A Practical and Scalable Synthesis of GTx-134, an IGF-1R Inhibitor

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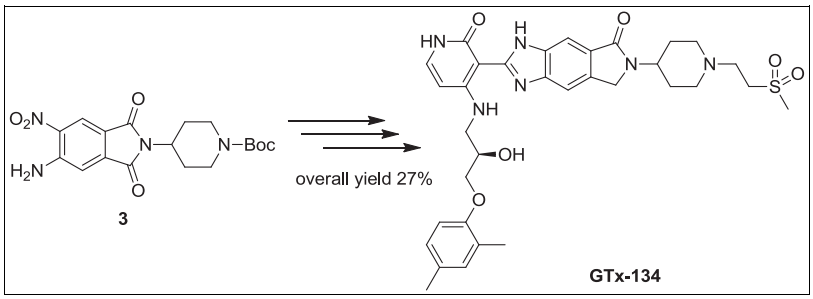
This work was dedicated in memory of Dr. Vlad E. Gregor (1954–2010) who made great contributions to medicinal chemistry and drug discovery.



A practical and scalable synthesis of GTx-134, a novel small-molecule inhibitor of insulin-like growth factor 1 receptor showing antitumor activity in preclinical models of multiple myeloma, is reported.

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INTRODUCTION

Multiple myeloma (MM) is a cancer of plasma cells, a type of white blood cells normally responsible for producing antibodies [2]. In MM, collections of abnormal plasma cells accumulate in the bone marrow where they receive proliferative, survival, and migratory signals from the bone marrow microenvironment [2]. MM remains the most part incurable, although the therapeutic landscape for the management of MM has been changed by the advent of autologous stem cell transplantation and several novel therapeutics such as the proteomsome inhibitor, bortezomib and the immunomodulatory drugs, thalidomide, and lenalidomide [2]. Insulin-like growth factor 1 receptor (IGF-1R) is a receptor tyrosine kinase widely expressed in normal tissues where it functions in growth regulation [3]. IGF-1R is activated by IGF-1 and by the related growth factor IGF-2. It is well known that IGF-1R stimulates the proliferation and survival of MM cells as well as their migration, adhesion, and invasion [3]. Therefore, inhibition of IGF-1R represents an attractive therapeutic target for MM [3,4].

GTx-134 (**1**) (Fig. 1), a novel small-molecule IGF-1R inhibitor, was discovered in our lab through structure-based drug design [5,6]. GTx-134 (**1**) reduced autophosphorylation of its target receptor and inhibited signaling through the PI3k/Akt pathway, inducing apoptosis of primary patient MM cells that led to an antitumor efficacy in a human melanoma cell lines (HMCL) xenografts model [7]. Encouraged by the growing interests in compound **1** and its close analogs [8], herein, we report an optimized, practical, and scalable synthetic route toward compound **1** [9].

RESULTS AND DISCUSSION

The synthetic work began from commercially available 4-amino-5-nitrophthalimide (**2**). According to various research reports [10], Mitsunobu reaction could be a very convenient method to make piperidine phthalimide **3** from phthalimide **2** using *tert*-butyl 4-hydroxypiperidine-1-carboxylate (**4**) (Scheme 1). Mitsunobu reaction requires stoichiometric quantities of an azodicarboxylate such as diethyl azodicarboxylate or diisopropyl azodicarboxylate and phosphorane reagents such as triphenylphosphine, which not only adds additional cost but also increases complexity of purification of the product. Thus, we were inclined to develop a cost-effective method which is more suitable for larger scale synthesis of phthalimide **3**. Inspired by a method described in a patent [11], we found that a simple heating of **2** with *tert*-butyl 4-aminopiperidine-1-carboxylate (**5**) in the presence of imidazole in 1,4-dioxane in a sealed pressure vessel gave phthalimide **3** in good yield (54%) (Scheme 1).

Stepwise synthesis of 1. The step by step synthesis of **1** from **3** was illustrated in Scheme 2. This linear synthesis began from the hydrogenation of **3** which was performed smoothly to give diaminophthalimide **6** in almost quantitative yield (>99%). The reaction of **6** with 4-iodo-2-methoxynicotinic aldehyde (**7**) was carried out in methanol in the presence of HOAc [12,13]. An oxidizing reagent is usually required for the formation of benzyl imidazole [14], but we used O_2 in the air as the oxidizing reagent in order to avoid introducing additional chemicals into the reaction mixture. The reaction, carried out in a flask open to the air, reached completion after 72 h to

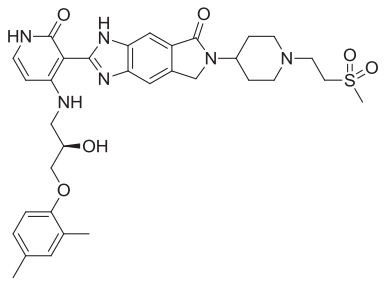
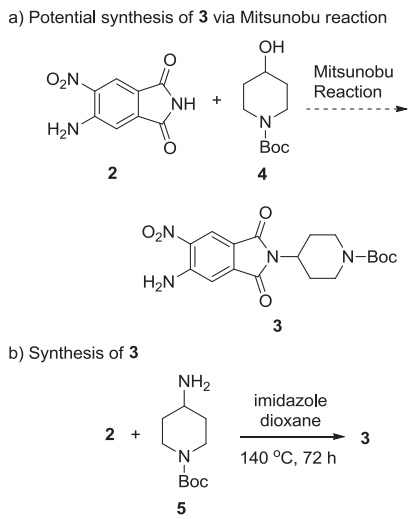


Figure 1. Structure of GTx-134 (**1**).

afford tricyclic compound phthalimido imidazole **8** in 92% yield. Demethylation of 2-methoxypyridine **8** using HCl solution (4.0 M in 1,4-dioxane) resulted in a mixture of iodopyridone **9** and chloropyridone **10** as the iodine in both **8** and **9** could be replaced by chlorine under the reaction condition [15]. Both iodide **9** and chloride **10** are very insoluble, which made the separation of them from the crude difficult, so the crude material was used in the next step directly. The ratio of iodide **9** and chloride **10** in the crude was proved to be 0.5:1 by ^{1}H NMR analysis (Fig. 2).

