Identification of a fluorometabolite from Streptomyces sp. MA37: (2R3S4S)-5-fluoro-2,3,4-trihydroxypentanoic acid

Long Ma,‡a Axel Bartholome,a Ming Him Tong,a Zhiwei Qin,b Yi Yu,c Thomas Shepherd,d Kwaku Kyeremeh,e Hai Deng*b and David O’Hagan*a

(2R3S4S)-5-Fluoro-2,3,4-trihydroxypentanoic acid (5-FHPA) has been discovered as a new fluorometabolite in the soil bacterium Streptomyces sp. MA37. Exogenous addition of 5-fluoro-5-deoxy-D-ribose (5-FDR) into the cell free extract of MA37 demonstrated that 5-FDR was an intermediate to a range of unidentified fluorometabolites, distinct from fluorooacetate (FAc) and 4-fluorothreonine (4-FT). Bioinformatics analysis allowed identification of a gene cluster (fdr), encoding a pathway to the biosynthesis of 5-FHPA. Over-expression and in vitro assay of FdrC indicated that FdrC is a NAD+-dependent dehydrogenase responsible for oxidation of 5-FDR into 5-fluoro-5-deoxy-lactone, followed by hydrolysis to 5-FHPA. The identity of 5-FHPA in the fermentation broth was confirmed by synthesis of a reference compound and then co-correlation by 19F-NMR and GC-MS analysis. The occurrence of 5-FHPA proves the existence of a new fluorometabolite pathway.

Introduction

The introduction of fluorine into organic molecules can substantially modulate their physicochemical properties.1 About 30% of current drugs, including many top sellers, contain at least one fluorine atom.2 In contrast to man-made molecules, fluorinated natural products are extremely rare.3

Fluoroacetate (FAc) 1 is the most ubiquitous fluorometabolite found as a toxic self-defence agent in many tropical and sub-tropical plants.4 In 1986, the actinomycete soil bacterium Streptomyces cattleya was found to secrete FAc 1 and 4-fluorothreonine (4-FT) 2 as part of its metabolic profile when grown in the presence of inorganic fluoride.5 Over the last decade, intermediates on the fluorometabolite pathway in S. cattleya have been uncovered and are shown in Scheme 1. The formation of the C–F bond is catalysed by the fluorinase, which mediates the biotransformation of S-adenosyl-L-methionine (SAM) 3 and inorganic fluoride into 5’-fluoro-5-deoxy-adenosine (5’-FDA) 4.7 5’-FDA is then phosphorylated generating 5-fluoro-5-deoxy-ribose phosphate (5-FDRP) 5,8 followed by ring opening to generate 5-fluoro-5-deoxy-ribulose-phosphate (5-FDRulp) 6.8 An aldolase catalyses a retro-aldol reaction to generate fluoroacetaldehyde 7, the last common intermediate on the pathway.10 Fluoroacetaldehyde 7 is either oxidised to FAc 1,11 or biotransformed into 4-FT 2 catalysed by a PLP-dependent transaldolase.12 The fluorinase gene remained a sole representative in the genome databases for a decade, however between 2012–2014, three fluorinase genes appeared in the genomes of sequenced microorganisms (Streptomyces sp. MA37, Actinoplanes sp. N902-109,13 Norcardia brasiliensis14,15). PCR amplification of these genes from genomic DNA, or the expression of synthetic genes in E. coli demonstrated that these were all functional fluorinases. Most recently, the marine actinomycete Streptomyces xinghaiensis (NRRL B-24674) was also shown to have a functional fluorinase.15 S. xinghaiensis was found to produce FAc 1 only (no 4-FT 2) and FAc 1 production is sea-salt dependent.15 S. xinghaiensis is the first fluorometabolite producer from a marine microorganism. Most importantly, all fluorinase genes described after the disclosure of that obtained from S. cattleya have greater than 80% sequence identity to the original fluorinase. In culture, N. brasiliensis was unable to produce a trace of fluorometabolite under laboratory culture conditions.15,14

