A Scalable Synthesis of the Difluoromethyl-*allo*-threonyl Hydroxamate-Based LpxC Inhibitor LPC-058

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Abstract

The difluoromethyl-*allo*-threonyl hydroxamate-based compound **LPC-058** is a potent inhibitor of UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase (LpxC) in Gram-negative bacteria. A scalable synthesis of this compound is described. The key step in the synthetic sequence is a transition metal/base-catalyzed aldol reaction of methyl isocyanoacetate and difluoroacetone, giving rise to 4-(methoxycarbonyl)-5,5-disubstituted 2-oxazoline. A simple NMR-based determination of enantiomeric purity is also described.

Graphical abstract

The alarming rise of antimicrobial resistance[1–3] of Gram-negative pathogens has become a serious threat to public health and highlights the urgent need for antibiotics with novel modes of action. The UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase (LpxC) is a novel, validated antibiotic target against Gram-negative pathogens.[4] The enzyme catalyzes the committed step in the biosynthesis of lipid A, the membrane anchor of lipopolysaccharide and the predominant lipid component of the outer leaflet of the Gram-negative outer membrane. We have previously reported the design of potent LpxC inhibitors containing a difluoromethyl-*allo*-threonyl hydroxamate headgroup.[4] This series of compounds, represented by **LPC-058**, displays broad-spectrum antibiotic activity against antibiotic resistant Gram-negative pathogens, thereby highlighting the therapeutic potential

of this class of LpxC inhibitors. Herein, we describe a scalable and stereoselective synthesis of **LPC-058**, which sets the stage for preclinical *in vivo* studies of this novel series.[4]

The initial medicinal chemistry route for the synthesis of **LPC-058** is shown in Scheme 1. The diacetylene carboxylic acid **4** was prepared in two steps from methyl-4-ethynyl benzoate (**1**). The first step employed modified Glaser reaction[5,6] conditions under which an excess of 4-ethylnylbenzenamine **2** (5 equiv) and copper(II) acetate (2 equiv) in the presence of piperidine in dichloromethane (DCM) provided **3** in a low yield (35%).[7] Subsequent alkaline hydrolysis of ester **3** provided the diacetylene carboxylic acid **4** in quantitative yield.[7]

We recently published the medicinal chemistry route for (2*S*,3*S*)-3-difluoromethylthreonine methyl ester **11**, which was generated in six steps starting from (*S*)-1-methyl-4-(methylsulfinyl)benzene (Scheme 1).[4] Our synthetic strategy for amino ester **11** was adapted from a published procedure for the corresponding monofluoro analogue.[8] The copper(I)-catalyzed [2+3] cycloaddition reaction between methyl isocyanoacetate and (*S*)-1,1-difluoro-3-(*p*-tolylsulfinyl)propan-2-one (**6**) provided oxazoline **7** as a mixture of diastereomers. The desired diastereoisomer of oxazoline **7** (i.e., 4*S*,5*S*,*S_S*) is the less polar spot on TLC with $R_f \sim 0.35$ [EtOAc/hexanes (2:3)] and was isolated by flash chromatography [EtOAc/hexanes (0–40%)] as the major product in 45% yield. Oxazoline **7** was readily hydrolyzed in a chloroform/water mixture at room temperature to give the amido ester **8** in near quantitative yield. Sulfoxide **8** was reduced to corresponding sulfide **9** by using trifluoroacetic anhydride with sodium iodide in acetone at –40 °C.[9] Desulfurization of **9** with a Raney nickel–sodium hypophosphite system provided the (2*S*,3*S*)-3-difluoromethyl-threonine derivative (**10**), which upon mild acidic hydrolysis afforded (2*S*, 3*S*)-3-difluoromethylthreonine methyl ester **11**.[10]

Amino ester **11** was reacted with diacetylene carboxylic acid **4** under standard coupling conditions to give amide ester **12**. Treatment of **12** with hydroxylamine under alkaline conditions afforded desired hydroxamate **LPC-058**.[4]

A number of factors made this approach unsuitable for delivering substantial quantities (>100 g) of **LPC-058**. The major drawback of this strategy is the tedious chromatographic separation of oxazoline **7** from its diastereomers. Furthermore, chiral sulfoxide **5** is very expensive or has to be prepared.[11] Finally, the copper-catalyzed cross-coupling of methyl 4-ethynylbenzoate **1** with excess 4-ethylnylbenzenamine **2** is complicated by the formation of homocoupled products that require tedious chromatographic separation. Therefore, we directed our attention to developing efficient and scalable routes to the key intermediates (2*S*,3*S*)-3-difluoromethylthreonine methyl ester **11** and diacetylene carboxylic acid **4**.

Synthesis of unsymmetrical diynes has recently been the subject of extensive research. A survey of the literature indicates that diynes can be formed under various conditions, and many useful methods have been reported.[12–16] In 2010, Balaraman et al. improved the synthesis of symmetrical and unsymmetrical 1,3-diynes by employing catalytic amounts (0.1 equiv) of copper(II) acetate in the presence of a stoichiometric amount of piperidine in 1,2-dichloroethane under aerobic conditions.[12] With this method, the yield of unsymmetrical

1,3-diyne **3** was >60% (an improvement over that described above); however, unfortunately, it still involved 5 equiv of 4-ethlnylbenzenamine **2**, and homocoupled byproducts remained a problem.

