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Synthesis of Fluorinated Proline Analogs for the Potential Inhibition of Proline Oxidase

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for the Potential Inhibition of Proline Oxidase

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Synthesis of Fluorinated Proline Analogs for Potential Inhibition of Proline Oxidase

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Acknowledgments

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Introduction

The focus of this project centers on the metabolism of L-proline to glutamic acid, specifically concerning the first step involving proline oxidase. It has been shown that 4,4-difluoro-L-proline acts as an inhibitor for proline oxidase by irreversibly inhibiting the proline oxidase in some unknown manner.[1] It is suspected that it is a substrate for the enzyme and that it is chemically modifying the enzyme in some manner. This preliminary report requires duplication and would lead to further insight into the mechanism of oxidation. In addition, the choice of suitably fluorinated proline analogs may help to clarify the mechanism of the conversion from proline to glutamic acid involving proline oxidase. Thus, the reported inhibition of proline oxidase is of significant interest.

Past synthetic routes to the fluorinated analogs were often low yielding. Thus, a new synthetic pathway will be attempted in an effort to obtain the fluorinated analogs in higher yields.

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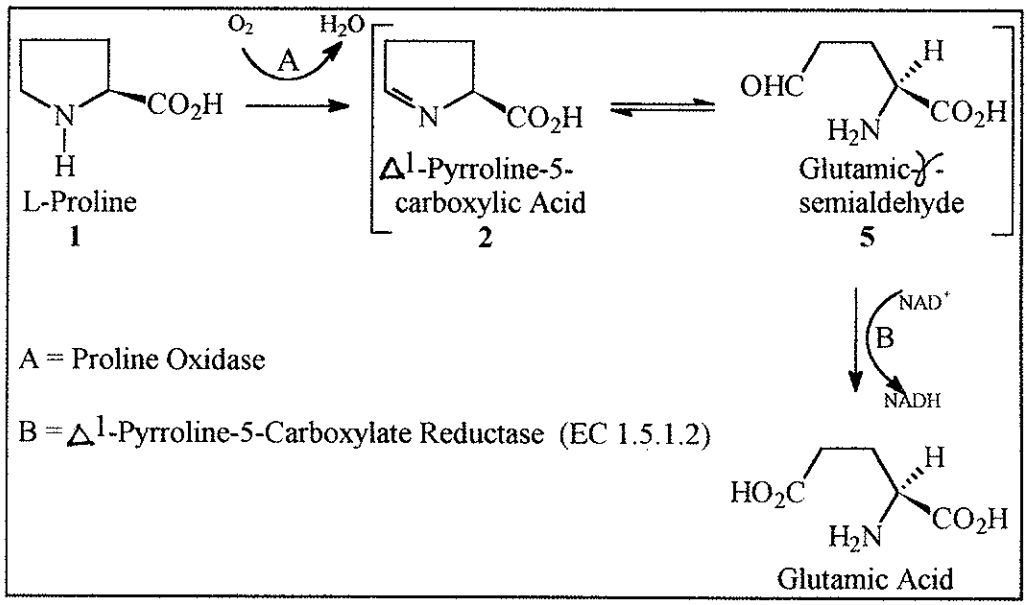
[1]Muldoon, W. P. Thesis: University of Minnesota. **1980**.

Synopsis of Current Knowledge of Proline Metabolism

It is believed that proline oxidase is the enzyme acting in the first step of the mechanism for proline oxidation to glutamic acid.[2] The overall pathway for the oxidation of L-proline to glutamic acid is shown in **Scheme 1**.[1]



Scheme 1

Proline oxidase uses molecular oxygen, not NAD^+ or FAD, during the catalysis of the oxidation of L-proline **1** to Δ^1-pyrroline-5-carboxylic acid **2** as demonstrated in **Scheme 2**.[1]

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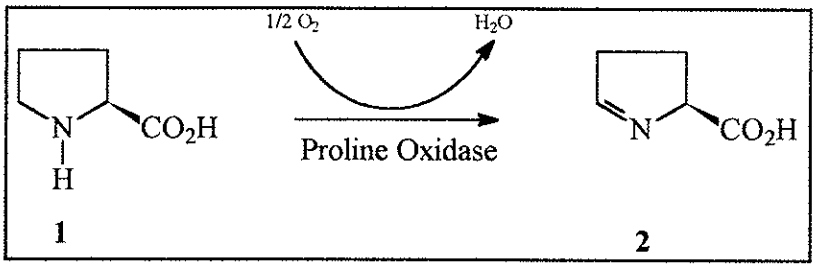
[2]Johnson, A. B.; Strecker, H. J. *J. Biol. Chem.* **1962**, *237*, 1876.



Scheme 2

Brunner and Neupert located proline oxidase in the inner mitochondrial membrane of rat liver tissue.[3] Isolation and subsequent assays found proline oxidase to be particle bound. Early attempts at purification of proline oxidase failed.[4,5]

The oxidation of **1** to **2** via proline oxidase requires molecular oxygen and cytochrome c.[2] This oxidation results in the reduction of ubiquinone,[6] which along with phospholipids support the transfer of electrons in mitochondria.[7] Oxidation of proline was restored upon addition of these cofactors to depleted mitochondria.[1]

Substrate specificity of proline oxidase has been investigated by many researchers. In general, it has been found that there are specific stereo-requirements at

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[3]Brunner, G.; Neupert, W. *FEBS Lett.* **1969**, *3*, 283.

