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SYNTHESIS OF β-DIFLUORINE-CONTAINING AMINO ACIDS

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Abstract: A convenient strategy was developed to prepare several β-difluoroamino acids. As exemplified by the synthesis of 3,3-difluoro-L-homocysteine, 3,3-difluoro-L-homoserine and 3,3-difluoro-L-methionine, the reaction sequence all started from L-isoascorbic acid. This approach holds potential to be extended to make other β-difluorine-containing amino acids.

The fluorine-containing amino acids and peptides are an important class of compounds displaying a broad spectrum of interesting and potent biological activities.[3] While few of them are naturally occurring,[4] the fluoroamino acids and peptides have attracted greater attention than the more widely known chlorinated and brominated counterparts due to their high clinical potential as enzyme inhibitors/chemotherapeutic agents.[3] Some of the features that make the incorporation of fluorine(s) and/or fluorinated substituent(s) into therapeutic agents particularly attractive include: the unique effects of fluorination on the bond strength, the increased stability of reactive intermediates, the ability to form a hydrogen bonding network, and the favorable steric interactions toward the biological targets. In fact, it has been well demonstrated that when fluorine(s) or fluorinated substituent(s) are strategically introduced at appropriate loci in amino acids or peptides, the chemical properties and biological activities of the resulting compounds could be significantly altered.[5] The successive incorporation of unnatural amino acids into proteins by in vitro suppression of the corresponding amber mutants with a chemically aminoacylated suppressor *t*-RNA derived from yeast *t*-RNA^{Phe},[6] has expanded the utility of fluoroamino acids as possible probes to investigate the role of a key residue of an enzyme in its catalysis, or the role of a specific residue of a protein on holding its conformation.[7] In recognition of their vast potential applications, a diverse array of fluorine-containing amino acids have been prepared and their biological activities evaluated. Among which, the β-fluoroamino acids have attracted special interest owing to their antibacterial, antihypertensive, cancerostatic and cytotoxic activities.[3a] The inhibitory effects of β-fluoroamino acids against coenzyme B_6 dependent catalysts have also been widely documented.[8] In support of our ongoing research directed toward elucidating the catalytic mechanism of a few PLP/PMP dependent enzymes, it has been necessary for us to synthesize several 3,3-difluoro-substituted amino acids as possible irreversible or suicide inhibitors. To achieve our goal, we have developed a convenient strategy to synthesize these desired molecules. Herein we report the versatility of this method as exemplified by the preparation of 3,3-difluoro-substituted L-homocysteine, L-homoserine, and L-methionine.

The first phase of our synthesis called for the construction of (2*S*)-3,3-difluoro-2-hydroxy-butan-1,4-diol with its C-4 hydroxyl group protected (**6**). Preparation of this compound starting from a erythritol derivative (**2**), a chiral precursor derived from L-isoascorbic acid (**1**),[9] is summarized in Scheme I. The four-step reaction involved benzylation of 4-OH with one equivalent of benzyl bromide in the presence of sodium hydride (62%), Swern oxidation of 3-OH (86%), difluorination of the 3-oxo group with diethylaminosulfur

