Supporting Information
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Synthetic approach to argpyrimidine as a tool for investigating non-enzymatic posttranslational modification of proteins

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Materials and Methods

Chemicals. 9-Fluorenlymethoxycarbonyl (Fmoc)-protected amino acid derivatives, 2-(1H-benzotriazol-1-yl)-1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and preloaded Fmoc-Lys-Wang resin were purchased from Novabiochem (Nottingham, UK). N,N-dimethylformamide (DMF), dichloromethane (DCM), acetonitrile (ACN) and trifluoroacetic acid (TFA) for SPPS were obtained from Biosolve (Valkenswaard, The Netherlands). All other chemical reagents and solvents were purchased in the highest available quality from the following companies if not noted otherwise: Sigma-Aldrich (Taufkirchen, Germany), Fisher-Scientific (Schwerte, Germany), TCI-Europe (Zwijndrecht, Belgium), VWR (Darmstadt, Germany), Roth (Karlsruhe, Germany), Merck (Darmstadt, Germany), Invitrogen (Darmstadt, Germany), J.T.Baker (Griesheim, Germany), NeoLab (Heidelberg, Germany) or Omnilab (Bremen, Germany).

NMR spectroscopy. Unless otherwise specified, proton (^1H) and carbon (^13C) NMR spectra were recorded at 25 °C on Bruker AVIII 400 or AVIII 600 spectrometers operating at 400 MHz or 600 MHz for proton and 100 MHz or 150 MHz for carbon nuclei. ^1H NMR data are recorded as follows: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral] where multiplicity is defined as: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet or combinations of the above. The residual protio-forms of CDCl₃ (δ 7.26), CD₃OD (δ 3.31), (CD₃)₂SO (δ 2.50) and D₂O (δ 4.79) were used as references for ^1H NMR spectra, and the central peak (δ 77.0) of the CDCl₃ triplet, the central peak (δ 49.0) of the CD₃OD heptet and the central peak (δ 39.5) of the (CD₃)₂SO heptet were used as references for proton-decoupled ^13C NMR spectra.

General procedures for organic synthesis. Reactions were monitored either by analytical LC-MS (see below) or analytical thin layer chromatography (TLC) that was performed on glass-backed silica gel 60 F254 plates as supplied by Merck. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included phosphomolybdic acid : ceric sulfate : sulfuric acid (conc.) : water (37.5 g : 7.5 g : 37.5 g : 720 ml) and ninhydrin : ethanol (33 g : 1000 ml). The retention factor (Rf) was quoted to the nearest 0.1. Flash column chromatography was performed using silica gel 60 (230-400 mesh, 0.04–0.063 mm, Macherey-Nagel (Duren, Germany)) as the stationary phase and the analytical
reagent- or HPLC-grade solvents indicated. C18 Strata™ solid phase extraction (SPE) cartridges (Giga Tubes), Phenomenex (Aschaffenburg, Germany), or Polygopre ™ 60-50 C18 powder, Macherey-Nagel (Duren, Germany), were used as the stationary phase in RP flash chromatography. A Finnigan MAT95 mass spectrometer was used to obtain low-resolution electron impact (EI) mass spectra. High-resolution electrospray ionization (ESI) mass spectra were obtained on a Bruker MaXis HD ESI-QTOF Instant Expertise mass spectrometer operating in positive ionization mode. Optical rotations were measured at 25 °C with a Perkin Elmer 341 polarimeter at the sodium-D line (589 nm) and the concentrations (c) (g/100 ml) indicated using spectroscopic grade solvents. The measurements were carried out in a cell with a path length (l) of 1 dm. Specific rotations [α]D were calculated using the equation [α]D = 100.a/(c.l) and are given in 10–1.deg.cm2.g–1. Melting points were measured on a Reichert hot-stage microscope apparatus and are uncorrected. X-ray crystallographic analysis was carried out at the University of Vienna X-Ray Structure Analysis Centre (see below). Specific experimental procedures are reported below.

2,4-Dioxopentan-3-yl acetate (5).

According to the published procedure1 with minor modifications,2 anhydrous sodium acetate (43.6 g, 531 mmol) was added, portionwise, to a magnetically stirred solution of 3-chloro-2,4-pentanedione (20 ml, 177 mmol) in anhydrous dimethyl sulfoxide (350 ml) maintained under an atmosphere of argon at 25 °C. The ensuing suspension was stirred at this temperature, and when 1H NMR indicated that all the starting material had been consumed (3 h) the reaction mixture was treated with diethyl ether (250 ml) and transferred into a separating funnel containing NH4Cl (250 ml of a saturated aqueous solution) and H2O (250 ml). The separated aqueous phase was extracted with diethyl ether (2 x 200 ml) and the combined organic fractions were washed with H2O (3 x 250 ml) then brine (300 ml) before being dried (MgSO4), filtered and concentrated under reduced pressure (680 mbar, 28 °C) to afford the volatile title compound 5 (24.5 g, ca. 87%) as a pale yellow oil. This material contained traces of diethyl ether but was otherwise pure at the NMR limit of detection and was used without further purification and
without delay in the next step of the reaction sequence. A portion of the material was purified by column chromatography (silica, 50% v/v hexane/DCM), and concentration of the relevant fractions ($R_f = 0.5$ in DCM) provided an analytically pure sample of compound 5\textsuperscript{1,3} as a clear, colorless volatile oil that was characterized as an equilibrating mixture of keto- and enol-tautomers (~2.8:1) (lit.\textsuperscript{3a} ~2.7:1). $^1$H NMR (400 MHz, CDCl$_3$) (keto-form): $\delta$ 5.47 (s, 1H), 2.28 (s, 6H), 2.23 (s, 3H). $^1$H NMR (400 MHz, CDCl$_3$) (enol-form): $\delta$ 14.41 (s, 1H), 2.25 (s, 3H), 2.01 (s, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) (keto-form): $\delta$ 199.0, 169, 85.1, 27.3, 20.4. $^{13}$C NMR (100 MHz, CDCl$_3$) (enol-form): $\delta$ 184.7, 169.5, 128.1, 20.6, 20.3. MS (EI): $m/z$ 158 (M$^+$, 2.5%), 116 (72), 74 (100). HRMS (ESI): $m/z$ [M + Na]$^+$ calcd. for C$_7$H$_{10}$O$_4$, 181.0471; found, 181.0470.