Streptomyces sp. MA37, an isolate from a Ghanaian soil sample, produces FAc 1 and 4-FT 2 in culture.13 Unlike S. cattleya and S. xinghaiensis, Streptomyces sp. MA37 also

Received 16th November 2014
Accepted 26th November 2014
DOI: 10.1039/c4sc03540b
www.rsc.org/chemicalscience

† Electronic supplementary information (ESI) available: The fdr gene cluster was deposited in EMBL Nucleotide Sequence Database under the accession no: LN612605. See DOI: 10.1039/c4sc03540b
‡ Current address: School of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, P. R. China.

produced a range of unidentified fluorometabolites in addition to FAC 1 and 4-FT 2, as determined by $^{19}$F-NMR of a supernatant extract.14 The identity of these novel fluorometabolites is of interest given that this class of natural products is so rare.

Here we report that 5-FDRP 5 is a branch point metabolite between two pathways. Exogenous addition of 5-fluoro-5-deoxyribose (5-FDR) 8 to cell free extracts was found to support biosynthesis predominantly of the unidentified fluorometabolites and could not significantly support FAC 1 and 4-FT 2 biosynthesis. Genomic-driven analysis allowed identification of the fdr gene cluster, encoding elements of the new pathway. In vitro assay of over-expressed FdRC demonstrated that the protein is a NAD+ dependent dehydrogenase that oxidizes 5-FDR 8 to 5-fluoro-5-deoxy-$\alpha$-ribo-$\gamma$-lactone (5-FRL) 9, followed by lactone hydrolysis to (2R3S4S)-5-fluoro-2,3,4-tri-hydroxypentanoic acid (5-FHPA) 10. This sequence from 5-FDRP forms the first steps of a new fluorometabolite pathway.

**Results and discussion**

5-FDRP 5 is known to be an intermediate in the biosynthesis of FAC 1 and 4-FT 2 in Streptomyces cattleya.8 In that study it was established that the de-phosphorylated free ribose 5-FDR 8 was unable to recover fluorometabolite biosynthesis in cell free extracts. It was unable to support fluorometabolite biosynthesis, and it was also unable to become phosphorylated to regenerate 5 and channel back into the fluorometabolite pathway to generate FAC 1 or 4-FT 2.8,15 Interestingly in the marine microorganism Salinispora tropica, a closely related pathway operates as illustrated in Scheme 2.17 This bacterium produces salinosporamide A 11 a chlorinated metabolite which has received attention as an anti-cancer therapeutic.18 Investigations into salinosporamide-A 11 biosynthesis have shown that the chlorine is introduced by a SAM-dependent chlorinase to generate 5'-ClDA 12, an enzyme closely analogous to the fluorinase of S. cattleya.14 The two pathways also share the second step. In S. cattleya, depurination of 5-FDA 1 generates 5-FDRP 5 and analogously in S. tropica 5'-CIRP 13 is generated. The two pathways appear to diverge at this point.17 For salinosporamide-A 11, SalN catalyses dephosphorylation of 5-CIRP 13 to generate the free sugar 5-chloro-5-deoxy-$\alpha$-ribose (5-CIR) 14 as a key intermediate on the biosynthetic pathway to salinosporamide A 11. The free sugar does not appear to be relevant in S. cattleya. Interestingly, 5-FDR 8 was also found to support this biotransformation to form the analogous fluorinated derivative of salinosporamide A, demonstrating that the salinosporamide-A pathway can channel fluoromethyl as well as chloromethyl intermediates.20

