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SYNTHESIS OF PROBE MOLECULES, 6-(DIMETHYLAMINO)-2-PHENYLISOINDOLIN-1-ONES, FOR MECHANISTIC STUDIES OF FIREFLY LUCIFERASE INHIBITION

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Abstract – Firefly luciferase is used in high-throughput screening based on the detection of chemiluminescence. It catalyzes an esterification reaction of luciferin with adenosine 5'-triphosphate (ATP) followed by decarbonylation with oxygen and concomitance of light. Previously, we reported that firefly luciferase also possesses acyl-CoA synthetase activity and catalyzes an aromatic carboxylic acid group of F-53, using ATP, Mg^{2+} and coenzyme A (CoA), to produce F-53 covalently attached to active-site lysine-529 residue of firefly luciferase through the formation of an amide group. The amidation of lysine-529 resulted in a deactivation of luciferase. In order to probe firefly luciferase inhibition's mechanism, we synthesized two probe molecules **1** and **2**, mimicking F-53. Molecule **1** contains an azido-appended side chain in the aromatic ring of F-53, while **2** possesses an azido and a carboxylic acid group appended side chains. Both synthetic schemes are readily amenable to large-scale syntheses. Molecule **1** was made from 2-allylaniline, which was derived from a thermal-induced aromatic-Claisen rearrangement of *N*-allylaniline. The azido-appended side chain of **2** was installed from a Horner-Wadsworth-Emmons reaction and the carboxylic acid side chain from a Sonogashira reaction.

INTRODUCTION

High-throughput screening (HTS) often uses a light-based method such as fluorescence or chemiluminescence for the detection of activities of a library of molecules targeting specific proteins or cells. Fluorescence assay detects the excited state of a fluorophore molecule through the absorption of irradiated light while chemiluminescence assay detects the emitted light through the energy produced from an exothermic chemical reaction.[1] In the latter assay, no assay background is present since no light introduces to the system. Although the brighter fluorescence light detection is preferred over lower light emitted chemiluminescence, a number of issues including higher assay background and presence of interfering fluorophores in the biological samples or in the library compounds, have made chemiluminescence detection an alternative.

Firefly luciferase, a 61-kDa enzyme, is commonly used in the chemiluminescence assay. It activates substrate luciferin (Figure 1) with adenosine 5′-triphosphate (ATP) co-enzyme to give luciferyl-AMP as an enzyme-bound complex, which subsequently reacts with oxygen to form oxyluciferin, a high-energy excited state enzyme-bound intermediate.[1] The high-energy state species undergoes relaxation to the ground state and concomitantly yields yellow-green light, a chemiluminescence. In addition to the aforementioned light emitting reaction, firefly luciferase also catalyzes fatty acyl-CoA synthesis.[2] It promotes the coupling of long-chain fatty acids and ATP in the presence of Mg^{2+} to give fatty acyl-AMP, which undergoes thioesterification with CoASH.[2,3] The resulting fatty acyl-CoA intermediate can react with various nucleophiles in the cells thereby producing acylated materials.[4,5] Suggestively, luciferase catalyzes the thioesterification of certain carboxylic acids *via* a mechanism similar to that of acyl-CoA synthetase.[6] Previously, it was found that molecule F-53 (Figure 1) inactivated luciferase by acylating the regulatory lysine residue 529 in live mammalian cells and it didn’t inactivate luciferase by acylating the regulatory lysine residue 529 *in vitro* reconstruction system.[7] Presumably, the carboxylic acid group of F-53 activates by luciferase to give the corresponding thioester F53-CoA, which in turn reacts with the amino function of lysine-529 residue of luciferase to give an amide, F53-Lys529(luciferase), catalyzed by some transferase(s) of acyl-CoA in cells.[7] Since Lys529 is needed in the enzymatic reactions,[8] this acylation resulted in an inhibition of luciferase. Carboxylic acid containing drugs including valproic acid, a drug for the treatment of bipolar disease, can be catalyzed by acyl-CoA synthetase using ATP, Mg^{2+} and CoASH to give the corresponding acyl-CoA thioester, which has been suggested to produce serious adverse effects.[9] However, the transferase(s) of acyl-CoA, active site(s), and mechanism remain to be unveiled. We therefore designed and synthesized two probe molecules, **1** and **2** (Figure 1), mimicking F-53, to investigate the interactive factors of firefly luciferase and acyl-CoA synthetase. We envision that the azido function of probe molecules will be used to react with an alkyne containing magnetic beads through a click coupling reaction. The resulting beads will be treated with target proteins or/and protein(s) responsible to the acyl-CoA transfer. Proteins that interact with the beads will be subjected to LC-MS-MS analysis for identification. Molecule **1**, a possible negative control, does not contain a carboxylic acid group and thereby may not bind to luciferase and/or acyl-CoA synthetase, while molecule **2**, possessing a carboxylic acid group, may bind to the enzymes. Herein, we described the synthesis of molecules **1** and **2**.

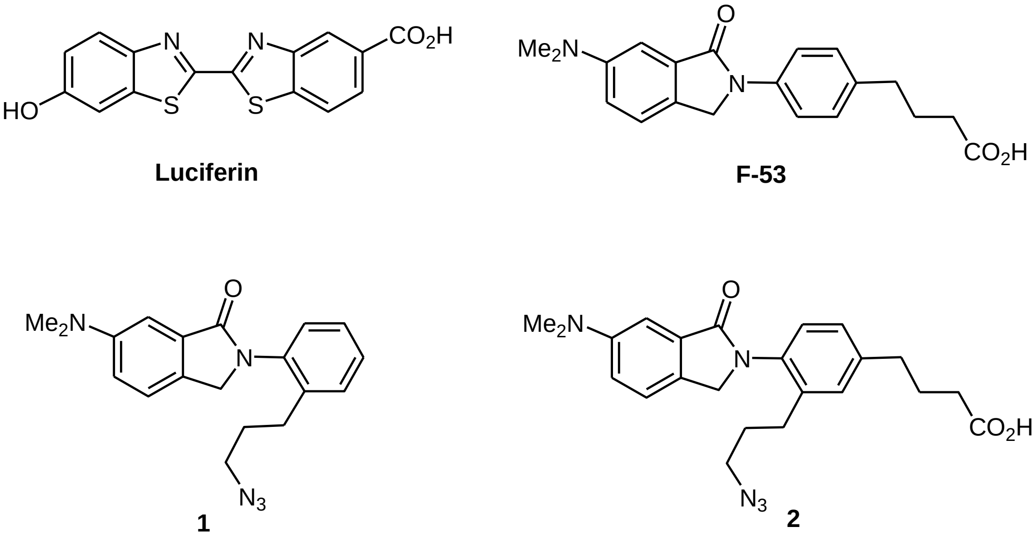

Luciferin **F-53** **1** **2**

Figure 1. Structures of luciferin, F-53, and designed probe molecules **1** and **2**

RESULTS AND DISCUSSION

A reported synthesis[7] of the isoindolinone ring was modified for the construction of the core fragment of **1** and **2**. However, installation of the two side chains on the aryl ring fragment of **2** exposed a challenge. The synthesis of **1** is straightforward and a retrosynthesis is outlined in Figure 2, where the 3-azidopropyl moiety on the aryl fragment of **1** would be prepared from **3**, containing an allyl group, which could readily be made from a coupling reaction of methyl 2-bromomethyl-5-nitrobenzoate (**4**) and 2-allylaniline (**5**).

The incompatibility of the C2’ and C4’ groups on the aryl fragment of **2** in the synthetic sequence led us to devise a retrosynthetic route depicted in Figure 2, where the C2’ 3-azidopropyl group would be made from 3-hydroxylpropyl and the C4’ 3-(hydroxycarbonyl)propyl group from 4-(*t*-butyldimethylsilyloxy)-1-butynyl group, as depicted in **6** and **7**, respectively. The attachment of C2’ 3-hydroxylpropyl group could be made from a Horner-Wadsworth-Emmons reaction of an aldehyde, derived from an alcohol, such as that in **8**. The C4’ 4-(*t*-butyldimethylsilyloxy)-1-butynyl group of **8** could be installed from a Sonogashira reaction of an aryl halide and 4-(*t*-butyldimethylsilyloxy)-1-butyne (**14**), and the isoindolinone ring of compound **8** could be synthesized from the coupling of **4** and 2-hydroxymethyl-4-iodoaniline (**9**).