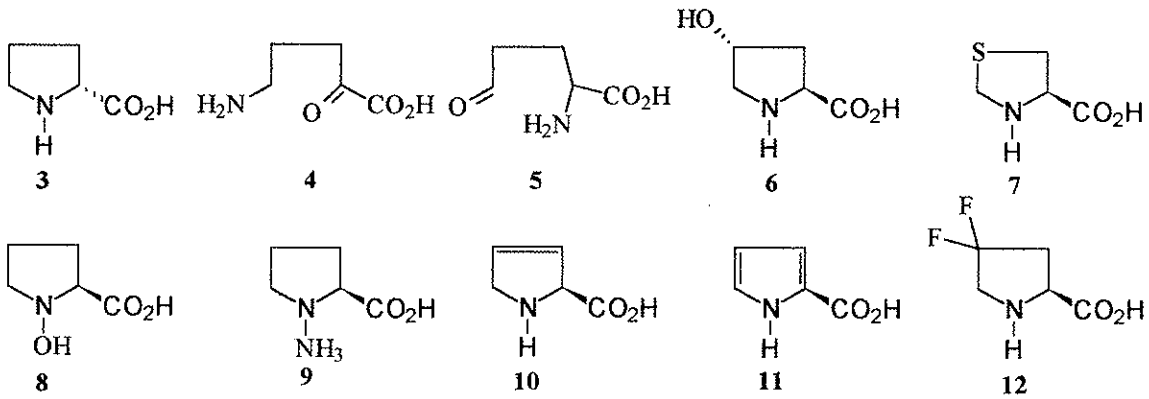
[4]Strecker, H. J. "Methods in Enzymology," 17 B, H. Tabor and C. W. Tabor, Eds., Academic Press, New York, **1971**, 251.

[5]Kramar, R. *Enzymol.* **1967**, *33*, 33.

[6]Erecińska, M. *Acta Biochim. Pol.* **1966**, *13*, 209.

[7]Lester, R. L.; Fleisher, S. F. *Biochim. Biophys. Acta.* **1961**, *47*, 358.

the alpha carbon. D-Proline **3** was shown to be oxidized at 20 % of the rate of L-proline.[8] Contrary to this, Muldoon has shown that **3** is not a substrate for proline oxidase.[1] This indicates that absolute stereochemistry of the alpha carbon is required. Lang and Lang may have been observing D-amino acid oxidase. In a paper by Lang and Schmid, **3** was shown to be oxidized to δ-amino-alpha-ketovaleric acid **4** rather than glutamic-γ-semialdehyde **5** which is produced by L-proline oxidation.[9] In addition, proline oxidase has been shown to be different from non-specific L-amino acid oxidases as demonstrated by the observation that DL-phenylalanine, leucine, glycine, lysine, valine, threonine, methionine, arginine, alanine, histidine, and tryptophan were unable to reduce cytochrome c.[2]



Trans-4-hydroxy-L-proline **6** has been shown to be a substrate for proline oxidase with approximately 20 % of the activity of proline **1**.[5] L-Thiazolidine-4-carboxylic acid **7**

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[8]Lang, K.; Lang, H. *Biochem. Z.* **1958**, *329*, 577.

[9]Lang, K.; Schmid, G. *Biochem. Z.* **1951**, *322*, 1.

was also shown to be oxidized by liver preparations.[10,11] In addition, Muldoon found that L-Thiazolidine-4-carboxylic acid **7**, 1-hydroxy-L-proline **8**, and 1-amino-L-proline **9** were all substrates for proline oxidase to varying degrees.[1]

Numerous compounds inhibit proline oxidation. L-3,4-dehydroproline **10** has been shown to inhibit proline oxidation competitively.[12] Consequently, 3,4-dehydrofluorination is not an issue in the irreversible inhibition of proline oxidase. It was shown that **10** is metabolized to pyrrole-2-carboxylic acid **11**, which is itself a non-competitive inhibitor of proline oxidase, by an enzyme different from proline oxidase.[12] In addition, the product of proline oxidation, Δ^1-pyrroline-5-carboxylic acid **2**, acts as an inhibitor of proline oxidase.[2]

Johnson and Strecker have shown that cyanide acts as a non-specific inhibitor of proline oxidation, although the type of inhibition is unknown.[2] In addition, Johnson and Strecker have shown that there are other compounds which inhibit proline oxidase. Among these are antimycin A, sodium azide, and amytal.[2] Concentrations of salt on the order of 1×10^{-2} M also inhibits proline oxidase. Inhibitory effects of Cu^{2+}, and of the sulfhydryl reagents Ag^{+}, BAL, and salyrgan were reported by Lang and Lang.[8] Also, Kramar and Fitscha reported that ethacrynic acid inhibits proline oxidase.[13] This

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[10]Cavallini, D.; DeMarco, C.; Mondovi, B.; Trasarti, F. *Biochim. Biophys. Acta.* **1956**, *22*, 558.

[11]Debey, H. J.; Mackenzie, J. B.; Mackenzie, C. G. *J. Nutr.* **1958**, *66*, 607.

[12]Dashman, T.; Lewinson, T. M.; Felix, A. M.; Schwartz, M. A. *Res. Commun. Chem. Pathol. Pharmacol.* **1979**, *24*, 143.

[13]Kramar, R.; Fitscha, P. *Enzymol.* **1970**, *39*, 101.

inhibitor suggests the need for a sulfhydryl group in the enzyme.[1] These inhibitors demonstrate the significance of oxidative phosphorylation in proline oxidation. Additional inhibitors are ethanol and acetaldehyde,[14] although the type and mechanism of inhibition are unclear.

To determine the activity of an enzyme on a particular substrate or inhibitor, a method is required to analyze the activity of substrates. The assay of choice utilizes iodonitrotetrazolium chloride [INT = 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride] as an electron acceptor.[1,5] The reduced INT is measured at 500 nm using an extinction coefficient of 11.5×10^6.[2]

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[14]Kiessling, K. H.; Fellenius, E.; Iwanicka-Kjellberg, A.; Carlgren, H. "Alcohol and Aldehyde Metabolizing Systems," R. G. Thurman, T. Yonetani, J. R. Williamson, and B. Chance, Eds., Academic Press, New York: **1974**, 417.

