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Development of a Practical Synthesis of STA-5312, a Novel Indolizine Oxalylamide Microtubule Inhibitor

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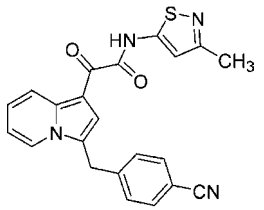
Abstract:

An efficient synthesis of the novel microtubule inhibitor STA-5312 (3-[(4-cyanophenyl)methyl]-*N*-(3-methyl-5-isothiazolyl)-α-oxo-1-indolizineacetamide) was developed. A novel DMF/Me_2SO_4 directed regioselective synthesis of the 3-(4-cyanobenzoyl)-indolizine (4) was a critical transformation within the four-step process. Alternatively, a CuCl mediated synthesis of 3-(4-cyanobenzyl)indolizine (5) was also developed. All intermediates were obtained in high quality and were used directly for the next step without extensive purification. The drug substance itself was purified by recrystallization from a mixture of THF and water, resulting in a high purity product (HPLC >98%). The process was applied successfully in the manufacturing of kilograms of GMP API.

Introduction

The development of multidrug resistance (MDR) is one of the leading causes of chemotherapy failure in cancer treatment.[1] The search for new chemical entities that can overcome MDR remains an active research area in anticancer drug discovery and development.[2]

3-[(4-Cyanophenyl)methyl]-*N*-(3-methyl-5-isothiazolyl)-α-oxo-1-indolizineacetamide, STA-5312 (**1**), possesses potent antitumor activity both in vitro and in vivo through its binding to a novel binding site in the microtubules.[3] More remarkably, STA-5312 demonstrates similar potency in inhibiting tumor growth in tumor xenograph animal models with both wild type and MDR type cancers.[4] As a novel microtubule inhibitor, STA-5312 was selected as a clinical development candidate.



STA-5312 (**1**)

Results and Discussion

The medicinal chemistry approach to the synthesis of STA-5312 is depicted in Scheme 1. 4-Cyanophenacyl bromide (**2**) was treated with 2 equiv of pyridine in acetonitrile at room temperature.[5] The resulting pyridinium salt was easily isolated in quantitative yield by collecting the precipitates from the reaction mixture, which, after drying in vacuo, was treated with 3-butyn-2-one in the presence of potassium carbonate as a base at room temperature in THF.[6] Removal of inorganic salt, concentration of the organic solution, and flash column purification with silica gel resulted in 1-acetyl-3-(4-cyanobenzoyl)indolizine (**3**) in 72% yield. All attempts to transform the acetyl group to the desired oxalyl functionality or its equivalent were not successful. However, when compound **3** was refluxed in benzene together with 3 equiv of ethylene glycol and 2 equiv of *p*-toluenesulfonic acid monohydrate an unprecedented regioselective deacetylation (retro Friedel−Crafts reaction) occurred leading to the formation of 3-(4-cyanobenzoyl)-indolizine **4**. This interesting transformation, however, was difficult to scale up and required silica gel chromatographic purification to provide pure product **4** in yields ranging from 30% to 60%. Reduction of the carbonyl group of **4** with $NaBH_3CN$ and TMSCl gave the key 3-(4-cyanobenzyl)-indolizine **5** in 56% yield after purification by silica gel chromatography.[7] Oxalylation of compound **5** with 1.2 equiv of oxalyl chloride was carried out in anhydrous diethyl ether at 0 °C. The removal of excessive oxalyl chloride and solvent required careful control of the external bath temperature to be below 25 °C in order to avoid decomposition. The crude oxalyl monochloride derivative **5A** was then used in the amidation reaction without purification. Thus, the THF solution of compound **5A** was treated with 2 equiv of 5-amino-3-methylisothiazole 0 °C. After silica gel chromatographic purification, STA-5312 (**1**) was isolated as a yellow

