

**Unnatural Lysines with Reduced Sidechain *N*-Basicity. Synthesis of *N*-trifluoroethyl
Substituted Lysine and Homologs**

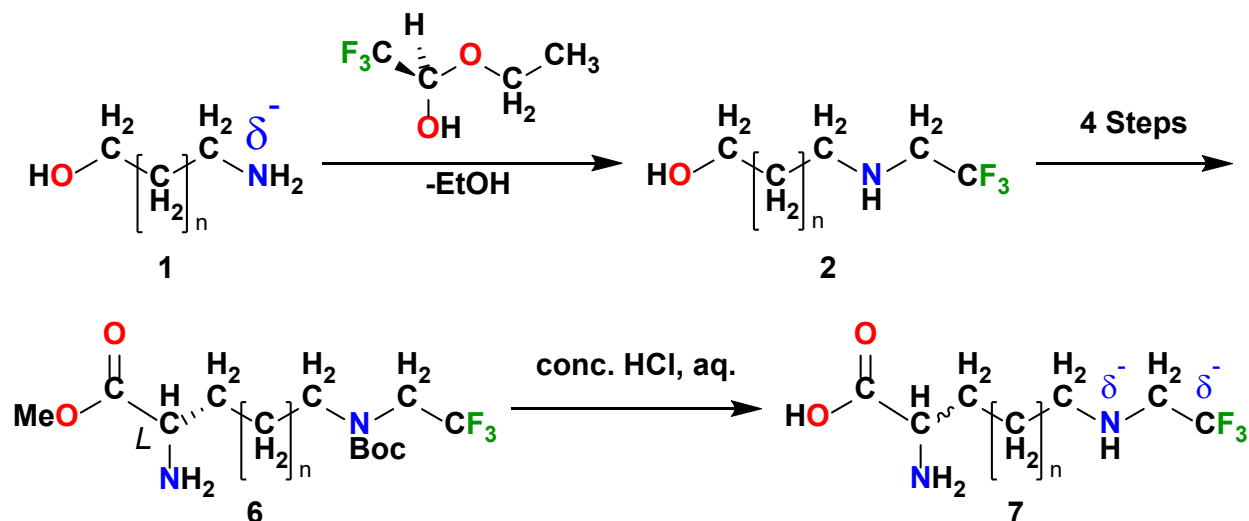
Brian Jameson and Rainer Glaser*[a]

[a] Prof. Dr. Rainer Glaser*

Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri 65401,
United States

Email: glaserr@umsystem.edu

Graphical Abstract

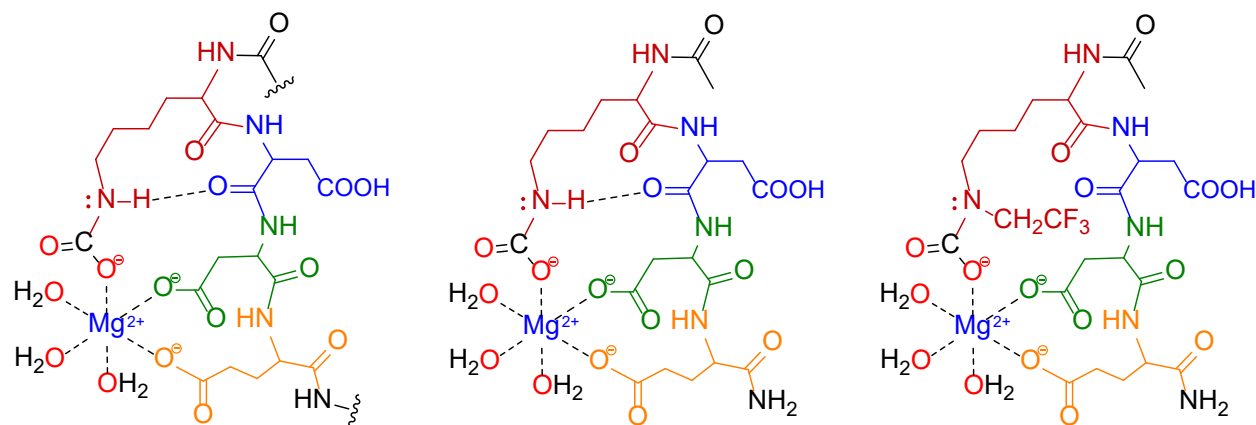


Abstract

The syntheses are reported of N^ϵ -(2,2,2-trifluoroethyl)-*D,L*-lysine ($^{\text{tF}}\text{K}$) and N^ζ -(2,2,2-trifluoroethyl)-*D,L*-homolysine ($^{\text{tF}}\text{K}_{+1}$) from amino alcohols $\text{HO}-(\text{CH}_2)_n-\text{NH}_2$. The syntheses involve reductive amination, Appel bromination, and the stereoselective bond formation between C_α of the amino acid and the fluorinated alkyl chain in the Schöllkopf bislactim amino acid synthesis. The methyl esters of the fluorinated amino acids are the relevant substrates for oligopeptide synthesis. With the *R*-Schöllkopf reagent, we stereoselectively generated methyl N^ϵ -*boc*- N^ϵ -(2,2,2-trifluoroethyl)-*L*-lysinate and methyl N^ζ -*boc*- N^ζ -(2,2,2-trifluoroethyl)-*L*-homolysinate. Products and intermediates were characterized by ^1H NMR, ^{13}C NMR, COSY, HSQC, and LCMS. A variety of *N*-functionality may be introduced by reacting hemiacetals with different appendages. This fluorine modification reduces the sidechain *N*-basicity by combined -I effect of the three fluorines. This effect increases the [amine]/[ammonium ion] ratio of the sidechain amine in lysine to facilitate carbamylation at lower pH conditions.