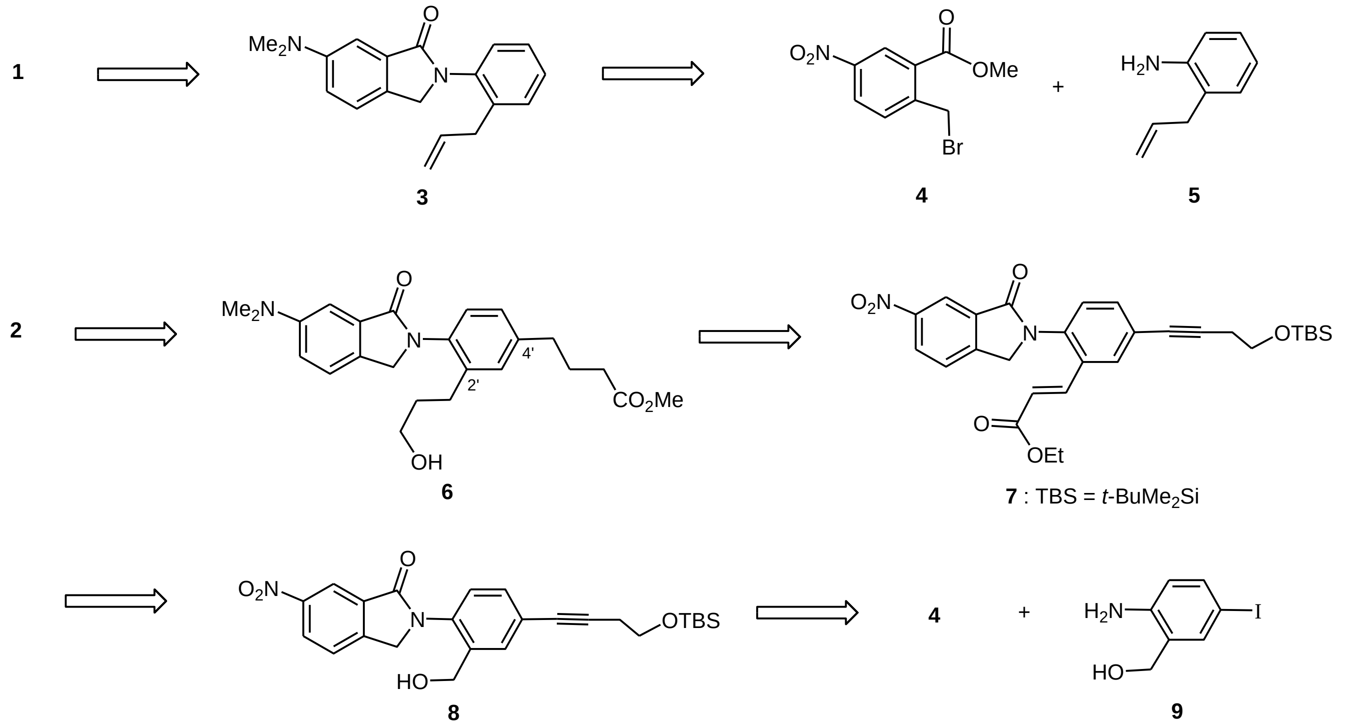
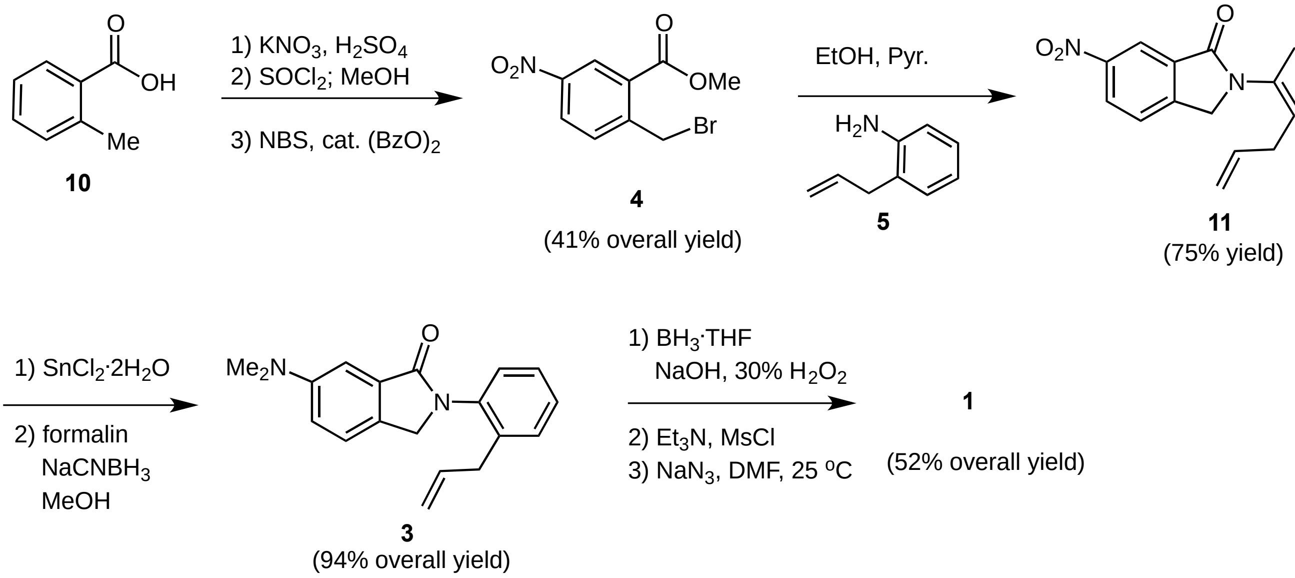


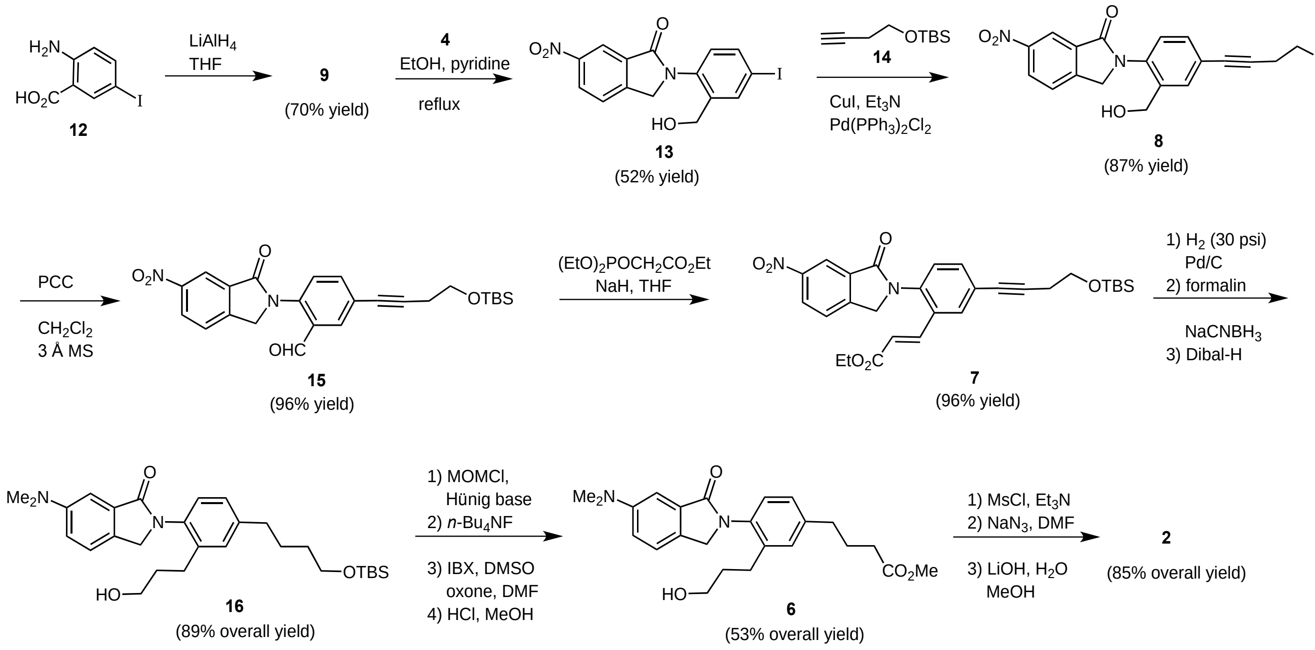
Figure 2. Retrosyntheses of molecules **1** and **2**

