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Development of a Practical Synthetic Method for Clinical Candidate 3-(2-{3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}phenyl)propanoic acid (P218) and Its Hydroxylated Metabolites

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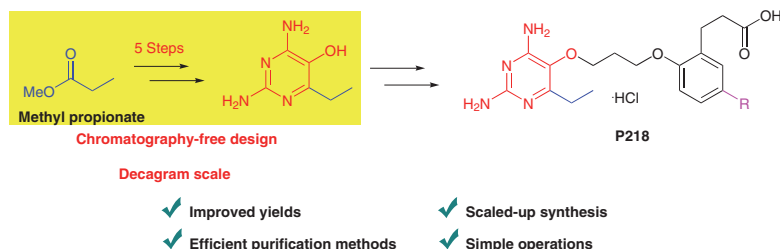
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Methyl propionate — 5 Steps — P218

Chromatography-free design

Decagram scale

✓ Improved yields ✓ Scaled-up synthesis
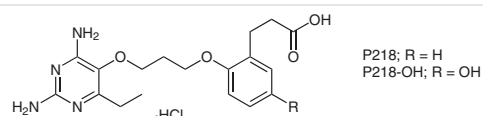✓ Efficient purification methods ✓ Simple operations

Abstract 3-(2-[3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy]phenyl)propanoic acid, known as P218, has demonstrated great potency and safety in preclinical and human studies. However, the previous synthetic methods for P218 gave low yields and required hazardous reagents and challenging procedures. In this study, we have successfully developed a decagram-scale synthetic route for P218 with practical and scalable methods for large-scale production. Furthermore, this is also a first report of a novel synthetic approach for P218-OH, a hydroxylated metabolite of P218, by modification of our discovery route. Our synthetic procedures for P218 and P218-OH are a significant advancement in drug development processes, including manufacturing processes and drug metabolism studies.

Key words P218, pyrimidine, antimalarial drugs, process development
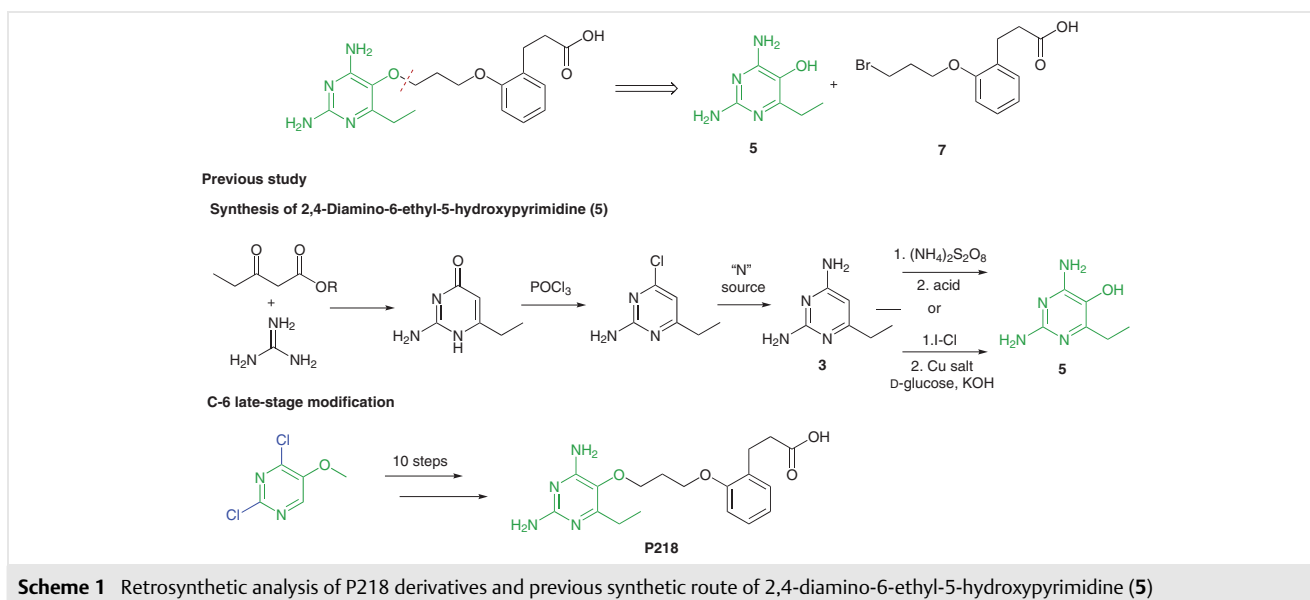
Malaria is a communicable disease caused by *Plasmodium parasites* and can be life-threatening, especially in tropical and subtropical countries such as Africa and Asia, including Thailand.[1–4] In 2020, during the COVID-19 pandemic, the World Health Organization (WHO) reported approximately 241 million cases of malaria and more than 627,000 deaths worldwide, with an 11% increase in the mortality rate compared to the previous year – two thirds of which were due to the COVID-19 disruption.[5] Severe malaria is more likely to develop in children under 5 years old and individuals with immune failures. *Plasmodium falciparum* (*P. falciparum*) is the most dangerous species to human life.[5–8] Despite the availability of several antimalarial drugs, the emergence of drug resistance poses a significant threat to human existence.[9,10] Moreover, affordable treatment and low production cost are necessary in view of the economic status of the most affected people.[11] Therefore, the development of new antimalarial candidates and a practical process to produce them are urgently needed.

