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PRACTICAL SYNTHESIS OF 1-SUBSTITUTED 5-AMINOPYRAZOLO[4,3-*d*]PYRIMIDIN-7-ONES USING INTRAMOLECULAR FRIEDEL-CRAFTS TYPE CYCLIZATION AND ITS APPLICATION TO THE SYNTHESIS OF PHARMACEUTICALLY ACTIVE COMPOUNDS

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Abstract – Intramolecular Friedel–Crafts type cyclization is known as an efficient method of synthesizing fused bicyclic pyrimidones. However, the synthesis of 1-substituted pyrazolo[4,3-*d*]pyrimidin-7-ones using this cyclization method has not been achieved. Herein, we describe the synthesis of various 1-substituted pyrazolo[4,3-*d*]pyrimidin-7-ones using practical intramolecular Friedel–Crafts type cyclization, which was carried out in *N*, *N*-dimethylformamide in the presence of chlorotrimethylsilane. A hypoxia-inducible factor prolyl hydroxylase domain (HIF-PHD) inhibitor was efficiently synthesized by this method.

INTRODUCTION

Efficient synthesis of bicyclic pyrimidones, which are commonly used as biologically active structures, is a key component in the process of drug discovery.[1] Many intramolecular Friedel–Crafts type cyclizations have been investigated for the synthesis of various fused pyrimidones (Figure 1). [2–4]

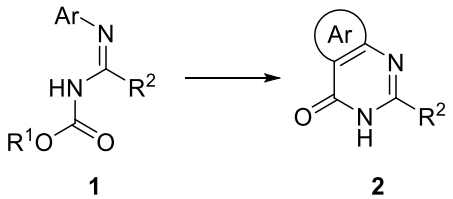
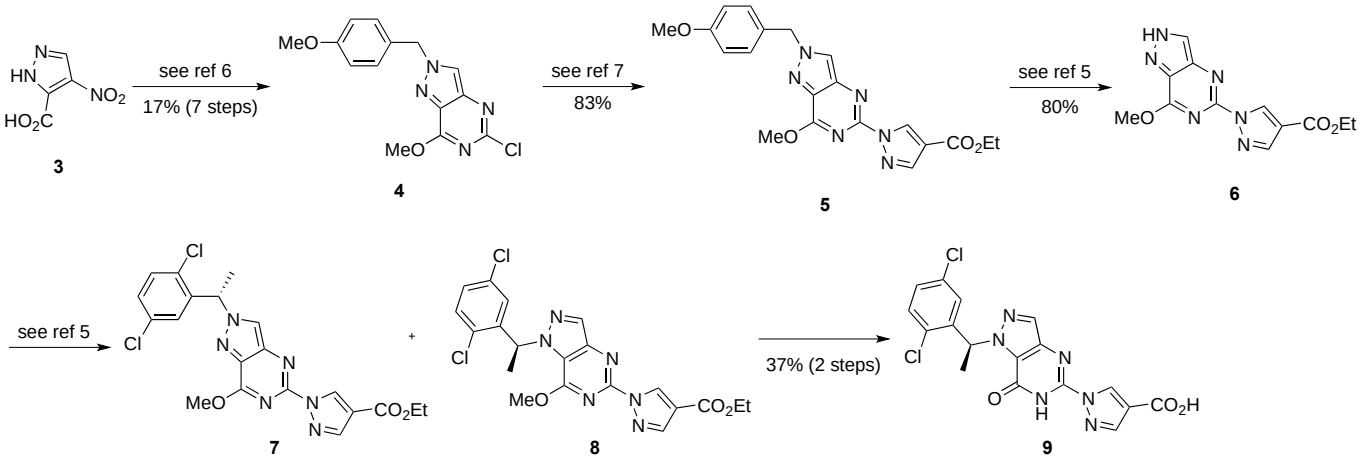


