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Design and Synthesis of Potent and Selective Azaindole-Based Rho Kinase (ROCK) Inhibitors

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*Rho kinase plays a pivotal role in several cellular processes such as vasoregulation, making it a suitable target for the treatment of hypertension and related disorders. We discovered a new compound class of Rho kinase (ROCK) inhibitors containing a 7-azaindole hinge-binding scaffold tethered to an aminopyrimidine core. Herein we describe the structure–activity relationships elucidated through biochemical and functional assays. The introduction of suitable substituents at the 3-position of the bicyclic moiety led to an increase in activity, which was required to design compounds with favorable pharmacokinetic profile. Azaindole **32** was identified as a highly selective and orally available ROCK inhibitor able to cause a sustained blood pressure reduction in vivo.*

Introduction

The Rho-associated coiled-coil containing protein kinase (ROCK) is a ~160 kDa serine/threonine kinase from the AGC kinase family. There are two isoenzymes that share ~90% homology in the kinase domain, ROCK-1 (alternatively called ROKβ) and ROCK-2 (also known as ROKα).[1] Both isoforms are ubiquitously expressed, but ROCK-1 is more pronounced in lung, spleen, testis, liver, and kidney, whereas ROCK-2 is preferentially expressed in brain, heart and skeletal muscle.[2] ROCK is an effector of the small GTP-binding protein RhoA,[3] and is implicated in a multitude of fundamental cellular processes[4] including smooth muscle contraction, cell growth and migration,[5] endothelial barrier maintenance,[6] and apoptosis.[7] Increased ROCK activity contributes to hypertension, coronary vasospasm, vascular inflammation, artherosclerosis, erectile dysfunction, cardiac hypertrophy, ischemia-reperfusion injury, glaucoma, and cerebral ischemia. Moreover, ROCK plays a critical role in the induction of neurite retraction and growth cone collapse, and therefore small-molecule inhibitors might be therapeutically effective in the promotion of axonal regeneration after spinal cord and other nerve injuries.[8] Recently it has been reported that a Rho kinase inhibitor diminishes the dissociation-induced apoptosis of human embryonic stem cells.[9]

The pivotal role of ROCK in vascular smooth muscle contraction has been intensely examined; ROCK mediates the phosphorylation of the regulatory myosin-binding subunit (MBS) of myosin light chain (MLC) phosphatase. Phosphorylated MBS inhibits the phosphatase activity causing an increase in the level of phosphorylated MLC and the contractile tone of the vascular smooth muscle apparatus independently of any change in intracellular Ca^{2+} concentration, a phenomenon known as "calcium sensitization".[10] The central function of ROCK in the control of smooth muscle contraction makes it a suitable target for broadly efficacious anti-hypertensive agents and the treatment of other cardiovascular diseases.[11]

A small number of ROCK inhibitors have been reported in the literature (Figure 1).[12] Amongst them, fasudil (HA-1077, **1**)[13] is the most fully characterized compound, and has been used in Japan since 1995 for the treatment of cerebral vasospasm after subarachnoidal bleeding.[14] However, results obtained with this compound should be interpreted with caution

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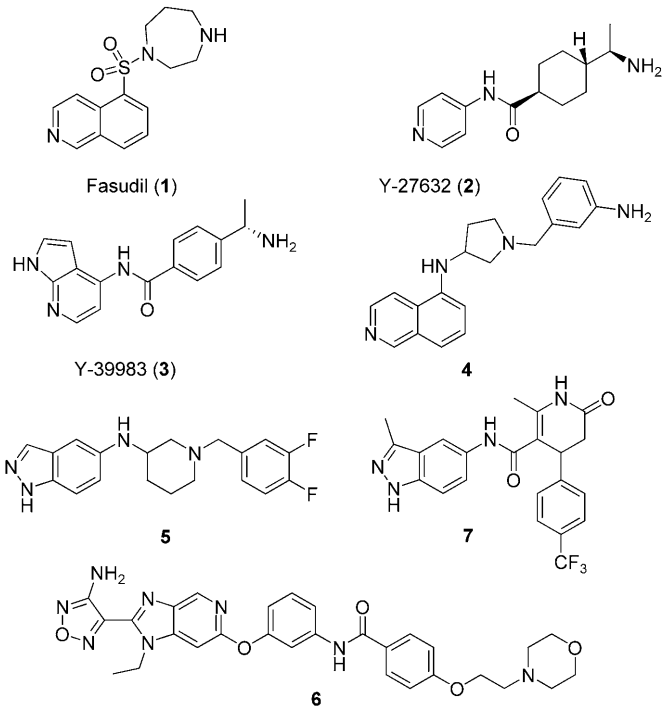


Figure 1. Known ROCK inhibitors.

since the selectivity for ROCK over other members of the AGC family of protein kinases is low.[15] The structural analogues Y-27632 (**2**)[16] and Y-39983 (**3**)[17] have proven important tool compounds in the development of ROCK inhibitors. Compounds such as analogues **4** and **5**[18] have been described by researchers from Kirin Brewery as potent ROCK inhibitors in vitro. GlaxoSmithKline disclosed two distinct ROCK inhibitor series, aminofurazan-azabenzimidazoles[19] and dihydropyridone indazole amides;[20] compounds **6** and **7** are shown as representative example, respectively.

In our efforts to develop potent, selective ROCK-1/ROCK-2 inhibitors for use as antihypertensive agents, we identified the azaindole-containing compound **32** as a highly potent, very selective, and orally active ROCK inhibitor.[21] Herein we describe the development of analogue **32** from the initial screening hit, disclosing the synthesis of compound **32** and derivatives, and describing the structure–activity relationships elucidated in the course of the study.

Results and Discussion

Screening hit and lead generation

An internal screening effort led to the discovery of diaminopyrimidine **8** as a lead compound with submicromolar inhibition of ROCK-2 (IC_{50} = 800 nM).[22] The compound consists of a 4-pyridyl head group (A) tethered by a thioether to a central benzene ring (B); the latter serves as a spacer moiety to the N-linked diaminopyrimidine (C) that is substituted with a methyl "foot" group (D) (Figure 2).

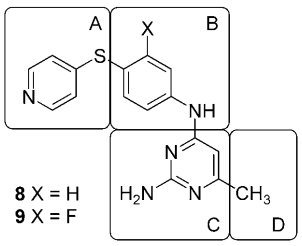


Figure 2. Screening hit **8** and lead compound **9**.

Initial studies showed the dramatic effect of fluorine substitution on the central aromatic moiety *ortho* to the thioether (X = F), which led to a 9-fold increase in activity (compound **9**, IC_{50} = 90 nM). The effect was less pronounced with a chloro atom instead, whilst a methyl or trifluoromethyl group, or a substituent *ortho* to the amino tether, significantly decreased the activity.

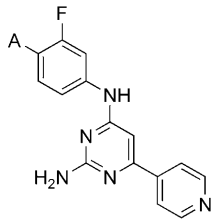
The aminopyrimidine (C) was mandatory for activity; removal of the primary amino group or replacement of the pyrimidine with a pyridine resulted in a complete loss of activity (data not shown). The replacement of the methyl "foot" group (D) by a 4-pyridyl residue further enhanced the potency of the compound (**10**, IC_{50} = 11 nM) as measured against isolated ROCK-2 enzyme (Table 1). The effect was confirmed in a functional assay using rings of rabbit saphenous arteries.[22] The phenylephrine mediated contraction of the vessel rings was antagonized by compound **10** with an IC_{50} value of 500 nM (Table 1).

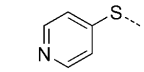
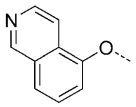
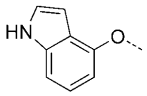
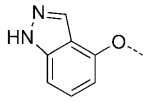
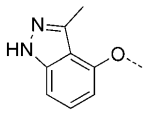
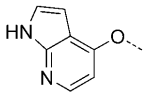
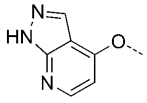
In hypertensive rats the thioethers **8** and **9** effectively lowered blood pressure.[22] However, the effect was short-lived due to the poor pharmacokinetic properties of this compound class. Investigations on the metabolism in rat hepatocytes revealed the rapid oxidation and cleavage of the sulfur tether. Replacement of this metabolically labile linker with the corresponding ether or methylene tether as well as longer spacer moieties such as an ethylene, amide, aminomethylene, or methyleneoxy group failed to give active ROCK inhibitors. The attempt to shield the sulfur atom from oxidation by a second *ortho* fluoro substituent was also unsuccessful. However, a slight increase in activity was observed (data not shown).

Design of 7-azaindole-based ROCK inhibitors

In a subsequent synthetic campaign we sought to replace the head moiety (A) along with the thioether. Numerous oxygen tethered mono and bicyclic heterocycles were introduced (Table 1). The fasudil-like isoquinoline **11** retained some activity (IC_{50} = 200 nM), while the analogous 4-tethered indole **12** was inactive. Indazoles are known hinge-binding motifs present in other ROCK inhibitors (Figure 1).[20,23] However, the 4-tethered compound **13** was twofold less active than the isoquinoline derivative **11**. Interestingly with regard to our findings in the 7-azaindole series, the 3-methyl substitution of the indazole head (derivative **14**) was not tolerated. Ultimately, we discovered that a 4-tethered 7-azaindole head group boosts the activity to give an IC_{50} value in the low, single digit nM range

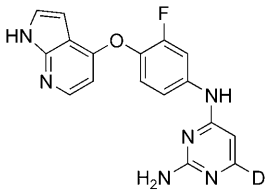
Table 1. SAR data for the ether-tethered heterocyclic head moieties (A).

