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ENZYMATIC RESOLUTION OF 2-TRIFLUOROMETHYLALANINE

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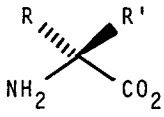
SUMMARY: Hog kidney aminoacylase catalyzes hydrolysis of N-trifluoroacetyl-R(+)2-trifluoromethylalanine with 99.1% enantioselectivity.

Beta-fluorinated amino acids have gained prominence as mechanism-based inhibitors of amino acid decarboxylases and transaminases.[1] Synthetic routes to the optical isomers of these compounds have generally relied on fluorination of an optically active precursor[2] or resolution by chemical means.[3] We report here preparation of the optical isomers of 2-trifluoromethylalanine 1 by partial hydrolysis of the racemic N-trifluoroacetyl



R(+)1: R = CH_3, R' = CF_3

S(-)1: R = CF_3, R' = CH_3

derivative with hog kidney aminoacylase (HKA)(EC 3.5.1.14). Racemic 1 is a powerful irreversible inhibitor of Pseudomonas cepacia 2,2-dialkylglycine decarboxylase (EC 4.1.1.64).[4]

In a typical procedure racemic N-trifluoroacetyl-1[5] (3.58 g, 14.9 mmol) and imidazole (0.608 g, 8.94 mmol) in 60 mL water (adjusted to pH 7.5 with concd ammonia) were treated with HKA (Sigma grade I, 0.72 g) for 67 h at 25°C. After protein removal by heating (pH 4, 100°C, 5 min) and centrifugation, the amino acid was isolated by chromatography on Dowex 50 (H form, water eluant) to give after recrystallization from 2-propanol 0.615 g (+)1 (53%), mp 254.0-254.1°C, $[\alpha]_D$+13.2°, NMR(D_2O) δ1.8(s). Hydrolysis of the remaining amide in 2 M HCl (100°C, 15 h) gave 0.26 g (-)1 (22%) after the same workup, mp 250.2-251.0°C, $[\alpha]_D$-13.2°.

Optical purities of the isolated amino acids were high. HPLC analysis (column: 0.46 x 25 cm C18; eluant: 0.25 mM copper (II) acetate,

0.50 mM L-phenylalanine, pH 4.5, 20% v/v methanol[6]) showed that the (+) and (-) products had optical purities of 98.2% and 97.0%, respectively.[7] Under these chromatographic conditions racemic 1 exhibited two equal area peaks at retention volumes of 19.7 mL (+) and 17.0 mL (-).

The stereochemical preference of hog kidney aminoacylase is to hydrolyze amino acid amides bearing the larger C-2 substituent in the pro-S position.[8] The enzymatic hydrolysis of 1 follows this trend. We have shown in other work that (+)1 obtained from aminoacylase hydrolysis of racemic N-chloroacetyl-1 has the R configuration,[9] in which the larger trifluoromethyl group occupies the pro-S position. Likewise, Bosch et al. found that N-acyl S-isovalines (R = CH_3, R' = CH_3CH_2) are selectively hydrolyzed by the same enzyme.[10]

The ability of the aminoacylase to discriminate between methyls and fluorinated methyls is limited. For example, we found no enantioselectivity in the enzymatic hydrolysis of N-trifluoroacetyl 2-fluoromethylalanine (R = CH_3, R' = CH_2F). Perhaps rotational flexibility in the 2-fluoromethyl group allows the S isomer to evade a steric barrier at the enzyme active site which otherwise prevents hydrolysis of N-acyl S(-)1 and N-acyl R-isovaline. We are continuing to study the scope of enzymatic enantioselective hydrolyses of N-acyl 2-substituted alanines.

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