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General experimental

Air and moisture sensitive reactions were carried out under an atmosphere of argon in oven-dried glassware. Room temperature (rt) refers to 18-25 °C. All evaporations and concentrations were performed under reduced pressure (*in vacuo*). All reagents (from Sigma Aldrich UK, Fluka UK, Alfa Aesar UK, Acros UK or Fisher UK) were of synthetic grade and were used without further purification, unless stated otherwise. When necessary, reagents were dried or purified prior to use according to standard methods.[1] Anhydrous solvents (DCM, THF, Et_2O) were obtained from MBraun MB SPS-800 solvent purification system by passage through two drying columns and dispensed under an argon atmosphere. Anhydrous MeOH and MeCN were distilled from calcium hydride in a recycling still.[1]

The course of reactions was followed by thin-layer chromatography (TLC) using aluminium plates coated with silica gel ($60F_{245}$ Merck). TLC plates were examined under UV light (254 nm and 366 nm) before being visualised with ammonium heptamolybdate, anisaldehyde-sulfuric acid, alkaline potassium permanganate or ceric sulfate-sulfuric acid and developed by heating. Column chromatography was performed using silica gel 60 (40–3 µm) under a positive pressure of compressed air.

NMR spectra were recorded on Bruker Advance 300, 400 or 500 instruments. ^{1}H spectra were recorded using deuterated solvent as the lock and the residual solvent signal as the internal standard and for ^{13}C NMR spectra signal of deuterated solvent was used as the internal standard. Chemical shifts are reported in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). The abbreviations for the multiplicity of the proton, carbon and fluorine signals are as follows: s singlet, d doublet, dd doublet of doublets, ddd doublet of doublet of doublets, t triplet, dt doublet of triplets, q quartet, m multiplet, br s broad singlet.

High resolution electrospray ionisation mass spectra were obtained on a Micromass LCT or ThermoFisher Excalibur Orbitrap spectrometers operating in positive or negative mode, from solutions in MeOH, MeCN or water by the Mass Spectrometry Service at the University of St Andrews. HPLC analyses/semi-preparations were performed using a Shimadzu Prominence (SIL-20A HT autosampler, CL-20AT ternary pump, DGU-20A3R solvent degasser, SPD 20A UV detector and CBM-20A controller module) with reverse phase column as indicated in individual experiment.

Protein concentrations were measured on a NanoDrop 1000 spectrophotometer at 280 nm using an extinction coefficient of 10.42 as calculated by ExPASy ProtParam tool.[2] SDS-PAGE was run using NuPAGE 4–12% BisTris gels (Novex) in MOPS SDS running buffer. Proteins were visualised using Instant Blue Coomassie-based gel stain (Expedion).

Compound preparation

5′-Chloro-5′-deoxy-adenosine (1)

5′-Chloro-5′-deoxy-adenosine **1** was synthesised using previously reported methods[3]; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.31 (s, 1H, Ade H-8), 8.23 (s, 1H, Ade H-2), 6.01 (d, *J* = 5.1 Hz, 1H, ribose H-1′), 4.82 (dd, *J* = 5.2, 5.1 Hz, ribose H-2′), 4.40 (dd, *J* = 5.2, 4.2 Hz, ribose H-3′), 4.31 – 4.27 (m, 1H, ribose H-4′), 3.97 (dd, *J* = 11.8, 5.0 Hz, 1H, ribose H-5′a), 3.86 (dd, *J* = 11.9, 5.0 Hz, 1H, ribose H-5′b); m/z (ES^{+}) 286 $[M+H]^{+}$, 309 $[M+Na]^{+}$.

5′-Fluoro-5′-deoxy-adenosine (3)

5′-Fluoro-5′-deoxy-adenosine **3** was synthesised using previously reported methods[4];^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.20 (s, 1H, Ade H-8), 8.12 (s, 1H, Ade H-2), 6.09 (d, *J* = 4.4 Hz, 1H, ribose H-1′), 4.77 (ddd, *J* = 45.8, 10.6, 2.6 Hz, 1H, ribose H-5′a), 4.68 (ddd, *J* = 41.9, 10.9, 2.9 Hz, 1H, ribose H-5′b), 4.31 – 4.25 (m, 1H, ribose H-2′), 4.42 (dd, *J* = 5.2, 5.2 Hz, 1H, ribose H-3′), 4.31 – 4.25 (m, 1H, ribose H-4′); ^{19}F NMR (471 MHz, *d4*-MeOD) δ -232.0 (ddd, *J* = 48.2, 48.2, 30.8 Hz, 1F, $CH_2\mathit{F}$); HRMS calculated for $C_{10}H_{13}FN_5O_3$ $[M+H]^{+}$ 269.0924 found 269.0922.