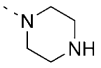
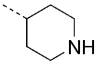
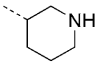


Compound	Head moiety (A)	ROCK-2[22] IC_{50} [nM][a]	Arteria saphena[22] IC_{50} [nM]
10		11	500
11		200	–
12		>3000	–
13		380	–
14		680	6800
15		2	270
16		>3000	–

[a] Standard error of the mean < 5%.

Table 2. SAR data for the foot moiety (D) in 3-unsubstituted azaindoles.



Compound	Foot moiety (D)	ROCK-2[22] IC_{50} [nM][a]	Arteria saphena[22] IC_{50} [nM]
17		17	1500
18		9	1100
19		11	1700
20		7	710
21		16	940
22		6	410
23		2	570
24		2	230
15		2	270
25		10	370
26		7	460

[a] Standard error of the mean < 5%.

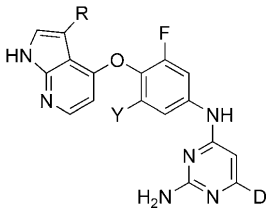
(compound **15**, IC_{50} = 2 nM). This bicyclic system is also the hinge binding moiety in Y-39983 (**3**, Figure 1). Conversely, the pyrazolopyridine analogue **16** is completely inactive.

The phenylephrine-mediated contraction of isolated arteria saphena vessels was inhibited by derivative **15** with an IC_{50} value of 270 nM. In anaesthetized rats, we observed a strong blood pressure lowering effect with doses up to 3 mg kg^{-1} administered intravenously. However, the aqueous solubility of derivative **15** (3 mg L^{-1}) was considered to be critically low. We observed both a strong inhibition of CYP3A4 (IC_{50} = 2.3 μM, Table 3), and poor pharmacokinetic characteristics. The blood clearance in mice was high, with a half-life time of 0.3 h, and the oral bioavailability was limited to 18% (Table 4).

In an attempt to further optimize the properties of the compounds, and with the narrow SAR of the head moiety (A) in hand, we decided to focus on the variation of the foot group (D), thought to be broadly tolerant towards substitution. Small substituents such as a hydrogen (compound **17**), a chloro atom (compound **18**), or a methyl group (compound **19**) resulted in a decrease in inhibition in the enzyme assay and a weakening of the vessel relaxant effect (Table 2). The introduction of a trifluoromethyl group in derivative **20** restored some activity, but this compound was inferior by a factor of 2–3 compared with the 4-pyridyl derivative **15**. The incorporation of a nitrogen-linked pyrrolidine (analogue **21**) was tolerated, but no improvement in inhibitory activity was observed. Some progress was made with the introduction of basic amines, such as piperazine **22**. The corresponding carbon-linked piperidine **23** was similarly active, and the regioisomer **24** slightly exceeded the potency of derivative **15** in the functional assay with an IC_{50} value of 230 nM. However, the slight improvement in vitro was gained at the expense of in vivo absorption. Whilst the introduction of a basic amine enhanced the aqueous solubility, no blood pressure lowering effect could be observed after oral dosing, which was attributed to a slow penetration and active transport phenomenon, as detected with Caco-2 cells (data not shown). Regioisomeric pyridyl compounds **25** and **26** were less potent than the *para*-substituted analogue **15**.

Since modification of the foot moiety only resulted in a fine-tuning of compound properties rather than a significant improvement in potency, we focused on variations of the 7-aza-

Table 3. SAR data for the foot moiety (D) in 3-substituted azaindoles.



Cmpd	R	Y	D	ROCK-2[22] IC_{50} [nM][a]	Arteria saphena[22] IC_{50} [nM]	ΔBP [mmHg][b]	CYP 3A4[d] IC_{50} [μM]
15	H	H	4-pyr	2	270	−65	2.3
27	Me	H	4-pyr	3	89	−70	2.7
28	Et	H	4-pyr	8	1200	–	–
29	Me	H	H	4	84	–	30
30	Me	H	Cl	7	100	–	34
31	Me	F	H	2	56	–	–
32	Me	F	Cl	3	65	−35[c]	41
33	Cl	F	CF_3	2	82	–	–
34	CN	F	H	0.9	20	–	–

[a] Standard error of the mean $<5\%$. [b] Maximum blood-pressure-lowering effect of a single intravenous administration (3 mg kg^{-1}) on the mean arterial blood pressure in anaesthetized rats.[22] [c] Administration of 0.3 mg kg^{-1}. [d] Inhibitory effect on the metabolism of midazolam using microsomes.

Table 4. Pharmacokinetic results for compounds **15**, **29**, **31** and **32** in Wistar rats.[a]

Compound	CL_b [L h^{-1} kg^{-1}][b]	$T_{1/2}$ [h]	F[c] [%]
15	5.3	0.3	18
29	2.1	0.5	n.d.
31	1.6	0.9	n.d.
32	1.7	1.2	48

[a] Intravenous administration of 0.5 mg kg^{-1} in 1% DMSO and 99% plasma; oral administration of 1.0 mg kg^{-1} in EtOH/Solutol/H_2O (1:2:7).[22] [b] Blood clearance. [c] Oral bioavailability.

indole head group. Whilst substituents in the 2- or 6-position were not tolerated (data not shown), we discovered that a methyl group in the 3-position of the heterobicyclic moiety resulted in a threefold enhancement of activity in the functional assay (compound **27**, Table 3). After intravenous administration of compound **27** (3 mg kg^{-1}), we observed a pronounced and enduring decrease in blood pressure in anaesthetized rats. However, the CYP3A4 inhibition was similar to that observed for compound **15**.

The 3-ethyl analogue **28** was significantly less active. In contrast to the azaindoles lacking substitution at the 3-position, the activity of 3-methyl compounds did not depend on a bulky pyrimidine foot moiety (D). Opposing our previous SAR observations, compound **29**, containing a 3-methyl azaindole head moiety and a simple hydrogen foot group, proved to be equipotent with compound **27**. Similar potencies were achieved with the chloro-substituted analogue **30**. The second fluoro atom (Y = F) in compounds **31** and **32** imparted some additional activity. In accordance with our assumption that the 4-pyridyl residue was responsible for the strong CYP3A4 enzyme inhibition, the analogues lacking this moiety (**29**, **30**, **32**) showed augmented selectivity for ROCK-2 over CYP3A4 (Table 3). Both a chloro atom (compound **33**) and especially a cyano group (compound **34**) in the 3-position of the 7-azaindole increased activity, but the aqueous solubility of these substrates was very low. A blood pressure lowering effect of 35 mmHg was achieved using compound **32** in anaesthetized rats at a low dose (0.3 mg kg^{-1}) proving the excellent in vivo efficacy of this compound (Table 3).

Considering the pharmacokinetics of our most potent compounds in rats (Table 4), compound **32** was selected as the best candidate with the longest half-life time (1.2 h) and the highest oral bioavailability (48%), and was shown to be a medium clearance drug in mice and dogs.[21] Compound **32** was further characterized in vitro and in vivo as described elsewhere.[21]

Selectivity

ROCK is known to be unique amongst protein kinases in terms of the combination of the AGC characteristic residue Phe360 (ROCK-1 numbering system) and the mutations Ile82, Met156, Ala215, and Asp160, which are important shape determinants for the ATP-binding cavity.[24] For this reason, it should be possible to achieve selective inhibition of ROCK over other kinases, and we therefore thoroughly examined the profile of azaindole **32**.

We challenged the possibility that other vasoactive mechanisms apart from ROCK inhibition contribute to the observed vessel relaxant effect. Myosin light chain kinase (MYLK, also called MLCK) was only moderately inhibited by azaindole **32** with an IC_{50} value of 7.4 μM. Zipper-interacting protein kinase (ZIP kinase, also known as DAP-like kinase or Dlk), is a member of the death-associated protein (DAP) kinase family, modulating myosin phosphatase activity and participating in the calcium sensitization of smooth muscle cells.[25] Azaindole **32** only slightly inhibited ZIP kinase with an IC_{50} value of 4.1 μM.

In collaboration with Millipore Corp. (Billerica, MA, USA), the specificity against a number of other kinases was explored. Compound **32** was tested at a concentration of 10 μM against 112 different kinases. The azaindole **32** was found to be inactive against 89 kinases, and only weakly active against an additional 21 kinases, including ALK, Aurora-A, Axl, CDK7, c-Raf, Fms, Met, MSK1, MST2, p70S6K, TrkB, PKA, as well as PKBα, -β, and -γ, with IC_{50} values in the range of 1–10 μM (data not shown). Only the receptor tyrosine kinases TrkA and Flt3 were inhibited at submicromolar concentrations with IC_{50} values of 252 and 303 nM, respectively. However, the target kinases, human ROCK-1 and ROCK-2, were inhibited with IC_{50} values of 0.6 nM and 1.1 nM, respectively, demonstrating the excellent selectivity of compound **32**.

The activity of derivative **32** against 63 cardiovascular relevant enzymes and receptors was explored in collaboration with MDS Pharma Services (Taipei, Taiwan). The azaindole **32** moderately inhibited the L-type calcium channel and sodium channel with IC_{50} values of 6.7 and 6.8 μM, respectively. The remaining 60 receptors and enzymes were not significantly af-

fected by compound **32** at a concentration of 10 μM (data not shown). Analogue **32** was shown not block the hERG channel K^+ current as measured using a whole-cell voltage-clamp technique.

