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Graphical Abstract

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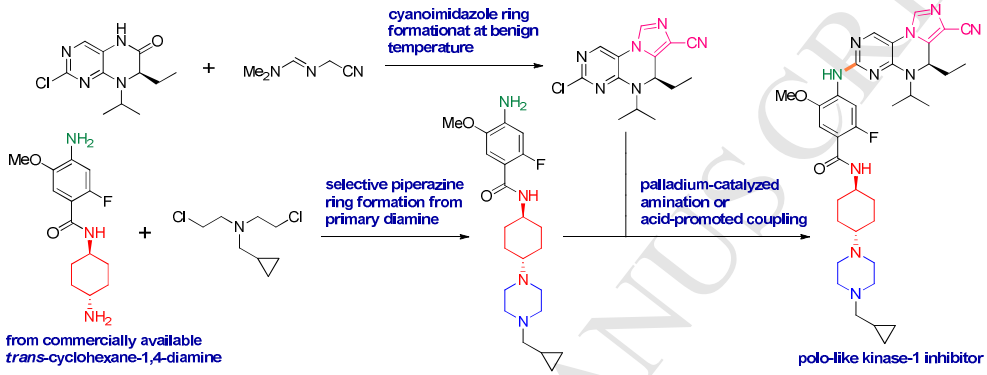

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A practical chromatography-free synthesis of a 5,6-dihydroimidazolo[1,5-*f*]pteridine derivative as a polo-like kinase-1 inhibitor

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ABSTRACT

A practical chromatography-free synthesis of a potent polo-like kinase-1 inhibitor possessing a unique 5,6-dihydroimidazolo[1,5-*f*]pteridine structure has been developed. We showed that key cyanoimidazole ring formation could be conducted at benign temperature and obtained a chiral 5,6-dihydroimidazolo[1,5-*f*]pteridine derivative in good yield without epimerization. An aniline derivative containing a *trans* 1,4-cyclohexyl diamine structure was prepared by a synthesis that makes use of defined stereocenters of commercially available trans-cyclohexane-1,4-diamine via selective piperazine ring formation from a primary diamine. A coupling reaction of the 3-chloro-5,6-dihydroimidazolo[1,5-*f*]pteridine derivative and the aniline derivative in the endgame was closely investigated, and good yields were achieved both by palladium-catalyzed amination and acid-promoted coupling under benign reaction conditions. As a result of these investigations, the polo-like kinase-1 inhibitor was successfully obtained in a practical way without concern for generation/separation of stereoisomers.

1. Introduction [i]

Polo-like kinases (PLKs) were identified as one of the kinase families that play an essential role in various cell-cycle related processes.[1] Amongst five known PLKs (PLK1–5), PLK1 has attracted much attention as an important target for a medicine, and inhibitors of PLK1 have been developed and evaluated as anticancer drugs in clinical studies.[2] Compound **19** is a potent PLK1 inhibitor that has strong enzyme and cellular activities against PLK1, and also exhibits oral bioavailability.[3]

Compound **19** is characterized by its unique 5,6-dihydroimidazolo[1,5-*f*]pteridine core structure, and medicinal chemists have synthesized **19** as described in Scheme 1 and Scheme 2.[3] Unnatural amino acid **1** was converted to methyl ester hydrochloric acid salt **2·HCl**, and reductive amination of **2·HCl** with acetone followed by an S_NAr reaction with 2,4-dichloro-5-nitropyrimidine **4** gave **5** (Scheme 1). Reduction of the nitro group of **5** led to cyclization to give **6**. Activation of the amide group of **6** by phosphorylation was followed by addition of an anion derived from **7**, and the subsequent acid-promoted cyclization provided in one pot **8**, which has the 5,6-dihydroimidazolo[1,5-*f*]pteridine structure. Aniline **15** was synthesized starting from commercially available ketone **9**. Amino ketone **10·HCl** prepared by deprotection of **9** was condensed with benzoic acid **11** to give ketone **12**. Imine formation of **12** with piperazine **13** followed by reduction using $NaBH_4$ provided *trans* **14** as the major product and *cis* isomer **14'** as the minor product, respectively. Hydrogenation of the mixture of **14** and **14'** provided aniline **15**.

For the synthesis of **19** from **8**, two routes have been reported (Scheme 2). In one route (Route A), compound **8** was reacted with aniline **15** in an acidic condition to afford **19**. In the other route (Route B), compound **19** was synthesized by sequential reactions: compound **8** was first reacted with aniline **16** using a palladium catalyst under a microwave condition, and then condensed with known amine **18**[4].

Three key issues need to be overcome for scale-up of the medicinal chemistry syntheses. First, the cryogenic condition (−78 °C) used for the synthesis of **8** from **6** is difficult to perform in large-scale production. Second, construction of the *trans* diamine structure on the cyclohexyl ring of **15**/**18** is a synthetic challenge. In the patent filed by the medicinal chemists, [3a] ratio of **14** to **14'** synthesized from **12** and **13** by reductive amination is not obviously mentioned. A mixture of **14** and **14'** was hydrogenated, and after simple workup, the obtained crude aniline **15** was used for the final coupling with **8** without further purification. Removal of the undesired cis isomer **19'** seemingly necessitated cumbersome workup procedures including column chromatography separation (Route A) or HPLC separation

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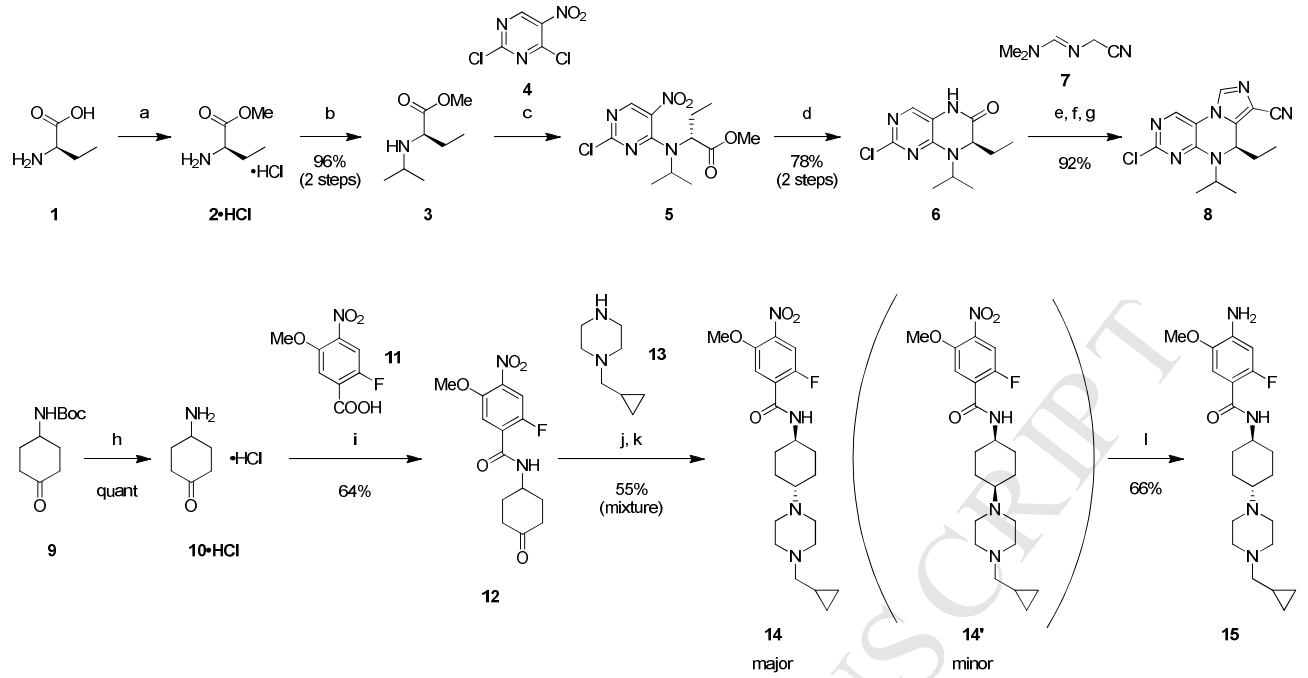
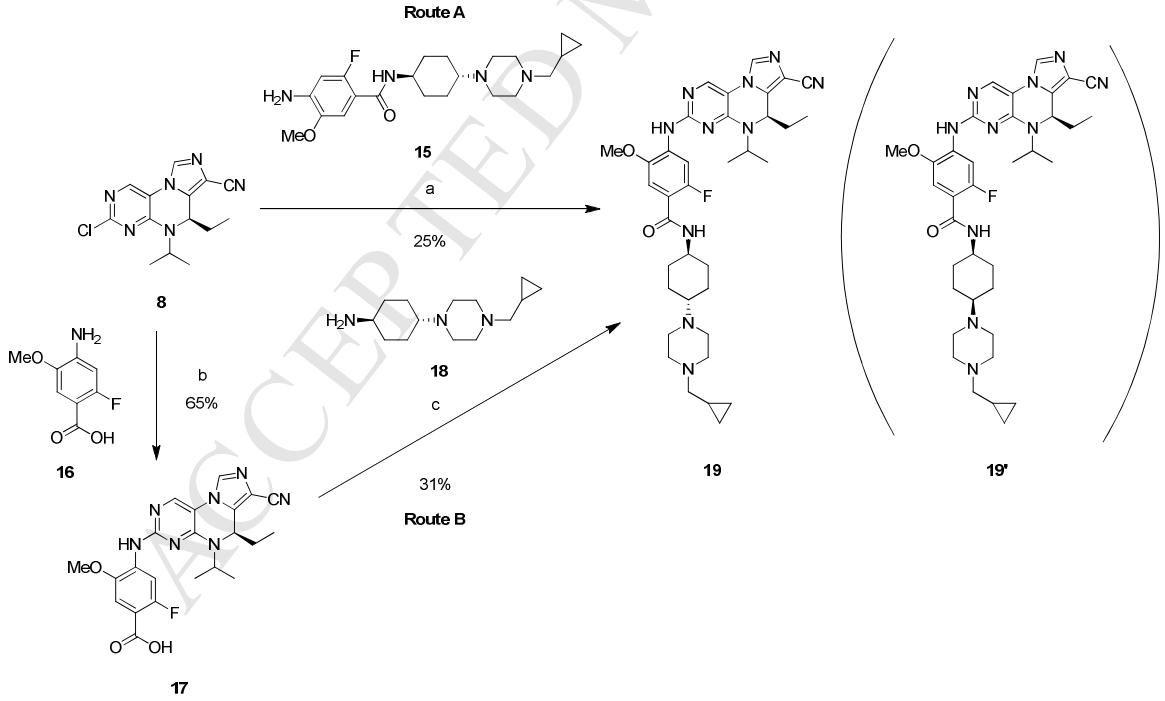


Scheme 1. Medicinal chemistry synthesis of **8** and **15**.