5′-Bromo-5′-deoxy-adenosine (4)

5′-Bromo-5′-deoxy-adenosine **4** was synthesised using previously reported methods[5]; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.52 (s, 1H, Ade H-8), 8.40 (s, 1H, Ade H-2), 6.11 (d, *J* = 5.2 Hz, 1H, ribose H-1′), 4.80 (dd, *J* = 5.2, 5.1 Hz, ribose H-2′), 4.39 (dd, *J* = 5.3, 4.2 Hz, ribose H-3′), 4.32 – 4.29 (m, 1H, ribose H-4′), 3.83 (dd, *J* = 11.1, 5.2 Hz, 1H, ribose H-5′a), 3.73 (dd, *J* = 11.0, 5.2 Hz, 1H, ribose H-5′b); HRMS calculated for $C_{10}H_{13}BrN_5O_3$ $[M+H]^{+}$ 330.0201 found 330.0190.

5′-Iodo-5′-deoxy-adenosine (5)

5′-Iodo-5′-deoxy-adenosine **5** was synthesised using previously reported methods[6]; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.34 (s, 1H, Ade H-8), 8.23 (s, 1H, Ade H-2), 6.03 (d, *J* = 5.3 Hz, 1H, ribose H-1′), 4.92 (m, 1H, ribose H-2′), 4.33 (dd, *J* = 5.3, 4.1 Hz, ribose H-3′), 4.11 – 4.08 (m, 1H, ribose H-4′), 3.65 (dd, *J* = 10.7, 5.9 Hz, 1H, ribose H-5′a), 3.53 (dd, *J* = 10.7, 5.8 Hz, 1H, ribose H-5′b); m/z (ES^{+}) 478 $[M+H]^{+}$, 500 $[M+Na]^{+}$; HRMS calculated for $C_{10}H_{13}IN_5O_3$ $[M+H]^{+}$ 378.006 found 378.005.

Fluorinase over-expression in *E. coli* and purification

E. coli BL21 (DE3) Gold cells bearing the pET-28a(+)-flA vector[7] were grown while shaking (180 rpm) in autoinduction medium[8] (4 × 400 mL in 2 L flasks) containing kanamycin (100 µg/mL) at 37 °C for 48 h. Cells were then harvested by centrifugation (6500 rpm, 15 min), and the cell pellet resuspended in buffer (100 mL, 150 mM NaCl, 20 mM TRIS pH8, 20 mM imidazole), before protease inhibitor cocktail (1 × Complete Mini, EDTA-free, Roche) and bovine pancreatic DNAse (Sigma, 5 mg) were added. The cells then were lysed using a cell disruptor (Constant Systems) at 30 kPSI, the lysate was centrifuged (20 000 rpm, 30 min) and the pellet discarded before being filtered (0.45 µm). The recombinant protein was purified from the lysate by passing the supernatant though a Ni^{2+} affinity column (Ni Sepharose 6 Fast Flow, GE, 5-7 mL bed volume) which was pre-equilibrated with the lysis buffer. The bound protein was washed (3 × 15 mL, 20 mM TRIS pH 8, 20 mM imidazole, 500 mM NaCl) before being eluted (3 × 15 mL, 20 mM TRIS pH 8, 400 mM imidazole, 500 mM NaCl). After SDS-PAGE analysis, fractions containing the desired protein were pooled, adenosine deaminase (ADA) suspension (50 µL calf spleen ADA, Sigma Aldrich) was added, and the mixture dialysed against buffer (20 mM TRIS pH 8, 7 L or 20 mM phosphate buffer pH 7.8) overnight. Adenosine removal was monitored by HPLC of samples of fluorinase which had been adjusted to the same protein concentration, denatured by heating at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 10 min). Samples of the supernatant (60 µL) were removed for analysis by HPLC (performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm). The non-His-tagged ADA enzyme was then separated from the fluorinase by passing the supernatant though another Ni^{2+} affinity column (Ni Sepharose 6 Fast Flow, GE, 5-7 mL bed volume, using the same procedure, the resultant eluate was dialysed against buffer (20 mM TRIS pH 8, 7 L or 20 mM phosphate buffer pH 7.8) overnight. The solution was concentrated by centrifugation (Amicon Ultra-15, 10 000 kDa cutoff) to give recombinant fluorinase (5-20 $mg.mL^{-1}$). The enzyme solution was then aliquotted into portions (5 mg or 10 mg, based on fluorinase), before being lyophilised and stored at −78 °C until needed.

