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SYNTHESIS AND RESOLUTION OF 7-FLUOROTRYPTOPHANS[1]

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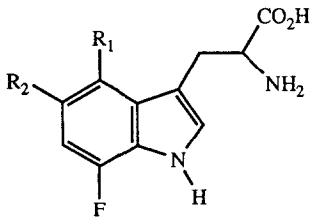
Abstract: 7-Fluoro-DL-tryptophan (**1a**), 4,7-difluoro-DL-tryptophan (**1b**) and 5,7-difluoro-DL-tryptophan (**1c**) were prepared by Fischer indole cyclization. The resolution was achieved by treatment of the N-trifluoroacetyl derivatives with carboxypeptidase A. All 7-fluorotryptophans are slow substrates for tryptophan indole-lyase from *E. coli*.

Fluorinated analogues of amino acids are useful to study amino acid reactions and metabolism.[2] Recently, there has been an increasing interest in the incorporation of fluoroamino acids in large biomolecules.[3] F-Phe-, F-Trp-, and F-Tyr-labeled proteins have been used in ^{19}F NMR studies to characterize fuctionally significant conformational changes.[4] Among fluorotryptophans, 4-, 5- and 6-fluorotryptophan are commercially available, and used extensively.[2c,3b,5] 4, 5, 6, 7-Tetrafluorotryptophan was synthesized and tested for enzyme inhibition.[6] However, there is only one report for preparation of **1a** and **1c**, which were not resolved,[6e] and the preparation of **1b** has not been previously reported. We report here the synthesis of 7-fluorotryptophans **1a-c**, their resolution, and their reactions with tryptophan indole-lyase (tryptophanase) from *E. coli*.