Figure 1. Reported intramolecular Friedel–Crafts type cyclization

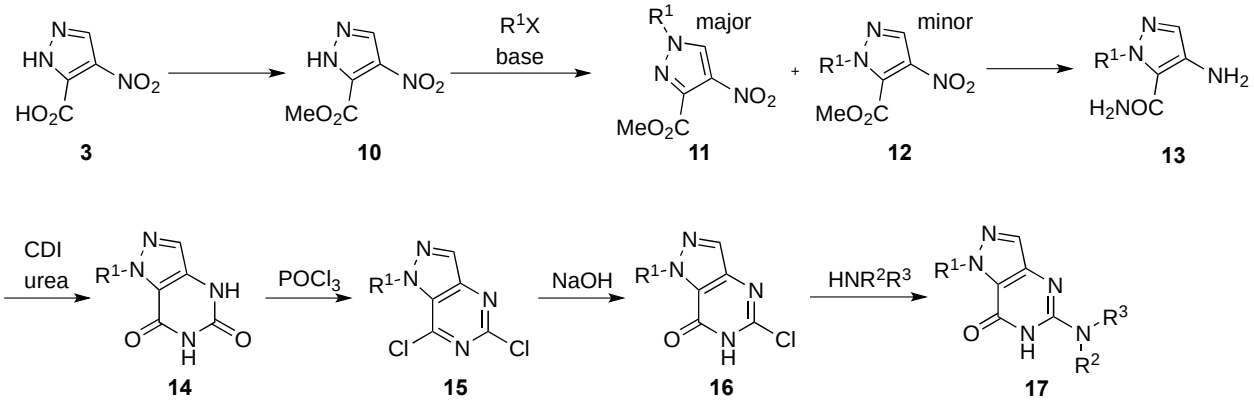
Ar = heteroaromatic ring or aromatic ring; R^1 = alkyl; R^2 = trihalomethyl, primary aliphatic amine

Recently, we reported that 1-substituted 5-aminopyrazolo[4,3-*d*]pyrimidin-7-ones have hypoxia-inducible factor prolyl hydroxylase domain (HIF-PHD) inhibitory activity.[5] According to the reported methodologies using commercially available pyrazole **3** as a starting material, the inhibitor **9** was prepared in low yield (4%). This low yield was due to the multiple steps involved in its synthesis and its lack of selectivity for benzylation (**7**/**8** = 1/1) (Scheme 1).[5–7] The most widely used synthesis of 5-aminopyrazolo[4,3-*d*]pyrimidin-7-ones is the reaction of pyrazole **13** with urea or CDI (1,1'-carbonyldiimidazole) to form a dihydropyrimidinedione ring, followed by chlorination, hydrolysis, and the introduction of an amino group (Scheme 2).[8] However, this approach has issues similar to those of inhibitor **9** with the selectivity of pyrazole alkylation (Scheme 2).[6, 9]

Herein, we report the development of an efficient method of synthesizing various 1-substituted 5-aminopyrazolo[4,3-*d*]pyrimidin-7-ones using intramolecular Friedel–Crafts type cyclization, with which the synthesis of pyrazolo[4,3-*d*]pyrimidin-7-ones has not been achieved previously.



Scheme 1. Synthesis of HIF-PHD inhibitor **9**

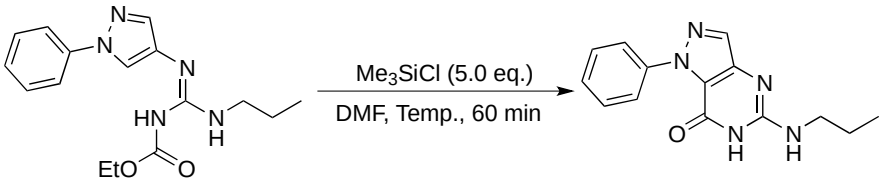


Scheme 2. The most common method of synthesis of 5-aminopyrazolo[4,3-*d*]pyrimidin-7-ones

RESULTS AND DISCUSSION

Initially, guanidine **18**, which was easily prepared from 1-phenyl-4-aminopyrazole in a one-pot reaction,[10] was used to investigate the conditions of intramolecular Friedel–Crafts type cyclization. The reaction was carried out under the conditions reported for synthesizing quinazolone using the intramolecular Friedel–Crafts reaction,[3] but the reaction was not completed and **18** remained unchanged (Table 1, entry 1). To complete the reaction, the temperature was increased (Table 1, entries 2 and 3). As a result, the reaction came close to completion at 120 ºC and fully completed by extending the reaction time to 90 min (Table 1, entry 4). The use of microwave irradiation, which has been reported to accelerate Friedel–Crafts cyclization,[4] was also examined. Although microwave irradiation was not effective at 80 ºC (Table 1, entry 5), the acceleration and completion of the reaction at ≥ 100 ºC were observed. The desired product **19**, which was reacted selectively at the more electron-rich position of the pyrazole, was obtained in 77% yield (Table 1, entries 6 and 7).

Table 1. Optimization of cyclization conditions



18 **19**

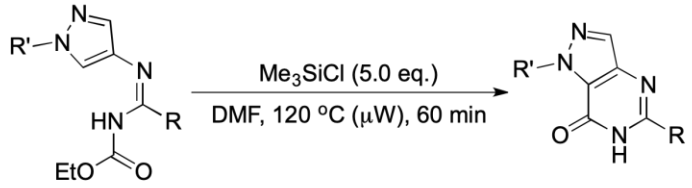
Entry	Temp. (ºC)	Conversion[a] (%)
1	80	33
2	100	59
3	120	78
4[b]	120	100
5[c]	80	26
6[c]	100	100 (77%[d])
7[c]	120	100 (77%[d])

[a]Determined by UV (254 nm) area of HPLC analysis of the reaction mixture. [b]Reaction time was 90 min. [c]Heated by microwave irradiation. [d]Isolated yield.

