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Biomimetic synthesis of 1-aryl-2,5-dimethyl pyrroles using egg white nano-ovalbumin at room temperature under solvent-free conditions

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ABSTRACT

Ovalbumin, as the major component of egg-white, is a globular, biocompatible, nontoxic and biodegradable phosphoglyco protein. This protein with the molecular weight of 44.5 kDa, contains 385 residues of amino acids with isoelectric point (pI) of 4.5. Many purification procedures have been reported for egg-white proteins such as gel permeation and anion exchange chromatography. In this study, we have reported a new inexpensive protocol using acetic acid and sodium chloride for extraction and purification of egg white nano-ovalbumin. The effective performance of this protein as a biocatalyst was proved through synthesis of *N*-substituted pyrrole derivatives. This reported innovative biomethodology has some advantages such as less pollution, mild reaction conditions, reusability of biocatalyst and excellent yields.

***Keywords**: Egg white nano-ovalbumin, Biocatalyst, N-substituted pyrroles, Paal-Knorr reaction.*

1. Introduction

Development of bio-catalyzed processes using proteins and enzymes have received considerable attention in chemical synthesis. These non-toxic metal free biocatalysts have high specificity and efficiency with less waste generation. Using biocatalysts under solvent-free conditions provides a new tool for extending environmentally benign and economical methodology [1-8].

Ovalbumin, as major component of egg-white, is a globular, biocompatible, nontoxic and biodegradable phosphoglyco protein. This protein with the molecular weight of 44.5 kDa, contains 385 residues of amino acids with the isoelectric point (pI) of 4.5 [9]. Many purification procedures have been reported for egg-white proteins such as gel permeation and anion exchange chromatography [10], Q sepharose fast flow column [11] and salting out precipitation using ammonium sulfate and sodium sulfate at a specific salt concentration, pH, and temperature [12,13]. In this study, we have reported a new inexpensive protocol using acetic acid and sodium chloride for extraction and purification of egg white ovalbumin.

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Albumin, has many catalytic sites to promote various organic reactions. Recently, bovine serum albumin (BSA) and human serum albumin (HSA) have accelerated Kemp elimination [1], Morita–Baylis–Hillman (MBH) reaction [2], aldol reaction [3], henry reaction [4], thio-michael addition [5], ketone reduction [6], Gewald condensation [7] and Biginelli reaction [8].

Pyrroles have been highlighted as the important biologically active scaffolds because of their presence in numerous therapeutically active compounds such as fungicides, antibiotics, anti-inflammatory drugs [14], cholesterol reducing drugs [15], antitumor agents [16], heme and vitamin B_{12} [17]. The Paal–Knorr reaction is one of the common approaches of the pyrroles synthesis *via* condensation of 1,4-dicarbonyl compounds and primary amines [18]. Previously, the Paal–Knorr condensation was promoted using various catalysts such as xanthan sulfuric acid (XSA) [19], silica sulfuric acid [20], $Bi(NO_3)_3.5H_2O$ [21], indium(III) salts [22], trichloroisocyanuric acid (TCCA) [23], copper iodide on activated carbon (CuI/C) [24], montmorillonite KSF [25], polystyrenesulfonate [26], microwave irradiation [27], $Sc(OTf)_3$ [28], zirconium sulfophenyl phosphonate [29], vitamin B_1 [30], β-CD [31], $ZrCl_4$ [32], $TiCl_2$/nano-γ-Al_2O_3 [33] and $SbCl_5.SiO_2$ [34].

In the current study, we have reported a simple new protocol for extraction of nano-ovalbumin from egg white, and apply it for promotion of Paal–Knorr reaction using aromatic amines. The recyclability of the biocatalyst, mild reaction conditions and easy work-up are some advantages of this new methodology which emphatically demonstrates the benign nature of this protocol.

