ARTICLE

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4-Fluorinated L-lysine analogs as selective i-NOS inhibitors: methodology for introducing fluorine into the lysine side chain†‡

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In the literature, the introduction of fluorine into bioactive molecules has been known to enhance the biological activity relative to the parent molecule. Described in this article is the synthesis of 4*R*-fluoro-L-NIL (**12**) and 4,4-difluoro-L-NIL (**23**) as part of our iNOS program. Both **12** and **23** were found to be selective iNOS inhibitors as shown in Table 2 below. Secondarily, methodology to synthesize orthogonally protected 4-fluoro-L-lysine and 4,4-difluoro-L-lysine has been developed.

Introduction

Since the pioneering work of Fried and Sabo,[1] it has been known that the regio- and stereo-selective introduction of fluorine into bioactive substrates has the potential to produce molecules that have biological properties that are significantly more potent than the parent.[1,2] The introduction of fluorine into bioactive molecules can, in addition to profoundly enhancing biological potency such as in the case of Taxol,[2b] alter pharmacodynamics and pharmacokinetics and translation across the lipid bilayer.[2] Stereochemically the carbon–fluorine bond van der Waals radius of 1.4 Å affords a minimal change relative to the carbon–hydrogen bond whose van der Waals radius is 1.1 Å. Yet, the electronegativity of fluorine causes significant modulation of the electronic characteristics of a bioactive molecule. A recent review describes pharmaceutical agents or potential agents in which fluorine is incorporated illustrating the benefits of inclusion of fluorine into biologically active molecules.[2] Soloshonok *et al*. have reported on selective incorporation of fluorine into α-amino acids[3] and the enantioselective introduction of fluorine into organic molecules.[4]

Elevated levels of nitric oxide (NO) generated by the action of induced nitric oxide synthase (iNOS) on L-arginine and the resulting NO derived-metabolites cause cellular cytotoxicity and tissue damage and are thought to contribute to the pathophysiology of a number of human diseases.[5] Under normal physiological conditions, the constitutive forms of a NOS generate low, transient levels of NO in response to increases in intracellular calcium concentrations. These low levels of NO act to regulate blood pressure, platelet adhesion, gastrointestinal motility, bronchomotor tone and neurotransmission.[6] During the course of our research on selective inhibitors of induced nitric oxide synthase (iNOS), iminoethyl-L-lysine (L-NIL, **1**)[7] was identified as a potent selective inhibitor of the iNOS.[8] L-NIL is an analog of lysine (Lys, **2**) where the ε-amine has been functionalized as an amidine (Chart 1). The amidine moiety is a structural isostere for the guanidine group of arginine which is the substrate of the NOS enzymes. Attempting to understand the stereochemical and electronic requirements of the arginine binding site of iNOS, fluorine was introduced at C-4 of the lysine side chain of L-NIL. The fluorine should alter the electronic characteristics of the side chain while minimally changing its stereochemical properties. Secondarily, we hoped to develop methodology to synthesize orthogonally protected 4-fluoro-L-lysine and 4,4-difluoro-L-lysine. A number of strategies were explored to incorporate, regioselectively, fluorine at carbon-4 (C-4) of the lysine framework. Installing fluorine at C-4 would have the least impact on the polar functionalities at C-2 and C-6 of lysine; whereas, the introduction of fluorine at C-5 would reduce the basicity of the C-6 amidine. Introduction of fluorine at C-3 would impact the α-amino acid functionalities enhancing the acidity of the carboxyl group while attentuating the basicity of the α-amino moiety. Research on the introduction of fluorine at C-5 will be reported elsewhere.[9] Synthesis of 3-fluoro-L-NIL proved troublesome and has been put aside for the time being. Described, herein, are the syntheses of 4-fluoro-L-NIL and 4,4-difluoro-L-NIL and the effect of the introduction of fluorine at C-4 of the lysyl moiety of L-NIL on iNOS inhibition. Additionally, methods for the syntheses of orthogonally protected 4-fluoro-L-lysine and 4,4-difluoro-L-lysine protected are illustrated.

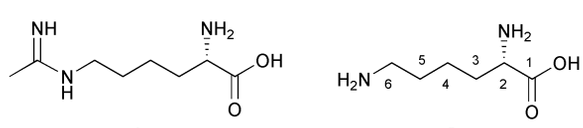

Chart 1 L-NIL (**1**) and L-Lys (**2**).

Results and discussion

In order to introduce fluorine into C-4, methodologies needed to be developed to achieve the desired regio- and stereoselective introduction of fluorine. 4-Fluoro-L-lysine has been described once in the patent literature using drastic reaction conditions to incorporate fluorine.[10] Approaches considered for the introduction of fluorine were electrophilic or nucleophilic fluorination and the use of commercially available fluorinated building blocks.

The introduction of fluorine at C-4 using Wadsworth–Emmons chemistry was explored. Initially, the assembly of the protected 4-fluoro-lysine was attempted by side chain

† Electronic supplementary information (ESI) available. See http://www.rsc.org/suppdata/ob/b3/b307563j/

‡ This article is dedicated to the memory of Professor Josef Fried of the University of Chicago, 1914–2001.

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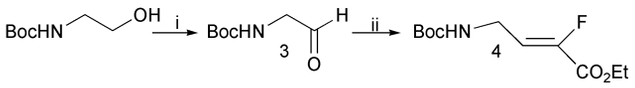
Table 1 Oxidation of amino alcohol

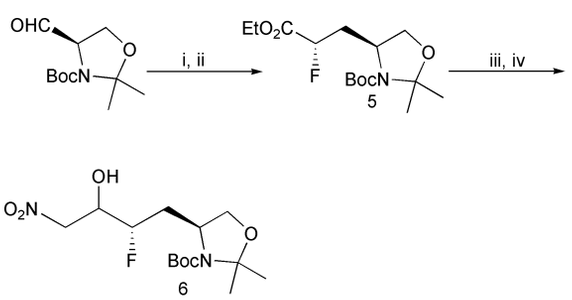
Compound	Oxidant	Solvent	Time	Temperature	Results
9, **10**	PDC	DMF	18 h	Ambient	Nothing recognizable
10	$KMnO_4$, $MgSO_4$	H_2O, sonication	4.5 h	Ambient	Product, s.m, by-products
10	$KMnO_4$, $MgSO_4$	H_2O–THF (1 : 1), sonication	18 h	45–50 °C	Black tar
10	$NaIO_4$, $RuCl_3 \cdot H_2O$ (cat)	H_2O–CH_3CN (1 : 1)	2.5 h	Ambient	77% product

homologation starting with *tert*-butyloxycarbonyl-glycinal[11] (**3**) which proved to be unstable under reaction conditions. As a result, the yields of **4** employing Wadsworth–Emmons reaction conditions were quite variable as illustrated in Scheme 1.



Scheme 1 Boc-Gly-ol approach to 4-fluorolysine: (i) SO_3–pyr, TEA, CH_2Cl_2; (ii) triethyl 2-fluoro-2-phosphonoacetate, NaH, −40 to r.t., 25%.

