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Preparation of an Electrophilic 3-Methylindole Derivative: Difficulties in Forming a Stable, Suitable Material for the Preparation of Tryptophan

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Abstract

In an attempt to prepare stereoselectively beta-deuterated tryptophan, N-protected indole-3-methanol compounds were prepared with model studies being done on undeuterated material. Conversion of these compounds to electrophilic species proved exceptionally difficult and resulted in very low yields or recovered starting material only. A summary of the current results utilizing N-tosyl indole-3-methanol will be presented as well as efforts using N-Boc indole-3-methanol.

Introduction

In recent years, nuclear magnetic resonance (NMR) spectroscopy has become an important tool in the determination of the three-dimensional structure of proteins in solution (Wuthrich, 1989), complementing X-ray diffraction structures obtained in the solid state. The advent of 2-D and higher-dimensional NMR techniques (Bax, 1989; Clore and Gronenborn, 1991) have permitted this approach to develop, providing solution phase structures of moderate size (ca. 30 kDa) proteins. Of particular utility in NMR-based structure solutions are methods which utilize selective (Otting and Wuthrich, 1990) or general (Fesik and Zuiderweg, 1990; Hansen et al. 1992) stable isotope labeling of the protein amino acids.

The amino acids which we were trying to prepare were leucine and tryptophan as shown in Fig. 1. Both of these amino acids as well as other amino acids bearing a beta methylene group have a pair of diastereotopic hydrogens at the beta position. Assignment of the two prochiral hydrogens is simplified by the stereospecific introduction of deuterium at either the pro-R or pro-S position while leaving the remaining hydrogen unchanged. Conformational analysis using coupling constants and the Karplus relationship to predict dihedral angles or using nuclear Overhauser effect (NOE) experiments to determine through-space proximity of atoms is facilitated by stereospecific deuteration of the diastereotopic prochiral hydrogens. Previous synthetic work toward the preparation of isotopically labeled amino acids involved the preparation of chiral deuterated glycine containing a 15-N label (Curley et al., 1994). Additional synthetic studies had utilized the alkylation of an enolate derived from ethyl hippurate (prepared as the 15-N and 13-C labeled compound from labeled 13-C ethyl bromoacetate and 15-N labeled potassium phthalimide followed by deprotection to afford uniformly 13-C, 15-N labeled glycine then reprotection) to prepare uniformly labeled cysteine (Fig. 2; Panigot et al., 1995). One advantage of this approach is that any of the carboxyl carbon, the alpha carbon, the beta carbon, and the nitrogen can be selectively labeled or not

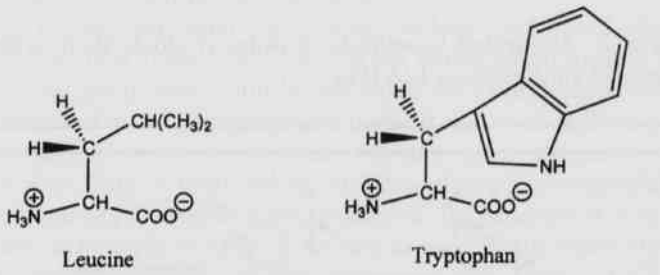

Fig. 1. Synthetic targets leucine and tryptophan. Note the prochiral hydrogens shown on the beta carbon using wedge-dashnotation.

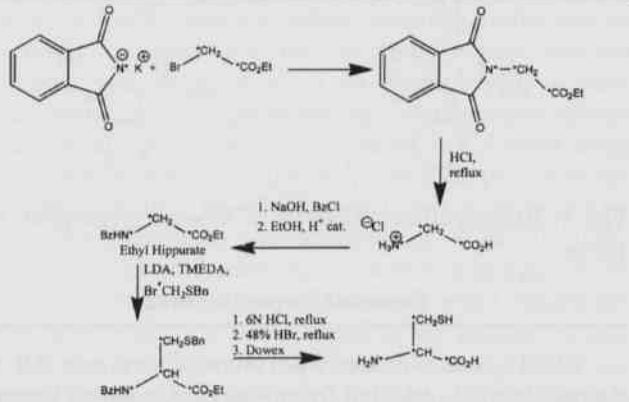

Fig. 2. Synethesis of labeled cysteine by alkylation of ethyl hippurate

Journal of the Arkansas Academy of Science, Vol. 54 [2000], Art. 7

labeled depending on the choice of starting materials. In trying this approach with leucine, efforts to prepare the known deuterated aldehyde (Piart-Goypiron et al., 1991) failed to provide usable material due to solvent impurities present and/or unexpected overoxidation of the dideuterated isobutyl alcohol to isobutyric acid instead of isobutyraldehyde-d (Fig. 3). We then focused our efforts on the preparation of tryptophan. The disconnection of tryptophan (Fig. 4) shows that methyl indole-3-carboxylate would be a reasonable starting material.

