc:MeOH, 95:5) to afford **33** (290 mg, 80%) as an



33

off-white powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.22; Mp 110-113 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.70 (s, 1H, H11), 8.50 (s, 1H, H13), 8.43 (d, J = 7.5 Hz, 1H, H19), 8.22 (s, 1H, H10), 7.81-7.72 (m, J = 7.5 Hz, 3H, H3, H6, H2), 7.42 (dd, J = 7.4 Hz, 2H, H7, H8), 7.33 (t, J = 7.4 Hz, 1H, H1), 7.04 (t, J = 7.5 Hz, 1H, H17), 6.95 (t, J = 7.5 Hz, 1H, H18), 6.81 (d, J = 7.5 Hz, 1H, H16), 4.52 (s, 2H, H29), 3.70-3.30 (m, J = 7.11, 4H, H23, H24), 3.1 (s, 1H, H27), 2.95 (s, 1H, H26), 1.41 (s, 9H, H22); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 168.2 (C28), 160.1 (C22), 157.3 (C12), 153.2 (C15), 146.2 (C9), 137.4 (C5), 134.5 (C7), 132.2 (C4), 130.0 (C14), 128.6 (C17), 127.6 (C3), 126.4 (C1), 124.2 (C6), 122.9 (C2), 121.9 (C18), 119.7 (C19), 119.2 (C8), 114.4 (C10), 111.8 (C16), 79.6 (C36), 73.2 (C21), 55.9 (C26), 55.4 (C23), 52.3 (27), 46.8 (C30), 36.0 (C29), 28.5 (C37, C38, C39), 27.5 (C22); IR (neat) ν 3363, 2977, 2930, 2361, 1704, 1648, 1629, 1601, 1533, 1506, 1454, 1249, 1229, 1193, 1155, 1155, 1115 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{28}H_{34}N_4O_5]$ 506.2529, found 506.2518.

1-(2-Hydroxyphenyl)-3-(naphthalen-2-yl)urea (51)

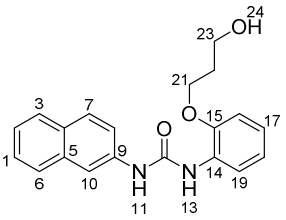


51

O-Aminophenol (4.0 g, 36.7 mmol) was added to a solution of 2-naphthaleneisocyanate **50** (4.00 g, 23.6 mmol) in dry CH_2Cl_2 (100 mL) in one portion. The reaction was stirred at room temperature for 48 hr while monitored by TLC (Pet. spirit:EtOAc, 50:50). The white precipitate was filtered, washed with dry CH_2Cl_2:Et_2O, 50:50 (2 x 100 mL) and dried *in vacuo*. The residue was additionally washed with 1M aqueous HCl in cases where *O*-aminophenol was observed in the ^{1}H NMR spectrum of the product. Compound **51** (5.20 g, 80%) was obtained as an off-white powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.48; Mp 163-165 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 9.98 (s, 1H, H20), 9.50 (s, 1H, H11), 8.24 (s, 1H, H13), 8.12 (s, 1H, H10), 8.08 (d, 1H, J = 9.8 Hz, H3), 7.82 (d, J = 10.0 Hz, 1H, H6), 7.80 (d, J = 10.0 Hz, 1H, H19), 7.78 (d, J = 10.0 Hz, 1H, H8), 7.45, 7.40 (dd, J = 10.0 Hz, 2H, H1,

H2), 7.33 (d, *J* = 9.8 Hz, 1H, H17), 6.86 (d, *J* = 10.0 Hz,1H, H7), 6.96, 6.98 (dd, *J* = 10.0 Hz, 2H, H16, H18); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 153.3 (C12), 146.3 (C15), 138.3 (C9), 134.5 (C5), 129.7 (C4), 129.1 (C3), 128.5 (C14), 128.1 (C17), 127.6 (C1), 127.0 (C6), 122.5 (C2), 120.1 (C19), 119.3 (C8), 115.1 (C10), 113.6 (C16); IR (neat) ν 3669, 3316, 2978, 2923, 1701, 1654, 1602, 1534, 1460, 1250, 1220 cm^{-1}; HRESI-MS: *m/z* calcd for $[C_{17}H_{14}N_2O_2]$ 278.1055, found 278.1073.

1-(2-(3-Hydroxypropoxy)phenyl)-3-(naphthalen-2-yl)urea (52)



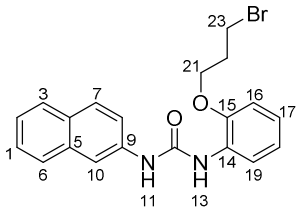
52

Phenolic urea **51** (1.0 g, 3.6 mmol) and KOH (1.20 g, 8.63 mmol) were added to a 50 mL oven-dried round bottom flask under argon. Anhydrous DMF (7 mL) was added and the mixture cooled to 0 ^{o}C before adding 3-bromopropanol (600 mg, 4.3 mmol) over 20 min. The reaction was stirred at room temperature for 16 hr while monitoring by TLC (Pet. spirit:EtOAc, 50:50). DMF was evaporated and the residue purified by silica gel column chromatography using a gradient of EtOAc:Pet. spirit 30:70 to 100 % EtOAc. The product isolated was redissolved in EtOAc (250 mL) and washed with 1 M KOH and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to yield **52** (904 mg, 75%) as an off-white powder. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.32; Mp 145-146 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 9.62 (s, 1H, H11), 8.18 (d, *J* = 7.5 Hz, 1H, H19), 8.15 (s, 1H, H13), 8.14 (s, 1H, H10), 7.84 (d, *J* = 7.4 Hz, 1H, H6), 7.80 (dd, J = 7.5 Hz, 2H, H8, H3), 7.51 (d, J = 7.5 Hz, 1H, H7), 7.43 (t, *J* = 7.5 Hz, 1H, H2), 7.34 (t, *J* = 7.5 Hz, 1H, H1), 7.02 (d, *J* = 7.5 Hz,1H, H16), 6.92 (dd, *J* = 7.5 Hz, 2H, H17, H18), 4.70 (s, *J* = 6.5 Hz, 1H, H24), 4.27 (t, *J* = 6.5 Hz, 2H, H21), 3.65 (t, *J* = 6.5 Hz, 2H, H23), 1.97 (q, *J* = 6.5 Hz, 2H, H22); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 155.4 (C15), 149.9 (C12), 140.3 (C9), 136.6 (C5), 131.9 (C6), 131.6 (C14), 131.3 (C7), 130.3 (C17), 129.85 (C4), 129.2 (C3), 126.8 (C1),

124.8 (C2), 123.3 (C18), 122.4 (C8), 121.7 (C19), 116.2 (C10), 114.6 (C16) 68.4 (C21), 60.3 (C23) 34.9 (C22); IR (neat) ν 3672, 3318, 2977, 2921, 1699, 1653, 1601, 1534, 1461, 1251, 1222 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{20}H_{20}N_2O_3$] 336.1474, found 336.1482.

