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Catalyst-Free and Scalable Process for Synthesis of Novel MAP4K4 Inhibitor DMX-5804 and Its Glyco-Conjugates

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ABSTRACT: DMX-5804 is a potent and selective mitogen-activated protein kinase kinase kinase kinase-4 (MAP4K4) inhibitor, which is currently under evaluation for the treatment of myocardial infarction. Here, we report the process development of scalable and practical synthesis of DMX-5804. Process optimization resulted in the following: (1) removal of transition metals from the process and reduced duration of reactions, (2) a streamlined process with significantly improved yields using low-cost raw materials, (3) importantly, microwave-assisted reactions were removed, (4) column purification avoided throughout the process, and (5) crystallized products showed over 95% purity in each step.

KEYWORDS: *process development, MAP4K4 inhibitor, DMX-5804, glyco-conjugates*

INTRODUCTION

Mitogen-activated protein kinase kinase kinase kinase-4 (MAP4K4), also known as HGK (hematopoietic progenitor kinase/germinal center kinase-like kinase) or NIK (Nck interacting kinase, the mouse ortholog), is a serine/threonine kinase. It plays an essential role in signal transduction by modulating gene transcription in the nucleus in response to changes in the cellular environment. It belongs to the mammalian family of Ste20 protein kinases, with a molecular target of TGFβ-activated kinase-1 (TAK1 or MAP3K7) which is a key activator of c-Jun N-terminal kinase (JNK) pathway. Recent work on MAP4K4 has projected it as a novel and promising drug development target in cancer.[1,2] Because of its relatively high expression in the brain and the crucial role of downstream p38 MAPK and JNK signaling in apoptosis, a few studies have also reported the beneficial role of MAP4K4 inhibition in neuronal recovery post-injury.[3,4] Our interest in MAP4K4 signaling originates from its recently reported participation in myocardial injury.[5] Myocardial MAP4K4 is activated in end-stage heart failure regardless of cause, that is, dilation, hypertrophism, ischemia, and anthracycline-induced cardiomyopathy.[6] Moreover, MAP4K4 also plays a defining role in pathologies that are precursors to myocardial infarction; for example, MAP4K4 expression and/or activity is increased in the aortas of mice and humans with atherosclerosis, and reduction of endothelial MAP4K4 expression ameliorated atherosclerotic lesion development and inflammatory signaling in a mouse model.[7]

Given that MAP4K4 was only recently recognized for its regulatory role in myocardial injury and tissue recovery, research on the development of its small-molecule inhibitors is relatively nascent. Researchers from Imperial College of London (ICL, London, UK) demonstrated that oxidative stress-induced cardiomyocyte death requires MAP4K4 activation and its pharmacologic inhibition by a novel molecule DMX-5804 reduces cardiomyocyte death both *in vitro* and *in vivo*.[8] Using human stem cell-derived cardiomyocytes as a platform for target validation and compound development, the ICL group identified two potent inhibitors of MAP4K4 (Figure 1) viz.,

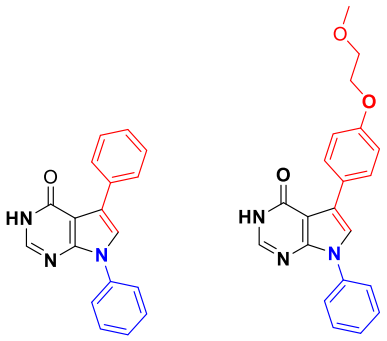

Figure 1. Selective small-molecule inhibitors of MAP4K4.

5,7-diphenyl-3,7-dihydro-4*H*-pyrrolo[2,3-d]pyrimidin-4-one (F1386-0303, IC_{50} 34 nm) and 5-(4-(2-methoxyethoxy)-phenyl)-7-phenyl-3,4a,7,7a-tetrahydro-4*H*-pyrrolo[2,3-d]-pyrimidin-4-one (DMX-5804, IC_{50} 3 nm), the latter showing significant reduction of infarct size in a mouse model.[8] DMX-5804 also exhibited better bioavailability *in vivo* as compared to F1386-0303.