In this context it appeared appropriate to explore a role for 5-FDR 8 in Streptomyces sp. MA37 metabolism to establish if any of the additional unknown metabolites are derived from the free sugar. To this end, 5-FDR 8 was prepared by synthesis and was incubated with a cell-free extract (CFE) of Streptomyces sp. MA37. Products were monitored by $^{19}$F-NMR. The usual dominance of FAC 1 and 4-FT 2, found in full whole cell incubations with added fluoride ion was no longer the observed profile. Instead these signals were substantially diminished and some of the minor unknowns now dominated the $^{19}$F-NMR spectrum (Fig. 1). These unknown signals were not observed in control experiments using boiled CFE incubated with 5-FDR 8 or the CFE alone. Therefore 5-FDR 8 appears to be an intermediate to some of the fluorometabolites in Streptomyces.
A homolog search of the *Streptomyces* sp. MA37 genome revealed an open reading frame (orf) fdrA that is predicted to encode a metal-dependent phosphoesterase, sharing high sequence identity (56%) with SaIN of the salinosporamide biosynthetic pathway. Notably, immediately downstream of fdrA are two orfs, fdrB and fdrC, that are divergently transcribed. These are FdB and FdC which also share high sequence identities with SaIH (68% identity) and SaIM (69% identity), respectively (Fig. 2 and S1†) and are predicted to encode a dihydroxy-acid dehydratase and a short chain dehydrogenase (SDRs). SaIM was found to catalyse the NAD⁺ dependant oxidation of 5-CIR 14 to 5-chlororibolactone 15, which is then hydrolysed to 5-chlororibonate 16 during studies exploring the biosynthesis of salinosporamide A 11. 21

It was an objective in this study to explore the function of fdrC. Over-expression of a codon-optimized synthetic gene for fdrC in *E. coli*, with a His₆ tag and a TEV protease cleavage site added, allowed isolation and purification of the coded protein. The resultant FdC appeared on SDS-page with an estimated mol. wt ~31 kDa (Fig. S2†). Incubations of the recombinant enzyme with 5-FDR 8 and NAD⁺ or NADP⁺, were followed by 19F NMR spectroscopy. When the assay was conducted in the absence of NAD⁺ or in the presence of NADP⁺, there was no turnover (Fig. 3A and B, respectively). Reactions with NAD⁺ however resulted in an efficient conversion of 5-FDR 8 to a new organofluorine compound (19F-NMR; –233.15 ppm, dt, J_{HF} = 25 Hz, J_{HF} = 47 Hz) demonstrating that FdC is a NAD⁺ dependent enzyme (Fig. 3C). Given the similarity of this pathway to that in salinosporamide-A 11, it was anticipated that the enzymatic product might be carboxylic acid 10. This would arise by oxidation of 5-FDR 8 to lactone 5-FRL 9 and then hydrolysis to the ring opened carboxylic acid 5-FHPA 10. The identity of 5-FHPA 10 in the FdC reaction mixture was confirmed by comparison with a synthetic sample. A synthesis of 5-FHPA 10 was carried out following the protocol22 illustrated in Scheme 3.

The 2′- and 3′-hydroxyl groups of D-ribose 18 were protected by preparation of acetonide 19. 22 Oxidation of the anomic alcohol to give lactone 20 was efficiently accomplished using iodine in DCM in the presence of potassium carbonate, 23 and then fluorination with Deoxofluor® afforded fluorolactone 21 in good yield. 24 Acetonide hydrolysis using TFA in water gave 5-FRL 9 and finally hydrolysis was accomplished with aqueous LiOH to give 10. This synthetic 5-FHPA 10 was found to be identical by 19F-NMR to the enzyme (FdC) reaction product.

The 19F-NMR signals became coincident when a sample of the enzymatic product was spiked into the supernatant of the *Streptomyces* sp. MA37 fermentation (Fig. 4C). The synthetic reference of 5-FHPA 10 was also used to confirm this species as

---

**Fig. 2** Organisation and proposed functions of the encoded proteins of the identified fluorometabolite gene cluster (8.55 kb; accession no. LN612605) predicted to direct the biosynthesis of 5-FHPA 10 in *Streptomyces* sp. MA37.

