Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 7, pp. 875-878, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00128-X

Two Syntheses of 2S-[2-^{2}H]-Kynurenine

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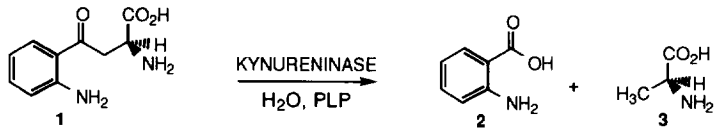
Abstract: The synthesis of 2S-[2-^{2}H]-kynurenine is described using two different routes. Diacetylation/racemisation of racemic kynurenine in deuterium oxide followed by acylase catalysed resolution is the most direct route. The alternative is to prepare 2S-[2-^{2}H]-tryptophan by a similar procedure and then convert this through to 2S-[2-^{2}H]-kynurenine *via* ozonolysis.

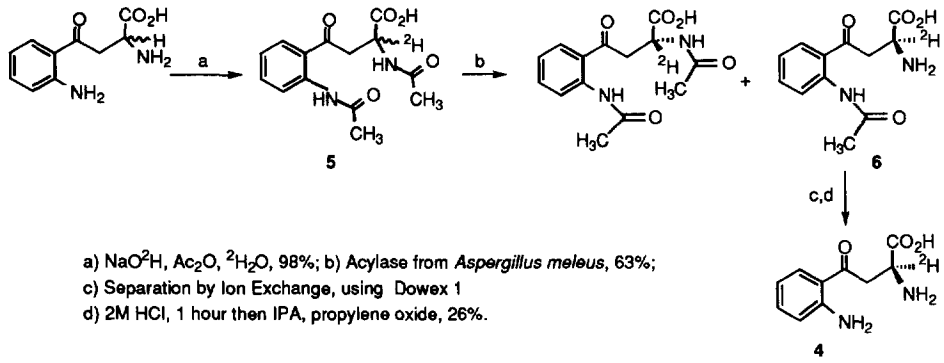
Kynureninase (EC 3.7.1.3) is a pyridoxal 5'-phosphate (PLP) dependent enzyme which catalyses the β,γ-cleavage of kynurenine **1** to give anthranilic acid **2** and L-alanine **3** (Scheme 1)[1]. Kynureninase plays a key regulatory role on the neurologically important tryptophan metabolic pathway[2]. Quinolinic acid, which is a potent neurotoxin, is one of the biosynthetic products of the pathway and has been implicated as an important etiological factor in various neurodegenerative disorders including epilepsy, Huntington's disease and AIDS-related dementia.[3] As part of our mechanistic studies on this enzyme we required 2S-[2-^{2}H]-kynurenine in order to determine the primary deuterium isotope effect.



Scheme 1

The synthesis of 2S-[2-^{2}H]-kynurenine **4** had not been previously reported. Acetylation and racemisation of amino acids in deuterium oxide affords a simple method of introducing deuterium into the C-2 position. This procedure was carried out using commercial R,S-kynurenine (Scheme 2). Under the reaction conditions both of the amino groups were acetylated to give racemic [2-^{2}H]-diacetyl R,S-kynurenine **5**.[4] Resolution was then achieved using the acylase from *Aspergillus meleus*, reported to be more effective with aromatic amino acids than pig liver acylase.[5] This was the first time diacetyl kynurenine had been employed as a substrate for this acylase. The reaction proceeded smoothly and as expected the enzyme showed its normal specificity for the 2S-amino acid.[6] Interestingly it did not hydrolyse the aromatic acetyl group and therefore gave N'-acetyl-2S-[2-^{2}H]-kynurenine **6** as the product in 63% isolated yield. Separation was carried out using ion-exchange (Dowex 1), the acetyl group was removed using 2M HCl and the free amino acid isolated using

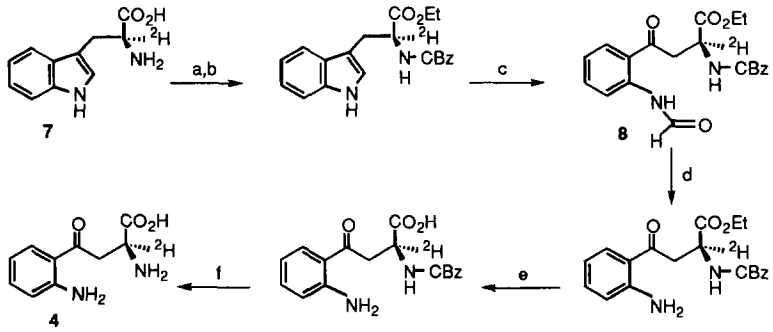
propylene oxide to trap excess HCl.[7] Chiral hplc showed an enantiomeric excess of 98% (see Figure).