1-(2-(3-Bromopropoxy)phenyl)-3-(naphthalen-2-yl)urea (53)

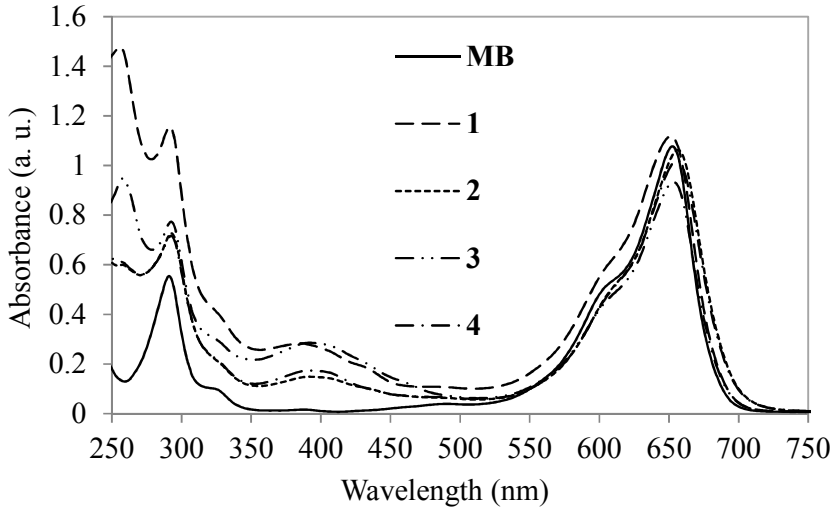
CBr_4 (3.10 g, 9.0 mmol) was added to a solution of alcohol **52** (800 mg, 2.4 mmol) in dry THF (30mL) in a 50 mL flame-dried round bottom flask under Ar. After 30 min stirring at room temperature PPh_3 (2.50 g, 9.5 mmol) was added in one portion and stirring continued at room temperature for a further 16 hr. The solvent was removed under reduced pressure and the residue redissolved in CH_2Cl_2 (45 mL). The organic layer was washed with 1% KOH, water and brine and dried over anhydrous Na_2SO_4. The crude residue was purified by silica gel column chromatography using Pet. spirit:EtOAc 90:10 to afford **53** (800 mg, 85%) as grey crystals. TLC R_f (Pet. spirit:EtOAc, 50:50) = 0.76; Mp 122-123 ºC; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 9.58 (s, 1H, H11), 8.17 (d, J = 7.5 Hz, 1H, H19), 8.13 (s, 1H, H13), 8.11 (s, 1H, H10), 7.84 (d, J = 7.5 Hz, 1H, H6), 7.81 (dd, J = 7.4 Hz, 2H, H8, H3), 7.52 (d, J = 7.5 Hz, 1H, H7), 7.45 (t, J = 7.5 Hz, 1H, H2), 7.36 (t, J = 7.5 Hz, 1H, H1), 7.05 (d, J = 7.6 Hz, 1H, H16), 6.94 (dd, J = 7.4 Hz, 2H, H17, H18), 4.18 (t, J = 6.5 Hz, 2H, H21), 3.80 (t, J = 6.5 Hz, 2H, H23), 2.35 (q, J = 6.5 Hz, 2H, H22); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 156.1 (C15), 149.7 (C12), 139.9 (C9), 136.3 (C5), 132.8 (C6), 131.9 (C14), 131.0 (C7), 129.5 (C17), 129.0 (C4), 128.8 (C3), 126.9 (C1), 124.7 (C2), 123.0 (C18), 121.9 (C8), 120.9 (C19), 115.8 (C10), 114.01 (C16) 64.8 (C21), 34.5 (C23) 29.1 (C22); IR (neat) ν 3317, 2996, 2927, 1696, 1655, 1602, 1534, 1250, 1221 cm^{-1}; HRESI-MS: *m/z* calcd for [$C_{20}H_{19}{}^{79}BrN_2O_2$] 397.0552, found 397.0566.



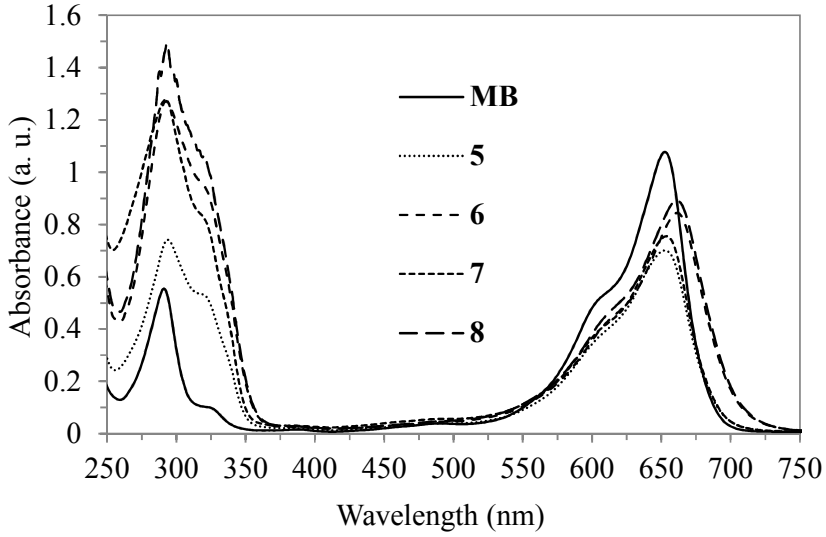
53

Section 2 – UV/Visible spectroscopy

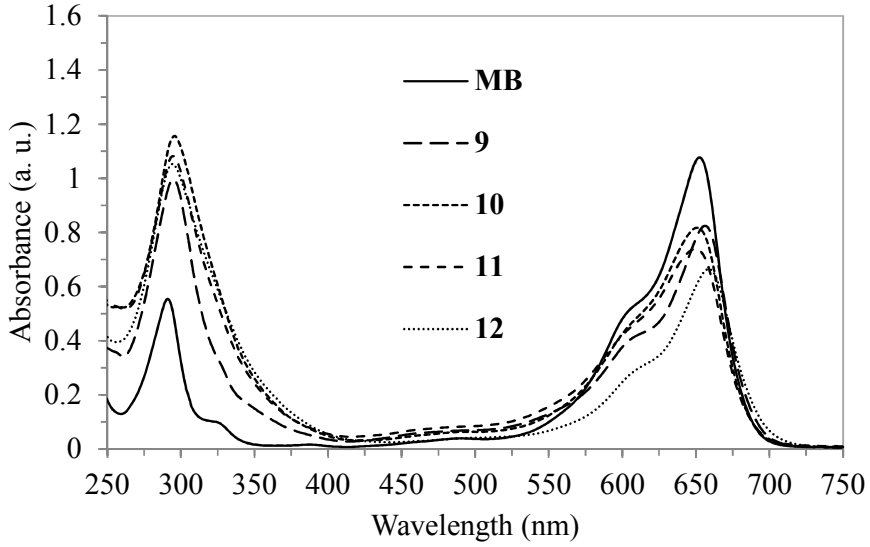
(a)