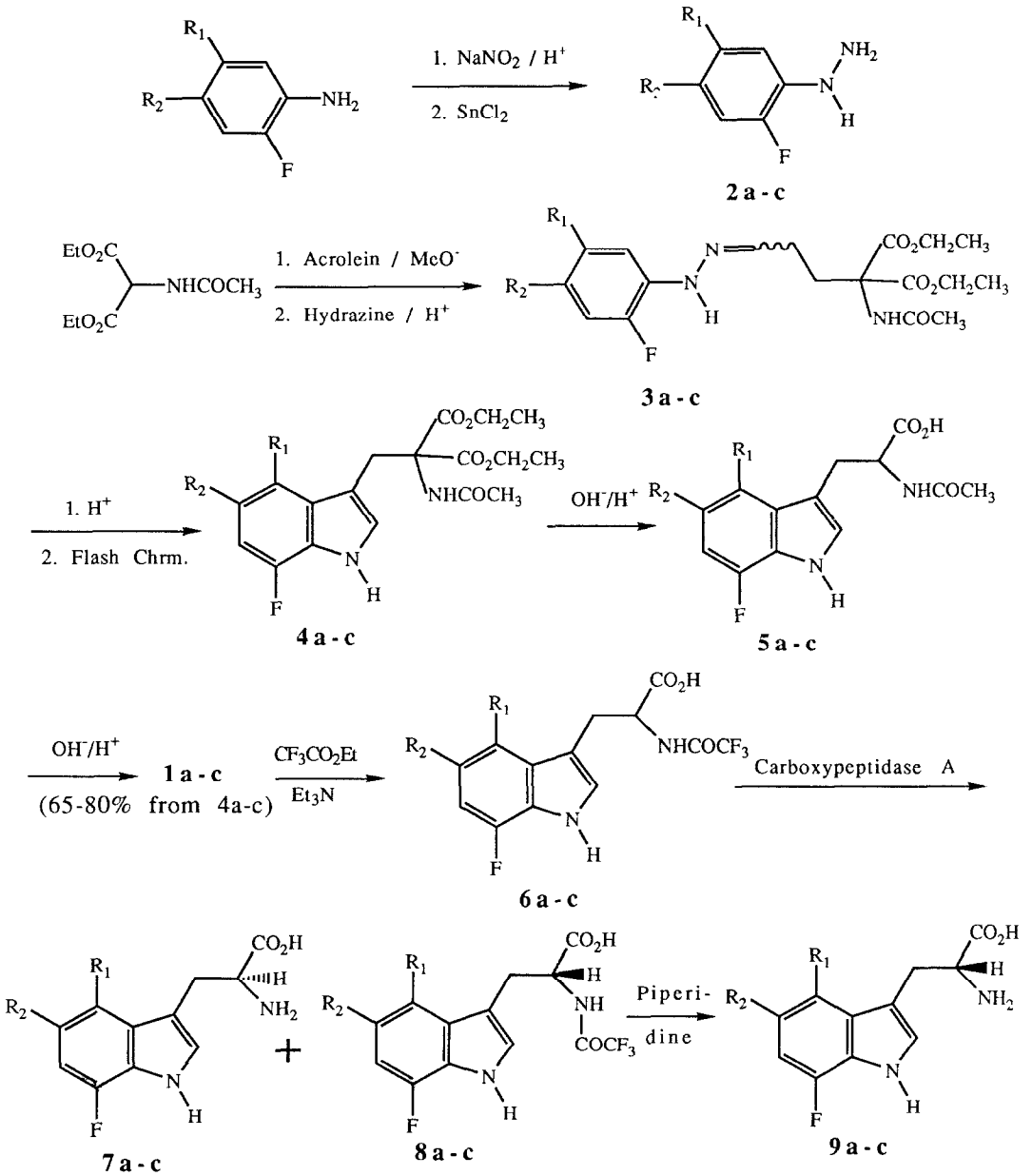


1a; $R_1=R_2=H$

1b; $R_1=F$, $R_2=H$

1c; $R_1=H$, $R_2=F$

Fluoroanilines were diazotized, reduced with $SnCl_2$, and the resulting HCl salts were neutralized with 4N NaOH solution to give fluorophenylhydrazines **2a-c** in 67-85% yields.[7] After the reaction of diethyl acetamidomalonate with acrolein and sodium methoxide in benzene,[8] addition of the fluorophenylhydrazine gave the crystalline hydrazones **3a-c**. (85-90%) The predominant stereochemistry of the hydrazones across the imine double bond was determined to be E, based on the results of the proton NMR.[9] Reflux of **3a-c** with dilute sulfuric acid (5-10%) for 5 hours afforded



indoles **4a-c**.[10] The yields were dependent on the number and position of fluorines, and were 38%, 36% and 12% for **4a**, **4c** and **4b**, respectively. Saponification and decarboxylation of **4a-c** were achieved by refluxing with 1.2 equivalent NaOH in aqueous dioxane (1:1).[11] The resulting N-acetyltryptophans **5a-c** were hydrolyzed

with 4 equivalents of NaOH to give **1a-c**.[12] **1a-c** were converted to the N-trifluoroacetyl derivatives[13] and were treated with carboxypeptidase A to obtain the **7a-c** and **8a-c**.[14] The N-TFA-D-7-fluorotryptophans were treated with 1M aqueous piperidine to give the D-7-fluorotryptophans **9a-c**. The optical purity of each enantiomer was measured by HPLC using a chiral column, and was greater than 99% e.e.[15] We have examined the kinetics of the reaction of *Escherichia coli* tryptophan indole-lyase with compounds **7a-c**.[16] All of them are slower substrates than L-tryptophan.

Table 1. Kinetic data for **7a-c** with tryptophan indole-lyase

Compound	K_m(mM)	V_{max}(rel.)	k_{cat}/K_m(rel.)
L-tryptophan	0.26	100%	100%
7 a	0.64	29%	14%
7 b	1.7	42%	6%
7 c	0.49	58%	30%