2. Experimental

2.1. Materials and methods

FT-IR spectra were run on a Bruker, Equinox 55 spectrometer. A Bruker (DRX-400 Avance) NMR was used to record the ^{1}HNMR spectra. Melting points were determined by a Buchi melting point B-540 B.V.CHI apparatus and were uncorrected. MALDI-TOF measurement was carried out by the Applied Biosystems (AB) Model 4800 MALDI-TOF/TOF mass spectrometer. The thermal gravimetric analysis (TGA) was done with NETZSCH TG 209 F1 Iris instrument. The products were characterized by FT-IR, ^{1}H-NMR, and a comparison of their physical properties with those reported in the literature. Field Emission Scanning Electron Microscopy (FESEM) was obtained on a Mira 3-XMU. Energy-dispersive X-ray Spectroscopy (EDS) was recorded using Phenom pro X.

2.2. Preparation of egg white nano-ovalbumin

In a beaker containing 33 g of egg-white, 50 ml of water, 9 ml of concentrated acetic acid and 3 g of NaCl were added and mixed to obtain a fatty solid of nano-ovalbumin which was filtered and washed with water. The fatty solid was purified as a white solid (4.5 g) by washing with acetone and then water at room temperature.

2.3. General procedure for the synthesis of N-aryl-2,5-dimethyl pyrroles

To a mixture of 2,5-hexandione (1.2 mmol) and primary aromatic amine (1 mmol) in a mortar, egg white nano-ovalbumin (0.07 g) was added and the resulting mixture was ground for denoted times in table 2. After the reaction was completed (monitored by TLC, hexane: EtOAc (80:20)), ethanol was added and the catalyst was separated from the product. Then the filtrated solution was poured into cold water and the products were isolated as pure crystals.

3. Results and Discussion

In this research, we report synthesis of *N*-aryl pyrroles using egg white nano-ovalbumin under mild and biocompatible conditions. First, egg white nano-ovalbumin was prepared by a new, simple, low cost and convenient method from egg white (Fig. 1) and identified by FT-IR, MALDI-TOF, FESEM, XRD, EDS and TGA techniques.

Secondly, *N*-arylpyrroles were synthesized in the presence of nano-ovalbumin under grinding conditions (Scheme 1).

The FT-IR spectrum of nano-ovalbumin exhibited a broad band at 3200–3500 cm^{-1} corresponding to stretching vibration of NH and OH (Fig. 2). Two bands at 1622 cm^{-1} and 1532 cm^{-1} correspond to the C=O and C–N stretching vibrations, respectively. The signal at 1073 cm^{-1} is assigned to P–O stretching vibration. Therefore, the FT-IR spectrum of obtained nano-ovalbumin is similar to the previously reported one [35].

The purity and molecular weight of nano-ovalbumin were determined by te MALDI-TOF/TOF tandem mass spectrometry with a single peak at 44.7 kDa (m/z) (Fig. 3) that is in agreement with literatures [9].

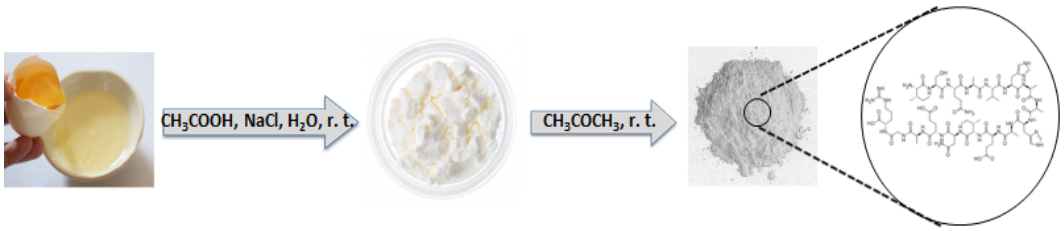
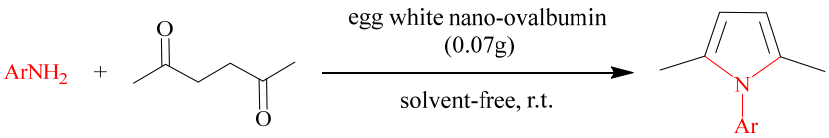


Fig. 1. Preparation of egg white nano-ovalbumin.



Scheme 1. Egg white nano-ovalbumin-catalyzed Paal-Knorr reaction.

