Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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The fluorinase from *Streptomyces cattleya* is also a chlorinase

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Materials

L-Se-methionine, L-methionine, L-[^{13}C-*methyl*]-methionine and SAM were purchased from the Sigma Chem. Co. Ltd, UK. 5'-fluoro-5'-deoxyadenosine (5'-FDA) (*Angew Chemie Int. Ed., English*, 2002, **41**, 3193-3195) and the recombinant fluorinase (*Nature*, 2004, **427**, 561 – 565) were prepared as previously described.

The fluorinase assay

Enzymatic activity was assayed at 25^{o}C, monitoring 5'-FDA consumptions using a Varian HPLC system (Varian 325 UV-Vis Detector, 400 Autosampler, 230 Solvent delivery module, 800 interface box, 350 reflective index). For the kinetic studies of the reverse reaction, recombinant fluorination enzyme (1.9 x 10^{-4} unit, unit = umol/min) was incubated at 37^{O}C with 5'-FDA (2 mM) and L-methionine (20 mM) in phosphate buffer (20mM, pH=7.8), at a final volume of 0.2 mL. The kinetic parameters for L-methionine (Km = 4.5 mM, Vmax = 0.74 nmol 5'-FDA consumption/mg protein/min) and 5'-FDA (Km = 0.14 mM) were evaluated for both substrates. For substrate specificity, fluorinase (1.9 x 10-4 unit) was incubated at 37^{O}C with SAM (1 mM) and fluoride or chloride ion (20 mM) in phosphate buffer (20 mM, pH = 7.8), at a final volume of 0.2 ml.

Samples for HPLC analysis were boiled at 95 ^{O}C (3 min) and the precipitated protein removed by centrifugation. An aliquot (20 µL) of the clear supernatant was used for HPLC analysis on a Hypersil 5 µM C-18 column (250x4.6 mm, Macherey-Nagel) equilibrated with KH_2PO_4 (50 mM) and acetonitrile (95:5 v/v). Runs were monitored by UV at 260 nm by gradient elution from a starting mobile phase of KH_2PO_4 (50 mM) and acetonitrile (95:5 v/v) to a final mobile phase consisting of KH_2PO_4 (50 mM) and acetonitrile (80:20 v/v). Samples were introduced through a Varian 400 Autosampler fitted with a 20-µL loop and the flow rate was maintained at 1.0 mL/min with a total time of elution of 20 min. The concentration of 5'-FDA was determined against a standard curve derived using synthetic 5'-FDA.