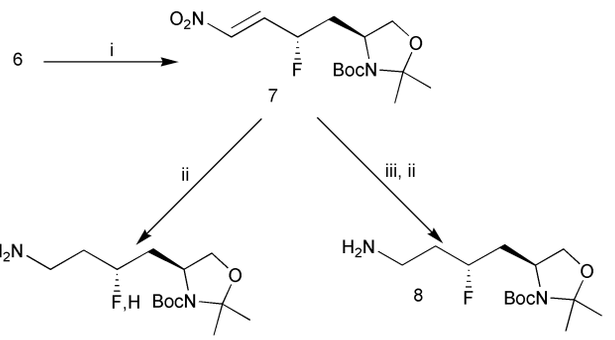
The poor yields in the side chain homologation towards 4-fluoro-L-NIL resulted in a change in strategy. Use of the Garner aldehyde employing Wadsworth–Emmons conditions was explored as an appropriate starting point as shown in Scheme 2. Initially, it was treated with the anion of triethyl 2-fluoro-2-phosphonoacetate generated either with sodium hydride, which was quite sluggish, or lithium hexamethyldisilazide. The resulting olefin was hydrogenated to yield **5**. The reduction was stereoselective to give predominantly the *R*-isomer of fluorine at C-4. When the olefin was reduced with sodium borohydride in the presence of nickel(II) chloride both fluorine diastereomers were obtained which could be seen in ^1H NMR. The ester was reduced by sodium borohydride to give the aldehyde and its hemiacetal. The resulting aldehyde–hemiacetal mixture was subjected to Henry reaction conditions using nitromethane to provide the necessary elements of the lysine side chain as shown in Scheme 2 resulting in **6**.



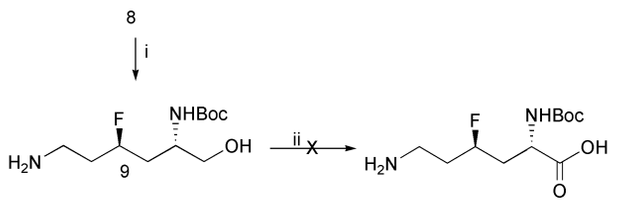
Scheme 2 Garner aldehyde synthesis: (i) triethyl 2-fluoro-2-phosphonoacetate, NaH, −40 to r.t., THF, overnight, 87%; (ii) H_2, 60 psi, Pd/C, EtOH, 90%; (iii) $NaBH_4$, MeOH, −50–0 °C; (iv) CH_3NO_2, Na_2CO_3, THF, 2 days, r.t., 100%.

The Henry product was treated with methanesulfonyl chloride which, not surprisingly, gave directly the desired unsaturated nitro product **7** as illustrated in Scheme 3. Catalytic hydrogenation of **7** using Pd/C resulted in the partial loss of fluorine. However, a two step process of sodium borohydride reduction of the olefin followed by catalytic hydrogenation reduction of the nitro group gave the desired amine **8**.

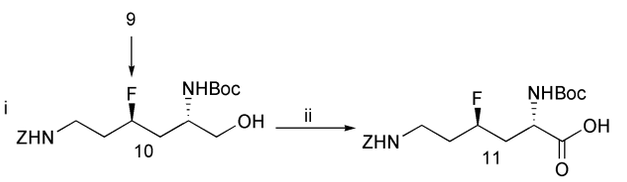
Shown in Scheme 4, unmasking the protected amino alcohol with aqueous acetic acid gave the desired amino alcohol **9**. Several oxidation conditions were attempted. It became necessary to protect the ε-amine with the benzyloxycarbonyl group as attempted oxidations of the alcohol were unsucessful in the presence of the free amine. Protected **10**, as shown in Scheme 5 and Table 1, was successfully oxidized using sodium periodate with ruthenium(III) chloride as the catalyst. This provided an orthogonally protected 4-fluoro-lysine **11** which could be used



Scheme 3 Reduction of the nitroolefin: (i) MsCl, TEA, CH_2Cl_2, DMAP cat, −70 °C to ambient, 2 h, 80%; (ii) H2, Pd/C, EtOH, 90%; (iii) $NaBH_4$, MeOH–H_2O, 98%.



Scheme 4 Attempted oxidation of the amino alcohol: (i) 3 : 1 AcOH–H_2O, r.t., 3 days; (ii) see Table 1.

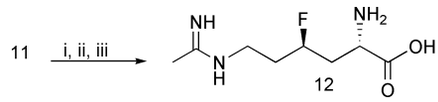


Scheme 5 Oxidation of the amino alcohol: (i) ZOSuc, $NaHCO_3$, 66%; (ii) see Table 1.

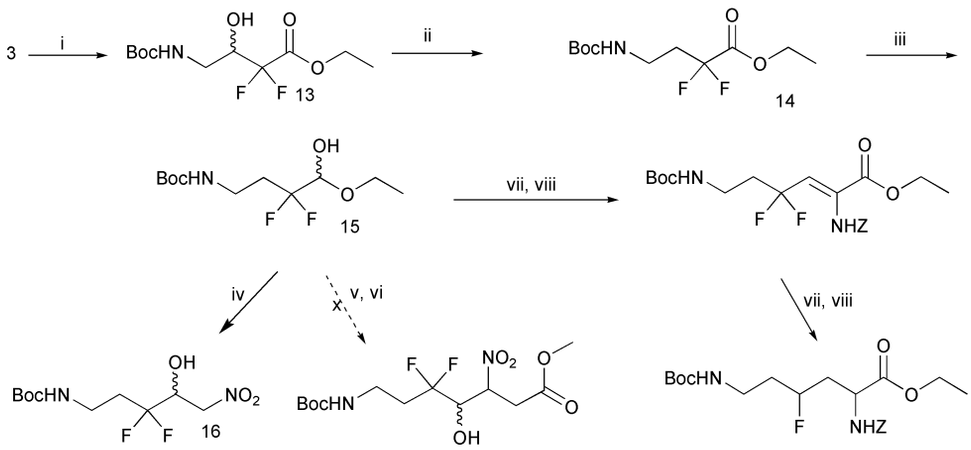
in other chemistries such as the incorporation into peptides or a precursor in the synthesis of peptidomimetics.

The completion of the synthesis of 4*R*-fluoro-L-NIL shown in Scheme 6 involved removal of the benzyloxycarbonyl group of **11** by treatment under catalytic hydrogenation conditions, followed by amidination using conditions described previously.[12] As improved reaction conditions were explored, ethyl acetimidate hydrochloride was found to be more easily handled and a more stable amidinating reagent than methyl acetamidate hydrochloride. For ease of purification by reverse phase separation on a YMC ODS AQ column, the *tert*-butyloxycarbonyl protecting group was left on. We also found that the amidine could be formed on the unprotected amino acid. Treatment of the protected amidine **11** with anhydrous hydrochloric acid yielded *4*R-fluoro-L-NIL (**12**).