Synopsis of Current Knowledge

of the Synthesis of Fluorinated Proline Analogs

The first synthesis of a fluorinated proline analog involved the conversion of the hydroxyl group in *trans*-4-hydroxy-L-proline **6** to a tosylate ester. This was followed by an S_N2 displacement of the *trans* tosylate with KF to form *cis*-fluoro-L-proline **13**.[15] This research utilized the carbobenzyloxy protecting group used by Patchett and Witkop in their study of hydroxyproline.[16] When fluorination was performed on the *trans* tosylate ester, a mixture of 83% *cis* to 17% *trans*-4-fluoro-L-proline was obtained in 62 % overall yield.[15] The *cis* **13** and *trans* **14** fluorinated isomers could be separated as the N-trifluoroacetyl derivatives using gas chromatography on a 4 % NGS column.[15]

In order to obtain the *cis*-4-hydroxy-L-proline isomer **15** from the natural occurring *trans*-4-hydroxy-L-proline isomer **6**, the natural isomer was oxidized to 4-keto-L-proline **16** using the Jones Reagent, and then selectively reduced using $NaBH_4$.[16] Fluorination of the *cis* tosylate yielded enantiomerically pure *trans*-4-fluoro-L-proline **14** in 56 % overall yield.[15]

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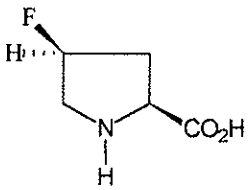
[15]Gottlieb, A. A.; Fujita, Y.; Udenfriend, S.; Witkop, B. *Biochemistry.* **1965**, *4*, 2507.

[16]Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.



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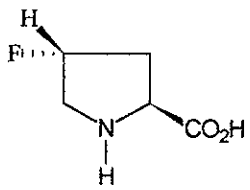

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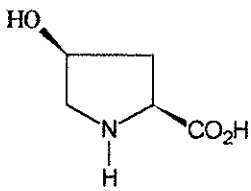

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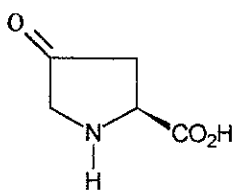

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The conformation of *cis*-4-fluoro-L-proline **13** and *trans*-4-fluoro-L-proline **14** in aqueous solution was studied using ^{1}H and ^{19}F NMR.[17] They used the method of Gottlieb et al to synthesize the *cis* and *trans* mono-fluorinated isomers.

A reagent showing useful properties for the fluorination of alcohols and ketones is DAST, diethylaminosulfur trifluoride. DAST is a mild fluorinating reagent used for the fluorination of alcohols to mono-fluorinated derivatives and for the difluorination of carbonyls. In addition, DAST is easily handled and employs mild reaction conditions. It is presumed that the mechanism acts in an S_N2 fashion. A series of other derivatives of DAST has also been utilized, including morpholino-DAST. These derivatives are used with the hope of increasing yield and stereoselectivity.

A method recently published approaches the synthesis of the mono-fluorinated products by utilizing morpholino-DAST.[18] Morpholinosulfur trifluoride fluorinated the appropriately protected *trans*-4-hydroxy-L-proline **6** and *cis*-4-hydroxy-L-proline **15** under mild conditions in up to 50% yield.[18] In addition, the products were close to

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[17]Gerig, J. T.; McLeod, R. S. *J. Am. Chem. Soc.* **1973**, *95*, 5725.

[18]Panasik, Jr. N.; Eberhardt, E. S.; Edison, A. S.; Powell, D. R.; Raines, R. T. *Int. J. Peptide Protein Res.* **1994**, *44*, 262.

enantiomerically pure, although it did not specify to what degree they were pure.

Shirota, Nagasawa, and Elberling were the first to synthesize the 4,4-difluoro-L-proline **12**.[19] A diketopiperazine **17** was utilized to protect the stereochemistry of the alpha carbon. Oxidation of **17** to the optically active diketonic tricyclic piperazinedione **18** was accomplished using dicylcohexylcarbodiimide and dimethylsulfoxide. Fluorination was performed with SF_4 in a Parr bomb, followed by deprotection which resulted in the isolation of 4,4-difluoro-L-proline **12**. The major drawbacks to this synthetic approach include low reaction yields of around 36 %, and a difficult fluorination involving high pressure.

An improved synthesis of 4,4-difluoro-L-proline **12** was reported by Sufrin, Balasubramanian, Vora, and Marshall.[20] Protection of the nitrogen and oxidation to 4-keto-L-proline **16** utilized the work of Patchett and Witkop.[16] The acid function was converted to the benzyl ester following the procedure of Gison.[21] Fluorination of the ketone utilized DAST, which resulted in a 44 % yield of **12**.

A 5-fluoro-L-proline **19** derivative has been reported by Kagel, Kofron, and Rich.[22] Fluorination of 5-hydroxy-L-proline **20** utilized DAST.

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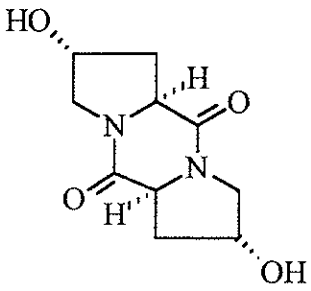
[19]Shirota, F. N.; Nagasawa, H. T.; Elberling, J. A. *J. Med. Chem.* **1977**, *20*, 1176.

[20]Sufrin, J. R.; Balasubramanian, T. M.; Vora, C. M.; Marshall, G. R. *Int. J. Peptide Protein Res.* **1982**, *20*, 438.

[21]Gison, B. F. *Helv. Chem. Acta.* **1973**, *56*, 1476.

