

Incorporation of Fluoroacetate into an Aromatic Polyketide and Its Influence on the Mode of Cyclization**

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The introduction of fluorine into organic molecules can substantially modulate their physicochemical properties, such as lipophilicity and basicity, as well as slow down their metabolic degradation. This effect has been exploited by the pharmaceutical industry because fluorinated compounds frequently show enhanced binding affinity to target proteins. Well over 20% of current drugs in clinical trials contain fluorine, thus demonstrating the importance of this modification.^[1] In contrast, there are only a handful of natural products that contain fluorine,^[2,3] and this has been attributed to two factors: the inherent low solubility of mineral fluorine, which means only a small fraction of the total mineral fluoride is available to organisms, and the high redox potential required for the oxidation of fluoride probably prevents it from being activated through a similar mechanism to that used by enzymes to effect chlorination.^[4]

The most abundant naturally occurring fluorinated compound is fluoroacetate, which is found in the leaves and seeds of some plants.^[2,3,5] Despite much interest in the biosynthesis of fluoroacetate little progress was made in elucidating the biosynthesis of fluorinated compounds by higher plants.^[2,3] Only two species of bacteria are known to produce fluorine-containing metabolites. Nucleocidin was originally isolated from *Streptomyces calvus* in 1956^[6,7] and showed modest antibiotic properties. Unfortunately, more recent attempts to isolate the compound from this species have failed, and hence, prevented investigation of the biosynthetic route to this interesting natural product.^[7] The discovery in 1986 that *Streptomyces cattleya* was capable of producing both fluoroacetate and 4-fluorothreonine provided a more accessible system for the study of the mechanism of biological fluorination.^[8] In 2002 the group of O'Hagan isolated the first

enzyme capable of incorporating fluoride into an organic molecule.^[9] The fluorinase was shown to catalyze the attack of fluoride on *S*-adenosylmethionine (SAM) to yield 5'-fluoro-5'-deoxyadenosine (5'-FDA). The gene encoding the fluorinase was identified and successful heterologous expression in *E. coli* led to the crystallization of the protein.^[10] The structure of the fluorinase supported a simple S_N2 attack on SAM. Very recently our group has found the gene cluster for fluorometabolite biosynthesis in *Streptomyces cattleya*.^[11] This discovery potentially opens the way to engineering new fluorinated compounds.

Acetate is the basic building block for the formation of many interesting natural products, such as polyketides,^[12] therefore we decided to investigate methods to incorporate fluoroacetate into such compounds. Indeed some of the tropical plants that produce fluoroacetate also produce fluoro fatty acids,^[2] suggesting that fluoroacetate can be activated to fluoroacetyl-CoA (**9**), and that it is taken as a substrate by the fatty acid synthase of the plant. To investigate the possibility of making fluorinated polyketides from fluoroacetate we used the type II polyketide synthase (PKS) from *Streptomyces coelicolor* that is responsible for forming actinorhodin (**8**) (Scheme 1 A).^[13] This PKS was chosen because its mode of cyclization is well characterized.^[14,15] If fluoroacetate were incorporated then the mode of cyclization of the polyketide chain might be affected.

The carbon backbone of actinorhodin (**8**), which is composed of 16 carbon atoms, is formed from eight malonyl-CoA (**1**) molecules by using an acyl carrier protein (ACP; actI-ORF3 (open reading frame 3)) and a keto synthase complexed to a chain length factor complex (KS-CLF, also referred to as KS α -KS β ; actI-ORF1, ORF2). The malonyl group of malonyl-CoA (**1**) has been shown, in vitro, to be loaded onto the ACP either by malonyl-CoA:ACP transacylase (MCAT, from the fatty acid synthase found in *S. coelicolor*) or catalyzed directly by the ACP itself.^[14,16–18] The starter acetyl group is formed by decarboxylation of malonyl-ACP. The resulting acetyl unit is then transferred onto the KS. Seven rounds of condensation with malonyl ACP produces the octaketide which is tailored to actinorhodin by a ketoreductase (ActIII), a cyclase (ActIV), an aromatase (ActVIII), and three other enzymes whose function has so far not been fully characterized (Scheme 1 A). If these tailoring enzymes are omitted then the shunt products SEK4 (**6**) and SEK4b (**7**) are produced by what has been named the actinorhodin minimal PKS (*act*-minimal PKS; consisting of MCAT, ACP, KS-CLF).^[18,19] Since the minimal PKS produces mainly these two compounds it has been proposed, based on the crystal structure, that the KS-CLF catalyzes the first ring cyclization between C7 and C12 to produce SEK4. Alter-