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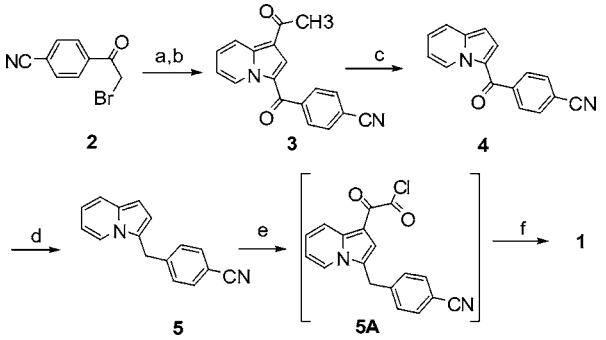
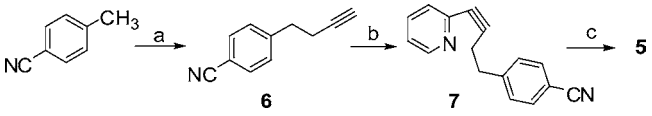
Scheme 1[a]



[a] Reagents and conditions: (a) pyridine, CH_3CN, rt, quantitative; (b) K_2CO_3, 3-butyn-2-one, THF, rt, 72%; (c) $HOCH_2CH_2OH$, *p*-TsOH·H_2O, benzene, reflux, 60%; (d) $NaBH_3CN$, TMSCl, CH_3CN, rt, 56%; (e) $(COCl)_2$, Et_2O, 0 °C; (f) 5-amino-3-methylisothiazole (2 equiv), THF, 0 °C, 78% (two steps).

Scheme 2[a]



[a] Reagents and conditions: (a) LDA, DMPU, propargyl bromide, THF, −78 to −40 °C, 92%; (b) 2-bromopyridine, $Pd(PPh_3)_2Cl_2$, CuI, TEA, THF, 60 °C, 80%; (c) CuCl, TEA, DMA, 130 °C, 90%.

crystalline solid (78% yield, HPLC purity >98%). However, we encountered significant scale-up challenges when the reaction was carried out at >10 g scales. Only moderate yield (30−40%) was achieved and often required multiple runs of column purification to achieve a purity of >95%.

In order to implement a process that is transferable for large scale GMP manufacturing, significant efforts were required to resolve the major drawbacks such as the uses of expensive reagents (i.e., 3-butyn-2-one), toxic reagent and solvent (i.e., $NaBH_3CN$ and benzene, respectively), low yield, and requirement of silica gel chromatographic purification in key steps.

With the recognition that the transformation of compound **3** to the key intermediate **4**, via the selective removal of the pendent 1-acetyl group is not atomically economic, we initiated our process development by exploring new routes that could lead to more direct formation of the key intermediate **4** or **5**.

A publication from the group of Gevorgyan described a novel synthesis of 3-alkylindolizines via a CuCl mediated cycloisomerization of 2-alkynylpyridine.[8] Thus, when 2-hex-1-ynylpyridine was heated to 130 °C in the presence of 50 mol % of CuCl, 3-*n*-propylindolizine was synthesized in 91% yield.[8] We were able to successfully apply this novel approach to the synthesis of compound **5** in up to a 50 g scale (Scheme 2). α-Deprotonation of *p*-tolunitrile with freshly prepared LDA (*n*-BuLi and diisopropylamine) at −78 °C in THF was followed by slow addition of neat propargyl bromide. The reaction temperature was raised to −40 °C and then quenched with saturated NH_4Cl solution. 4-(Butyn-3-yl)benzonitrile (**6**) was easily purified and isolated as a solid in 92% yield following standard aqueous washings, removal of organic solvents, and drying under a vacuum.

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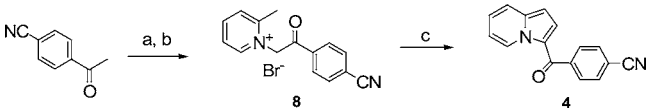
Scheme 3[a]



[a] Reagents and conditions: (a) Br_2, EtOAc, rt; (b) picoline, MeCN, rt, 83% (two steps); (c) $DMF(O\text{-}Bu^t)_2$ (10 equiv), DMF, 130−135 °C, 80%.

