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A Highly Stereospecific and Efficient Synthesis of Homopentafluorophenylalanine

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Abstract: A short and efficient synthesis of homopentafluorophenylalanine (**6**) from oxazolidine aldehyde **1** in 57% overall yield and in >98% ee is described. The enantiomeric excess of the product was determined by ^{19}F NMR analysis of the coupling product derived from **5** and L-Ser(O-*t*-Bu)-OCH_3, by comparison to a dipeptide obtained from racemic **5**.

Fluorinated amino acids have recently emerged as an important class of structural and functional building blocks.[1,2] A number of research groups[3] including ours[4] have recently described the successful incorporation of fluorinated amino acids into peptides and proteins, including the concomitant effects on structure and function of the resulting ensembles.

Our laboratory has reengineered model coiled coil proteins to assess the effect of π-cation interactions in determining the stability of these ubiquitous folds.[5] Extensive statistical analysis of available protein structures reveals that π-cation interactions are manifested in the pairing of the cationic side chains of lysine or arginine with the aromatic amino acids phenylalanine, tyrosine, or tryptophan.[6]

Coiled coils are superhelical ensembles consisting of two or more α-helices. Their assembly is made possible by a heptad repeat in the primary sequence $(abcdefg)_n$, where the *a* and *d* residues are largely shielded from solvent water and are typically hydrophobic.[7] Residues at sites flanking the hydrophobic core, namely the *e* and *g* positions, are frequently seen to form interhelical electrostatic contacts, providing a secondary specificity motif. Analysis of the distances between C_α carbons of the *i* and $(i' + 5)$ residues in coiled coil structures suggests a range from 9.5 to 11 Å.[8,9] This distance is readily spanned by lysine and homophenylalanine (homoPhe) residues to provide the appropriate geometry for interhelical π-cation interactions between the alkylammonium group (Lys) and the center of the aromatic ring (homoPhe). Substitution of the aromatic ring hydrogens with fluorine atoms provides a nearly isosteric compound differing only its ability to participiate in π-cation interactions (Figure 1). Our design, therefore, required large quantities of homopentafluorophenylalanine (homo-Pf-Phe) in chiral form for solid-phase peptide synthesis.

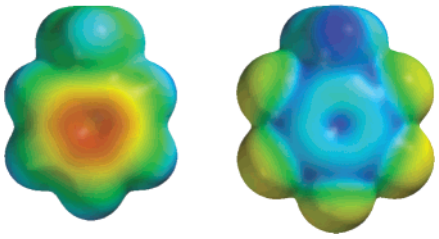

FIGURE 1. Electrostatic potential surfaces for toluene (left) and pentafluorotoluene (right): red codes for negative electrostatic potential and blue codes for positive potential. Calculations were carried out on fully geometry-optimized structures using the 6-31G** basis set as described by Mecozzi et al.[10]

Our synthesis of homo-Pf-Phe commenced from the configurationally stable[11] Garner aldehyde **1** which was synthesized according to literature procedures (Scheme 1).[12] The pentafluorobenzyl moiety was introduced by Wittig olefination of aldehyde **1** with pentafluorobenzyl bromide and potassium *tert*-butoxide as the base to yield oxazolidine-olefin **2**. The reaction produced exclusively the *trans*-olefin **2** in 92% isolated yield.[13] The open-chain

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(13) Configuration of the double bond was assigned on the basis of the $^1H-^1H$ coupling constants of the vinylic protons in **2** (J = 16.44 Hz) and **3** (J = 16.50 Hz).