[22]Kagel, J. R.; Kofron, J. L.; Rich, D. H. *Pept.: Chem. Biol., Proc. Am. Pept. Symp., 12th.* J. A. Smith, J. E. Rivier, Eds. ESCOM, Leiden, Netherlands: **1992**, 818.



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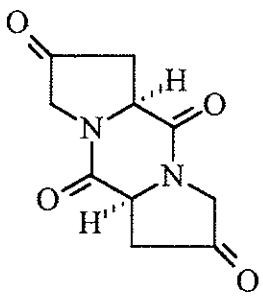

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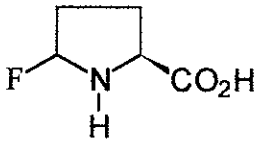

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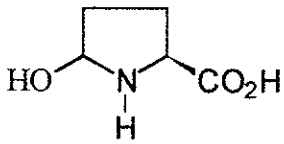

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Rationale

There are two likely sites proposed for the oxidation of L-proline **1** by proline oxidase. Two of these mechanisms involve direct hydroxylation of the substrate. The first potential mechanism involves α^1-hydroxylation followed by the loss of water across the first and fifth atoms. The second possible mechanism comprises of N-hydroxylation also followed by the loss of water. Both ultimately result in the cyclic Schiff's base, **2**.

Several reports have already been cited which demonstrate that proline oxidase oxidizes L-proline **1** to Δ^1-pyrroline-5-carboxylic acid **2** as outlined in **Scheme 2**. Muldoon chose several suitably substituted proline residues to test with proline oxidase.[1] Of these, 4,4-difluoro-L-proline **12** exhibited strange behavior in that it essentially destroyed the catalytic activity of proline oxidase such that addition of proline resulted in no reaction. Therefore, it seems worthwhile to explore this particular result in hopes of explaining the unusual behavior further, as well as developing additional insight into the mechanism of oxidation.

Muldoon reported that substrates for proline oxidase must be five membered rings with a carboxylic acid group on the alpha carbon having an absolute stereochemistry of "R".[1] Substitution at any position besides the nitrogen reduces the binding affinity of the substrate to the enzyme, presumably due to steric factors.

The synthesis of the suitably fluorinated proline analogs in a good yield is the first concern of this endeavor. Following the synthesis, we hope to evaluate the analogs for

inhibition of proline oxidase, and concomitantly develop a mechanism for the inhibition as well as for the oxidation.

Since the atomic radius of fluorine is not significantly larger than that of hydrogen, .64 Å to .37 Å,[23] it has been assumed that steric factors would not be an issue for fluorinated proline derivatives.

Muldoon reported that 4,4-difluoro-L-proline **12** inhibited proline oxidase and also prevented retention of activity with addition of proline **1**, the natural substrate.[1] Separate experiments ruled out inhibitory effects of fluoride anions since they showed no effect on the oxidation of L-proline at a concentration of up to 30 μM.[1]

Since preliminary studies showed the irreversible inhibition of proline oxidase in the presence of **12**,[1] these results will need to be duplicated. It is possible that the fluorine atoms could be hydrogen bonding strongly enough to proline oxidase to prevent the exchange of substrates. To test this hypothesis, the fluorinated analogs would have to be tested with the enzyme in varying concentrations, including making the enzyme more abundant than the substrate. In this particular case, activity should be regained upon the addition of proline to the non-active mixture. The *cis* **13** and *trans* **14** mono-fluorinated isomers may also give insight into stereochemical considerations of the inhibition.

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[23]Zumdahl, S. S. Chemistry, 2nd ed. (Lexington: Heath, 1989) 309.

Results and Discussion

The first potential product chosen for this project was 4,4-difluoro-L-proline **12**, since this was the actual product in question in Muldoon's work. However, it is recognized that both *cis* **13** and *trans* **14** mono-fluorinated isomers, as well as 5-fluoro-L-proline **19** are suitable choices. Originally, a hemiacetal **24** was to be used to protect the chirality of the alpha carbon and the nitrogen atom[24] as shown in **Scheme 3**. However, difficulty in reducing the ester moiety to an alcohol forced the synthesis to be abandoned.

