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Synthesis and Cytotoxicity Studies of Achiral Azaindole-Substituted Titanocenes

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ABSTRACT: *From the reaction of 1-methyl-1H-pyrrolo[2,3-b]pyridine (***1a***),1-(methoxymethyl)-1H-pyrrolo[2,3-b]pyridine (***1b***), 1-isopropyl-1H-pyrrolo[2,3-b]pyridine* **(1c***), and 1-(4-methoxybenzyl)-1H-pyrrolo [2,3-b]pyridine (***1d***) under Vilsmeier–Haak conditions, the corresponding aldehydes in position 3 (***2a–2d***) were synthesized. These aldehydes were transformed in the corresponding fulvenes (***3a–3d***) by the Knoevenagel condensation and treated with Li[BEt$_3$H] to obtain the corresponding lithiated cyclopentadienide intermediates (***3′a–3′d***). These intermediates were, finally transmetallated to titanium with TiCl$_4$ to yield the 7-azaindol-3-yl-substituted titanocenes bis {[(1-methyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl] cyclopentadienyl} titanium(IV) dichloride (***4a***), bis{[(1-methoxymethyl-1-H - pyrrolo[2,3-b]pyridin-3-yl)methyl]cyclopentadienyl} titanium(IV)dichloride (***4b***), bis{[(1-Isopropyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl]cyclopentadienyl} titanium(IV) dichloride (***4c***), and bis{[(4-methoxybenzyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl]cyclopentadienyl} titanium(IV) dichloride (***4d***). All the titanocenes had their cytotoxicity investigated through MTT-based preliminary in vitro testing on the Caki-1 cell lines to determinate their IC$_{50}$ values. Titanocenes* **4a–4c** *were found to have IC$_{50}$ values of 120 ± 10, 83 ± 13, and 54 ± 12, μM respectively, whereas* **4d** *showed no cytotoxic activity.* © 2011 Wiley Periodicals, Inc. Heteroatom Chem 22:148–157, 2011; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20668

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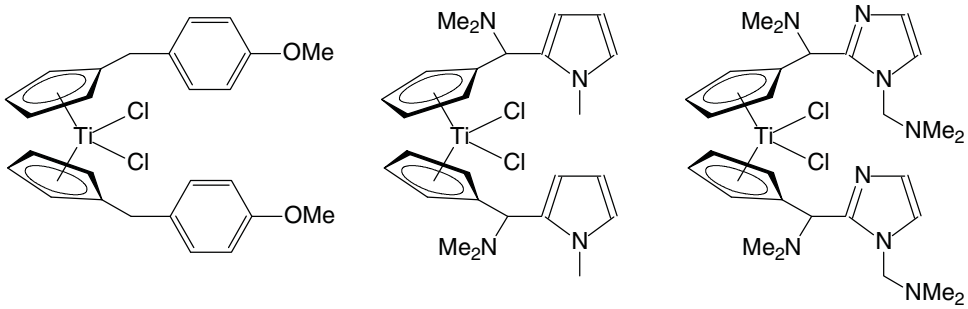
INTRODUCTION

In the last few years, important results have been reported about the cytotoxicity of titanium-based reagents by showing their significant potential against solid tumour, which makes them promising anticancer drug candidates.

Titanocene Y (bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride), which was synthesized through hydridolithiation of *p*-anisyl fulvene with superhydride (Li[BEt$_3$H]) followed by transmetallation with TiCl$_4$, is an example for these compounds [1]. This titanocene showed an IC$_{50}$ value of 21 μM when tested on the LLC-PK (epithelial pig kidney) cell line, which has proven to be a good mimic of a kidney carcinoma cell line and a reliable tool for the optimization of titanocenes against this type of cancer [1].

A further step in the field of these compounds was the preparation and evaluation of new anticancer drugs candidates including heteroaryl-substituted and dimethylamino groups in the structure of titanocenes [3–5]. Two examples are Titanocene C [6] and Titanocene M [7], which showed IC$_{50}$ values of 5.5 and 5.4 μM, respectively, when tested on the LLC-PK cell line, and their structures are presented in Fig. 1.



Titanocene Y **Titanocene C** **Titanocene M**

FIGURE 1 Molecular structures of Titanocene Y, Titanocene C, and Titanocene M.

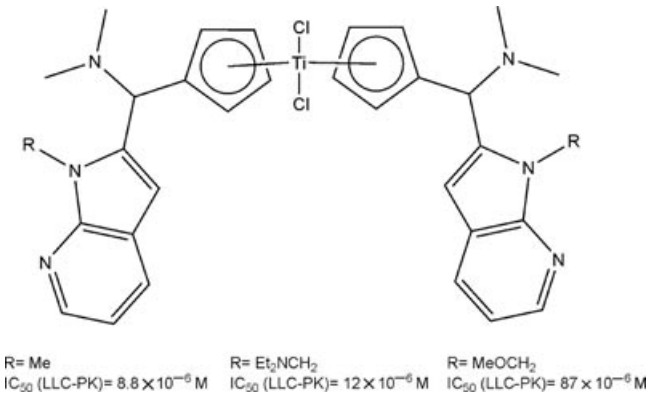


FIGURE 2 Structure and IC_{50} values of azaindole-substituted titanocenes.

More recently, a variety of new dimethylamino-functionalized and azaindole-substituted titanocenes were synthesized and tested for their cytotoxicity against LLC-PK cells [8]; their structures are presented in Fig. 2. Structurally, these compounds present 7-azaindoles substituted at position 1 linked to a Cp ring by a bridge carbon in position 2. This bridge carbon is substituted with an *N*,*N*-dimethylamino group.

These titanocenes showed an IC_{50} value from 8.8 to 87 μM when tested on the LLC-PK cell line [8].

This paper is presenting the synthesis and cytotoxicity of new 7-azaindolyl-substituted titanocenes in comparison with the results obtained in the previous work. These titanocenes, which are various achiral 7-azaindole-substituted at position 1, differ from those previously reported by linking Cp rings at position 3 (instead of position 2) and removing the *N*,*N*-dimethylamino group at the bridging carbon. The main idea of this article is to present a new series of 7-azaindol-3-yl-substituted titanocenes, their synthesis, preliminary cytotoxicity studies, and to compare with the results obtained for 7-azaindol-2-yl-substituted and (dimethylamino)-functionalized titanocenes.