The discovery synthesis of DMX-5804 reported by the ICL group was straightforward in terms of bond construction

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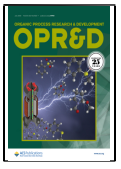
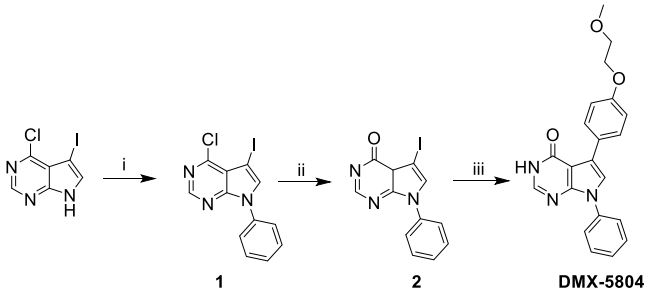


strategy.[8] However, there is significant room for improvement in reaction conditions and process optimization to enable large-scale production. Gram to kilogram quantities of this new chemical entity will be needed for pre-clinical and clinical studies. In this article, we describe a process development that resulted in an easy, practical, and scalable procedure for the synthesis of crystalline DMX-5804 in excellent yields. Compared to the discovery synthesis method, the reported method eliminates the need for transition-metal catalysts, anhydrous solvents, commercially unavailable borate intermediate, and the microwave-assisted reaction step. Moreover, our method is not dependent on column purification of intermediates and high-performance liquid chromatography (HPLC) purification of the final product which are required steps in the discovery method. The new method affords quantitative yields of DMX-5804 in a reproducible manner and provides an easy three-step route to create a novel library of compounds of the MAP4K4 inhibitor class.

RESULTS AND DISCUSSION

Discovery Synthetic Route to DMX-5804.[8] Synthesis of DMX-5804 *via* the discovery route is shown in Scheme 1. The researchers used *Chan*−*Lam*-like amination with excessive copper(II) acetate monohydrate and 2-phenyl-1, 3,2-dioxoborinone (commercially not available and no synthetic scheme reported) at 60 °C for 24 h. Following the required work-up, the crude compound was purified by reverse-phase preparative HPLC-MS to afford 4-chloro-5-iodo-7-phenyl-7*H*-pyrrolo[2,3-d]pyrimidine (**1**) in 33% yield.[8] The chloropyrimidine derivative was converted to 5-iodo-7-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-d]pyrimidin-4-one (**2**) by refluxing in acetic acid in the presence of sodium acetate for 15 h, reportedly with 97% yield. Compound **2** was coupled with 2-(4-(2-methoxyethoxy)-phenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolan by using microwave-assisted *Suzuki*−*Miyaura* cross-coupling reaction at 120 °C for 3 h in the presence of $Pd(dppf)Cl_2$. The resultant DMX-5804 was reported at an 18% yield. The overall yield of this discovery method was reported as 22%. Importantly, all stages required purification by preparative HPLC.

Scheme 1. Discovery Route for Synthesis of DMX-5804[a]

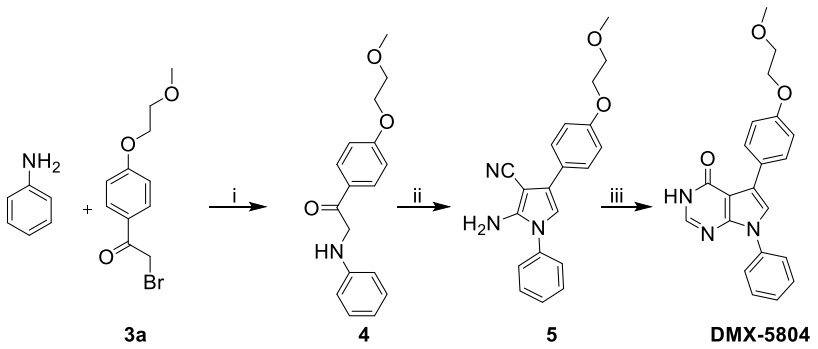


[a]Reagents and conditions: (i) phenylboronic acid, cupric acetate, dimethylformamide, 60 °C, 15−33%; (ii) Na-acetate, acetic acid, 100 °C, 15 h; (iii) 2-(4-(2-methoxyethoxy)phenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolan, $Pd(dppf)Cl_2$, potassium carbonate, microwave reactor, 120 °C, 18% yield.