Because the Boc protective group on piperidine N in **8** was eliminated during the demethylation, it had to be added to piperidines in **9** and **10** to provide iodide **11** and chloride **12**, respectively [16]. Iodide **11** and chloride **12** were isolated together from the reaction crude, although separation of one from the other was not successful. ^{1}H NMR spectra in both DMSO-d_6 and $CDCl_3$ indicated that the ratio of iodide **11** to chloride **12** in the product was 0.5:1, which is consistent with that of **9** to **10** (Fig. 3). The yield of the two steps from **8** to **11/12** was 83%, which was calculated based on the weight average molecular weight 528.41 of the 0.5:1 mixture of **11** and **12**.

Scheme 1

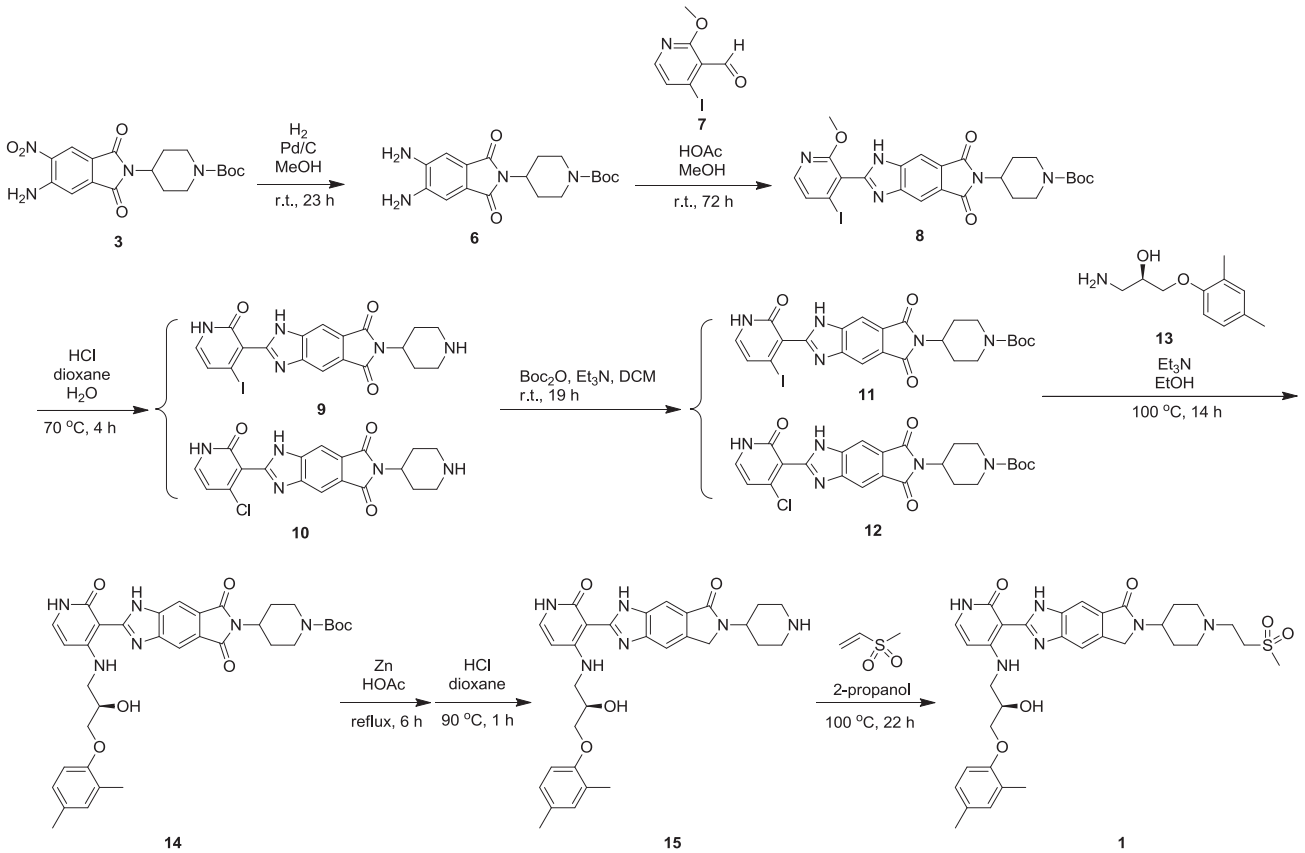


The amination of both iodide **11** and chloride **12** amine **13** was completed by heating in EtOH in the presence of triethylamine to afford aminopyridone **14** in 71.2% yield [17]. The reduction of phthalimide **14** using zinc dust in acetic acid, followed by removing Boc protective group with 4.0 M HCl in 1,4-dioxane, gave amide **15** in 63.4% yield [5,18]. The addition of the piperidine in **15** to methyl vinyl sulfone was carried out by heating in a mixed solvent of EtOH and isopropanol (v/v, 1:1), providing the final target GTx-134 (**1**) in 78.5% yield [19]. The overall yield of the linear route from **3** to **1** is 26.8%. Compound **1** has two conformational isomers **1a** and **1b**, possibly owing to tautomeric property of the imidazole moiety in **1** and hydrogen bond between imidazole proton and pyridone O (Fig. 4), which was evidenced by the two sets of peaks in ^{1}H NMR and ^{13}C NMR (DMSO-d_6), one set for each of the conformational isomers. In order to prove the two sets of the peaks in NMR spectra belong to the two conformational isomers **1a** and **1b**, not two different compounds, compound **1** was converted to di-hydrochloride salt (**1** · 2HCl) which showed only one set of peaks in both ^{1}H NMR and ^{13}C NMR obtained in DMSO-d_6, an unambiguous evidence of single compound.

As shown in Scheme 3, we had also attempted to use chloronicotinic aldehyde **16** [20] as a replacement for its iodo analog **7** to access compound **14**. Aldehyde **16** was treated with diamine **6** in MeOH in the presence of HOAc. The reaction proceeded well but only afforded the desired cyclized product **17** in 43% yield, much lower than the cyclization reaction of **7** with **6** (92%, Scheme 2), probably because of different reactivity of aldehyde moiety. With compound **17** in hand, de-methylation and subsequent Boc protection generated compound **12** in 83% yield over two steps. The following displacement of chlorine with amine **13** in Et_3N/EtOH afforded compound **14** in 72% yield. This synthetic route was deprioritized because of the low reaction yield from the cyclization reaction of diamine **6** and chloronicotinic aldehyde **16**.