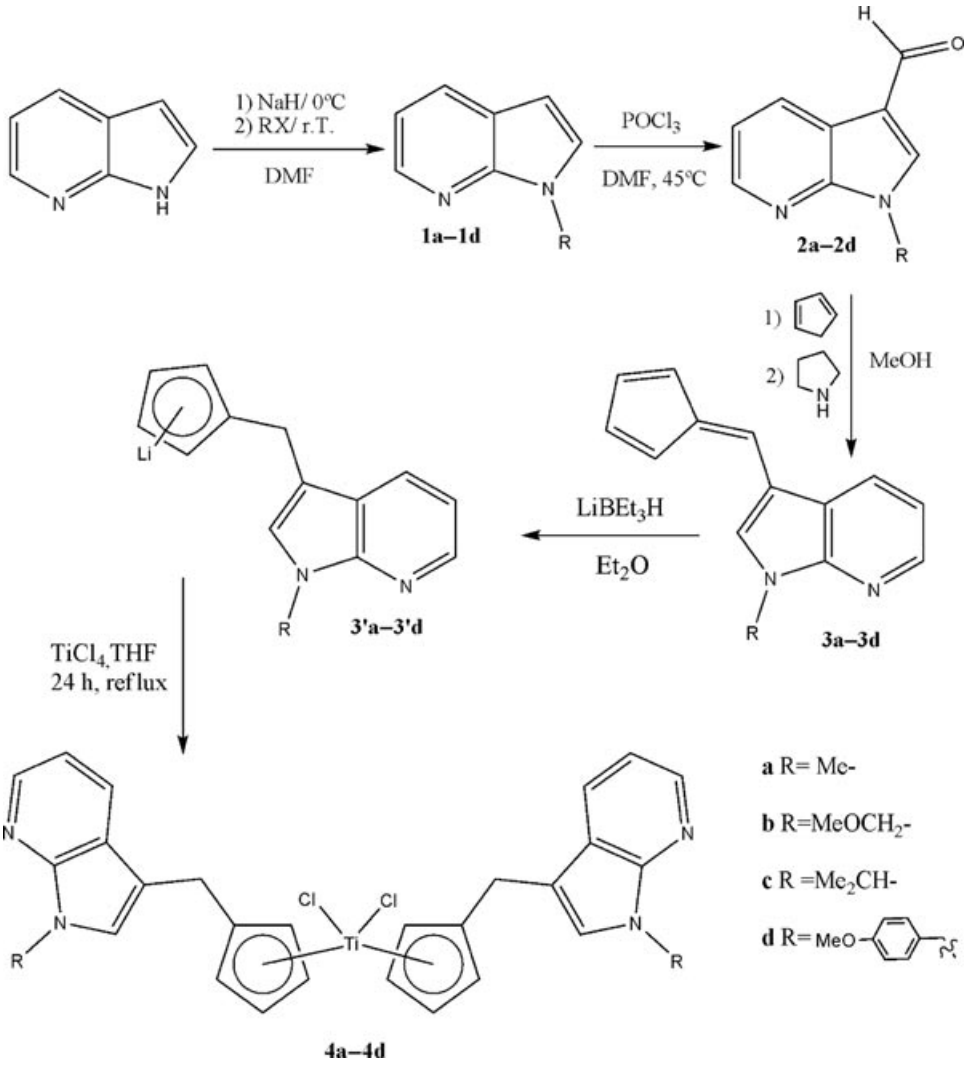
RESULTS AND DISCUSSION

The complete synthesis of titanocenes **4a–4d** is shown in Scheme 1. The syntheses of azaindoles **1a–1d** were made by the alkylation with the corresponding alkyl halide [8,9]. Then, aldehydes **2a–2d** were prepared using the Vilsmeier–Haak reaction where phosphoryl chloride and *N*,*N*-dimethylformamide were used to introduce an aldehyde group in position 3 of the corresponding 7-azaindole derivative. The fulvenes **3a–3d** were synthesized from these aldehydes and freshly cracked cyclopentadiene under Knoevenagel condensations using pyrrolidine as the base of choice in methanol as a protic solvent. Fulvenes were treated with $LiBEt_3H$ in dry ether to give the lithiated cyclopentadienide intermediates (**3′a–3′d**); no specific synthetic problems were encountered, when fulvenes derived from alkylated 7-azaindoles were used in the hydridolithiation reactions. And the last step was the transmetallation reaction of these lithiated intermediates with $TiCl_4$ (0.5 mol equivalents) under reflux for a day in THF to give titanocenes **4a–4d** as dark solids.

Cytotoxicity Studies

Titanocenes **4a–4d** were tested against the human renal cell line, Caki-1 and exhibited IC_{50} values of 120 ± 10, 83 ± 13, and 54 ± 12 μM, whereas **4d** showed no cytotoxicity as seen in Fig. 3.

These results shows that **4b** has a similar value comparing with its 7-azaindol-2-yl-substituted analogue, which has a IC_{50} of 87 μM when tested on the LLC-PK cell line [8], but in the case of **4a** the value has a 100-fold increase in magnitude in terms of IC_{50} value with respect to its 7-azaindol-2-yl-substituted analogue.



SCHEME 1 Synthesis of titanocenes **4a-4d**.

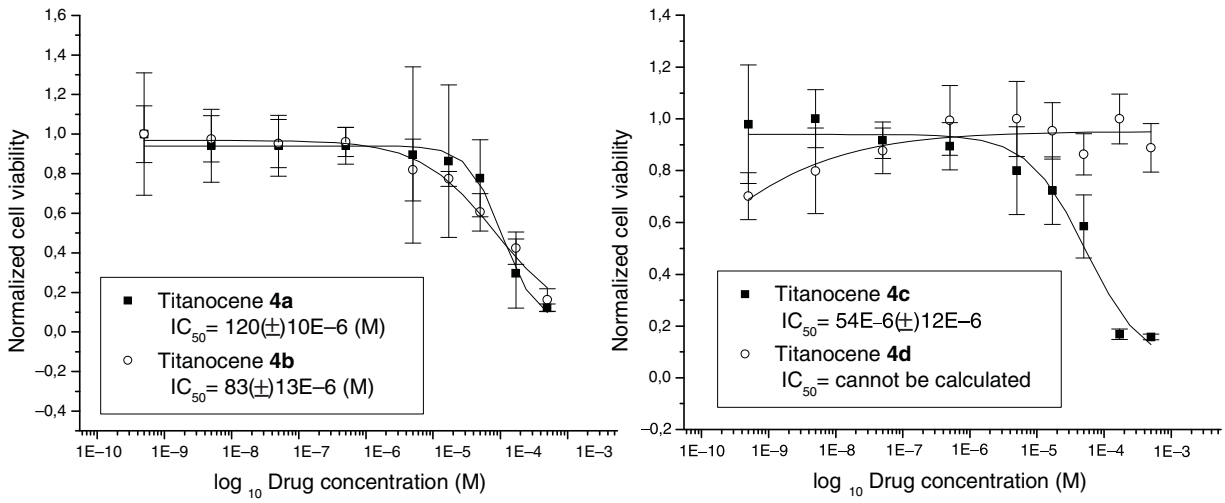


FIGURE 3 Cytotoxicity studies of titanocenes **4a–4d** against Caki-1 cells.

