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An improved synthetic strategy for the multigram-scale synthesis of the PLK1 inhibitor plogosertib

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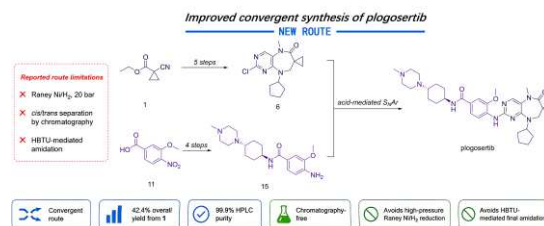
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Abstract

This paper describes the development of a practical and chromatography-free route for the multigram-scale synthesis of plogosertib, a polo-like kinase 1 (PLK1) inhibitor. Compared with the synthetic route disclosed in Cyclacel's patent, the present procedure employed an improved convergent strategy, in which pyrimido-diazepine intermediate **6** and *trans*-configured aminobenzamide intermediate **15** were prepared independently and then coupled via an acid-mediated nucleophilic aromatic substitution reaction. In the pyrimido-diazepine sequence, nitrile reduction was achieved using NaBH₄/CoCl₂·6H₂O under mild conditions, avoiding Raney Ni-catalyzed hydrogenation under pressurized hydrogen. Regioselectivity in the preparation of intermediate **4** was improved by controlling the S_NAr reaction temperature. In the aminobenzamide sequence, the *cis*-isomeric impurity formed during reductive amination was effectively removed by MTBE slurry purification without column chromatography. The final acid-mediated S_NAr reaction also replaced the

coupling-reagent-mediated amidation used in the reported process. Key transformations were systematically optimized with respect to reaction efficiency, selectivity, and operational practicality. The optimized process afforded plogosertib in 42.4% overall yield from compound **1** with 99.9% HPLC purity, demonstrating its suitability for practical multigram-scale preparation.

Graphical abstract



Keywords Plogosertib · PLK1 inhibitor · Multigram-scale synthesis · Process optimization

Introduction

Polo-like kinase 1 (PLK1) is a highly conserved serine/threonine protein kinase at both the structural and functional levels. It participates in multiple cell-cycle events, including mitotic entry, centrosome maturation, spindle assembly, chromosome segregation, and cytokinesis (Archambault and Glover 2009). PLK1 is frequently overexpressed in many cancer types, and its abnormal expression is closely associated with tumor proliferation and poor prognosis (Strebhardt and Ullrich 2006). Therefore, PLK1 has been regarded as an attractive target for anticancer drug discovery (Weiß and Efferth 2012).

In recent years, a number of small-molecule PLK1 inhibitors have been developed. According to their target domains, PLK1 inhibitors are broadly classified into kinase domain (KD)-targeting inhibitors and polo-box domain (PBD)-targeting inhibitors (Xu et al. 2013; Stafford et al. 2023). KD-targeting inhibitors mainly act as ATP-competitive inhibitors by occupying the ATP-binding pocket of PLK1, thereby directly suppressing its catalytic activity. Several ATP-competitive PLK1 inhibitors have advanced into clinical evaluation, including BI 2536 and GSK461364 in phase I studies, volasertib in a phase III study, and onvansertib in phase II studies. These clinical

investigations indicate the continued therapeutic interest in ATP-competitive PLK1 inhibitors as anticancer agents (Zhang et al. 2022; Raab et al. 2024).

Plogosertib (Fig. 1), also known as CYC140, is a highly selective, potent, and orally active ATP-competitive PLK1 inhibitor developed by Cyclacel Pharmaceuticals (Lee et al. 2015a; Zhang et al. 2022). Plogosertib has entered a phase I clinical study in patients with advanced solid tumors and lymphoma (ClinicalTrials.gov 2022), highlighting its potential as a PLK1-targeted anticancer agent. To support further preclinical and clinical development, a reliable and scalable process is required to provide sufficient quantities of high-purity material. However, the disclosed route still leaves room for improvement in the practicality of key transformations and the assembly of key fragments. Therefore, the development of an improved process for plogosertib is of practical importance.

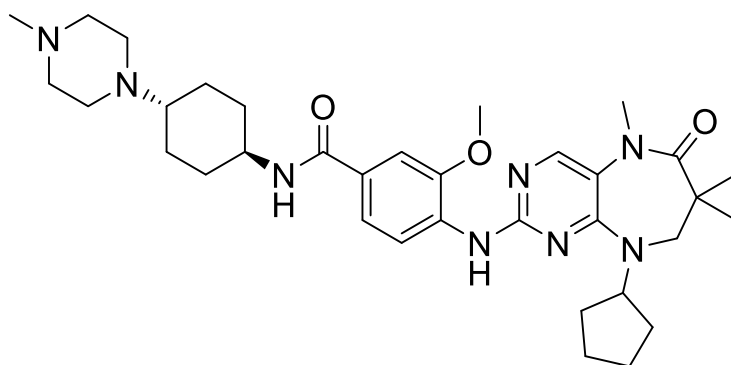
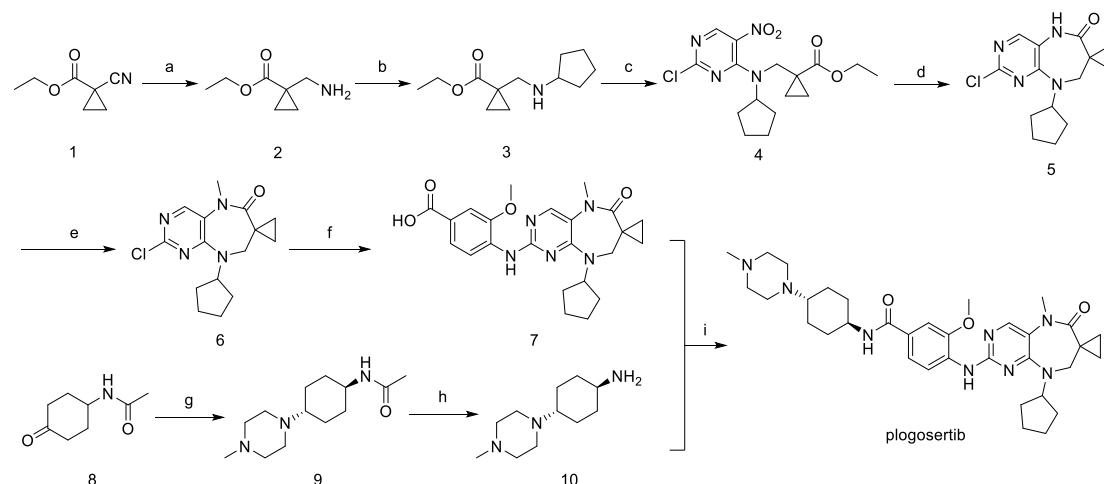


Fig. 1 Structure of the PLK1 inhibitor plogosertib

To date, the only disclosed synthetic route to plogosertib is that reported in Cyclacel's patent WO2021032958A1 (Skead et al. 2021). As shown in Scheme 1, ethyl 1-cyanocyclopropane-1-carboxylate (**1**) was used as the starting material. The pyrimido-diazepine intermediate **7** was prepared through sequential nitrile reduction, reductive amination, nucleophilic substitution, reductive cyclization, *N*-methylation, and nucleophilic substitution. The side-chain intermediate **10** was synthesized from *N*-(4-oxocyclohexyl)acetamide (**8**) through reductive amination followed by acid-mediated *N*-deacetylation. Finally, amide coupling of intermediate **7** with intermediate **10** afforded plogosertib.

