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Chemoenzymatic Synthesis of Each Enantiomer of Orthogonally Protected 4,4-Difluoroglutamic Acid: A Candidate Monomer for Chiral Brønsted Acid Peptide-Based Catalysts

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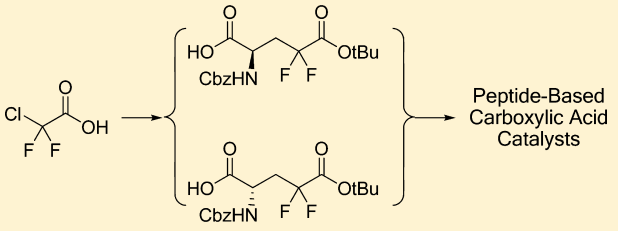
Supporting Information

ABSTRACT: We have accomplished an asymmetric synthesis of each enantiomer of 4,4-difluoroglutamic acid. This α-amino acid has been of interest in medicinal chemistry circles. Key features of the synthesis include highly scalable procedures, a Reformatsky-based coupling reaction, and straightforward functional group manipulations to make the parent amino acid. Enantioenrichment derives from an enzymatic resolution of the synthetic material. Conversion of the optically enriched compounds to orthogonally protected forms allows for the selective formation of peptide bonds. 4,4-Difluoroglutamic acid, in a suitably protected form, is also shown to exhibit enhanced catalytic activity in both an oxidation reaction and a reduction reaction, in comparison to the analogous glutamic acid derivative.



INTRODUCTION

One of the foci of the research in our laboratory is the use of amino acids and peptides as catalysts for synthetic organic transformations.[1] Central to the function of these peptides are specific catalytically active amino acids that, in and of themselves, may mediate the chemical transformation of interest. In some respects, this strategy may be analogous to the roles of catalytic residues in enzymes.[1c,2] Recently, we have been studying the potential catalytic activity of peptides containing carboxylic acid side chains, in the forms of aspartic or glutamic acids, and have found their applications in the epoxidation of olefins[3] and the Baeyer−Villiger oxidation of ketones.[4] In these processes, we have gained evidence that the carboxylic acid side chain may engage in transient conversion to a peracid intermediate (Figure 1a).[5] Notably, aspartic and glutamic acid moieties could also be relevant for the development of chiral Brønsted acid-based catalysts, a subfield of asymmetric catalysis that has seen explosive interest in recent years.[6] Of course, the role of these amino acid side chains in enzymatic proton transfer catalysis, and as H-bond donors or acceptors, is also ubiquitous.[7] The most successful non-enzymatic Brønsted acid catalysts reported to date appear to exhibit pK_a values that are lower than typical aspartic or glutamic acid residues.[8] Therefore, we wondered if 4,4-difluoroglutamic acid, by virtue of the inductive effect of the fluorine atoms,[9] could provide a boost in catalytic activity for Brønsted acid-catalyzed processes, over its proteinogenic glutamic counterpart. Such a residue then might be inserted into peptide libraries, facilitating the search for new chiral Brønsted acid catalysts (Figure 1b).

The compound of interest 4,4-difluoroglutamic acid has been synthesized before, in both racemic and optically pure forms.[10] Excellent as these pioneering precedents are, we felt that we needed more rapid access to large quantities of the material and unambiguously in each enantiomeric form. Additionally, the parent compound 4,4-difluoroglutamic acid *per se* cannot be incorporated into a peptide directly, without being protected orthogonally. This unglamorous, though necessary, aspect was not well-described in the chemical literature.

RESULTS AND DISCUSSION

We were very much attracted to the simplicity and robustness of the racemic synthesis developed by Tsukamoto and Coward[10b] and therefore based our work on this efficient route (Scheme 1). Our synthesis began from chlorodifluoroacetic acid **1**, an inexpensive commercially available acid. Its diethylamide derivative **2** was subjected to a Reformatzky reaction to give an *N*,*O*-acetal **3**. For this step, we found that the original procedure,[11] which heats acid-washed zinc powder with reactants at 65 °C, was not suitable for large-scale synthesis. The zinc insertion into **2** is highly exothermic, yet it does not take place unless being heated to ∼50 °C; consequently, the reaction is prone to thermal runaway. We examined experimentally various physical and chemical methods of zinc activation,[12] including ultrasound, Zn/Cu couple, Zn/Ag couple, TMSCl, and 1,2-dibromoethane as additives. None were particularly effective. Eventually, we found that adding a catalytic amount of $CeCl_3$ allowed the reaction to proceed smoothly at room temperature[13] and gave an acceptable yield of **3** in 53%. This modification should make for a safer procedure and one that is more easily scalable to the desired 20 g level. The *N*,*O*-acetal **3** was subsequently