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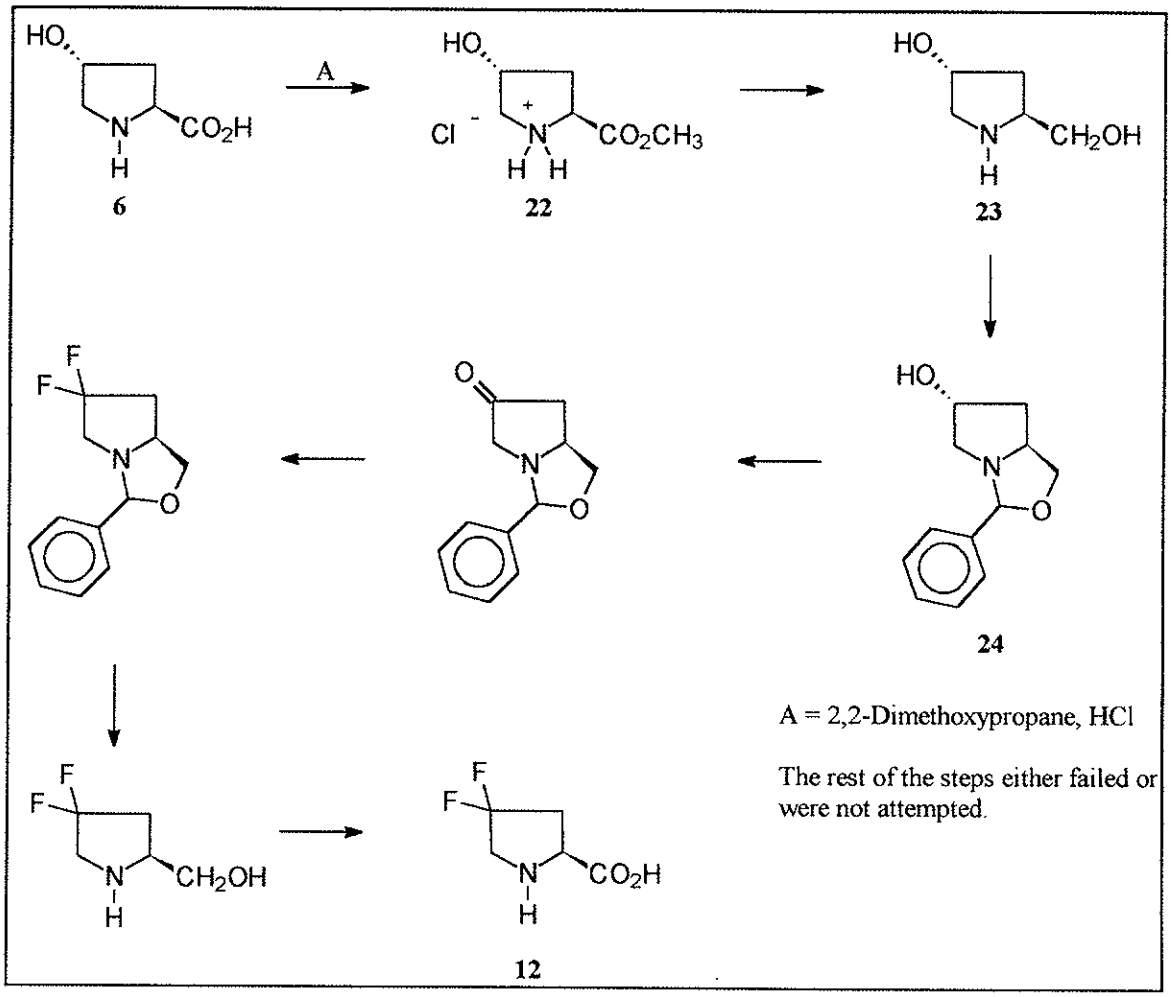
[24]Thottathil, J. K.; Moniot, J. L.; Mueller, R. H.; Wong, M. K. Y.; Kissick, T. P. *J. Org. Chem.* **1986**, *51*, 3140.



Scheme 3

Keeping in mind that preservation of the stereochemistry of the alpha carbon is essential, another suitable protecting group was needed for the second pathway. It was reported by Rapoport and others that N-(9-(9-phenylfluorenyl)) **21** derivatives of amino acids preserve the stereochemistry of the alpha carbon.[25, 26, 27, 28]

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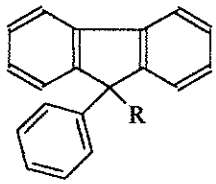
[25]Christie, B. D.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 1239.

[26]Koskinen, A. M. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1859.

[27]Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1987**, *109*, 236.

[28]Lubell, W. D.; Jamison, T. F.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 3511.

Since it is necessary to protect the nitrogen, and preserve the stereochemistry of the alpha carbon, 9-bromo-9-phenylfluorene was chosen as the protecting group. Note that R is the compound being protected.



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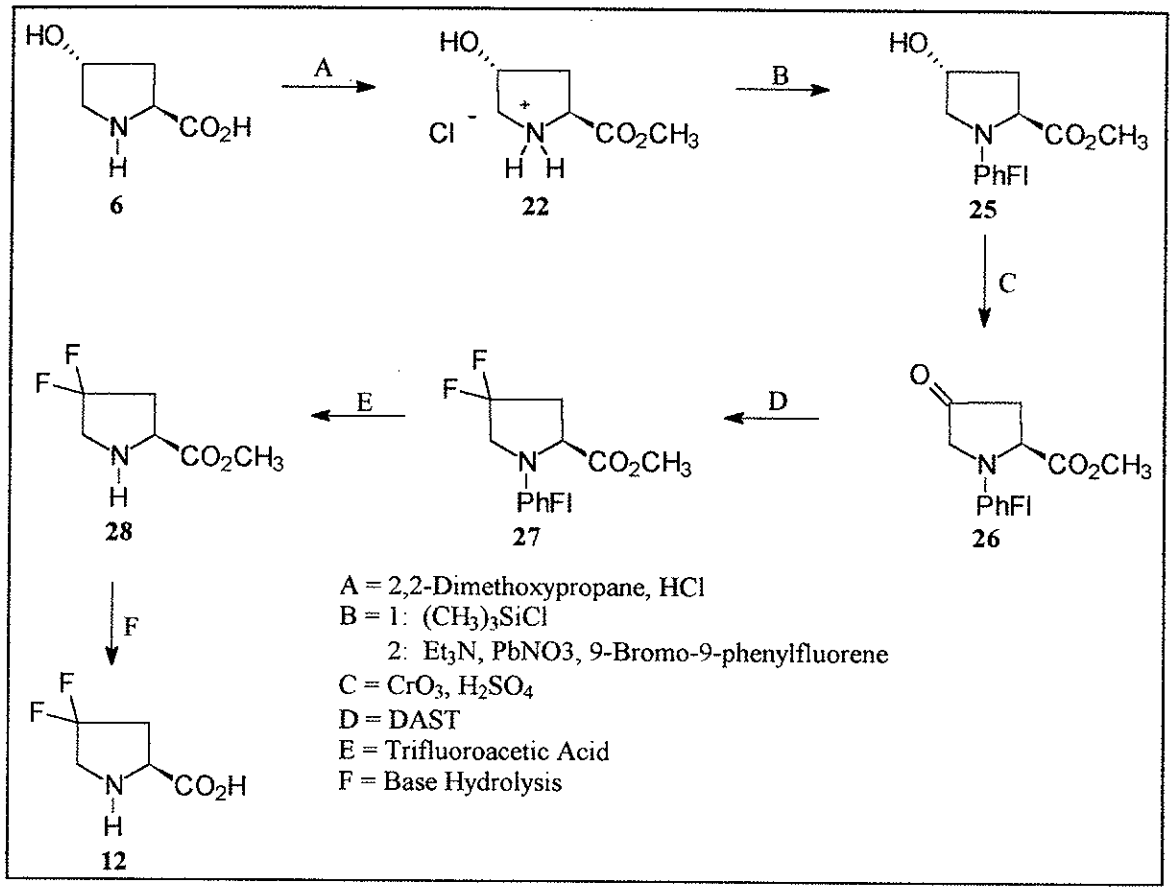
Since DAST will difluorinate ketones. Jones oxidation of suitably protected secondary alcohols reported by Patchett and Witkop was utilized to oxidize the protected 4-hydroxy-L-proline **25** to the protected 4-keto-L-proline **26**.[16] This enabled a quick approach to the fluorination reaction that utilized DAST which will difluorinate ketones. In fact, a similar scheme was performed by Sufrin, Balasubramanian, Vora, and Marshall.[20] The pathway utilized in this project is shown in **Scheme 4**. Yields were moderate leading up to the fluorination reaction. However, the N-(9-phenylfluoren-9-yl)-4-keto-L-proline **26** appeared to decompose during storage and during the attempted fluorinations. In addition, the fluorination reactions resulted in mixtures which were difficult to purify. Since the reaction was monitored by TLC, it was noticed that many products were forming. As the reaction proceeded, the number of spots increased. This development made it difficult to identify the product. Furthermore, this evidence caused us to realize that a significant amount of time was necessary to work out parameters making this reaction useful. A timely publication documenting the synthesis of the