Efficient syntheses of unsymmetrical diynes using an alkynyl halide and a terminal alkyne have been reported.[14,15] To reduce the need for excess 4-ethylnylbenzenamine **2**, we replaced methyl 4-ethynylbenzoate **1** with methyl 4-(bromoethynyl)-benzoate **16**[17] as described in Scheme 2. Treatment of methyl 4-ethynylbenzoate **1** with *N*-bromosuccinimide in the presence of silver trifluoroacetate as a catalyst provided 4-(bromoethynyl)-benzoate **16**[17] in 83% yield. The reaction of **16** with a near-stoichiometric amount of 4-ethynylbenzenamine **2** (1.05 equiv) and copper(II) acetate (0.05 equiv) as a catalyst in the presence of piperidine proceeded efficiently to give diyne **3** (92% yield). In the scale-up process, crude intermediate **3** was hydrolyzed to give diacetylene acid **4** without purification.

We next turned our attention to the large scale synthesis of (2*S*,3*S*)-3-difluoromethylthreonine methyl ester **11**. The chiral sulfoxide-controlled stereoselective synthesis of optically pure (2*S*,3*S*)-3-difluoromethylthreonine methyl ester **11** (Scheme 1) is efficient and useful on a small scale. However, on a large scale, this approach has significant drawbacks, including (1) the high cost of (*S*)-1-methyl-4-(methylsulfinyl)benzene, (2) the fact that there was only a single crystalline intermediate in the sequence (i.e., chiral difluoroacetone **6**), and (3) the low boiling point of trifluoroacetic anhydride that can be a safety concern when it is used in large scale reactions. Taken together, these concerns led us to re-evaluate our strategy. During the development of an alternative scalable route, our main objectives were (a) to address the safety issues, (b) to enhance the robustness of the synthetic route, and (c) to devise a sequence that had the potential for additional crystalline intermediates to facilitate purification by recrystallization. We took advantage of the well-documented transition metal/base-catalyzed aldol reaction to construct oxazolines in a stereo-selective manner.[18] It is worth mentioning that the authors in that work have included entries of monofluoroacetone and trifluoroacetone reacting with methyl α-isocyanoacetate to yield corresponding oxazolines stereoselectively in ratios of 71:29 and 99:1, respectively. Inspired by their results, we had hoped to obtain stereoselectivity somewhere between the aforementioned ratios when reacting difluoroacetone with methyl α-isocyanoacetate as described in Scheme 3.

Difluoroacetone **17** was reacted with methyl α-isocyanoacetate **18** to give the diastereomeric mixture of oxazolines **19a** and **19b**. The reaction was performed in dry 1,1-dichloroethane (DCE) at ambient temperature in the presence of 4 mol % CuCl and 5 mol % TEA. The ratio of oxazoline diastereomers **19a** and **19b** is 87:13, which was determined by NMR chemical shifts of the crude reaction mixture after removal of the catalyst and solvent. Replacing DCE with DCM as the reaction solvent also provided similar results in terms of chemical yield and diastereoselectivity. Hydrolysis of the diastereomeric mixture of oxazolines **19a** and **19b** in methanolic HCl at room temperature afforded the corresponding amino acid hydrochloride salts **20a** and **20b**. Desired diastereomer **20a** was obtained in >99% purity after recrystallizaion from a MeOH/MTBE solvent at room temperature with a chemical yield of 42%. Alternatively, recrystallization can be accomplished from hot acetonitrile to provide **20a** in 56% yield and >99% purity. To obtain optically pure **21**, amino acid

hydrochloride salt **20a** was neutralized by Na_2CO_3 and the racemic free base was reacted with equimolar amounts of *R*-(−)-camphorsulfonic acid [*R*-(−)-CSA] at room temperature in acetone. When the mixture was cooled, the desired amino ester **21** was obtained in 60% yield based on a single enantiomer with an optical purity of >99%. Optical purity was established using ^{1}H NMR and ^{19}F NMR as follows: 1H NMR and 19F NMR of racemic hydrochloride salt in the presence of a 10-fold excess of (+)-tartaric acid in CD_3OD yielded a spectrum with well-resolved peaks. In particular, the α-CH, ester CH_3, and ^{19}F of the racemic methyl ester (Figure 1A,D) were well-resolved and readily identified when the spectra were recorded in the presence of excess tartaric acid (Figure 1B,E). It should be noted that ^{1}H NMR and ^{19}F NMR of a recrystallized optically pure sample of **21** in the presence of a 10-fold excess of (+)-tartaric acid in CD_3OD displayed only one set of peaks corresponding to the desired enantiomer (Figure 1C,F). The modified route to chiral amino ester **21** was shorter and cheaper than that described earlier for the corresponding hydrochloride salt **11** and was deemed suitable for large scale synthesis.