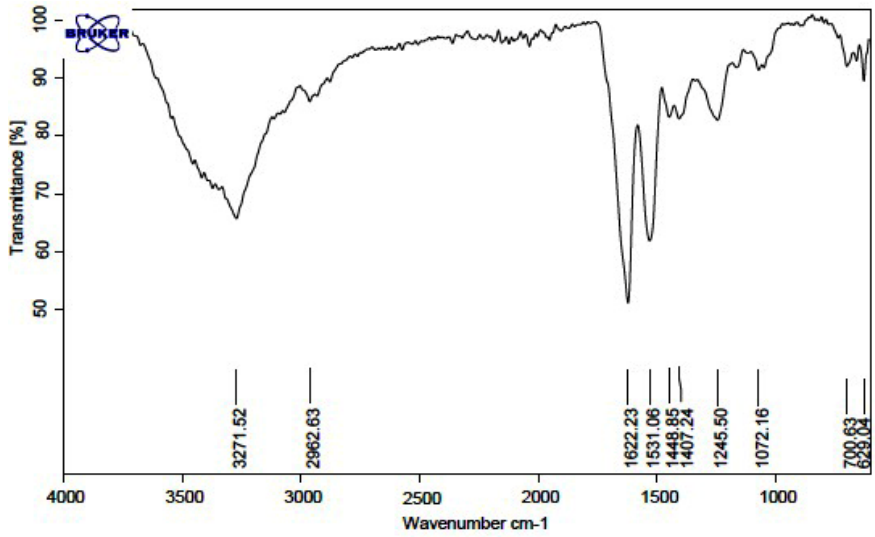


Fig. 2. FT-IR (ATR) spectra of nano-ovalbumin.

The thermo gravimetric results of nano-ovalbumin in the temperature range of 50 °C to 800 °C are presented in Fig. 4. A slight weight loss was attributed to removal of moisture from the catalyst (endothermic effect at 50–110 °C, 1% weight loss). The main weight loss (19 %) at the temperature range of 110–817 °C is due to protein degradation. The char yield of the catalyst in 817 °C is 81.44 %. Therefore, it was found that nano-ovalbumin is appropriate to promote organic reactions at temperatures below 100 °C.

The particles size of nano-ovalbumin was investigated by the FESEM. This image indicates that nanoparticles dimensions are approximately 60 nm on average (Fig. 5).

Fig. 6 depicts the X-ray diffraction (XRD) pattern of nano-ovalbumin in 2θ = 10–80˚. A broad peak observed at 2θ =20-50° describes an amorphous structure for nano-ovalbumin, being in accordance with the previously reported patterns [36].

The existence of the expected elements in the structure of the nano-ovalbumin was approved by energy-dispersive X-ray spectroscopy EDS (EDX) analysis (Fig. 7). The EDS results clearly confirm the presence of C, O, S and Cl elements in the catalyst.

To optimize the reaction conditions, the condensation of aniline with 2, 5-hexandione was done under various conditions (Table 1). The results in table 1 exhibit the optimum conditions of this reaction are the use of 0.07 g of catalyst with 1 mmol of amine under solvent free grinding (Table 1, Entry 10).

It was observed that the catalyst could be reused at least four times with a marginal decrease of its catalytic activity (Table 1, Entries 12-14). In table 1, the catalytic efficiency of egg white nano-ovalbumin in this biomimetic synthesis is compared to those of other previously reported methods at room temperature. The results show that egg white nano-ovalbumin is among the most appropriate ones with respect to efficiency in comparison with the other catalysts.