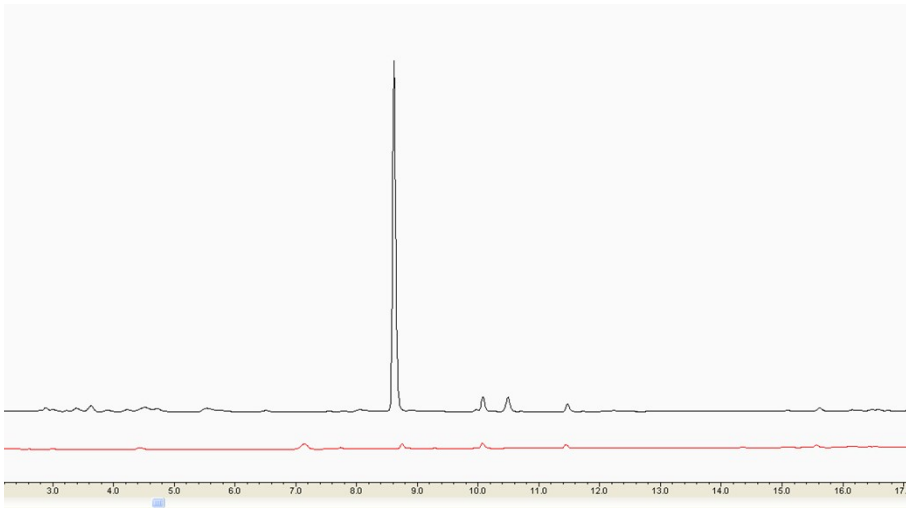


Figure S1. HPLC traces of fluorinase: pre (black) and post treatment with adenosine deaminase. Adenosine (t_R = 8.6 min) confirmed with reference sample. Reaction and HPLC conditions: See above.

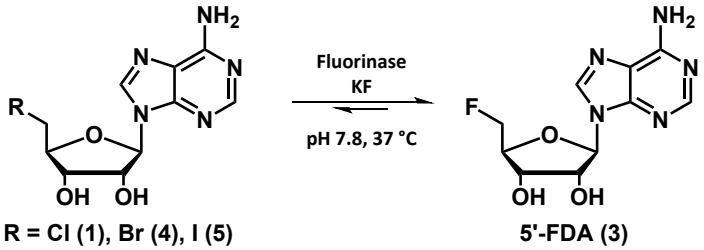
Comparative enzymatic rate assays

HPLC analysis

Analyses of samples were performed on a shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Injection volume: 30 µL; mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear gradient: 0% solvent B to 5% solvent B over 5 min, 5% solvent B to 25% solvent B over 10 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm).

5′-ClDA, 5′-BrDA and 5′-IDA to 5′-FDA

Time course experiments were conducted (in triplicate) using the following reaction conditions:



Scheme S1. Fluorinase-catalysed direct fluorination of 5'-ClDA (**1**), 5'-BrDA (**4**) and 5'-IDA (**5**) 5′-halogenated-5′-deoxy-adenosine substrates to FDA **3**.

Direct fluorination*:* in a total reaction volume of 600 µL (in 40 mM phosphate buffer, at pH 7.8), substrate **1**, **4** or **5** (0.15 mM), KF (80 mM) and recombinant fluorinase (125 mM) were incubated at 37 °C. Samples of the supernatant (60 µL) were periodically removed at 0.5 h, 1 h, 2 h, 4 h, 6 h and 20 h, the protein precipitated by heating at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 5 min).