**Scheme 3** Reagents and conditions: (a) Acetone, conc. H₂SO₄ (cat.), 2 h, 24%; (b) I₂, K₂CO₃, DCM, 2 h, 97%; (c) Deoxo-fluor®, DCM, 40 °C, 30 min, 52%; (d) TFA, water, 5 h, 96%; (e) LiOH, water, 3 day, quant.
a component of the product mixture from *Streptomyces* sp. MA37. This was achieved by global persilylation of the components of the CFE using N-methyl-N-(trimethylsilyl)-tri-fluoroacetamide (MSTFA). The extract was then analysed by GC-MS (Fig. 3 and S3†). Comparison with the persilylated derivative of synthetic 5-FHPA 10 revealed a constituent of the CFE with an identical fragmentation pattern and retention time (Fig. S3†). Therefore these studies are consistent with 5-FHPA 10 as the product of FdrC enzyme, and as an end product in fluorometabolite biosynthesis in *Streptomyces* sp. MA37.

Incubation of synthetic 5-FRL 9 with FdrC (Fig. 3D) resulted in its complete conversion to 10, suggesting lactone hydrolysis is also catalysed by the enzyme. Two step enzymatic catalysis is probably also the case for SalM (conversion of 15 to 16) on the salinosporamide A 11 pathway.25

A study of the reaction kinetics for FdrC mediated NAD⁺ oxidation indicates that FdrC has a higher affinity for D-ribose over 5-FDR 8 as measured by *Kₘ*, but overall both riboses oxidise with a similar efficiency (kcat/Km) of less than 0.4 μM⁻¹ min⁻¹ (Table 1 and Fig. S4†). This study identifies 10 as a novel fluorometabolite and extends the very small collection of this rare class of natural products. Organic chemists have only identified five unique fluorine containing natural products so far.24 The pathway to 10 branches from that already established for FAC 1 and 4-FT 2 biosynthesis. The branch point occurs at 5-FDRP 5, whereby phosphorolysis generates 5-FDR 8. This sugar is then oxidised by FdrC to 5-FRL 9, which undergoes hydrolysis to generate 5-FHPA 10 (Scheme 4).

We finish with a commentary on the genes that surround *fdr*A-C in the gene cluster (Fig. 2). The *fdr* cluster was found located in a different scaffold of the draft genome assembly of the MA37 strain, indicating that it is physically remote to the *fl*

| Vmax  | Km  | kcat | Specificity constant (kcat/Km) |
|-------|-----|------|-------------------------------|
| 5-FDR | 4.96±0.25 | 2.73±0.73 | 0.58 | 0.21 |
| D-ribose | 2.73±0.05 | 0.84±0.14 | 0.32 | 0.38 |

Table 1  Kinetic data of FdrC mediated oxidation using 5-FDR 8 and D-ribose 18 as substrates, respectively.

Fig. 4 ¹⁹F{¹H}-NMR spectroscopic analyses of (insert) fluorometabolites in the supernatant of the culture medium from *Streptomyces* sp. MA37. (A) the ¹⁹F NMR expansion showing two ¹⁹F NMR signals of 4-FT 2 and 5-FHPA 10; (B) the FdrC enzymatic reaction after overnight incubation with NAD⁺ and 5-FDR 8; (C) co-addition of the A and B samples showing the identical chemical shifts; (D) co-addition of synthetic 5-FHPA 10 and sample A with (¹H) couplings; (E) co-addition of synthetic 5-FHPA 10 and sample A with (¹H) decoupling.

Scheme 4 The proposed fluorometabolite pathway toward the synthesis of 5-FHPA 10 from SAM 3 in *Streptomyces* sp. MA37. Bracket: 9, although synthesized, is an unstable intermediate in the aqueous solution and was not observed in the supernatant of the MA37 fermentation during the ¹⁹F NMR analysis.
steps and structure of the other fluorometabolites on this branch pathway.