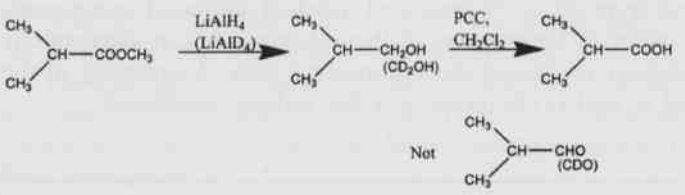

Fig. 3. Attempted synethesis of isobutyr(aldehyde-d) with model studies using $LiA1H_4$.

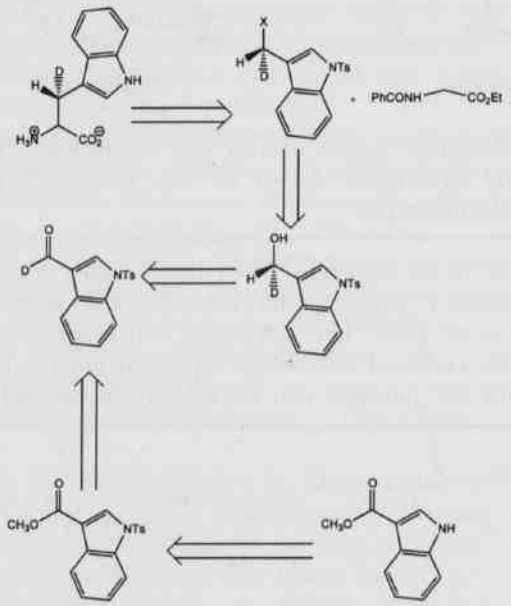

Fig. 4. Retrosynthetic analysis of labeled tryptophan synthesis.

General Experimental

$LiAlD_4$ was obtained from Acros Chemicals. All other chemicals were obtained from Aldrich Chemical Company and used as received. Solvents were used as received. Anhydrous ether was obtained from Fisher Chemical Company and used as received. Reactions using moisture-sensitive reagents were run in oven-dried glassware under nitrogen. NMR spectra were obtained on a Hitachi R-1200 60 MHz NMR using $CDCl_3$ as solvent and TMS as internal standard. Chemical shift values are given in parts per million (δ) downfield from TMS. FT-IR spectra were obtained on a Nicolet 5PC FT-IR spectrophotometer with samples deposited as films evaporated from $CHCl_3$ on NaCl plates. Column chromatography was performed using Merck 7734-4 silica gel.

N-Tosyl Methyl Indole-3-carboxylate **(1)** was prepared in a manner analogous to that used for other N-tosylations of indoles and imidazoles (Greene and Wuts, 1999).

N-Tosyl Indole-3-Methanol **(2):** A suspension of 11.04 g (33.5 mmol) of **1** was suspended in 100 mL of anhydrous ether and cooled to 0 °C. After 10 min. at 0 °C, $LiAlH_4$ (1.28 g, 33.5 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched by the addition of 1.28 mL H_2O, 1.28 mL of 15% NaOH solution, and another 3.84 mL of water. The mixture was filtered, dried ($MgSO_4$), and concentrated to yield 7.10 g (70%) of the hydroxymethyl indole. NMR 7.0 - 8.0 (m, 9H), 4.2 (s, 2H), 2.7 (s, 3H). IR 3300 cm^{-1} (br s).

N-Tosyl Indole-3-Carbaldehyde **(3):** To a solution of 3.03 g (10 mmol) of **2** in 50 mL of CH_2Cl_2 which was cooled to 0 °C was added 3.23g (15 mmol) PCC. The mixture was allowed to warm to room temperature and stir overnight. It was filtered through Celite, concentrated, and chromatographed (20% ethyl acetate / hexane) to yield aldehyde **3**. NMR 9.5 (s, 1H), 7.0 - 8.0 (m, 9H), 2.7 (s, 3H). IR 2720 cm^{-1}, 1705 cm^{-1}.

Sample Attempt to Prepare N-tosyl Indole-3-Methanol Tosylate: A solution of 0.301 g of **2** and tosyl chloride (0.381 g) in pyridine (5 mL) was allowed to stand at 4 °C overnight. On extraction into ether, the only organic material present was unreacted **2**. Other variations (room temperature, shorter reaction times at low temperature) also led to recovered starting material.