Synthesis of probe molecule **1**. Molecule **1** was thereby synthesized as depicted in Scheme 1. Following a reported procedure,[10] nitration of *o*-toluic acid (**10**) with potassium nitrate in concentrated sulfuric acid gave a mixture of 2-methyl-5-nitrobenzoic acid and 2-methyl-3-nitrobenzoic acid in a ratio of 7:3 (99% yield; an inseparable mixture), which was directly methylated with thionyl chloride in methanol[11] to give the corresponding methyl esters. The isomeric methyl esters were separated by recrystallization in hexane to give pure methyl 2-methyl-5-nitrobenzoate. It was brominated by *N*-bromosuccinimide (NBS) and a catalytic amount of benzoyl peroxide in refluxing dichloromethane to give **4** in a 41% overall yield from **10**. 2-Allylaniline (**5**) was prepared in 65% yield from a thermal-induced aromatic amino-Claisen rearrangement or [3,3]-sigmatropic rearrangement of *N*-allylaniline in refluxing *o*-xylene in the presence of 1 equivalent of boron trifluoride•diethyl ether complex at 140 °C for 6 hours.[12] Coupling of benzoate **4** and allylaniline **5** along with pyridine under refluxing ethanol gave isoindolinone **11** in a 75% yield. It was converted into dimethylamine **3** *via* a reduction of the nitro function with stannous chloride dihydrate[13] in refluxing ethanol following by dimethylation with 37% formalin and sodium cyanoborohydride in methanol in a 94% overall yield. Hydroboration of the alkenyl moiety of **3** followed by oxidation with NaOH and 30% H_2O_2, mesylation of the resulting primary alcohol with methanesulfonyl chloride (MsCl) and triethylamine in dichloromethane, and displacement with sodium azide in DMF afforded a 52% overall yield of probe molecule **1**. All the synthetic steps are relatively straightforward, and each steps have been repeated and the reactions were reproducible.



Scheme 1. Synthesis of probe molecule **1**

Synthesis of probe molecule **2**. After few attempts for the installation of C2' and C4' appendages in the aryl ring of molecule **2**, we settled on the functionalization of isoindolinone **13**, containing a C2'-hydroxylmethyl and C4'-iodide groups (Scheme 2). Accordingly, commercially available 2-amino-5-iodobenzoic acid (**12**) was reduced with lithium aluminum hydride in THF to give 2-amino-5-iodobenzyl alcohol (**9**) (70% yield), which was coupled with **4** using similar reaction conditions as described above to produce compound **13** in a 52% yield. Due to the presence of a C2'-hydroxymethyl group in the aryl ring of **13**, *t*-butyldimethylsilyl (TBS) protected hydroxyl alkenyl organometallic reagents for the installment of C4' side chain were investigated. Initially, Suzuki coupling reactions of **13** with either 4-(*t*-butyldimethylsiloxy)-1-butenyl-pinacolborane or –catecholborane were carried out, yields of the desired coupling product were inconsistent ranging from 32 – 68% yield. Hence, Sonogashira reaction of **13** and acetylene **14** was carried out by the treatment with 3.6 mol% each of cuprous iodide and $Pd(PPh_3)_2Cl_2$ along with triethylamine to give an 87% yield of **8**. Oxidation of **8** with pyridinium chlorochromate (PCC) in dichloromethane following by Horner-Wadsworth-Emmons reaction of resulting aldehyde **15** with triethyl phosphonoacetate and sodium hydride at 25 °C gave compound **7** in a 96% overall yield. The remaining steps for the conversion of **7** to **2** are relatively straightforward although lengthy owing to the needs of transforming C2' ester function to alcohol and C4' alcohol function to carboxylic acid. Global reductions of the alkene, alkyne, and nitro functions of **7** with 2 atmospheric of H_2 and a catalytic amount of 10% Pd/C following by dimethylation of the resulting amino function with 40% formalin and sodium cyanoborohydride, and reduction of the ester with diisobutylaluminum hydride (Dibal-H) afforded **16** in an 89% overall yield. Compound **16** was transformed into **6** by a sequence of reactions: 1) protection of the hydroxyl group with methoxymethyl chloride (MOMCl) and diisopropylethylamine (Hünig base); 2) removal of the TBS protecting group with tetra-*n*-butylammonium fluoride in THF; 3) oxidation of the resulting primary alcohol with iodoxybenzoic acid (IBX) in DMSO following oxone in DMF; and 4) hydrolysis of the MOM protecting group with HCl in methanol. Finally, compound **6** was treated with MsCl followed by sodium azide, and basic hydrolysis with lithium hydroxide in water and methanol to furnish probe molecule **2** in an 85% overall yield in three steps.



Scheme 2. Synthesis of probe molecule **2**

CONCLUSION

Two probe molecules **1** and **2** were designed to study the mechanism of inhibition of firefly luciferase. These two molecules, containing an *ortho*-(3-azidopropyl) group at N-2 of the aryl ring of 2-phenylisoindolin-1-ones, with and without a *para*-butyric acid group at the aryl ring, were synthesized from coupling reactions of methyl 2-bromomethyl-5-nitrobenzoate (**4**) with 2-allylaniline (**5**) and 2-hydroxymethyl-4-iodoaniline (**9**), separately. Compound **5** was conveniently prepared from an aromatic amino-Claisen rearrangement reaction of *N*-allylaniline, while compound **9** prepared from 2-amino-5-iodododobenzoic acid (**12**). The *para*-butyric acid group was installed by a Sonogashira reaction of iodide intermediate **13** and 3-(*tert*-butyldimethylsiloxy)-1-butyne (**14**) in a very good yield. The synthetic methods are general and can be applied to the syntheses of other regioisomers, which may be needed for the mechanistic investigation. Biological studies of **1** and **2** are being conducted and will be reported in due course.

EXPERIMENTAL

Chemicals were purchased from Fisher Scientific, VWR international LLC, and Chem-Impex International, Inc. All solvents were dried over appropriated drying agent such as CaH_2 for DMF, dichloromethane, and acetonitrile, Na/benzophenone for THF and Et_2O followed by distillation. Column chromatography was carried out on silica gel (200 - 400 mesh). ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Varian Unity plus 400-MHz Spectrometer or a Bruker Avance Neo 400-MHz NMR spectrometer. The chemical shift data for each signal on ^{1}H NMR are given in units of δ relative to TMS (δ = 0 ppm) or $CHCl_3$ (δ = 7.26 ppm) and on ^{13}C NMR are given in units of δ relative to $CDCl_3$ (δ = 77.0 ppm). Mass spectra were obtained from an API 2000-triple quadrupole ESI-MS/MS mass spectrometer (Applied Biosystems). High-resolution mass spectra were obtained from a LCT Premier (Waters Corp., Milford MA) time of flight mass spectrometer.

2-Methyl-5-nitrobenzoic acid and 2-methyl-3-nitrobenzoic acid.[10] To a solution of 15.0 g (0.11 mol) of *o*-toluic acid (**10**) in 90 mL of concentrate sulfuric acid at 0 °C, a solution of 15.0 g (0.14 mol) of potassium nitrate in 90 mL of concentrate sulfuric acid was added dropwise over 1 h. The reaction mixture was added to 100 g of ice chips and stirred vigorously until all ice melted. The precipitated solid was collected and dissolved in Et_2O, washed with water, dried (anhydrous Na_2SO_4), filtered, and concentrated under vacuum to give 19.86 g (99.5% yield) of a mixture of 2-methyl-5-nitrobenzoic acid and 2-methyl-3-nitrobenzoic acid in a ratio of 7:3 (based on ^{1}H NMR spectrum) as white solids. Attempts to separate this mixture of regioisomers using silica gel column chromatography failed. The mixture was used in the subsequent step without purification. For 2-methyl-5-nitrobenzoic acid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1 H), 8.30 (d, *J* = 8.6 Hz, 1H), 7.51 – 7.49 (m, 1H), 2.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.7 (C=O), 146.8, 145.2, 132.5, 131.2, 126.1, 125.2, 20.9 (CH_3).