Reagents and Conditions: (a) $SOCl_2$, MeOH; (b) acetone, $NaBH(OAc)_3$, NaOAc, AcOH, THF; (c) **4**, THF, rt; (d) 5% Pt/C, H_2 (75 psi), NH_4VO_3, $P(OPh)_3$, 5 h, rt; (e) LHMDS, $ClPO(OEt)_2$, THF, −78 °C; (f) **7**, LHMDS, THF, −78 °C; (g) AcOH, THF; (h) 4 M HCl in dioxane, rt, 6 h; (i) **11**, HATU, DIPEA, DMF, rt, 2.5 h; (j) **13**, cat. methanesulfonic acid, toluene, reflux with a Dean-Stark reflux condenser, 5 h; (k) $NaBH_4$, EtOH, rt, overnight; (l) Pd/C, H_2 (balloon), MeOH/AcOH (5:1), rt, 8 h.



Scheme 2. Medicinal chemistry synthesis of **19** from **8**.

Reagents and Conditions: (a) conc. HCl, 2-propanol, 95 °C, 2 days; (b) $Pd(OAc)_2$, Xantphos, Cs_2CO_3, *N*,*N*-dimethylacetamide/dioxane, microwave, 160 °C, 15 min; (c) HATU, DIPEA, DMF, rt, 2 h.

(Route B).[5] Finally, the syntheses of **19** from **8** required long reaction time or the use of microwave irradiation, and suffered from low yields. The acid-promoted coupling of **8** with **15** in 2-propanol using concentrated HCl required 2 days for the reaction, providing **19** in low yield (25%, Route A). The palladium-catalyzed amination of **8** with aniline **16** was conducted using the microwave system (160 °C, 15 min), and the subsequent coupling with **18** gave **19**, also in low yield (31%, Route B).

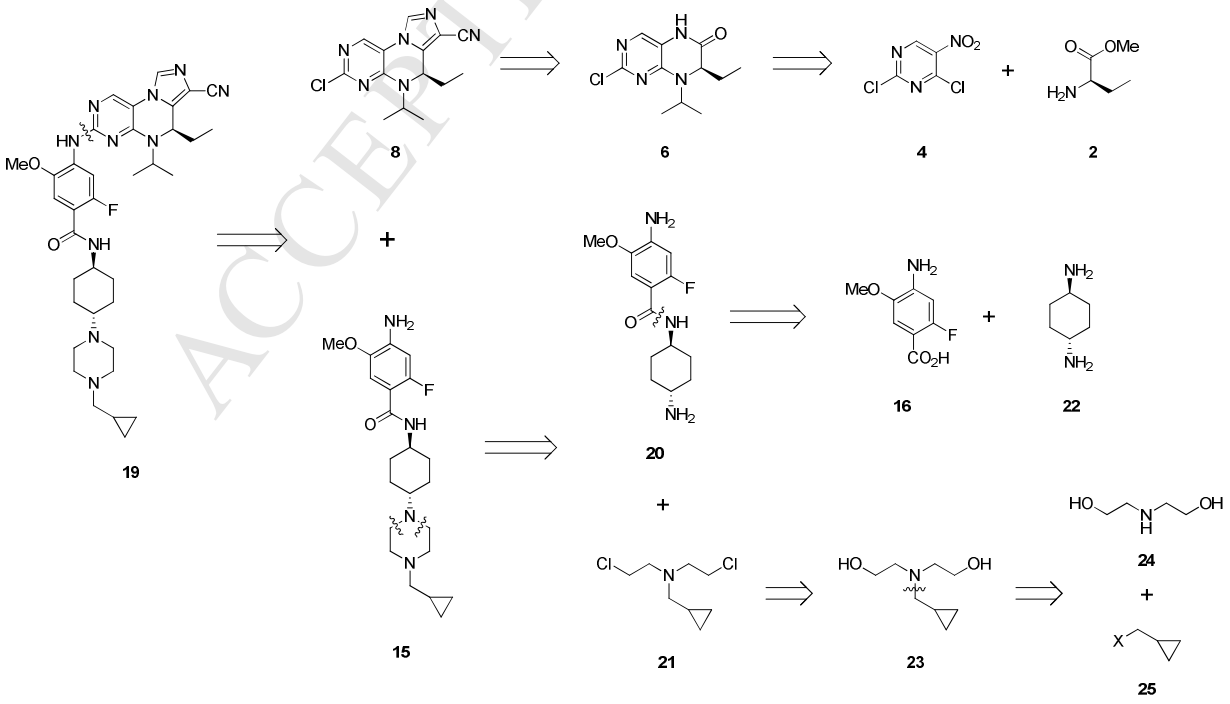
As a first step toward a future scale-up synthesis to supply **19** to preclinical/clinical studies, we initiated development of a practical column chromatography-free/HPLC separation-free synthesis of **19** that addresses these three key issues. Retrosynthetic analysis of **19** is shown in Scheme 3. Since it is known that a convergent synthesis is generally preferable from a standpoint of cost and efficiency,[6] we planned to conduct the coupling reaction of **8** with **15** in the last step, and to obtain **19** in a convergent manner. Compound **8** was expected to be synthesized from **2** and **4** as in the medicinal chemistry synthesis, though the synthesis was modified for scale-up, including revisit of reaction conditions for the preparation of **8** from **6**. For the synthesis of **15**, we adopted another synthetic route that makes use of defined *trans* stereocenters of commercially available *trans* diamine **22**.[7] Aniline **15** was envisioned to be synthesized from **20** and **21** via piperazine ring formation. Aniline **20** was anticipated to be synthesized from aniline **16** and **22**, and **21** was envisaged to be prepared by chlorination of diol **23** synthesized by alkylation of diol **24** with **25**.

2. Results and Discussion

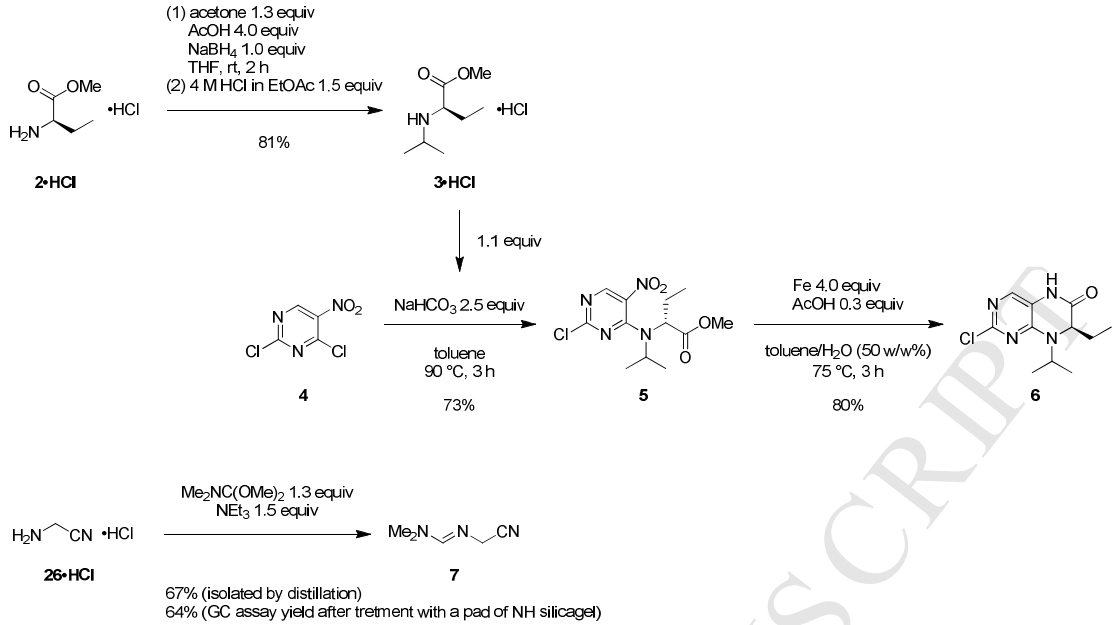
Lactam **6** was prepared from commercially available **2·HCl** and **4** with a modified procedure (Scheme 4).[8] The reductive amination of **2·HCl** with acetone using $NaBH_4$ instead of expensive $NaBH(OAc)_3$ as the reducing agent proceeded smoothly, and **3** was isolated as a hydrochloric acid salt in 81% yield. The subsequent S_NAr reaction of **4** with **3·HCl** in toluene using $NaHCO_3$ as the base gave **5** in 73% yield.

For preparation of **6**, reduction of the nitro group of **5** using inexpensive iron with acetic acid was examined to avoid the use of an expensive Pt catalyst[9]. When the reduction was conducted using acetic acid as the solvent, rapid and huge exotherm was observed. During the course of investigation, addition of a catalytic amount of AcOH was found to be sufficient for completion of the reaction. The use of a catalytic amount of AcOH decreased the reaction rate, which made the reaction proceed without rapid exotherm. Also, it is noteworthy that addition of a small amount of water was effective for improving fluidity of the reaction mixture, presumably because water helped acetic acid salt of **6** to dissolve into the reaction mixture. The reaction was carried out in the presence of 4.0 equiv of iron, 0.3 equiv of acetic acid, and water (50 w/w% to **5**) in toluene at 75 °C for 3 h, leading to the reduction of the nitro group and the cyclization to provide **6** without notable exotherm. Upon completion of the reaction, 6 M HCl was added to the reaction mixture to dissolve iron. Following extraction with EtOAc, and washing with water, brine, and saturated aqueous $NaHCO_3$, lactam **6** was crystallized from $EtOH/H_2O$ and isolated in 80% yield.

