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A practical synthesis of (2*R*)-3,5-di-*O*-benzoyl-2-fluoro-2-C-methyl-D-ribono-γ-lactone

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ABSTRACT

The title compound was synthesized in 23% overall yield using only one purification in four chemical steps. The key features of this practical synthesis include an asymmetric aldol condensation and an enzymatic hydrolysis to remove the major undesired isomer.

1. Introduction

1-(2-Deoxy-2-fluoro-2-C-methyl-β-D-ribofuranosyl)-cytosine **1** is a potent and selective anti-HCV agent[1], and is currently in clinical trials for the treatment of hepatitis C. 3,5-Di-*O*-benzoyl-2-fluoro-2-C-methyl-D-ribono-γ-lactone **2** is a key intermediate for the synthesis of **1**.[2] A number of synthetic routes for preparing **2** have been disclosed.[3] However, none of these syntheses are deemed suitable for scaling up to commercial scale manufacturing due to the shortcomings of very low overall yield, lack of robustness, the use of expensive and toxic reagents, chromatographic isolation, etc. Although in the latest version of the synthesis[4] most of the scale-up issues have been resolved, the manufacturing cost is still a concern, and continued efforts to pursue a more cost effective and scalable alternative synthesis are warranted. Herein, we report a practical synthesis of **2** using a combination of an asymmetric aldol condensation and an enzymatic resolution.[5]

Our synthetic strategy is outlined in Figure 1. The target lactone could be prepared from intermediate **3**, which, in turn, could be formed via an asymmetric aldol condensation reaction between 2,3-*O*-isopropylidene-D-glyceraldehyde **4** and ethyl 2-fluoropropionate **5**. This approach has been used many times in the literature for the preparation of γ-lactones.[6] For example, Heathcock et al.[7] reported that the reaction of **4** with the enolate of methyl propionate **6** led to the formation of a 3:2 mixture of **7** and **8** (Scheme 1). Treatment of the mixture with 60% acetic acid at room temperature overnight resulted in a 3:2 mixture of lactones **9** and **10**. We reasoned that similar results should be observed for ester **5**, and if so, the desired adduct **3** should be the major product. If the major isomer could be isolated at some point in the process, a practical synthesis for **2** would be achievable.

2. Results and discussion

The initial aldol condensation reaction between **4** and **5** was carried out by first treating **5** with LDA at 0 °C in THF for half an

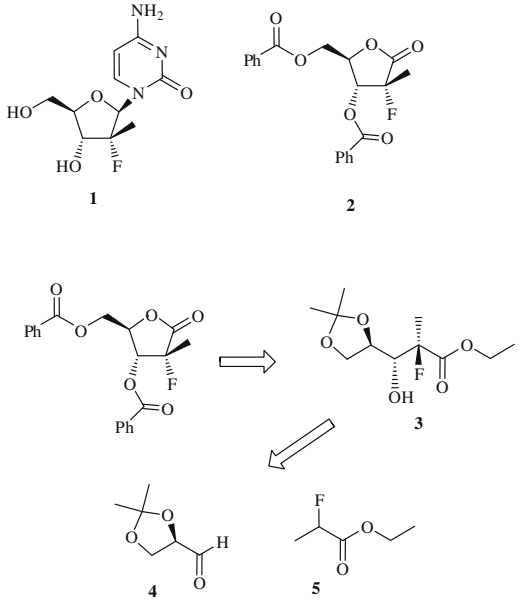

Figure 1.

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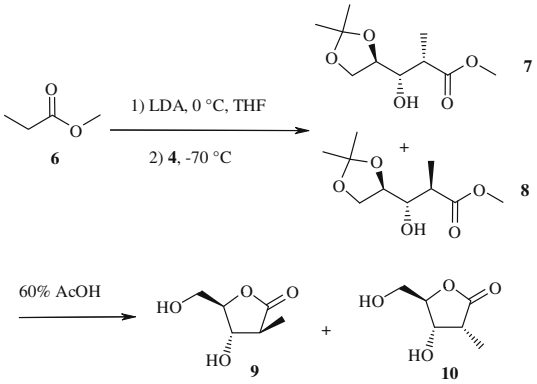
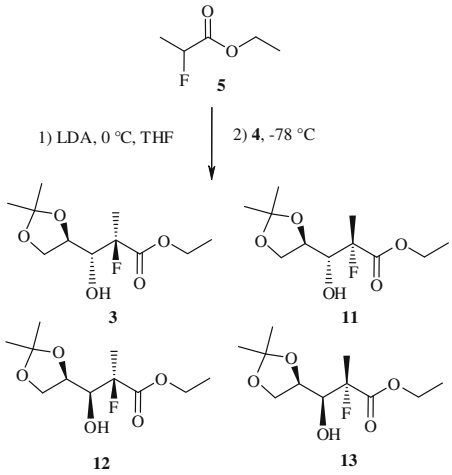
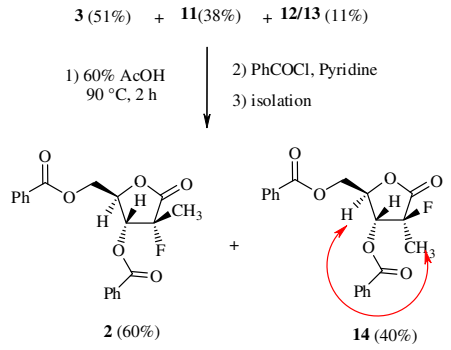


Scheme 1. A literature example.



Scheme 2. The condensation of **5** with **4**.

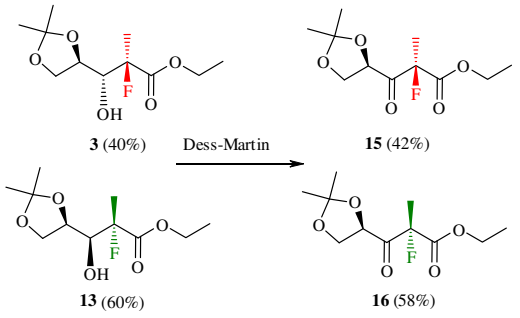
hour, and then cooling the mixture to around −78 °C, followed by the addition of **4** as a THF solution. GC analysis revealed that the crude product was a mixture of three components, **3** (51%), **11** (38%), **12,** and/or **13** (11%) (Scheme 2), a result similar to that observed in Scheme 1. The initial assignment of the structures was conducted via a tedious process. The attempts to isolate individual isomers using column chromatography were unsuccessful. Treatment of the crude mixture with 60% acetic acid at 90 °C for 2 h resulted in complete conversion to a mixture of un-protected lactones, which was reacted with an excess amount of benzoyl chloride in pyridine (Scheme 3). Two major products with a ratio of approximately 60:40 were detected in the reaction mixture, which corresponded to the ratio of the two major products in the



Scheme 3. Conversion of aldol adducts to lactones.