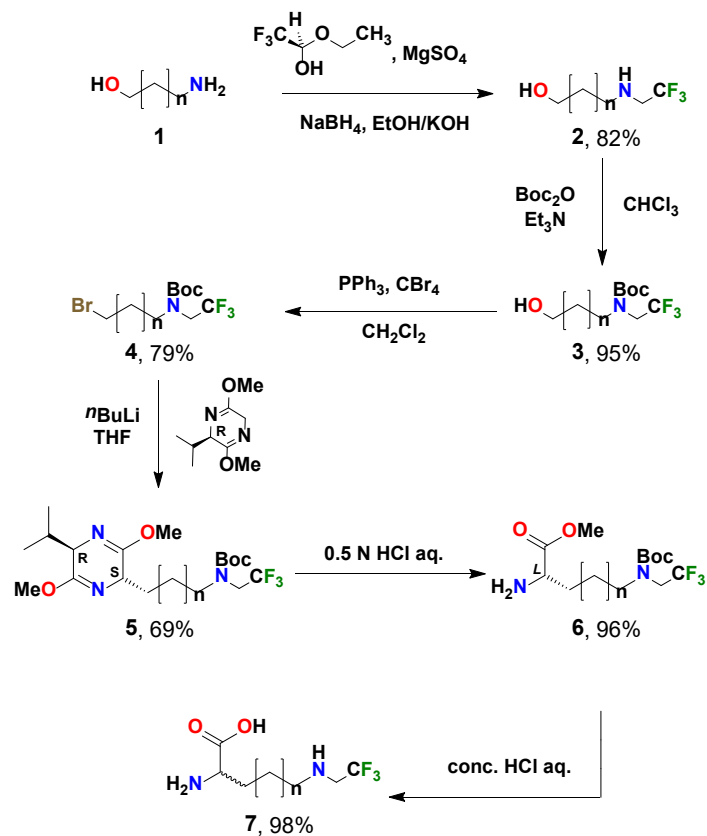
Introduction

Structural modification and labeling of amino acids are among the most studied topics in biochemistry, and the incorporation of these analogs has been extensively used to investigate the functionality of biological systems and enzymes.^[1-5] Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is a cornerstone of atmospheric CO₂ fixation, catalyzing the formation of 3-phospho-D-glycerate from ribulose 1,5-bisphosphate RuBP.^[6,7] The active site of this spinach RuBisCO contains the amino acid sequence Lys²⁰¹-Asp²⁰²-Asp²⁰³-Glu²⁰⁴ (KDDE) with the Asp-Glu moiety complexed by a Mg²⁺ ion, and it is activated by the addition of an “activator” CO₂ to the lysine side chain amino group to form a carbamate (Scheme 1).^[8-10] An important feature of this active site is that activation requires a two-pronged attack on CO₂, first by the nucleophilic amino group of the lysine sidechain and then by the Mg²⁺ ion Lewis acid for carbamate stabilization.^[11,12] Capture is complicated further by the competing carboxylase/oxygenase reaction pathways and the H₂O/CO₂ replacement penalty around the Mg²⁺ coordination sphere.^[13,14] Despite these limitations CO₂ capture utilizing this activation reaction is of great interest because it offers the advantages of quantitative capture at ambient temperatures and of energy-efficient reversibility.^[15,16]



Scheme 1. The Lys-Asp-Asp-Glu sequence in the active site of RuBisCO (left), the capped KDDE tetramer (middle), and the capped ^{tF}KDDE tetramer with the fluorinated unnatural lysine ^{tF}K (right).

Several RuBisCO-inspired studies have aimed to investigate the carbamylation pathway of the active site. We proposed to utilize the carbamylation involved in RuBisCO activation as a reversible CO₂ capture and release (CCR) system,^[8,14] and we focus on the smallest possible active site model, a capped KDDE tetramer (Scheme 1). Yang and Schell investigated the CO₂ capture mechanism and experimentally quantified the extent of capture of this capped KDDE tetramer system that closely models the RuBisCO active site.^[17-19] Schell et al. investigated the thermochemistry of several small alkylamine systems to provide a framework for corresponding experimental solution phase studies.^[20] The active site of RuBisCO has also inspired related CO₂ capture frameworks. Langer et al. developed an iridium(I) complex featuring an enamido phosphine anion which possesses the unique ability to form an octahedral complex by binding two CO₂ molecules.^[21]



Scheme 2. Synthesis of *N*-trifluoroethyl substituted lysine analogs.

We have focused on utilizing a capped KDDE tetramer for CO₂ capture (Scheme 1). RuBisCO possesses the unique ability to depress the pK_a of the lysine's ammonium ion such that lysine becomes available as a nucleophile at lower pH conditions.^[22] However, the capped KDDE tetrapeptide model does not retain this structural feature.^[17] We have employed chemical means to lower the pK_a of the protonated amino group in smaller amine systems by *N*-alkylation with a 2,2,2-trifluoroethyl group.^[23] With success in the smaller model systems, we are now employing this concept in oligopeptide capture systems featuring this fluorine modification on lysine. We describe here the synthesis of *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-lysine (**7**, n = 2, ^{tF}K) as outlined in Scheme 2. The synthesis begins with reductive amination of **1** leading to fluorinated **2**.^[24] Subsequent protection and Appel bromination of **3** leads to the alkyl bromide **4**.^[25] The key step for the amino acid synthesis involves the Schöllkopf bislactim reaction to stereo-selectively form the bond between C_α of the amino acid and the fluorinated alkyl chain.^[26,27]

Although ^{tF}K (n = 2) was the primarily desired synthetic product, a variety of extended lysine homologs with the trifluoroethyl substituted sidechain may be synthesized by starting with amino alcohols of varying alkyl lengths. Accordingly, *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-homolysine (^{tF}K₊₁, n = 3), the one carbon homolog of ^{tF}K, was generated as proof of concept. The synthetic route and pertinent ¹H NMR data is described here only for the synthesis of *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-lysine from 4-amino-1-butanol, however the same synthetic methods were applied to synthesize *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-homolysine from 5-amino-1-pentanol and the NMR spectra for this product and intermediates can be found in the supporting information. Pertaining to CO₂ capture, by varying the alkyl length of the fluorinated ^{tF}K_{+n}, we can explore the conformational effects of carbamylation of our active site ^{tF}KDDE model as compared to ^{tF}K_{+n}DDE.