In our lab, we tried to reproduce this route to synthesize DMX-5804; however, we faced several obstacles in the process. First, we could not find any commercial source for 2-phenyl-1, 3,2-dioxoborinone, and no reference was available to make this intermediate. To overcome this issue, we performed the stage 1 reaction with phenylboronic acid as a substitute for phenyl dioxoborinone and used copper(II) acetate monohydrate in DMF at 60 °C. However, this strategy yielded only trace amounts of the product even after 24 h. Arnold *et al.* reported the same reaction for the synthesis of **1** using phenylboronic acid in dichloromethane, but the reaction duration was 12 d at room temperature, which is time-consuming and not meant for commercial production purposes.[9] With trace amounts of **1** in hand, we proceeded further to synthesize **2** using the conditions reported in the discovery method. This reaction proceeded smoothly and afforded excellent yields of **2** (>90% yield). However, the microwave reaction in stage iii (Scheme 1) to synthesize DMX-5804 was again challenging. We observed significant charring, and the desired compound was in trace amounts (<5%). We tried this reaction without microwave assistance, but no product formation occurred. It is noteworthy that we used a conventionally available kitchen microwave for this reaction, which might have significant bearing on the way the reaction proceeded. Regardless, the reaction appears to require substantial energy input to drive this reaction forward. Based on these experiences, we decided to establish a *de novo* synthetic route for DMX-5804.

***De Novo* Synthesis of DMX-5804.** The discovery synthetic route is capable of supplying medicinal chemists with quantities of DMX-5804 sufficient for initial *in vitro* and *in vivo* studies; however, it is apparent that this route needs significant modifications to obtain meaningful amounts of DMX-5804 in a reproducible manner. We identified the following drawbacks of the discovery method, which make it very challenging, if not impossible, to translate to a commercial scale: (a) the

Scheme 2. New Strategy for Synthesis of DMX-5804[a]



[a]Reagents and conditions: (i) $NaHCO_3$, DMF, 50 °C (ii) malononitrile, KOH, methanol reflux; (iii) 95% formic acid, reflux.

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requirement of transition-metal catalysts, (b) high cost of boronate intermediate, (c) microwave-assisted reaction, (d) need of preparative HPLC for purification of intermediates and the final product, (e) poor yields with reproducibility challenges in cross-coupling reactions, and (f) safety concerns in discarding metallic complexes.

Because of the high cost and inaccessibility of boronates in stage 1, we sought to start synthesis from readily available and inexpensive starting materials. As shown in Scheme 2, we identified substituted 2-bromo-1-(4-(2-methoxyethoxy)-phenyl)ethan-1-one (**3a**) as a suitable starting material for DMX-5804. It was reacted with aniline in DMF at room temperature in the presence of a mild base for 3 h to produce 1-(4-(2-methoxyethoxy)phenyl)-2-(phenylamino)ethan-1-one (**4**) in quantitative yields (90%). The product was recrystallized in isopropyl alcohol. In the next step, a pyrrole derivative (**5**) was synthesized *via* Knoevenagel condensation of malononitrile. We screened diisopropylethylamine, trimethylamine, KOH, and potassium-*tert*-butoxide as bases for this reaction. The reaction with KOH provided >95% of **5**, with 80−86% isolated yield; the other bases yielded approximately 70% conversion.

With 2-amino-pyrrole-3-carbonitrile derivative (**5**) in hand, we turned our focus to the assembly of the pyrimidine ring in the structure of DMX-5804. Compound **5** was refluxed in 95% formic acid for 8−10 h. After cooling, the reaction mixture was poured in ice-water to precipitate the crude compound. The crude compound was dissolved in a mixture of chloroform/methanol (1:5), decolorized with activated charcoal, and recrystallized from chloroform/methanol mixture to obtain DMX-5804 with 99% purity as analyzed by HPLC (Figure 2). It