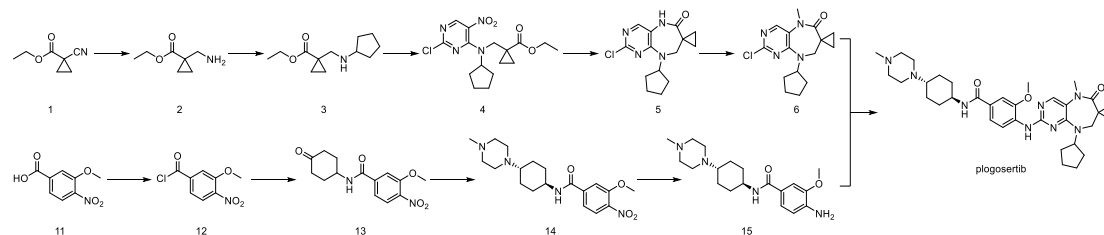


Scheme 1. Reported synthetic route to plogosertib. Reagents and conditions: **a** Raney Ni, H₂, EtOH, 20 bar, rt, overnight, 94%; **b** cyclopentanone, NaBH(OAc)₃, DCM, rt, overnight, 98%; **c** 2,4-dichloro-5-nitropyrimidine, K₂CO₃, acetone, 0–5 °C, 71%; **d** Fe, AcOH, 25–30 °C, overnight, crude; **e** *t*-BuOK, MeI, MeCN, 0–10 °C, overnight, 77.5%; **f** 4-amino-3-methoxybenzoic acid, NMP, 115–125 °C, 36 h, 89.7%; **g** *N*-methylpiperazine, MeSO₃H, toluene, reflux; then NaBH₄, EtOH, 15–20 °C, 25%; **h** conc. HCl, H₂O, 95–105 °C, 17 h, 97.9%; **i** HBTU, DIPEA, DCM, 15–25 °C, 78.8%.

During our preliminary evaluation of the route disclosed in Cyclacel's process patent, several process-related limitations were identified that could hinder further scale-up. ①The preparation of intermediate **2** involved Raney Ni-catalyzed hydrogenation under pressurized hydrogen, which required specialized equipment and strict safety controls for large-scale manufacturing. ②The preparation of side-chain intermediate **10** was inefficient because reductive amination of *N*-(4-oxocyclohexyl)acetamide (**8**) afforded a mixture of *cis/trans* isomers, and purification on basic alumina followed by recrystallization was required to obtain the desired *trans*-isomer. This resulted in a low isolated yield and increased operational complexity. ③The amide coupling of intermediate **7** with intermediate **10** was performed using HBTU/DIPEA in DCM, which increased the reagent cost and complicated the work-up. These limitations made the reported route less attractive for multigram-scale preparation.

To address these limitations, an improved convergent route was designed by changing the sequence of fragment assembly. In this route, the benzamide side chain was constructed before the final C–N bond formation, allowing the *cis*-isomeric

impurity generated during reductive amination to be controlled at an earlier intermediate stage. Accordingly, the Raney Ni-catalyzed hydrogenation was replaced by a Lewis acid-assisted NaBH₄ reduction for the preparation of the aminomethyl intermediate **2**, and the HBTU-mediated final amidation was replaced by a late-stage acid-mediated nucleophilic aromatic substitution reaction. As shown in Scheme 2, the pyrimido-diazepine intermediate **6** was prepared from ethyl 1-cyanocyclopropane-1-carboxylate (**1**) through sequential Lewis acid-assisted nitrile reduction, reductive amination, nucleophilic substitution, reductive cyclization, and *N*-methylation. In parallel, aminobenzamide intermediate **15** was synthesized from 3-methoxy-4-nitrobenzoic acid (**11**) through acid chloride formation, amidation, reductive amination, and nitro reduction. Finally, plogosertib was assembled via acid-mediated nucleophilic aromatic substitution between chloro-substituted intermediate **6** and aminobenzamide intermediate **15**. The key transformations leading to intermediates **2**, **4**, **5**, **6**, **13**, and **14**, as well as the final assembly of plogosertib, were then systematically optimized.



Scheme 2. Improved convergent synthetic route to plogosertib

Results and discussion

Preparation of intermediate **2**

The synthesis of intermediate **2** involved selective reduction of the nitrile group in compound **1** to afford aminomethyl intermediate **2**. In the reported route, this transformation was performed by Raney Ni-catalyzed hydrogenation under pressurized hydrogen. Although the desired amine could be obtained, the use of Raney Ni under pressurized hydrogen raised safety concerns during scale-up. Therefore, alternative reduction systems were investigated to develop a milder and safer process (Table 1).

Initial screening showed that catalytic hydrogenation over Pd/C and reduction with NaBH₄ alone were unsatisfactory, affording intermediate **2** in yields of only 41.1% and

39.8%, respectively (entries 1 and 2). Considering that Lewis acid-assisted NaBH_4 systems have been reported for the reduction of nitriles to primary amines, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ were subsequently evaluated as additives (Khurana and Kukreja 2002). Both metal salts markedly improved the reaction efficiency compared with NaBH_4 alone. In particular, the $\text{NaBH}_4/\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ system afforded intermediate **2** in 83.6% yield within 4 h (entry 7), whereas the corresponding $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ system gave a lower yield of 75.2% after 6 h (entry 3). Therefore, $\text{NaBH}_4/\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ was selected as the preferred reduction system for further optimization.

The effects of reagent loading and solvent were then investigated. Reducing the amount of NaBH_4 from 3.0 to 2.0 equiv. decreased the yield from 83.6% to 69.7% (entries 7 and 4), whereas reducing the amount of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ from 1.0 to 0.5 equiv. decreased the yield to 62.7% (entries 7 and 5). These results indicated that 3.0 equiv. of NaBH_4 and 1.0 equiv. of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ were required for efficient conversion. Solvent screening showed that EtOH afforded intermediate **2** in 75.6% yield, whereas MeOH increased the yield to 83.6% (entries 6 and 7). Accordingly, NaBH_4 (3.0 equiv.) and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (1.0 equiv.) in MeOH were selected as the optimal conditions. The reaction was subsequently performed on a 50.00 g scale of compound **1**, affording 43.01 g of intermediate **2** in 83.6% isolated yield.

Table 1 Optimization of the nitrile reduction of compound **1** to intermediate **2**

Entry	Reduction system	Equiv.	Solvent	Time (h)	Yield (%)
1	H_2 , Pd/C	0.1	MeOH	12	41.1
2	NaBH_4	3.0	MeOH	11	39.8
3	$\text{NaBH}_4/\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	3.0/1.0	MeOH	6	75.2
4	$\text{NaBH}_4/\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	2.0/1.0	MeOH	6	69.7

5	NaBH ₄ /CoCl ₂ ·6H ₂ O	3.0/0.5	MeOH	6	62.7
6	NaBH ₄ /CoCl ₂ ·6H ₂ O	3.0/1.0	EtOH	4	75.6
7	NaBH₄/CoCl₂·6H₂O	3.0/1.0	MeOH	4	83.6

Conditions: compound **1** was used as the substrate; all reactions were performed at room temperature unless otherwise stated. Yields refer to isolated yields of intermediate **2**.

