Synthesis of *N*-acetyl-tryptophan glucoside

A dissertation

Submitted for the partial fulfilment of the Degree

of

MASTERS OF SCIENCE (CHEMISTRY)

Submitted by

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PATIALA-147004

DECLARATION

I, hereby declare that the dissertation entitled **"Synthesis of *N*-acetyl-tryptophan glucoside"** being submitted in the partial fulfilment of the requirements for the award of degree **of Master of Science in Chemistry** to **School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala** is a record of my own work carried out under the supervision of **Dr. Amjad Ali**, and **Dr. Kamaldeep Paul** from Jan-July2023. Further, any work of this dissertation has not been submitted to any other University for the award of any other degree or diploma.

Vidhi Tyagi

Vidhi Tyagi

Date: 15/07/2023

Place: Patiala

CERTIFICATE

This is to certify that the dissertation entitled **"Synthesis of *N*-acetyl-tryptophan glucoside"** being submitted by **Vidhi Tyagi** to **School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Chemistry** is an authentic record of the work carried out by the candidate under our guidance and supervision. She has fulfilled the requirements for the submission of this dissertation, which to our knowledge has reached the requisite standard.

The results embodied in the dissertation have not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

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ABSTRACT

Tryptophan derivatives, whether produced in our body or chemically, both are equally resourceful. One such example of a derivative is *N*-acetyl tryptophan glucoside which has been investigated for its potential to protect macrophages against gamma-ray irradiation. Previously, the compound was obtained through isolation from bacteria. However, due to the limited quantity obtained, we now attempted to synthesize it chemically. The reaction to synthesize *N*-acetyl-L-tryptophan comes under the category of condensation reaction where water is eliminated and a single product is formed from two different molecules. It is tough to synthesize the *N*-glucoside as *glycol*-tetrahydro-β-carbolines and *C*-glycosyl conjugates always get synthesized at elevated temperatures. Numerous attempts have been made to synthesize the glucoside under various conditions in this thesis starting from L-tryptophan as a reactant.

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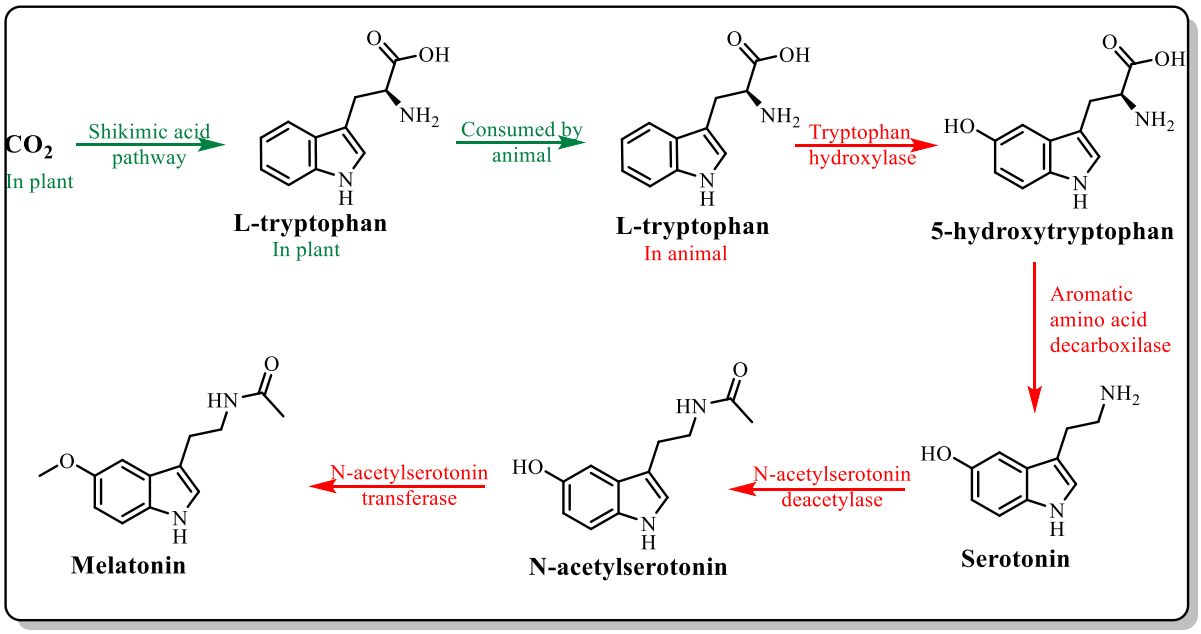
INTRODUCTION

One of the essential amino acid tryptophan has many biological properties whether it is used by the liver to produce vitamin B3 or to produce melatonin and serotonin by the pineal gland. Tryptophan-5-hydroxylase and 5-hydroxytryptamine decarboxylase enzymatically convert this amino acid to 5-hydroxytryptamine, also known as serotonin, and then melatonin is enzymatically produced by serotonin *N*-acetyltransferase and hydroxy indole-O-methyltransferase from serotonin, which is then secreted by the pineal gland (**scheme 1**).[1] In total four enzymes are used in conversion of the L-tryptophan to serotonin and lastly to melatonin.[2] These neurotransmitters regulate our sleep cycle and mood. Our body should maintain the minimum level of tryptophan; as it is not produced inside, we must consume it orally. In some countries, it is sold as a prescription drug for insomnia, depression, and mood disorder, but as there can be side effects of direct oral intake, it is advised to consume it through our diet. Dairy products, turkey, oat, tuna, and banana are good L-tryptophan sources.[3]

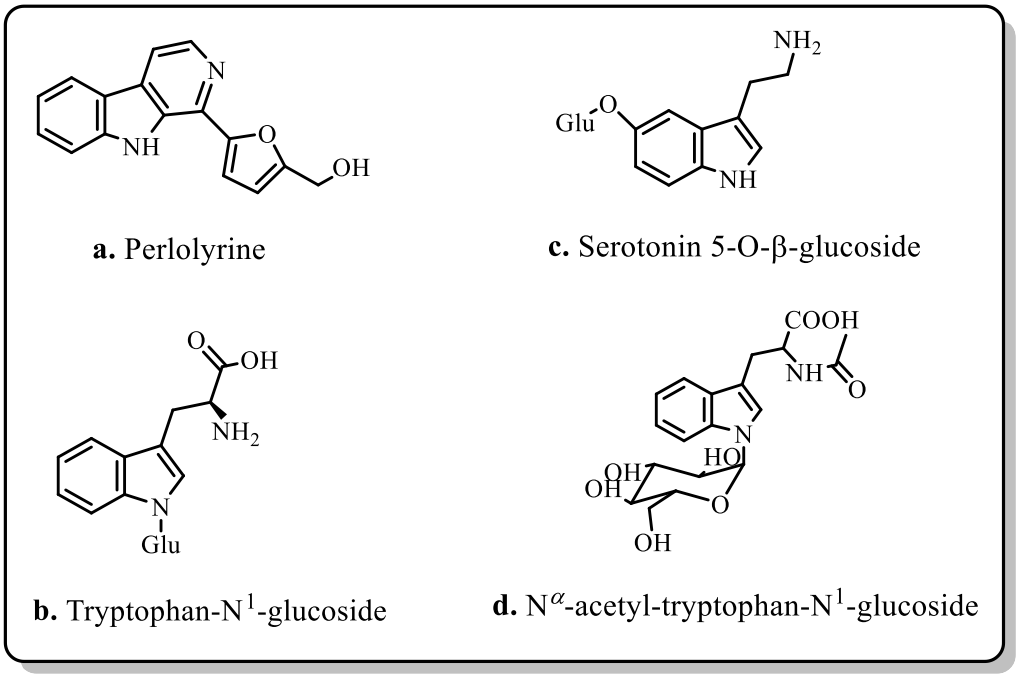


Scheme 1. Pathway used to synthesize serotonin and melatonin

Tryptophan derivatives are equally crucial for the development of new drugs. These compounds show many biological activities, antiviral activities against viruses like TMV and broad-spectrum fungicidal activities.[4] L-tryptophan reacts with aromatic aldehydes to give rise to tetrahydro-β-carboline and β-carboline alkaloids. These have been found in several food items, fruit-based products, and drinks, whether it is alcoholic or non-alcoholic. These alkaloids get produced during processing under mild conditions. Some of these β-carboline show good

antioxidant properties.[5] Even one of the tetrahydro-β-carboline i.e., tetrahydropentoxyline was found in human urine.[6]

When discussing tryptophan derivatives, tryptophan Maillard reaction products show various properties. Millard reaction mainly occurs between sugar and amino acid and can be seen during cooking and is responsible for the browning of food when cooked. This non-enzymatic reaction gives rise to products that show antioxidation, antiproliferation, and anti-inflammatory[7] activities. One such example is when fructose and tryptophan are heated at 130 °C for 2 hrs. it gives rise to perlolyrine[8] (**figure 1a**). The glycosylated form of tryptophan is also found in nature in many forms like tryptophan-N^1-glucoside (**figure 1b**) and serotonin 5-O-β-glucoside (**figure 1c**) which has been found in Robusta coffee species in a high level, while in Arabica coffee species, is present lower in level.[9] It is also found in many fruit and fruit juices.[10,11] Several ways have been tried to synthesize tryptophan derivatives like glycoconjugates of tryptophan in the past, whether it is C-derivative or N-derivative. Tryptophan-C-glycosyl was also detected in human urine.[12] One such tryptophan derivative is used as a gamma protector like N^{α}-acetyl-tryptophan-N^1-glucoside (**figure 1d**).



a. Perlolyrine

c. Serotonin 5-O-β-glucoside

b. Tryptophan-N^1-glucoside

d. N^{α}-acetyl-tryptophan-N^1-glucoside

Figure 1. Tryptophan derivatives

Gamma rays have a very short wavelength, making them penetrate the human body. Prolonged contact can kill cells, can cause mutation, or even can cause cancer. Although radiation plays a significant role in the treatment of cancer, but it can also cause damage to DNA and normal tissue that depress the immune system and cause inflammation.[13] Macrophages live in the body's tissues and play a role in both health and disease.[14] To achieve whole-body radioprotection, macrophages must be protected since, when exposed to radiation, they produce cytokines that deactivate the macrophages and may cause tissue damage and inflammation.[15,16] It has been found that the novel radioprotective compound *N*-acetyl tryptophan glucoside (NATG) exhibits high radioprotective efficacy. In murine macrophages, NATG pre-treatment was revealed to be essential for protecting DNA and antioxidant enzymes from gamma radiation-induced damage.[17] Epigallocatechin gallate (EGCG) which is a polyphenol that has been found in green tea shows antioxidant properties[18]. Similarly, antioxidants were found in many vegetables and fruits[19]. In this project, we have tried to make the *N*-acetyl tryptophan derivatives using different methodologies. Starting from tryptophan, we first acetylated it and then reactions were performed to add glucose to *N*-acetylated tryptophan in various acidic conditions. It was a challenging task to make the desired glucoside of tryptophan as the indole moiety in tryptophan as shown in **figure 2** has four distinct sites which include C3, C2-C3 π bond, C2-N sigma bond, and nitrogen atom. With the help of acid, these positions can easily be protonated. Protonation at C3 occurs more readily than nitrogen of indole.[20] Although α-NH_2 is protected by acetyl group but α-acid group of tryptophan is an active site for reactions like esterification.