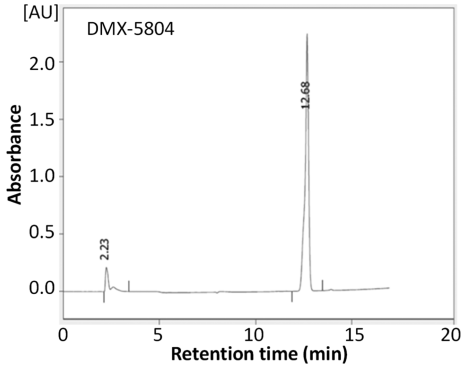

Figure 2. HPLC profile of DMX-5804.

is noteworthy that the reaction mixture and conditions did not impact the stability of DMX-5804. This observation is in contrast to the discovery method where significant charring and loss of the final product were found.

General New Route for Synthesis of DMX-5804 and Its Analogues. After obtaining reproducible yields of DMX-5804 from Scheme 2 reaction, we focused our attention on generalizing this scheme to synthesize novel analogues of DMX-5804. We modified O-alkoxy functionality because modification in any other place in the structure of DMX-5804 has been reported detrimental to the purported MAP4K4 inhibitory activity. Here, we replaced compound **3a** (of Scheme 2) with 2-bromo-1-(4-methoxyphenyl)ethan-1-one (**3b** in Scheme 3) to obtain a compound with a phenolic functional group (**6**) which is hypothesized to serve as an intermediate for the synthesis of DMX-5804 or its analogues (Scheme 3).

First, we attempted base-catalyzed O-alkylation in a variety of conditions (Table 1). Initially, we performed the reaction in acetonitrile using potassium carbonate at 60 °C for 12 h, which resulted in only trace amounts of DMX-5804 but approximately 20% compound **7** (pyrimidine O-alkylation) and unreacted starting material. We also screened various solvents combined with different bases to get DMX-5804 but failed. The reaction involving potassium carbonate in DMF resulted in the quantitative consumption of the starting material. However, DMX-5804 was obtained only in small amounts (5−7%), whereas compound **7** was isolated as the major product (88%). To check the stability of compound **7**, we refluxed it in dioxane−HCl for 6 h. Compound **7** was stable in acidic conditions, and its solubility in polar solvents was significantly better than that of DMX-5804. Also, its HPLC retention time was identical to that of DMX-5804 (data not shown).

In order to shift alkylation to the desired phenolic site, we changed the reaction conditions to acidic by using boron trifluoride diethyl etherate as a Lewis acid (entries 9−11 of Table 1). We found that in the presence of boron trifluoride diethyl etherate (BF_3OEt_2) in dichloromethane, the reaction resulted in phenolic alkylation (entry 9). Hypothesizing that the relatively low separated yields of this reaction are due to the insolubility of phenolic derivative in dichloromethane, we decided to perform this reaction in neat conditions with BF_3OEt_2. This modification produced satisfactory yields of DMX-5804 (entry 10). Furthermore, we added an excess of methyl glycol as a solvent as well as a reactant in the presence of BF_3OEt_2 to obtain quantitative yields of DMX-5804 in good time (entry 11). It is worth mentioning that in these acidic conditions, the other regio-isomer (pyrimidine alkylated compound **7**) was completely absent. In addition to the discovery compound DMX-5804, we employed this route for the synthesis of ribose- and glucose-conjugated analogues. These sugar analogues were particularly interesting because of their increased solubility in an aqueous medium compared to virtually insoluble DMX-5804. More work is needed to study the mechanistic basis of preferential phenolic alkylation in the presence of BF_3OEt_2. Previously, Coyle *et al.*, have reported the use of BF_3OEt_2 for phenolic alkylation.[10] It has been recently reported that BF_3OEt_2 promotes the formation of carbocation with aliphatic −OH groups by coordination of the oxygen atom, followed by the transfer of the alkyl moiety.[11,12] Thus, a possibility of activation of aliphatic −OH in methoxyethanol by BF_3OEt_2 exists. Simultaneously, Lewis acid BF_3OEt_2 can also deactivate the pyrimidinone ring by preventing keto→enol conversion, secondary to its interaction with −NH group.

