Biosynthesis of fluoroacetate and 4-fluorothreonine in *Streptomyces cattleya*. Incorporation of oxygen-18 from [2-^{2}H,2-^{18}O]-glycerol and the role of serine metabolites in fluoroacetaldehyde biosynthesis

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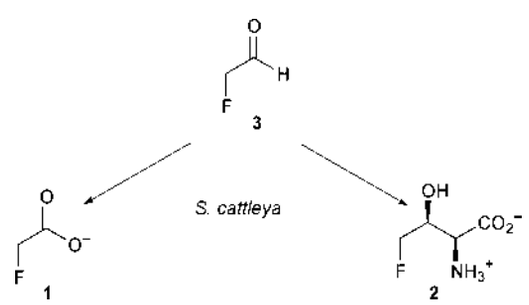
A series of isotope labelling experiments was carried out to investigate the biosynthesis of fluoroacetate and 4-fluorothreonine in resting cells of *Streptomyces cattleya*. Previous studies have shown that fluoroacetaldehyde is a precursor to both of these metabolites and the experiments were conducted to explore in greater detail the metabolic origin of fluoroacetaldehyde in *S. cattleya*. Ethanolamine and cysteamine are C_2 metabolites of serine and cysteine respectively and these two metabolites emerged as candidate precursors to fluoroacetaldehyde in *S. cattleya*. However feeding experiments with [1,1-$^{2}H_2$]-ethanolamine and [1,1-$^{2}H_2$]-cysteamine did not indicate incorporation into the fluorometabolites, suggesting that these compounds are not relevant precursors to fluoroacetaldehyde in *S. cattleya*. Upon feeding [2-^{2}H,2-^{18}O]-glycerol to resting cells of *S. cattleya*, the deuterium atom was not incorporated into 4-fluorothreonine, however the oxygen-18 atom became incorporated into the carboxylate group of fluoroacetate and into the C(3)-O oxygen atom of 4-fluorothreonine. This observation indicates that there is an oxidation at C-2 of glycerol, but that the oxygen atom is formally retained from glycerol during the biosynthesis. In overview, the data suggest that fluoroacetaldehyde is derived from a C_3 glycolytic intermediate rather than a C_2 amino acid metabolite.

Introduction

The actinomycete *Streptomyces cattleya* biosynthesises fluoroacetate **1** and the amino acid 4-fluorothreonine **2** as secondary metabolites.[1] The bacterium is one of only two bacteria[1,2] which are known to elaborate fluorometabolites, and more generally *S. cattleya* belongs to a select group of organisms which biosynthesise fluorine containing natural products.[3,4] The metabolic pathways involved in fluorometabolite production have been investigated using isotopically labelled precursors and enzyme purification studies.[5,6] From these studies fluoroacetaldehyde **3** has emerged as a common biosynthetic intermediate to both fluoroacetate **1** and 4-fluorothreonine **2** in the organism (Scheme 1).[7]



Scheme 1 Fluoroacetaldehyde **3** is a common precursor of fluoroacetate **1** and 4-fluorothreonine **2** in *S. cattleya*.

Feeding experiments administering [1-$^{2}H_1$]-fluoroacetaldehyde **3a** to resting cell preparations of *S. cattleya* resulted in a significant level of incorporation (30%) of deuterium into 4-fluorothreonine **2** indicating that fluoroacetaldehyde **3** directly contributes C-3 and C-4 of this amino acid.[7] More recently the biochemical process for the conversions of fluoroacetaldehyde **3**, to fluoroacetate **1** and 4-fluorothreonine **2** has been revealed.

A pyridoxal phosphate (PLP)-dependent transaldolase has been identified[8] in *S. cattleya* which mediates an aldol reaction between L-threonine and fluoroacetaldehyde **3** to generate 4-fluorothreonine **2** and acetaldehyde **4** (Scheme 2). This novel enzyme catalyses the final step in 4-fluorothreonine biosynthesis. The conversion of fluoroacetaldehyde **3** to fluoroacetate **1** is also on a firm biochemical footing. An NAD^+ dependent aldehyde dehydrogenase[9] which efficiently oxidises fluoroacetaldehyde **3** to fluoroacetate **1** has recently been purified to homogeneity. This enzyme is expressed during the secondary phase of cell growth and oxidises fluoroacetaldehyde **3** more efficiently than acetaldehyde **4** suggesting it has a specific role to play in fluoroacetate biosynthesis. Thus isotope labelling experiments and enzyme isolation support the role of fluoroacetaldehyde **3** as an intermediate to both fluoroacetate **1** and 4-fluorothreonine **2** in *S. cattleya*. However, the initial biochemical steps from inorganic fluoride leading to the biosynthesis of fluoroacetaldehyde **3** remain unclear.

Previous isotope labelling experiments[5,6] have indicated that glycine **5** is converted to serine **6** before it becomes incorporated into the fluorometabolites **1** and **2**. This was evident from the substantial double labelling of the resultant metabolites after feeding experiments with [2-^{13}C]-glycine **5a**. The recombination of the C-2 of glycine **5** is attributed to the *in vivo* biosynthesis of [2,3-$^{13}C_2$]-serine **6a** from two molecules of [2-^{13}C]-glycine **5a**, catalysed by serine hydroxymethyl transferase (SHMT) as shown in Scheme 3.

