Pergamon

Tetrahedron Letters 39 (1998) 1169–1172

TETRAHEDRON LETTERS

Practical Synthesis of Boc and Fmoc Protected 4-Fluoro and 4-Difluoroprolines from *Trans*-4-Hydroxyproline

Luc Demange, André Ménez and Christophe Dugave*

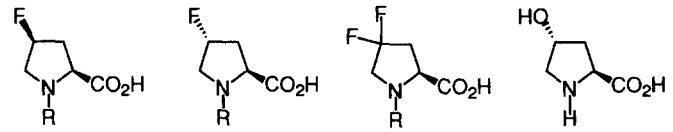
Département d'Ingénierie et d'Etude des Protéines (DIEP), CEA / Saclay, 91191 Gif-sur-Yvette, France

Received 22 October 1997; accepted 6 December 1997

Abstract: Boc-*cis*-4-fluoro-L-proline and 4-difluoro-L-proline, usable in classical peptide synthesis, were obtained in respectively 71% (3 steps) and 65% (4 steps) overall yields from the readily available *trans*-4-hydroxy-L-proline methyl ester. The corresponding fluorinated *trans*-isomer was isolated in 24% yield (5 steps). Transformation of Boc-protected compounds to their Fmoc-equivalents was performed in high yields.

Proline residues confer unique structural constraints in a peptide chain and hence may play a major role in protein folding, structure and function. Fluorinated prolines therefore, in particular 4-fluoro and 4-difluoroprolines **1-3** (chart 1, R = H), are useful tools for investigating protein-peptide or protein-protein interactions as well as conformational transitions.[1]

Chart 1



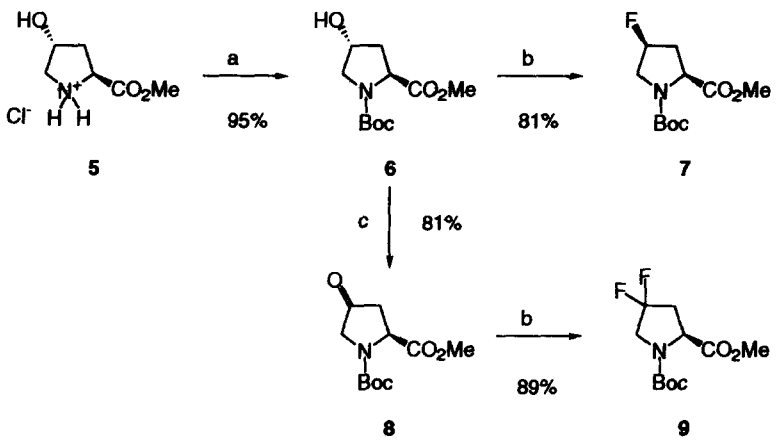
R = Boc **1a** Fmoc **1b** | **2a** **2b** | **3a** **3b** | **4**

Several methods describing the preparation of fluoro and difluoroprolines starting from N-acetyl-[2], benzoyl-[3] and Z-hydroxyproline[4] methyl esters or hydroxyproline-derived diketopiperazine[5] and oxazolidinone[6] have been reported. However, to our knowledge, no simple synthesis of compounds **1-3** , suitably protected for solid phase peptide synthesis, has been formally described. In the present letter, we report a straighforward synthesis of Boc- and Fmoc-protected *cis*- and *trans*-4-fluoro-L-prolines (respectively **1** and **2**) and 4-difluoro-L-proline **3** starting from the commercially available *trans*-4-hydroxy-L-proline **4**.

One example of direct fluorination of Boc-protected 4-hydroxypyroglutamic acid has been described in moderate yield (40%).[7] We observed that Boc-protected compound **6** readily obtained from compound **4** was easily converted into *cis*-4-fluoroproline **7** in good yield (81%) with DAST.[8] Oxidation of compound **6** with PDC[9] and difluorination gave difluoroproline **9** in 72% yield (2 steps) (scheme 1).