3-(2-{3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}phenyl)propanoic acid (P218) (Figure 1) was discovered by Yuthavong et al.[12,13] P218, prepared in hydrochloride salt form, has demonstrated high potency against wild-type and resistant *P. falciparum*.[12] Recently, first-in-human and sporozoites challenge clinical studies have shown favorable safety and pharmacokinetic profiles of P218, as well as its chemoprotective antimalarial activity against *P. falciparum*.[14,15] P218 and its hydroxylated metabolite P218-OH (Figure 1), together with their glucuronide forms were identified.[15,16] Consequently, standard P218 and metabolites are needed for subsequent clinical and related studies, and the development of a practical synthetic route for P218 and its metabolites is required.


P218; R = H
P218-OH; R = OH

Figure 1 Structure of P218 and P218-OH as hydrochloride salt

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Scheme 1 Retrosynthetic analysis of P218 derivatives and previous synthetic route of 2,4-diamino-6-ethyl-5-hydroxypyrimidine (**5**)

Retrosynthetic analysis of P218 envisioned 2,4-diamino-6-ethyl-5-hydroxypyrimidine (**5**) and the bromo-substituted derivative **7** as the common core (Scheme 1). However, the synthesis of **5** has encountered challenges,[17–20] including poor overall yields, poor reproducibility, the use of hazardous reagents such as phosphorus oxychloride (POCl₃), and the need for extensive purification processes (Scheme 1). Recently, an alternative synthetic route for P218 was proposed through C-6 late-stage modification starting from commercially available 2,4-dichloro-5-methoxypyrimidine (Scheme 1). However, this synthetic route also poses significant challenges due to the use of complex, expensive chemical reagents and extensive purification methods.[21] The disadvantages of both synthetic routes have raised concerns regarding the high cost of production. Therefore, a simple and robust synthetic route is required for producing P218 to serve as a medicine at low cost.

To avoid the aforementioned challenges during scale-up, we report here an alternative and more practical method for the synthesis of P218 and its derivatives, with significant improvements in the synthetic processes and overall yields of the products. We synthesized the key intermediate **5** in parallel, followed by conjugation of two bromo-substituted derivatives. In particular, a chromatography-free synthetic method for 2,4-diamino-6-ethyl-5-hydroxypyrimidine (**5**), as a key intermediate for P218 and P218-OH, has been developed. This method is scalable, up to multigram scale, allowing access to key intermediate **5**, which has been a bottleneck in previous methods.[21] The synthetic procedure allowed us to produce P218 and its metabolite P218-OH in ten and twelve steps, respectively. Moreover, our development of these synthetic procedures will be a foundation for further derivatization and optimization of antimalarial drugs and other antifolate agents.[22–24]

The synthesis of 2,4-diamino-6-ethyl-5-hydroxypyrimidine (**5**) began with a low-cost commercially available material, methyl propionate, using a chromatography-free synthetic method on a 60-gram scale, as depicted in Scheme 2. Methyl propionate underwent nucleophilic attack by acetonitrile in a presence of sodium hydride as a base in anhydrous tetrahydrofuran. The starting material was initially prepared at a low temperature of –78 °C and then refluxed at 70 °C, resulting in a crude of 3-oxopentanenitrile (**1**). Trimethoxybenzene of known purity was chosen as internal standard for ¹H NMR quantitative analysis (qHNMR). The purity of compound **1** was obtained by ¹H NMR (73.18%, STD = 0.02). To establish a more viable synthetic procedure, the temperature for the preparation step was optimized by varying it from –78 to 0 °C. The reaction was also optimized at –10 °C, followed by reflux at 70 °C, and it still showed a similar yield. Subsequently, nitrile **1** was transformed into a crude mixture of 3-methoxypent-2-enenitrile (**2a**) and 3,3-dimethoxypentanenitrile (**2b**) in the presence of trimethyl orthoformate under acidic conditions. The crude containing impurities was quantified by using trimethylbenzene as internal standard. The yields of **2a** and **2b** (53:47) in the crude mixture were obtained by ¹H qNMR (86.64%, STD = 0.04). The enol ether **2a** and acetal **2b** were subjected to guanidine, affording 6-ethylpyrimidine-2,4-diamine (**3**) in low yield (22% over 3 steps). Pyrimidine **3** underwent a Boyland–Sims oxidation reaction in the presence of ammonium persulfate under basic condition, leading to the formation of 2,4-diamino-6-ethylpyrimidin-5-yl hydrogen sulfate (**4**) in high yields (84%). The resulting sulfate ester **4** was further hydrolyzed under concentrated acidic conditions to provide 2,4-diamino-6-ethyl-5-hy-
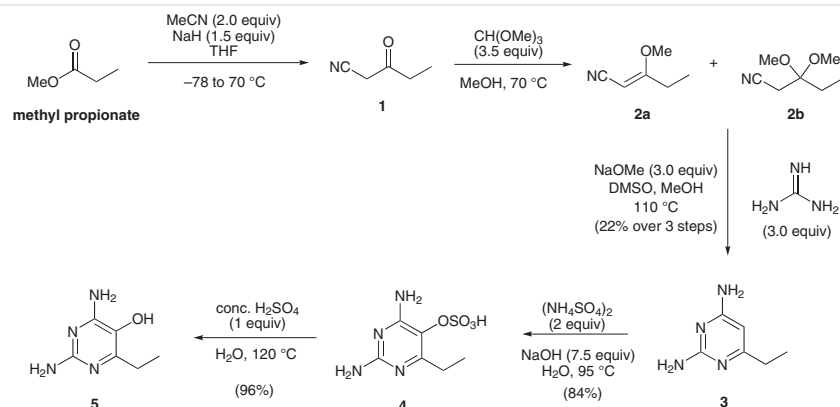
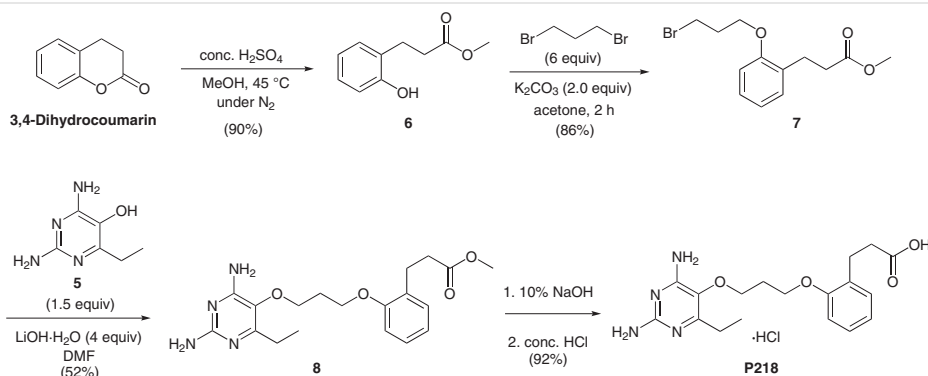**Scheme 2** Synthesis of 2,4-diamino-6-ethyl-5-hydroxypyrimidine (**5**)