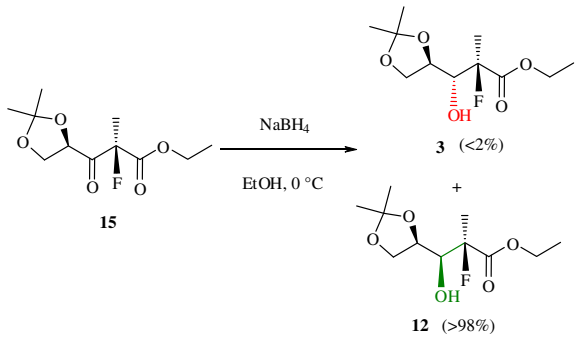
aldol mixture. The two products were isolated, and the major isomer was confirmed to be **2** by NMR studies and comparison with an authentic sample. The minor isomer was confirmed to be **14** by extensive NMR studies. One NMR feature clearly distinguishing the two compounds was a clear NOE detected between the methyl group and the C-4 proton in **14**, whereas no such NOE was detected in **2**. This work confirmed that the two major compounds present in the crude aldol condensation product (Scheme 2) were **3** and **11**.

The third component in the GC chromatogram of the aldol mixture must be **12** and/or **13**. At this point, a 3:2 mixture of this component and compound **3** was obtained through flash column chromatography. Oxidation of this mixture with Dess–Martin reagent produced a mixture of two isomers in the same 3:2 ratio. This result clearly indicated that C-2 configuration in compound **3** and the third component was different, and thus, the third component must be **13** (Scheme 4).



Scheme 4. Confirmation of compound **13**.

The fourth possible isomer, **12**, was prepared by reduction of **15** with $NaBH_4$ in ethanol at 0 °C. The reaction was surprisingly stereoselective, as indicated in Scheme 5. Unfortunately, **12** and **13** co-eluted in our GC method, and thus, it was not conclusive regarding the ratio of these two isomers in the mixture of the aldol product.



Scheme 5. Preparation of compound **13**.

After the establishment of the stereochemistry the reaction was optimized, so as to achieve better stereoselectivity and chemical yield. Our studies have indicated that the solvent affected the stereoselectivity, but the concentration did not. Better selectivities were observed in TBME or toluene (typically, 56:38:6) than in THF and 2-MeTHF (typically, 50:39:11). Two bases, LDA and LiHMDS, were evaluated for the reaction. The batches using LiHMDS demonstrated better selectivities (e.g., 64:32:4), but offered lower conversions and crude yields. In one experiment, ester

5 was added to a THF solution of LiHMDS at −78 °C, and analysis of the mixture after 10 min at the temperature revealed that more than 60% of the ester had been converted to a condensed product via Claisen condensation. Our explanation is that LiHMDS is a relatively weak base. Therefore, in the reaction mixture there was a significant amount of the ester (in equilibrium with its enolate), and thus Claisen condensation had occurred. For the same reason, the addition order is very important. For instance, addition of the LDA to an ester solution should be avoided. The best way to conduct the reaction is to add a solution of both **4** and **5** in toluene to a solution of LDA at low temperature. Similar selectivity was observed when the reaction was carried out at temperatures from −78 to −20 °C. Deterioration in both stereoselectivity and yield was experienced at higher temperatures. With the optimized conditions, a selectivity of 62:34:4 was achieved. It is also worth mentioning that aldehyde **4** tends to polymerize. Freshly distilled **4** is a liquid. It can turn into a gel in just a few hours. For our reaction, both freshly cracked liquid and polymerized gel gave similar results.

At this point, it seemed to us that further improvement of selectivity would be hard to achieve using the lithium enolate of **5**. Thus, an evaluation of other types of enolates was attempted. It is clear that the selectivity at C-2 and C-3 is controlled by different factors. The selectivity at C-3 is dictated by the steric center in **4** and follows the Felkin–Anh rule.[8] The fact that the ratio of **3**+**11**/**12**+**13** was ~9:1 indicated a fairly favorable selectivity at C-3. The control of selectivity at C-2 is more complicated. A *syn* addition from a *Z*-enolate[9] is required to form **3**.[10] There are many enolates in the literature that demonstrates high *syn* selectivity in aldol condensations; examples include **17**,[11] **18**,[12] **19**,[13], and **20**[14] (Fig. 2).

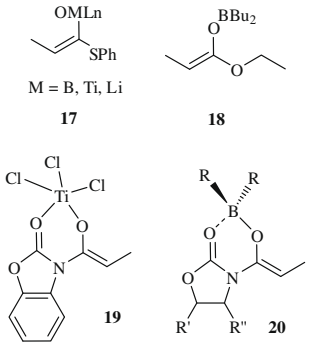


Figure 2. Enolates demonstrated *syn* selectivities.

Thus, the corresponding fluorine-substituted substrates, **21**[15] and **22**,[16] were prepared, and enolates **23**,[17] **24**,[17] **25**,[18] **26**,[19] and **27**[20] were generated accordingly (Fig. 3). The aldol condensation of these enolates with aldehyde **4** was then conducted. The crude products were treated with 60% acetic acid at ~90 °C for 2 h, and the ratio of the resulting lactones **28**–**29** was measured as an indicator of the *syn*/*anti* selectivity in the aldol reaction. Some results are listed in Table 1. The data indicate that the selectivity is not easily predictable. Enolates **23** and **24** offered reversed selectivity, despite the facts that both enolates existed almost exclusively as *Z*-isomers, and excellent *syn* selectivity was observed in their reactions with certain aldehydes.[15,17] No reactions were detected for enolates **25** and **27**. The best selectivity was observed with enolate **26**; however, the reaction gave a very low isolated yield. It appears to us that the effects of a fluorine atom on the enolates are profound; it alters the stereoselectivity of the reactions;[21] it decreases the reactivity of the enolates, and thus, the boron enolates are no longer reactive toward the aldehyde; and it makes the substrates and the corresponding products susceptible to hydrolysis, which accounts for the low isolated yield of the reactions.

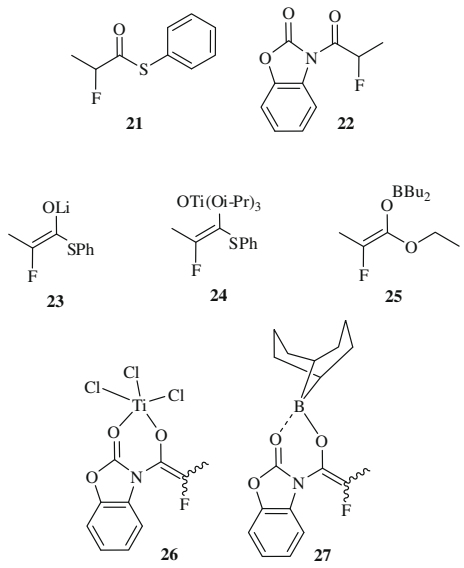
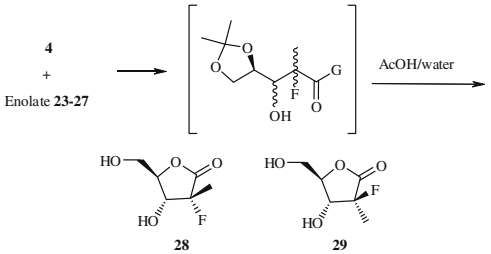


Figure 3. Fluorine-substituted substrates and their enolates.