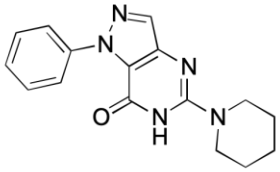
Next, the scope of cyclization was studied under optimal conditions. **21a** was obtained in a high yield. This example is the first reported synthesis of bicyclic pyrimidone with the secondary amine using Friedel–Crafts type cyclization. Cyclization of **20b** afforded the desired product **21b** and the byproduct **21b'** cyclized at the 2-position of *p*-toluidine. Compound **21c**, which has a similar structure to the

HIF-PHD inhibitor, was obtained in a high yield. **21d** was obtained in an excellent yield when *N*-methyl-2-pyrrolidone (NMP) was used as a solvent because the substitution of imidazole in **20d**, which was similar to CDI, occurred easily with dimethylamine generated by degradation of *N*, *N*-dimethylformamide (DMF) in the reaction. This result showed that NMP was also suitable solvent for Friedel–Crafts cyclization. **21e** with a benzyl side chain and thienopyrimidone **21f** were also obtained in high yields.

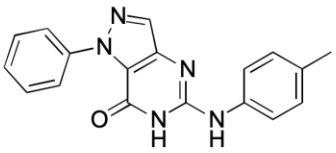
Table 2. The scope of the cyclization reaction



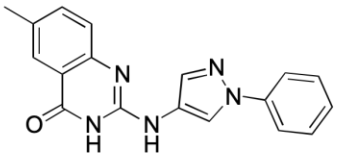
20 **21**



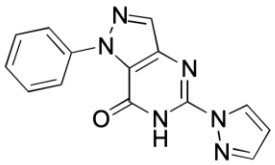
21a, 90%



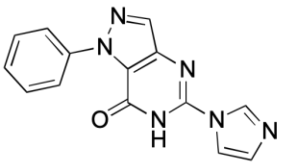
21b, 53%[a]



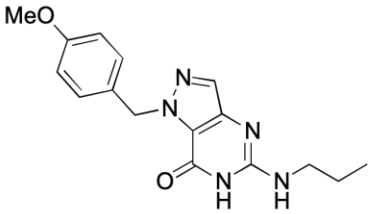
21b', 44%[a]