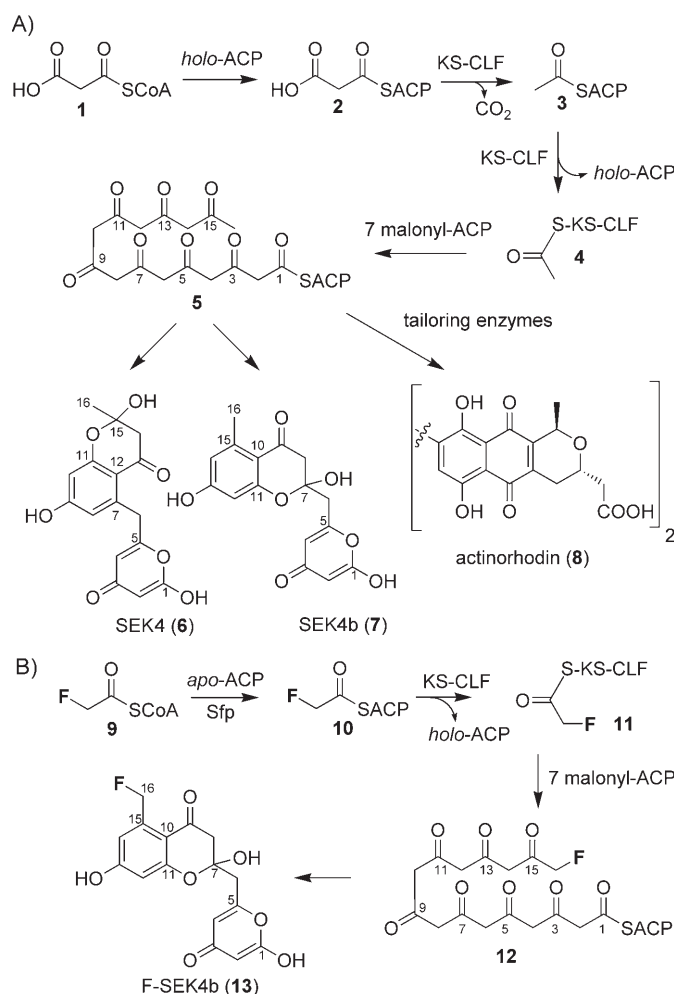
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natively the oxygen atom of an enol at the C11 ketone attacks C7, which then eventually leads to SEK4b (Scheme 1 A).^[20] To load the minimal PKS with a fluoroacetate starter unit, we took advantage of the surfactin synthetase-activating enzyme Sfp from *Bacillus subtilis*, which is a phosphopantetheinyl transferase shown to have broad specificity. This enzyme is well documented to not only transfer the 4'-phosphopantetheinyl moiety of coenzyme A (CoA) to a conserved serine residue in ACPs and PCPs (peptidyl carrier proteins), but also to accept a wide range of CoA derivatives, such as acetyl-CoA and malonyl-CoA (**1**).^[21,22] We synthesized fluoroacetyl-CoA (**9**)^[11] and found that it could act as a substrate for Sfp and *apo*-ACP (ACP without phosphopantetheine prosthetic group) to yield fluoroacetyl-ACP (**10**; Figure 1).