The Sonogashira coupling reaction[9] of **6** with 2-bromopyridine proceeded smoothly in THF at 60 °C to afford the desired 2-alkynylpyridine derivative **7** as an oil in 80% yield and excellent purity of >98% (HPLC, 230 nm) after routine workup and removal of organic solvents. The CuCl mediated cyclization reaction was carried out in TEA/DMA (1:7 volume ratio) at 130 °C with 1 equiv of CuCl. We found in our hand that, by using 1 mol equiv of CuCl, the cyclization reaction proceeded quickly and took only a few hours to reach completion at a scale of 10−50 g. After cooling to room temperature, the reaction mixture was added to water and ethyl acetate. Treatment of the organic solution with charcoal and removal of solvent afforded the desired key indolizine compound **5** as an off-white solid in 90% yield with an HPLC purity of >95%.

Being aware of the regulatory requirement to eventually achieve a ppm level of transition metals in drug substances, we decided that before we further optimized this route that utilized Pd and Cu, we would also explore alternative routes that do not employ transition metals. In our laboratory, we were able to dramatically improve the chemoselectivity of a reported method by using DMF di-*tert*-butyl acetal (Scheme 4).[10] Under the newly established reaction condition, reaction of picolinium salt **8**, which was easily prepared by treating picoline with 4-cyanophenacyl bromide (**2**),[11] with a large excess (10−15 equiv) of DMF di-*tert*-butyl acetal at 130 °C with DMF as a solvent, resulted in the isolation of the indolizine **4** in good yield (Scheme 3). However, the high cost of DMF di-*tert*-butyl acetal makes it less attractive for large scale production. Hence we set out to explore a more affordable reagent to replace the acetal and hopefully to improve the selectivity further.[12]

The intermolecular cyclization promoted by DMF acetal presumably proceeds via the formation of the iminium intermediate (**I**). Apparently, the bulky *tert*-butyl group accelerates the in situ formation of its corresponding iminium intermediate (**I**, R = *t*-Bu) and therefore facilitates the intermolecular cyclization reaction. We rationalized that a preformed iminium intermediate should further favor the formation of the intermolecular cyclization. It is reported that the formation of the DMF solution of methoxymethylene-dimethylammonium methylsulfate (**I**, R = Me) was achieved via the adduction of DMF with methyl sulfate at elevated temperatures.[13] The adduct **I** had been applied in the

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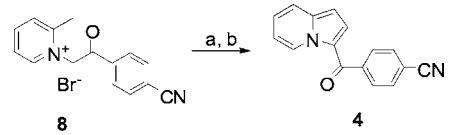
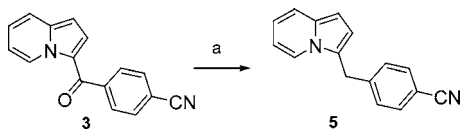
Scheme 4[a]



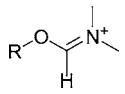
[a] Reagents and conditions: (a) Me_2SO_4/DMF, 60−80 °C; (b) **8**, Et_3N, DMF, 0 °C to rt, 76%.

Scheme 5[a]



[a] Reagents and conditions: (a) BH_3−THF, MeOH/MeCN, 50 °C, 52%.

preparation of dioxolanes and dioxanes[14] and in the Beckmann rearrangement of cyclohexanone oxime to its corresponding ϵ-caprolactam.[15]



(**I**)

To our delight, when the picolinium salt **8** was treated at room temperature with a preformed DMF solution of methoxymethylenedimethylammonium methylsulfate in the presence of Et_3N as a base, the desired indolizine product **4** was readily formed as the major product (Scheme 4). The reaction was easy to scale up. We obtained reproducible yields and purities at scales ranging from 20 to 100 g (75−85%). Isolation was readily achieved by adding water to the reaction mixture resulting in the precipitation of product **4** which was collected, washed with water, and vacuum-dried.

The reduction of the 3-acylindolizine **4** to the key intermediate **5** was achieved by using BH_3−THF as an alternative reducing agent to the toxic sodium cyanoborohydride used during the earlier stage of the program (Scheme 5).[16] The use of MeCN as solvent was crucial in preventing the reduction of the cyano functional group. Interestingly, we found that a small amount of MeOH was beneficial to the reaction and resulted in a much cleaner reaction. The best ratio for **4**/BH_3−THF/MeOH was found to be 1/4/2. The optimal reaction temperature was 50 °C. A higher temperature often led to a much lower yield, while for temperatures lower than 50 °C sluggish reactions occurred. Subsequent investigation determined that contaminants from boranic side products in compound **4** often caused messy and unpredictable reactions when **4** was treated with oxalyl chloride. Pure product **5** (HPLC purity >95%) was obtained as an off-white solid by filtering the crude product through a short silica gel plug using DCM and hexanes as eluents to remove the contaminants. Although the yield of this step, after purification, is moderate (50−60%), the low cost of its precursor compound **4** made it attractive to large scale manufacturing.