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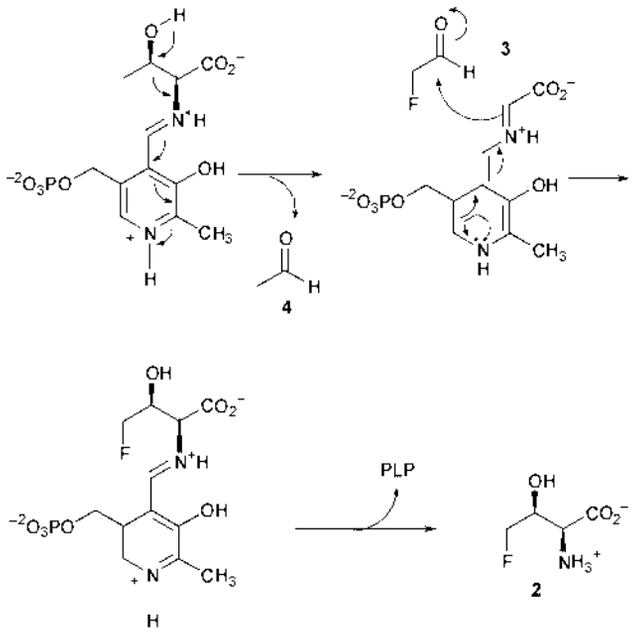
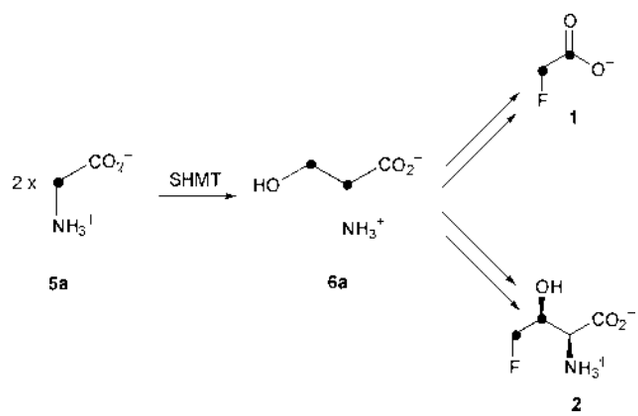


Scheme 2 Proposed mechanism for the enzymatic formation of 4-fluorothreonine **2** *via* threonine transaldolase.[8]



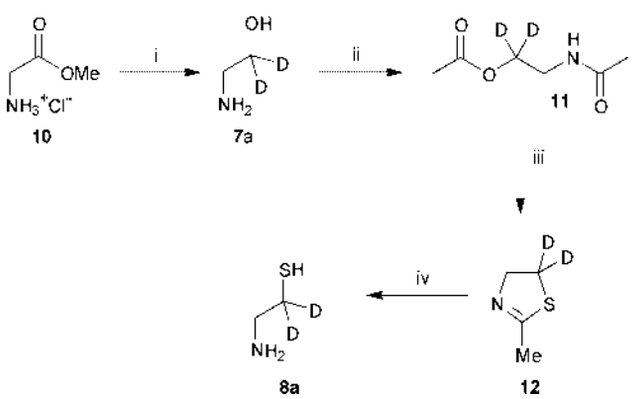
Scheme 3 Proposed pathway accounting for the observed labelling patterns of the fluorometabolites by [2-^{13}C]-glycine **5a**.

Carbons C-2 and C-3 of serine **6** then contribute to the C_2 unit required for the biosynthesis of fluoroacetate **1** and for C-3 and C-4 of 4-fluorothreonine **2**. Experiments with [1-^{13}C]-glycine **5b** did not significantly label the fluorometabolites, consistent with their origin primarily from C-2 and not C-1 of glycine **5** *via* C-2 and C-3 of serine **6**. Investigations with appropriate isotopically labelled serine, pyruvate and glycerol have indicated that these compounds were also good precursors of the fluorometabolites and it was proposed that the carbon substrate for fluorination was derived from a phosphorylated intermediate of the glycolytic pathway present between glycerol and pyruvate.[4] This analysis requires that serine contributes to the glycolytic pathway *via* its metabolism to pyruvate. However, extensive cell-free studies with the proteins of *S. cattleya* have failed to demonstrate the conversion of glycolytic-phosphate intermediates to organofluorine compounds.[10,11] Therefore, alternative biosynthetic pathways were considered to explain the previous data. Instead of metabolism into the glycolytic pathway *via* pyruvate it was considered that serine **6** may become metabolised to ethanolamine (2-aminoethanol) **7**, which as a C_2 compound emerged as a putative precursor to fluoroacetaldehyde **3**. Likewise, if serine is converted to cysteine in the usual manner then decarboxylation would deliver cysteamine **8**, another C_2 candidate precursor to fluoroacetaldehyde **3**. The origin of ethanolamine **7** or cysteamine **8** from serine and then conversion of one of these C_2 intermediates to fluoroacetaldehyde **3**, would be consistent with all of the previously reported labelling studies. It therefore became an objective to explore if either of these compounds had a role to play in fluorometabolite biosynthesis. Accordingly the synthesis of [1,1-$^2H_2$]-ethanolamine **7a** and [1,1-$^2H_2$]-cysteamine **8a**, and the results of feeding experiments with these compounds to cells of *S. cattleya*, are reported.