N-Tosyl 3-Chloromethylindole **(4):** Compound **2** (0.30 g, 1 mmol) and triphenylphosphine (0.30 g, 1.14 mmol) were combined in 25 mL CCl_4 and allowed to stir for 3 days. The mixture was concentrated and chromatographed using 20% ethyl acetate / hexane as eluant yielding 0.04 g (12%) of **4**. NMR 7.0 - 8.0 (m, 9H), 4.3 (s, 2H), 2.7 (s, 3H). IR showed no absorbance at 3300 cm^{-1}.

N-Tosyl 3-Bromomethylindole **(5):** A mixture of **2** (0.10 g, 0.33 mmol), triphenylphosphine (0.105 g, 0.4 mmol), and carbon tetrabromide (0.133g, 0.4 mmol) was allowed to stir in 5 mL DMF for 4 days. The mixture was poured into 50 mL water and extracted with 50 mL ether. The ether extract was dried ($MgSO_4$), concentrated, and chromatographed (20% ethyl acetate / hexane) to yield 0.054g

(45%) of **5**. NMR 7.0 - 8.0 (m, 9H), 4.2 (s, 2H), 2.7 (s, 3H). IR showed no absorbance at 3300 cm^{-1}.

Results and Discussion

The planned synthesis of the O-tosylate of indole-3-methanol, to be alkylated with ethyl hippurate dianion to provide tryptophan after protecting group removal, is outlined in Fig. 5. The initial attempt at the preparation of indole-3-methanol from methyl indole-3-carboxylate by $LiAlH_4$ reduction gave rise to a material which was less polar than the starting material using thin- layer chromatography (TLC). In reviewing the known reactivity of 3-substituted indoles with $LiAlH_4$ (Silverstein et al., 1954; Leete and Marion, 1953) it was found that $LiAlH_4$ reacted with indole aldehydes and esters to provide 3-methylindole (Fig. 6). Reaction of $NaBH_4$ with indole-3- carbaldehyde would provide indole-3-methanol, however, this approach was unsatisfactory for the preparation of the dideuterio alcohol required. Additionally, as expected, $NaBH_4$ failed to reduce the methyl ester. Thus, an alternative synthetic approach was required.

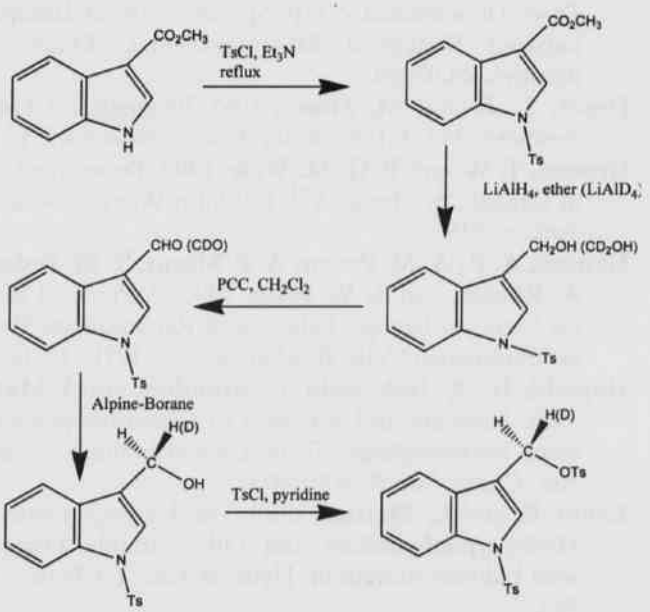

Fig. 5. Planned synthesis of indole-3-methanol tosylate

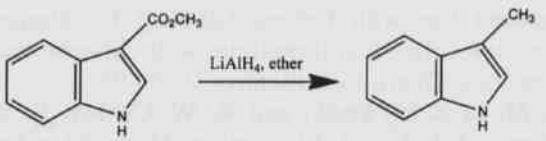

Fig. 6. Reaction of methyl indole-3-carboxcylate to give 3-methylindole instead of the desired indole-3-methanol

In considering the mechanism of formation of 3-methylindole by $LiAlH_4$ reduction, one possible approach to avoiding elimination would be to decrease the electron density of the indole nitrogen. One possible means of doing this would be to make a sulfonamide derivative of the indole ester. N-Tosyl methyl indole-3-carboxylate was prepared by reacting the indole ester with tosyl chloride in triethylamine as outlined for the corresponding imidazole (Greene and Wuts, 1999). The sulfonamide derivative was chosen due both to its electron withdrawing ability and its stability toward $LiAlH_4$ reduction. The compound was successfully reduced with $LiAlH_4$ to N-tosyl indole-3-methanol and oxidized to the corresponding aldehyde using PCC to demonstrate the viability of the methodology before incorporation of the deuterium label using $LiAlD_4$. Having demonstrated the viability of this synthetic approach, the compound was then reduced with $LiAlD_4$ and oxidized to provide the deuterated aldehyde. The deuterated aldehyde was reduced using Alpine-Borane under standard conditions to provide a chiral deuterated primary alcohol.