Scheme 4

monofluorinated isomers **13** and **14** appeared and we chose to work in that direction.

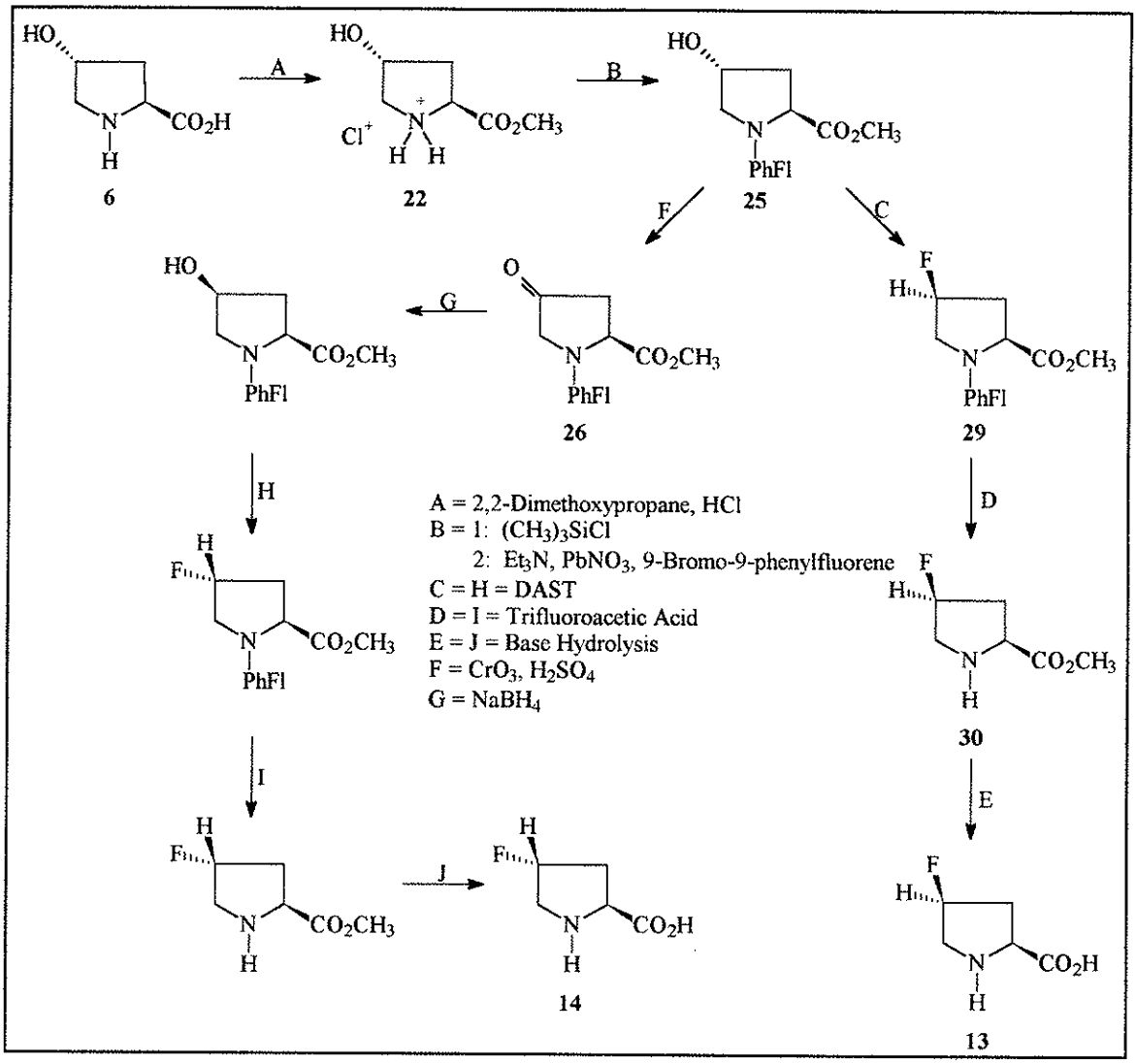
Coincidentally, a recent paper by Panasik Jr., Eberhardt, Edison, Powell, and Raines appeared,[18] which turned out to be a fortuitous route for the synthesis of the mono-fluorinated proline isomers **13** and **14**. 9-Bromo-9-Phenylfluorene was used as the protecting group again, although the publication utilized the N-acetyl derivatives. Morpholinosulfur trifluoride was used in a five fold excess to enantioselectively fluorinate the protected *trans*-4-hydroxy-L-proline **25**.[18] Fluorination with morpholinosulfur trifluoride results in the inversion of the stereochemical center at the fourth position.[18] The quenched reaction mixture was purified using flash

chromatography. Mass spectrometry was utilized to detect possible fluorinated products. Fractions with a molecular ion peak of 387 m/z were pooled and submitted for high field NMR analysis at the University of Minnesota. The ^{1}H NMR data suggests that the enantioselectivity was not as complete as eluded to in the publication. **Table 1** contains the interpretation of the spectral chemical shifts and a comparison to literature sources.