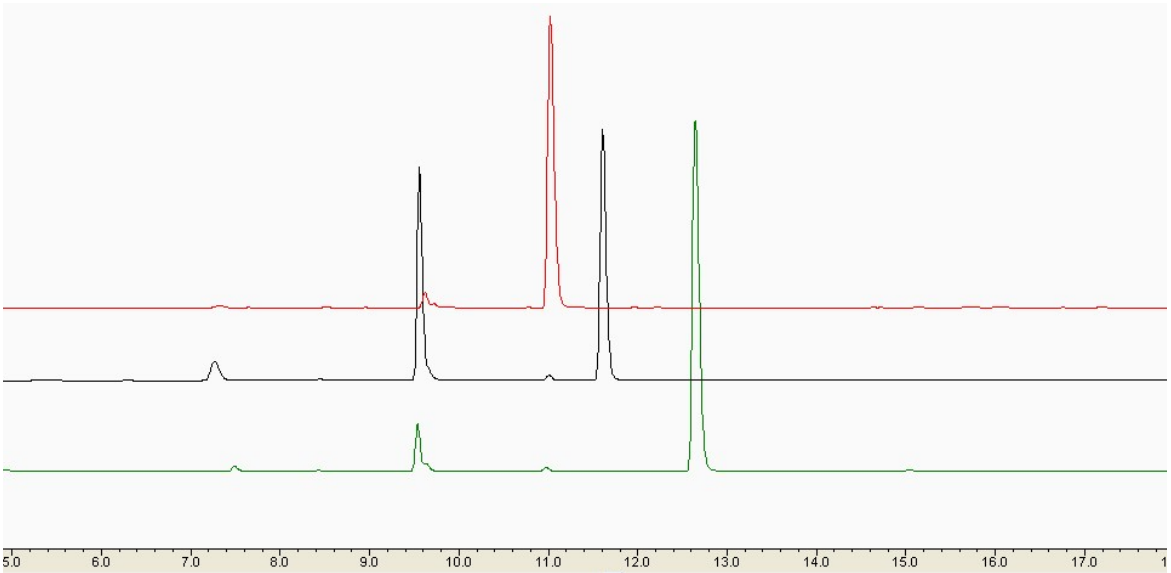
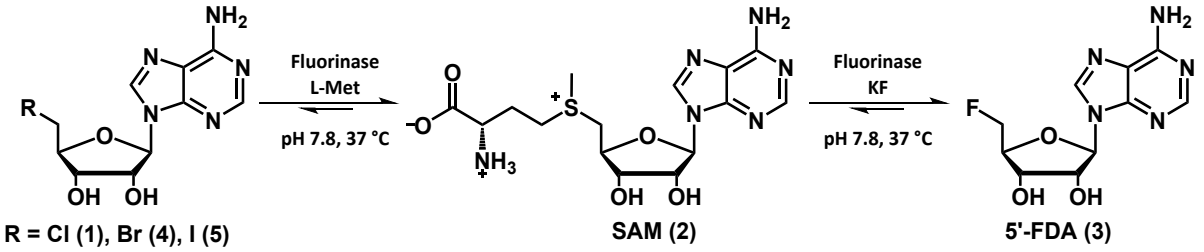


Figure S2. Analytical HPLC of fluorinase catalysed conversion of 5'-ClDA (**1**) (red), 5'-BrDA (**4**) (black) and 5'-IDA (**5**) (green) to FDA (**3**, t_R = 9.5 min) after 20 h *via* direct fluorination. Reaction and HPLC conditions: See Above.



Scheme S2. Fluorinase-catalysed two-step fluorination of 5'-ClDA (**1**), 5'-BrDA (**4**) and 5'-IDA (**5**) to 5'-FDA (**3**) via SAM (**2**).

***'Two-step' fluorination**:* in a total reaction volume of 600 µL (in 40 mM phosphate buffer, at pH 7.8), substrate **1**, **4** or **5** (0.15 mM), $_{L}$-methonine 0.1 mM, KF (80 mM) and recombinant fluorinase (125 mM) were incubated at 37 °C. Samples of the supernatant (60 µL) were periodically removed at 0.5 h, 1 h, 2 h, 4 h, 6 h and 24 h, the protein precipitated by heating at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 5 min).