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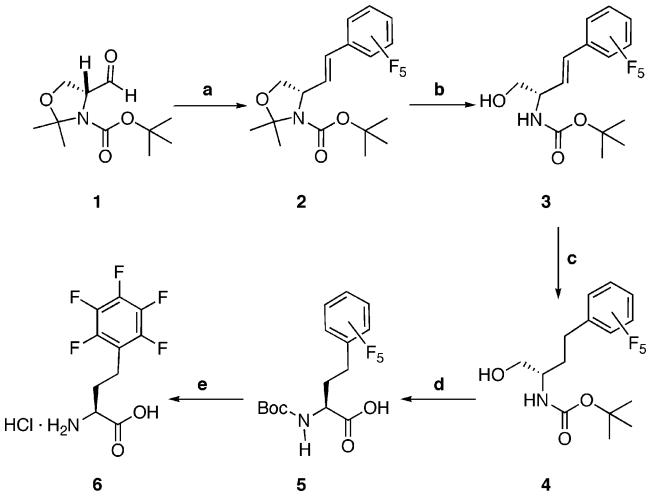
SCHEME 1[a]

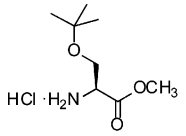


[a] Reagents and conditions: (a) $C_6F_5CH{=}PPh_3$, THF, −78 °C, 14 h, 92%; (b) (i) CH_3COCl, CH_3OH, 0 °C, 0.5 h, (ii) $(t\text{-Boc})_2O$, TEA, CH_2Cl_2, 4 h, 98%; (c) Pd-black, EtOH, H_2, 98%; (d) PDC, DMF, rt, 24 h, 64%; (e) HCl, CH_3OH, rt, quant.

saturated alcohol **4** can, in principle, be obtained by reduction of the oxazolidine-olefin **2**, followed by ring opening. However, attempts to hydrogenate the double bond in the cyclic compound **2** using several hydrogenation catalysts proved only marginally successful. We suspected that the double bond might be inaccessible to the catalyst surface because of geometric constraints imposed by the oxazolidine ring. Thus, an alternative strategy with ring opening followed by hydrogenation was employed to obtain compound **4**. Several literature reports that describe the removal of acetonide protection in oxazolidine compounds similar to **2** utilize methanol and *p*-toluenesulfonic acid[14] to accomplish solvolysis.

While this method generally yields the intact *N*-*t*-Boc compound, with **2** the reaction resulted in incomplete conversion with significant *N*-*t*-Boc deprotection, necessitating chromatographic purification. Thus, the acetonide in the oxazolidine-olefin was cleaved by HCl generated in situ by using an acetyl chloride/methanol couple, which also resulted in the removal of the *t*-Boc group.[15] The amino alcohol so obtained was *t*-Boc protected in a subsequent step in the same pot. This method gave near-quantitative conversion of oxazolidine-olefin **2** to the *t*-Boc protected olefin-alcohol **3**, which could be used without further purification. Reduction of the double bond in the olefin-alcohol **3** was achieved by catalytic hydrogenation using Pd-black (10% by wt) in ethanol at room temperature in 12 h (98%). The resulting alcohol **4** was subjected to oxidation using pyridinium dichromate in DMF to deliver *t*-Boc-protected 2*S*-homo-Pf-Phe **5** in 64% yield, which was further processed to yield the hydrochloride salt of homo-Pf-Phe (**6**).

The optical purity of **6** was verified by coupling **5** to a protected methyl ester of L-serine (**7**) and the resulting dipeptide **8** was analyzed using ^{19}F NMR spectroscopy. In the case of the dipeptide obtained from racemic **5**,[16] two different sets of ^{19}F signals were observed, whereas **5** from the present synthesis yielded a dipeptide with signals in the ratio 117:1 indicating an enantiomeric excess of 98.3%.[17]



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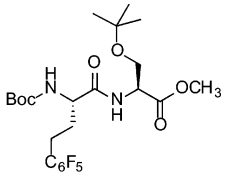

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In summary, we have developed a short and efficient route for conversion of Garner aldehyde **1** to 2*S*-homo-Pf-Phe in 57% overall yield. The construction of 2*R*-homo-Pf-Phe is similarly achieved from the corresponding oxazolidine aldehyde derived from L-serine. Studies detailing the incorporation of **5** into peptides and its effect on secondary and quaternary structure will be reported shortly.