The target tetrapeptide with the *N*-trifluoroethylated lysine could be obtained efficiently by reductive amination of an oligopeptide containing alloc-lysine. We did not choose that route for several reasons. The synthesis of ^{tF}KDDE by attachment of Boc protected ^{tF}K to the DDE

tripeptide via solution phase amidation is significantly less costly than the direct approach using expensive protected KDDE as starting material. Second, only our approach allows for the syntheses of longer chain lysine analogs or analogs with additional functionality in the lysine chain. Third and from an experimental perspective, the separation of the product ^{19}F -KDDE from KDDE proved to be difficult.

Experimental Section

Materials and Instrumentation

All reagents used in our synthetic methods were purchased from Sigma Aldrich (>95% pure). Anhydrous solvents (EtOH, THF) were procured from Fisher Scientific and deuterated solvents (CDCl_3 , D_2O) were acquired from Sigma Aldrich for NMR characterization. Reactions were monitored by NMR and all spectra were acquired using a Bruker 400 MHz Avance III HD Liquid State NMR. The products and intermediates were effectively characterized by using ^1H water suppression NMR with pre-saturation techniques, (Bruker pulse program *noesygppr1d*), gradient ^1H - ^1H COSY (Bruker pulse program *cosygpppqf*), proton decoupled ^{13}C NMR (Bruker pulse program *zgpg30*), and phase sensitive gradient HSQC (Bruker pulse program *hscetgpsi2*). ^1H and ^{13}C NMR data is reported alongside each product for the synthesis of N^{ϵ} -(2,2,2-trifluoroethyl)-*D,L*-lysine ^{19}F -K which includes chemical shift (ppm), splitting (singlet 's', doublet 'd', triplet 't', etc.), integration, and J -coupling constants (Hz). The reported ^{13}C NMR chemical shifts refer to the centers of the quartet signals of the carbons at positions 1' and 2' which are caused by $J(^{13}\text{C}, ^{19}\text{F})$ coupling, and the presence of two *E/Z*-rotamers. The $J(^{13}\text{C}, ^{19}\text{F})$ coupling constants for compounds **2-7** ($n = 2$) are reported alongside the ^{13}C NMR chemical shift data. Rotamer splitting caused by the presence of the carbamate protecting group in compounds **2-6** ($n = 2, 3$) is not addressed in the experimental section but evidence of rotamer splitting may be found in the supporting information and is discussed in greater detail for compounds **7** (*vide*

infra). Additional NMR spectra including ^{13}C NMR, COSY, and HSQC is provided in the supporting information both for $^{\text{tF}}\text{K}$ (Figures S1-S24) and $^{\text{tF}}\text{K}_{+1}$ (Figures S25-S45).

The HPLC system used was equipped with a 168 diode array detector, a 507e auto injector and used a 32 KARAT software package (Gold System from Beckmann Coulter, Fullerton, CA). The HPLC system was coupled with an ion trap mass spectrometer (LCQ Fleet from Thermo Fisher, Waltham, MA) and equipped with an ESI ionization source. The gradient solvent system used for the LC system was 10% acetonitrile to 50% acetonitrile over 30 minutes with a flow rate of 1 ml/min. The column used for the characterization was a BetaBasic C18, 150 Å, 0.46 cm $\times$ 15 cm, 5 μm column from Thermo Fisher.

When necessary, column chromatography was performed to purify the crude reaction mixtures in which a hexane/ethyl acetate gradient was used for each column separation from 100% hexane to 100% ethyl acetate in 5% increments. The same gradient technique was used in each purification, and total volumes used in each separation were proportional to the amount of product being separated (100.0 ml of total solvent per 5.0 g of crude product). High purity 60 Å silica gel procured from Sigma-Aldrich was used for the solid phase in each separation. 10.0 ml fractions were collected in each separation, dried at room temperature, and characterized by NMR for purity. The yield for each column separation was based on the combination of fractions containing pure product.

Synthetic Procedures

4-((2,2,2-Trifluoroethyl)amino)butan-1-ol (2). A tri-necked flask was fitted with distillation glassware to the center neck and a rubber septum and penny head stopper to the two remaining necks. To the distillate reservoir a vacuum adaptor was attached, and tubing from the vacuum adaptor was attached to an oil bubbler. Each connecting joint was greased and the glassware was purged with flame drying and gentle nitrogen flow. The glassware was cooled to room temperature before adding the reactants. A 1:1.3 mol ratio of 8.0 g (89.8 mmol) 4-amino-butan-1-ol and 13.8 ml (116.9 mmol) of trifluoroacetaldehyde ethyl hemiacetal were mixed and

heated to 90° C with a small amount of MgSO₄ to remove water generated during the formation of the imine intermediate. The reaction was stirred for 16 hours (neat), removing ethanol as a byproduct by distillation. The reaction mixture was held at 90° C for the duration of the reduction, where a solution containing 6.1 g (1.8 mol eq.) NaBH₄ and 0.1 g KOH in 40.0 ml of ethanol was added, and the reaction was refluxed overnight. The resulting residue was neutralized with two 20.0 ml portions of distilled water and extracted with Et₂O. The resulting organic layer was washed with two 20.0 ml portions of NaCl brine and dried in vacuo to obtain the product as a clear oil in 82.1% yield. ¹H NMR (400 MHz, CDCl₃): δ = 3.56 (t, *J* = 5.7 Hz, 2 H), 3.14 (q, *J* = 9.4 Hz, 2 H), 2.71 (t, *J* = 6.13 Hz, 2 H), 1.56 (m, *J* = 5.45 Hz, 4 H) ppm. ¹³C NMR (400 MHz, CDCl₃): δ = 125.46 (q, 277.87 Hz), 62.35, 50.38 (q, 31.08 Hz), 62.35, 30.91, 27.27, 49.36 ppm.