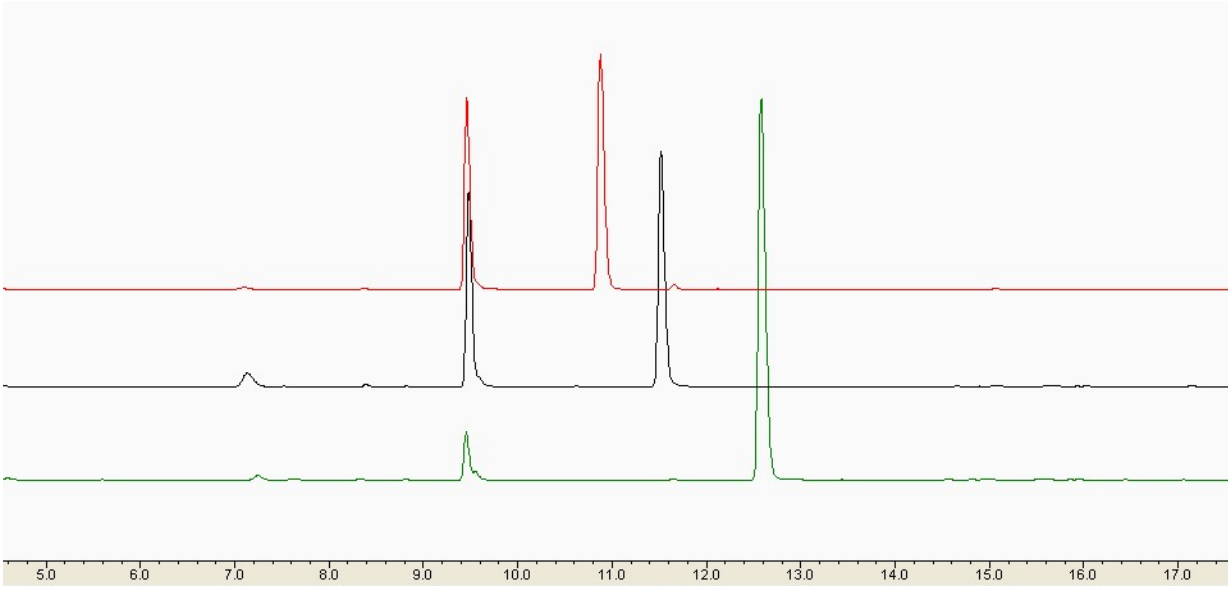
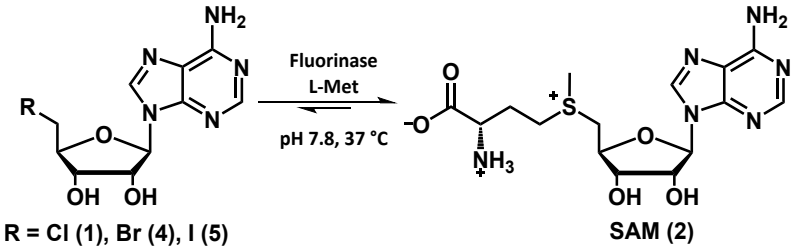


Figure S3. Analytical HPLC of fluorinase catalysed conversion of 5'-ClDA (**1**, red), 5'-BrDA (**4**, black) and 5'-IDA (**5**, green) to FDA (**3**, t_R = 9.5 min) after 24 h via 'two-step' fluorination. Reaction and HPLC conditions: See Above.

5′-ClDA, 5′-BrDA and 5′-IDA to SAM



Scheme S3. Fluorinase-catalysed conversion of 5'-ClDA (**1**), 5'-BrDA (**4**) and 5'-IDA (**5**) to SAM (**2**).

Single time point experiments were conducted (in triplicate) using the following reaction conditions: in a total reaction volume of 80 µL (in 40 mM phosphate buffer, at pH 7.8), substrate **1**, **4** or **5** (0.15 mM), ($_L$-methonine 0.1 mM), and recombinant fluorinase (125 mM) were incubated at 37 °C. Samples of the supernatant (60 µL) were removed at 4 h and the protein precipitated by heating at 95°C for 1 min, before being clarified by centrifugation (13 000 rpm, 5 min).