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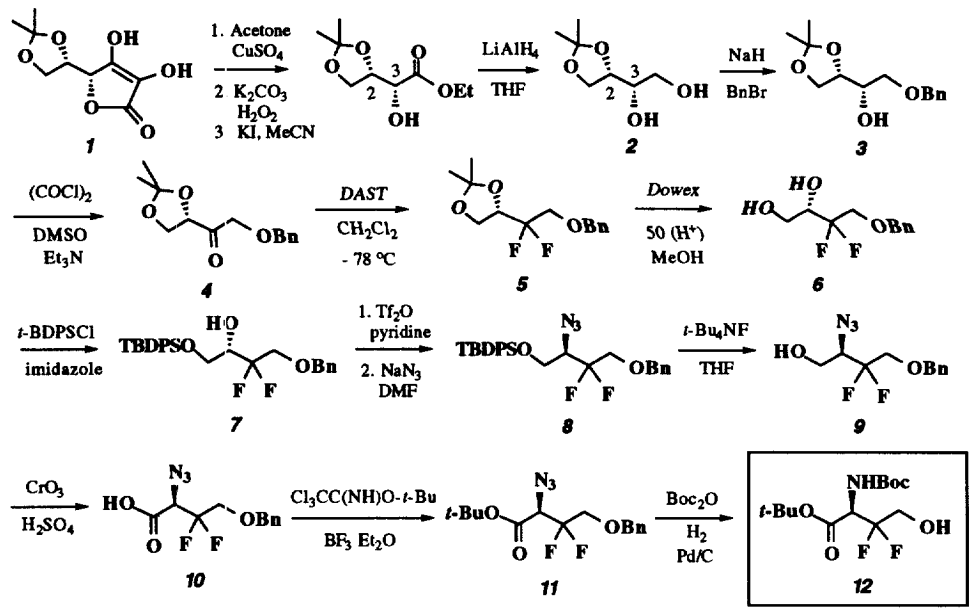
trifluoride (DAST) (67%),[10] and acid hydrolysis to remove the isopropylidene protecting group (56%). The overall yield of these four step was 20%. The primary alcohol of **6** was then selectively protected to give **7** in 95% yield. Introduction of the nitrogen functionality at C-2 was achieved by a two-step sequence initiated by the conversion of the C-2 hydroxyl group to its triflate followed by in situ treatment with sodium azide in DMF.[11] This two-step S_N2 displacement proceeded swiftly with inversion of configuration in 80% yield.[12] The resulting **8** was treated with *tert*-butylammonium fluoride to remove the silyl protecting group and the resulting product **9** was subjected to Jones oxidation to give **10** in 48% overall yield. The acid **10** was further protected with *tert*-butyl-2,2,2-trichloroacetimidate in the presence of boron trifluoride diethyl etherate (61%).[13] Reduction of the azido moiety, hydrogenolysis of the benzyl group,[15] and the protection of the amino group with *tert*-butyloxycarbonyl (Boc) were all accomplished in one pot to furnish **12** in 56% yield.

Scheme I

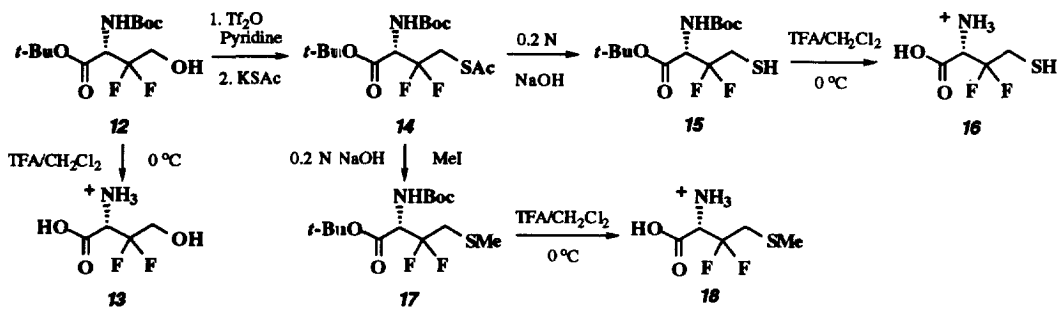


This central intermediate **12** (readily prepared in gram quantities) could be deprotected in a mixture of trifluoroacetic acid (TFA) and methylene chloride (1:3) to give 3,3-difluoro-L-homoserine **13** (Scheme II). The isolated yield, after purification by HPLC (C_{18} column, gradient elution with MeCN/H_2O and 0.1% TFA), was 40%. Oxidization of **13** to obtain the corresponding aspartic acid derivative was attempted using both Jones reagent and a milder two-step method with tetra-*n*-propylammonium perruthenate (TDAP) and buffered sodium chlorite. Unfortunately, formation of a complicated mixture accompanied by the elimination of fluorine was observed in both cases.