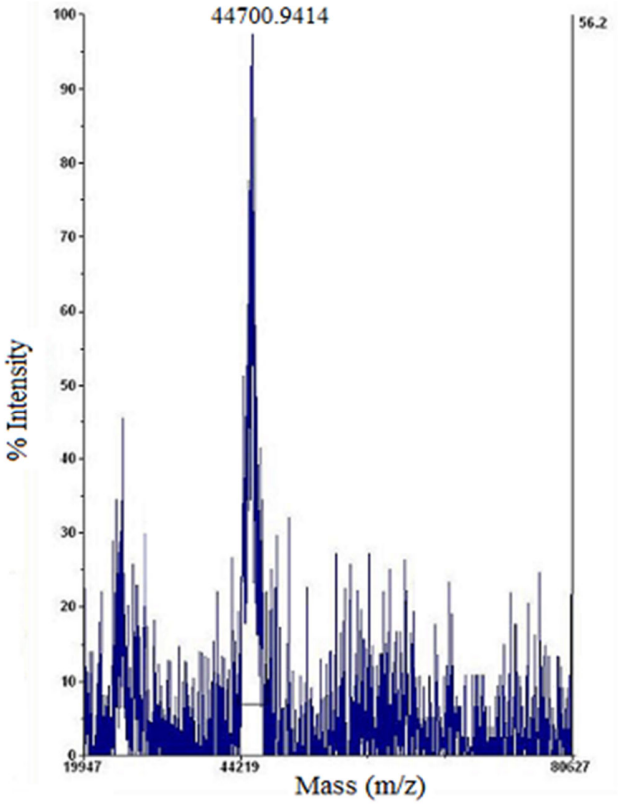


Fig. 3. MALDI-TOF/TOF mass spectrum of nano-ovalbumin (0.016 g of nano-ovalbumin was dissolved in 0.5 ml of 7.0 M aqueous solution of guanidinium chloride).

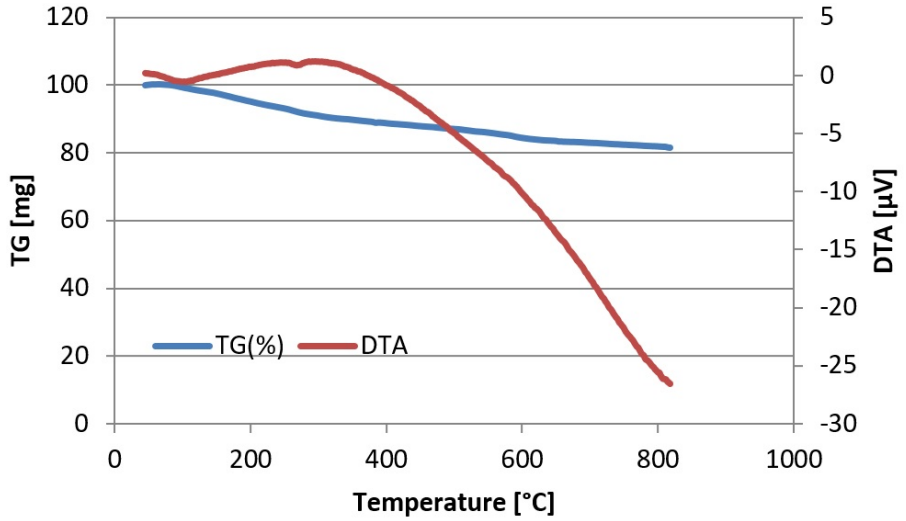


Fig. 4. Thermal gravimetric analysis (TG-DTG) pattern of egg white nano-ovalbumin.

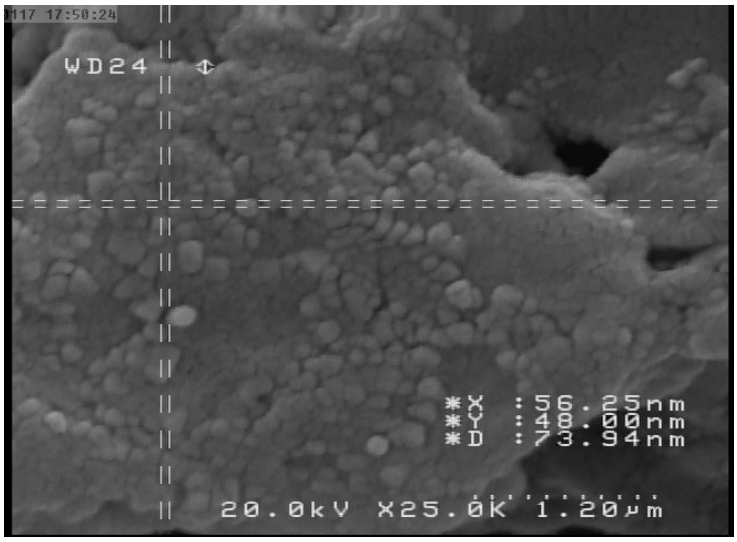


Fig. 5. FESEM image of nano-ovalbumin.