Finally, optimization of the oxalylation of compound **5** and subsequent amidation with 2-amino-4-methylthiazole led to the establishment of a one pot process in THF that not only significantly improved the reaction efficiency but also simplified the isolation of high purity production while eliminating the necessity of chromatographic purification. When THF was used as solvent, treatment of **5** with oxalyl chloride (1.2 equiv) at −40 °C was followed by sequential addition into the reaction mixture of Et_3N (1.2 equiv), DMAP (10 mol %), and 2-amino-4-methylthiazole (1.2 equiv as in THF). In order to minimize the formation of impurities, it was imperative to maintain the reaction temperature below −40 °C during the oxalylation reaction and below −25 °C during the subsequent amidation reaction. The use of 10 mol % of DMAP allowed the amidation to proceed smoothly while the reaction temperature was maintained below −25 °C which significantly reduced the formation of impurities.

After the reaction proceeded to completion (monitored by TLC), the reaction was quenched with about 1.5 volume equiv of 3% $NaHCO_3$ aqueous solution which led to the precipitation of the product STA-5312 while most of the impurities remained in the solution phase. After washing the collected solid with THF/water (1:2) and 80% EtOH, the crude product was then dissolved in THF. Attempts to recrystallize the product with the addition of an organic antisolvent, such as heptane, EtOH, *i*-PrOH, or EtOAc, led to the formation of an oily paste. On the other hand, adding water to the THF solution resulted in the precipitation of the product as fine powders with a purity of >98%.

Conclusions

We developed an efficient four-step process for the synthesis of STA-5312, a novel indolizine type microtubule inhibitor. All reaction intermediates were obtained in high purities which were used directly for the next step without extensive purification. The drug substance itself was purified by recrystallization from THF and water. The novel DMF/Me_2SO_4 directed synthesis of mono 3-substituted indolizines formed the key transformation of the process. This chemical process was applied successfully to the multi-kilogram GMP manufacturing of the API (Scheme 6). When comparing the new route to the original route, we were able to not only shorten the synthesis from six steps to four steps but also eliminate the use of expensive reagents, hazardous solvents, and chromatographic purifications.

Experimental Section

General Procedures: Melting points were obtained from DSC Q100, TA Instrument (with heating ramp 10.0 °C/min, nitrogen purge) and were uncorrected. NMR data were obtained at 300 MHz using a Varian instrument with TMS as an internal standard. FTIR analysis was performed with a PERKIN ELMER FT−IR spectrometer (Spectrum 1000), Norwalk, CT. HPLC analyses were performed on a Hewlett-Packard series 1100 reversed-phase liquid chromatograph equipped with a UV detector and an Agilent Eclipse XDB-C18, 150 mm × 4.6 mm, 5 μm column. LCMS data were acquired from a Finnigan LCQ LC/MS system (equipped

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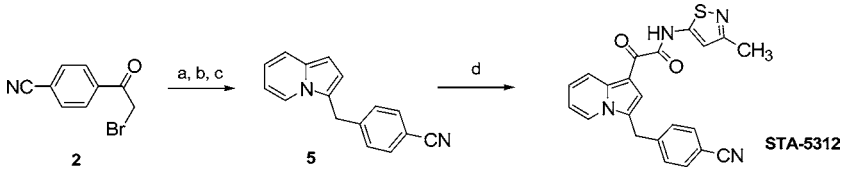
(16) Mlotkowska, B. L.; Cushman, James A.; Harwood, J.; Lam, W. W.; Ternay, A. L., Jr. *J. Heterocycl. Chem.* **1991**, *28*, 731.