droxypyrimidine (**5**) in excellent yields (96%). In summary, hydroxypyrimidine (**5**) was prepared in five steps in 18% yield without chromatographic purification (Scheme 2).

On the other hand, the key bromo-substituted intermediate **7** was prepared (Scheme 3). The process began with the acidic hydrolysis of 3,4-dihydrocoumarin, producing ring-opened methyl 3-(2-hydroxyphenyl)propanoate (**6**) in excellent yield (90%) without column chromatography. To reduce the costs of chemical reagents used in the Mitsunobu reaction as described in the previous study,[12] e.g., 3-bromopropan-1-ol, triphenylphosphine, and diisopropyl azodicarboxylate, a modified process was developed. A simple O-alkylation of the phenol group of **6** with an excess of 1,3-dibromopropane under basic condition was performed, resulting in methyl propanoate **7** in good yield (86%) without the observation of di-O-alkylated side product (Scheme 3).

The bromo-substituted intermediate **7** was then subjected to nucleophilic attack by the hydroxyl group of the prepared pyrimidine **5** under basic conditions (Scheme 3). This led to the formation of the desired ester intermediate **8** in moderate yield (50%). The observed preferential formation of ester **8** indicates that O-alkylation was favored. No-

tably, the N-alkylated side product was not observed. This might be due to the low reactivity of the amine moiety of the pyrimidine ring and the use of LiOH as a base. Consequent hydrolysis of the methyl ester under basic conditions, followed by precipitation in a hydrochloric acid solution, resulted in the formation of the final product P218 as a hydrochloride salt in excellent yield (92%) (Scheme 3).

To prepare the hydroxylated metabolite P218-OH, we unsuccessfully attempted to perform late-stage modification of P218 under various conditions (unpublished data). Therefore, P218-OH was totally synthesized from intermediate **5** and a new counterpart (Scheme 4). 4-(Benzyloxy)phenol, commercially available, was used as the starting material to produce P218-OH in a multistep reaction (12 steps), including 5 steps to form intermediate **5**. First, 5-(benzyloxy)-2-hydroxybenzaldehyde (**9**) was obtained in good yield (72%) by formylation by using paraformaldehyde and magnesium chloride (Scheme 4). The Wittig coupling reaction of aldehyde **9** with ethyl (triphenylphosphoranylidene)acetate was then carried out. Subsequently, the two subsequent alkylation reactions were performed as previously described for P218, resulting in ethyl acrylate **12** in satisfactory yield (40% over 3 steps). Then, alkene **12** was

Scheme 3 Synthesis of 3-(2-[3-[(2,4-diamino-6-ethylpyrimidin-5-yl)oxy]propoxy]phenyl)propanoic acid (P218)