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KS-CLF, MCAT, and malonyl-CoA (**1**) were added to the fluoroacetyl-ACP (**10**) and the mixture was incubated at 30 °C for 4 hours. Formation of the products was monitored by LC-UV-MS analysis. LC-UV-MS analysis revealed the formation of SEK4 (**6**) with a $[(M-H_2O)+H]^+$ ion at m/z 301 and SEK4b (**7**) with a $[M+H]^+$ ion at m/z 319, as well as a new compound exhibiting a $[M+H]^+$ ion at m/z 337 that eluted later from the column (Figure 2). High-resolution mass analysis on this new compound (**13**) gave a molecular weight of 337.0712 ($[M+H]^+$), consistent

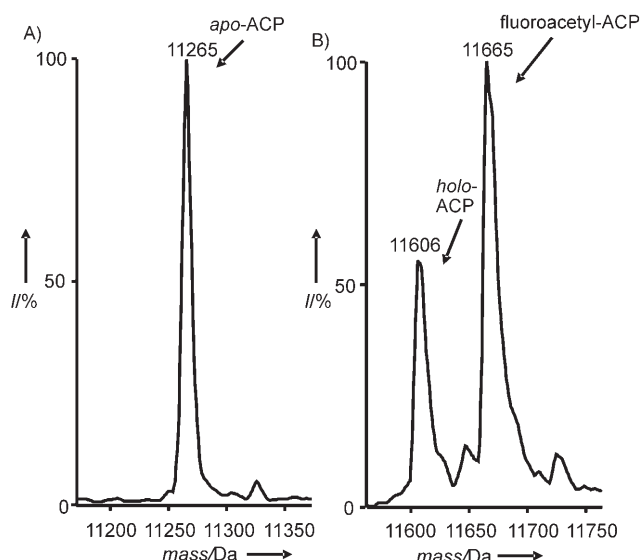


Figure 1. Generation of fluoroacetyl-ACP (**10**) from fluoroacetyl-CoA (**9**), *apo*-ACP, and Sfp. A) *apo*-ACP; B) *holo*- and fluoroacetyl-ACP (**10**). The formation of *holo*-ACP is because of the free CoA generated from hydrolysis of fluoroacetyl-CoA (**9**).

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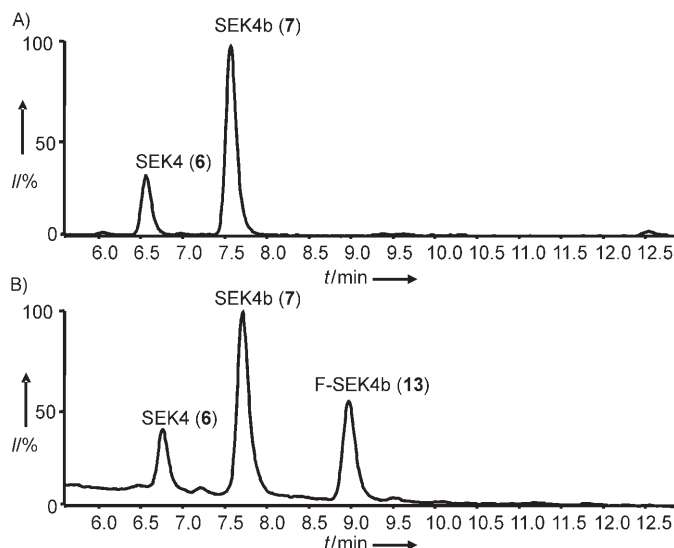


Figure 2. LC-UV trace of polyketide production monitored at 218 nm. A) In vitro assay with *holo*-ACP, KS-CLF, MCAT, and malonyl-CoA (**1**). SEK4 (**6**) and SEK4b (**7**) were produced as reported before.^[14,15] B) In vitro assay using fluoroacetyl-ACP, KS-CLF, MCAT, and malonyl-CoA (**1**). A fluorinated compound that eluted later off the column was observed.

with the molecular formula $C_{16}H_{13}O_7F$ ($[M+H]^+$: 337.0718). For the fluorinated compound, not only was the quasi molecular ion $[M+H]^+$ at m/z 337 clearly observed, but the ions at $[M+NH_4]^+$ at m/z 354 and $[M+Na]^+$ at m/z 359 were also found (Figure 3 A). The collision induced MS/MS analysis of the $[M+H]^+$ ion at m/z 337 produced the base peak of the fragment ion for the loss of HF at m/z 317, which additionally confirms the incorporation of fluorine into the molecule (Figure 3 B). The ratio of SEK4 (**6**) to SEK4b (**7**) and to the fluorinated compound was approximately 1:4:2 as judged by their UV peak areas. Careful examination of the LC-MS trace showed that only one fluorine-containing compound was produced, indicating that the fluorine atom might be able to influence the mode of cyclization of the octaketide intermediate.