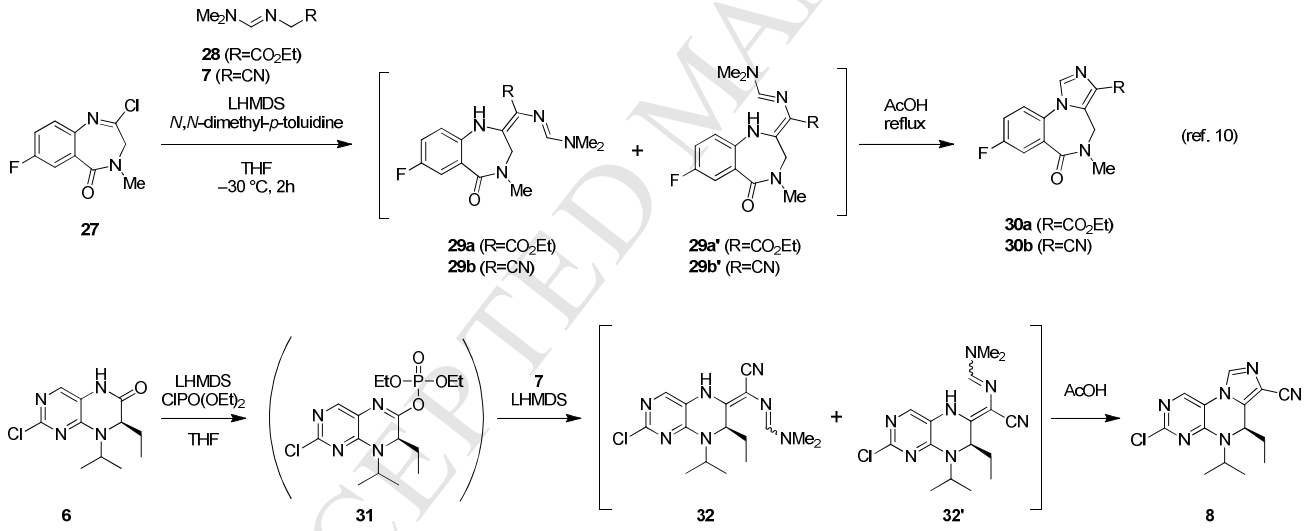
Compound **7**[3a, 10] was prepared by the reaction of aminoacetonitrile hydrochloride **26·HCl** with *N*,*N*-dimethylformamide dimethyl acetal in the presence of triethylamine in THF, and isolated in 67% yield by distillation (5.6 mmHg, 90 °C). During investigation into a workup procedure, we found that treatment with a pad of NH silica gel was effective for removing byproducts generated during the reaction. After the reaction was completed, the solvent was switched from THF to diisopropyl ether, and the mixture was passed through a pad of NH silica gel (50 w/w% to **26·HCl**), which removed precipitated triethylamine hydrochloride salt and brown tarry byproducts. Following evaporation of the solvent, **7** was obtained in 64% yield. It was possible to use the obtained **7** for the next reaction with **6** without further purification, which allowed us to avoid time-consuming distillation.



Scheme 3. Retrosynthetic analysis of **19**.



Scheme 4. Synthesis of **6** and **7**.



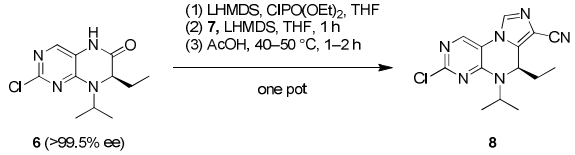
Scheme 5. Precedent work and synthesis of **8** from **6**.

Next, the preparation of **8** from **6** was investigated. There have been some precedents for this interesting imidazole ring formation reaction (Scheme 5). Rogers-Evans and co-workers reported cyanoimidazole ring formation from an iminochloride.[10] Addition of an anion of **28**/**7** generated by LHMDS to iminochloride **27** gave intermediates **29** and **29'**, and the subsequent acid-promoted isomerization of **29** to **29'** and cyclization provided **30**.[11] Fryer et al. reported that addition of an anion of methyl ester analogue of **28** to an iminophosphate gave a methoxycarbonyl imidazole.[12] The medicinal chemists used these chemistries in a very excellent manner and conducted the three reactions (phosphorylation, addition of **7**, and isomerization/cyclization) in one pot. In these works, addition of the anions to the iminochloride/iminophosphate was conducted at –78 °C or at –30 °C, which largely restrict a manufacturing facility for scale-up. To confirm whether this reaction could be carried out under more benign conditions, we began by examining the reaction conditions closely (Table 1).

When we conducted the addition of the anion of **7** to **31** at −70 °C, **8** was obtained in 56% yield (entry 1). To our surprise, when the temperature for the addition of the anion of **7** was increased to −35 °C and −5 °C, the yield increased to 72% and 77%, respectively (entries 2, 3). Next, we conducted the phosphorylation of **6** at −5 °C. Under this condition, **8** was obtained in good yield (71%, entry 4) comparable to the yield of entry 3, where the phosphorylation was conducted at −70 °C.[13]

Under theses reaction conditions, phosphorylation of **6** did not reach complete conversion, and 5–10% (HPLC area%) of **6** remained unreacted. On the basis of these results, we concluded that both the phosphorylation and the addition of the anion of **7** could be performed at −5 °C. Fixing the temperatures for the phosphorylation and the addition of the anion of **7** at −5 °C, we

Table 1 Synthesis of **8** from **6**.



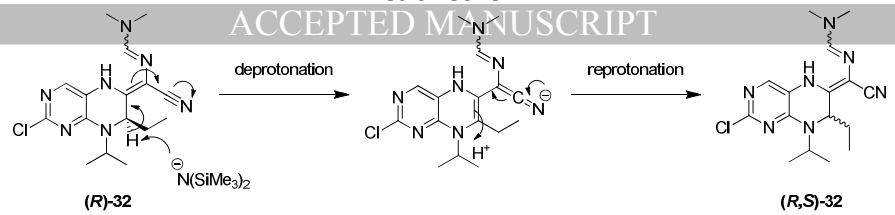
entry	phosphorylation				addition of **7**			yield[a] (%)	optical purity (% ee)
	LHMDS (equiv)	$ClPO(OEt)_2$ (equiv)	temp (°C)	time (h)	**7** (equiv)	LHMDS (equiv)	temp (°C)		
1[b]	1.1	2	−70 → 0	2.5	3	3	−70	56	-
2[b]	1.1	2	−70 → 0	2.5	3	3	−35	72	-
3[b]	1.1	2	−70 → 0	2.5	3	3	−5	77	-
4	1.1	2	−5	1	3	3	−5	71	99.8
5	1.1	2	−5	1	2	3	−5	74	99.8
6	1.1	1.5	−5	1	3	3	−5	74	99.6
7	1.1	1.5	−5	1	2	3	−5	75	97.3
8	1.1	1.5	−5	1	2	2.5	−5	60	99.8
9	1.1	1.5	−5	1	2	2	−5	44	-
10	1.1	1.2	−5	1	2	3	−5	56	98.5

[a]Isolated yield. [b]Compound **7** isolated by distillation was used for the reaction.

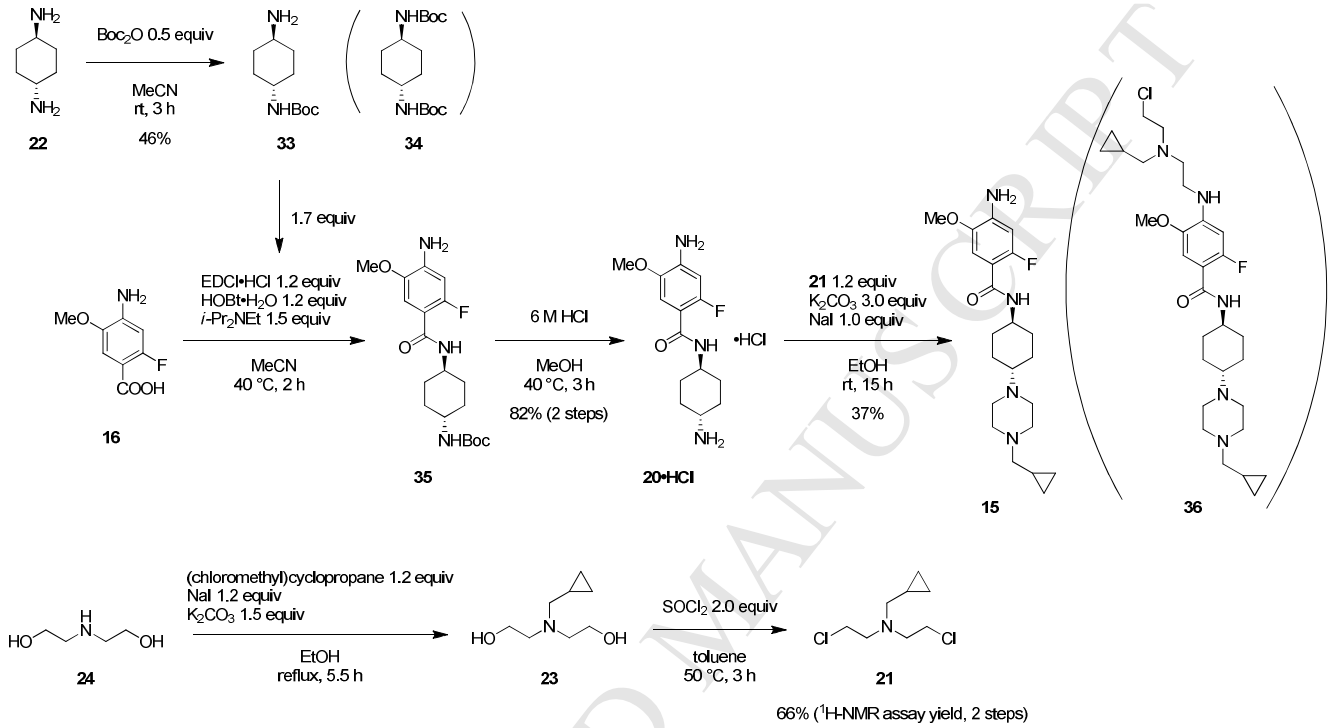
then tried to decrease the amounts of reagents. Whereas decrease of the amount of diethyl chlorophosphate from 2.0 equiv (entry 5) to 1.5 equiv did not affect the yield (75%, entry 7), decrease to 1.2 equiv caused large yield deterioration (56%, entry 10). Decrease of the amount of **7** from 3.0 equiv (entry 4) to 2.0 equiv was found to be acceptable (74%, entry 5). In contrast, decrease of the amount of LHMDS used for the deprotonation of **7** had crucial effects on the yields. When the amount of LHMDS for the deprotonation of **7** was decreased from 3.0 equiv (entry 7) to 2.5 equiv and 2.0 equiv, the yield decreased to 60% and 44%, respectively (entries 8, 9). Isolation of **8** was conducted by crystallization, and typically 3–5% (HPLC assay yield) of **8** was included in mother liquor.

