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Scalable, Economical, and Practical Synthesis of 4-(Difluoromethyl)pyridin-2-amine, a Key Intermediate for Lipid Kinase Inhibitors

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Supporting Information

ABSTRACT: A new, scalable, rapid, high yielding, and practical synthesis of 4-(difluoromethyl)pyridin-2-amine provides a key intermediate for the preparation of numerous protein kinase inhibitors and clinical candidates targeting phosphoinositide 3-kinase (PI3K) and the mechanistic target of rapamycin (mTOR) kinase. Starting from 2,2-difluoroacetic anhydride, an efficient five-step and two-pot procedure to prepare 4-(difluoromethyl)pyridin-2-amine (**1**) has been developed. Noteworthy aspects of this strategy include the avoidance of an amination process using a sealed vessel. Each step of the synthetic route has been optimized, and key intermediates have been isolated and characterized prior to the final two-pot procedure, which has been successfully applied for large-scale production.

KEYWORDS: *upscaling, 4-(difluoromethyl)pyridin-2-amine, pyridine ring closure, difluoroacetic acid anhydride, telescope process, chromatography-free process*

INTRODUCTION

Phosphoinositide 3-kinase (PI3K) and the phosphatidylinositol 3-kinase-related protein kinase mechanistic target of rapamycin (mTOR) are key regulators of metabolism, cell growth, and survival. In cancer, this pathway is frequently overactivated by mutated surface receptors, oncogenes, loss of 5′-lipid phosphatase (phosphatase and tensin homolog), or mutation of the catalytic subunit of PI3Kα.[1−5] Through protein kinase B (PKB/Akt), PI3K attenuates tuberous sclerosis complex, which triggers the assembly of an active mTOR complex 1 (TORC1).[4,6] Rapamycin (sirolimus) and its derivatives (rapalogs) RAD001 (everolimus) as well as CCI-779 (temsirolimus) are allosteric inhibitors of TORC1.[7,8] Rapalogs have been used extensively as immunosuppressive agents to prevent organ transplant rejection.[9] Moreover, temsirolimus has been approved in renal cell carcinoma, and everolimus is in phase III in advanced renal cell carcinoma and in use in breast cancer.[10]

The first PI3K inhibitor, wortmannin,[11] has been shown to covalently modify PI3K and, at a higher concentration, mTOR kinase.[12] Since the discovery of wortmannin, a large number of PI3K[1−3,5,13] and mTOR kinase inhibitors[7,14] have been deployed.

PQR309 (bimiralisib, see Scheme 1)[15] with a triazine core and BKM120 (buparlisib)[16] with a pyrimidine core are pan-PI3K inhibitors substituted with two morpholino groups and one 4-(trifluoromethyl)pyridin-2-amine. Chemical modifications on the heteroaromatic ring of PQR309 have been investigated,[17] and the replacement of the trifluoromethyl group by the difluoromethyl augmented mTOR kinase affinity, as confirmed by multiple structure−activity relation (SAR) studies. Recently, we reported the discovery and preclinical characterization of 5-[4,6-bis({3-oxa-8-azabicyclo[3.2.1]octan-8-yl})-1,3,5-triazin-2-yl]-4-(difluoromethyl)pyridin-2-amine (**PQR620**), a highly potent and selective inhibitor of mTOR,[18,19] and the dual PI3K/mTOR kinase inhibitor (*S*)-4-(difluoromethyl)-5-(4-(3-methylmorpholino)-6-morpholino-1,3,5-triazin-2-yl)pyridin-2-amine (**PQR530**).[19,20] Both **PQR620** and **PQR530** have a 4-(difluoromethyl)pyridin-2-amine as an essential binding feature for activity and selectivity.

In order to perform further studies on these compounds in vivo, we optimized their synthesis to approach large-scale preparation. While scaling up the production of **PQR620** and **PQR530**, we recognized the limited access to the essential building block 4-(difluoromethyl)pyridin-2-amine (**1**) (Scheme 1). Herein, we report a new, efficient synthetic strategy and the development of a practical and scalable procedure to access 4-(difluoromethyl)pyridin-2-amine (**1**), which can be exploited in the large-scale production of kinase inhibitors.

Currently Available Synthetic Strategies. Previous literature reports are available for the synthesis of 4-(difluoromethyl)pyridin-2-amine (**1**) and are summarized in Scheme 2. All three synthetic routes led to compound **1** using diverse strategies, among which routes B and C showed particularly good overall yields. Nevertheless, these reported routes were previously carried out in small scale (<5 g), and in

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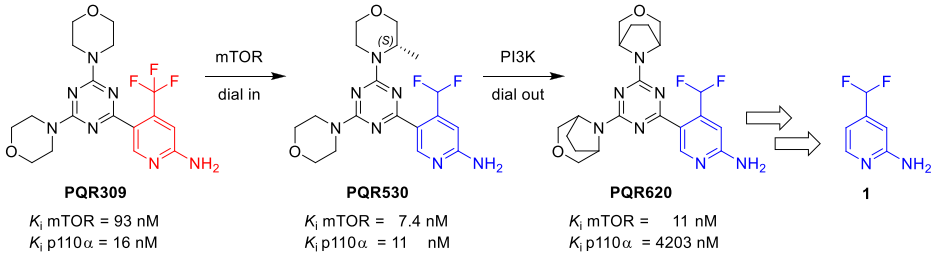
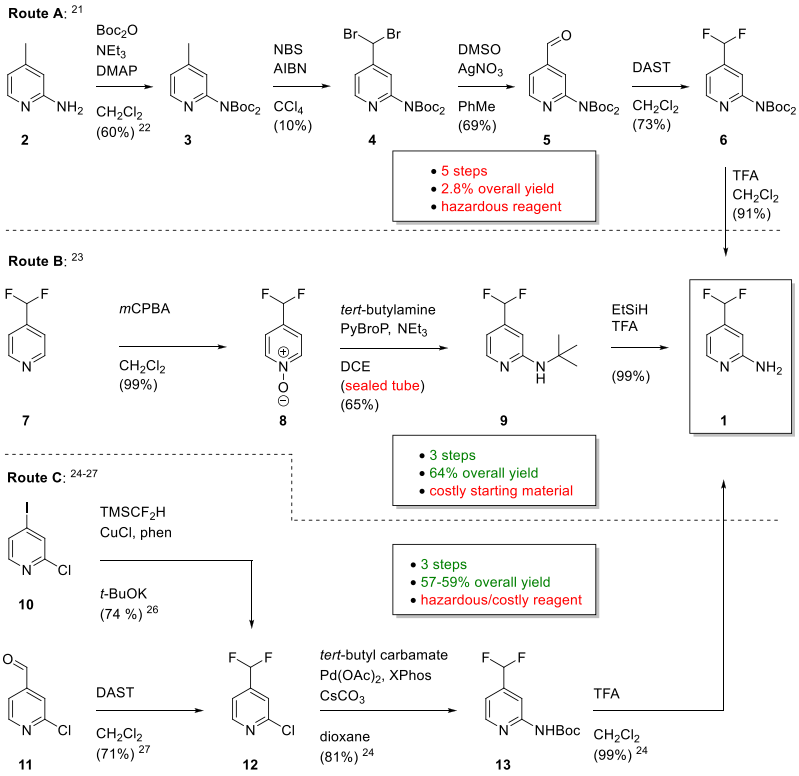
Scheme 1. 4-(Difluoromethyl)pyridin-2-amine (1) as the Key Component in PI3K and mTOR Inhibitors[a]