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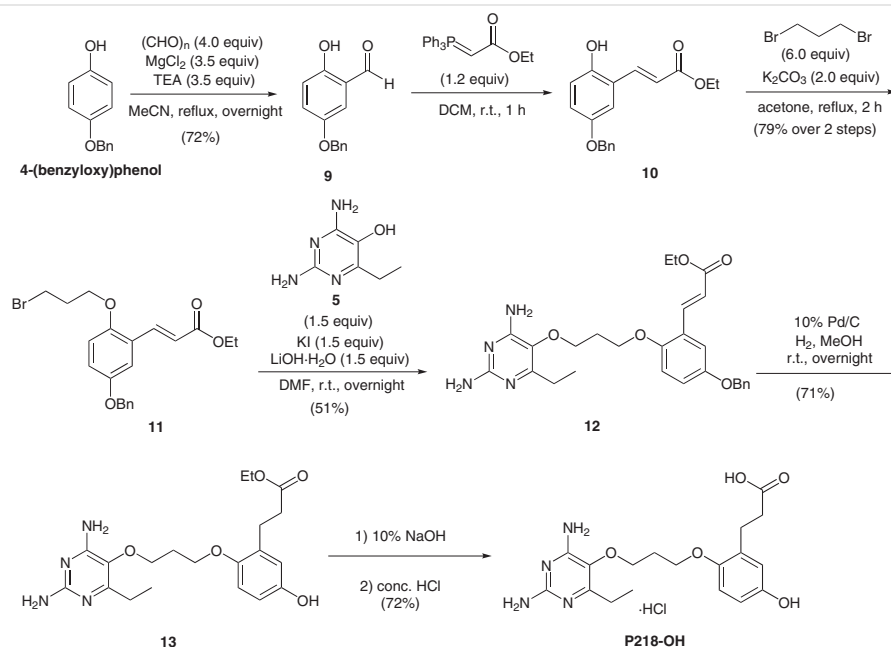
Scheme 4 Synthesis of 3-(2-[3-[(2,4-diamino-6-ethylpyrimidin-5-yl)oxy]propoxy]-5-hydroxyphenyl)propanoic acid (P218-OH)

hydrogenated and the benzyl protecting group was removed, resulting in the ethyl propanoate intermediate **13** in good yield (71%). Finally, the desired final product, P218-OH, was obtained through hydrolysis under basic conditions and subsequent precipitation with concentrated hydrochloric acid; this led to the formation of P218-OH as a hydrochloride salt in good yield (72%) (Scheme 4).

In summary, this study presents an efficient synthetic procedure for the crucial dihydrofolate reductase-targeting moiety, 2,4-diamino-6-ethyl-5-hydroxypyrimidine (**5**), to overcome the limitations of previous methods. The optimized procedures resulted in high yields of P218 and its metabolite, P218-OH, and offer a practical approach for further large-scale studies and manufacturing. This could have significant implications for the development of pyrimidine derivatives for combating malaria as a major global health issue.

All chemicals used for the synthesis were purchased from commercial suppliers and used without further purification. Reactions were monitored by TLC. The products were collected by either precipitation or column chromatography as indicated in the procedures. ^{1}H and ^{13}C NMR spectra were obtained on Bruker DRX400 or AV500D spectrometers (100 or 125 MHz for ^{13}C NMR). Quantitative ^{1}H NMR spectroscopy (qNMR) was applied for purity assessment. Mass spectra were obtained on an Agilent 6540 UHD Q-TOF LC/MS spectrometer. Melting points were recorded using Electrothermal IA9100 digital melting-point apparatus.

3-Oxopentanenitrile (1)

Anh MeCN (72 mL, 1.37 mol) was added dropwise to a suspension of NaH (24.5 g, 1.02 mol, 60% dispersion in mineral oil) in anh THF (300 mL) at –78 °C for 1 h under a N_2 atmosphere. A solution of methyl propionate (60.0 g, 0.68 mol) in anh THF (100 mL) was added to the reaction mixture, which was then heated to 70 °C overnight. After completion of the reaction, the mixture was acidified to pH ~2–3 with 3 M aq HCl. The solution was then extracted with DCM (2×), dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure; this gave crude product **1** as a yellow-brown oil (66.37 g) (the yields were determined by weighing the product obtained after removal of the mineral oil). Crude product **1** was directly used on the same day for the next step without further purification.

^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.45 (s, 2 H), 2.65 (q, J = 7.2 Hz, 2 H), 1.13 (t, J = 7.2 Hz, 3 H).

^{13}C NMR (100 MHz, CDCl$_3$): δ = 197.9, 113.6, 35.3, 31.3, 7.0.

ESI-HRMS: m/z [M]$^+$ calcd for C$_5$H$_7$NO: 97.0528; found: 97.0545.

3-Methoxypent-2-enenitrile (2)

Conc. H$_2$SO$_4$ (8 mL, 0.18 mol) was added slowly to a solution of **1** (66.37 g, 0.69 mol) and trimethyl orthoformate (264 mL, 2.4 mol) in anh MeOH (180 mL); the mixture was stirred at 70 °C overnight. The reaction mixture was basified to pH ~8–9 with K$_2$CO$_3$. MeOH was then removed by evaporation under reduced pressure. The mixture was diluted with H$_2$O and extracted with EtOAc (2×). The organic layer was washed with sat. aq NaCl, dried over Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure; this gave crude product **2** as a brown oil (80.0 g). The crude mixture of product **2** was directly used for the next step without purification.