After screening the reaction conditions, we analyzed optical purity of the obtained **8** and found that the optical purity of **8** prepared under the reaction conditions of entry 7 and entry 10 decreased to 97.3% ee and 98.5% ee, respectively. A plausible mechanism for the optical purity deterioration is described in Scheme 6. It is assumed that a strong base such as LHMDS could bring about deprotonation at the γ position of the cyano group of **32** at −5 °C to give an anion, and reprotonation of the anion would lead to the optical purity decrease. Under the reaction conditions of entry 7 and entry 10, the total amounts of diethyl chlorophosphate and **7** were less than the total amounts under the conditions of entries 4, 5, and 6. This situation would result in an increased amount of remaining active LHMDS in the reaction mixture and would cause the deprotonation of **32**, leading to the optical purity decrease of **8**. We also examined the possibility of racemization of compound **6** by treating it with 1.1 equiv of LHMDS in THF at –5 °C for 5 h. A small portion of the reaction mixture was quenched with MeCN/20 mM aqueous KH_2PO_4 (30:70) and analyzed by chiral HPLC, which showed that the optical purity of **6** (>99.7% ee) did not change during 5 h under this reaction condition. Considering these results, the reaction conditions of entry 5 were finally selected as the best reaction conditions.

Aniline **15** was prepared by the synthesis described in Scheme 7. Mono *N*-Boc protected *trans* diamine **33**[14] was prepared from commercially available *trans*-cyclohexane-1,4-diamine **22** by treatment with Boc_2O in MeCN. The obtained crude **33** containing di-Boc byproduct **34** (approximately 3:1) was used for condensation with **16** without purification. The condensation of **16** with **33** was carried out using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) and 1-hydroxybenzotriazole monohydrate ($HOBt·H_2O$) in MeCN, giving **35** exclusively without self-condensation of **16**. Addition of water to the reaction mixture led to crystallization, which enabled isolation of **35** by filtration. The obtained **35** was dissolved in MeOH, and the Boc group was deprotected with 6 M HCl. After pH was adjusted to 7.8 with 8 M NaOH, the mixture was treated with activated carbon. Following evaporation of MeOH, filtration of the resulting aqueous slurry gave **20·HCl** in 82% yield for 2 steps from **16**. The di-Boc byproduct **34** was completely removed during isolation of **20·HCl**.



Scheme 6. Plausible mechanism for optical purity decrease of **8**.



Scheme 7. Synthesis of **15**.

Compound **21** was synthesized via alkylation of **24** with (chloromethyl)cyclopropane followed by chlorination of the hydroxyl groups. Diol **24** was reacted with (chloromethyl)cyclopropane in the presence of NaI and K_2CO_3 in refluxing EtOH. After evaporation of EtOH, **23** was extracted with EtOAc and washed with saturated brine, and the solvent was switch to toluene. The obtained toluene solution of **23** was treated with 2.0 equiv of $SOCl_2$ at 50 °C for 3 h. On completion of the reaction, the reaction mixture was neutralized with slow addition of 8 M NaOH. Following phase separation, filtering off insoluble matter with a pad of silica gel, and evaporation of the solvent, the obtained **21** (66% for 2 steps from **24**, determined by ^{1}H NMR) was used for the piperazine ring formation without further purification.

There have been many examples of piperazine ring formation from a primary amine by a reaction with bis(2-chloroethyl)amine derivatives.[15] However, preparation of a piperazine ring from an unsymmetrical primary diamine by reacting one of the primary amino groups selectively with bis(2-chloroethyl)amine derivatives without protection seems to be difficult. To the best of our knowledge, there has been only one report (patent) that includes this selective piperazine ring formation from an unsymmetrical primary diamine, and the reported yield was quite low (12%).[16] We thought that protection of the aromatic amino group of **20·HCl** was not indispensable for the reaction with **21**, considering difference of nucleophilicity between an aromatic amino group and an alkyl amino group.

For the synthesis of **15** from **20·HCl** by the selective piperazine ring formation, we screened several bases ($NaHCO_3$, K_2CO_3, NaOAc, *N*,*N*-diisopropylethylamine) and solvents (EtOH, THF, acetone, MeCN) using 1.2 equiv of **21** at 50 °C for 3 h. The reaction was rather slow, and in addition, generation of many byproducts was observed, which resulted in low conversion of the reaction. Among the screened solvents, EtOH was found to be the best solvent; the conversion exceeded 50% only when EtOH was used as the solvent. As for the bases, K_2CO_3 and *N*,*N*-diisopropylethylamine gave good results. The best result was achieved when K_2CO_3 and EtOH were used for the reaction with NaI as an additive. The S_N2 reaction of **20·HCl** with **21** using 1.0 equiv of NaI and 3.0 equiv of K_2CO_3 in EtOH mainly occurred not at the aromatic amino group, but at the alkyl amino group on the cyclohexyl ring, and provided **15** as expected. During the reaction, generation of several byproducts was observed, and HPLC assay yield was found to be moderate (67%). Dialkylated product **36** (the structure was suggested by LC-MS analysis) was identified as the most notable byproduct for this reaction, and even under the best reaction condition, approximately 17% (HPLC area%) of **36** was observed. After workup, **15** was once isolated as a hydrochloric acid salt, and then dissolved in water and treated with activated carbon. The activated carbon was

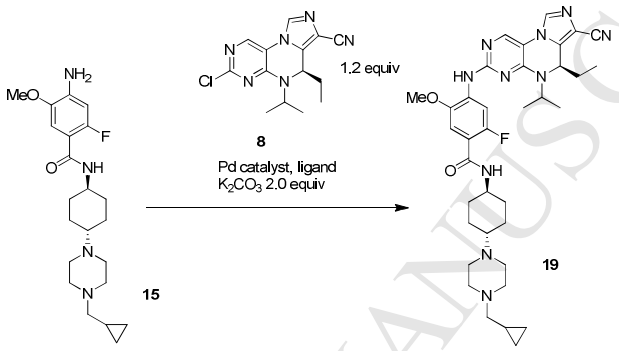
filtered off, and pH was adjusted to 9.0 with 25% aqueous NH_3. Filtration of the resulting slurry afforded pure **15** (**36**: 0.4 HPLC area%) in 37% isolated yield with 17% of **15** in the mother liquor.

Having obtained **8** and **15** in hand, the last coupling reaction of **8** with **15** was examined. Other than the patent filed by the medicinal chemists[3a], there has been only one report (patent) that describes a coupling reaction of 3-halo-5,6-dihydroimidazo[1,5-*f*]pteridine with an amine, and the reported yield was only moderate (70 mg scale reaction, 52%).[17] As described previously, the acid-promoted coupling reaction required long reaction time (>24 h) and the palladium-catalyzed amination needed high reaction temperature (microwave, 160 °C), and in addition, the yields were quite low; therefore, more benign reaction conditions that afford the coupling product in good yield are highly demanded for scale-up.

First, we tried to conduct the coupling reaction using a base; however, the reaction did not proceed well in a basic condition. When *N*,*N*-diisopropylethylamine was used for the reaction in *N*,*N*-dimethylacetamide (DMAC) at 100 °C, the reaction did not proceed at all. Additionally, when Cs_2CO_3 or *t*-BuOK was used for the reaction in DMAC at 100 °C, decomposition of **8** was mainly observed with a small amount of **19**. Thus, we turned our attention to the palladium-catalyzed amination[18] (Table 2) and the acid-promoted coupling reaction (Table 3).

Screening of palladium catalysts and ligands using *t*-BuOH as the solvent showed that the combination of $Pd(OAc)_2$ and Xantphos was good for the reaction (Table 2, entries 1–4). When the reaction was conducted in *t*-BuOH using 10 mol% of $Pd(OAc)_2$ and 10 mol% of Xantphos in the presence of 2.0 equiv of K_2CO_3, the reaction reached full conversion in 1 h. HPLC assay yield was 86%, and after workup, **19** was isolated in 61%

Table 2 Synthesis of **19** by palladium-catalyzed amination.



entry	catalyst (mol%)	ligand (mol%)	solvent	temp (°C)	time (h)	yield[a] (%)
1	Pd_2dba_3 (10)	Xphos (10)	*t*-BuOH	reflux	3	82
2	$Pd(OAc)_2$ (10)	Xantphos (10)	*t*-BuOH	reflux	1	86 (61)[b]
3	$Pd(dppf)Cl_2$ (10)	-	*t*-BuOH	reflux	4	35
4	$PdCl_2[P(o\text{-}Tol)_3]_2$ (10)	-	*t*-BuOH	reflux	4	<3
5	$Pd(OAc)_2$ (10)	Xantphos (10)	THF	reflux	5	57
6[c]	$Pd(OAc)_2$ (10)	Xantphos (10)	*s*-BuOH	reflux	3	81
7[c]	$Pd(OAc)_2$ (10)	Xantphos (10)	*s*-BuOH	85	1	90 (78)[b]
8[c]	$Pd(OAc)_2$ (1)	Xantphos (1)	*s*-BuOH	90	3	N.D.[d]

[a]HPLC assay yield. [b]Isolated yield in parentheses. [c]1.1 equiv of **8** was used. [d]Not detected.

yield (entry 2). Several kinds of other solvents were screened for the reaction. While the use of THF as the solvent decreased the yield (57%, entry 5), *s*-BuOH was found to be a good solvent for the reaction. HPLC assay yield reached 90%, and **19** was isolated in 78% yield (entry 7). Although we tried to decrease the amounts of $Pd(OAc)_2$ and Xantphos to 1 mol%, the reaction completely stalled (entry 8). This was probably because **8** and/or **15** contained a small amount of impurities that deactivated the palladium catalyst.