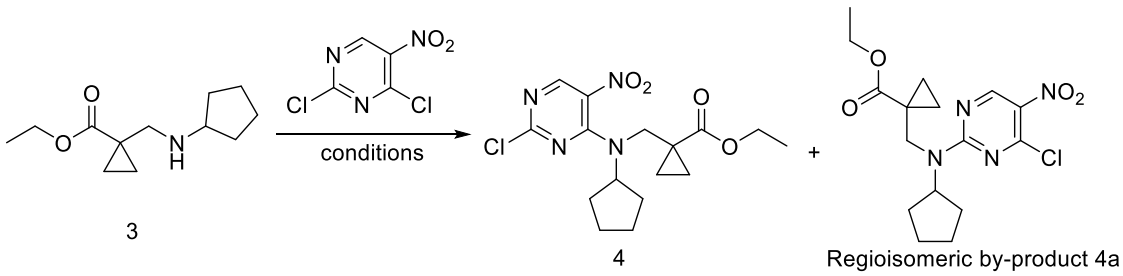
Preparation of intermediate **4**

Intermediate **2** was first converted into secondary amine intermediate **3** by reductive amination with cyclopentanone in the presence of NaBH(OAc)₃, affording intermediate **3** in 93.1% yield. Intermediate **4** was then prepared by nucleophilic aromatic substitution (S_NAr) of 2,4-dichloro-5-nitropyrimidine with intermediate **3**. The electron-withdrawing nitro group at the C-5 position activates the adjacent C-4 position toward nucleophilic substitution (Lee et al. 2015b). However, the C-2-substituted regioisomer **4a** was isolated from the reaction mixture and assigned on the basis of its spectroscopic data. Therefore, the reaction conditions were optimized with the aim of improving the regioselectivity and controlling the formation of this by-product (Table 2).

Several solvents were initially screened using K₂CO₃ as the base. DMF and 1,4-dioxane gave relatively higher levels of **4a** (entries 1 and 2). In contrast, acetone and MeCN showed better regioselectivity, giving HPLC area percentages of 84.0% and 84.5%, respectively, for intermediate **4** (entries 3 and 4). Since the difference in regioselectivity between these two solvents was small, acetone was selected for further study, considering its lower cost and greater suitability for scale-up. The influence of the base was then examined in acetone. Organic bases, including Et₃N and DIPEA (entries 5 and 6), were less favorable for regioselectivity, whereas the inorganic carbonates K₂CO₃ and Na₂CO₃ afforded lower levels of **4a** (entries 3 and 7). Considering the results of solvent and base screening, the acetone/K₂CO₃ system was retained for subsequent temperature optimization.

Temperature was found to be a key factor for this regioselective S_NAr reaction. Although increasing the temperature accelerated the reaction, it also promoted the formation of **4a**. When the reaction was carried out at 40 °C, the reaction was completed within 1 h, but the level of **4a** increased to 19.9% (entry 8). Lowering the temperature effectively improved the regioselectivity. At 0 °C, intermediate **4** accounted for 90.8% of the HPLC area, while the level of **4a** was reduced to 9.1% (entry 9). Further lowering the temperature to −15 °C did not provide additional benefit and prolonged the reaction time (entry 10). Accordingly, the optimized conditions for the mild and scalable synthesis of intermediate **4** were established as follows: 2,4-dichloro-5-nitropyrimidine and intermediate **3** in a molar ratio of 1:1.05, K_2CO_3 as the base, acetone as the solvent, and 0 °C for 3 h. Although residual **4a** remained in the crude product, it could be efficiently removed by recrystallization from EtOH (3 volumes), affording intermediate **4** in 82.3% isolated yield with 99.6% HPLC purity.

Table 2 Optimization of the regioselective S_NAr reaction for the synthesis of intermediate **4**

 Reaction scheme showing the synthesis of intermediate 4 from compound 3 and 2,4-dichloro-5-nitropyrimidine under various conditions. The reaction yields intermediate 4 and a regioisomeric by-product 4a.						
Entry	Solvent	Base	Temp. (°C)	Time (h)	HPLC area (%)	
					4	4a
1	DMF	K_2CO_3	25	2	76.0	22.9
2	1,4-Dioxane	K_2CO_3	25	3	74.2	24.7
3	Acetone	K_2CO_3	25	2	84.0	15.0
4	MeCN	K_2CO_3	25	2	84.5	14.5
5	Acetone	Et_3N	25	3	77.4	21.5
6	Acetone	DIPEA	25	3	79.0	20.0

7	Acetone	Na ₂ CO ₃	25	3	80.6	18.3
8	Acetone	K ₂ CO ₃	40	1	79.1	19.9
9	Acetone	K₂CO₃	0	3	90.8	9.1
10	Acetone	K ₂ CO ₃	-15	5	91.0	9.0

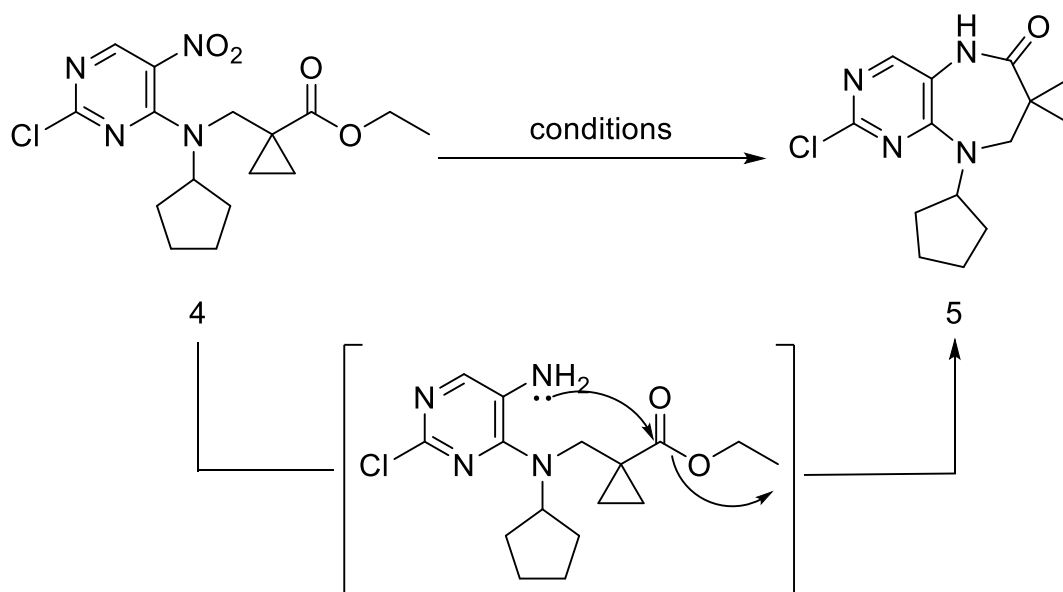
Conditions: 2,4-dichloro-5-nitropyrimidine was used as the limiting reagent; intermediate **3** (1.05 equiv.), base (1.2 equiv.), and the indicated solvent were used at the indicated temperature. HPLC areas were determined at 254 nm before purification. The peak of the 2-substituted by-product was assigned based on isolation and spectroscopic characterization of a representative sample.

