Synthesis S. J. Mattingly et al. Paper

Synthesis of 2-Fluoroacetoacetic Acid and 4-Fluoro-3-hydroxybutyric Acid

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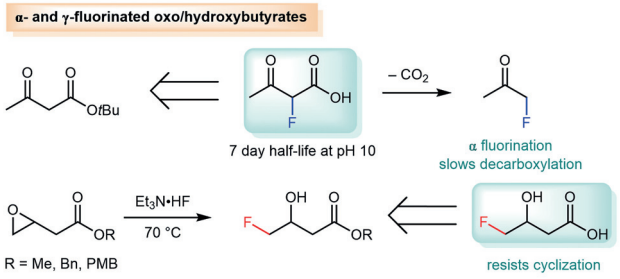

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Abstract The butyric acid scaffold is the base structure of several human metabolites that serve diverse and prominent biochemical roles including as oxidative sources of cellular energy and as substrates for biosynthesis. Derivatization of metabolites through incorporation of fluorine often alters bioactivity and can facilitate detection and analysis by nuclear magnetic resonance or positron emission tomography depending upon the fluorine isotope employed. We describe the synthesis of two new fluorinated butyric acids (and three related esters) that are derivatives of the metabolites acetoacetic acid and 3-hydroxybutyric acid. 4-Fluoro-3-hydroxybutyric acid is prepared from epoxy ester precursors via ring opening by triethylamine trihydrofluoride. 2-Fluoroacetoacetic acid is prepared by electrophilic fluorination of an acid-labile β-keto ester. The gradual pH-dependent decarboxylation of 2-fluoroacetoacetic acid is investigated by ^{19}F NMR spectroscopy.

Key words fluorination, ketone bodies, acetoacetate, acetoacetic acid, 3-oxobutanoic acid, 3-hydroxybutanoic acid, β-hydroxybutyrate

The strategic incorporation of fluorine into the structures of biologically active compounds often confers unique physiochemical properties and can favorably influence the pharmacokinetic profiles of medicinal compounds and alter the metabolism of biomolecules.[1] Consequently, there is growing interest in the development of fluorinated derivatives of small molecule metabolites for applications such as magnetic resonance spectroscopy (MRS), using the NMR-active and naturally abundant isotope ^{19}F, and positron emission tomography (PET), using the positron-emitting isotope ^{18}F.[2]

An important focus of our research is the investigation of cancer metabolism through the development and application of radiofluorinated metabolite derivatives for *in vivo* imaging by PET.[2c,3] Due to the intrinsic limitations of radioisotope production, PET radiotracers are prepared in extremely small quantities and are difficult to characterize by standard analytical techniques. A radiotracer's identity is typically confirmed through comparison of its chromatographic behavior against a chemically identical nonradioactive standard. Often the stable isotope standards are themselves new compounds and require synthetic method development. Here we describe our synthetic approach to the preparation of five novel fluorinated derivatives of butyric acid to lay the groundwork for translation of this research to radiochemistry.

Oxidized forms of butyric acid serve as the base structure of several human metabolites from diverse biochemical pathways including the tricarboxylic acid (TCA) cycle, amino acid catabolism, neurotransmitter synthesis, and ketone body metabolism. Consequently, this class of biomolecule represents a worthwhile target for applications related to bioimaging. We have focused on acetoacetic acid (**1**) and 3-hydroxybutyric acid (**2**), as they represent important energy substrates and biosynthetic precursors,[4] and have prepared novel fluorinated derivatives **3** and **4a–d** (Figure 1). Targeting these small polyfunctional structures through chemical modification of plausible precursors presented several synthetic challenges including rearrangement phenomena, sensitivity to acidic/basic pH and chromatography, and tendencies toward cyclization or decarboxylation. When fluorine is successfully incorporated, however, some of these labile properties are mitigated. For example, compound **3**, which was expected to rapidly decarboxylate, exhibited greater stability than its non-fluorinated counterpart **1**. Intrigued by this unanticipated result, we studied the rate of decarboxylation of **3** in aqueous solutions under various pH using ^{19}F NMR and discuss a rationale for the trend observed.

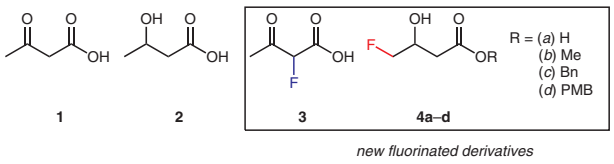
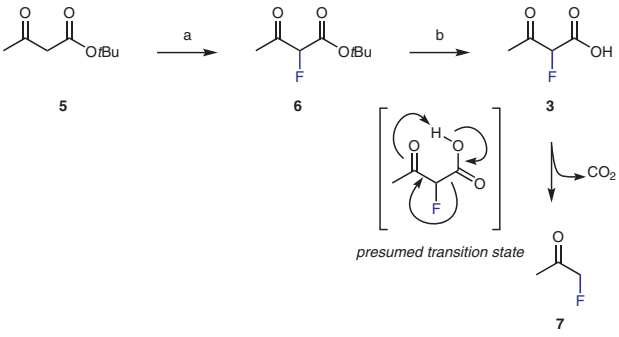


Figure 1 Acetoacetic acid (**1**), 3-hydroxybutyric acid (**2**), 2-fluoroacetoacetic acid (**3**), 4-fluoro-3-hydroxybutyric acid (**4a**) and esters (**4b–d**)

Ester derivatives of **3** are well known in the literature, therefore it is likely that the free acid has been prepared by other groups previously; however, no characterization data for this compound are described, nor is there a report of its stability. Halogenated β-keto acids are understood to spontaneously decarboxylate,[5] but it may be useful to study these short-lived species, particularly with regard to the fluorinated forms, since short lifetimes do not necessarily preclude imaging modalities like MRS and PET which operate on timescales of minutes or hours.

The ethyl ester of **3**, ethyl 2-fluoroacetoacetate, is an inexpensive and widely available derivative (see toxicity caution in the experimental section), but generation of free acid **3** from this ester is not facile. Base-mediated deprotection conditions generate an enolate anion which promotes undesired side reactions.[6] We found that *tert*-butyl ester **6** is a convenient precursor for preparing **3** (Scheme 1) as the *t*-butyl group is cleaved under acidic conditions. Ester **6** was prepared from acetoacetate *t*-butyl ester (**5**) using the electrophilic fluorination agent Selectfluor®.[7] Deprotection of **6** with TFA generated acid **3** which gradually decarboxylated to afford fluoroacetone **7**.



Scheme 1 *Reagents and conditions*: (a) Selectfluor®, MeCN, rt, 72 h, 71%; (b) TFA/CH_2Cl_2 (1:1), rt, 1 h, 86%.