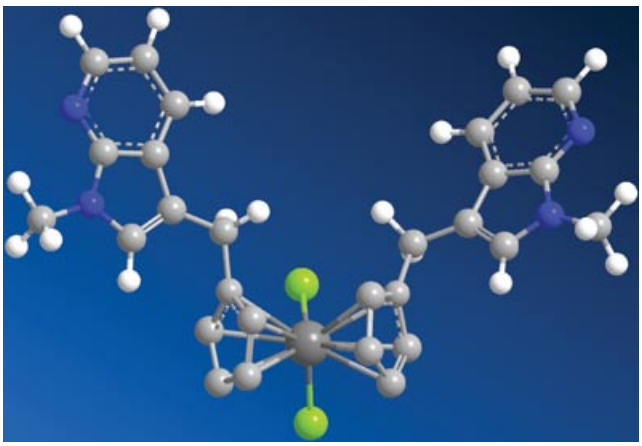


FIGURE 4 DFT-calculated structure of **4a**.

On the other hand, the best cell testing result of these compounds belongs to **4c** that shows the lower IC_{50} value of the group and its cytotoxicity is relatively close to Titanocene Y ($IC_{50} = 21$ μM), whereas **4d** showed no cytotoxicity for concentrations as high as 500 μM.

One more time these results expose the possible intramolecular stabilization of the mono or dication in 7-azaindol-2-yl-substituted and dimethylamino-functionalized titanocenes that is believed to play a role in the cytotoxicity of these compounds [8].

*Structural DFT Studies of Dichloridobis{(1, 2, 3, 4,5-η)- 1-[(1-methyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl]cyclopenta - 2,4 - dien - 1 - yl} titanium (IV) [$TiCl_2\{\eta^5$-C_5H_4-$CH(C_7H_4N_2$-$CH_3\}_2$] (**4a**)*

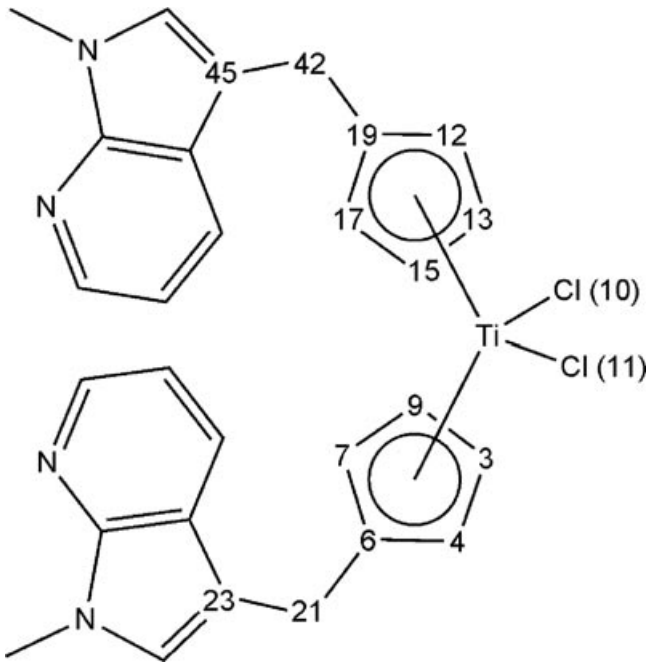
DFT calculations for titanocene **4a** were made at the B3LYP level, using the 6-31G(d, p) basis set (see Fig. 4).

The length of the bond between Ti and the cyclopentadienyl C(19) and C(6) is 246.2 and 250.7 pm, respectively, whereas the C—C bonds of the cyclopentadienyl rings have bond lengths between 140.6 and 142.9 pm (see Table 1). The bond lengths between C(19)–C(42) and C(6)–C(21) are very similar 150.4 and 150.2 pm, respectively. The optimization shows a distance between C(42) and C(21) of 485.1 pm, and the calculated bond length for C(42)–C(45) and C(21)–C(23) are also very similar, 150.9 and 151.0 pm, respectively. Finally, the bond lengths between Ti and Cl (10) and Cl (11) are 235.0 and 234.0, respectively.

The Cl(10)–Ti–Cl(11) angle is calculated to be 95.6°. and the angles formed between C(19)–C(42)–C(45) and C(6)–C(21)–C(23) are 113.2° and 112.8°; whereas C(19)–Ti–C(6) is 114.3°.

TABLE 1 Selected Bond Lengths [pm] from the DFT-Calculated Structure of Titanocene **4a**.

Bond	*Length (pm)*	*Bond*	*Length (pm)*
Ti–C(19)	246.2	Ti–C(6)	250.7
Ti–C(12)	241.3	Ti–C(4)	240.0
Ti–C(13)	236.8	Ti–C(3)	237.8
Ti–C(15)	243.1	Ti–C(9)	241.0
Ti–C(17)	245.8	Ti–C(7)	245.8
C(19)–C(12)	141.4	C(6)–C(4)	142.0
C(12)–C(13)	142.2	C(4)–C(3)	142.6
C(13)–C(15)	141.9	C(3)–C(9)	140.9
C(15)–C(17)	140.6	C(9)–C(7)	142.1
C(17)–C(19)	142.9	C(7)–C(6)	141.6
C(19)–C(42)	150.4	Ti–Cl(10)	235.0
C(6)–C(21)	150.2	Ti–Cl(11)	234.0
C(42)–C(21)	485.1		
C(42)–C(45)	150.9	C(21)–C(23)	151.0



Conclusions and Outlook

The Vilsmeier–Haak formylation reaction of 7-azaindoles in position 3 has been found very effective to obtain precursors to make suitable fulvene groups at this position and prepare new cytotoxic 7-azaindole-substituted titanocene dichloride complex. On the Caki-1 cell line, complexes **4a–4c** gave IC_{50} values of 120 ± 10, 83 ± 13, and 54 ± 1 2 μM, whereas **4d** showed no toxicity. These results show that 7-azaindol-3-yl-*N*-substituted titanocenes complex present, in general, lower cytotoxicity than 7-azaindol-2-yl-substituted and dimethylamino-functionalized titanocenes. This shows that the possible intramolecular stabilization of the mono- or dication by the dimethylamino-substituent in these titanocenes could play an important role in the cytotoxicity of these compounds.