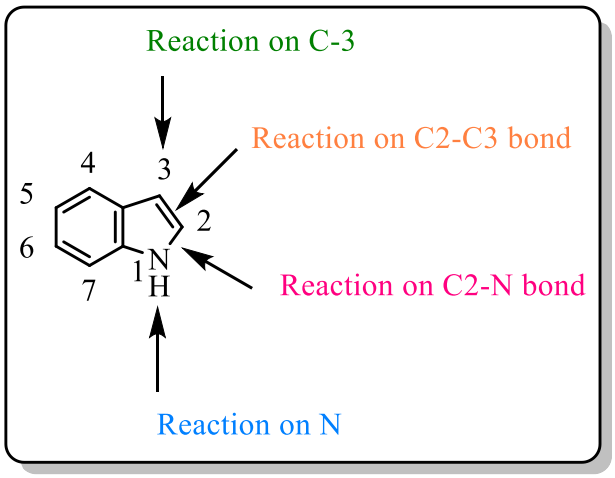
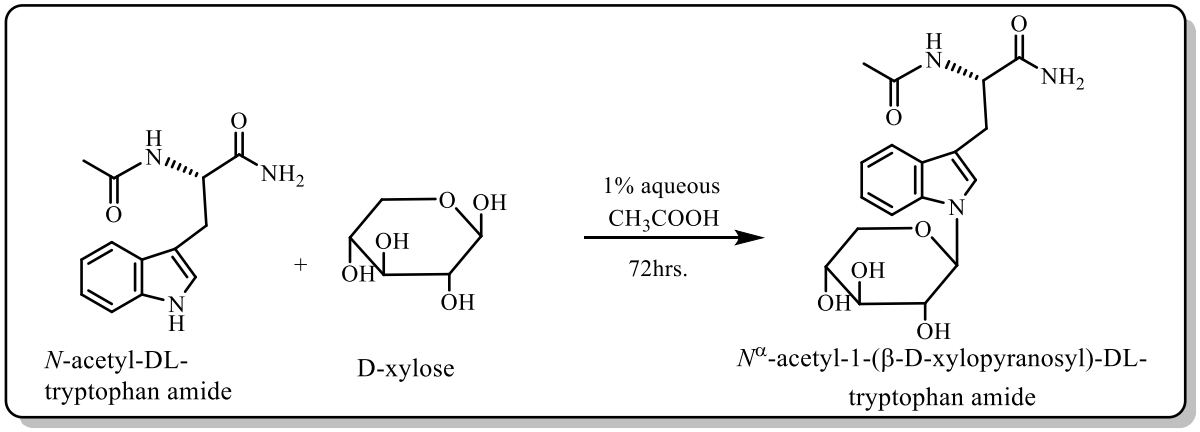


Figure 2. Active sites of indole for reaction[20]

LITERATURE REVIEW

Chemical formation of tryptophan derivatives

Nyhammar *et al.*[21] did the reaction between *N*-acetyl-DL-tryptophan amide and sugar like D-xylose and D-glucose in acidic conditions provided by acetic acid and refluxed for about 72hrs. In the case of D-xylose, 1.0% aq. acetic acid was added (**scheme 2**) giving a yield of 20% N^{α}-acetyl-1-(β-D-xylopyranosyl)-DL-tryptophan amide while in the case of glucose, 50% of aq. acid was added giving a yield of 10% N^{α}-acetyl-1-(β-D-glucopyranosyl)-DL-tryptophan amide. Despite the fact that the HPLC and NMR spectra suggested just one component, the result produced was a diastereomeric combination of both D- and L-tryptophan derivatives.



Scheme 2. Synthesis of N^{α}-acetyl-N^{1}- (D-xylopyranosyl)-DL-tryptophan amide

It was found out that on changing the concentration of acetic acid there is not much change in the isolated yield but at going to more acidic conditions the time of reaction has shortened. Low pH and rise in temperature blocked the tryptophan's indole nitrogen by reducing carbohydrates.

Yaylayan *et al.*[22] studied the kinetic of the reaction between tryptophan and glucose or mannose and then studied the role of deglycation in the Maillard reaction. When it comes to mannose tryptophan is consumed more than glucose. The rate of tryptophan disappearance is observed to be 1.3 times higher with mannose, when the temperature is 110 °C, and 1.8 times higher when the temperature is further raised to 140 °C. The amount of reactive free sugars present and the generation of Amadori products determine the degree of Maillard browning. It has been seen that Amadori products have a tendency to undergo reactions with a second molecule of sugar, giving the diglycated products, provided that the sugar exhibits sufficient reactivity. Additionally, these products were also subject to decomposition reactions. When there was a

lower concentration of reactive sugars, it was observed that only Amadori products underwent decomposition. Diglycated Amadori products have been observed to exhibit significantly higher reactivity compared to Amadori products in the formation of brown coloration.

Gutsche *et al.*[12] synthesized *C*-glycosyl derivatives and *N*-glycosyl derivatives of tryptophan, but polyol-tetrahydro-β-carbolines were also produced in the reaction.[12] For both the derivatives reaction conditions were optimized. For *C*-glycosyl, the pH of the reaction was set at 1 with the help of 1M H_2SO_4 and for *N*-glycosyl derivative the pH was set at 2 with 1M HCl. The rest of the reaction conditions were the same for both the model reactions like L-tryptophan and the aldohexose were dissolved in 10ml of water and after setting the pH, the contents were heated at 80 °C for 12 days. **Figure 3** summarised the probable tryptophan-aldohexoses products in relation to the indole moiety's sensitivity towards electrophilic attack. The isolation of these products was done with the help of Lichroprep C_{18} column, and then structure elucidation was done by ^{1}H-NMR, ^{13}C-NMR spectroscopic techniques.

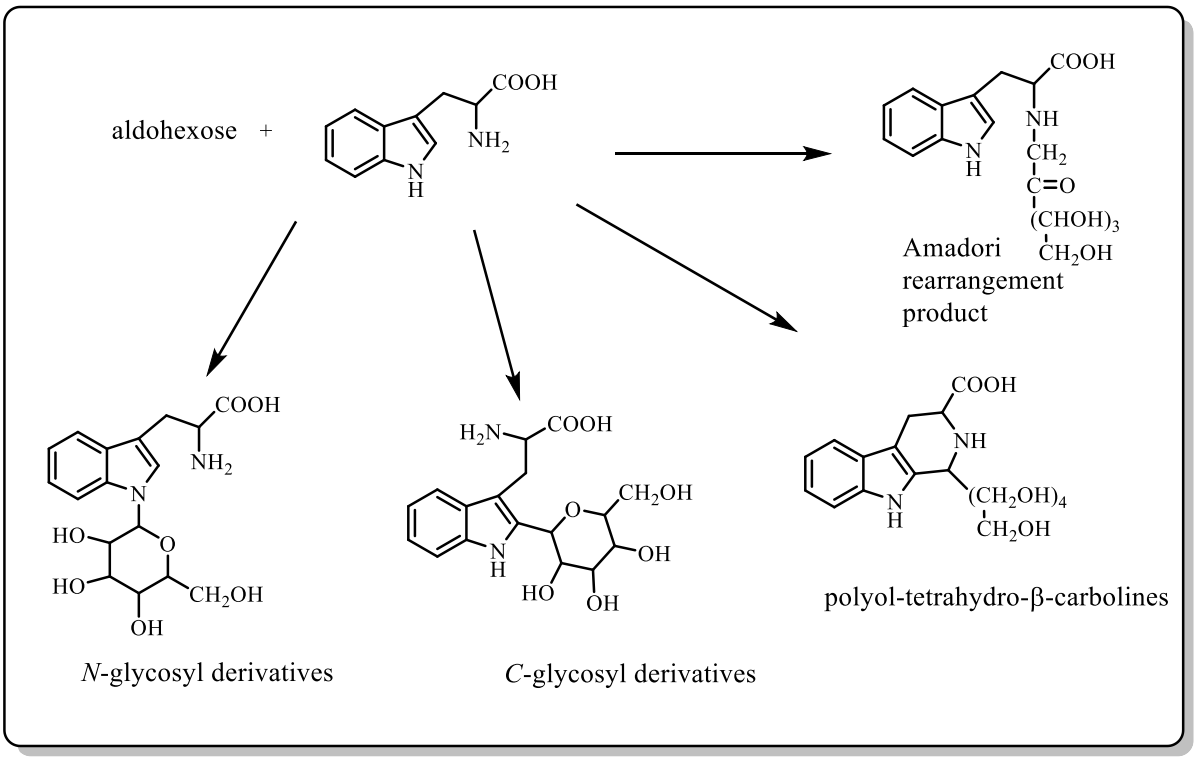
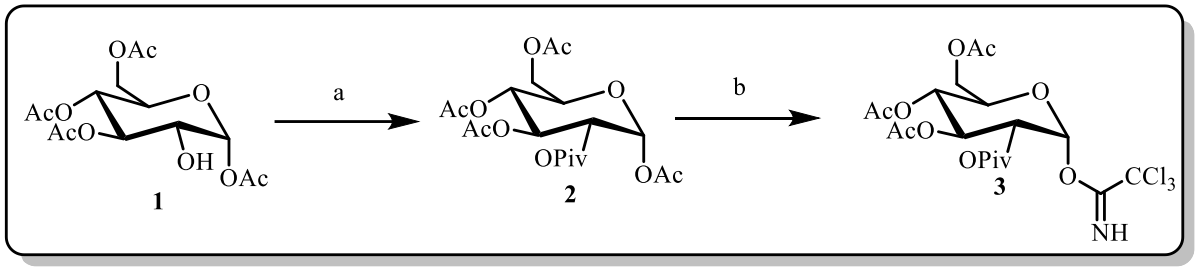