Decarboxylation of β-keto acids is known to depend on solution pH. Consequently, we were interested to look at the decarboxylation rate of **3** in aqueous conditions over a range of pH (pH 3, 5, 7, and 10). The percentage of decarboxylated **3** was tracked over a time course using ^{19}F NMR by comparing the disappearance of the signal from the α-fluorine of **3** with the appearance of signals corresponding to the decomposition product, fluoroacetone **7**. Figure 2a provides example spectra from this kinetic experiment and illustrates the coupling of these signals (for all related spectra, see the Supporting Information). Here, the two signals related to fluoroacetone **7** are integrated together; the smaller of these signals (also present in the purchased authentic standard) we presume to be a hydrate form. The tabulated results for all pH conditions (Figure 2b) demonstrate that acidic conditions (pH 3) promoted rapid decarboxylation, as is the case for non-fluorinated β-keto acids.[8] Mildly acidic (pH 5) and neutral conditions (pH 7) exhibited similar gradual rates of decarboxylation; this represents a conveniently wide margin around physiological pH for potential biological applications. Basic conditions (pH 10) hindered the loss of CO_2 significantly. This trend can be explained on the basis that decarboxylation of β-keto acids is

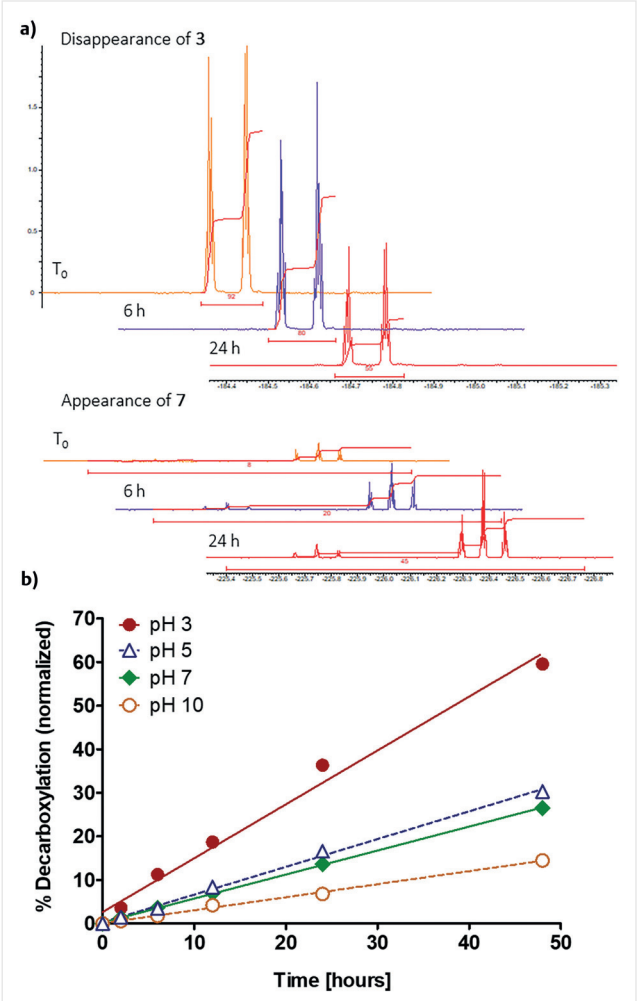


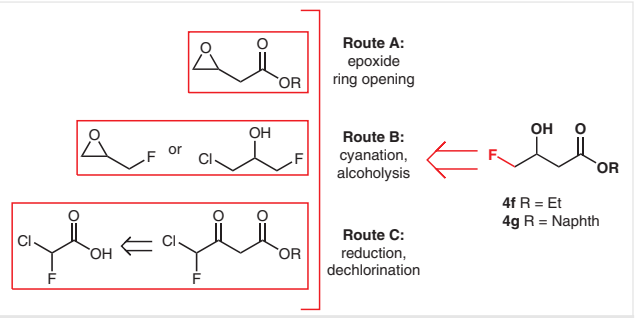
Figure 2 (a) Stacked ^{19}F NMR spectra illustrating reduction in signal intensity from compound **3** over 24 hours in pH 3 aq solution concurrent with increasing signal intensity from fluoroacetone **7**; x-axis: chemical shift (ppm), y-axis: fixed scale for all spectra (absolute intensity). (b) Decarboxylation of **3** in buffers of pH 3, 5, 7, and 10 over 48 h

understood to proceed most rapidly through a concerted mechanism (illustrated in Scheme 1) in which a protonated carboxyl donates its proton to the carbonyl oxygen via a formally uncharged cyclic six-membered transition state, ultimately resulting in an enol product and CO_2.[9] Under basic conditions (i.e., pH 10) decarboxylation of the carboxylate in anionic form proceeds slowly.[10]

A comparison of the half-lives of acetoacetic acid (**1**) and fluorinated derivative **3** gives an indication of the increase in stability attained through fluorination: the half-life of acetoacetic acid is reported to be 11.7 hours (aq conditions, 25 °C),[8] whereas for acid **3** the half-life is just under 40 hours, even under the most acidic conditions assessed. After 48 hours at pH 10, where the slowest rate of CO_2 loss was observed, **3** was only 14% decarboxylated. The greater resistance of **3** to decarboxylation as compared to **1** may be ascribable to the fact that α fluorination increases the acidity of carboxylic acid protons (e.g., pK_a acetic acid = 4.76, pK_a fluoroacetic acid = 2.59).[11] A lower pK_a for **3** would result in a smaller proportion of the free acid—the species capable of decarboxylation by the fast concerted mechanism—in solution. These results raise the question as to whether or not α fluorination of β-keto acids confers a unique stability among the halides, as is the case for acyl fluorides.[12] It also engenders the possibility of a shelf-stable silyl ester derivative of **3** for applications such as decarboxylative alkylations, which are challenging using classic β-keto acids.[13]

There is precedent in the literature for compounds similar to **4a–d** (Figure 1). For example, 4-fluoro-3-hydroxybutyrate ethyl and naphthyl esters (**4f** and **4g**) (Scheme 2) have been prepared previously.