To confirm that the observation of one fluorinated compound is a result of the influence of fluorine itself rather than some other experimental factors, we synthesized $CD_3^{13}COSCoA$, by using $CD_3^{13}COONa$, CoA, and acetyl-CoA ligase, and used it to replace fluoroacetyl-CoA (**9**) in the assay. Two peaks with the retention time corresponding to SEK4 (**6**) and SEK4b (**7**) were observed. Analysis of their ESI-MS spectra indicated that each peak contained both labeled and unlabeled products in similar ratios. As expected, the incorporation of labeled acetate in the starter unit shifted the mass of $[(M-H_2O)+H]^+$ ion from 301 amu to 305 amu for SEK4 (**6**), and the mass of the $[M+H]^+$ ion from 319 amu to 323 amu for SEK4b (**7**) (see the Supporting Information). Therefore, the labeling experiments suggest that it was the fluorine that affected the cyclization of the octaketide.

To rigorously determine the structure of the fluorinated compound we accumulated sufficient material for analysis by 1H NMR spectroscopy. The spectra revealed that the new compound had a structure very similar to that of SEK4b (**7**) (Figure 4 and Table 1, see the Supporting Information for 1H NMR data of SEK4 (**6**)).

The signals of the protons on C2, C4, C6, and C8 are almost unchanged. However, the C16 methyl signal in SEK4b (**7**) (singlet at $\delta = 2.55$ ppm) is not present in the spectra for the new compound (Figure 4 B), and the protons on C12 and C14 on the new compound were shifted downfield compared to their counterparts in SEK4b (**7**). The most significant signals in the new compound were the multiplets at around $\delta = 5.75$ ppm, which were not present in the spectrum of SEK4b (**7**). There were two sets of overlapping signals representing the diastereotopic C16 methylene protons in the new compound, with coupling constants of 49.1 and 15.5 Hz. The larger of the observed coupling constants is consistent with the introduction of fluorine at C16, which also explains the downfield shift of the protons on C14 and C12. Total correlation spectroscopy (TOCSY) experiments provided additional evidence of the connectivity between C12, C14, and C16. We therefore proposed the structure of the newly formed fluorinated compound as shown in Scheme 1 B, and named it fluoro-SEK4b (F-SEK4b, **13**).

It is very interesting that the incorporation of a fluorine atom has such a profound effect on the mode of cyclization, such that the formation of fluoro-SEK4F (SEK4) is completely prevented. The presence of the fluorine atom will

