FLUORINE-CONTAINING AMINO ACIDS AND THEIR DERIVATIVES. 5.[1] SYNTHESIS OF NOVEL FLUORINATED ANALOGUES OF THE ANTITUMOR AGENT, METHOTREXATE

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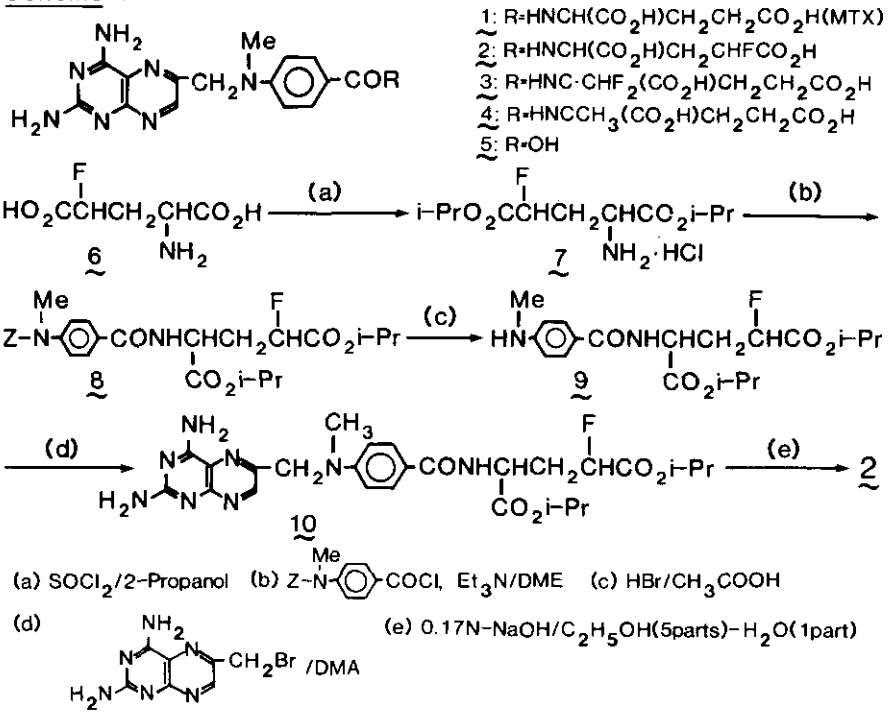
Abstract — Novel methotrexate analogues with γ-fluoro- or α-difluoromethyl-substitued-glutamic acid were synthesized for antitumor screening.

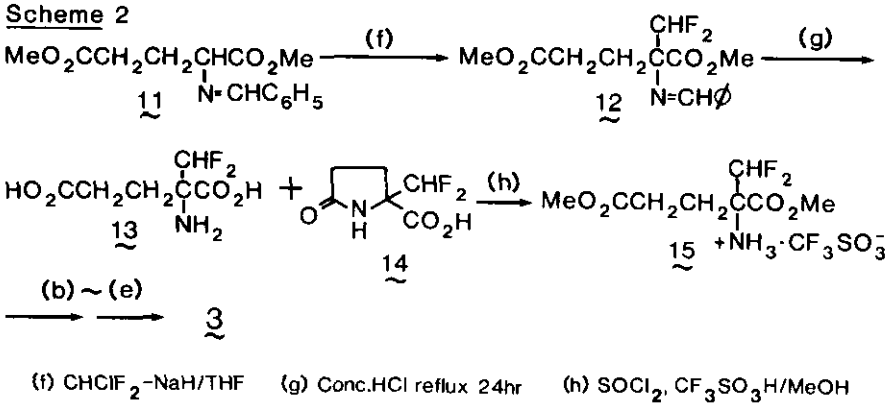
Methotrexate (MTX), a potent inhibitor of dihydrofolate reductase, is an antitumor agent in wide clinical use against various types of cancers.[2] Progress in pharmacological and physicochemical research on this drug and dihydrofolate reductase itself has led to an understanding of the fundamentals of the mode of action and the mechanism of transport and metabolism, enabling more accurate drug design based on the structure-activity relationships.[3] Recently, increasing attention is being paid to chemical modification of the glutamic acid moiety in the search for less toxic analogues for use in high-dose treatments.[4] A few studies have already been attempted based on the idea that acidity enhancement of the γ-carboxylic acid group might diminish the in vivo polyglutamate formation and hence lower its toxicity to meet the requirements of high-dose treatment.[5] Along the same line we synthesized two novel methotrexate analogues (2 and 3) containing fluorinated glutamic acids.