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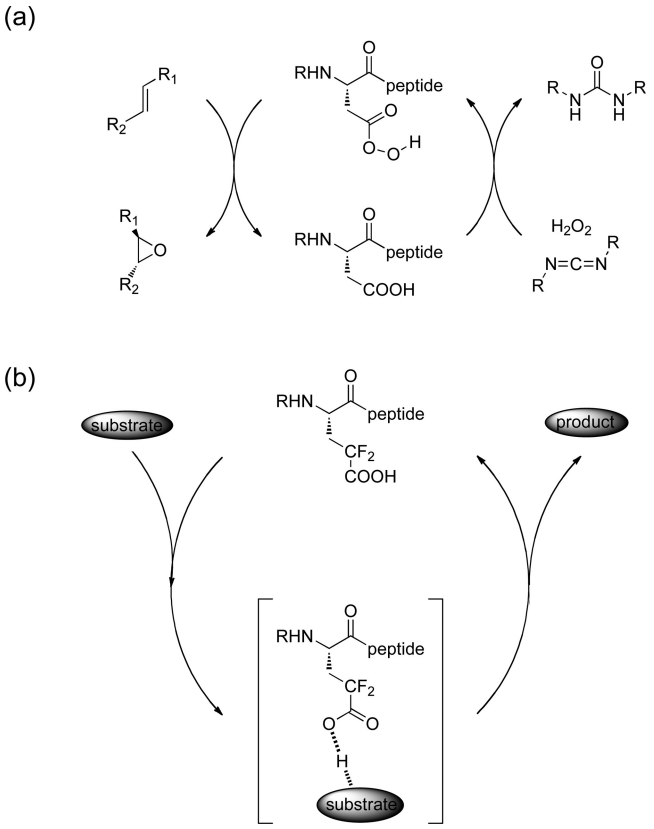
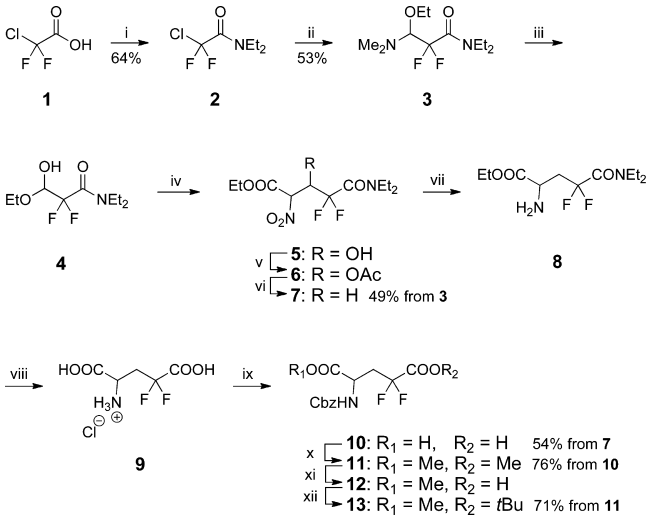


Figure 1. (a) Acid–peracid catalytic cycle. (b) Brønsted acid-catalyzed reactions.

Scheme 1. Synthesis of DL-13[a]



[a]Reagents and conditions: (i) $SOCl_2$, DMF, 50–90 °C; Et_2NH, Et_2O, 0–23 °C; (ii) Zn, $CeCl_3$, Et_2SO_4, DMF, 0–65 °C; (iii) Amberlyst 15 H, EtOH/H_2O, rt; (iv) $CH_2NO_2(COOEt)$, Et_2NH, THF, 0–23 °C; (v) Ac_2O, cat. H_2SO_4, DCM, 55 °C; (vi) $NaBH_4$, THF, 0 °C; (vii) Raney Ni, 1 atm H_2, EtOH, rt; (viii) 12 M HCl, 100 °C; (ix) CbzCl, $NaHCO_3$, H_2O, 0–23 °C; (x) $TMSCHN_2$, PhMe/MeOH, rt; (xi) LiOH, MeOH/H_2O, 0 °C; (xii) *t*BuBr, K_2CO_3, $BnNEt_3Cl$, $AcNMe_2$, 55 °C.

hydrolyzed to give the corresponding hemiacetal **4**, by employing a strong cationic exchange resin Amberlyst 15 as the catalyst.[14] The hemiacetal **4** was then transformed according to the multistep procedure developed by Tsukamoto and Coward to give 4,4-difluoroglutamic acid **9**. We have made two minor modifications to their procedure to suit our needs. First the reaction temperature for the acetylation of intermediate **5** was raised by 30 °C from room temperature, which shortened the reaction time from overnight as reported to half an hour. Secondly we also chose to convert the crude hydrochloride salt **9** to the Cbz-protected amino acid **10** directly, thus bypassing a laborious ion-exchange chromatography step.

From intermediate **10**, the key to the eventual orthogonal protection of 4,4-difluoroglutamic acid was the differential functionalization of the two carboxylic acids. While we were unable to find conditions that could selectively esterify the carboxylic acids in **10**, we found that the dimethyl ester derivative **11** could be readily hydrolyzed to give **12** with total regioselectivity, due to the activating effect of the geminal difluoro group.[15] From **12**, a *tert*-butyl ester was installed to give an orthogonally protected difluoroglutamic acid **13**.[16]

We next endeavored to resolve the racemic amino acid ester **13** into the individual enantiomers by enzymatic hydrolysis (Scheme 2). A survey of the literature revealed that subtilisin, an inexpensive commercially available enzyme, was often employed for this purpose.[17] There were two issues, however, that needed to be addressed. First, the substrate **13** is not appreciably soluble in water, the preferred solvent for enzymatic reaction; second, while the serine protease subtilisin Carlsberg is optimal under alkaline conditions, substrate **13** is sensitive to base. After experimenting unsuccessfully with organic–aqueous biphasic conditions, we eventually decided to develop conditions with a homogeneous reaction medium, which was buffered at neutral pH, and with a water-miscible cosolvent added to improve the solubility of the substrate. From a screen of cosolvents (Table 1), we found that the enzyme was the most active with DMSO as the cosolvent.

Using DMSO as the preferred cosolvent, we next attempted to optimize other reaction variables (Table 2). The amount of the sodium phosphate buffer salts, which was critical to the reaction, was found to be optimal at 1.5 equiv (entries 1–3), yielding the highest selectivity factor. The enzymatic reaction was also very sensitive to the water content of the reaction medium. Using the aforementioned phosphate buffer, we found that a 60:40 ratio of DMSO to aqueous buffer was optimal (entry 1). Higher water content caused lower selectivity, presumably caused by higher background hydrolysis rate (entry 4), while higher DMSO content led to enzyme denaturation (entry 5). Furthermore, we found that the enzyme was functioning more selectively at lower temperatures, albeit at a lower reaction rate (entries 6 and 7), which could be compensated by increasing the catalyst loading (entry 8). Finally, we were pleased to discover that the outcome of the reaction improved when conducted on a larger scale (entry 9). Under the optimized resolution conditions, the amino acid ester **13** was obtained in ≥99% ee and the amino acid **14** was obtained in >90% ee. The recovery of the two compounds was close to the theoretical limit of 50%. The reaction has since been run on multigram scale without difficulty (Scheme 2).