Furthermore, in order to shed some light on the origin of the oxygen atoms of fluoroacetate and that at C-3 of 4-fluorothreonine, an experiment with [2-^{2}H,2-^{18}O]-glycerol **9a** was conducted. It seemed likely that the oxygen atom of fluoroacetaldehyde **3** would contribute to both of these positions, however this remained to be determined. Additionally the incorporation of oxygen-18 from [2-^{2}H,2-^{18}O]-glycerol **9a** into the carboxylate of fluoroacetate **1** or at C-3 of 4-fluorothreonine **2**, would rule out the involvement of an intermediate from amino acid metabolism, such as ethanolamine or cysteamine **8** as labelled oxygen would become liberated during transamination to generate serine. Thus the biosynthetic experiments with ethanolamine/cysteamine **7/8** and glycerol **9** were designed to complement each other. Together they should reveal if the metabolic branch towards fluoroacetaldehyde **3** occurs between glycerol **9** and serine **6** or whether fluoroacetaldehyde **3** is derived from a C_2 product of serine or cysteine catabolism.

Results

The synthesis of [1,1-$^2H_2$]-ethanolamine **7a** followed a previous protocol[12] involving the reduction of glycine methyl ester hydrochloride **11** with $LiAl^2H_4$ (Scheme 4). The reaction conveniently



Scheme 4 *Reagents and conditions*: i, $LiAl^2H_4$, THF, reflux, 3 h (73%); ii, Ac_2O (84%); iii, P_4S_{10} (73%); iv, 10 M HCl, reflux, 48 h (71%).

generated the required isotopically labelled [1,1-$^2H_2$]-ethanolamine **7a** in a single step. This material was used directly in a biosynthetic experiment but it also served as a labelled intermediate for the synthesis of [1,1-$^2H_2$]-cysteamine **8a**. The synthetic route to [1,1-$^2H_2$]-cysteamine **8a** from [1,1-$^2H_2$]-ethanolamine **7a** is shown in Scheme 4 and follows a modification of a previously described method to this labelled compound.[13] It was found that for the relatively small scale preparation of isotopically labelled material it was advantageous to isolate and purify the acetamide **11** instead of following the larger scale one pot reaction of **7** to **12**. In particular if acetamide **11** was not neutralised with sodium bicarbonate solution and then purified by chromatography it was sufficiently contaminated with acetic acid and the P_4S_{10} mediated conversion to **12** proved low yielding. The purification step proved to be an important modification.

The synthesis of [2-^{2}H,2-^{18}O]-glycerol **9a** (Scheme 5) required the introduction of both isotopic labels in an economical manner. This was achieved by efficient exchange of the carbonyl oxygen of dihydroxyacetone **13** in [^{18}O]-water and in otherwise dry acidic conditions. Exchange was monitored initially using [^{18}O]-water enriched to 10 atom%. When a set of

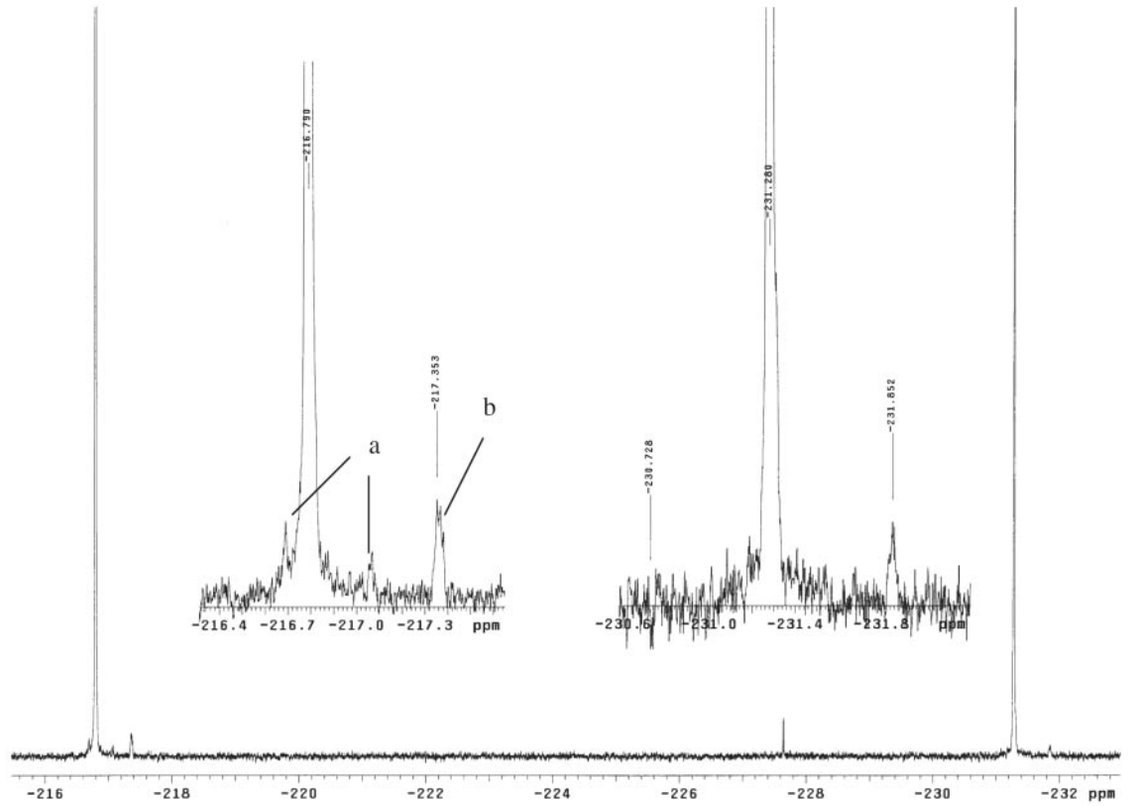
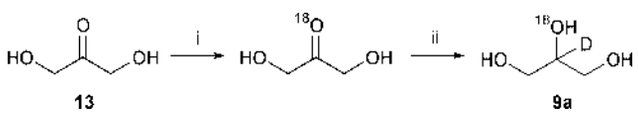