Compound **12** is also a convenient precursor for homocysteine and methionine. As depicted in Scheme II, the free hydroxyl group of **12** could be activated by triflation and then converted in situ with potassium

thioacetate to a thioacetate moiety (**14**, 76% yield).[15] Upon treatment with 0.2 N NaOH, **14** was converted to *N*-*t*-Boc-3,3-difluoro-L-homocysteine-O*t*Bu (**15**) in 78% yield. Removal of the protecting groups in a mixture TFA:CH_2Cl_2 (1:3) at room temperature led to 3,3-difluoro-L-homocysteine (**16**) in 33% yield. Preparation of the corresponding methionine derivative (**18**) was achieved via an analogous sequence, except for a methylation reaction with methyl iodide performed prior to the final deprotection step.[16] The overall yield from **14** to **18** was 21%. The reported yields were calculated based on the products (**16** and **18**) purified by HPLC (C_{18} column, gradient elution with MeCN/H_2O and 0.1% TFA).

Scheme II



In conclusion, we have developed a versatile method for the preparation of several 3,3-difluoro-L-amino acids.[17] Despite the great promise of β-fluoroamino acids as bioregulators and/or enzyme inhibitors, few β-difluoro-substituted amino acids have been synthesized. A few examples include: 3,3-difluoroalanine,[18] 2-amino-3,3-difluorobutyric acid,[19] 3,3-difluorophenylalanine,[19] 3,3-difluoromethylornithine,[20] 3,3-difluoroaspartic acid,[21] 3,3-difluoroasparagine,[21] and 3,3-difluoroglutamic acid.[22] Preparation of 3,3-difluorohomoserine and 3,3-difluorohomocysteine in their protected forms have also been reported.[23] The general and efficient preparation sequence presented in this paper should be a valuable addition to the existing arsenal of organic chemistry to make this class of biologically important molecules.

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1. These two authors contributed equally to this paper. K. L. was responsible for the design of Scheme I.
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11. Compound **7**: ^{1}H NMR ($CDCl_3$) δ 7.68-7.29 (15 H, m, aromatic Hs), 4.60 (2H, s, PhCH_2), 4.06 (1H, m, 2-H), 3.90 (2H, m), 3.88 (1H, ddd, J = 12.9, 11.4, 9.0 Hz), 3.69 (1H; ddd, J = 12.9, 11.1, 9.6), 1.06 (9H, s, CH_3s); ^{13}C NMR ($CDCl_3$) δ 137.1-127.7 (aromatic Cs), 120.8 (t, J = 245 Hz; C-3), 73.9, 70.4 (dd, J = 30, 25), 68.4 (dd, J = 34, 27), 61.9, 26.8 (CH_3s), 19.2; high resolution MS; calc for $C_{27}H_{36}F_2NO_3Si$ $(M + NH_4)^+$ 488.2433, found 488.2428; $[\alpha]_D$ +4.3 ($CHCl_3$, c 0.02). Compound **8**: ^{1}H NMR ($CDCl_3$) δ 7.68-7.26 (15 H, m, aromatic Hs), 4.56 (2H, s, PhCH_2), 4.06-3.86 (3H, m), 3.76 (1H, m), 3.60 (1H; ddd, J = 11.4, 10.8, 10.8), 1.07 (9H, s, CH_3s); ^{13}C NMR ($CDCl_3$) δ 136.7-127.8 (aromatic Cs), 120.4 (t, J = 248 Hz; C-3), 73.9, 68.4 (dd, J = 33, 29), 63.9 (dd, J = 28, 24), 61.9, 26.7 (CH_3s), 19.1 (CMe_3); high resolution MS; calc for $C_{27}H_{35}F_2N_4O_2Si$ $(M + NH_4)^+$ 513.2497, found 513.2494; $[\alpha]_D$ -8.5 ($CHCl_3$, c 0.02).
12. The stereochemical course of the azide displacement step (**7** → **8**) proceeded with clean S_N2 inversion and was established by determining the enantiomeric purity of the Mosher ester of **9** which gave only one set of signals in ^{19}F NMR ($CDCl_3$, no internal reference) δ 120.3 (s), 82.8 (d, J = 272 Hz), 76.7 (d, J = 272).
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17. The spectral properties of the final difluoroamino acids are all consistent with the literature data.
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