^{1}H NMR (500 MHz, CDCl$_3$): δ = 4.29 (s, 1 H), 3.63 (s, 3 H), 3.21 (s, 6 H), 2.65 (s, 2 H), 2.48 (q, J = 7.6 Hz, 2 H), 1.83 (q, J = 7.6 Hz, 2 H), 1.14 (t, J = 7.6 Hz, 3 H), 0.93 (t, J = 7.6 Hz, 2 H).

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^{13}C NMR (100 MHz, CDCl$_3$): δ = 179.9, 118.3, 116.5, 101.1, 68.8, 56.2, 48.5, 27.2, 26.8, 22.9, 11.4, 7.9.

ESI-HRMS: m/z [M + Na]$^+$ calcd for C$_6$H$_9$NO: 134.0582; found: 134.0580.

ESI-HRMS: m/z [M + Na]$^+$ calcd for C$_7$H$_{13}$NO$_2$: 166.0844; found 166.0834.

6-Ethylpyrimidine-2,4-diamine (3)

A mixture of guanidine hydrochloride (207.1 g, 2.16 mol) and NaOMe (116.6 g, 2.16 mol) in anh MeOH (300 mL) was left stirring at r.t. for 15 min under a N$_2$ atmosphere. The reaction mixture was filtered under vacuum and the filtrate was added to a solution of **2** (80 g, 0.72 mol) in anh DMSO (480 mL). The mixture was heated at 110 °C overnight. MeOH was then removed by evaporation in vacuo. The mixture was subsequently extracted with EtOAc (2×). The organic layer was washed with sat. aq NaCl, dried over Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure; this gave the crude product as a brown oil. DMSO was removed after distillation under reduced pressure. The crude product was crystallized with Et$_2$O; this gave **3** as a white solid; yield: 20.8 g (22%); mp 161.1–164.8 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 6.08 (s, 2 H), 5.69 (s, 2 H), 5.56 (s, 1 H), 2.26 (q, J = 7.6 Hz, 2 H), 1.08 (t, J = 7.6 Hz, 3 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.5, 164.7, 163.2, 91.9, 29.8, 12.7.

ESI-HRMS: m/z [M + H]$^+$ calcd for C$_6$H$_{10}$N$_4$: 139.0984; found: 139.0987.

2,4-Diamino-6-ethylpyrimidin-5-yl Hydrogen Sulfate (4)

A suspension of **3** (20.8 g, 0.15 mol) and 5 N aq NaOH solution (226 mL, 1.13 mol) was stirred at r.t. for 30 min and then cooled to 0 °C. A solution of ammonium persulfate (69 g, 0.3 mol) in H$_2$O (93 mL) was added and the mixture was stirred at 0 °C for 1 h. The mixture was left at r.t. for 1 h and heated at 95 °C overnight. The mixture was cooled to 0 °C and acidified to pH 4.5 with 3 M aq HCl. The precipitate obtained was collected by filtration and washed with H$_2$O; this gave **4** as a yellow solid; yield: 29.7 g (84%); mp 220 °C (decomp).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.75 (br s, 1 H), 8.33 (br s, 1 H), 7.26 (br s, 3 H), 2.61 (q, J = 7.4 Hz, 2 H), 1.14 (t, J = 7.5 Hz, 3 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 161.6, 153.1, 149.0, 123.2, 20.7, 11.7.

ESI-HRMS: m/z [M + Na]$^+$ calcd for C$_6$H$_{10}$N$_4$O$_4$SNa: 257.0320; found: 257.0316.

2,4-Diamino-6-ethyl-5-hydroxypyrimidine (5)

A solution of conc. H$_2$SO$_4$ (6.72 mL, 0.127 mol) in H$_2$O (10 mL) was heated to 120 °C for 30 min. Compound **4** (29.68 g, 0.127 mol) was then added to the solution, which was then stirred for 20 min. After completion of the reaction, the mixture was cooled to 0 °C, and then basified to pH 8 with K$_2$CO$_3$. A pale brown solid precipitated; it was washed with H$_2$O; this gave **5**; yield: 18.8 g (96%); mp 220 °C (decomp).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.36 (br s, 1 H) 5.96 (br s, 2 H), 5.35 (br s, 2 H), 2.42 (q, J = 7.6 Hz, 2 H), 1.08 (t, J = 7.6 Hz, 3 H).

^{1}H NMR (400 MHz, MeOD): δ = 2.64 (q, J = 7.6 Hz, 2 H), 1.24 (t, J = 7.6 Hz, 3 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 159.2, 154.9, 147.9, 125.2, 22.0, 12.5.

ESI-HRMS: m/z [M + H]$^+$ calcd for C$_6$H$_{11}$N$_4$O: 155.0933; found: 155.0936.