4-(Boc(2,2,2-trifluoroethyl)amino)butan-1-ol (3). To a dry round bottom flask fitted with a jacketed condenser, 8.53 g (49.9 mmol) of 4-((2,2,2-trifluoroethyl)amino)butan-1-ol (**2**) was dissolved in 50.0 ml of CHCl₃. To this, a catalytic amount of triethylamine (Et₃N) was added and stirred at room temperature. 12.0 g (1.1 mol eq.) of di-*tert*-butyl-dicarbonate (Boc₂O) was added, and the resulting solution was stirred for 24 hours at room temperature. The solution was dried in vacuo and purified with column chromatography to obtain **3** as a clear oil in 95.0% yield. ¹H NMR (400 MHz, CDCl₃): δ = 3.82 (m, *J* = 8.8 Hz, 2 H), 3.66 (t, *J* = 5.7 Hz, 2 H), 3.33 (m, *J* = 6.1 Hz, 2 H), 1.62 (m, *J* = 7.2 Hz, 2 H), 1.54 (m, *J* = 6.0 Hz, 2 H), 1.45 (s, 9 H) ppm. ¹³C NMR (400 MHz, CDCl₃): δ = 155.33, 126.15 (q, 282.89 Hz), 81.12, 62.38, 48.02 (q, 36.91 Hz), 47.73, 29.66, 24.60, 28.25, 24.6 ppm.

4-Bromo-*N*-boc-*N*-(2,2,2-trifluoroethyl)butan-1-amine (4). 1.0 g (3.7 mmol) of 4-(boc(2,2,2-trifluoroethyl)amino)butan-1-ol (**3**) was dissolved in anhydrous THF in a flame dried tri-neck flask. A jacketed condenser was fixed to the tri-necked flask, and a bubbler was attached to the condenser. The temperature of the solution was reduced to 0° C under nitrogen flow and 2.5 g of CBr₄ (2 mol eq.) and 2.0 g of PPh₃ (2 mol eq.) were added sequentially. The reaction

mixture was allowed to warm to room temperature and to react for 16 hours. Hexane was added to fully precipitate O=PPh₃ formed in the reaction. Excess solid CBr₄/PPh₃ and O=PPh₃ were removed by vacuum filtration, the filtrate was washed several times with hexane, and the resulting dark orange crude product **4** was dried in vacuo. The residue was purified by column chromatography to obtain a pale-yellow oil of **4** in 78.5% yield. ¹H NMR (400 MHz, CDCl₃): δ = 3.82 (m, *J* = 8.8 Hz, 2 H), 3.41 (t, *J* = 6.5 Hz, 2 H), 3.33 (m, *J* = 6.1 Hz, 2 H), 1.83 (m, *J* = 7.0 Hz, 2 H), 1.70 (m, *J* = 7.5 Hz, 2 H), 1.45 (s, 9 H) ppm. ¹³C NMR (400 MHz, CDCl₃): δ = 155.33, 124.71 (q, 284.12 Hz), 81.11, 62.38, 48.02 (q, 33.09 Hz), 29.66, 24.33 ppm.

4-((2*R*,5*S*)-5-Isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)-*N*-boc-*N*-(2,2,2-trifluoroethyl)butan-1-amine (5**).** A tri-necked flask, fitted with a condenser and bubbler, was flame dried and purged with nitrogen. To the flask, a 1:1 mol ratio of 312.7 μl (1.1 mol eq.) of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine was combined with 697.9 μl of 2.5M *n*BuLi (1.1 mol eq.) sequentially in anhydrous THF and allowed to stir for 15 min at -78° C. 528.0 mg (1.6 mmol) of 4-bromo-*N*-boc-*N*-(2,2,2-trifluoroethyl)butan-1-amine (**4**) was added slowly and the reaction stirred for 8 hours, allowing the solution to rise to room temperature. The reaction was slowly quenched with water and separated with Et₂O/H₂O liquid extraction. The organic layer was dried in vacuo, and the residue was purified by column chromatography, resulting in a pale-yellow oil in 68.9% yield. ¹H NMR (400 MHz, CDCl₃): δ = 3.99 (m, *J* = 10.13, 1 H), 3.92 (s, 1 H), 3.82 (m, *J* = 8.8 Hz, 2 H), 3.67 (s, 3 H), 3.65 (s, 3 H), 3.25 (m, *J* = 6.1 Hz, 2 H), 2.25 (m, *J* = 3.3 Hz, 1 H), 1.75 (m, *J* = 5.0 Hz, 2 H), 1.53 (m, *J* = 7.6 Hz, 2 H), 1.44 (s, 9 H), 1.21 (m, *J* = 8.2 Hz, 2 H), 1.02 (d, *J* = 7.0 Hz, 3 H), 0.66 (d, *J* = 6.7 Hz, 3 H) ppm. ¹³C NMR (400 MHz, CDCl₃): δ = 163.77, 163.70, 155.15, 124.79 (q, 279.96 Hz), 80.77, 60.86, 55.32, 52.36, 48.22 (q, 35.55 Hz), 47.97, 33.86, 31.82, 28.24, 28.07, 21.80, 19.12, 16.63 ppm.