Figure 3: Suggested reaction products[12]

Ronner *et al.*[23] proposed that during food processing and preparation the reaction between L-tryptophan and glucose gave the glucose-tryptophan Amadori product as well as polyol-tetrahydro-β-carbolines. The reaction were done for different time frames and at various temperatures in the presence of oxidants which are added to food and in air. In addition to

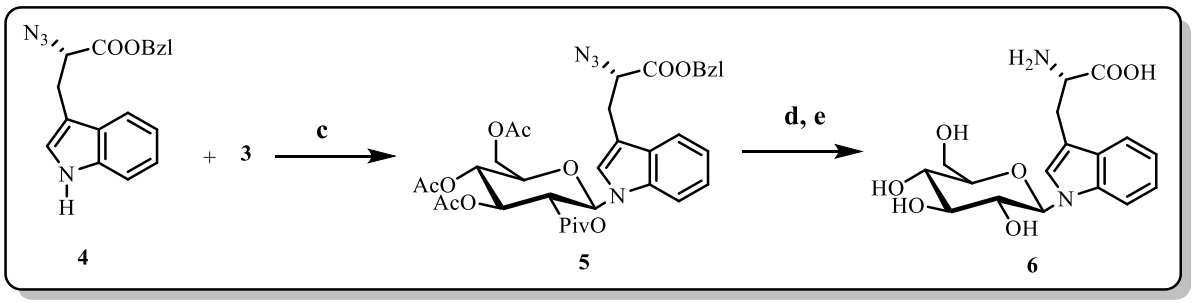
Amadori product and polyol-tetrahydro-β-carbolines at temperatures over 100 °C under the applied circumstances, a compound with a UV spectrum distinct from the other products was also found which was identified as acetyl-β-C. At a temperature of 120 °C Amadori product, polyol-tetrahydro-β-carbolines, and acetyl-β-C were formed up to 2-2.5% but at 150 °C neither of the first two was formed and the only product which was identified as acetyl- β-C.

Schnabel *et al*.[24] reported the synthesis of tryptophan N-glucoside **6** from tetra-acetyl-glucosylimidate **3** and Cbz-Trp-OBzl **4**.[24] The required **3** was synthesized from tetra-acetyl-glucose **1**. This is a two-step process, firstly pivaloyation of **1** is done with PivCl in the presence of pyridine and $CHCl_3$ at OH-2 and then the acetyl group at the anomeric carbon was cleaved with hydrazine acetate, gave giving rise to **3** as shown in **scheme 3.**



Scheme 3: Reagents and conditions: (a) 5 equivalent PivCl, pyridine, $CHCl_3$ rt; (b) (i) 1.5 equivalent $H_2N–NH_3OAc$, DMF, rt; (ii) 0.1 equivalent DBU, 5 equivalent CCl_3CN, 0 °C

The required tryptophanyl-N-glucoside was produced by glycosylating α-azido-tryptophan-benzyl ester **4** with **3** activated by TMSOTf which after purification by flash chromatography gave a yield of 5 in 43%. The deprotection of **5** was done by palladium oxide-hydrate which simultaneously reduced the azido group and deacetylation in 40% aq methylamine giving **6** as shown in **scheme 4.**



Scheme 4: Reagents and conditions: (c) 0.1 equivalent TMSOTf, DCM, -15 °C; (d) $PdO–H_2O$, Hydrogen, Methanol, rt; (e) 40% aq $MeNH_2$ in water, rt.

Xu *et al.*[25] did the synthesis of (-)-indolactam V (**figure 4**) which was accomplished either through an 8-step process (giving yield of 49%) using the known 4-nitrotryptophan derivative, or through a 12-step process (giving yield of 18%) starting from L-glutamic acid. The synthesis presented involved the utilization of Pd-catalysed reactions for the synthesis of 4-nitrotryptophan and 4-nitrotryptophanol. This method permitted the addition of both a 4-substitution indole and a C-9 stereocenter to IL-V, thereby enhancing the lactamization processes. Additionally, it allowed the quick and successful synthesis of aldehyde. The synthetic strategy allowed for the preparation of (-)-IL-V on a gram-scale, taken into account the practical aspects involved.

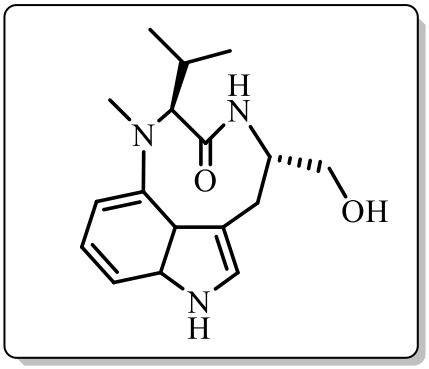


Figure 4. (-)-indolactam V

Li *et al.*[4] synthesized a collection of tryptophan derivatives incorporating 2,5-diketopiperazine and acyl hydrazine functional groups. The synthesized compounds were subjected to systematic bioassays, revealing moderate to good levels of activity against TMV. Synthesized compounds shown in **figure 5** exhibited greater antiviral activity as compared to other synthesized compounds in terms of inactivation, curative effects, and protection *in vivo* compared to ribavirin, and ningnanmycin. The majority of the compounds showed a wide range of effectiveness when evaluated against different types of phytopathogenic fungi.

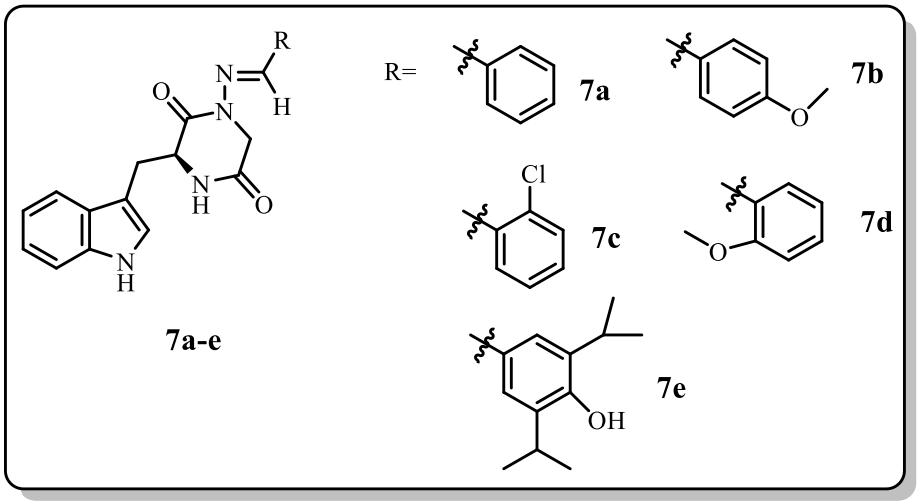


Figure 5. Some of synthesized compounds which showed higher antiviral activity

Tryptophan glycoconjugates that are isolated from nature

Hofsteenge *et al.*[26] demonstrated that a protein can have numerous tryptophan residues that are *C*-mannosylated. Proteins that include *N*- or *O*-linked oligosaccharides were seen from very long time but this time *C*-linked proteins have been found out. Proteins containing TSR modules and cytokine receptors of class 1 appear to be among the most probable candidates for *C*-mannosylation. *C*-mannosylation involves the *C*-glycosidic attachment of a mannose sugar residue to the tryptophan indole moiety. Interleukin 12 and Human RNase 2 are the first tryptophan found which are selectively *C*-mannosylated in motif WXXW.

Diem *et al.* detected the tryptophan-*N*-glucoside in fruit and fruit juices with the help of HPLC[10]. The amount of glucoside found in samples are shown in **table 1**.

Table 1: Amount of tryptophan-*N*-glucoside found in samples

n[a]	Sample	Tryptophan-N-glucoside [mg/L]
3	Pear juice	13.5±1.7
2	Pear fruit	7.3±1.4
2	Apple juice	0.7±0.2
1	Apricot fruit	0.3±0.0
3	Apple fruit	0.2±0.1
2	Peach fruit	0.2±0.0
2	Raspberry fruit	0.2±0.0
1	Kiwi fruit	≥0.1
1	Plum fruit	≥0.1

[a] number of samples analyzed

To get a deeper understanding, deuterium labelled tryptophan has been injected into the pear fruit and then stored at rt for 2-15 days. The analyses of the samples were done by HPLC-MS/MS. This has been the first to describe naturally produced N^1-(β-D-glucopyranosyl-$^4C_1$)-L-tryptophan as a novel intermediary of tryptophan in plants.