With multigram quantities of amino ester **21** and diacetylene carboxylic acid **4** in hand, we proceeded to the large scale synthesis of **LPC-058** (Scheme 4). Amide coupling under EDC/HOBt conditions provided diacetylene ester **12**. Treatment of **12** with hydroxylamine and sodium methoxide at 0 °C provided hydroxamate **LPC-058** in 67% yield over two steps. It is noted that no epimerization at the α-carbon was observed by LC–MS or NMR under these conditions.

To determine the absolute configuration of **11** and **21**, they were crystallized in methanol/MTBE and acetone solvents, respectively. The absolute configuration was determined by the measurement of the Flack parameter, which is calculated during the structural refinement.[19,20] If the value is near 0, the crystal is optically pure, in which case the absolute structure of the molecule can be determined with certainty by the X-ray data. In our study, the final refinement of the Cu Kα data of the crystal of **11** and **21** resulted in Flack parameters of 0.0(0) and −0.0(1), allowing an unambiguous assignment of the absolute structure as shown in Figure 2. The two chiral centers of **11**, C-2 and C-3, and the four chiral centers of **21**, C-2, C-3, C-8, and C-11, were thus determined to be *S* and *S* and *S*, *S*, *R*, and *S*, respectively. In summary, a short and scalable synthesis of hydroxamic acid derivative **LPC-058** is described. The syntheses of key intermediates diacetylene carboxylic acid **4** and difluorothreonine methyl ester **21** were accomplished without any chromatography steps.

EXPERIMENTAL SECTION

Methyl 4-ethynylbenzoate, 4-ethylnylbenzenamine, 1,1-difluoroacetone, and methyl α-isocyanoacetate were purchased from commercial vendors and used without further purification. LC–MS analysis was conducted on a HPLC instrument with a quadrapole mass analyzer and reverse phase C18 column (4.6 mm × 50 mm, 1.8 μm) with a water/acetonitrile [each with 0.2% (v/v) formic acid] gradient at a flow rate of 0.5 mL/min. The HRMS analyses were conducted on a TOF mass spectrometer using ESI mode. [1]H, ^{13}C, and ^{19}F spectra were recorded on 300 (or 400) MHz, 75 (or 100) MHz, and 282.4 (or 376.5) MHz spectrometers, respectively. Column chromatography was conducted using either silica gel flash chromatography or prepacked silica gel cartridges. All moisture-sensitive reactions

were conducted using dry solvents under a slight pressure of ultrapure quality argon. Glassware was dried in an oven at 140 °C for at least 12 h prior to being used and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reaction mixtures were stirred magnetically using Teflon-coated magnetic stirring bars. Commercially available disposable syringes were used for transferring reagents and solvents.

Methyl 4-(Bromoethynyl)benzoate (16).[17]

To a solution of methyl 4-ethynylbenzoate **1** (10.00 g, 62.5 mmol, 1.0 equiv) in acetone (100 mL) were added NBS (12.24 g, 68.8 mmol, 1.05 equiv) and CF_3CO_2Ag (0.53 g, 0.31 mmol, 0.05 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 2 h. The resulting solution was concentrated to remove acetone. The residue was diluted with water (100 mL) and extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with water (2 × 60 mL) and brine (60 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the crude product that was purified by silica gel chromatography (eluting with EtOAc in hexane, 0–10%) to give **16** as a white solid (12.4 g, 83% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.9 (s, 3H), 7.50 (d, J = 8.1 Hz, 2H), 7.97 (d, J = 6.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.3, 53.4, 79.4, 127.3, 129.5, 129.9, 131.9, 166.3. MS (LC/MS, ESI): m/z 240 [M + H]$^+$.

Methyl 4-[(4-Aminophenyl)buta-1,3-diyn-1-yl]benzoate (3).[7]

Copper(II) acetate (0.45 g, 0.50 mmol, 0.05 equiv) was added at room temperature under a stream of argon to a stirred solution of methyl (4-bromoethylnyl)benzoate (12.0 g, 50.0 mmol, 1.00 equiv), 4-ethynylaniline **2** (6.20 g, 52.5 mmol, 1.05 equiv), and piperidine (12.77 g, 150.0 mmoL, 3.00 equiv) in MeOH (200 mL, degassed with argon). The reaction mixture was stirred at room temperature for 20 h. The resulting suspension was diluted with water (400 mL) and stirred for 30 min at room temperature. The mixture was filtered, and the filtered solid was washed with water (2 × 200 mL). The solid obtained was dried to afford crude **3** (13.5 g) as a yellow solid that was carried on to the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H), 5.85 (s, 2H), 6.54 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 53.0, 71.8, 78.3, 80.5, 87.0, 105.7, 114.3, 126.7, 130.1, 130.3, 133.0, 134.8, 151.6, 166.2. MS (LC/MS, ESI): m/z 276 [M + H]$^+$.