MAP4K4 Inhibition. We submitted DMX-5804, ribose-analogue **9**, and regio-isomer **7** to examine their MAP4K4 inhibitory activity in a cell-free assay using recombinant MAP4K4 enzyme and myelin basic protein (MBP) as its substrate. The results of the assay are given in Figure 3. We found that the concentration for 50% inhibition of MAP4K4 (IC_{50}) of DMX-5804 was 205.9 nM. Compound **7**, where the alkoxy group was O-conjugated in pyrimidine ring, showed no inhibitory activity in the concentration range tested. However, ribose-analogue **9** showed inhibitory activity (IC_{50} = 255.8 nM) comparable to that of DMX-5804. These data demonstrate that it is possible to modify the phenolic group for targeting purposes and/or to alter the pharmacokinetic and pharmacodynamic profiles of this class.

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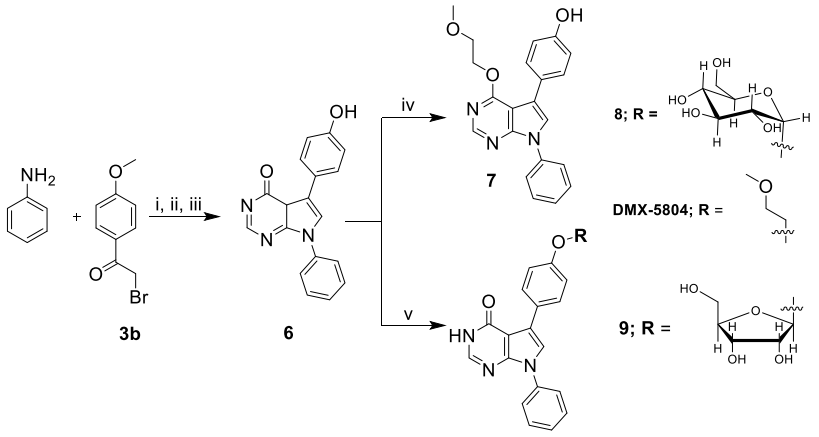
Scheme 3. General Scheme for Synthesis of DMX-5804 Analogues[a]



[a]Reagents and conditions: reagents and conditions: (i) $NaHCO_3$, DMF, 50 °C (ii) malononitrile, KOH, MeOH reflux; (iii) 95% formic acid, reflux; boron tribromide in dichloromethane (iv) base, 1-bromo-2-methoxyethane, appropriate solvent; (v) BF_3OEt_2, 2-methoxyethan-1-ol, at 60−70 °C.

Table 1. Reaction Conditions for Base-Catalyzed and Acid-Catalyzed Synthesis of DMX-5804

entry[a]	solvent	base/acid	time (h)	temp (°C)	DMX-5804 (%)	7 (%)
1	acetonitrile	K_2CO_3	12	reflux	trace	20
2	acetone	K_2CO_3	15	reflux	Trace	<10
3	DMF	K_2CO_3	12	80	5−7	88
4	DMF	$CsCO_3$	12	80	10	70
5	DMSO	K_2CO_3	15	60	trace	55
6	DMF	TEA	15	80	trace	60
7	THF	NaH	5	10	trace	20
8	DMF	NaH	8	10	trace	15
9	DCM	BF_3OEt_2	10−12	40	25−30	
10		BF_3OEt_2	10−12	60	60−70	
11	methyl glycol	BF_3OEt_2	6−8	70−80	80−85	

[a]For entries 1−8, 1-bromo-2-methoxyethane was used as a reactant; for entries 9−10, 2-methoxyethan-1-ol was used as a reactant (Scheme 3).