Later, Diem *et al.*[27] identified and isolated the pentose derivatives of tryptophan in food samples like soy sauce, fish, tomato juice, red wine and many more in different concentrations. This depends on the ingredients used and processing conditions. Out of all the tryptophan-

derived glycoconjugates, pentose-derived glycoconjugates were found in greatest concentration in the seasoning sauce (55%) and soy sauce (25%) sample. In comparison to results from with D-glucose, it was observed that there has been a much quicker reduction in the tryptophan content in model studies when pentose was present. While 66% of the tryptophan in the presence of D-glucose remained unaltered, the concentration of tryptophan in ribose system was decreased to 6% within 48 hours. When compared to D-glucose, ribose adopted a markedly greater concentration of the ring-open aldehyde form, which helps to explain its significantly higher reactivity towards electrophilic alteration of tryptophan.

Gutsche *et al.*[12] isolated *C*-glycosyl tryptophan (**figure 6**) from human urine. From seven healthy individuals human urine was collected and freeze-dried. After concentrating the samples and adjusting the pH to 9-10 these were passed through anion exchange chromatography. The collected sample was then applied to HPLC-ESI-MS were *C*-glycosyl derivative was found. Structure elucidation was done by ^{1}H-NMR, ^{13}C-NMR, and ESI-MS where *m/z* peak at 367 $[M+H]^+$ confirmed the presence of tryptophan *C*-glycosyl derivative.

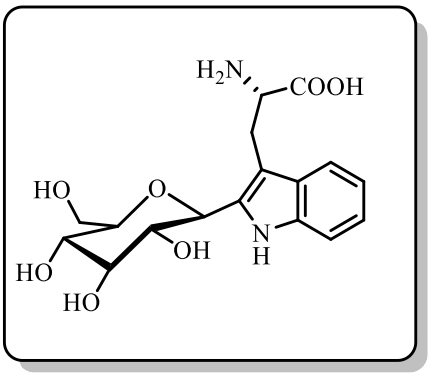
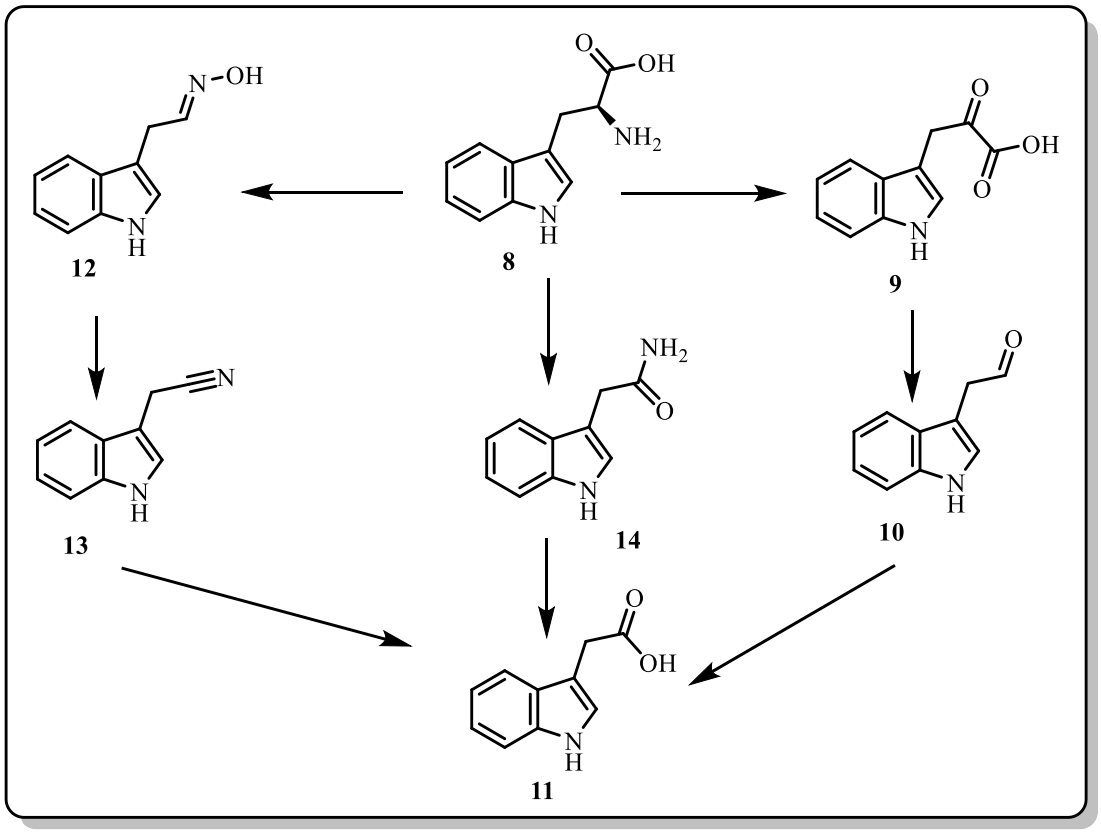


Figure 6. Tryptophan glycoconjugate found in human urine.

Malhotra *et al.*[28] isolated *N*-acetyl-tryptophan glucoside from the radioresistant bacterium *Bacillus sp.* INM-1. A 24-hour-old culture of *Bacillus sp.* INM-1 was exposed to gamma radiation (5k Gy) and allowed to develop in incubation for 24 hours at 32 °C. 1 L of sterile TYG culture was inoculated with a new inoculum (5%) from the irradiated (5k Gy) *Bacillus sp.* INM-1 broth, and the mixture was then incubated for 96 hours at 32 °C with constant shaking. After incubation, the development of the bacteria was stopped, and to separate out the bulk of the microorganisms from the broth, it was centrifuged. After centrifugation, the supernatant was lyophilized. Following lyophilization, the powder was subjected to a fractionation procedure based on organic solvents, utilizing a series of organic solvents in the

order from nonpolar to polar and at last water was used. Finally, the water-soluble fraction was separated and lyophilized. The methanol-water mobile phase was used in a reverse-phase cartridge to further process this water fraction. With ethyl acetate, methanol, and water (1:1.5:1) serving as the mobile phase, all fractions were verified on TLC and seen under UV light. The fraction with the bright spot on the TLC was collected, and its purity was then verified by HPLC using a C18 column with a gradient of methanol: water for 45 minutes as the mobile phase. The characterization of compound was then done by several spectroscopic techniques. The study was conducted to assess the efficacy of a newly discovered molecule, NATG, in providing protection against radiation in J774A.1 cells, which are murine macrophages.

Herderich *et al*.[29] summarised the discovery of auxin, which took place during the early 20th century. Auxin helps in plant growth, specifically focusing on its involvement in cell elongation, root development, and tropic responses. Indole-3-acetic acid **11** (auxin) can be derived from the amino acid tryptophan **8.** It has been observed that diverse plant species possess the ability to synthesize auxin through multiple pathways as shown in **scheme 5**.



Scheme 5. Different pathways to synthesize **11**

The catalytic synthesize of **9** from **8** is well documented to be facilitated by transaminating enzymes across various species. A distinct L-tryptophan dehydrogenase activity has been identified in pea, maize, and tomato. In Brassicaceae, the synthesis of **11**, either through **14** pathway or *via* the intermediate indole-3-acetaldoxime **12** which then converted to indole-3-acetonitrile **13** through the enzymatic conversion. This enzymatic conversion has been seen in Chinese cabbage.

RESEARCH GAP AND OBJECTIVES

Many tryptophan derivatives have been synthesized and used for medical purposes over many years. Tryptophan-*N*-glycoconjugate has been isolated and synthesized times earlier but *N*-acetyl-tryptophan glucoside has rarely been synthesized through chemical method. So, the main objectives which are completed in the present dissertation work are as follows:

(I) To chemically synthesize the *N*-acetyl-tryptophan glucoside as which was earlier extracted from the bacterial *Bacillus sp.* INM-1.

(II) Optimize the reaction to obtain the desired product.

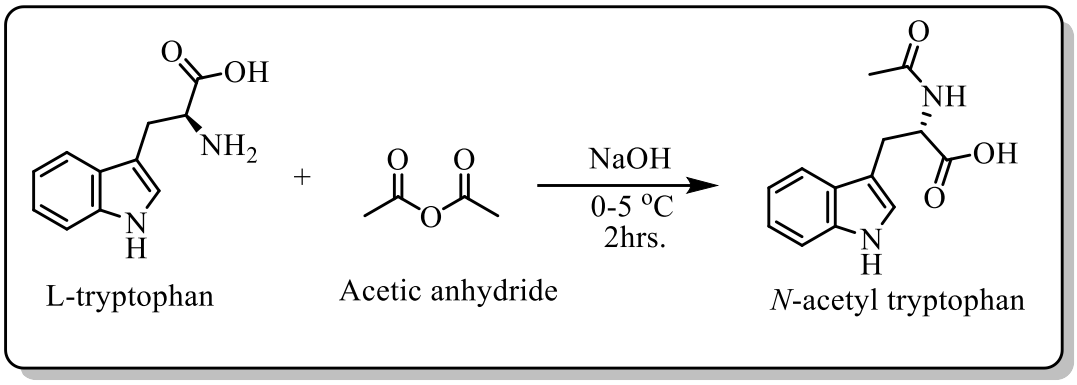
(III) To characterize the product through different spectroscopic techniques.

EXPERIMENTAL SECTION

Chemicals and instruments used: Tryptophan, *N*-acetyl-DL-tryptophan, Dextrose, and all the solvents used were purchased from Loba Chemie Pvt. Ltd. For column chromatography, silica gel mesh 60-120 was used. The NMR was recorded in $CDCl_3$ or DMSO as per the solubility of the synthesized compound on JEOL 400 MHz NMR instrument. High-Resolution Mass Spectroscopy (HRMS) was carried out on Waters with QTOF mass spectrometer with UPLC and PDA detector.