Molecular modeling

Docking studies using the X-ray co-crystal structure of ROCK-1 with fasudil (2esm.pdb, subunit A)[15b] suggest that the 7-azaindole head group serves as both a H-bond donor and acceptor, making two key hydrogen bonds with the hinge region (Glu 154 and Met 156) of the ATP-binding site (Figure 3 a). The secondary amino group, which links the central difluorobenzene ring and the pyrimidine foot group, forms an H-bond with Asp 160, while the terminal, primary amino group can form an H-bond to Asp 202. Modeling studies indicate that a small substituent at the 3-position on the azaindole can be accommodated (R group, Table 3). Optimal substituents are methyl, chloro, and nitrile, which all form beneficial hydrophobic interactions with the protein. Additionally, the nitrile group is predicted to form a water mediated H-bond to Asp 216 (Figure 3 b). Recently Cai et al.[26] described a series of 7-azaindoles as c-Met kinase inhibitors, which are similar to our ROCK inhibitors with respect to the A and B moieties, but different with respect to the C moiety. From the X-ray co-crystal structure described by Cai et al.[26] it is apparent that these 7-azaindoles bind to the inactive form of c-Met kinase (DFG-out), fundamentally different to the proposed binding mode of our ROCK inhibitors.

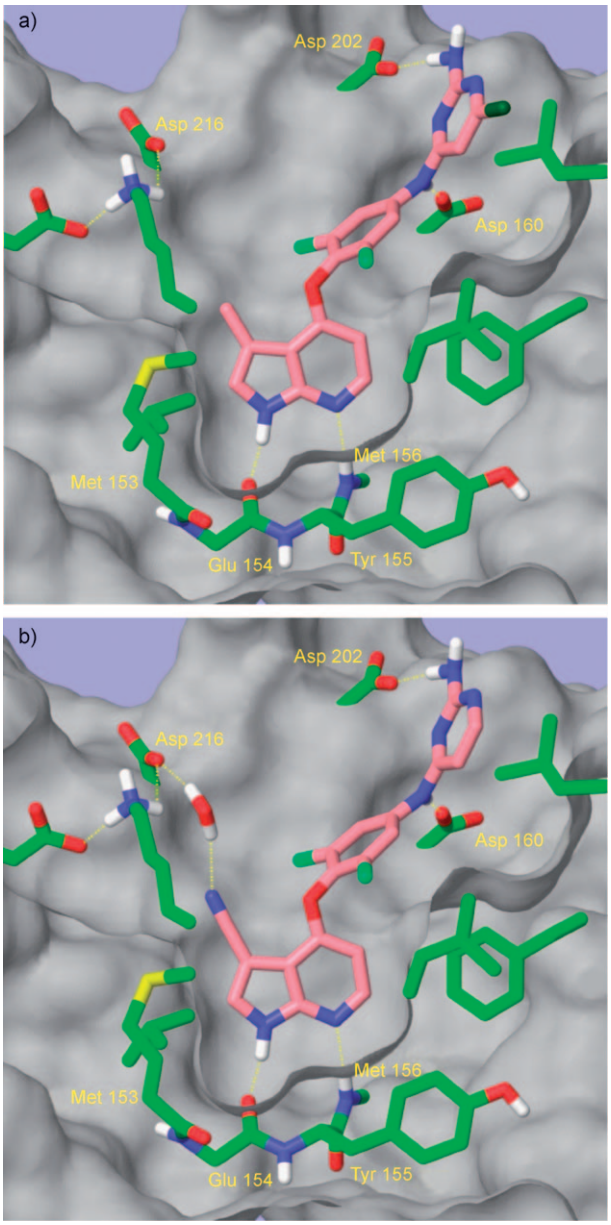


Figure 3. Molecular modeling images of inhibitors docked and minimized into ROCK-1 (2esm.pdb, subunit A, Asp 216 side chain minimized): a) Compound **32**; b) Compound **34**. The inhibitors are shown in pink, while the protein surface is given in grey with key amino acids labeled and highlighted in green. Heteroatoms and polar hydrogens are color coded (N, blue; O, red; S, yellow; F, light green; Cl, dark green; polar H, white) and hydrogen bonds are shown as yellow dotted lines.

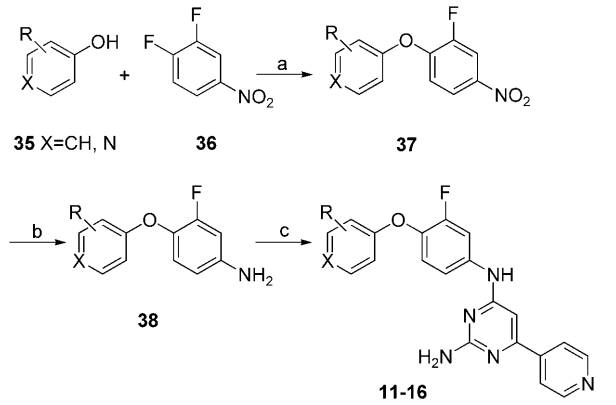
Syntheses

The ROCK inhibitors were synthesized by first making the top aniline fragment, comprised of the head group (A) and the central benzene ring (B), and the bottom chloropyrimidine fragment comprised of the aminopyrimidine moiety (C) and the foot group (D), and subsequently coupling the two fragments together via an acid catalyzed nucleophilic aromatic substitution to give the desired compounds.

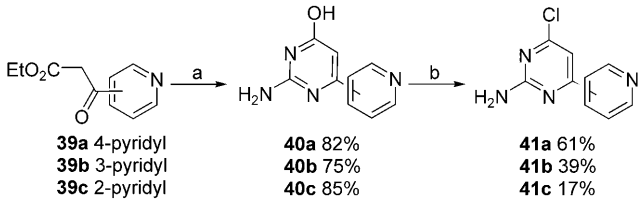
The general synthetic route for the preparation of compounds **11**–**16** is shown in Scheme 1. The appropriate bicyclic phenol **35** was reacted with 3,4-difluoronitrobenzene (**36**) to give the corresponding aryl ether **37**. The nitro group was catalytically reduced to aniline **38**, which was in turn coupled with pyrimidine **41 a** following a standard procedure for the introduction of a pyrimidine moiety involving aqueous hydrochloric acid (Scheme 2) to afford the target compounds **11**–**16**.

The chloropyrimidine intermediates **41 a–c**[27] were synthesized in 2 steps from the β-ketoesters **39 a–c** (Scheme 2), and used in the synthesis of ROCK inhibitors **15** and **25**–**28**. The β-ketoesters **39 a–c** underwent cyclization with guanidinium carbonate, and the resulting hydroxypyrimidines **40 a–c** were chlorinated with phosphorylchloride. In the case of intermediate **41 a**, we discovered that two equivalents of acetic acid were required for a clean transformation.

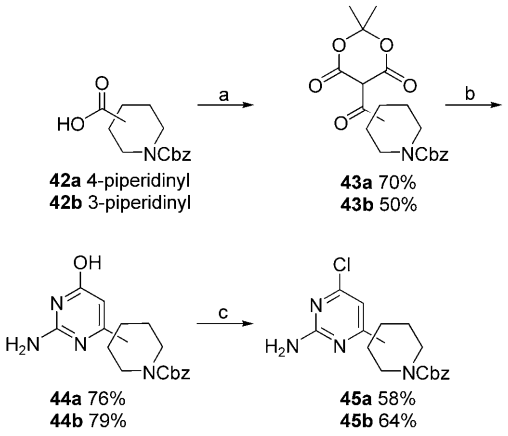
The carbon-linked, piperidine-substituted chloropyrimidines were introduced as Cbz-protected moieties before deprotection to give the ROCK inhibitors **23** and **24**. The Cbz-protected piperidines were synthesized from carboxylic acids **42 a** and **42 b**. These acids were reacted with Meldrum's acid to yield intermediates **43 a** and **43 b**, which were then subjected to the same conditions used in the preparation of **40** (Scheme 2). Chlorination conditions using phosphoryl chloride in acetonitrile and an ammonium salt as a phase-transfer catalyst, as described by Robins et al.,[28] were used to obtain the chloropyrimidine fragments **45 a** and **45 b** (Scheme 3).



Scheme 1. Synthesis of ROCK inhibitors **11**–**16**. Reagents and conditions: a) K_2CO_3, DMF, 40 °C; b) H_2 (1 atm), PtO_2 (cat.), EtOH, RT; c) **41a**, aq HCl, H_2O, reflux.

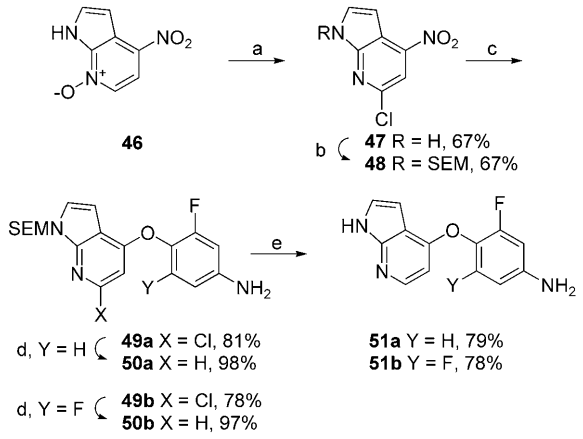


Scheme 2. Synthesis of intermediates **41a–c**. Reagents and conditions: a) Guanidinium carbonate, aq HCl, EtOH, reflux, o/n; b) $POCl_3$, HOAc (**41a** only), $PhNMe_2$, 100 °C, 3 h.



Scheme 3. Synthesis of intermediates **45a** and **45b**. Reagents and conditions: a) 2,2-Dimethyl-1,3-dioxolan-4,6-dione, EDC, DMAP, CH_2Cl_2, 0 °C→RT, o/n; b) Guanidinium carbonate, aq HCl, EtOH, reflux, 7 h; c) $POCl_3$, iPr$_2$NEt, $BnNEt_3^+Cl^-$, MeCN, 0 °C→RT, o/n.