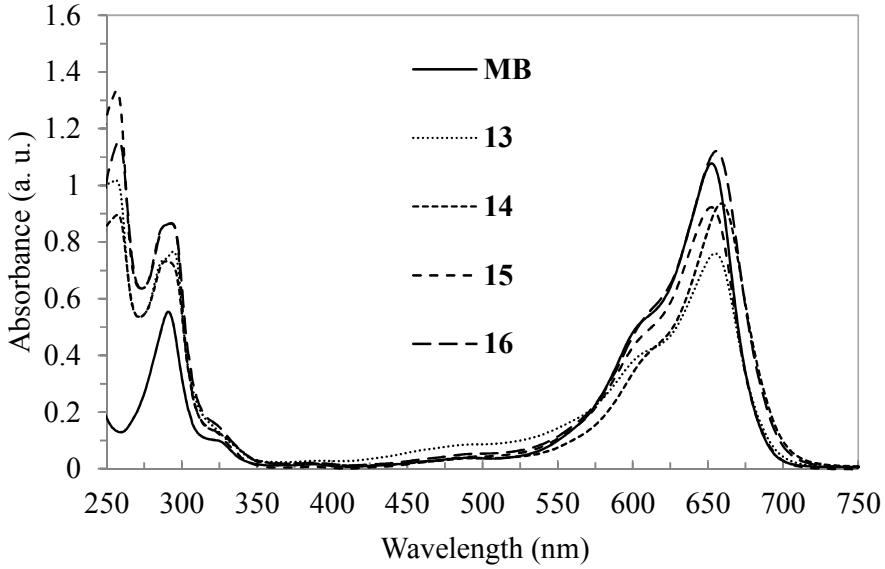
(b)



(c)



(d)

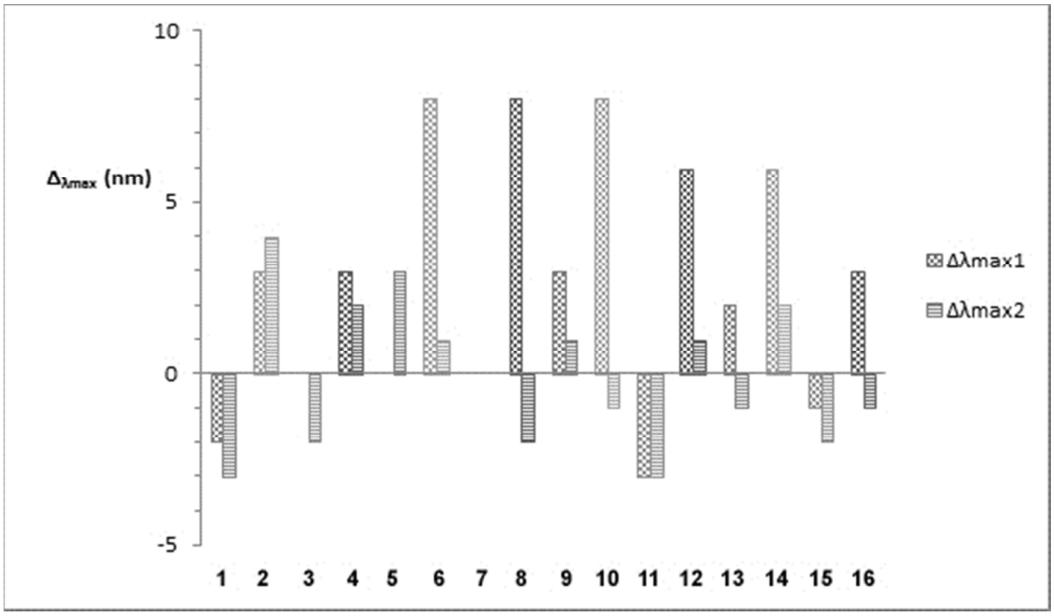


Supplementary Figure 5. UV/visible absorption spectra of **(a)** reserpine-EPI-MBs **1-4 (b)** pterostilbene-EPI-MBs **5-8 (c)** INF55-EPI-MBs **9-12** and **(d)** INF271-EPI-MBs **13-16**. Spectra were recorded on 10 μM solutions at 23 ^{o}C in MeOH.

(a)

Compound	λ_{max1} [nm](log ε)	λ_{max2} [nm](log ε)
MB	653 (4.04)	612 (3.73)
1	651 (4.05)	609 (3.79)
2	656 (4.02)	616 (3.74)
3	653 (3.97)	610 (3.68)
4	656 (4.00)	614 (3.72)
5	653 (3.84)	615 (3.65)
6	661 (3.93)	613 (3.61)
7	653 (3.91)	612 (3.68)
8	661 (3.95)	610 (3.64)
9	656 (3.91)	613 (3.68)
10	651 (3.91)	611 (3.69)
11	650 (3.86)	609 (3.66)
12	659 (3.82)	613 (3.48)
13	655 (3.88)	611 (3.62)
14	659 (3.97)	614 (3.63)
15	652 (3.96)	610 (3.68)
16	656 (4.05)	611 (3.74)

(b)