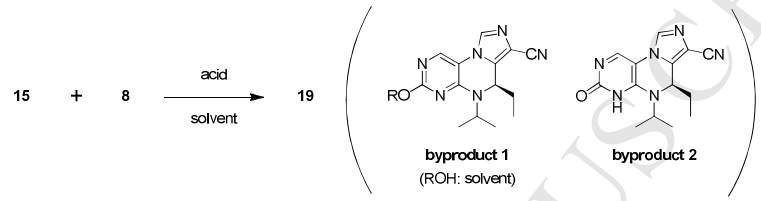
In preliminary experiments of the acid prompted coupling reaction of **8** with **15**, several kinds of acids (concentrated HCl, MsOH, $TsOH{\cdot}H_2O$, H_2SO_4, H_3PO_4, 6 M HCl, 48% aqueous HBr, AcOH) and solvents (alcohols, DMF, DMAC, *N*-methylpyrrolidone, toluene, dioxane, *n*-BuOAc) were screened. Among the screened solvents, alcohols gave relatively good conversion. Whereas the reaction in 2-propanol (bp: 82 °C) with concentrated HCl was very slow and reached as little as 6% conversion after 8 h at reflux condition (Table 3, entry 1), it was found that the use of MsOH and $TsOH{\cdot}H_2O$ largely promoted the conversion. When MsOH and $TsOH{\cdot}H_2O$ were used for the reaction, the conversion reached 44% and 58% after 8 h, respectively (entries 2, 3). To achieve faster conversion, alcohols that have a higher boiling point than 2-propanol were used for the reaction. When the reaction was conducted in primary alcohol *i*-BuOH (bp: 108 °C), a large amount of byproduct was formed by addition of *i*-BuOH to **8** (byproduct 1), and a small amount of hydrolyzed form of **8** (byproduct 2) was observed, which resulted in moderate conversion (48%, entry 4). Although tertiary alcohol *t*-amylalcohol (bp: 102 °C) did not give byproduct 1, the reaction in *t*-amylalcohol was not so fast and conversion after 8 h was also moderate (59%, entry 5). Fortunately, secondary alcohol *s*-butanol (bp: 100 °C) was found to be a good solvent for this reaction. The reaction in *s*-butanol with $TsOH{\cdot}H_2O$ reached up to

81% conversion after 8 h (entry 6). Furthermore, increase of the amount of **8** to 2.0 equiv and 2.4 equiv led to higher conversions (entries 7, 8). We also confirmed that the reaction did not proceed in *s*-BuOH without acid (entry 9).

In the initial screening of acids, concentrated HCl was not found to be effective for the reaction (entry 1, 10). During the investigation, we happened to find that the reaction employing concentrated HCl as the acid was largely accelerated by addition of a small amount of water. When the reaction was carried out in *s*-BuOH/H_2O (95:5) using 3.0 equiv of concentrated HCl, the reaction speed largely increased and became comparable to that of the reaction using TsOH·H_2O (entry 11, 12). We presume that addition of a small amount of water helps hydrochloric acid salt of **15** formed by addition of concentrated HCl to dissolve in the solvent, which led to acceleration of the reaction. Finally, the reaction conditions of entry 12 were selected as the best conditions, taking into account that complete removal of 3.0 equiv of TsOH was found not to be easy.

Isolation of pure **19** required elaborated workup procedures. On completion of the reaction, *s*-butanol was evaporated, and the product was extracted with 1 M HCl and washed with EtOAc. After pH of the aqueous extract was adjusted to approximately 9 with 8 M NaOH, the product was extracted with EtOAc and washed with 5% aqueous $NaHCO_3$. Following solvent switch to 2-propanol, **19** was crystallized from 2-propanol/*n*-heptane (1:3). The obtained crude product was dissolved in MeOH and treated with activated carbon (Darco® G-60) at 50 °C, which was very effective for removing red color of the crude product. The activated carbon was filtered off, and solvent was switched to 2-propanol, and crystallization from 2-propanol gave pure **19** in 71% yield as an off-white solid (entry 12). The obtained **19** was

Table 3 Synthesis of **19** by acid-promoted coupling reaction.



entry	**8** (equiv)	acid (equiv)	solvent	temp (°C)	time (h)	conversion (%)	yield[a] (%)
1[b]	0.83	c.HCl (3.0)	2-propanol (bp: 82 °C)	reflux	8	6	-
2	1.5	MsOH (3.0)	2-propanol	reflux	8	44	-
3	1.5	TsOH (3.0)	2-propanol	reflux	8	58	-
4	1.5	TsOH (3.0)	*i*-BuOH (bp: 108 °C)	reflux	8	48	-
5	1.5	TsOH (3.0)	*t*-amylalcohol (bp: 102 °C)	reflux	8	59	-
6	1.5	TsOH (3.0)	*s*-BuOH (bp: 100 °C)	reflux	8	81	-
7	2.0	TsOH (3.0)	*s*-BuOH	reflux	10	89	85 (56)[c]
8	2.4	TsOH (3.0)	*s*-BuOH	reflux	13	88	88 (60)[c]
9	1.5	-	*s*-BuOH	reflux	8	N.D.[d]	-
10	2.0	c.HCl (3.0)	*s*-BuOH	reflux	8	26	-
11	2.0	c.HCl (3.0)	*s*-BuOH/H_2O 95:5	reflux	8	79	-
12	2.4	c.HCl (3.0)	*s*-BuOH/H_2O 95:5	reflux	11	87	86 (71)[c]

[a]HPLC assay yield. [b]Reagents were used based on the amount of **8** (**15**: 1.2 equiv, c.HCl: 3.0 equiv). [c]Isolated yield in parentheses. [d]Not detected.

analyzed by chiral SFC, which showed that the obtained **19** did not contain three other potential stereoisomers ((*S*)-enantiomer, (*R*)-cis isomer, (*S*)-cis isomer).

3. Conclusions

A practical chromatography-free synthesis of **19** was successfully developed. We studied cyanoimidazole ring formation closely and showed that the cryogenic condition was not imperative for the reaction provided that appropriate care was taken for the potential epimerization of **8**. This result will allow us to conduct this reaction on a large scale using a normal manufacturing facility. An alternative synthesis of **15** utilizing the advantage of the defined stereochemistry of commercially available *trans* diamine **22** was also developed. The piperazine ring was successfully constructed from primary diamine **20·HCl** without protection. Although the piperazine ring formation requires some improvements to achieve higher yield, this synthesis provided **15** in a simple way without concern for generation of stereoisomers. The last coupling reaction of 3-chloro-5,6-dihydroimidazolo[1,5-*f*]pteridine **8** with aniline **15** was scrutinized to find feasible reaction conditions and to improve the low yields of the medicinal chemistry synthesis. Consequently, much higher yields were achieved both by the palladium-catalyzed amination and the acid-promoted coupling under benign reaction conditions. Although the acid-promoted coupling seems to be preferable for scale-up from the viewpoint of cost at this point, future development of the palladium-catalyzed amination might enable further decrease of the amount of a palladium catalyst and render the palladium-catalyzed amination a more cost-effective procedure. The obtained **19** did not include stereoisomers, and column chromatography/HPLC separation was not required. These findings will lead to a successful future scale-up synthesis and development of a potent anticancer drug candidate **19**, and at the same time, will

contribute to syntheses of related 5,6-dihydroimidazolo[1,5-*f*]pteridine derivatives.

4. Experimental section

4.1 General

All materials were purchased from commercial suppliers and used without any additional purification. NH silica gel (CHROMATOREX) was purchased from Fuji Silysia Chemical Ltd. Silica gel (Silica Gel 60, spherical) was purchased from Nacalai Tesque, Inc. Celite® (No. 500) and DARCO® (G-60) were purchased from Wako Pure Chemical Industries, Ltd. Melting points were determined on a Stanford Research Systems OptiMelt MPA 100, and are uncorrected unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 600 spectrometer or a BRUKER AVANCE 500 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are shown in ppm. HPLC analysis of the compounds and reaction monitoring was carried out on a Shimadzu LC-2010C$_{HT}$. High-resolution mass spectrometry (HRMS) data was obtained on a Shimadzu Prominence UFLC system with a Thermofisher LTQ Orbitrap Discovery. IR spectra were recorded on a Thermo Electron FT-IR Nicolet 4700 (ATR). Data of elemental analyses, data of melting points for **35** and **20·HCl** (determined by differential scanning calorimetry: DSC), data of HRMS for **7**, **23** and **21**, and IR spectra of **7**, **35**, **20·HCl**, **23**, **21** and **19** were obtained by Sumika Chemical Analysis Service, Ltd.

4.2 HPLC, Chiral HPLC, chiral SFC, and GC conditions

HPLC conditions. (A) Inertsil ODS-3 column, 5 µm, 150 mm × 4.6 mm i.d.; UV detector at 254 nm; isocratic elution with MeCN/50 mM aqueous KH_2PO_4 (50:50) at 1.0 mL/min flow rate; column temperature: 25 °C. Retention times: **6** (4.0 min), **31** (10.2 min).

(B) YMC A-313 column, 5 µm, 250 mm × 6.0 mm i.d.; UV detector at 254 nm; isocratic elution with MeCN/10 mM aqueous $AcONH_4$ (40:60) at 1.0 mL/min flow rate; column temperature: 25 °C. Retention times: **15** (8.3 min), **20·HCl** (5.4 min), **36** (41 min).

Chiral HPLC conditions. (A) CHIRALCEL OJ-RH, 5 µm, 150 mm × 4.6 mm i.d.; UV detector at 254 nm; isocratic elution with MeCN/20 mM aqueous KH_2PO_4 (40:60) at 1.0 mL/min flow rate; column temperature: 25 °C. Retention times: **5** (11.8 min), ***ent*-5** (15.1 min).

(B) CHIRALCEL OJ-RH, 5 µm, 150 mm × 4.6 mm i.d.; UV detector at 254 nm; isocratic elution with MeCN/20 mM aqueous KH_2PO_4 (30:70) at 1.0 mL/min flow rate; column temperature: 25 °C. Retention times: **6** (8.2 min), ***ent*-6** (5.7 min).

(C) CHIRALCEL OD-RH, 5 µm, 150 mm × 4.6 mm i.d.; UV detector at 254 nm; isocratic elution with MeOH at 0.7 mL/min flow rate; column temperature: 25 °C. Retention times: **8** (5.0 min), ***ent*-8** (6.5 min).

Chiral SFC condition. CHIRALPAK AD-H, 5 µm, 250 mm × 4.6 mm i.d.; UV detector at 220 nm; isocratic elution with CO_2/EtOH/diethylamine (700:300:3) at 3.0 mL/min flow rate; pressure: 100 bar; column temperature: 35 °C. Retention times: **19** (20.3 min), ***ent*-19** (13.3 min), cis isomer of **19** (9.3 min), cis isomer of ***ent*-19** (7.7 min).

GC condition. SUPELCO SPB®-5, Capillary GC Column, 30 m × 0.53 mm i.d., 5 µm film; FID detector; He carrier gas (approximately 6 mL/min); oven heating 90 °C for 5 min, 3 °C/min to 140 °C, 30 °C/min to 200 °C for 5 min. Retention time: **7** (19.7 min).