2-Amino-4,6-dimethylpyrimidin-5-ol (6).

According to the published procedure,\textsuperscript{2} to a magnetically stirred solution of guanidine sulfate (4) (4.1 g, 38 mmol) in methanesulfonic acid (32 ml, 1.2 M) maintained at 25 °C was added crude diketone 5 (12 g, ca. 76 mmol), resulting in a mildly-exothermic reaction. After 3 h, another portion (6 g, ca. 38 mmol) of compound 5 was added, followed by the final portion (6 g, ca. 38 mmol) after 3 h, whereupon no exothermic reaction was observed. The ensuing dark brown viscous reaction mixture was stirred for 16 h before being cooled to 0 °C and diluted with H$_2$O (85 ml) followed by the dropwise addition of NH$_4$OH (28-30% aqueous solution, 70 ml). The ensuing mixture (pH 7-8) was stirred at 25 °C for 30 min before being filtered through a plug of cotton wool using additional H$_2$O (60 ml) to remove the brown waxy solid consisting of relatively non-polar organic impurities. The combined orange filtrates were concentrated under reduced pressure with heating to 60 °C then treated with ethyl acetate and MeOH (500 ml of a 1:1 mixture) and stirred at 35 °C for 30 min to dissolve the desired compound 6. The resulting suspension was dried with MgSO$_4$ then filtered through filter paper using an additional portion (100 ml) of the same solvent to remove the salts. The filtrates were treated with silica (ca. 14 g) then concentrated under reduced pressure (40 °C, 100 mbar). Subjection of the ensuing
material to column chromatography (silica, 1→5→8% v/v MeOH/DCM gradient elution) and concentration of the relevant fractions \((R_f = 0.2\) in 10% v/v MeOH/DCM) afforded the title compound \(6^4\) (4.5 g, 85%) as a pale-tan powder. A portion of this material was crystallized from a 1:1 mixture of ethyl acetate and ethanol to obtain off-white needles, \(\text{mp: } 170-180 ^\circ\text{C}\) (subl.) [lit.\(^4c\) 208 °C (decomp.)]. \(^1\)H NMR (600 MHz, \((\text{CD}_3)_2\text{SO})\): \(\delta 7.80\) (s, 1H, OH), 5.74 (s, 2H, NH\(_2\)), 2.16 (s, 6H). \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta 2.28\) (s, 6H). \(^13\)C NMR (150 MHz, \((\text{CD}_3)_2\text{SO})\): \(\delta 157.3, 155.0, 139.0, 18.7\). \(^13\)C NMR (100 MHz, CD\(_3\)OD): \(\delta 158.3, 157.7, 141.2, 18.6\). HRMS (ESI): \([m/z\) \([\text{M} + \text{H}]^+\) calcd. for C\(_6\)H\(_9\)N\(_3\)O, 140.0818; found, 140.0817.

2-amino-4,6-dimethylpyrimidin-5-yl tert-butyl carbonate \((7)\).

A magnetically stirred solution of 2-aminopyrimidinol \(6\) (410 mg, 2.95 mmol) in t-BuOH (20 ml) and NaOH (7 ml of a 1 M aqueous solution) maintained at 25 °C under an argon atmosphere was treated with Boc\(_2\)O (1.16 g, 5.3 mmol). After 16 h at this temperature, the reaction mixture was diluted with H\(_2\)O (50 ml) and ethyl acetate (50 ml), and the separated aqueous phase was extracted with ethyl acetate (2 x 50 ml). The combined organic phases were washed with brine (100 ml) before being dried (MgSO\(_4\)), filtered and concentrated under reduced pressure to give a tan solid. Subjection of this material to column chromatography (silica, 1.5% v/v MeOH/DCM) and concentration of the relevant fractions \((R_f = 0.2\) in 5% v/v MeOH/DCM) afforded compound \(7\) (529 mg, 75%) as an off-white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 5.03\) (broad s, 2H, NH\(_2\)), 2.23 (s, 6H), 1.54 (s, 9H). \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta 160.1, 159.8, 151.1, 136.6, 84.1, 27.6, 18.6\). HRMS (ESI): \([m/z\) \([\text{M} + \text{Na}]^+\) calcd. for C\(_{11}\)H\(_{17}\)N\(_3\)O\(_3\), 262.1162, found: 262.1157; \([m/z\) \([\text{M} + \text{H}]^+\) calcd. for C\(_{11}\)H\(_{17}\)N\(_3\)O\(_3\), 240.1343, found: 240.1337.