Scheme 6[a]



[a] Reagents and conditions: (a) picoline, MeCN, rt, 83%; (b) (i) Me_2SO_4, DMF, 60 °C; (ii) Et_3N, 0 °C to rt, 76%; (c) BH_3−THF, MeOH/MeCN, 50 °C, 52%; (d) (i) oxalyl chloride, THF, −50 to −40 °C; (ii) 5-amino-3-methylisothiazole, TEA, DMAP, THF, −30 to −25 °C, 2 h, 50%.

with an Agilent 1100 series HPLC). Microanalysis was performed by Atlantic Microlab, Inc., Norcross, GA.

All reagents and solvents were commercially available and were used without further purification unless otherwise stated. All anhydrous reactions were conducted in oven-dried glassware under the protection of nitrogen. Air and moisture sensitive reagents were transferred via syringes. Column chromatographic purification was performed with EM silica gel 60 under slightly pressured air or nitrogen.

3-(4-Cyanobenzoyl)indolizine (4). To a solution of 4-acetylbenzonitrile (34 g, 235 mmol) in EtOAc (320 mL) was added Br_2 (neat, 11.9 mL, 320 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After the solvent was removed under reduced pressure, the resulting residue was dissolved in CH_3CN (210 mL). To it was added picoline (50 mL, 500 mmol). After the reaction mixture was stirred for 2 h at room temperature and then 0.5 h at 0 °C, EtOAc (70 mL) was added to the mixture. The resulting precipitates were collected by filtration and washed with EtOAc to give 2-methyl-1-(4-cyano)-phenacylpyridinium bromide (60 g, 88%) which was used directly in the next step. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.05−8.03 (m, 8H), 6.78 (s, 2H), 2.74 (s, 3H). To a stirred suspension of the above picolinium salt (50 g, 120 mmol) in DMF (500 mL) was added DMF−Me_2SO_4 (400 mL) (pre-prepared in a separate reaction flask by stirring a mixture of equal mole equivalents of DMF and Me_2SO_4 at 60−80 °C for 3 h, then cooled to room temperature). After the addition, the reaction mixture was stirred at room temperature for 15 min. To it was then added Et_3N (500 mL) while the inner temperature was kept between 25 and 40 °C. After stirring at room temperature for 2 h, the reaction mixture was charged with ice water (2000 mL) with stirring which led to the formation of slurry. The precipitates were collected, washed with water, and dried under a vacuum to give 29 g (76%) of 3-(4-cyanobenzoyl)indolizine. R_f 0.3 (10% ethyl acetate in hexanes); mp 156−157 °C (recrystallized from ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.98 (d, J = 6.6 Hz, 1H), 7.89−7.77 (m, 4H), 7.60 (d, J = 11 Hz, 1H), 7.30−7.22 (m, 2H), 7.01 (m, 1H), 6.57 (d, J = 6.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 181.75, 144.91, 140.17, 132.12, 129.41, 129.23, 126.92, 125.43, 122.09, 118.88, 118.42, 114.60, 114.12, 103.61. ESMS calcd for $C1_6H_{10}N_2O$, 246.1; found, 247.1 $(M + H)^+$. Anal. Calcd for $C_{16}H_{10}N_2O$: C, 78.03; H, 4.09; N, 11.38. Found: C, 77.82; H, 3.92; N, 11.11.

3-(4-Cyanobenzyl)indolizine (5). To a solution of 3-(4-cyanobenzoyl)indolizine (24.6 g, 100 mmol) in CH_3CN (600 mL) containing MeOH (9 mL) was added BH_3−THF (1 M, 450 mL), and the resulting mixture was stirred at 50 °C for 1 h. The reaction mixture was cooled to ∼10 °C, quenched with ice water (70 mL) carefully (∼8 mL/min). To the mixture was then added EtOAc (200 mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and diluted with heptane (30 mL), filtered through a short silica gel funnel (110 g of silica gel packed in 1:1 CH_2Cl_2/hexanes), and eluted with 1:1 CH_2Cl_2/hexanes (∼600 mL), and the product fraction was collected and evaporated to give 12.4 g of 3-(4-cyanobenzyl)indolizine (51% yield, 96% purity by HPLC) as a white solid. R_f 0.3 (10% ethyl acetate in hexanes); mp (DSC): 76.3 °C. IR (cm^{-1}): 2224, 1604, 1503, 1434, 1363, 1316, 1174, 1026, 838, 819, 755. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm), 4.09 (s, 2H), 6.36 (m, 2H), 6.56 (m, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 9.3 Hz, 1H), 7.45 (m, 3H); ^{13}C NMR ($CDCl_3$): δ (ppm) 143.8, 133.6, 130.7, 130.1, 128.1, 120.1, 118.4, 118.1, 117.2, 115.1, 112.4, 110.2, 99.8, 32.4. Anal. Calcd for $C_{16}H_{12}N_2$ (232.1): C, 82.73; H, 5.21; N, 12.06. Found: C, 82.61; H, 5.10; N, 12.00. ESMS calcd for $C_{16}H_{12}N_2$, 232.1; found, 233.1 $(M + H)^+$.