Scalable synthesis of 1. The aforementioned stepwise synthetic procedure was then modified into a scalable procedure in which only column chromatography in the reaction sequence from **3** to **1** was performed at the last step. The synthesis started from hydrogenation of nitro compound **3** to give diaminophthalimide **6** which was reacted with nicotinic aldehyde **7** to give crude tricyclic compound phthalimido imidazole **8**. Demethylation of **8** followed by evaporation and basification resulted in a crude containing both iodopyridone **9** and chloropyridone **10**, which was then reacted with Boc_2O to afford a crude mixture of **11** and chloride **12**. The amination of crude iodide **11** and chloride **12** by heating with chiral amine **13** in EtOH afforded crude aminopyridone **14**, which was reduced with Zn dust in HOAc to give crude amide **15**. The transformation of **15** to **1** was carried out by heating

Scheme 2



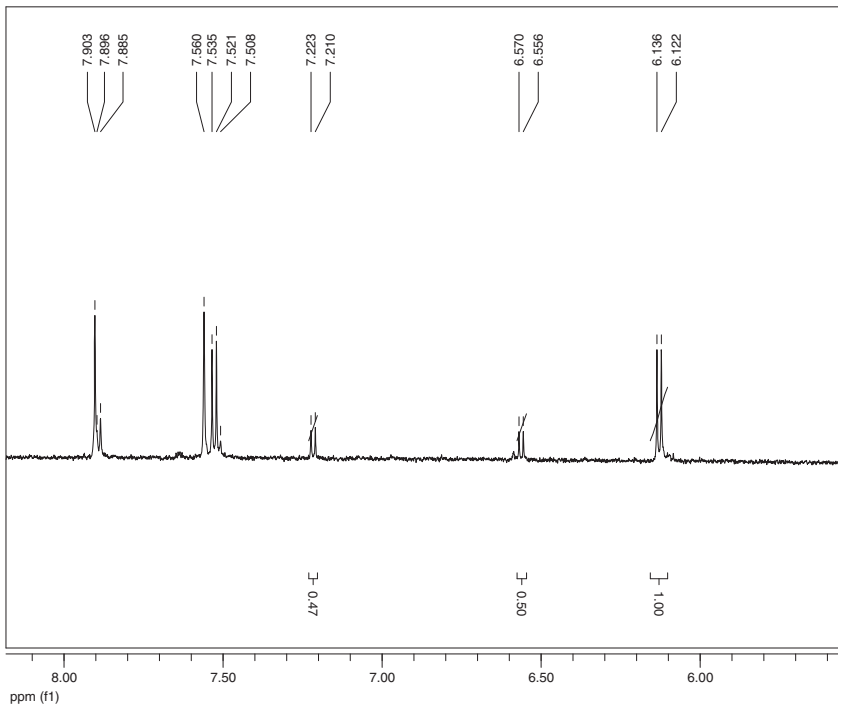


Figure 2. ^{1}H NMR (DMSO-d_6) of the crude [**9** + **10**]. The peaks at 6.56 (d, J = 5.6 Hz, 1H), 7.22 (d, J = 5.6 Hz, 1H), 7.50 (s, 1H), and 7.88 (s, 1H) belong to **9**, and the ones at 6.13 (d, J = 5.6 Hz, 1H), 7.53 (d, J = 5.6 Hz, 1H), 7.56 (s, 1H), and 7.90 (s, 1H) to **10**.