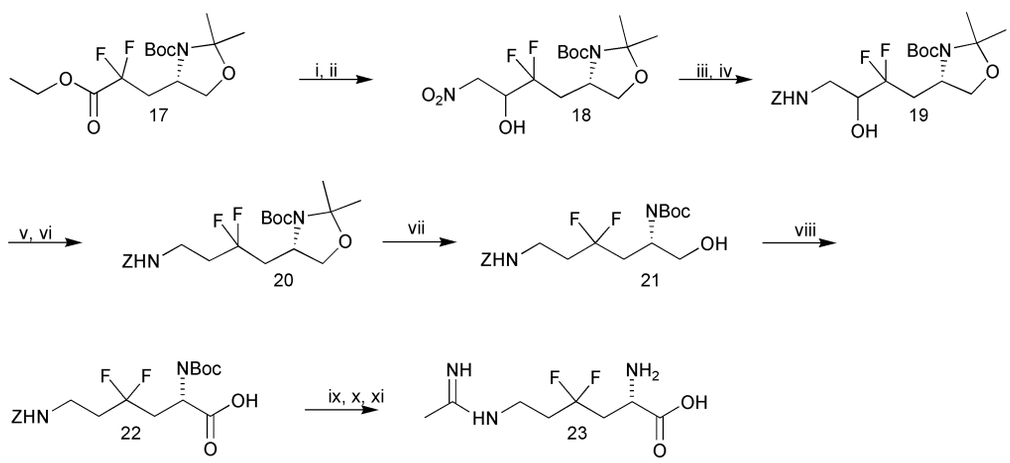
In parallel with our efforts to synthesize 4-fluoro-L-NIL, 4,4-difluoro-L-NIL was synthesized. No report in the literature on



Scheme 6 Amidine synthesis: (i) Pd/C, MeOH, 5 psi, r.t., 100 %; (ii) ethyl acetimidate hydrochloride, NaOH, r.t., EtOH, 36 %; (iii) 4 M HCl–dioxane, HOAc, quantitative.

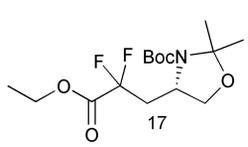


Scheme 7 Attempted synthesis of 4,4-difluoro-L-NIL using the Henry reaction. (i) Zn, ethyl bromodifluoroacetate, THF, Δ, 4 h, 42%; (ii) a. thiocarbonyldiimidazole, cat. DMAP, DCM, b. Et_3SiH, benzoyl peroxide, toluene, Δ, 84%; (iii) $NaBH_4$, MeOH, −60 °C, 78%; (iv) nitromethane, K_2CO_3, THF, 18 h, r.t., 75%; (v) ethyl nitroacetate, K_2CO_3, THF, 18 h, r.t., no reaction; (vi) ethyl nitroacetate; DBU, 18 h, r.t., no reaction; (vii) triethyl Z-phosphono-glycine, base; (viii) H_2, a variety of catalysts.



Scheme 8 Synthesis of 4,4-difluoro-L-NIL: (i) 1 M DIBAL-H in toluene, toluene, 1.5 h, −78 °C, EtOH quench, 100%; (ii) nitromethane, K_2CO_3, 20 h, r.t., 80%; (iii) 20% $Pd(OH)_2$/C, HOAc, 3 h, r.t., 91%; (iv) CBZ–OSuccinimide, $NaHCO_3$, Acetone–H_2O (1 : 1), 18 h, 0 °C to r.t., 78%; (v) thiocarbonyldiimidazole, DMAP, DCM, 1 h, r.t., 81%; (vi) benzoyl peroxide, triethylsilane, toluene, 3 h, reflux, 71%; (vii) acetic acid–H_2O (4 : 1); (viii) $NaIO_4$, cat. $RuCl_3$, ACN–H_2O (1 : 1), 2.5 h, r.t., 75%; (ix) Pd/C, MeOH, 5 psi, r.t.; (x) ethyl acetimidate hydrochloride, NaOH, EtOH, r.t., 35%; (xi) glacial HOAc, 4 M HCl in dioxane, 1 h, 95%.

the synthesis of 4,4-difluoro-L-lysine was found. We used published substrates, among them were 3,3-difluoro-L-pyroglutamol,[13] 4,4-difluoro-L-glutamic acid,[14] and 1-benzyl 6-methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-4-oxohexanedioate,[15–17] towards the synthesis of 4,4-difluoro-L-NIL. Ultimately compound **17**, described by Kim and Qian[18] in their synthesis of 4,4-difluoro-L-arginine was the pivotal intermediate needed to further homologate the fluorinated lysine side chain. This compound was synthesized using Garner's aldehyde which was further elaborated under Reformatsky reaction conditions[19] followed by deoxygenation.



As described in the synthesis of 4*R*-fluoro-L-NIL, side chain elaboration was initially attempted as shown in Scheme 7. The Reformatsky reaction using Boc-glycinal and ethyl bromodifluoroacetate proceeded smoothly resulting in **13** with careful handling needed in order to avoid loss of ester. Chromatography frequently led to lower yields. Once characterized, chromatography of **13** was avoided after the Reformatsky reaction because **13** was sufficiently pure to carry into the next reaction and the yields were greatly improved. Deoxygenation using Barton conditions yielded **14**. With the side chain skeleton in hand, the ester **14** was reduced using sodium borohydride and quenching with ethanol to give **15**. To demonstrate that the Henry reaction would be a reasonable approach, a model system using the hemiacetal **15** and nitromethane was attempted. This proved successful in yielding **16**. Introduction of the incipient fragments of the amino acid was attempted with ethyl nitroacetate as the α-amino acid presursor. This failed to give desired nitro ester. An attempt to introduce the amino acid fragment using triethyl *N*-benzyloxycarbonylphosphonoglycinate and **15** gave a mediocre yield of α,β-unsaturated amino acid. What caused us to abandon this approach was the inability to find reduction conditions, either chiral or achiral, that did not result in the loss of one fluorine.

With a few modifications, the synthesis of 4,4-difluoro-L-NIL was completed in a similar manner to **12** as shown in Scheme 8. The ester of oxazolidine **17**[18] was reduced as described previously for **15**, using either sodium borohydride or Dibal-H. Treatment of the hemiacetal with nitromethane gave the desired hydroxynitro adduct **18**. Attempts to deoxygenate **18** under mesylation conditions followed by elimination prior to the reduction of the nitro group were unsuccessful. Reduction of the nitro group and subsequent protection of the amine **19** was necessary before successful deoxygenation to give **20**. The remaining steps were the same as described for **12** to yield the desired amidine **23**.