Having obtained the protected amino acid L-**14**, its enantiomer D-**14** could be made by removing the methyl ester in D-**13**. In a quite surprising event, unforeseen by us, the presence of the geminal difluoro group in D-**13** made the *tert*-butyl ester more susceptible to hydrolysis than the methyl ester. We have screened a number of reagents and conditions for the

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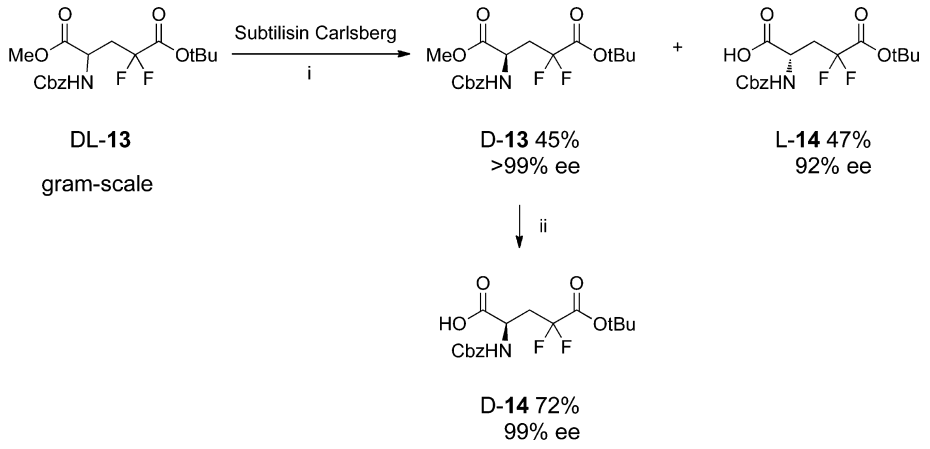
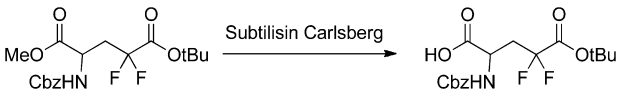
Scheme 2. Enzymatic Kinetic Resolution of DL-13[a]



[a]Reagents and conditions: (i) subtilisin Carlsberg, DMSO/phosphate buffer, pH 7.0, rt; (ii) Me_3SnOH, 1,2-DCE, 70 °C.

Table 1. Screening of Cosolvent for Enzymatic Resolution[a]



13 14

cosolvent	yield[b] %	cosolvent	yield[b] %
*t*BuOH	7	MeCN	3
THF	1	DMF	10
1,4-dioxane	1	DMSO	39
acetone	4	[bmim][BF_4]	12

[a]Conditions: 26 mM substrate in 40:60 pH 7.0 sodium phosphate buffer/cosolvent mixture, 0.77 equiv buffer salts, 73U enzyme/mmol substrate, 37 °C, 20 h. [b]Uncalibrated yield measured by integrating UV 215 nm absorption in HPLC trace.

deprotection of the methyl ester, including LiOH, K_2CO_3, TMSOK, and LiI. None were high yielding and selective for this substrate. Finally, we discovered that trimethyltin hydroxide could remove the methyl ester in high yield and without racemization,[19] thus giving the other enantiomer of the protected amino acid. While the entire synthetic sequence is perhaps lengthy, it only makes uses of four chromatographic purification steps. Most of the intermediates are carried forward without purification.

With a robust and scalable synthesis of the monomeric amino acid in hand, the stage was set for the investigation of its reactivity and potential catalytic utilities. While these studies are underway in our laboratory, we wish to present here two reactions that illustrate the benefit from the increased acidity of the difluoroglutamic acid: the Baeyer–Villiger oxidation of cyclobutanones[20] and the transfer hydrogenation of imines.[21] Both processes are known to be catalyzed by Brønsted acids. In the experiments presented below, we found that the difluoroglutamic catalyst gave substantial rate acceleration over its corresponding identical glutamic counterpart, thus confirming our initial hypothesis. For example, as shown in Scheme 3, we observe the 3-phenyloxetanone **16a** undergoes oxidative ring expansion in the presence of glutamic acid derivative **15a** (10 mol %) to give lactone **16b** in only 28% yield within 18 h. On the other hand, the difluorinated catalyst analogue **15b** delivers the product in 79% yield within the same time frame. By the same token, glyoximine derivative **17** is reduced upon exposure to the Hantszsch ester and catalyst **15a** (10 mol %) to afford phenylglycine derivative **18** in 20% yield (Scheme 4). Once again, catalyst **15b** exhibits greater activity, with **18** produced in 68% yield under the analogous conditions. The products of these reactions, **16b** and **18**, are not surprisingly racemic, given the simplicity of the catalyst. However, once this amino acid is incorporated into peptide

Table 2. Optimizing the Reaction Conditions for Enzymatic Resolution[a]

entry	enzyme loading[b] (U/mmol)	phosphate[c] (equiv)	DMSO (vol %)	temp (°C)	yield[d] (%)	ee of SM (%)	k_{rel}[e]
1	77	1.5	60	37	53	82	16
2	77	3.0	60	37	70	94	7
3	77	6.0	60	37	67	98	12
4	77	1.5	50	37	67	90	7
5	77	1.5	70	37	7	4	3
6	77	1.5	60	30	46	76	40
7	77	1.5	60	23	39	60	58
8	269	1.5	60	23	52	92	40
9[f]	269	1.5	60	23	53	>99	>80
10	0	1.5	60	23	4	ND[g]	

[a]Reactions were run using 13 mM substrate in DMSO/pH 7.0 sodium phosphate buffer mixture under the specified conditions for 12–17 h. Reactions were conducted on 5–10 mg scale. [b]The amount of enzyme used, in enzyme unit, per mmol of substrate. [c]The equivalents of phosphates relative to substrate. [d]Uncalibrated yield measured by integrating UV 215 nm absorption in HPLC trace. [e]Nominal selectivity factor calculated using formula by Kagan.[18] [f]Reaction was run at 100 mg scale. [g]Not determined.