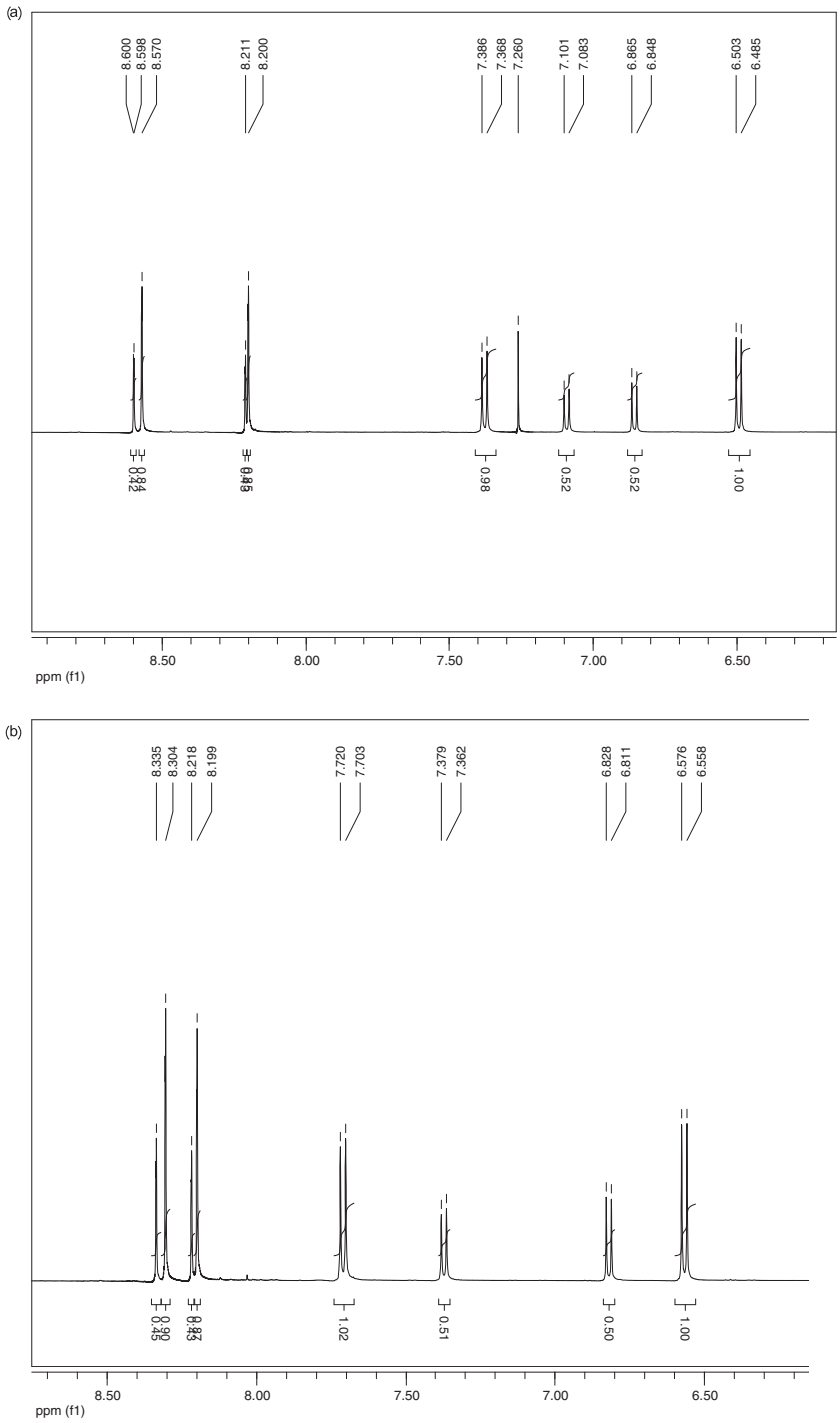


Figure 3. ^{1}H NMR of the mixture of [**11** + **12**]: (a) in $CDCl_3$. The peaks at 6.86 (d, $J = 7.0$ Hz), 7.09 (d, $J = 7.0$ Hz), 8.21 (s), and 8.60 (s) belong to **11**, while ones at 6.49 (d, $J = 7.0$ Hz), 7.38 (d, $J = 7.0$ Hz), 8.20 (s), and 8.57 (s) to **12**. (b) in DMSO-d_6. The peaks at 6.82 (d, $J = 6.8$ Hz), 7.37 (d, $J = 6.8$ Hz), 8.22 (s), and 8.34 (s) belong to **11**, while the ones at 6.57 (d, $J = 6.8$ Hz), 6.82 7.71 (d, $J = 7.2$ Hz), 8.20 (s), and 8.31 (s) to **12**.

15 with methyl vinyl sulfone in a mixed solvent of EtOH and 2-propanol. The reaction crude was purified to afford final product **1** by chromatography which was the only column chromatography employed from **3** till the last step. The overall yield of this scalable procedure is 27.2% from **3**, very similar to that the stepwise procedure (26.8%), but the separations and purifications in the stepwise synthesis were eliminated except in the last step.

In summary, we have described a practical and scalable synthesis of GTx-134 (**1**) as well as stepwise synthetic

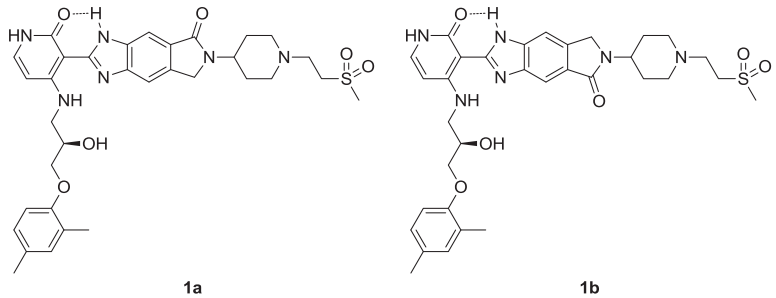
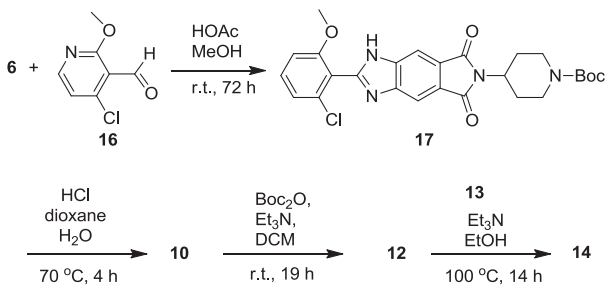


Figure 4. Two conformational isomers: **1a** and **1b**.

Scheme 3



procedures with characterization of all key intermediates. This novel small-molecule inhibitor of IGF-1R was prepared from commercially available chemicals via a nine-step reaction sequence in which only two chromatographic purifications were employed.

EXPERIMENTAL

All commercial reagents and solvents were used without purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer using TMS as internal standard set at 0 ppm. Thin-layer chromatography analysis was carried out on silica gel 60 F254 precoated aluminum sheets, and UV light was used for detection. Separation by flash column chromatography was performed on Merck silica gel (230–400 mesh). Low-resolution mass spectra (ESI) were obtained with 1100LCMSD VL Series (Agilent Technologies, Santa Clara, CA) spectrometer. The CHN analysis was performed by Numega Resonance Labs, San Diego, CA 92121.

4-(5-Amino-6-nitro-1,3-dioxo-1,3-dihydro-isoindol-2-yl)-piperidine-1-carboxylic acid tert-butyl ester (3). A mixture of 5-amino-6-nitro-isoindole-1,3-dione (**2**) (5.18 g, 25.00 mmol), *tert*-butyl 4-amino-piperidine-1-carboxylate (**5**) (6.01 g, 30.0 mmol), and imidazole (4.08 g, 60.0 mmol) in 1,4-dioxane (200 mL) was sealed in a ChemGlass pressure vessel. After it was heated at 140°C for 72 h, the reaction mixture was evaporated to dryness at 95°C (the bath temperature) under reduced pressure. The chromatography of the residue with DCM/MeOH/28% aqueous NH_3H_2O (320:10:1) afforded **3** (5.27 g, 54%). ^{1}H NMR ($CDCl_3$) $\delta = 1.48$ (s, 9H), 1.64–1.68 (2H), 2.31–2.42 (2H), 2.74–2.80 (2H), 4.19–4.27 (3H), 7.03 (br s, 2H, NH), 7.33 (s, 1H), 8.59 (s, 1H). ^{1}H NMR (DMSO-d_6) $\delta = 1.42$ (s, 9H), 1.63–1.66 (2H), 2.04–2.15 (2H), 2.80 (br, 2H), 4.03 (br, 2H) 4.15 (m, 1H), 7.38 (s, 1H), 8.23 (s, 1H), 8.36 (br s, 2H, NH). ^{13}C NMR ($CDCl_3$) $\delta = 28.4$, 28.7, 43.4, 49.4, 79.9, 114.0, 118.0, 123.1, 133.7, 137.2, 149.2, 154.6, 166.1, 166.2. ^{13}C NMR (DMSO-d_6) $\delta = 28.0$, 28.2, 43.5, 48.3, 78.7, 114.2, 115.6, 121.9, 131.6, 136.2, 150.1, 153.7, 165.8, 165.9. MS (ESI): $m/z = 391$ [M + H$^+$].