Methyl *N*^ε-boc-*N*^ε-(2,2,2-trifluoroethyl)-*L*-lysinate (6**).** 50.0 mg of 4-((2*R*,5*S*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)-*N*-boc-*N*-(2,2,2-trifluoroethyl)butan-1-amine (**5**) was combined with 800.0 μl of 0.3M hydrochloric acid and 1.0 ml of acetonitrile and stirred

at 0° C for 2 hours. The desired methyl ester (**6**) and by-product valine methyl ester were extracted using CHCl₃ and rinsed twice with two 20 ml portions of NaCl brine. The organic layer was dried over NaSO₄, and then dried in vacuo at 110° C for 1 hour. The residue was purified by LCMS, generating **6** as a clear oil in 96.0% yield. ¹H NMR (400 MHz, CDCl₃): δ = 3.82 (m, *J* = 8.8 Hz, 2 H), 3.66 (s, 3 H), 3.38 (t, *J* = 6.5 Hz, 1 H), 3.23 (m, *J* = 6.1 Hz, 2 H), 1.69 (m, *J* = 7.9 Hz, 2 H), 1.52 (m, *J* = 7.6 Hz, 2 H), 1.42 (s, 9 H), 1.31 (m, *J* = 7.7 Hz, 2 H) ppm. ¹³C NMR (400 MHz, CDCl₃): δ = 170.00, 155.10, 124.72 (q, 283.59 Hz), 80.95, 53.12, 48.22 (q, 32.77 Hz), 47.78, 30.03, 28.18, 27.28, 22.26 ppm. LCMS (ESI): *m/z* = 342.88 (M⁺, 100%), 237.07 (29%).

***N*^ε-(2,2,2-Trifluoroethyl)-*D,L*-lysine (**7**).** 50.0 mg (0.15 mmol) of methyl *N*^ε-*tert*-boc-*N*^ε-(2,2,2-trifluoroethyl)-*L*-lysinate was combined with 100.0 μl of concentrated hydrochloric acid (37% aq.) and heated to reflux for 1 hour in a fume hood with no condenser. The solution was cooled to room temperature and placed under vacuum with slight heating to remove excess water, concentrating the product as a yellow oil in 98% yield. ¹H NMR (400 MHz, 90% H₂O/10% D₂O): δ = 3.92 (q, *J* = 8.8 Hz, 2 H), 3.83 (t, *J* = 6.1 Hz, 1 H), 3.18 (t, *J* = 8.1 Hz, 2 H), 1.88 (m, *J* = 8.3 Hz, 2 H), 1.75 (m, *J* = 7.8 Hz, 2 H), 1.45 (m, *J* = 6.7 Hz, 2 H) ppm. ¹³C NMR (400 MHz, CDCl₃): δ = 175.13, 124.93 (q, 277.30 Hz), 55.76, 50.85, 49.96 (q, 34.84 Hz), 32.03, 27.49, 24.06 ppm.

Results and Discussion

Description of the Synthesis

The overall synthesis is outlined in Scheme 2 and we discuss here some strategic aspects of the approach. The discussion focusses on the synthesis of *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-lysine ^{tF}K (**7**, *n* = 2) from 4-amino-1-butanol. The homolog *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-homolysine ^{tF}K₊₁ (**7**, *n* = 3) was synthesized and characterized in the same manner using 5-amino-1-pentanol under the same conditions giving similar yields. All naming conventions for intermediates and

products were generated in accordance with IUPAC standardized naming conventions.^[28] All spectra for ^{19}F and its intermediate are found in the supporting information.

The primary amino alcohol **1** was converted to the fluorinated secondary amino alcohol **2** by reductive amination. After heating, the presence of the intermediate imine was confirmed by ^{13}C NMR, and the spectrum showed the trifluoroacetaldehyde ethyl hemiacetal starting material and the hemi-aminal intermediate in equilibrium with the desired imine (Figure 1). First described in good yield forming hydrazones,^[24] we used the same solvent free approach to form our fluorinated amino alcohol **2**. Several reaction conditions were explored including the use of a *p*-toluenesulfonic acid (PTSA) catalyst and dried molecular sieves for H_2O removal, neither of which increased product yield. Exchanging sodium borohydride with alternate reducing agents, namely water-stable sodium cyanoborohydride, did not enhance product formation. Amines **1** and **2** were separated by column chromatography but led to severe product loss even with the addition of dilute basic or acidic cosolvents. The ^{13}C NMR spectrum of pure **2** is shown in Figure 1 and the ^1H NMR, COSY, and HSQC spectra of pure **2** are provided in the supporting information. The yield of **2** was determined by integration of the $-\text{CH}_2\text{CF}_3$ to $\text{HOCH}_2\text{CH}_2-$ ^1H NMR signals.