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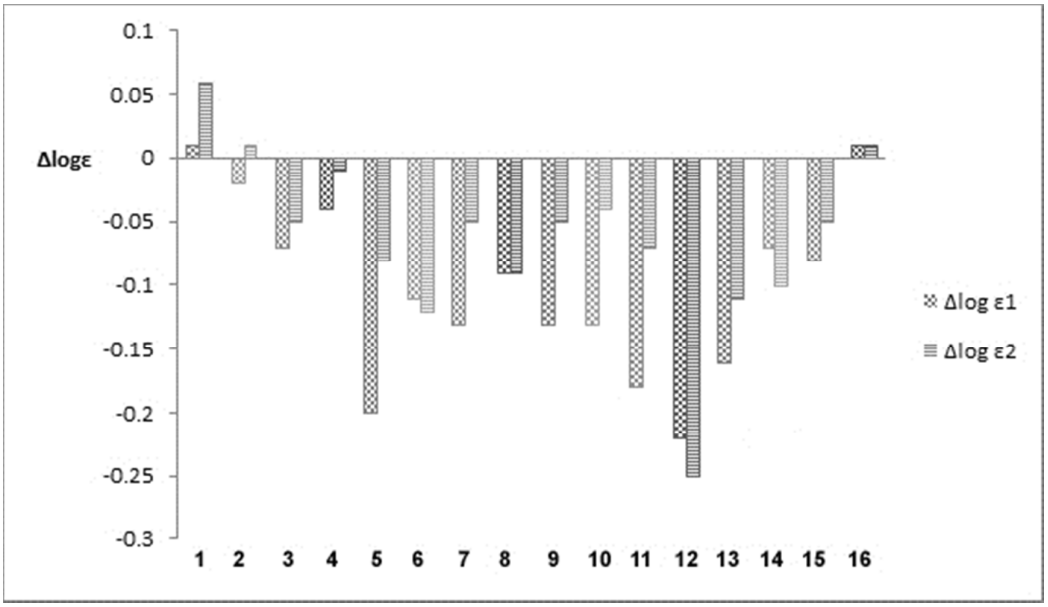
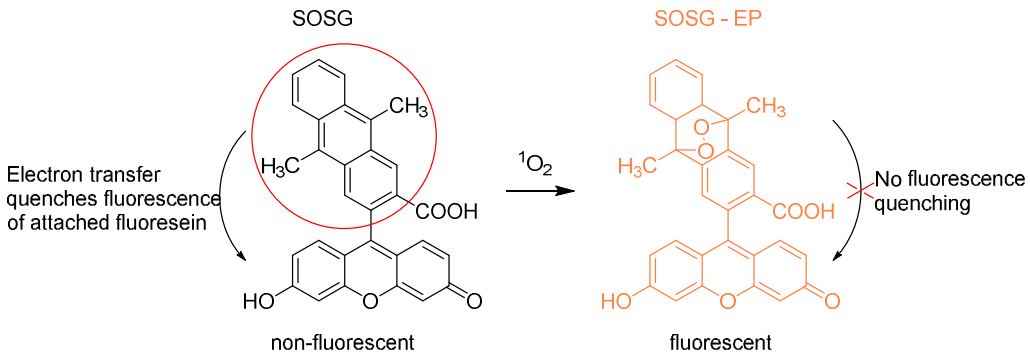


Supplementary Figure 6. (a) λ_{max1}, λ_{max2} wavelength and extinction coefficient (log ε) data for MB and hybrids **1-16**. **(b)** $\Delta\lambda_{max1}$ and $\Delta\lambda_{max2}$ data for **1-16** relative to MB. $\Delta\lambda$ values were calculated by subtracting the λ_{max1} and λ_{max2} wavelengths for MB from the corresponding hybrid values. **(c)** ΔLog ε data for **1-16** relative to MB. ΔLog ε values were calculated by subtracting the log ε values for MB at λ_{max1} and λ_{max2} (i.e. log ε1 = 4.04, log ε2 = 3.73) from the corresponding values for hybrids.

Section 3 – $^1O_2$ and •OH experiments

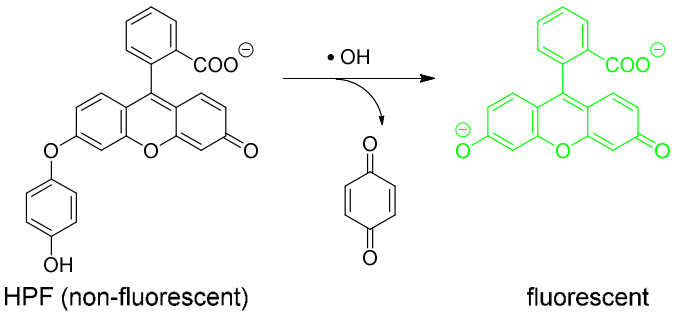
The SOSG probe shows high selectivity for singlet oxygen and is water soluble at mM concentrations. The structure of the probe has not been released by the supplier (Invitrogen) but is believed to be bichromophoric, invoking a photoinduced electron transfer (PET) design comprising a dimethyl anthracene moiety attached to fluorescein (**Supplementary Figure 7**). Electron transfer processes quench fluorescence emission from the fluorescein portion of SOSG. In the presence $^1O_2$, the anthracene moiety undergoes reaction to form an SOSG endoperoxide (SOSG-EP) that disrupts the conjugated *p*-dimethylaryl system, allowing the attached photoexcited fluorescein to relax via fluorescence. The reaction is irreversible and the fluorescence signal is proportional to the singlet oxygen concentration in solution.[9]



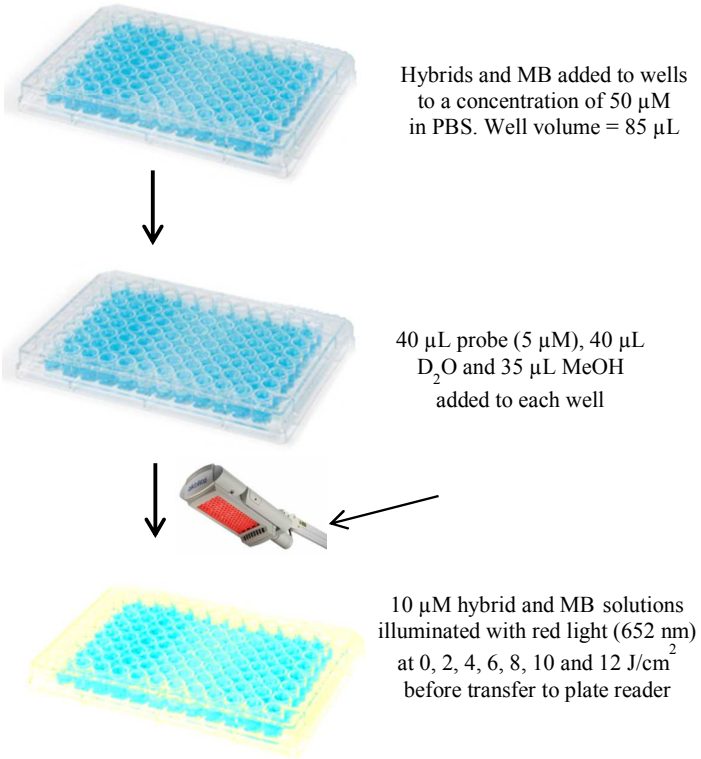
Supplementary Figure 7. SOSG fluoresces in the presence of $^1O_2$ after forming SOSG - EP.