EXPERIMENTAL

MTT-Based Assay

Preliminary in vitro cell tests [10] were performed on the cell line Caki-1 to compare the cytotoxicity of the compounds presented in this work. These cell lines were chosen based on their regular and long-lasting growth behavior. They were obtained from the ATCC (American Tissue Cell Culture Collection) and maintained in Dulbecco's modified eagle medium containing 10% (v/v) FCS (fetal calf serum) 1% (v/v) penicillin streptomycin and 1% (v/v) L-glutamine. Cells were seeded in 96-well plates containing 200-μL microtiter wells at a density of 5000 cells/200 μL of medium and were incubated at 37°C for 24 h to allow for exponential growth. Then, the compounds tested were dissolved in 70 μL of DMSO and diluted with medium to obtain stock solutions of 5×10^{-4} M in concentration. The cells were then treated with various concentrations of the compounds and incubated for 48 h at 37°C. Then the solutions were removed from the wells; and the cells were washed with PBS (phosphate buffer solution), and fresh medium was added to the wells. Following a recovery period of 24-h incubation at 37°C, individual wells were treated with a 200-μL of a solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) in medium (solution of 20 mg of MTT in 40 mL of medium). The cells were incubated for 3 h at 37°C. The medium was then removed, and the purple formazan crystals were dissolved in 200 μL of DMSO per well. Absorbance was then measured at 540 nm by a Wallac–Victor (multilabel HTS counter) plate reader. Cell viability was expressed as a percentage of the absorbance recorder for control wells. The values used for the dose response curves represent the values obtained from four consistent MTT-based assays for each compound tested. The data were analyzed using the programme package Origin and delivered IC_{50} values and standard deviations.

General

$TiCl_4$ (1.0 M in toluene) and ^{t}BuLi (1.7 M in pentane) were obtained commercially from Sigma Aldrich (St. Louis, MO). Manipulations of air- and moisture sensitive compounds were done by using standard Schlenk techniques under Ar. UV/vis spectra: *Unicam UV4 spectrometer*; λ_{max} in nm (ε in $dm^3\ mol^{-1}\ cm^{-1}$) in CH_2Cl_2. IR spectra: Perkin–Elmer Paragon-1000 FT-IR spectrometer; in KBr disk. ^{1}H- and ^{13}C-NMR spectra: Varian 300 or 400 spectrometer; δ in ppm rel. to Me_4Si as an internal standard, J in Hz. MS: quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Water's Corp., USA); Solutions were made in acetonitrile/H_2O 1:1; in *m/z*. Elemental analyses: anal. Exeter-CE-440 elemental analyzer for C, H, and N.

*Synthesis of 1-Methyl-1H-pyrrolo[2,3-b]pyridine (**1a**).* In a 250-mL round-bottom flask, 50 mL of dry DMF was added dropwise over a suspension of 7-azaindole (4.00 g, 33.9 mmol, 1.00 equiv) and NaH (60% in oil) (1.63 g, 40.6 mmol, 1.20 equiv), under N_2 at 0°C during 5 min. The mixture was then stirred for 15 min at 0°C, and a solution of MeI (2.11 mL, 33.89 mmol, 1 equiv) in dry DMF (10 mL) was added dropwise over 5 min. The mixture was stirred at RT for 3 h. A brown solution was obtained, and DMF was evaporated to yield a brown solid that was redissolved in 50 mL of water and extracted with CH_2Cl_2 (3×50 mL), organic layers were washed with brine (50 mL) and water (30 mL).

The organic layer was dried over Na_2SO_4filtered and concentrated to give brown oil with a very intense smell (4.05 g, 30.6 mmol, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.32 (d, J = 4.4, 1H), 7.89 (d, J = 7.8, 1H), 7.16 (d, J = 3.4, 1H), 7.03 (dd, J = 7.8, 4.7, 1H), 6.43 (d, J = 3.4, 1H), 3.88 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.0, 143.0, 129.2, 128.9, 120.7, 115.9, 99.5, 31.5 ES-MS (pos.): *m/z* 133.1 ($[M + H]^+$)

*Synthesis of 1-Methyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (**2a**).* Under N_2, (4.69 mL, 50.4 mmol, 1.10 equiv) of $POCl_3$ were added dropwise over 30 mL of dry DMF under N_2 for ten minutes at RT and the mixture was stirred for five minutes. Then 1-methyl-7-azaindole (6.05 g, 45.8 mmol, 1.00

equiv) in dry DMF was added dropwise at 0°C for 5 min. The formation of a brown solid was observed, and the mixture was stirred at 40°C for 2 h. After this time, 10 g of ice was added and the reaction mixture (pH = 3) was basified with 60 mL of 40% NaOH. The mixture was refluxed for 5 min, and a red solution was observed. DMF was evaporated in vacuo, and the brown solid obtained was redissolved in water (200 mL). This aqueous layer was extracted with CH_2Cl_2 (4 × 50 mL); the organic layers were combined, washed with brine (50 mL), dried over Na_2SO_4, and concentrated to give (3.25 g, 20.2 mmol, 44.5%) a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.96 (s, 1H), 8.54 (dd, J = 7.8, 1.6, 1H), 8.43 (dd, J = 4.8, 1.6, 1H), 7.83 (s, 1H), 7.26 (dd, J = 4.2, 3.6, 1H), 3.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 184.41, 148.68, 145.06, 138.88, 130.38, 118.81, 117.60, 116.25, 31.72. ES-MS(pos.) *m/z*: 161.1 $[M + H]^+$.

*Synthesis of 3-(Cyclopenta-2,4-dienylidenemethyl)-1-methyl-1H-pyrrolo [2,3-b]pyridine (***3a***).* 1-Methyl-7-azaindole-3-carboxaldehyde (3.25 g, 20.3 mmol, 1.00 equiv) was dissolved in dry Me(OH) (40 mL) under N_2, and freshly cracked cyclopentadiene (3.40 mL, 50.7 mmol, 2.50 equiv) was added at 0°C. The solution was stirred for 5 min, and pyrrolidine (2.54 mL, 30.4 mmol, 1.5 equiv) in dry MeOH (10 mL) was added, and mixture was stirred for 90 min at RT. After this time, a red solution was observed, AcOH (2.32 mL, 40.6 mmol, 2.00 equiv) was added, and solution was stirred at RT for another 10 min.

At this point, 50 mL of Et_2O and 50 mL of water were added to the flask. An aqueous phase was extracted with Et_2O (3 × 50 mL), the organic layers were combined, washed with brine (2 × 50 mL), dried over Na_2SO_4 filtered, and concentrated to yield a dark red solid (3.40 g, 16.3 mmol, 80.4%)

^{1}H NMR (400 MHz, $CDCl_3$) δ; 8.39 (dd, J = 4.7, 1.4, 1H), 8.11 (dd, J = 7.9, 1.4, 1H), 7.64 (s, 1H), 7.34 (s, 1H), 7.17 (dd, J = 7.9, 4.7, 1H), 6.73 (d, J = 5.2, 1H), 6.64 (m, 1H), 6.47 (d, J = 5.0, 1H), 6.38 (m, 1H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 148.03, 144.03, 141.39, 133.84, 131.53, 129.16, 129.13, 127.53, 126.82, 120.29, 119.53, 116.93, 111.63, 31.74. ES-MS(pos.) *m/z*: 209.43 $[M + H]^+$.