Methyl 2-methyl-5-nitrobenzoate.[11] To a 7:3 mixture of 2-methyl-5-nitrobenzoic acid and 2-methyl-3-nitrobenzoic acid (6.88 g, 38.01 mmol) in 80 mL of MeOH at 0 °C was added dropwise of 5.52 mL (76.0 mmol) of thionyl chloride. The reaction solution was stirred for 4 h under reflux (65 °C), cooled to room temperature, and concentrated on a rotary evaporator. The resulting solid was diluted with 100 mL of EtOAc and ~50 mL of saturated aqueous sodium hydrogencarbonate by adjusting the pH to 8. The mixture was separated on a separatory funnel and the water layer was extracted twice with EtOAc. The combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), concentrated, and crystallized from hexane to obtain 4.78 g (69% yield) of methyl 2-methyl-5-nitrobenzoate as a white solid; mp 66 – 67 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 2.7 Hz, 1H), 8.25 (dd, *J* = 8.4, 2.7 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 3.96 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.8 (C=O), 147.8, 145.9, 132.7, 130.4, 126.0, 125.6, 52.3 (OCH_3), 21.7 (CH_3); MS (ESI; positive mode): *m/z* calcd for $C_9H_{10}NO_4$ $(M+H)^+$: 196.0, found 196.2. The spectral data is in agreement with that reported.[11] The mother liquor of the crystallization contained impure methyl 2-methyl-3-nitrobenzoate.

Methyl 2-(bromomethyl)-5-nitrobenzoate (4).[7] A solution of 2.0 g (10.6 mmol) of methyl 2-methyl-5-nitrobenzoate, 2.07 g (11.7 mmol) of *N*-bromosuccinimide, and 51 mg of benzoyl peroxide in 80 mL of CH_2Cl_2, under argon was heated under reflux for 15 h. The reaction mixture was neutralized with saturated aqueous sodium hydrogencarbonate, and the organic and aqueous layers were separated. The water layer was extracted twice with CH_2Cl_2 and the combined organic layers were washed with brine, dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and EtOAc (9:1) as an eluent to give 1.66 g (59% yield) of **4** as a white solid; mp 77 – 78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, *J* = 2.5 Hz, 1H), 8.34 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 5.01 (s, 2 H, CH_2Br), 4.02 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 165.1 (C=O), 147.4, 146.1, 133.0, 130.3, 127.0, 126.5, 53.1 (OCH_3), 29.3 (CH_2Br). The spectral data is in agreement with that reported.[7]

2-Allylaniline (5).[12] To a solution of 2.5 g (18.7 mmol) of *N*-allylaniline in 10 mL of *o*-xylene under argon was added 2.4 mL (18.7 mmol) of $BF_3{\bullet}Et_2O$, and the solution was stirred for 8 h at 140 °C in a sealed tube. After cooled to room temperature, the reaction solution was diluted with 10% (by weight) aqueous NaOH solution to pH 10 and extracted three times with EtOAc. The combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and EtOAc (20:1) as an eluent to obtain 1.62 g (65% yield) of 2-allylaniline (**5**) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 – 6.98 (m, 2H), 6.76 (t, *J* = 7.4 Hz, 1H), 6.73 – 6.63 (m, 1H), 5.96 (ddt, *J* = 11, 8, 6 Hz, 1H, =CH), 5.19 – 5.05 (m, 2H, $=CH_2$), 3.66 (br. s., 2H, NH_2), 3.32 (d, *J* = 6 Hz, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 144.6, 135.7, 129.8, 127.2, 123.6, 118.3, 115.7, 115.4, 36.0.

2-(2-Allylphenyl)-6-nitroisoindolin-1-one (11). To a solution of 0.70 g (2.54 mmol) of methyl 2-bromomethyl-5-nitrobenzoate (**4**) and 0.34 g (2.54 mmol) of 2-allylaniline (**5**) in 25 mL of EtOH under argon, was added 0.27 mL (3.30 mmol) of pyridine. The reaction solution was heated under reflux for 24 h, cooled to room temperature, diluted with aqueous sodium hydrogencarbonate, and extracted twice with EtOAc. The combined organic layer was washed with 50 mL of brine, dried (anhydrous Na_2SO_4), concentrated under reduced pressure, and purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 0.56 g (75% yield) of **11** as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.49 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.42 – 7.32 (m, 3H), 7.26 – 7.24 (m, 1H), 5.88 (ddt, *J* = 17, 10, 6 Hz, 1H, =CH), 4.98 – 4.87 (m, 2H, $=CH_2$), 4.82 (s, 2H), 3.40 (d, *J* = 6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 165.4 (C=O), 148.3, 147.4, 138.1, 136.1, 135.9, 133.7, 130.6, 128.7, 127.6, 127.5, 126.5, 124.2, 119.3, 116.1, 53.4, 36.2; MS (ESI; positive mode): *m/z* calcd for $C_{17}H_{14}N_2O_3Na$ $(M+Na)^+$: 317.0, found 317.1. HRMS (ESI) calcd for $C_{17}H_{14}N_2O_3Na^+$ $[M + Na]^+$: 317.0897, found 317.0901.

2-(2-Allylphenyl)-6-aminoisoindolin-1-one. A mixture of 0.52 g (1.77 mmol) of **11** and 2.0 g (8.86 mmol) of $SnCl_2•2H_2O$ in 30 mL of EtOH under argon was heated under reflux for 1h. The solution was cooled to room temperature, diluted with EtOAc, and neutralized with saturated aqueous sodium hydrogencarbonate. The resulting mixture was extracted twice with EtOAc, and the combined organic layers were washed with 50 mL of brine, dried (anhydrous Na_2SO_4), and concentrated to obtain 0.467 g (99% yield) of 2-(2-allylphenyl)-6-aminoisoindolin-1-one as a light yellow solid; mp 154 – 156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.22 (m, 5H), 7.20 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.0, 2.2 Hz, 1H), 5.89 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H, =CH), 5.02 (d, *J* = 16 Hz, 1H, *trans*- =C*H*H), 4.88 (d, *J* = 10.2 Hz, 1H, *cis*- =C*H*H), 4.60 (s, 2H), 4.0 – 3.75 (br. s., 2H, NH_2), 3.38 (d, *J* = 6.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 168.2 (C=O), 147.1, 138.6, 137.2, 136.6, 133.6, 131.5, 130.6, 128.4, 128.0, 127.5, 123.5, 119.2, 116.2, 109.4, 53.4, 36.3; MS (positive mode): *m/z* calcd for $C_{17}H_{16}N_2ONa$ $(M+Na)^+$: 287.0, found 287.0 $(M+Na)^+$. HRMS (ESI) calcd for $C_{17}H_{17}N_2O^+$ $[M+H]^+$: 265.1335, found 265.1330.

2-(2-Allylphenyl)-6-(dimethylamino)isoindolin-1-one (3). To a solution of 0.77 g (2.92 mmol) of 2-(2-allylphenyl)-6-aminoisoindolin-1-one in 5 mL of MeOH, were added 4.75 g (58.4 mmol) of formalin (37% W/W) and 1.1 g (17.5 mmol) of sodium cyanoborohydride. The solution was stirred for 24 h at 25 °C, neutralized with saturated aqueous sodium hydrogencarbonate, and extracted three times with EtOAc. The combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), concentrated under reduced pressure, and column chromatographed on silica gel using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 0.82 g (96% yield) of compound **3** as a white solid; mp 151 – 152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 4H), 7.6 – 7.18 (m, 2H), 6.98 (dd, *J* = 8.4, 2.3 Hz, 1H), 5.90 (ddt, *J* = 16.8, 10.2, 6.8 Hz, 1H, =CH), 4.95 (dd, *J* = 6.8, 2 Hz, 1H, *cis*- =C*H*H), 4.90 (dd, *J* = 16.8, 2 Hz, 1H, *trans*- =C*H*H), 4.60 (s, 2H), 3.40 (d, *J* = 6.8 Hz, 2H), 3.03 (s, 6H, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 168.8 (C=O), 151.2, 138.6, 137.4, 136.7, 133.4, 130.7, 129.4, 128.4, 128.1, 127.5, 123.2, 116.7, 116.3, 106.9, 53.4, 41.0 (2C, NCH_3), 36.3; MS (ESI) *m/z* calcd for $C_{19}H_{21}N_2O^+$ $(M+H)^+$: 293.1, found 293.3. HRMS (ESI) calcd for $C_{19}H_{21}N_2O^+$ $[M + H]^+$: 293.1648, found 293.1642.

6-(Dimethylamino)-2-[2-(3-hydroxypropyl)phenyl]isoindolin-1-one. To a solution of 0.72 g (1.30 mmol) of **3** in 5 mL of THF under argon was added 1.97 mL (1.97 mmol; 1M THF solution) of $BH_3•THF$.