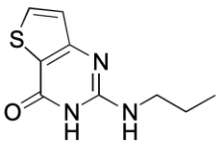
21c, 91%



21d[b], 97%



21e, 85%



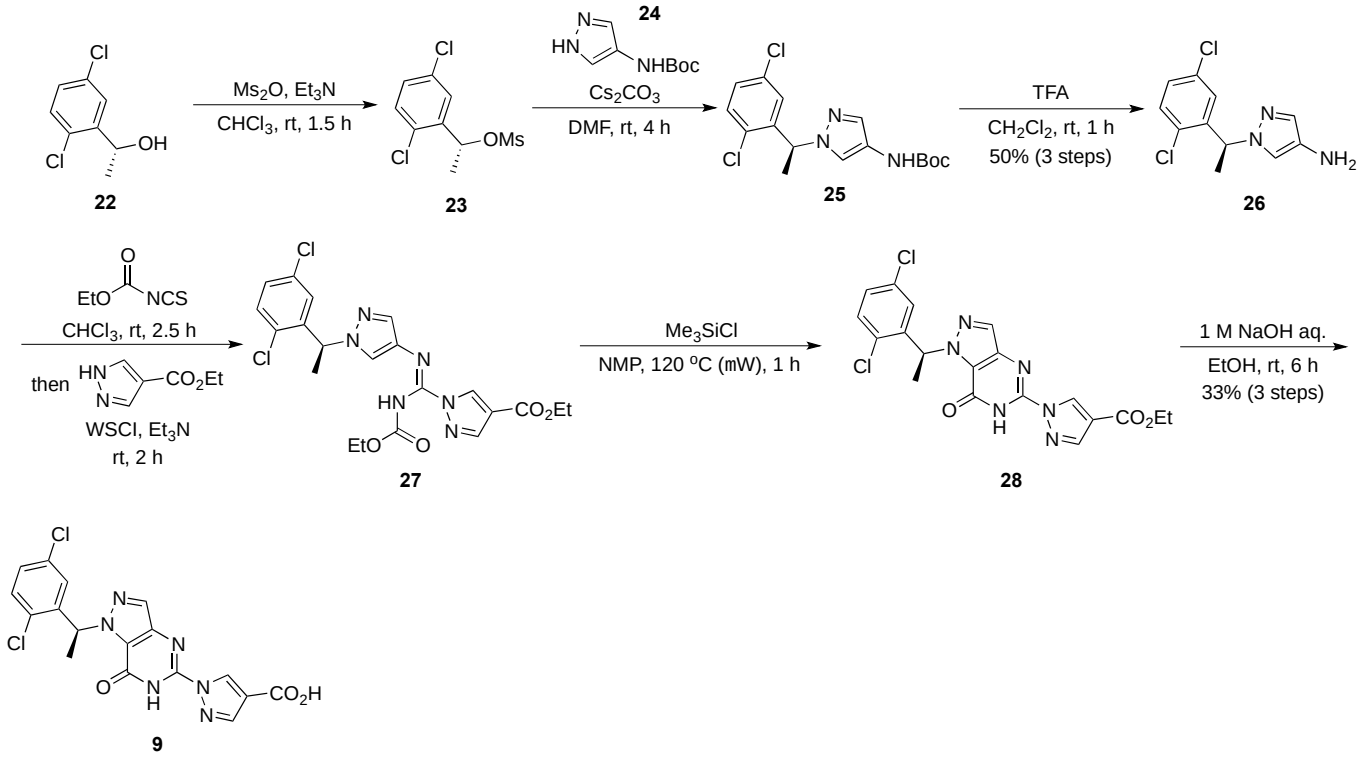
21f, 90%

[a]The yields were calculated by a mixture of ^{1}H NMR spectra. [b]NMP was used as a solvent.

To demonstrate the application of cyclization, we attempted to synthesize the HIF-PHD inhibitor **9** (Scheme 3). We could overcome the selectivity in this manner because pyrazole **24** could be used for the benzylation. Compound **25** was obtained by the benzylation of **24** under basic conditions using methanesulfonylated benzyl alcohol **23**, which was synthesized from commercially available chiral benzyl alcohol **22** (93% enantiomer excess). Subsequent deprotection of the Boc group gave the desired aminopyrazole **26** in 50% yield from **22**. The cyclization precursor **27** was obtained by transformation of **26** and intramolecular Friedel–Crafts type cyclization was carried out under the conditions which we had developed. NMP was used as a solvent for the cyclization because the slight substitution of pyrazole observed in the synthesis of **21c**. **28** was hydrolyzed to give the desired product **9** with high enantiomer

excess (99% ee). We achieved an improvement in the overall yield and a reduction in the number of steps required compared to Scheme 1 (17%, 6 steps in Scheme 3 vs 4%, 11 steps in Scheme 1).

In conclusion, we have achieved the first efficient synthesis of 1-substituted 5-aminopyrazolo[4,3-*d*]pyrimidin-7-ones using intramolecular Friedel–Crafts type cyclization. The usefulness of the cyclization was also demonstrated by its application to the HIF-PHD inhibitor **9**. This methodology is expected to be applicable to process chemistry and to enable easy access to a wide variety of 5-aminopyrazolo[4,3-*d*]pyrimidin-7-ones, which are attractive structures in the area of medicinal chemistry.



Scheme 3. Synthesis of HIF-PHD inhibitor **9** using intramolecular Friedel–Crafts type cyclization

EXPERIMENTAL

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 UltraShield Plus. Chemical shifts in the NMR spectra are reported in parts per million (ppm) with relative to tetramethylsilane (δ = 0.00 ppm) as the internal standard. The following abbreviations are used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (*J*) are in hertz. High resolution mass spectra (HRMS) were recorded on a LTQ Orbitrap Velos Pro mass spectrometer equipped with an ESI Lockspray source for accurate mass values.

Ethyl *N*-[*N'*-(1-Phenylpyrazol-4-yl)-*N*-propylcarbamimidoyl]carbamate (**18**)

Ethoxycarbonyl isothiocyanate (120 mg, 0.912 mmol) was added to a solution of 1-phenylpyrazol-4-amine (132 mg, 0.829 mmol) in $CHCl_3$ (3.0 mL) at room temperature. After being stirred at room temperature for 1 h, WSCI (191 mg, 1.20 mmol), Et_3N (0.346 mL, 2.49 mmol), and *n*-propylamine (0.0818 mL, 0.995 mmol) were added, also at room temperature. After being stirred at room temperature for 4 h, water was added to the reaction mixture and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/EtOAc = 90/10–50/50) to give **18** as a pale yellow gum (207 mg, 79%). IR (ATR) 3249, 3153, 2964, 2932, 2874, 1723 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J*=7.5 Hz, 3H), 1.34 (t, *J*=7.1 Hz, 3H), 1.53 (sextet, *J* = 7.3 Hz, 2H), 3.35 (q, *J* = 7.1 Hz, 2H), 4.17 (q, *J*=7.1 Hz, 2H), 4.71 (brs, 1H), 7.39–7.32 (m, 1H), 7.52–7.45 (m, 2H), 7.66 (s, 1H), 7.71–7.63 (m, 2H), 7.94 (s, 1H), 10.35 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 14.7, 22.8, 42.9, 60.9, 119.0, 120.2, 123.7, 127.2, 129.6, 138.2, 139.7, 159.7, 164.8; HRMS (ESI) *m/z* calcd for $C_{16}H_{22}N_5O_2$ $[M+H]^+$: 316.1768, found: 316.1762.