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References and Notes

1. Reported in part at the 1990 Southeast/Southwest Combined Regional Meeting of the American Chemical Society, New Orleans, LA, December 5-7, 1990. Abstract No. 430
2. (a) Woolridge, E. M. and Rokita, S. E., *Biochemistry*, **1991**, 30, 1852 (b) Phillips, R. S., Fletcher, J. G., Von Tersch, R. L., and Kirk, K. L., *Arch. Biochem. Biophys.*, **1990**, 276, 65 (c) Miles, E. W., Phillips, R. S., Yeh, H. J. C., and Cohen, L. A., *Biochemistry*, **1986**, 25, 4240
3. (a) Imperialli, B. In Advances in Biotechnological Process, Vol. 10, Synthetic Peptides in Biotechnology, Mizrahi, A. Ed.; Alan, R. Liss Inc.; New York, 1988, pp 97-131 (b) Luck, L. A. and Falke, J. J., *Biochemistry*, **1991**, 30, 4248
4. (a) Jenkins, B. G., *Life Sci.*, **1991**, 48, 1227 (b) Li, E., Qian, S. J., Winter, N. S., d'Avignon, A., Levin M. S., and Gordon, J. I., *J. Biol. Chem.*, **1991**, 266, 3622 (c) Peersen, O. B., Pratt, E. A., Truong, H. T. N., Ho, C., and Rule, G. S., *Biochemistry*, **1990**, 29, 3256
5. (a) Hott, J. L. and Borkman, R. F., *Biochem. J.*, **1989**, 264, 297 (b) Pazoutova, S., Kren, V., and Sajdl, P., *Appl. Microbiol. Biotechnol.*, **1990**, 33, 330 (c) Niven, G. W., Kerby, N. W., Rowell, P., Foster, C. A., and Stewart, W. D. P., *J. Gen. Microbiol.*, **1988**, 134, 689
6. for synthesis; (a) Petrova, T. D., Savchenko, T. I., Ardyukova, T. F., Yakobson, G. G., *Khim. Geterotsikl. Soedin*, **1971**, 2, 213 (b) Rajh, H. M., Uitzetler, J. H., Westerhuis, L. W., Dries, C. L., Tesser, G. I., *Peptide Protein Res.*, **1979**, 14, 68 (c) Ojima, I., Kato, K., Nakahashi, K., Fuchikami, T., and Fujita, M., *J. Org. Chem.*, **1989**, 54, 4511 for enzyme reaction; (d) Knorre, D. G., Lavrik, O. I., Petrova, T. D., Savachenko, T. I., Yakobson, G. G., *FEBS Lett.*, **1971**, 12, 204 (e) Nevinskii, G. A., Favorova, O. O., Lavrik, O. I., Petrova, T. D., Kochkina L. L. and Savchenko, T. I., *FEBS Lett.*, **1974**, 43, 135