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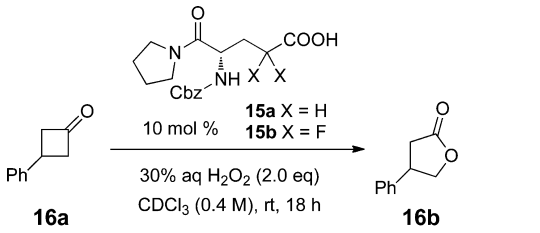
Scheme 3. Difluoroglutamic Acid-Catalyzed Baeyer–Villiger Oxidation



structures, there are ample opportunities for creating highly enantioselective catalysts, as we have demonstrated numerous times before in related systems.[1a−c,3] These preliminary observations confirm our original hypothesis and provide the basis of our ongoing studies with the title compound.

■ CONCLUSIONS

We have developed a robust and scalable synthesis of a key amino acid for studies in asymmetric Brønsted acid catalysis. Of additional significance, 4,4-difluoroglutamic acid derivatives have proven of interest in medicinal chemistry settings,[22] and the synthesis we report here may prove useful to chemists beyond the context of our specific goals. In this context, we have synthesized both enantiomers of orthogonally protected 4,4-difluoroglutamic acids in a protected form suitable for use in peptide coupling. The synthesis yields products with high optical purity and requires few purification steps. Preliminary experiments have demonstrated that the difluoroglutamic acid is a promising strong Brønsted acid catalyst. We are currently engaged in the study of peptides incorporating this amino acid.

■ EXPERIMENTAL SECTION

General Information. Anhydrous $CeCl_3$ was prepared by slowly flame drying the commercially available heptahydrate under vacuum and then cooled to rt under vacuum. Purchased zinc powder was purified by first washing with 2 M HCl, then rinsing with water, acetone, and diethyl ether, and finally flame-dried under vacuum. All other reagents and solvents were used as received without purification, unless otherwise noted.

The enzyme subtilisin Carlsberg (EC 3.4.21.62) from *Bacillus licheniformis* was purchased and was used as received. Enzyme activity was assayed by the manufacturer. One unit enzyme is defined by the manufacturer as the amount of enzyme that can hydrolyze casein to produce 1.0 μmol of tyrosine per minute at pH 7.5 and 37 °C, as detected colorimetrically by Folin–Ciocalteu reagent.

Unless otherwise noted, all reactions were carried out at benchtop conditions without exclusion of air or moisture.

Proton and carbon NMR chemical shifts were recorded relative to TMS and were calibrated to either internally added TMS or residual solvent signal. Fluorine NMR chemical shifts were recorded relative to $FCCl_3$ and were calibrated automatically by the spectrometer using solvent deuterium lock signal. Fluorine NMR spectra were recorded without proton decoupling. All NMR data were acquired at ambient temperature.

Infrared spectra were acquired on a Fourier transform spectrometer equipped with a diamond crystal for attenuated total reflectance measurement. Only peaks in the carbonyl region are reported.

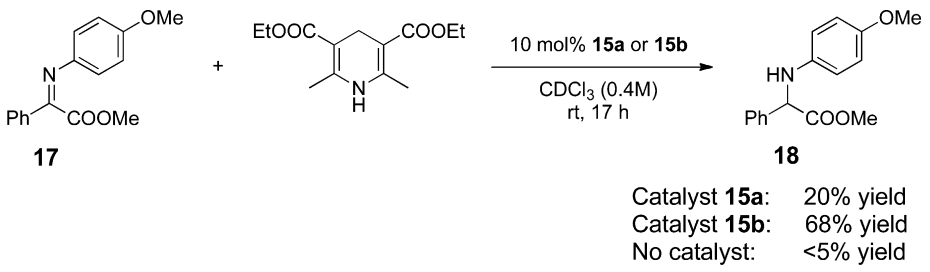
The procedures leading up to the preparation of **13** and **14** are described for representative experiments repeated several times on convenient scale. We have since used this procedure to successfully make more than 20 g of **13** per batch and have resolved DL-**13** on 5 g scale.

2-Chloro-*N*,*N*-diethyl-2,2-difluoroacetamide (2). See Supporting Information for a diagram of the apparatus used for this reaction. Thionyl chloride (65 mL, 0.89 mol) was slowly added from an addition funnel to a solution of chlorodifluoroacetic acid (50 mL, 0.58 mol) in dry DMF (400 mL). The solution was then heated in an oil bath to 50 °C with stirring. Chlorodifluoroacetic chloride was produced as a gas (bp 26 °C), which was condensed via a dry ice cooled condenser to another flask containing a solution of diethylamine (210 mL, 2.02 mol) in dry diethyl ether (500 mL) at 0 °C. The temperature of the oil bath was gradually increased from 50 to 90 °C to maintain a steady rate of chlorodifluoroacetic chloride production. After 2 h, the oil bath was removed. The reactions were left stirring overnight. Afterward, the ethereal solution was filtered. The filtrate was washed with water (3 × 500 mL) and 1 M HCl (3 × 500 mL), dried over magnesium sulfate, and then evaporated in vacuo to dryness to give the product as a pale yellow-green liquid (68.7 g, 64%). Proton and fluorine NMR spectra were recorded in $CDCl_3$ and were consistent with literature data.[11]

3-(Dimethylamino)-3-ethoxy-*N*,*N*-diethyl-2,2-difluoropropanamide (3). [*Caution: this is a highly exothermic reaction, and therefore it is very important to monitor the reaction temperature closely and apply cooling swiftly if the temperature rises rapidly above 70 °C in order to avoid thermal runaway.*] A solution of diethyl sulfate (50 mL, 374 mmol) in dry DMF (90 mL) was heated under nitrogen at 90 °C for 2 h and then cooled with an ice bath to <10 °C. To the solution α-chloroamide **2** (34.7 g, 187 mmol), zinc powder (25.0 g, 375 mmol), and finally anhydrous cerium trichloride (1.86 g, 7.55 mmol) were added. After stirring under nitrogen at <10 °C for 25 min, the reaction was taken out of the ice bath. As the reaction mixture warmed up to rt, the reaction gradually became highly exothermic. An ice bath was used to cool the reaction mixture when necessary, such that the temperature never exceeded 60 °C. After the exotherm subsided, the reaction mixture was further stirred under nitrogen at rt for 2 h. After adding more anhydrous cerium trichloride (516 mg, 2.09 mmol) to the reaction mixture, it was heated in a 65 °C oil bath for 1 h and then filtered to give a viscous dark brown opaque solution. The solution was continuously extracted with pentane (250 mL) overnight.