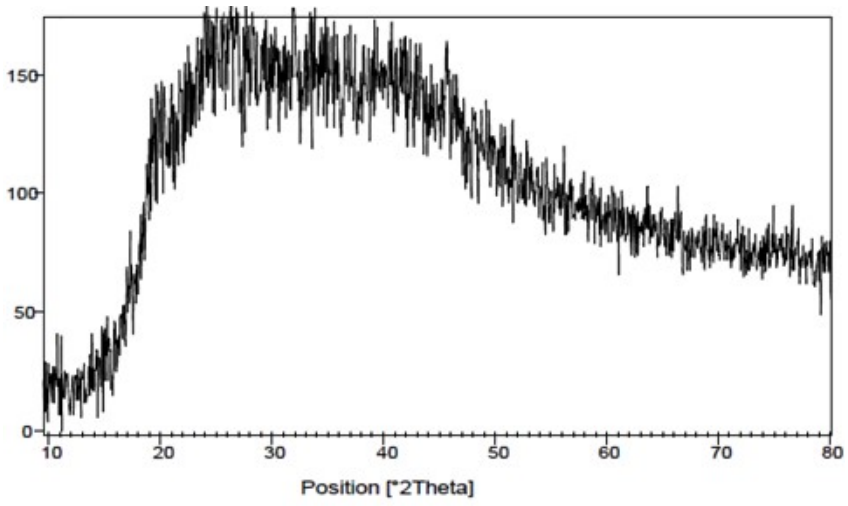


Fig. 6. XRD pattern of nano-ovalbumin.

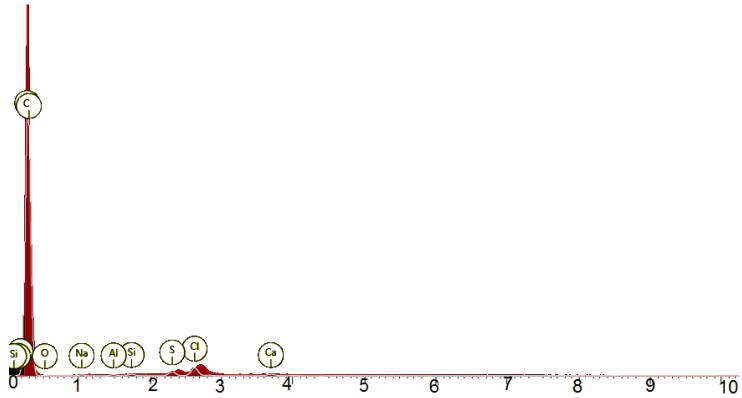
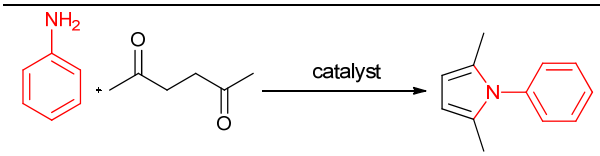


Fig. 7. EDX patterns of nano-ovalbumin.