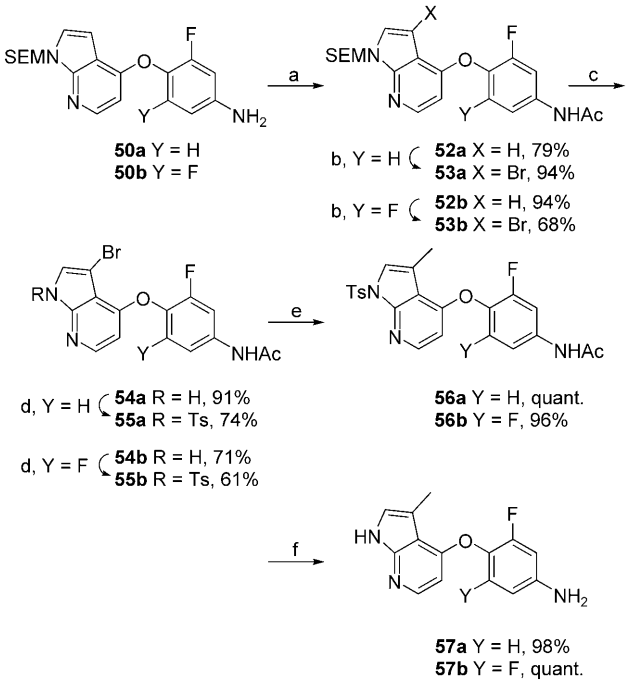
In the synthesis of ROCK inhibitors containing a 7-azaindole hinge-binding group, generation of a *p*-aminophenol ether in the 4-position of the bicyclic moiety proved problematic. Initial attempts to generate the ethers by the substitution of 4-chloro- or 4-nitro-7-azaindole with *p*-aminophenol failed,[29] and a rearranged product was isolated as the major component in accordance with the literature.[30] The palladium-catalyzed coupling of 4-chloro-7-azaindole derivatives worked with simple phenols, but failed in the case of *p*-nitro- and *p*-aminophenol, whilst N-protected aminophenols were coupled in modest yields. For example, the Pd-catalyzed reaction of SEM-protected 4-chloro-7-azaindole with *N*-(3-fluoro-4-hydroxyphenyl)acetamide afforded only 22% of the ether **52a**.[31] Eventually, we developed an uncatalyzed method giving access to the required ether substituted azaindoles. Based on the notion that an electron-withdrawing substituent in 6-position of 4-nitro-7-azaindole enhances the electrophilicity of the bicyclic system, and that side reactions initiated by N1 deprotonation can be avoided by chemical protection of this position, we designed the SEM-protected 7-azaindole **48** (Scheme 4).[32] Com-



Scheme 4. Synthesis of intermediates **51a** and **51b**. Reagents and conditions: a) HMDS, $ClCO_2Me$, THF, RT, o/n; b) NaH, DMF, 10 °C→RT; SEMCl, RT, o/n; c) 4-Amino-2-fluorophenol (for **49a**) or 4-amino-2,6-difluorophenol (for **49b**), K_2CO_3, DMSO, 120 °C, 3 h; d) H_2 (1 atm), Pd/C (cat.), TEA, EtOH, RT, 8 h; e) 1. TFA (50% in CH_2Cl_2), RT, 2 h; 2. NaOAc, EtOH, RT, 30 min.

pound **48** was synthesized from the known azaindole-7-oxide **46**[33] in two steps, and proved to be a versatile reagent for nucleophilic substitution in 4-position. The phenyl ethers **49a** and **49b** were isolated in 81% and 78% yield, respectively, while in the case of the unprotected azaindole **47**, the yield of the ether bond formation did not exceed 50% and dropped on large scale. The activating 6-chloro atom was removed by catalytic hydrogenation to yield key intermediates **50a** and **50b**. Additionally, the reduction step benefited from the SEM protection through enhanced solubility of the substrates in organic solvents. The N1 deprotection required two manipulations since the acid-mediated activation of the SEM group resulted in stable hemiaminals, requiring slightly basic conditions for complete deprotection to give anilines **51a** and **51b**.

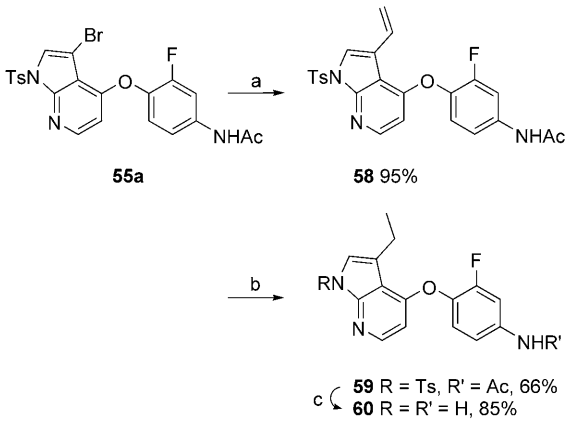
Substitution at the 3-position of the 7-azaindole ring required protection of the aniline with an acetate group (Scheme 5). Subsequent bromination of intermediates **52a** and **52b** proceeded better in the presence of the SEM group due to the solubilizing effect. Cleavage of the SEM group and subsequent tosylation of N1 gave compounds **55a** and **55b**. The 3-methyl group was introduced in a Negishi reaction using dimethylzinc as one-carbon source to give products **56a** and **56b** in almost quantitative yield. This Pd-catalyzed step worked less reliably using the SEM protected substrate **53a**



Scheme 5. Synthesis of intermediates **57a** and **57b**. Reagents and conditions: a) AcCl, TEA, CH_2Cl_2, RT, 3 h; b) Br_2, CH_2Cl_2, 0 °C, 10 min; c) 1. HCl (4 N in dioxane), RT, o/n; 2. aq. LiOH, THF, RT, o/n; d) *n*BuLi, TsCl, THF, −70 °C→RT, 2 h; e) Me_2Zn, cat $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, dioxane, 100 °C, 30 min; f) NaOH, H_2O, EtOH, 90 °C, 15 h.

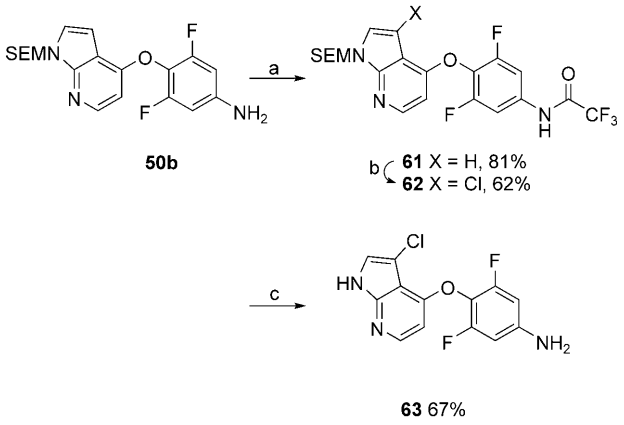
directly, and the total yield was inferior compared with the pathway containing a change in the N1 protecting group. Hydrolytic cleavage of the acetate group in compounds **56a** and **56b** yielded the desired fragments **57a** and **57b**.

Similarly, the N-tosylated substrate **55a** was used to introduce a two-carbon moiety through a Suzuki–Miyaura coupling of this compound with vinylboronic acid di-*n*-butylester gave compound **58** in a 95% yield. The catalytic reduction of the double bond afforded the ethyl derivative **59**, which in turn gave the deprotected, 3-ethyl substituted azaindole **60** (Scheme 6).



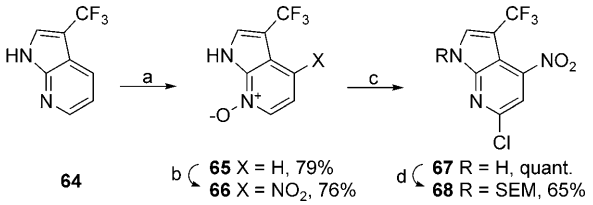
Scheme 6. Synthesis of intermediate **60**. Reagents and conditions: a) Vinylboronic acid di-*n*-butylester, cat $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, $NaHCO_3$, DME/H_2O (3:1), 90 °C, 2 h; b) H_2 (1 atm), cat Pd/C, EtOH, RT, o/n; c) NaOH, H_2O, EtOH, 90 °C, o/n.

Contrary to the previous preparations, we used a more labile aniline protecting group in the synthesis of 3-chloroazaindole **63** from key intermediate **50b** (Scheme 7). The trifluoroacetanilide **61** reacted with NCS in tetrachloromethane to give the chloro derivative **62** in a 62% yield, and deprotection was achieved in 67% yield.



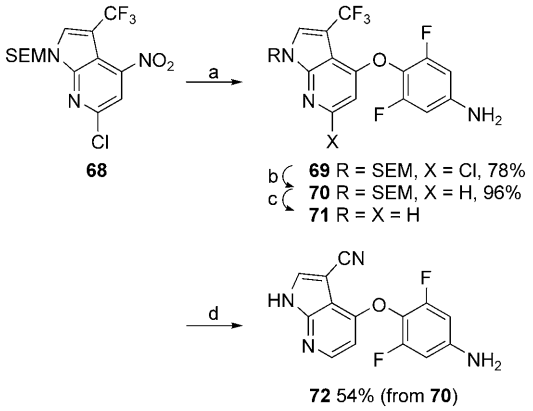
Scheme 7. Synthesis of intermediate **63**. Reagents and conditions: a) TFAA, TEA, CH_2Cl_2, 0 °C, 1 h; b) NCS, CCl_4, 60 °C, 1 h; c) 1. TFA, CH_2Cl_2, RT, 3 h; 2. aq LiOH, THF, RT, 72 h.