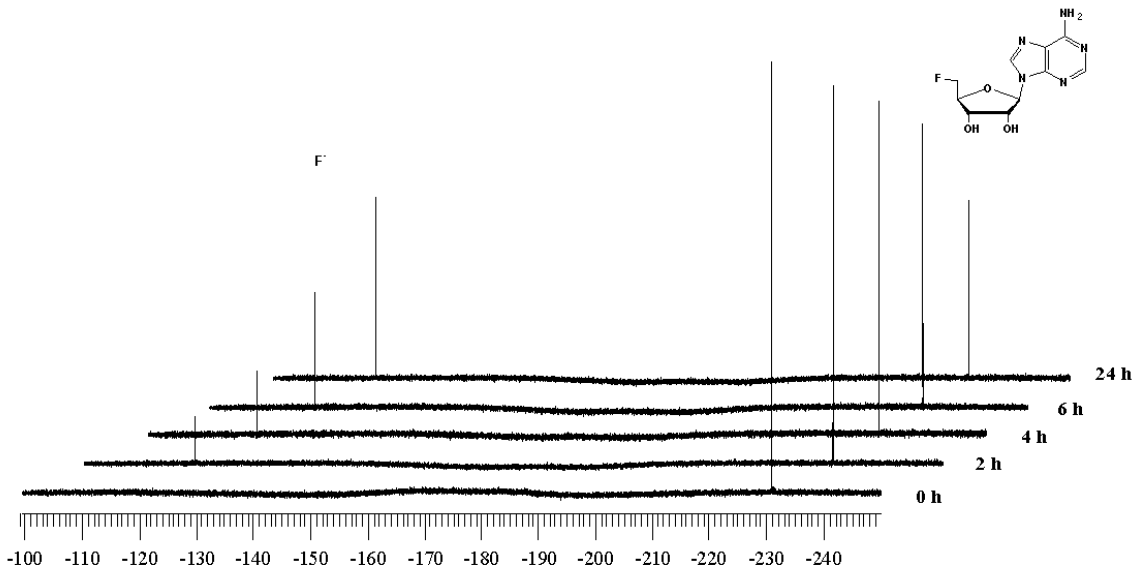
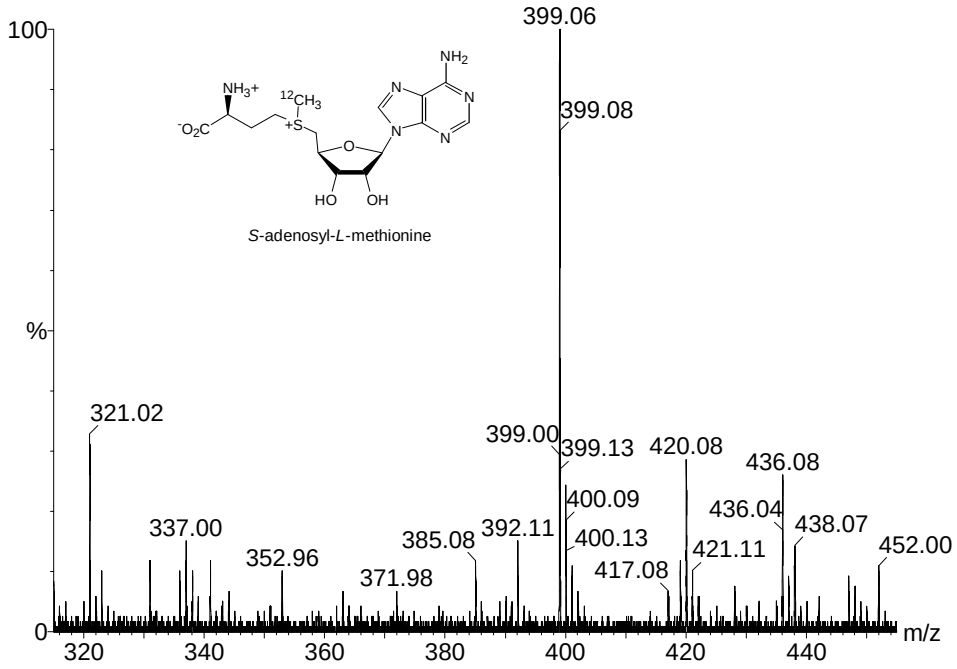


Figure 1 The fluorinase operating in reverse. $^{19}F\{^{1}H\}$-NMR (500MHz) study monitoring the course of the conversion of 5'-FDA (d= -230.87 ppm) and L-methionine to generate F^- (d=-120.0 ppm) and SAM. 5-FDA (2 mM) and L-methionine (20mM) were incubated with the fluorinase ($2.3x10^{-2}$ unit) in a phosphate buffer (20 mM) at pH 7.8.

(a)



(b)