*4.3 Methyl (2R)-2-(Propan-2-ylamino)butanoate Hydrochloride (1:1) (**3·HCl**)*

To a mixture of methyl (2*R*)-2-aminobutanoate hydrochloride (1:1) (**2·HCl**) (14.7 g, 95.7 mmol) in THF (59 mL) were added acetic acid (23.0 g, 383 mmol) and acetone (7.23 g, 124 mmol), and the mixture was stirred at 20–30 °C for 1 h under N_2 atmosphere. To the mixture was added sodium borohydride (3.62 g, 95.7 mmol) portionwise at 10 °C, maintaining the temperature below 20 °C. After being stirred at 20–30 °C for 2 h, the mixture was cooled to 0–10 °C, and H_2O (44 mL) was slowly added. To the mixture was added 8 M NaOH (54 mL), and pH was adjusted to 9.0–9.5, maintaining the temperature below 30 °C. Toluene (74 mL) was added, and the layers were separated. The organic layer was filtered and insoluble matter was washed with toluene (7 mL). To the combined filtrate were added seed crystals of **3·HCl** (15.0 mg) at 0–10 °C. To the mixture was added 4 M HCl in EtOAc (35.9 mL, 144 mmol) dropwise, and the mixture was stirred at 0–10 °C for 1 h. The resulting solids were collected by filtration and washed with EtOAc (29 mL) and dried *in vacuo* at 50 °C to give **3·HCl** (15.1 g, 81%) as a white solid. Mp 155–157 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.92 (t, *J* = 7.6 Hz, 3H), 1.28 (dd, *J* = 6.4, 4.2 Hz, 6H), 1.89 (dquin, *J* = 14.4, 7.5 Hz, 1H), 1.98–2.08 (m, 1H), 3.28 (br s, 1H), 3.78 (s, 3H), 4.04 (br s, 1H), 9.18 (br s, 1H), 9.87 (br s, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 9.2, 18.1, 19.1, 22.6, 49.0, 52.8, 57.3, 169.3; IR (ATR) 2969, 2650, 2504, 1736, 1561, 1470, 1436, 1389, 1314, 1264, 1219, 1165, 1117, 1011, 959, 924, 852, 798, 753, 653, 535, 455 cm^{-1}; Anal. Calcd for $C_8H_{18}ClNO_2$: C, 49.10; H, 9.27; Cl, 18.12; N, 7.16; O, 16.35. Found: C, 49.08; H, 9.03; N, 7.35.

*4.4 Methyl (2R)-2-[(2-Chloro-5-nitropyrimidin-4-yl)(propan-2-yl)amino]butanoate (**5**)*

To a mixture of 2,4-dichloro-5-nitropyrimidine (**4**) (50.0 g, 258 mmol) and **3·HCl** (55.5 g, 284 mmol) in toluene (500 mL) was added $NaHCO_3$ (54.1 g, 644 mmol), and the mixture was stirred at 85–95 °C for 3 h under N_2 atmosphere. After cooling to room temperature, the mixture was filtered and insoluble matter was washed with toluene (50 mL). To the combined filtrate was added H_2O (250 mL), and the layers were separated. To the organic layer were added EtOAc (250 mL) and 1 M HCl (250 mL), and the layers were separated. The organic layer was concentrated *in vacuo* until the weight of the mixture became approximately 100 g. To this mixture was added 2-propanol (250 mL), and the mixture was concentrated *in vacuo* again until the weight of the mixture became approximately 100 g. To the resulting mixture were added 2-propanol (200 mL) and seed crystals of **5** (50.0 mg), and the mixture was stirred at 20–30 °C for 1 h. The mixture was cooled to 0–10 °C and stirred for 1 h, and then filtrated. Wet solids were washed with 2-propanol/H_2O (1:1, 100 mL) and dried *in vacuo* at 50 °C to give **5** (59.7 g, 73%) as a yellow solid. Mp 89–91 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.07 (t, *J* = 7.6 Hz, 3H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.36 (d, *J* = 6.4 Hz, 3H), 1.86–2.04 (m, 1H), 2.37–2.56 (m, 1H), 3.55 (dt, *J* = 13.1, 6.5 Hz, 1H), 3.75 (s, 3H), 3.78 (t, *J* = 6.8 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 12.0, 19.5, 21.6, 23.2, 52.5, 53.7, 58.8, 131.0, 153.5, 156.5, 159.4, 170.8; IR (ATR) 1739, 1572, 1543, 1513, 1466, 1439, 1374, 1345, 1311, 1215, 1197, 1180, 1165, 1084, 993, 912, 866, 767, 559, 445 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{12}H_{18}ClN_4O_4$, 317.1011; found, 317.1008. Optical purity: 99.9% ee (chiral HPLC condition A).

*4.5 (7R)-2-Chloro-7-ethyl-8-(propan-2-yl)-7,8-dihydropteridin-6(5H)-one (**6**)*

To a mixture of **5** (20.0 g, 63.1 mmol) and reduced iron (14.1 g, 253 mmol) in toluene (100 mL) were added AcOH (1.14 g, 18.9 mmol) and H_2O (10 mL) in this order, and the mixture was stirred at 70–80 °C for 3 h under N_2 atmosphere. To the mixture was added 6 M HCl (100 mL) with vigorous stirring, and the mixture was stirred at 70–80 °C for 1 h. After cooling to room temperature, the mixture was filtered and insoluble matter was washed with H_2O (40 mL) and EtOAc (40 mL). To the combined filtrate were added EtOAc (160 mL) and H_2O (60 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 200 mL), and the combined organic layer was washed with 10% aqueous NaCl (2 × 100 mL) and saturated aqueous $NaHCO_3$ (200 mL). The organic layer was concentrated *in vacuo* until the weight of the mixture became approximately 60 g. EtOH (100 mL) was added and the mixture was concentrated *in vacuo* until the weight of the mixture became approximately 60 g. EtOH (100 mL) was added and the mixture was concentrated *in vacuo* again until the weight of the mixture became approximately 60 g. To the resulting mixture was added EtOH (20 mL), and H_2O (90 mL) was added dropwise. The mixture was cooled to 0–10 °C and stirred for 1 h, and then filtrated. Wet solids were washed with EtOH/H_2O (1:2, 40 mL) and dried *in vacuo* at 50 °C to give **6** (12.7 g, 80%) as a white solid. Mp 200–201 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.6 Hz, 3H), 1.37 (d, *J* = 6.8 Hz, 3H), 1.41 (d, *J* = 6.8 Hz, 3H), 1.81 (dt, *J* = 14.5, 7.5 Hz, 1H), 1.99 (ddd, *J* = 14.5, 7.5, 3.0 Hz, 1H), 4.28 (dd, *J* = 7.4, 3.2 Hz, 1H), 4.59 (spt, *J* = 6.8 Hz, 1H), 7.71 (s, 1H), 9.80 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 8.6, 19.8, 20.9, 27.7, 49.2, 58.6, 117.8, 139.1, 151.9, 154.4, 165.8; IR (ATR) 3229, 2964, 1692, 1655, 1604, 1476, 1410, 1365, 1269, 1237, 1194, 1155, 1127, 1088, 1002, 932, 773, 688, 558, 411, 401 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{11}H_{16}ClN_4O$, 255.1007; found, 255.1007. Optical purity: 99.4% ee (chiral HPLC condition B).

*4.6 N'-(Cyanomethyl)-N,N-dimethylimidoformamide (**7**)*

To a mixture of aminoacetonitrile hydrochloride (1:1) (**26·HCl**) (10.0 g, 108 mmol) in THF (100 mL) were added *N*,*N*-dimethylformamide dimethyl acetal (16.7 g, 141 mmol) and triethylamine (16.4 g, 162 mmol), and the mixture was stirred at 45–55 °C for 5 h. After cooling to room temperature, the mixture was concentrated *in vacuo* until the weight of the mixture became approximately 40 g. Diisopropyl ether (50 mL) was added and the mixture was concentrated *in vacuo* again until the weight of the mixture became approximately 40 g. To the resulting mixture was added diisopropyl ether (100 mL), and the mixture was passed through a pad of NH silica gel (100–200 mesh, 5.00 g), followed by washing with IPE (2 × 100 mL). The combined filtrate was concentrated *in vacuo* to give **7** (8.75 g, 88.1 w/w%, 64% GC assay yield) as a pale yellow oil. The obtained **7** was used for the next reaction without further purification. A small portion of the product was purified by distillation (5.6 mmHg, 90 °C) to obtain a sample for analysis. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.90 (br s, 6H), 4.18 (s, 2H), 7.44 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 34.6 (br s), 40.0 (br s), 41.5, 118.1, 157.5; IR (ATR) 2917, 1639, 1489, 1432, 1415, 1377, 1326, 1260, 1233, 1114, 1066, 1042, 1017, 984, 970, 903, 865 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_5H_{10}N_3$, 112.0869; found, 112.0875.

*4.7 (6R)-3-Chloro-6-ethyl-5-(propan-2-yl)-5,6-dihydroimidazo[1,5-f]pteridine-7-carbonitrile (**8**)*