	Major Isomer		Minor Isomer	
Proton #	Reported[18]	Actual	Reported[18]	Actual
H^1	4.7	3.72	4.45	3.61
H^2	2.32	2.2 - 1.9	3.45	2.2 - 1.9
H^3	2.43	3.09	3.64	2.75
H^4	5.23	5.0	5.17	5.32
H^5	3.87 - 3.62	3.285	3.87 - 3.62	?
H^6	3.87 - 3.62	3.53	3.87 - 3.62	3.41
Me ester	3.67	3.52	3.71	3.42

Table 1

Thus the compound analyzed is a mixture of the isomeric mono-fluorinated protected prolines **13** and **14** is a 70 to 30 ratio respectively. This new synthetic pathway is shown in **Scheme 5**.


A = 2,2-Dimethoxypropane, HCl
B = 1: $(CH_3)_3SiCl$
2: Et_3N, $PbNO_3$, 9-Bromo-9-phenylfluorene
C = H = DAST
D = I = Trifluoroacetic Acid
E = J = Base Hydrolysis
F = CrO_3, H_2SO_4
G = $NaBH_4$

Scheme 5

Conclusion

The original plan of synthesizing 4,4-difluoro-L-proline **12** in improved yield was abandoned during the course of the project, and thus still remains a target. The synthesis of *cis*-4-fluoro-L-proline **13** appears close to completion. However, the *trans* isomer **14** still needs to be synthesized, or separated from the other isomer. In addition, the biological evaluation of the compounds in question still needs to be performed in order to elucidate a mechanism of inhibition.

Experimental

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Infrared spectroscopy was performed on a Mattson Galaxy series FTIR 5000. ^{1}H NMR spectra were obtained with a 60 MHz Varian EM360A with TMS as an internal reference except where noted. Mass spectra were obtained using a Hewlett Packard 5988A mass spectrometer. Kieselgel 60 from Merck was used for both TLC and for column chromatography. The solvent systems used are described where applicable.

***trans*-4-Hydroxy-L-proline methyl ester hydrochloride salt**[29] (**22**). 4-Hydroxy-L-proline (**6**) (11.947 g , 91.1 mmol) was dissolved in 2,2-dimethoxypropane (400 ml, 3.25 mol) and 37% HCl (90 ml, 2.96 mol). The solution was stirred at room temperature for 24 h. The volume was reduced under reduced pressure. Recrystallization in MeOH and Et_2O gave 14.1 g (86.1%) of (**22**) as white crystals: mp 156.5-158 °C; ^{1}H NMR, D_2O, δ from TMS) 2.55 (m, H-R), 3.55 (s, H-OR), 3.90 (s, CH_3O-); IR (KBr, cm^{-1}) 1742 (ester carbonyl), 2200-3600 (OH, amine).

Attempted reduction to diol (23)

Using $LiAlH_4$. A flask was charged with (**22**) (0.500 g, 2.75 mmol), THF (6.0

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[29]Rachele, J. R. *J. Org. Chem.* **1963**, *28*, 2898.

ml, 74 mmol), and a stir bar. $LiAlH_4$ (0.2 g, 4.13 mmol) was added with stirring. The solution was stirred for 19 h at room temperature before being quenched with 20% acetic acid (15 ml). After standing for 2 h, the solution was placed on a Dowex 50 x 8 (50-100 mesh) ion exchange column and washed with 200 ml of H_2O. Following volume reduction under reduced pressure, a small amount of brown tar was recovered.

Using $LiBH_4$.[30] A 1 L flask was charged with a stir bar, $LiBH_4$ (3.986 g, 183 mmol), THF (200 ml, 2.466 mol), and (**22**) (10 g, 55.06 mmol), and flushed with N_2. A solution of I_2 (9.645 g, 76 mmol) dissolved in THF (50 ml) was added dropwise over a 1/2 h at 0 °C. Upon completion of H_2 evolution, the reaction was refluxed for 18 h. A black tar resulted after evaporation under reduced pressure.

Using $NaBH_4$.[31] A 1 L flask was charged with (**22**) (8.056 g, 44.3 mmol), THF (200 ml, 2.466 mol), and a stirring bar. The flask was flushed with N_2, and a solution of I_2 (9.644 g, 76 mmol) dissolved in THF (50 ml) was added dropwise with stirring over 1/2 h at 0 °C. The reaction was then refluxed for 18 h. An additional 100 ml of THF was added to prevent it from refluxing dry. After refluxing, MeOH (400 ml) was added, and then the solution was evaporated under reduced pressure. The residue was dissolved in 20 % KOH (200 ml) and extracted with CH_2Cl_2 (3 x 100).

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[30]Silverman, R. B.; Levy, M. A. *J. Org. Chem.* **1980**, *45*, 815.

[31]McKennon, M. J.; Meyers, A. I. *J. Org. Chem.* **1993**, *58*, 3568.