[a]PQR309 is a pan-PI3K inhibitor with a moderate affinity for mTOR kinase,[15] **PQR530**[20] is a potent dual PI3K/mTOR inhibitor, and **PQR620**[18] is a highly specific mTOR kinase inhibitor.

Scheme 2. Previously Reported Routes to 4-(Difluoromethyl)pyridin-2-amine (1)



some cases, hazardous reagents or potentially dangerous methods as well as expensive reagents/starting materials were used. The three strategies were reported with isolation of all intermediates. In Scheme 2, drawbacks and disadvantages of these reported synthetic pathways are highlighted in red.

In the first report, Terauchi et al.[21] reported the synthesis of 4-(difluoromethyl)pyridin-2-amine (**1**) from inexpensive 4-methylpyridin-2-amine (**2**) in five steps (route A, Scheme 2). The free amino group of compound **2** was protected,[22] and the resulting bis-Boc-protected compound **3** transformed into the corresponding *gem*-dibromomethyl-pyridine **4** using a radical bromination reaction. However, the yield of this bromination step was only 10%. Exposure of compound **4** to silver nitrate and dimethyl sulfoxide gave access to aldehyde **5** in 69% yield. Subsequent fluorination of compound **5** using (diethylamino)-sulfur trifluoride (DAST) and final deprotection of the 2-amino group gave the desired 4-(difluoromethyl)pyridin-2-amine (**1**), both steps in excellent yield (73−91%). This five-step synthesis was reported for a 1−2 g scale. Most of the steps afford the desired intermediate in high yield, but the low yield of the bromination reduces the overall yield of compound **1** to 2.8%. In addition to the low overall yield, the use of the hazardous reagent DAST makes this route difficult to scale up.

Cohen et al.[23] reported a procedure, which involves a shorter three-step gram-scale synthesis of 4-(difluoromethyl)-pyridin-2-amine (**1**) from 4-(difluoromethyl)pyridine (**7**) (route B, Scheme 2). In the first step, the corresponding pyridine-*N*-oxide **8** was obtained from pyridine **7** by oxidation using *meta*-chloroperbenzoic acid in 99% yield. Then, an amination procedure, involving the phosphonium salt, bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), was used to obtain *N*-(*tert*-butyl)-4-(difluoromethyl)pyridin-2-amine (**9**) in 65% yield. Finally, the deprotection of the *tert*-butyl group was achieved by the use of triethylsilane in the presence of trifluoroacetic acid. All steps gave the desired products in high yields (>65%). However, two aspects of route B would need to be addressed prior to scale-up, namely, (i) the

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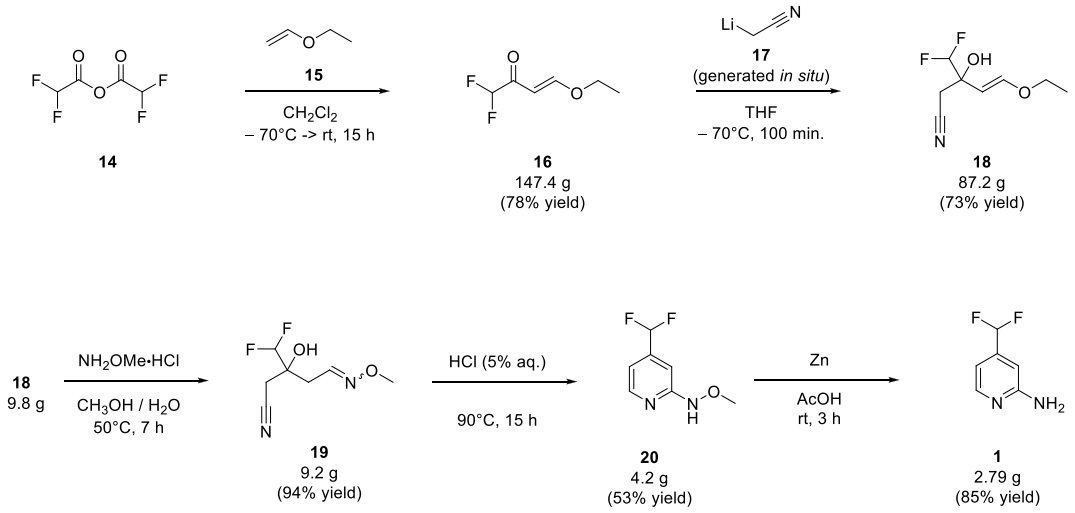
Scheme 3. Novel, Practical, and Scalable Synthesis of 4-(Difluoromethyl)pyridin-2-amine (1)



price of 4-(difluoromethyl)pyridin-2-amine (**7**) and (ii) the use of a sealed pressure tube for the amination reaction.

