Biosynthesis of fluoroacetate and 4-fluorothreonine by *Streptomyces cattleya*. The stereochemical processing of glycerol

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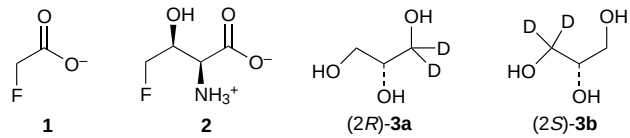
When both (2*R*)-[1-$^2H_2$]- and (2*S*)-[1-$^2H_2$]-glycerol are incubated with resting cell suspensions of *S. cattleya*, only the 2*R*-enantiomer labels the fluoromethyl groups of fluoroacetate and 4-fluorothreonine, with retention of both deuterium atoms, placing metabolic and mechanistic limitations on the process of biological fluorination.

Streptomyces cattleya is one of very few biological systems which have been identified with the capability of biosynthesising organofluorine natural products.[1] We are currently investigating[2–5] the pathways by which fluorometabolite biosynthesis is achieved in *S. cattleya* and have shown[4] the efficient incorporations of glycine and pyruvate into fluoroacetate **1** and 4-fluorothreonine **2** by resting cell suspensions of *S. cattleya*.

The incorporations are consistent with the conversion of glycine to serine and then serine to pyruvate,[4,5] and the labelling patterns indicate that C-2 and C-3 of pyruvate contribute C-1 and C-2 of fluoroacetate **1** and C-3 and C-4 of 4-fluorothreonine **2** respectively. Tamura *et al*.[6] have shown that [2-^{13}C]glycerol is efficiently incorporated into fluoroacetate **1** by cell suspensions of *S. cattleya*, the isotope becoming regiochemically located at C-1 of fluoroacetate. Together these findings suggest that, prior to incorporation into the fluorometabolites, pyruvate and glycerol enter a common pathway and the glycolytic pathway emerges as the most obvious candidate.

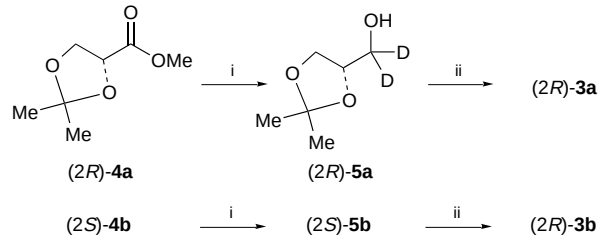
To shed further light on the role of glycerol we have investigated the stereochemical processing of glycerol during fluorometabolite biosynthesis in *S. cattleya*. Only two of the three carbon atoms of glycerol can contribute the carbons of fluoroacetate **1** and C-3 and C-4 of 4-fluorothreonine **2**, thus one of the prochiral hydroxymethyl groups of glycerol is cleaved during the biosynthetic process. In order to probe the cryptic stereochemistry of glycerol metabolism in *S. cattleya*, both (2*R*)-[1-$^2H_2$]- **3a** and (2*S*)-[1-$^2H_2$]-glycerol **3b** were prepared such that the prochiral hydroxymethyl groups of glycerol were independently labelled.

Isotopically enriched [1-$^2H_2$]glycerols **3a** and **3b** were synthesised as shown in Scheme 1 following a previously reported[7] protocol. This involved $LiAlD_4$ reduction of either (*R*)- or (*S*)-methyl 2,2-dimethyl-1,3-dioxolane-4-carboxylate, **4a** or **4b** {$[\alpha]_D$ −9.9 (neat) lit.,[8] −10.1 (neat)} respectively, to generate the corresponding alcohols **5a** and **5b** {$[\alpha]_D$ −12.2 (neat), lit.,[7] −14.9 (neat)}. Deprotection using aqueous mineral acid generated the desired glycerols **3a** and **3b**‡ in good overall yield as summarised in Scheme 1. The enantiomeric glycerols **3a** and **3b** were administered in separate experiments to resting cell suspension cultures[5] of *S. cattleya* at a final concentration of 7.5 mm. The level of isotope incorporation into the resultant fluoroacetate **1** was determined by GC–MS analysis[5] after 48 h in each case. The data indicated that deuterium had only become incorporated into fluoroacetate **1** from (2*R*)-[1-$^2H_2$]glycerol **3a** (M + 2 = 28%, M + 1 = 5%). No significant incorporation of isotope from (2*S*)-[1-$^2H_2$]glycerol **3b** was apparent.



This conclusion was confirmed by $^{19}F\{^1H\}$ NMR analysis of the supernatants from each experiment. The presence of a deuterium atom geminal to fluorine is readily diagnosed by an induced shift to lower frequency in the ^{19}F NMR spectrum.[9] The effect is additive and each deuterium atom induces a shift of *ca*. 0.6 ppm in magnitude. The resultant $^{19}F\{^1H\}$ NMR spectrum from the (2*R*)-[1-$^2H_2$]glycerol **3a** feeding experiment is shown in Fig. 1.