Methods:

Acetylation of tryptophan: L-tryptophan (1g, 4.89 mmol) was dissolved in 1.5 ml water and 2.5 ml of 2N NaOH. 1.2 ml of acetic anhydride, and 12 ml of NaOH were added in eight portions over the course of 15 minutes. Throughout the procedure, the reaction mixture was kept in an ice bath. After 20 minutes at ambient temperature, 6 ml of 6 N H_2SO_4 was added and the solution was cooled in an ice bath. The precipitated white compound was filtered and then liberated of any residual tryptophane by rinsing with 25 ml of 0.2 N HCl and eventually with water (**scheme 6**). Yield: 88%



Scheme 6. Acetylation of tryptophan

Spectral Data: ^{1}H-NMR (400 MHz, DMSO-d6) δ 10.80 (s, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 2.3 Hz, 1H), 7.04 – 6.99 (m, 1H), 6.96 – 6.92 (m, 1H), 4.41 (td, *J* = 8.4, 5.1 Hz, 1H), 3.11 (dd, *J* = 14.6, 5.0 Hz, 1H), 2.94 (dd, *J* = 14.6, 8.8 Hz, 1H), 1.76 (s, 3H).

Synthesis of tryptophan glycoconjugates: Tryptophan glycoconjugates were synthesized with L-tryptophan (250 mg, 1.22 mmol) and D-glucose (450 mg, 2.49 mmol) in 10 ml water. With the help of 0.5 HCl pH was set at 2. The reaction mixture was heated for 12 days at 80 °C with continuous stirring. The expected products were the *N*- or *C*-glycoconjugates of tryptophan as shown in **figure 3**.

Synthesis of *N*-acetyl tryptophan glucoside:

Method 1: *N*-Acetyl-DL-tryptophan (500 mg, 2.03 mmol) and D-glucose (900 mg, 5.01 mmol) were dissolved together in 10 ml water and 15 ml methanol in an RBF, and pH was adjusted to 2 with the help of 0.5 M **HCl**. The contents of RBF were heated for 12 days at 80 °C with continuous stirring. The reaction progress was checked from time to time by TLC. After the work up with ethyl acetate, the product is separated with column chromatography using chloroform and methanol as eluents. Isolated Yield: 12%

Spectral data: ^{1}H-NMR (400 MHz, DMSO-d6) δ 12.57 (s, 1H), 10.81 (s, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.09 (s, 1H), 7.05 – 7.00 (m, 1H), 6.96 – 6.92 (m, 1H), 4.41 (td, *J* = 8.4, 5.1 Hz, 1H), 3.11 (dd, *J* = 14.6, 4.9 Hz, 1H), 2.94 (dd, *J* = 14.6, 8.8 Hz, 1H), 1.76 (s, 3H).

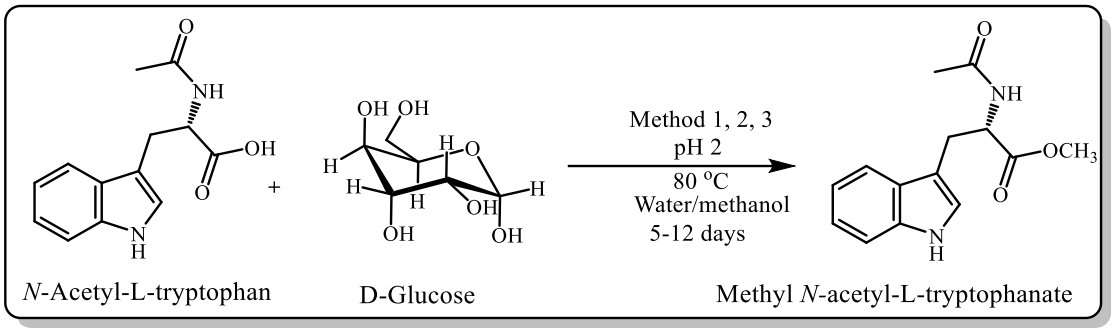
Method 2: *N*-Acetyl-DL-tryptophan (250 mg, 1.015 mmol) and D-glucose (182 mg, 1.015 mmol) were dissolved together in 10 ml water and 15 ml methanol in RBF, and pH was adjusted to 2 with the help of **acetic acid**. The contents of RBF were heated for 9 days at 80 °C with continuous stirring. The reaction progress was checked from time to time by TLC. The reaction mixture was extracted with water and ethyl acetate and then separated with column chromatography using chloroform and methanol as eluents (**scheme 7**). Yield: 11%

Spectral data: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.18 (t, *J* = 7.0 Hz, 1H), 7.11 (t, *J* = 7.1 Hz, 1H), 6.96 (s, 1H), 6.02 (s, 1H), 4.94 (dt, *J* = 7.9, 5.3 Hz, 1H), 3.69 (s, 3H), 3.36 – 3.25 (m, 2H), 1.94 (s, 3H). ^{13}C- NMR (101 MHz, $CDCl_3$) δ 172.52, 169.88, 136.18, 127.82, 122.76, 122.35, 119.81, 118.60, 111.38, 110.14, 53.15, 29.78, 27.66, 23.33

Method 3: *N*-Acetyl-DL-tryptophan (250 mg, 1.015 mmol) and D-glucose (182 mg, 1.015 mmol) were dissolved together in 10 ml water and 15 ml methanol in RBF. The pH was adjusted to 2 with the help of **TFA**. The contents of RBF were heated for 5 days at 80 °C with

continuous stirring. The reaction progress was checked from time to time by TLC. The mixture was extracted with water and ethyl acetate, and then separated with column chromatography using chloroform and methanol as eluents (**scheme 7**). Yield: 20%

Spectral data: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.21 – 7.15 (m, 1H), 7.13 – 7.08 (m, 1H), 6.96 (s, 1H), 6.02 (d, *J* = 7.6 Hz, 1H), 4.94 (dt, *J* = 7.9, 5.2 Hz, 1H), 3.69 (s, 3H), 3.51 –3.14 (m, 2H), 1.94 (s, 3H)



Scheme 7. Synthesis of *N*-acetyl tryptophan glucoside. Reagents and conditions: (1) HCl, 12 days; (2) Acetic Acid, 9 days; (3) TFA, 5 days

Method 4: *N*-Acetyl-DL-tryptophan (250 mg, 1.015 mmol) and D-glucose (182 mg, 1.015 mmol) were dissolved together in 10 ml water and 15 ml ethanol in RBF. The pH was adjusted to 2 with the help of TFA. The contents of RBF were heated for 7 days at 80 °C with continuous stirring. The reaction progress was checked from time to time by TLC. The pure product was extracted from the reaction mixture with ethyl acetate after neutralizing it with $NaHCO_3$. Yield: 17%

Spectral data: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.19 – 7.13 (m, 1H), 7.12 – 7.07 (m, 1H), 6.96 (d, *J* = 2.1 Hz, 1H), 6.09 (d, *J* = 7.8 Hz, 1H), 4.92 (dt, *J* = 7.9, 5.4 Hz, 1H), 4.21 – 4.05 (m, 2H), 3.42 – 3.18 (m, 2H), 1.93 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 3H).

RESULTS AND DISCUSSION

Acetylation of tryptophan has been done by adding acetic anhydride with continuous stirring in the alkaline solution of tryptophan as shown in **scheme 6**. The ^{1}H-NMR (**figure 7**) of the product obtained gave a peak at δ 1.76 ppm which corresponds to methyl and the peak at δ 10.80 ppm corresponding to amide hydrogen giving the integration of 1H confirming the addition of acetyl group on α-NH_2 of tryptophan.

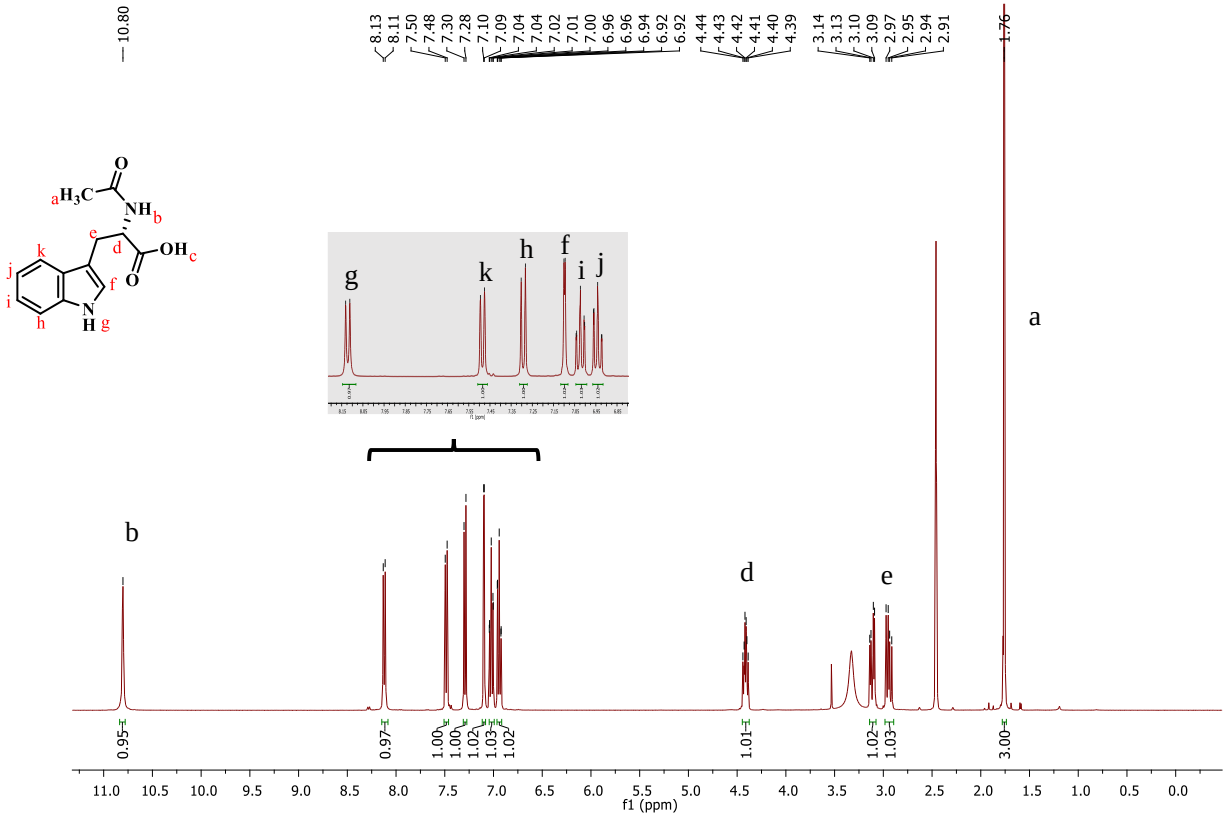


Figure 7: ^{1}H-NMR spectrum of *N*-acetyl-L-tryptophan

Tryptophan glucoside was tried to synthesize by refluxing both reactants for 12 days at 80 °C after setting the pH at 2 with HCl. TLC showed a mixture of products in 120 mg. HRMS of the same showed the peak at *m/z* 393 $[M+Na+4H]^{+}$ which says that the glucose did attach to tryptophan but at which position this couldn't be confirmed.