*Synthesis of bis{[(1-Methyl-1-H-pyrrolo[2, 3-b] pyridin-3-yl)methyl]cyclopentadienyl }titanium(IV) dichloride [$TiCl_2\{\eta^5$-C_5H_4-$CH(C_7H_4N_2$-$CH_3\}_2$] (***4a***).* 14.0 mL (13.7 mmol, 1.30 equiv) of a solution of superhydride ($LiBEt_3H$) in THF (1 M) was concentrated by the removal of the solvent by heating it to 60°C under reduced pressure of 10^{-2} mbar for 40 min and then to 90°C for 20 min in a Schlenk flask to remove most of the THF. The concentrated superhydride was dissolved in 200 mL of dry diethyl ether to give a cloudy yellow suspension. 2.20 g (10.5 mmol, 1.00 equiv) of the red solid fulvene was added to a Schlenk flask and was dissolved in 90-mL dry diethyl ether to give a red solution. Then this solution was transferred to the superhydride solution via cannula. The solution was left to stir for 16 h at RT to give a light yellow precipitate. This solid (lithiated intermediate) was isolated on a Schlenk frit under N_2, yielding a yellow solid (2.04 g., 9.40 mmol, 89.4%). This solid was dissolved in dry THF (60 mL) under N_2, then 3.15 mL (3.15 mmol, 0.50 equiv) of a 1 M solution of $TiCl_4$ in toluene was added and the mixture was stirred for 16 h in a Schlenk flask under reflux. THF was evaporated in vacuo, and a dark black solid was obtained. This solid was redissolved in CH_2Cl_2, and it was filtered with Celite by low pressure. An organic layer was collected (brown-red liquid), and the solvent was eliminated to yield a red oil, which was washed with pentane and a dark red solid was obtained (2.08 g., 41.0%)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.41–7.07 (m, 8H $C_7H_4N_2$), 6.35 (m, 8H C_5H_4), 4.31 (s, 4H), 4.11 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 138.35, 136.15, 134.11, 131.87, 131.30, 129.27, 128.22, 127.10, 125.25, 123.37, 114.91, 114.39 (C_5H_4, $C_7H_4N_2$); 33.06 ($C_5H_4CH_2$); 26.25 (CH_3–). Anal. calcd for $C_{28}H_{26}Cl_2N_4Ti$ (536.10): C 62.68, H 4.85, Cl 13.43, N 10.44; found: C 59.15, H 5.31, Cl 16.28, N 8.65. ES-MS(neg) *m/z*: 519.1 $[M - H - Cl - H_2O]^-$. IR absorptions (KBr, cm^{-1}): 3402, 3083, 2923, 2853, 2654, 2190, 1637, 1524, 1459, 1262, 1131, 1038, 798, 747. UV–vis (CH_2Cl_2, nm): λ 290 (ε 19051), λ 256 (ε 18962), λ 233 (ε 28035).

*Synthesis of 1-(methoxymethyl)-1H-pyrrolo[2,3-b]pyridine (***1b***).* 7-Azaindole (2.00 g, 16.9 mmol, 1.00 equiv) was mixed with NaH (60% dispersion in mineral oil) (0.81 g, 20.3 mmol, 1.20 equiv) in a 100-mL round-bottom flask under N_2 at 0°C, and dry DMF (40 mL) was added dropwise within 10 min and the mixture was then stirred for a further 30 min at 0°C. Then a solution of methoxy methyl chloride (1.28 mL, 16.9 mmol, 1.00 equiv) in DMF (5 mL) was added dropwise at 0°C under $N_2$for 5 min, and the mixture was stirred at room temperature for 3 h. After this, a light yellow suspension was observed. 25 mL of water was added over the reaction mixture, and the solvent was eliminated under reduced pressure to yield a yellow oil. 30 mL of water was added to the oil; and the mixture was extracted with CH_2Cl_2 (3 × 25 mL), the organic layer was combined and dried over $Na_2SO_4$and concentrated to give 2.28 g (14.0 mmol, 83.1% yield) of a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.32 (dd, $J = 4.7$, 1.5, 1H), 7.89 (dd, $J = 7.8$, 1.6, 1H), 7.30 (d, $J = 3.6$, 1H), 7.07 (dd, $J = 7.8$, 4.7, 1H), 6.49 (d, $J = 3.6$, 1H), 5.62 (s, 2H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.1, 143.2, 128.8, 127.8, 120.6, 116.4, 101.1, 74.7, 56.2. ES-MS(pos.) m/z 163.1 $[M + H]^+$.

*Synthesis of 1-(Methoxymethyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (***2b***).* In a 100-mL round-bottom flask, under N_2, 15 mL of dry DMF was stirred with $POCl_3$ (1.31 mL, 14.0 mmol, 1.00 equiv) at 0°C for 5 min. After this, a solution of 1-(methoxymethyl)-1H-pyrrolo[2,3-*b*]pyridine (2.28 g., 14.0 mmol, 1.00 equiv) in DMF (10 mL) was added dropwise at 0°C and then the mixture was stirred for 2 h at 40°C. A yellow precipitate was observed in the flask. The solvent was eliminated in vacuo, and a yellow solid was obtained. The solid was redissolved in water (150 mL), and the solution was extracted with CH_2Cl_2 (4 × 35 mL). Organic layers were combined and washed with brine (1 × 30 mL), dried over Na_2SO_4, filtered, and concentrated to yield a yellow solid (2,13 g., 11.1 mmol, 79.5%)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.00 (s, 1H), 8.55 (dd, $J = 7.9$, 1.4, 1H), 8.42 (dd, $J = 4.7$, 1.4, 1H), 7.97 (s, 1H), 7.27 (dd, $J = 7.9$, 4.7, 1H), 5.68 (s, 2H), 3.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 185.01, 148.62, 145.46, 137.18, 130.75, 119.32, 117.61, 117.48, 75.44, 56.87. ES-MS(pos.) *m/z*: 160.3 $[M - MeOH + H]^+$

*3-(Cyclopenta-2,4-dienylidenemethyl)-1-(methoxymethyl)-1H-pyrrolo[2,3-b]pyridine (***3b***).* In a 100-mL round-bottom flask, 3-(cyclopenta-2,4-dienylidenemethyl)-1-(methoxymethyl)-1*H*-pyrrolo [2,3-*b*]pyridine (2.13 g., 11.2 mmol, 1.00 equiv) was dissolved in dry MeOH (30 mL) under N_2 at 0°C. Then freshly cracked cyclopentadiene (1.88 mL, 27.9 mmol, 2.50 equiv) was added dropwise to the mixture and the solution was stirred for 5 min. Then 1.40 mL of pyrrolidine in 10.0 mL of MeOH was added dropwise and the mixture was stirred for 90 min at RT.