3-[(4-Cyanophenyl)methyl]-*N*-(3-methyl-5-isothiazolyl)-α-oxo-1-indolizineacetamide (STA-5312). To a stirred solution of oxalyl chloride (22.7 mL, 0.26 mol) in anhydrous THF (750 mL) at −50 to −40 °C (inner temp) was added a solution of 3-(4-cyanobenzyl)indolizine (50.24 g, 0.22 mol) in anhydrous THF (100 mL). The addition rate was controlled at ∼10 mL/min so that the inner temperature was maintained below −40 °C. The resulting mixture was kept at ∼ −40 °C for 1 h. To it were successively added triethylamine (36.2 mL, 0.26 mol), DMAP (2.65 g, 0.02 mol), and a THF solution (50 mL) of 5-amino-3-methylisothiazole (33.18 g, 0.29 mol), while the inner temperature was maintained below −30 °C. After the addition the mixture was then stirred at ∼ −25 °C for 2 h, quenched with 3% sodium bicarbonate (1.5 L), and stirred at 0 °C for 4 h. The precipitate was collected and washed with THF/water (1:2, 0 °C, ∼3 L) and 80% ethanol (0 °C, 1 L) to give the crude product (HPLC purity: 94%, 230 nm) as a brown solid. The crude product was dissolved in THF (800 mL) and refluxed for 15 min. After filtration to remove any insoluble particles, the filtrate was cooled to 0 °C, and to it water (1 L) was added with stirring. The mixture was let stand at 0 °C overnight. The precipitate was collected, washed with THF/water, (1:2, 0 °C) and dried under a vacuum to give 41.9 g (47.6% yield, 99% purity by HPLC) of 2-[3-(4-cyanobenzyl)-indolizin-1-yl]-*N*-(3-methylisothiazol-5-yl)-2-oxoacetamide (**STA-5312**) as a yellow crystalline powder. Mp (DSC) 233.5 °C; IR (cm^{-1}) 3292 (m), 3149 (m), 3028 (w), 2969 (w), 2841 (w), 2229 (m), 1670 (m), 1550 (s), 1505 (s), 1477 (s), 1354 (s); ^{1}H NMR ($CDCl_3$) δ (ppm), 2.46 (s, 3H), 4.33 (s, 2H),

6.78 (s, 1H), 6.97 (t, 1H, $J = 6$ Hz), 7.33 (d, 2H, $J = 9$ Hz), 7.42 (t, 1H, $J = 7.5$ Hz), 7.62 (d, 2H, $J = 9$ Hz), 7.80 (d, 1H, $J = 6$ Hz), 8.17 (s, 1H), 8.66 (d, 1H, $J = 9$ Hz), 10.44 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm), 19.3, 32.4, 108.5, 109.8, 111.4, 115.6, 118.5, 119.9, 121.4, 124.0, 124.5, 127.1, 129.0, 132.8, 140.5, 141.8, 158.6, 160.8, 163.7, 174.9. ESMS calcd for $C_{22}H_{16}N_4O_2S$, 400.1; found, 401.1 $(M + H)^+$. Anal. Calcd for $C_{22}H_{16}N_4O_2S$: C, 65.98; H, 4.03; N, 13.99; O, 7.99; S, 8.01. Found: C, 65.80; H, 4.12; N, 13.85; O, 8.06; S, 8.03.

