Synthesis of α-[^{14}C]Methyl and α-[^{3}H]Methyl-L-tryptophan

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Summary

The title compounds were synthesized starting from dimethyl (2S,3aR,8aS)-(+)-8-(phenylsulfonyl)hexahydropyrrolo[2,3-b]indole-1,2-dicarboxylate. This compound on treatment with LDA followed by reaction with [^{14}C]methyl iodide or [^{3}H]methyl iodide gave the methyl substituted derivative which on treatment with trifloroacetic acid followed by hydrolysis with alkali gave the desired α-[^{14}C]methyl- or [^{3}H]methyl-L-tryptophan, respectively, in 50-60% radiochemical yield.

Key Words: α-[^{14}C]methyl-L-tryptophan, α-[^{3}H]methyl-L-tryptophan, serotonin, enantiospecific synthesis, autoradiography, positron emission tomography

Introduction

An efficient synthesis of radioactively labelled α-methyl-L-tryptophan is of great interest because of its importance in the in vivo study of the biosynthesis of the neurotransmitter serotonin, using both autoradiography[1] and positron emission tomography[2]. The alteration of the level of brain serotonin and probably its synthesis rate have been implicated in a number of neurological diseases[3] and disorders[4]. We have therefore been interested in developing a reliable and high yield synthesis of radioactively labelled α-methyl-L-tryptophan.

To date several different asymmetric syntheses of α-methyl-L-tryptophan have been reported. Two different approaches have been used. The first approach reported by

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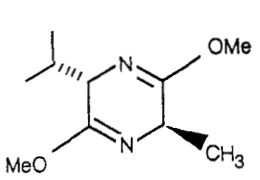

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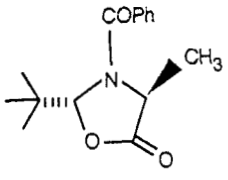

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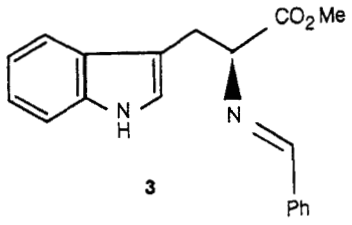

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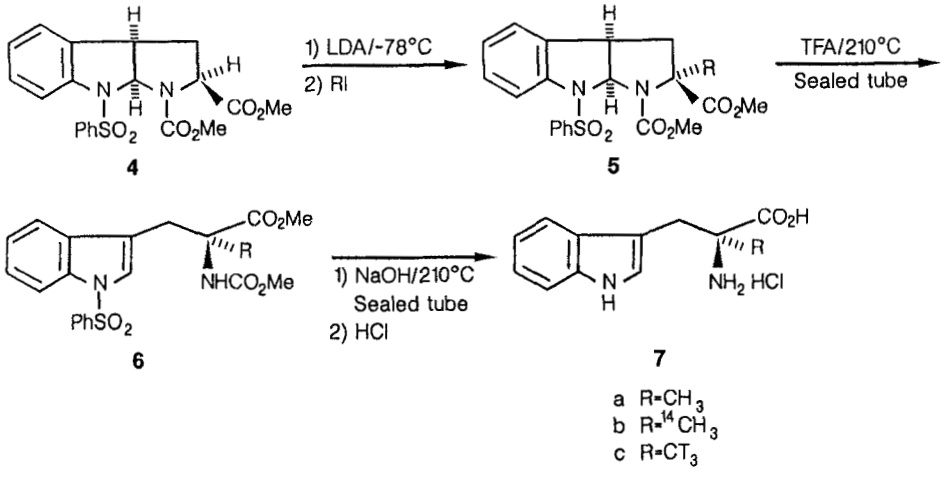
Schöllkopf[5] involved the alkylation of the methylated bislactim **1** with N-protected indolylmethyl bromide. In a similar fashion Seebach[6] also alkylated the methylated oxazolidinone **2** with N-protected indolylmethyl bromide. The second approach which was reported by Brana et al.[7] involved the methylation of enolate of N-benzylidene tryptophan methyl ester **3**. The same method was adopted by Chaly et al.[8] to prepare α-[^{11}C]methyl-L-tryptophan. The approach using N-benzylidene tryptophan methyl ester has been found to have poor reproducibility[9,10].