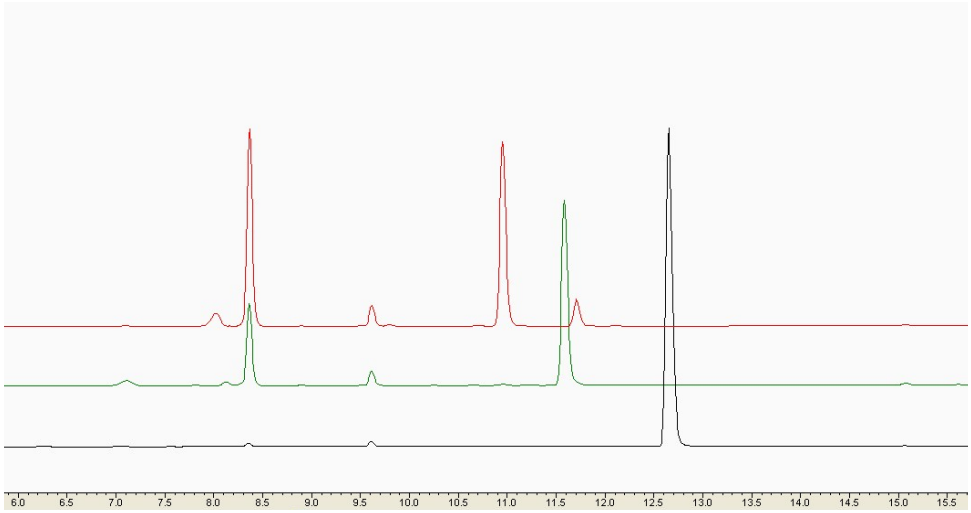
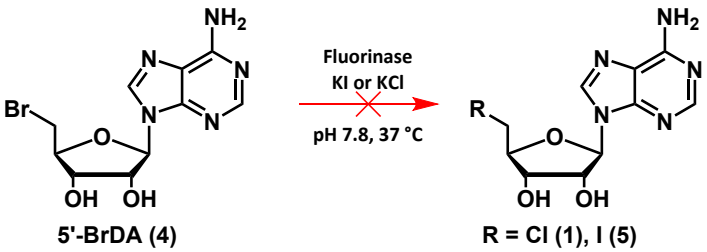


Figure S4. Analytical HPLC of fluorinase catalysed conversion of 5'-ClDA (**1**, red), 5'-BrDA (**4**, black) and 5'-IDA (**5**, green) to SAM (**2**, t_R = 8.4 min) after 4 h. Reaction and HPLC conditions: See Above.

5′-BrDA to 5′-ClDA and 5′-IDA

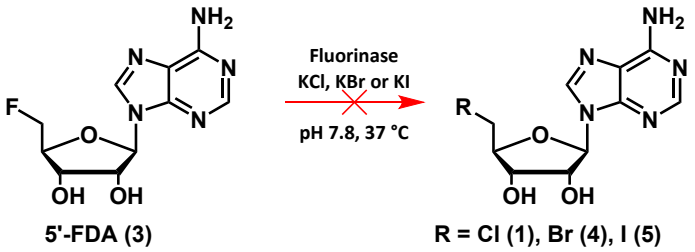


Scheme S4. Fluorinase-catalysed direct halogenation of 5′-BrDA (**4**) to 5′-ClDA (**1**) or 5′-IDA (**5**).

Direct chloro/iodo-halogenation of 5′-BrDA: in a total reaction volume of 100 µL (in 40 mM phosphate buffer, at pH 7.8), substrate **4** (0.15 mM), KI or KCl (80 mM) and recombinant fluorinase

(125 mM) were incubated at 37 °C. Samples of the supernatant (50 µL) were periodically removed at 4 h and 24 h, the protein precipitated by heating at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 5 min). No visible FDA production was observed *via* HPLC analysis.