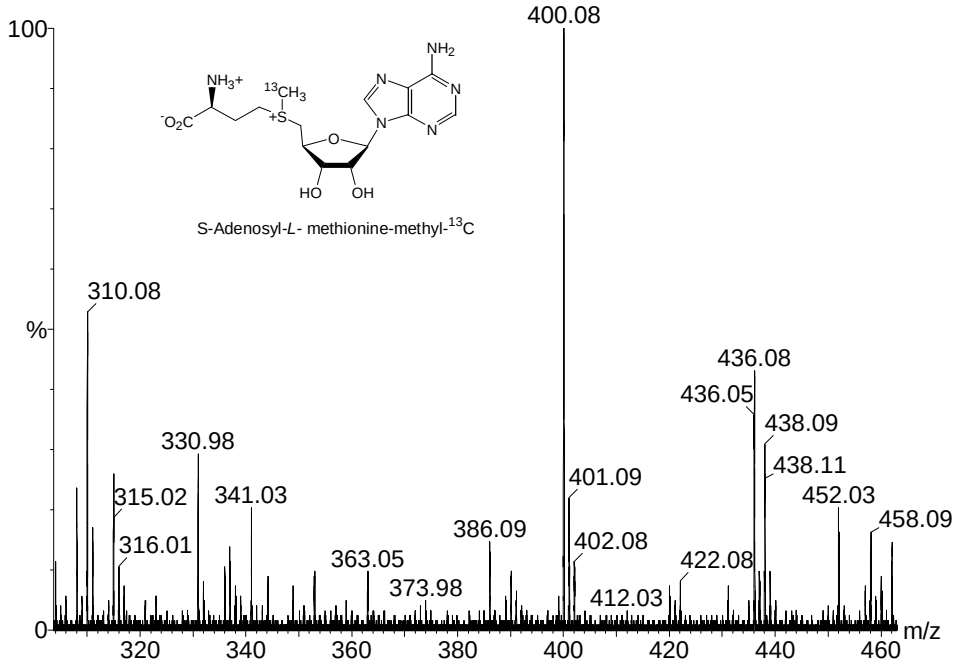


Figure 2. ESI-MS spectra (Micromass LCT) of **(a)** SAM (M+H: 399) and **(b)** L-[^{13}C-*methyl*]-SAM (M+H: 400) generated as products after the conversion of 5'-FDA and either **(a)** L-methionine or **(b)** L-[^{13}C-*methyl*]-methionine, catalysed by the fluorinase in the reverse direction.
5-FDA (1 mM) and L-methionine (20 mM) or L-[^{13}C-*methyl*]-methionine (20 mM) were incubated with the fluorinase (1.4×10^{-3} unit) in a phosphate buffer (20 mM) at pH 7.8. The spectrum was recorded after 12 hrs.

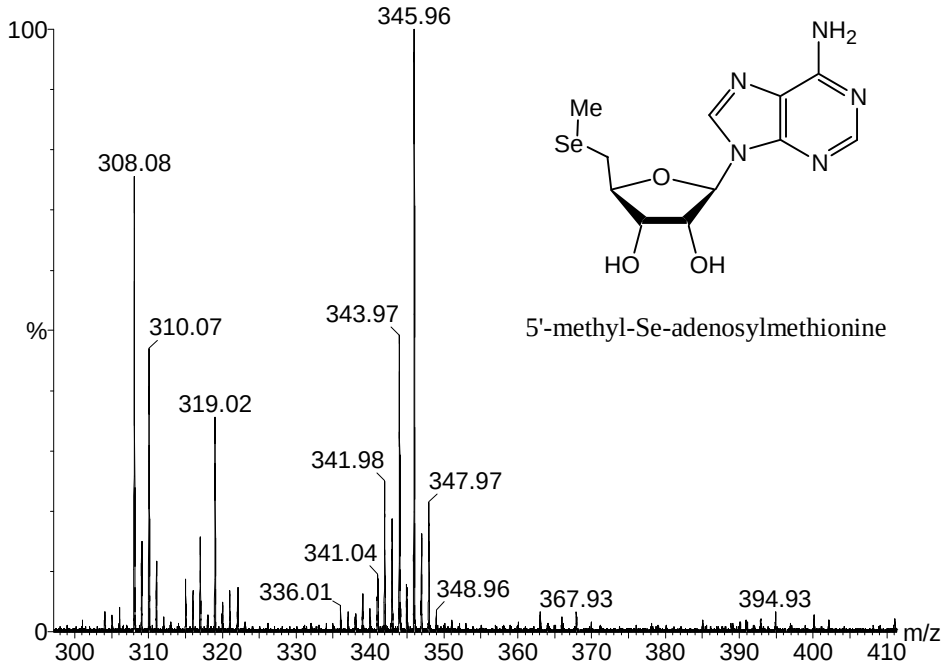


Figure 3. ESI-MS spectrum (Micromass LCT) of with the characteristic isotope signature of selenium catalysed by the fluorination enzyme from 5'-FDA and seleno-methionine.

ESI-MS spectra (Micromass LCT) of 5'-[MeSe]-SAM, (M+H: 345.96) generated as a product after the conversion of 5'-FDA and L-Se-methionine catalysed by the fluorinase in the reverse direction. 5'-FDA (1 mM) and L-Se-methionine (5 mM) were incubated with the fluorinase (1.4×10^{-3} unit) in a phosphate buffer (20mM) at pH 7.8. The spectrum was recorded after 12 hrs.