4-[(4-Aminophenyl)buta-1,3-diyn-1-yl]benzoic Acid (4).[7]

To a solution of crude compound **3** (13.5 g, 50.0 mmol, 1.00 equiv) in methanol (100 mL) was added 1 N NaOH (100 mL, 100.0 mmol, 2.00 equiv) at room temperature under a stream of argon. The reaction mixture was heated to reflux for 1 h. The solution was diluted with water (100 mL) and acidified with concentrated HCl to pH ~3. The precipitate was filtered, washed with water, and dried under vacuum to give crude acid **4** (11.5 g, two steps, 88% yield for two steps), which was carried to the next step without purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.95 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 73.0, 77.2, 81.2, 85.0,

112.0, 118.8, 125.8, 130.2, 131.9, 133.1, 134.6, 144.2, 167.2. MS (LC/MS, ESI): m/z 262 $[M + H]^+$.

(4*S*,5*S*)-Methyl 5-(Difluoromethyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (19a)

Methyl 2-isocyanoacetate (20.00 g, 201.8 mmol, 1.00 equiv) was added slowly to an ice-cold suspension of 1,1-difluoroacetone (22.78 g, 242.20 mmol, 1.20 equiv), CuCl (0.80 g, 8.10 mmol, 0.04 equiv), and TEA (1.41 mL, 10.10 mmol, 0.05 equiv) in anhydrous DCM (330 mL) under argon. The reaction mixture was stirred at 0 °C and gradually warmed to room temperature overnight (20 h). The resulting solution was diluted with DCM (150 mL). The mixture was washed with 10% aqueous ammonia (3 × 100 mL), water (100 mL), and brine (100 mL) and dried (anhydrous Na_2SO_4). Evaporation of the solvent afforded a crude mixture (86:14) of diastereomers **19a** and **19b** (brown liquid, 35.5 g, 91% yield), which was carried to the next step without further purification. ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.35 (s, 3H), 1.54 (s, 0.5H), 3.67 (s, 0.5H), 3.74 (s, 3H), 4.47 (s, 0.17H), 4.78 (s, 1H), 5.69 (t, J_{HF} = 56 Hz, 1H), 5.88 (t, J_{HF} = 56 Hz, 0.17H), 6.94 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 15.4, 43.4, 52.5, 114.2 (t, J_{CF} = 494 Hz), 168.7. ^{19}F NMR (376.5 MHz, CDCl$_3$): δ −132.83 (d, J_{HF} = 56 Hz, major), −131.40 (ABq, d, 2F, J_{HF} = 56 Hz, J_{FF} = 300 Hz, minor). MS (LC/MS, ESI): m/z 194 $[M + H]^+$.

Methyl 2-Amino-4,4-difluoro-3-hydroxy-3-methylbutanoate Hydrochloride (racemic 20a)

To a stirred solution of oxazoline **19a** and **19b** (35.30 g, 182.70 mmol, 1.00 equiv) in methanol (180 mL) kept at room temperature in a water bath under argon was added dropwise concentrated HCl (36 mL). The reaction mixture was stirred at room temperature for 14 h. The resulting solution was concentrated to dryness. The residue was diluted with MTBE (400 mL) and stirred vigorously for 2 h. The suspension was concentrated to afford crude **20a** and **20b** as a light brown solid. ^{1}H NMR (400 MHz, CD$_3$OD): δ 1.30 (s, 3H), 1.48 (s, 0.5H), 3.85 (s, 0.5H), 3.86 (s, 3H), 4.15 (s, 0.17H), 4.16 (s, 1H), 5.95 (t, J_{HF} = 56 Hz, 0.17H), 5.99 (t, J_{HF} = 56 Hz, 1H). ^{19}F NMR (376.5 MHz, CD$_3$OD): δ −134.25, −135.15 (ABq, d, 2F, J_{HF} = 56 Hz, J_{FF} = 301 Hz). ^{13}C NMR (100 MHz, CD$_3$OD): δ 15.9, 52.6, 55.9, 71.2 (t, $^2J_{CF}$ = 42.5 Hz), 115.4 (t, J_{CF} = 491 Hz), 166.7. MS (LC/MS, ESI): m/z 184 $[M + H]^+$. The crude product was dissolved to ~55 mL of methanol and MTBE (~270 mL) at room temperature. Then the solution was left at −20 °C for 2 days to give the racemic **20a** (17.00 g, 42% yield) as an off-white solid. Alternatively, a crude mixture of **20a** and **20b** can be recrystallized from hot acetonitrile (~200 mL) to provide racemic **20a** in 56% yield. Mp: 142 °C. ^{1}H NMR (400 MHz, CD$_3$OD): δ 1.30 (s, 3H), 3.87 (s, 3H), 4.16 (s, 1H), 5.99 (t, J_{HF} = 56 Hz, 1H). ^{19}F NMR (376.5 MHz, CD$_3$OD): δ −134.25, −135.15 (ABq, d, 2F, J_{HF} = 56 Hz, J_{FF} = 301 Hz). ^{13}C NMR (100 MHz, CD$_3$OD): δ 15.9, 52.6, 56.0, 71.2 (t, $^2J_{CF}$ = 42.5 Hz), 115.4 (t, J_{CF} = 490 Hz). ^{19}F NMR (376 MHz, CDCl$_3$): δ −134.70. HRMS (ESI/TOF): m/z $[M + H]^+$ calcd for $C_6H_{12}F_2NO_3$ 184.0785, found 184.0776.

