Nature of Invention: Chemical molecule and synthesis route

Applicant: ChemiEvolve Industries

Inventors: Ansh Sethi, Raunak Jalan

Chemical Formula: $C_6H_9N_3O_2$

Chemical Name: 2'-13C-L-Histidine

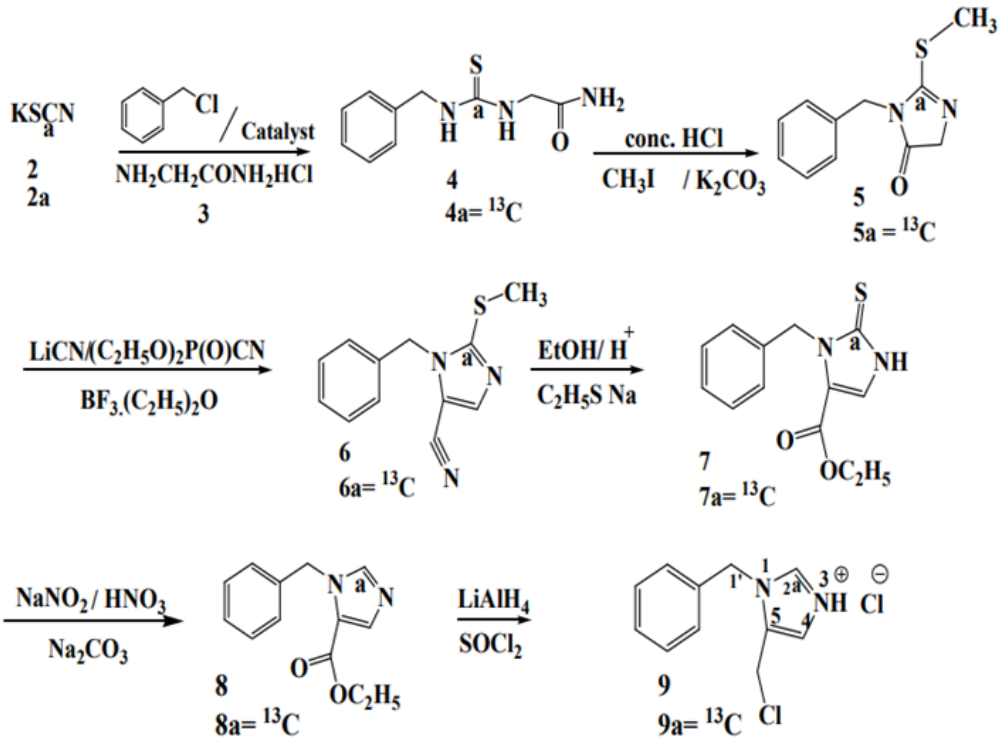
Chemical synthesis routes:

RAW MATERIALS

- Synthesis of Benzyl Thiourea (4)
  - Potassium thiocyanate
  - Benzyl chloride
  - O- dichlorobenzene
  - Glycine amide HCl
- Cyclization of Benzyl Thiourea (4)
  - Acetone
  - HCl (aq.)
- Conversion to 1-Benzyl-2-(Methylthio)-Imidazol-5-Ketone (5)
  - Methyl iodide
  - Potassium Iodide
- Conversion to Aromatic Nitrile (6)
  - Diethyl phosphorocyanidate (DEPC)
  - Lithium cyanide in THF (Tetrahydrofuran)
  - Boron trifluoride etherate ($BF_3O(C_2H_5)_2$)
  - Benzene
  - Concentrated Sulphuric Acid (H_2SO_4)
- Conversion to Intermediate Compound (7)
  - Ethanol
  - Sodium ethyl thiolate
  - DMF (Dimethyl formamide)
  - Palladium (Pd)