The latest contribution to the synthesis of 4-(difluoromethyl)pyridin-2-amine (**1**) is its formal synthesis from 2-chloro-4-(difluoromethyl)pyridine (**12**) (route C, Scheme 2).[24] This synthesis afforded the desired product **1** via Buchwald–Hartwig amination from halide **12** and *tert*-butyl carbamate in 81% yield and subsequent Boc-deprotection in the presence of trifluoroacetyl in 99% yield. However, halide **12** is expensive and not readily available as the starting material, which requires its synthesis from (i) 2-chloro-4-iodopyridine (**10**) or (ii) 2-chloroisonicotinaldehyde (**11**). The copper-mediated direct difluoromethylation of aryl iodide **10** requires the use of two equivalents of costly $TMSCF_2H$,[25] and the fluorination of 2-chloroisonicotinaldehyde (**11**) involves the use of the hazardous reagent DAST;[26] therefore, both approaches to intermediate **12** are not prone to scale-up.

RESULTS AND DISCUSSION

Synthetic Strategy. Currently available synthetic strategies have limitations for the production of kilogram quantities of building block **1**, when bulk amounts of drugs for clinical development studies are required. Our aim was to develop a novel strategy for the 4-(difluoromethyl)pyridin-2-amine synthesis starting from a cheaper starting material and avoiding harsh conditions. Herein, we describe a practical five-step synthesis of compound **1**, which, compared to previously reported procedures, has the following advantages: (i) avoids the use of fluorinating agents and sealed vessels and (ii) uses the commercially available 2,2-difluoroacetic anhydride as the starting reagent (**14**; Scheme 3).

As depicted in Scheme 3, the first step allowed the preparation of (*E*)-4-ethoxy-1,1-difluorobut-3-en-2-one (**16**) in 78% yield from ethyl vinyl ether (**15**) and 2,2-difluoroacetic anhydride (**14**). Similarly, the synthesis of the commercially available (*E*)-4-ethoxy-1,1,1-trifluorobut-3-en-2-one has been reported.[27] This reaction was validated on 90 g scale, and the product **16** was used immediately after isolation in order to avoid hydrolysis upon storage.

For the next step, acetonitrile was deprotonated in situ using *n*-butyllithium to generate a solution of the nucleophile **17** in tetrahydrofuran. Upon nucleophilic attack of the enone **16**, the desired nitrile **18** was obtained in 73% yield. This procedure was conducted similar to the one reported for the trifluoromethyl analogue of enone **16** using tetrahydrofuran as solvent instead of 1,2-dimethoxyethane.[28]

Intermediate **18** represents an ideal precursor for cyclization and introduction of an amine.[28] As previously reported, the obvious approach to 2-aminopyridine is reacting the nitrile derivative **18** with ammonia. Cyclization of compound **18** to the desired 2-aminopyridine **1** in presence of ammonia was achieved in 33% yield (Table S1, see the Supporting Information). The major drawback of this reaction is the use of a sealed tube and the high pressure that builds up during the reaction. Our attempts to optimize this reaction by screening various ammonia equivalents are summarized in Table S1. Amines such as *tert*-butylamine, benzylamine, methoxyamine, and hydroxylamine were tested as a replacement for ammonia. Most of the tested conditions gave no desired product, either due to no conversion or decomposition of the starting material, except for benzylamine, where traces of the desired product were observed. Interestingly, reacting the nitrile **18** with methoxyamine in the absence of a base gave the methoxyimine **19** as a *E*/*Z*-mixture (~1:1) in 94% yield (Scheme 3 and entry 13, Table S1 in the Supporting Information).

Consequently, the cyclization of methoxyimine **19** was carried out under acidic conditions. The cyclized pyridine **20** was obtained in 53% yield, when the reaction was performed on 9.2 g scale. Reduction of compound **20** with zinc in acetic acid gave the product **1** in 85% yield. Common reducing agents, such as $LiAlH_4$ and $NaBH_4$, were also tested but performed poorly in affording the desired pyridine **1** (see Table S2 in the Supporting Information). The structure of *N*-(4-(difluoromethyl)pyridin-2-yl)-*O*-methylhydroxylamine **20** was confirmed by X-ray analysis (Figure 1).

Overall, the reaction sequence presented herein allows for the preparation of 4-(difluoromethyl)pyridin-2-amine (**1**) from 2,2-difluoroacetic anhydride (**14**) in five consecutive steps. This sequence was optimized for a batch of ~250 g of difluoroacetic anhydride and afforded compound **1** with 24% overall yield.

Optimization toward a One-Pot Procedure for Cyclization. The transformation of compound **18** into *E*/*Z*-methoxyimine **19** using *O*-methylhydroxylamine was performed in methanol/water as solvent. The subsequent

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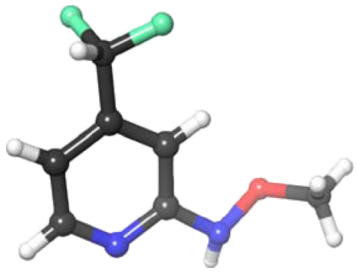


Figure 1. Crystal structure of **20** from the cyclization reaction of the methoxyimine **19** in hydrochloric acid (red O, blue N, gray C, white H). Crystallographic data for **20** have been deposited with the Cambridge Crystallographic Data Center (ccdc.cam.ac.uk) under deposition number 1939418.