Table 1
Diastereoselectivity of different enolates[a]



Entry	Enolate	Ratio **28/29**[b]
1	**23**	28/72
2	**24**	32/68
3	**26**	78/22
4	**25**	No reaction
5	**27**	No reaction

[a] The enolates were generated according to literature procedures. The aldol reactions were quenched with 6% HCl solution and extracted with dichloromethane. The organic solution was concentrated to dryness, the residue was mixed with 60% acetic acid solution, and the resulting mixture was stirred at ~88 °C for 2 h.
[b] GC area ratio.

One thought was that increasing the bulk of either the aldehyde or the ester might be beneficial to the diastereoselectivity of the reaction. Thus, aldehyde **30**[22] and esters **31**,[23] **32**,[24] and **33**[25] were prepared (Fig. 4). Unfortunately, similar selectivities were observed for the reactions of these esters **31**–**33** with aldehyde **4**. The larger esters did offer one benefit—higher crude yield. However, as will be discussed later, the products from these larger esters are not good substrates for the enzymatic resolution. Worse selectivities (48:41:11) were observed for the condensation of **30** with ester **5**.

As expected, the isolation of the desired isomer, **3**, from the crude product using conventional methods was not successful.

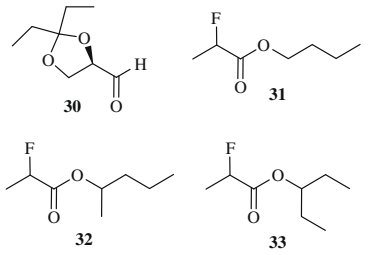
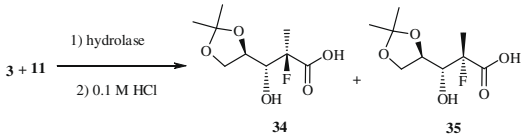


Figure 4. Aldehyde and esters tested for the aldol condensation.

Therefore, we turned our attention to an enzymatic diastereoselective hydrolysis approach. Selective hydrolysis of one of the major isomers to the corresponding carboxylic acid, coupled with an extractive workup, could be a very efficient separation method.[26] Thus, a mixture (~73:27) of **3** and **11** was isolated from a crude aldol product. Hydrolases were screened for the hydrolysis of this mixture using a pH-indicator, well plate assay. Among the 214 commercial hydrolases tested, 17 demonstrated activity and were analyzed for their diastereoselectivity (see Table 2). Eight of them showed either no selectivity or a slight preference for substrate **3**. Eight of them demonstrated different degrees of selectivity for **11**. One widely used enzyme, CALB[27] (*Candida antarctica* lipase, form *B*), was outstanding. In the experiment it hydrolyzed only substrate **11**. Therefore, this enzyme was selected for an in depth reaction optimization. First, 1 M Na_2SO_4 solution, 0.1 M KPI (potassium phosphate) buffer, and 10% sorbitol solution were identified as the best media for the reaction. Then, the reaction was optimized using a batch of crude aldol product (containing 40.8% **3**, 34.6% **11**, and 7.7% **12/13**), and some of the results are listed in Table 3. In the experiments, the reaction was stopped when isomer **11** was completely hydrolyzed. In an ideal situation no amount of **3** would be hydrolyzed at this point. In reality, a portion of isomer **3** was hydrolyzed in all the experiments. Therefore, the primary objective for the reaction optimization was to determine the optimal combination of minimal hydrolysis of **3** and reasonable reaction time. As indicated in the table, the pH of the reaction media had a minor effect on the reaction rate, but did affect the percentage of **3** hydrolysis (entries 2 and 5–7). The percentage was significantly higher at pH of 7.5. The reaction temperature clearly affected both the reaction rate and hydrolysis of **3** (entries 7–10). When the temperature changed from 35 °C to 50 °C, the percent-

Table 2
Diastereoselectivity of hydrolases[a]



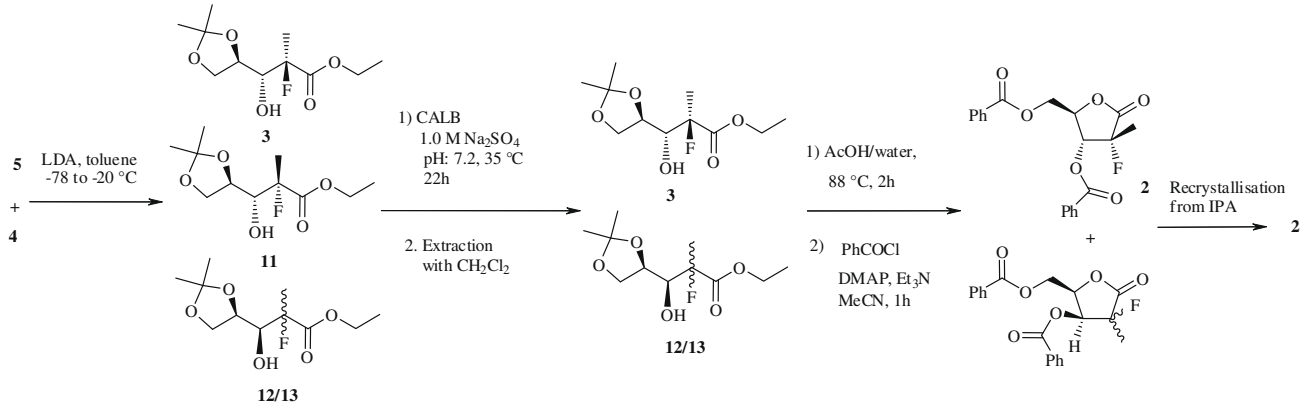
Enzyme	Esters[b]		Acids[c]	
	3	**11**	**34**	**35**
CALB	**96.9**	**3.1**	**0.0**	**100.0**
TOY	89.3	10.7	17.3	82.7
L6	80.3	19.7	17.4	82.6
HLE	78.2	21.8	18.4	81.6
PLE	80.0	20.0	43.6	56.4
CLE	80.3	19.7	44.2	55.8
L1	74.8	25.2	45.8	54.2
LCV	73.7	26.3	57.9	42.1
SP524	71.3	28.7	79.6	20.4
MME	69.2	30.8	84.9	15.1
RME	69.1	30.9	83.3	16.7
SP523	72.8	27.2	68.3	31.7
RMM	71.6	28.4	80.1	19.9
F10	72.0	28.0	86.0	14.0
OF	71.6	28.4	79.5	20.5
LAO	71.4	28.6	86.9	13.1