tert-Butyl 4-(5,6-diamino-1,3-dioxoisoindolin-2-yl)piperidine-1-carboxylate (6). To a mixture of nitro compound **3** (2.54 g, 6.51 mmol) and 10% Pd/C (254.0 mg) in 2-propanol (5 mL) was added MeOH (80 mL). After it was stirred under atmospheric hydrogen for 18 h, the reaction mixture was filtered through a pad of Celite and evaporated to give **6** (2.33 g, 99%). ^{1}H NMR ($CDCl_3$) $\delta = 1.47$ (s, 9H), 1.62–1.65 (2H), 2.31–2.41 (2H), 2.75 (br, 2H), 4.11–4.34 (3H), 7.06 (s, 2H). ^{1}H NMR (DMSO-d_6) $\delta = 1.42$ (s, 9H), 1.55–1.59 (2H), 2.03–2.13 (2H), 2.67–2.88 (2H), 3.99–4.07 (3H), 5.60 (br s, 4H, NH), 6.83 (s, 2H). ^{13}C NMR ($CDCl_3$) $\delta = 28.4$, 29.1, 43.0, 48.6, 79.7, 110.2, 124.6, 139.4, 154.7, 168.8. ^{13}C NMR (DMSO-d_6) $\delta = 28.6$, 28.8, 43.0, 47.5, 78.0, 106.6, 120.9, 139.8, 153.7, 168.6. MS (ESI): $m/z =$ 361 [M + H$^+$].

tert-Butyl 4-(2-(4-iodo-2-methoxypyridin-3-yl)-5,7-dioxoimidazo[4,5-f]isoindol-6(1H,5H,7H)-yl)piperidine-1-carboxylate (8). To a solution of 1,2-diamino compound **6** (2.23 g, 6.20 mmol) in MeOH (100 mL) was added 4-iodo-2-methoxynicotinic aldehyde (**7**) (1.71 g, 6.51 mmol) and HOAc (5.0 mL) resulting in a mixture which was stirred at the room temperature for 72 h, and evaporated under reduced pressure (the bath temperature from 60°C till 95°C). The chromatography of the residue with DCM/MeOH/28% aqueous NH_4OH (240:10:1) furnished **8** (3.44 g, 92%). ^{1}H NMR ($CDCl_3$) $\delta = 1.43$ (s, 9H), 1.67–1.70 (2H), 2.38–2.45 (2H), 2.69–2.83 (2H), 3.84 (s, 3H), 4.18–4.33 (3H), 7.48 (d, $J = 5.4$ Hz, 1H), 7.87 (d, $J = 5.4$ Hz, 1H), 8.03 (br, 2H). ^{1}H NMR (DMSO-d_6) $\delta = 1.43$ (s, 9H), 1.69–1.71 (2H), 2.14–2.24 (m, 2H), 2.74–2.92 (2H), 3.82 (s, 3H), 4.00–4.15 (2H), 4.24 (m, 1H), 7.66 (d, $J = 5.4$ Hz, 1H), 8.02 (2H), 8.03 (d, $J = 5.4$ Hz, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 28.4$, 28.9, 44.0,

48.9, 54.6, 80.1, 111.0, 118.5, 126.8, 128.0, 149.0, 151.9, 154.8, 161.5, 168.1, 174.8. ^{13}C NMR (DMSO-d_6) δ = 28.1, 28.6, 43.0, 48.1, 54.1, 78.8, 107.7, 113.1, 114.8, 119.9, 125.3, 126.0, 127.1, 137.7, 146.7, 148.8, 152.8, 153.9, 161.2, 167.8, 172.0. MS (ESI): m/z = 604 [M + H$^+$].

2-(4-Iodo-2-oxo-1,2-dihydropyridin-3-yl)-6-(piperidin-4-yl) imidazo[4,5-f]isoindole-5,7(1H,6H)-dione (9) and 2-(4-chloro-2-oxo-1,2-dihydropyridin-3-yl)-6-(piperidin-4-yl)imidazo[4,5-f] isoindole-5,7(1H,6H)-dione (10). A mixture of 2-methoxypiridine compound (**8**) (3.38 g, 5.60 mmol), HCl in 1,4-dioxane (4 M, 200 mL), and H_2O (1.80 mL) was heated at 70°C for 4.0 h, and evaporated under reduced pressure (the bath temperature < 60°C) to result in a residual crude which was azeotropically distilled with 2-propanol under reduced pressure (the bath temperature < 98°C). The residue was diluted with a mixed solvent of MeOH and DCM (1:4, v/v, 250 mL), basified with 28% aqueous NH_3H_2O (5 mL), sonicated for 10 min, and evaporated to dryness to provide a crude product which contained both **9** and **10**. ^{1}H NMR (DMSO-d_6) δ = 1.26–1.36 (2H), 1.76-1.80 (2H), 2.37–2.60 (2H), 2.90–2.94 (2H), 3.68–3.77 (1H), 6.13 (d, J = 5.4 Hz, 2/3H of **10**), 6.56 (d, J = 5.4 Hz, 1/3H of **9**), 7.22 (d, J = 5.4 Hz, 1/3H of **9**), 7.50 (s, 1/3H of **9**), 7.53 (s, J = 5.4 Hz, 2/3H of **10**), 7.56 (s, 2/3H of **10**), 7.88 (s, 1/3H of **9**), 7.90 (s, 2/3H of **10**). MS (ESI): m/z = 490 and 398 [M + H$^+$].

tert-Butyl 4-(2-(4-iodo-2-oxo-1,2-dihydropyridin-3-yl)-5,7-dioxoimidazo[4,5-f]isoindol-6(1H,5H,7H)-yl)piperidine-1-carboxylate (11) and tert-butyl 4-(2-(4-chloro-2-oxo-1, 2-dihydropyridin-3-yl)-5,7-dioxoimidazo[4,5-f]isoindol-6(1H,5H,7H)-yl)piperidine-1-carboxylate (12). To a solution of the crude product of the last step (mixture of **9** and **10**) in DCM (200 mL) was added Et_3N (1.92 mL, 1.4 g, 13.8 mmol) at 0°C under N_2, followed by the addition of the solution of Boc_2O (1.52 g, 6.96 mmol) in DCM (50 mL). After the reaction mixture was stirred at 0°C for 1 h and at the room temperature for 19 h, MeOH (5.0 mL) was added slowly with at 0°C. The reaction mixture was then evaporated under reduced pressure at 55°C (the bath temperature) to afford a crude residue which was subjected to chromatography with DCM/MeOH/28% aqueous NH_3H_2O (115:10:1) to afford a mixture of **11** and **12** (2.46 g, yield 83% for two steps, which was calculated based on the weight average molecular weight 528.41 of the mixture of **11** and **12** in 1:2, the ratio determined by ^{1}H NMR analysis). ^{1}H NMR ($CDCl_3$) δ = 1.47 (s, 9H), 1.69–1.72 (2H), 2.37–2.48 (2H), 2.72–2.82 (2H), 4.25–4.33 (3H), 6.49 (d, J = 7.0 Hz, 2/3H of **12**), 6.86 (d, J = 7.0 Hz, 1/3H of **11**), 7.09 (d, J = 7.0 Hz, 1/3H of **11**), 7.38 (d, J = 7.0 Hz, 2/3H of **12**), 8.20 (s, 2/3H of **12**), 8.21 (s, 1/3H of **11**), 8.57 (s, 2/3H of **12**), 8.60 (s, 1/3H of **11**). ^{1}H NMR (DMSO-d_6) δ = 1.43 (s, 9H), 1.71–1.74 (2H), 2.12–2.22 (2H), 2.76–2.92 (2H), 4.00–4.11 (2H), 4.27–4.35 (1H), 6.57 (d, J = 6.8 Hz, 2/3H of **12**), 6.82 (d, J = 6.8 Hz, 1/3H of **11**), 7.37 (d, J = 6.8 Hz, 1/3H of **11**), 7.71 (d, J = 7.2 Hz, 2/3H of **12**), 8.20 (s, 2/3H of **12**), 8.22 (s, 1/3H of **11**), 8.31 (s, 2/3H of **12**), 8.34 (s, 1/3H of **11**). MS (ESI): m/z = 590 and 498 [M + H$^+$].