N-acetyl tryptophan glucoside was tried to synthesize three different conditions, each time giving acidic conditions provided by different acids viz., HCl, acetic acid, and trifluoracetic acid. In method 1 to synthesize NATG, the reaction of *N*-acetyl tryptophan (NAT) and glucose in the acidic conditions provided by HCl gave a mix of two compounds. But it could not be

separated by column chromatography as the quantity of product was very less and reactant was recovered in excess amount. The ^{1}H-NMR of the mixture proved the excess of reactant NAT. HRMS of the same showed the peak at *m/z* 283 which is of methyl *N*-acetyl-L-tryptophanate with sodium ion.

Method 2 to synthesize *N*-acetyl tryptophan glucoside (NATG) was done by giving acidic conditions with acetic acid for 9 days. The yield obtained was 153 mg and it contained a mixture of two compounds one of which matches with the reactant, confirmed with TLC. Through column chromatography, the product was separated from the reactant and was characterized with ^{1}H-NMR, ^{13}C-NMR, and HRMS. In proton NMR (**figure 8**), the peaks observed matched with the reactant NAT except for one singlet at δ 3.60 ppm corresponding to 3H . Other peaks are δ 1.76 ppm singlet of 3H showed the N^{α}-acetyl group, at δ 3.36 – 3.25 ppm multiplet of 2 diastereomeric CH_2 of tryptophan. A multiplet at δ 4.4 ppm corresponds to the CH of tryptophan. All 5 aromatic hydrogens and indole NH peak lay in the aromatic region. The peak at δ 8.23 ppm corresponds to the amide NH.

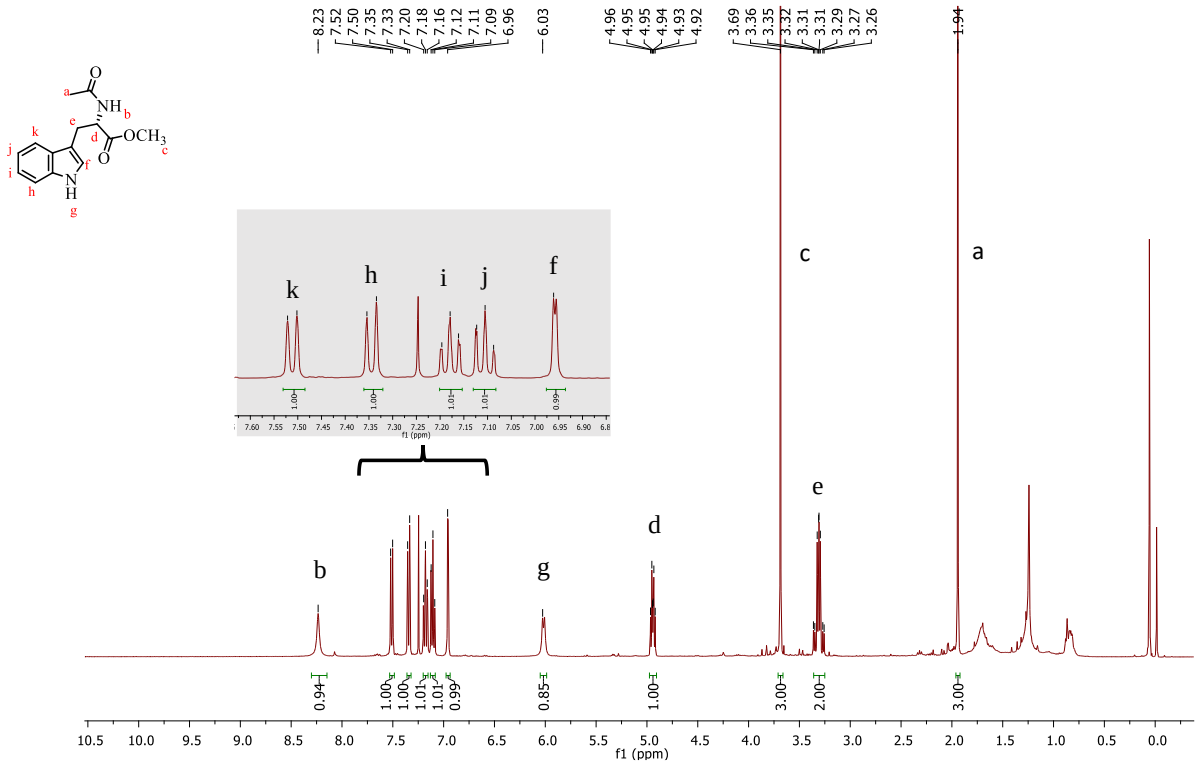


Figure 8. ^{1}H-NMR spectrum of methyl *N*-acetyl-L-tryptophanate

In ^{13}C-NMR (**Figure 9**), the peaks at δ 52.46 and 53.15 represents the CH_3 carbon. This shows the addition of one new carbon corresponding to CH_3.

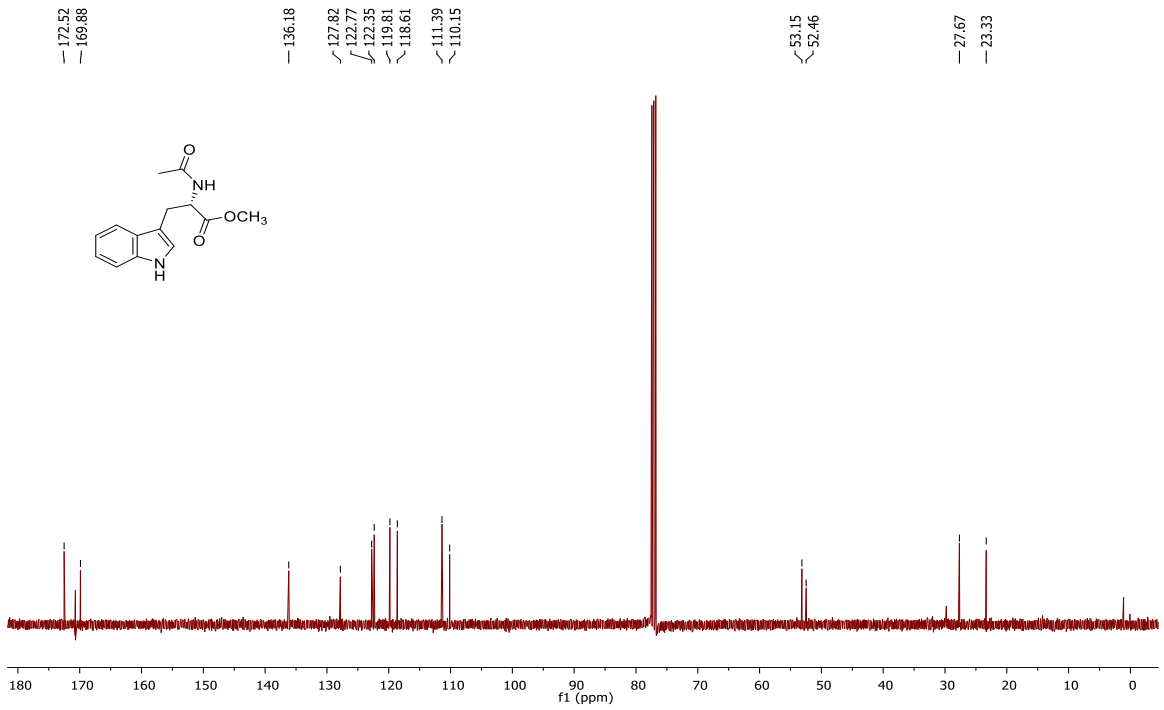


Figure 9. ^{13}C-NMR spectrum of methyl *N*-acetyl-L-tryptophanate

HRMS of the product (**Figure 10**) also showed the peaks at *m/z* 261 $[M+H]^+$ and *m/z* 283 $[M+Na]^+$. This concludes the addition of CH_3 on the reactant and not the addition of glucose as desired. The methyl group gets attached to the α-COOH of the amino acid giving the product an ester which is confirmed as methyl *N*-acetyl-L-tryptophanate.