The extract was evaporated in vacuo to remove pentane. To the residual liquid was added saturated aqueous sodium bicarbonate solution (100 mL) and diethyl ether (100 mL). The resulting heterogeneous mixture was filtered. The organic layer was separated

Scheme 4. Difluoroglutamic Acid-Catalyzed Transfer Hydrogenation



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out. The aqueous layer was extracted with diethyl ether (2 × 100 mL). The combined ethereal extract was washed with water (2 × 50 mL) and brine (50 mL), dried over sodium sulfate, and concentrated to give a dark red viscous oil as the crude product. The crude product was distilled under vacuum (1.9 Torr). Product **3** was collected at 80 °C as a clear colorless liquid (24.8 g, 53%). Proton and fluorine NMR spectra were recorded in $CDCl_3$ and were consistent with literature data.[11]

4-Ethoxycarbonyl-*N*,*N*-diethyl-2,2-difluoro-4-nitrobutanamide (7). To a solution of *N*,*O*-acetal **3** (41.5 g, 164 mmol) in ethanol (370 mL) was added Amberlyst 15 resin (33.0 g, 4.99 mequiv H^+/g) and water (6.0 mL, 333 mmol). After stirring at rt overnight, the mixture was filtered. The resulting brown solution was concentrated in vacuo to dryness and then redissolved in diethyl ether (300 mL). A copious amount of anhydrous potassium carbonate was added to the solution, which was then vigorously stirred for 3 h. The mixture was filtered through Celite and then concentrated in vacuo to give the crude hemiacetal **4** as a clear yellow oil (36.7 g).

The oil **4** was dissolved in dry THF (200 mL). To the solution in an ice bath, ethyl nitroacetate (18.5 mL, 167 mmol) and diethylamine (24.0 mL, 230 mmol) were added. The solution was stirred at rt under nitrogen overnight. Afterward, the volatile components in the reaction solution were evaporated in vacuo. In an ice bath, to the residual oil was added 0.6 M HCl solution (400 mL). The mixture was extracted with EtOAc (3 × 200 mL). The organic extract was washed with 0.5 M HCl solution (3 × 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give crude alcohol **5** as a viscous red oil (50.9 g). [*N.B.: thorough removal of diethylamine from the crude product mixture by vacuum drying is critical for the subsequent acetylation reaction.*]

The oil **5** was dissolved in dry DCM (150 mL). To it acetic anhydride (20.5 mL, 211 mmol) and 18 M sulfuric acid (0.45 mL, 8.1 mmol) were added. The resulting solution was heated to reflux (55 °C). After 30 min, the reaction solution was poured into water (300 mL) and extracted with EtOAc (3 × 200 mL) and then DCM (200 mL). The combined organic phase was washed with brine (3 × 100 mL), dried over $MgSO_4$, and concentrated in vacuo to give acetylated alcohol **6** as a viscous red oil.

The oil **6** was dissolved in dry THF (750 mL). To the solution at 0 °C, sodium borohydride (6.32 g, 165 mmol) was added. The resulting solution was stirred under nitrogen at 0 °C. After 2 h, the reaction solution was concentrated in vacuo to dryness. The residual material was redissolved in EtOAc (200 mL). To the solution was added carefully 1 M HCl (300 mL). After vigorous stirring, the organic phase was separated out. The aqueous phase was extracted with EtOAc (2 × 200 mL). The combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give crude product **7** as a viscous yellow oil, which was purified by flash column (i.d. 13 cm × L 26 cm) chromatography on silica gel, eluting with 16:100 to 20:100 EtOAc/hexanes. The purified product was a clear colorless oil (23.9 g, 49% from **3**). Proton and fluorine NMR spectra were recorded in $CDCl_3$ and were consistent with literature data.[10b]

***N*-Benzyloxycarbonyl-4,4-difluoro-DL-glutamic Acid (10).** A slurry of Raney nickel in water (35 mL wet volume) was centrifuged. The supernatant water was removed. The metallic residue was resuspended in absolute ethanol (35 mL). The centrifugation−suspension cycle was repeated two more times to obtain a suspension of Raney nickel in ethanol. The suspension was added to a solution of nitro compound **7** (23.9 g, 80.7 mmol) in absolute ethanol (200 mL). The mixture was stirred at rt overnight under a balloon of hydrogen. The reaction mixture was then filtered through Celite to give a cloudy green solution, which was evaporated in vacuo to dryness to give crude amide **8** as a viscous green oil.

The oil **8** was dissolved in 12 M HCl (300 mL) and then stirred at 100 °C overnight. Hydrochloric acid was removed by vacuum distillation, at temperature not exceeding 100 °C. A yellow oil was obtained after the distillation. A small amount of water was added to dissolve the oil. The solution was then evaporated in vacuo to dryness. This step was repeated several times to remove as much HCl as possible. In the end, a viscous yellow green oil was obtained as the crude difluoroglutamic acid **9**.