(R)-tert-Butyl 4-(2-(4-((3-(2,4-dimethylphenoxy)-2-hydroxypropyl) amino)-2-oxo-1,2-dihydropyridin-3-yl)-5,7-dioxoimidazo[4,5-f] isoindol-6(1H,5H,7H)-yl)piperidine-1-carboxylate (14). To a suspension of a mixture of iodide **11** and chloride **12** (**11**/**12** = 1:2, mol/mol, 2.41 g, 4.56 mol) in EtOH (50 mL) was added (*R*)-1-amino-3-(2,4-dimethyl-phenoxy)-propan-2-ol (**13**) (1.34 g, 6.84 mmol) and Et_3N (1.27 mL, 922.9 mg, 9.12 mmol) resulting a mixture which was heated in a ChemGlass pressure vessels at 100°C for 14 h and then concentrated. The residues was subjected to chromatography with DCM/MeOH/28% aqueous NH_3H_2O (150:10:1) to give **14** (2.13 g, 71%). ^{1}H NMR (DMSO-d_6) δ = 1.30 (s, 9H), 1.66–1.70 (2H), 2.12–2.18 (2H), 2.18 (s, 3H), 2.20 (s, 3H), 2.72–2.90 (2H), 3.55 (m, 1H), 3.70 (m, 1H) 3.95–4.09 (4H), 4.11–4.24 (2H), 5.54 (d, J = 5.2 Hz, 1H, OH), 6.20 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.96 (s, 1H), 7.37 (m, 1H), 7.63 (s, 1H), 8.08 (s, 1H), 10.94 (1H, NH), 11.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ = 15.9, 20.2, 28.0, 28.6, 45.6, 47.9, 67.6, 69.6, 78.7, 90.9, 94.6, 107.7, 111.1, 111.8, 124.4, 124.9, 125.5, 127.0, 129.0, 131.1, 135.8, 136.4, 145.1, 153.8, 154.3, 155.8, 157.0, 162.3, 168.1 (one C is missing). MS (ESI): m/z = 657 [M + H$^+$].

(R)-2-(4-((3-(2,4-dimethylphenoxy)-2-hydroxypropyl)amino)-2-oxo-1,2-dihydropyridin-3-yl)-6-(piperidin-4-yl)-6,7-dihydroimidazo [4,5-f]isoindol-5(3H)-one (15). A suspension of phthalimide **14** (2.0 g, 3.05 mmol) and zinc dust (2.0 g, 30.6 mmol) in HOAc (40 mL) was heated till refluxing (bath temperature 120°C) for 2 h and concentrated to result in a crude, which was then mixed with HCl in 1,4-dioxane (4.0 M, 40 mL), heated at 90°C for 1 h and evaporated. The residue was diluted with a mixed solvent of MeOH and DCM (1:1, v/v, 50 mL), basified with aqueous NH_3H_2O (28% in H_2O, 10 mL), concentrated, and loaded on silica gel to subject a chromatographic purification with DCM/MeOH/28% aqueous NH_3H_2O (90:10:1) to furnish **15** (1.05 g, 63.4%). ^{1}H NMR (MeOH-d_4) δ = 2.11–2.21 (4H), 2.24 (s, 3H), 2.25 (s, 3H), 3.17–3.24 (2H), 3.54–3.57 (2H), 3.67 (m, 1H), 3.80 (m, 1H), 4.03–4.11 (2H), 4.27 (m, 1H), 4.43 (m, 1H), 4.52 (s, 2H), 6.32 (d, J = 7.6 Hz, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.96 (s, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.49 (br s, 1H), 7.86 (s, 1H). ^{13}C NMR (MeOH-d_4) δ = 16.5, 20.7, 28.1, 44.9, 46.7, 47.6, 70.1, 70.7, 93.6, 97.0, 112.3, 113.8, 116.8, 119.7, 122.4, 127.0, 127.6, 128.2, 131.0, 132.5, 136.4, 137.0, 156.1, 159.0, 164.7, 171.3 (two Cs are missing). MS (ESI): m/z = 543 [M + H$^+$].

2-{4-[(R)-3-(2,4-Dimethyl-phenoxy)-2-hydroxy-propylamino]-2-oxo-1,2-dihydro-pyridin-3-yl}-6-[1-(2-methanesulfonyl-ethyl)-piperidin-4-yl]-6,7-dihydro-3H-1,3,6-triaza-s-indacen-5-one (1). To a solution of piperidine compound **15** (1.00 g, 1.84 mmol) in a mixed solvent of EtOH (25 mL) and 2-propanol (25 mL) was added methyl vinyl sulfone