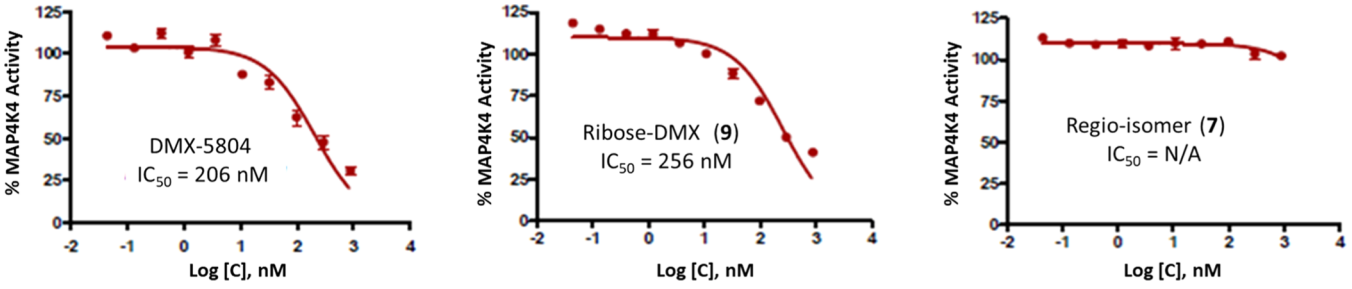


Figure 3. Biological MAP4K4 inhibitory activity of DMX-5804, compound **7**, and compound **9**.

CONCLUSIONS

The new route described above (Scheme 2) to synthesize DMX-5804 was effectively used for a large-scale manufacturing process which generated API grade (>99.99 by HPLC area %) in excellent (80−85%) yield. In the optimized route, 1-(4-(2-methoxyethoxy)phenyl)-2-(phenylamino)ethan-1-one (**1**) eliminated the need of using a transition-metal catalyst and expensive borates. In addition, this intermediate made it easy to access various DMX-5804 analogues. In comparison, the initial discovery route generated DMX-5804 in only a 22% yield. It is worth noting that this three-step sequence did not require purification steps using column chromatography. The general scheme reported here takes 6−8 h to complete and also eliminates the use of anhydrous solvents and the microwave-assisted reaction step, which is very conducive to commercial-scale synthesis of DMX-5804. A potential limitation of this method is the sequential use of both acidic and basic reaction steps, which makes it difficult to choose an appropriate protection strategy for the synthesis of certain analogues. However, this limitation does not affect the synthesis of DMX-5804.

EXPERIMENTAL SECTION

General Information. All reagents and solvents were purchased from commercial suppliers and used without further purification. ^1H and ^{13}C NMR spectra were recorded on a Varian spectrometer in suitable deuterated solvents. The solvents and measurement frequency used are indicated for

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each experiment. The signal of the residual deuterated solvent relative to tetramethylsilane was used as the internal reference. All spectra are reported in supplemental data as follows: chemical shift δ in parts per million (multiplicity, coupling constant J in hertz, and number of protons). The resonance multiplicity is described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), doublet of triplets (dt), or triplet of doublets (td). Product purity was determined by HPLC. For reaction monitoring, analytical thin-layer chromatography (TLC) was performed on Merck Silica gel 60 F254 strips and visualization was accomplished by irradiation with UV light (254 or 366 nm).

HPLC. HPLC was performed using an Azura P 6.1L HPLC system (Knauer, Berlin, Germany) with a UV-1 detector set at λ = 254 nm. The samples were separated on a Sonoma C18, 10 μm, 4.6 × 250 mm using water-acetonitrile gradient 5−95% at 1.5 mL/min.

*2-Bromo-1-(4-methoxyphenyl)ethan-1-one (**3**).* To a solution of 4-hydroxyacetophenone (25 g) in dimethylformamide, potassium carbonate (38 g) and 1-bromo-2-methoxyethane (30 g) were added. The reaction mixture was heated to 90 °C for 12−20 h and monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature, poured into ice-water, and extracted with ethyl acetate. The organic extract was dried and evaporated. The residue was purified by flash column using hexanes/ethyl acetate (1:9). The obtained 1-(4-(2-methoxyethoxy)phenyl)ethan-1-one (71%) intermediate was brominated in diethyl ether to get **3** (90%). The conditions of this bromination reaction using bromine have been previously reported.[13]

*1-(4-(2-Methoxyethoxy)phenyl)-2-(phenylamino)ethan-1-one (**4**).* A round-bottom flask fitted with a magnetic stir bar was charged with aniline (1.07 mol), sodium bicarbonate (1.28 mol), and *N*,*N*′-dimethylformamide (DMF, 300 mL). The slurry was stirred while adding 2-bromo-1-(4-(2-methoxyethoxy)phenyl)ethan-1-one (1.18 mol, dissolved in DMF) dropwise. The mixture was heated to 50 °C and stirred for 5 h. The reaction mixture was cooled to room temperature and poured on to ice-water to obtain a precipitate which was filtered and recrystallized from isopropyl alcohol as a light yellow solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.7 Hz, 2H), 7.23 (m, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 7.7 Hz, 3H), 4.57 (s, 2H), 4.20 (d, J = 4.5 Hz, 2H), 3.78 (d, J = 4.5 Hz, 2H), and 3.46 (s, 3H).