Fig. 1 $^{19}F\{^{1}H\}$-NMR spectrum of fluoroacetate **1** (−216.8 ppm) and 4-fluorothreonine **2** (−231.3 ppm) after [1,1-$^{2}H_2$]-ethanolamine **7a** (10 mM) was incubated with resting cells of *S. cattleya*. The signals (a) represent satellites due to natural abundance ^{13}C–^{19}F coupling. The signal (b) represent a deuterium induced isotope shift due to molecules containing a mono-labelled fluoromethyl group ($CF^{2}HH$).



Scheme 5 *Reagents and conditions*: i, $H_2{}^{18}O$, 1 M HCl, stir, 48 h; ii, $NaB^{2}H_4$, MeOH (84%).

conditions had been optimised the exchange was then carried out with [^{18}O]-water enriched to 90 atom%. The glycerol was prepared by adding dry methanol and mediating a reduction of the carbonyl with $NaB^{2}H_4$. This method proved efficient and afforded [2-^{2}H,2-^{18}O]-glycerol **9a** with a final isotope content of ^{2}H at 96 atom% and ^{18}O at 64 atom% as determined by GC/MS analysis.

Biosynthetic feeding experiments to *S. cattleya*

[1,1-$^{2}H_2$]-Ethanolamine **7a** was added at two different concentrations (10 mM and 20 mM) to resting cell preparations of *S. cattleya* in 50 mM MES buffer (pH 6.5) (33 mg wet weight ml^{-1}), supplemented with NaF (2 mM) and the biotransformation was incubated for 48 hours. Incorporation of label into the resultant fluorometabolites **1** and **2** was determined by $^{19}F\{^{1}H\}$ NMR and GC/MS analyses as previously described.[5] Deuterium incorporation into C-2 of fluoroacetate **1** and into C-3 and C-4 of 4-fluorothreonine **2** was diagnosed directly by heavy isotope induced shifts in the resultant $^{19}F\{^{1}H\}$ NMR spectrum. The ^{19}F-NMR signals for the fluorometabolites containing a single deuterium ($F^{2}HHC$-) atom become shifted to lower frequency by *ca.* 0.6 ppm. If the fluoromethyl group contains two deuterium atoms ($F^{2}H_2C$-) the effect is additive and the ^{19}F-NMR signal is shifted to lower frequency by *ca.* 1.2 ppm relative to the unlabelled species. The resultant $^{19}F\{^{1}H\}$ NMR spectrum after the feeding experiment with [1,1-$^{2}H_2$]-ethanolamine **7a** at 10 mM is shown in Fig. 1. The signal at −217.4 ppm (induced shift of *ca.* 0.6 ppm) represents a population of fluoroacetate **1** molecules (~2%) bearing a fluoromethyl group with a single deuterium. The asymmetric satellites most evident around the fluoroacetate **1** signal at −216.79 ppm arise from ^{19}F–^{13}C coupling due to the presence of natural abundance (~1%) carbon-13. The asymmetry arises due to the heavy atom (^{13}C) induced shift. Deuterium incorporation of a similar magnitude and pattern to that found in **1** was obvious also in the ^{19}F-NMR signal (−231.3 ppm) for 4-fluorothreonine **2**. This mirroring of the isotopic labelling patterns from isotopically labelled precursors, into both of the fluorometabolites is consistent with all previously reported experiments. Notably there was no evidence of a population of fluorometabolites carrying two deuterium atoms on the fluoromethyl group and therefore no evidence for any direct incorporation of [1,1-$^{2}H_2$]-ethanolamine into **1** or **2**. GC/MS analysis of the resultant monolabelled fluoroacetate was determined to be 1.0% and 2.0% when [1,1-$^{2}H_2$]-ethanolamine **7a** was administered to cells of *S. cattleya* at concentrations of 10 mM and 20 mM respectively (Tables 1 and 2). The low level of incorporation of single label into **1** and **2** from [1,1-$^{2}H_2$]-ethanolamine **7a** may be explained by the isomerisation of [1,1-$^{2}H_2$]-ethanolamine **7a** *via* the coenzyme B_{12} dependent enzyme, ethanolamine ammonia lyase. This enzyme converts ethanolamine **7** to acetaldehyde **4**[11] and mediates a formal vicinal interchange process with migration of the amine NH_2 in one direction and a hydrogen (deuterium) in the other direction (Scheme 6) yielding after aminal collapse to