Having prepared the N-tosyl indole-3-methanol, the focus shifted to the conversion of the hydroxyl group into a leaving group that could be alkylated with ethyl hippurate dianion. The initially proposed method was to directly convert the alcohol to the tosylate. All attempts to prepare the tosylate from N-tosyl indole-3-methanol were unsuccessful and starting materials were recovered. Preparation of tosylates of benzylic systems is known to be difficult under the standard tosylation conditions (Fieser and Fieser, 1967) using pyridine and tosyl chloride at 0 ^{o}C overnight (Tipson, 1944). It is believed this may also be a problem in this case due to the benzylic character of the hydroxymethyl group. Additionally, efforts to prepare other sulfonates were also unsuccessful. The reaction with methanesulfonyl chloride led to recovered starting material. Attempted triflate preparation using trifluoromethanesulfonic anhydride led to undetermined decomposition products.

The preparation of halides from alcohols as shown in Fig. 7 became the next target. Fearing reaction or decomposition of the highly sensitive indole structure under acid or base conditions, halide preparations under neutral reaction conditions, such as reaction of an alcohol with a tetrahalomethane in the presence of triphenylphosphine (Hayashi et al., 1973) were the principal aim. Efforts using carbon tetrachloride and triphenylphosphine provided the chloromethyl indole. However, yields after purification by column chromatography were usually quite poor (10 - 15%). The chloromethylated compound failed to undergo alkylation on treatment with the dianion of ethyl hippurate. Similar difficulties were encountered in the attempt to prepare cysteine by alkylation of an alkyl chloride; however, the more reactive alkyl bromide could be alkylated in that instance. As a more reactive halide might be more reactive toward alkylation in this instance as well, an attempt to pre-

pare the bromomethyl indole was undertaken. Reaction of indole-3-methanol with carbon tetrabromide with triphenylphosphine again provided the desired halide in poor yield after column chromatography. Attempted alkylation of ethyl hippurate dianion with the bromide failed in this instance.

At this point efforts using an alternative protecting group on nitrogen were begun to determine whether the sulfonamide group played a role in the poor yields and low reactivity. One of the few other protecting groups capable both of electron withdrawal and stability toward $LiAlH_4$ is the tert-butoxycarbonyl (Boc) group. Research currently underway involves the preparation of N-Boc methyl indole-3-carboxylate and reduction to N-Boc indole-3-methanol with $LiAlH_4$ (Fig. 8).

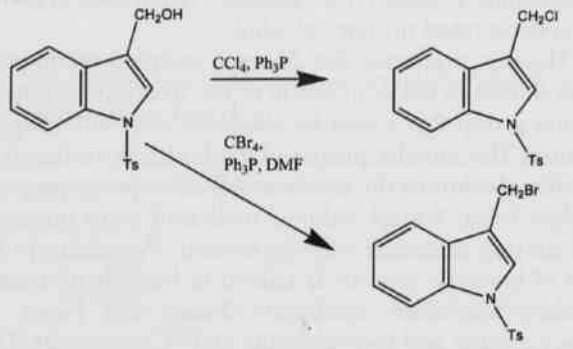

Fig. 7. Synthesis of 3-chloromethyl- and 3-bromomethyl N-tosyl indole

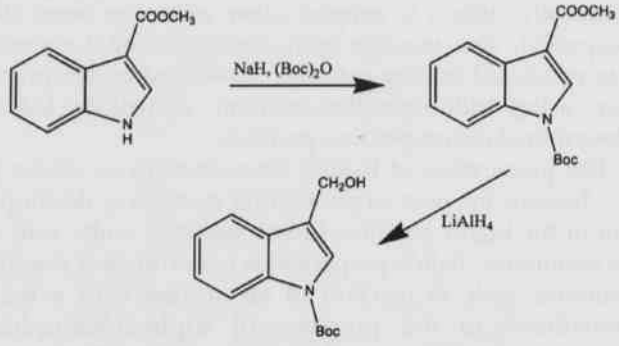

Fig. 8. Current effprts utilizing N-Boc protected methyl indole-3-carboxylate

Conclusions

The preparation of leucine via this route was unsuccessful due to problems with purification and oxidation. Preparation of a suitable electrophilic 3-methylindole derivative proved to be elusive. In those cases where small amounts of electrophile were prepared, alkylation of ethyl hippurate dianion failed to occur. Alternate approaches to the synthesis of labeled tryptophan and preparation of labeled histidine will be investigated.

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