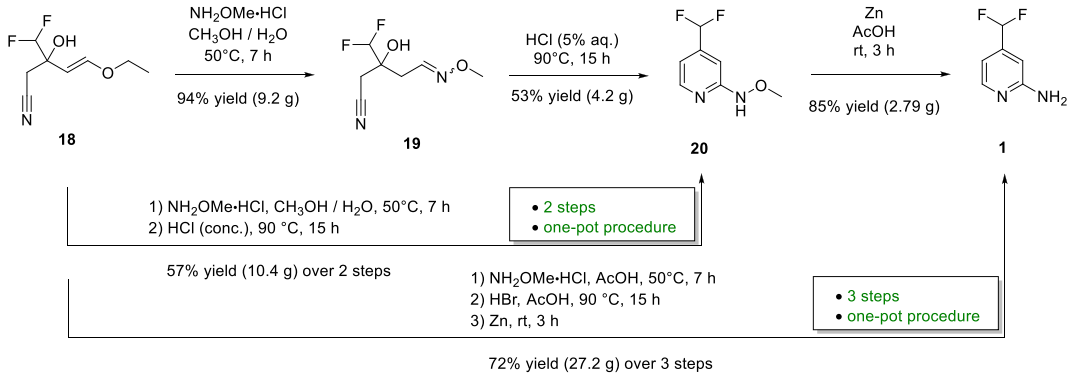
cyclization reaction to pyridine **20** also proceeded in aqueous medium. We exploited the opportunity to combine these two steps in a one-pot fashion (Scheme 4). Initial results are summarized in Table S3 (see the Supporting Information). After completion of the reaction, the mixture was basified to obtain compound **20** as a free base. When carried out on a 3 g scale, the reaction mixture was extracted using an organic solvent (EtOAc), and the desired compound was isolated in a satisfying 64% yield over two steps after a plug filtration over silica gel (entry 1, Table S3 in the Supporting Information). Increasing the scale from 3 to 20 g, the extraction and silica gel plug filtration were omitted, and after basification, the precipitate was collected by simple Buchner filtration. The latter procedure is more suitable for the development of a large-scale process and led to the preparation of compound **20** in the analogous yield of 57% over two steps (entry 3, Table S3 in the Supporting Information).

Church et al. reported the formation of a pyridine ring by cyclization of a nitrile via addition of hydrobromic acid.[29] Since this reaction as well as the reduction of *O*-methylhydroxylamine **20** with zinc (Scheme 3), proceeds in acetic acid, we attempted a one-pot procedure for the transformation of compound **18** into pyridine **1** over three steps. In our first attempt, this reaction proceeded with a satisfying yield of 65% on a 400 mg scale (entry 1, Table S4 in the Supporting Information). Increasing progressively the reaction scale, this one-pot three-step procedure was finally performed on a 50 g batch of nitrile **18** (entry 4, Table S4). The desired product **1** was obtained in 72% yield over three steps in a one-pot procedure (Scheme 4). In comparison, the three-step procedure described in Scheme 3 showed a 42% overall yield from compound **18** to compound **1**. This optimized one-pot procedure was exploited to perform the large-scale synthesis of 4-(difluoromethyl)pyridin-2-amine (**1**).

Large-Scale Synthesis: Two Subsequent One-Pot Procedures. The large-scale synthesis of 4-(difluoromethyl)-pyridin-2-amine (**1**) depicted in Scheme 5 was carried out on two separate batches of 3.6 kg of 2,2-difluoroacetic anhydride (**14**). In the first step, enone **16**, obtained from the nucleophilic attack of the vinyl ether **15** on carbonyl **14** in the presence of base, was not isolated. The reaction solution was (i) washed with water to remove the excess of pyridine and (ii) with an aqueous sodium bicarbonate solution to remove the excess of 2,2-difluoroacetic acid. After an additional wash with brine, the organic solution was dried over sodium sulfate, concentrated, and used in the next step without further purification. In parallel, to a solution of acetonitrile in tetrahydrofuran, *n*-butyllithium was added to form (cyanomethyl)lithium (**17**) in situ as a colorless suspension, and then the above solution of (*E*)-4-ethoxy-1,1-difluorobut-3-en-2-one (**16**), diluted with tetrahydrofuran, was added at −70 °C. The addition of **17** to the carbonyl group of **16** was completed within <3 h, and nitrile **18** was isolated in 77% yield. This procedure was performed in two separate batches, which were combined for work-up and isolation affording 3.06 kg of compound **18** as a brownish liquid. In summary, the synthesis of nitrile **18** was achieved in a three-step one-pot procedure in excellent overall yield, overcoming the issues of decomposition of enone **16**, prone to decomposition upon isolation.

Then, the optimized three-step one-pot procedure described in Scheme 4 was used to convert intermediate **18** into the final compound **1**. (*E*)-3-(Difluoromethyl)-5-ethoxy-3-hydroxy-pent-4-enenitrile (**18**) was treated with *O*-methoxylamine hydrochloride in acetic acid to form intermediate **19**, which was not isolated. After completion of the reaction, hydrobromic acid was added to the reaction mixture. The cyclization to pyridine **20** was completed after 12 h, and its reduction was conducted in situ using zinc as reducing agent. The work-up procedure was optimized in order to avoid purification over silica gel. The reaction mixture was (i) filtered over Celite, (ii) concentrated to reduce the solvent volume, and (iii) basified with NH_4OH to precipitate the product, which was (iv) extracted with dichloromethane. The combined organic layers were (v) dried over sodium sulfate, (vi) treated with activated charcoal, and then (vii) concentrated. Product **1** was precipitated from heptane and isolated as a pale brownish solid. Using our optimized three-step one-pot procedure for

Scheme 4. Optimizations toward a One-Pot Synthesis of 4-(Difluoromethyl)pyridin-2-amine (1)