1-Phenyl-5-(propylamino)-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**19**)

A mixture of **18** (50 mg, 0.16 mmol) and chlorotrimethylsilane (0.10 mL, 0.79 mmol) in DMF (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc three times. The combined organic phases were washed with brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/MeOH = 100/0–92/8) to give **19** (33 mg, 77%) as a colorless solid. mp 206–208 °C; IR (ATR) 3220, 2972, 2858, 1685, 1629 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, *J*=7.5 Hz, 3H), 1.55 (sextet, *J*=7.3 Hz, 2H), 3.24 (q, *J*=6.8 Hz, 2H), 6.16 (brs, 1H), 7.40–7.33 (m, 1H), 7.54–7.43 (m, 2H), 7.75–7.66 (m, 2H), 7.92(s, 1H), 10.94 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.2, 22.0, 42.1, 119.9, 123.5, 126.8, 128.3, 133.3, 139.4, 144.7, 151.3, 153.2; HRMS (ESI) *m/z* calcd for $C_{14}H_{16}N_5O$ $[M+H]^+$: 270.1349, found: 270.1346.

1-Phenyl-5-(1-piperidyl)-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**21a**)

Ethoxycarbonyl isothiocyanate (92 mg, 0.70 mmol) was added to a solution of 1-phenylpyrazol-4-amine (101 mg, 0.634 mmol) in $CHCl_3$ (2.0 mL). After being stirred at room temperature for 1 h, WSCI (146 mg, 0.761 mmol), Et_3N (0.27 mL, 1.90 mmol), and piperidine (0.075 mL, 0.76 mmol) were added to the mixture at room temperature. After being stirred at room temperature for 3 h, water was added and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/EtOAc =

90/10–25/75) to give **20a** (147 mg, 68%). A mixture of **20a** (50 mg, 0.15 mmol) and chlorotrimethylsilane (0.094 mL, 0.73 mmol) in DMF (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc and EtOAc/THF (1/1) three times respectively. The combined organic phases were washed with brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by NH silica gel column chromatography (EtOAc/MeOH = 100/0–93/7–90/10–85/15) to give **21a** (39 mg, 90%, 61% from 1-phenylpyrazol-4-amine) as a colorless solid. mp 219 °C; IR (ATR) 3136, 3076, 2920, 2848, 1660 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d*$_6$) δ ^1H NMR (400 MHz, DMSO-*d*$_6$) δ 1.67–1.47 (m, 6H), 3.64–3.45 (m, 4H), 7.41–7.34 (m, 1H), 7.55–7.44 (m, 2H), 7.77–7.66 (m, 2H), 7.95 (s, 1H), 11.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 23.8, 24.9, 46.6, 119.8, 123.5, 126.9, 128.4, 133.5, 139.4, 144.2, 151.8, 154.0; HRMS (ESI) *m/z* calcd for $C_{16}H_{18}N_5O$ $[M+H]^+$: 296.1506, found: 296.1470.

5-(4-Methylanilino)-1-phenyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**21b**)
6-Methyl-2-[(1-phenylpyrazol-4-yl)amino]-3*H*-quinazolin-4-one (**21b'**)

Ethoxycarbonyl isothiocyanate (91 mg, 0.69 mmol) was added to a solution of 1-phenylpyrazol-4-amine (100 mg, 0.628 mmol) in $CHCl_3$ (2.0 mL). After the reaction was stirred at room temperature for 1 h, ethoxycarbonyl isothiocyanate (41 mg, 0.31 mmol) was added again and the mixture was stirred at room temperature for an additional 1 h. After the reaction was completed, WSCI (145 mg, 0.754 mmol), Et_3N (0.26 mL, 1.88 mmol), and *p*-toluidine (81 mg, 0.75 mmol) were added to the mixture at room temperature. After being stirred at room temperature overnight, water was added and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure and the residue was purified by NH silica gel column chromatography (hexane/EtOAc = 95/5–75/25–70/35). The resulting residue was crystallized with EtOAc to give crude **20b** as a colorless solid (192 mg, 84%). A mixture of **20b** (51 mg, 0.14 mmol) and chlorotrimethylsilane (0.090 mL, 0.70 mmol) in DMF (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc/THF (1/1) three times. The combined organic phases were concentrated under reduced pressure and the residue was purified by NH silica gel column chromatography (EtOAc/MeOH = 100/0–80/20–70/30) to give the mixture of **21b** and **21b'** (43 mg, 97%, 81% from 1-phenylpyrazol-4-amine) as a colorless solid. **21b**: mp 291–292 °C; IR (ATR) 3207, 3048, 2940, 2868, 1693, 1616 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d*$_6$) δ 2.28 (s, 3H), 7.21–7.11 (m, 2H), 7.44–7.36 (m, 1H), 7.60–7.46 (m, 4H), 7.78–7.69 (m, 2H), 8.05 (s, 1H), 8.48 (s, 1H), 10.96 (s, 1H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 20.3, 119.1, 120.8, 123.7, 127.1, 128.4, 129.1, 131.2, 133.8, 136.4, 139.3, 143.6, 147.9, 152.7; HRMS (ESI) *m/z* calcd for