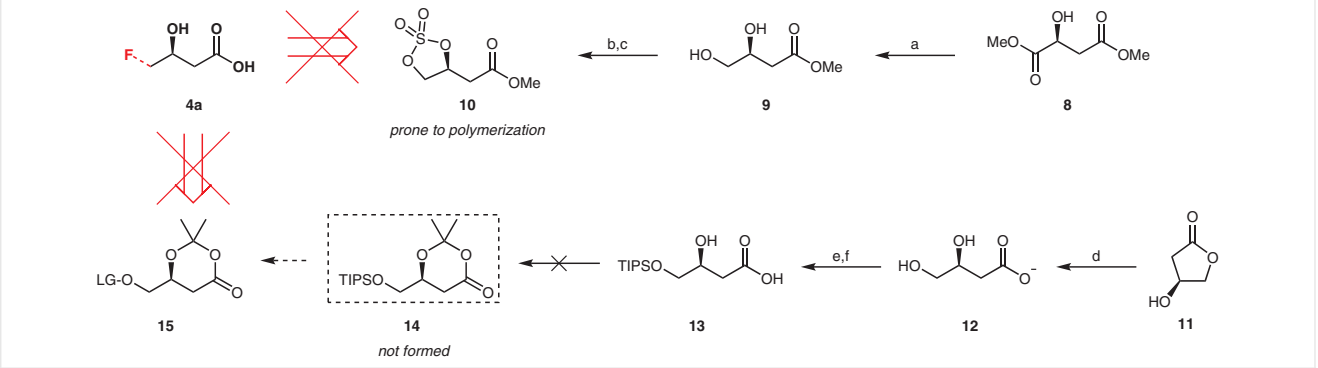
Graham et al. achieved the naphthyl ester **4g** through enantioselective epoxide ring opening (Route A, Scheme 2) using a (salen)Co catalyst; in this way they prepared both ^{19}F and ^{18}F versions of ester **4g** with high *ee* values.[14] This route relies on fluorination of an esterified precursor, but in other approaches fluorinated starting materials were esterified. Bergmann et al.[15] and later Liu and coworkers[16] used cyanation of, respectively, an epoxide or a geminal chlorofluoro dihalide (Route B) to generate a fluorohydrin-functionalized nitrile intermediate which was then converted into the ethyl ester **4f** by ethanolysis. A dihalide precursor was also employed in a synthesis of **4f** by Shibatomi et al. (route C);[17] a Claisen condensation of ethyl acetate with 2-chloro-2-fluoroacetic acid resulted in a β-keto dihalide that was then subjected to two reduction steps, a borohydride-mediated reduction of the keto moiety followed by reductive dehalogenation with tributyltin hydride to achieve the final fluorinated ester **4f**.



Scheme 2 Published routes for the preparation 4-fluoro-3-hydroxybutyrate ethyl and naphthyl esters

Given our outlook toward radiofluorination, we were interested to devise a route to **4a** that involved late-stage installation of a nucleophilic form of fluoride. We initially tested two synthetic routes that were unsuccessful but merit discussion; the first involved a cyclic sulfate precursor and the second, a cyclic dioxanone (compounds **10** and **15**, respectively) (Scheme 3).

Cyclic sulfates[18] (e.g., **10**) behave similarly to epoxides and react rapidly with nucleophilic fluoride, including [^{18}F]fluoride,[19] with moderate regioselectivity for primary over secondary carbons.[20] They are a dual-purpose functionality in that they simultaneously activate a carbon for nucleophilic substitution while protecting a hydroxy group



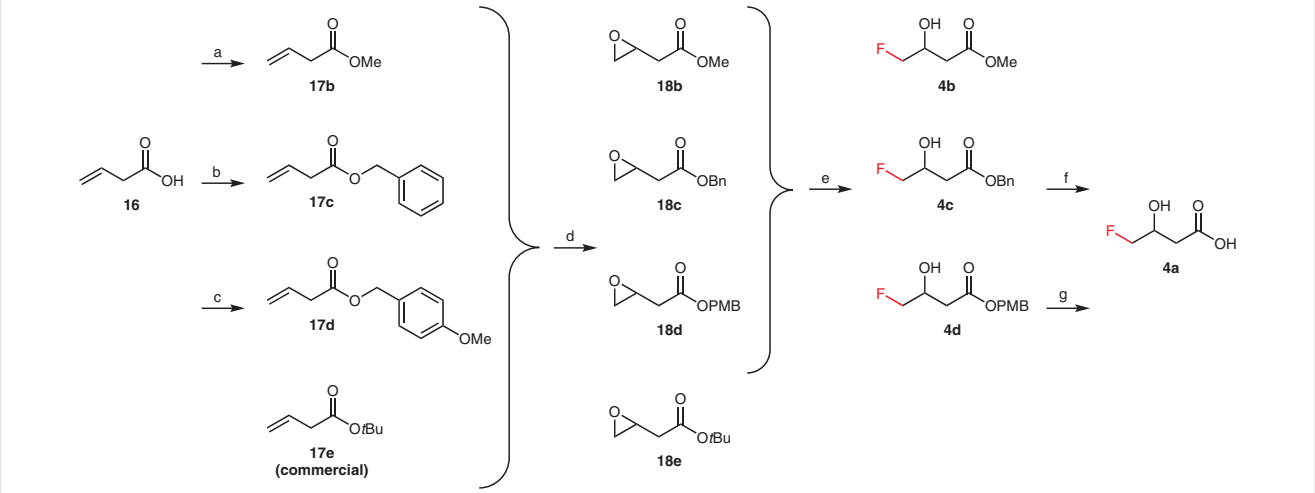
Scheme 3 Retrosynthetic routes that failed to provide 4-fluoro-3-hydroxybutyric acid (**4a**). *Reagents and conditions*: (a) $BH_3{\cdot}SMe_2$, $NaBH_4$, THF, 0 °C to rt, 16 h, 95%; (b) $SOCl_2$, THF, 0 °C to rt, 16 h, 60%; (c) $NaIO_4$, $RuCl_3$ (cat.) CH_2Cl_2/H_2O, 0 °C, 2.5 h, 88%; (d) NaOH, MeOH/H_2O, 50 °C, 2.5 h (carried over as crude); (e) TIPSCl (2 equiv), imidazole, DMF, 0–50 °C, 2 h, 64% (2 steps); (f) DMF/H_2O (20:1), 70 °C, 3 h, 75%.

1 to 3 carbons away. After nucleophilic ring opening, the resultant anionic sulfate intermediate can be hydrolyzed under acidic conditions to a hydroxy moiety. They are generally formed in two steps from diols, which, if chiral, dictate the stereochemistry of the heterocycle and, consequently, the 1,2-fluorohydroxy product. Cyclic sulfate **10** (Scheme 3) was prepared using a modified version of a published method.[21] Dimethyl-(*S*)-(–) malate (**8**) was chemoselectively reduced[22] with borane–dimethyl sulfide complex and catalytic sodium borohydride to generate chiral diol **9**.[23] Thionyl chloride was used to convert **9** into a cyclic sulfite intermediate (not shown) which was oxidized with $RuCl_3/NaIO_4$ to cyclic sulfate **10**. This product was stable to chromatography but polymerized upon solvent evaporation. Some intrinsic instability of cyclic sulfates has been reported previously.[24] Variations to the carboxylate protecting group, a methyl ester in the case of **10**, were not investigated but could have an impact on the overall stability of related compounds.