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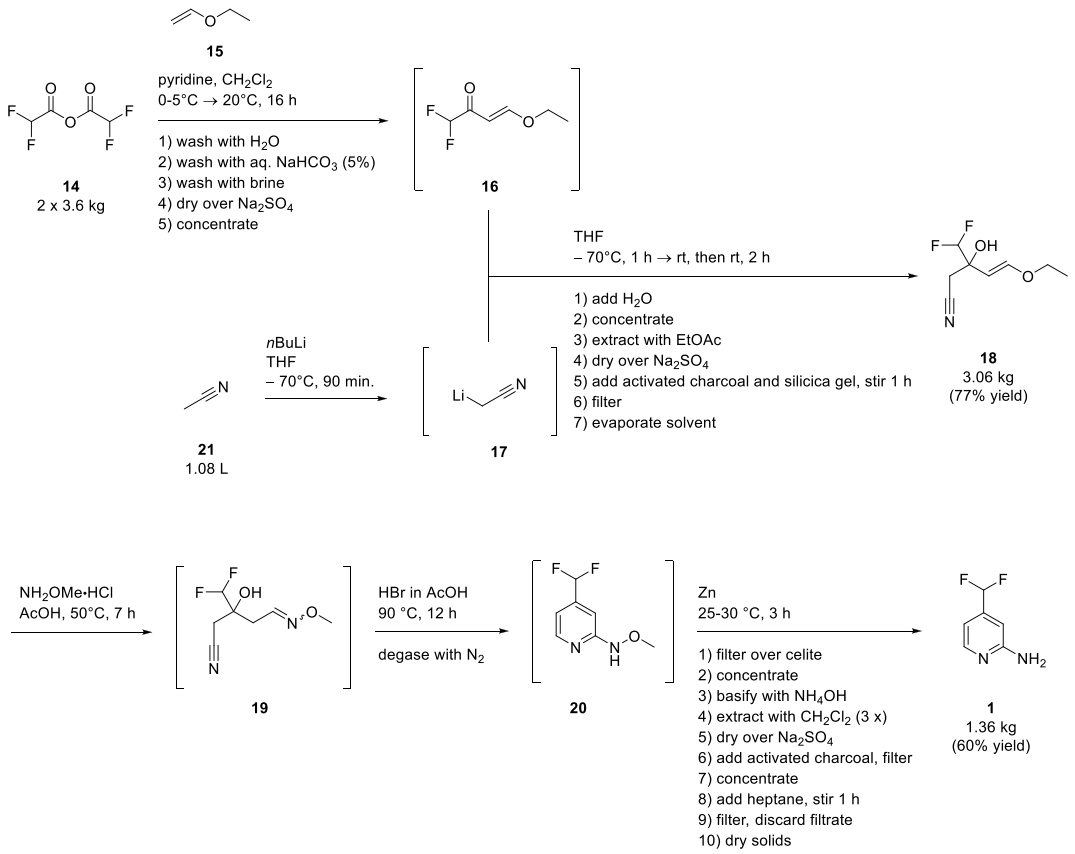
Scheme 5. Kilogram-Scale Process of 4-(Difluoromethyl)pyridin-2-amine (1) via Two One-Pot Procedures



cyclization of nitrile **18** into 2-aminopyridine **1**, we obtained 1.36 kg (60% yield). Considering the transformation from commercially available 2,2-difluoroacetic anhydride (**14**), the desired product was obtained using a five-step two-pot procedure in a 46% overall yield.

CONCLUSIONS

In summary, an efficient and practical synthesis for 4-(difluoromethyl)pyridin-2-amine (**1**) has been developed, avoiding the use of potentially dangerous techniques and hazardous or expensive reagents. Altogether, the described improvements have provided a fast and practical access to **1**, a key intermediate in the large-scale production of PI3K and mTOR kinase inhibitors. The novel process based on a five-step two-pot procedure has been validated by preparing a 1.36 kg batch of 4-(difluoromethyl)pyridin-2-amine (**1**) with 46% overall yield. This material allowed large-scale synthesis of 5-[4,6-bis({3-oxa-8-azabicyclo[3.2.1]octan-8-yl})-1,3,5-triazin-2-yl]-4-(difluoromethyl)pyridin-2-amine (**PQR620**),[18] a highly selective mTOR kinase inhibitor with potential application in the treatment of cancer[30] and neurodegenerative diseases,[19] as well as the dual PI3K/mTOR kinase inhibitor (*S*)-4-(difluoromethyl)-5-(4-(3-methylmorpholino)-6-morpholino-1,3,5-triazin-2-yl) pyridin-2-amine (**PQR530**), a preclinical candidate in oncology.[20]

EXPERIMENTAL SECTION

General Information. Reagents were purchased at the highest commercial quality from Sigma-Aldrich or Fluorochem and used without further purification. Solvents were purchased from Acros Organics. Silica gel except otherwise noted was purchased from Merck KGaA (pore size 60 Å, 230−400 mesh particle size). Melting points (mp) were determined on a Stuart SMP20 apparatus and are uncorrected. ^{1}H, ^{19}F, and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. NMR spectra were obtained in deuterated solvents, such as $(CD_3)_2SO$. The chemical shifts (δ values) are reported in parts per million and corrected to the signal of the deuterated solvents: 2.50 ppm (^{1}H NMR) and 39.52 ppm (^{13}C NMR) for $(CD_3)_2SO$. ^{19}F NMR spectra are calibrated relative to $CFCl_3$ (δ = 0 ppm) as external standard. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), dt (doublet of triplets), t (triplet), q (quartet), m (multiplet), and br (broadened). Coupling constants, when given, are reported in Hertz (Hz). High-resolution mass spectroscopy (HRMS) spectra were recorded on a Thermo Fisher Scientific LTQ Orbitrap XL (nanoESI-MS) spectrometer. The chromatographic purity of final compounds was determined by high-performance liquid chromatography (HPLC) analyses on an UltiMate 3000 SD System from Thermo Fisher with a LPG-3400SD pump system, an ACC-3000 autosampler, a column oven, and a DAD-3000 diode array detector. An Acclaim 120 C18 reversed-phase column from Thermo Fisher was used as the stationary phase. Gradient elution (5:95 for 0.2 min, 5:95 → 100:0 over 10 min, 100:0 for 3 min) of the mobile phase consisting of $CH_3CN/MeOH/H_2O_{(10:90)}$ was used at a flow rate of 0.5 mL/min at 40 °C. CHN elemental analysis was performed using a vario MICRO cube (Elementar, Germany).