The crude **9** was dissolved in water (550 mL) at 0 °C. To it was added sodium bicarbonate (54.5 g, 647 mmol) portionwise. To the alkaline suspension, benzyl chloroformate (24 mL, 163 mmol) was then added. The suspension was stirred at rt overnight. Afterward, the reaction suspension was then washed with chloroform (100 mL × 5). The aqueous phase was acidified in an ice bath with 12 M HCl (70 mL) and then extracted with EtOAc (150 mL × 4). The aqueous phase was saturated with brine (250 mL) and then extracted again with EtOAc (150 mL). The combined organic extract was washed with brine (100 mL), dried over magnesium sulfate, and evaporated in vacuo to dryness to give a clear yellow oil. The oil gradually solidified under vacuum to give dicarboxylic acid **10** as a pale red-orange solid (13.74 g, 54% from **7**). Proton and fluorine NMR spectra were recorded in CD_3OD. The product was ∼90% pure by visual inspection of its NMR spectra. The compound's proton and fluorine NMR spectra, as well as its mass spectrum, were consistent with literature data.[15]

Dimethyl *N*-Benzyloxycarbonyl-4,4-difluoro-DL-glutamate (11). To a solution of dicarboxylic acid **10** (5.95 g, 18.8 mmol) in 5:2 toluene/methanol (175 mL) was added dropwise an excess amount (∼25 mL) of a 2 M solution of trimethylsilyldiazomethane in hexanes, until gas evolution ceased, and the reaction solution stayed golden yellow in color. Acetic acid was then added dropwise to the reaction solution, until gas evolution ceased and the golden yellow color disappeared. The reaction solution was concentrated to give a viscous oil, which was then diluted with ethyl acetate (300 mL), washed with saturated aqueous solution of sodium bicarbonate (100 mL) and brine (100 mL), dried over magnesium sulfate, and evaporated to give a viscous oil. The oil was purified by flash chromatography on silica gel (8−66% ethyl acetate in hexanes) to give diester **11** as a clear colorless oil, which gradually solidified into a white waxy solid (4.89 g, 76%). R_f 0.62 (2:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40−7.28 (m, 5H), 5.44 (d, J = 8.3 Hz, 1H), 5.13, 5.10 (ABq, J = 12.2 Hz, 2H), 4.61 (td, J = 7.8, 4.8 Hz, 1H), 3.774 (s, 3H), 3.766 (s, 3H), 2.79 (qd, J = 15.5, 4.7 Hz, 1H), 2.64 (qd, J = 15.4, 7.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 164.2 (t, $^2J_{CF}$ = 32.3 Hz), 155.6, 136.0, 128.5, 128.2, 128.1, 114.8 (t, $^1J_{CF}$ = 251.5 Hz), 67.1, 53.4, 52.8, 48.9 (t, $^3J_{CF}$ = 4.9 Hz), 36.1 (t, $^2J_{CF}$ = 23.5 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ −104.12 (t, $^3J_{HF}$ = 15.6 Hz). IR (FT-ATR): 1769, 1732, 1685 cm^{-1}. MS (ESI) calculated for $[C_{15}H_{17}F_2NO_6 + H]^+$ 346.1102; found 346.1105.

5-*tert*-Butyl 1-Methyl *N*-benzyloxycarbonyl-4,4-difluoro-DL-glutamate (DL-13). To a solution of diester **11** (4.89 g, 14.2 mmol) in methanol (180 mL) at 0 °C was added a solution of lithium hydroxide monohydrate (0.606 g, 14.2 mmol) in water (60 mL) portionwise. After stirring for 2 min, 2 M hydrochloric acid (25 mL) and brine (350 mL) were added to the reaction solution, which was then extracted with EtOAc (3 × 150 mL). The organic phase was washed with water (2 × 100 mL) and brine (100 mL), dried over magnesium sulfate, and evaporated to dryness to give crude monoester **12** as a pale yellow oil, which was then dissolved in *N*,*N*-dimethylacetamide (110 mL). Benzyltriethylammonium chloride (3.26 g, 14.2 mmol), potassium carbonate (49 g, 351 mmol), and *tert*-butyl bromide (79 mL, 687 mmol) were added to the solution. The mixture was stirred at 55 °C for 18 h. Afterward, the reaction mixture was partitioned between water (500 mL) and ethyl acetate (500 mL). The organic phase was washed with water (3 × 250 mL) and brine (100 mL), dried over magnesium sulfate, and evaporated to dryness to give a yellow oil, which was purified by flash chromatography on silica gel (6−50% ethyl acetate in hexanes), to afford *tert*-butyl ester DL-**13** as a white solid (3.92 g, 71% from **11**). R_f 0.39 (3:1 hexanes/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38−7.27 (m, 5H), 5.51 (d, J = 8.1 Hz, 1H), 5.10 (s, 2H), 4.61 (td, J = 7.9, 4.7 Hz, 1H), 3.75 (s, 3H), 2.70 (qd, J = 15.6, 4.7 Hz, 1H), 2.59 (qd, J = 15.4, 7.6 Hz, 1H), 1.49 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.1, 162.4 (t, $^2J_{CF}$ = 31.4 Hz), 155.7, 136.1, 128.6, 128.3, 128.2, 114.8 (t, $^1J_{CF}$ = 252.3 Hz), 85.2, 67.3, 53.0, 49.3 (t, $^3J_{CF}$ = 4.4 Hz), 36.1 (t, $^2J_{CF}$ = 23.6 Hz), 27.7. ^{19}F NMR (376 MHz, $CDCl_3$): δ −103.9,

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−104.3 (ABX_2, $^2J_{FF}$ = 266 Hz, $^3J_{HF}$ = 15.7 Hz). IR (FT-ATR): 1750, 1713, 1693 cm^{-1}. MS (ESI) calculated for $[C_{18}H_{23}F_2NO_6 + H]^+$ 388.1572; found 388.1582.

Kinetic Resolution of DL-13. To a solution of DL-**13** (1.00 g, 2.58 mmol) in DMSO (120 mL), sodium phosphate buffer (pH 7.0, 400 mM, 10 mL), water (50 mL), and a solution of subtilisin (59.9 mg) in water (20 mL) were added sequentially. An ice bath was used to keep the reaction mixture at rt during the addition process. The solution was stirred at rt for 13 h, then acidified with 10% citric acid solution in water (300 mL), and saturated with brine (300 mL). The solution was extracted with ethyl acetate (4 × 200 mL). The organic extract was washed with water (5 × 300 mL) and brine (300 mL), dried over sodium sulfate, and evaporated to dryness to yield a clear colorless oil. The oil was purified by flash column chromatography. The column was first eluted with 75:25:1 hexanes/EtOAc/AcOH to collect 5-*tert*-butyl 1-methyl *N*-benzyloxycarbonyl-4,4-difluoro-D-glutamate (D-**13**) and then with 50:50:1 hexanes/EtOAc/AcOH to collect *N*-benzyloxycarbonyl-5-*tert*-butyl-4,4-difluoro-L-glutamic acid (L-**14**).