Table 2 NOS inhibition (IC_{50}, μM)

Compound no.	i-NOS	e-NOS	n-NOS	Selectivity e-NOS/i-NOS	Selectivity n-NOS/i-NOS
L-NIL (**2**)	4.94 ± 1.65	127 ± 25	47.1 ± 11	26	10
12	6.09	477	88.7	78	15
23	7.08	512	80.6	72	11

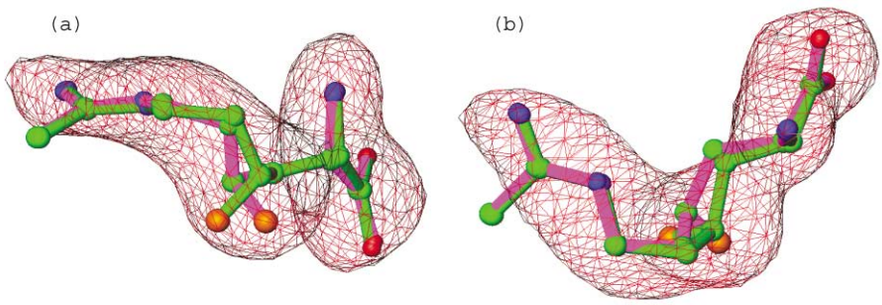


Fig. 1 The red nets represent the difference electron density map, calculated without including the contribution from the inhibitor. The refined models, *R*-configuration in green and *S*-configuration in magenta, are docked into this unbiased map. The fluorine atoms in both configurations are orange. Fig. 1a clearly indicates that the fluorine atoms in both configurations are docked nicely into the electron density. Fig. 1b is obtained by rotating the horizontal axis a 90° of Fig. 1a. The CB atom of the *S*-configuration is a bit out of the electron density in this figure.

Bioassay

Enzyme inhibition assays were performed with the human NOS isoforms as described previously.[20] Introduction of fluorine at C-4 of L-NIL provided compounds with similar enzyme inhibition of iNOS isozyme as L-NIL as shown in Table 2. However, both **12** and **23** showed improved selectivity for the iNOS isozyme *versus* eNOS enzyme of 78 and 72 respectively and iNOS *versus* nNOS enzyme of 15 and 11.

X-Ray crystallography

When miNOS became available in-house, compounds that had previously shown promising biological activity were co-crystallized with miNOS.

The crystal structure of the oxygenase domain (residues 66–498) of murine iNOS in complex with compound **12** has been determined with 2.3 Å resolution. The protein production and purification, and the crystallization and structure determination have been described by Tainer *et al*.[21] In compound **12**, there are two chiral centers at C-4 and C-2. The stereochemistry at C-2 was set as "*S*" because the synthesis was effected by the use of chiral Garner aldehyde and no loss of stereochemistry was observed. As noted above, a single isomer was prepared. Alternative chemistry that provided the saturated fluorinated side chain produced a diastereomeric pair. Although the unbiased difference Fourier map (without including the inhibitor contribution) showed that the *R*-configuration molecule fitted slightly better than the *S*-configuration molecule (Fig. 1), the refinement statistics, both having same *R*free and *R*, did not distinguish the two configurations.

If we consider the amino acid end of the compound **12** molecule is the tail part, the chain the middle part and the amidine group the head part, the dominant feature of the interactions is the bi-dentate interaction of Glutamate 371 toward the amidine group (guanidine group in arginine) of the inhibitor. Glutamate 371 also binds to the amino group of the tail part of the inhibitor. These three hydrogen bonds essentially tie down the head and tail parts of the inhibitor molecule and anchor the whole molecule in place. As shown in Fig. 2, the amino group of the inhibitor molecule is surrounded by a cluster of polar residues such as Arg382, Gln257, Asp376, Tyr367 and a series of water molecules (W). The polar interactions of these arginine-like molecules in the head and tail parts with iNOS are all very similar. The middle part of the inhibitor molecule exhibits a certain degree of flexibility as shown by the variations between compound **12** and L-NIL (Fig. 2). These conformational

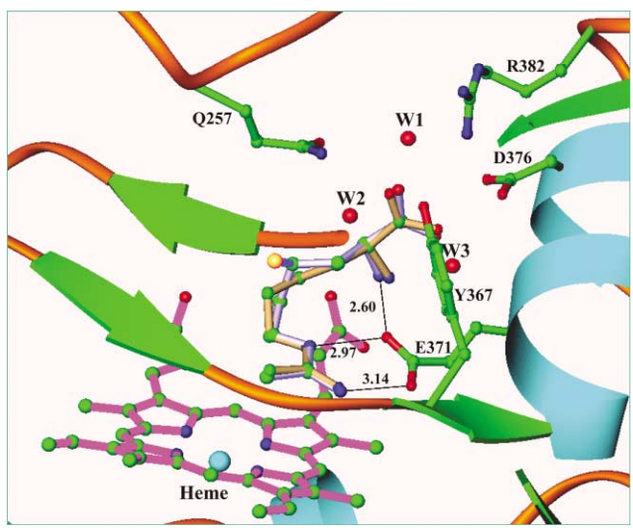


Fig. 2 This is a ribbon representation of the arginine binding pocket of the structure of oxygenase (residues 66–498) domain of mouse iNOS in complex with compound **12**. The L-NIL molecule is also docked into this pocket for comparison. Compound **12** has the bonds in very light blue while L-NIL in light orange and heme in magenta. Some important adjacent residues around the inhibitor are depicted. The fluorine atom is shown in bright orange. The three neighboring water molecules are labeled W1, W2 and W3.

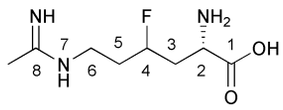
variations are summed up in the Table 3. These kinds of flexibility offer opportunities for inhibitor design. So far, none of these arginine (or L-NIL) like molecules have been found to have direct interaction with the heme molecule. In this case, the nearest group, the methyl group of the amidine group, exhibits a 4.2 Å distance toward the heme iron atom in compound **12** and 4.4 Å in L-NIL.

The crystal structure determination did not distinguish the absolution configuration of the inhibitor. On one hand, it may be due to the limitation of the data resolution. Although the fluorine atom points to different direction for each diastereomer, the pocket is large enough to accommodate both *R* and *S* fluorine atoms as shown in Fig. 2.

Conclusion

The introduction of fluorine into the lysine side chain at C-4 has yielded highly selective iNOS inhibitors with potencies comparable to L-NIL and greater selectivity than NIL *versus*

Table 3 The formula, numbering system and torsion angles for compound **12** and L-NIL



	N=8–7–6	8–7–6–5	7–6–5–4	6–5–4–3	5–4–3–2	4–3–2–1	3–2–1=*O*
12	159	173	−66	94	87	125	60
NIL	162	116	67	−102	−178	161	122

eNOS and nNOS. The X-ray crystallography data shows the arginine binding site can accommodate modification of the lysine side chain. In addition, we have developed methods to synthesize orthogonally protected 4-fluoro and 4,4-difluoro-L-lysine.