Table 1 GC/MS determined incorporation levels into fluoroacetate **1** after feeding [1,1-$^{2}H_2$]-ethanolamine **7a** to resting cell of *S. cattleya*

	^{2}H-Incorporation (%)		
	M + 1		*M* + 2
Precursor	C-1	C-2	C-1 + C-2
[1,1-$^{2}H_2$]-ethanolamine (10 mM)	<0.5	1.1 ± 0.15	<0.5
[1,1-$^{2}H_2$]-ethanolamine (20 mM)	<0.5	2.1 ± 0.31	<0.5

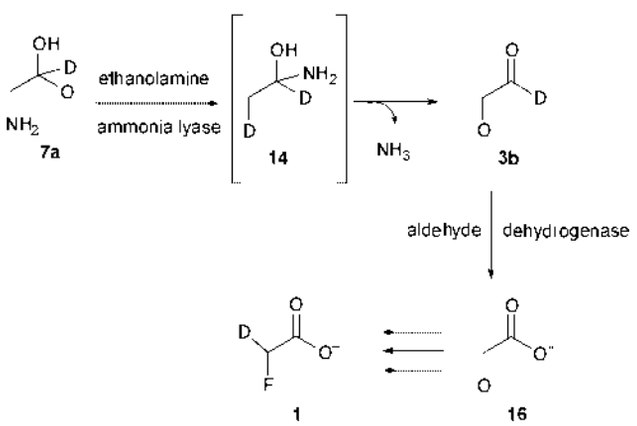
Table 2 GC/MS determined incorporation levels into 4-fluorothreonine **2** after feeding [1,1-$^2H_2$]-ethanolamine **7a** to resting cells of *S. cattleya*

	^{2}H-Incorporation (%)			
	(C-1 + C-2)		(C-2 + C-3 + C-4)	
Precursor	M + 1	M + 2	M + 1	M+2
[1,1-$^2H_2$]-ethanolamine (10 mM)	<0.5	<0.5	1.0 ± 0.06	<0.5
[1,1-$^2H_2$]-ethanolamine (20 mM)	<0.5	<0.5	2.3 ± 0.19	<0.5

Table 3 Isotope incorporation levels into fluoroacetate **1** after feeding [2-$^2H_1$,2-^{18}O]-glycerol **9a** to *S. cattleya*. The experiment was carried out in triplicate and incorporations were determined by GC/MS analysis

	^{18}O,^{2}H-Incorporation (%)				
	(C-1 + C-2)			C-2	
Precursor	M + 1	M + 2	M + 3	M + 1	M+2
Exp. 1 [2-$^2H_1$,2-^{18}O]-glycerol	<0.5	1.3 ± 0.21	n.d.[a]	<0.5	<0.5
Exp. 2 [2-$^2H_1$,2-^{18}O]-glycerol	<0.5	1.4 ± 0.03	n.d.	<0.5	<0.5
Exp. 3 [2-$^2H_1$,2-^{18}O]-glycerol	<0.5	1.7 ± 0.28	n.d.	<0.5	<0.5

[a] n.d. not detectable



Scheme 6 Proposed metabolism of [1,1-$^2H_2$]-ethanolamine to account for the observed labelling of isotope into fluoroacetate (and 4-fluorothreonine).

an *in vivo* pool of [1,2-$^2H_2$]-acetaldehyde **3b**. Oxidation by an acetaldehyde dehydrogenase will generate an *in vivo* pool of [2-$^2H_1$]-acetate **16**, which is known to label the fluorometabolites **1** and **2** in the observed manner, after processing around the citric acid cycle and then entry into the glycolytic pathway.[5]

[1,1-$^2H_2$]-Cysteamine **8a** was incubated with resting cells of *S. cattleya* at two concentrations (1 mM and 3 mM) in a similar manner to that described for [1,1-^{2}HH]-ethanolamine. In a series of control experiments it was found that when unlabelled cysteamine **8** was administered to resting cells at concentrations higher than 3 mM, then cysteamine significantly inhibited fluorometabolite production. In the event there was no evidence by GC/MS or ^{19}F-NMR analysis of any isotope incorporation into either of the fluorometabolites **1** and **2** after feeding experiments with [1,1-$^2H_2$]-cysteamine at 1 mM and 3 mM. Finally [2-$^2H_1$,2-^{18}O]-glycerol **9a** was incubated with resting cells of *S. cattleya* for 48 h at a final concentration of 10 mM. The experiment was carried out in triplicate and the level of isotope incorporation into the resultant fluorometabolites **1** and **2** was determined by GC/MS analysis (Tables 3 and 4).