The solution was stirred at 25 °C for 8 h, cooled to 0 °C, and added a basic solution of hydrogen peroxide, consisted of 2.5 mL (0.025 mmol) of 30% H_2O_2 and 5.9 mL (5.9 mmol) of 1*N* NaOH. The solution was stirred at 0 °C for 1 h, extracted with EtOAc twice, and the combined organic layer was washed with brine (50 mL), dried (anhydrous Na_2SO_4), and concentrated to dryness under reduced pressure to obtain 0.414 g (54% yield) of 6-(dimethylamino)-2-(2-(3-hydroxypropyl)phenyl)isoindolin-1-one as a yellow solid; mp 171 – 172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.26 (m, 4H), 7.25 – 7.19 (m, 2H), 6.98 (dd, *J* = 8.4, 2.5 Hz, 1H), 4.63 (s, 2H, NCH_2), 3.58 (q, *J* = 7 Hz, 2H, OCH_2), 3.03 (s, 6H, $N(CH_3)_2$), 2.68 (t, *J* = 7 Hz, 2H, ArC*H*$_2$), 2.21 (t, *J* = 7 Hz, 1H, OH), 1.87 (quintet, *J* = 7 Hz, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 169.4 (C=O), 151.2, 140.2, 137.3, 133.2, 129.8, 129.3, 128.5, 128.2, 127.1, 123.2, 116.8, 106.9, 61.6 (OCH_2), 53.7, 41.0 (2C, NCH_3), 32.7, 26.8; MS (positive mode): *m/z* calcd for $C_{19}H_{22}N_2O_2Na^+$ $(M+Na)^+$: 333.1, found 333.2. HRMS (ESI) calcd for $C_{19}H_{23}N_2O_2^+$ $(M+H)^+$: 311.1754, found 311.1746.

2-(2-(3-Azidopropyl)phenyl)-6-(dimethylamino)isoindolin-1-one (1). To a solution of 0.20 g (0.64 mmol) of 6-(dimethylamino)-2-(2-(3-hydroxypropyl)phenyl)isoindolin-1-one and 0.13 mL (0.90 mmol) of triethylamine in 10 mL of dry CH_2Cl_2 at 0 °C under argon, was added 95 mg (0.83 mmol) of methanesulfonyl chloride, and the resulting solution was stirred for 2 h at 0 °C. After, it was extracted with CH_2Cl_2 twice, and the combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), and concentrated to dryness under reduced pressure to obtain 0.25 g (100% yield) of the corresponding mesylate product as a yellow oil. It was used in the following reaction without purification. To a solution of the mesylate (0.250 g, 0.64 mmol) in 3 mL of DMF (distilled over CaH_2 under reduced pressure) under argon, was added 0.17 g (2.56 mmol) of NaN_3. The solution was stirred at 25 °C for 24 h, diluted with water, and extracted three times with CH_2Cl_2. The combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of CH_2Cl_2 and MeOH (70:1) as an eluent to obtain 0.21 g (98% yield) of **1** as a yellow oil; IR (neat) 2900, 2850, 2100 (N_3), 1675, 1650, 1500, 1390, 1350, 1200 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.24 (m, 6H), 6.99 (dd, *J* = 8.2, 1.95 Hz, 1H), 4.63 (s, 2H), 3.25 (t, *J* = 6.8 Hz, 2H, CH_2N_3), 3.04 (s, 6H, $N(CH_3)_2$), 2.68 (t, *J* = 6.8 Hz, 2H, $ArCH_2$), 1.89 (quint, *J* = 6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 169.0 (C=O), 151.2, 139.5, 137.4, 133.2, 130.0, 129.2, 128.5, 128.3, 127.5, 123.2, 116.7, 106.9, 53.5, 51.0 (CH_2N_3), 41.0 (2C, NCH_3), 29.3, 28.6; MS (positive mode): *m/z* calcd for $C_{19}H_{22}N_5O^+$ $(M+H)^+$: 336.1, found 335.8; calcd for $C_{19}H_{21}N_5ONa^+$ $(M+Na)^+$: 358.0, found 357.9. HRMS (ESI) calcd for $C_{19}H_{22}N_5O^+$ $(M+H)^+$: 336.1824, found 336.1844.

2-Hydroxymethyl-4-iodoaniline (9). To a cold (0 °C) solution of 3.0 g (11.4 mmol) of 2-amino-5-iodobenzoic acid (**12**) in 100 mL of THF was added 0.44 g (11.4 mmol) of $LiAlH_4$ in portion over 20 min, and the mixture was stirred at 25 °C for 3 h to give a yellow solution. The progress of the reaction was monitored by TLC and upon completion the reaction solution was diluted with 3 mL each of

H_2O and 10% aqueous NaOH solution. The precipitated solids were filtered and washed three times with EtOAc (50 mL each). The filtrate was concentrated and chromatographed on a silica gel column using a gradient mixture of hexane and Et_2O as eluents to give 1.96 g (70% yield) of **9** as a light yellow solid; mp 181 – 183 °C; ^{1}H NMR[14] (400 MHz, $CDCl_3$) δ 7.40 – 7.35 (m, 2H), 6.48 (d, *J* = 8 Hz, 1H), 4.61 (d, *J* = 2.8 Hz, 2H, OCH_2), 4.25 – 4.15 (br. s., 1H, OH); ^{13}C NMR ($CDCl_3$) δ 147.2, 138.0, 136.4, 128.6, 117.5, 76.6, 62.0. The spectral data is in agreement with that reported.

2-[2-(Hydroxymethyl)-4-iodophenyl]-6-nitroisoindolin-1-one (13). To a solution of 4.0 g (16 mmol) of **9** and 4.37 g (16 mmol) of **4** in 200 mL of EtOH under argon was added 1.64 g (20.8 mmol) of pyridine. The solution was heated at 80 °C for 30 h. After cooling to 25 °C, it was neutralized with saturated aqueous sodium hydrogencarbonate, and extracted three times with EtOAc. The combined organic layers were washed with brine (100 mL), dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 1.92 g (52% yield) of compound **13** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.2 Hz, 1H), 8.49 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.42 – 7.32 (m, 3H), 4.62 (s, 4H, NCH_2 and OCH_2); ^{13}C NMR ($CDCl_3$) δ 166.7 (C=O), 151.8, 148.6, 139.2, 134.2, 130.9, 129.3, 128.1, 128.0, 126.4, 124.9, 119.8, 88.6, 61.2 (OCH_2), 53.1 (NCH_2) ppm. HRMS (ESI) calcd for $C_{15}H_{12}IN_2O_4$ $(M+H)^+$: 410.9842, found 410.9839.

3-(*tert*-Butyldimethylsilyloxy)-1-butyne (14).[15] To a solution of 2.8 g (40 mmol) of 3-butyn-1-ol in 200 mL of CH_2Cl_2 were added 5.8 g (80 mmol) of imidazole and 9.68 g (60 mmol) of *t*-butyldimethylsilyl chloride and the solution was stirred under argon at 25 °C for 12 h. The resulting solution was washed twice with water (50 mL each) and brine (50 mL), dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and EtOAc (5:1) as an eluent to obtain 6.90 g (97% yield) of the title compound as a colorless oil; ^{1}H NMR[15] (400 MHz, $CDCl_3$) δ 3.74 (t, *J* = 7.2 Hz, 2H, OCH_2), 2.45 – 2.33 (m, 2H, CH_2), 1.96 (s, 1H, ≡CH), 0.10 (s, 9H, $C(CH_3)_3$), 0.07 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 82.0, 70.1, 61.8, 26.1 (3C, $C(CH_3)_3$), 24.3, 18.6, -5.4 (2C, $Si(CH_3)_2$). The spectral data is in agreement with that reported.

