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A CONVENIENT SYNTHETIC ACCESS TO β,β-DIFLUORO-γ-KETO-α-AMINO ACIDS. APPLICATION TO THE SYNTHESIS OF A POTENTIAL INHIBITOR OF KYNURENINASE

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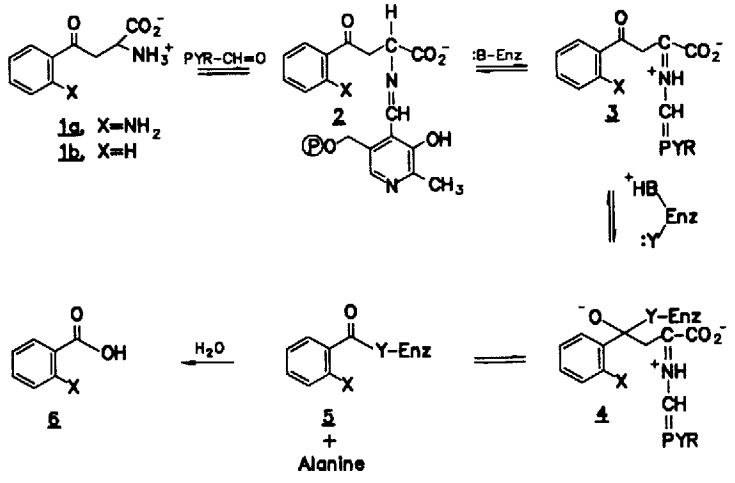
Abstract: A convenient synthesis of a novel class of kynureninase inhibitors, difluoroketoamino acid (11) is described.

Recent reports implicating the excitatory amino acid quinolinic acid in the etiology of Huntington's chorea and other neurodegenerative diseases[1,2] have triggered interest[3] in the inhibition of the biosynthesis of quinolinic acid from L-tryptophan.[4] Kynureninase (EC 3.7.1.3) is a key enzyme in this pathway; it catalyzes the conversion of kynurenine (1a) to anthranilic acid (6a) which is further metabolized to quinolinic acid.[5] Kynureninase belongs to a rare category of pyridoxal phosphate (PYR-CH=O) dependent enzymes which cleaves the carbon-carbon bond β,γ to the amino group of the substrate[5] (see Scheme 1 and ref. 6 for a discussion of the mechanism). Interestingly, the ortho-amino group of kynurenine is not necessary for activity since desaminokynurenine (1b)[5a] and even β-chloro-L-alanine and analogues, the only known inhibitors,[7] are substrates for this enzyme. We report the design and synthesis of α-amino-β,β-difluoro-γ-oxobenzenebutanoic acid (2,2-difluoro-2-benzoyl alanine) (11) as a potential new inhibitor of kynureninase.

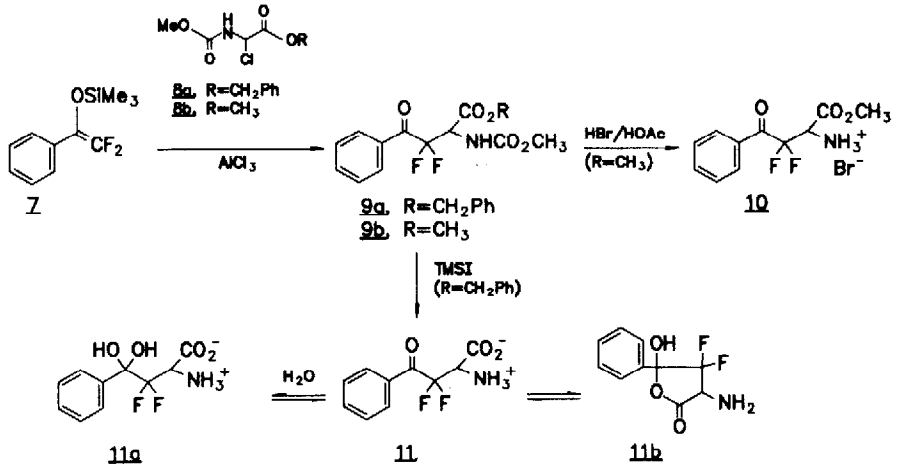
Scheme 1. Proposed mechanism for the carbon-carbon bond cleavage of kynurenine by kynureninase.[6]