It is clear from the M + 2 intensities in the GC/MS data presented in Tables 3 and 4 that there is a significant incorporation of oxygen-18 from [2-$^2H_1$,2-^{18}O]-glycerol **9a** found in both fluoroacetate **1** (1.3–1.7%, M + 2 in Table 3) and 4-fluorothreonine **2** (1.8–2.9%, M + 2 in Table 4). However, there is no evidence that the deuterium atom from [2-$^2H_1$, 2-^{18}O]-glycerol **9a** was also incorporated into C-3 of 4-fluorothreonine **2**, as there is no significant enhancement of the M + 1 (^{2}H-only) or M + 3 (^{2}H + ^{18}O) ions (Table 4). The data force the conclusion that the C(2)–H bond of glycerol is broken at some stage along the biosynthetic pathway to 4-fluorothreonine **2** whereas the oxygen at C-2 of glycerol is retained and becomes incorporated into both the fluoroacetate **1** carboxylate group and into the C-3 hydroxy group of 4-fluorothreonine **2**. Metabolic oxidation to a carbonyl intermediate such as dihydroxyacetone phosphate would satisfy these observations. There was no evidence for a direct incorporation of isotope from [1,1-$^2H_2$]-ethanolamine **7a**, although there was an indirect incorporation most probably *via* [2-$^2H_1$]-acetate generated *in vivo*. Also there was no evidence of any isotope incorporation into **1** and **2** from [1,1-$^2H_2$]-cysteamine **8a**. These experiments demonstrate that glycerol **9** contributes to a carbonyl intermediate of the glycolytic pathway but that this intermediate is not metabolised to an amino acid such as serine **6** or cysteine, as the original C-2 oxygen atom would be lost during transamination. These biosynthetic relationships and a current overview of fluorometabolite biosynthesis is outlined in Scheme 7.

Experimental

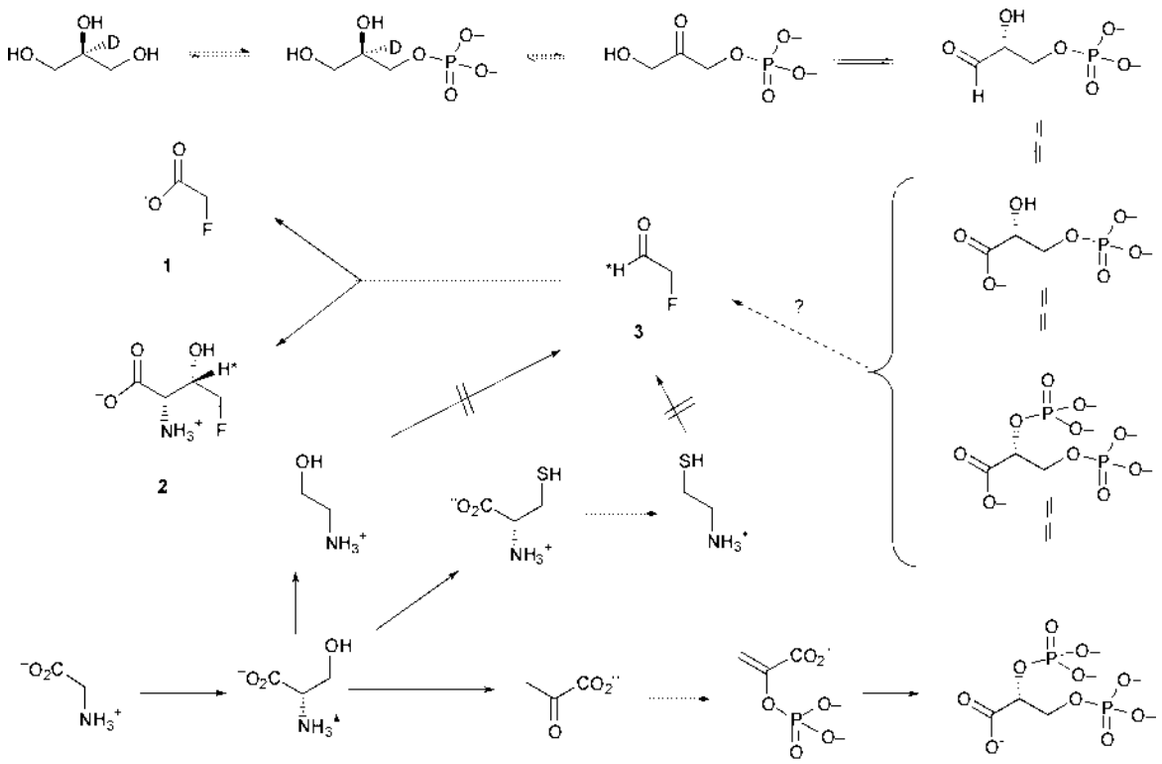
General

Cultures were incubated in a temperature controlled Gallenkamp orbital incubator and resting cells on a temperature controlled Innova 2000 platform shaker. NMR spectra were recorded on Bruker AC 300, Varian VXR-400S, Varian Unity 300, Varian Mercury 200 or Varian Oxford 300 spectrometers. Melting points were measured on a Gallenkamp variable heater and are uncorrected. GC/MS analysis was performed on a Agilent 5973 mass spectrometer equipped with a HP-5MS capillary column (30 m × 250 μm × 0.25 μm) connected to a Agilent 6890 series oven. Reaction glassware was dried in an oven at 200 °C and cooled in a dry atmosphere of nitrogen. Reaction solvents were dried and freshly distilled prior to use. Reactions requiring anhydrous conditions were conducted under an atmosphere of dry nitrogen and thin layer chromatography was carried out on Merck, Kieselgel 60, F_{254} aluminium and glass backed plates. Visualisation of plates was achieved by using a UV lamp or permanganate stains. Column chromatography was carried out over silica gel Merck, Kieselgel 60, 230–400 mesh.