For target compound **4a**, we also considered a dioxanone[25] precursor, an example is shown in Scheme 3 (compound **15**) with a generic leaving group (LG) on carbon 4. We intended a dioxanone heterocycle to serve several purposes: preserve the stereochemistry of the β-hydroxy, protect the carboxylate, and simultaneously mask all protic functionalities. After leaving group displacement with fluoride, the dioxanone would have been cleaved under acidic conditions restoring the β-hydroxy acid. Towards this end, as shown in Scheme 3, chiral lactone **11** (commercially available or easily prepared from carnitine[26]) was opened with sodium hydroxide[27] and directly silylated with excess TIPS-chloride to a bis-protected silyl ester and primary silyl ether for ease of chromatography and to prevent recyclization. The silyl ester was chemoselectively deprotected with simple heating in a DMF/water mixture using a strategy laid out by Chen at al.[28] to achieve acid **13**. Although an atom inefficient strategy, this represents a new route to **13**, a useful differentially protected chiral diol. The prior report for the synthesis of **13** follows a different synthetic path in which compound **9** is silylated and the methyl ester deprotected.[29]

Unfortunately, in our hands, **13** could not be converted into dioxanone **14** using 2-methoxypropene as has been described,[30] or using 2,2-dimethoxypropane, a reagent used for similar reactions.[31] Rather, unreacted starting material was recovered or a side product (the methyl ester of **13**) was formed, a phenomenon which has been documented previously.[32] This was the case as well for similar attempts at dioxanone formation with a derivative of **13** consisting of a benzyl ether in place of TIPS.

Epoxyesters **18b–d** (Scheme 4) are suitable precursors for preparing 4-fluoro-3-hydroxybutyrates **4**. 3-Butenoic acid (**16**) is an inexpensive starting material for this purpose. To protect the carboxylic acid functionality, esterification using mild conditions such as Steglich esterification or trichloroacetimidate reagents is beneficial as these alkylations avoid the double bond migrations known to occur with β,γ-unsaturated esters.[33] Incidentally, alkenes **17** could be viewed as precursors for diol esters such as **9** (see Scheme 3); unfortunately, an attempt at Sharpless asymmetric dihydroxylation of **17d** resulted in loss of the ester protecting group, likely through cyclization. Fortunately, epoxidation of esters **17** with MCPBA generated racemic epoxides **18**, which can be resolved, if desired, to favor either enantiomer through hydrolytic kinetic resolution using a (salen)Co Jacobsen catalyst.[34] Epoxides **18** are acid and base sensitive, and without delicate treatment can isomerize to α,β-unsaturated γ-hydroxyesters.[35] Furthermore, compounds **18** are also sensitive to normal-phase chromatogra-



Scheme 4 *Reagents and conditions*: (a) MeI, DBU, MeCN, rt, 2 h, 28%; (b) benzyl alcohol, DMAP, DCC, CH_2Cl_2, 0 °C to rt, 16 h, 97%; (c) PMB trichloroacetimidate, CH_2Cl_2, 0 °C to rt, 16 h, 99%; (d) MCPBA, CH_2Cl_2, 16–48 h, 57% (**18b**), 60% (**18c**) 69% (**18d**) 98% (**18e**); (e) $Et_3N{\cdot}HF$, 70–80 °C, 6 h, 22% (**4b**), 76% (**4c**), 72% (**4d**); (f) H_2/Pd/C, MeOH/EtOAc, 14 h, rt, 90%; (g) TFA, CH_2Cl_2, 0 °C, 2 h, 80%.

phy to greater or lesser degrees; we found that methyl ester **18b** was most labile whereas benzyl and *p*-methoxybenzyl esters **18c** and **18d** were more tolerant of chromatography without great loss of yield. Ring opening under the fairly mild conditions of triethylamine trihydrofluoride resulted in γ fluorination for all but *t*-butyl epoxide **18e** which did not withstand the reaction conditions. Ester deprotection of fluoroesters **4c** and **4d** with H_2/Pd/C or TFA, respectively, produced 4-fluoro-3-hydroxybutyric acid (**4a**), which could be purified by normal- or reverse-phase chromatography, did not cyclize in aqueous conditions, and is stable to storage at –18 °C.

In conclusion, we have isolated and characterized 2-fluoroacetoacetic acid (**3**) and observed that it decarboxylates gradually at a rate that is pH dependent and slower than that of the non-fluorinated analog acetoacetic acid (**1**). This loss of CO_2 is most rapid at low pH. At physiological pH its half-life is calculated to be >90 hours. For the introduction of a fluorohydrin motif onto the terminal position of the butyrate structure, epoxides **18b–d**, although somewhat labile, are suitable precursors that undergo ring opening with triethylamine trihydrofluoride. In this manner we prepared novel fluorinated esters **4b–d**. The γ-fluorinated free acid **4a** was isolated by ester deprotection and, notably, does not readily cyclize.

When fluorinated molecular imaging probes serve as bioisosteres for endogenous compounds, they can provide a window into cellular metabolism and have applications from basic biology investigations to clinical medicine. Despite the biological significance of compounds like acetoacetic acid (**1**) and 3-hydroxybutyric acid (**2**), the preparation of fluorinated 3-hydroxy/oxobutyrates has received little attention in recent literature. This likely stems from concerns regarding toxicity. Esters of **3** are known to be toxic compounds metabolized through a pathway that converges with that of the pesticide/rodenticide fluoroacetate. The mechanism of toxicity for such species involves a 'lethal synthesis' beginning with formation of fluoroacetyl coenzyme A and leading, ultimately, to a defluorinated metabolite, 4-hydroxy-*trans*-aconitate, which strongly inhibits the TCA cycle enzyme aconitase.[36] Compounds **4** could also share this toxic pathway. Even so, concerns over toxicity have little relevance with regard to potential PET applications as radiofluorinated compounds are clinically administered at non-pharmacological doses. Moreover, fluorinated hydroxy/oxobutyrates have interesting physical properties of synthetic utility and can be applied to the construction of larger structures with medicinal rather than toxic properties.

Caution! Given the toxicity of ester derivatives of **3** and the likely toxicity of compounds **3** and **4a–d**, caution should be used when handling these chemicals, including avoiding skin contact. Experimental procedures and characterization data for reported compounds **6**,[7] **9**,[22,37] **10** (modified procedure),[21] **17b**,[38] **17c**,[39] **17d**,[40] **18b**,[41] **18c**,[42] **18e**,[34b] and NMR spectra for all new compounds are given in the Supporting Information. Selectfluor® (>95%), *tert*-butyl acetoacetate (**5**) (reagent grade, 98%), *tert*-butyl 3-butenoate (**17e**) (>98%), and fluoroacetone (**7**) (98%), were purchased from Millipore Sigma and used without further purification. The pH of buffers were verified on a Fisher Scientific Accumet basic ab15 pH meter. Column chromatography was performed using SiliaFlash F60 silica gel (230-400 mesh); PAA = *p*-anisaldehyde. FTIR were recorded using a Thermo Nicolet 8700 FTIR spectrophotometer. NMR spectra were acquired on either an Agilent/Varian 500 MHz spectrometer or a Bruker Ascend 600 MHz spectrometer. High-resolution mass spectra were acquired on either an Agilent Technologies 6220 oaTOF (ESI) or a Kratos Analytical MS-50 instrument (EI).