Recently Crich et al.[11-13] used L-tryptophan as a chiron for enantiospecific methylation to obtain α-methyl-L-tryptophan. In this method N-α-methoxycarbonyl-L-tryptophan methyl ester was cyclised to the hexahydropyrrolo[2,3-b]indole followed by N-protection with phenylsulfonyl to give **4**. Methylation of the enolate of **4** with methyl

Scheme 1



iodide proceeded enantiospecifically. The hexahydropyrrolo[2,3-b]indole was reopened by treatment with trifluoroacetic acid to give the protected α-methyl-L-tryptophan without any racemisation. However, the use of either sodium in liquid ammonia[11,12] or photolysis[14] for desulfonylation makes the method impractical for radiosynthesis. We report here an improvement of the synthesis and extend it to the synthesis of [^{14}C] and [^{3}H] labelled α-methyl-L-tryptophan. This adaptation makes the method the best to date for the synthesis of [^{14}C] and [^{3}H] labelled α-methyl-L-tryptophan. Furthermore, the important starting material which is the hexahydropyrrolo[2,3-b]indole **4** is readily available commercially (Aldrich Chemical) at a reasonable price and has an excellent stability.

Results and Discussion

The reaction of the hexahydropyrrolo[2,3-b]indole **4** with lithium diisopropylamine at -78°C gave the enolate as a distinctive orange-red solution. The enolate was then treated with a solution of [^{14}C]methyl iodide in tetrahydrofuran. After 2 hours the solution turned brownish at which time the reaction was complete as indicated by radio TLC and comparison of the TLC with that of an authentic cold material of **5a** which had earlier been prepared using the same method and had been characterized by ^{1}H-NMR. As has been clearly demonstrated by Crich[15] using single crystal X-ray crystallography, the quenching by methyl iodide occurs stereospecifically in the *exo* face of the enolate of **4**. Treatment of **5a** with trifluoroacetic acid and heating the resulting solution in a sealed tube at 210°C achieved a rapid ring opening of the hexahydropyrrolo[2,3-b]indole to the protected α-methyl-L-tryptophan **6a**.

The final stage was the removal of the protecting groups. Crich et al.[11,12] used a two-step process which involved first the desulfonylation by either reaction with sodium in liquid ammonia[11,12] or by photolysis[14] followed by hydrolysis of the methyl carbamate and the methyl ester with potassium hydroxide. To optimize the yield and to make the synthesis more attractive for the introduction of the radiolabelled methyl group, we instead sought a single step and single pot removal of all the protecting groups. Hibino[16]

and Cribble[17,18] recently reported the removal of the N-phenylsulfonyl group in indole containing heterocycles by using alkaline conditions. Indeed, when **6a** was treated with 5N potassium hydroxide and heated at 210°C in a sealed tube all the protecting groups were cleaved within 15 minutes. Since the product of this synthesis, α-[^{14}C]methyl-L-tryptophan **7b**, must be suitable for the study of serotonin synthesis in vivo[19], the presence of the potassium ion in the final radiopharmaceutical is not recommended. Consequently, it was found that 8N sodium hydroxide is a good replacement, however, it requires 2 hours for removal of the protecting groups.

Finally, the product was purified by reversed phase chromatography by loading the solution of α-methyl-L-tryptophan at pH 5.8 (isoelectric point of L-tryptophan) onto a C18 silica gel column. The phenylsulfonic acid and the inorganic salts were washed out using water. The change of elution solvent to aqueous acid methanol afforded pure labelled α-methyl-L-tryptophan. The radiochemical and stereochemical purity of the product was determined by HPLC using a WH analytical chiral column (see details in Materials and Methods) and a radioactivity detector. As shown in Fig. 1A, only the L-enantiomer (98%) was produced in the synthesis; no D-enantiomer was detected. Further proof of the enantiomeric purity of the nonradioactive α-methyl-L-tryptophan, prepared using the same method, was confirmed by ^{1}H-NMR (95% detection limit) using a chiral lanthanide shift reagent[20]. Liquid scintillation measurement of an aliquot was used to estimate the radiochemical yield (60%) of the synthesis.

Similarly, when the procedure was repeated using [^{3}H]methyl iodide, pure α-[^{3}H]methyl-L-tryptophan **7c** was obtained in 50% radiochemical yield. The syntheses were repeated to evaluate reproducibility of the yields for both α-[^{14}C]methyl- and α-[^{3}H]methyl-L-tryptophan.

The major advantages of the present method are that the starting material is commercially available, it has good stability, and the reactions can be carried out in a single pot thus reducing radiochemical losses due to transfers. We are presently trying to adapt this approach to the synthesis of α-[^{11}C]methyl-L-tryptophan.