5'-FDA to 5'-ClDA, 5'-BrDA and 5'-IDA



Scheme S5. Fluorinase-catalysed direct halogenation of 5'-FDA **3** to **1**, **4** or **5**.

***Direct chloro/iodo/bromo-halogenation of 5'-FDA**:* in a total reaction volume of 100 µL (in 40 mM phosphate buffer, at pH 7.8), substrate **4** (0.15 mM), KBr, KCl or KI (80 mM) and recombinant fluorinase (125 mM) were incubated at 37 °C. Samples of the supernatant (50 µL) were periodically removed at 4 h and 24 h, the protein precipitated by heating at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 5 min). No visible production of **1**, **4** or **5** in each case was observed via HPLC analysis.

Control experiments

To ensure that the production of FDA observed in the 'direct fluorination' is a result of a one step fluorination reaction mediated by the fluorinase enzyme a number of control experiments were performed. To reject any influence of endogenous FDA or background conversion of FDA from any endogenous SAM, experiments were performed using the same reaction conditions (as above) except without the addition of **1**, **4** and **5** or L-methionine (Fig. S5 A and B, t=0 h and 18h). In order to ensure any endogenous L-methonine was not the cause of FDA production, experiments were performed with fluorinase enzyme which had been incubated with L-amino acid oxidase (Fig. S5C), the same experiments were conducted with additional L-methonine (Fig. S5D) and without LAAO (to confirm LAAO activity in the control experiments, Fig. S5E). Additionally, experiments were conducted with each substrate (**1**, **4** and **5**) in the presence of KF but the absence of the fluorinase enzyme (Fig S5 F, G and H for **1**, **4** and **5** respectively). In each case samples were taken after 24h heated at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 5 min) and analysed by HPLC.

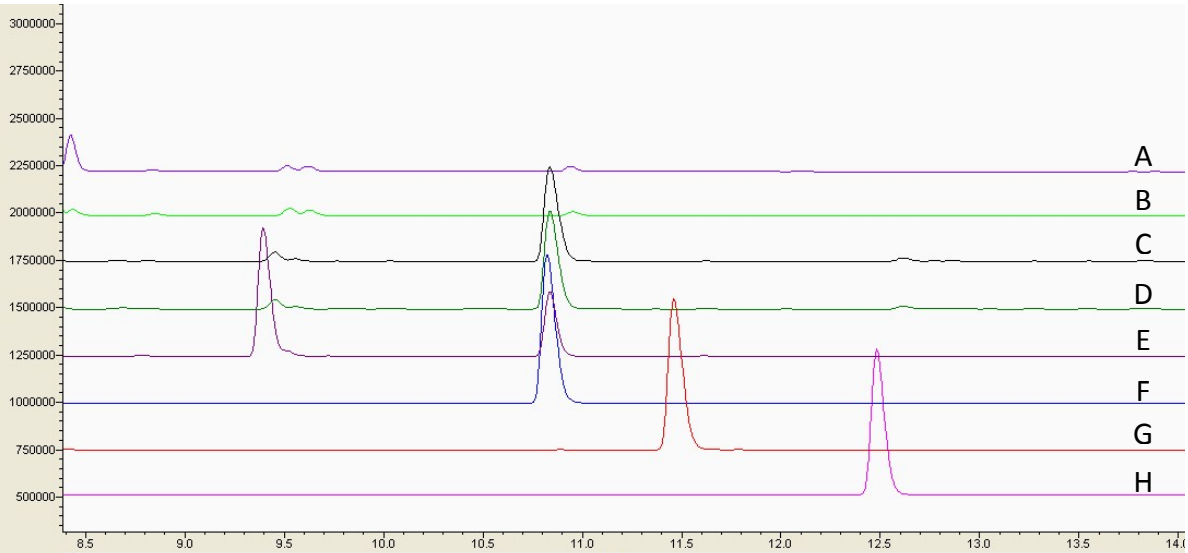


Figure S5. Analytical HPLC of control experiments (after 24h). Reaction and HPLC conditions: See Above.