The HPF probe was developed by Setsukinai *et al* as a tool for stable and selective detection of •OH.[10] Although a related 3′-*p*-(aminophenyl) fluorescein (APF) probe produces ~ 5-fold greater fluorescence than HPF in the presence of •OH, HPF is substantially less sensitive to $^1O_2$ making it more selective for •OH and better suited for use in our

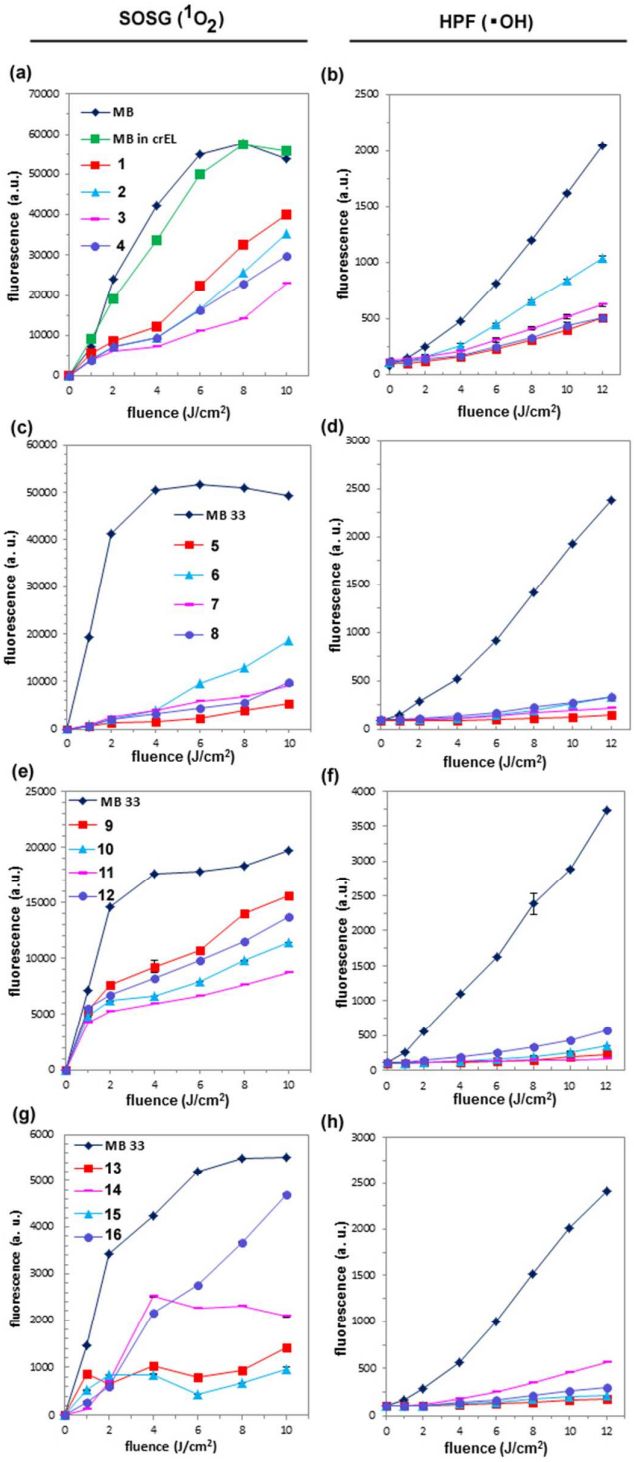
experiments.[11] In the presence of •OH, the non-fluorescent HPF molecule is oxidised, leading to expulsion of *p*-benzoquinone and a bright green fluorophore (Supplementary Figure 8).



Supplementary Figure 8. Reaction of HPF with •OH to produce a green fluorophore.



Supplementary Figure 9. Schematic summary of experiments for measuring $^1O_2$ and •OH generation by MB and hybrids **1-16** following illumination with 652 nm red light.



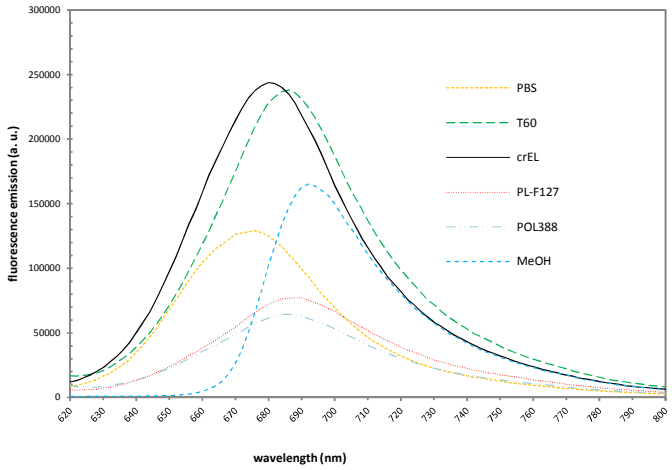
Supplementary Figure 10. Fluorescence measurements of $^1O_2$ and •OH generation following illumination (652 nm) of SOSG and HPF probes in the presence of: (a,b) reserpine-EPI-MBs **1-4**, (c,d) pterostilbene-EPI-MBs **5-8**, (e,f) INF55-EPI-MBs **9-12** and (g,h) INF271-EPI-MBs **13-16**. Data for MB are shown for comparison. $^1O_2$ production from MB formulated in cremophore EL® (CrEL) is also shown in **(a)**. Data represent the mean of 4-6 independent experiments ± SEM. Panels (a), (c), (e) and (g) show evidence for MB

photobleaching (i.e. signal plateau forming) at fluence >4 J/cm^2 in the SOSG experiments. Photobleaching was also apparent for compound **14**. No photobleaching was observed for MB or any hybrids in the HPF experiments over the fluence range tested.

Section 4 – *In vitro* screening of hybrids for photodynamic inactivation of MRSA USA300

(a) Identification of CrEL as a sutable aqueous vehicle

Solubilisation of reserpine-EPI-MB **3** into aqueous micelles was examined using the surfactants tween 60 (T60), cremophor EL (CrEL) and triblock copolymers F127 (PL-F127) and poloxamer 388 (POL388). Compound **3** was dissolved in CH_2Cl_2 at 2-3 mM and the solution was added to a separate solution of each detergent in CH_2Cl_2 (final concentration 100 mg/mL). The combined mixtures were sonicated until a homogenous solution was obtained. The organic solvent was removed under reduced pressure and the remaining thin films redissolved in PBS to create homogenous 500 μM aqueous micellar solutions. Fluorescence emission spectra of 10 μM micellar solutions of **3** were recorded. A large increase in emission intensity at 680 nm (excitation 600 nm) was observed with **3** formulated in CrEL and T60 compared to the other detergents, PBS or MeOH. The increased fluorescence observed with CrEL and T60 suggested that these surfactants minimise aggregation, making them suitable vehicles for aqueous solubilisation of **3** and, by inference, all other hybrids.