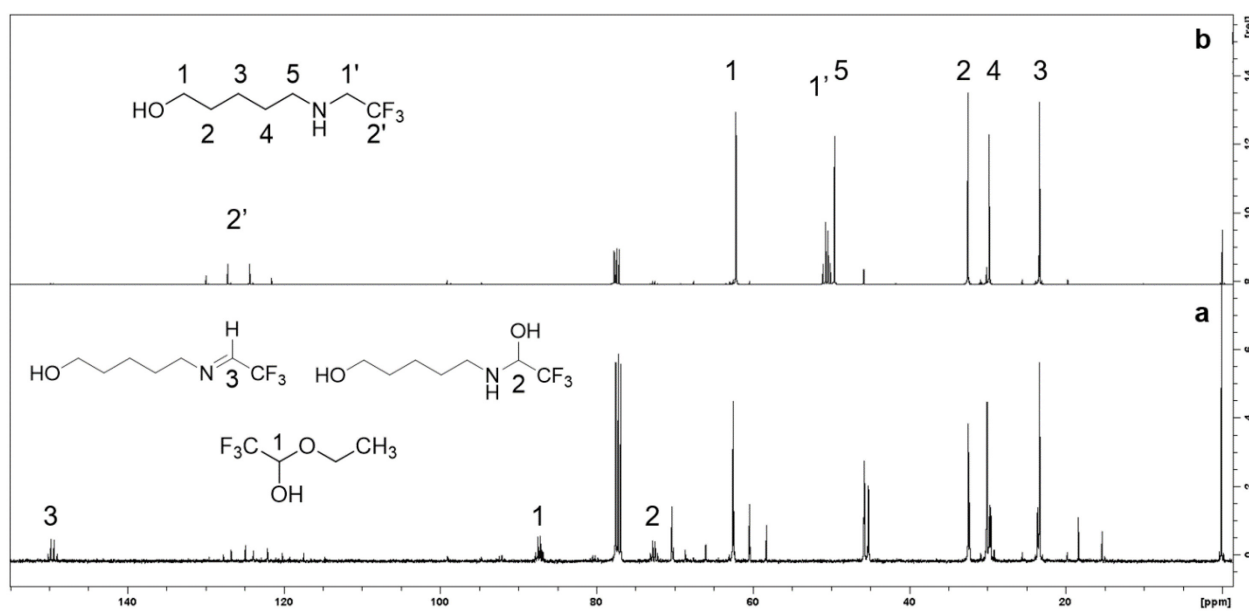


Figure 1. a) Observed equilibrium of trifluoroacetaldehyde ethyl hemiacetal with the imine and hemiaminal intermediates. b) 2° amine after NaBH₄ reduction and separation.

The fluorinated secondary amine **2** was protected using di-*tert*-butyl dicarbonate (Boc₂O) under conventional room temperature reaction conditions to form the tertiary carbamate **3**. This protecting group was chosen not only to reduce the nucleophilicity of the nitrogen but also because of its stability in basic media which was necessary for the Schöllkopf bislactim addition using *n*BuLi. Boc protection was performed using the reaction mixture of primary amino alcohol **1** and secondary fluorinated amino alcohol **2**. While the separation of the unprotected amines by column chromatography led to severe product loss, the separation of the boc protected amines by column chromatography was facile. In the NMR spectra for the tertiary carbamate **3** a loss of ¹H NMR signal resolution at the α position on either side of the nitrogen is observed due to the large protecting group, and a distinct change in chemical shift due to the electron withdrawing carbonyl in the boc protecting group.

The alcohol functionality in **3** was converted to a bromide in **4** using Appel bromination under anhydrous conditions^[25] to introduce a more favorable leaving group for the

stereoselective ring substitution in the subsequent step. Excess CBr₄ and PPh₃ were removed by simple vacuum filtration using hexane which also separates the by-product triphenylphosphine oxide O=PPh₃ due to low solubility. Alkyl bromide **4** degrades easily in the presence of humidity and therefore was always used within one week.

The amino acid moiety is introduced via the Schöllkopf bislactim reaction using the bislactim reagent (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (Scheme 2). To ensure that *n*BuLi would not prematurely couple with **4**, *n*BuLi and the bislactim were mixed in a 1:1 equimolar ratio. *n*BuLi generates the conjugate carbanion of the bislactim in a stereoselective manner. This deprotonation is accompanied by a color change of the solution from pale yellow to deep red.^[26] This anionic species couples to the brominated tertiary carbamate **4** to form cyclic intermediate **5** and precipitating LiBr.^[26]

For convenient separation and retention of the C_α stereocenter, the imine functionalities of **5** were selectively hydrolyzed to the methyl ester **6** and valine methyl ester. This hydrolysis kept the boc protecting group in place because of the use dilute strong acid. The byproduct was removed by l/l extraction and rotatory evaporation^[29] to afford pure **6**. Further hydrolysis of **6** with concentrated hydrochloric acid generated the de-protected amino acid **7**. The ¹H NMR spectra of the Schöllkopf products **6** (*n* = 2, 3) do not provide any indication of the presence of the minor enantiomer, that is, the enantiomeric excess (e.e.) is at least 90% and, according to literature,^[26] it is likely to be much higher. It has been shown that racemization of amino acids occurs slowly during strong acid hydrolysis.^[30-32] Although the stereochemistry of **6** can be guaranteed, the same cannot be said for **7** and, hence, **7** is shown as a racemic mixture in Schemes 1 and 2. LC-MS analysis of **6** and discussion of the NMR spectra of **7** are presented in the following sections.

LC-MS Analysis of Boc Protected Fluorinated **6**

The two protected products **6**, methyl *N*^ε-(2,2,2-trifluoroethyl)-*L*-lysinate (*n* = 2) and methyl *N*^ζ-(2,2,2-trifluoroethyl)-*L*-homolysinate (*n* = 3), were separated and characterized by LCMS. With a view to the unprotected amino acid functional group in **7**, it was preferable to characterize product **6** which contains a methyl ester protecting group. LCMS traces and MS spectra for these two compounds can be found in the supporting information (Figures S47 and S48). Both methyl *N*^ε-(2,2,2-trifluoroethyl)-*L*-lysinate and methyl *N*^ζ-(2,2,2-trifluoroethyl)-*L*-homolysinate show a fragment with *m/z* = 56. The appearance of this fragment was explained by a McLafferty rearrangement of the Boc protecting group (Figure S49), generating a carbamic acid and isobutene (*m/z* = 56).^[33]

Fluorine Coupling in ¹H and ¹³C NMR Spectra of Deprotected **7**

The ¹⁹F nucleus is nearly monoisotopic and possess a half integer spin which causes strong *J*-coupling to ¹H nuclei.^[34] The ¹⁹F nucleus splits the signals of neighboring hydrogen in ¹H NMR spectra and de-shields adjacent ¹H environments due to fluorine's inductive electron withdrawing effect. The *J*(¹H, ¹⁹F) coupling constants (Hz) for the hydrogen at the N-CH₂CF₃ (1') position of *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-lysine ^{tF}K and *N*^ζ-(2,2,2-trifluoroethyl)-*D,L*-homolysine ^{tF}K₊₁ are 8.85 Hz and 8.82 Hz, respectively.