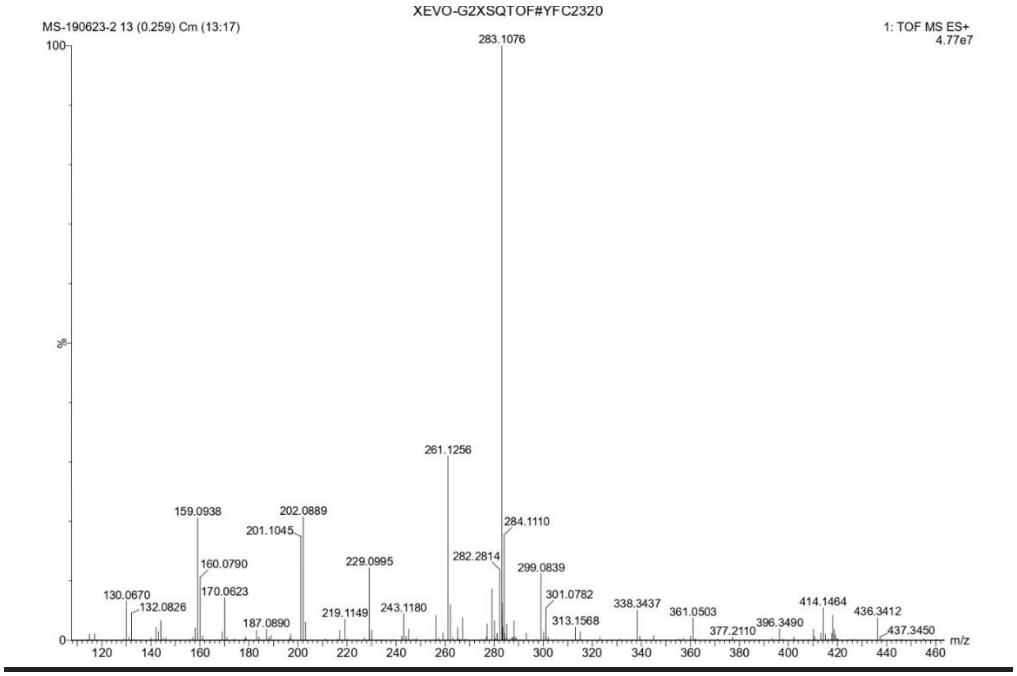


Figure 10. HRMS spectrum of methyl *N*-acetyl-L-tryptophanate

On following method 3 in which TFA is used to provide acidic conditions, the product formed was characterized with ^{1}H-NMR. The spectra came out to be the same as the one with acetic acid. With this, it is concluded that the product formed in this case is also methyl *N*-acetyl-L-tryptophanate.

In method 4 when ethanol is used as a solvent to dissolve NAT instead of methanol, it has been found out that instead of methoxy ethoxy group is getting attached giving the product as ethyl *N*-acetyl-L-tryptophanate. **Figure 11** shows the ^{1}H-NMR of the product in which the multiplet at δ 4.21 – 4.05 ppm corresponds to 2H of ethoxy CH_2, and the triplet at δ 1.21 ppm corresponds to ethoxy CH_3.

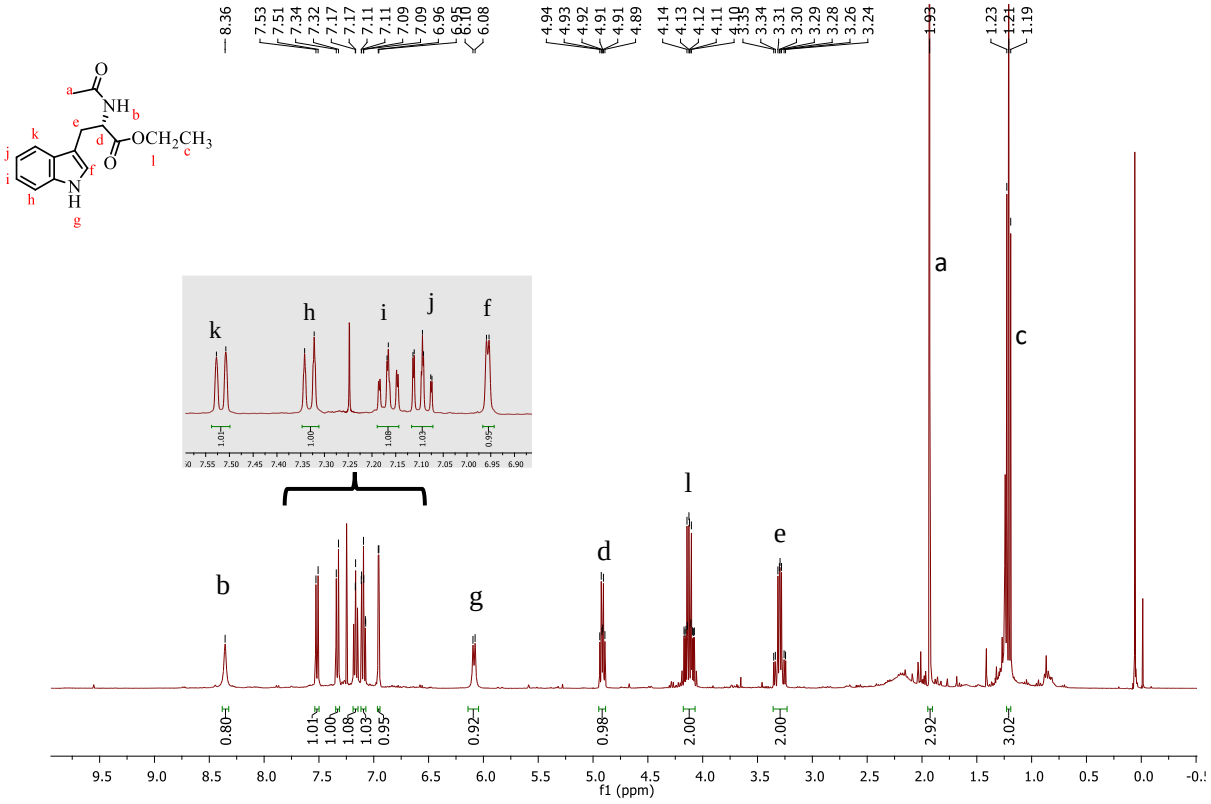
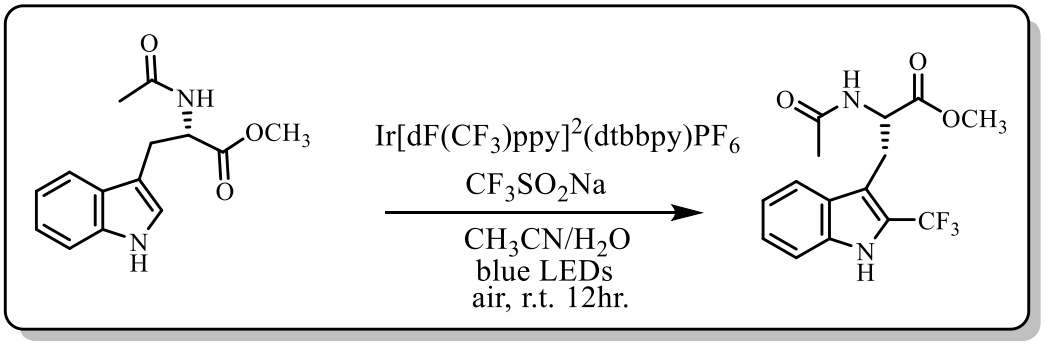


Figure 11. ^{1}H-NMR spectrum of ethyl *N*-acetyl-L-tryptophanate

Methyl *N*-acetyl-L-tryptophanate has a wide variety of applications. It shows less antioxidant activity against ROS but is used to synthesize a number of its derivatives that shows high screening test for $ROO^{\cdot}$, HOCl, and $^1O_2$[30]. Similarly, the said ester shows quenching in fluorescent intensity. When a CF_3 get binds to the tryptophan residue in the polypeptide chain from some source, fluorescent intensity gets quenched. CF_3SO_2Na is used as a

trifluoromethylation source for polypeptides containing tryptophan under visible light as shown in **scheme 8**.[31]As this ester have N-unprotected indole, it can be used for altering drugs and peptides which show bioactivity.[32]



Scheme 8. Trifluoromethylation of methyl *N*-acetyl-L-tryptophanate

Table 2: Reaction conditions tried to synthesize *N*-acetyl-DL-tryptophan

S.No.	Solvent	Reaction Conditions	Product Formed
1.	Water + methanol	0.5 HCl to set pH 2	Methyl *N*-acetyl-L-tryptophanate
2.	Water + methanol	Glacial acetic acid to set pH 2	Methyl *N*-acetyl-L-tryptophanate
3.	Water + methanol	TFA to set pH 2	Methyl *N*-acetyl-L-tryptophanate
4.	Water + ethanol	TFA to set pH 2	Ethyl *N*-acetyl-L-tryptophanate

CONCLUSION

N-acetyl-L-tryptophan has been tried to synthesize with many methods starting from L-tryptophan. Acetylation of L-tryptophan was done by acetic anhydride in basic conditions giving a pure product with a good yield. After the acetylation, glycosylation was tried with D-glucose in acidic conditions provided by different acids. As *N*-acetyl tryptophan was not soluble in water, methanol or ethanol was added to make it soluble. Through ^{1}H-NMR, ^{13}C-NMR and HRMS structure elucidation has been done and it was found that alcohol being a protic acid donates the proton and gives the product as alkyl *N*-acetyl-L-tryptophanate instead of the desired glucoside. Further studies are in progress to synthesize the *N*-acetyl tryptophan glucoside.

REFRENCES

(1) Baenas, N.; García-Viguera, C.; Domínguez-Perles, R.; Medina, S. Winery By-Products as Sources of Bioactive Tryptophan, Serotonin, and Melatonin: Contributions to the Antioxidant Power. *Foods* **2023**, *12* (8). DOI: 10.3390/foods12081571.

(2) Lee, B. H.; Hille, B.; Koh, D.-S. Serotonin Modulates Melatonin Synthesis as an Autocrine Neurotransmitter in the Pineal Gland. *Proc. Natl. Acad. Sci. USA.* **2021,** *118* (43), e2113852118. DOI: 10.1073/pnas.2113852118

(3) Dougherty, D. M.; Richard, D. M.; Dawes, M. A.; Mathias, C. W.; Acheson, A.; Hill-Kapturczak, N. L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. *Int J Tryptophan Res.* **2009**, *2* (1), 45-60. DOI: 10.4137/ijtr.s2129

(4) Li, L.; Yang, R.; Liu, J.; Zhang, J.; Song, H.; Liu, Y.; Wang, Q. Design, Synthesis, and Bioactivities of Novel Tryptophan Derivatives Containing 2,5-Diketopiperazine and Acyl Hydrazine Moieties. *Molecules* **2022**, *27*, 5758. DOI:10.3390/molecules27185758.