Fluoroketone containing peptides are proteolytic enzyme inhibitors. These compounds form stable hydrates or hemiketals which are thought to inhibit proteolytic enzymes by mimicking the tetrahedral transition state of the amide bond cleavage. On the basis of this precedent, the intermediacy of hemiketal 4 in the kynureninase catalyzed reaction depicted in Scheme 1 made a difluoro analogue of desamino kynurenine (i.e. 11) an attractive target for the inhibition of kynureninase.

No β,β-difluoro-γ-keto-α-amino acids similar to structure 11 have been reported. None of the methods described for the synthesis of fluorinated amino acids and fluorinated ketones were of direct use for the synthesis of 11.[8,9,10] Yamana[11] described the synthesis of 1,1-difluoro-2-trimethylsiloxy-2-phenylethylene (7) and related silyl enol ethers and their use to prepare α,α-difluoro-β-hydroxyketones. We have found that these fluorosilyl enol ethers can also be used to form amino acid 11. Treatment of 7 with benzyl or methyl N-methoxycarbonyl-2-chloroglycinate (8a or 8b)[12] and aluminum chloride provided the α,α-difluoroketone 9a and 9b in 40% and 49% yield, respectively. Hydrogen bromide (32%) in glacial acetic acid converted 9b into the methyl ester 10, which upon treatment with either aqueous acid or base under a number of conditions failed to provide 11. However, deprotection of benzyl ester 9a to 11 was readily accomplished with trimethylsilyl iodide in 68% yield (see Scheme 2).

Scheme 2.



The experimental procedure for the two-step transformation is as follows:

Preparation of benzyl ester 9a. To a mixture of $AlCl_3$ (1.0 g, 7.3 mmol) and dry CH_2Cl_2 (10 mL) cooled to -5°C under argon was added a combined solution of 7[11] (1.67 g, 7.3 mmol) and 8a[12b] (7.62 g, 29.6 mmol) in dry CH_2Cl_2 (25 mL) dropwise over 30 min. The reaction was allowed to warm to 25°C, stir for 48 h, quenched with sat'd aq $NaHCO_3$ and filtered through celite. The aqueous layer was extracted with additional CH_2Cl_2 (2x25 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to give 6.0 g of yellow oil. Purification by flash chromatography (30% EtOAc/hexane) gave 1.1 g (40%) 9a as a white solid, mp 87-90°C (toluene); IR (KBr) 3382, 1752, 1716 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.01 (2H, d, J=8), 7.64 (1H, t, J=8), 7.48 (2H, t, J=8), 7.34 - 7.23 (5H, m), 5.58 (1H, d, J=9), 5.35 (1H, ddd, J=14, 9, 9), 5.17 (2H, s), 3.74 (3H, s); ^{19}F NMR (282 MHz, $CDCl_3$, versus $CFCl_3$) -101.8 (1F, dd, J=289, 9), -107.8 (1F, dd, J=289, 14); ^{13}C NMR (75 MHz, $CDCl_3$) 187.7 (t, J=29), 166.0, 156.6, 134.7, 134.3, 131.5 (t, J=2), 130.1 (t, J=3), 128.8, 128.6, 128.6, 128.4, 115.7 (dd, J=264, 261), 68.2, 56.8 (t, J=25), 53.0; MS (CI/CH_4) m/e 378 (MH^+). Anal. Calcd for $C_{19}H_{17}F_2NO_5$: C, 60.48; H, 4.54; N, 3.72. Found: C, 60.39; H, 4.51; N, 3.74.