Methyl 3-(2-Hydroxyphenyl)propanoate (6)

3,4-Dihydrocoumarin (20 mL, 157.8 mmol) was dissolved in anh MeOH (300 mL); then conc. H$_2$SO$_4$ (2 mL, 37.3 mmol) was added. The reaction mixture was heated to 45 °C overnight under a N$_2$ atmosphere. MeOH was removed under vacuum, and then the mixture was neutralized with K$_2$CO$_3$. The mixture was diluted with H$_2$O and extracted with DCM (2×). The organic layer was collected and washed with sat. aq NaCl and dried over Na$_2$SO$_4$. The solution was filtered and concentrated under reduced pressure to dryness. The crude product was further purified by precipitation with cooled hexane; this gave **6** as a white solid; yield: 25.9 g (90%); mp 39.4–41.1 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.35 (s, 1 H), 7.03 (d, J = 6.3 Hz, 1 H), 7.03–6.99 (m, 1 H), 6.77 (d, J = 7.9 Hz, 1 H), 6.69 (t, J = 7.4 Hz, 1 H), 3.57 (s, 3 H), 2.76 (t, J = 7.7 Hz, 2 H), 2.55 (t, J = 7.7 Hz, 2 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.9, 155.1, 129.6, 127.2, 126.4, 118.8, 114.8, 51.2, 33.3, 25.4.

ESI-HRMS: m/z [M + Na]$^+$ calcd for C$_{10}$H$_{12}$O$_3$: 203.0684; found: 203.0684.

Methyl 3-[2-(3-Bromopropoxy)phenyl]propanoate (7)

A solution of **6** (12.0 g, 66.6 mmol) in acetone (200 mL) was stirred at r.t. and then 1,3-dibromopropane (40.5 mL, 398.7 mmol) and K$_2$CO$_3$ (18.2 g, 131.7 mmol) were added. The reaction mixture was heated to 65 °C under reflux for 2 h. After completion of the reaction, the mixture was subjected to solvent removal under vacuum. The reaction mixture was then quenched with H$_2$O and extracted with EtOAc (2×). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to give the crude product which was further purified by column chromatography (silica gel, 10% EtOAc/hexane); this gave **7** as a pale yellow oil; yield: 17.2 g (86%).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.13–7.19 (m, 2 H), 6.86 (m, 2 H), 4.12 (t, J = 6.1 Hz, 2 H), 3.68 (s, 3 H), 3.64 (t, J = 6.1 Hz, 2 H), 2.96 (t, J = 7.8 Hz, 2 H), 2.62 (t, J = 7.8 Hz, 2 H), 2.35 (qt, 2 H).

^{13}C NMR (100 MHz, CDCl$_3$): δ = 173.7, 156.4, 129.9, 128.9, 127.6, 120.7, 111.1, 65.0, 51.5, 34.1, 32.3, 30.1, 26.1.

ESI-HRMS: m/z [M + Na]$^+$ calcd for C$_{13}$H$_{17}$BrO$_3$: 323.0259; found: 323.0277.

Methyl 3-(2-{3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}phenyl)propanoate (8)

The pale brown solid of **5** (3.4 g, 18.7 mmol) and LiOH·H$_2$O (2.1 g, 50.0 mmol) were dissolved in anh DMF (25 mL) at r.t. under a N$_2$ atmosphere. After the mixture had stirred for 1 h, **7** (3.75 g, 12.5 mmol) and KI (3.1 g, 18.7 mmol) were added, and the mixture was then stirred overnight. After completion of the reaction, DMF was removed under reduced pressure. The residue was diluted with H$_2$O, followed by extraction with EtOAc (2×). The organic layer was collected and dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure; this gave the crude product. The crude product was purified by column chromatography (silica gel, 2% MeOH/DCM); this gave **8** as a pale yellow oil; yield: 2.4 g (52%).

^{1}H NMR (500 MHz, MeOD-d_4): δ = 7.20–7.16 (m, 1 H), 7.14 (dd, J = 7.1, 1.6 Hz, 1 H), 6.97 (brd, J = 7.4 Hz, 1 H), 6.87–6.84 (m, 1 H), 4.23 (t, J = 6.0 Hz, 2 H), 3.95 (t, J = 6.0 Hz, 2 H), 3.61 (s, 3 H), 2.92 (t, J = 7.7 Hz, 2 H), 2.59 (t, J = 7.7 Hz, 2 H), 2.45 (q, J = 7.6 Hz, 2 H), 2.29–2.24 (m, 2 H), 1.10 (t, J = 7.6 Hz, 3 H).

^{13}C NMR (125 MHz, MeOD-d_4): δ = 175.4, 160.5, 157.9, 130.9, 130.3, 129.9, 128.8, 121.7, 112.3, 70.9, 65.3, 52.0, 35.1, 31.0, 27.2, 24.9, 13.3.

ESI-HRMS: m/z [M + H]$^+$ calcd for $C_{19}H_{27}N_4O_4$: 375.2032; found: 375.1960.

3-(2-{3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}phenyl)propanoic Acid Hydrochloride (P218·HCl)

Ester intermediate **8** was then hydrolyzed by 10% NaOH in EtOH and heated to 90 °C. After 2 h, EtOH was removed under reduced pressure; this gave the crude product which was then acidified to pH 2–3 with conc. HCl, affording a salt form of P218 without the need for column chromatography; this gave P218·HCl as a white solid; yield: 2.2 g (92%); mp 195.4–197.6 °C. The NMR data are in accordance with the literature,[12] with the signal of the quaternary carbon on the pyrimidine ring missing.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.45 (br s, 1 H), 12.09 (br s, 1 H), 8.31 (br s, 1 H), 7.84 (br s, 1 H), 7.44 (br s, 2 H), 7.19–7.14 (m, 2 H), 6.98 (d, J = 8.0 Hz, 1 H), 6.85 (t, J = 7.3 Hz, 1 H), 4.15 (t, J = 5.9 Hz, 2 H), 3.90 (t, J = 6.3 Hz, 2 H), 2.78 (t, J = 7.7 Hz, 2 H), 2.48–2.45 (m, 2 H), 2.24–2.19 (m, 2 H), 1.12 (t, J = 7.6 Hz, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 173.9, 161.1, 156.2, 153.3, 147.3, 129.5, 128.6, 127.5, 120.3, 111.3, 70.7, 63.9, 33.7, 29.1, 25.3, 19.8, 11.9.