(1.95 g, 18.4 mmol), and the resulting mixture which was heated at 100°C for 22 h and concentrated. Chromatography of the crude residue with DCM/MeOH/28% aqueous NH_4OH (190:10:1) provided title compound (937.1 mg, 78.5%). ^{1}H NMR (DMSO-d_6) (two conformational isomers **1a** and **1b**) δ = 1.68–1.81 (4H), 2.10 (m, 2H), 2.15 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.73 (m, 2H), 2.99 (m, 2H), 3.07 (s, 3H, CH_3), 3.31 (m, 2H), 3.55 (m, 1H), 3.70 (m, 1H), 3.93–4.07 (3H), 4.13 (m, 1H), 4.43 (s, 1H), 4.47 (s, 1H), 5.48 (d, J = 6 Hz, 0.5H, exchangeable H), 5.53 (d, J = 6 Hz, 0.5H, exchangeable H), 6.20 (d, J = 7.6 Hz, 0.5H), 6.21 (d, J = 7.6 Hz, 0.5H), 6.77 (d, J = 8.4 Hz, 0.5H), 6.78 (d, J = 8.4 Hz, 0.5H), 6.90 (d, J = 8.4 Hz, 0.5H), 6.91 (d, J = 8.4 Hz, 0.5H), 6.92 (s, 0.5H), 6.94 (s, 0.5H), 7.30–7.347 (1.5H), 7.68 (s, 0.5H), 7.80 (s, 0.5H), 7.95 (s, 0.5H), 11.11–11.18 (1H, exchangeable NH), 11.22–11.23 (1H, exchangeable NH). ^{13}C NMR (DMSO-d_6) (two conformational isomers) δ = 15.92, 20.04, 29.66, 41.53, 45.53, 45.48, 45.54, 45.61, 48.63, 51.04, 51.11, 52.13, 67.73, 67.76, 69.55, 69.71, 91.40, 91.45, 94.53, 94.58, 105.96,, 110.67, 110.72, 111.21, 125.54, 125.61, 126.23, 126.36, 127.03, 128.84, 128.93, 131.11, 131.82, 134.50, 134.88, 134.91, 135.72, 135.80, 141.22, 144.26, 153.81, 154.33, 154.40, 156.58, 156.74, 162.27, 167.45, 167.49. MS (ESI): m/z = 649 [M + H$^+$]. Anal. Calcd for $C_{33}H_{40}N_6O_6S$: C, 61.09; H, 6.21; N, 12.95, S 4.94; Found: C, 60.85; H, 6.17; N, 12.87, S 4.85.

Preparation and NMR analysis of dihydrochloride salt of 1. To a solution of compound **1** (108.5 mg, 0.20 mmol) in a mixed solvent of MeOH and DCM (200 mL) (3:1, v/v) was added a solution of HCl (1.0 M, 0.42 mL) resulting a clean solution which was stirred for 5 min at room temperature, and evaporated to provide **1** · 2HCl (144.4 mg, 100%). ^{1}H NMR (DMSO-d_6) δ = 1.95–1.99 (2H), 2.10 (m, 2H), 2.17 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.20–2.30 (2H), 3.14 (s, 3H, CH_3), 3.17–3.26 (2H), 3.47–3.57 (3H), 3.60–3.72 (3H), 3.80–3.84 (2H), 3.95–4.05 (2H), 4.12 (m, 1H), 4.37 (m, 1H), 4.45 (s, 2H), 6.21 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.96 (s, 1H), 7.35 (d, J = 7.6 Hz, 1H), (1.5H), 7.60 (s, 1H), 7.85 (s, 1H), 10.93 (br, 1H, exchangeable NH), 10.93 (d, J = 6.0 Hz, 1H, exchangeable NH), 11.36 (br, 1H, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ = 15.9, 20.1, 26.8, 40.8, 45.4, 45.9, 46.0, 48.0, 48.6, 51.0, 67.7, 69.7, 90.7, 94.7, 108.1, 108.8, 111.2, 125.6, 126.7, 127.1, 128.9, 131.1, 134.8, 135.5, 136.7, 138.7, 152.6, 154.4, 157.0, 161.9, 167.4.

tert-Butyl 4-(2-(4-chloro-2-methoxypyridin-3-yl)-5,7-dioxoimidazo[4,5-f]isoindol-6(1H,5H,7H)-yl)piperidine-1-carboxylate (17). To a solution of 1,2-diamino compound **6** (2.23 g, 6.20 mmol) in MeOH (100 mL) was added 4-chloro-2-methoxynicotinic aldehyde (**7**) (1.12 g, 6.51 mmol) and HOAc (5.0 mL) resulting in a mixture which was stirred at the room temperature for 72 h, and evaporated under reduced pressure (the bath temperature from 60°C till 95°C). The chromatography of the residue with CH_2Cl_2/MeOH/28% aqueous NH_4OH (240:10:1) gave **17** (1.35 g, 42.5%). ^{1}H NMR (DMSO-d_6) δ = 1.34 (s, 9H), 1.68–1.72 (2H), 2.13–2.24 (m, 2H), 2.74–2.90 (2H), 3.85 (s, 3H), 4.00–4.15 (2H), 4.24 (m, 1H), 7.37 (d, J = 5.6 Hz, 1H), 8.02 (2H), 8.34 (d, J = 5.6 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ = 28.1, 28.7, 43.0, 48.0, 54.3, 78.8, 111.2, 113.8, 118.2, 125.2, 142.8, 144.8, 149.6, 153.8, 162.4, 167.9, 172.3. MS (ESI): m/z = 512 [M + H$^+$].

2-(4-Chloro-2-oxo-1,2-dihydropyridin-3-yl)-6-(piperidin-4-yl)imidazo[4,5-f]isoindole-5,7(1H,6H)-dione (10). A mixture of 2-methoxypiridine compound **17** (1.28 g, 2.5 mmol), HCl in 1,4-dioxane (4.0 M, 60 mL) and H_2O (0.5 mL) was heated at 70°C for 4.0 h, and evaporated under reduced pressure (the bath temperature < 60°C) to result in a residual crude, which was azeotropically distilled with 2-propanol under reduced pressure (the bath temperature < 98°C). The residue was diluted with a mixed solvent of MeOH and DCM (1:4, v/v, 250 mL), basified with 28% aqueous NH_3H_2O (5 mL), sonicated for 10 min, and evaporated to dryness to furnish crude product **10**. ^{1}H NMR (DMSO-d_6) δ = 1.26–1.35 (2H), 1.74–1.80 (2H), 2.37–2.60 (2H), 2.90–2.94 (2H), 3.75 (m, 1H), 6.13 (d, J = 5.2 Hz, 1H), 7.53 (s, J = 5.2 Hz, 1H), 7.62 (s, 1H), 7.85 (s). MS (ESI): m/z = 398 [M + H$^+$].

