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SYNTHESIS AND MECHANISM STUDY OF NEW BIVALENT β-CARBOLINE DERIVATIVES

(Kajian Sintesis dan Mekanisma Derivatif Bivalen β-karbolin Baharu)

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Abstract

This study reports simple and straightforward methods for synthesizing new bivalent β-carboline compounds using L-tryptophan as a starting material with 1,4-dibromobutane as a dimerization linker. The synthetic route began with coupling L-tryptophan with formaldehyde via Pictet-Spengler condensation to afford tetrahydro-β-carboline, **T1** as the key intermediate. The reaction proceeded with decarboxylation of **T1** using potassium dichromate with acetic acid to afford β-carboline, **T2**. Subsequent alkylation of **T2** using 1,4-dibromobutane as the linker yielded intermediate **T3**, followed by dimerization to furnish the new bivalent β-carboline, **T4**. ^{1}H and ^{13}C NMR confirmed all the synthesized compounds. In addition, this study includes the proposed mechanism for the synthesis of a new bivalent β-carboline compound.

Keywords: synthesis, bivalent β-carboline, L-Tryptophan, Pictet-Spengler condensation, dimerization

Abstrak

Kajian ini melaporkan kaedah mudah dan secara terus untuk mensintesis sebatian baru bivalen β-karbolin menggunakan L-tryptofan sebagai bahan pemulaan dengan 1,4-dibromobutana sebagai penghubung dimerisasi. Laluan sintesis bermula dengan gandingan L-tryptofan dengan formaldehid melalui pemeluwapan Pictet-Spengler untuk mendapatkan tetrahidro-β-karbolin, **T1** sebagai kunci perantaraan. Tindak balas diteruskan dengan pendekarboksilan **T1** menggunakan kalium dikromat dengan asid asetik untuk menghasilkan β-karbolin, **T2**. Seterusnya alkilasi **T2** menggunakan 1,4-dibromobutana sebagai penghubung menghasilkan perantara **T3**, diikuti dengan dimerisasi untuk menghasilkan bivalen β-karbolin baharu, **T4**. Semua sebatian yang disintesis disahkan dengan ^{1}H NMR dan ^{13}C NMR. Sebagai tambahan, kajian ini merangkumi mekanisma yang dicadangkan untuk sintesis sebatian bivalen β-karbolin baharu.

Kata kunci: sintesis, bivalen β-Karbolin, L-Tryptofan, pemeluwapan Pictet-Spengler, dimerisasi

Introduction

β-carbolines are derived from a large group of heterocyclic compounds known as Norharmane (Figure 1), which have a 9H-pyrido[3,4-*b*]indole structural unit [1]. Furthermore, β-carboline can be classified as a synthetic and naturally occurring indole alkaloid with varying degrees of aromaticity that possess a planar tricyclic pyrido[3,4-*b*]indole ring system [2,3,4]. The core skeleton of β-carboline consists of a pyridine ring fused to an indole backbone (Figure 1).

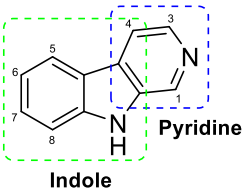


Figure 1. Core skeleton of β-carboline structure

β-carboline is a natural constituent found in plants, human tissues, body fluids, marine life, insects, and mammals [1]. Furthermore, β-carboline is also distributed in fungi and foods with broad pharmacological properties, including sedative, antithrombin, antimalarial, anti-HIV, and anti-inflammatory [5,6]. In addition, previous studies have reported that β-carboline, with its widespread biological properties, is commonly studied for its antitumor activity [7].

β-carboline alkaloid can be isolated from the seeds of a plant named *Peganum harmala,* which belongs to the family of Zygophyllaceae [4,7,8]. Other names for *Peganum harmala* include harmal, African rue, and Syrian rue. It is a perennial herbaceous, glabrous plant that can grow in semi-arid rangeland and sandy soils, especially in the Mediterranean region of North Africa and the Middle East [9]. β-carboline derivatives extracted from this plant are used mainly to treat various diseases, including asthma and jaundice. Despite its toxicity level, *Peganum harmala* has been used to treat a range of human ailments, as the health benefits outweigh the detrimental effects [10].