For the ¹³C NMR spectra, we used the pulse program *zgpg30* which applies ¹H-¹³C decoupling but does not negate *J*(¹⁹F, ¹³C) coupling, allowing for the large *J*-coupling between these two nuclei to confirm the presence of the fluorinated appendage in the final product. The carbons at the N-CH₂CF₃ (1') and N-CH₂-CCF₃ (2') positions each couple to the same three fluorine nuclei in the appendage, causing each of these ¹³C NMR signals to appear as quartets. The magnitude of the ¹*J*-coupling is largest for the 2' carbon since it is directly connected to all three fluorine. The ¹³C NMR spectrum for *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-lysine ^{tF}K is compared

to native lysine, highlighting the coupling effect of fluorine in the $-\text{CH}_2\text{CF}_3$ appendage (Figure 3). The coupling constants (Hz) at the $\text{N}-\underline{\text{C}}\text{H}_2\text{CF}_3$ (1') and $\text{N}-\text{CH}_2\underline{\text{C}}\text{F}_3$ (2') positions for N^ϵ -(2,2,2-trifluoroethyl)-*D,L*-lysine $^{\text{tF}}\text{K}$ are $^2J(^{13}\text{C}, ^{19}\text{F}) = 34.84$ Hz and $^1J(^{13}\text{C}, ^{19}\text{F}) = 277.30$ Hz, respectively. Similarly, the $J(^{13}\text{C}, ^{19}\text{F})$ coupling constants (Hz) for N^ζ -(2,2,2-trifluoroethyl)-*D,L*-homolysine $^{\text{tF}}\text{K}_{+1}$ at the 1' and 2' positions are $^2J(^{13}\text{C}, ^{19}\text{F}) = 34.85$ Hz and $^1J(^{13}\text{C}, ^{19}\text{F}) = 277.66$ Hz, respectively.

In intermediates **3-6**, the ^{13}C NMR is of great interest due to the presence of *two* sets of $-\text{CF}_3$ quartets in the ^{13}C NMR spectrum. This was caused by the presence of two ensembles of non-equilibrating rotational isomers caused by the boc carbamate protecting group.^[35] The $-\text{CF}_3$ group in these compounds acts as a probe to observe the two rotamer ensembles, and the apparent signal strength of the $-\text{CF}_3$ signal is severely diminished since it is split into both a quartet by the ^{19}F nuclei and into two distinct rotamer populations.

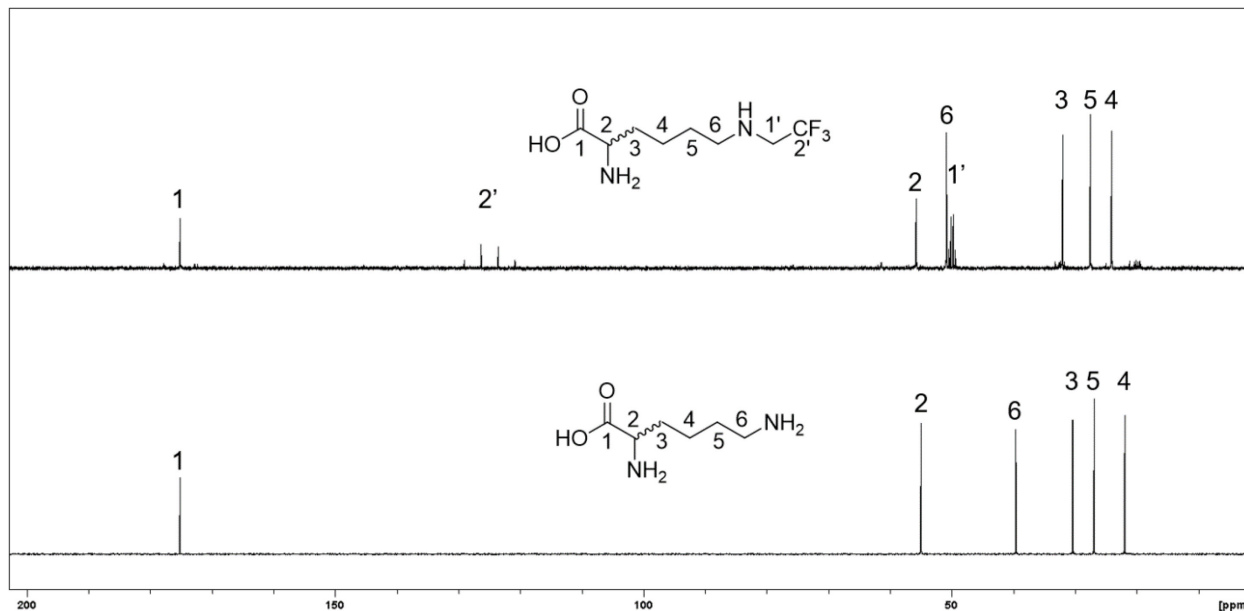


Figure 3. ^{13}C NMR of *D,L*-lysine (bottom) and N^ϵ -(2,2,2-trifluoroethyl)-*D,L*-lysine (**7**, $n = 2$, top) (pulse prog. *zgpg30*, 90% H_2O /10% D_2O , 400 MHz).