(5) Herraiz, T.; Galisteo, J.; Chamorro, C. L-Tryptophan Reacts with Naturally Occurring and Food-Occurring Phenolic Aldehydes to Give Phenolic Tetrahydro-β-Carboline Alkaloids: Activity as Antioxidants and Free Radical Scavengers. *J. Agric. Food Chem.* **2003**, *51* (8), 2168−2173 DOI:10.1021/jf0210066.

(6) Horiuchi, K.; Yonekawa, O.; Iwahara, K.; Kanno, T.; Kurihara, T.; Fujise, Y. A Hydrophilic Tetrahydro-13-Carboline in Human Urine; *J. Biochem.***1994**, *115* (2), 362-366 DOI: 10.1093/oxfordjournals.jbchem.a124343

(7) Qin, D.; Li, L.; Li, J.; Li, J.; Zhao, D.; Li, Y.; Li, B.; Zhang, X. A New Compound Isolated from the Reduced Ribose-Tryptophan Maillard Reaction Products Exhibits Distinct Anti-Inflammatory Activity. *J. Agric. Food Chem.* **2018**, *66* (26), 6752–6761. DOI: 10.1021/acs.jafc.8b01561

(8) Lee, S. H.; Jeong, S. J.; Jang, G. Y.; Kim, M. Y.; Hwang, I. G.; Kim, H. Y.; Woo, K. S.; Hwang, B. Y.; Song, J.; Lee, J.; Jeong, H. S. Isolation and Identification of an Antiproliferative Compound from Fructose-Tryptophan Maillard Reaction Products. *J Agric Food Chem* **2016**, *64* (15), 3041–3047. DOI: 10.1021/acs.jafc.6b00157.

(9) Servillo, L.; Giovane, A.; Casale, R.; Cautela, D.; D'Onofrio, N.; Balestrieri, M. L.; Castaldo, D. Glucosylated Forms of Serotonin and Tryptophan in Green Coffee Beans. *LWT* **2016**, *73*, 117–122. DOI: 10.1016/j.lwt.2016.06.001.

(10) Diem, S.; Bergmann, J.; Herderich, M. Tryptophan-N-Glucoside in Fruits and Fruit Juices. *J. Agric. Food Chem.* **2000**, *48* (10), 4913–4917. DOI:10.1021/jf0003146.

(11) Pu, S. min; Chen, W. dong; Zhang, Y. jun; Li, J. hua; Zhou, W.; Chen, J.; Chen, M. shun; Liu, C. mei. Comparative Investigation on the Phytochemicals and Biological

Activities of Jackfruit (Artocarpus Heterophyllus Lam.) Pulp from Five Cultivars. *Plant Foods Hum. Nutr*. **2023**, *78* (1), 76–85. DOI: 10.1007/s11130-022-01018-6.

(12) Gutsche, B.; Grun, C.; Scheutzow, D.; Herderich, M. Tryptophan Glycoconjugates in Food and Human Urine *Biochem J*. **1999**, 343, 11-19

(13) Poljšak, B.; Fink, R. The Protective Role of Antioxidants in the Defence against ROS/RNS-Mediated Environmental Pollution. *Oxid. Med. Cell Longev.* **2014**, *2014*:671539, DOI: 10.1155/2014/671539.

(14) Epelman, S.; Lavine, K. J.; Randolph, G. J. Origin and Functions of Tissue Macrophages. *Immunity*. **2014**, *41* (1), 21–35. DOI: 10.1016/j.immuni.2014.06.013.

(15) Sologuren, I.; Rodríguez-Gallego, C.; Lara, P. C. Immune Effects of High Dose Radiation Treatment: Implications of Ionizing Radiation on the Development of Bystander and Abscopal. Effects. *Transl. Cancer Res.* **2014**, *3* (1), 18–31. DOI: 10.3978/j.issn.2218-676X.2014.02.05.

(16) Gallin Elaine K.; Green Spencer W.; Sheehy Paul A. Enhanced Activity of the Macrophage-like Cell Line J774.1 Following Exposure to Gamma Radiation. *J. Leukoc. Biol.* **1985**, *38* (3), 369–381.

(17) Malhotra, P.; Gupta, A. K.; Singh, D.; Mishra, S.; Singh, S. K.; Kumar, R. N-Acetyl-Tryptophan Glucoside (NATG) Protects J774A.1 Murine Macrophages against Gamma Radiation-Induced Cell Death by Modulating Oxidative Stress. *Mol. Cell Biochem.* **2018**, *447* (1–2), 9–19. DOI:10.1007/s11010-018-3289-9.

(18) Tiwari, M.; Dixit, B.; Parvez, S.; Agrawala, P. K. EGCG, a Tea Polyphenol, as a Potential Mitigator of Hematopoietic Radiation Injury in Mice. *Biomed. Pharmacother.***2017**, *88*, 203–209. DOI: 10.1016/J.BIOPHA.2016.12.129.

(19) Shaban, S.; El-Husseny, M. W. A.; Abushouk, A. I.; Salem, A. M. A.; Mamdouh, M.; Abdel-Daim, M. M. Effects of Antioxidant Supplements on the Survival and Differentiation of Stem Cells. *Oxid. Med. Cell Longev.* **2017**. 2017,5032102. DOI: 10.1155/2017/5032102.

(20) Mohammadi Ziarani, G.; Moradi, R.; Ahmadi, T.; Lashgari, N. Recent Advances in the Application of Indoles in Multicomponent Reactions. *RSC Adv.* **2018**, *8* (22), 12069–12103. DOI:10.1039/c7ra13321a.

(21) Nyhammar, T.; Pernemalm, A. Reaction of N^{α}-Acetyl-DL-Tryptophan Amide with D-Xylose or D-Glucose in Acidic Solution. *Food Chem.* **1985**, *17* (4),289-296 DOI: 10.1016/0308-8146(85)90037-8

(22) Yaylayan', V.; Forage, N. G. Determination of the Kinetics and Mechanism of Decomposition of Tryptophan Amadori Rearrangement Product by RP-HPLC Analysis; *J. Agric. Food Chem.* **1991**, *39* (2), 364–369 DOI: 10.1021/jf00002a028

(23) Rönner, B.; Lerche, H.; Bergmüller, W.; Freilinger, C.; Severin, T.; Pischetsrieder, M. Formation of Tetrahydro-β-Carbolines and β-Carbolines during the Reaction of L-Tryptophan with D-Glucose. *J. Agric. Food Chem.* **2000**, *48* (6), 2111–2116. DOI: 10.1021/jf991237l.

(24) Schnabel, M.; Römpp, B.; Ruckdeschel, D.; Unverzagt, C. Synthesis of Tryptophan N-Glucoside. *Tetrahedron Lett.* **2004**, *45* (2), 295–297. DOI: 10.1016/j.tetlet.2003.10.190.

(25) Xu, Z.; Zhang, F.; Zhang, L.; Jia, Y. Total Synthesis of (-)-Indolactam v. *Org. Biomol. Chem.* **2011**, *9* (7), 2512–2517. DOI: 10.1039/c0ob01115k.

(26) Hofsteenge, J.; Blommers, M.; Hess, D.; Furmanek, A.; Miroshnichenko, O. The Four Terminal Components of the Complement System Are C-Mannosylated on Multiple Tryptophan Residues. *J. Biol. Chem.* **1999**, *274* (46), 32786–32794. DOI: 10.1074/jbc.274.46.32786.

(27) Diem, S.; Albert, J.; Herderich, M. Reactions of Tryptophan with Carbohydrates: Identification of Pentose-Derived Tryptophan Glycoconjugates in Food. *Eur. Food Res. Technol.* **2001**, *213* (6), 439–447. DOI: 10.1007/s002170100376.

(28) Malhotra, P.; Adhikari, M.; Singh, S. K.; Kumar, R. N-Acetyl Tryptophan Glucopyranoside (NATG) Provides Radioprotection to Murine Macrophage J774A.1 Cells. *Free Radic. Res.* **2015**, *49* (12), 1488–1498. DOI: 10.3109/10715762.2015.1095295.

(29) Herderich, M.; Gutsche, B. Tryptophan-Derived Bioactive Compounds in Food. *Food Rev. Int.* **1997**, *13* (1), 103–135. DOI: 10.1080/87559129709541100.

(30) Estevão, M. S.; Carvalho, L. C.; Ribeiro, D.; Couto, D.; Freitas, M.; Gomes, A.; Ferreira, L. M.; Fernandes, E.; Marques, M. M. B. Antioxidant Activity of Unexplored Indole Derivatives: Synthesis and Screening. *Eur. J. Med. Chem.* **2010**, *45* (11), 4869–4878. DOI: 10.1016/j.ejmech.2010.07.059.

(31) Ding, B.; Weng, Y.; Liu, Y.; Song, C.; Yin, L.; Yuan, J.; Ren, Y.; Lei, A.; Chiang, C. W. Selective Photoredox Trifluoromethylation of Tryptophan-Containing Peptides. *Eur. J. Org. Chem.* **2019**, *2019* (46), 7596–7605. DOI:10.1002/ejoc.201901572.

(32) Weng, Y.; Ding, B.; Liu, Y.; Song, C.; Chan, L. Y.; Chiang, C. W. Late-Stage Photoredox C-H Amidation of N-Unprotected Indole Derivatives: Access to N-(Indol-2-Yl)Amides. *Org. Lett.* **2021**, *23* (7), 2710–2714. DOI: 10.1021/acs.orglett.1c00609.

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