Preparation of pyrimido-diazepine intermediate **5**

The conversion of intermediate **4** to intermediate **5** proceeded via reductive cyclization, in which reduction of the nitro group is presumed to generate an aminopyrimidine ester intermediate that subsequently undergoes intramolecular aminolysis to afford pyrimido-diazepine intermediate **5**. Therefore, several common nitro reduction systems were first evaluated for this one-pot transformation (Table 3). Na₂S₂O₄ and SnCl₂·2H₂O did not afford the desired cyclized intermediate **5** under the tested conditions (entries 1 and 2). Zn/AcOH and Fe/NH₄Cl promoted the reductive cyclization, but only gave intermediate **5** in 58.8% and 47.1% yields, respectively (entries 3 and 4). In contrast, Fe/AcOH was more suitable for this transformation, affording intermediate **5** in 83.9% yield at 60 °C (entry 5).

After Fe/AcOH was selected as the preferred reduction system, the effect of temperature was further investigated. Lowering the temperature from 60 to 40 °C slightly improved the yield from 83.9% to 85.7% (entries 5 and 6). At 25 °C, intermediate **5** was obtained in the highest yield of 89.2% with 99.5% HPLC purity (entry 7). Therefore, Fe/AcOH at 25 °C was identified as the optimal condition for the preparation of intermediate **5**.

Table 3 Optimization of the reductive cyclization of intermediate **4** to intermediate **5**



Entry	Reduction system	Temp. (°C)	Time (h)	Yield (%)
1	Na ₂ S ₂ O ₄	60	4	ND
2	SnCl ₂ ·2H ₂ O	60	5	ND
3	Zn/AcOH	60	5	58.8
4	Fe/NH ₄ Cl	60	5	47.1
5	Fe/AcOH	60	4	83.9
6	Fe/AcOH	40	4	85.7
7	Fe/AcOH	25	4	89.2

Conditions: intermediate **4** was used as the substrate with the indicated reduction system. Yields refer to isolated yields after work-up. ND = no desired cyclized intermediate **5** was detected.

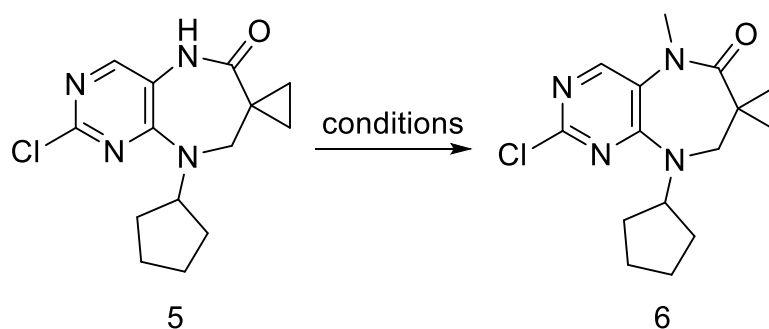
Preparation of pyrimido-diazepine intermediate **6**

With intermediate **5** in hand, its *N*-methylation was investigated for the preparation of pyrimido-diazepine intermediate **6**. Representative *N*-methylation conditions are summarized in Table 4. Dimethyl carbonate and dimethyl sulfate were initially evaluated as methylating reagents; however, both reactions proceeded slowly and afforded intermediate **6** in only 55.2% and 67.2% yields, respectively, even at 60 °C

(entries 1 and 2). In contrast, methyl iodide markedly improved the reaction efficiency and enabled the methylation to proceed rapidly at 25 °C (entries 3–6).

The effect of the base was subsequently examined using methyl iodide as the methylating reagent. Cs₂CO₃ provided intermediate **6** in 93.2% yield within 2 h, whereas NaH and Na₂CO₃ gave lower yields of 85.2% and 83.1%, respectively (entries 3, 5, and 6). The highest yield of 94.8% was obtained with *t*-BuOK after 3 h (entry 4), and this system was therefore selected for scale-up. On a 40.00 g scale of intermediate **5**, the substrate and *t*-BuOK were stirred in THF at 0 °C for 30 min, followed by dropwise addition of CH₃I. The mixture was then warmed to 25 °C and stirred for 3 h, affording 39.70 g of intermediate **6** in 94.8% isolated yield with 99.4% HPLC purity.

Table 4 Optimization of the *N*-methylation of intermediate **5** to intermediate **6**



Entry	Methylating reagent	Base	Temp. (°C)	Time (h)	Yield (%)
1	DMC	<i>t</i> -BuOK	60	6	55.2
2	DMS	<i>t</i> -BuOK	60	7	67.2
3	CH ₃ I	Cs ₂ CO ₃	25	2	93.2
4	CH₃I	<i>t</i>-BuOK	25	3	94.8
5	CH ₃ I	NaH	25	3	85.2
6	CH ₃ I	Na ₂ CO ₃	25	3	83.1

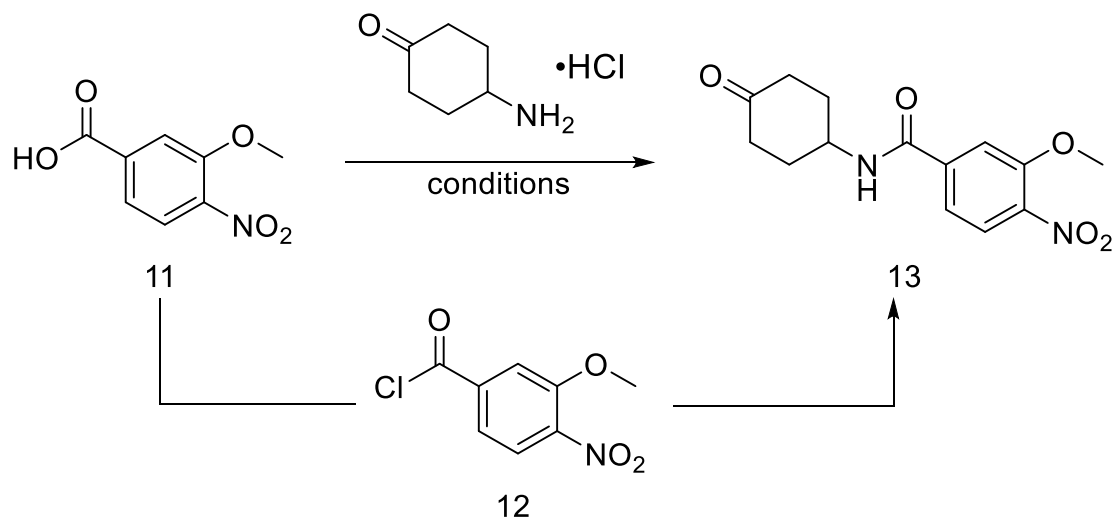
Conditions: intermediate **5** was used as the limiting reagent; methylating reagent (1.1 equiv.) and base (1.1 equiv.) were used in THF at the indicated temperature. Yields refer to isolated yields after work-up. DMC = dimethyl carbonate; DMS = dimethyl sulfate.

Preparation of benzamide intermediate **13**

Benzamide intermediate **13** was prepared by amide bond formation between 3-methoxy-4-nitrobenzoic acid (**11**) and 4-aminocyclohexanone hydrochloride. Representative activation and amidation conditions are summarized in Table 5. Direct amidation using representative coupling reagents was first evaluated. CDI and EDCI/HOBt afforded intermediate **13** in only 54.9% and 63.2% yields, respectively (entries 1 and 2). Although HATU in the presence of DIPEA increased the yield to 67.8%, the reaction still required 10 h, and the use of an expensive coupling reagent was considered less attractive for scale-up (entry 3). Therefore, conversion of acid **11** into the corresponding acid chloride was investigated as an alternative strategy.