Conclusions

A synthetic route has been developed for generating *N*-trifluoroethyl functionalized lysine and homologs with varying alkyl chain lengths. Two unnatural amino acids *N*^ε-(2,2,2-trifluoroethyl)-*D,L*-lysine (**7**, *n* = 2, ¹⁹FK) and *N*^ζ-(2,2,2-trifluoroethyl)-*D,L*-homolysine (**7**, *n* = 3, ¹⁹FK₊₁) were synthesized with this synthetic route. The stereochemistry at the C_α of the amino acid is determined by the chirality of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine employed in the Schöllkopf bislactim synthesis and the thermodynamic addition favored the *S*-enantiomer. The boc-protected methyl ester **6** is of particular interest for its retention of the C_α stereocenter which allows this modified lysine to be incorporated into larger peptide systems.³⁶ Intermediate **6** was characterized by LCMS and featured a fragmentation pattern that produces the corresponding carbamic acid and isobutene from the boc protecting group. Intermediates **3-6** feature *two* sets of –CF₃ quartets in their ¹³C NMR spectra which demonstrate the presence of two ensembles of non-equilibrating rotational isomers caused by the boc carbamate protecting group. The –CH₂CF₃ appendage was confirmed in the final product **7** by ³*J*(¹⁹F, ¹H) coupling in the ¹H NMR spectrum and ¹*J*(¹⁹F, ¹³C) and ²*J*(¹⁹F, ¹³C) coupling in the ¹³C NMR spectrum.

The synthetic route described here for the synthesis of unnatural lysine derivatives, including fluorinated derivatives, proceeds under mild reaction conditions with high yields and offers versatility in the composition of the final product. This method allows for the synthesis of extended lysine derivatives by starting with amino alcohols HO–(CH₂)_{*n*}–NH₂ of varying chain length and a variety of *N*-functionality may be introduced by varying the hemiacetal reagent during the reductive amination reaction.

Conflicts of interest

There are no conflicts to declare.

Supporting Information Available

NMR spectra of *N*-trifluoroethyl lysine ^{tr}K and its homolog ^{tr}K₊₁ and of the intermediates **2-6** along their synthetic paths. LMCS data and a scheme to support the fragmentation pattern of compounds **6**. This supporting information (28 pages) is available free of charge at URL ____.

Acknowledgments

LCMS carried out by Dr. Fabio Gallazzi at the Molecular Interaction Core (MIC) at the University of Missouri. This work was supported by Grant No. 1665487 from the National Science Foundation.

Keywords

Fluorinated Amino Acid Synthesis; Lysine; Homolysine; 2,2,2-Trifluoroethyl Group; Stereospecific Synthesis

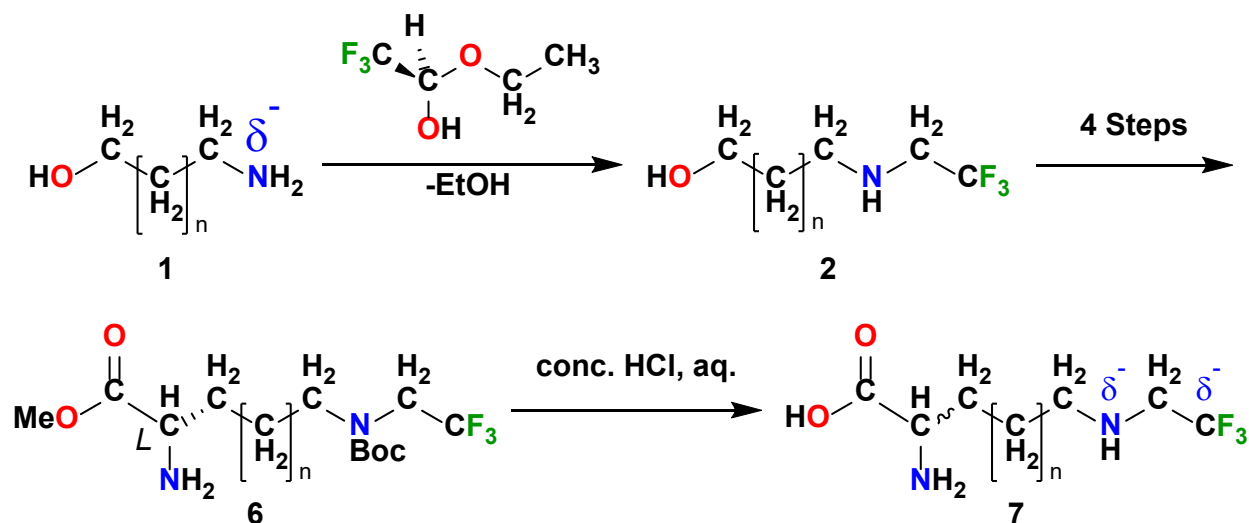
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ToC Graphic



ToC Text

The syntheses are reported of N^ϵ -(2,2,2-trifluoroethyl)- D,L -lysine ($^{\text{tF}}\text{K}$) and N^ζ -(2,2,2-trifluoroethyl)- D,L -homolysine ($^{\text{tF}}\text{K}_{+1}$). Stereoselectively formed N^ϵ -boc- N^ϵ -(2,2,2-trifluoroethyl)- L -lysinate and methyl N^ζ -boc- N^ζ -(2,2,2-trifluoroethyl)- L -homolysinate are relevant substrates for oligopeptide synthesis. This fluorine modification reduces the sidechain N -basicity and increases the [amine]/[ammonium ion] ratio of the sidechain amine in lysine derivatives.