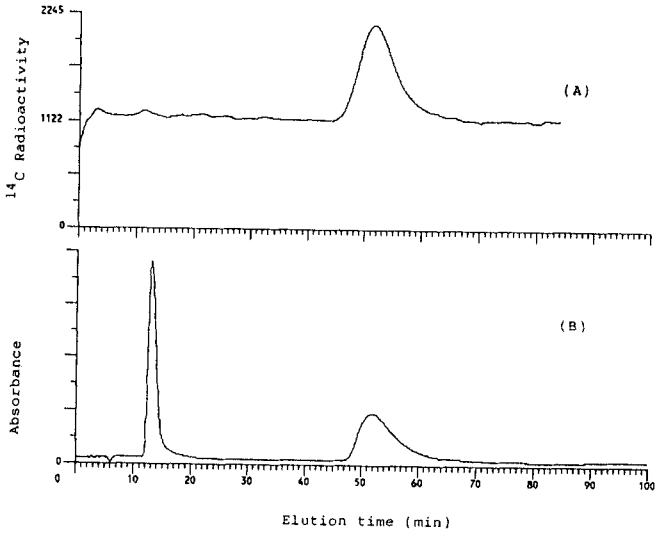


Fig. 1

HPLC runs of (A) α-[^{14}C]methyl-L-tryptophan and (B) α-methyl-D,L-tryptophan

Experimental

Materials and Method

[^{14}C]Methyl iodide, [1.85 GBq(50 mCi); 2.15 GBq/mmol (58.0 mCi/mmol)] and [^{3}H] methyl iodide, [1.85 GBq (50 mCi); 3.15 TBq/mmol (85.0 Ci/mmol)] were obtained from Amersham International. Dimethyl 8-(phenylsulfonyl)-(2S, 3aR, 8aS)-(+)-hexahydropyrrolo[2,3-b]indole-1,2-dicarboxylate, n-butyllithium(2.5M),diisopropylamine, anhydrous tetrahydrofuran, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium (III) derivative and trifloroacetic acid were obtained from Aldrich Chemical Company. α-Methyl-D,L-tryptophan and α-methyl-D,L-tryptophan methyl ester hydrochloride were from the Sigma Chemical Company. α-Methyl-L-tryptophan was obtained from Bis Chem Inc., Montreal, Canada. Reversed phase (C18) Octadecyl silica gel (40μ) was purchased from the American Chemical Company. Diisopropylamine was freshly distilled over CaH_2 under argon just before use. HPLC analysis was conducted using Bio-Rad model 1350 HPLC pump, multiple wavelength detector and recorder. ^{14}C and ^{3}H Radiochemical purity of the

final product was measured by Radiomatic FLO-ONE/Beta A-500 series multichannel radioactive detector interfaced with Bio-Rad HPLC system. Yttrium silicate cell (500μL) was used as detector. Radio TLC's were done using Packard model 7220/21 radiochromatogram scanner. Liquid scintillation measurements were performed on a LKB-1219 instrument using Scintanalyzed Universal cocktail (Fisher Scientific). Enantiomeric purity of the final product after synthesis was determined using a Daicel Chiralpak WH analytical column (24 x 0.4cm) and 5.0mM $CuSO_4$:MeOH (90:10) (1.5ml/min) as eluent, and UV detector(Bio-Rad) set at 254nm and the temperature of the column maintained at 49°C. TLC's were carried out on Analtech Uniplate TLC plates (Cat.No.47521) using 1:1 mixture of EtOAc:Hexane for the ester and 2:1:0.5 mixture of CH_2Cl_2:MeOH:NH_4OH for final α-methyl tryptophan hydrochloride respectively. In the case of nonradioactive synthesis the products were also analysed using 400MHZ Varian NMR instrument using CD_3OD/DCl as solvent. The temperature during synthesis was maintained by a Flexicool cooling probe immersed in acetone solvent.

Synthesis of dimethyl (2S, 3aR, 8aS)-2-[^{14}C]methyl-8-(phenylsulfonyl)hexahydro-pyrrolo[2,3-b]indole-1,2-dicarboxylate 5b.

To a two-neck 50mL ground jointed (19/24) flask with septum under argon was added using a syringe anhydrous THF(5mL) followed by freshly distilled diisopropylamine (0.18mL, 1.2 mmol) and the contents were stirred and cooled to -78°C in an acetone bath into which a cooling probe was immersed. n-BuLi(0.5mL, 1.2 mmol) was added and the contents stirred for 30 minutes to form lithium diisopropylamide. Dimethyl 8-(phenyl-sulfonyl)(2S, 3aR, 8aS)-(+)-hexahydropyrrolo[2,3-b]indole-1,2-dicarboxylate **4** (525mg, 1.25mmol) dissolved prior in anhydrous THF (10mL) was added slowly through the septum using a syringe. An orange-red coloured anion was formed after the addition of the compound. The mixture was allowed to stir for additional 30 min at that temperature when a deep reddish colour developed.