2-{4-[4-(*tert*-Butyldimethylsilyloxy)but-1-ynyl]-2-(hydroxymethyl)phenyl}-6-nitroisoindolin-1-one (8). To a yellow solution of 1.9 g (2.5 mmol) of iodide **13** in 200 mL of triethylamine (distilled over CaH_2) under nitrogen in a dry box were added 17.6 mg (0.09 mmol) of CuI and 65 mg (0.09 mmol) of bis(triphenylphosphine)palladium chloride. The solution was stirred under argon for 20 min and added 0.94 g (5.1 mmol) of 4-(*t*-butyldimethylsilyloxy)-1-butyne (**14**), resulting in a green solution. It was stirred at 25 °C for 12 h, concentrated under reduced pressure, and diluted with 100 mL of EtOAc and 50 mL of H_2O. The organic layer was separated and aqueous layer was extracted twice with EtOAc (100 mL each). The combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 1.87 g (87% yield) of compound **8** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.2 Hz, 1H), 8.50 (dd, *J* = 8, 2.2 Hz, 1H),7.87 (d, *J* = 8 Hz, 1H), 7.60 (d, *J* = 8 Hz, 1H), 7.31 (d, *J* = 8 Hz, 1H), 7.14 (s, 1H), 4.62 (s, 4H), 3.79 (t, *J* = 7.2 Hz, 2H, $SiOCH_2$), 2.59 (t, *J* = 7.2 Hz, 2H), 1.7 – 1.4 (bs., 1H, OH), 0.90 (s, 9H, $C(CH_3)_3$), 0.09 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 166.4 (C=O), 153.2, 140.3, 137.2, 135.6, 133.1, 129.8, 128.1, 127.3, 125.4, 123.0, 120.8, 118.1, 116.0, 82.4, 63.6, 61.2, 54.4, 27.6 (3C, $C(CH_3)_3$), 24.0, 19.0, -5.1 (2C, $Si(CH_3)_2$). HRMS (ESI) calcd for $C_{25}H_{31}N_2O_5Si$ $(M+H)^+$: 467.2002, found 467.2013.

5-(4-(*tert*-Butyldimethylsilyloxy)but-1-ynyl)-2-(6-nitro-1-oxoisoindolin-2-yl)benzaldehyde (15). To a solution of 1.90 g (4.07 mmol) of **8** in 50 mL of CH_2Cl_2 under argon were added 2.0 g of 4 Å molecular sieve and 1.05 g (4.88 mmol) of pyridinium chlorochromate, and the mixture was stirred at 25 °C for 3 h, resulting a dark green mixture. The reaction mixture was filtered through Celite and washed with 100 mL of CH_2Cl_2. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 1.82 g (96% yield) of compound **15** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (s, 1H, O=CH)), 8.80 (d, *J* = 2.2 Hz, 1H), 8.49 (dd, *J* = 8.0, 2.2 Hz, 1H), 8.38 (d, *J* = 4.8 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 7.40 (d, *J* = 4.8 Hz, 1H), 4.62 (s, 2H, NCH_2), 3.79 (t, *J* = 7.2 Hz, 2H, OCH_2), 2.60 (t, *J* = 7.2 Hz, 2H, CH_2), 0.90 (s, 9H, $C(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 195.0 (HC=O), 162.1 (NC=O), 153.1, 141.9, 139.6, 139.2, 132.1, 131.4, 128.6, 120.3, 118.6, 117.6, 114.2, 102.6, 88.2, 81.2, 61.8, 60.4, 26.0 (3C, $C(CH_3)_3$), 24.0, 18.8, -5.1 (2C, $Si(CH_3)_2$). HRMS (ESI) calcd for $C_{25}H_{29}N_2O_5Si$ $(M+H)^+$: 465.1846, found 465.1862.

Ethyl 3-{5-[4-(*tert*-butyldimethylsilyloxy)but-1-ynyl]-2-(6-nitro-1-oxoisoindolin-2-yl)phenyl}-2-propenoate (7). To a cold (0 °C) solution of 0.88 g (3.92 mmol) of triethyl phosphonoacetate in 30 mL of dried THF under argon was added 98 mg (3.92 mmol) of NaH in portion and the resulting mixture was stirred at 25 °C for 2 h to give a colorless solution. To it, a solution of 1.82 g (3.92 mmol) of **15** in 30 mL of THF was added *via* a cannula, and the solution was stirred for additional 2 h, diluted with 20 mL of H_2O, and extracted three times with 50 mL each of CH_2Cl_2. The combined organic layer was washed with brine (20 mL), dried (anhydrous Na_2SO_4), concentrated, and purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:2) as an eluent to obtain 2.0 g (96% yield) of compound **7** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d , *J* = 2.2 Hz, 1H), 8.49 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.70 (d, *J* = 3 Hz, 1H), 7.60 (d, *J* = 10 Hz, 1H, ArCH=), 7.5 (d, *J* = 2 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 6.67 (s, 1H), 6.35 (d, *J* = 10 Hz, 1H, O=C-CH=), 4.62 (s, 2H), 4.23 (q, *J* = 6.8 Hz, 2H, CO_2CH_2), 3.78 (t, *J* = 7.2 Hz, 2H, $SiOCH_2$), 2.58 (t, *J* = 7.2 Hz, 2H, CH_2), 1.30 (t, *J* = 6.8 Hz, 3H, CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.07 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 167.5 (OC=O), 163.2 (NC=O), 152.4, 138.9, 137.1, 134.2, 133.2, 130.8, 129.6, 125.8, 124.3, 122.6, 121.4, 116.8, 114.8, 111.0, 88.2, 81.6, 61.8, 60.6, 34.8, 27.2 (3C, C(*C*H$_3$)$_3$), 24.1, 23.9, 19.0, -5.0 (2C, Si(CH$_3$)$_2$). HRMS (ESI) calcd for $C_{29}H_{35}N_2O_6Si$ (M+H)$^+$: 535.2264, found 535.2259.

Ethyl 3-{2-(6-amino-1-oxoisoindolin-2-yl)-5-[4-(*tert*-butyldimethylsilyloxy)butyl]phenyl}propanoate. To a solution of 2.0 g (3.76 mmol) of **7** in 30 mL of EtOAc was added 50 mg of 10% palladium/carbon and the mixture was shaken under 30 psi. of hydrogen gas for 12 h on a hydrogenator. The hydrogen gas was released and the reaction mixture was filtered through Celite and concentrated under reduced pressure to give 1.91 g (99% yield) of the title compound as a yellow oil. This molecule was used in the subsequent step without purification; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.30 – 6.90 (m, 6H), 4.62 (s, 2H, NCH$_2$), 4.20 (2H, q, *J* = 6.8 Hz, 2H, CO$_2$CH$_2$), 3.64 (t, *J* = 7.2 Hz, 2H, SiOCH$_2$), 2.92 – 2.86 (m, 2H, ArCH$_2$), 2.64 – 2.60 (m, 4H, O=CCH$_2$, ArCH$_2$), 1.70 – 1.55 (m, 4H, CH$_2$-CH$_2$), 1.30 (t, *J* = 6.8 Hz, 3H, CH$_3$), 0.90 (s, 9H, C(CH$_3$)$_3$), 0.07 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR (CDCl$_3$) δ 168.0 (OC=O), 165.0 (NC=O), 147.6, 138.4, 136.1, 131.4, 130.6, 128.4, 128.0, 127.2, 124.0, 119.6, 116.2, 109.8, 63.0, 61.2, 34.0 (NCH$_2$), 31.2, 29.9, 28.6, 27.4, 26.6, 25.4 (3C, C(CH$_3$)$_3$), 24.8, 18.0 (SiC), -5.4 (2C, Si(CH$_3$)$_2$). HRMS (ESI) calcd for $C_{29}H_{43}N_2O_4Si$ (M + H)$^+$: 511.2992, found 511.3001.

Ethyl 3-(5-(4-(*tert*-butyldimethylsilyloxy)butyl)-2-(6-(dimethylamino)-1-oxoisoindolin-2-yl)phenyl)-propanoate. To a solution of 1.91 g (3.76 mmol) of ethyl 3-{2-(6-amino-1-oxoisoindolin-2-yl)-5-[4-(*tert*-butyldimethylsilyloxy)butyl]phenyl}propanoate in 20 mL of MeOH, were added 6.11 g (37.6 mmol) of formalin (37% W/W) and 1.42 g (22.5 mmol) of sodium cyanoborohydride, and the solution was stirred for 24 h at 25 °C. It was neutralized with saturated sodium hydrogencarbonate and extracted three times with EtOAc. The combined organic layer was washed with brine (70 mL), dried (anhydrous Na$_2$SO$_4$), concentrated, and purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 1.92 g (95% yield) of the title compound as a yellow oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35 – 6.90 (m, 6H), 4.58 (s, 2H, NCH$_2$), 4.20 (q, *J* = 6.8 Hz, 2H, CO$_2$CH$_2$), 3.61 (t, *J* = 7.2 Hz, 2H, SiOCH$_2$), 2.99 (s, 6H, N(CH$_3$)$_2$), 2.84 – 2.80 (m, 2H, ArCH$_2$), 2.60 – 2.56 (m, 4H, O=CCH$_2$, ArCH$_2$), 1.70 – 1.55 (m, 4H, CH$_2$CH$_2$), 1.30 (t, *J* = 6.8 Hz, 3H, CH$_3$), 0.86 (s, 9H, C(CH$_3$)$_3$), 0.04 (s, 6H, Si(CH$_3$)$_2$); ^{13}C NMR (CDCl$_3$) δ 168.2 (OC=O), 165.0 (NC=O), 148.2, 140.1, 138.2, 132.3, 131.9, 129.8, 129.6, 126.7, 120.9, 115.6, 113.0, 106.4, 62.8, 60.1, 41.8 (2C, N(CH$_3$)$_2$), 34.6, 32.0, 29.6, 27.3, 26.9, 26.1, 25.8 (3C, C(*C*H$_3$)$_3$), 24.9, 18.0 (CSi), -5.2 (2C, Si(CH$_3$)$_2$). HRMS (ESI) calcd for $C_{31}H_{47}N_2O_4Si$ (M+H)$^+$: 539.3305, found 539.3305.