After that, 1.30 mL of AcOH was added to the mixture and a gas formation was observed, while the mixture was stirred for a further 10 min.

50 mL of water and 50 mL of Et_2O were added to the flask, and the aqueous layer was extracted in Et_2O (3 × 50 mL). Organic layers were combined, washed with brine (2 × 35 mL), dried over Na_2SO_4, filtered, and concentrated to yield a red solid (2.05 g, 8.61 mmol, 77.0%)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.39 (dd, $J = 4.7$, 1.5, 1H), 8.11 (dd, $J = 7.9$, 1.5, 1H), 7.75 (s, 1H), 7.33 (s, 1H), 7.20 (dd, $J = 7.9$, 4.7, 1H), 6.75–6.36 (m, 4H), 5.68 (s, 2H), 3.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 148.39, 144.31, 142.26, 134.25, 129.93, 129.73, 128.58, 127.65, 126.76, 120.28, 119.72, 117.54, 113.11, 75.05, 56.72. ES-MS(pos.) *m/z*: 239.5 $[M + H]^+$

Synthesis of bis{[(1-Methoxymethyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl]cyclopentadienyl} titanium(IV) Dichloride [$TiCl_2\{\eta^5$-C_5H_4-$CH(C_7H_4N_2$-CH_2-O-$CH_3\}_2$*] (***4b***).* In a 250-mL Schlenk flask under reduced pressure, 10 mL of a 1 M solution of superhydride ($LiBEt_3H$) in THF was stirred at 60°C for 20 min and at 90°C for 30 min at a pressure of 10^{-2} mbar to remove most of the THF.

In another Schlenk flask under N_2, 3-(cyclopenta-2,4-dienylidenemethyl)-1-(methoxymethyl)-1*H*-pyrrolo[2,3-*b*]pyridine (1.80 g., 7.50 mmol, 1.00 equiv) was dissolved in dry Et_2O (200 mL) and the red solution formed added to the $LiBEt_3H$ solution. While stirring overnight at RT, an orange solution was obtained. It contained precipitated yellow lithiated intermediate. This solid was isolated using a Schlenk frit under N_2, yielding a yellow solid (1.47 g, 5.95 mmol, 79.3%)

This intermediate was redissolved under N_2, in 40 mL of dry THF, and 3 mL (3 mmol, 0.5 equiv) of a 1 M solution of $TiCl_4$ in toluene was added and the mixture was stirred overnight under reflux, which resulted in a black solution. THF was removed in vacuo at RT, and a dark green solid was obtained. The solid was redissolved in CH_2Cl_2, and the solution was filtered through a plug of Celite®. The organic layer was collected, the solvent evaporated in vacuo to yield a red solid, which was washed with pentane (1.20 g, 2.01 mmol, 34.0%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40- 7.10 (m, 4H, $C_7H_4N_2$), 6.34–6.44 (m, 8H, C5H4), 5.65 (s, 4H), 4.26 (s, 4H), 3.32 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 129.80, 129.00, 128.14, 126.51, 126.45, 126.36, 125.26, 122.93, 116.14, 114.95, 113.30 (C_5H_4,$C_7H_4N_2$); 75.17, 56.48 ($MeOCH_2$) 34.07 ($C_5H_4CH_2$). Anal. calcd for $C_{30}H_{30}Cl_2N_4O_2Ti$ (596.12): C 60.32, H 5.06, Cl 11.74, N 9.39; found: C 56.05, H 5.26, Cl 11.74, N 7.56. ES-MS(neg) *m/z*: 577.3 $[M - H - Cl + H_2O]^-$. IR absorptions (KBr, cm^{-1}): 3397, 3092, 2929, 1635, 1434, 1384, 1338, 1183, 1125, 1038, 800, 777. UV–vis (CH_2Cl_2, nm): λ 290 (ε 17416), λ 260 (ε 15794), λ 232 (ε 27636), λ 229 (ε 25633)

*Synthesis of 1-Isopropyl-1H-pyrrolo[2,3-b]pyridine (***1c***).* In a 250-mL round-bottom flask, 60 mL of dry DMF was added dropwise over a suspension of 7-azaindole (4.00 g, 33.9 mmol, 1.00 equiv) and NaH (60% in oil) (1.63 g, 40.6 mmol, 1.20 equiv), under N_2 at 0°C for 5 min. During this addition, H_2gas evolved and a pale suspension was formed;

the mixture was stirred at RT for another 10 min. Then isopropyl bromide (3.80 mL, 40.6 mmol, 1.20 equiv) was added, and the solution was stirred at RT overnight (after 1 h, the solution turned yellow). In the workup, the reaction mixture was dissolved in 400 mL of ice-water and then extracted with AcOEt (3 × 30 mL), the organic layers were combined, washed with brine (2×30 mL), filtered, and dried in vacuo to yield a yellow oil (5.36 g, 98%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.30 (dd, J = 4.7, 1.5, 1H), 7.87 (dd, J = 7.8, 1.5, 1H), 7.30 (d, J = 3.6, 1H), 7.02 (dd, J = 7.8, 4.7, 1H), 6.44 (d, J = 3.6, 1H), 5.20 (m, 1H), 1.50 (d, J = 6.8, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 146.96, 142.41, 128.60, 124.05, 120.69, 115.57, 99.43, 45.19, 22.89. ES-MS(pos.) m/z: 161.38 $[M + H]^+$.

*Synthesis of 1-Isopropyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (***2c***).* In a 250-mL round-bottom flask, $POCl_3$ (5.01 mL, 54.0 mmol, 1.50 equiv) was dissolved in dry DMF (40 mL) and the mixture was stirred for 10 min under N_2 at RT. Then a solution of 1-isopropyl-1*H*-pyrrolo[2,3-*b*]pyridine (5.76 g., 36.0 mmol, 1.00 equiv) in DMF (10.0 mL) was added dropwise under N_2 at 0°C for 3 min and the solution was stirred at 40°C overnight during which the solution turned brown and a white precipitate was observed. The mixture was basified with a solution of 40% NaOH and poured into 300 mL of water-ice. The white solid was filtered off, and the brown solution was extracted with AcOEt (3 × 40 mL). The organic layers were combined, washed with brine (2 × 40 mL), filtered, and concentrated to yield a dark brown solid (5.15 g, 27.3 mmol, 76.0%)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.94 (s, 1H), 8.50 (dd, J = 7.8, 1.6, 1H), 8.37 (dd, J = 4.7, 1.6, 1H), 7.93 (s, 1H), 7.21 (dd, J = 7.8, 4.7, 1H), 5.19 (m, 1H), 1.55 (d, J = 6.8, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 184.48, 148.12, 144.76, 134.79, 130.36, 118.91, 117.93, 116.44, 46.55, 22.65. ES-MS(pos.) m/z: 189.41 $[M + H]^+$.