Table 4 Isotope incorporation levels into 4-fluorothreonine **2** after feeding [2-$^2H_1$,2-^{18}O]-glycerol **9a** to *S. cattleya*. The experiment was carried out in triplicate and incorporations were determined by GC/MS analysis

	^{18}O,^2H-Incorporation (%)				
	(C-1 + C-2)		(C-2 + C-3 + C-4)		
Precursor	*M* + 1	*M* + 2	*M* + 1	*M* + 2	*M*+3
Exp. 1 [2-$^2H_1$,2-^{18}O]-glycerol	<0.5	<0.5	<0.5	2.9 ± 0.35	n.d.[a]
Exp. 2 [2-$^2H_1$,2-^{18}O]-glycerol	<0.5	<0.5	<0.5	2.1 ± 0.02	n.d.
Exp. 3 [2-$^2H_1$,2-^{18}O]-glycerol	<0.5	<0.5	<0.5	1.8 ± 0.02	n.d.

[a] n.d. not detectable



Scheme 7 The metabolic relationships between various amino acids and glycerol, and their contributions to fluorometabolite biosynthesis.

Culture conditions

Streptomyces cattleya NRRL 8057 was obtained from United States Department of Agriculture, Agricultural Research Service, Midwest Area Northern Regional Research Laboratories, Peoria, Illinois, USA. Cultures were maintained on agar slants containing soybean flour (2% w/v), mannitol (2% w/v), agar (1.5% w/v) and tap water, grown at 28 °C until sporualtion could be detected. The resultant static cultures were stored at 4 °C.

Seed cultures were prepared by transfer of spores and aerial mycelia from a static culture, into a conical flask (500 ml), plugged with cotton wool and containing chemically defined medium (90 ml).[14] After shaking for 4 days at 28 °C and 180 rpm an aliquot (0.3 ml) of the vegetative mycelium was used to inoculate the batch cultures. The cultures were incubated at 28 °C and rotated at 180 rpm for 6–8 days. Cells were harvested by centrifugation (25 minutes, 14000 rpm, 25 °C) and the pellet was washed three times with 50 mM sterile MES buffer pH 6.5. After the final wash the bacterial pellet was resuspended in 50 mM MES buffer pH 6.5 at a concentration of 0.176 wet wt ml^{-1}.

For resting cell experiments the cell suspension (5 ml) was supplemented with the filter sterilised putative precursor and NaF (2 mM). The total volume was adjusted to 23 ml with 50 mM MES buffer (pH 6.5) and the suspension was incubated in conical flasks (75 ml) plugged with cotton wool at 28 °C on an orbital shaker at 200 rpm for 48 hours.

^{19}F NMR analysis of resting cell experiments

Isotope incorporation. Supernatants of resting cell experiments were lyophilised and the residues resuspended in ^2H_2O (0.7 ml). Particulate matter was filtered by passing the solutions through a cotton wool plug before transferring to an NMR tube.

[1,1-$^2H_2$]-Ethanolamine 7a [12]

Glycine methyl ester hydrochloride (5.0 g, 40.0 mmol) was added slowly over 15 min to a stirred solution of $LiAl^2H_4$ (2.13 g, 50.7 mmol) in dry THF (50 ml) at 0 °C. The mixture was heated under reflux for 3 h and then the reaction was cooled to ambient temperature. The reaction was quenched by the dropwise addition of saturated Na_2SO_4 solution until effervescence ceased and a white solid was formed. After stirring for a further 30 min, the precipitate was collected by filtration and extracted into tetrahydrofuran (600 ml) by Soxhlet extraction for 18 hours to give a pale yellow solution. The solvent was removed *in vacuo* to give a yellow–brown viscous oil, which was then purified by Kugelrohr distillation (100 °C, 10 mmHg), to yield [1,1-$^2H_2$]-ethanolamine **7a** as a pale yellow oil (1.86 g, 73%).

δ_H (200 MHz; ^2H_2O) 2.54 (2 H, s, CH_2N); δ_C (50.3 MHz, ^2H_2O) 42.4 (*C*H_2NH_2), 62.5 (quintet, *J* 21.8, *C*^2H_2OH); *m/z* (EI) 63 ([M]$^+$, 7.0%), 33 ([C^2H_2OH]$^+$, 5.5%), 30 ([CH_2NH_2]$^+$, 100%).

N,*O*-Diacetyl-[1-$^2H_2$]-ethanolamine 11[15]

Acetic anhydride (6 ml, 64 mmol) was slowly added to [1,1-$^2H_2$]-ethanolamine (2 g, 32 mmol) and heated with stirring at 130 °C for 1 h. After cooling to room temperature, chloroform (50 ml) and water (50 ml) were added to the reaction mixture. The aqueous layer was basified to pH 10 with potassium carbonate and then the products were extracted into chloroform (10 × 50 ml). The organic layers were combined and dried ($MgSO_4$). The solvent was removed *in vacuo* to give the crude product which was purified by column chromatography over silica (EtOAc–MeOH, 9 : 1) to yield **11** as a clear, brown oil (3.96 g, 84%).