In our synthesis, we did not use the commonly employed coupling reaction of 5[6] but adopted instead the method developed by Piper and Montgomery,[7] which is shown in Scheme 1, because fluoroamino acids are often unstable under strongly basic conditions. The starting material, γ-fluoroglutamic acid (6), was prepared by a known method[8] involving Michael addition of diethyl fluoromalonate[9] to ethyl 2-acetamido-acrylate and decarboxylation of the resulting adduct. Unfortunately, the methyl ester of 6 could not be used in step b because it quickly cyclized to a 2-pyrrolidone-5-carboxylic acid derivative under the reaction conditions employed. Furthermore, taking into account the deprotection procedure of the final step (Step e), we chose isopropyl ester (7) as a suitable reactant for this scheme.[10] This ester was then reacted with N-benzyloxycarbonyl-N-methyl-p-aminobenzoyl chloride in DME in the presence of triethylamine to afford

Scheme 1



Scheme 2



the product (8) in 90% overall yield from 6. After deprotection of the benzyloxycarbonyl group by treatment with HBr-CH_3COOH, the resulting amine (9) was coupled with 2,4-diamino-6-(bromomethyl)pteridine in dimethylacetamide and the product (10) was obtained in 70% yield from 8.[11] Mild alkaline hydrolysis of 10 without hydrolysis of the 4-amino group afforded the desired product (2) in 84% isolated yield.[10] After careful purification,[12] the analytically pure sample

was obtained and identified as the desired compound.[11]

Next, an another fluorine-containing analogue of methotrexate, the α-difluoromethylated derivative (3), was synthesized as shown in Scheme 2. The Schiff base of dimethylglutamate (11) was reacted with difluorocarbene generated in situ by a known method[13] to give the α-difluoromethylated product (12). This product was hydrolyzed, without purification, to a mixture of free acids, 13 and 14, which were subsequently separated by chromatography using AG 50W-X8 ion-exchange resin, giving isolated yields of 14% and 24%, respectively. Here we discovered that these two cyclized and noncyclized acids, 14 and 13 can be almost completely interconverted from one to another under the proper conditions.[14] Thus, the desired noncyclized ester (15) was exclusively obtained from both acids 13 and 14 by treatment with thionyl chloride in methanol in the presence of trifluoromethanesulfonic acid. Surprisingly, the use of a very strong acid like trifluoromethanesulfonic acid was required to prevent internal cyclization, because of the markedly diminished basicity of 15. In the following acylation step of 15 (b in Scheme 2), this diminished basicity again required a large excess of N-benzyloxycarbonyl-N-methyl-p-aminobenzoyl chloride to make the sluggish acylation reaction proceed faster than the internal cyclization. The subsequent reactions of 15 which led to the desired final product (3) were done in almost the same way as for 2. The α-methyl derivative (4) was also prepared as a reference compound of 3 for antitumor screening. Antitumor screening of these novel fluorine-containing methotrexate analogues is in progress and their results will be reported elsewhere in the near future.

Note: While this paper was being prepared, we were informed that a similar work was to be reported by Coward et al. at the 188th ACS Meeting.[15]

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10. The alkaline hydrolysis of the isopropyl ester proceeded smoothly under the almost same conditions as those for the methyl ester.