Preparation of α-Amino-β,β-difluoro-γ-oxobenzenebutanoic Acid (11). Benzyl ester 9a (2.5 g, 6.62 mmol), trimethylsilyl iodide (4.22 g, 21.08 mmol) and $CHCl_3$ (20 mL) were heated at 40°C for 48 h. The orange reaction was concentrated and diluted with water (2 mL) and MeOH (10 mL). The resulting solid was collected by filtration and washed with ether to give 1.03 g (68 %) of a yellow solid. Recrystallization from MeOH/water provided 11 as an analytically pure crystalline sesquihydrate, mp 175-178°C (dec): IR (KBr) 3435, 3105, 1632, and 1480 cm-1; ^1H NMR (300 MHz, CD_3OD), major form: 7.61 - 7.55 (2H, m), 7.41 - 7.34 (3H, m), 4.77 (1H, dd, J=28, 1); minor form: 7.99 (2H, d, J=8), 7.65 (1H, t, J=8), 7.51 (2H, t, J=8), 4.67 (1H, dd, J=25, 5); ^{19}F NMR (282 MHz, CD_3OD, versus $CFCl_3$) major form: -105.2 (1F, bd, J=260), -119.9 (1F, dd, J=260, 28); minor form: -101.4 (1F, bd, J=267), -116.2 (1F, dd, J=267, 25); ^{19}F NMR (282 MHz, CD_3OD plus D_2O, versus $CFCl_3$); new form: -106.3 (1F, bd, J=258), -119.6 (1F,m dd, J=258, 23); ^{13}C NMR (75 MHz, CD_3OD) major form:[15] 168.0 (bd, J=6), 136.5, 130.5 (bd, J=2), 130.0, 128.6, 118.9 (dd, J=258, 255), 57 (dd, J=30, 18): MS (CI/CH_4) m/e 230 (MH^+). Anal. Calcd for $C_{10}H_9F_2NO_3$ $1.5H_2O$: C, 46.88; H, 4.72; N, 5.47. Found: C, 47.27; H, 4.35; N, 5.57.

Examination of the spectral data for 11 indicated the compound exists as an equilibrium between two forms. The tentative assignment for the major form (ca. 85%) is the cyclic hemiketal 11b.[15] The second component (ca. 15%) observed by ^1H and ^{19}F NMR exhibited chemical shifts in the aromatic region consistent with the keto form (11) (see ^1H NMR of 9a for comparison). The addition of D_2O to the NMR solution led to the observation of a third component by ^{19}F NMR, presumably the hydrated-keto form 11a. Gelb et al.[9d] reported a similar equilibration for a 4-oxo-5,5,5-trifluoromethylpentanoic acid derivative where a cyclic hemiketal form exists in organic solvents and a hydrated keto form exists in the presence of water. Compound 11 is a competitive inhibitor of kynureninase in vitro and this data will be reported elsewhere.[14] In summary, a novel inhibitor of kynureninase was obtained utilizing a new method to prepare difluoroketoamino acid 11. The general synthetic utility of this reaction is under investigation for the preparation of fluorinated ketoamines and amino acids.

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12. (a) Bernstein, Z.; Ben-Ishai, D. Tetrahedron 1977, 33, 881. (b) Benzyl N-methoxycarbonyl-α-chloroglycinate (8b) was prepared in a similar manner as 8a, starting with benzyl glyoxylate monohydrate,[13] and was isolated as a white solid, mp 75-79°C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 3.90 (3H, s), 5.47 (2H, s), 6.40 (1H, br s), 7.65 (5H, s); MS (CI) m/e 222 (M^+-HCl); IR (nujol) 3300, 1740, 1710 cm^{-1}. The full experimental procedure will be provided on request.
13. Prepared from glyoxylic acid $\cdot H_2O$ and benzyl alcohol (toluene, p-TsOH, Dean-Stark trap). For an alternative synthesis, see Jung, M.E.; Schishido, K.; Davis, L.H. J. Org. Chem. 1982, 47, 891.
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15. No signal was observed near 188 ppm in the ^{13}C NMR spectrum indicating the absence of the ketone carbonyl in the major component. The hemiketal carbon signal could not be distinguished from the weak signals observed for the minor component.

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