The $^{14}CH_3I$ ampoule was taken inside an argon glove bag and cooled with liquid nitrogen in a dewar for 30 min to ensure the content was frozen inside. Anhydrous THF

(4-5mL) was then introduced through the septum and using a needle the seal on the ampoule was broken and the THF was sucked into the ampoule dissolving the [^{14}C] methyl iodide. Meanwhile the flask containing the anion was taken out of the cooling bath and cooled once again in liquid nitrogen to obtain a frozen solid. The flask was transferred carefully inside the glove bag and the ampoule containing the methyl iodide was connected to the flask by tilting it at an angle of 45°C. The contents of the ampoule was allowed to drip slowly into the reaction flask. The flask was maintained at -78°C with dry acetone bath for 2h and left to stir over night at room temperature. A deep reddish brown solution resulted after the reaction. A radio TLC of the reaction mixture was taken and compared with that of cold authentic methylated compound and it showed methylation yielding the product **5b**.

Synthesis dimethyl (2S, 3aR, 8aS)-2-methyl-8-(phenylsulfonyl)hexahydro-pyrrolo[2,3-b]indole-1,2-dicarboxylate 7a

The material was prepared using the same procedure as that of the ^{14}C-labelled analogue **5** (see above); R_f 0.38 (hexane-EtoAc, 1:1); ^{1}H-NMR ($CDCl_3$) δ 7.60 (d, 1H, J = 8 Hz, 4-H), 7.44 (m, 2H, Ph), 7.32 (t, J = 6.8 Hz, 1H, 6-H), 7.18 (t, 3H, Ph), 7.01 (t, J = 8 Hz, 1H, 5-H), 6.94 (d, J = 6.8 Hz, 1H, 7-H), 6.24 (d, J = 5.6 Hz, 1H, 8a-H), 3.55 (s, 3H, 1-OMe), 3.36 (br t, J = 8 Hz, 1H, 3a-H), 3.00 (s, 3H, 2-OMe), 2.68 (d, J = 13.6 Hz, 1H, 3-H), 2.15 (dd, J = 8, 13.6 Hz, 1H, 3-H), 1.62 (s, 3H, 2-Me).

Synthesis of Nα-methoxycarbonyl-1-phenylsulfonyl-α-[^{14}C]methyl-L-tryptophan methyl ester 6b.

Compound **5b** was transferred along with THF to a pressure tube and rotary evaporated to yield a sticky brown coloured mass. To this was added trifloroacetic acid (1.5mL) and the pressure tube was sealed and heated in a sand bath maintained at 210°C for 1h resulting in a dark coloured mixture. The tube was cooled to room temperature and the excess trifloroacetic acid was evaporated under reduced pressure to finally yield a

dark brown gummy solid. Radio TLC of the above mass EtOAc:Hexane (1:1) showed evidence of product formation. At this stage it was felt that further characterization of the compound was not essential and this was used in the next step without any purification.

Synthesis of α-[^{14}C]methyl-L-tryptophan 7b.

To compound **6b** in the pressure tube was added either 5N KOH (4mL) or 8N NaOH (4mL) and the pressure tube was again sealed and heated in a sand bath at 210°C for 1-2h when the sticky mass completely dissolved and formed a brown solution. The tube was allowed to cool to room temperature and the solvent removed by rotary evaporation resulting in a light brown solid. Water (1-2 mL) was added to the solid residue and acidified using 6N HCl or 10N HCl (depending on whether KOH or NaOH was used for hydrolysis) when a light yellowish orange solution resulted. Radio TLC as well as radio HPLC clearly showed formation of the α-[^{14}C]methyl-L-tryptophan **7b** with complete stereospecificity (Fig. 1A). The yield of the product was found to be 60% based on the final radioactivity determined by liquid scintillation method.

Removal of K^+ ion and $PhSO_3H$

The above hydrochloride solution of the α-[^{14}C]methyl-L-tryptophan was adjusted to a pH of 5.89 (isoelectric point for α-methyl-tryptophan) using a pH Stat. This was loaded onto a C18 silica gel column (4 x 25 cm) and washed with water when both K^+ ion and $PhSO_3H$ were removed leaving the amino acid on the column which was later eluted with 0.25 N HCl in 90% aqueous methanol.

Synthesis of α-[^{3}H]methyl-L-tryptophan 7c

This compound was prepared using an identical procedure described for the preparation of α-[^{14}C]methyl-L-tryptophan. The yield was 50% in two sets of experiments. No direct specific activity measurements of the products were done. The specific activities of the products were assumed to be the same as the specific activities of

[^{14}C]methyl iodide (58 mCi/mmol) and [^{3}H]methyl iodide (85 Ci/mmol). This assumption seems to us to be reasonable since there was no other source of CH_3I present in the reaction vessels.

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