Compared with the direct coupling methods, the acid chloride route gave higher conversion and a cleaner reaction profile. Oxalyl chloride also promoted the formation of the desired amide, but SOCl_2 gave a higher yield and a shorter reaction time (entries 4 and 5). Thus, SOCl_2 was selected for the preparation of the corresponding acid chloride. After the activation step was established, the base used in the subsequent amidation was further optimized. The K_2CO_3 -mediated reaction gave a lower yield and required a longer reaction time than the corresponding reactions using soluble tertiary amine bases, which may be attributed to the poor solubility of K_2CO_3 in DCM and its limited ability to liberate the free amine from the amine hydrochloride salt (entries 5–7). In addition, neutralization of HCl by K_2CO_3 may generate water in the reaction system, which could promote partial hydrolysis of the acid chloride and decrease the effective concentration of the acylating reagent. In contrast, soluble tertiary amine bases could more efficiently release the free amine and scavenge HCl under homogeneous conditions. Among the tested tertiary amine bases, DIPEA gave the best result (entry 7). Finally, 3-methoxy-4-nitrobenzoic acid was converted into the corresponding acid chloride with SOCl_2 in toluene at 70 °C, followed by coupling with 4-aminocyclohexanone hydrochloride in the presence of DIPEA in DCM at 25 °C. Benzamide intermediate **13** was obtained in 88.7% overall yield from 3-methoxy-4-nitrobenzoic acid with 99.3% HPLC purity.

Table 5 Optimization of the activation and amidation conditions for the synthesis of intermediate **13**



Entry	Activation system	Base	Time (h)	Yield (%)
1	CDI	—	10	54.9
2	EDCI/HOBt	—	12	63.2
3	HATU	DIPEA	10	67.8
4	(COCl) ₂	K ₂ CO ₃	9	74.8
5	SOCl ₂	K ₂ CO ₃	6	79.3
6	SOCl ₂	Et ₃ N	5	87.0
7	SOCl₂	DIPEA	5	88.7

Conditions: 3-methoxy-4-nitrobenzoic acid was used as the limiting reagent; 4-aminocyclohexanone hydrochloride (1.05 equiv.), the indicated activation system, and the indicated base were used under the specified reaction conditions. Yields refer to the overall isolated yields of intermediate **13** from 3-methoxy-4-nitrobenzoic acid.

Preparation of aminobenzamide intermediate **15**

Aminobenzamide intermediate **15** was prepared from benzamide intermediate **13** through reductive amination followed by nitro reduction. In the reported side-chain strategy, the *trans*-4-(4-methylpiperazin-1-yl)cyclohexylamine fragment was prepared

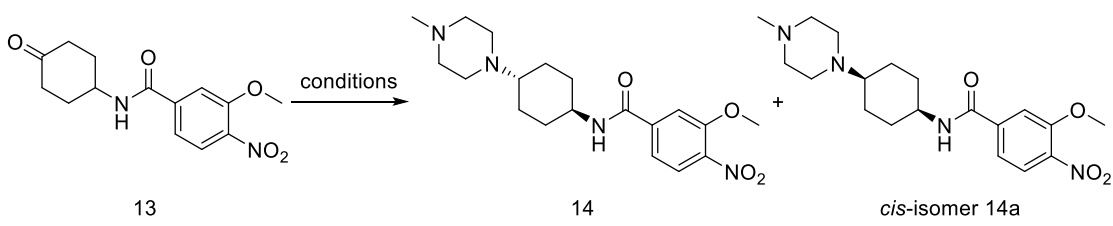
before its coupling with the benzoic acid fragment. However, this route suffered from low yield and operational complexity because the desired *trans*-isomeric precursor had to be isolated from a *cis/trans* mixture by chromatographic purification followed by recrystallization. Therefore, in the present route, benzamide intermediate **13** was used directly as the ketone substrate, and the *N*-methylpiperazine group was introduced at a later stage by reductive amination. This design shifted the stereochemical separation to the nitrobenzamide intermediate **14**, for which a more practical non-chromatographic purification method could be developed.

The reductive amination of intermediate **13** with *N*-methylpiperazine proceeds via iminium formation followed by hydride reduction. Because the iminium carbon on the cyclohexane ring is prochiral, hydride attack from either face can generate both the desired *trans*-isomer **14** and the corresponding *cis*-isomer **14a**. Related studies on 4-substituted cyclohexane-1-amines have shown that the *trans*-diastereomers are thermodynamically favored over the corresponding *cis*-diastereomers (Farkas et al. 2024). Accordingly, the reaction was optimized by monitoring the HPLC areas of **14** and **14a** in the crude reaction mixture (Table 6). Among the tested reducing agents, NaBH(OAc)₃ gave a better crude reaction profile than NaBH₄ and NaBH₃CN in THF at 40 °C (entries 1–3), increasing the HPLC area of **14** to 61.7% while reducing the formation of **14a**. Solvent screening further showed that MeCN and DMF were less suitable for this transformation, whereas DCM gave the highest proportion of the desired *trans*-isomer, with **14** accounting for 65.9% of the HPLC area at 40 °C (entries 4–6).

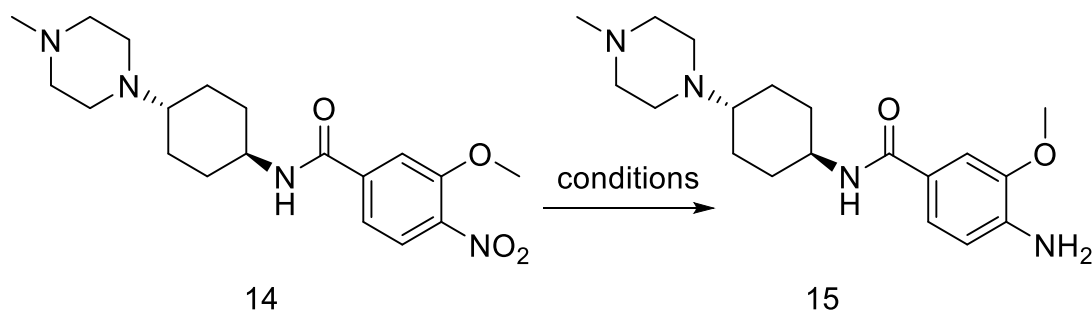
The reaction temperature had a clear influence on both conversion and stereochemical outcome. In DCM, increasing the temperature to 60 °C decreased the HPLC area of **14** and promoted the formation of **14a**, indicating that elevated temperature was unfavorable for this reductive amination. Lowering the temperature to 25 °C gave the best crude profile, with HPLC area percentages of 68.1% for **14** and 30.7% for **14a**. Further cooling to 0 °C resulted in incomplete conversion of intermediate **13**, with HPLC area percentages of 38.9% for **14** and 15.6% for **14a**. Thus, DCM and 25 °C were selected as the preferred solvent and temperature, respectively

(entry 8). During the subsequent purification study, the *cis*-isomer **14a** was found to be readily soluble in methyl *tert*-butyl ether (MTBE), whereas the desired *trans*-isomer **14** was almost insoluble in this solvent. Based on this pronounced solubility difference, slurring the crude product in MTBE selectively removed the *cis*-isomeric impurity **14a** and afforded intermediate **14** in 57.6% isolated yield with 99.4% HPLC purity.