LAB-SCALE SYNTHESIS OF 2'-13C-L-HISTIDINE

REACTION STEPS (PRODUCT YIELD): -



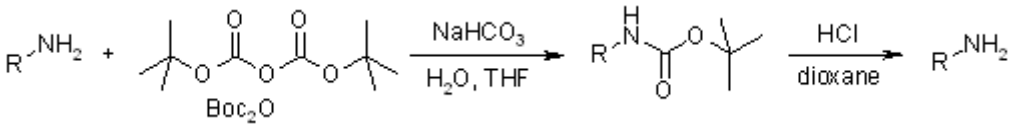
- Synthesis of Benzyl Thiourea (4) :
  - Potassium thiocyanate (2) was reacted with benzyl chloride in o-dichlorobenzene using bis(triphenylphosphoranylidene)ammonium chloride as a catalyst.
  - A mixture containing benzyl isothiocyanate and some benzyl thiocyanate was formed.
  - The mixture was then treated with glycine amide HCl at pH 8.5.
  - The product was recrystallized from acetone to obtain benzyl thiourea (4).
- Cyclization of Benzyl Thiourea (4):
  - Compound 4 was dissolved in acetone and aqueous HCl.
  - It was then subjected to extraction to obtain 1-benzyl-2-thioxoimidazol-4-one.
- Conversion to 1-Benzyl-2-(Methylthio)-Imidazol-5-Ketone (5):
  - 1-benzyl-2-thioxoimidazol-4-one was treated with methyl iodide and potassium carbonate, resulting in the formation of 1-benzyl-2-(methylthio)imidazol-5-ketone (5).
- Conversion to Aromatic Nitrile (6):
  - Compound 5 was treated with diethyl phosphorocyanidate (DEPC) and LiCN in THF.
  - The resulting mixture was then subjected to extraction to obtain the cyanohydrin phosphate corresponding to 5.
  - Next, the cyanohydrin phosphate was treated with boron trifluoride etherate in benzene to eliminate diethyl phosphate, yielding aromatic nitrile 6.
  - Finally, aromatic nitrile 6 was refluxed in ethanol with concentrated sulfuric acid to obtain ethyl-1-benzyl-2-ethylthio-5-imidazole carboxylate.
- Conversion to Intermediate Compound (7):

- Compound 6 was treated with acidified ethanol to convert the nitrile function into the ethyl carboxyl function, resulting in the formation of ethyl-1-benzyl-2-ethylthio-5-imidazole carboxylate.
- Subsequently, ethyl-1-benzyl-2-ethylthio-5-imidazole carboxylate was treated with sodium ethyl thiolate in DMF, leading to the formation of ethyl-1-benzyl-2,3-dihydro-2-thioxo-5-imidazole-carboxylate (7).

- Conversion to 1-Benzyl-5-Chloromethylimidazolium Chloride (9):
  - Compound 7, according to Scheme 2, was treated with tert-butyl (N-diphenylmethylene) glycinate (10) under O'Donnell conditions.
  - This reaction resulted in the formation of the protected histidine derivative 11.

SEPARATION STEPS (FINAL PURITY)

- Cleavage and Deprotection to Obtain L-Histidine (1):
  - To carefully remove the protecting groups from the protected histidine derivative 11 without altering the structure of L-histidine, especially its imidazole ring, the following steps were taken:
  - The tert-butyl ester protecting group was removed by acid catalysis using acids such as trifluoroacetic acid (TFA). TFA cleaved the ester bond, releasing the carboxylic acid group of histidine. The reaction was carried out under controlled conditions to avoid any undesired alteration to the imidazole ring.
  - Subsequently, the amino protecting group was removed through hydrogenolysis. Palladium on activated charcoal served as a catalyst for this reaction. Hydrogen gas reacted with the protected amino group, effectively removing the protecting group and restoring the free amino group of L-histidine. This process was carried out carefully to ensure the imidazole ring remained intact.
  - By following these steps, L-histidine (1) was obtained with high chemical and optical purity, preserving the crucial structure of its imidazole ring, which is essential for its biological activity.



References:

https://www.researchgate.net/publication/229484314_Synthesis_of_Lhistidine_specifically_labelled_with_stable_isotopes

Cleavage, Deprotection, and Isolation of Peptides after Fmoc Synthesis (thermofisher.com)

Boc-Protected Amino Groups (organic-chemistry.org)

List the contributions of each author:

- **ANSH SETHI** carried out the literature search and found the reaction steps, and **product yield**.
- **RAUNAK JALAN** and **ANSH SETHI** found necessary separation steps to achieve desired product purity.

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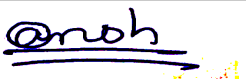
Ansh Sethi	220167	
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