Experimental Section

(*S*,*E*)-*tert*-Butyl 2,2-Dimethyl-4-(perfluorostyryl)oxazolidine-3-carboxylate (2). To a solution of PPh_3 (3.15 g, 12 mmol) in dry THF (20 mL) pentafluorobenzyl bromide (3.13 g, 12 mmol) was added with a syringe in one portion. The mixture was stirred for 15 min, the resulting solution of phosphonium salt was taken into a syringe and added dropwise over a period of 15 min to a flask containing potassium *tert*-butoxide (1.32 g, 11.7 mmol) in dry THF (15 mL) at 0 °C. The mixture was stirred for 1.5 h at 0 °C following which the flask was cooled to −78 °C in a dry ice-acetone bath. To this a solution of Garner aldehyde **1** (458 mg) in dry THF (10 mL) was added over a period of 15 min. The resulting pale yellow suspension was stirred for 6 h at −78 °C followed by 8 h stirring at room temperature. THF was removed under vacuum and the oily suspension obtained was dissolved in Et_2O and salts were removed by filtration. The filtrate was concentrated to 30 mL to which 150 mL of hexane was added to precipitate triphenylphosphine oxide that was removed by filtration. The filtrate was concentrated and the residue was subjected to flash chromatography with 5% EtOAc in hexane as eluant to give oxazolidine-pentafluorophenyl-olefin **2** as a white solid (721 mg, 92%): ^1H NMR (300 MHz, $CDCl_3$) δ 6.50−6.37 (m 2H), 4.59−4.44 (2m 1H), 4.14 (dd, 1H, J = 6.2 Hz, 9.0 Hz), 3.86 (dd, 1H, J = 1.7 Hz, 9.1 Hz), 1.75−1.35 (m, 15H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 151.9, 146.5, 143.1, 139.4, 138.3, 137.7, 136.1, 115.6, 112.0, 94.5, 80.7, 80.1, 74.5, 68.0, 60.0, 52.0, 28.5, 27.4, 26.7, 24.7, 23.7; ^{19}F NMR (282.6 MHz, $CDCl_3$) δ −145.69 and −146.03 (2m, 2F), −159.17 and −159.63 (2m, 1F), −165.87 and −166.38 (2m, 2F); mp 74 °C; $[\alpha]^{25}_D$ = +66.6 (*c* 1, $CHCl_3$); ES HRMS *m*/*z* for $C_{18}H_{20}NO_3F_5Na^+$ calcd 416.1261, obsd 416.1265. Anal. Calcd for $C_{18}H_{20}F_5NO_3$: C, 54.96; H, 5.12; N, 3.56; F, 24.15. Found: C, 55.02; H, 5.10; N, 3.44; F, 24.28.

(*S*,*E*)-*tert*-Butyl 1-Hydroxy-4-(perfluorophenyl)but-3-en-2-ylcarbamate (3). To methanol (6 mL) at 0 °C was added acetyl chloride (0.6 mL, 8.4 mmol) with stirring. After 10 min, olefin **2** (315 mg, 0.8 mmol) was added in one portion to this solution. The reaction was stirred for 0.5 h. Removal of methanol yielded a white solid. To this were added *t*-Boc_2O (262 mg, 1.2 mmol), triethylamine (0.42 mL, 3 mmol), and methylene chloride (5 mL), and the reaction was stirred at rt for 3 h. Methylene chloride was removed under vacuum, and to the residue were