Proton decoupling simplifies the ^{19}F NMR signals of the two fluorometabolites and allows a more accurate assessment of the level of incorporation. The signals of both fluoroacetate **1** (−214.6 ppm) and 4-fluorothreonine **2** (−229.2 ppm) have an associated signal shifted to higher frequency, by 1.2 ppm. This



Scheme 1 *Reagents and conditions*: i, $LiAlH_4$, Et_2O, 30 min., 77%; ii, HCl (2%), 2 h, room temp., 100%

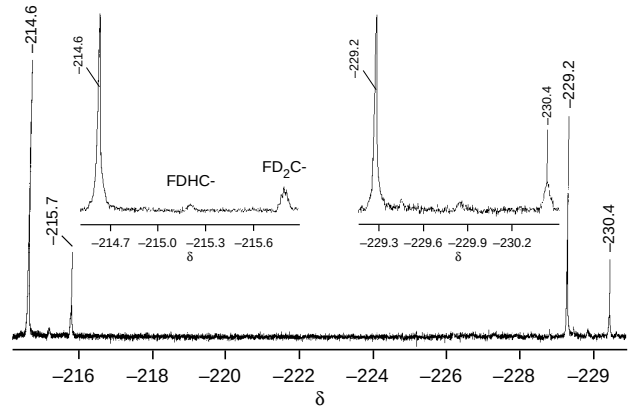


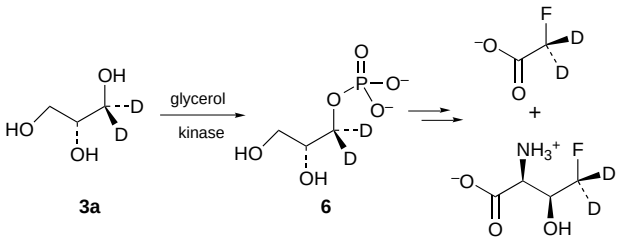
Fig. 1 $^{19}F\{^1H\}$ NMR spectrum of fluoroacetate **1** (−214.6 ppm) and 4-fluorothreonine **2** (−229.2 ppm) formed on incubation of resting cell suspensions of *S. cattleya* with (2*R*)-[1-$^2H_2$]glycerol **3a**. Insets show expansions of the relevant regions of the spectrum. Both signals have deuterium-induced upfield shifted (1.2 ppm) signals associated with the incorporation of geminal di-deuterium labelled fluoromethyl groups (FD_2C). A shifted (0.6 ppm) signal of lower intensity indicates a population of molecules containing a single deuterium atom on the fluoromethyl groups (FDHC).

is assigned in each case to that population (*ca*. 28%) of molecules containing two deuterium atoms on the fluoromethyl group (FD_2C). There is also a minor signal in each case shifted to higher frequency by 0.6 ppm which corresponds to a smaller population (< 5%) of molecules carrying a single deuterium atom on the fluoromethyl group (FDHC). It should be noted that the levels and patterns of deuterium incorporation into fluoroacetate **1** and 4-fluorothreonine **2** are very similar, reinforcing the conclusion[4,5] that the fluoromethyl groups in the metabolites have a common origin and that there is consequently a single fluorinating enzyme in *S. cattleya*.

Only (2*R*)-[1-$^2H_2$]glycerol **3a** was incorporated into the fluorinated metabolites and therefore only the *pro-R* hydroxymethyl carbon of glycerol contributes C-2 of fluoroacetate **1** and C-4 of 4-fluorothreonine **2**. The *pro-S* hydroxymethyl group of glycerol must be cleaved and lost during fluorometabolite biosynthesis. The nature of this stereochemical processing allows certain conclusions to be drawn about the pathway leading to the substrate for fluorination. Glycerol is phosphorylated by glycerol kinase at its *pro-R* hydroxymethyl group to generate *sn*-glycerol-3-phosphate **6**, prior to its entry into the glycolytic pathway, as illustrated in Scheme 2.

Whilst these experiments do not reveal the substrate for the fluorination enzyme they do indicate that the carbon atom of glycerol which becomes activated by phosphorylation *in vivo* is also that one which is fluorinated downstream. Also the predominantly labelled populations in each of the fluorinated metabolites retained both deuterium atoms from (2*R*)-[1-$^2H_2$]glycerol **3a**, thus we can conclude that no obligatory oxidation is required at this carbon centre prior to fluorination.

The retention of both deuteriums is consistent with two minimal mechanistic hypotheses. The fluorination event may involve nucleophilic substitution by fluoride at the original phosphorylated (or enzymatically activated) carbon atom of *sn*-glycerol-3-phosphate **6** or of a metabolite derived from **6** on the glycolytic pathway to pyruvate. Alternatively fluoride may be introduced by an elimination–addition process on a glycolytic intermediate. This latter hypothesis intuitively appears less likely; however it is not ruled out by the labelling pattern observed. These results provide the first, albeit tentative, mechanistic insight into the process of biological fluorination based on empirical observation.



Scheme 2 Observed incorporation (28%) of **3a** into fluoroacetate and 4-fluorothreonine in *S. cattleya*

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Footnotes

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‡ The glycerols **3a** and **3b** were purified over silica gel and showed identical ^1H NMR spectra to those previously reported.[7] The isotope content determined by FAB mass spectrometry was 99.4 atom% (double label) for each sample.

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