0040-4039/98/$19.00
PII: S0040-4039(97)10793-6

Scheme 1

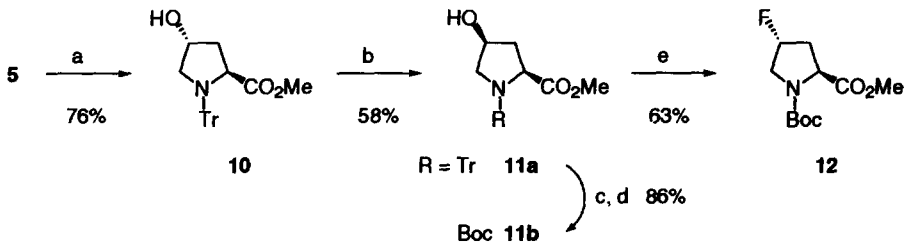


a: Boc_2O, NEt_3 / CH_2Cl_2, r.t.; b: DAST / dry CH_2Cl_2 -78°C to r.t.; c: PDC / CH_2Cl_2, r.t., 3 Å molecular sieves

Inversion of configuration at C-4 was not obvious. The diastereoselective alcohol inversion of Z-hydroxyproline reported by Patchett and Witkop[10] could not be transposed to the Boc-methyl ester series since borohydride reduction of ketone **8** led to a mixture of *cis/trans*-Boc-hydroxyproline methyl esters. Treatment of compound **6** in Mitsunobu conditions[11] gave a mixture of N- and C-deprotected by-products. This suggests either a participation of the Boc-carbonyl group via an intramolecular substitution[12] or a saponification of the methyl ester during hydrolysis of the intermediary benzoyl ester.

Inversion of the alcohol configuration was finally performed in 58% yield starting from N-tritylhydroxyproline **10** (scheme 2).[13] Further deprotection / protection as *tert*-butyl carbamate and fluorination as reported above gave *trans*-4-fluoroproline **12** in 54% overall yield (2 steps).

Scheme 2



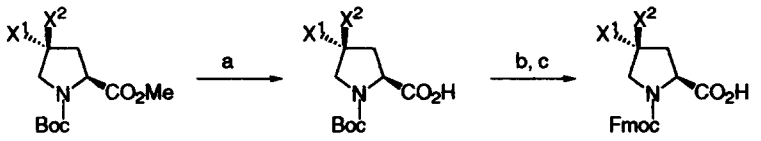
a: Tr-Cl, NEt_3 / $CHCl_3$, 0°C; b: PPh_3, DEAD, Ph-CO_2H / toluene then KOH / MeOH; c: HCO_2H / $ClCH_2$-CH_2Cl; d: Boc_2O, NEt_3 / CH_2Cl_2, r.t.; e: DAST / dry CH_2Cl_2 -78°C to r.t..

Final saponification afforded Boc-protected compounds **1a-3a** in very good yields.[14,15] Acidic deprotection and treatment with Fmoc-Su in methanol[16] led to the corresponding Fmoc-protected equivalents **1b-3b** in 93-99% yields (scheme 3).[17]

Even though Young and coworkers demonstrated that fluorination with DAST occurs with complete inversion of configuration,[7] we assessed that our sequences of reactions did not epimerise any asymmetric center. Comparison of ^1H, ^{13}C and ^{19}F NMR data for compounds **1ab** and **2ab** clearly showed numerous

significative differences. For each compound, no trace of the other diastereomer were detected. This confirmed that no epimerisation occured at C-2 or C-4 position. Moreover, diastereomeric purity of Fmoc-fluoroprolines was also checked by TLC (D.e. > 99%; **1b**: Rf = 0.40; **2b**: Rf : 0.31; 0.25 mm silica gel 60; eluant: chloroform:methanol:acetic acid 98:1:1).