To a mixture of **6** (5.00 g, 19.6 mmol) in THF (50 mL) was added LHMDS (1.1 M THF solution, 19.6 mL, 21.6 mmol) dropwise at −5 °C, and the mixture was stirred at −5 °C for 0.5 h under N_2 atmosphere. To the mixture was added diethyl chlorophosphate (6.76 g, 39.2 mmol) dropwise at −5 °C, and the mixture was stirred at −5 °C for 0.5 h. To the mixture was added **7** (4.96 g, 88.1 wt%, 39.3 mmol), and then LHMDS (1.1 M THF solution, 53.5 mL, 58.9 mmol) was added dropwise at −5 °C, and the mixture was stirred at −5 °C for 1 h. To the mixture was added AcOH (30 mL) dropwise, maintaining the temperature below 20 °C, and the mixture was stirred at 40–50 °C for 3 h. After cooling to room temperature, H_2O (50 mL), EtOAc (50 mL) and THF (50 mL) were added, and the layers were separated. The organic layer was washed with 1 M NaOH (3 × 50 mL) and H_2O (50 mL), and concentrated *in vacuo* until the weight of the mixture became approximately 30 g. EtOAc (50 mL) was added, and the mixture was concentrated *in vacuo* until the weight of the mixture became approximately 25 g. EtOAc (50 mL) was added and the mixture was concentrated *in vacuo* again until the weight of the mixture became approximately 25 g. To the mixture was added diisopropyl ether (75 mL) dropwise at 40–50 °C, and the mixture was stirred at 40–50 °C for 1 h. The mixture was gradually cooled to 0–10 °C and stirred for 1 h, and then filtrated. Wet solids were washed with diisopropyl ether (25 mL) and dissolved in THF (150 mL). To the mixture was added activated carbon (500 mg) at 40–50 °C, and the mixture was stirred at 40–50 °C for 1 h. Activated carbon was filtered off and washed with THF (10 mL). The combined filtrate was concentrated *in vacuo* until the weight of the mixture became approximately 25 g. EtOH (50 mL) was added and the mixture was concentrated *in vacuo* again until the weight of the solution became approximately 25 g. To the resulting mixture was added EtOH (20 mL), and then H_2O (50 mL) was added dropwise at 20–30 °C. The mixture was stirred at 20–30 °C for 1 h and then filtrated. Wet solids were washed with H_2O (25 mL) and dried *in vacuo* at 50 °C to give **8** (4.40 g, 74%) as a brown solid. Mp 252–253 °C (decomp); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.84 (t, *J* = 7.4 Hz, 3H), 1.44 (d, *J* = 6.8 Hz, 3H), 1.48 (d, *J* = 6.8 Hz, 3H), 1.84 (dquin, *J* = 14.6, 7.5 Hz, 1H), 1.97 (ddd, *J* = 14.4, 7.6, 3.4 Hz, 1H), 4.68 (quin, *J* = 6.9 Hz, 1H), 5.11 (dd, *J* = 8.3, 3.4 Hz, 1H), 8.02 (s, 1H), 8.36 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 8.6, 20.1, 20.8, 30.6, 50.6, 51.6, 109.9, 113.4, 114.3, 131.6, 133.1, 141.2, 152.8, 158.2; IR (ATR) 3106, 2970, 2938, 2223, 1605, 1536, 1492, 1419, 1402, 1377, 1362, 1341, 1331, 1236, 1142, 1114, 1081, 962, 755, 651, 586, 507, 463 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{16}ClN_6$, 303.1119; found, 303.1118. Optical purity: 99.8% ee (chiral HPLC condition C).

*4.8 tert-Butyl (trans-4-Aminocyclohexyl)carbamate (**33**)*[14]

To a mixture of *trans*-cyclohexane-1,4-diamine (**22**) (150 g, 1.31 mol) in MeCN (2.7 L) was added $(Boc)_2O$ (143 g, 657 mmol) in MeCN (300 mL) maintaining the temperature below 30 °C, and the mixture was stirred at 20–30 °C for 3 h. The resulting slurry was concentrated *in vacuo*. To the residue was added saturated aqueous $NaHCO_3$ (3 L), and the mixture was stirred at 20–30 °C for 1 h, and then filtrated. Wet solids were washed with H_2O (400 mL) and dried *in vacuo* at 50 °C to give **33** (**33**:**34** = approximately 3:1, 129 g, 46%) as a white solid. The obtained **33** was used for the next reaction without further purification.

*4.9 4-Amino-N-(trans-4-aminocyclohexyl)-2-fluoro-5-methoxybenzamide Hydrochloride (1:1) (**20·HCl**)*

To a mixture of 4-amino-2-fluoro-5-methoxybenzoic acid **16** (65.8 g, 355 mmol), **33** (129 g, 604 mmol), *N*,*N*-diisopropylethylamine (91.2 mL, 533 mmol) and 1-hydroxybenzotriazole monohydrate (65.2 g, 426 mmol) in MeCN (1.32 L) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (81.7 g, 426

mmol), and the mixture was stirred at 40 ˚C for 2 h. To the mixture was added H_2O (1.32 L) dropwise, and the mixture was stirred at 20–30 °C for 1 h, and then filtrated. Wet solids were washed with H_2O (526 mL) and dissolved in MeOH (1.32 L). To the mixture was added 6 M HCl (526 mL), and the mixture was stirred at 40 °C for 3 h. After cooling to 5 °C, 8 M NaOH (324 mL) was added dropwise and pH of the mixture was adjusted to 7.8, maintaining the temperature below 20 °C. To the mixture was added activated carbon (6.60 g), and the mixture was stirred at 20–30 °C for 0.5 h. Activated carbon was filtered off and washed with MeOH (132 mL). The combined filtrate was concentrated *in vacuo* to remove MeOH. The resulting slurry was stirred at 20–30 °C for 0.5 h, and then filtrated. Wet solids were washed with acetone (132 mL) and dried *in vacuo* at 50 °C to give **20·HCl** (92.0 g, 82% for 2 steps) as a brown solid. Mp 309 °C (decomp.) (DSC); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.29–1.53 (m, 4H), 1.84–1.92 (m, 2H), 2.01 (br d, J = 10.4 Hz, 2H), 2.92 (ddd, J = 11.4, 7.5, 4.1 Hz, 1H), 3.60–3.72 (m, 1H), 3.77 (s, 3H), 5.61 (s, 2H), 6.40 (d, J = 13.2 Hz, 1H), 7.03 (d, J = 6.9 Hz, 1H), 7.32–7.54 (m, 1H), 8.30 (br s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 29.6 (2C), 30.4 (2C), 47.6, 49.1, 56.3, 99.8 ($^2J_{CF}$ = 29.0 Hz), 108.3 ($^2J_{CF}$ = 13.9 Hz), 111.4 ($^3J_{CF}$ = 5.0 Hz), 142.4, 142.9 ($^3J_{CF}$ = 12.6 Hz), 156.0 ($^1J_{CF}$ = 239.4 Hz), 163.3 ($^3J_{CF}$ = 2.5 Hz); IR (ATR) 3428, 3310, 2926, 1622, 1607, 1541, 1510, 1319, 1256, 1217, 1169, 1022, 874, 853, 768, 729 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{21}FN_3O_2$ (free form), 282.1612; found, 282.1610. A sample of *tert*-butyl {*trans*-4-[(4-amino-2-fluoro-5-methoxybenzoyl)amino]cyclohexyl}carbamate (**35**) for analysis was prepared by the reaction of **20·HCl** with 1.0 equiv of Boc_2O in EtOAc/5% aqueous $NaHCO_3$. Data for **35**: Mp 248 °C (DSC); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22–1.38 (m, 4H), 1.44 (s, 9H), 2.01–2.08 (m, 2H), 2.08–2.16 (m, 2H), 3.45 (br s, 1H), 3.88 (s, 3H), 3.90–3.99 (m, 1H), 4.26 (s, 2H), 4.43 (br d, J = 6.3 Hz, 1H), 6.36 (d, J = 13.2 Hz, 1H), 6.50 (br dd, J = 15.0, 7.7 Hz, 1H), 7.44 (d, J = 6.9 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.4 (3C), 31.8 (2C), 32.1 (2C), 48.0, 49.0, 56.0, 79.2, 100.7 ($^2J_{CF}$ = 30.2 Hz), 108.9 ($^2J_{CF}$ = 11.3 Hz), 111.8 ($^3J_{CF}$ = 3.8 Hz), 141.1 ($^3J_{CF}$ = 12.6 Hz), 143.2, 155.3, 156.4 ($^1J_{CF}$ = 239.4 Hz), 163.1 ($^3J_{CF}$ = 2.5 Hz); IR (ATR) 3497, 3468, 3317, 2941, 1686, 1620, 1508, 1450, 1366, 1315, 1275, 1261, 1213, 1157, 1072, 1020, 766, 727, 652 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{19}H_{29}FN_3O_4$, 382.2137; found, 382.2131.

*4.10 2-Chloro-N-(2-chloroethyl)-N-(cyclopropylmethyl)ethanamine (**21**)*

To a mixture of 2,2'-iminodiethanol **24** (400 g, 3.80 mol) in EtOH (4 L) were added NaI (684 g, 4.57 mol), (chloromethyl)cyclopropane (413 g, 4.57 mol), and K_2CO_3 (789 g, 5.71 mol), and the mixture was stirred under reflux for 5.5 h. After cooling to room temperature, the resulting solids were filtered off and washed with EtOAc. The combined filtrate was concentrated *in vacuo*. To the residue were added EtOAc (8 L) and saturated brine (1.6 L), and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 4 L), and to the combined organic layer was added Na_2SO_4 (400 g, 2.82 mol), and the mixture was stirred at room temperature for 10 min. The mixture was filtrated and insoluble matter was washed with EtOAc. The combined filtrate was concentrated *in vacuo* to give 2,2'-[(cyclopropylmethyl)imino]diethanol **23** (472 g, pale yellow oil). The obtained **23** was used for the next reaction without further purification. To a solution of **23** (300 g, 1.88 mol) in toluene (3 L) was added thionyl chloride (448 g, 3.77 mol) dropwise maintaining the temperature below 10 °C, and the mixture was stirred at 50 °C for 3 h. After cooling to room temperature, the mixture was neutralized by slow addition of 8 M NaOH (1.2 L), and the layers were separated. To the organic layer were added Na_2SO_4 (500 g, 3.52 mol) and activated carbon (30 g), and the mixture was stirred at room temperature for 30 min. The mixture was filtrated and insoluble matter was washed with toluene, and the combined filtrate was concentrated *in vacuo*. To the residue was added toluene (300 mL), and the mixture was passed through a pad of silica gel, and insoluble matter was washed with toluene (200 mL). The combined filtrate was concentrated *in vacuo* to give **21** (343 g, 91.6 wt% determined by ^{1}H NMR using diphenylmethane as an internal standard, 66% for two steps from **24**, pale brown oil). The obtained **21** was used for the next reaction without further purification. A small portion of the obtained **21** was purified by distillation (4 mmHg, 88 °C) to obtain a sample for analysis. ^{1}H NMR (500 MHz, $CDCl_3$) δ; 0.06–0.19 (m, 2H), 0.47–0.59 (m, 2H), 0.85 (ttt, J = 8.1, 6.5, 5.0 Hz, 1H), 2.49 (d, J = 6.6 Hz, 2H), 2.96 (t, J = 7.3 Hz, 4H), 3.54 (t, J = 7.3 Hz, 4H); ^{13}C NMR (126 MHz,$CDCl_3$) δ 3.9 (2C), 9.2, 42.0 (2C), 56.5 (2C), 59.8; IR (ATR) 3079, 3001, 2960, 2817, 1462, 1447, 1299, 1251, 1102, 1051, 1019, 936, 828, 802, 765, 726, 661 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_8H_{16}Cl_2N$, 196.0654; found, 196.0630. A small portion of the obtained **23** was purified by distillation (3 mmHg, 134 °C) to obtain a sample for analysis. Data for **23**: ^{1}H NMR (500 MHz, $CDCl_3$) δ; 0.08–0.16 (m, 2H), 0.45–0.60 (m, 2H), 0.89 (ttt, J = 8.1, 6.6, 5.0 Hz, 1H), 2.45 (d, J = 6.6 Hz, 2H), 2.73–2.79 (m, 4H), 3.57–3.71 (m, 4H); ^{13}C NMR (126 MHz,$CDCl_3$) δ 4.0 (2C), 8.8, 56.0 (2C), 59.6, 59.6 (2C); IR (ATR) 3347, 2939, 2876, 2820, 1033, 888, 873, 828 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_8H_{18}NO_2$, 160.1332; found, 160.1335.