[a] The hydrolase screening was based on a color change of the pH-indicator of bromothymol blue. The reactions were carried out using ready to use 96 well MTPs preloaded with an enzymes solution of 20–40 μl containing roughly 1 mg lyophilisate per well. The assay was carried out by the addition of 10 μl of a 10% ethanolic substrate solution into the reaction wells prefilled with 170 μl 7.5 mM potassium phosphate buffer at pH ~7.2 including 1.5 μg pH indicator. The acid formed in the reaction decreased the pH and resulted in a color change from green to yellow, which was monitored by an UV microplate reader. The activity hits—emerging yellow wells—were acidified to pH < 2 with 1 ml 0.1 M HCl and extracted with 500 μl ethyl acetate. After dramatization with diazomethane solution, the ethyl acetate solutions were analyzed on GC for [b]**3**/**11** ratio and [c]**34**/**35** ratio.

Table 3
Optimization of CALB-catalyzed diastereoselective hydrolysis[a]

Entry	*S*/*E*[b]	*C*[c] (%)	Buffer	pH	*T* (°C)	Time (h)	*A* (%) **3**[d]	% **3** Hydrolyzed[e]	Yield[f] (%)
1	10	5	3 mM PKI, 1 M Na_2SO_4	7.2	35	63	61.4	15.3	62.9
2	10	5	100 mM KPI	7.2	35	42	56.9	11.4	64.9
3	10	5	200 mM KPI	7.2	35	42	51.8	11.7	62.2
4	10	5	500 mM KPI	7.2	35	72	52.8	10.3	57.4
5	10	5	100 mM KPI	6.5	35	46	45.7	11.4	52.3
6	10	5	100 mM KPI	7.0	35	42	51.4	11.2	65.9
7	10	5	100 mM KPI	7.5	35	42	52.7	12.7	66.7
8	10	5	100 mM KPI	7.5	40	39	55.7	13.9	64.9
9	10	5	100 mM KPI	7.5	45	24	53.6	14.5	60.8
10	10	5	100 mM KPI	7.5	50	23	47.8	16.4	47.2
11	10	5	10% Sorbitol	7.5	45	42	66.0	13.7	66.4
12	10	5	10% Sorbitol, 1 M Na_2SO_4	7.5	45	42	50.8	12.2	66.7
13	5	10	10% Sorbitol	7.5	45	18	56.3	9.0	76.3
14	5	10	10% Sorbitol, 1 M Na_2SO_4	7.5	45	18	53.1	11.3	70.8
15	5	10	1 M Na_2SO_4	7.5	45	18	53.4	13.2	71.1
16	5	10	10% Sorbitol	7.5	45	15	48.6	13.3	60.3
17	10	10	10% Sorbitol	7.5	45	23	55.0	8.8	65.7
18	20	10	10% Sorbitol	7.5	45	64	76.7	14.0	53.1

[a] CALB was the technical formulation Lipozyme CALB L*K from Novozyme.
[b] wt/wt.
[c] as wt %.
[d] GC area% in the isolated crude product.
[e] Calculated based on area% of **3** and **34** [**34**/(**34** + **3**) × 100].
[f] Estimated with the area% of **3** and the weight of isolated crude product.

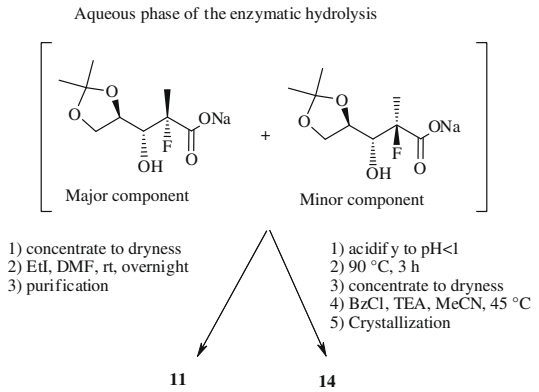


Scheme 6. The complete process for the synthesis of **2**.

age of hydrolyzed **3** increased from 12.7% to 16.4% while the reaction time decreased from 42 h to 23 h. Increasing the enzyme load helped to boost the reaction rate (comparing entries 16–18). The concentration of the substrate also affected the reaction rate. The hydrolysis of **3** was largely caused by its reaction with the medium. This is consistent with the fact that the percentage of **3** hydrolysis was correlated to reaction time, temperature, and pH of the reaction media. A 'placebo' experiment (same conditions without enzyme) further confirmed this conclusion. The reaction was further optimized. As a compromise between the reaction rate, enzyme load, and minimized non-enzymatic hydrolysis, our final reaction conditions were pH 7.2, $T = 35$ °C, $s/e = 6.6$, and 5% substrate concentration in 1.0 M Na_2SO_4 buffer. Under these conditions, the reaction was usually complete within approximately 20 h.

Our overall process is illustrated in Scheme 6. Upon completion of the enzymatic hydrolysis, the reaction mixture was extracted with dichloromethane. The organic solution was dried over anhydrous $MgSO_4$ and filtered to remove residual enzyme. Concentration of the solution afforded a crude mixture that contained **3** and **12/13**. This mixture was stirred in ~60% acetic acid solution at 88 °C for 2 h, and was then concentrated to dryness. The resulting residue was dissolved in acetonitrile and benzoylated with benzoyl chloride and triethylamine. The crude lactone mixture was recrystallized from 2-propanol to afford pure **2** in ~23% overall yield.

It is also worth noting that pure **11** and **14** were prepared, respectively, starting from the aqueous phase of the enzymatic hydrolysis that contained mainly the sodium salts of **35** and **34**. Thus, the solution was concentrated to dryness. The resulting solid was suspended in DMF and reacted with an excess amount of iodoethane. The crude product was a mixture of **3**, **11**, and **12/13** in a GC area ratio of 28:70:2. Pure **11** was isolated from this mixture (Scheme 7). In a different experiment, the aqueous solution was acidified with 31% HCl to pH < 1 and heated at 90 °C for 3 h. The resulting mixture was then concentrated to dryness. The residue was suspended in acetonitrile and reacted with an excess amount of benzoyl chloride in the presence of triethylamine. After workup and purification, pure **14** was isolated.



Scheme 7. Preparation of **11** and **14**.

3. Conclusion

The aldol condensation of **4** and **5** afforded a mixture of at least three isomeric products. The desired isomer **3** was the major component (~60%). Treatment of the crude mixture with CALB led to the hydrolysis of the major by-product **11** to its acid, which was then removed by extraction. This approach allows the production of **2** with only one recrystallization. The mild reaction conditions, coupled with the availability and inexpensiveness of the enzyme, make the process scalable and practical. Our internal cost analysis has concluded that this process is by far the most cost effective one.

4. Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer. IR spectra were recorded on a Nicolet/Magna 550 instrument. Mass spectra were recorded on a Thermo Fisher TSQ Quantum Ultra AM mass spectrometer. Optical rotations were measured at 25 °C using a JASCO P-1010 polarimeter. Melting Point was taken using a TA DSC Q2000 at a heating rate of 10 °C/min. 2,3-Isopropylidene-D-glyceraldehyde **4**[28] and ethyl 2-fluoropropionate **5**[29] were prepared according to literature procedures.

4.1. Aldol condensation of ethyl 2-fluoropropionate 5 and 2,3-*O*-isopropylidene-D-glyceraldehyde 4

To a 1.6 M MeLi solution in ethyl ether (164 mL, 261 mMol) at <−10 °C was slowly added diisopropylamine (26.4 g, 261 mMol). After the addition the mixture was cooled to −78 °C. To the mixture was slowly added a solution of **4** (20 g, 154 mMol) and **5** (28 g, 231 mMol) in toluene (100 mL), while maintaining the temperature below −70 °C. After the addition, the mixture was stirred at −78 °C for 10 min, and was slowly warmed to ambient temperature in 1 h. The resulting mixture was slowly poured into a 28% KH_2PO_4 solution (210 mL) while maintaining the temperature be-

low 20 °C. After stirring briefly, the mixture was transferred to a separatory funnel. The aqueous phase was separated and extracted twice with ethyl acetate (2 × 100 mL). The combined organic solutions were dried over $MgSO_4$, filtered, and concentrated to dryness to give 33.5 g of crude product as a thick oil. GC chromatogram of the product showed a mixture of 56% **3**, 40% **11**, and 4% **12/13**.

4.2. Preparation of (2*R*)-3,5-di-*O*-benzoyl-2-fluoro-2-C-methyl-D-ribono-γ-lactone 2

A 4-neck round-bottomed flask, equipped with a mechanic stirrer, a thermo couple, a pH probe, and a base dosing pump inlet, was charged with 1.0 M sodium sulfate buffer (620 mL), and the crude aldol product (33.0 g) was prepared in Section 4.1. The mixture was stirred at 35 °C to become a clear solution. Next, CALB solution[30] (3.3 g) was added. The mixture was stirred at the temperature, while the pH was maintained at 7.2 by the addition of 1.0 M NaOH solution via a pH pump. After 5 h, additional CALB (1.7 g) was added, and the reaction was continued overnight. The reaction was stopped by the addition of dichloromethane (60 mL). The mixture was cooled to ambient temperature and transferred to a separatory funnel. The aqueous phase was separated and extracted with dichloromethane twice (2 × 50 mL). The combined organic solution was dried over $MgSO_4$ for at least 1 h, filtered, and concentrated to give a thick oil (20.2 g). This oil (20 g) was mixed with acetic acid (120 mL) and water (64 mL). The mixture was stirred at 90 °C for 2 h and then concentrated to dryness. The residue was mixed with toluene (50 mL) and concentrated to dryness. The residue was dissolved in acetonitrile (100 mL). To this solution were added DMAP (0.2 g) and benzoyl chloride (33.7 g, 240 mMol). Triethylamine (26.4 g, 264 mMol) was slowly added while maintaining the temperature <40 °C. After the addition, the mixture was stirred at ambient temperature for 1 h, diluted with ethyl acetate (100 mL) and water (100 mL), transferred to a separatory funnel. The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic solution was washed with saturated $NaHCO_3$ (50 mL), dried over $MgSO_4$, filtered, and concentrated to a thick oil. A mixture of the oil and 2-propanol (160 mL) was heated to ~70 °C, at which point a clear solution formed. The solution was then slowly cooled to ambient temperature overnight. The solid was filtered, washed with 2-propanol, and dried under vacuum at 50 °C for at least 5 h to give 12.7 g (23% overall yield from **4**) pure **2** as an off-white solid: mp 132.9–133.1 °C; $[\alpha]_D^{25} = +124.6$ (*c* 1.0, CH_2Cl_2); FTIR ν_{max} (neat, cm^{-1}): 1790, 1731, 1711; ^1H NMR (500 MHz, $CDCl_3$): δ 8.10–8.05 (m, 2H), 8.00–7.95 (m, 2H), 7.66–7.60 (m, 1H), 7.58–7.53 (m, 1H), 7.51–7.44 (m, 2H), 7.43–7.37 (m, 2H), 5.51 (dd, *J* = 7.3, 17.6 Hz, 1H), 4.99 (ddd, *J* = 3.6, 5.1, 7.3 Hz, 1H), 4.75 (dd, *J* = 3.6, 12.5 Hz, 1H), 4.59 (dd, *J* = 5.2,12.5 Hz, 1H), 1.75 (d, *J* = 25 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.18, 169.00, 165.78, 165.29, 134.15, 133.53, 130.12, 129.73, 129.00, 128.69, 128.52, 128.03, 91.72, 90.23, 77.44, 72.40, 72.28, 62.29, 18.86, 18.66; MS (ESI): *m/z* 373 ($[M+1]^+$).

4.3. Isolation of ethyl (2*R*,3*R*,4*R*)-4,5-*O*-isopropylidene-2-fluoro-3-hydroxy-2-methylvalerate 3

A sample of crude product after the enzymatic hydrolysis (2.0 g) was dissolved in *t*-butyl methyl ether (TBME, 20 mL). To the solution was slowly added hexane until the mixture was cloudy. After the solids formed, hexane was added to maximize precipitation. The mixture was aged at ambient temperature for 2 h. The solid was filtered, washed with hexanes, and dried in the air to give a solid. The solid was mixed with 10 mL TBME. The mixture was heated to become a clear solution, and was then cooled to ambient temperature overnight. The solid was filtered, washed with TBME, and dried under vacuum at 40 °C overnight to give **3** (0.8 g) as a white solid: mp 62.8–64.5 °C; $[\alpha]_D^{25} = +16.7$ (*c* 1.0, CH_2Cl_2); FTIR ν_{max} (neat, cm^{-1}): 3425, 1732; ^1H NMR (500 MHz, $CDCl_3$): δ 4.42–4.17 (m, 2H), 4.14 (dd, *J* = 6.2, 12.4 Hz, 1H), 4.11–4.06 (m, 1H), 4.02 (dd, *J* = 6.0, 8.7 Hz, 1H), 3.94 (dd, *J* = 6.4, 23.9 Hz, 1H), 1.66 (d, *J* = 22.3 Hz, 3H), 1.38 (s, 3H), 1.33 (s, 3H), 1.32 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.49, 170.29, 109.40, 96.09, 94.58, 74.82, 74.76, 74.73, 74.65, 66.29, 66.26, 61.85, 26.31, 25.27, 20.87, 20.68, 14.04; MS (ESI): *m/z* 251 ($[M+1]^+$).