Interestingly, bivalent β-carboline alkaloids (Figure 2) elicited substantially greater bioactivity than the corresponding monomers. Thus, this suggests that bivalent compounds have better therapeutic potential and thus should be studied in future research [11].

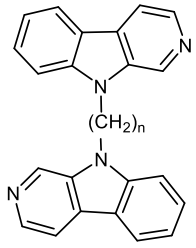


Figure 2. Example of a bivalent β-carboline structure

The bivalent β-carboline structure has two β-carboline monomers linked at positions-1, -2, -3, and -9 by carbon chains, heteroatoms, heterocyclic, amide esters, or amino-groups [11]. In addition, a previous study discovered that bivalent β-carboline elicited anticancer properties via a suitable linker, significantly improving activity by 100- to 500-fold over the corresponding monomers [5,12]. The improvement in anticancer activity might have occurred as the bivalent compound was more favourable to intercalating into DNA [5]. Therefore, bivalent β-carboline was expected to have significantly higher antitumor potency *in vitro* and *in vivo* than the corresponding monomers [12].

There are numerous reports of bivalent β-carboline derivatives. Daoud et al. investigated the anticancer activity of the bivalent β-carboline derivative B-9-3 (**1**) (Figure 3), which demonstrated potent anticancer activity against three cancer cell lines with IC_{50} values ranging from 3.58 μM–10.89 μM [13]. Chen et al. reported the synthesis and anticancer activity of a series of novel N^9-heterobivalent β-carboline (**2**) and (**3**) with strong cytotoxic activities against six cancer cell lines with IC_{50} values lower than 20 μM (Figure 3) [1].

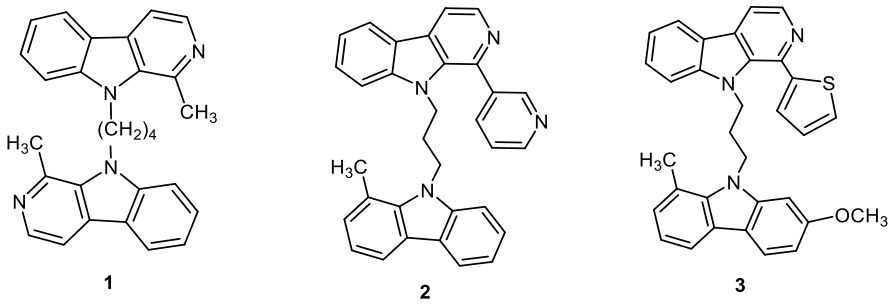


Figure 3. Reported bivalent β-carboline derivatives

Bivalent β-carboline, linked by suitable linkers, could significantly improve DNA-binding affinity as the dimerized compounds can bind to DNA through bis-intercalation mode, causing significant changes in DNA double helix structure [7,14,15]. The structural-activity relationships of the bivalent β-carboline compound indicated a few factors that determined antitumor activity; the common β-carboline moiety was very crucial for antitumor activity, the length of the linker affected antitumor potencies, four to six methylene units were more favourable, and the linker position in positions-1,-2,-3, and -9 were beneficial [14,16]. The problem statement that this study addresses is the importance of developing new anticancer drugs as cancer is currently one of the leading causes of major health problems worldwide. Thus, this study aims to synthesize a new bivalent β-carboline compound using the suitable length of 1,4-dibromobutane in position-9 in the four-step method using commercially available L-tryptophan as a starting material. The bivalent β-carboline was characterized using ^{1}H- and ^{13}C-NMR.

Materials and Methods

This section will describe a sequential decarboxylation and aromatization of tetrahydro-β-carboline-3-carboxylic acid **T1** to furnish β-carboline **T2**. This was followed by the dimerization reaction of the monomer β-carboline to form a new bivalent β-carboline **T4**.

Synthesis of tetrahydro-β-carboline (T1)

Tetrahydro-β-carboline-3-carboxylic acid (**T1**) was prepared through Pictet-Spengler condensation of L-tryptophan (**1**, 2.0 g, 9.8 mmol) with 37% formaldehyde (**2**, 0.98 mL, 32.63 mmol). The reaction started with 3 hours of stirring of L-tryptophan (**1**) and NaOH (0.4 g, 10 mmol) in a specific amount of water (200 mL). Then, 37% formaldehyde (**2**) was added to the solution, and it continued to reflux for 3 hours at room temperature. Upon completion, the reaction mixture was neutralized with glacial acetic acid, and the product was filtered off, washed well with water, and dried overnight.