*4.11 4-Amino-N-{trans-4-[4-(cyclopropylmethyl)piperazin-1-yl]cyclohexyl}-2-fluoro-5-methoxybenzamide (**15**)*

To a mixture of **20·HCl** (10.0 g, 31.5 mmol), K_2CO_3 (13.1 g, 94.8 mmol) and NaI (4.72 g, 31.5 mmol) in EtOH (50 mL) was added **21** (7.40 g, 37.7 mmol), and the mixture was stirred at 20–30 °C for 15 h. To the mixture were added EtOAc (50 mL), THF (50 mL) and H_2O (100 mL), and the layers were separated. To the organic layer were added 10% aqueous NaCl (90 mL) and 12 M HCl (13 mL) maintaining the temperature below 30 °C, and the layers were separated. The aqueous layer was concentrated *in vacuo* to remove EtOH and THF. To the resulting aqueous layer was added activated carbon (1.00 g), and the mixture was stirred at 20–30 °C for 0.5 h. Activated carbon was filtered off, and washed with H_2O (20 mL). To the combined filtrate were added EtOAc (50 mL) and THF (50 mL). To the mixture was added 25% aqueous NH_3 (18 mL) dropwise maintaining the temperature below 30 °C, and the layers were separated. The organic layer was washed with saturated aqueous NaCl (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. To the residue was added EtOH (60 mL), and 15% HCl in EtOH (40 mL) was added dropwise at 20–30 °C. The resulting slurry was stirred at 20–30 °C for 1 h, and 5–10 °C for 1 h, and then filtrated. Wet solids were washed with EtOH (15 mL), and dissolved in H_2O (65 mL). To the mixture was added activated carbon (600 mg), and the mixture was stirred at 20–30 °C for 0.5 h. Activated carbon was filtered off, and washed with H_2O (10 mL). To the combined filtrate was added 25% aqueous NH_3 (3.1 mL), and pH was adjusted to 9.0. The resulting slurry was stirred at 20–30 °C for 1 h and then filtrated. Wet solids were washed with H_2O (13 mL) and dried *in vacuo* at 50 °C to give **15** (4.73 g, 37%) as a white solid. Mp 184–185 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.10 (q, J = 4.9 Hz, 2H), 0.45–0.57 (m, 2H), 0.82–0.92 (m, 1H), 1.20–1.31 (m, 2H), 1.43 (qd, J = 12.5, 3.0 Hz, 2H), 1.97 (br d, J = 12.5 Hz, 2H), 2.16 (br d, J = 11.3 Hz, 2H), 2.25 (d, J = 6.8 Hz, 2H), 2.27–2.32 (m, 1H), 2.64 (br s, 8H), 3.87 (s, 3H), 3.89–3.95 (m, 1H), 4.27 (s, 2H), 6.36 (d, J = 13.2 Hz, 1H), 6.50 (br dd, J = 14.9, 7.7 Hz, 1H), 7.45 (d, J = 7.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ

3.9 (2C), 8.3, 27.3 (2C), 32.3 (2C), 48.7, 49.0 (2C), 53.7 (2C), 56.0, 62.7, 63.8, 100.6 ($^2J_{CF}$ = 31.7 Hz), 108.9 ($^2J_{CF}$ = 12.1 Hz), 111.7 ($^3J_{CF}$ = 3.0 Hz), 141.0 ($^3J_{CF}$ = 13.6 Hz), 143.1, 156.4 ($^1J_{CF}$ = 238.6 Hz), 163.0 ($^3J_{CF}$ = 4.5 Hz); IR (ATR) 3481, 3346, 2937, 2809, 1738, 1619, 1512, 1451, 1309, 1236, 1214, 1160, 1005, 871, 836, 797, 766, 729, 584, 523 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{22}H_{34}FN_4O_2$, 405.2660; found, 405.2659.

*4.12 Synthesis of 4-{[(6R)-7-Cyano-6-ethyl-5-(propan-2-yl)-5,6-dihydroimidazo[1,5-f]pteridin-3-yl]amino}-N-{trans-4-[4-(cyclopropylmethyl)piperazin-1-yl]cyclohexyl}-2-fluoro-5-methoxybenzamide (**19**) by coupling of **8** with **15** using a palladium catalyst*

To a mixture of **15** (1.00 g, 2.47 mmol), **8** (823 mg, 2.72 mmol) and potassium carbonate (683 mg, 4.94 mmol) in *s*-butanol (10 mL) were added Xantphos (143 mg, 0.247 mmol) and palladium(II) acetate (55.5 mg, 0.247 mmol), and the mixture was stirred at 85 °C for 1 h. After cooling to room temperature, H_2O (15 mL) and 6 M HCl (3 mL) were added, and the mixture was filtered through a pad of Celite® (No. 500, 2.00 g). To the filtrate was added EtOAc (10 mL), and the layers were separated. To the aqueous layer was added 8 M NaOH (1.5 mL) and pH was adjusted to 9.5. The resulting aqueous layer was extracted with EtOAc/THF (3:2, 25 mL). The organic layer was washed with 25% aqueous NH_3 (10 mL) and saturated aqueous NaCl (10 mL). The organic layer was filtrated to remove insoluble matter, and the filtrate was concentrated *in vacuo*. To the residue was added 2-propanol (10 mL), and the mixture was concentrated *in vacuo*. To the residue was added 2-propanol (5 mL), and the mixture was stirred at 20–30 °C for 1 h, and then filtrated. Wet solids were washed with 2-propanol (2 mL) and H_2O (2 mL), and dried *in vacuo* at 50 °C to give **19** (1.30 g, 78%) as a pale yellow solid. Mp 240–242 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.07–0.14 (m, 2H), 0.47–0.55 (m, 2H), 0.81–0.92 (m, 4H), 1.21–1.35 (m, 2H), 1.44–1.50 (m, 5H), 1.54 (d, *J* = 6.8 Hz, 3H), 1.76–1.87 (m, 1H), 1.93 (dtd, *J* = 14.2, 7.3, 3.4 Hz, 1H), 1.99 (br d, *J* = 12.5 Hz, 2H), 2.15–2.22 (m, 2H), 2.26 (d, *J* = 6.8 Hz, 2H), 2.28–2.34 (m, 1H), 2.65 (br s, 8H), 3.90–3.96 (m, 1H), 3.97 (s, 3H), 4.79 (spt, *J* = 6.8 Hz, 1H), 5.08 (dd, *J* = 8.3, 3.4 Hz, 1H), 6.61 (br dd, *J* = 14.9, 7.7 Hz, 1H), 7.58 (d, *J* = 6.8 Hz, 1H), 7.86 (s, 1H), 7.96 (s, 1H), 8.30–8.37 (m, 1H), 8.43 (d, *J* = 15.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 3.9 (2C), 8.3, 8.8, 20.2, 21.4, 27.3 (2C), 30.6, 32.3 (2C), 48.8, 49.1 (2C), 49.1, 50.6, 53.7 (2C), 56.3, 62.7, 63.8, 104.9 ($^2J_{CF}$ = 36.2 Hz), 109.2, 109.8, 111.1 ($^3J_{CF}$ = 4.5 Hz), 112.2 ($^2J_{CF}$ = 13.6 Hz), 114.0, 130.8, 133.0, 133.2 ($^3J_{CF}$ = 13.6 Hz), 141.2, 143.7, 152.4, 155.6 ($^1J_{CF}$ = 238.6 Hz), 157.2, 162.5 ($^3J_{CF}$ = 4.5 Hz); IR (nujol) 3406, 2227, 1637, 1623, 1607, 1550, 1521, 1501, 1490, 1447, 1313, 1275, 1252, 1204, 1176, 1154, 1065, 1022, 1007, 964, 875, 780 cm^{-1}; HRMS (ESI): $[M+H]^+$ calcd for $C_{36}H_{48}FN_{10}O_2$, 671.3940; found, 671.3943. Optical purity: >99.9% ee (chiral SFC condition).

*4.13 Synthesis of 4-{[(6R)-7-Cyano-6-ethyl-5-(propan-2-yl)-5,6-dihydroimidazo[1,5-f]pteridin-3-yl]amino}-N-{trans-4-[4-(cyclopropylmethyl)piperazin-1-yl]cyclohexyl}-2-fluoro-5-methoxybenzamide (**19**) by coupling of **8** with **15** in an acidic condition*

To a mixture of **15** (3.00 g, 7.42 mmol) and **8** (5.39 g, 17.8 mmol) in *s*-BuOH/H_2O (95:5, 30 mL) was added concentrated hydrochloric acid (2.25 g, 22.3 mmol), and the mixture was stirred at reflux for 11 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. To the residue were added EtOAc (45 mL) and 1 M HCl (60 mL), and the layers were separated. The organic layer was extracted with 1 M HCl (30 mL). To the combined aqueous layer was added 8 M NaOH (30 mL) and pH was adjusted to 9. The resulting aqueous layer was extracted with EtOAc (60 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ (3 × 60 mL) and concentrated *in vacuo*. To the residue was added 2-propanol (30 mL), and the mixture was stirred at 40–50 °C for 1 h. The mixture was gradually cooled to 0–10 °C and stirred for 1 h, and then filtrated. Wet solids were washed with 2-propanol/*n*-heptane (1:3, 9 mL) and dissolved in MeOH (60 mL). To the mixture was added DARCO® (G-60, 600 mg), and the mixture was stirred at 40–50 °C for 1.5 h. DARCO® was filtered off, and the filtrate was concentrated *in vacuo*. To the residue was added 2-propanol (15 mL), and the mixture was stirred at 40–50 °C for 1 h. The mixture was gradually cooled to 0–10 °C and stirred for 1 h, and then filtrated. Wet solids were washed with *n*-heptane (9 mL) and dried *in vacuo* at 50 °C to give **19** (3.54 g, 71%) as a pale gray solid.

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Appendix A. Supplementary Data

Supplementary data related to this article can be found at

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