4.4. Preparation of ethyl (2*R*,4*R*)-4,5-isopropylidene-2-fluoro-3-keto-2-methylvalerate 15

A mixture of Dess–Martin reagent (1.7 g, 4.0 mMol) in dichloromethane (50 mL) was stirred at ambient temperature. A couple of drops of pyridine was added to help the dissolution. To the mixture was added a solution of **3** (500 mg, 2.0 mMol) in dichloromethane (10 mL). The resulting mixture was stirred overnight during which time, heavy precipitation formed. The reaction was quenched by the addition of saturated $Na_2S_2O_3$ solution (20 mL) and saturated $NaHCO_3$ solution (20 mL). The organic phase was separated, and the aqueous phase was extracted with dichloromethane. The combined organic solution was dried over $MgSO_4$, filtered, and concentrated to give the crude product, which was purified with flash column chromatography (silica gel, eluting with dichloromethane) to give **15** (320 mg, 64%) as a clear liquid: $[\alpha]_D^{25} = +13.3$ (*c* 1.0, CH_2Cl_2); FTIR ν_{max} (neat, cm^{-1}): 1737 (br); ^1H NMR (500 MHz, $CDCl_3$): δ 4.96 (ddd, *J* = 2.6, 5.6, 8.0 Hz, 1H), 4.35–4.19 (m, 3H), 4.06 (ddd, *J* = 1.5, 5.6, 8.8 Hz, 1H), 1.73 (d, *J* = 22.6 Hz, 3H), 1.46 (s, 3H), 1.40 (s, 3H), 1.30 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 200.45, 200.22, 166.32, 166.12, 111.28, 97.84, 96.32, 77.67, 65.99, 65.95, 62.79, 25.60, 25.06, 20.27, 20.10, 13.92.

4.5. Preparation of ethyl (2*R*,3*S*,4*R*)-4,5-isopropylidene-2-fluoro-3-hydroxy-2-methylvalerate 12

A solution of **15** (100 mg, 0.40 mMol) in ethanol (10 mL) was cooled to 0 °C. To the solution was added $NaBH_4$ (15 mg, 0.40 mMol). The mixture was stirred at 0 °C for 1 h and then concentrated to dryness on a rotavapor. The residue was partitioned between water (2 mL) and TBME (50 mL). The organic solution was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give the crude product (80 mg), which was a mixture of **12** and **3** in a ratio of 98:2. The crude product was purified with flash column chromatography (silica gel, eluting with dichloromethane/ethyl acetate, 8:1) to give **12** (60 mg) as a clear liquid: $[\alpha]_D^{25} = -5.7$ (*c* 1.0, CH_2Cl_2); FTIR ν_{max} (neat, cm^{-1}): 3514 (br), 1761, 1735; ^1H NMR (500 MHz, $CDCl_3$): δ 4.40 (tt, *J* = 1.9, 7.3 Hz, 1H), 4.32–4.19 (m, 2H), 4.08 (dd, *J* = 6.8, 8.0 Hz, 1H), 3.90 (t, *J* = 7.7 Hz, 1H), 3.64–3.57 (m, 1H), 2.97 (d, *J* = 10.9 Hz, 1H), 1.67 (d, *J* = 14.6 Hz, 3H), 1.40 (s, 3H), 1.35 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.83, 170.64, 109.90, 94.88, 93.37, 73.84, 73.63, 72.97, 66.36, 61.98, 26.11, 25.40, 21.87, 21.69, 14.03; MS (ESI): *m/z* 251 ($[M+1]^+$).

4.6. Preparation of ethyl (2*S*,3*R*,4*R*)-4,5-isopropylidene-2-fluoro-3-hydroxy-2-methylvalerate 11

Approximately, one-sixth (170 g) of the aqueous mother liquor (pH ~ 7.0 by paper) from experiment **4.2** was concentrated to dryness on a rotavapor. The residual solid was mixed with toluene (250 mL), and the mixture was concentrated to dryness. The residual solid was mixed with acetonitrile (250 mL), and the mixture was concentrated to dryness. The resulting solid was mixed with DMF (50 mL) and iodoethane. The mixture was stirred at ambient temperature overnight. The mixture was concentrated on a rotava-

por to almost dryness. The resulting solid was partitioned between water (50 mL) and dichloromethane (250 mL). The organic solution was washed with water (10 mL), dried over $MgSO_4$, filtered, and concentrated to give the crude product (1.4 g), which was a mixture of **3** (28%), **11** (70%), and **12/13** (2%). Column chromatography (silica gel, eluting with hexanes/MTBE, 2:1) of the crude product afforded pure **11** (470 mg) as a colorless oil: $[\alpha]_D^{25} = +11.0$ (*c* 1.0, CH_2Cl_2); FTIR ν_{max} (neat, cm^{-1}): 3462 (br), 1738; ^1H NMR (500 MHz, $CDCl_3$): δ 4.34–4.20 (m, 3H), 4.13–3.96 (m, 3H), 2.54 (d, J = 6.2 Hz, 1H), 1.62 (t, J = 15 Hz, 3H), 1.41 (s, 3H), 1.37 (s, 3H), 1.33 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.48, 170.28, 109.25, 6.94, 95.44, 74.59, 74.35, 74.18, 65.93, 65.89, 61.99, 26.42, 25.50, 19.90, 19.71, 14.08; MS (ESI): *m/z* 251 ($[M+1]^+$).