Table 6 Optimization of the reductive amination conditions for the selective formation of *trans*-configured intermediate **14**

					
Entry	Reducing agent	Solvent	Temp. (°C)	HPLC area (%)	
				14	14a
1	NaBH ₄	THF	40	55.3	43.5
2	NaBH ₃ CN	THF	40	57.9	40.9
3	NaBH(OAc) ₃	THF	40	61.7	37.1
4	NaBH(OAc) ₃	MeCN	40	53.8	45.2
5	NaBH(OAc) ₃	DMF	40	50.1	48.5
6	NaBH(OAc) ₃	DCM	40	65.9	33.2
7	NaBH(OAc) ₃	DCM	60	51.2	47.6
8	NaBH(OAc)₃	DCM	25	68.1	30.7
9	NaBH(OAc) ₃	DCM	0	38.9	15.6

Conditions: intermediate **13** (1.0 equiv.), *N*-methylpiperazine (1.2 equiv.), AcOH (0.05 equiv.), reducing agent (2.0 equiv.), and solvent (10 volumes). Intermediate **13**, *N*-methylpiperazine, and AcOH were stirred in the indicated solvent for 1 h, followed by addition of the reducing agent and further reaction for 2 h at the indicated temperature. HPLC areas of **14** and **14a** were determined from the crude reaction mixture before MTBE slurry purification.



Finally, catalytic hydrogenation of intermediate **14** over 10% Pd/C in MeOH at room temperature for 6 h afforded aminobenzamide intermediate **15** in 95.2% isolated yield with 97.9% HPLC purity.

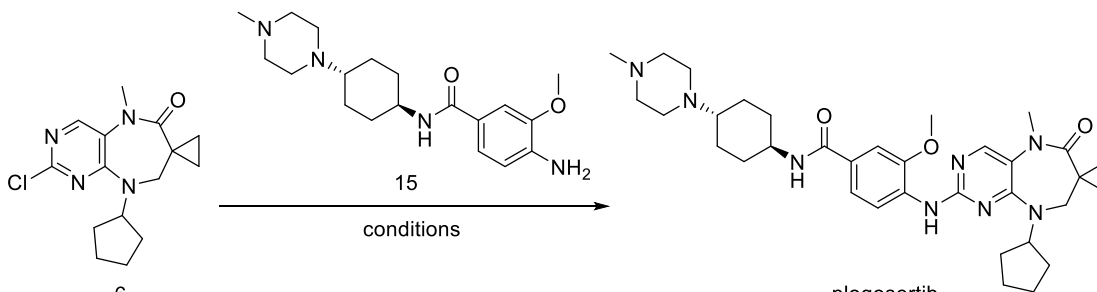
Preparation of plogosertib

With intermediates **6** and **15** in hand, the final C–N bond formation was investigated. Buchwald–Hartwig amination was initially evaluated for the coupling of intermediates **6** and **15**. However, the reaction proceeded with low conversion and generated a complex impurity profile, affording plogosertib in only 25.7% isolated yield after column chromatographic purification. Considering the low reaction efficiency and the need for chromatographic purification, this palladium-catalyzed approach was considered unsuitable for further process development. Therefore, a transition-metal-free, acid-mediated S_NAr reaction was investigated as a more practical alternative.

The acid-mediated S_NAr reaction was subsequently optimized with respect to solvent and acid, and the results are summarized in Table 7. When HCl was used as the acid, *n*-BuOH gave the best result among the tested solvents, affording crude plogosertib in 84.6% yield after 15 h (entry 5). Other solvents, including *i*-PrOH, 2-MeTHF, *t*-BuOH, and DMF, gave lower yields under their respective reflux conditions (entries 1–4). Different acids were then evaluated in *n*-BuOH. Trifluoroacetic acid (TFA) and *p*-toluenesulfonic acid (*p*-TsOH) gave relatively low yields, whereas methanesulfonic acid (MSA) improved the yield to 77.3% (entries 6–8). Finally, HCl was identified as the optimal acid for this transformation. After completion of the reaction under the optimized conditions, the mixture was basified, and crude plogosertib was isolated without column chromatography in 84.6% yield with 95.6%

HPLC purity. Recrystallization from EtOH/H₂O (4:1, v/v) afforded plogosertib in 78.2% isolated yield with 99.9% HPLC purity.

Table 7 Optimization of the acid-mediated S_NAr conditions for the synthesis of plogosertib

				
Entry	Solvent	Acid	Time (h)	Yield (%)
1	<i>i</i> -PrOH	HCl	23	56.7
2	2-MeTHF	HCl	24	40.1
3	<i>t</i> -BuOH	HCl	22	61.9
4	DMF	HCl	19	54.2
5	<i>n</i>-BuOH	HCl	15	84.6
6	<i>n</i> -BuOH	TFA	30	59.9
7	<i>n</i> -BuOH	<i>p</i> -TsOH	25	55.4
8	<i>n</i> -BuOH	MSA	23	77.3

Conditions: intermediate **15** (1.0 equiv.), intermediate **6** (1.1 equiv.), acid (2.0 equiv.), and solvent (10 volumes) at reflux. HCl was used as 12 M aqueous HCl. Yields refer to the isolated yields of crude plogosertib after work-up and before final recrystallization.

Conclusions

In summary, an improved convergent process for the multigram-scale synthesis of plogosertib was established. The use of NaBH₄/CoCl₂·6H₂O enabled the selective reduction of the nitrile group under mild conditions, avoiding Raney Ni-catalyzed hydrogenation under pressurized hydrogen. Regioselective formation of intermediate **4**

was achieved by controlling the S_NAr reaction at 0 °C, and the C-2-substituted impurity was removed by recrystallization. For the benzamide fragment, the *cis*-isomeric impurity generated during reductive amination was effectively controlled by MTBE slurry purification without column chromatography. The final assembly of plogosertib was accomplished via a late-stage acid-mediated S_NAr reaction between intermediates **6** and **15**, replacing the HBTU-mediated amide coupling used in the reported process. The optimized process afforded plogosertib in 42.4% overall yield from compound **1** with 99.9% HPLC purity, enabling practical multigram-scale preparation of plogosertib.

Experimental

General

All commercially available reagents and solvents were used without further purification unless otherwise noted. Melting points were measured on a Büchi Melting Point B-540 apparatus. TLC analyses were performed on silica gel GF254 plates (Qingdao Ocean Chemicals, Qingdao, China). Mass spectra were recorded in electrospray ionization mode on an Agilent 1100 LC–MS system (Agilent, Palo Alto, CA, USA). ¹H and ¹³C NMR spectra were recorded on Bruker AVANCE III HD 400- and 600-MHz NMR spectrometers equipped with Bruker Ascend magnets (Bruker BioSpin, Rheinstetten, Germany), using TMS as an internal standard.