*Synthesis of 3 - (Cyclopenta - 2, 4 - dienylidenemethyl)-1-(isopropyl)-1H-pyrrolo[2,3-b]pyridine(***3c***).* 1-Isopropyl - 1*H* - pyrrolo[2,3 - *b*]pyridine - 3 - carbaldehyde (5.15 g, 27.3 mmol, 1 equiv.) and freshly cracked cyclopentadiene (4.58 mL, 68.4 mmol, 2.5 equiv) were dissolved in MeOH (110 mL) in a 250-mL round-bottom flask under N_2 at 0°C. Then pyrrolidine (3.43 mL, 41.0 mmol, 1.50 equiv) was added dropwise during 2 min and the resulting solution was stirred overnight. As a result, a dark red solution was obtained, which was neutralized with acetic acid (3.12 mL, 54.7 mmol, 2.00 equiv).

100 mL of H_2O and 100 mL of AcOEt were added to the solution, and the aqueous layer was extracted in AcOEt (3 × 50 mL), then organic phases were combined, washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered, and concentrated to yield a dark red oil. (3.48 g, 14.7 mmol, 55.0%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37 (dd, J = 4.7, 1.5, 1H), 8.11 (dd, J = 7.9, 1.5, 1H), 7.76 (s, 1H), 7.38 (s, 1H), 7.16 (dd, J = 7.9, 4.7, 1H), 6.77 – 6.36 (m, 4H), 5.23 (m, 1H), 1.57 (d, J = 6.8, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 147.49, 143.73, 141.11, 133.68, 129.61, 128.96, 127.45, 127.28, 126.83, 120.48, 119.55, 117.10, 111.89, 46.06, 22.77. ES-MS(pos.) m/z: 237.46 $[M + H]^+$.

Synthesis of bis{[(1-Isopropyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl] cyclopentadienyl} titanium (IV) dichloride [$TiCl_2\{\eta^5$-C_5H_4-$CH(C_7H_4N_2$-$CH(CH_3)_2\}_2$*] (***4c***).* In a 250-mL Schlenk flask, a 1 M solution of $LiBEt_3H$ in THF (18.15 mL, 18.15 mmol, 1.30 equiv) was heated at 60°C for 20 min and at 90°C for 30 min under vacuum of 10^{-2} mbar to remove most of the THF. In another 250-mL Schlenk flask, 3-(cyclopenta-2,4-dienylidenemethyl)-1-(isopropyl)-1*H*-pyrrolo[2,3-*b*]pyridine (3.30 g., 13.9 mmol, 1.00 equiv) was dissolved in dry Et_2O (200 mL) under N_2 and the solution was added to the concentrated $LiBEt_3H$ solution via cannula. Then the mixture was stirred overnight at RT and a light yellow precipitate of the lithiated intermediate was obtained. The yellow precipitate was isolated using a Schlenk frit under N_2, yielding (1.54 g, 6.30 mmol, 45.0%) of a light yellow solid.

In a 250-mL Schlenk flask, the lithiated intermediate (1.54 g., 6.30 mmol, 1.00 equiv) was dissolved in dry THF (100 mL) under N_2.At this point, (3.15 mL, 3.15 mmol, 0.50 equiv) of a 1 M solution of $TiCl_4$ in toluene was added and the mixture was kept under refluxed for 16 h. A red solution was obtained, and THF was eliminated in vacuo at RT using to yield a red oil, which was redissolved in CH_2Cl_2 and filtered through a plug of Celite®. The solvent was evaporated in vacuo, and a dark red solid was obtained (1.55 g., 2.60 mmol, 42.0%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.57–6.84 (m, 8H, $C_7H_4N_2$), 6.44–6.06 (m, 8H, C_5H_4), 5.31–5.24 (m, 2H), 4.24 (s, 4H), 1.51 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 137.24, 134.41, 134.21, 132.29, 131.34, 129.01, 128.21, 127.30, 123.05, 122.42, 115.53, 115.17 (C_5H_4, $C_7H_4N_2$); 45.61 26.66 (Me_2CH); 22.88 ($C_5H_4CH_2$). Anal. calcd for $C_{32}H_{34}Cl_2N_4Ti$ (592.16): C 64.86, H 5.74, Cl 11.86, N 9.49; found: C 68.00, H 6.25, Cl 8.53., N 9.29. ES-MS(neg) m/z: 573.66 $[M - H - Cl + H_2O]^-$. IR absorptions (KBr, cm^{-1}): 3427, 3092, 3047, 2971, 2927, 2872, 1595, 1436, 1277, 1230,

1199, 1124, 801, 770. UV–vis (CH_2Cl_2, nm): λ 290 (ε 21262), λ 259 (ε 20779), λ 233 (ε 43100).

*Synthesis of 1-(4-Methoxybenzyl)-1H-pyrrolo[2,3-b]pyridine (***1d***).* In a 250-mL round-bottom flask, a mixture of 7-azaindole (3.90 g, 33.0 mmol, 1.00 equiv) and NaH (60% in mineral oil) (1.98 g, 49.5 mmol, 1.50 equiv) was stirred in DMF (50 mL) under N_2 at 0°C for 10 min and then the mixture was allowed to warm up to RT during another 15 min. To this light yellow solution, 4-methoxybenzylbromide (5.80 mL, 59.4 mmol, 1.20 equiv) was added dropwise over 5 min and the resulting yellow solution was stirred for 16 h at RT.

The crude reaction mixture was poured into ice-water (250 mL) and was extracted with AcOEt (3 × 50 mL); the organic layers were combined, washed with brine (1 × 50 mL), filtered, and concentrated to yield a yellow oil. The oil was purified by chromatography on a column (SiO_2) with AcOEt as the eluent to give **1d** (7.40 g, 31.0 mmol, 91.4%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$)δ: 8.39 (dd, J = 4.7, 1.5, 1H), 7.93 (dd, J = 7.8, 1.5, 1H), 7.20–7.15 (m, 3H), 7.09 (dd, J = 7.8, 4.7, 1H), 6.85–6.82 (m, 1H), 6.48 (d, J = 3.5, 1H), 5.46 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.11, 147.66,142.95, 129.89, 128.96, 128.78, 127.76, 120.56, 115.80, 114.08, 99.97, 55.22, 47.28. ES-MS(pos.) *m/z*: 239.43 $[M + H]^+$.