Synthesis of β-carboline (T2)

Next, we attempted the decarboxylation-aromatization of tetrahydro-β-carboline-3-carboxylic acid (**3**, 1 g, 4.6 mmol) by stirring it in water (100 mL) at 100°C. The reaction continued to stir and heat with $K_2Cr_2O_7$ (9.5 g, 32 mmol) and acetic acid as a base (10 mL). Then, the reaction mixture was cooled by running it under tap water. Upon completion, sodium sulfite was added as a removing oxidizing agent. This was followed by the addition of 2.5 M NaOH drop by drop until it reached pH 7. The solution was extracted with ethyl acetate, and the combined organic layer was collected, washed with water and brine, and dried over anhydrous sodium sulfate. Lastly, the solution was evaporated. Yellowish solid, yield 47%, m.p. 199°C. ^{1}H-NMR (400 MHz, MeOD-d_4): δ 8.77 (s, 1 H), 8.26 (d, J = 5.5 Hz, 1 H), 8.17 (d, J = 7.8 Hz, 1 H), 8.08-8.07 (m, 1 H), 7.55-7.54 (m, 2 H), 7.27-7.23 (m, 1 H); ^{13}C NMR (101 MHz, MeOD-d_4): δ 137.0, 132.7, 129.1, 128.4, 121.4, 120.8, 119.5, 114.8, 111.5.

Synthesis of intermediate bivalent β-carboline (T3)

The intermediate **T3** was prepared by stirring β-carboline (**T2,** 0.5 g, 2.96 mmol) in anhydrous DMF (15 mL) at room temperature for 30 minutes, then 60% NaH dispersed in mineral oil (0.23 g, 5.92 mmol) and

appropriate 1,4-dibromobutane (0.7 mL, 5.92 mmol) was added. The reaction mixture continued to stir at room temperature, and upon completion, the mixture was poured into water, extracted with ethyl acetate, and washed with water and brine. Next, it was dried under anhydrous sodium sulfate, filtered, and evaporated. The final result of the reaction was an oily product. Dark brownish oil product, yield 98%, m.p. 188°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (d, *J* = 5.9 Hz, 1 H), 8.31-8.27 (m, 2 H), 7.85 (d, *J* = 7.3 Hz, 1 H), 7.69 (d, *J* = 8.7 Hz, 2 H), 7.49 (d, *J* = 7.8 Hz, 1 H), 3.50 (d, *J* = 12 Hz, 2 H), 3.43 (d, *J* = 5.9 Hz, 2 H), 2.03-2.00 (m, 4 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 144.6, 136.2, 132.9, 132.3, 130.8, 123.5, 122.6, 119.6, 117.3, 111.2, 60.3, 44.4, 33.9, 29.7.

Synthesis of bivalent β-carboline (T4)

β-carboline (**T2**, 0.18 g, 1.09 mmol) in anhydrous DMF (6 mL) was added by stirring to a solution of intermediate (**T3**, 0.5 g, 1.64 mmol), 60% NaH dispersed in mineral oil (0.21 g, 5.49 mmol), potassium iodide (0.91 g, 5.49 mmol) in anhydrous DMF (10 mL). Next, intermediate **T3** was further reacted to form bivalent β-carboline **(T4)**. The reaction mixture was stirred at room temperature until the reaction was completed, and then it was poured into ice-cold water. After that, the reaction mixture was extracted with ethyl acetate and washed with water and brine. The combined organic layer was dried over anhydrous sodium sulfate, Na_2SO_4, and filtered. The purification process was conducted using column chromatography (DCM/MeOH 100:1 as the eluent) to furnish bivalent β-carboline **(T4)**. Dark brownish oil product, yield 13%, m.p. >270°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 2 H), 8.45 (d, *J* = 5.5 Hz, 2 H), 8.19 (d, *J* = 8.2 Hz, 2 H), 8.10 (d, *J* = 5.5 Hz, 2 H), 7.70-7.66 (m, 2 H), 7.52 (d, *J* = 8.4 Hz, 2 H), 7.36 (t, *J* = 7.5 Hz, 2 H), 5.06-5.00 (m, 4 H), 4.47 (t, *J* = 7.3 Hz, 4 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 143.3, 138.1, 137.5, 133.8, 132.0, 129.9, 122.6, 120.7, 118.5, 115.5, 110.1, 43.5.