3-(4-Cyanobenzyl)indolizine (5, Scheme 2). To a stirred solution of *N*,*N*-diisopropylamine (62.0 mL, 0.22 mol) in anhydrous THF (300 mL) in a one-neck round-bottom flask (1 L) at −78 °C was added *n*-BuLi (122.0 mL, 3.6 M, 0.44 mol) over 10 min. The reaction temperature rose up to −65 °C. The mixture was stirred at −78 °C for 30 min. DMPU (53.2 mL, 0.44 mol) was added in one portion. The resulting mixture was stirred at −78 °C for 1 h. Some slurry was observed. *p*-Tolunitrile (48.0 mL, 0.4 mol) was diluted with THF (20 mL) and added dropwise to the above mixture at −78 °C within 40 min. Stirring was continued for 1 h. The reaction mixture became yellowish red. It took 20 min to add propargyl bromide (80%, Aldrich, 22.2 mL, 0.2 mol) to the reaction mixture, which was subsequently stirred at −78 °C for 1.5 h. Reaction was allowed to warm up to −40 °C and quenched with saturated NH_4Cl (120 mL), water (60 mL), and ethyl acetate (400 mL). In a 2 L separatory funnel, the organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 200 mL). The combined organic layers were washed with water (40 mL) and brine (40 mL), dried over sodium sulfate, and concentrated in vacuo to yield an oil residue, which gradually turned into a yellow solid at room temperature. The solid was then washed with hexanes to give 4-(butyn-3-yl)benzonitrile (28.6 g, 92.4%), which was used for the next step without further purification (purity 97.4%, 230 nm HPLC). ^{1}H NMR ($CDCl_3$): δ (ppm) 7.60 (m, 2H); 7.36 (m, 2H); 2.90 (t, 2H, $J = 7.2$ Hz, 2H); 2.51 (m, 2H); 1.99 (t, $J = 2.7$ Hz, 1H). ESMS calcd ($C_{11}H_9N$), 155.1; found, 156.1 $(M + H)^+$. To a 1 L round-bottom flask were added THF (250 mL), 4-(butyn-3-yl)benzonitrile (49.38 g, 0.318 mol), TEA (250 mL, 1:1 TEA/THF), bromopyridine (33.3 mL, 0.35 mol), Pd $(PPh_3)_2Cl_2$ (4.47 g, 6.0 mmol), and CuI (1.21 g, 6.0 mmol). The reaction mixture was stirred at 60 °C for 2 h. The reaction was then cooled to room temperature and quenched with water (100 mL) and ethyl acetate (100 mL). The organic layer was separated and washed with water (50 mL) and brine (30 mL), dried over sodium sulfate, and concentrated in vacuo to give a brown solid, which was subsequently washed with heptane (2 × 50 mL) to afford 4-(4-(pyridin-2-yl)butyn-3-yl)benzonitrile as an off-white solid (58.73 g, 79.6%) with a purity of 98.4% (HPLC, 230 nm). ^{1}H NMR ($CDCl_3$) δ (ppm) 8.54 (m, 1H); 7.59 (m, 3H); 7.39 (m, 2H); 7.31 (m, 1H); 7.19 (m, 1H); 3.01 (t, $J = 6.9$ Hz, 2H); 2.76 (t, $J = 6.9$ Hz, 2H). ESMS calcd ($C_{16}H_{12}N_2$), 232.1; found, 233.1 $(M + H)^+$. The crude product (58.73 g, 0.25 mol) was dissolved in DMA (490 mL) in a round-bottom flask (1 L). To it were added TEA (70 mL, 1:7 TEA/DMA) and CuCl (25.05 g, 0.25 mol). The resulting mixture was stirred at room temperature for 10 min and then kept stirring at 130 °C for an additional 3 h. After cooling to room temperature, the reaction mixture was quenched with water (300 mL) and ethyl acetate (300 mL). The dark brown mixture was then filtered through Celite to give a clear two-layer mixture. The organic phase was charged with decolorizing carbon (10 g) and stirred at room temperature for 20 min, followed by filtration through Celite and concentration to give desired 3-(4-cyanobenzyl)indolizine as an oil, which transformed into a solid after refrigeration (−4 °C) overnight (52.63 g, 89.6%) with purity 94.9% (230 nm, HPLC). Analytical data are identical to those of an authentic sample.

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