Alternatively, a magnetically stirred suspension of crude 2-aminopyrimidinol \(6\) (174 mg, \(\leq 0.92\) mmol) containing the salts from the MeSO\(_3\)H-mediated condensation reaction (above) and Boc\(_2\)O (280 mg, 1.29 mmol) in t-BuOH (6 ml) was stirred at 60 °C under an argon atmosphere. After 16 h at this temperature, another portion of Boc\(_2\)O was added (200 mg, 0.92 mmol), and
after further 6 h a final portion of Boc₂O (200 mg, 0.92) was introduced. After 48 h at 60 °C, the
now clear reaction mixture was concentrated under reduced pressure before being dissolved
using ethyl acetate (5 ml) and H₂O (5 ml) then poured into NaHCO₃ (5 ml of a saturated
aqueous solution). The separated aqueous phase was extracted with ethyl acetate (2 x 5 ml),
and the combined organic phases were washed with NaHCO₃ (10 ml of a saturated aqueous
solution) then brine (10 ml) before being dried (MgSO₄), filtered and concentrated under
reduced pressure to afford a brown solid. Subjection of this material to column chromatography
(silica, 0.5→1→2% v/v MeOH/DCM gradient elution) and concentration of the relevant fractions
(\(R_f = 0.2\) in 5% v/v MeOH/DCM) provided compound 7 (157 mg, 71% over two steps from
guanidine 4) as an off-white solid. This material was identical in all respects to compound 7
described above.

**tert-Butyl (5-((tert-butoxycarbonyl)oxy)-4,6-dimethylpyrimidin-2-yl)carbamate (8).**
A magnetically stirred solution of amine 7 (255 mg, 1.07 mmol) and Boc₂O (233 mg of a 97% reagent, 1.03 mmol) in anhydrous THF (4.3 ml) maintained at 0 °C under an argon atmosphere was treated with NaHMDS (2.13 ml of a 1 M solution in THF, 2.13 mmol), dropwise over 15 min. The resulting reaction mixture was taken out of the ice-water bath and allowed to warm to 25 °C. After 2.5 h, the ensuing yellow reaction mixture was concentrated under reduced pressure at 25 °C to give a slurry, diluted with H₂O (25 ml) and ethyl acetate (50 ml), then poured into NH₄Cl (25 ml of a saturated aqueous solution). The separated aqueous phase was extracted with ethyl acetate (2 x 50 ml), and the combined organic phases were washed with brine (100 ml) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give a yellow oil. Subjection of this material to column chromatography (silica, 0.5% v/v MeOH/DCM) and concentration of the relevant fractions (\(R_f = 0.3\) in 2% v/v MeOH/DCM) afforded compound 8 (235 mg, 65%) as a white foam. ¹H NMR (400 MHz, CDCl₃): \(\delta\) 7.95 (broad s, 1H, NH), 2.34 (s, 6H), 1.52 (s, 9H), 1.48 (s, 9H). ¹³C NMR (100 MHz, CDCl₃): \(\delta\) 160.7, 154.0, 150.7, 150.4, 139.2,
84.4, 81.1, 28.2, 27.5, 18.8. HRMS (ESI): m/z [M + Na]+ calcd. for C_{16}H_{25}N_{3}O_{5}: 362.1686, found: 362.1689; m/z [M + H]+ calcd. for C_{16}H_{25}N_{3}O_{5}: 340.1867, found: 340.1869.

**Benzyl (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-hydroxypentanoate (2).**

According to the published procedure, a magnetically stirred solution of Fmoc-Glu-OBn S3 (5.05 g, 11.0 mmol) in anhydrous THF (60 ml) maintained at ca. -10 °C (ice-salt cooling bath) under an argon atmosphere was treated, dropwise, with N-methylmorpholine (1.21 ml, 11.0 mmol) then isobutyl chloroformate (1.44 ml, 11.0 mmol). The resulting reaction mixture was stirred at -10 °C for 30 min °C then filtered through a short pad of Celite, which was washed with ice-cold anhydrous THF (32 mL). The combined filtrates, magnetically stirred at -10 °C, were treated with sodium borohydride (567 mg, 15.0 mmol) in one portion. The ensuing mixture was then treated with ice-cold MeOH (120 ml), dropwise over 30 min at 0 °C, then stirred at this temperature for 30 min. The resulting clear solution was diluted with EtOAc (300 mL) then poured into HCl (200 ml of a 1 M aqueous solution). The separated aqueous phase was extracted with EtOAc (2 x 200 ml), and the combined organic phases were washed with NaHCO3 (300 ml of a saturated aqueous solution) then brine (300 ml) before being dried (MgSO4), filtered and concentrated under reduced pressure. Subjection of the ensuing material to column chromatography (silica, 0.5→1% v/v MeOH/DCM gradient elution) and concentration of the relevant fractions (Rf = 0.5 in 5% v/v MeOH/DCM) afforded compound 2 (3.98 g, 81%) as a white gum. This material was identical, in all respects, with the authentic sample. 1H NMR (400 MHz, CDCl3): δ 7.76 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.3 Hz, 2H), 7.42 – 7.29 (m, 9H), 5.53 (d, J = 7.9 Hz, 1H, NH), 5.21 (d, J = 12.2, 1H), 5.16 (d, J = 12.2 Hz, 1H), 4.47 (dd, J = 12.8, 7.6 Hz, 1H), 4.41 (d, J = 6.9, 2H), 4.21 (broad t, J = 6.8, 1H), 3.63 (broad s, 2H), 1.96 (m, 1H), 1.79 (m, 1H), 1.62-1.53 (m, 2H). 13C NMR (100 MHz, CDCl3): δ 172.1, 156.0, 143.9 and 143.7 (splitting of signals for Fmoc quaternary C10 and C13 due to the presence of rotamers), 141.3, 135.2, 128.6, 128.5, 128.3, 127.7, 127.0, 125.1, 120.0 and 119.9 (splitting of signals for Fmoc
CHs due to the presence of rotamers), 67.2, 67.0, 62.0, 53.6, 47.2, 29.3, 28.1. MS (ESI) m/z 446 [M + H]+.

