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Stereospecific Synthesis of 4-Fluoroglutamic Acids

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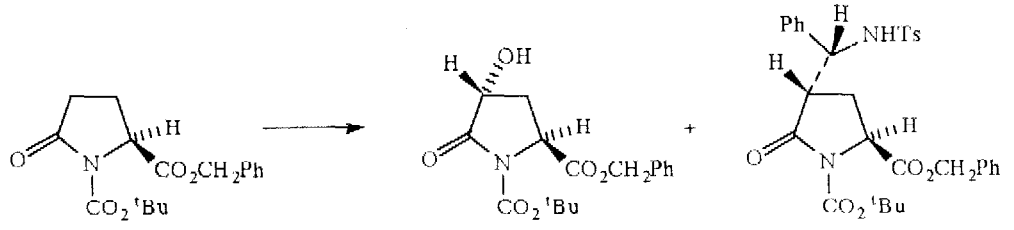
Key Words: 4-fluoroglutamate; stereochemistry; conformational isomerism; inhibitor; amino acid.

Abstract : A stereospecific route to 4-fluoroglutamic acids from pyroglutamic acid has been devised and variable temperature ^{1}H-nmr spectroscopic studies have explained apparent inconsistencies in the stereochemical outcome of steps in a synthesis of 4-fluoroglutamic acids from hydroxyprolines.

We have long been interested both in the enzyme dihydrofolate reductase,[1-3] which has been shown to be inhibited by the 4-fluoroglutamate analogue of methotrexate,[4,5] and in the enzymatic γ-carboxylation of glutamic acid in proteins in the blood clotting cascade, where 4-fluoroglutamic acid has been used to investigate the stereochemistry of the carboxylation process.[6] This has led us to devise a stereospecific synthesis of *erythro*- and *threo*- 4-fluoroglutamic acids.

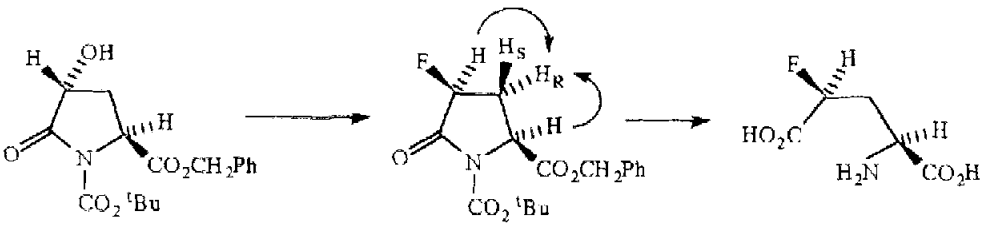
When we began our studies, the only synthesis of the diastereoisomeric 4-fluoroglutamates were non-stereospecific, giving racemic mixtures of the *erythro*- and *threo*- forms.[5,7-11] These mixtures were later separated by a variety of methods.[5,6,12-15] During the course of our studies, however, a stereospecific synthesis of D-*erythro*- and L-*threo*- 4-fluoroglutamic acids was reported by Hudlicky and Merola.[16] We now wish to report our studies on this synthesis, some of which will provide an explanation for apparent inconsistencies in the stereochemistry of the reactions involved in Hudlicky and Merola's synthesis.

L-Pyroglutamic acid was converted directly to benzyl N-*tert*-butoxycarbonyl-L-pyroglutamate **(1)** by esterification followed by urethane formation.[17,18] Ohta *et al.*[19] had converted this compound to the (2S,4R) alcohol **(2)** by stereospecific reaction with lithium hexamethyldisilazide and 2-toluenesulphonyl-3-phenyloxaziridine. When we prepared 2-toluenesulphonyl-3-phenyloxaziridine[20] and repeated this reaction, we obtained only a small yield of the desired alcohol together with considerable quantities of a 5 : 1 ratio of the compound **(3)** and its C-6 epimer.[18] These were evidently formed from aldol reaction of the imine byproduct formed on transfer of oxygen from the oxaziridine. We were able to improve the yield of the alcohol to 30% following correspondence with Dr Ohta.[21]