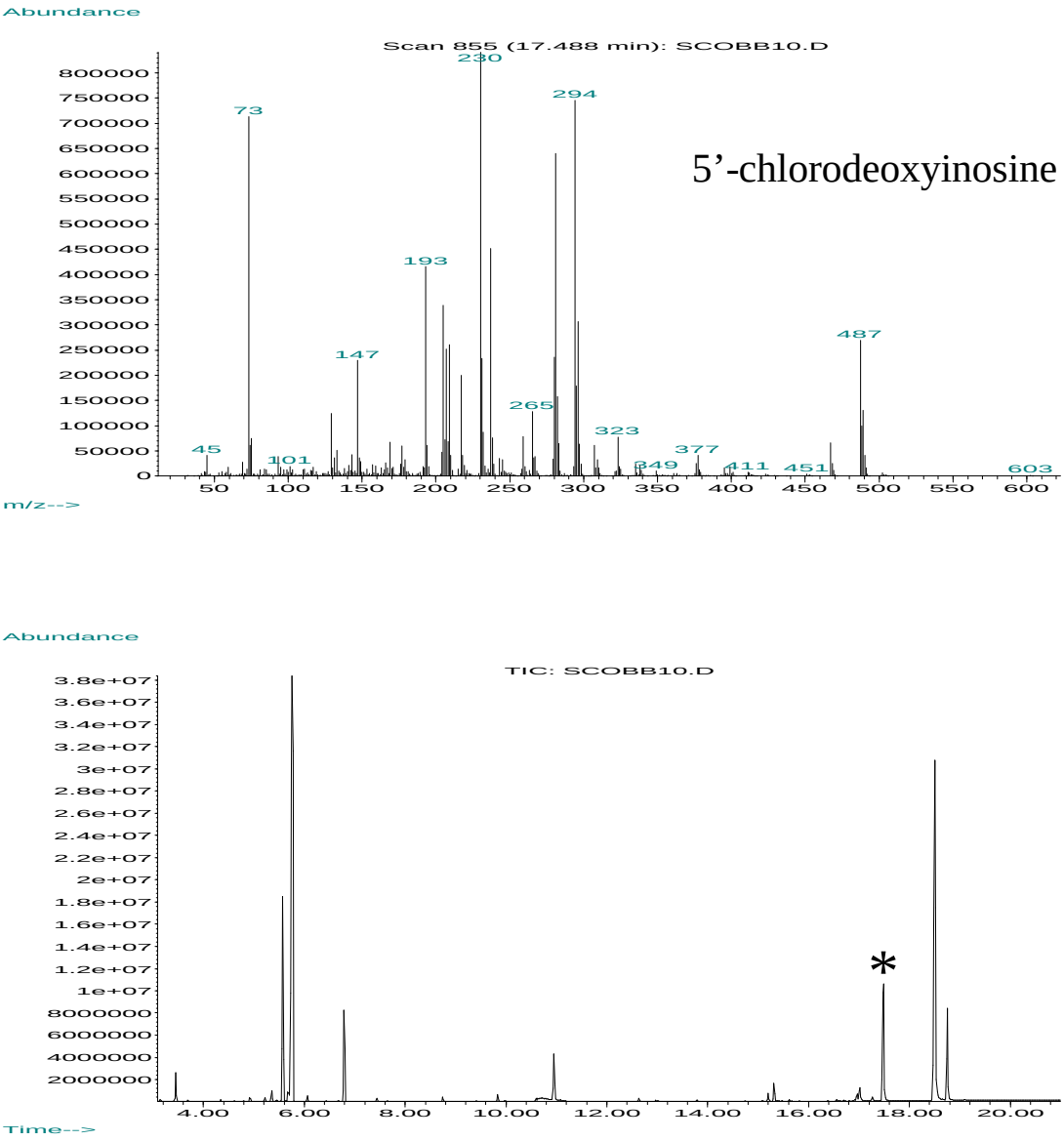


Figure 4 GC/MS trace of the MSTFA derivative of 5'-ClDI

SAM (1mM), chloride ion (20 mM), fluorinase ($1.4x10^{-3}$ unit) and adenosyl acid deaminase (0.05 unit) were incubated in a phosphate buffer at pH 7.8. The spectrum was recorded after12 h.
m/z 487 (M $-CH_3$, 31%), 294 (M $C_8H_{11}N_4OSi$,88) and 230 (230).