1-((9H-Fluoren-9-yl)methyl) 2-benzyl (S)-pyrrolidine-1,2-dicarboxylate (10) and
diisopropyl (S)-1-((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(benzyloxyl)-5-oxopentyl)hydrazine-1,2-dicarboxylate (11).

In a typical procedure, a magnetically stirred solution of alcohol 25 (20 mg, 0.044 mmol), aryl carbamate 8 (21 mg, 0.090 mmol) and triphenylphosphine (18 mg, 0.067 mmol) in anhydrous THF (0.45 ml, 0.1 M) maintained at 0 °C under an argon atmosphere was treated, dropwise, with diisopropyl azodicarboxylate (DIAD) (13 μl, 0.067 mmol). The ensuing yellow solution was allowed to warm to 25 °C and stirred 24 h, at which point TLC analysis still indicated the presence of the unreacted alcohol 2. The solvent was evaporated under reduced pressure, and the ensuing residue was subjected to column chromatography (silica, 0→0.1→0.25→0.5% v/v MeOH/DCM gradient elution). Concentration of the relevant fractions provided varying amounts of compounds that were tentatively assigned as 10 and 11.

Compound 10. Rf = 0.7 in 2% v/v MeOH/DCM. 1H NMR (700 MHz, CDCl3) (complex mixture of rotamers): δ 7.80 – 7.23 (m, app. 9H, 13H), 5.26 – 5.08 (m, 2H), 4.51 – 4.34 (m, 2H), 4.29 (m, 1H), 3.78 – 3.64 (m, 2H), 3.56 (m, 1H), 2.33 – 1.91 (m, 4H). 13C NMR (176 MHz, CDCl3) (complex mixture of rotamers): δ 173.3 (C), 144.41 (C), 143.92 (C), 141.82 (C), 141.67 (C), 140.61 (C), 130.03 (CH), 128.55 (CH), 128.35 (CH), 128.20 (CH), 128.07 (CH), 127.73 (CH), 127.65 (CH), 127.05 (CH), 125.22 (CH), 125.15 (CH), 125.10 (CH), 124.96 (CH), 67.50 and 67.46 (CH2, rotamers), 66.86 and 66.80 (CH2, rotamers), 59.33 and 58.93 (CH, rotamers), 47.24
and 47.20 (CH, rotamers), 47.00 and 46.49 (CH₂, rotamers), 31.07 and 29.89 (CH₂, rotamers), 24.38 and 23.42 (CH₂, rotamers). MS (ESI) m/z 877 [(M₂ + Na)⁺, 25%]; 450 [(M + Na)⁺, 62%]; 428 [(M + H)⁺, 100%]. HRMS (ESI): m/z [M + Na]⁺ calcd. for C₂₇H₂₅NO₄: 450.1676, found: 450.1677.


Benzyl (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-iodopentanoate (S₄).

A magnetically stirred solution of triphenylphosphine (33 mg, 0.13 mmol) and imidazole (11 mg, 0.16 mmol) in anhydrous DCM (0.6 ml) maintained at 0 °C under an argon atmosphere was treated with iodine (32 mg, 0.13 mmol). The resulting reaction mixture was taken out of the cooling bath and stirred for 30 min before being cooled to 0 °C and treated with a solution of alcohol 2 (37.7 mg, 0.085 mmol) in anhydrous DCM (0.5 ml). The ensuing mixture was stirred at 0 °C for 1 h then allowed to warm to 25 °C and stirred for 2 h before being passed through a short plug of silica, which was washed with additional DCM. The combined filtrates were concentrated under reduced pressure then subjected to column chromatography (silica, 10→20% v/v ethyl acetate/hexanes gradient elution). Concentration of the relevant fractions (Rᵣ = 0.15 in 10% v/v ethyl acetate/hexanes) afforded compound S₄ (46 mg, 97%) as a clear, colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.42 – 7.30 (m, 9H), 5.32 (d, J = 8.0 Hz, 1H, NH), 5.22 (d, J = 12.3, 1H), 5.17 (d, J = 12.1 Hz, 1H), 4.42 (m, 3H), 4.21 (broad t, J = 6.7, 1H), 3.14 (broad m, 2H), 1.99 (m, 1H), 1.83 (m, 3H). MS (ESI) m/z 556 [M + H]⁺.
tert-Butyl (4,6-dimethyl-2-((4-nitrophenyl)sulfonamido)pyrimidin-5-yl) carbonate (12).