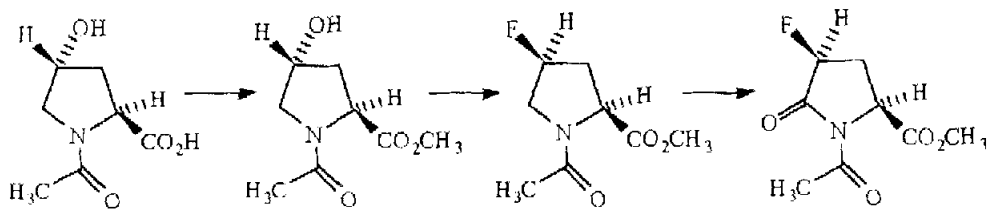
(1) **(2)** **(3)**

The alcohol (2) was now reacted with diethylaminosulphur trifluoride (DAST) to give a 40% yield of the 4-fluoride (4), $C_{17}H_{20}NO_5F$, mp 123-124 °C, $[\alpha]_D$ -70.9 ° (c 0.165, $CHCl_3$). The *cis* nature of the hydrogen atoms at C-2 and C-4 was shown by the fact that irradiation of these protons in the ^1H-nmr spectrum caused an n.O.e in the ***same*** C-3 hydrogen (H_{3R}), verifying that the reaction of the alcohol (2) with DAST had proceeded with inversion of stereochemistry. The intention was now to hydrolyse the fluoride (4) to yield L-*threo*- 4 fluoroglutamic acid (5) but at this point Hudlicky and Merola's synthesis was published.[16] In this work, a protected analogue (9) of the fluoride (4) , prepared by a different route, was hydrolysed to L-*threo*- 4 fluoroglutamic acid (5), and so, in principle, our synthesis had been completed



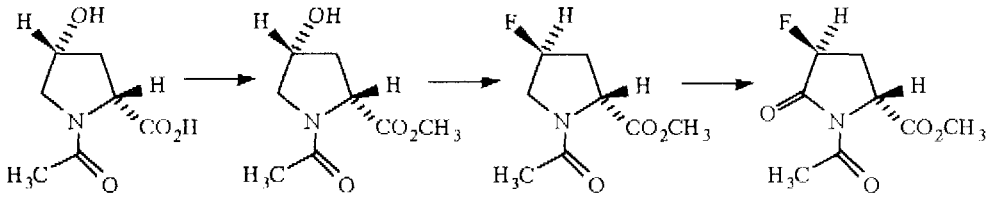
(2) (4) (5)

In this synthesis, L-*threo* - 4-fluoroglutamic acid (5) was prepared from commercially available L-*trans*-4-hydroxyproline by acetylation to (6), esterification to methyl 1-acetyl-4-hydroxyprolinate (7), reaction with DAST, oxidation and hydrolysis. Hudlicky and Merola claimed that the product of the fluorination reaction was a mixture containing *ca.* 60% of methyl *trans* - 4-fluoroprolinate and *ca.* 40% of the *cis* epimer (8). This implied loss of stereochemistry in the DAST reaction. However when the "mixture" of 4-fluoroprolinates was oxidised to the pyroglutamate with ruthenium tetroxide, "sterically almost uniform" methyl *cis*- 1 acetyl-4-fluoropyroglutamate (9) was obtained. It was suggested that this strange occurence might be due to enolisation of the pyroglutamate carbonyl group in the oxidation step. This would imply thermodynamic control in the process, with isomer (9) being the more stable of the 4 epimers.