HPLC method

HPLC purity was determined by area normalization using a Symmetry C18 column (250 × 4.6 mm, 5 μm). The detection wavelength was 254 nm, the column temperature was maintained at 30 °C, and the flow rate was 1.0 mL/min. Mobile phase A was a 0.03 M sodium dihydrogen phosphate buffer containing 5% methanol, adjusted to pH 2.53. Mobile phase B was methanol. Samples were dissolved in MeOH at a concentration of 1 mg/mL. The injection volume was 10 μL. The gradient elution conditions were as follows:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	5	95

25	95	5
30	95	5

Ethyl 1-(aminomethyl)cyclopropane-1-carboxylate (2)

Ethyl 1-cyanocyclopropane-1-carboxylate (50.00 g, 0.36 mol) and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (85.49 g, 0.36 mol) were added to MeOH (500 mL), and the mixture was cooled to 0 °C. NaBH_4 (40.77 g, 1.08 mol) was added in five portions. After the addition was complete, the reaction mixture was stirred at room temperature for 4 h. The reaction was quenched with 4 M aqueous HCl (400 mL), and MeOH was removed under reduced pressure. The residue was adjusted to pH 12 with 4 M aqueous NaOH and stirred for 20 min. The mixture was filtered, and the filter cake was washed with DCM (100 mL). The phases of the filtrate were separated, and the aqueous layer was extracted with DCM (500 mL $\times$ 2). The combined organic layers were washed with brine (800 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to afford intermediate **2** as a colorless oil (43.01 g, 83.6% yield). ESI-MS m/z : 144.2 $[\text{M} + \text{H}]^+$.

^1H NMR (400 MHz, CDCl_3) δ 4.07 (q, $J = 7.1$ Hz, 2H), 2.69 (s, 2H), 1.49 (s, 2H), 1.20–1.15 (m, 5H), 0.69 (q, $J = 4.0$ Hz, 2H).

Ethyl 1-((cyclopentylamino)methyl)cyclopropane-1-carboxylate (3)

Intermediate **2** (40.00 g, 0.28 mol) and cyclopentanone (24.67 g, 0.29 mol) were added to DCM (400 mL) and stirred at room temperature for 30 min. $\text{NaBH}(\text{OAc})_3$ (88.81 g, 0.42 mol) was slowly added, and the resulting mixture was stirred at room temperature for 4 h. After completion, saturated aqueous NaHCO_3 (500 mL) was added to quench the reaction. The phases were separated, and the aqueous layer was extracted with DCM (300 mL $\times$ 3). The combined organic layers were washed with brine (800 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to give intermediate **3** as a yellow oil (54.95 g, 93.1% yield). ESI-MS m/z : 212.1 $[\text{M} + \text{H}]^+$.

^1H NMR (400 MHz, CDCl_3) δ 4.04 (q, $J = 7.1$ Hz, 2H), 3.05–2.95 (m, 1H), 2.62 (s, 2H), 1.95 (s, 1H), 1.81–1.70 (m, 2H), 1.67–1.56 (m, 2H), 1.50–1.40 (m, 2H), 1.32–1.23 (m, 2H), 1.22–1.19 (m, 2H), 1.16 (t, $J = 7.1$ Hz, 3H), 0.75 (q, $J = 4.1$ Hz, 2H).

Ethyl 1-(((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)methyl)cyclopropane-1-carboxylate (4)

Intermediate **3** (50.00 g, 0.24 mol) was dissolved in acetone (250 mL), and K_2CO_3 (37.36 g, 0.27 mol) was added at 0 °C. A solution of 2,4-dichloro-5-nitropyrimidine (43.71 g, 0.23 mol) in acetone (250 mL) was added dropwise over 1 h, and the mixture was stirred at 0 °C for 3 h. After completion, the solvent was removed under reduced pressure. Water (500 mL) was added to the residue, and the mixture was extracted with DCM (400 mL $\times$ 2). The combined organic layers were washed with brine (700 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to afford the crude product (85.82 g). The crude product was dissolved in hot EtOH (260 mL) and then gradually cooled to room temperature. The precipitated solid was collected by filtration and dried to give intermediate **4** as a yellow solid (68.63 g, 82.3% yield, 99.6% HPLC purity). m.p. 114.5–114.7 °C; ESI-MS m/z : 369.0 $[M + H]^+$.

^1H NMR (400 MHz, DMSO- d_6) δ 8.89 (s, 1H), 3.96–3.87 (m, 3H), 3.49 (s, 2H), 1.89–1.75 (m, 6H), 1.49 (s, 2H), 1.20–1.15 (m, 2H), 1.14–1.07 (m, 2H), 0.96 (t, J = 7.1 Hz, 3H).

2'-Chloro-9'-cyclopentyl-8',9'-dihydrospiro[cyclopropane-1,7'-pyrimido[4,5-*b*][1,4]diazepin]-6'(5'*H*)-one (5)

Glacial AcOH (420 mL) was heated to 40 °C, and iron powder (42.41 g, 0.76 mol) was added. The suspension was stirred at 40 °C for 30 min and then cooled to 25 °C. Intermediate **4** (70.00 g, 0.19 mol) was slowly added, and the reaction mixture was stirred at 25 °C for 4 h. After completion, the reaction mixture was filtered through Celite, and the filter cake was washed with MeOH (100 mL). The filtrate was concentrated under reduced pressure. Water (600 mL) was added to the residue, and the suspension was stirred for 30 min. The solid was collected by filtration and dried to give intermediate **5** as a white solid (49.56 g, 89.2% yield, 99.5% HPLC purity). m.p. 246.3–247.6 °C; ESI-MS m/z : 293.0 $[M + H]^+$.

^1H NMR (400 MHz, DMSO- d_6) δ 9.84 (s, 1H), 7.93 (s, 1H), 4.91–4.79 (m, 1H), 3.41 (s, 2H), 1.87–1.74 (m, 2H), 1.73–1.63 (m, 2H), 1.61–1.59 (m, 2H), 1.47–1.34 (m, 2H), 1.16 (q, J = 3.9 Hz, 2H), 0.90 (q, J = 4.0 Hz, 2H).

2'-Chloro-9'-cyclopentyl-5'-methyl-8',9'-dihydrospiro[cyclopropane-1,7'-pyrimido[4,5-*b*][1,4]diazepin]-6'(5'*H*)-one (6)

Intermediate **5** (40.00 g, 0.14 mol) and *t*-BuOK (16.86 g, 0.15 mol) were added to THF (240 mL). The mixture was stirred at 0 °C for 30 min, and CH₃I (21.32 g, 0.15 mol) was added dropwise. After the addition was complete, the reaction mixture was warmed to 25 °C and stirred for 3 h. The solvent was removed under reduced pressure, and water (200 mL) was added to the residue. The mixture was extracted with DCM (200 mL × 3). The combined organic layers were washed with brine (800 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to afford intermediate **6** as a white solid (39.70 g, 94.8% yield, 99.4% HPLC purity). m.p. 144.9–145.1 °C; ESI-MS *m/z*: 307.0 [M + H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.04 (s, 1H), 4.75 (m, 1H), 3.53 (s, 2H), 3.17 (s, 3H), 1.89–1.78 (m, 2H), 1.71–1.61 (m, 2H), 1.57–1.43 (m, 4H), 0.94 (q, *J* = 4.4 Hz, 2H), 0.71 (q, *J* = 4.5 Hz, 2H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ 172.2, 157.1, 154.3, 150.9, 124.4, 58.6, 52.1, 37.0, 27.6, 24.0, 23.8, 11.5.