2-{4-[4-(*tert*-Butyldimethylsilyloxy)butyl]-2-(3-hydroxypropyl)phenyl}-6-(dimethylamino)isoindolin-1-one (16). To a cold (-78 °C) solution of 1.92 g (3.5 mmol) of ethyl 3-(5-(4-(*tert*-butyldimethylsilyloxy)butyl)-2-(6-(dimethylamino)-1-oxoisoindolin-2-yl)phenyl)propanoate in 40 mL of dried THF under argon was added dropwise 9.0 mL (9.0 mmol; 1M in toluene) of diisobutylaluminum hydride. After adding, the solution was stirred at -30 °C for 2 h until no starting material remained (monitored by TLC), diluted with 20 mL of H_2O, filtered, and extracted the filtrate three times with EtOAc (60 mL each). The combined organic layers were washed with brine, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:2) as an eluent to obtain 1.68 g (95% yield) of compound **16** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 4.60 (s, 2H), 3.62 – 3.60 (m, 4H, OCH_2 and $SiOCH_2$), 2.99 (s, 6H, $N(CH_3)_2$), 2.69 (t, *J* = 6.4 Hz, 2H), 2.55 (t, *J* = 8.4 Hz, 2H), 1.86 (quint, *J* = 6.4 Hz, 2H), 1.78 – 1.60 (m, 4H, CH_2CH_2), 1.45 (quint, *J* = 8.4 Hz, 2H, CH_2), 0.88 (s, 9H, $C(CH_3)_3$), 0.03 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 168.8 (C=O), 149.8, 139.1, 136.5, 131.6, 129.0, 128.7, 128.2, 127.9, 126.5, 122.2, 116.0, 105.1, 63.8, 60.1, 53.8, 50.4, 39.8 (2C, $N(CH_3)_2$), 37.5, 35.2, 28.4, 27.3, 26.2 (3C, $C(CH_3)_3$), 18.4 (SiC), -5.1 (2C, $Si(CH_3)_2$). HRMS (ESI) calcd for $C_{29}H_{45}N_2O_3Si$ $(M+H)^+$: 497.3199, found 497.3221.

2-{4-[4-(*tert*-Butyldimethylsilyloxy)butyl]-2-[3-(methoxymethoxy)propyl]phenyl}-6-(dimethylamino)-isoindolin-1-one. To a solution of 1.68 g (3.38 mmol) of **16** in 100 mL of CH_2Cl_2 under argon at 25 °C were added 1.09 g (8.45 mmol) of *N,N*-diisopropylethylamine and 0.544 g (6.76 mmol) of chloromethyl methyl ether. The reaction solution was heated at 40 °C for 12 h, cooled to 25 °C, and diluted with 100 mL of CH_2Cl_2. The resulting solution was washed sequentially with 50 mL of 10% aqueous citric acid solution, 50 mL of saturated aqueous sodium hydrogencarbonate solution, and 50 mL of brine, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:1) as an eluent to obtain 1.70 g (93% yield) of the title compound as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 5.22 (s, 2H, OCH_2O), 4.57 (s, 2H, NCH_2), 3.97 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.62 – 3.60 (m, 2H, $SiOCH_2$), 3.61 (s, 3H, OCH_3), 2.99 (s, 6H, $N(CH_3)_2$), 2.61 – 2.57 (m, 4H, 2 $ArCH_2$), 1.90 – 1.75 (m, 2H, CH_2), 1.70 – 1.50 (m, 4H, CH_2CH_2), 0.87 (s, 9H, $C(CH_3)_3$), 0.02 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 168.7 (C=O), 149.7, 139.1, 136.5, 131.9, 129.1, 128.8, 128.3, 127.9, 126.5, 122.2, 116.1, 105.1, 99.9 (OCH_2O), 63.8, 62.1, 60.6, 53.8, 50.4, 39.8 (2C, $N(CH_3)_2$), 35.1, 32.2, 28.4, 27.3, 26.2 (3C, $C(CH_3)_3$), 18.5 (SiC), -4.9 (2C, $Si(CH_3)_2$). HRMS (ESI) calcd for $C_{31}H_{49}N_2O_4Si$ $(M+H)^+$: 541.3462, found 541.3459.

6-(Dimethylamino)-2-{4-(4-hydroxybutyl)-2-[3-(methoxymethoxy)propyl]phenyl}isoindolin-1-one. To a solution of 1.70 g (3.18 mmol) of 2-{4-[4-(*tert*-butyldimethylsilyloxy)butyl]-2-[3-(methoxymethoxy)propyl]phenyl}-6-(dimethylamino)isoindolin-1-one in 20 mL of THF under argon at 25 °C was added 3.18 mL (1M solution in THF) of tetra-*n*-butylammonium fluoride. The resulting solution was stirred for 4 h and TLC of an aliquot of the solution indicated absence of the starting material. It was diluted with 100 mL of EtOAc and the solution was washed twice with water (20 mL each) and brine (20 mL), dried (anhydrous Na_2SO_4), and concentrated under vacuum to give 1.34 g (99%

yield) of the title compound as a yellow oil. This material was used in the subsequent step without purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 5.23 (s, 2H, OCH_2O), 4.60 (s, 2H, NCH_2), 3.97 (t, J = 6.4 Hz, 2H, OCH_2), 3.61 – 3.59 (m, 2H, OCH_2), 3.60 (s, 3H, OCH_3), 2.99 (s, 6H, $N(CH_3)_2$), 2.60 – 2.56 (m, 4H, 2 $ArCH_2$), 1.88 – 1.56 (m, 6H, 3 CH_2); ^{13}C NMR ($CDCl_3$) δ 169.9 (C=O), 151.5, 140.1, 137.5, 133.2, 129.9, 129.5, 128.7, 128.2, 127.5, 123.2, 117.1, 106.3, 99.9 (OCH_2O), 63.8, 62.1, 53.8, 50.2, 41.0 (2C, $N(CH_3)_2$), 36.4, 35.1, 31.6, 28.8, 25.1. HRMS (ESI) calcd for $C_{25}H_{35}N_2O_4$ $(M+H)^+$: 427.2597, found 427.2612.

4-{4-[6-(Dimethylamino)-1-oxoisoindolin-2-yl]-3-[3-(methoxymethoxy)propyl]phenyl}butanoic acid. To a solution of 1.07 g (2.50 mmol) of 6-(dimethylamino)-2-{4-(4-hydroxybutyl)-2-[3-(methoxymethoxy)propyl]phenyl}isoindolin-1-one in 20 mL of dried DMSO under argon at 25 °C was added 1.05 g (3.75 mmol) of IBX and the resulting solution was stirred for 12 h. It was diluted with 200 mL of EtOAc and the solution was washed three times with water (50 mL each). The organic layer was concentrated and the residue was dissolved in 30 mL of DMF. To it, Oxone ($2KHSO_5·KHSO_4·K_2SO_4$), 0.57 g (3.75 mmol), was added and the mixture was stirred at 25 °C for 6 h. The mixture was basified with 100 mL of saturated aqueous sodium hydrogencarbonate, diluted with 50 mL of CH_2Cl_2, acidified with 1N HCl until the pH reached 4, and extracted four times with EtOAc (100 mL each). The combined organic layers were washed with brine (50 mL), dried (anhydrous Na_2SO_4), and concentrated under vacuum to obtain 0.711 g (65% yield) of the title compound as a pale yellow solid; mp 191–192 °C. This material was used in the subsequent step without purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 5.21 (s, 2H, OCH_2O), 4.60 (s, 2H, NCH_2), 3.97 (t, J = 6.4 Hz, 2H, OCH_2), 3.61 (s, 3H, OCH_3), 2.99 (s, 6H, $N(CH_3)_2$), 2.60 – 2.56 (m, 4H, 2 $ArCH_2$), 2.39 (t, J = 6.4 Hz, 2H, $O{=}CCH_2$), 1.90 – 1.86 (m, 2H) 1.55 – 1.53 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.3 (CO_2H), 169.6 (NC=O), 151.3, 140.2, 137.6, 132.6, 129.9, 129.6, 129.1, 128.6, 127.5, 123.1, 117.1, 106.7, 99.8 (OCH_2O), 62.0, 53.8, 50.2, 40.8 (2C, $N(CH_3)_2$), 37.8, 33.2, 31.2, 27.0, 25.1. HRMS (ESI) calcd for $C_{25}H_{32}N_2O_5$ $(M+H)^+$: 440.2311, found 440.2317.