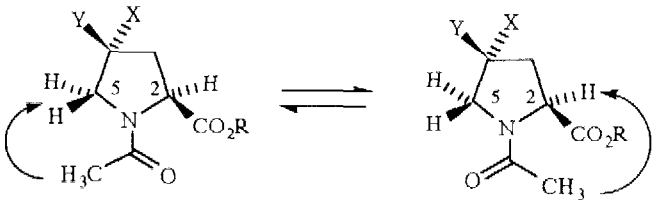
(6) (7) (8) (9)

When D-*cis*- 4-hydroxyproline was taken through the same sequence of reactions, the product of the DAST reaction was reported to be a mixture containing *ca.* 80 % of the *trans* -isomer (12) together with *ca.* 20 % of the *cis* - epimer.[16] The constitution of the mixture did not change after several recrystallisations from hexane and benzene. Again, on oxidation to the pyroglutamate (13), a single diastereoisomer was obtained. The stereochemistry of the products (9) and (13) was proven by hydrolysis to L-*threo*- and D-*erythro*- 4-fluoroglutamic acids respectively and these were subjected to X-ray crystal structure analysis. Since (9) and (13) cannot ***both*** represent the thermodynamically more stable products of the oxidation reactions and since these products are the expected products from a 'normal' sequence of events in which the DAST reaction occurred with inversion of stereochemistry, it seemed to us that an alternative explanation must account for the results.



(10) (11) (12) (13)

Work on compounds in the pyroglutamic acid and proline series has shown that, whilst there is little conformational isomerism of N-acyl derivatives in the former compounds, such isomerism is well known in the proline series.[22] It therefore seemed that the most plausible explanation for Hudlicky and Merola's results would be that the apparent mixtures of epimers from the DAST reactions were in fact single epimers exhibiting conformational isomerism in the ^{1}H-nmr spectra. We therefore repeated the syntheses of the compounds **(6)**, **(7)**, **(8)**, **(10)**, **(11)**, and **(12)** and examined their ^{1}H-nmr spectra in [$^2H_6$]-dmso at various temperatures. The ratios of the two isomers for each compound are reported in the table together with free energy differences calculated from the Boltzmann equation. It was found that, in all cases, the two isomers were exchanging on the nmr time scale. Coalescence temperatures are reported in the table for the acetyl methyl groups, with the exception of compound **(8)** where it was found to be more convenient to use the OMe signals. Free energies of activation were calculated from the coalescence temperatures by the graphical method of Shanan-Atidi and Bar-Eli.[23] NOe experiments were performed on all six compounds. In some cases it was necessary to use C^2HCl_3 as solvent and reduced temperatures of between - 50 ° C and - 30 ° C to avoid saturation transfer effects. In all cases it was found that irradiation of the major acetyl methyl signal led to enhancements of the two protons on C-5, whilst irradiation of the corresponding minor signal led to enhancement of the proton on C-2. Thus we can say that, in each case, the major isomer is **(A)** and the minor is isomer **(B)**.



(A) (B)

Table Free Energy Differences and Barriers (KJ mol^{-1}, 293 K) for the equilibrium **(A)** $\rightleftharpoons$ **(B)** in the compounds **(6)** - **(8)** and **(10)** **(12)**

Compound	Ratio **(A)** : **(B)** (293 K)	Coalescence Temperature (K)	Free Energy Difference (± 0. 4)	Free Energy Barrier **(A)** → **(B)**
(6) (trans)	0.78 : 0.22	373 ± 2	3. 08	75. 4
(7) (trans)	0.85 : 0.15	404 ± 2	4. 23	84. 2
(12) (trans)	0.82 : 0.18	373 ± 2	3. 69	78. 9
(10) (cis)	0.63 : 0.37	405 ± 2	1.30	83. 0
(11) (cis)	0.67 : 0.33	383 ± 2	1. 73	78. 31
(8) (cis)	0.56 : 0.41	376 + 2	0. 76	75. 0

It is of interest to note that conformation **(A)** is very much preferred in the *trans* series of compounds **(6)**, **(7)** and **(12)**. This same preference is also shown, but is less pronounced in the *cis* series **(10)**, **(11)** and **(8)**. The apparent inconsistancies in the synthesis of Hudlicky and Merola have now been explained as being due to the conformational isomerism which is exhibited by compounds in the proline series but not by compounds in the pyroglutamic acid series.

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