(2*S*,3*S*)-Methyl 2-Amino-4,4-difluoro-3-hydroxy-3-methylbutanoate (−)-CSA (21)

To an ice bath-cooled solution of racemic **20** (16.70 g, 76.00 mmol, 1.00 equiv) in water (100 mL) was added Na$_2$CO$_3$ (24.17 g, 228.00 mmol, 3.00 equiv) under argon. The reaction mixture was stirred at room temperature for 1 h. Then the resulting solution was extracted

with EtOAc (6 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the free amine (14.0 g) as a light brown liquid. The free amine (13.50 g, 73.7 mmol, 1.00 equiv) was dissolved in acetone (100 mL), and (1R)-(−)-10-camphorsulfonic acid (17.12 g, 73.70 mmol, 1.00 equiv) was added. The mixture was stirred at room temperature overnight. The white solid was filtered and washed with acetone (20 mL) and dried to give (−)-CSA salt **21** (8.40 g, 28% yield). Mp: 160 °C. $[a]^D_{25} = -24.1$ (c = 0.4; MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 0.83 (s, 3H), 1.09 (s, 3H), 1.30 (s, 3H), 1.37–1.42 (m, 1H), 1.57–1.64 (m, 1H), 1.88 (d, J = 18.4 Hz, 1H), 2.00–2.04 (m, 2H), 2.29–2.35 (m, 1H), 2.57–2.61 (m, 1H), 2.76 (d, J = 14.8 Hz, 1H), 3.86 (s, 3H), 4.21 (s, 1H), 5.98 (t, J_{HF} = 56 Hz, 1H). ^{19}F NMR (376.5 MHz, CD_3OD): δ −134.23, −135.13 (ABq, d, 2F, J_{HF} = 56 Hz, J_{FF} = 301 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 5.8, 18.7, 19.0, 24.3, 26.4, 42.2, 42.6, 46.8, 52.6, 56.0, 58.2, 71.2 (t, $^2J_{CF}$ = 42.5 Hz), 115.4 (t, J_{CF} = 491 Hz), 166.7, 218.1. ^{19}F NMR (376 MHz, $CDCl_3$): δ −134.68 (t, J_{HF} = 56 Hz). HRMS (ESI/TOF): m/z [M + H]+ calcd for $C_6H_{12}F_2NO_3$ 184.0785, found 184.0781.

(2S,3S)-Methyl 2-{4-[(4-Aminophenyl)buta-1,3-diyn-1-yl]-benzamido}-4,4-difluoro-3-hydroxy-3-methylbutanoate (12).[4]

To a solution of diacetylene carboxylic acid **4** (3.00 g, 12.49 mmol) in anhydrous DMF (25 mL) were added amino ester **21** (5.40 g, 13.11 mmol, 1.05 equiv), EDC-HCl (2.64 g, 13.80 mmol, 1.2 equiv), and HOBt (1.87 g, 13.80 mmol, 1.2 equiv) at room temperature under argon. The mixture was cooled to 0 °C, and DIEA (8.1 mL, 45.90 mmol, 4.00 equiv) was added. The reaction mixture was stirred at 0 °C for 2 h and then allowed to warm to room temperature for 14 h. The yellow solution was then concentrated to dryness. The residue was treated with water (100 mL) and extracted with EtOAc (3 × 80 mL). The combined extracts were washed with water (80 mL) and brine (80 mL) and dried (anhydrous Na_2SO_4). The crude product was purified by silica gel chromatography (eluting with MeOH in DCM, 0–2.5%) to give **12** as a yellow solid (4.23 g, 80%). Mp: 167–168 °C dec. $[a]^D_{25} = +28.2$ (c = 0.2; MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 1.37 (d, J = 2.1 Hz, 3H), 3,76 (s, 3H), 4.267 (dd, J = 9.3, 19.5 Hz, 1H), 4.42 (dd, J = 9.3, 19.8 Hz, 1H), 4.81 (s, 1H), 5.85 (t, J_{HF} = 56 Hz, 1H), 6.61 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.1 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H). ^{19}F NMR (400 MHz, CD_3OD): δ −131.06, −137.61 (ABq, d, 2F, J_{HF} = 56 Hz, J_{FF} = 300 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 20.5, 51.7, 70.9, (71.9, 72.2), 76.9, 79.1, 84.7, (85.5, 87.8), 108.3, 114.3, 126.2, 127.2, 127.6, 132.2, 133.6, 133.9, 150.2, 168.2, 170.6. MS (LC/MS, ESI): m/z 427 [M + H]+. HRMS (ESI/TOF): m/z [M]+ calcd for $C_{23}H_{20}F_2N_2O_4$ 426.1391, found 426.1392.