Methyl 4-{4-[6-(dimethylamino)-1-oxoisoindolin-2-yl]-3-(3-hydroxypropyl)phenyl}butanoate (6). To a solution of 0.71 g (1.61 mmol) of 4-{4-[6-(dimethylamino)-1-oxoisoindolin-2-yl]-3-[3-(methoxymethoxy)propyl]phenyl}butanoic acid in 20 mL of MeOH was added 2 mL of conc. HCl and the resulting solution was stirred at 25 °C for 24 h. To it, was added 50 mL of saturated aqueous sodium hydrogencarbonate solution, and a majority of MeOH was removed on a rotary evaporator. The residue was extracted four times with EtOAc (50 mL each) and the combined organic layers were washed with brine (20 mL), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:1) as an eluent to give 0.584 g (88% yield) of **6** as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 4.57 (s, 2H, NCH_2), 3.71 (s, 3H, OCH_3)), 3.61 (t, *J* = 6 Hz, 2H, OCH_2), 2.99 (s, 6H, $N(CH_3)_2$), 2.60 – 2.56 (m, 4H, 2 $ArCH_2$), 2.39 (t, *J* = 6.4 Hz, 2H, $O{=}CCH_2$), 1.90 – 1.86 (m, 2H, CH_2) 1.56 – 1.52 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 174.8 (OC=O), 169.8 (NC=O), 151.1, 140.1, 137.6, 132.5, 129.9, 129.6, 129.2, 128.4, 127.5, 123.1, 117.1, 106.6, 61.4, 53.8, 51.2, 40.8 (2C, $N(CH_3)_2$), 37.8, 32.2, 31.8, 27.1, 25.1. HRMS (ESI) calcd for $C_{24}H_{31}N_2O_4$ $(M+H)^+$: 411.2284, found 411.2279.

Methyl 4-{4-[6-(dimethylamino)-1-oxoisoindolin-2-yl]-3-[3-(methylsulfonyloxy)propyl]phenyl}-butanoate. To a solution of 0.6 g (1.47 mmol) of **6** and 0.31 mL (2.19 mmol) of triethylamine in 20 mL of CH_2Cl_2 under argon at 0 °C was added dropwise 0.25 g (2.19 mmol) of methanesulfonyl chloride (freshly distilled). The resulting solution was stirred for 8 h at 25 °C, diluted with 5 mL of water, and extracted three times with CH_2Cl_2 (50 mL each). The combined organic layers were washed with brine (20 mL), dried (anhydrous Na_2SO_4), and concentrated under vacuum. The residue was purified by silica gel column chromatography using a mixture of hexane and EtOAc (1:1) as an eluent to give 0.713 g (99% yield) of the title compound as a yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 4.61 (s, 2H, NCH_2), 4.31 (t, *J* = 6.4 Hz, 2H, $SOCH_2$), 3.72 (s, 3H, OCH_3), 3.07 (s, 3H, CH_3S), 2.99 (s, 6H, $N(CH_3)_2$), 2.60 – 2.56 (m, 4H, 2 $ArCH_2$), 2.40 (t, *J* = 6.4 Hz, 2H, $O{=}CCH_2$), 1.88 (quint, *J* = 6.4 Hz, 2H) 1.60 (quint, *J* = 7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 174.8 (OC=O), 169.8 (NC=O), 151.2, 140.1, 137.6, 132.4, 130.2, 129.6, 128.6, 128.2, 127.5, 123.0, 117.1, 106.4, 68.2, 53.8, 51.2, 41.2 (2C, $N(CH_3)_2$), 37.6, 32.2, 31.8, 31.4, 27.0, 25.3. HRMS (ESI) calcd for $C_{25}H_{33}N_2O_6S$ $(M+H)^+$: 489.2059, found 489.2023.

Methyl 4-{3-(3-azidopropyl)-4-[6-(dimethylamino)-1-oxoisoindolin-2-yl]phenyl}butanoate. To a solution of 0.71 g (1.46 mmol) of methyl 4-{4-[6-(dimethylamino)-1-oxoisoindolin-2-yl]-3-[3-(methylsulfonyloxy)propyl]phenyl}butanoate in 30 mL of dry DMF under argon at 25 °C was added 0.379 g (5.83 mmol) of NaN_3. The resulting solution was stirred for 18 h and then diluted with 20 mL of saturated aqueous sodium hydrogencarbonate solution. The mixture was extracted three times with CH_2Cl_2 (50 mL each) and the combined organic layers were washed with water (50 mL) and brine (20 mL), dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a mixture of hexane and EtOAc (1:1) as an eluent to give 0.617 g (97% yield) of the title compound as a yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.90 (m, 6H), 4.61 (s, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 3.25 (t, *J* = 6.4 Hz, 2H, N_3CH_2), 3.01 (s, 6H, $N(CH_3)_2$), 2.60 – 2.56 (m, 4H, 2 $ArCH_2$), 2.38 (t, *J* = 6.4 Hz, 2H, $O{=}CCH_2$), 1.90 – 1.86 (m, 2H, CH_2) 1.61 – 1.57 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 176.2 (OC=O), 169.9 (NC=O), 151.6, 140.8, 138.1, 134.6, 132.8, 131.1, 130.6, 130.2, 129.8, 126.0, 115.2, 105.0, 53.8, 53.4, 50.9, 40.6 (2C, $N(CH_3)_2$), 35.0, 32.4, 31.8, 29.3, 28.7. HRMS (ESI) calcd for $C_{24}H_{30}N_5O_3$ $(M+H)^+$: 436.2349, found 436.2361.

4-{3-(3-Azidopropyl)-4-[6-(dimethylamino)-1-oxoisoindolin-2-yl]phenyl}butanoic acid (2). To a solution of 0.62 g (1.42 mmol) of methyl 4-{3-(3-azidopropyl)-4-[6-(dimethylamino)-1-oxoisoindolin-

2-yl]phenyl}butanoate in 30 mL of MeOH was added 2 mL of 10% aqueous LiOH solution, and the resulting solution was stirred at 25 °C for 6 h. The solution was concentrated on a rotary evaporator to remove most of MeOH and the residue was diluted with 50 mL of CH_2Cl_2, and acidified with 10% aqueous HCl to pH ~4. The solution was concentrated under reduced pressure and purified by a short silica gel column using a gradient mixture of EtOAc and methanol as eluent to give 0.532 g (89% yield) of probe molecule **2** as a light yellow solid; mp 183-186 °C; IR (solid) 3250 – 2700 (b.s., OH), 2100 (s, N_3), 1650 (s, C=O), 1600 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 – 6.95 (m, 6H), 4.62 (s, 2H, NCH_2), 3.24 (t, *J* = 6.4 Hz, 2H, N_3CH_2), 3.02 (s, 6H, $N(CH_3)_2$), 2.60 – 2.56 (m, 4H, 2 $ArCH_2$)), 2.39 (t, *J* = 8.0 Hz, 2H, $O{=}CCH_2$), 1.92 – 1.88 (m, 2H, CH_2), 1.61 – 1.57 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 178.2 (OC=O), 169.1 (NC=O), 150.6, 139.2, 137.6, 133.4, 129.8, 129.2, 128.7, 128.3, 127.6, 124.3, 117.5, 106.4, 53.6, 50.9, 40.8 (2C, $N(CH_3)_2$), 35.0, 32.4, 31.8, 29.5, 28.9. HRMS (ESI) calcf for $C_{23}H_{28}N_5O_3$ $(M+H)^+$: 422.2192, found 422.2187.

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