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added ethyl acetate (60 mL) and brine (20 mL). The layers were separated, and the organic layer was washed sequentially with brine (20 mL × 2), 5% aq $KHSO_4$ (20 mL × 2), and brine (20 mL × 2). The organic layer was dried over anhydrous Na_2SO_4 and the solvents removed to yield olefinol **3** (276 mg, 97.6%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.60−6.54 (dd, 1H, J = 0.9 Hz, 16.5 Hz), 6.53−6.48 (d, 1H, J = 16.2 Hz), 5.03 (bm, 1H), 4.44 (bm, 1H), 3.80 (bm, 2H), 2.03 (bs, 1H), 1.47 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 155.9, 146.6, 143.4, 136.3, 116.1, 80.4, 65.1, 28.5, 27.9; ^{19}F NMR (282.6 MHz, $CDCl_3$) δ −145.83 (m, 2F), −159.06 (t, 1F, J = 20.6 Hz), −166.00 (m, 2F); mp 105 °C; $[\alpha]^{25}_D$ = +20.85 (*c* 0.84, $CHCl_3$); ES HRMS *m*/*z* for $C_{15}H_{16}NO_3F_5Na^+$ calcd 376.0948, obsd 376.0944. Anal. Calcd for $C_{15}H_{16}F_5NO_3$: C, 51.00; H, 4.56; N, 3.96; F, 26.89. Found: C, 50.97; H, 4.63; N, 3.82; F, 26.62.

(*S*)-*tert*-Butyl 1-Hydroxy-4-(perfluorophenyl)butan-2-ylcarbamate (4). To a solution of olefin **3** (247 mg, 0.7 mmol) in 5 mL of ethanol was added Pd-black (30 mg) and the suspension stirred under H_2 atmosphere (using a balloon) for 12 h. Upon removal of Pd-black by filtration and concentration of the filtrate under reduced pressure, compound **4** was obtained in pure form (246 mg, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, 1H, J = 5.1 Hz), 3.70−3.59 (m, 3H), 2.79 (t, 2H, J = 8.2 Hz), 2.31 (bs, 1H), 1.82 (m, 1H), 1.67 (m, 1H), 1.52 (2s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.5, 146.8, 143.6, 139.5, 136.0, 114.7, 80.1, 65.5, 52.4, 31.1, 28.5, 19.4; ^{19}F NMR (282.6 MHz, $CDCl_3$) δ −147.35 (dd, 2F, J = 8 Hz, 22.2 Hz), −160.61 (t, 1F, J = 20.3 Hz), −165.67 (m, 2F); mp 107 °C; ES HRMS calcd for $C_{15}H_{18}NO_3F_5Na^+$ 378.1105, obsd 378.1110; $[\alpha]^{25}_D$ = +1.5 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{15}H_{18}F_5NO_3$: C, 50.71; H, 5.11;, N, 3.94; F, 26.74. Found: C, 50.55; H, 5.06; N, 3.89; F, 27.02.

(*S*)-2-(*tert*-Butoxycarbonyl)-4-(perfluorophenyl)butanoic Acid (5). To a mixture of alcohol **4** (142 mg, 0.4 mmol) and pyridinium dichromate (602 mg, 1.6 mmol) under argon was added dry DMF (3 mL), and the mixture was stirred overnight at rt. To the reaction mixture was added 50 mL of water, the aqueous mixture was extracted with Et_2O (50 mL × 3), and the organic layers were dried over anhydrous Na_2SO_4. Ether was removed, to the resulting residue was added 20 mL of 2 N NaOH, and the mixture was then stirred for 15 min after which it was washed with Et_2O (20 mL × 3). The aqueous layer was acidified to pH 2 with solid $KHSO_4$ and was extracted with Et_2O (20 mL × 3). The crude product was purified by flash chromatography (1:9 CH_3OH/CH_2Cl_2 with 0.1% AcOH) and **5** recovered as a white solid (95 mg, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.10 (bs, 1H), 7.04 (d, 0.5H, J = 7 Hz), 5.17 (d, 0.5H, J = 8.1 Hz), 4.40−4.22 (2m, 1H), 2.83−2.65 (m, 2H), 2.20 (m, 1H), 1.98 (m, 1H), 1.46 and 1.36 (2s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 175.2, 174.3, 155.8, 154.8, 154.5, 145.7, 142.4, 138.1, 134.8, 112.6, 81.2, 79.6, 53.0, 51.9, 30.3, 27.2, 17.4; ^{19}F NMR (282.6 MHz, $CDCl_3$) δ −146.88 to −147.54 (m, 2F), −160.30 to −160.63 (m, 1F), −165.64 to −166.01 (m, 2F); $[\alpha]^{25}_D$ = +22.9 (*c* 1.87, $CHCl_3$); ES HRMS *m*/*z* calcd for $C_{15}H_{16}NO_4F_5Na^+$ 392.0897, obsd 392.0893. Anal. Calcd for $C_{15}H_{16}F_5NO_4$: C, 48.79; H, 4.37; N, 3.79, F, 25.72. Found: C, 47.06; H, 4.26; N, 3.55; F, 24.55.