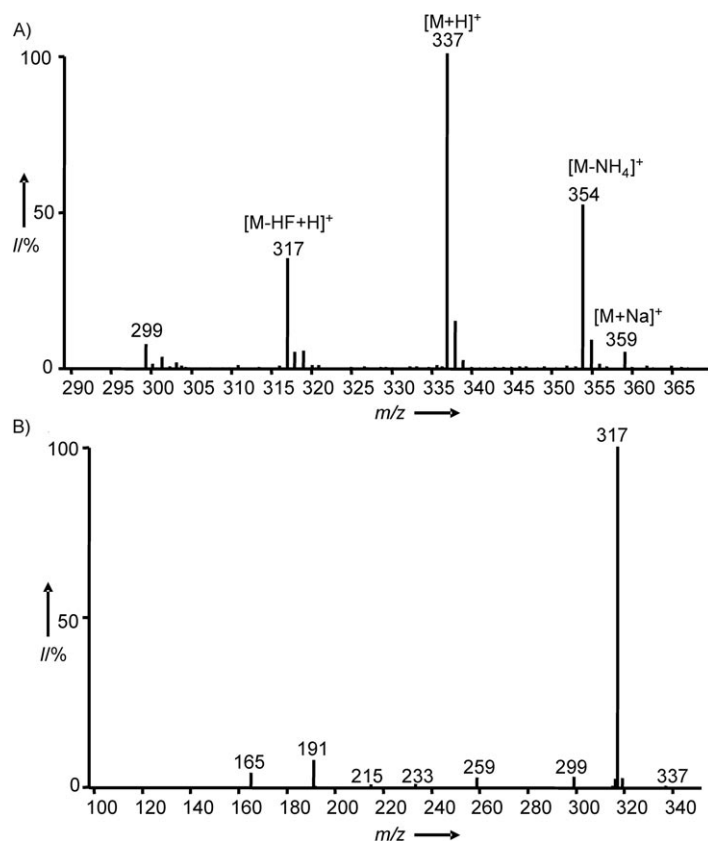


Figure 3. Mass spectrometric analysis of F-SEK4b (**13**). A) Full scan spectrum showing the quasi molecular ion $[M+H]^+$ at m/z 337, $[M+NH_4]^+$ ion at m/z 354 and $[M+Na]^+$ ion at m/z 359. B) MS/MS spectrum of $[M+H]^+$ at m/z 337, showing the major fragment peak at m/z 317 resulting from the loss of HF from the parent ion.

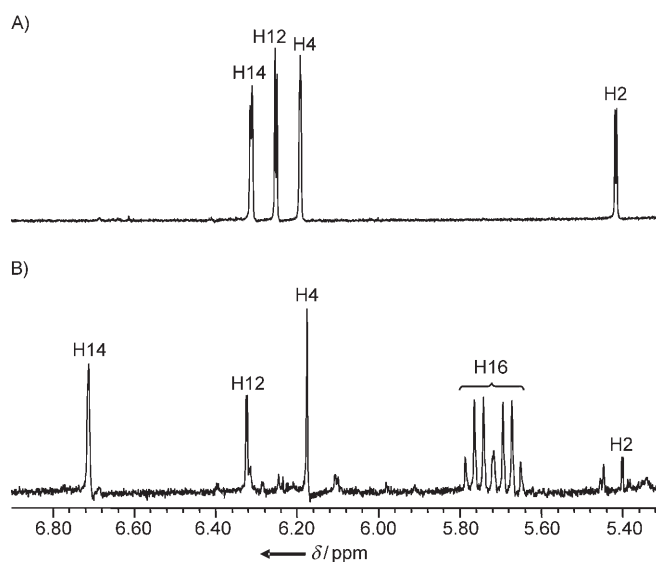


Figure 4. 1H NMR spectra of A) SEK4b (**7**) and B) fluoro-SEK4b (**13**) ($[D_4]CH_3OH$, 700 MHz, 300 K).

increase the electrophilicity of the C15 carbonyl group of the octaketide intermediate (**12**), possibly leading to the C10/C15 aldol condensation, which possibly out-competes the more

Table 1: ^1H NMR data for SEK4b (**7**) and F-SEK4b (**13**).^[a]

Carbon ^[b]	^1H NMR of 7 δ [ppm]	^1H NMR of 13 δ [ppm]
2	5.41 (d, $J = 2.1$ Hz, 0.5 H) ^[c]	5.40 (d, $J = 2.2$ Hz, 0.5 H) ^[c]
4	6.19 (s, 1 H) ^[c]	6.18 (s, 1 H) ^[c]
6	3.07 (d, $J = 14.4$ Hz, 1 H)	
3.13 (d, $J = 14.4$ Hz, 1 H)	3.07 (d, $J = 14.4$ Hz, 1 H)	
8	2.73 (d, $J = 16.1$ Hz, 1 H)	
3.04 (d, $J = 16.1$ Hz, 1 H)	2.72 (d, $J = 16.3$ Hz, 1 H)	
3.03 (d, $J = 16.3$ Hz, 1 H)		
12	6.25 (d, $J = 2.4$ Hz, 1 H)	6.32 (d, $J = 2.3$ Hz, 1 H)
14	6.31 (d, $J = 2.4$ Hz, 1 H)	6.71 (d, $J = 2.3$ Hz, 1 H)
16	2.55 (s, 3 H)	5.75 (m, $J = 15.5$, 49.1 Hz, 1 H)
5.68 (m, $J = 15.5$, 49.1 Hz, 1 H)		