^{19}F NMR Decarboxylation Study

Aqueous buffer solutions (1 M) were prepared at four pH values and were verified to within 0.06 units. Buffers for pH 3, 5, and 7 were prepared from a citric acid monohydrate/dibasic sodium phosphate system; pH 10 buffer was prepared from a sodium hydrogen carbonate/sodium carbonate system. For each pH condition studied a 10 μL aliquot of acid **3** was dissolved in 60 μL of D_2O followed by 540 μL of the relevant buffer solution. After buffer addition, the pH was rechecked. The solutions were transferred to NMR tubes and maintained at 21–22 °C for the full course of the NMR experiment. Spectra were acquired over a time course (initial scan, 2 h, 6 h, 12 h, 24 h, 48 h). Data were processed by integrating the ^{19}F signals related to either fluoroacetone (**7**) or 2-fluoroacetoacetic acid (**3**), after which the ratio of each species at the various time points was tabulated. The data were normalized to reflect 0% decarboxylation at their initial time points. The predominant ^{19}F signal related to **3** was a doublet at –184 ppm with long-range secondary splitting. Neither the enol nor enolate of **3** were apparent from the ^{19}F spectra at any pH. For the case of fluoroacetone (**7**), the predominant signal occurs at –226.4 ppm appearing as a triplet with secondary splitting due to long-range coupling. The secondary ^{19}F signal for **7**, a triplet at –224.9, constituting ≈11% of the larger signal was presumed to be the hydrate form (K_H = 0.167 in D_2O according to Buschmann et al.)[43] Enol/enolate signals for **7** were occasionally observable but not large enough for integration. As a control, ^{19}F spectra of authentic standard fluoroacetone (**7**) were acquired in similar buffer/D_2O mixtures. In pH 10 buffer solution, two doublets with a combined intensity of 0.9% at –195.0 ppm and –196.5 ppm were observed in the ^{19}F NMR spectrum of **7**; we attribute these signals to *E* and *Z* isomers of the fluoroacetone enol/enolate.

2-Fluoroacetoacetic Acid (3)

To *tert*-butyl 2-fluoroacetoacetate (**6**) (0.322 g, 1.82 mmol) was added CH_2Cl_2 (1 mL) and TFA (1 mL). The mixture was stirred at rt for 1 h before solvent removal by rotary evaporation (acetonitrile in several aliquots was added as a carrier solvent to remove residual TFA). The final crude product was isolated as an oil (0.189 g, 86%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.31 (d, *J* = 48.9 Hz, 1 H), 2.42 (d, *J* = 4.1 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 199.2 (d, *J* = 23.1 Hz, *C*), 167.5 (d, *J* = 21.9 Hz, *C*), 90.8 (d, *J* = 196.8 Hz, *C*H), 26.2. Also apparent in the ^{13}C NMR spectrum are fluoroacetone signals: δ 25.7 and δ 85.0, in agreement with literature values.[44]

^{19}F NMR (564.8 MHz, $CDCl_3$): δ = –191.6 (dq, *J* = 48.1, 3.9 Hz).

HRMS (ESI): *m*/*z* [M – H]$^-$ calcd for $C_4H_4FO_3$: 119.0150; found: 119.0148.

4-Fluoro-3-hydroxybutyric Acid (4a)

Compound **4a** was prepared from either of two starting materials: **4c** or **4d**.

*From p-Methoxybenzyl Ester **4d***

p-Methoxybenzyl ester **4d** (0.06 g, 0.25 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and chilled to 0 °C. TFA (150 μL, 1.95 mmol) was added, and the solution was stirred for 2 h. The solvent was evaporated under vacuum and the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH gradient elution from 1 to 7% MeOH). The product is a clear, colorless oil (0.024 g, 80%). R_f = 0.15 (CH_2Cl_2/MeOH, 9:1, PAA stain).

*From Benzyl Ester **4c***

Benzyl ester **4c** (0.147 g, 0.691 mmol) was dissolved in MeOH/EtOAc (1:1, 3.4 mL total vol) and Pd/C (10%, 0.015 g) was added. The mixture was stirred under 1 atm of H_2 for 14 h then filtered over Celite. The solvents were evaporated and the residue was purified as above yielding 0.076 g, (90%).

FTIR (DCM cast film): 2900–3600 (br), 2965, 1717, 1411, 1249, 1180, 1090, 1019 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.47 (ddd, *J* = 47.2, 13.7, 4.2 Hz, 1 H), 4.42 (ddd, *J* = 47.2, 9.5, 5.1 Hz, 1 H), 4.36–4.27 (m, 1 H), 2.65 (d, *J* = 6.2 Hz, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.4 (*C*), 85.2 (d, $^1J_{C-F}$ = 170.4 Hz, *C*H$_2$), 66.7 (d, $^2J_{C-F}$ = 20.0 Hz, *C*H), 36.6 (*C*H$_2$).

^{19}F NMR (470 MHz, $CDCl_3$): δ = –230.0 (td, *J* = 47.3, 18.7 Hz).

HRMS (ESI): *m*/*z* [M – H]$^-$ calcd for $C_4H_6FO_3$: 121.0306; found: 121.0306.

Methyl 4-Fluoro-3-hydroxybutyrate (4b)

To methyl 3,4-epoxybutyrate (**18b**) (0.249 g, 2.145 mmol) was added neat Et_3N·HF (6.917 g, 42.9 mmol) in one portion at rt. The mixture was heated to 70 °C and stirred for 6 h. The reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 (40 mL), and quenched with chilled sat. aqueous $NaHCO_3$ (40 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed by rotary evaporation. The residue was purified by silica gel chromatography (hexanes/EtOAc, 1:2, isocratic). The product is a clear, colorless oil (0.065 g, 22%). R_f = 0.59 (hexanes/EtOAc, 1:2, PAA stain).