4-Nitrobenzenesulfonyl chloride (150 mg, 0.67 mmol) was added to a magnetically stirred solution of amine 7 (107 mg, 0.45 mmol) in anhydrous pyridine and DCM (0.9 ml of a 1:1 mixture, 0.5 M) maintained at 0 °C. The ensuing reaction mixture was allowed to warm to 25 °C and stirred for 3 h before being diluted with DCM (20 ml) and poured into a separating funnel containing NH₄Cl and H₂O (20 ml of a 1:1 mixture). The separated aqueous phase was extracted with DCM (2 x 10 ml), and the combined organics were washed with NH₄Cl and H₂O (20 ml of a 1:1 mixture) then NaHCO₃ (20 ml of a saturated aqueous solution) and brine (20 ml) before being dried with MgSO₄, filtered and concentrated under reduced pressure. The resulting dark-yellow oil was subjected to column chromatography (silica, 0.25→0.35% v/v MeOH/DCM gradient elution) to afford, after the concentration of the relevant fractions (Rᵢ = 0.5 in 2% v/v MeOH/DCM), the title compound 12 (123 mg, 65%) as a bright-yellow foam. ¹H NMR (600 MHz, CDCl₃): δ 8.35 (s, 4H), 2.30 (s, 6H), 1.53 (s, 9H). ¹³C NMR (150 MHz, CDCl₃): δ 161.4, 152.1, 150.3, 145.1, 139.8, 130.2, 123.7, 85.0, 27.5, 18.6 (one signal obscured or overlapping). HRMS (ESI): m/z [M + H]+ calcd. for C₁₇H₂₀N₄O₇S: 447.0945, found: 447.0949.
Benzyl (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((N-(5-((tert-butoxycarbonyl)oxy)-4,6-dimethylpyrimidin-2-yl)-4-nitrophenyl)sulfonamido)pentanoate (13).

A magnetically stirred solution of alcohol 2 (463 mg, 1.04 mmol), sulfonamide 12 (923 mg, 2.17 mmol) and triphenylphosphine (408 mg, 1.56 mmol) in anhydrous THF (10.5 ml, 0.1 M) maintained at 0 °C under an argon atmosphere was treated with DIAD (307 μl, 1.56 mmol), dropwise. The ensuing yellow solution was allowed to warm to 25 °C in the ice/water bath over 16 h before being concentrated under reduced pressure. The resulting residue was subjected to column chromatography (silica, 0→0.1→0.15→0.25% v/v MeOH/DCM gradient elution), and concentration of the relevant fractions (Rf = 0.5 in 1% v/v MeOH/DCM or 30% v/v ethyl acetate/hexanes) provided a yellow foam. Subjection of this material to column chromatography (silica, 5% v/v ethyl acetate/toluene) and concentration of the relevant fractions afforded the title compound 13 as a clear, bright-yellow foam (818 mg, 92%). [α]D = +0.2 (c 0.4, CHCl3). 1H NMR (400 MHz, CDCl3): δ 8.30 (d, J = 9.0 Hz, 2H), 8.22 (d, J = 9.0 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.41 – 7.26 (m, 9H), 5.59 (d, J = 8.5 Hz, 1H, NH), 5.21 (s, 2H), 4.59 (dd, J = 12.0, 7.0 Hz, 1H), 4.45 (d, J = 7.0 Hz, 2H), 4.26 – 4.13 (m, 3H), 2.23 (s, 2H), 2.06 – 1.86 (m, 4H), 1.55 (s, 9H). 13C NMR (100 MHz, CDCl3): δ 172.1, 160.3, 155.9, 153.5, 150.5, 149.9, 146.8, 143.8 and 143.7 (splitting of signals for Fmoc quaternary C10 and C13 due to the presence of rotamers), 141.3, 139.5, 135.2, 130.0, 128.6, 128.5, 128.2, 127.7, 127.0, 125.0, 123.4, 119.9, 84.8, 67.3, 67.0, 53.7, 47.2, 47.0, 29.3, 27.5, 25.6, 18.5. HRMS (ESI): m/z [M + Na]+ calcd. for C44H45N5O11S: 874.2728, found, 874.2729.
Benzyl (S)-2-amino-5-((N-(5-hydroxy-4,6-dimethylpyrimidin-2-yl)-4-nitrophenyl)sulfonamido)pentanoate (14).

Compound 13 (66.5 mg, 0.078 mmol) was treated with piperidine (2 ml of a 20% v/v solution in DMF) and the resulting solution was stirred at 25 °C for 2.5 h then diluted with ethyl acetate (20 ml) and poured into NH₄Cl (20 ml of a saturated aqueous solution). The separated aqueous phase was extracted with ethyl acetate (2 x 20 ml), and the combined organic phases were washed with NH₄Cl (2 x 20 ml of a saturated aqueous solution) then brine (25 ml) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give a yellow gum. Subjection of this material to column chromatography (silica, 0→0.1→1→3% v/v MeOH/DCM gradient elution) and concentration of the relevant fractions (Rᵉ = 0.1 in 30% v/v ethyl acetate/hexanes) afforded the title compound 14 as a clear, bright-yellow foam (30 mg, 73%).

\[ \alpha \delta = +2.2 \text{ (c 0.5, CHCl}_3\text{)} \]

\(^1\)H NMR (400 MHz, CDCl₃): \( \delta \) 8.27 (d, \( J = 9.0 \text{ Hz, 2H} \)), 8.18 (d, \( J = 9.0 \text{ Hz, 2H} \)), 7.39 – 7.28 (m, 5H), 5.15 (s, 2H), 4.11 (m, 2H), 3.62 (dd, \( J = 7.0, 3.8 \text{ Hz, 1H} \)), 2.18 (s, 6H), 1.91 (m, 3H), 1.76 - 1.70 (m, 1H). \(^{13}\)C NMR (100 MHz, CDCl₃): \( \delta \) 174.9, 154.1, 149.8, 149.6, 147.0, 143.9, 135.4, 129.8, 128.6, 128.5, 128.2, 123.4, 67.0, 53.8, 47.2, 31.2, 25.7, 18.5.

HRMS (ESI): \( m/z \) [M + H]⁺ calcd. for C\(_{24}\)H\(_{27}\)N\(_5\)O\(_7\)S: 530.1704, found: 530.1692.
Benzyl (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((N-(5-hydroxy-4,6-dimethylpyrimidin-2-yl)-4-nitrophenyl)sulfonamido)pentanoate (15).