*2-Amino-4-(4-(2-methoxyethoxy)phenyl)-1-phenyl-1H-pyrrole-3-carbonitrile (**5**).* A round-bottom flask fitted with a magnetic stir bar was charged with **4** (0.31 mol), potassium hydroxide (0.94 mol) dissolved in water (50 mL), malononitrile (0.34 mol), and methanol (450 mL). The mixture was heated to 80 °C for 4 h. Afterward, the reaction mixture was cooled and evaporated to 200 mL. The precipitate was filtered and recrystallized in cold isopropyl alcohol to obtain 2-amino-4-(4-(2-methoxyethoxy)phenyl)-1-phenyl-1*H*-pyrrole-3-carbonitrile **5** as a brown solid (88% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (m, 4H), 7.41 (m, 4H), 6.95 (d, J = 8.7 Hz, 2H), 4.14 (d, J = 4.6 Hz, 2H), 3.76 (d, J = 4.6 Hz, 2H), and 3.45 (s, 3H).

5-(4-(2-Methoxyethoxy)phenyl)-7-phenyl-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (DMX-5804). Compound **5** (0.22 mol) was dissolved in 85% formic acid (200 mL) and refluxed for 8−10 h. After cooling to room temperature, the mixture was poured onto ice-water to obtain a precipitate. The precipitate was filtered, dried, and recrystallized from dichloromethane/ethanol (1:2) to afford DMX-5804 as a white solid (80−85% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.30 (s, 1H), 7.94 (s, 1H), 7.79 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.53 (t, J = 7.8 Hz, 2H), 7.41 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 8.6 Hz, 2H), 4.17 (t, J = 4.7 Hz, 2H), 3.77 (t, J = 4.7 Hz, 2H), and 3.46 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.74, 158.09, 148.33, 143.07, 137.26, 129.79, 129.44, 127.67, 125.73, 124.80, 121.78, 121.41, 114.59, 106.35, 71.07, 67.30, and 59.20; mp 158−160 °C;

*5-(4-(2-Hydroxy)phenyl)-7-phenyl-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (**6**).* To a cold (−30 °C) solution of 5-(4-methoxyphenyl)-7-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-d]pyrimidin-4-one (300 mg) in dichloromethane was added BBr_3 (Scheme 3). The temperature of the reaction mixture was raised to room temperature by stirring for 1 h. The solvent was evaporated and to the residue was added saturated ice-cold $NaHCO_3$ solution. The precipitate was filtered to obtain the title compound **6** (286 mg, 100% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H), 9.34 (s, 1H), 7.92 (d, J = 2.99 Hz, 1H), 7.77 (d, J = 8.59 Hz, 2H), 7.74 (d, J = 7.55 Hz, 2H), 7.61 (s, 1H), 7.53 (t, J = 7.83 Hz, 2H), 7.39 (t, J = 7.39 Hz, 1H), and 6.75 (d, J = 8.59 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 159.04, 156.62, 148.09, 144.61, 137.72, 130.02, 129.58, 127.43, 124.91, 124.59, 121.53, 120.67, 115.23, and 106.43.

Base-Catalyzed Alkylation. To a solution of compound **6** (25 g) in DMF (150 mL) was added 1-bromo-2-methoxyethane, or any alkyl halide. After stirring at 80−90 °C for 12−15 h, the reaction mixture was cooled to room temperature and poured in crushed ice-water mixture. After stirring for 1 h, the precipitate was collected and recrystallized from DCM/ethanol (1:2).