$C_{18}H_{16}N_5O$ $[M+H]^+$: 318.1349, found: 318.1345. **21b'**: mp 207–210 °C; IR (ATR) 3117, 2966, 2595, 2310, 1706, 1683, 1631 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.38 (s, 3H), 7.35–7.25 (m, 1H), 7.59–7.39 (m, 4H), 7.92–7.78 (m, 3H), 7.96 (s, 1H), 8.80 (s, 1H), 9.98 (brs, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 20.5, 88.4, 116.7, 117.6, 117.6, 124.7, 125.0, 125.2, 125.5, 129.4, 131.0, 133.4, 134.9, 139.7, 148.7, 169.2; HRMS (ESI) *m/z* calcd for $C_{18}H_{16}N_5O$ $[M+H]^+$: 318.1349, found: 318.1344.

1-Phenyl-5-pyrazol-1-yl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**21c**)

Ethoxycarbonyl isothiocyanate (151 mg, 1.15 mmol) was added to a solution of 1-phenylpyrazol-4-amine (152mg, 0.955 mmol) in $CHCl_3$ (3.0 mL). After being stirred at room temperature for 2 h, WSCI (219 mg, 1.15 mmol), Et_3N (0.40 mL, 2.86 mmol), and pyrazole (78 mg, 1.15 mmol) were added to the mixture at room temperature. After being stirred at room temperature for 5 h, water was added and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/EtOAc = 95/5–55/45) to give crude **20c** (137 mg, 44%). A mixture of **20c** (50 mg, 0.15 mmol) and chlorotrimethylsilane (0.099 mL, 0.77 mmol) in DMF (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to room temperature, the mixture was purified by HPLC (Capcellpak C18 UG80 Φ20 mm*250 mm 5 μm, 0.05%TFA-H_2O/0.05%TFA-MeCN = 58/42–48/52) to give **21c** (39 mg, 91%, 40% from 1-phenylpyrazol-4-amine) as a colorless solid. mp 179–180 °C; IR (ATR) 3308, 3143, 3104, 1699, 1608 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 6.69 (dd. J = 2.7, 1.7 Hz, 1H), 7.50–7.43 (m, 1H), 7.59–7.51 (m, 2H), 7.77–7.69 (m, 2H), 7.96 (d, J = 1.0 Hz, 1H), 8.30 (s, 1H), 8.63 (dd, J = 2.7, 0.64 Hz, 1H), 12.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 109.7, 123.4, 124.4, 127.7, 128.5, 128.9, 134.7, 138.9, 140.8, 143.0, 143.3, 152.3; HRMS (ESI) *m/z* calcd for $C_{14}H_{11}N_6O$ $[M+H]^+$: 279.0989, found: 279.0989.

5-Imidazol-1-yl-1-phenyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**21d**)

Ethoxycarbonyl isothiocyanate (199 mg, 1.52 mmol) was added to a solution of 1-phenylpyrazol-4-amine (201 mg, 1.26 mmol) in $CHCl_3$ (4.0 mL). After the reaction was stirred at room temperature for 1 h, ethoxycarbonyl isothiocyanate (83 mg, 0.63 mmol) was added again and the mixture was stirred at room temperature for an additional 1 h. After the reaction was completed, WSCI (145 mg, 0.754 mmol), Et_3N (0.26 mL, 1.88 mmol), and imidazole (103 mg, 1.52 mmol) were added to the mixture at room temperature. After being stirred at room temperature for 2.5 h, water was added and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/MeOH = 100/10–90/10) to give crude **20d** (265 mg, 65%). A mixture of **20d** (50 mg, 0.15 mmol) and chlorotrimethylsilane (0.099 mL, 0.77 mmol) in NMP (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was

cooled to room temperature, Et_3N (0.11 mL, 0.77 mmol) was added and the resulting mixture was purified by silica gel column chromatography (EtOAc/MeOH = 100/0–50/50, and EtOAc/MeOH = 100/0–65/35) to give **21d** (44 mg, 97%, 63% from 1-phenylpyrazol-4-amine) as a colorless solid. mp 235 °C (decomp); IR (ATR) 3192, 3158, 3085, 1658, 1593 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.15 (s, 1H), 7.46–7.38 (m, 1H), 7.58–7.47 (m, 2H), 7.80–7.71 (m, 2H), 7.97 (s, 1H), 8.25 (s, 1H), 8.59 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 117.3, 123.7, 124.2, 127.2, 128.4, 128.9, 134.4, 135.7, 139.3, 142.2, 144.8, 155.8; HRMS (ESI) *m/z* calcd for $C_{14}H_{11}N_6O$ $[M+H]^+$: 279.0989, found: 279.0985.