Scheme 3

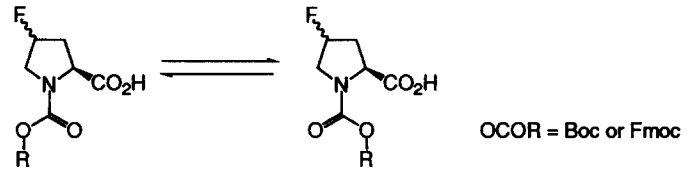


7 $X^1 = H, X^2 = F$ — **1a** 92% — **1b** 99%
12 $X^1 = F, X^2 = H$ — **2a** 99% — **2b** 96%
9 $X^1 = F, X^2 = F$ — **3a** 95% — **3b** 93%

a: 2 equiv. LiOH / CH_3CN-H_2O 3-1 r.t.; b: TFA / CH_2Cl_2; c: Fmoc-Su NEt_3 / MeOH.

^1H, ^{13}C and ^{19}F NMR spectra of the title compounds clearly showed significative doubling of several signals. This clearly demonstrates the existence of *cis*- and *trans*- conformers of the Boc- and Fmoc-fluoroprolines as already observed with Boc-[17] and Fmoc-prolines urethane bonds (scheme 4) and for the aminoacyl-proline[19] and N-acetylproline amide bonds. [7, 20] These results unambiguously demonstrate that reaction of Boc-protected 4-hydroxyproline derivatives with DAST occurs via a S_N^2 process. This has been very recently discussed by Patino and coworkers who reported the synthesis of compounds **1b** and **2b**.[20] The authors asserted that doubling of ^{19}F NMR signals (-172.8 and -173.8 ppm) reflects an epimerisation at C-4. However, in our hands, compound **1b** gave two singlets at 173.7 ppm (40%) and 174.8 ppm (60%) corresponding to the mixture of *cis*- and *trans*-carbamate conformers, whereas compound **2b** appeared as two singlets at 178.4 ppm (65%) and 179.0 ppm (35%).

Scheme 4



Trans - conformer *Cis* - conformer

In conclusion, we described in this paper a convergent and straighforward preparation of optically pure Boc- and Fmoc-prolines fluorinated on C-4, starting from the unexpensive *trans*-hydroxyproline. These compounds, used in classical peptide synthesis, displayed stabilities and reactivities similar to their hydrocarbon patterns.

Acknowledgments: We gratefully thank Dr. Leïla Sergent (DBCM/CEA/Saclay) for ^{19}F NMR spectra. We express our thanks to Dr. Nadège Jamin (DIEP/CEA/Saclay) for helpful advice and discussions.