N-(9-Phenylfluoren-9-yl)-4-hydroxy-L-proline methyl ester[25-28] **(25).** **(22)** (0.9077 g, 4.998 mmol) was dissolved in $CHCl_3$ (80 ml) and CH_3CN (16 ml). The flask was flushed with N_2 and chlorotrimethylsilane (0.65 ml, 5.122 mmol) was added dropwise with stirring at room temperature. The solution was refluxed for 2 h. Et_3N (1.5 ml, 10.762 mmol) was added dropwise, followed by $PbNO_3$ (2.98 g, 8.998 mmol). A solution of 9-bromo-9-phenylfluorene (1.630 g, 5.07 mmol) dissolved in $CHCl_3$ (32 ml) was added slowly. The solution was stirred at room temperature for 28 h, at which time it was quenched with MeOH (1 ml). The solution was filtered and the filtrate was evaporated resulting in a dark amber oil. The oil was partitioned between Et_2O (250 ml) and 5 % citric acid (250 ml). Purification via flash chromatography (1:1 EtOAc:Hex) resulted in recovery of 1.079 g (56 %) of white crystals: Rf .356 in 1:1 EtOAc:Hex; mp 55-56 °C; ^1H NMR ($CDCl_3$, δ from TMS) 1.2 (t, 2H, H-R on C3), 1.75 (m, 2H, H-R on C5), 2.0 (s, 1H, H-OR), 3.2 (s, 3H, methyl ester), 4.1 (dd, 1H, **H**-R-OH, C4), 4.4 (m, 1H, H-R-N-, C2), 7.5 (m, 14H, H-ar); IR (KBr, cm^{-1}) 3447 (H-O), 2949, 2872 (sp^2 C-H), 1730 (C=O).

Attempted oxidation of hydroxyl via PCC on alumina. **(25)** (50 mg, 0.13 mmol) was dissolved in hexane (4 ml). PCC on Alumina (1.056 g, 0.814 mmol) was added, and the solution stirred for 5 h. The solution was filtered and washed with Et_2O (3 x 10), and then evaporated. TLC demonstrated that the reaction did not occur.

N-(9-Phenylfluoren-9-yl)-4-keto-L-proline methyl ester (26).[16] **(25)** (782.5 mg,

2.03 mmol) was dissolved in acetone (15 ml). This mixture was titrated with a solution of CrO_3 (13.4 g, 0.134 mol) dissolved in H_2SO_4 (11.5 ml, 0.216 mol) and H_2O (until titrant volume totaled 50 ml). The reaction was stirred for 25 min and was quenched with MeOH (3 ml). This solution was filtered and the filtrate diluted with $CHCl_3$ (25 ml), which was then washed with brine (3 x 30). The organic solution was then evaporated under reduced pressure. The resulting residue was dissolved in Et_2O and recrystallized with petroleum ether resulting in 0.5876 g (75.5 %) of off-white crystals: mp decomp.; ^{1}H NMR ($CDCl_3$, δ from TMS) 1.2 (m, 2H, H-R, C3), 2.0 (m, 2H, H-R, C5), 3.6 (s, methyl ester), 4.1 (m, 1H, H-R, C2), 7.3 (m, 14H, H-ar); IR (KBr, cm^{-1}) 2959 (sp^2 C-H), 1748 (ester C=O), 1669 (C=O), lack of OH stretch at 2500-3500.

Attempted Difluorination to 4,4-Difluoro-L-proline (27)

Using DAST in benzene.[20] (**26**) (0.105 g, 0.274 mmol) was dissolved in dry benzene (2 ml), and the solution was stirred with cooling in a dry ice bath. DAST (0.15 ml, 1.135 mmol) was added dropwise. Following the addition, the reaction was returned to room temp and stirred for 7 days at which time it was quenched by pouring the mixture over ice. It was extracted with CH_2Cl_2 (3 x 2) which was then dried with Na_2SO_4. Evaporation yielded 0.14 g of crude tar. It was realized that the starting material had decomposed.

Using DAST in CH_2Cl_2.[20] (**26**) (0.188 g, 0.49 mmol) was dissolved in CH_2Cl_2 (5 ml). The solution was cooled in a dry ice bath with stirring while DAST (0.15 ml, 1.135 mmol) was added dropwise. The reaction was quenched after 7 days by pouring the

mixture over ice. The aqueous solution was extracted with CH_2Cl_2 (3 x 5). The organic extract was dried with $MgSO_4$ and evaporated yielding a crude tar containing several different products according to t.l.c. Several attempts were made at purification without much success. This time, the starting material hadn't decomposed, except during the reaction.

N-(Phenylfluoren-9-yl)-4(S)-fluoro-L-proline methyl ester (29).[18]

Morpholinosulfur trifluoride (0.564 g, 3.22 mmol) was added dropwise with stirring over ten minutes to a solution (5 ml) at -80 °C under nitrogen of CH_2Cl_2 containing **25** (0.120 g, 0.645 mmol). The reaction was warmed to room temperature, and stirred for 48 h. The mixture was evaporated, quenched with water (2 ml), concentrated again, submitted to flash chromatography (ethyl acetate-hexanes, 1:6) to yield several unknown fractions. These were analyzed by mass spectrometry to locate possible product and a molecular ion peak at 387 was located. Potential product (30 mg 24.6 %) was located in two fractions with an R_f of 0.647 in ethyl acetate neat. ^1H NMR (300 MHz Bruker, $CDCl_3$, ppm), 7.15-7.75 (m, H-ar) 5.0 (dt, 1H, C4, minor isomer at 5.32), 3.72 (dd, 1H, C1, minor isomer at 3.61), 3.53 (m, 1H, C5, minor isomer at 3.41), 3.285 (dd, 1H, C5), 3.52 (s, 3H, minor isomer at 3.42), 3.09 (ddd, 1H, C3, minor isomer at 2.75), 2.2 - 1.9 (m, 1H, C3)