[a] The ^1H NMR spectra were recorded in $[\text{D}_4]\text{CH}_3\text{OH}$ (700 MHz, 300 K). [b] Carbon atoms are labeled according to their numbers in the polyketide backbone (see Scheme 1). [c] It was observed that the proton on C2 exchanged with deuterium from $[\text{D}_4]\text{CH}_3\text{OH}$ to 50% within 30 min resulting in the proton at C4 appearing as a singlet. When using $[\text{D}_3]\text{CH}_3\text{CN}$ as solvent, the integration of the proton on C2 is one, and the coupling to the proton on C4 is observed as a doublet with a coupling constant of 2.1 Hz for SEK4b (**7**).

commonly observed C7/C12 reaction to generate SEK4-type compounds. Recently, the crystal structure of KS-CLF was reported and the site in which the polyketide elongation takes place was identified as an 17 Å amphipathic tunnel at the heterodimer interface.^[20] The authors postulate that a water molecule (WAT1), which binds to the backbone amides of Trp 135' and Phe 136', might play a catalytic role in the cyclization. WAT1 lies in an oxyanion hole at the mid-point of the tunnel and is therefore in the correct position to act as a general acid to assist the C7/C12 aldol condensation. Alternatively it could also promote the formation of SEK4b (**7**) by aiding the attack on the C7 carbonyl carbon center by the enolate oxygen atom at C11. If the mode of cyclization were determined solely in this manner, then the introduction of fluorine remote to this position should not have had such a dramatic influence. Computational modeling studies, also reported in the same paper, predicted that the longest linear chain that could fit into the tunnel while covalently attached to the KS was a heptaketide. These data led to the suggestion that the mature octaketide chain could only fit into the tunnel by buckling. If this is the case then it seems possible that the increased reactivity of the starter unit containing fluorine might promote cyclization before the chain is fully matured, and direct product formation to F-SEK4b (**13**). The fact that the introduction of fluorine can influence the mode of cyclization raises the possibility that new compounds could be made if fluorine can be successfully incorporated during biosynthesis of other aromatic polyketides.

In conclusion we have demonstrated for the first time that a fluorinated polyketide can be formed by using fluoroacetate as a starter unit. The presence of fluorine in the growing polyketide was shown to profoundly affect the mode of cyclization, possibly indicating that it might be another useful tool for combinatorial biochemistry.

Experimental Section

Preparation of labeled acetyl-CoA ($\text{CD}_3^{13}\text{COSCoA}$): $\text{CD}_3^{13}\text{COONa}$ (13 mg; Amersham, UK), free CoA (10 mg), ATP (66 mg), and MgCl_2 (120 μL of a 1 M solution) were added to acetyl-CoA ligase (500 μg ; Sigma) in 1 mL tris buffer (125 mM, pH 7.5). The mixture was incubated at 30°C for 1 h and then chloroform was added to precipitate the protein. The resulting supernatant was analyzed by the LC-MS first, and subsequently purified by HPLC using a semipreparative column (Phenomenex Prodigy C18, 250 mm $\times$ 10 mm, 10 μm) with a gradient of 5% B to 25% B over 20 min, where A was H_2O with 0.1% TFA and B was acetonitrile with 0.1% TFA.

ESI-MS data: acetyl-CoA, $[M+H]^+$ 810; labeled acetyl-CoA, $[M+H]^+$ 814.

Preparation of fluoroacetyl-CoA (**9**): Fluoroacetyl-CoA was synthesized as previously described.^[11]

Expression and purification of actinorhodin minimal PKSs: KS-CLF, MCAT, and *holo*-ACP were expressed and purified as previously described.^[17] *apo*-ACP was expressed as His₆-tag protein in *E. coli* BL21.