4-[(4-Aminophenyl)buta-1,3-diyn-1-yl]-N-[(2S,3S)-4,4-difluoro-3-hydroxy-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl]benzamide (LPC-058).[4]

To an ice-cold solution of **12** (4.00 g, 9.4 mmol) dissolved in anhydrous MeOH (15 mL) and THF (15 mL) was added hydroxylamine hydrochloride (3.27 g, 46.9 mmol, 5.0 equiv) followed by 25% sodium methoxide in a methanol solution (16.0 mL, 70.50 mmol, 7.5 equiv). The reaction mixture was stirred under argon at 0 °C for 2 h and then allowed to warm to ambient temperature while stirring was continued overnight (14 h). The resulting yellow suspension was condensed to dryness with a rotary evaporator, and the residue obtained was treated with water (200 mL) and saturated NH_4Cl (80 mL) and extracted with

EtOAc (3×100 mL). The combined extracts were washed with water (80 mL) and brine (80 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the crude product, which was purified by silica gel chromatography (eluting with 0–5% MeOH in DCM) to afford the title compound as a yellow solid (3.29 g, 82% yield). Mp: 155–157 °C dec. $[a]^D_{25}$ = +61.2 (c = 0.4; MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 1.36 (s, 3H), 4.73 (s, 1H), 5.80 (t, J_{HF} = 56 Hz, 1H), 6.61 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H). ^{19}F NMR (400 MHz, CD_3OD): δ −130.00, −137.91 (ABq, d, 2F, J_{HF} = 56 Hz, J_{FF} = 300 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 16.5, 54.9, 70.8, (72.6, 72.9, 73.2), 76.8, 79.0, 84.7, 108.4, (112.7, 116.0, 119.2), 114.3, 126.3, 127.5, 132.2, 133.5, 133.8, 150.2, 166.5, 167.5. MS (LC/MS, ESI): m/z 428.2 [M + H]$^+$. HRMS (ESI/TOF): m/z [M]$^+$ calcd for $C_{22}H_{19}F_2N_3O_4$ 427.1344, found 427.1346.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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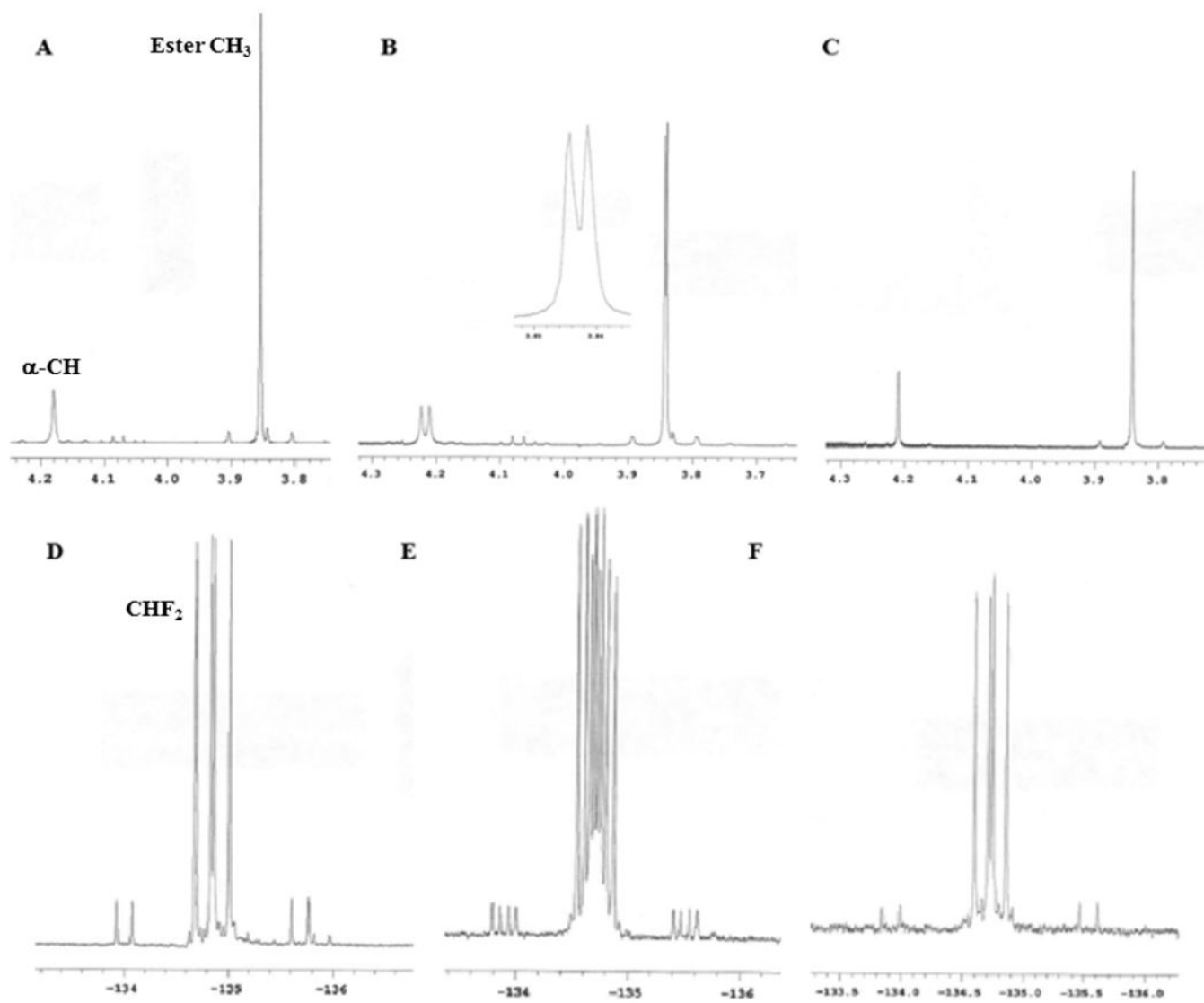20. Flack HD. Acta Crystallogr Sect A: Found Crystallogr. 1983; 39:876.