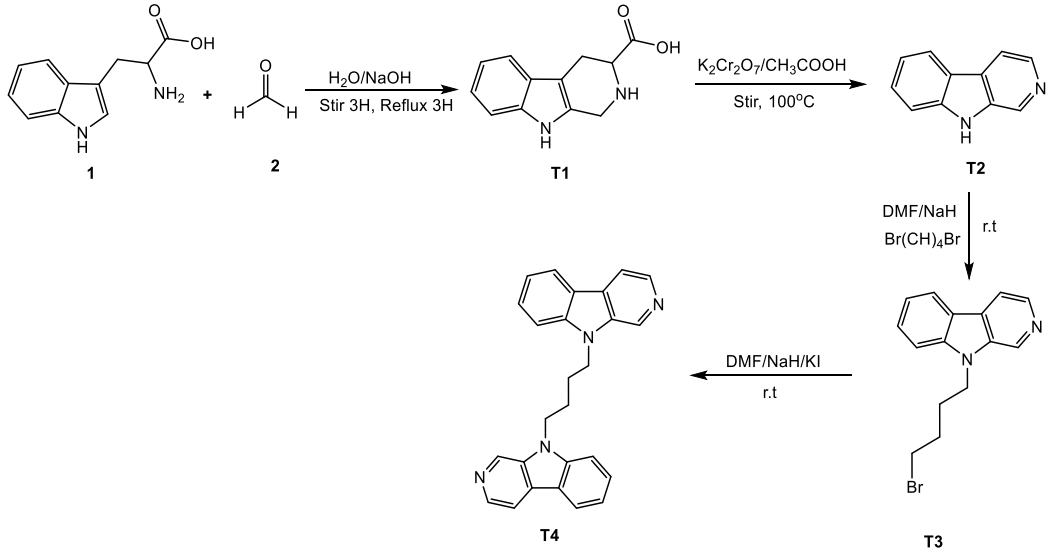
Results and Discussion

The desired bivalent β-carboline **(T4)** synthesis was performed in a four-step method starting from L-tryptophan **(1)** as outlined in Scheme 1. Firstly, the tetrahydro-β-carboline **(T1)** was prepared from the successful reaction of Pictet-Spengler condensation of L-tryptophan **(1)** with formaldehyde. The reaction was further reacted to form β-carboline **(T2)** with a 47% yield via decarboxylation by using potassium dichromate in the presence of acetic acid. Next, the β-carboline structure was alkylated at position-9 by the action of the strong base, NaH in anhydrous DMF, followed by the addition of 1,4-dibromobutane to successfully afford intermediate **T3** with a 98% yield. The intermediate **T3** was present in an oily product form. Finally, the intermediate **T3** was further reacted with β-carboline **(T2)** with a strong base, NaH in solvent anhydrous DMF, in the presence of potassium iodide. The reaction was carried out at room temperature to successfully afford the targeted compound, bivalent β-carboline **T4** (13%). The bivalent β-carboline **(T4)** underwent a purification process using column chromatography and furnished the oily product. ^{1}H NMR and ^{13}C NMR were utilized to characterize the chemical structures of all synthesized compounds in this study.