*(E)-4-Ethoxy-1,1-difluorobut-3-en-2-one (**16**).* To a cooled (−70 °C) solution of ethyl vinyl ether (**15**, 120 mL, 1.25 mol, 1 equiv) and pyridine (123 mL, 1.52 mol, 1.21 equiv) in

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dichloromethane (1 L), a solution of difluoroacetic anhydride (177 mL, 1.50 mol, 1.2 equiv) in dichloromethane (150 mL) was slowly added. Then, the reaction mixture was allowed to warm to room temperature over 15 h. The yellow reaction mixture was transferred into a separating funnel and washed with deionized H_2O (6 × 1.6 L) until the pH of the aqueous layer was neutral. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to afford the desired product **16** as an orange liquid (147.4 g, 78%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 7.93 (d, $^3J_{H,H}$ = 12.6 Hz, 1H), 6.34 (t, $^2J_{H,F}$ = 53.6 Hz, 1H), 5.87 (dt, $^3J_{H,H}$ = 12.6 Hz, J = 1.3 Hz, 1H), 4.14 (q, $^3J_{H,H}$ = 7.1 Hz, 2H), 1.28 (t, $^3J_{H,H}$ = 7.0 Hz, 3H). ^{19}F{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ −127.36 (s, 2F). ^{13}C{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ 186.9 (t, $^2J_{C,F}$ = 23.8 Hz), 167.1, 109.6 (t, $^1J_{C,F}$ = 247.9 Hz), 100.3, 68.6, 14.4. NSI-HRMS (m/z): $[M + H]^+$ calcd for $C_6H_9F_2O_2$, 151.0565; found, 151.0558.

*(E)-3-(Difluoromethyl)-5-ethoxy-3-hydroxy-pent-4-enenitrile (**18**).* To a cooled (−70 °C) solution of *n*-butyllithium (2.5 M in hexanes, 250.8 mL, 0.63 mol, 1 equiv) in tetrahydrofuran (1.06 L), acetonitrile (32.7 mL, 0.63 mol, 1 equiv) was added under the nitrogen atmosphere. The resulting colorless suspension was stirred at −70 °C for 1.5 h. Then, a solution of (*E*)-4-ethoxy-1,1-difluoro-but-3-en-2-one (**16**, 94.1 g, 0.63 mol, 1 equiv) in tetrahydrofuran (160 mL) was added to the suspension over 15 min. During addition, the reaction mixture first turned orange and then dark brown. The mixture was stirred at −70 °C for 1.75 h and was then allowed to warm slowly to room temperature. Deionized H_2O (800 mL) and EtOAc (600 mL) were added. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 1.2 L). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvents were evaporated under reduced pressure. Filtration over silica gel (cyclohexane/EtOAc 6:1) gave the desired product **18** as a reddish oil (87.2 g, 73%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 6.66 (d, $^3J_{H,H}$ = 12.8 Hz, 1H), 6.20 (s, 1H), 5.79 (t, $^2J_{H,F}$ = 55.8 Hz, 1H), 4.76 (d, $^3J_{H,H}$ = 12.8 Hz, 1H), 3.75 (q, $^3J_{H,H}$ = 7.0 Hz, 2H), 2.88 (d, $^2J_{H,H}$ = 16.8 Hz, 1H), 2.81 (d, $^2J_{H,H}$ = 16.7 Hz, 1H), 1.21 (t, $^3J_{H,H}$ = 7.0 Hz, 3H). ^{19}F{^{1}H} NMR (400 Hz, $(CD_3)_2SO$): δ −129.26 (d, $^2J_{F,F}$ = 274.2 Hz, 1F), −130.09 (d, $^2J_{F,F}$ = 274.2 Hz, 1F). ^{13}C{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ 150.3, 117.3, 116.1 (t, $^1J_{C,F}$ = 248.8 Hz), 100.4 (t, $^3J_{C,F}$ = 2.6 Hz), 71.2 (t, $^2J_{C,F}$ = 21.8 Hz), 64.8, 25.0 (t, $^3J_{C,F}$ = 3.1 Hz), 14.5. NSI-HRMS (m/z): $[M + H]^+$ calcd for $C_8H_{12}F_2NO_2$, 192.0831; found, 192.0822.

*3-(Difluoromethyl)-3-hydroxy-5-(methoxyimino)-pentanenitrile (E/Z-**19**).* To a solution of (*E*)-3-(difluoromethyl)-5-ethoxy-3-hydroxy-pent-4-enenitrile (**18**, 9.8 g, 0.051 mol, 1 equiv) in MeOH (5 mL), deionized H_2O (93 mL) and methoxylamine hydrochloride (12.8 g, 0.15 mmol, 3 equiv) were added. The mixture was stirred at 50 °C for 7 h. Then, the mixture was cooled down to room temperature, and EtOAc (120 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 120 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to afford the desired product **19** (∼1:1 mixture of *E*/*Z* stereoisomers) as a yellow oil (9.2 g, 94%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 7.45 (t, $^3J_{H,H}$ = 6.5 Hz, 1H, *E*-**19**), 6.96 (t, $^3J_{H,H}$ = 5.1 Hz, 1H, *Z*-**19**), 6.45 (s, 1H, *Z*-**19**), 6.43 (s, 1H, *E*-**19**), 5.97 (t, $^2J_{H,F}$ = 55.0 Hz, 1H, *Z*-**19**), 5.95 (t, $^2J_{H,F}$ = 54.9 Hz, 1H, *Z*-**19**), 3.81 (s, 1H, *Z*-**19**), 3.75 (s, 1H, *E*-**19**), 2.94−2.80 (m, 2H, *E*/*Z*-**19**), 2.67−2.62 (m, 2H, *Z*-**19**), 2.54−2.49 (m, 2H, *E*-**19**). ^{19}F{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ −130.3 (d, $^2J_{F,F}$ = 280.0 Hz, 1F, *Z*-**19**), −130.3 (d, $^2J_{F,F}$ = 280.5 Hz, 1F, *E*-**19**), −131.3 (d, $^2J_{F,F}$ = 280.5 Hz, 1F, *E*-**19**), −131.3 (d, $^2J_{F,F}$ = 280.0 Hz, 2F, *Z*-**19**). ^{13}C{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ 145.7 (*E*-**19**), 145.1 (*Z*-**19**), 117.6 (*Z*-**19**), 116.9 (*E*-**19**), 116.2 (t, $^1J_{C,F}$ = 248.3 Hz, *Z*-**19**), 116.2 (t, $^1J_{C,F}$ = 248.2 Hz, *E*-**19**), 71.0 (t, $^2J_{C,F}$ = 21.3 Hz, *E*-**19**), 70.4 (t, $^2J_{C,F}$ = 21.3 Hz, *Z*-**19**), 61.3 (*Z*-**19**), 61.0 (*E*-**19**), 33.9 (*E*-**19**), 30.1 (*Z*-**19**), 23.4−23.2 (m, *E*/*Z*-**19**). NSI-HRMS (m/z): $[M + H]^+$ calcd for $C_7H_{11}F_2N_2O_2$, 193.0783; found, 193.0779.