Reaction conditions with minimal PKS to produce SEK4 and SEK4b: The reaction mixture contained malonyl-CoA (**1**) (1 mM), *holo*-ACP (50 μM), MCAT (1.5 μM) and KS-CLF (6 μM) in a total volume of 100 μL of 200 mM potassium phosphate buffer (pH 7.4, 10% glycerol, 1 mM EDTA, 1 mM DTT). After a 2 h incubation at 30°C, HCl (7 μL of 6 N) was added to precipitate the proteins. The sample was centrifuged and the supernatant was directly subjected to LC-MS/MS analysis.

Reaction conditions with fluoroacetyl-CoA or labeled acetyl-CoA: Fluoroacetyl-CoA (2 μL of 100 mM) or labeled acetyl-CoA, MgCl_2 (2 μL of 0.1 M), Sfp (5 μL of 188 μM),^[22] and *apo*-ACP (1 μL of 16.5 mM) were added to 10 μL of 400 mM potassium phosphate buffer (pH 7.4). The mixture was incubated at 30°C for 5 min, and then 31 μL of 400 mM potassium phosphate buffer (pH 7.4) and 20 μL of 30 μM KS-CLF were added. After incubation at 30°C for 10 min, MCAT (3 μL of 50 μM) and malonyl-CoA (**1**) (1 μL of 100 mM) were added to the mixture. The final 100 μL mixture was incubated at 30°C for 4 h and then HCl (7 μL of 6 N) was added to precipitate the proteins. After centrifugation, the supernatant was directly subjected to LC-MS/MS analysis.

LC-MS/MS analysis of polyketide production from the enzyme reactions: LC-MS/MS analysis was carried out by using an Agilent HP1100 HPLC coupled to a Finnigan MAT LCQ mass spectrometer fitted with an ESI source. A Luna C18 column (Phenomenex, 250 $\times$ 4.6 mm, 5 μm) was used with a gradient elution of 25% B to 40% B over 25 min at a flow rate of 1 mL min⁻¹ where A was H_2O with 0.1% TFA and B was MeCN with 0.1% TFA. The mass spectrometer was run in positive ionization mode and scanned from 50 to 1000 m/z . Collision energy for MS/MS analysis was set to 22%.

Large scale preparation of F-SEK4b (**13**) and NMR analysis: A total of 40 individual 100 μL reactions were set up as described above. F-SEK4b (**13**) was purified from each 100 μL incubation by HPLC methods, combined, and lyophilized. The ^1H NMR spectrum was recorded with a Bruker Avance 700 MHz spectrometer that was equipped with a TXI cryoprobe.