HPLC chromatography of 'chlorinase' reactions

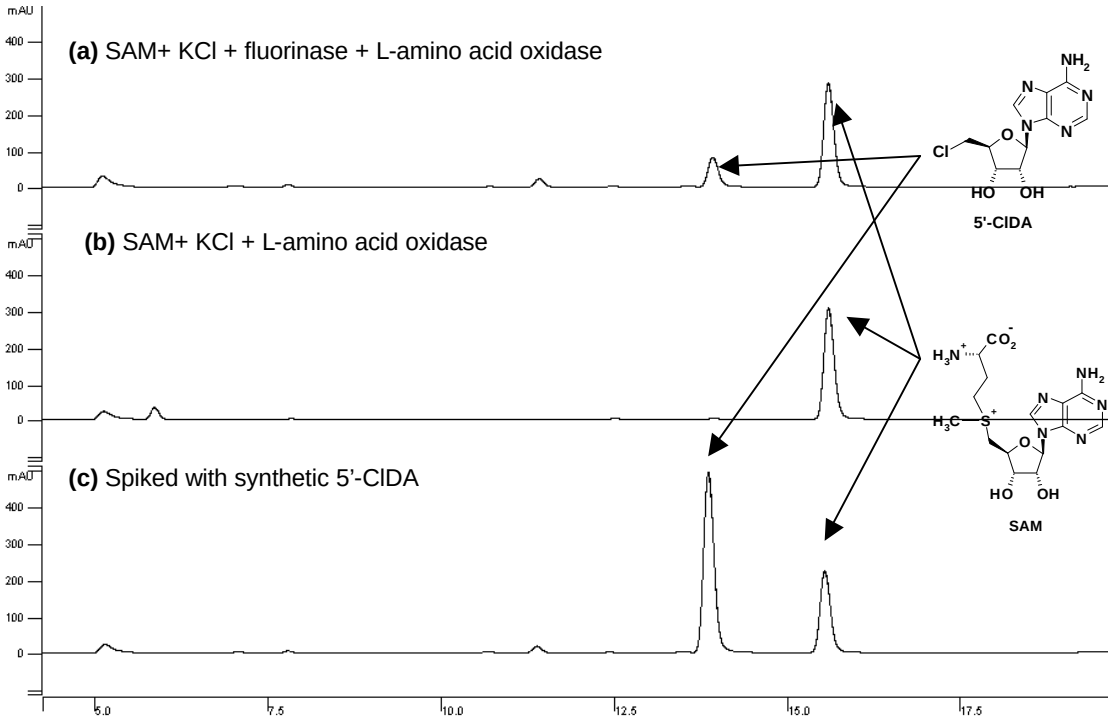


Figure 5 ***HPLC profiles after running the fluorinase reaction with and without the oxidase***

SAM (1mM), chloride ion (20 mM), fluorinase ($1.4x10^{-3}$ unit) and L-amino acid oxidase (ca. 0.03 unit) were incubated in a phosphate buffer (20mM) at pH 7.8. Each chromatograp was recorded after12 hrs. **(a)** is the result of thew fluorinase and amino acid oxidase combination, showing 5'-ClDA production, **(b)** is a control without fluorinase and **(c)** is the product of chromatograph (a) spiked with synthetic 5'-ClDA. The small peak at 11.5 minutes is 5'-chloro-5'-deoxyinosine (2'-ClDI) arising for a low level deaminase activity in the amino acid oxidase.

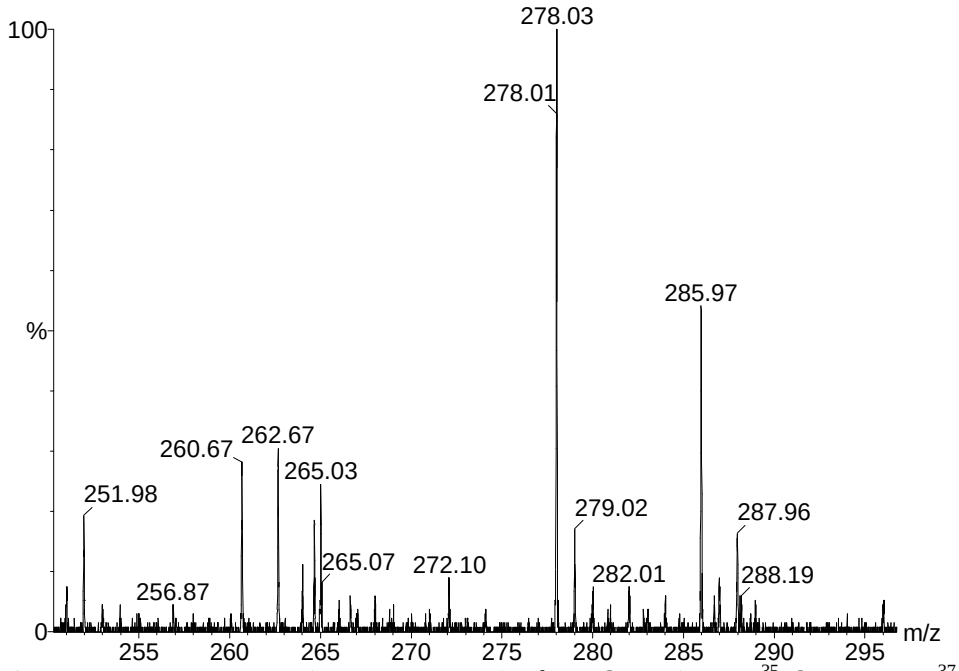


Figure 6 ESI-MS spectra (Micromass LCT) of 5'-ClDA, (M+H: ^{35}Cl = 285.97, ^{37}Cl = 287.96) generated as a product after the combination of SAM and chloride ion catalysed by the fluorinase and in combination with the L-amino acid oxidase.