Conclusions

In conclusion, 5-FHPA 10 is identified as a new fluorometabolite branching from the established fluorinase pathway to FAc 1 and 4-FT 2. In silico analysis enabled identification of a biosynthetically relevant gene cluster. In vitro assay of over-expressed FdrC demonstrated that it can oxidise (NAD⁺ dependent) 5-FDR 8 to its corresponding 5-FRL 9 followed by hydrolysis to generate 5-FHPA 10. FdrC was similarly active with D-ribose. GC-MS analysis and correlation of synthetic or enzymatically prepared 5-FHPA 10 with the product of the supernatant of Streptomyces sp. MA37 indicated identical products, demonstrating that 5-FHPA 10 is a new natural product of Streptomyces sp. MA37. This is the first secure identification of a new fluorinated natural product since 1998.26–29

Experimental section

19F-NMR analysis

The samples from enzyme reaction and the supernatants of Streptomyces sp. MA37 fermentation were subjected to 19F-NMR analysis. The 19F-NMR spectra were recorded with and without proton decoupling on a Bruker AV-500 MHz instrument (19F at 470.3 MHz). The chemical shifts of 19F-NMR were calculated with respect to CFCl3.

Cell-free extraction (CFE) reaction

The cells of Streptomyces sp. MA 37 was harvested by centrifugation (13 000 rpm × 20 min) after 8 day fermentation. The cell pellets were washed three times using Tris–HCl buffer (20 mM, pH 7.5) supplemented with 10 mM MgCl2 to remove remnant culture media. The cells were re-suspended in the same buffer (0.1 g wet-cell weight per mL). The cells were disrupted by ultrasonication (60% duty cycle for 30–60 s). Cell debris was removed by centrifugation (13 000 rpm, 30 min) and the resultant clear supernatant was used as the cell free extract for incubation experiments. The cell free extracts (1 mL) were supplemented with or without 5-FDR at 37 °C for 6 hours. At the end of the incubation period, protein was precipitated by heating the vial to 90 °C for 3 min and the protein was then removed by centrifugation. The supernatant was collected for 19F-NMR analysis.

Plasmid construction, over-expression and purification of Streptomyces sp. MA 37 FdRC enzyme

The plasmid encoding the chemically synthesised and codon-optimised fdrC gene was provided by the commercial supplier DNA 2.0 (CA, USA). AGGAGGTAAACAT was incorporated as the ribosome binding site (RBS). T7 promoter and kanamycin-resistance gene were incorporated. A His-tag containing peptide (Met-Ser-Tyr3-His6-Asp-Tyr-Asp-Ile-Pro-Thr2) was fused to the N-terminus of the enzyme. A TEV proteinase cleavage site (Pro-Val-Phe-Ser-Gly) was engineered into the synthetic gene to enable cleavage of the His-tag. Two restriction sites of NcoI and EcoRI were designed to locate upstream and downstream of the fdrC gene, respectively. The plasmid was transformed using the heat shock method. A mixture of chemically competent bacteria and plasmid DNA was placed at 42 °C for 90 s and then placed back on ice. E. coli BL21(DE3) Gold cells, transformed with the synthetic fdrC plasmids were grown in Lysogeny broth containing 50 ng mL⁻¹ kanamycin at 37 °C until cell density reached an absorbance at ~0.6 at 600 nm. The culture was then cooled on ice for 30 min. A final concentration of 0.3 mM isopropylthiogalactoside (IPTG) was added as induction procedure for FdrC over-expression. The incubation was continued at 25 °C for ~24 h. The resultant E. coli cells were collected and lysed. After centrifugation, the cell lysate was applied onto a bench-top column packed with Ni²⁺-charged His-Bind resin (Qiagen) for protein purification. Recombinant protein bound on the resin was firstly washed with a solution of Tris–HCl (20 mM, pH 8.0), imidazole (20 mM) and NaCl (0.5 M) buffer, followed by a further wash with a solution of Tris–HCl (20 mM, pH 8.0), imidazole (50 mM) and NaCl (0.5 M). The protein was eventually eluted with a solution of Tris–HCl (20 mM, pH 8.0), imidazole (400 mM) and NaCl (0.5 M). The protein concentration was measured by OD280 nm (Nanodrop). The extinction coefficient was determined using the ExPasy ProtParam tool. The identity of the protein was confirmed by both polyacrylamide gel electrophoresis and ESI-MS analysis (Fig. S2†).