Figure 1.
(A and D) Racemic hydrochloride salt **20a** in CD$_3$OD. (B and E) Racemic hydrochloride salt **20a** in the presence of a 10-fold excess of (+)-tartaric acid in CD$_3$OD. (C and F) Recrystallized optically pure sample of **21** in the presence of a 10-fold excess of (+)-tartaric acid in CD$_3$OD. (A–C) ^{1}H spectra and (D–F) ^{19}F spectra.
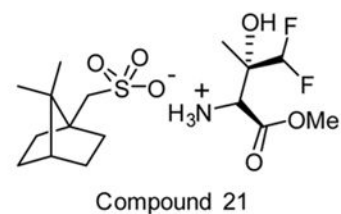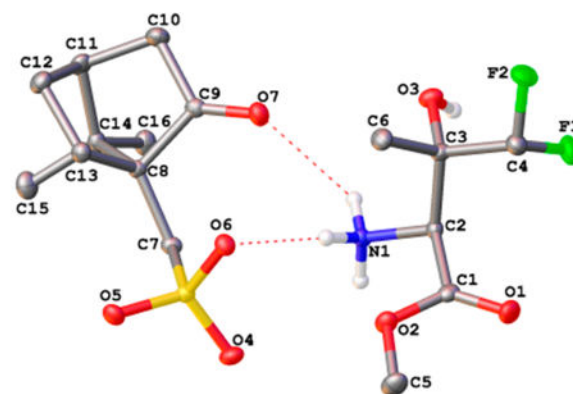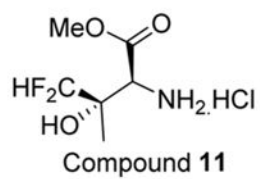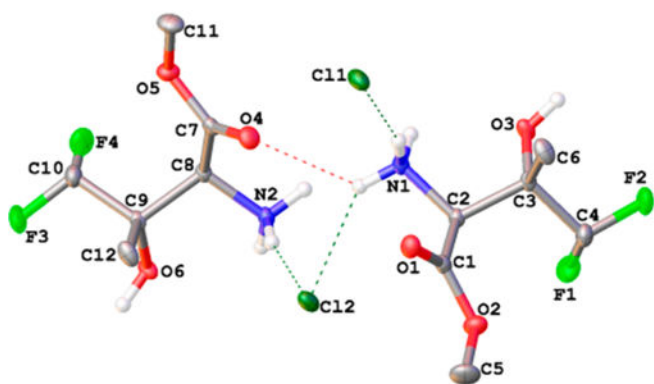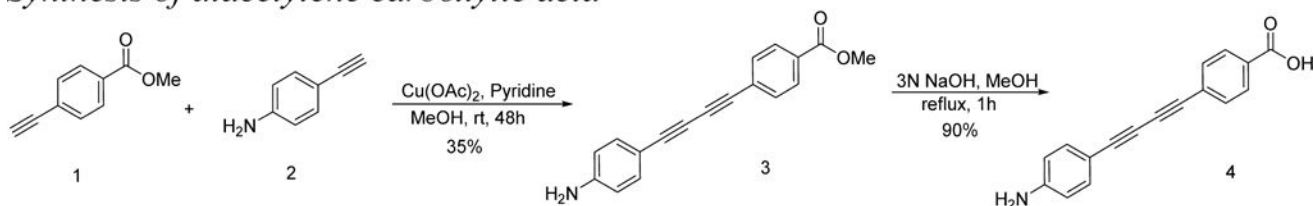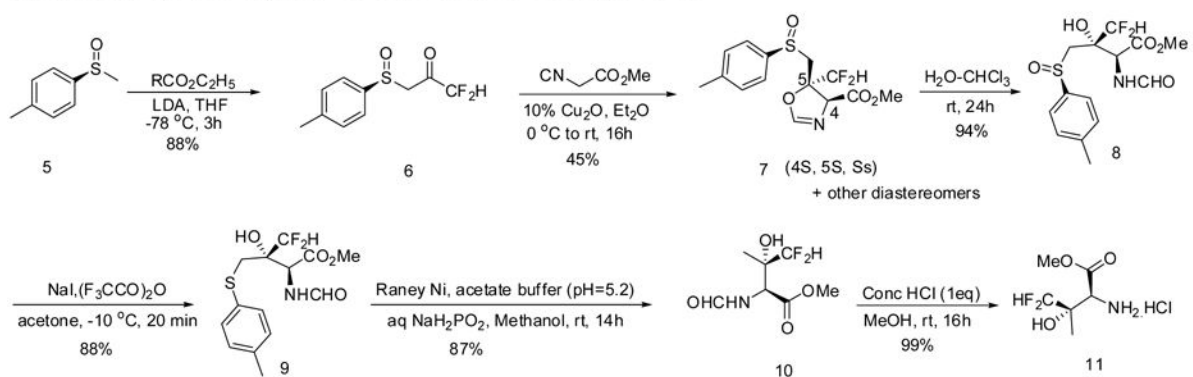
Figure 2.
X-ray crystallographic structures of **11** and **21**. The compounds are colored in the following atom colors: carbon, gray; nitrogen, blue; oxygen, red; sulfur, yellow; fluorine, green; chlorine, deep green.