*Synthesis of 1-(4-Methoxybenzyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (***2d***).* In a 250-mL round-bottom flask, $POCl_3$ (4.37 mL, 47.0 mmol, 1.50 equiv) was dissolved in anhydrous DMF (40 mL) under N_2 at 0°C and then a solution of 1-(4-methoxybenzyl)-1*H*-pyrrolo[2,3-*b*]pyridine (**1d**) (7.40 g., 31.1 mmol, 1.00 equiv) in 10 mL of dry DMF was added dropwise. A dark brown solution was observed, and the mixture was stirred at 45°C overnight. A brown solution was obtained.

The solution was basified with a solution of NaOH (40%) in water till pH 14, and it was poured onto 400 mL of ice-water. Then it was extracted with AcOEt (3 × 100 mL), and organic layers were dried over Na_2SO_4 and filtered. and the solvent was evaporated to yield a yellow-brown solid (7.12 g., 27.1, 87.3%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.91 (s, 1H), 8.56 (dd, J = 7.8, 1.3, 1H), 8.46 (dd, J = 4.7, 1.3, 1H), 7.76 (s, 1H), 7.29–7.25 (m, 3H), 6.90–6.87 (m, 2H), 5.46 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 184.63, 159.66, 148.57, 145.19, 137.63, 130.57, 129.64, 127.79, 119.03, 117.70, 116.67, 114.43, 55.31, 48.11. ES-MS(pos.) *m/z*: 267.14 $[M + H]^+$.

*Synthesis of 3-(Cyclopenta-2,4-dienylidenemethyl)-1-(4-methoxybenzyl)-1H-pyrrolo[2,3-b]pyridine (***3d***).* In a 500-mL round-bottom flask, 1-(4-methoxybenzyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde. (**2d**) (6.72 g., 25.2 mmol, 1.00 equiv) was dissolved in MeOH and freshly cracked CpH (4.23 mL, 63.1 mmol, 2.50 equiv) was added at RT. Then pyrrolidine (3.16 mL, 37.9 mmol, 1.50 equiv) was added dropwise and the mixture was stirred overnight at RT. After that time, a dark solution was obtained, AcOH (3.00 mL) was added, and the mixture was stirred for 5 min at RT. Then the solution was poured over 200 mL of water and it was extracted with AcOEt (3 × 200 mL) and organic layers were washed with brine (3 × 100 mL). After that, it was dried over Na_2SO_4 and filtered. The solvent was evaporated in vacuo to yield a dark red oil that was purified using a flash chromatography column (SiO_2/CH_2Cl_2/AcOEt 10%) to yield a red oil (5.73 g., 18.2 mmol, 72.2%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.41 (dd, J = 4.7, 1.5, 1H), 8.11 (dd, J = 7.9, 1.5, 1H), 7.60 (s, 1H), 7.33 (s, 1H), 7.25–7.14 (m, 3H), 6.87–6.81 (m, 2H), 6.67–6.33 (m, 4H), 5.45 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.39, 148.00, 144.19,141.53, 133.83, 130.35, 129.25, 129.21, 129.19, 128.78, 127.57, 126.82, 120.27, 119.61, 117.20, 114.29, 112.19, 55.29, 47.78. ES-MS(pos.) *m/z*: 315.51 $[M + H]^+$.

Synthesis of bis{[(4-Methoxybenzyl-1-H-pyrrolo[2,3-b]pyridin-3-yl)methyl]cyclopentadienyl} titanium(IV) dichloride [$TiCl_2\{\eta^5$-C_5H_4-$CH(C_7H_4N_2$-CH_2-$(C_6H_4$-$OCH_3)_2\}_2$*] (***4d***).* In a 250-mL Schlenk flask, a 1 M solution of $LiBEt_3H$ in THF (12.4 mL, 12.4 mmol, 1.30 equiv) was heated at 60°C for 20 min and at 90°C for 30 min under vacuum of 10^{-2} mbar to remove THF. In another 250-mL Schlenk flask, 3-(cyclopenta-2,4-dienylidenemethyl)-1-(4-methoxybenzyl)-1*H*-pyrrolo[2,3-*b*]pyridine (**3d**) (3.00 g, 9.55 mmol, 1.00 equiv) was dissolved in dry Et_2O (200 mL) under N_2 and the solution was added to the concentrated solution of $LiBEt_3H$ via cannula; the orange reaction mixture was stirred overnight at RT and resulted in a yellow suspension of the lithiated intermediate. The yellow solid was filtered using a Schlenk frit under N_2, yielding (2.24 g, 6.95 mmol, 72.7%) of a yellow powder.

This litiated intermediate was dissolved in dry THF (100 mL) in a 250-mL Schlenk flask under N_2 and a dark red solution was observed. It was stirred for a couple of minutes at RT and, then, a 1 M

solution of $TiCl_4$ in toluene (3.47 mL, 3.47 mmol, 0.50 equiv) was added dropwise under N_2. The mixture was refluxed overnight, which resulted in a dark red solution. THF was removed in vacuo, and a red solid oil was obtained. This was dissolved in CH_2Cl_2 and filtered through a plug of Celite®. After the solvent was evaporated in vacuo, a dark red solid was obtained (1.40 g, 1.87 mmol, 53.8%).

^{1}H NMR (400 MHz, CD_2Cl_2) δ: 8.18–8.14 (m, 2H), 7.98–7.72 (m, 2H), 7.16–6.65 (m, 12H), 6.36–5.92 (m, 8H, C_5H_4), 5.26 (s, 4H), 3.76–3.53 (m, 10H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ: 158.33, 147.13, 144.78, 141.92, 133.09, 131.40, 130.33, 129.63 129.61 127.94, 127.92, 126.65, 126.47, 126.22, 124.78, 114.28, 113.13, 46.26, 40.47, 25.20. Anal. calcd for $C_{42}H_{38}N_4O_2TiCl_2$ (749): C 67,30, H 5.11, Cl 9.46, N 7.47; found: C 67.48, H 5.30, Cl 5.27, N 6.85. UV–vis (CH_2Cl_2, nm): λ 229 (ε: 76,700), λ 232 (ε 78,600), λ 285 (ε 38,600). ES-MS (pos.) *m/z*: 750.12 $[M + H]^+$. IR absorptions (KBr, cm^{-1}): 3416, 3241, 3104, 3003, 2956, 2927, 2834, 2356, 1870, 1711, 1611, 1512, 1247, 1174, 1028, 799.

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