δ_H (300 MHz; ^2H_2O) 1.99 (3 H, s, CH_3), 2.10 (3 H, s, CH_3), 3.45 (2 H, s, CH_2C$^2H_2$); δ_C (75.5 MHz, C$^2HCl_3$) 20.8 (*C*H_3), 23.1 (*C*H_3), 62.6 (quintet, *J* 22.6, *C*$^2H_2$), 170.1 (NHCO*C*H_3), 170.3 (COO*C*H_3); *m/z* (CI+) 148 ([M + H]$^+$, 100%), 88 ([M − CH_3COO]$^+$, 36.5%), 30 ([$CH_3COH(OH)$]$^+$, 8.7%).

[5-$^2H_2$]-2-Methyl-2-thiazoline 12[13]

Phosphorus pentasulfide (0.45 g, 1.0 mmol) was added to *N*,*O*-diacetyl-[1-$^2H_2$]-ethanolamine (1.5 g, 10.2 mmol) and the mixture slowly heated to 100 °C until complete dissolution. The reaction mixture was transferred into a Kugelrohr distillation apparatus and heated to 200 °C for 1.5 h at atmospheric pressure. After this time, distillation was carried out under reduced pressure for a further 10 min to afford a yellow oil (825 mg) containing the product and acetic acid as an impurity. The oil (300 mg) was dissolved in chloroform (20 ml) and water (20 ml) was added. The aqueous layer was basified with potassium carbonate (pH 12) and then the products were extracted into chloroform (3 × 20 ml). The organic layers were combined and dried over $MgSO_4$. The solvent was removed *in vacuo* to give the product as a clear, yellow oil (281 mg, 73%).

δ_H (300 MHz; C$^2HCl_3$) 2.22 (3 H, t, ^5J 1.69, CH_3), 4.28 (2 H, br, CH_2); δ_C (75.5 MHz, C$^2HCl_3$) 20.8 (*C*H_3), 33.3 (quintet, *J* 21.9, *C*$^2H_2$), 62.7 (*C*H_2), 169.9 (N*C*S); *m/z* (EI) 103 ([M]$^+$, 63.3%), 83 (18.3%), 62 ([$CH_2C^2H_2S$]$^+$, 100%), 55 (21.7%).

[1,1-$^2H_2$]-Cysteamine 8a[13]

10 M HCl (5 ml) was added to [5-$^2H_2$]-2-methyl-2-thiazoline (250 mg, 2.43 mmol) and the mixture heated at 130 °C for 48 h. After cooling to ambient temperature, water (30 ml) was added and unreacted starting material was removed by extraction into chloroform (2 × 30 ml). The aqueous layer was concentrated under reduced pressure and freeze-dried to yield a pale yellow solid (199 mg, 71%).

Mp 66–68 °C (dec) (lit.[13] mp 70 °C); δ_H (300 MHz; ^2H_2O) 3.14 (2 H, br, CH_2N); δ_C (75.5 MHz, ^2H_2O) 42.3 (*C*H_2NH_2), 21.2 (quintet, *J* 21.9, *C*$^2H_2$); *m/z* (EI) 79 ([M]$^+$, 3.5%), 46 ([M − SH]$^+$, 8.8%), 49 ([C^2H_2SH]$^+$, 4.0%), 30 ([CH_2NH_2]$^+$, 100%).

[2-$^2H_1$,2-^{18}O]-glycerol

[^{18}O]-Water (0.5 ml) and 1 M HCl (0.01 ml) were added to dry dihydroxyacetone (500 mg, 2.8 mmol), and the solution stirred at room temperature for 48 h. The reaction mixture was diluted with freshly distilled MeOH (5 ml) and NaB^2H_4 (418 mg, 10 mmol) was added over 5 min to the stirring solution at 0 °C. The mixture was stirred at room temperature under a nitrogen atmosphere for 18 h. 6 M H_2SO_4 (2 ml) was added and the reaction mixture stirred for 2 h. A white precipitate formed and the solvent was removed under reduced pressure to give a white solid. The solid was vigorously shaken with ethyl acetate (3 × 20 ml) and the organic layers were combined. The solvent was removed *in vacuo* to give the product glycerol as a clear oil (446 mg, 85%). The level of ^{2}H and ^{18}O was 96 atom% and 64 atom% respectively, as determined by ^{13}C-NMR and GC/MS analyses. For GC/MS analysis the sample was derivatised by adding *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (0.1 ml) to a 1 mg ml^{-1} solution of [2-$^2H_1$,2-^{18}O]-glycerol in ethyl acetate (0.5 ml) and subsequent heating of the mixture at 80 °C for 1 h.

δ_H (300 MHz; ^2H_2O) 3.7, 3.8 (4 H, dd, ^4J 11.1 and 11.3, 2 × CH_2); δ_C (75.5 MHz, $^2H_4$-MeOH) 65.9 (*C*H_2), 74.9 (t, *J* 21.4, *C*^{2}HH); *m/z* (EI), MSTFA derivative, 296 ([M − CH_3]$^+$, 6.4%), 219 ([M − ^{18}OH − $SiMe_3$]$^+$, 52.5%), 208 ([M − CH_2O − $SiMe_3$]$^+$, 100%), 147 ([$CH_3C^2HH^{18}O$ − $SiMe_3$]$^+$, 19.5%), 120 ([CH_2O − $SiMe_3$]$^+$, 20.3%), 103 ([$CH_3CDH^{18}O$ − $SiMe_3$]$^+$, 19.5%), 73 ([$SiMe_3$]$^+$, 56.8%).

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