3-Methoxy-4-nitro-*N*-(4-oxocyclohexyl)benzamide (13)

3-Methoxy-4-nitrobenzoic acid (50.00 g, 0.25 mol), SOCl₂ (36.8 mL, 0.51 mol), and DMF (1.0 mL, 0.01 mol) were added successively to toluene (500 mL). The mixture was heated to 70 °C and stirred for 2 h. After completion, the reaction mixture was cooled to room temperature and concentrated under reduced pressure to give acid chloride **12** as a white solid (53.85 g, 98.5% yield), which was used directly in the next step. 4-Aminocyclohexanone hydrochloride (39.25 g, 0.26 mol) and DIPEA (80.71 g, 0.62 mol) were added to DCM (200 mL), and the mixture was stirred at room temperature for 30 min. A solution of acid chloride **12** (53.85 g, 0.25 mol) in DCM (200 mL) was added dropwise at 0 °C. The mixture was then warmed to 25 °C and stirred for 3 h. After completion, the reaction mixture was concentrated under reduced pressure. Water (500 mL) was added to the residue, and the suspension was stirred for 30 min. The solid was collected by filtration and dried under vacuum to afford

intermediate **13** as a white solid (65.78 g, 88.7% yield over two steps, 99.3% HPLC purity). m.p. 191.5–192.2 °C; ESI-MS m/z : 291.0 $[M - H]^-$.

^1H NMR (400 MHz, DMSO- d_6) δ 8.60 (d, $J = 7.5$ Hz, 1H), 7.97 (d, $J = 8.4$ Hz, 1H), 7.70 (d, $J = 1.6$ Hz, 1H), 7.57 (dd, $J = 8.4, 1.6$ Hz, 1H), 4.31 (m, 1H), 3.99 (s, 3H), 2.60–2.51 (m, 2H), 2.35–2.26 (m, 2H), 2.17–2.07 (m, 2H), 1.81 (qd, $J = 12.8, 4.6$ Hz, 2H).

3-Methoxy-*N*-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-4-nitrobenzamide (14)

Intermediate **13** (60.00 g, 0.21 mol), *N*-methylpiperazine (24.69 g, 0.25 mol), and glacial AcOH (0.6 mL, 0.01 mol) were added to DCM (600 mL). The mixture was stirred at room temperature for 1 h, and NaBH(OAc)₃ (87.07 g, 0.42 mol) was slowly added. After the addition was complete, the reaction mixture was stirred at 25 °C for 2 h. Saturated aqueous Na₂CO₃ (600 mL) was added to quench the reaction. The phases were separated, and the aqueous layer was extracted with DCM (500 mL $\times$ 3). The combined organic layers were washed with brine (800 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give the crude product as a yellow solid (93.66 g). MTBE (800 mL) was added, and the suspension was stirred at room temperature for 3 h. The solid was collected by filtration, washed with MTBE (80 mL), and dried to afford intermediate **14** as a white solid (44.51 g, 57.6% yield, 99.4% HPLC purity). m.p. 205.8–206.2 °C; ESI-MS m/z : 377.1 $[M + H]^+$.

^1H NMR (400 MHz, DMSO- d_6) δ 8.48 (d, $J = 7.8$ Hz, 1H), 7.94 (d, $J = 8.4$ Hz, 1H), 7.68 (d, $J = 1.6$ Hz, 1H), 7.54 (dd, $J = 8.4, 1.6$ Hz, 1H), 3.98 (s, 3H), 3.76–3.68 (m, 1H), 2.61–2.51 (m, 2H), 2.36–2.25 (m, 6H), 2.17 (s, 3H), 1.96–1.88 (m, 2H), 1.87–1.76 (m, 2H), 1.44–1.22 (m, 5H).

4-Amino-3-methoxy-*N*-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]benzamide (15)

Intermediate **14** (40.00 g, 0.11 mol) was added to MeOH (400 mL), followed by 10% Pd/C (4.00 g). The mixture was stirred under a hydrogen atmosphere at room temperature for 6 h. After completion, the mixture was filtered, and the filter cake was washed with MeOH (40 mL). The filtrate was concentrated under reduced pressure to

afford intermediate **15** as a white solid (35.04 g, 95.2% yield, 97.9% HPLC purity). m.p. 129.5–130.0 °C; ESI-MS m/z : 347.1 $[M + H]^+$.

^1H NMR (400 MHz, DMSO- d_6) δ 7.74 (d, $J = 7.9$ Hz, 1H), 7.34–7.23 (m, 2H), 6.59 (d, $J = 8.6$ Hz, 1H), 5.19 (s, 2H), 3.80 (s, 3H), 3.77–3.68 (m, 1H), 2.49–2.16 (m, 8H), 2.14 (s, 3H), 1.88–1.77 (m, 4H), 1.41–1.17 (m, 5H).

^{13}C NMR (101 MHz, DMSO- d_6) δ 165.9, 145.7, 141.2, 122.5, 121.5, 112.4, 110.0, 62.6, 55.9, 55.8, 55.7, 48.8, 48.5, 46.2, 32.1, 27.5.

4-[(9'-Cyclopentyl-8',9'-dihydro-5'-methyl-6'-oxospiro[cyclopropane-1,7'-[7H]pyrimido[4,5-*b*][1,4]diazepin]-2'(5'*H*)-yl)amino]-3-methoxy-*N*-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]benzamide (plogosertib)

Intermediate **6** (30.00 g, 98.00 mmol) and intermediate **15** (30.80 g, 88.89 mmol) were added to *n*-BuOH (300 mL). At 0 °C, 12 M HCl (14.81 mL, 177.78 mmol) was added dropwise. After the addition was complete, the reaction mixture was heated to reflux and stirred for 15 h. The mixture was cooled to room temperature and filtered, and the filter cake was washed with *n*-BuOH (10 mL $\times$ 2). The filter cake was suspended in water (150 mL), and the pH was adjusted to 11 with 4 M aqueous NaOH at 0 °C. The solid was collected by filtration to give crude plogosertib as a white solid (46.38 g, 84.6% yield, 95.6% HPLC purity). The crude product was added to EtOH/H₂O (4:1, v/v, 400 mL) and heated to 70 °C until complete dissolution. The solution was cooled to room temperature and allowed to crystallize for 3 h. The precipitated solid was collected by filtration and dried under vacuum to afford plogosertib as a white solid (42.87 g, 78.2% yield, 99.9% HPLC purity). m.p. 150.5–151.1 °C; ESI-MS m/z : 617.2 $[M + H]^+$.

^1H NMR (600 MHz, DMSO- d_6) δ 8.39 (d, $J = 8.4$ Hz, 1H), 8.04 (d, $J = 7.9$ Hz, 1H), 7.98 (s, 1H), 7.68 (s, 1H), 7.51–7.42 (m, 2H), 4.84 (p, $J = 8.7$ Hz, 1H), 3.94 (s, 3H), 3.71 (dp, $J = 11.8, 4.0$ Hz, 1H), 3.47 (s, 2H), 3.16 (s, 3H), 2.48 (s, 4H), 2.41–2.21 (m, 4H), 2.21–2.16 (m, 1H), 2.12 (s, 3H), 1.94–1.84 (m, 4H), 1.84–1.78 (m, 2H), 1.68 (t, $J = 5.4$ Hz, 2H), 1.59 (dq, $J = 9.6, 4.0$ Hz, 2H), 1.53–1.46 (m, 2H), 1.39–1.25 (m, 4H), 0.90 (q, $J = 4.4$ Hz, 2H), 0.67 (q, $J = 4.5$ Hz, 2H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ 172.2, 165.4, 156.6, 155.5, 150.3, 147.2, 132.4, 127.5, 120.4, 118.9, 116.5, 109.7, 62.5, 58.3, 56.5, 55.0, 52.7, 48.6, 48.3, 46.0, 45.5, 36.8, 31.8, 27.6, 27.2, 23.9, 23.7, 11.3, 9.5.

Statements and Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability The data supporting the findings of this study are available within the article and its supplementary information files.

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