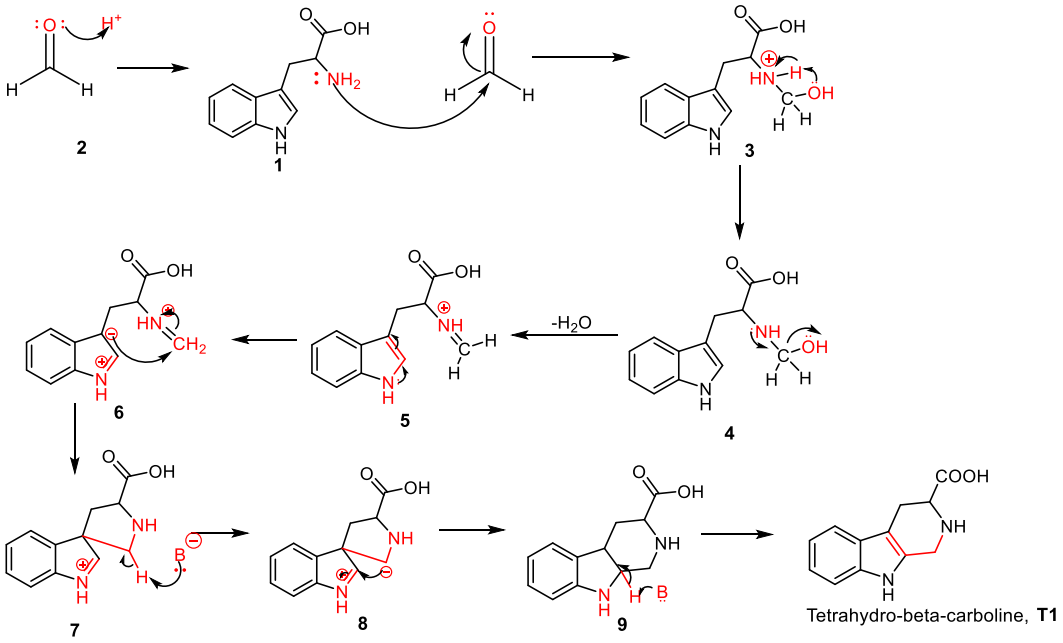
The proposed mechanism for the Pictet-Spengler condensation of L-tryptophan forming tetrahydro-β-carboline **T1** was outlined in Scheme 2. Initially, the mechanism started with the production of an iminium ion **3**, followed by the formation of an imine intermediate **5**, Schiff base, which removed H_2O. Next, the imine intermediate **5** underwent 6-endo-trig cyclizations involved in the base's action, forming a new ring, and the final product was tetrahydro β-carboline **(T1)**.

The proposed mechanism of the oxidation-decarboxylation of tetrahydro-β-carboline **(T1),** which produces β-carboline **(T2)**, is outlined in Scheme 3. Based on Scheme 3, the first step of the mechanism involved abstracting protons from tetrahydro-β-carboline **(T1)** by water. Then a new bond was formed: the double bond and functional group -COOH were removed, forming compound **10**. On the other hand, potassium dichromate that has been treated with acetic acid formed a chromic acid known as the common oxidizing agent for the oxidation reaction. Then, the strong nucleophile site of compound **10** attacked the electrophile site of chromic acid-water repeated proton abstraction in compound **11**. Next, the nucleophile site of -OH was abstracted proton from the α-carbon of compound **12**, forming a new bond. This double bond formed a new aromatic pyridine ring which successfully formed β-carboline **(T2)**.