ESIMS analysis of FdRC enzyme reaction

ESI-FT-MS was used to determine the identity of enzymatic products in the FdRc-mediated reactions. Reaction mixtures consisted of FdRC (0.625 mg mL⁻¹), NAD⁺ (5 mM) and MgCl2 (10 mM) in the presence of either 5-FDR (10 mM) or D-ribose (10 mM) in the Tris–HCl buffer (1 mL, 25 mM, pH 7.8). The reactions were conducted overnight at 37 °C. Protein was removed by heating the vial to 90 °C for 3 min, followed by centrifugation (133 000 rpm, 3 min). The supernatant was subjected for ESI-FT-MS analysis.

Enzyme kinetics measurements for FdRC

A UV-Vis spectroscopy-based assay was employed to monitor the enzyme activity of FdRC. The enzyme reactions were initiated by mixing FdRC (final concentration was 0.26 mg mL⁻¹) with either 5-FDR or D-ribose, supplemented with 1 mM magnesium chloride and 2.5 mM nicotinamide adenine dinucleotide (NAD⁺) in the Tris–HCl buffer (25 mM, pH 7.8). The concentrations of the substrates used in the assay ranged from 0.5 ìM to 200 ìM. After mixing with the substrate, the UV spectra were recorded immediately, scanning from 300 nm to 450 nm every 0.2 minutes for 3 min at ambient temperature. An increase in absorbance at 340 nm was attributed to the production of NADH. Initial FdRC reaction velocities were plotted against substrate concentration and a Michaelis–Menten plot was established (see Fig. S4†). Enzyme kinetics parameters Vmax, Km, and specificity constant were all calculated accordingly and are listed in Table 1.
Acknowledgements

DOH thanks EPSRC and the ERC for financial support. He also acknowledges the Royal Society for a Wolfson Merit Award. KK and HD thank the Leverhulme Trust; Royal Society Africa Award (AA090088).