FTIR (neat): 3450 (br), 2959, 2907, 1736, 1441, 1284, 1176, 1093, 1016 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.44 (ddd, *J* = 47.1, 9.5, 4.2 Hz, 1 H), 4.40 (ddd, *J* = 47.1, 9.5, 5.1 Hz, 1 H), 4.34–4.23 (m, 1 H), 3.73 (s, 3 H), 3.07 (d, *J* = 5.0 Hz, 1 H), 2.59 (d, *J* = 6.5 Hz, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.2 (*C*), 85.4 (d, *J* = 170.1 Hz, *C*H$_2$), 66.8 (d, *J* = 20.6 Hz, *C*H), 52.0 (*C*H$_3$), 36.6 (d, *J* = 6.3 Hz, *C*H$_2$).

^{19}F NMR (470 MHz, $CDCl_3$): δ = –229.9 (ddd, *J* = 47.3, 47.3, 18.7 Hz).

HRMS (EI): the parent molecular ion was not observed, however, fragments are indicative; *m*/*z* [M – H_2O]$^+$ calcd for $C_5H_7FO_2$: 118.0430; found: 118.0432; *m*/*z* [M – OCH_3]$^+$ calcd for $C_4H_6FO_2$: 105.0346; found: 105.0351.

Benzyl 4-Fluoro-3-hydroxybutyrate (4c)

To benzyl 3,4-epoxybutyrate (**18c**) (0.510 g, 2.654 mmol) was added neat Et_3N·HF (10.69 g, 66.36 mmol) in one portion at rt. The mixture was heated to 80 °C and stirred for 6 h. The reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 (60 mL), and quenched with chilled sat. aqueous $NaHCO_3$ (40 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed by rotary evaporation. The residue was purified by silica gel chromatography (hexanes/EtOAc, 2:1, isocratic). The product is a clear, pale yellow oil (0.426 g, 76%). R_f = 0.50 (hexanes/EtOAc, 3:2, PAA stain).

FTIR (DCM cast film): 3454 (br), 3035, 2957, 1732, 1456, 1274, 1170, 1089, 1017, 750, 698 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.42–7.33 (m, 5 H), 5.18 (s, 2 H), 4.44 (ddd, *J* = 47.1, 9.5, 4.4 Hz, 1 H), 4.40 (ddd, *J* = 47.1, 9.5, 5.1 Hz, 1 H), 4.35–4.26 (m, 1 H), 3.03 (br s, 1 H), 2.64 (d, *J* = 6.5 Hz, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6 (*C*), 135.4, 128.6 (*C*H), 128.3 (*C*H), 126.9 (*C*H), 85.3 (d, *J* = 170.5 Hz, *C*H$_2$), 66.9 (d, *J* = 20.6 Hz, *C*H), 66.8 (*C*H$_2$), 36.9 (d, *J* = 6.1 Hz, *C*H$_2$).

^{19}F NMR (470 MHz, $CDCl_3$): δ = –229.9 (ddd, *J* = 47.1, 47.1, 19.1 Hz).

HRMS (EI): *m*/*z* [M]$^+$ calcd for $C_{11}H_{13}FO_3$: 212.0849; found: 212.0848.

p-Methoxybenzyl 4-Fluoro-3-hydroxybutyrate (4d)

To *p*-methoxybenzyl 3,4-epoxybutyrate (**18d**) (0.215 g, 0.966 mmol) was added neat Et_3N·HF (7.78 g, 48.3 mmol) in one portion at rt. The mixture was heated to 80 °C and stirred for 6 h. The reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 (30 mL), and quenched with chilled sat. aqueous $NaHCO_3$ (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed by rotary evaporation. The residue was purified by silica gel chromatography (CH_2Cl_2/EtOAc, 5:1, isocratic). The product is a yellow oil (0.169 g, 72%). R_f = 0.63 (CH_2Cl_2/EtOAc, 5:1, PAA stain).

FTIR (neat): 3469 (br), 2958, 2839, 1732, 1613, 1516, 1248, 1170, 1113, 1030, 822 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.30 (d, *J* = 8.0 Hz, 2 H), 6.90 (d, *J* = 8.0 Hz, 2 H), 5.11 (s, 2 H), 4.43 (ddd, *J* = 46.9, 9.5, 4.2 Hz, 1 H), 4.39 (ddd, *J* = 46.9, 9.5, 5.3 Hz, 1 H), 4.34–4.24 (m, 1 H), 3.82 (s, 3 H), 3.05–3.00 (m, 1 H), 2.61 (d, *J* = 6.5 Hz, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.7 (*C*), 159.8 (*C*), 130.2 (*C*H), 127.5 (*C*), 114.0 (*C*H), 85.3 (d, *J* = 170.7 Hz, *C*H$_2$), 66.9 (d, *J* = 20.7 Hz, *C*H), 66.6 (*C*H$_2$), 55.3 (*C*H$_3$), 36.9 (d, *J* = 6.2 Hz, *C*H$_2$).

^{19}F NMR (470 MHz, $CDCl_3$): δ = –230.0 (ddd, *J* = 46.6, 46.6, 18.8 Hz).

HRMS (EI) *m*/*z* [M]$^+$ calcd for $C_{12}H_{15}FO_4$: 242.0954; found: 242.0949.

3-Hydroxy-4-{[tris(1-methylethyl)silyl]oxy}-(3*S*)-butanoic Acid (13)

According to a patent protocol,[27] to (*S*)-β-hydroxy-γ-butyrolactone (**11**) (0.843 g, 8.25 mmol) in MeOH (10 mL) was added 1 M aqueous NaOH (8.25 mL, 1 equiv). The mixture was stirred at 50 °C for 2.5 h. The crude product **12** was dried by azeotropic drying with acetonitrile and was used without further purification. The ^{1}H NMR was in agreement with that reported by Hollingsworth.[27]

^{1}H NMR (600 MHz, D_2O): δ = 4.03–3.97 (m, 1 H), 3.56 (dd, *J* = 11.7, 3.8 Hz, 1 H), 3.44 (dd, *J* = 11.9, 7.0 Hz, 1 H), 2.30 (dd, *J* = 15.1, 5.6 Hz, 1 H), 2.27 (dd, *J* = 15.1, 8.3 Hz, 1 H).

To the crude sodium salt (theoretical 8.25 mmol) was added DMF (8 mL) and imidazole (1.12 g, 16.5 mmol). The mixture was cooled to 0 °C. Triisopropylsilyl chloride (3.34 g, 17.3 mmol) was added. The mixture was allowed to come to rt then heated at 50 °C for 1 h. The mixture was poured into hexanes (50 mL) and H_2O (20 mL) was added. The hexane layer was washed with H_2O (2 × 10 mL) and brine (10 mL). The solvent was condensed by rotary evaporation. The intermediate product, a bis-protected primary silyl ether and silyl ester, was purified by silica gel chromatography (gradient elution: 0% hexanes to 15% EtOAc in hexanes) and isolated as a clear, colorless oil (2.284 g, 64%). R_f = 0.55 (EtOAc/hexanes, 1:4, $KMnO_4$ stain).