A magnetically stirred solution of compound 13 (514 mg, 0.60 mmol) in anhydrous DCM (6 ml, 0.1 M) maintained at 25 °C was treated with TFA (1.2 ml, 15 mmol). After 1.5 h at this temperature, the reaction mixture was concentrated under reduced pressure then subjected to column chromatography (silica, 0.25→0.5→1% v/v MeOH/DCM gradient elution). Concentration of the relevant fractions ($R_f = 0.2$ in 30% v/v ethyl acetate/hexanes) afforded the title compound 15 as a clear, bright-yellow foam (415 mg, 95%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.20 (d, $J = 9.0$ Hz, 2H), 8.12 (d, $J = 9.0$ Hz, 2H), 7.64 (d, $J = 7.5$ Hz, 2H), 7.48 (d, $J = 7.5$ Hz, 2H), 7.31 – 7.17 (m, 9H), 5.57 (d, $J = 8.5$ Hz, 1H, NH), 5.09 (s, 2H), 4.46 (m, 1H), 4.39 – 4.31 (m, 2H), 4.13 – 3.94 (m, 3H), 2.15 (s, 6H), 1.92 – 1.73 (m, 4H). MS (ESI) m/z 774 [(M + Na)$^+$, 30%]; 752 [(M + H)$^+$, 100%].
(S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((N-(5-hydroxy-4,6-dimethylpyrimidin-2-yl)-4-nitrophenyl)sulfonamido)pentanoic acid (16).

A magnetically stirred solution of benzyl ester 15 (225 mg, 0.30 mmol) in i-PrOH (42 ml) maintained at 25 °C was treated with a solution of NaOH (20.5 mg, 0.51 mmol, final concentration 0.08 M) and CaCl$_2$ (470 mg, 4.2 mmol, final concentration 0.66 M) in H$_2$O (1.9 ml). After 16 h at this temperature, the reaction mixture was acidified with 1 M HCl to pH 3-4 then extracted with ethyl acetate (3 x 50 ml). The organic fractions were washed with brine (100 ml), dried (MgSO$_4$), filtered and concentrated under reduced pressure to give a yellow solid. Subjection of this material to column chromatography (silica, 5→10% v/v MeOH/DCM gradient elution) and concentration of the relevant fractions ($R_f = 0.2$ in 5% v/v MeOH/DCM) afforded the title compound 16 as a bright-yellow glass (160 mg, 80%). $[\alpha]_D = +5.1$ (c 0.2, CHCl$_3$). $^1$H NMR (700 MHz, CDCl$_3$ with a drop of CD$_3$OD): $\delta$ 8.26 (d, $J = 7.8$ Hz, 2H), 8.17 (d, $J = 7.8$ Hz, 2H), 7.70 (d, $J = 7.0$ Hz, 2H), 7.55 (m, 2H), 7.34 (m, 2H), 7.24 (m, 2H), 4.38 (m, 3H), 4.17 (m, 1H), 4.07 (m, 2H), 2.23 (s, 6H), 1.95 (m, 1H), 1.85 (m, 2H), 1.80 (m, 1H). $^{13}$C NMR (176 MHz, CDCl$_3$ with a drop of CD$_3$OD): $\delta$ 156.3, 154.5, 149.8, 149.5, 146.7, 144.2, 143.8, 143.6, 141.2, 129.8, 127.7, 127.0, 125.0, 123.4, 119.9, 67.0, 53.6, 47.4, 47.1, 28.9, 25.5, 18.3. HRMS (ESI): $m/z$ [M + H]$^+$ calcd. for C$_{32}$H$_{31}$N$_5$O$_9$S: 684.1735, found: 684.1739.
4,6-Dimethyl-2-(tritylamino)pyrimidin-5-ol (19).

In a typical procedure, a magnetically stirred suspension of 2-aminopyrimidinol 6 (9.5 mg, 0.068 mmol) and K₂CO₃ (14 mg, 0.10 mmol) in anhydrous DMF (0.7 ml, 0.1 M) maintained at 25 °C under an argon atmosphere was treated with TrtCl (29 mg, 0.10 mmol). After 3 h at this temperature, when TLC analysis indicated that the starting material had been consumed, the reaction mixture was diluted with ethyl acetate (5 ml) then H₂O (5 ml), and the separated aqueous phase was extracted with ethyl acetate (2 x 5 ml). The combined organic phases were washed with NH₄Cl (3 x 5 ml of a saturated aqueous solution) then brine (5 ml) before being dried (MgSO₄), filtered and concentrated under reduced pressure to afford a tan solid. Subjection of this material to column chromatography (silica, 1% v/v MeOH/DCM) and concentration of the relevant fractions (Rf = 0.4 in 2% v/v MeOH/DCM) afforded compound 19 (14.3 mg, 55%) as a clear, pale-tan foam. ¹H NMR (400 MHz, CDCl₃): δ 7.27 – 7.05 (complex m, 15 H), 1.97 (broad s, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 168.8, 155.6, 145.7, 139.6, 129.2, 127.3, 126.3, 70.8, 18.2 (some of the signals for quaternary carbons were identified using 2D spectra). HRMS (ESI): m/z [M + H]⁺ calcd. for C₂₅H₂₃N₃O: 382.1914, found: 382.1920; Ph₃C⁺ calcd.: 243.1168; found: 243.1174. A sample of this material was crystallized from hot hexane to obtain pale-tan needles that were amenable to X-ray single crystal analysis.
(S)-2-amino-5-[(5-hydroxy-4,6-dimethylpyrimidin-2-yl)amino]pentanoic acid (Apy\textsuperscript{free}, 23).