7. Suschitzky, H., *J. Chem. Soc.*, **1953**, 3326.
8. Waner, D. T. and Moe, O. A., *J. Am. Chem. Soc.*, **1948**, 70, 2765.
9. Karabastos, G. J. and Taller, R. A., *J. Am. Chem. Soc.*, **1963**, 85, 3624
10. The fluoroindoles were isolated by flash chromatography with ethyl acetate/ hexane after extraction with ethyl acetate and drying.
11. Porter, J., Dykert, J. and Rivier, J., *Int. J. Peptide Protein Res.*, **1987**, 53, 13
12. Each compound was characterized by ^{1}H, ^{13}C and ^{19}F NMR, and elemental analysis. **1a**: mp 252-254 °C(lit. 254-256 °C) ; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.49 (d, 1H, J=8.0, C4-H), 7.23(s, 1H, C2-H), 6.98(td, 1H, J=4.7, 7.9Hz, C5-H), 6.84(dd, 1H, J=7.9, 11.5Hz, C6-H), 3.84(dd, 1H, J=4.1, 9.3Hz, αC-H), 3.49(dd, 1H, J=4.1, 15.3Hz, βC-H), 3.15(dd, 1H, J=9.3, 15.2Hz, βC-H) ^{13}C NMR (Methanol-D_4, 62.9 Mhz, decoupler on, ppm) 174.2(COO), 151.2(d, J_{C-F}=-242.9Hz, C7), 115.4(d, J_{C-F}=6.1Hz, C3a),126.3(d, J_{C-F}=13Hz, C7a), 126.2(C2), 120.4(d, J_{C-F}=6.2Hz, C5), 114.5(d, J_{C-F}=3.3Hz, C4), 110.7(C3), 107.3(d, J_{C-F}=16.4Hz, C6), 56.6(αC), 28.4(βC), ^{19}F NMR(Methanol-D_4, 282.4Mhz, ppm) -135.2(dd, J=4.7, 11.5Hz). Analysis. Calc.: C 59.46% H 4.95% N 12.6%; Found: C 59.36%, N 5.01%, H 12.58% **1b**: mp 264-265 °C; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.24(s, 1H, C2-H), 6.80(ddd, 1H, J=3.5, 8.5, 10.4Hz, C5-H)*, 6.67(ddd, 1H, J=3.1, 8.5, 10.5Hz, C6-H)*, 4.22(dd, 1H, J=5.0, 9.3Hz, αC-H), 3.60(dd, 1H, J=5.0, 15.0Hz, βC-H), 3.25(dd, 1H, J=9.3, 15.0Hz, βC-H) ^{13}C NMR (Methanol-D_4, 75Mhz, decoupler on, ppm) 171.4 (COO), 154.1(dd, J_{C-F}=1.9, -238.4Hz, C4), 147.6(dd, J_{C-F}=2.7, -239.2Hz, C7), 128.6(dd, J_{C-F}=11.6, 16.6Hz, C7a), 127.2(C2), 119.6(dd, J_{C-F}=22.5, 12.5Hz, C3a), 108.1(C3), 107.3(dd, J_{C-F}=8.9, 19.6Hz, C5)*, 104.6(dd, J_{C-F}=7.1, 23.0Hz, C6)*, 56.0 (αC), 28.6(βC). ^{19}F NMR(Methanol-D_4, 282.4Mhz, ppm) -129.8(ddd J=3.5, 10.5, 22.5, F4) -139.6(ddd, J=3.1, 10.4, 22.5, F7), Analysis. Calc.: C 55% H 4.2% N 11.66%; Found: C 54.92%, H 4.2%, N 11.62% * interchangable **1c**: mp 249-250 °C; ^{1}H NMR (Methanol-D_4, 300Mhz, ppm) 7.30 (s, 1H, C2-H), 7.26(dd, 1H, J=2.2, 9.4Hz, C4-H), 6.73(ddd, 1H, J=2.2, 9.6, 11.1Hz, C6-H), 3.82(dd, 1H, J=4.3, 8.7Hz, αC-H), 3.40(dd, 1H, J=4.3, 15.3Hz, βC-H), 3.15(dd, 1H, J=8.7, 15.3Hz, βC-H) ^{13}C NMR (Methanol-D_4, 62.9Mhz, decoupler on, ppm) 174.1(COO), 158.1(dd, J_{C-F}=9.7, -235.0Hz, C5), 150.2(dd, J_{C-F}=14.3, -246.1Hz, C7), 131.4(dd, J_{C-F}=6.7, 11.2Hz, C3a), 128.0(C2), 122.9(d, J_{C-F}= 13.5Hz, C7a), 110.9(C3), 100.4(dd, J_{C-F}=3.7, 23.8Hz, C4), 97.4(dd, J_{C-F}=20.9, 30.7Hz, C6), 56.4(αC), 28.1(βC), ^{19}F NMR(Methanol-D_4, 282.4Mhz, ppm) -122.1 (ddd, J=2.1, 9.4, 9.6Hz, F5) -131.3(dd, J=2.1, 11.1Hz, F7) Analysis. Calc.: C 55% H 4.2% N 11.66%; Found: C 54.94%, H 4.23%, N 11.57%.
13. Curphey, T. J., *J. Org. Chem.*, **1979**, 44, 2805
14. Phillips, R. S. and Cohen, L. A., *Tetrahedron Lett.*, **1983**, 24, 5555
15. Column: Cu-proline. Eluent: 10mM $CuSO_4$ Detector: 270nm L-isomer elutes last. **7a**; $[\alpha]^{25}_D$= -21.8 (c=0.7, DMSO) **7b**; $[\alpha]^{25}_D$= -44.1(c=0.74, formamide) **7c**; $[\alpha]^{25}_D$= -27.5 (c=1, methanol) **9a**; $[\alpha]^{25}_D$= +23.1(c=0.73 DMSO) **9b**; $[\alpha]^{25}_D$= +45.9 (c=0.73, formamide) **9c**; $[\alpha]^{25}_D$=+28.1 (c=1, methanol)
16. Tryptophan indole-lyase was purified from *E. coli* JM101 containg the *tnaA* gene on plasmid pMD6 (Phillips, R. S. and Gollnick, P. D., *J. Bio. Chem.*, **1989**, 264, 10627). Catalytic turnover with L-tryptophan and L-7-fluoro-tryptophans was measured by coupled assay with lactate dehydrogenase and NADH at 340nm. (Morino, Y., and Snell, E. E., *Methods Enzymol.*, **1970**, 17A, 439).