5-*tert*-Butyl 1-Methyl *N*-benzyloxycarbonyl-4,4-difluoro-D-glutamate (D-13). D-**13** was obtained as a clear colorless oil (447 mg, 45%), which gradually solidifies into a translucent white solid upon standing. Its mass spectrum, proton, carbon, and fluorine NMR spectra were identical to those of the racemic compound DL-**13**. R_f (75:25:1 hexanes/EtOAc/AcOH) 0.34. IR (FT-ATR): 1749, 1725, 1714 cm^{-1}. $[\alpha]_D^{20}$ = −1.4° (*c* = 1.0, chloroform). Chiral HPLC (Chiralpak OJ-H, 250 × 4.6 mm, 85:15 hexanes/EtOH, 1.0 mL/min, 25 °C, UV 215 nm): t_L = 10.4 min, t_D = 13.4 min, >99% ee.

***N*-Benzyloxycarbonyl-5-*tert*-butyl-4,4-difluoro-L-glutamic Acid (L-14).** L-**14** was obtained as a clear colorless oil. The oil was submerged in hexane and then scratched with a glass rod rapidly. The oil quickly solidifies into a white solid (451 mg, 47%). R_f (50:50:1 hexanes/EtOAc/AcOH) 0.34. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.03 (s, br), 7.38−7.26 (m, 5H), 5.56 (d, *J* = 8.3 Hz, 1H), 5.11, 5.10 (ABq, *J* = 12.3 Hz, 2H), 4.62 (td, *J* = 8.0, 4.4 Hz, 1H), 2.74 (qd, *J* = 15.6, 4.3 Hz, 1H), 2.62 (qd, *J* = 15.3, 7.7 Hz, 1H), 1.48 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 175.0, 162.4 (t, $^2J_{CF}$ = 31.4 Hz), 156.1, 135.8, 128.5, 128.3, 128.2, 114.6 (t, $^1J_{CF}$ = 252.3 Hz), 85.4, 67.5, 49.1 (t, $^3J_{CF}$ = 3.9 Hz), 35.6 (t, $^2J_{CF}$ = 23.6 Hz), 27.6. ^{19}F NMR (376 MHz, $CDCl_3$): δ −103.7, −104.0 (ABX_2, $^2J_{FF}$ = 266 Hz, $^3J_{HF}$ = 15.7 Hz). IR (FT-ATR): 3294, 1758, 1746, 1727, 1716, 1693 cm^{-1}. MS (ESI) calculated for $[C_{17}H_{21}F_2NO_6 + H]^+$ 374.1415, found 374.1419. $[\alpha]_D^{20}$ = +0.2° (*c* = 1.0, chloroform). Chiral HPLC (Chiralcel OD, 250 × 4.6 mm, 950:50:1 hexanes/iPrOH/TFA, 1.0 mL/min, 25 °C, UV 215 nm): t_L = 20.8 min, t_D = 25.6 min, 92% ee.

***N*-Benzyloxycarbonyl-5-*tert*-butyl-4,4-difluoro-D-glutamic Acid (D-14).** To a solution of D-**13** (447 mg, 1.15 mmol) in dry 1,2-dichloroethane (40 mL) under nitrogen at 70 °C, trimethyltin hydroxide (642 mg, 3.48 mmol) was added. After stirring for 3 h, the reaction solution was diluted with EtOAc (120 mL) and washed successively with 10% citric acid solution in water (3 × 50 mL) and brine (50 mL), dried over magnesium sulfate, and then evaporated to dryness to afford a clear colorless oil. The oil was purified by flash chromatography (50:50:1 hexanes/EtOAc/AcOH). After purification, the afforded product was submerged under hexanes and then scratched rapidly with a glass rod until all of the product solidified. The hexanes solvent was evaporated in vacuo to give the product D-**14** as a white powder (312 mg, 72%). Its mass spectrum, infrared spectrum, proton, carbon, and fluorine NMR spectra were identical to those of its enantiomer L-**14**. $[\alpha]_D^{20}$ = −1.1° (*c* = 1.0, chloroform). Chiral HPLC (Chiralcel OD, 250 × 4.6 mm, 950:50:1 hexanes/iPrOH/TFA, 1.0 mL/min, 25 °C, UV 215 nm): t_L = 21.2 min, t_D = 24.7 min, 99% ee.

(*S*)-4-Benzyloxycarbonylamino-5-oxo-5-(1-pyrrolidinyl)-pentanoic Acid (15a). A solution of Cbz-Glu(O*t*Bu)-OH (501 mg, 1.47 mmol), HOBt hydrate (583 mg, 3.73 mmol), EDC·HCl (724 mg, 3.70 mmol), and pyrrolidine (freshly distilled, 330 μL, 3.72 mmol) in DCM (5 mL) was heated by microwave at 60 °C for 20 min. Afterward, the solution was diluted with EtOAc (100 mL), washed with 10% aqueous citric acid solution (3 × 30 mL), saturated aqueous sodium bicarbonate solution (3 × 30 mL), and brine (30 mL), dried over sodium sulfate, and evaporated in vacuo to dryness to give a clear colorless oil, which was then dissolved in 1:1 DCM/TFA (5 mL) and stirred at rt. After 1 h, all volatile components in the reaction solution was evaporated in vacuo to give a pale yellow oil as the crude product, which was purified by flash column chromatography (85:15:1 EtOAc/hexanes/AcOH) on silica gel to afford the product as a white foam (456 mg, 93%). R_f (85:15:1 EtOAc/hexanes/AcOH) 0.23. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37−7.27 (m, 5H), 6.06 (d, *J* = 8.5 Hz, 1H), 5.09 (s, 2H), 4.59 (td, *J* = 8.8, 3.9 Hz, 1H), 3.65−3.34 (m, 4H), 2.55−2.38 (m, 2H), 2.11−1.77 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 176.7, 170.4, 156.6, 136.4, 128.6, 128.2, 128.0, 67.0, 51.8, 46.7, 46.3, 29.6, 27.6, 26.0, 24.2. IR (FT-ATR): 3278, 1702, 1605 cm^{-1}. $[\alpha]_D^{20}$ = +3.5° (*c* = 0.9, chloroform). MS (ESI) calculated for $[C_{17}H_{22}N_2O_5 + H]^+$ 335.1607; found 335.1605.