Experimental

Thin layer chromatograms were run on 0.25 mm EM precoated plates of silica gel 60 F254. Visualization was achieved by exposure to I_2 or phosphomolybdic acid or $Ca(ClO)_2$ followed by spraying with a 0.5% KI–0.5% potato starch solution. G.C. were run on a Shimadzu 9A using capillary columns from several vendors. Preparative column chromatograpy using flash chromatography conditions was performed on EM silica gel or a Biotage Flash-40 system. To successfully use the Biotage columns under flash chromatography conditions the optimal R_f needs to be 0.15–0.2 in contrast to an R_f of 0.35–0.4 as described by Still.[22] In general, eluting solvents for the normal phase columns were combinations of EtOAc–hexane (heptane for larger columns), EtOAc–DCM and EtOH–DCM. The solvent choice was made based on the compound lipophilicity and R_f. Hydrophilic compounds were purified by reverse phase column chromatography. Reverse phase chromatography was performed on a Rainin preparative HPLC system with a YMC ODS-AQ (10 μ) preparative column eluting with acetonitrile–water (0.05% HOAc) at 10 mL min^{-1}. HPLC chromatography was performed using a Hewlitt-Packard HP 1100 with a dual absorbance detector. Both chiral and achiral columns were acquired from several vendors. ^{1}H NMR spectra were taken in commercially available deuterated solvents on a Bruker 400 MHz Ultrashield. All chemical shifts are reported in parts per million (δ) downfield (positive) relative to Me_4Si (organic solvents) or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (D_2O) as an internal standard for ^{1}H and ^{13}C NMR. ^{13}C NMR spectra were obtained on a Bruker 400 MHz Ultrashield spectrometer at an observation frequency of 100 MHz. The observation frequency for ^{19}F NMR was 376 MHz and the chemical shifts in parts per million (δ) were reported upfield or downfield relative to fluorotrichloromethane (Freon-11). ^{19}F NMR spectra were proton decoupled. All reagents were purchased from Sigma-Aldrich and used as is. Solvents were purchased from Burdick & Jackson. Microanalyses and optical rotations were performed in-house.

3. Boc-glycinal

To an ice cooled stirred solution of Boc-glycinol (50.0 mmol, 8.06 g) in 150 mL of DCM was added TEA (150 mmol, 20.9 mL) and SO_3·pyridine (150 mmol, 23.96 g) in 100 mL of DMSO. The reaction changed from clear to yellow upon addition of the oxidant. After removal from the icebath, the reaction was stirred for 20 min. The reaction mixture was poured into 450 mL of ice cold brine. The aqueous layer was extracted with Et_2O (2 × 500 mL). The combined organic washes were washed with cold 1 M $KHSO_4$ (100 mL) and with cold brine (2 × 100 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered, and concentrated under vacuum. The residue was filtered through a pad of silica gel (90 × 50 mm). The aldehyde was eluted from the silica gel with 20% EtOAc in DCM. The yield of aldehyde **3** was 78%. The aldehyde is not particularly stable and needs to be either stored at −20 °C or used immediately.

5. *tert*-Butyl (4*S*)-4-(3-ethoxy-2-fluoro-3-oxopropyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

5a: To a stirred cooled (15–20 °C) suspension of NaH (6.6 mmol, 0.264 g) in 40 mL of THF was added triethyl 2-fluoro-2-phosphonacetate (6.6 mmol, 1.59 g) in 5 mL of THF. After stirring for 18 h at ambient temperature, the reaction was cooled to −40 °C to which was added Garner's aldehyde (4.4 mmol, 1.00 g) in 5 mL THF. After stirring for 2 h at 5 °C and 3 h at room temperature, an additional 2.2 mmol of the phosphonate anion was added to the reaction mixture. After 18 h, MTBE (50 mL) was added to the reaction. The organic layer was washed with brine. After drying over $MgSO_4$, the reaction mixture was filtered and concentrated under vacuum. The residue was purified by flash chromatography to yield 1.20 g (85%) of desired **5a**. δ_H (400 MHz; $CDCl_3$) 1.27 (6 H, s, 2 CH_3), 1.34 (3 H, t, J = 7.1 Hz, OCH_2CH_3), 1.45 (9 H, s, $C(CH_3)_3$), 4.16 (2 H, br t, CH_2O), 4.32 (2 H, q, J = 7.1 Hz, OCH_2CH_3), 4.93 (1 H, br s, $NCHCH_2$), 6.01 (1 H, dt, J = 7.2, 19.3 Hz, CH=CF). δ_C (100 MHz, $CDCl_3$) 14.5 (OCH_2CH_3), 24.1 (CH_3), 27.0 (CH_3), 28.8 ((CH_3)$_3$C)), 53.8 (CH), 62.1 (OCH_2), 69.1 (OCH_2), 80.2 ((CH_3)$_3$CO)), 92.9 ($OC(CH_3)_2N$), 117.4, 129.0 (d, J = 81.0 Hz, CH=CF), 149.2 (d, J = 261.4 Hz, CH=CF), 160.9 (d, J = 34.0 Hz, CH=CFC=O).

5: To a solution of **5a** (1.57 mmol, 0.50 g) in EtOH was added 5% Pd/C. After 18 h under H_2 at 60 psi at ambient temperature, the Pd/C was filtered from the reaction and the reaction mixture was concentrated under vacuum to quantitatively yield **5**. ^{1}H NMR confirmed reduction of olefin. δ_H (400 MHz; $CDCl_3$) 1.32 (3 H, t, J = 7.0 Hz, OCH_2CH_3), 1.49 (9 H, s, $C(CH_3)_3$), 1.55 (3 H, s, CH_3), 1.60 (3 H, s, CH_3), 2.01–2.38 (2 H, m, CH_2CHF); 3.77–4.20 (3 H, m, $NCHCH_2$, CH_2O), 4.27 (2 H, q, J = 7.0 Hz, OCH_2CH_3), 5.00 (1 H, ddd, J = 8.2, 35.2, 46.0 Hz, CHF). δ_F (376 MHz; $CDCl_3$) −156.2.

6. *tert*-Butyl (4*S*)-4-[(2*S*)-2-fluoro-3-hydroxy-4-nitrobutyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

6a: To a stirred solution cooled to −50 °C of **5** (1.57 mmol, 0.50 g) in 3.5 mL of MeOH was added $NaBH_4$ (3.13 mmol, 0.118 g) in four portions at 15 min intervals. After stirring for an additional hour, $KHSO_4$ (1 M, 0.5 mL) was added and the reaction was stirred with warming to 0 °C. To the reaction was added EtOAc (50 mL), which was washed with $KHSO_4$ (1 M, 50 mL), and brine (1 × 100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The yield of product was 0.46 g which was a mixture of hemiacetal and aldehyde.

6: To a stirred suspension of K_2CO_3 (0.75 g, 5.4 mmol) in 50 mL of THF cooled to 5 °C was added a solution ot **6a** (24.0 mmol, 7.85 g) and nitromethane (5.0 mL, 92.3 mmol) in 100 mL of THF. After warming to ambient temperature, the reaction was stirred overnight. To the reaction was added 0.5 M

$KHSO_4$ (50 mL) and MTBE (100 mL). The layers were separated and the organic layer was washed with saturated brine (2 × 100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum to yield 7.93 g (99%) of a white solid. (Found: C, 50.35; H, 7.86; N, 8.05. Calc. for $C_{19}H_{25}F_1N_2O_6$: C, 50.00; H, 7.49; N, 8.33%.) δ_H (400 MHz; $CDCl_3$) 1.50 (9 H, s, $C(CH_3)_3$), 1.52–1.64 (6 H, m, 2 CH_3), 1.92 (1 H, hept, J = 6.7 Hz, CH_2CHF), 2.07–2.38 (1 H, m, CH_2CHF), 3.75–3.92 (1 H, m, $NCHCH_2$), 3.91–4.09 (2 H, m, CH_2O), 4.21–4.49 (1 H, m, CHF), 4.46–4.75 (4 H, m, CH_2NO_2, CHF, OH).