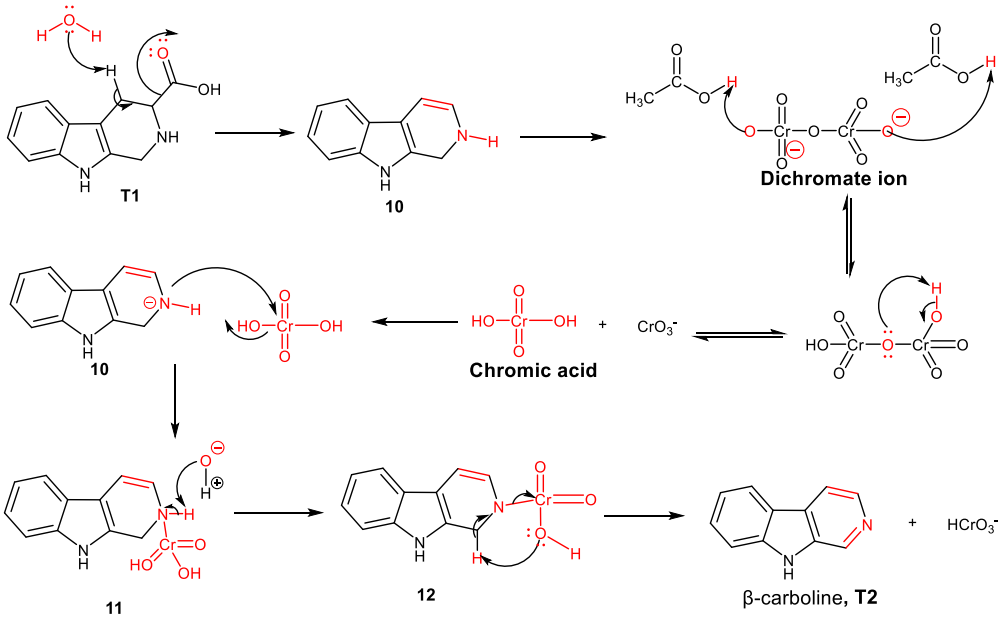
Lastly, the proposed mechanism for the new bivalent of β-carboline **(T4)** is outlined in Scheme 4. The mechanism started with the strong base, NaH, that abstracted protons from β-carboline (T2), producing a strong nucleophile site of compound **13**. Then, the strong nucleophile of N in compound **13** attacked the electrophile of C from the 1,4-dibromobutane, and it removed bromine since it is a good leaving group. Finally, the intermediate of bivalent β-carboline **(T3)** was successfully formed with NaBr. The intermediate bivalent β-carboline **(T3)** further reacted with the monomer β-carboline **(T2)** by the same reaction mechanism as the intermediate bivalent β-carboline **(T3)** to form a symmetrical structure of bivalent β-carboline **(T4)**.



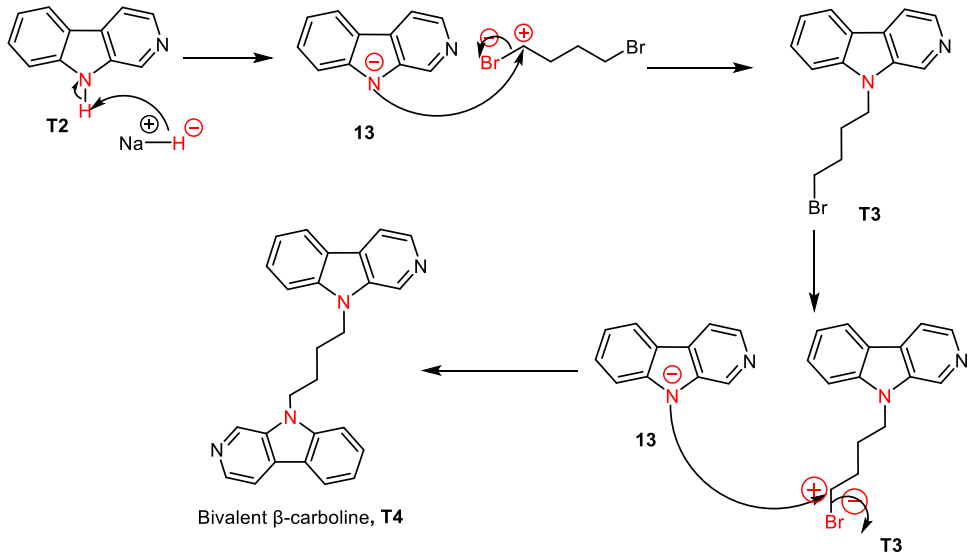
Scheme 1. Overall synthetic route for new bivalent β-carboline compound **T4**



Scheme 2. Proposed mechanism for the Pictet-Spengler condensation of L-tryptophan forming tetrahydro-β-carboline (**T1**)



Scheme 3. Proposed mechanism for the oxidation-decarboxylation of tetrahydro-β-carboline (**T1**) forming β-carboline (**T2**)



Scheme 4. Proposed mechanism for dimerization of β-carboline (**T4**)

Conclusion

In summary, this study has successfully synthesized a new bivalent β-carboline compound **(T4)** from the starting material L-tryptophan **(1)** with a 13% yield. The synthesis reaction is simple and straightforward, using commercially available, safe, and cheaper chemicals. Moreover, the mechanism for the synthesis route of the new bivalent β-carboline compound **(T4)** has been

proposed in this study for future reference. Future studies will evaluate the *in vitro* cytotoxicity potential of T4 in various cancer cell lines to determine its potential as an anticancer agent.

Acknowledgement

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