(*S*)-1-Carboxy-3-(perfluorophenyl)propan-1-aminium chloride (6): ^{1}H NMR (300 MHz, CD_3OH) δ 4.05 (t, 1H, J = 6.19 Hz), 3.27 (m, 2H), 2.24−2.04 (m, 2H); ^{13}C NMR (75.5 MHz, CD_3OH) δ 171.3, 148.3, 145.0, 143.1, 140.7, 137.4, 114.9, 53.5, 30.9, 19.4; ^{19}F NMR (282.6 MHz, CD_3OH) δ −147.35 (dd, 2F, J = 8 Hz, 22.2 Hz), −160.61 (t, 1F, J = 20.3 Hz), −165.67 (m, 2F); $[\alpha]^{25}_D$ = +31.3 (*c* 0.5, CH_3OH); ESI-MS calcd for $C_{10}H_9NO_2F_5^+$ 270.05, obsd 270.08. Anal. Calcd for $C_{10}H_9ClF_5NO_2$: C, 39.30; H, 2.97; N, 4.58; F, 31.08. Found: C, 38.01; H, 3.05; N, 4.32; F, 29.26.

(*S*)-Methyl 3-*tert*-Butoxy-2-((*S*)-2-(*tert*-butoxycarbonyl)-4-(perfluorophenyl)butanamido)propanoate (8). To *N*-*t*-Boc-homopentafluorophenylalanine (18.5 mg, 0.05 mmol), (*O*-*tert*-butyl)-2*S*-serinemethylester hydrochloride (13 mg, 0.06 mmol), and HBTU (23 mg, 0.06 mmol) in dry DMF was added 0.031 mL of diisopropylethylamine, and the mixture was stirred for 20 min. DMF was removed, and to the residue was added 10 mL of water followed by extraction with CH_2Cl_2. The organic layers were pooled and washed sequentially with brine (10 mL), 5% aq $KHSO_4$ (10 mL × 3), brine (10 mL), 5% aq Na_2CO_3 (10 mL × 3) ,and brine (10 mL). The organic layer was dried on anhydrous Na_2SO_4, and the solvent was removed to yield the dipeptide **8**, which was directly used for further analysis: ^{1}H NMR (300 MHz) δ 6.89 (d, 1H, J = 8.4 Hz), 5.41 (d, 1H, J = 9.45 Hz), 4.83 (m, 1H), 4.35 (m, 1H), 3.96 (dd, 1H, J = 2.8 Hz, 9.12 Hz), 3.74 (s, 3H), 3.57 (dd, 1H, 3.0 Hz, 9 Hz), 2.80 (s, 2H), 2.22 (m, 1H), 1.87 (m, 1H), 1.58 and 1.56 (2s, 9H), 1.26 and 1.25 (2s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.0, 169.5, 154.4, 145.7, 142.4, 140.4, 138.1, 137.1, 113.1, 79.1, 61.5, 60.6, 52.7, 51.9, 51.4, 38.8, 33.8, 27.2, 26.2, 17.5; ^{19}F NMR (282.6 MHz, $CDCl_3$) δ −144.24 (dd, 2F, J = 8.3 Hz, 22.4 Hz), −157.73 (t, 1F, J = 20.8 Hz), −162.95 (m, 2F).

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