7. *tert*-Butyl (4*S*)-4-[(2*S*,3*E*)-2-fluoro-4-nitrobut-3-enyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

To a stirred solution of **6** (13.3 mmol, 4.48 g) in 70 mL of DCM cooled to −70 °C was added TEA (3.7 mL, 26.6 mmol), methanesulfonyl chloride (1.07 mL, 13.8 mmol), and DMAP (5 mg). After stirring for 1 h, the cloudy yellow solution was allowed to warm to room temperature. The reaction mixture was filtered through a thin pad of EM silica gel and washed with 20% EtOAc–DCM. The filtrate was concentrated under vacuum yielding 4.14 g (99%) of product.

8. *tert*-Butyl (4*S*)-4-[(2*R*)-4-amino-2-fluorobutyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

8a: To a stirred solution of **7** (3.62 g, 11.5 mmol) in 115 mL of THF was added H_2O (23 mL) followed by $NaBH_4$ resulting in the evolution of gas. After 3 h, the reaction was concentrated under vacuum. The residue was treated with EtOAc (200 mL) and 1 M $KHSO_4$ (100 mL). After the layers were separated, the organic layer was washed with saturated brine (1 × 100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to yield 3.77 g (100%) of a pale yellow oil. δ_H (400 MHz; $CDCl_3$) 1.49 (9 H, s, $C(CH_3)_3$), 1.56 (3 H, s, CH_3), 1.59 (3 H, s, CH_3),1.96 (2 H, hept, J = 6.7 Hz, CH_2CHF), 2.20–2.49 (2 H, m, CH_2CHF), 3.78–3.88 (1 H, m, $NCHCH_2$), 3.90–4.08 (2 H, m, CH_2O), 4.47–4.59 (2 H, m, CH_2NO_2), 4.60–4.88 (1 H, m, CHF).

8: The product from **8a** (3.61 g, 11.3 mmol) in EtOH was treated with H_2 at 60 psi and 5% Pd/C for 24 h. After the catalyst was filtered, the filtrate was concentrated under vacuum to give 3.61 g of **8**.

9. 1,1-Dimethylethyl [(1*S*,3*R*)-5-amino-3-fluoro-1-(hydroxymethyl)pentyl]carbamate

A solution of **8** (3.30 g, 11.4 mmol) in 120 mL HOAc : H_2O (5 : 1) was stirred for 3 d at ambient temperature. The reaction mixture was concentrated under vacuum to quantitatively recover product.

10. Phenylmethyl [(3*R*,5*S*)-5-[[(1,1-dimethylethoxy)carbonyl]-amino]-3-fluoro-6-hydroxyhexyl]carbamate

To a stirred icebath cooled solution of **9** (3.60 g, 11.4 mmol) in 120 mL of acetone:H_2O (1 : 1) was added $KHCO_3$ (2.28 g, 22.8 mmol) and benzyloxycarbonylsuccinimde (2.84 g, 11.4 mmol). After 30 min, additional $KHCO_3$ (1.14 g, 5.7 mmol) was added to the reaction. The reaction was allowed to warm to room temperature and was stirred for 1 h. After concentrating the reaction under vacuum, EtOAc : MTBE (1 : 1, 100 mL) was added to reaction mixture. The organic layer was washed with 1 M $KHSO_4$ (100 mL), saturated solution of $KHCO_3$ (100 mL), and brine (100 mL). Following the washes the organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified using flash chromatography conditions yielding 2.90 g (66%) of a pale yellow solid. δ_H (400 MHz; $CDCl_3$) 1.42 (9 H, s, $C(CH_3)_3$), 1.64–1.90 (4 H, m, CH_2CHF), 3.24–3.38 (2 H, m, $NHCH_2$), 3.53–3.68 (2 H, m, CH_2O), 3.75–3.90 (1 H, m, $NHCH$), 4.58–4.80 (1 H, m, CHF), 5.04–5.15 (2 H, m, $PhCH_2O$), 7.29–7.38 (5 H, m, Ph).

11. (4*R*)-*N*-6-[(benzyloxy)carbonyl]-*N*-2-(*tert*-butoxycarbonyl)-4-fluoro-3-methyl-L-lysine

To a solution of **10** (0.92, 2.4 mmol) in 72 mL of CH_3CN : H_2O (1 : 1) was added $Ru(III)Cl_3 \cdot H_2O$. The reaction turned a greenish brown. To the stirred reaction was added $NaIO_4$ (2.08 g, 9.6 mmol). The stirring was stopped once the addition was complete. After 20 min, a white precipitate had formed. After 2 h, EtOAc : MTBE (1 : 1, 60 mL) and 10% $NaHSO_3$ solution (60 mL) were added to the reaction. The organic layer was washed with additional 10% $NaHSO_3$ (60 mL) and saturated brine (60 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered, and concentrated under vacuum to yield 0.74 g (77%) of the desired acid. δ_H (400 MHz; $CDCl_3$) 1.43 (9 H, s, $(CH_3)_3C$), 1.48–1.55 (2 H, m, CH_2CHF), 1.58–1.92 ((2 H, m, CH_2CHF), 3.01–3.21 (2 H, m, $NHCH_2$), 4.24–4.36 (1 H, m, $NHCHC{=}O$), 5.10 (2 H, CH_2O), 5.16–5.40 (1 H, m, CHF), 6.40 (1 H, br d, NH), 7.28–7.39 (5 H, m, Ph). δ_C (100 MHz, $CDCl_3$) 28.7 ($(CH_3)_3C$), 29.7 (CH_2CHF), 32.0 (CH_2CHF), 41.0 ($NHCH_2$), 53.5 ($NHCH$), 67.1 ($PhCH_2O$), 81.5 ($(CH_3)_3C$), 128.5 (Ph), 128.6 (Ph), 128.9 (Ph), 137.0 (Ph), 156.0 ($OC{=}O$), 157.0 ($OC{=}O$), 176.6 ($COOH$).

12. *N*-6-iminoethyl-4-fluoro-L-lysine dihydrochloride

12a: Removal of the benzyloxycarbonyl protecting group of **11** (0.43 g, 1.1 mmol) was achieved under catalytic hydrogenation conditions at 5 psi and Pd/C to quantitatively yield the free ε-amine.

12b: The α-N-Boc-4*R*-fluoro-L-lysine (0.37 g, 1.1 mmol, **12a**) in 5 mL of H_2O was treated with 1 M NaOH to pH 9.5. In three portions, ethyl acetimidate hydrochloride (0.424 g, 3.3 mmol) was added to the stirred lysine solution. After each addition the pH was adjusted to pH 9.5. After 15 min of stirring, the pH was adjusted to pH 2. The reaction mixture was concentrated under vacuum. The solids were triturated with EtOH and filtered. The concentrated EtOH filtrate was chromatographed on a YMC ODS AQ column to yield 0.133 g (36%) of Boc-protected amidine.