(*S*)-4-Benzyloxycarbonylamino-2,2-difluoro-5-oxo-5-(1-pyrrolidinyl)pentanoic Acid (15b). A solution of L-**14** (0.38 g, 1.0 mmol), HOBt hydrate (187 mg, 1.20 mmol), EDC·HCl (233 mg, 1.19 mmol), and pyrrolidine (212 μL, 2.39 mmol) in DCM (5 mL) was stirred overnight at rt. Afterward, the solution was diluted with EtOAc (100 mL), washed with 10% aqueous citric acid solution (2 × 30 mL), saturated aqueous sodium bicarbonate solution (30 mL), and brine (30 mL), dried over sodium sulfate, and evaporated in vacuo to dryness to give a clear colorless oil. The oil was purified by flash column chromatography (8:2 hexanes/acetone) to give a clear colorless oil (398 mg, 92%), a portion of which (299 mg) was then dissolved in 30:1:1 TFA/Et_3SiH/H_2O (9.6 mL) and stirred at rt. After 4 h, all volatile components in the reaction solution was evaporated in vacuo. The residual oil was purified by reverse phase chromatography on C_{18} column (2−50% MeCN in H_2O, with 0.1% TFA in both eluents). The product fractions were evaporated in vacuo to give a white solid. In order to remove the remaining trace amount of TFA, the product was dissolved in water and evaporated in vacuo for three times, followed by addition and evaporation of toluene for three times. It was then dissolved in water/MeOH and lyophilized to give a white powder (202 mg, 73% overall). The final product was verified to be free from TFA by fluorine NMR. The compound exhibits multiple conformers in NMR at rt, the dominant major conformer is as follows: ^{1}H NMR (500 MHz, DMSO): δ 14.7 (br, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.39−7.28 (m, 5H), 5.02 (s, 2H), 4.55 (td, *J* = 8.0, 5.3 Hz, 1H), 3.52 (dt, *J* = 9.8, 6.8 Hz, 1H), 3.43 (dt, *J* = 10.0, 6.8 Hz, 1H), 3.32−3.22 (m, 2H), 2.62−2.46 (m, 1H), 2.43−2.28 (m, 1H), 1.92−1.82 (m, 2H), 1.80−1.70 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.6, 164.8 (br), 155.9, 135.9, 128.3, 127.9, 127.7, 115 (v br), 67.0, 47.2, 46.8, 46.5, 36.5 (t, $^2J_{CF}$ = 23.1 Hz), 25.6, 23.8. ^{19}F NMR (376 MHz, DMSO): δ −103.5, −104.3 (ABX_2, $^2J_{FF}$ = 256 Hz, $^3J_{HF}$ = 16.8 Hz). IR (FT-ATR): 3277, 1708, 1610 cm^{-1}. $[\alpha]_D^{20}$ = −5.1° (*c* = 1.0, chloroform). MS (ESI) calculated for $[C_{17}H_{20}F_2N_2O_5 + H]^+$ 371.1419; found 371.1422.

Baeyer−Villiger Oxidation of Cyclobutanone. To a solution of cyclobutanone **16a**[23] (28.0 mg, 0.192 mmol) and catalyst **15b** (7.1 mg, 0.019 mmol) in $CDCl_3$ (0.50 mL), 30% aqueous hydrogen peroxide (39.1 μL, 0.383 mmol) was added. The mixture was vigorously stirred at rt for 18 h, before bromoform (16.8 μL, 0.192 mmol) was added to the reaction mixture. The reaction mixture was then examined by proton NMR. The product yield (79%) was determined by comparing the integration of the internal standard bromoform (singlet at 6.82 ppm) and the carboxylate methylene protons of the lactone product **16b** (dd at 4.67 and 4.27 ppm). The proton NMR spectrum of the product was consistent with literature data.[23] Chiral HPLC (Chiralpak IA, 250 × 4.6 mm, 99:1 hexanes/EtOH, 1.0 mL/min, 25 °C, UV 215 nm): *t* = 41.5, 48.4 min, racemic.

Transfer Hydrogenation of Imine. A mixture of imine **17**[24] (≥9:1 *Z*/*E* mixture of isomers, 53.4 mg, 0.198 mmol), diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (55 mg, 0.22 mmol), and a solution of catalyst **15b** (7.4 mg, 0.020 mmol) in $CDCl_3$ (0.50 mL) was stirred at rt. The mixture gradually became a homogeneous solution as it stirred overnight for 17 h. An NMR internal standard 1,3,5-trimethoxybenzene (11.8 mg, 0.0702 mmol) was added to the reaction solution, which was then examined by proton NMR. The product yield (68%) was determined by comparing the integration of

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the aryl protons of the internal standard (singlet at 6.08 ppm) and the *p*-methoxyphenyl protons of the product **18** (doublet at 6.53 ppm). The proton NMR spectrum of the product was consistent with literature data.[14] Chiral HPLC (Chiralpak IB, 250 × 4.6 mm, 90:10 hexanes/iPrOH, 0.5 mL/min, 25 °C, UV 245 nm): t = 14.5, 15.1 min, racemic.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra and HPLC traces for compounds **11**−**15**; a diagram of the apparatus to prepare compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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