During the optimization of the ROCK inhibitors, we developed a new synthesis of 7-azaindole **64**, which could be accomplished on a large scale in three steps from 2-fluoropyridine.[34] Analogously to the route used for the 3-unsubstituted azaindole, we activated the compound in an N-oxidation, nitration and reductive chlorination reaction sequence (Scheme 8). The SEM protection of compound **67** yielded de-



Scheme 8. Synthesis of building block **68**. Reagents and conditions: a) mCPBA, EtOAc, 0 °C, 1 h; b) HNO_3, TFA, 70 °C, 2 h; c) Cl_3CCOCl, HMDS, THF, 0 °C→RT, 2 h; d) SEMCl, NaH, DMF, RT, 45 min.

rivative **68** as reactive building block. Compound **68** was reacted with 4-amino-2,6-difluorophenol to give the corresponding ether **69** in 78% yield, and the hydrogenolytic elimination of the chloro atom to give compound **70** proceeded in almost quantitative yield (Scheme 9). Due to the sensitivity of the trifluoromethyl moiety to basic hydrolysis, acidic reaction conditions (50% trifluoroacetic acid in dichloromethane) were optimal for SEM group deprotection. Fortunately, the hemiaminal of compound **71** was less stable than those derived from compounds **53a** and **53b**, and no subsequent treatment with base was required. The corresponding 3-cyano compound **72** was accessed from **71** by aminolysis of the trifluoromethyl group with aqueous ammonia.[35]

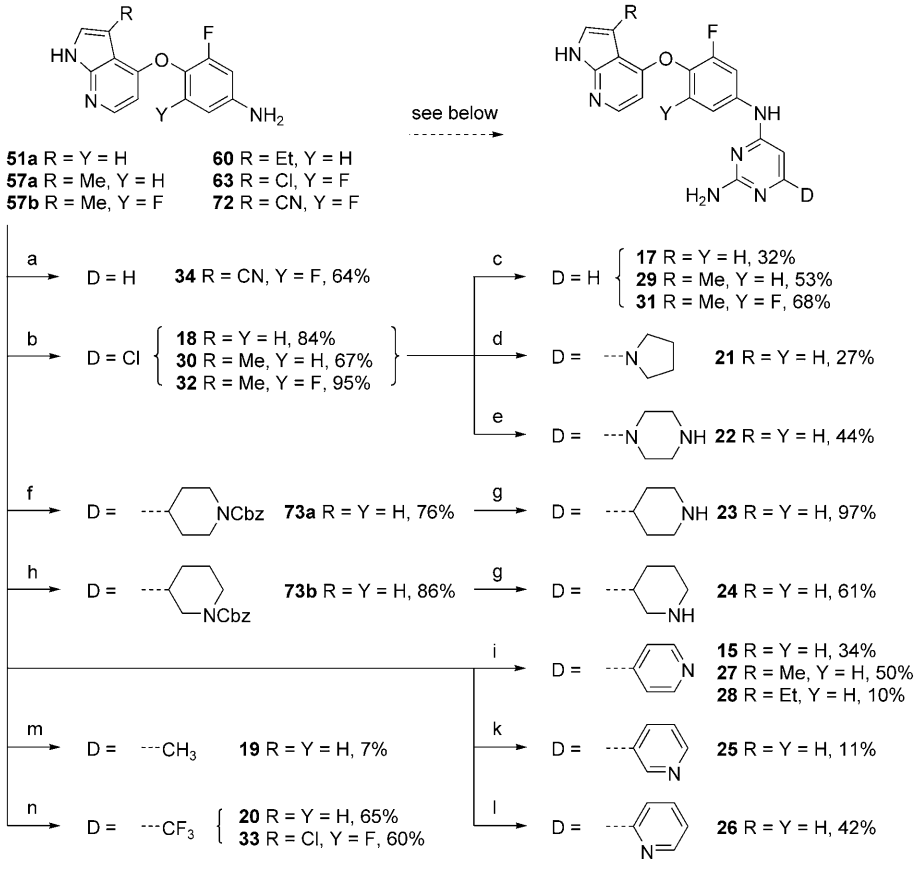


Scheme 9. Synthesis of intermediates **71** and **72**. Reagents and conditions: a) 4-Amino-2,6-difluorophenol, K_2CO_3, DMSO, 120 °C, 3 h; b) H_2 (1 atm), cat Pd/C, EtOH, RT, 24 h; c) TFA, CH_2Cl_2, RT, 3 h; d) NH_3 (aq.), dioxane, 60 °C, o/n.

With the desired anilines and chloropyrimidines in hand, the nucleophilic aromatic substitution was performed in aqueous hydrochloric acid as described in Scheme 1 with the yields given in Scheme 10. A chloro atom as foot moiety (D) in compounds **18**, **30**, and **32** allowed for further manipulations. Hydrogenolysis of the C–Cl bond in compounds **18**, **30**, and **32** yielded the corresponding unsubstituted derivatives **17**, **29**, and **31**, respectively. Substitution of the chloro group in compound **18** with secondary amines gave compounds **21** and **22**. The preparation of ROCK inhibitors **23** and **24** required hydrogenolytic cleavage of the Cbz protecting group in compounds **73a** and **73b**, respectively. All other compounds were synthesized directly from the respective aniline and chloropyrimidine without further manipulations.

Conclusions

Herein, we have described a reliable synthesis of compound **32** and its analogues and discussed the various SAR data elucidated in our studies. We developed a highly potent ATP competitive ROCK inhibitor with excellent selectivity over other protein kinases and cardiovascular relevant enzymes and receptors. Compound **32** is a medium clearance compound in different species and exerts a pronounced blood pressure lowering effect in vivo, and as such is a valuable therapeutic agent for further pharmacological investigations on the physiological role of ROCK.



Scheme 10. Assembly of ROCK inhibitors from the top and bottom fragments. Reagents and conditions: a) 2-Amino-4-chloropyrimidine, aq HCl, reflux, 2 h; b) 2-Amino-4,6-dichloropyrimidine, aq HCl, reflux, o/n; c) H_2 (1 atm), cat Pd/C, THF/MeOH/EtOH (1:1:10), TEA, RT, o/n; d) Pyrrolidine, *i*Pr_2NEt, 1-butanol, reflux, 6 h; e) Piperazine, *i*Pr_2NEt, 1-butanol, reflux, 5 h; f) **45a**, aq HCl, reflux, o/n; g) H_2 (1 atm), cat Pd/C, EtOH, RT, o/n; h) **45b**, aq HCl, reflux, 4.5 h; i) **41a**, aq HCl, reflux, o/n; j) **41b**, aq HCl, reflux, o/n; k) **41c**, aq HCl, reflux, o/n; l) Ethyl (2-amino-6-chloropyrimidin-4-yl)acetate,[36] aq HCl, reflux, o/n; m) 4-Chloro-6-(trifluoromethyl)pyrimidin-2-amine,[37] aq HCl, reflux, o/n.

Experimental Section

General methods and materials: ^{1}H NMR and ^{13}C NMR spectra were recorded in [D_6]DMSO at RT on Bruker Avance spectrometers operating at 300 MHz, 400 MHz and 500 MHz for ^{1}H NMR, and at 125 MHz for ^{13}C NMR. Flash chromatography was performed on silica gel 60 (0.063–0.200 mm) purchased from Merck KGaA (Germany). Preparative HPLC chromatography was performed on a 250 mm × 30 mm column packed with YMC gel ODS-AQ S-5/15 μm, with CH_3CN/H_2O as the eluent and UV-detection. Solvents for extraction and chromatography were reagent grade and used as received. Commercial reagents were used without purification. Petroleum ether (PE) refers to the fraction boiling in the range 40–60 °C.

6-Chloro-4-nitro-1*H*-pyrrolo[2,3-*b*]pyridine (47): 4-Nitro-1*H*-pyrrolo[2,3-*b*]pyridin-7-oxide[33] (76.0 g, 424 mmol) and hexamethyldisilazane (89.5 mL, 424 mmol) in THF (3.8 L) were treated with methyl chloroformate (164 mL, 2.12 mol) and stirred at RT overnight. The reaction mixture was filtered, and the volatile compounds were removed in vacuo. The resi-

due was triturated with Et_2O (500 mL) and the crystals were collected by suction filtration to give the desired compound (69.5 g, 67% yield, 80% pure). An analytical sample was purified by HPLC to yield a yellow solid; ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 7.00$ (dd, $J =$ 3.2, 1.9 Hz, 1H), 7.96 (s, 1H), 8.00 (dd, $J = 3.2$, 2.8 Hz, 1H), 12.79 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 100.1$, 109.7, 111.5, 132.8, 142.1, 145.9, 150.6 ppm; HRMS: m/z $[M]^+$ calcd for $C_7H_4ClN_3O_2$: 196.9992, found: 196.9983.

6-Chloro-4-nitro-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo-[2,3-*b*]pyridine (48): Azaindole **47** (77.1 g, 390 mmol) was dissolved in DMF (1.65 L) and the solution was cooled to 10 °C. NaH (15.6 g, 390 mmol, 60% in mineral oil) was added portionwise to the reaction. The mixture was warmed to RT and 2-(trimethylsilyl)-ethoxymethylchloride (68.3 g, 410 mmol) was added. After stirring overnight, the mixture was diluted with H_2O (8.3 L) and extracted with EtOAc (3×3.0 L). The combined organic layers were washed with brine (3.0 L), dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (PE/EtOAc, 9:1) to yield azaindole **48** as an oil (85.9 g, 67%). Unreacted starting material was eluted with CH_2Cl_2/MeOH 95:5 (6.1 g, 8%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = -0.09$ (s, 9H), 0.85 (t, $J = 8.1$ Hz, 2H), 3.54 (t, $J = 8.1$ Hz, 2H), 5.68 (s, 2H), 7.08 (d, $J = 3.5$ Hz, 1H), 8.05 (s, 1H), 8.15 ppm (d, $J = 3.5$ Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = -1.46$, 17.1, 65.9, 73.2, 100.3, 110.8, 112.2, 135.6, 142.8, 146.4, 149.6 ppm; HRMS: m/z $[M]^+$ calcd for $C_{13}H_{18}ClN_3O_3Si$: 327.0806, found: 327.0806.