4.7. Preparation of (2*S*)-3,5-di-*O*-benzoyl-2-fluoro-2-C-methyl-D-ribono-γ-lactone 14

Approximately, two-thirds (700 g) of the aqueous mother liquor from experiment **4.2** was acidified to pH < 1 with 31% hydrochloric acid. The mixture was stirred at 90 °C for 3 h, and was then concentrated to dryness on a rotavapor. The residual solid was mixed with toluene (500 mL), and the mixture was concentrated to dryness. This operation was repeated with toluene (300 mL) and acetonitrile (400 mL). The resulting solid was mixed with acetonitrile (150 mL). To the stirring mixture were added 4-dimethylaminopyridine (50 mg) and benzoyl chloride (21 g). The mixture was cooled to 10 °C, and triethylamine (21 g) was added while maintaining the batch temperature below 40 °C. After the addition, the mixture was stirred at 45 °C for 30 min and cooled to room temperature. The mixture was filtered, and the wet cake was washed with acetonitrile. The filtrate was concentrated to dryness to give an oil. The crude oil was mixed with ethyl acetate (~200 mL) and washed with 10% Na_2CO_3 solution, brine, dried over $MgSO_4$, filtered, and concentrated to give crude product (~15 g) as a thick oil. The crude product was purified with flash column chromatography (eluting with dichloromethane), followed by a recrystallization from 2-propanol (50 mL) and drying under vacuum at 45 °C overnight to give pure **14** (4.8 g) as a white solid: mp 95.2–99.4 °C; $[\alpha]_D^{25} = +23.6$ (*c* 1.0, CH_2Cl_2); FTIR ν_{max} (neat, cm^{-1}): 1802, 1735, 1712; ^1H NMR (500 MHz, $CDCl_3$): δ 8.03 (dd, J = 1.2, 8.3 Hz, 2H), 7.67–7.61 (m, 1H), 7.59–7.53 (m, 1H), 7.51–7.45 (m, 2H), 7.45–7.40 (m, 2H), 5.89–5.79 (m, 1H), 4.79–4.69 (m, 2H), 4.69–4.59 (m, 1H), 1.77 (d, J = 20 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.36, 170.16, 165.85, 164.86, 134.25, 133.49, 129.90, 129.82, 129.75, 129.73, 129.04, 128.80, 128.50, 128.47, 128.05, 94.43, 92.93, 78.53, 78.49, 74.62, 74.38, 62.96, 16.93, 16.73; MS (ESI): *m/z* 373 ($[M+1]^+$).

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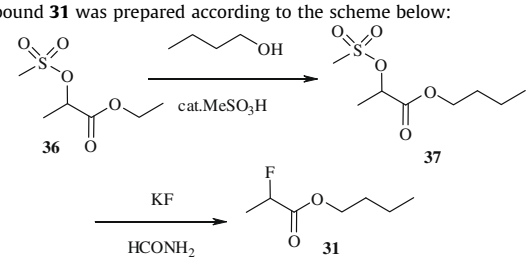
References

1. (a) Clark, J. PCT Int. Appl. WO 2005003147 A2, 2005.; (b) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. *J. Med. Chem.* **2005**, *48*, 5504–5508.
2. (a) Wang, P.; Stec, W.; Clark, J.; Chun, B.; Shi, J.; Du, J. PCT Int. Appl. WO 2006012440 A2, 2006.; (b) Chun, B.; Wang, P. PCT Int. Appl. WO 2006031725 A2, 2006.
3. Ref. 2a,b Mayes, B. A.; Moussa, A. PCT Int. Appl. WO 2007075876 A2, 2007.
4. Axt, S. D.; Sarma, K.; Vitale, J.; Zhu, J.; Ross, B.; Rachakonda, S.; Jin, Q.; Chun, B. PCT Int. Appl. WO 2008045419 A1, 2008.
5. (a) Cedilote, M.; Cleary, T.; Iding, H.; Zhang, P. U.S. Pat. Appl. Publ. US 2008145901 A1, 2008.; (b) Cedilote, M.; Cleary, T.; Zhang, P. U.S. Pat. Appl. Publ. US 2008177079 A1, 2008.
6. For example: (a) Bernrdi, A.; Beretta, M. G.; Colombo, L.; Gennari, C.; Poli, G.; Scolastico, C. *J. Org. Chem.* **1985**, *50*, 4442–4447; (b) Kita, Y.; Yasuda, H.; Tamura, O.; Itoh, F.; Ke, Y. Y.; Tamura, Y. *Tetrahedron Lett.* **1985**, *26*, 5777–5780; (c) Danilova, G. A.; Melnikova, V. I.; Pivnitsky, K. K. *Tetrahedron Lett.* **1986**, *27*, 2489–2490; (d) Kita, Y.; Tamura, O.; Itoh, F.; Yasuda, H.; Kishino, H.; Ke, Y. Y.; Tamura, Y. *J. Org. Chem.* **1988**, *53*, 554–561.
7. Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pirrung, M. C.; White, C. T.; Vanderveer, D. *J. Org. Chem.* **1980**, *45*, 3846–3856.
8. (a) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199–2204; (b) Anh, N. T.; Eisentein, O. *Nov. J. Chim.* **1977**, *1*, 61.
9. To simplify this discussion, the term '*Z*' or '*E*' describes only the relationship between the methyl group and metallated oxygen, and the influence of the heavier fluorine and sulfur atoms on the nomenclature is ignored.
10. Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099–3111.
11. For examples: (a) Nagase, R.; Matsumoto, N.; Hosomi, K.; Hidashi, T.; Funakoshi, S.; Misaki, T.; Tanabe, Y. *Org. Biomol. Chem.* **2007**, *5*, 151–159; (b) Tanabe, Y.; Matsumoto, N.; Funakoshi, S.; Manta, N. *Synlett* **2001**, 1959–1961; (c) Eames, J.; de las Heras, M. A.; Jones, R. V. H.; Warren, S. *Tetrahedron Lett.* **1996**, *37*, 4581–4584; (d) Annunziata, R.; Cinquini, M.; Cozzi, F.; Cozzi, P. G.; Consolandi, E. *Tetrahedron* **1991**, *47*, 7897–7910; (e) Corey, E. J.; Kim, S. S. *J. Am. Chem. Soc.* **1990**, *112*, 4976–4977; (f) Christlieb, M.; Davies, J. E.; Eames, J.; Hooley, R.; Warren, S. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2983–2996; (g) Danheiser, R. L.; Nowick, J. S. *J. Org. Chem.* **1991**, *56*, 1176–1185; (h) Corey, E. J.; Imwinkelried, R.; Pikul, S.; Xiang, Y. B. *J. Am. Chem. Soc.* **1989**, *111*, 5493–5495; (i) Patel, D. V.; VanMiddlesworth, F.; Donaubauer, J.; Gannett, P.; Sih, C. J. *J. Am. Chem. Soc.* **1986**, *108*, 4603–4604.
12. Tnoue, T.; Liu, J. F.; Buske, D. C.; Abiko, A. *J. Org. Chem.* **2002**, *67*, 5250–5256.
13. (a) Shaw, S. J.; Ashley, G. W.; Burlingame, M. A. PCT Int. Appl. WO 070536 A2, 2007.; (b) Ashley, G. W.; Burlingame, M.; Desai, R.; Fu, H.; Leaf, T.; Licari, P. J.; Tran, C.; Abbanat, D.; Bush, K.; Macielag, M. *J. Antibiot.* **2006**, *59*, 392–401; (c) Burlingame, M. A.; Mendoza, E.; Ashley, G. W. *Tetrahedron Lett.* **2004**, *45*, 2961–2964; (d) Santi, D. V.; Ashley, G.; Myles, D. C. WO 012534 A2, 2002.; (e) Ashley, G.; Chan-Kai, I.; Burlingame, M. A. WO 044717 A2, 2000.
14. (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2123–2129; (b) Evans, D. A.; Bartoli, J. *Tetrahedron Lett.* **1982**, *23*, 807–810.
15. Ishihara, T.; Ichihara, K.; Yamanaka, H. *Tetrahedron* **1996**, *52*, 255–262.
16. Prepared following literature procedure (Andrade, C. K. Z.; Rocha, R. O.; Vercillo, O. E.; Silva, W. A.; Matos, R. A. F. *Synlett* **2003**, 2351–2352): 74% yield as white solid: mp 139.5–140.1 °C; FTIR ν_{max} (neat, cm^{-1}): 1797, 1732; ^1H NMR (500 MHz, $CDCl_3$): δ 8.12–8.05 (m, 1H), 7.34–7.27 (m, 2H), 7.27–7.21 (m, 1H), 6.09 (dq, J = 6.6, 48.4 Hz, 1H), 1.72 (dt, J = 12.3, 24.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.6, 169.4, 150.6, 142.8, 127.2, 125.9, 125.2, 116.0, 110.2, 86.7, 85.2, 18.1, 17.9.
17. Ishihara, T.; Ichihara, K.; Yamanaka, H. *Tetrahedron Lett.* **1995**, *36*, 8267–8270.
18. Prepared following the literature procedure in Ref. 11
19. Prepared following the literature procedure in Ref. 12c.
20. Prepared following the literature procedure in Ref. 9
21. One possible cause for the low observed stereoselectivity in these reactions could be the formation of higher percentage of *E*-enolate favored by fluorine–metal interactions. Although fluorine-metal interactions were detected under certain circumstances (e.g., Takemura, H.; Kon, N.; Kotoku, M.; Nakashima, S.; Otsuka, K.; Yasutake, M.; Shimyozu, T.; Inazu, T. *J. Org. Chem.* **2001**, *66*, 2778; Barr, D.; Hutton, K. B.; Morris, J. H.; Mulvey, R. E.; Reed, D.; Snaith, R. *J. Chem. Soc., Chem. Commun.* **1986**, 127; Plenio, H. *ChemBioChem* **2004**, *5*, 650; and the references cited in these papers), no strong evidence for the existence of such interactions in α-fluoroenolates were reported in the literature. Some literature results might have indicated the lack of such interaction or the lack of influence of such an interaction on the *E*/*Z* ratio of enolates. For instance, ^{19}F NMR of the enolates of *N*,*N*-dimethylfluoroacetamide indicated an almost 1:1 mixture of *E*/*Z* isomers even at −85 °C (Welch, J. T.; Eswarakrishnan, S. *J. Org. Chem.* **1985**, *50*, 5403). Another example is that enolates **23** and **24** existed almost exclusively as *Z*-enolates under the reaction conditions (Refs. 15 and 17), indicating that the coordination between F–Li or F–Ti (if it exists) has little effect on the *E*/*Z* ratio.
22. Schmid, C. R.; Bradley, D. A. *Synthesis* **1992**, 587–590.
23. Compound **31** was prepared according to the scheme below:



To a solution of methanesulfonyl ethyl lactate **36** (purchased from Acros, 60 g, 306 mMol) in *n*-butanol (100 mL) was added methanesulfonic acid (1.4 g, 14.6 mMol). The mixture was gradually heated to ~120 °C, and the low boiling point components were distilled out. The distillation was continued until batch temperature reached ~136 °C. More *n*-butanol (30 mL) was added, and the

distillation was continued for ~2 h. GC analysis indicated ~96% conversion. The mixture was cooled to ambient temperature and diluted with ethyl acetate. The solution was washed with saturated Na_2CO_3 solution, brine, dried over $MgSO_4$, filtered, and concentrated to give crude product **37** (68.5 g).A flask equipped with a mechanic stirrer, an addition funnel, and a distillation outlet was charged with KF (70 g, 1200 mMol) and formamide (150 mL). The mixture was heated to 100 °C, and the vacuum for the distillation head was set at 40 mbar. Crude **37** (68 g, 300 mMol) was slowly added from the addition funnel for ~2.5 h. The product formed in the reaction mixture was distilled out and collected (total liquid collected: 36 g). The crude product was purified by fractional distillation, and pure product **31** (24 g, 55% overall yield) was collected at 52–64 °C/40 mbar, as a clear liquid (Fujisawa, H., Takeuchi, Y. *J. Fluorine Chem.* **2002**, *117*, 173–176): ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.98 (dq, J = 61, 8.5 Hz, 1H), 4.18 (t, J = 8.5 Hz, 2H), 1.70–1.60 (m, 2H), 1.57 (dd, J = 29.5, 8.5 Hz, 3H), 1.44–1.34 (m, 2H), 0.93 (t, J = 9 Hz, 3H).

24. Compound **32** was prepared following the same procedure as in Ref. 21, 44% overall yield as a clear liquid: IR ν_{max} (neat, cm^{-1}): 1757, 1737; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.07–4.99 (m, 1.5H), 4.95–4.89 (m, 0.5H), 1.69–1.60 (m, 1H), 1.69–1.60 (m, 1H), 1.56 (ddt, J = 2.7, 5.5, 10.4 Hz, 3H), 1.55–1.46 (m, 1H), 1.43–1.28 (m, 2H), 1.25 (dt, J = 4.4, 8.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.27, 170.09, 86.45, 85.00, 72.34, 37.93, 37.91, 19.92, 19.87, 18.61, 18.59, 18.47, 18.44, 18.29, 18.26, 13.83.
25. Compound **33** was prepared following the same procedure as in Ref. 21, 50% overall yield as a clear liquid: FTIR ν_{max} (neat, cm^{-1}): 1756, 1737; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.00 (dq, J = 6.9, 48.9 Hz, 1H), 4.90–4.84 (m, 1H), 1.66–1.56 (m, 4H), 1.59 (dd, J = 25.0, 10.0 Hz, 3H), 0.93–0.87 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.51, 170.32, 86.43, 84.99, 78.21, 26.46, 26.42, 18.58, 18.40, 9.53.
26. (a) Janes, L. E.; Cimpoia, A.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 9019–9029; (b) Aggarwal, V. K.; Astle, C. J.; Iding, H.; Wirz, B.; Rogers-Evans, M. *Tetrahedron Lett.* **2005**, *46*, 945–947.
27. Anderson, E. M.; Larsson, K. M.; Kirk, O. *Biocatal. Biotransform.* **1998**, *16*, 181–204.
28. Schmid, C. R.; Bryant, J. D.; Dowlatzedah, M.; Philips, J. L.; Prather, D. E.; Schantz, R. D.; Sear, N. L.; Vianco, C. S. *J. Org. Chem.* **1991**, *56*, 4056–4058.
29. Fritz-Langhals, E.; Schultz, G. *Tetrahedron Lett.* **1993**, *34*, 293–296.
30. CALB was a technical solution from BioCatalytics, Cat# SO L-101, Lot# CN02100.