Scheme 2

The only disadvantage of this procedure is the high cost of kynurenine, 50% of which is by necessity not used during the reaction. Therefore an alternative was also examined. The most convenient synthetic route to 2S-kynurenine is *via* the ozonolysis of protected 2S-tryptophan, which cleaves the double bond of the pyrrole ring to directly give the N'-formyl kynurenine.[8]

Firstly, 2S-[2-^{2}H]-tryptophan **7** was prepared from 2S-tryptophan using the racemisation/acetylation procedure followed by resolution with acylase as above, in 42% overall yield. This material was also analysed by chiral hplc and shown to have an enantiomeric excess of 99%. The carboxylic acid and amino groups were then protected as the ethyl ester and N-carbobenzyloxy derivatives, respectively, in 60% overall yield. Although the procedure for conversion of this material through to kynurenine has been reported, there are few experimental details[9] and so it was necessary to optimise the reaction conditions. Ozonolysis was performed in methanol solution at -78 °C for one hour to give the protected N'-formyl 2S-[2-^{2}H]-kynurenine **8** in 33% isolated yield. The three protecting groups could then be removed sequentially. The N-formyl group was removed under acid conditions and the ethyl ester hydrolysed using lithium hydroxide in a 1:1 mixture of THF



Scheme 3

and water. Finally the amino group was deprotected by hydrogenation over palladium on charcoal to afford the 2S-[2-^{2}H]-kynurenine **4**. The material was identical in all respects to that obtained from the first method, with an enantiomeric excess of 98%.

Two complementary routes are thus presented for the synthesis of 2S-[2-^{2}H]-kynurenine, both of which give material of comparable purity and enantiomeric excess. The most appropriate for a particular application will depend on the availability of kynurenine and whether further elaboration is required. Direct synthesis from racemic kynurenine is the shorter route, giving an overall yield of 8%, but the starting material is expensive. The synthesis of kynurenine from tryptophan is longer but begins from the much cheaper tryptophan. The overall yield is 8% from 2S-[2-^{2}H]-tryptophan, but 4% from 2S-tryptophan. It also offers the additional advantage that it produces a protected compound from which the three protecting groups can be selectively removed. The deuterated kynurenine prepared in this study will be used to measure the primary deuterium isotope effects for the kynureninase catalysed reaction and the results of these studies will be reported at a later date.

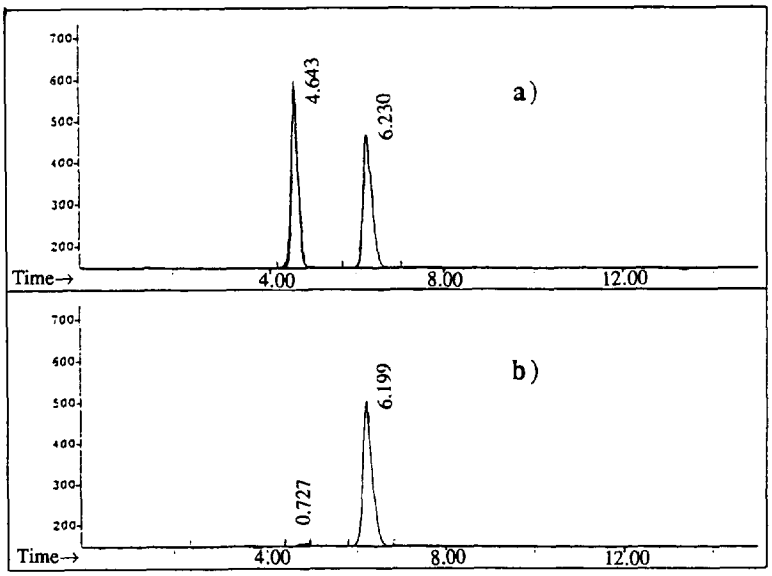


Figure: HPLC analysis of 2S-[2-^{2}H]-kynurenine. Conditions; Crownpak CR(+) (150 mm x 4 mm id, 5 μm) using 2.5 % methanol in aq. $HClO_4$ at pH2 (premixed) at 1 ml min^{-1} and 50° C.
a) Commercial R,S-kynurenine (Sigma Chemical Co. Ltd.);
b) 2S-[2-^{2}H]-kynurenine. Ratio of peak areas gives ee at 97.4%.

Acknowledgements: We wish to thank MSD for a CASE studentship (to FCR) and for performing the chiral hplc analyses, the Royal Society of Edinburgh for a Personal Research Fellowship (to NPB), the EPSRC Mass Spectrometry Service at Swansea, and the University of St. Andrews for their generous support.

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7. m.p. 146° C; m/z (Found: $[M + H]^+$, 210.0986. $C_{10}H_{11}N_2O_3{}^2H$ requires 210.0989); $[\alpha]_D{}^{20}$ +25.5° (c 0.005 in H_2O); ν_{max} (nujol)/ cm^{-1} 3320 (OH), 1720 (CO); δ_H (200MHz, ^2H_2O) 3.75 (2H, s, βCH_2), 7.30 (1H, d, H-3'), 7.40 (1H, t, H-5'), 7.62 (1H, t, H-4'), 8.02 (1H, d, H-6'); δ_C(74.76 MHz, ^2H_2O) 36.95 (s, C-3), 47.98 (s, C-2), 117.78 (s, C-3'), 117.89 (s,C-5'), 129.53 (s, C-6'), 133.80 (s, C-4'), 171.07 (s, C-1), 197.73 (s, C-4); m/z (FAB) 210 ($[M+H]^+$, 100%), 232 (35%, $[M+Na^+]$.
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(*Received in Belgium* 21 *November* 1995; *accepted* 1 *March* 1996)