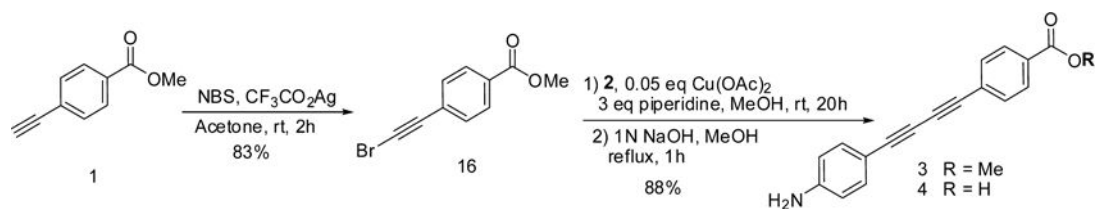## Synthesis of diacetylene carboxylic acid[7]
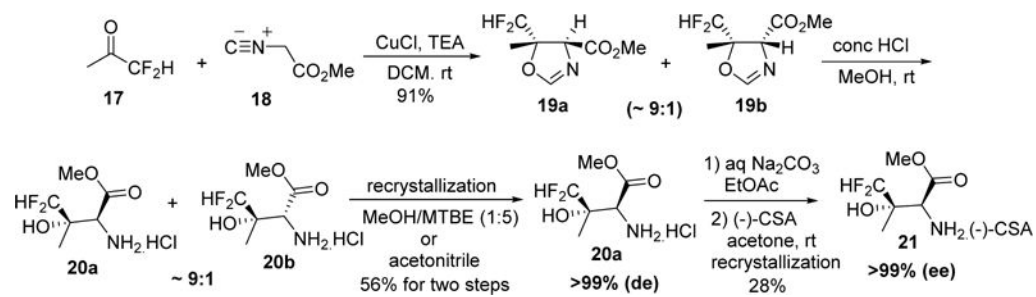

Synthesis of the difluoro-threonine methyl ester[4]

Synthesis of LPC-058[4]
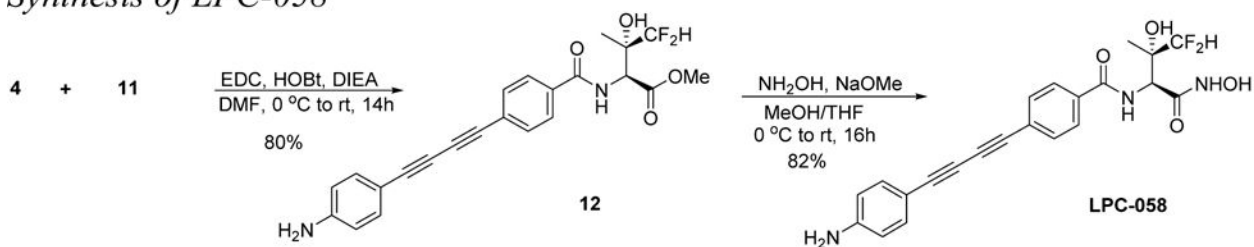
Scheme 1.
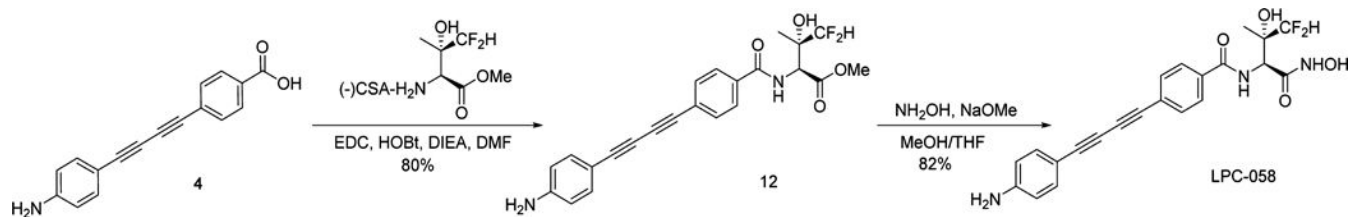Initial Route to LPC-058

Scheme 2.
Synthesis of Diyne Carboxylic Acid 4

Scheme 3.
Synthesis of Amine

Scheme 4.
Amide Coupling