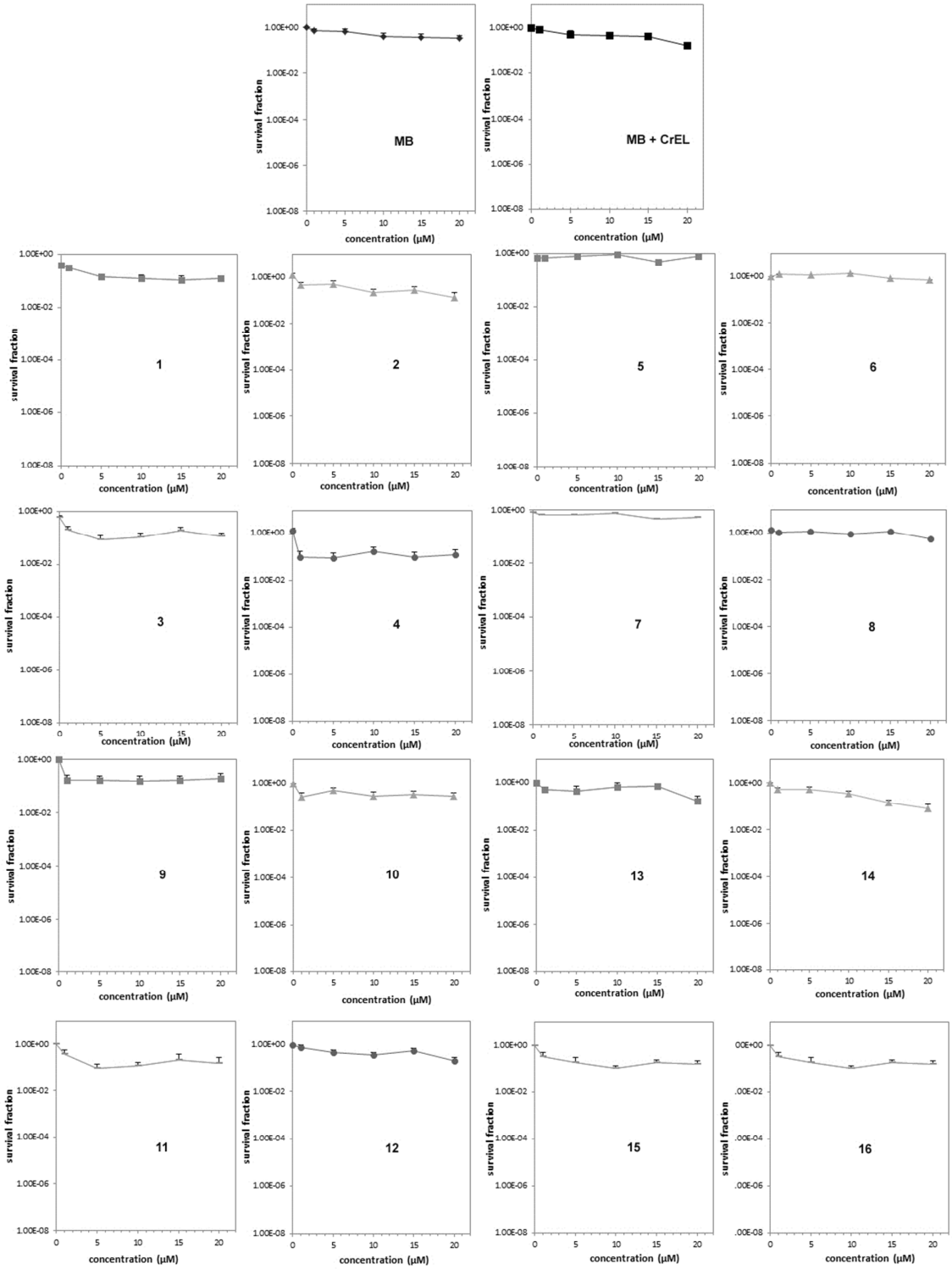


Supplementary Figure 11. Fluorescence emission spectra (excitation 600 nm) of reserpine-EPI-MB **3** formulated in aqueous micelles using tween 60 (T60), cremophor EL (CrEL), triblock copolymers F127 (PL-F127) and poloxamer 388 (POL388). Spectra recorded in PBS and MeOH are shown for comparison.

(b) Preparation of CrEL micellar solutions

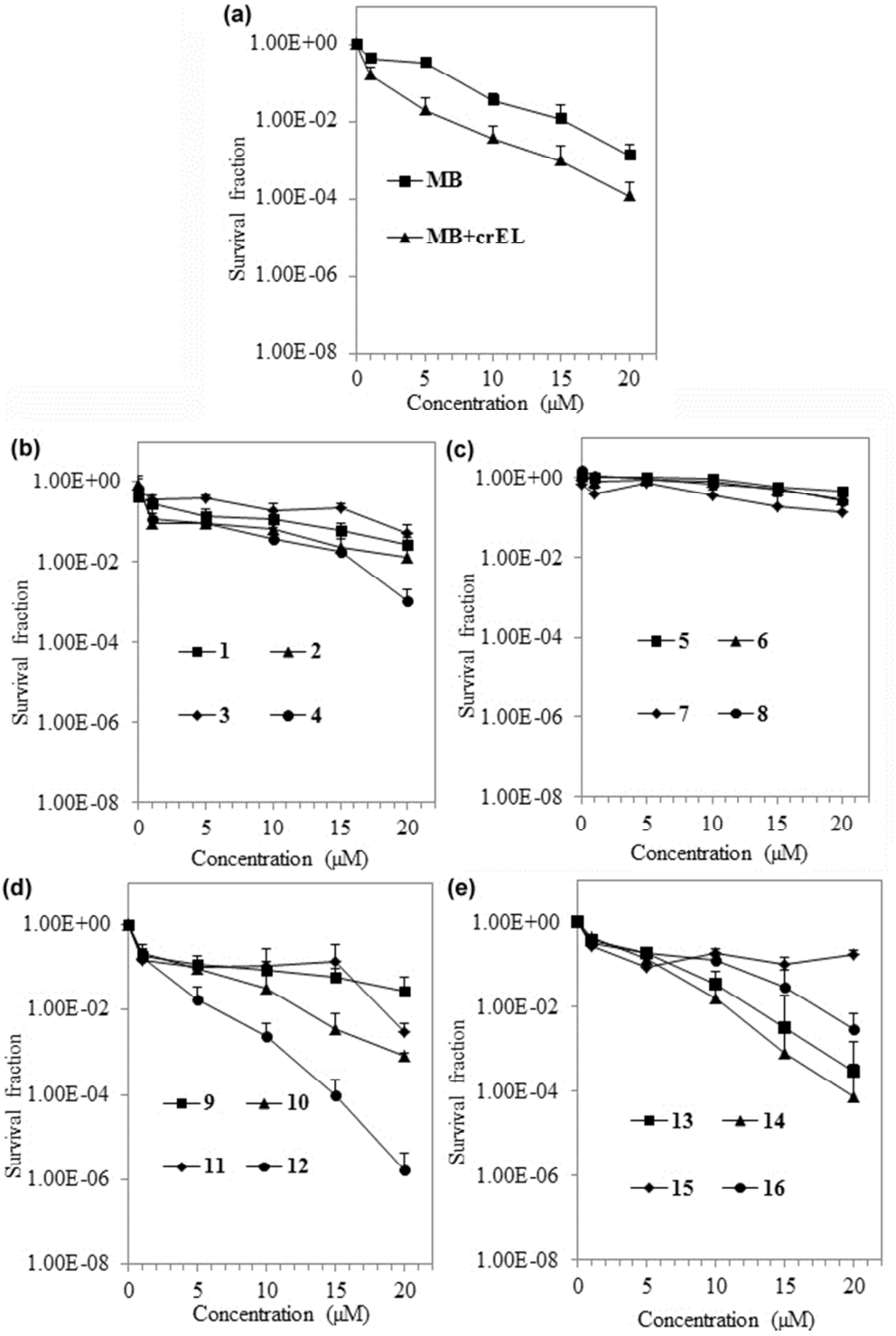
Mixtures were prepared by combining solutions of 2-3 mM hybrids **1-16** (or MB) in dry CH_2Cl_2 with 500 mL CrEL solutions (100 mg/mL in dry CH_2Cl_2).The combined mixture was stirred and sonicated until a homogenous solution was obtained. The solvent was removed *in vacuo* and the resulting dry film redissolved in 1 mL of sterile PBS, with agitation. The micellar hybrid solutions were filtered through a 0.22 µm mixed-cellulose-ester (MCE) filter under sterile conditions before addition to cellular and *in vivo* assays.