12. The above **12b** (012 g, 0.35 mmol) in 1.5 mL of HOAc was treated with 4 M HCl–dioxane (0.5 mL, 2 mmol) for 30 min. The reaction mixture was concentrated followed by lyophilization to yield 0.11 g (quantitative) of **12** as a white glassy solid. (Found: C, 32.21; H, 6.63; N, 13.72; Cl, 21.23. Calc. for $C_8H_{16}F_1N_3O_2 \cdot 1.8\ HCl \cdot 1.5\ H_2O$: C, 32.26; H, 7.04; N, 14.11; Cl, 21.42%). δ_H (400 MHz; D_2O) 1.82–2.00 (2 H, m, CH_2CH_2CF), 2.11 (3 H, s, CH_3), 2.20–2.31 (2 H, m, $CFCH_2CH$), 3.33 (2 H, t, J = 6.9 Hz, $NHCH_2$), 4.00 (1 H, dd, J = 3.9, 7.1 Hz, $NHCHC{=}O$). δ_C (100 MHz; D_2O) 18.7 (CH_3), 32.4 (d, J = 25 Hz, CH_2CHF), 35.0 (d, J = 25 Hz, CH_2CHF,), 38.4 (CH_2NH), 51.7 ($CHNH$), 90.2 (d, J = 205 Hz, CHF), 165.3 ($NHC{=}NH$), 173.0 ($COOH$). δ_F (376 MHz; D_2O) −185.1.

13. Ethyl 4-[(*tert*-butoxycarbonyl)amino]-2,2-difluoro-3-hydroxybutanoate

To a solution of **3** (0.56 g, 3.5 mmol) in 15 mL of THF was added etched Zn (0.46 g, 7.0 mmol) and ethyl bromo-difluoroacetate (1.42 g, 7.0 mmol). The reaction mixture was refluxed for 2 h. The reaction solution was decanted from unreacted Zn. To the reaction mixture was added a saturated solution of 1 M $KHSO_4$ (15 mL) and 15 mL of Et_2O. The organic layer was washed with NH_4Cl solution (2 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The reaction mixture was purified using flash chromatography conditions to yield 0.42 g (42%) of yellow oil **13**. Chromatography is not ideal for **13**. (Found: C, 46.40; H, 6.62; N, 5.26. Calc for $C_{11}H_{19}F_2NO_5$: C, 46.64; H, 6.76; N, 4.94%.)

14. Ethyl 4-[(*tert*-butoxycarbonyl)amino]-2,2-difluoro-3-[(1H-imidazol-1-ylcarbonothioyl)oxy]butanoate

14a: To a stirred solution of **13** (0.65 g, 2.3 mmol) in 10 mL was added 1,1-thiocarbonyldiimidazole (0.61 g, 3.4 mmol) followed by DMAP (0.024 g, 0.2 mmol). The yellow of the reaction changed upon the addition of DMAP. After 1 h, the reaction was filtered through a 30 × 20 mm pad of silica gel and was washed with 20% EtOAc–DCM. The solvent was removed under N_2 to yield **14a** (0.86 g, 96%), a pale yellow oil. (Found: C, 46.40; H, 6.62; N, 5.26. Calc. for: $C_{15}H_{21}F_2N_3O_5S$: C, 46.64; H, 6.76; N, 4.94.)

14: The product of **14a** was dissolved in 10 mL of toluene which was concentrated to 5 mL under vacuum. A solution of **14a** (0.79 g, 2.2 mmol) in 20 mL of toluene:Et_3SiH (1 : 1) was heated to reflux. To this solution was added benzoyl peroxide (0.53 g, 2.2 mmol) in 5 mL of toluene in four portions at 15 min intervals. After heating for 2 h, the reaction mixture cooled to room temperature was filtered through a 65 × 30 mm pad of silica gel washed with DCM followed by 20% EtOAc–DCM to yield 0.40 g (84%) of **14**. δ_H (400 MHz; $CDCl_3$) 1.37 (3 H, t, J = 7.1 Hz, OCH_2CH_3), 1.43 (9 H, s, $(CH_3)_3C$), 2.24–2.39 (2 H, m, CH_2CF_2), 3.38 (2 H, br q, OCH_2), 4.34 (2 H, q, J = 7.1 Hz, OCH_2CH_3), 4.71 (1 H, br s, NC*H*). δ_F (376 MHz, $CDCl_3$) −105.3.

15. *tert*-Butyl 4-ethoxy-3,3-difluoro-4-hydroxybutylcarbamate

To a solution cooled to −60 °C of **14** (0.27 g, 1.0 mmol) in 2 mL was added $NaBH_4$ (0.75 g, 2.0 mmol) in 4 equal portions 15 min apart. After 1 h, 1 M $KHSO_4$ (1 mL) and 15 mL Et_2O were added. When the reaction had warmed to 5 °C, 15 mL of saturated brine was added. The aqueous layer was extracted with Et_2O (2 × 5 mL). The combined organic layers were washed with saturated brine (2 × 20 mL). The organic layer was treated with Na_2SO_4, filtered, and stripped to recover **15** (0.21 g, 78%). δ_H (400 MHz; $CDCl_3$) δ 1.25 (3 H, t, J = 7.1 Hz, OCH_2CH_3), 1.44 (9 H, s, $(CH_3)_3C$), 2.07–2.30 (2 H, m, CH_2CF_2), 2.96 (1 H, br s, O*H*), 3.28–3.48 (2 H, m, $NHCH_2$), 3.49–3.58 (1 H, m, OCH_2CH_3), 3.85–3.94 (1 H, m, OCH_2CH_3), 4.66 (1 H, ddd, J = 5.0, 7.8, 9.9 Hz, C*H*OH), 4.80 (1 H, br s, N*H*).

16. *tert*-Butyl 3,3-difluoro-4-hydroxy-5-nitropentylcarbamate

On a 7.9 mmol scale, **16** was prepared as described in example **6** starting with the product of example **15**.

17. *tert*-Butyl (4*S*)-4-(3-ethoxy-2,2-difluoro-3-oxopropyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate [16]

Compound **17** was synthesized as described in literature reference 16.

18. *tert*-Butyl (4*S*)-4-(2,2-difluoro-3-hydroxy-4-nitrobutyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

18a: A solution of **17** in 10 mL of toluene was concentrated to 5 mL under vacuum. To a stirred solution of **17** (5.0 mmol, 1.69 g) at −70 °C was added Dibal-H in toluene (1.5 M, 10.5 mmol, 7.0 mL) while maintaining the temperature at or below −65 °C. After stirring the reaction for 30 min, the reaction was quenched with 2 mL of EtOH and warmed to −20 °C, to which was added 25 mL of a Rochelle salt solution. The reaction mixture was stirred for 30 min. To the reaction was added 25 mL of EtOAc. Once the emulsion had dissipated, the layers were separated. The aqueous layer was extracted with EtOAc (25 mL). The combined organic layers were washed with a saturated brine solution (3 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to yield quantitatively **18a**.