4-[(6-Chloro-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy]-3,5-difluoroaniline (49b). SEM-protected azaindole **48** (12.0 g, 36.6 mmol) was dissolved in DMSO (144 mL) and K_2CO_3 (15.2 g, 110 mmol) and 4-amino-2,6-difluorophenol (7.97 g, 54.9 mmol) were added. The mixture was heated for 3 h in a preheated oil bath at 120 °C. Subsequently, the mixture was poured into H_2O and extracted with EtOAc (2× 300 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (PE/EtOAc, 4:1) to yield the desired compound as yellow crystals (12.2 g, 78%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = -0.09$ (s, 9H), 0.83 (t, $J = 8.0$ Hz, 2H), 3.52 (t, $J = 8.0$ Hz, 2H), 5.56 (s, 2H), 5.87 (s, 2H), 6.38–6.40 (m, 2H), 6.42 (s, 1H), 6.49 (s, 1H), 7.61 ppm (d, $J = 3.5$ Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = -1.50$, 17.1, 65.6, 72.8, 96.9 (dd, $^2J_{C,F} = 19.4$, $^4J_{C,F} = 4.2$ Hz), 97.6, 100.3, 108.5, 117.7 (t, $^2J_{C,F} = 16.3$ Hz), 128.9, 144.6, 148.4, 148.5 (t, $^3J_{C,F} = 13.4$ Hz), 155.5 (dd, $^1J_{C,F} = 244$ Hz, $^3J_{C,F} = 6.9$ Hz), 159.1 ppm; HRMS: m/z $[M+H]^+$ calcd for $C_{19}H_{22}ClF_2N_3O_2Si$: 426.1211, found: 426.1210.

3,5-Difluoro-4-[(1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo-[2,3-*b*]pyridin-4-yl)oxy]aniline (50b): Aniline **49b** (47.0 g, 110 mmol) was dissolved in EtOH (570 mL) and palladium on charcoal (6.00 g, 10 wt.%) and TEA (13.4 g, 132 mmol) were added to the solution. The mixture was stirred under a H_2 atmosphere for 2 h. A further portion of palladium on charcoal (6.00 g, 10 wt.%) was added, and the reaction was stirred under H_2 for an additional 6 h. The mixture was filtrated and the filtrate concentrated in vacuo. The residue was redissolved in CH_2Cl_2, washed with H_2O and the organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo to yield of the title compound (41.8 g, 97%), which was used without further purification; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = -0.10$ (s, 9H), 0.81 (t, $J = 7.9$ Hz, 2H), 3.52 (t, $J = 7.9$ Hz, 2H), 5.61 (s, 2H), 5.80 (s, 2H), 6.36–6.42 (m, 3H), 6.45 (d, $J = 5.3$ Hz, 1H), 7.56 (d, $J = 3.2$ Hz, 1H), 8.13 ppm (d, $J = 5.3$ Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = -1.52$, 17.1, 65.3, 72.5, 96.8 (dd, $^2J_{C,F} =$ 19.3 Hz, $^4J_{C,F} = 4.6$ Hz), 97.0, 100.3, 109.1, 118.1 (t, $^2J_{C,F} = 16.4$ Hz), 128.1, 144.5, 148.0 (t, $^3J_{C,F} = 13.3$ Hz), 150.1, 155.6 (dd, $^1J_{C,F} = 243$ Hz, $^3J_{C,F} = 7.3$ Hz), 157.7 ppm; HRMS: m/z $[M]^+$ calcd for $C_{19}H_{23}F_2N_3O_2Si$: 391.1528, found: 391.1539.

***N*-{3,5-Difluoro-4-[(1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo-[2,3-*b*]pyridin-4-yl)oxy]phenyl}acetamide (52b)**: Aniline **50b** (42.1 g, 108 mmol) was dissolved in CH_2Cl_2 (975 mL) and cooled to 0 °C. TEA (21.8 g, 215 mmol) and acetylchloride (12.7 g, 161 mmol) were added, and the mixture was stirred at 0 °C for 3 h. Saturated aq $NaHCO_3$ (200 mL) was added, and the mixture was vigorously stirred for 10 min. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2 to 95:5) to yield the acetylated compound **52b** (44.0 g, 94%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = -0.10$ (s, 9H), 0.81 (t, $J = 8.0$ Hz, 2H), 2.10 (s, 3H), 3.52 (t, $J = 8.0$ Hz, 2H), 5.62 (s, 2H), 6.41 (d, $J = 3.7$ Hz, 1H), 6.50 (d, $J = 5.5$ Hz, 1H), 7.54 (d, $J = 10.3$ Hz, 2H), 7.60 (d, $J = 3.7$ Hz, 1H), 8.15 (d, $J = 5.5$ Hz, 1H), 10.42 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = -1.39$, 17.2, 24.1, 65.5, 72.7, 97.0, 100.7, 103.1 (dd, $^2J_{C,F} = 20.6$ Hz, $^4J_{C,F} = 5.1$ Hz), 109.3, 124.4 (t, $^2J_{C,F} = 15.7$ Hz), 128.6, 137.9 (t, $^3J_{C,F} = 12.8$ Hz), 144.7, 150.2, 155.0 (dd, $^1J_{C,F} = 246$ Hz, $^3J_{C,F} =$ 5.6 Hz), 157.0, 169.2 ppm; HRMS: m/z $[M+H]^+$ calcd for $C_{21}H_{25}F_2N_3O_3Si$: 434.1707, found: 434.1715.

***N*-{4-[(3-Bromo-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo-[2,3-*b*]pyridin-4-yl)oxy]-3,5-difluorophenyl}acetamide (53b)**: A solution of acetamide **52b** (32.1 g, 74.0 mmol) in CH_2Cl_2 (960 mL) was cooled to 0 °C and treated with a solution of bromine (13.0 g, 81.4 mmol) in CH_2Cl_2 (77 mL), and the reaction was stirred for 10 min at 0 °C. The mixture was then pored into an ice-cold 10% solution of $Na_2S_2O_3$ (1.3 L). The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (1.3 L). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product (74% pure), which was used without further purification (35.2 g, 68%). An analytical sample was purified by HPLC to yield a tan solid; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = -0.09$ (s, 9H), 0.83 (t, $J = 8.0$ Hz, 2H), 2.10 (s, 3H), 3.54 (t, $J =$ 8.0 Hz, 2H), 5.61 (s, 2H), 6.45 (d, $J = 5.4$ Hz, 1H), 7.56 (d, $J = 10.6$ Hz, 2H), 7.87 (s, 1H), 8.18 (d, $J = 5.4$ Hz, 1H), 10.44 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = -1.41$, 17.1, 24.1, 65.7, 72.7, 85.0, 100.7, 103.1 (dd, $^2J_{C,F} = 20.2$ Hz, $^4J_{C,F} = 4.9$ Hz), 108.0, 123.7 (t, $^2J_{C,F} = 16.2$ Hz), 128.3, 138.1 (t, $^3J_{C,F} = 12.7$ Hz), 146.0, 149.0, 154.7 (dd, $^1J_{C,F} = 246$ Hz, $^3J_{C,F} = 6.0$ Hz), 157.4, 169.1 ppm; HRMS: m/z $[M+H]^+$ calcd for $C_{21}H_{24}BrF_2N_3O_3Si$: 512.0812, found: 512.0790.

***N*-{4-[(3-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy]-3,5-difluoro-phenyl} (54b)**: Acetamide **53b** (35.2 g, 50.8 mmol) was stirred overnight in solution of HCl in dioxane (4 N, 850 mL). The resultant precipitate was collected by suction filtration and washed with Et_2O. The crystals were suspended in THF (1.2 L), treated with an aq solution of lithium hydroxide (2 N, 400 mL), and the mixture was stirred overnight. Subsequently, brine was added to the reaction, and the aqueous mixture was extracted with EtOAc (2× 300 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated to afford the deprotected compound (86% pure), which was used without purification (16 g, 71% yield). An analytical sample was purified by HPLC to yield a tan solid; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 2.10$ (s, 3H), 6.34 (d, $J = 5.4$ Hz, 1H), 7.55 (d, $J = 10.6$ Hz, 2H), 7.65 (s, 1H), 8.10 (d, $J = 5.4$ Hz, 1H), 10.43 (s, 1H), 12.22 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 24.1$, 84.0, 99.6, 103.1 (dd, $^2J_{C,F} = 20.6$ Hz, $^4J_{C,F} = 5.3$ Hz), 107.5, 123.8 (t, $^2J_{C,F} = 16.0$ Hz), 125.3, 137.9 (t, $^3J_{C,F} = 12.7$ Hz), 145.6, 149.6, 154.8

(dd, $^1J_{C,F}=246$ Hz, $^3J_{C,F}=6.2$ Hz), 157.2, 169.1 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{15}H_{10}BrF_2N_3O_2$: 381.9998, found: 381.9984.