1-[(4-Methoxyphenyl)methyl]-5-(propylamino)-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**21e**)

Ethoxycarbonyl isothiocyanate (155 mg, 1.18 mmol) was added to a solution of 1-[(4-methoxyphenyl)methyl]pyrazol-4-amine (200 mg, 0.984 mmol) in $CHCl_3$ (4.0 mL). After being stirred at room temperature for 1 h, WSCI (226 mg, 1.18 mmol), Et_3N (0.41 mL, 2.95 mmol), and *n*-propylamine (0.097 mL, 1.18 mmol) were added to the mixture at 50 ºC. After being stirred at room temperature for 1.5 h, water was added and the resulting mixture was extracted with $CHCl_3$ three times. The combined organic phases were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/EtOAc = 95/5–40/60) to give **20e** (296 mg, 78%). A mixture of **20e** (50 mg, 0.14 mmol) and chlorotrimethylsilane (0.089 mL, 0.70 mmol) in DMF (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc/THF (1/1) three times. The combined organic phases were washed with brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by NH silica gel column chromatography (EtOAc/MeOH = 100/0–90/10) to give **21e** (37 mg, 85%, 66% from 1-[(4-methoxyphenyl)methyl]pyrazol-4-amine) as a colorless solid. mp 182 °C; IR (ATR) 3230, 3001, 2963, 2935, 2865, 1685, 1631, 1610 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, *J* = 7.5 Hz, 3H), 1.51 (sextet, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.1 Hz, 2H), 3.70 (s, 3H), 5.52 (s, 2H), 6.03–5.94 (m, 1H), 6.90–6.81 (m, 2H), 7.23–7.14 (m, 2H), 7.59 (s, 1H), 10.76 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.2, 21.9, 42.1, 53.2, 55.0, 113.7, 120.2, 128.9, 129.5, 130.6, 142.3, 150.7, 153.9, 158.6; HRMS (ESI) *m/z* calcd for $C_{16}H_{20}N_5O_2$ $[M+H]^+$: 314.1612, found: 314.1606.

2-(Propylamino)-3*H*-thieno[3,2-*d*]pyrimidin-4-one (**21f**)

Ethoxycarbonyl isothiocyanate (232 mg, 1.77 mmol) was added to a solution of thiophen-3-amine hydrochloride (200 mg, 1.48 mmol) and Et_3N (0.225 mL, 1.62 mmol) in $CHCl_3$ (4.0 mL). After being stirred at room temperature for 1 h, WSCI (339 mg, 1.77 mmol), Et_3N (0.820 mL, 5.90 mmol), and *n*-propylamine (0.146 mL, 1.77 mmol) were added to the mixture at room temperature. After being stirred

at room temperature for 2 h, water was added and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure and the residue was purified by NH silica gel column chromatography (hexane/EtOAc = 95/5–70/30) to give **20f** (190 mg, 50%). A mixture of **20f** (53 mg, 0.21 mmol) and chlorotrimethylsilane (0.13 mL, 1.0 mmol) in DMF (2.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to room temperature, saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc/THF (1/1) three times. The combined organic phases were washed with brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by NH silica gel column chromatography (EtOAc/MeOH = 100/0–85/15) to give **21f** (39 mg, 90%, 45% from thiophen-3-amine) as a colorless solid. mp 209–210 °C; IR (ATR) 3248, 3163, 3108, 3065, 2954, 2931, 2902, 2865, 1656, 1613 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, *J* = 7.5 Hz, 3H), 1.54 (sextet, *J* = 7.3 Hz, 2H), 3.24 (dt, *J* = 7.1, 5.7 Hz, 2H), 6.26 (t, *J* = 5.7 Hz, 1H), 7.05 (d, *J* = 5.1 Hz, 1H), 7.95 (d, *J* = 5.3 Hz, 1H), 10.80 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.2, 22.0, 42.0, 112.1, 124.0, 134.2, 153.2, 157.9, 159.8; HRMS (ESI) *m/z* calcd for $C_9H_{12}N_3OS$ $[M+H]^+$: 210.0696, found: 210.0697.