(c) Dark toxicitiy against MRSA USA300



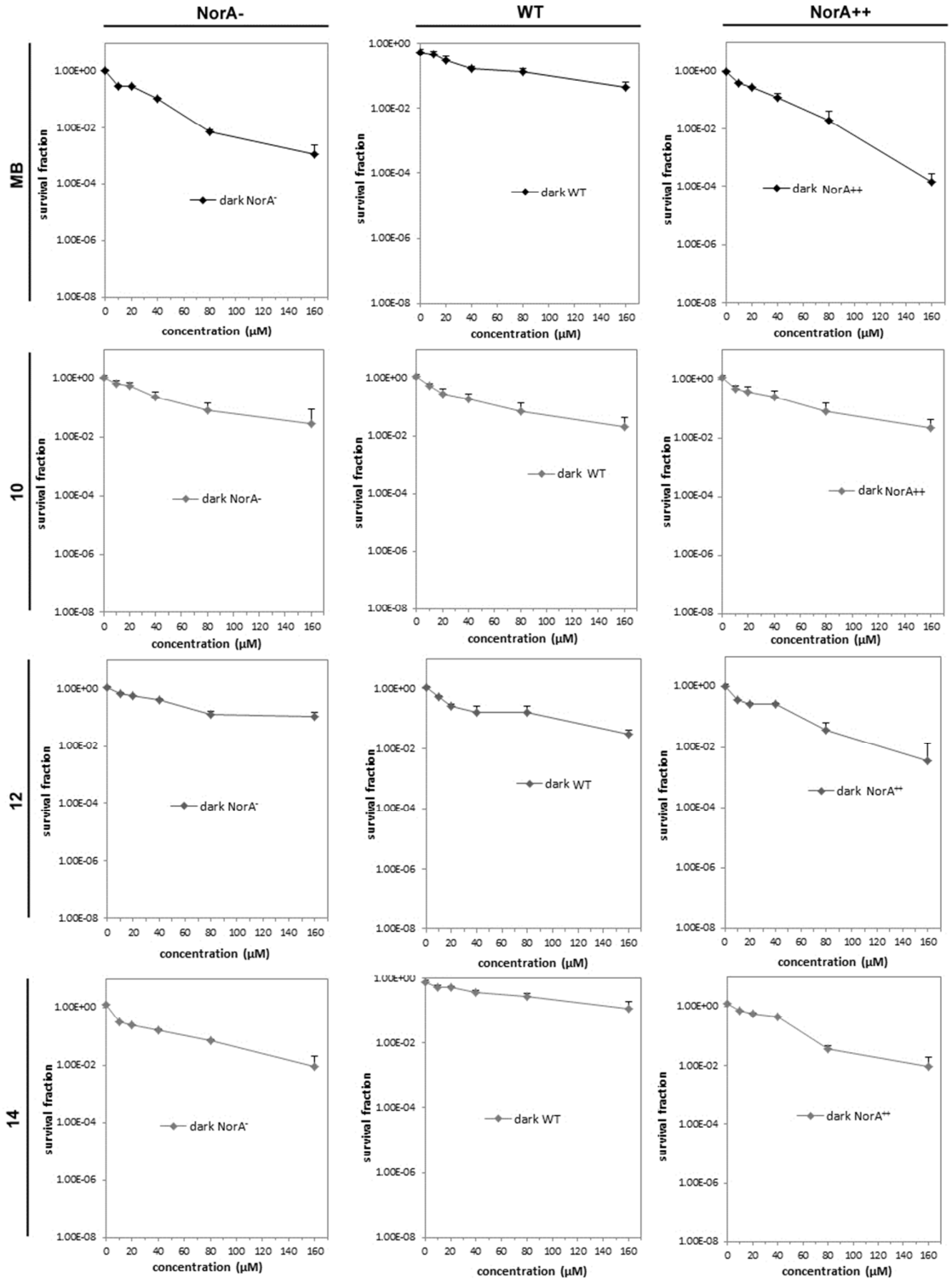
Supplementary Figure 12. Dark toxicity of MB and **1-16** against *S. aureus* MRSA USA300. Data represent the mean of three independent experiments ± SEM.

(d) Photodynamic inactivation of MRSA USA300



Supplementary Figure 13. Photodynamic inactivation of MRSA USA300 by hybrids: (a) MB in PBS/MeOH and CrEL (b) **1-4**, (c) **5-8** (d) **9-12** and (e) **13-16** over the concentration range 1-20 μM. Data represent the mean of three independent experiments ± SEM.

Section 5 – Photodynamic inactivation of *S. aureus* isogenic NorA efflux mutants