tert-Butyl 4-(2-(4-chloro-2-oxo-1,2-dihydropyridin-3-yl)-5,7-dioxoimidazo[4,5-f]isoindol-6(1H,5H,7H)-yl)piperidine-1-carboxylate (12). To a solution of the crude chloropyridone compound **10** in DCM (80 mL) was added Et_3N (0.77 mL, 557.2 mg, 5.51 mmol) at 0°C under N_2, followed by the addition of the solution of Boc_2O (820.0 mg, 3.75 mmol) in DCM (50 mL). After the reaction mixture was stirred at 0°C for 1 h and at the room temperature for 19 h, MeOH (2.0 mL) was added slowly with at 0°C. The reaction mixture was then evaporated under reduced pressure at 55°C (the bath temperature) to afford a crude residue which was subjected to chromatography with DCM/MeOH/28% aqueous NH_3H_2O (115:10:1) to afford **12** (1.03 g, yield 83% for two steps). ^{1}H NMR ($CDCl_3$) δ = 1.47 (s, 9H), 1.70–1.74 (2H), 2.37–2.49 (2H), 2.75–2.83 (2H), 4.25–4.33 (3H), 6.49 (d, J = 6.8 Hz, 1H), 7.39 (d, J = 6.8 Hz, 1H), 8.21 (s, 1H), 8.58 (s, 1H). ^{1}H NMR (DMSO-d_6) δ = 1.43 (s, 9H), 1.71–1.74 (2H), 2.12–2.22 (2H), 2.76–2.92 (2H), 4.00–4.11 (2H), 4.25 (m, 1H), 6.57 (d, J = 7.0 Hz, 1H), 7.71 (d, J = 7.0 Hz, 1H), 8.22 (s, 1H), 8.31 (s,1H). ^{13}C NMR ($CDCl_3$) δ = 28.4, 29.0, 43.8, 49.3, 79.7, 109.4, 111.4, 116.3, 123.0, 128.0, 128.7, 135.8, 136.7, 146.4, 146.8, 148.5, 148.8, 154.6, 162.9, 167.8. ^{13}C NMR (DMSO-d_6) δ = 28.0, 28.7, 43.5, 48.3, 78.8, 107.0, 109.8, 115.5, 122.0, 127.5, 128.1, 135.9, 137.6, 145.8, 146.6, 149.3, 153.8, 160.4, 167.1, 167.2. MS (ESI): m/z = 498 [M + H$^+$].

Preparation of 14 from 12. To a suspension of chloride **12** (747.0 mg, 1.50 mol) in EtOH (25 mL) was added (*R*)-1-amino-3-(2,4-dimethyl-phenoxy)-propan-2-ol (**13**) (439.3 mg, 2.25 mmol) and Et_3N (0.42 mL, 3.4.7 mg, 3.0 mmol) resulting a mixture which was heated in a ChemGlass pressure vessels at 100°C for 14 h and then concentrated. The residues was subjected to chromatography with CH_2Cl_2/MeOH/28% aqueous NH_3H_2O (150:10:1) to provide **14** (711.3 mg, 72%).

Scalable synthetic procedure of 1 from 3. To a mixture of nitro compound **3** (2.54 g, 6.51 mmol) and 10% Pd/C (254.0 mg) was added 2-propanol (5.0 mL), and then MeOH (80.0 mL). After it was stirred under atmospheric hydrogen for 18 h, the reaction mixture was diluted with MeOH (40.0 mL) and HOAc (5.0 mL), sonicated for 30 min, and filtered over Celite. The filtrate was mixed with aldehyde **7** (1.76 g, 6.69 mmol), stirred at room temperature for 72 h, and evaporated under reduced pressure (the bath temperature from 60°C till 95°C) to afford an oil which was mixed with 4.0 M HCl in1,4-dioxane (200.0 mL) and H_2O (2.0 mL), heated at 70°C for 4.0 h and evaporated under reduced pressure (the bath temperature < 60°C), and the residual crude was azeotropically distilled with 2-propanol under reduced pressure (the bath temperature < 98°C) and pumped at 25°C for 16 h, affording 3.64 g crude product. To a solution of the crude in DCM (100 mL) was added Et_3N (3.00 mL, 2.17 g, 21.44 mmol) 0°C under N_2, followed by the addition of the solution of Boc_2O (2.40 g, 11.00 mmol). After the reaction mixture was stirred at 0°C for 1 h and at the room temperature for 19 h, MeOH (5 mL) was added slowly with at 0°C and then evaporated under reduced pressure at 55°C (the bath temperature) to afford a crude residue which was pumped for 16 h. The residue was then mixed with amine **13** (2.00 g, 10.24 mmol) and Et_3N (1.20 mL, 867.0 mg, 8.57 mmol) in EtOH (50 mL) resulting a mixture that was heated in a ChemGlass pressure vessel at 100°C for 14 h and then concentrated to give a fluorescent crude product, which was mixed with zinc dust (425.0 mg, 65.0 mmol) and HOAc (50 mL). After it was heated till refluxing (bath temperature 120°C) for 2 h, the reaction mixture was cooled to room temperature, diluted with a mixed solvent of MeOH (120 mL) and DCM (20 mL), and filtered. The filtrate was concentrated at 70°C (the bath temperature) under reduced pressure, and further dried azotroplically with isopropanol. The resulting residue was dissolved in a mixed solvent of DCM and MeOH (1:1, v/v, 200 mL) at room temperature, basified with aqueous NH_3H_2O (28% in H_2O, 50 mL) at 0°C, stirred for 30 min at room temperature, and then poured into water (150 mL). The aqueous phase was extracted with DCM (3 × 60 mL), and then with 60 mL of mixed solvent DCM and MeOH (4:1, v/v). The combined organic extracts were washed with saturated NaCl solution, dried over $MgSO_4$, and concentrated to give a crude product **15**, which was heated together with methyl vinyl sulfone (2.0 g, 16.9 mmol) in a mixed solvent of EtOH (25 mL) and 2-propanol (25 mL) at 100°C for 22 h. The reaction mixture was concentrated to result in a crude residue, which was subjected to chromatography (CH_2Cl_2 /MeOH/28% aqueous NH_3H_2O) (190:10:1) to afford **1** (1.15 g, 27.2%).

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[9] We claim that the purpose of this report is providing detailed synthetic procedures as references for chemists to use in their own work on similar reactions, and there is neither an intent of commercial use of the product nor interest in further research work on the application of it.

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[20] 4-Chloro-2-methoxy-1,2-dihydropyridine-3-carbaldehyde (**16**), the lowest price in June, 2014: $65.0/mmol ($380/g) (Broadpharm, San Diego, CA, 92121); 4-Iodo-2-methoxy-1,2-dihydropyridine-3-carbaldehyde (7), the lowest price in November, 2014: $65.8/mmol ($250/g) (Key Organics, Camelford, Cornwall, PL32 9RA, UK).