ESI-HRMS: m/z [M + H]$^+$ calcd for $C_{18}H_{24}N_4O_4$: 361.1847; found: 361.1876.

5-(Benzyloxy)-2-hydroxybenzaldehyde (9)

A round-bottomed flask was charged with 4-(benzyloxy)phenol (10.0 g, 50.0 mmol), anh MgCl$_2$ (15.5 g, 175.0 mmol), and paraformaldehyde (6.0 g, 200 mmol); the addition of anh MeCN followed (0.5 M) at 0 °C. The resulting mixture was stirred at r.t. for 30 min, before triethylamine (26.3 mL, 175 mmol) was added. The reaction mixture was then heated to reflux for 18 h. After completion of the reaction, the resulting mixture was cooled to r.t. and treated with 1 M aq HCl. The two phases were separated and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to give the crude product, which was further purified by column chromatography (silica gel, 5% EtOAc/hexane); this gave **9** as a white solid; yield: 8.2 g (72%); mp 95.5–97.6 °C.

^{1}H NMR (500 MHz, CDCl$_3$): δ = 10.67 (s, 1 H), 9.83 (s, 1 H), 7.45–7.38 (m, 4 H), 7.37–7.32 (m, 1 H), 7.22 (dd, J = 9.0, 3.1 Hz, 1 H), 7.08 (d, J = 3.1 Hz, 1 H), 6.94 (d, J = 9.0 Hz, 1 H), 5.07 (s, 2 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 196.1, 156.2, 151.8, 136.5, 128.7 (2C), 128.2, 127.5 (2C), 126.1, 120.0, 118.7, 116.8, 71.0.

ESI-HRMS: m/z [M – H]$^+$ calcd for $C_{14}H_{12}O_3$: 227.0714; found: 227.0709.

Ethyl (E)-3-[5-(Benzyloxy)-2-(3-bromopropoxy)phenyl]acrylate (11)

To a stirred solution of **9** (10.7 g, 46.8 mmol) in DCM (0.2 M) at r.t. was added ethyl (triphenylphosphoranylidene)acetate (19.6 g, 56.2 mmol) and the reaction mixture was stirred for 1 h. After completion of the reaction, the mixture was quenched with water and the two phases were separated. The aqueous layer was extracted with DCM (2×). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to afford the crude product, which was used in next step without further purification. To the resulting mixture of **9** in acetone (0.1 M) was added 1,3-dibromopropane (280.8 mmol, 28.5 mL) and K$_2$CO$_3$ (12.9 g, 93.6 mmol) and the mix-

ture was stirred at r.t. The reaction mixture was then heated to reflux, at which it was stirred for 2 h. Then the acetone was removed under reduced pressure. The reaction residue was then added to water and extracted with EtOAc (2×). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to give the crude product, which was further purified by column chromatography (silica gel, 5% EtOAc/hexane); this gave **11** as a white solid; yield: 15.5 g (79% over two steps); mp 57.1–59.9 °C.

^{1}H NMR (500 MHz, CDCl$_3$): δ = 7.97 (d, J = 16.2 Hz, 1 H), 7.45–7.37 (m, 4 H), 7.36–7.31 (m, 1 H), 7.15 (d, J = 3.0 Hz, 1 H), 6.97 (dd, J = 9.0, 3.0 Hz, 1 H), 6.87 (d, J = 9.0 Hz, 1 H), 6.44 (d, J = 16.1 Hz, 1 H), 5.04 (s, 2 H), 4.26 (q, J = 7.1 Hz, 2 H), 4.12 (t, J = 5.8 Hz, 2 H), 3.63 (t, J = 6.3 Hz, 2 H), 2.36 (p, J = 6.1 Hz, 2 H), 1.34 (t, J = 7.1 Hz, 3 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 167.2, 152.9, 151.8, 139.3, 136.8, 128.6 (2C), 128.0, 127.4 (2C), 124.4, 118.9, 118.1, 114.1, 113.8, 70.7, 66.6, 60.4, 32.3, 30.0, 14.3.

ESI-HRMS: m/z [M + Na]$^+$ calcd for $C_{21}H_{23}BrNaO_4$: 441.0672; found: 441.0666.