Notes and references

1 D. O'Hagan, J. Fluorine Chem., 2010, 131, 1071–1081.
2 V. A. Brunet and D. O'Hagan, Angew. Chem., Int. Ed., 2008, 47, 1179–1182.
3 (a) H. Deng and D. O'Hagan, Chem. Rev., 2014, DOI: 10.1021/cr500209t; (b) H. Deng, D. O'Hagan and C. Schaffrath, Nat. Prod. Rep., 2004, 21, 773–784; (c) C. D. Murphy, Nat. Prod. Rep., 2006, 23, 147–152.
4 D. O'Hagan, R. Perry, J. M. Lock, J. J. M. Meyer, L. Dasaradhi, J. T. G. Hamilton and D. B. Harper, Phytochemistry, 1993, 33, 1043–1046.
5 M. Sanada, T. Miyano, S. Iwadare, J. M. Williamson, B. H. J. Arison, L. Smith, A. W. Douglas, J. M. Liesch and E. Inamine, J. Antibiot., 1986, 39, 259–265.
6 H. Deng and D. O'Hagan, Curr. Opin. Chem. Biol., 2008, 12, 582–592.
7 (a) D. O'Hagan, C. Schaffrath, S. L. Cobb, J. T. G. Hamilton and C. D. Murphy, Nature, 2002, 416, 279; (b) C. Schaffrath, H. Deng and D. O'Hagan, FEBS Lett., 2003, 547, 111–114.
8 S. L. Cobb, H. Deng, J. T. G. Hamilton, R. P. McGlinchey and D. O'Hagan, Chem. Commun., 2004, 592–593.
9 M. Onega, R. P. McGlinchey, H. Deng, J. T. G. Hamilton and D. O'Hagan, Bioorg. Chem., 2007, 35, 375–385.
10 S. J. Moss, C. D. Murphy, D. O'Hagan, C. Schaffrath, J. T. G. Hamilton, W. C. McRoberts and D. B. Harper, Chem. Commun., 2000, 2281–2282.
11 C. D. Murphy, S. J. Moss and D. O'Hagan, Appl. Environ. Microbiol., 2001, 67, 4919–4921.
12 (a) C. Murphy, D. O’Hagan and C. Schaffrath, Angew. Chem., Int. Ed., 2001, 4479–4481; (b) H. Deng, S. M. Cross, R. P. McGlinchey, J. T. G. Hamilton and D. O’Hagan, Chem. Biol., 2008, 15, 1268–1276.
13 H. Deng, L. Ma, N. Bandaranayaka, Z. Qin, G. Mann, K. Kyereme, Y. Yu, T. Shepherd, J. H. Naismith and D. O’Hagan, ChemBioChem, 2014, 15, 364–368.
14 Y. Wang, Z. Deng and X. Qu, F1000Research, 2014, 61, 1−14.
15 S. Huang, L. Ma, M. H. Tong, Y. Yu, D. O’Hagan and H. Deng, Org. Biomol. Chem., 2014, 12, 4828–4831.
16 S. L. Cobb, H. Deng, J. T. G. Hamilton, R. P. McGlinchey, D. O’Hagan and C. Schaffrath, Bioorg. Chem., 2005, 33, 393–401.
17 A. S. Eustáquio, R. P. McGlinchey, Y. Liu, C. Hazzard, L. L. Beer, G. Florova, M. Alhamadsheh, A. Lechner, A. J. Kale, Y. Kobayashi, K. Reynolds and B. S. Moore, Proc. Natl. Acad. Sci. U. S. A., 2009, 106, 12295–12300.
18 W. Fenical, P. R. Jensen, M. Palladino, K. S. Lam, G. K. Lloyd and B. C. Potts, Bioorg. Med. Chem., 2009, 17, 2175–2180.
19 A. S. Eustáquio, F. Pojer, J. P. Noel and B. S. Moore, Nat. Chem. Biol., 2008, 4, 69–74.
20 A. S. Eustáquio, D. O’Hagan and B. S. Moore, J. Nat. Prod., 2010, 73, 378–382.
21 A. J. Kale, R. P. McGlinchey and B. S. Moore, J. Biol. Chem., 2010, 285, 33710–33717.
22 Y. H. Jin and C. K. Chu, Tetrahedron Lett., 2002, 43, 4141–4143.
23 M. B. Fusaro, V. Chagnault, S. Josse and D. Postel, Tetrahedron, 2013, 69, 5880–5883.
24 P. Nasomjai, D. O’Hagan and A. M. Z. Slawin, Beilstein J. Org. Chem., 2009, 5, A37.
25 A. Lechner, A. S. Eustáquio, T. A. M. Gulder, M. Hafner and B. S. Moore, Chem. Biol., 2011, 18, 1527–1536.
26 Recently a fluoroaromatic was claimed as a new metabolite from a Streptomyces isolate,27 however it was subsequently shown to be an insecure identification.28 Also some derivatives of 5-fluorouracil were claimed29 as isolates from a sponge in the South China Sea, however these most likely arise from industrial contamination and then bio-accumulation. The last bona fide fluorometabolites to be identified are derivatives of ω-fluoro-oleate from seeds of the West African plant Dichapetalum toxicarium.30
27 N. Jaivel, C. Uvarani, R. Rajesh, D. Velmurugan and P. Marimuthu, J. Nat. Prod., 2014, 77, 2–8.
28 M. S. Ayoub, D. B. Cordes, A. M. Z. Slawin and D. O’Hagan, J. Nat. Prod., 2014, 77, 1249–1251.
29 X. H. Xu, G. M. Yao, Y. M. Li, C. J. Lin, X. Wang and C. H. Kong, J. Nat. Prod., 2003, 66, 283–288.
30 W. W. Christie, J. T. G. Hamilton and D. B. Harper, Chem. Phys. Lipids, 1998, 97, 41–47.