18: To an ice bath cooled stirred suspension of K_2CO_3 (1.0 mmol, 0.14 g) in 10 mL of THF was added dropwise a solution of **18a** (5.0 mmol, 1.70 g) and nitromethane (7.5 mmol, 0.45 g, 0.4 mL) in 15 mL of THF. After stirring for 20 h with the icebath removed, 25 mL of Et_2O was added to the reaction. The organic layer was washed with 1 M $KHSO_4$ (15 mL) and saturated brine (2 × 15 mL). After treating the organic layer as described in **18a**, the residue was chromatographed using flash chromatography conditions. Although the diastereomers were separated, they were recombined to yield 1.42 g (80%) of **18**. δ_H (400 MHz; $CDCl_3$) 1.47 (3 H, s, CH_3), 1.49 (9 H, s ($(CH_3)_3C$), 1.55 (3 H, s, CH_3), 2.22–2.38 (1 H, m, CH_2CF_2), 2.54 (1 H, ddd, J = 9.1, 11.1, 24.0 Hz, CH_2CF_2), 3.82–3.88 (1 H, m, NC*H*), 4.01–4.07 (2 H, m, CH_2O), 4.55–4.65 (m, 2 H, CH_2NO_2), 4.81 (1 H, dhext, J = 4.0, 33.6 Hz, C*H*OH), 5.52 (1 H, br d, O*H*). δ_F (376 MHz, $CDCl_3$) −108.6, −109.8.

19. *tert*-Butyl (4*S*)-4-(4-{[(benzyloxy)carbonyl]amino}-2,2-difluoro-3-hydroxybutyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

19a: Under catalytic hydrogenation conditions at 60 psi, EtOH–HOAc and ambient temperature with 20% $Pd(OH)_2$/C, **18** (1.7 mmol, 0.62 g) was reduced to give 0.50 g (91%) of the desired amine **19a**. δ_H (400 MHz; DOAc) 1.50 (3 H, s, CH_3), 1.52 (9 H, s, $(CH_3)_3C$), 1.60 (3 H, s, CH_3), 2.18–2.62 (2 H, m, CH_2CF_2), 3.20–3.49 (2 H, m, CH_2NH), 3.93–4.11 (2 H, m, CH_2O), 4.2–4.4 (2 H, m, C*H*NH, C*H*OH). δ_C (100 MHz, DOAc) (major diastereomer) 24.7 (CH_3), 27.8 (CH_3), 28.7 (($CH_3)_3C$), 36.7 (t, J = 25 Hz, CH_2CF_2), 40.6 (CH_2NH), 52.8 (N*C*H), 68.2 (CH_2O), 72.3 (t, J = 25 Hz, *C*HOH), 81.5 (($CH_3)_3CO$), 93.4 (N*C*O), 124.0 (t, CF_2), 152.7 (*C*=O). δ_F (376 MHz, DOAc) −108.6, −109.1.

19: On a 1.5 mmol scale, **19** was prepared in the same manner as described in example 10 starting with **19a**. δ_H (400 MHz; $CDCl_3$) 1.45 (3 H, s, CH_3), 1.48 (9 H, s, $(CH_3)_3C$), 1.54 (3 H, s, CH_3), 2.2–2.5 (2 H, m, CH_2CF_2), 3.2–3.4 (2 H, m, CH_2NH), 3.4–4.0 (4 H, m, CH_2O, C*H*NH, C*H*OH), 5.1–5.3 (2 H, m, OCH_2Ph), 7.3–7.4 (5 H, m, Ph).

20. *tert*-Butyl (4*S*)-4-(4-{[(benzyloxy)carbonyl]amino}-2,2-difluorobutyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate

On a 1.4 mmol scale, compound **20** was prepared as described in example 14.

δ_H (400 MHz; $CDCl_3$) δ 1.42 (6 H, s, CH_3), 1.53 (9 H, s, $C(CH_3)_3$), 1.97–2.29 (4 H, m, $CH_2CF_2CH_2$), 3.39–3.52 (2 H, m, CH_2NH), 3.88–4.02 (2 H, m, CH_2O), 4.08–4.20 (m, 1 H, NC*H*), 5.10 (2 H, br s, $PhCH_2O$), 7.36 (5 H, br s, Ph).

21. *N*-6-[(benzyloxy)carbonyl]-*N*-2-(*tert*-butoxycarbonyl)-4,4-difluoro-L-lysinol

On a 1.2 mmol scale, compound **21** was synthesized as exemplified in example 10 starting with **20**.

22. *N*-6-[(benzyloxy)carbonyl]-*N*-2-(*tert*-butoxycarbonyl)-4,4-difluoro-L-lysine

On a 0.42 mmol scale, compound **22** was synthesized as described in example 11 starting with **21**. δ_H (400 MHz; $CDCl_3$) 1.46 (9 H, s, $(CH_3)_3C$), 2.02–2.20 (2 H, m, CH_2CF_2), 2.24–2.58 (2 H, m, CH_2CF_2), 3.30–3.46 (2 H, m, $NHCH_2$), 4.45–4.59 (1 H, m, NHC*H*), 5.16 (2 H, br s, $PhCH_2O$), 7.37 (5 H, br s, Ph). δ_C (100 MHz; $CDCl_3$) 28.8 (($CH_3)_3C$), 35.2 (CH_2NH), 37.1 (t, J = 25 Hz, CH_2CF_2), 38.5 (t, J = 25 Hz, CH_2CF_2), 49.5 (NH*C*H), 80.9 ($(CH_3)_3CO$), 128.8 (Ph), 128.9 (Ph), 130.5 (Ph), 136.7 (Ph), 156.0 (*C*=O), 157.0 (*C*=O), 175.4 (*C*OOH).

23. *N*-6-iminoethyl-4,4-difluoro-L-lysine dihydrochloride

On a 0.26 mmol scale, compound **23** was synthesized following the method of example **12** starting with **22**. (Found: C, 30.17; H, 6.60; N, 13.41; Cl, 22.33. Calc. for $C_8H_{15}F_2N_3O_2 \cdot 2\,HCl \cdot 1.4\,H_2O$: C, 29.90; H, 6.21; N, 13.08; Cl, 22.06%). δ_H (400 MHz;

D_2O) 1.96 (3 H, s, C=NHCH_3), 2.10–2.60 (4 H, m, CH_2CF_2-CH_2), 3.57 (2 H, t, J = 6.8 Hz, CH_2NH), 4.08 (1 H, dd, J = 3.7, 8.3 Hz, NHCH). δ_C (100 MHz, D_2O) 18.8 (CH$_3$), 34.2 (t, J = 24 Hz, CH$_2$CF_2), 36.0 (CH$_2$NH), 37.2 (t, J = 24 Hz, CH$_2$CF_2), 49.8 (NHCH), 123.9 (t, J = 241.5 Hz, CF$_2$), 165.3 (C=NH), 173.4 (COOH). δ_F (376 MHz, D_2O) −98.2.

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