An adaptation of the original published procedure was employed.\textsuperscript{3b,6} A magnetically stirred solution of L-arginine (22) (3.0 g, 17.2 mmol) in methanesulfonic acid (12 ml, 1.5 M) maintained at 25 °C was treated with crude diketone 5 (3.5 g, ca. 22 mmol), resulting in a mildly-exothermic reaction. Further portions of compound 5 (2 x 2.7 g, ca. 17 mmol each) were added after 3 and 6 h, respectively. The ensuing dark brown viscous mixture was stirred for 48 h then cooled to 0 °C and neutralized by the dropwise addition of NH\textsubscript{4}OH (ca. 20 ml of a 28-30% aqueous solution). The resulting brown-orange mixture (pH ~7) was stirred at 25 °C for 30 min then diluted with H\textsubscript{2}O (25 ml) and loaded, using additional H\textsubscript{2}O, onto a column of C\textsubscript{18}-reversed phase silica gel (10 x 10 cm) that had been equilibrated with MeOH then H\textsubscript{2}O. Elution with 0→10→20% v/v MeOH/H\textsubscript{2}O and concentration of the relevant fractions containing fluorescent material (R\textsubscript{f} = 0.2 in 1:2:7 v/v/v H\textsubscript{2}O/\texttextit{i}-PrOH/ethyl acetate) afforded the title compound 23 (2.83 g, 65%) as a white powder. A portion of this material was lyophilized from TFA (0.1% in H\textsubscript{2}O) to obtain compound 23 (zwitterion, white fluffy powder) that was used for characterization and all spectroscopic measurements. mp: 192-196 °C (decomp.) [lit. for HCl salt\textsuperscript{6} 207 °C (decomp.)]. [\protect\textalpha]\textsubscript{D} = +28.1 (c 0.3, H\textsubscript{2}O) [lit.\textsuperscript{6} +17.5 (c 0.5, 1 M HCl)]. \textsuperscript{1}H NMR (600 MHz, D\textsubscript{2}O): \delta 3.73 (t, J = 6.1 Hz, 1H), 3.44 (t, J = 6.8 Hz, 2H), 2.39 (s, 6H), 1.93-1.84 (m, 2H), 1.75-1.60 (m, 2H). \textsuperscript{1}H NMR (600 MHz, \texttextit{d}_{3}\textsubscript{2}SO): \delta 8.14 (broad s, 2H, NH\textsubscript{2}), 7.87 (broad s, 1H, OH), 6.38 (s, 1H, NH), 3.86 (t, J = 6.2 Hz, 1H), 3.18 (dd, J = 12.0, 6.2 Hz, 2H), 2.18 (s, 6H), 1.84-1.71 (m, 2H), 1.63-1.49 (m, 2H). \textsuperscript{13}C NMR (150 MHz, D\textsubscript{2}O): \delta 174.4, 150.8, 137.7, 54.3, 40.4, 27.5, 23.9, 16.8 (due to H/D exchange of the phenolic OH, the corresponding \textit{ipso}-carbon is not visible in the spectrum). \textsuperscript{13}C NMR (150 MHz, \texttextit{d}_{3}\textsubscript{2}SO): \delta 171.2, 156.3, 155.1, 138.8, 52.1, 40.3, 27.8, 24.9, 19.0. HRMS (ESI): m/z [M + H]\textsuperscript{+} calcd. for C\textsubscript{11}H\textsubscript{18}N\textsubscript{4}O\textsubscript{3}, 255.1452; found, 255.1448.

On 0.6 mmol scale and using pre-packed C\textsubscript{18} cartridges for purification, compound 23 was obtained in 82% yield.
Solid phase peptide synthesis. Peptide 17 was synthesized by automated Fmoc-based SPPS starting with Fmoc-Lys-Wang resin (0.2 mmol) and using a Tribute synthesizer (Protein Technologies, Inc., USA) according to the manufacture’s protocols. Briefly, Fmoc was removed with piperidine (20% v/v in DMF) in cycles of 3 and 7 min. All amino acids (2.5 eq.) were coupled using HBTU (2.38 eq.) and diisopropylethylamine (DIPEA, 5 eq.) in 30 min. The amino acids used in the synthesis carried orthogonal side-chain protecting groups as follows: Arg(Pbf), Asn(Trt), Asp(tBu), Cys(Trt), Gln(Trt), Glu(tBu), Lys(Boc), Ser(tBu) and Thr(tBu). Double couplings were performed for each proline residue, and the synthesis quality and yields were maximized by using pseudoproline building blocks to reduce aggregation7 as indicated: AQLGGPEAKSDETAAK. Following the attempted final couplings and on-resin Fmoc deprotection, the resin was washed with DCM then dried. Resin-immobilized, side chain-protected peptides were treated with an acidic cocktail containing TFA/triisopropylsilane/H2O in a 90 : 5 : 5 v/v/v ratio, and the ensuing suspension was gently agitated for 3.5 h at 25 °C. Thereafter, the resin was filtered and washed with TFA, and the combined cleavage solutions were treated with three volumes of cold diethyl ether with subsequent centrifugation. The precipitated crude peptides were washed twice with ether, dissolved in an acidic aqueous buffer consisting of ACN and H2O (50% v/v mixture containing 0.1% TFA), lyophilized then subjected to LC-MS.