11. Some of the compounds characterized are as follows. Compound (9) showed: mp 101-104°C; IR ($CHCl_3$) ν_{max} 3440, 1730, 1650, 1610, 1105 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13-1.37 (12H, m, $CH\underline{Me}_2$), 2.23-3.03 (2H, m, $C\underline{H}_2CHF$), 2.87 (3H, s, NCH_3), 3.39 (1H, br. m, $N\underline{H}Me$), 4.63-5.40 (4H, m, $C\underline{H}CO_2Pr^i$, $C\underline{H}F$ and $CHMe_2$), 6.58 and 7.70 (4H, ABq, J = 9.0 Hz, arom. H), 6.83 (1H, d, J = 6.0 Hz, $CON\underline{H}$); ^{19}F NMR (acetone-d_6) δ ($C_6\underline{F}_6$) -26.17 to -29.00 (m); MS m/z 382 (M^+), 323 ($M^+ - C_4H_{11}$), 134 (C_8H_8ON). Compound (2) showed: mp ~250°C (dec.); IR (KBr disc) ν_{max} (significant peaks only) 1640, 1600 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.00-2.63 (2H, m, $C\underline{H}_2CHF$), 3.20 (3H, s, $NC\underline{H}_3$), 4.27-5.40 (2H, m, $NHC\underline{H}$ and $CH_2C\underline{H}F$), 4.78 (2H, s, $C\underline{H}_2N$), 6.82 and 7.73 (4H, ABq, J = 9.0 Hz, arom. H), 7.03 and 7.77 (br. s, $N\underline{H}_2$), 8.36 (1H, d, J = 7.5 Hz, $CON\underline{H}$), 8.59 (1H, s, arom. H); ^{19}F NMR (DMSO - d_6) δ ($C_6\underline{F}_6$) -22.00 to -26.00 (m); single peak in HPLC analysis (C_{18}-μ Bondapak column, 15% CH_3CN in 0.1 M NaOAc, pH = 3.6). Compound (3) showed: mp 205-207°C (dec.); IR (KBr disc) ν_{max} 1635, 1600 cm^{-1}; ^1H NMR δ 2.32 (4H, br. s, $C\underline{H}_2C\underline{H}_2$), 3.21 (3H, s, $NC\underline{H}_3$), 4.81 (2H, s, $C\underline{H}_2N$), 6.43 (1H, t, J = 56.5 Hz, CHF_2), 6.84 and

7.66 (4H, AB q, J = 9.0 Hz, arom. H), 7.47 (2H, s, NH_2), 8.12 (2H, br. s, NH_2), 8.20 (1H, s, CONH), 8.62 (1H, s, arom. H); ^{19}F NMR (DMSO-d_6) δ 35.33 (d, $^2J_{HF}$ = 56.5 Hz); Compound (4) showed: mp 192-198°C (dec.); IR (KBr disk) ν_{max} 1635, 1600 cm^{-1}; ^1H NMR δ 1.40 (2H, br. s, CH_2CH_2COOH), 2.17 (2H, br. s, CH_2CH_2COOH), 3.20 (3H, s, NMe), 4.77 (2H, s, NCH_2), 6.67 (2H, s, NH_2), 6.75 and 7.60 (4H, AB q, J = 9.0 Hz, arom. H), 7.53 (2H, br. s, NH_2), 7.85 (1H, s, CONH), 8.52 (1H, s, arom. H). Compound (13) showed: mp 129-131°C; IR (KBr disc) ν_{max} 1730, 1500, 1240, 1080 cm^{-1}; ^1H NMR (D_2O) δ (ext. TMS) 2.47-3.17 (4H, m, CH_2CH_2), 6.82 (1H, dd, $^2J_{HF}$ = 54.9 Hz, $^2J_{HF}$ = 52.3 Hz, CHF_2); ^{19}F NMR (D_2O) δ (ext. C_6F_6) 34.24 (1F, dd, $^2J_{FF}$ = 279.4 Hz, $^2J_{HF}$ = 54.9 Hz), 39.52 (1F, dd, $^2J_{FF}$ = 279.4 Hz, $^2J_{HF}$ = 52.3 Hz); mass spectrum, m/z 198 (MH^+), 179 ($M^+ - H_2O$), 152 ($M^+ - CO_2H$), 146 ($M^+ - CHF_2$), 134 ($M^+ - CO_2H - H_2O$), 128 ($M^+ - CHF_2 - H_2O$). Compound (14) showed: mp 180-181°C; IR (KBr disc) ν_{max} 1730, 1650, 1255, 1040 cm^{-1}; ^1H NMR (D_2O) δ (ext. TMS) 2.93 (4H, br. s, CH_2CH_2), 6.78 (1H, dd, $^2J_{HF}$ = 53.3 Hz, $^2J_{HF}$ 52.5 Hz, CHF_2). ^{19}F NMR (D_2O) δ (ext. C_6F_6) 32.24 (1F, dd, $^2J_{FF}$ = 279.4 Hz, $^2J_{HF}$ = 53.3 Hz), 39.14 (1F, dd, $^2J_{FF}$ = 279.4 Hz, $^2J_{HF}$ = 52.5 Hz); mass spectrum, m/z 179 (M^+), 134 ($M^+ - CO_2H$), 128 ($M^+ - CHF_2$). All these compounds gave satisfactory results on elementary analysis.

12. We employed a slightly modified procedure of that used by Piper et al.[7] The details will be reported elsewhere.

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