FTIR (neat): 3477 (br), 2945, 2893, 2868, 1719, 1465, 1123, 883, 681 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.14–4.07 (m, 1 H), 3.74 (dd, *J* = 9.8, 5.3 Hz, 1 H), 3.69 (dd, *J* = 9.8, 6.0 Hz, 1 H), 2.94 (d, *J* = 4.9 Hz, 1 H), 2.62 (dd, *J* = 16.2, 5.3 Hz, 1 H), 2.57 (dd, *J* = 16.6, 7.5 Hz, 1 H), 1.31 (sept, *J* = 7.1 Hz, 3 H), 1.14–1.04 (m, 39 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.2 (*C*), 68.8 (*C*H), 66.3 (*C*H$_2$), 39.4 (*C*H$_2$), 17.9 (*C*H$_3$), 17.7 (*C*H$_3$), 11.9 (*C*H).

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{22}H_{48}NaO_4Si_2$: 455.2983; found: 455.2979.

Anal. Calcd for $C_{22}H_{48}O_4Si_2$: C, 61.06; H, 11.18. Found: C, 61.08; H, 11.11.

Following a chemoselective deprotection strategy outlined by Chen et al.,[28] the bis-protected intermediate (1.78 g, 4.11 mmol) was dissolved in DMF/H_2O (20:1, 21 mL) and heated at 70 °C for 3 h. The reaction was cooled to rt, diluted with Et_2O (80 mL), washed with sat. aqueous NH_4Cl (2 × 30 mL) and brine (2 × 30 mL), and dried over Na_2SO_4. The solvent was evaporated by rotary evaporation and the crude residue was purified by silica gel chromatography (hexanes/EtOAc, 1:1). The product was isolated as a clear, yellow oil (0.848 g, 75%). R_f = 0.17 (hexanes/EtOAc, 1:1, $KMnO_4$ stain). The ^{1}H NMR was in agreement with Cooksey et al.[30]

^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.17–4.10 (m, 1 H), 3.77 (dd, *J* = 9.8, 4.5 Hz, 1 H), 3.68 (dd, *J* = 9.8, 6.0 Hz, 1 H), 2.64 (dd, *J* = 16.2, 4.5 Hz, 1 H), 2.58 (dd, *J* = 16.2, 7.9 Hz, 1 H), 1.17–1.09 (m, 3 H), 1.09–1.05 (m, 18 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 177.1 (*C*), 68.5 (*C*H), 66.3 (*C*H$_2$), 37.8 (*C*H$_2$), 17.9 (*C*H$_3$), 11.8 (*C*H).

p-Methoxybenzyl 3,4-Epoxybutyrate (18d)

p-Methoxybenzyl 3-butenoate (**17d**) (0.477 g, 2.312 mmol) was dissolved in CH_2Cl_2 (12 mL) and chilled to –78 °C. Reagent grade ≤77% MCPBA (1.061 g, 4.624 mmol) was added in one portion. The mixture was stirred for 48 h before being allowed to come to rt. The precipitated solids were removed by filtration. The solution was quenched with sat. aqueous $K_2S_2O_3$ (8 mL) and filtered again. The filtrate was washed with sat. aqueous $K_2S_2O_3$ (8 mL), H_2O (10 mL), and brine (10 mL), dried over Na_2SO_4, and condensed by rotary evaporation. The crude residue was purified by silica gel chromatography. The column was pretreated with 2% Et_3N in hexanes/acetone (2:1). The product was eluted with hexanes/acetone (2:1, isocratic). The product was isolated as an amber oil (0.355 g, 69%). R_f = 0.37 (hexanes/acetone, 2:1, with a 2% Et_3N pretreated plate).

FTIR (neat): 3003, 2958, 2838, 1735, 1612, 1516, 1303, 1248, 1170, 1032, 823 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.31 (d, *J* = 8.5 Hz, 2 H), 6.90 (d, *J* = 8.5 Hz, 2 H), 5.11 (s, 2 H), 3.82 (s, 3 H), 3.32–3.27 (m, 1 H), 2.84 (dd, *J* = 5.0, 5.0 Hz, 1 H), 2.59 (dd, *J* = 6.3, 6.3 Hz, 2 H), 2.56 (dd, *J* = 5.0, 2.5 Hz, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.2 (*C*), 159.7 (*C*), 130.1 (*C*H), 127.7 (*C*), 114.0 (*C*H), 66.5 (*C*H$_2$), 55.2 (*C*H$_3$), 47.9 (*C*H), 46.6 (*C*H$_2$), 38.1 (*C*H$_2$).

HRMS (EI): *m*/*z* [M]$^+$ calcd for $C_{12}H_{14}O_4$: 222.0892; found: 222.0893.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1610695.

References

(1) Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443.
(2) (a) Van Hee, V. F.; Grasso, D.; Sonveaux, P.; Labar, D.; Gregoire, V.; Dehon, G.; Muccioli, G. G.; Frederick, R. *Oncotarget* **2017**, *8*, 24415. (b) Li, C.; Liu, H.; Duan, D.; Zhou, Z.; Liu, Z. *Nucl. Med. Biol.* **2018**, *64-65*, 34. (c) Wuest, M.; Hamann, I.; Bouvet, V.; Glubrecht, D.; Marshall, A.; Trayner, B.; Soueidan, O.-M.; Krys, D.; Wagner, M.; Cheeseman, C.; West, F.; Wuest, F. *Mol. Pharmacol.* **2018**, *93*, 79. (d) Ko, I. O.; Jung, K.-H.; Kim, M. H.; Kang, K. J.; Lee, K. C.; Kim, K. M.; Noh, I.; Lee, Y. J.; Lim, S. M.; Kim, J. Y.; Park, J.-A. *Contrast Media Mol. Imaging* **2017**, 3981358/1. (e) Pisaneschi, F.; Witney, T. H.; Iddon, L.; Aboagye, E. O. *MedChemComm* **2013**, *4*, 1350.
(3) (a) De Silva, A.; Wuest, M.; Wang, M.; Hummel, J.; Mossman, K.; Wuest, F.; Hitt, M. M. *Am. J. Nucl. Med. Mol. Imaging* **2012**, *2*, 88. (b) Hamann, I.; Krys, D.; Glubrecht, D.; Bouvet, V.; Marshall, A.; Vos, L.; Mackey, J. R.; Wuest, M.; Wuest, F. *FASEB J.* **2018**, *32*, 5104. (c) Krys, D.; Hamann, I.; Mattingly, S.; Wuest, M.; Wuest, F. *Membrane Transporters in Breast Cancer and their Importance in PET Imaging*, In *Membrane Proteins in Health and Disease, 61st Annual Meeting of the Canadian Society for Molecular Biosciences*; Banff: Canada, **2018**, Poster 53.
(4) (a) Webber, R. J.; Edmond, J. *J. Biol. Chem.* **1977**, *252*, 5222. (b) Laffel, L. *Diabetes Metab. Res. Rev.* **1999**, *15*, 412.
(5) *Peroxidases in Chemistry and Biology, Vol. 2*; Everse, J.; Everse, K. E.; Grisham, M. B., Ed.; CRC Press: Boca Raton (FL), **1990**, 304.
(6) Bergmann, E. D.; Szinai, S. *J. Chem. Soc.* **1956**, 1521.
(7) Huber, D. P.; Stanek, K.; Togni, A. *Tetrahedron: Asymmetry* **2006**, *17*, 658.
(8) Hay, R. W.; Bond, M. A. *Aust. J. Chem.* **1967**, *20*, 1823.
(9) Jencks, W. P. *Catalysis in Chemistry and Enzymology - McGraw-Hill Series in Advanced Chemistry*; McGraw-Hill: New York, **1969**, 644.
(10) Hanson, R. W. *J. Chem. Educ.* **1987**, *64*, 591.
(11) Dean, J. A. *Handbook of Organic Chemistry* 1987.
(12) Olah, G. A.; Kuhn, S. J. *J. Org. Chem.* **1961**, *26*, 225.
(13) Chen, Y.; Tian, S.-K. *Chin. J. Chem.* **2013**, *31*, 37.