Low-res mass spectrometry and HPLC analysis. Analytical LC-MS was performed on a Waters Auto Purification HPLC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager and 2489 UV/Visible Detector). Mass spectra were acquired by electrospray ionization (ESI) operating in positive ion mode. Separation was achieved with a Kromasil C4 column (300-5-C4, 150 × 4.6 mm, 5 μm particle size) or a Kromasil C18 column (300-5-C18, 150 × 4.6 mm, 5 μm particle size) at a flow rate of 1 ml/min running a linear gradient of 5 to 65% of buffer B (ACN + 0.05 % TFA) in buffer A (ddH2O + 0.05 % TFA) over 10 min. Analytical RP-HPLC was conducted on a Dionex Ultimate 3000 instrument using a Kromasil C4 column (300-5-C4, 150 x 4.6 mm, 5 μm particle size) or Thermo Fisher Scientific C4 column (BioBasic-4, 150 x 4.6, 5 μm particle size) at a flow rate of 1 ml/min running a linear gradient of 5 to 65% of buffer B (ACN + 0.08% TFA) in buffer A (ddH2O + 0.1% TFA) over 30 min, monitoring absorption at 214 and 280 nm.
**UV-Vis spectroscopy.** UV-Vis spectra were recorded on a SAFAS UVmc2 spectrophotometer from SAFAS (Monaco) using Apy$^\text{free}$ (93 μM) dissolved in 40 mM HEPES·KOH (pH 7.5) at 25 °C in 1 nm wavelength increments. The raw data were exported from SAFAS software as CSV and processed using OriginPro.

**Fluorescence spectroscopy.** The fluorescence spectra of Apy$^\text{free}$ (25 μM) in 40 mM HEPES·KOH (pH 7.5) were recorded using a FluoroMax-4 spectrofluorometer (Horiba Scientific, Japan) at 25 °C. The excitation spectrum was recorded from 290 to 380 nm using an emission wavelength of 380 nm. The emission spectrum was recorded from 340 to 480 nm using an excitation wavelength of 320 nm. Data were collected in 0.5 nm wavelength increments and with a slit width of 1 nm. The raw data were exported from FluorEssence software as CSV and processed using OriginPro.
References


NMR spectra

400 MHz $^1$H NMR of compound 5 in CDCl$_3$
100 MHz $^{13}$C NMR of compound 5 in CDCl$_3$
400 MHz \textsuperscript{1}H NMR of compound 6 in (CD\textsubscript{2})\textsubscript{2}SO
400 MHz $^1$H NMR of compound 7 in CDCl$_3$
400 MHz $^1$H NMR of compound 8 in CDCl$_3$
400 MHz $^1$H NMR of compound 2 in CDCl$_3$
100 MHz $^{13}$C NMR of compound 2 in CDCl$_3$
700 MHz $^1$H NMR of compound 10 in CDCl$_3$
176 MHz $^{13}$C NMR of compound 10 in CDCl$_3$
400 MHz $^1$H NMR of compound S4 in CDCl$_3$
600 MHz \textsuperscript{1}H NMR of compound 12 in CDCl\textsubscript{3}
150 MHz $^{13}$C NMR of compound 12 in CDCl$_3$
400 MHz $^1$H NMR of compound 13 in CDCl$_3$
400 MHz $^1$H NMR of compound 14 in CDCl$_3$
100 MHz $^1$H NMR of compound 14 in CDCl$_3$
400 MHz $^1$H NMR of compound 15 in CDCl$_3$
700 MHz NMR $^1$H NMR of compound 16
in CDCl$_3$ with a drop of CD$_3$OD

16
176 MHz $^{13}$C NMR of compound 16 in CDCl$_3$
with a drop of CD$_3$OD
400 MHz $^1$H NMR of compound 19 in CDCl$_3$
100 MHz $^{13}$C NMR of compound 19 in CDCl$_3$
150 MHz $^{13}$C NMR of arpyrimidine (23) in D$_2$O

Signal for CF$_3$ of residual TFA
150 MHz $^1$C NMR of arpyrimidine (23) in $(\text{CD}_2)_2\text{SO}$

Signal for $\text{CF}_3$ of residual TFA
X-Ray analysis

The X-ray intensity data was measured on Bruker D8 Venture diffractometer equipped with multilayer monochromators, Mo Kα INCOATEC micro focus sealed tube and Kryoflex II cooling device. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted at calculated positions and refined with a riding model respectively as rotating groups. The following software was used: Bruker SAINT software package using a narrow-frame algorithm for frame integration, SADABS for absorption correction, OLEX2 for structure solution, refinement, molecular diagrams and graphical user-interface, Shelx for refinement and graphical user-interface SHELXS-2013 for structure solution, SHELXL-2013 for refinement, Platon for symmetry check. Experimental data and CCDC-code can be found in Table 1. Crystal data, data collection parameters, and structure refinement details are given in Tables 2 and 3. Molecular structure in “Ortep View” is displayed in Figure 1. Packing is shown in Figure 2.

Table 1 Experimental parameter and CCDC-Code.

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4,6-Dimethyl-2-(tritylamino)pyrimidin-5-ol [19]

Figure 1 Asymmetric Unit of [19], drawn with 50% displacement ellipsoids. Bond precision: C-C=0.0015 Å.

Table 2 Data collection and structure refinement of [19].

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Table 3 Sample and crystal data of [19].

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Figure 2 Packing of [19]. The layered system is influenced by one moderate hydrogen bond (light orange shaded). The corresponding bond length between O1H-N1 is 2.7441(12) Å, the angle is 167.9(18) °. The light green shaded area highlights nonpolar interactions in plane a-b.

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