SAM and chloride ion were incubated in a phosphate buffer at pH 7.8 and at a final concentration of 1 mM for SAM and 20 mM for chloride ion. The fluorinase was added at 6 (mg/ml) and the oxidase at 2 mg/ml. The spectrum was recorded after stopping the reaction after 12 hours.

Crystal Data collection parameters

Data collection	5'Cl-FDA
Wavelength (Å)	0.933
Resolution (Highest Shell, Å)	45-2.0 (2.11 -2.0)
Space group	$C222_1$
Cell constants (Å)	a= 75.5Å b = 129.2 Å c= 183.1 Å
Unique reflections	56675 (8767)
Average redundancy	4.7 (4.8)
I/s	13.6 (2.4)
Completeness (%)	98 (100)
R_{merge} (%)	9.5 (45.4)
Refinement	
R	19.0 (25.4)
R_{free}	24.7 (33.3)
rmsd bonds (Å) / angles (º)	0.009 / 1.21
Residues in Ramachandran Core (%)	87%
PDB accession code	2c2w

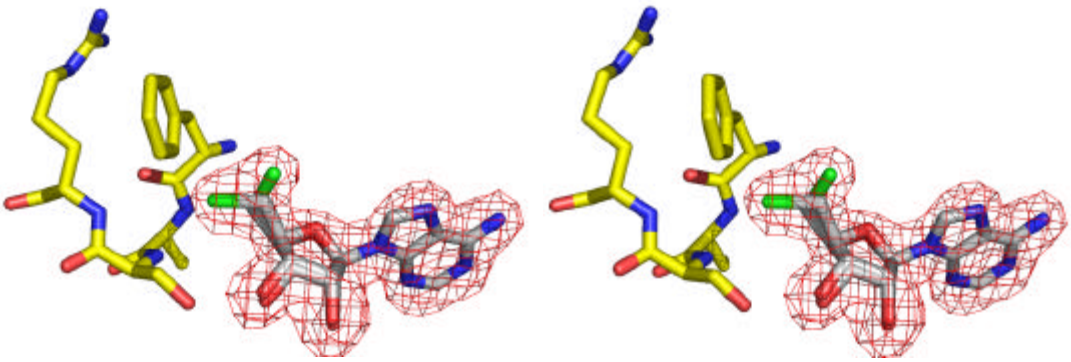


Figure 7 The unbiased Fo-Fc electron density for 5Cl-FDA. Both conformation of the chlorine atom are visible.

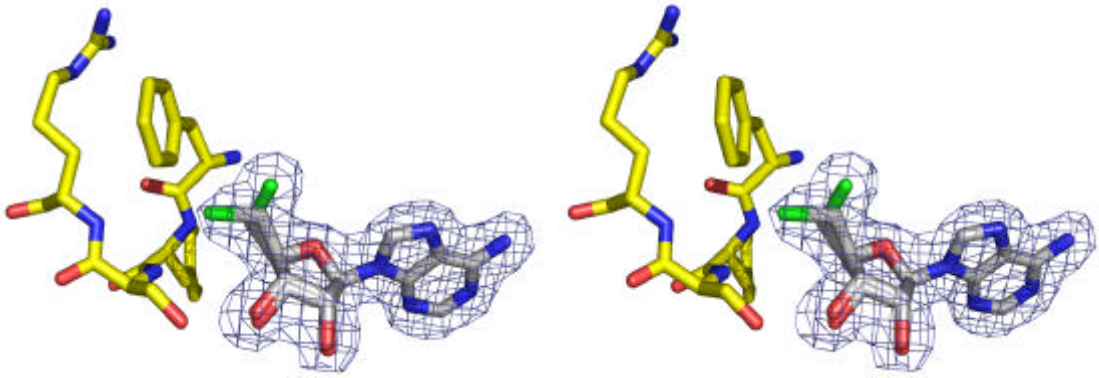


Figure 8 The final 2Fo-Fc electron density for 5Cl-FDA. Both conformation of the chlorine atom are visible.