***N*-[4-({3-Bromo-1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}oxy)-3,5-difluorophenyl]acetamide (55b)**: Acetamide **54b** (16.0 g, 86% pure, 36.0 mmol) was dissolved in THF (920 mL) and cooled to −70 °C. A solution of *n*BuLi in hexane (2.5 M, 18.4 mL, 41.9 mmol) was added dropwise, and the mixture was stirred for 15 min. Subsequently, a solution of *p*-TsCl (8.78 g, 46.1 mmol) in THF (46 mL) was added to the reaction. The mixture was warmed to RT and stirred for 2 h. The mixture was poured into a saturated $NaHCO_3$ solution (4.6 L) and was extracted with EtOAc (2×2.3 L). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/acetone, 9:1) to yield the title compound as off-white crystals (11.7 g, 61%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=2.10$ (s, 3H), 2.36 (s, 3H), 6.65 (d, $J=5.6$ Hz, 1H), 7.45 (d, $J=8.2$ Hz, 2H), 7.54 (d, $J=10.5$ Hz, 2H), 8.04 (d, $J=8.2$ Hz, 2H), 8.15 (s, 1H), 8.26 ppm (d, $J=5.6$ Hz, 1H), 10.45 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=21.1$, 24.1, 91.0, 103.1 (dd, $^2J_{C,F}=20.2$ Hz, $^4J_{C,F}=4.9$ Hz), 103.6, 110.1, 123.1 (t, $^2J_{C,F}=15.9$ Hz), 125.3, 127.8, 130.1, 133.9, 138.3 (t, $^3J_{C,F}=12.7$ Hz), 146.1, 147.3, 147.9, 154.5 (dd, $^1J_{C,F}=246$ Hz, $^3J_{C,F}=6.1$ Hz), 157.6, 169.1 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{22}H_{16}BrF_2N_3O_4S$: 536.0086, found: 536.0075.

***N*-[3,5-Difluoro-4-({3-methyl-1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}oxy)-phenyl]acetamide (56b)**: A degassed solution of 3-bromoazaindole **55b** (12.0 g, 22.4 mmol) in dioxane (240 mL) was treated with a solution of dimethylzinc in toluene (2 M, 33.6 mL, 67.1 mmol) and Pd(dppf)Cl$_2$·CH$_2$Cl$_2$ (914 mg, 1.11 mmol) and the mixture was heated at 100 °C for 30 min. After cooling to RT, EtOAc and HCl (1 N) were added (250 mL). The aqueous phase was extracted with EtOAc (250 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated to yield a reddish residue (12.8 g, 96% yield), which was used without purification (80% pure). An analytical sample was purified by HPLC; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=2.09$ (s, 3H), 2.35 (s, 3H), 2.41 (s, 3H), 6.53 (d, $J=5.6$ Hz, 1H), 7.42 (d, $J=8.1$ Hz, 2H), 7.53 (d, $J=10.5$ Hz, 2H), 7.67 (s, 1H), 7.97 (d, $J=8.1$ Hz, 2H), 8.17 (d, $J=5.6$ Hz, 1H), 10.43 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=11.8$, 21.1, 24.1, 102.9, 103.1 (dd, $^2J_{C,F}=21.1$ Hz, $^4J_{C,F}=4.4$ Hz), 112.1, 113.8, 122.7, 123.5 (t, $^2J_{C,F}=16.1$ Hz), 127.5, 130.0, 134.6, 138.2 (t, $^3J_{C,F}=12.8$ Hz), 145.6, 146.8, 148.8, 154.7 (dd, $^1J_{C,F}=246$ Hz, $^3J_{C,F}=6.1$ Hz), 158.5, 169.1 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{23}H_{19}F_2N_3O_4S$: 472.1138, found: 472.1118.

3,5-Difluoro-4-[(3-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy]aniline (57b): A solution of acetamide **56b** (12.8 g, 80% pure, 21.6 mmol) in EtOH (360 mL) was treated with a 20% aqueous NaOH solution (220 mL) and the mixture was heated to 90 °C for 15 h. The mixture was concentrated in vacuo, and the remaining residue was diluted with H_2O and EtOAc (200 mL). The aqueous phase was separated and extracted with EtOAc (250 mL). The combined organic layers were dried ($MgSO_4$), stirred with charcoal, filtered, and concentrated to give the crude product as a colorless oil (6.6 g, quant. yield), which was used without purification (91% pure). An analytical sample was purified by HPLC; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=2.42$ (s, 3H), 5.77 (s, 2H), 6.17 (d, $J=5.4$ Hz, 1H), 6.40 (d, $J=10.7$ Hz, 2H), 7.13 (s, 1H), 7.98 (d, $J=5.4$ Hz, 1H), 11.36 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=12.2$, 97.1 (dd, $^2J_{C,F}=19.3$ Hz, $^4J_{C,F}=4.6$ Hz), 98.5, 108.0, 109.2, 118.2 (t, $^2J_{C,F}=16.4$ Hz), 122.2, 144.4, 148.0 (t, $^3J_{C,F}=13.2$ Hz), 151.1, 155.9 (dd, $^1J_{C,F}=243$ Hz, $^3J_{C,F}=7.3$ Hz), 159.3 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{14}H_{11}F_2N_3O$: 276.0943, found: 276.0948.

6-Chloro-*N*^{4}-{3,5-difluoro-4-[(3-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy]phenyl} pyrimidin-2,4-diamine (32): Aniline **57b** (3.50 g, 86% pure, 10.9 mmol) and 4,6-dichloropyrimidine-2-amine (1.97 g, 12.0 mmol) were suspended in H_2O (45 mL) and the mixture was treated with HCl (1 N, 5.5 mL). The mixture was heated at reflux overnight, cooled and treated with a solution of NaOH (1 N, pH 10). DMF was added, and the aqueous phase was extracted with EtOAc (150 mL). The organic layer was washed with H_2O (2× 70 mL), dried (Na_2SO_4) and concentrated. The crude product was triturated with a small volume of ice-cold MeOH. The precipitate was collected by suction filtration and washed with CH_2Cl_2. The filtrate was concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/MeOH, 100:4 to 10:1) to give the title compound as a tan solid (3.1 g, 70% combined yield); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=2.44$ (s, 3H), 6.04 (s, 1H), 6.21 (d, $J=5.4$ Hz, 1H), 6.99 (br s, 2H), 7.16 (s, 1H), 7.74 (d, $J=10.6$ Hz, 2H), 7.99 (d, $J=5.4$ Hz, 1H), 9.77 (s, 1H), 11.43 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=12.0$, 94.3, 98.3, 103.1 (dd, $^2J_{C,F}=21.3$ Hz, $^4J_{C,F}=4.8$ Hz), 107.7, 108.9, 122.3, 123.0 (t, $^2J_{C,F}=16.1$ Hz), 138.6 (t, $^3J_{C,F}=13.0$ Hz), 144.1, 151.1, 154.9 (dd, $^1J_{C,F}=245$ Hz, $^3J_{C,F}=6.8$ Hz), 158.3, 158.4, 161.3, 162.6 ppm; HRMS: *m/z* $[M+H]^+$ calcd for $C_{18}H_{13}ClF_2N_6O$: 403.0881, found: 403.0865.

3-(Trifluoromethyl)-1*H*-pyrrolo[2,3-*b*]pyridine 7-oxide (65): A solution of *m*-chloroperbenzoic acid (335 g, 1.45 mol) in EtOAc (3 L) was dried (Na_2SO_4) and cooled to 0 °C. Compound **64**[34] (180 g, 969 mmol) was added to the solution in portions. The mixture was stirred for 1 h during which time white crystals precipitated. The solid was collected by suction filtration and washed with EtOAc (600 mL) to yield the desired N-oxide as a white crystalline solid (155 g, 79%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.25$ (dd, $J=8.0$, 6.2 Hz, 1H), 7.67 (d, $J=8.0$ Hz, 1H), 8.16 (s, 1H), 8.31 (d, $J=6.2$ Hz, 1H), 13.40 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=105.4$ (q, $^1J_{C,F}=37.4$ Hz), 117.5 118.5, 119.5 (q, $^3J_{C,F}=2.2$ Hz), 123.5 (q, $^1J_{C,F}=266$ Hz), 127.5 (q, $^1J_{C,F}=5.0$ Hz), 132.7, 138.5 ppm; HRMS: *m/z* $[M]^+$ calcd for $C_8H_5F_3N_2O$: 202.0354, found: 202.0348.

4-Nitro-3-(trifluoromethyl)-1*H*-pyrrolo[2,3-*b*]pyridine 7-oxide (66): A solution of compound **65** (9.00 g, 44.5 mmol) in TFA (117 mL) was heated to 70 °C. Nitric acid (65%, 6.2 mL, 89 mmol) was added within 10 min and the reaction was stirred at this temperature for 2 h. The reaction was stopped by pouring the mixture into an ice/water mixture. The precipitate was collected by suction filtration and washed with H_2O. The product was dried in vacuo to yield the title compound as an off-white solid (8.32 g, 76%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=8.09$ (d, $J=6.9$ Hz, 1H), 8.46 (s, 1H), 8.49 (d, $J=6.9$ Hz, 1H), 14.2 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=105.5$ (q, $^2J_{C,F}=37.9$ Hz), 110.7, 115.4, 122.6 (q, $^1J_{C,F}=266$ Hz), 132.4, 132.7 (q, $^3J_{C,F}=6.5$ Hz), 137.0, 141.3 ppm; HRMS: *m/z* $[M]^+$ calcd for $C_8H_4F_3N_3O_3$: 247.0205, found: 247.0209.