Kinetic assays

In order to determine the kinetic parameters for of 5'-BrDA **4** a series of enzymatic assay were set up with the fluorinase enzyme: in a total reaction volume of 200 µL (in 40 mM phosphate buffer, at pH 7.8), substrate **4** (at a range of concentrations: 10 – 500 µM), KF (200 mM) and recombinant fluorinase (8 µM) were incubated at 37 °C. Samples of the supernatant (40 µL) were periodically removed (at 5 – 30 min), the protein precipitated by heating at 95°C for 5 min, before being clarified by centrifugation (13 000 rpm, 5 min). Analyses of samples were performed on a shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Injection volume: 30 µL; mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear gradient: 0% solvent B to 5% solvent B over 5 min, 5% solvent B to 25% solvent B over 10 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm). Quantification of 5'-FDA produced was performed using a standard curve of the peak area derived from synthetic 5'-FDA, which was prepared by running known concentrations using the same HPLC method. The initial velocity of reaction was plotted against substrate **4** concentrations using a best-fit model based on Michaelis-Menten equation usingGraphPad Prism 7 (GraphPad Software).

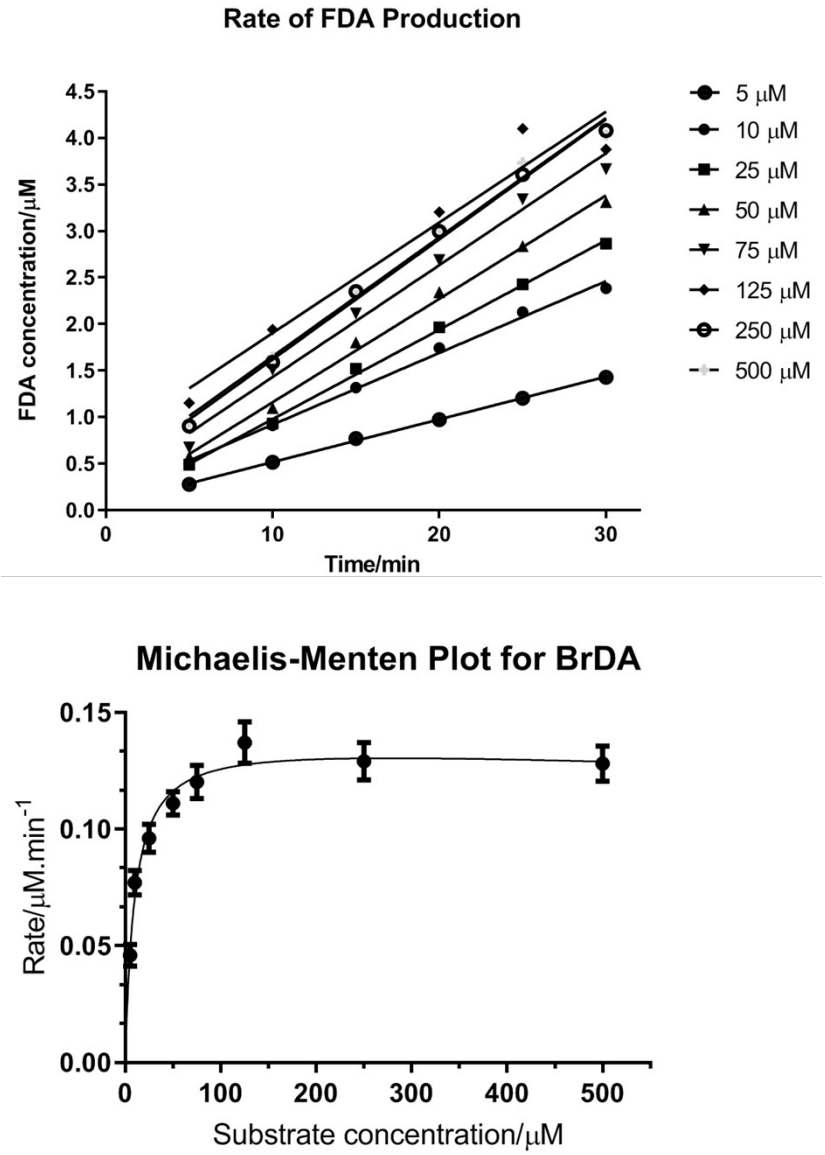


Figure S5. Kinetic assays of the fluorinase mediated direct fluorination of 5′-BrDA (**4**).

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