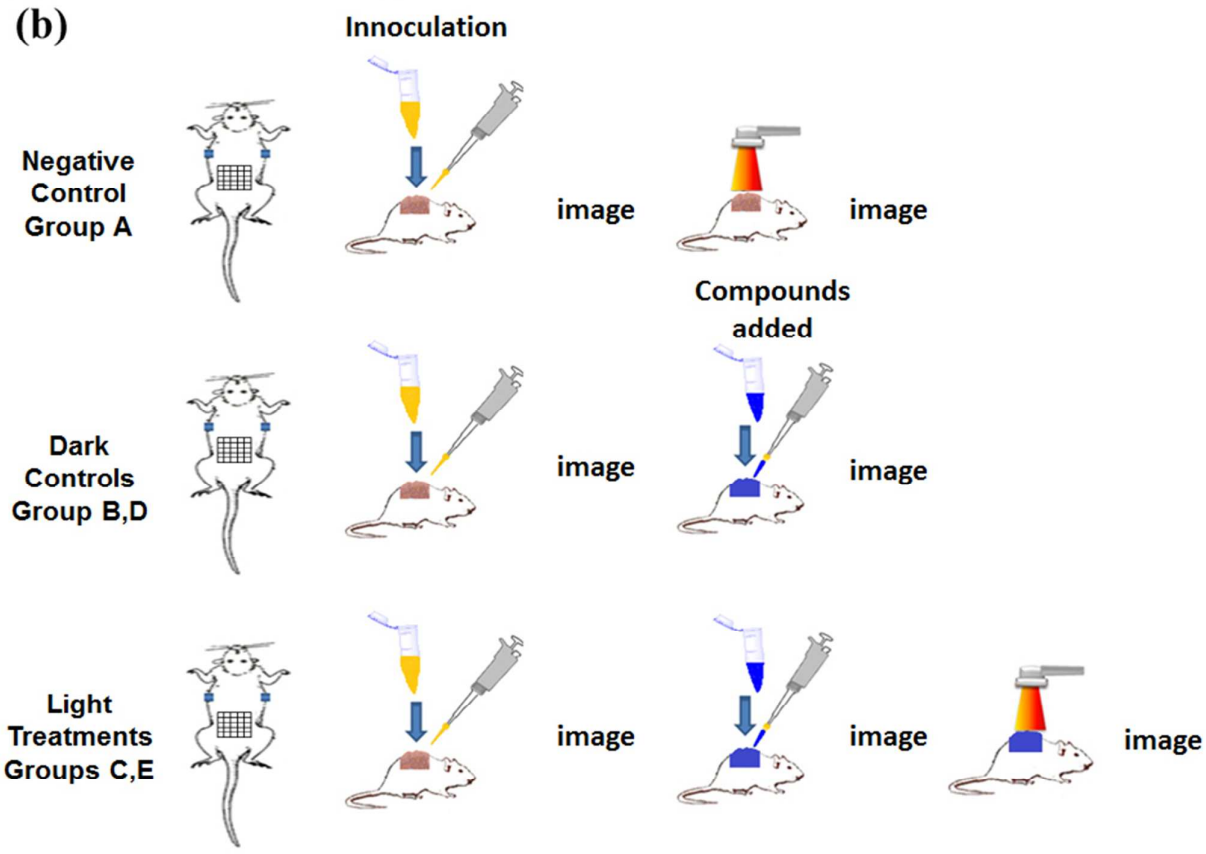
Supplementary Figure 14. Dark toxicity of MB, **10**, INF55(Ac)en-MB **12** and **14** against NorA-, WT and NorA++ *S. aureus* strains. Data represent the mean ± SEM from three independent experiments.

Section 6 – Murine MRSA wound infection aPDI model

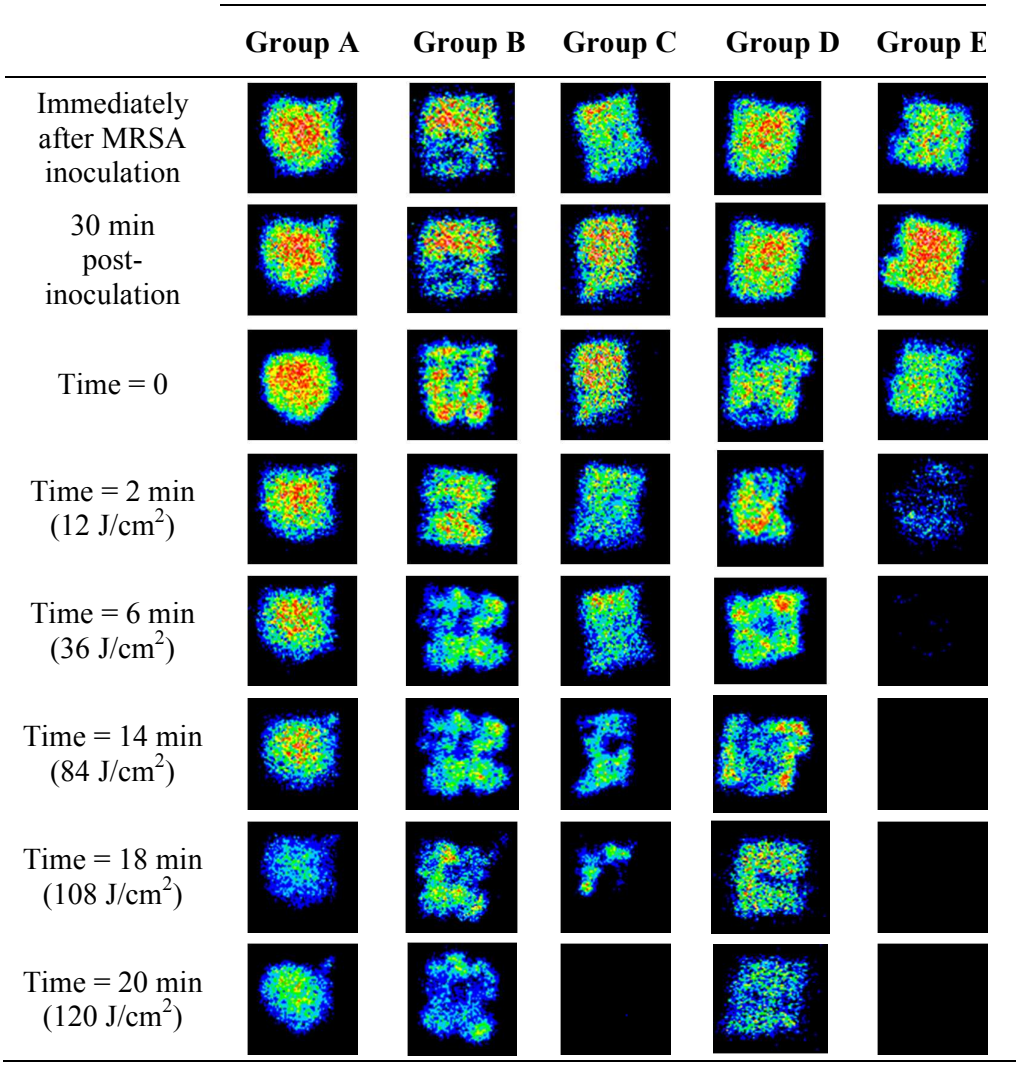
(a)

Group	Animals	Treatment
A Negative control	6	No compound + light (12, 36, 84, 108, 120 J/cm^2)
B MB dark control	6	MB (40 µL of 200 µM soln.) + no light
C aPDT with MB	6	MB + light (12, 36, 84, 108, 120 J/cm^2)
D 12 Dark control	6	12 (40 µL of 200 µM soln.) + no light
E aPDT with 12	6	12 + light (12, 36, 84, 108, 120 J/cm^2)

(b)



Supplementary Figure 15. (a) Animal cohorts and treatments used in murine MRSA wound infection experiments. (b) Illustrated summary of the experiment.



Supplementary Figure 16. Bioluminescence images captured from representative animals in Groups A-E during the 'light-treatment' phase of the experiment. Images were recorded at the time of MRSA Xen30 inoculation and at the intervals indicated. Application of 652 nm light aliquots at the times indicated corresponded to fluences shown in parentheses.

Supplementary Figure 17. Bioluminescence images captured from representative animals in Groups A-E during 10 day post-treatment monitoring.

Supporting Information − References

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