Ethyl (E)-3-(5-(Benzyloxy)-2-{3-[(2,4-diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}phenyl)acrylate (12)

To a stirred solution of **11** (7.1 g, 16.9 mmol) in DMF (0.5 M) at r.t. was added 2,6-diamino-4-ethyl-5-hydroxypyrimidin-1-ium chloride (**5**·HCl; 4.8 g, 25.4 mmol), KI (4.2 g, 25.4 mmol), and LiOH·H$_2$O (1.1 g, 25.4 mmol); then the mixture was stirred for 18 h. After completion of the reaction, EtOAc and water were added and the two phases were separated. The aqueous layer was extracted with EtOAc (2×). The combined organic layers were extracted many times with water to remove the DMF and dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure, to give the crude product, which was further purified by column chromatography (silica gel, 2% MeOH/DCM); this gave **12** as a white foamy solid; yield: 4.2 g (51%).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.88 (d, J = 16.1 Hz, 1 H), 7.49–7.42 (m, 2 H), 7.41–7.36 (m, 3 H), 7.34–7.28 (m, 1 H), 7.07 (s, 2 H), 6.66 (d, J = 16.1 Hz, 1 H), 6.11 (s, 2 H), 5.56 (s, 2 H), 5.10 (s, 2 H), 4.19 (t, J = 5.9 Hz, 2 H), 4.16 (q, J = 7.1 Hz, 2 H), 3.78 (t, J = 6.1 Hz, 2 H), 2.30 (q, J = 7.6 Hz, 2 H), 2.18 (t, J = 6.1 Hz, 2 H), 1.22 (t, J = 7.1 Hz, 3 H), 0.97 (t, J = 7.6 Hz, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.5, 159.7, 159.0, 158.2, 152.3, 151.7, 138.9, 137.2, 128.4 (2C), 128.2, 127.8, 127.8 (2C), 123.1, 119.0, 118.6, 114.0, 113.8, 69.8, 68.9, 65.4, 60.0, 29.4, 23.5, 14.2, 12.6.

ESI-HRMS: m/z [M + H]$^+$ calcd for $C_{27}H_{33}N_4O_5$: 493.2445; found: 493.2449.

Ethyl 3-(2-{3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}-5-hydroxyphenyl)propanoate (13)

Anh and degassed MeOH (0.1 M) was added to a round-bottomed flask charged with **12** (3.5 g, 7.1 mmol) and 10% Pd/C (10% w/w, 636.3 mg) at r.t. The reaction mixture was allowed to stir under a H$_2$ atmosphere. After completion of the reaction, the mixture was filtered through Celite in a Buchner funnel. The solution was concentrated under reduced pressure, to give the crude product, which was further purified by column chromatography (silica gel, 4% MeOH/DCM); this gave **13** as a white foamy solid; yield: 2.0 g (71%).

^{1}H NMR (500 MHz, MeOD-d_4): δ = 6.80 (d, J = 8.3 Hz, 1 H), 6.69–6.52 (m, 2 H), 4.14 (t, J = 5.8 Hz, 2 H), 4.07 (q, J = 7.1 Hz, 2 H), 3.94 (t, J = 6.2 Hz, 2 H), 2.85 (t, J = 7.7 Hz, 2 H), 2.56 (dd, J = 8.2, 7.1 Hz, 2 H), 2.47 (q, J = 7.6 Hz, 2 H), 2.22 (p, J = 6.0 Hz, 2 H), 1.19 (t, J = 7.1 Hz, 3 H), 1.12 (t, J = 7.6 Hz, 3 H).

^{13}C NMR (125 MHz, MeOD-d_4): δ = 175.0, 161.3, 160.6, 160.1, 152.1, 151.3, 131.1, 130.3, 118.0, 114.5, 113.8, 71.0, 66.0, 61.5, 35.4, 31.1, 27.2, 24.9, 14.5, 13.3.

ESI-HRMS: m/z [M + H]$^+$ calcd for $C_{20}H_{29}N_4O_5$: 405.2132; found: 405.2137.

3-(2-{3-[(2,4-Diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}-5-hydroxyphenyl)propanoic Acid Hydrochloride (P218-OH·HCl)

To afford the final product (P218-OH), the intermediate **13** (180.9 mg, 0.45 mmol) was then hydrolyzed by 10% NaOH (4 mL). After 10 min, EtOH was removed from the mixture under reduced pressure, to give the crude product, which was then acidified to pH 2–3 with concentrated HCl, affording a salt form of P218-OH, with no need for column chromatography; this gave a white solid; yield: 142.2 mg (72%); mp 208.3–212.8 °C.

^{1}H NMR (400 MHz, MeOD): δ = 6.81 (d, J = 8.6 Hz, 1 H), 6.67–6.57 (m, 2 H), 4.14 (t, J = 5.7 Hz, 2 H), 4.04 (t, J = 6.2 Hz, 2 H), 2.84 (dd, J = 8.5, 7.1 Hz, 2 H), 2.65–2.50 (m, 4 H), 2.27 (p, J = 6.0 Hz, 2 H), 1.18 (t, J = 7.6 Hz, 3 H); .

^{13}C NMR (100 MHz, MeOD): δ = 177.0, 163.5, 155.0, 152.2, 151.2, 149.1, 131.3, 129.7, 117.9, 114.4, 113.7, 72.3, 65.7, 35.3, 30.8, 27.2, 21.5, 12.5.

ESI-HRMS: m/z [M + H]$^+$ calcd for $C_{18}H_{24}O_4N_5$: 377.1825; found: 377.1817.

Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

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