*4-(4-(2-Methoxyethoxy)-7-phenyl-7H-pyrrolo[2,3-d]-pyrimidin-5-yl)phenol (Regio-Isomer **7**).* A crystalline white solid obtained in 80% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.37 (s, 1H), 8.16 (s, 1H), 7.74 (m, 4H), 7.63 (s, 1H), 7.54 (d, J = 7.9 Hz, 2H), 7.41 (d, J = 7.4 Hz, 1H), 6.76 (d, J = 8.6 Hz, 2H), 4.16 (t, J = 5.7 Hz, 2H), 3.58 (d, J = 5.2 Hz, 2H), and 3.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 158.01, 156.68, 147.78, 147.36, 137.55, 130.16, 129.64, 127.48, 124.82, 124.45, 121.42, 121.18, 115.17, 105.60, 69.83, 58.55, and 45.67; mp 221−224 °C.

Acidic O-Alkylation. To a mixture of compound **6** (25 g) and $BF_3{\cdot}OEt_2$ (50 mL) was added a solution of an appropriate substrate (2-methoxyethan-1-ol, β-D-glucose pentaacetate, or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-beta-D-ribofuranose). In one condition, the solutions of substrate were prepared in dichloromethane, whereas in another condition, the substrates were added solid to the reaction mixture (neat). After stirring at 25 °C for 18 h, the reaction mixture was evaporated. The residue was washed with saturated $NaHCO_3$ (50 mL) and extracted with chloroform (50 mL × 2). The combined organic phase was dried over anhydrous $MgSO_4$, concentrated, and crystallized from ethanol.

*7-Phenyl-5-(4-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (**8**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ (d, J = 3.6 Hz, 1H), 7.95 (d, J = 3.7 Hz, 3H), 7.75 (m, 3H), 7.55 (t, J = 5.28 Hz, 2H), 7.41 (t, J = 7.42 Hz, 1H), 7.04 (dd, J = 2.94 Hz, 2H), 4.88 (d, J = 7.39 Hz, 1H), 3.96 (br s, 5H), 3.70 (dd, J = 5.28 Hz, 1H), 3.47 (dd, J = 5.86 Hz, 1H), 3.34 (m, 1H), 3.26 (m, 2H), and 3.17 (t, J = 3.17 Hz, 1H); mp 149−152 °C.

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*5-(4-((3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)oxy)phenyl)-7-phenyl-3,7-dihydro-4H-pyrrolo[2,3-d]-pyrimidin-4-one (**9**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.11 (d, J = 3.6 Hz, 1H), 7.95 (d, J = 3.7 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.76 (d, J = 7.4 Hz, 2H), 7.71 (s, 1H), 7.54 (t, J = 7.9 Hz, 2H), 7.41 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 8.8 Hz, 2H), 5.48 (s, 1H), 5.31 (s, 1H), 4.98 (s, 1H), 4.69 (s, 1H), 4.02 (s, 2H), 3.89 (m, 1H), 3.55 (dd, J = 5.0 Hz, 1H), and 3.37 (m, 1H); mp 155−158 °C.

MAP4K4 Inhibition Assay. MAP4K4 assay for inhibition concentration (IC_{50}) determination was performed using a kit by BioAssay Systems Services (Hayward, CA). Briefly, MAP4K4 in assay buffer (40 ng in 10 μL) was incubated with varying concentrations of test compounds (5 μL in dimethylsulfoxide) for 15 min at 37 °C. The compounds were titrated from 900 nM (final reaction concentration) in 3× steps. Compound dilutions were made in assay buffer with 0.036% dimethylsulfoxide. Reactions were initiated by adding 5 μL of the reaction mix (2.6 μg/μL MBP, 193 μM ATP; final reaction concentration of 13 μg MBP, 48 μM ATP) and run at 37 °C for 20 min. These kinase reactions were stopped with an ADP detection reagent and fluorescence was measured at $530_{Ex}/590_{Em}$. IC_{50} was computed using GraphPad Prism Nonlinear Sigmoidal Dose-Response fitting.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00130.

^{1}H NMR of **4**, **5**, **6**, **7**, **8**, **9**, and DMX-5804; ^{13}C NMR of **7** and DMX-5804; mass spectra of **7**, **8**, **9**, and DMX-5804; and HPLC profile of compound **6** (PDF)

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Notes

The authors declare no competing financial interest.

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