(14) Graham, T. J. A.; Lambert, F. R.; Ploessl, K.; Kung, H. F.; Doyle, A. G. *J. Am. Chem. Soc.* **2014**, *136*, 5291.
(15) Bergmann, E. D.; Cohen, S.; Shahak, I. *J. Chem. Soc.* **1961**, 3448.
(16) Liu, Y.; Hazzard, C.; Eustaquio, A. S.; Reynolds, K. A.; Moore, B. S. *J. Am. Chem. Soc.* **2009**, *131*, 10376.
(17) Shibatomi, K.; Kobayashi, F.; Narayama, A.; Fujisawa, I.; Iwasa, S. *Chem. Commun.* **2012**, *48*, 413.
(18) Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.
(19) Roemer, J.; Steinbach, J.; Kasch, H. *Appl. Radiat. Isotopes* **1996**, *47*, 395.
(20) (a) Berridge, M. S.; Franceschini, M. P.; Rosenfeld, E.; Tewson, T. J. *J. Org. Chem.* **1990**, *55*, 1211. (b) Megia-Fernandez, A.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Curr. Org. Chem.* **2011**, *15*, 401.
(21) Griffiths, J.-P.; Nie, H.; Brown, J. R.; Day, P.; Wallis, J. D. *Org. Biomol. Chem.* **2005**, *3*, 2155.
(22) Saito, S.; Ishikawa, T.; Kuroda, A.; Koga, K.; Moriwake, T. *Tetrahedron* **1992**, *48*, 4067.
(23) Pospisil, J.; Marko, I. E. *Tetrahedron Lett.* **2008**, *49*, 1523.
(24) Robinson, R. I.; Woodward, S. *Tetrahedron Lett.* **2003**, *44*, 1655.
(25) Seebach, D.; Imwinkelried, R.; Stucky, G. *Angew. Chem.* **1986**, *98*, 182.
(26) Calvisi, G.; Catini, R.; Chiariotti, W.; Giannessi, F.; Muck, S.; Tinti, M. O.; De Angelis, F. *Synlett* **1997**, 71.
(27) Hollingsworth, R. L. EP513430A1, **1992**.
(28) Chen, B.; Sun, H.-X.; Qin, J.-F.; Wang, B. *Tetrahedron Lett.* **2016**, *57*, 253.
(29) Christopher, J. A.; Kocienski, P. J.; Kuhl, A.; Bell, R. *Synlett* **2000**, 463.
(30) Cooksey, J.; Gunn, A.; Kocienski, P. J.; Kuhl, A.; Uppal, S.; Christopher, J. A.; Bell, R. *Org. Biomol. Chem.* **2004**, *2*, 1719.
(31) Pounder, R. J.; Dove, A. P. *Biomacromolecules* **2010**, *11*, 1930.
(32) Rachele, J. R. *J. Org. Chem.* **1963**, *28*, 2898.
(33) (a) Ilangovan, A.; Anandhan, K.; Kaushik, M. P. *Tetrahedron Lett.* **2015**, *56*, 1080. (b) Alcock, S. G.; Baldwin, J. E.; Bohlmann, R.; Harwood, L. M.; Seeman, J. I. *J. Org. Chem.* **1985**, *50*, 3526.
(34) (a) Su, Q.; Dakin, L. A.; Panek, J. S. *J. Org. Chem.* **2007**, *72*, 2. (b) Cook, C.; Liron, F.; Guinchard, X.; Roulland, E. *J. Org. Chem.* **2012**, *77*, 6728.
(35) (a) Larcheveque, M.; Henrot, S. *Tetrahedron* **1990**, *46*, 4277. (b) Faissat, L.; Martin, K.; Chavis, C.; Montero, J.-L.; Lucas, M. *Bioorg. Med. Chem.* **2003**, *11*, 325. (c) Degenhardt, C. R. *J. Org. Chem.* **1980**, *45*, 2763.
(36) (a) Proudfoot, A. T.; Bradberry, S. M.; Vale, A. J. *Toxicol. Rev.* **2006**, *25*, 213. (b) Lauble, H.; Kennedy, M. C.; Emptage, M. H.; Beinert, H.; Stout, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13699.
(37) White, J. D.; Lincoln, C. M.; Yang, J.; Martin, W. H. C.; Chan, D. B. *J. Org. Chem.* **2008**, *73*, 4139.
(38) Rao, G. C. *Org. Prep. Proced. Int.* **1980**, *12*, 225.
(39) Miao, B.; Ma, S. *Chem. Commun.* **2014**, *50*, 3285.
(40) Shah, J. P.; Russo, C. M.; Howard, K. T.; Chisholm, J. D. *Tetrahedron Lett.* **2014**, *55*, 1740.
(41) Mohr, P.; Roesslein, L.; Tamm, C. *Tetrahedron Lett.* **1989**, *30*, 2513.
(42) Hoff, B.; Anthonsen, T. *Tetrahedron: Asymmetry* **1999**, *10*, 1401.
(43) Buschmann, H. J.; Fueldner, H. H.; Knoche, W. *Ber. Bunsen Ges. Phys. Chem.* **1980**, *84*, 41.
(44) Abraham, R. J.; Jones, A. D.; Warne, M. A.; Rittner, R.; Tormena, C. F. *J. Chem. Soc., Perkin Trans. 2* **1996**, 533.