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- [1] P. Kirsch, *Modern Fluoroorganic Chemistry, Synthesis, Reactivity, Applications*, Weinheim, **2004**; R. D. Chambers, *Fluorine in Organic Chemistry*, Blackwell Publishing, Oxford, **2004**.
- [2] C. D. Murphy, C. Schaffrath, D. O'Hagan, *Chemosphere* **2003**, 52, 455–461.
- [3] D. B. Harper, D. O'Hagan, *Nat. Prod. Rep.* **1994**, 11, 123–133.
- [4] H. Deng, D. O'Hagan, C. Schaffrath, *Nat. Prod. Rep.* **2004**, 21, 773–784; D. G. Fujimori, C. T. Walsh, *Curr. Opin. Chem. Biol.* **2007**, 11, 553–560; C. S. Neumann, D. G. Fujimori, C. T. Walsh, *Chem. Biol.* **2008**, 15, 99–109; F. H. Vaillancourt, E. Yeh, D. A. Vosburg, S. Garneau-Tsodikova, C. T. Walsh, *Chem. Rev.* **2006**, 106, 3364–3378.
- [5] N. Grobbelaar, J. J. M. Meyer, *J. Plant Physiol.* **1990**, 135, 550–553.
- [6] S. O. Thomas, V. L. Singleton, J. A. Lowery, L. M. Sharpe, L. M. Pruess, J. N. Porter, J. H. Mowat, N. Bohonos, *Antibiot. Annu.* **1956**, 4, 716–721; G. O. Morton, J. E. Lancaster, G. E. van Lear, W. Fulmor, W. E. Meyer, *J. Am. Chem. Soc.* **1969**, 91, 1535–1537.
- [7] D. O'Hagan, D. B. Harper, *J. Fluorine Chem.* **1999**, 100, 127–133.
- [8] M. Sanada, T. Miyano, S. Iwadare, J. M. Williamson, B. H. Arison, J. L. Smith, A. W. Douglas, J. M. Liesch, E. Inamine, *J. Antibiot.* **1986**, 39, 259–265.
- [9] D. O'Hagan, C. Schaffrath, S. L. Cobb, J. T. G. Hamilton, C. D. Murphy, *Nature* **2002**, 416, 279–279.
- [10] C. J. Dong, F. L. Huang, H. Deng, C. Schaffrath, J. B. Spencer, D. O'Hagan, J. H. Naismith, *Nature* **2004**, 427, 561–565.
- [11] F. L. Huang, S. F. Haydock, D. Spiteller, T. Mironenko, T. L. Li, D. O'Hagan, P. F. Leadlay, J. B. Spencer, *Chem. Biol.* **2006**, 13, 475–484.
- [12] J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **2001**, 18, 380–416; A. M. Hill, *Nat. Prod. Rep.* **2006**, 23, 256–320.
- [13] D. A. Hopwood, *Chem. Rev.* **1997**, 97, 2465–2497.
- [14] R. McDaniel, S. Ebert-Khosla, D. A. Hopwood, C. Khosla, *Science* **1993**, 262, 1546–1550; R. McDaniel, S. Ebert-Khosla, H. Fu, D. A. Hopwood, C. Khosla, *Proc. Natl. Acad. Sci. USA* **1994**, 91, 11542–11546.
- [15] T. P. Nicholson, C. Winfield, J. Westcott, J. Crosby, T. J. Simpson, R. J. Cox, *Chem. Commun.* **2003**, 686–687; C. Hertweck, A. Luzhetskyy, Y. Rebets, A. Bechthold, *Nat. Prod. Rep.* **2007**, 24, 162–190.
- [16] C. Bisang, P. F. Long, J. Cortes, J. Westcott, J. Crosby, A. L. Matharu, R. J. Cox, T. J. Simpson, J. Staunton, P. F. Leadlay, *Nature* **1999**, 401, 502–505; T. S. Hitchman, J. Crosby, K. J. Byrom, R. J. Cox, T. J. Simpson, *Chem. Biol.* **1998**, 5, 35–47.
- [17] A. L. Matharu, R. J. Cox, J. Crosby, K. J. Byrom, T. J. Simpson, *Chem. Biol.* **1998**, 5, 699–711.
- [18] J. Dreier, A. N. Shah, C. Khosla, *J. Biol. Chem.* **1999**, 274, 25108–25112.
- [19] H. Fu, S. Ebert-Khosla, D. A. Hopwood, C. Khosla, *J. Am. Chem. Soc.* **1994**, 116, 4166–4170; H. Fu, D. A. Hopwood, C. Khosla, *Chem. Biol.* **1994**, 1, 205–210.
- [20] A. Keatinge-Clay, D. A. Maltby, K. F. Medzihradszky, C. Khosla, R. M. Stroud, *Nat. Struct. Mol. Biol.* **2004**, 11, 888–893.
- [21] L. E. N. Quadri, P. H. Weinreb, M. Lei, M. M. Nakano, P. Zuber, C. T. Walsh, *Biochemistry* **1998**, 37, 1585–1595; H. D. Mootz, R. Finking, M. A. Marahiel, *J. Biol. Chem.* **2001**, 276, 37289–37298.
- [22] K. Reuter, M. R. Mofid, M. A. Marahiel, R. Ficner, *EMBO J.* **1999**, 18, 6823–6831.