6-Chloro-4-nitro-3-(trifluoromethyl)-1*H*-pyrrolo[2,3-*b*]pyridine (67): A solution of compound **66** (8.60 g, 34.8 mmol) in THF (150 mL) was treated with hexamethyldisilazane (7.34 mL, 34.8 mmol) and cooled to 0 °C. The reaction was then treated dropwise with trichloroacetyl chloride (15.8 g, 87 mmol) and subsequently warmed to RT and stirred for 2 h. The reaction was poured into H_2O (750 mL) and extracted with EtOAc (2×300 mL). The combined organic layers were washed with brine (200 mL), dried (Na_2SO_4), filtered and concentrated. The residue was triturated with PE and the product was collected by suction filtration to give the desired compound as a yellowish solid in quantitative yield (9.2 g);

^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.08 (s, 1H), 8.63 (s, 1H), 13.62 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 102.5 (q, $^2J_{C,F}$ = 38.2 Hz), 105.2 (q, $^3J_{C,F}$ = 1.8 Hz), 111.6, 129.9 (q, $^1J_{C,F}$ = 266 Hz), 133.6 (q, $^3J_{C,F}$ = 5.7 Hz), 144.3, 148.5, 149.9 ppm; HRMS: *m/z* [*M*]$^+$ calcd for $C_8H_3ClF_3N_3O_2$: 264.9866, found: 264.9872.

6-Chloro-4-nitro-3-(trifluoromethyl)-1-{[2-(trimethylsilyl)ethoxy]-methyl}-1*H*-pyrrolo[2,3-*b*]pyridine (68): A solution of compound **67** (204 g, 634 mmol) and [2-(chloromethoxy)ethyl](trimethyl)silane (116 g, 697 mmol) in DMF (2.5 L) was treated with NaH (25.4 g, 634 mmol, 60% suspension in mineral oil), and the solution was stirred at RT for 45 min. The mixture was poured into brine and extracted twice with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (PE/EtOAc, 95:5) to yield the title compound as a colorless oil (162 g, 65%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = −0.10 (s, 9H), 0.84 (t, *J* = 8.0 Hz, 2H), 3.58 (t, *J* = 8.0 Hz 2H), 5.70 (s, 2H), 8.16 (s, 1H), 8.83 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = −1.51, 17.0, 66.4, 73.9, 102.7 (q, $^2J_{C,F}$ = 38.6 Hz), 105.8 (q, $^3J_{C,F}$ = 1.9 Hz), 112.8, 122.7 (q, $^1J_{C,F}$ = 266 Hz), 136.1 (q, $^3J_{C,F}$ = 6.0 Hz), 145.1, 148.8, 148.9 ppm; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{17}ClF_3N_3O_3Si$: 396.0753, found: 396.0753.

4-[(6-Chloro-3-(trifluoromethyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo[2,3-*b*]-pyridin-4-yl)oxy]-3,5-difluoroaniline (69): SEM-protected 7-azaindole **68** (71.6 g, 181 mmol) was dissolved in DMSO (0.7 L) under an argon atmosphere. The solution was treated with K_2CO_3 (75.0 g, 543 mmol) and 4-amino-2,6-difluorophenol (39.4 g, 271 mmol) and heated to 120 °C for 3 h. The mixture was poured into H_2O (3 L) and extracted twice with EtOAc (2 L). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (PE/EtOAc, 4:1) to yield the desired product as a colorless oil (69.5 g, 78%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = −0.09 (s, 9H), 0.84 (t, *J* = 8.0 Hz, 2H), 3.58 (t, *J* = 8.0 Hz, 2H), 5.62 (s, 2H), 5.90 (s, 2H), 6.40 (d, *J* = 11.0 Hz, 2H), 6.53 (s, 1H), 8.39 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = −1.50, 17.0, 66.1, 73.4, 96.9 (dd, $^2J_{C,F}$ = 23.1 Hz, $^4J_{C,F}$ = 4.0 Hz), 101.3, 102.9 (q, $^2J_{C,F}$ = 38.8 Hz), 104.7 (q, $^3J_{C,F}$ = 1.6 Hz), 117.0 (t, $^2J_{C,F}$ = 16.4 Hz), 123.1 (q, $^1J_{C,F}$ = 266 Hz), 130.2 (q, $^3J_{C,F}$ = 5.6 Hz), 146.7, 148.3, 148.7 (t, $^3J_{C,F}$ = 13.4 Hz), 155.2 (dd, $^1J_{C,F}$ = 244 Hz, $^3J_{C,F}$ = 6.9 Hz), 159.2 ppm; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{20}H_{21}ClF_5N_3O_2Si$: 494.1085, found: 494.1086.

3,5-Difluoro-4-[(3-(trifluoromethyl)-1-{[2-(trimethylsilyl)ethoxy]-methyl}-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)oxy]aniline (70): A solution of aniline **69** (660 mg, 1.33 mmol) in EtOH (10 mL) was treated with palladium on charcoal (142 mg, 0.134 mmol, 10 wt.%) and stirred for 24 h under a H_2 atmosphere. The reaction was filtered through Celite and the plug was washed with EtOH (20 mL). The filtrate was concentrated in vacuo and the residue was redissolved in CH_2Cl_2 (20 mL). The organic phase was washed with saturated $NaHCO_3$ solution (2×), dried ($MgSO_4$) and concentrated in vacuo. The colorless oil obtained was used without further purification (589 mg, 96%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = −0.10 (s, 9H), 0.83 (t, *J* = 8.0 Hz, 2H), 3.57 (t, *J* = 8.0 Hz, 2H), 5.67 (s, 2H), 5.82 (s, 2H), 6.40 (d, *J* = 10.7 Hz, 2H), 6.53 (d, *J* = 5.1 Hz, 1H), 8.27 (d, *J* = 5.1 Hz, 1H), 8.31 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = −1.50, 17.0, 65.8, 73.0, 96.8 (dd, $^2J_{C,F}$ = 23.2 Hz, $^4J_{C,F}$ = 4.0 Hz), 101.3, 102.4 (q, $^2J_{C,F}$ = 38.5 Hz), 105.2 (q, $^3J_{C,F}$ = 1.5 Hz), 117.5 (t, $^2J_{C,F}$ = 16.4 Hz), 123.1 (q, $^1J_{C,F}$ = 265 Hz), 129.5 (q, $^3J_{C,F}$ = 5.7 Hz), 146.8, 148.2 (t, $^3J_{C,F}$ = 13.3 Hz), 149.8, 155.4 (dd, $^1J_{C,F}$ = 244 Hz, $^3J_{C,F}$ = 7.2 Hz, 157.8 ppm; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{20}H_{22}F_5N_3O_2Si$: 460.1474, found: 460.1476.

4-(4-Amino-2,6-difluorophenoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbonitrile (72): A suspension of compound **70** (150 mg, 0.33 mmol) in CH_2Cl_2 (1.0 mL) was treated with an excess of TFA (1.0 mL, 1.5 mol) and stirred for 3 h at RT. The volatile components were removed in vacuo, and the residue was redissolved in *tert*-butyl methyl ether (10 mL). The organic phase was washed with saturated $NaHCO_3$ (2× 5 mL), dried (Na_2SO_4), filtered and concentrated in vacuo to yield intermediate **71** (73 mg, 87%, 77% purity), which was used without purification. Aqueous concd NH_3 (5.0 mL) was added, and dioxane was added until complete dissolution of the solid. The solution was heated in a sealed tube at 60 °C overnight. The aqueous mixture was extracted with EtOAc (4×) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by preparative HPLC to yield the title compound as a tan solid (51 mg, 54%); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 5.83 (br s, 2H), 6.41 (d, *J* = 10.8 Hz, 2H), 6.47 (d, *J* = 5.4 Hz, 1H), 8.22 (d, *J* = 5.4 Hz, 1H), 8.40 (s, 1H), 12.91 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 81.3, 96.9 (dd, $^2J_{C,F}$ = 19.5 Hz, $^4J_{C,F}$ = 4.0 Hz), 100.8, 107.9, 116.0, 117.3 (t, $^2J_{C,F}$ = 16.1 Hz), 135.1, 147.0, 148.3 (t, $^3J_{C,F}$ = 13.3 Hz), 149.7, 155.4 (dd, $^1J_{C,F}$ = 244 Hz, $^3J_{C,F}$ = 7.1 Hz), 157.9 ppm; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_8F_2N_4O$: 287.0744, found: 287.0738.

4-{4-[(2-Aminopyrimidin-4-yl)amino]-2,6-difluorophenoxy}-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbonitrile (34): Aniline **72** and 2-amino-4-chloro-pyrimidine were reacted following the same procedure described for the preparation of compound **32**. The title compound was obtained as a tan solid in a 64% yield; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 6.07 (d, *J* = 5.7 Hz, 1H), 6.55 (d, *J* = 5.5 Hz, 1H), 6.64 (br s, 2H), 7.83 (d, *J* = 11.2 Hz, 2H), 7.91 (d, *J* = 5.7 Hz, 1H), 8.25 (d, *J* = 5.5 Hz, 1H), 8.46 (s, 1H), 9.75 (s, 1H), 13.00 ppm (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 81.4, 97.2, 101.1, 102.9 (dd, $^2J_{C,F}$ = 20.9 Hz, $^4J_{C,F}$ = 5.0 Hz), 108.0, 116.0, 122.0 (t, $^2J_{C,F}$ = 16.5 Hz), 135.4, 139.6 (t, $^3J_{C,F}$ = 13.1 Hz), 147.1, 149.8, 154.7 (dd, $^1J_{C,F}$ = 245 Hz, $^3J_{C,F}$ = 6.5 Hz), 156.0, 157.4, 160.4, 162.2 ppm; HRMS: *m/z* [*M*+H]$^+$ calcd for $C_{18}H_{11}F_2N_7O$: 380.1066, found: 380.1061.

Molecular modeling: Maestro (v. 8.0, Schrödinger, Portland, OR) was used for inhibitor docking and modeling picture generation. Energy minimization was performed with the following MacroModel settings: Force field, OPLS 2005; Inhibitor, side chain of Asp216 and in the case of compound **34** one added water molecule flexible, all other protein coordinates fixed; Solvent, water; Convergence on gradient, convergence threshold = 0.2.

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