References and notes

1. "Fluorine-containing amino acids; synthesis and properties"; Kukhar', V. P. & Soloshonok, V. A. Ed.; John Wiley and Sons Ltd., 1995, Chischester, England.
2. (a) Hudlicky, M.; Merola, J.; *Tetrahedron Lett.* **1990**, *31*, 7403-7406; (b) Hudlicky, M.; *J. Fluorine Chem.* **1993**, *60* , 193-210.
3. Kronenthal, D. R.; Mueller, R. H.; Kuester, P. L.; Kissick, T. P.; Johnson, E. J.; *Tetrahedron Lett.* **1990**, *31*, 1241-1244.
4. Gottlieb, A.; Fujita, Y. Undenfriend, S.; Witkop, B.; *Biochemistry* **1965**, *4*, 2507-2513.
5. Shirota, F. N.; Nagasawa, H. T.; Elberling, J. A.; *J. Med. Chem.* **1977**, *20*, 1176-1181.
6. Hart, B. P.; Coward, J. K.;. *Tetrahedron Lett.* **1993**, *34*, 4917-4920.
7. Avent, A. G.; Bowler, A. N.; Doyle, P. M.; Marchand, C. M.; Young, D. W.; *Tetrahedron Lett.* **1992**, *33*, 1509-1512.
8. Middleton, W. J.; *J. Org. Chem.* **1975**, *40*, 574-578.
9. Czerneki, S.; Georgoulis, C.; Stevens, C. L.; Vijayakumaran, K.; *Tetrahedron Lett.* **1985**, *26*, 1699-1702.
10. Patchett, A. A.; Witkop, B.; *J. Am. Chem. Soc* **1957**, *79*, 185-192.
11. Marshall, J. A.; Lebreton, J.; DeHoff, B. S.; Jenson, T. M.; *J. Org. Chem.* **1987**, *52* , 3883-3889.
12. Ducrocq, C.; Righini-Tapie, A.; Azerad, R.; Green, J. F.; Friedman, P. A.; Beaucourt, J. P.; Rousseau, B.; *J. Chem. Soc. Perkin Trans. I* **1986**, 1323-1328.
13. Applegate, H. E.; Cimarusti, C. M.; Dolfini, J. E.; Funke, P. T.; Koster, W. H.; Puar, M. S.; Slucharchyk, W. A.; Young, M.G.;*J. Org. Chem.* **1979**, *44* , 811-818.
14. Guibé, E.; Decottignies-Le Maréchal, P.; Le Maréchal, P; Azerad, R.; FEBS Lett. **1984**, *177*, 265-268.
15. **1a**: Mp = 160-162°C; $[\alpha]_D^{20}$ = -54 (c = 1.0, methanol); ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ (ppm) 8.56 (b, 1H, CO_2H, 5.21 (dm, $^2J_{H\text{-}F}$ = 52 Hz, 1H), 4.51 (bm, 1H), 3.88-3.52 (m, 2H), 2.71-2.29 (m, 2H), 1.49 (s, major) + 1.44 (s, minor) (9H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ (ppm) 176.9 (major) + 174.8 (minor), 155.2 (minor) + 153.7 (major), 91.8 (major, d, $^1J_{C\text{-}F}$ = 178 Hz) + 91.0 (minor, d, $^1J_{C\text{-}F}$ = 177 Hz), 81.4 (minor) + 80.8 (major), 57.7, 53.0 (apparent t), 37.2 (major, d, $^2J_{C\text{-}F}$ = 23 Hz) + 35.6 (minor, d, $^2J_{C\text{-}F}$ = 20 Hz), 28.2; ^{19}F NMR ^{1}H-^{19}F dec (282.4 MHz, $CDCl_3$, C_6F_6): δ (ppm) -173.9 (minor), -175.9 (major); MS (DCI, NH_3): m/z 251 (MNH_4^+, 100%), 234 (MH^+, 33%), 195 (MNH_4^+ - C_4H_8, 95%); Anal. calcd for $C_{10}H_{16}FNO_4$: C, 51.50; H, 6.91; F, 8.14 N, 6.00. Found: C, 51.07; H, 6.91; F, 7.65; N, 5.82. **2a**: Mp = 114-117°C; $[\alpha]_D^{20}$ = -48 (c = 1.0, methanol); ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ (ppm) 8.79 (b, 1H), 5.23 (dm, $^2J_{H\text{-}F}$ = 52.3 Hz, 1H), 4.53 (t, $^3J_{H\text{-}H'}$ = 8.5 Hz), 4.42 (t, $^3J_{H\text{-}H'}$ = 8.0 Hz) (1H), 4.00-3.73 (m, minor) + 3.72-3.44 (dm, major) (2H), 2.68-2.52 (m) + 2.5-2.10 (complex m, 2H), 1.49 (s, major) + 1.44 (s, minor) (9H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ (ppm) 178.0 (major) + 174.5 (minor), 156.1 (major) + 153.7 (minor), 91.3 (major, d, $^1J_{C\text{-}F}$ = 178.6 Hz) + 91.0 (minor, d, $^1J_{C\text{-}F}$ = 179 Hz), 82.3 (major) + 81.2 (minor), 57.7 (major) + 57.6 (minor), 53.5 (major, d, $^2J_{C\text{-}F}$ = 23 Hz) + 53.0 (minor, d, $^2J_{C\text{-}F}$ = 23 Hz), 37.5 (minor, d, $^2J_{C\text{-}F}$ = 23 Hz) + 35.7 (major, d, $^2J_{C\text{-}F}$ = 22.0 Hz), 28.3 (major) + 28.1 (minor); ^{19}F NMR ^{1}H-^{19}F dec (282.4 MHz, $CDCl_3$, C_6F_6): δ (ppm) -178.5 (major), -178.8 (minor); MS (DCI, NH_3): m/z 251 (MNH_4^+, 74%), 234 (MH^+, 90%), 195 (MNH_4^+ - C_4H_8, 100%)%); Anal. calcd for $C_{10}H_{16}FNO_4$: C, 51.50; H, 6.91; F, 8.14 N, 6.00. Found: C, 51.67; H, 6.96; F, 7.69; N, 5.92. **3a**: Mp = 119-121°C; $[\alpha]_D^{20}$ = -49 (c = 1.0, methanol); ^{1}H NMR (250 Mhz, $CDCl_3$, TMS): δ (ppm) 8.51 (b, 1H), 4.60 (dd, major, $^3J_{H\text{-}H'}$ = 5.8 Hz, $^3J_{H\text{-}H''}$ = 8.4 Hz) + 4.48 (dd, minor, $^3J_{H\text{-}H'}$ = 5.4 Hz, $^3J_{H\text{-}H'}$ = 9.2 Hz) (1H), 3.83 (apparent dq, 2H), 2.68-2.52 (m) + 2.82-2.51 (m) (2H), 1.49 (s, major) + 1.44 (s, minor) (9H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ (ppm) 176.3 (major) + 174.5 (minor), 154.7 (major) + 153.2 (minor), 126.1 (major, t, $^1J_{C\text{-}F}$ = 250 Hz) + 125.6 (minor, t, $^1J_{C\text{-}F}$ = 249 Hz), 82.2 (major) + 81.7 (minor), 56.9 (minor) + 56.7 (major), 53.4 (major, t, $^2J_{C\text{-}F}$ = 32 Hz) + 52.9 (minor,t , $^2J_{C\text{-}F}$ = 33 Hz), 38.5 (minor, t, $^2J_{C\text{-}F}$ = 26 Hz) + 37.4 (major, t, $^2J_{C\text{-}F}$ = 25 Hz), 28.2 (major) + 28.1 (minor); ^{19}F NMR ^{1}H-^{19}F dec (282.4 MHz, $CDCl_3$, C_6F_6): δ (ppm) -97.1 (minor, AB, $^1J_{F\text{-}F\ gem.}$= 235 Hz, δ_A = -94.7, δ_B = -99.5), -100.88 (major, AB, δ_A close to δ_B); MS (DCI, NH_3): m/z 269 (MNH_4^+, 100%), 252 (MH^+, 15%), 213 (MNH_4^+ -C_4H_8, 15%); Anal. calcd for $C_{10}H_{15}F_2NO_4$: C, 47.81; H, 6.02; F, 15.12 N, 5.57. Found: C, 48.29; H, 6.08; F, 14.56; N, 5.51.
16. Lapatsanis, L.; Milias, G.; Froussios, K.; Kovolos, M.; *Synthesis* **1983**, 671-674.
17. Hondrelis, J.; Lonergan, G.; Voliotis, S.; Matsoukas, J.; *Tetrahedron Lett.* **1992**, *33*, 1509-1512.
18. Weißhoff, H.; Frost, K.; Brandt, W.; Henklein, P.; Mügge, C.; Frömmel, C.; *FEBS Lett.* **1995**, *372*, 203-209.
19. Panasik Jr., N.; Eberhardt, E. S.; Edison, A. S.; Powell, D. R.; Raines, R. T.; *Int. J. Peptide Protein Res.* **1994**, *44*, 262-269.
20. Dugave, C.; Demange, L.; Ménez, A.; **1997**, unpublished results.
21. Tran, T. T.; Patino, N.; Condom, R.; Frogier, T.; Guedj, R.; *J. Fluorine Chem.* **1997**, *60* , 193-210.