*4-(Difluoromethyl)-N-methoxy-pyridin-2-amine (**20**).* A flask was charged with 3-(difluoromethyl)-3-hydroxy-5-(methoxyimino)pentanenitrile (**19**) (8.8 g, 0.046 mol, 1 equiv) and an aqueous HCl-solution (5%) (115 mL, 0.19 mol, 4.05 equiv). The mixture was stirred at 90 °C for 15 h. Completion of the reaction was confirmed by thin-layer chromatography analysis. The reaction mixture was cooled to 0 °C, and NaOH (2 M, 100 mL) was added until pH ≈ 9−11. A light brownish solid was precipitated. The desired product **20** was collected by filtration as a light brownish solid (4.2 g, 53%). Crystals suitable for X-ray diffraction were obtained by crystallization from dichloromethane layered with *n*-pentane. mp 65 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 9.81 (s, 1H), 8.27 (d, $^3J_{H,H}$ = 5.0 Hz, 1H), 7.03 (t, $^2J_{H,F}$ = 55.3 Hz, 1H), 6.94 (d, $^3J_{H,H}$ = 5.0 Hz, 1H), 6.92 (s, 1H), 3.68 (s, 3H). ^{19}F{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ −114.73 (s, 2F). ^{13}C{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ 161.7, 148.9, 143.7 (t, $^2J_{C,F}$ = 22.9 Hz), 113.7 (t, $^1J_{C,F}$ = 237.6 Hz), 111.7 (t, $^3J_{C,F}$ = 5.3 Hz), 103.1 (t, $^3J_{C,F}$ = 6.8 Hz), 62.7. NSI-HRMS (m/z): $[M + H]^+$ calcd for $C_7H_9F_2N_2O$, 175.0677; found, 175.0674. HPLC: t_R = 5.52 min (99.3% purity).

*4-(Difluoromethyl)pyridin-2-amine (**1**).* A round-bottom flask was charged with 4-(difluoromethyl)-*N*-methoxy-pyridin-2-amine (**20**, 3.97 g, 22.8 mmol, 1 equiv) under the nitrogen atmosphere. Then, degassed acetic acid (40 mL, 10 vol) was added. To the resulting brownish solution, zinc (4.37 g, 68.4 mmol, 3 equiv) was added portionwise, while the temperature of the reaction mixture was maintained at 16−20 °C. The resulting greenish mixture was stirred at room temperature for 3 h and filtered over a short pad of Celite. The filter cake was washed with EtOAc, and the filtrate was concentrated under reduced pressure. The thick yellowish residue was then cooled down with an ice/water bath to 16−20 °C, and ammonium hydroxide (33% in H_2O, 40 mL) was slowly added under strong stirring. Precipitation of a brownish solid was observed. The mixture was extracted with dichloromethane (3 × 70 mL), the combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. Solvent switch from dichloromethane (30 mL) to heptanes (30 mL) was performed, and the flask was kept for 15 h at 4 °C. The desired product **1** was collected by filtration as a light brownish solid (2.79 g, 85%). mp 96 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.02 (d, $^3J_{H,H}$ = 5.2 Hz, 1H), 6.89 (t, $^2J_{H,F}$ = 55.6 Hz, 1H), 6.57 (d, $^3J_{H,H}$ = 5.3 Hz, 1H), 6.55 (s, 1H), 6.25 (br s, 2H). ^{19}F{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ −114.96 (s, 2F). ^{13}C{^{1}H} NMR (400 MHz, $(CD_3)_2SO$): δ 160.1, 148.9, 142.7 (t, $^2J_{C,F}$ = 22.5 Hz), 113.9 (t, $^1J_{C,F}$ = 237.3 Hz), 107.5 (t, $^3J_{C,F}$ = 5.5 Hz), 104.1 (t, $^3J_{C,F}$ = 6.8 Hz). NSI-HRMS (m/z): $[M + H]^+$ calcd for $C_6H_7F_2N_2$, 145.0572; found, 145.0567. HPLC: t_R = 4.31 min (97.9% purity). Anal. Calcd for

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$C_6H_6F_2N_2$: C, 50.00; H, 4.20; N, 19.44. Found: C, 49.61; H, 4.34; N, 19.89.

*One-Pot Synthesis of 4-(Difluoromethyl)pyridin-2-amine (**1**) from Nitrile **18**.* To a solution of (*E*)-3-(difluoromethyl)-5-ethoxy-3-hydroxy-pent-4-enenitrile (**18**, 50 g, 0.26 mol, 1 equiv) in acetic acid (500 mL), methoxylamine hydrochloride (66 g, 0.78 mol, 3 equiv) was added, and the resulting yellowish reaction mixture was stirred at 50 °C for 7 h. Then, the mixture was cooled down to room temperature, and HBr in acetic acid (33%, 89 mL, 0.51 mol, 1.9 equiv) was added. The resulting reaction mixture was stirred at 90 °C for 15 h. The dark orange reaction mixture was allowed to cool down to room temperature, degassed, and placed under the nitrogen atmosphere. Zinc (50.2 g, 0.78 mol, 3 equiv) was added portionwise over 15 min at room temperature (the temperature was maintained with an ice/water bath during addition), and the resulting greenish reaction mixture was stirred at room temperature for 3 h. Then, the mixture was filtered over a short pad of Celite, the filter cake was washed with EtOAc, and the mixture was concentrated under reduced pressure. The thick yellow residue was then cooled down in an ice/water bath to 16−20 °C, and ammonium hydroxide (33% in H_2O, 370 mL) was slowly added under vigorous stirring. Precipitation of a colorless solid was observed. The mixture was extracted with dichloromethane (3 × 900 mL), the combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. Solvent switch from dichloromethane (300 mL) to heptane (250 mL) by rotatory evaporation of dichloromethane at 500 mbar was operated, and the flask was kept for 15 h at 4 °C. The desired product **1** was obtained by filtration as a light brownish solid (27.2 g, 72%). The spectroscopic data are consistent with the product isolated from the procedure reported above.