1-[(1*S*)-1-(2,5-Dichlorophenyl)ethyl]pyrazol-4-amine (**26**)

Et_3N (0.725 mL, 5.21 mmol) and methanesulfonic anhydride (681 mg, 3.91 mmol) were added to a solution of **22** (498 mg, 2.61 mmol, 93.38% ee) in $CHCl_3$ (10 mL) at room temperature. After being stirred at room temperature for 2 h, 1 M HCl was added and the resulting mixture was extracted with $CHCl_3$ twice. The combined organic phases were concentrated under reduced pressure to give crude **23** as a yellow gum. *tert*-butyl *N*-(1*H*-pyrazol-4-yl)carbamate **24** (525 mg, 2.87 mmol) and cesium carbonate (2.55 g, 7.82 mmol) were added to a solution of **23** in DMF (10 mL) at room temperature. After being stirred at room temperature for 2 h, water was added and the resulting mixture was extracted with EtOAc there times. The combined organic phases were washed with brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane/EtOAc = 95/5–75/25) and NH silica gel column chromatography (hexane/EtOAc = 95/5–80/20) to give crude **25**. TFA (4.0 mL) was added to a solution of **25** in CH_2Cl_2 (4.0 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated *in vacuo* and saturated aqueous $NaHCO_3$ was added. The resulting mixture was extracted with $CHCl_3$ three times and the combined organic phases were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 50/50–0/100) to give **26** (326 mg, 50%). IR (ATR) 3400, 3324, 3222, 3097, 3060, 2984, 2938, 1585 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.82 (d, *J* = 7.1 Hz, 3H), 2.93 (s, 2H), 5.74 (q, *J* = 7.1 Hz, 1H), 6.98 (d, *J* = 2.6 Hz, 1H), 7.10 (d, *J* = 0.90 Hz, 1H), 7.16 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.25 (d, *J* = 0.77 Hz, 1H), 7.28 (d, *J* = 8.6 Hz, 1H); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 19.9, 57.5, 117.8, 127.3, 128.9, 129.2, 130.2, 130.7, 131.9, 133.4, 142.0; HRMS (ESI) *m/z* calcd for $C_{11}H_{12}N_3Cl_2$ $[M+H]^+$: 256.0403, found: 256.0403.

1-[1-[(1*S*)-1-(2,5-Dichlorophenyl)ethyl]-7-oxo-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl]pyrazole-4-carboxylic acid (9)

Ethoxycarbonyl isothiocyanate (197 mg, 1.50 mmol) was added to a solution of **26** (321 mg, 1.25 mmol) in $CHCl_3$ (6.0 mL) at room temperature. After being stirred at room temperature for 2.5 h, WSCI (288 mg, 1.50 mmol), Et_3N (0.523 mL, 3.76 mmol), and ethyl 4-pyrazolecarboxylate (211 mg, 1.50 mmol) were added. After being stirred at room temperature for 2 h, the reaction mixture was purified by silica gel column chromatography (hexane/EtOAc = 95/5–50/50) to give crude **27** (351 mg). A mixture of **27** (100 mg) and chlorotrimethylsilane (0.123 mL, 1.01 mmol) in NMP (3.0 mL) was stirred at 120 °C under microwave irradiation for 1 h. After it was cooled to ambient temperature, saturated aqueous $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc there times. The combined organic phases were washed with brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give **28**. 1 M NaOH aq. (1.01 mL, 1.01 mmol) was added to a solution of **28** in THF (1 mL) and EtOH (1 mL) at room temperature. After being stirred at room temperature for 6 h, the mixture was concentrated *in vacuo*. The residue was dissolved in water and 1 M HCl was added to the solution. The precipitate was collected by filtration, which was then suspended in EtOH at 50 ºC to produce **9** (50 mg, 33%) as a colorless powder. mp 233–235 °C; IR (ATR) 3284, 3137, 3115, 1731, 1682, 1620 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.86 (d, *J*=7.1 Hz, 3H), 6.75 (q, *J*=6.9 Hz, 1H), 7.16 (d, *J*=2.6 Hz, 1H), 7.42 (dd, *J*=8.6, 2.6 Hz, 1H), 7.53(d, *J*=8.6 Hz, 1H), 8.16 (s, 1H), 8.22 (s, 1H), 8.86 (s, 1H), 12.96 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.9, 56.1, 118.3, 124.0, 127.3, 129.3, 130.5, 131.3, 131.7, 132.1, 133.2, 138.6, 141.0, 142.6, 143.1, 153.0, 162.9; HRMS (ESI) *m/z* calcd for $C_{17}H_{13}N_6O_3Cl_2$ $[M+H]^+$: 419.0421, found: 419.0426; Enantiomeric Excess: 98.93% ee (t_R = 9.678 min, column: Daisel CHIRALPAK IC-3 (4.6×150 mm), eluent: hexane/EtOH/AcOH=70/30/0.5).

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