*Large-Scale Synthesis of (E)-3-(Difluoromethyl)-5-ethoxy-3-hydroxy-pent-4-enenitrile (**18**) from Anhydride (**14**).* Step 1. To a cooled (0−5 °C) solution of pyridine (1.97 kg, 25.0 mol, 1.2 equiv) in dichloromethane (15 L), ethyl vinyl ether (1.5 kg, 20.8 mol, 1 equiv) was added, followed by a solution of difluoroacetic anhydride (3.6 kg, 20.8 mol, 1 equiv) in dichloromethane (2 L). The mixture was slowly warmed up to 20 °C and stirred at this temperature for 16 h. The reaction mixture was washed with deionized H_2O (2 × 15 L) (pH of the aqueous layer: 4−5). The organic layer was then washed with a 5% aqueous $NaHCO_3$ solution (15 L) (pH of the aqueous layer: 7−8) and with brine (15 L). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure below 30 °C to around 3−3.5 vol stage (∼4.8 L). Step 2. To a cooled (−70 °C) solution of *n*-butyllithium (2.5 M in hexanes, 8.3 L, 20.8 mol, 1 equiv) in tetrahydrofuran (15 L) was added acetonitrile (1.08 L, 20.8 mol, 1 equiv). A colorless suspension was formed and stirred at −70 °C for 90 min. The solution of (*E*)-4-ethoxy-1,1-difluorobut-3-en-2-one (4.8 L stage from the previous step, 20.8 mol, 1 equiv) in tetrahydrofuran (1.5 L) was added to the colorless suspension. The reaction mixture turned orange. The resulting mixture was stirred at −70 °C for 1 h, slowly warmed to room temperature, and then stirred for 2 h. The above procedure was conducted in two separate batches. These two batches were then combined for the work-up. The reaction mixture was cooled to 0 °C, quenched with deionized H_2O (15 L), and concentrated under reduced pressure 40 °C. EtOAc (15 L) was added, and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 15 L), and the combined organic layers were dried over anhydrous Na_2SO_4. Activated charcoal (0.2 w/w) and silica gel (0.4 w/w, 60−120 mesh) were added, and the mixture was stirred for 1 h before filtration through Celite. The Celite bed was washed with EtOAc (4.5 L). The filtrate was concentrated under reduced pressure below 55 °C to afford the desired product **18** as a brownish liquid (3.06 kg, 77%). The spectroscopic data are consistent with the product isolated from the procedure reported above.

*Large-Scale Synthesis of 4-(Difluoromethyl)pyridin-2-amine (**1**) from Nitrile **18**.* To a solution of (*E*)-3-(difluoromethyl)-5-ethoxy-3-hydroxy-pent-4-enenitrile (**18**, 3.0 kg, 15.7 mol, 1 equiv) in acetic acid (30 L), *O*-methoxylamine hydrochloride (3.9 kg, 47.1 mol, 3 equiv) was added. The reaction mixture was stirred at 50 °C for 7 h. Then, it was cooled to 25 °C, and hydrobromic acid in acetic acid (33%, 5.5 L, 31.4 mol, 2 equiv) was added. After addition, the resulting reaction mixture was stirred at 90 °C for 12 h. The mixture was degassed with nitrogen and cooled to 20 °C, and zinc (2.42 kg, 47.1 mol, 3 equiv) was added. The mixture was stirred at 25−30 °C for 3 h before it was filtered over a Celite bed. The Celite bed was washed with EtOAc (15 L) and acetic acid (6 L). The filtrate was concentrated to around 3 vol stage and basified with aqueous NH_4OH solution (25%). The aqueous layer was extracted with dichloromethane (3 × 30 L). The combined organic layers were dried over Na_2SO_4, treated with activated charcoal (0.1 w/w), and filtered through Celite. The Celite bed was washed with dichloromethane. The filtrate was concentrated under reduced pressure, and heptane (12 L) was added. The resulting mixture was stirred at 25−30 °C for 1 h. The suspension was then filtered, and the solid was dried under vacuum. The desired product **1** was obtained as a pale brownish solid (1.36 kg, 60%). The spectroscopic data are consistent with the product isolated from the procedure reported above.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.oprd.9b00312.

> Optimization of cyclization and varying amine equivalents; optimization of reaction conditions for the reduction of compound **20**; optimization of reaction conditions for the one-pot procedure to compound **20**; optimization of reaction conditions for the one-pot procedure to compound **1**; crystal data for **20**; ^{1}H NMR spectra; ^{19}F{1H} NMR spectra; ^{13}C{1H} NMR spectra; NSI-HRMS spectra; and HPLC chromatograms (PDF)

Accession Codes

Crystallographic data for **20** have been deposited with the Cambridge Crystallographic Data Center (ccdc.cam.ac.uk) under deposition number 1939418.

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Notes

The authors declare the following competing financial interest(s): ADA, FB and PHe are past employees of PIQUR Therapeutics AG, Basel; and PHe and MPW are shareholders of PIQUR Therapeutics AG.

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■ ABBREVIATIONS

DCM, dichloromethane; FKBP12, FK506 binding protein 12; mTOR, mechanistical (or mammalian) target of rapamycin; TORC1, mTOR complex 1; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; Rheb, ras homolog enriched in brain; SAR, structure−activity relationship; DAST, (diethylamino)sulfur trifluoride; PyBroP, bromo-tris-pyrrolidino-phosphonium hexafluorophosphate

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