Table 1. Synthesis *N*-phenyl-2,5-dimethyl pyrrole under various conditions.[a]



Entry	Catalyst (g)	Solvent (Condition)	Time (min)	Yield (%)[b]	Ref.
1	-	- (grinding)	45	-	
2	Nano-ovalbumin (0.07)	Chloroform (r.t.)	45	50	
3	Nano-ovalbumin (0.07)	Ethanol (r.t.)	45	60	
4	Nano-ovalbumin (0.07)	Petroleum ether (r.t.)	45	94	
5	Nano-ovalbumin (0.07)	n-hexane (r.t.)	45	93	
6	Nano-ovalbumin (0.07)	H_2O (r.t.)	45	5	
7	Nano-ovalbumin (0.07)	C_2H_5OH/H_2O (r.t.)	45	55	
8	Nano-ovalbumin (0.03)	- (grinding)	15	85	
9	Nano-ovalbumin (0.05)	- (grinding)	15	93	
10	**Nano-ovalbumin (0.07)**	**- (grinding)**	**15**	**94**	
11	Nano-ovalbumin (0.1)	- (grinding)	15	94	
12	Nano-ovalbumin (0.07), 2nd run	- (grinding)	15	86	
13	Nano-ovalbumin (0.07), 3rd run	- (grinding)	15	78	
14	Nano-ovalbumin (0.07), 4th run	- (grinding)	15	76	
15	XSA (0.10)	-	15	90	[19]
16	SSA (0.15)	-	15	90	[20]
17	$Bi(NO_3)_3.5H_2O$ (1 mmol)	CH_2Cl_2 (r.t.)	10 h	96	[21]
18	$SbCl_5/SiO_2$ (0.30)	Hexane (r.t.)	1 h	98	[34]
19	Vitamin B_1 (5 mol %)	Ethanol (r.t.)	1 h	96	[30]
20	TCCA (0.02 mmol)	CH_3CN (r.t.)	2 h	90	[23]
21	β-CD (0.15)	Water (60 °C)	24 h	86	[31]
22	$Sc(OTf)_3$ (0.1)	-.(30 °C)	25	93	[28]
23	$ZrCl_4$ (0.1)	-.(40 °C)	7	98	[32]
24	$TiCl_2$/nano-γ-Al_2O_3 (0.02	- (grinding)	10	98	[33]
25	I_2 (0.025)	THF (r.t.)	9 h	89	[37]

[a]Reaction conditions: Amine (1 mmol) and 2,5-hexanedione (1.2 mmol).
[b]Isolated yield.

After providing the optimal conditions for the synthesis of *N*-phenyl-2,5-dimethyl pyrrole, the scope and efficiency of the ovalbumin were explored for various aromatic amines (Table 2).

As expected, the reaction of aromatic amines with electron-donating groups gave higher yields than that with electron-withdrawing groups such as NO_2. Moreover, it was found that ortho- and meta-substituted anilines require more reaction time than para substituted anilines; this may be due to an increase of steric hindrance around the amine group. Additionally, the less nucleophilic aromatic amines such as 1-amino naphthalene reacted with 2, 5-hexan-dione in 65% yield. 1,4-diaminobenzene (Table 2, Entry 13) did not give the corresponding bispyrrole and gave monopyrrole as the chief product, but 4,4′-methylendianiline affords the corresponding bispyrrole as the major product (Table 2, Entry 14)

According to the mentioned results, a plausible mechanism is proposed in scheme 2 for the synthesis of 1-aryl-2,5-dimethyl pyrroles in the presence of nano-ovalbumin. The catalytic activity of nano-ovalbumin is due to acidic properties of the side chain ammonium and carboxylic acid groups in some of amino acid residues such as Arg, Lys, Asp and Glu. The pH value of solution of egg white nano-ovalbumin in the guanidinium chloride (7 M) confirms this issue (pH=3.75).

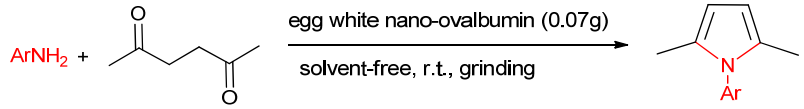
4. Conclusions

Egg white nano-ovalbumin as an environmentally benign biocatalyst was prepared from egg white by a simple protocol. The catalytic efficiency of nano-ovalbumin for the promotion of *N*-aryl-2,5-dimethyl pyrroles synthesis was shown under mild and green conditions. Short reaction times, solvent-less, moderate temperature, reusability of eco-friendly metal-free biocatalyst and excellent yields are advantages of this protocol.

Acknowledgments

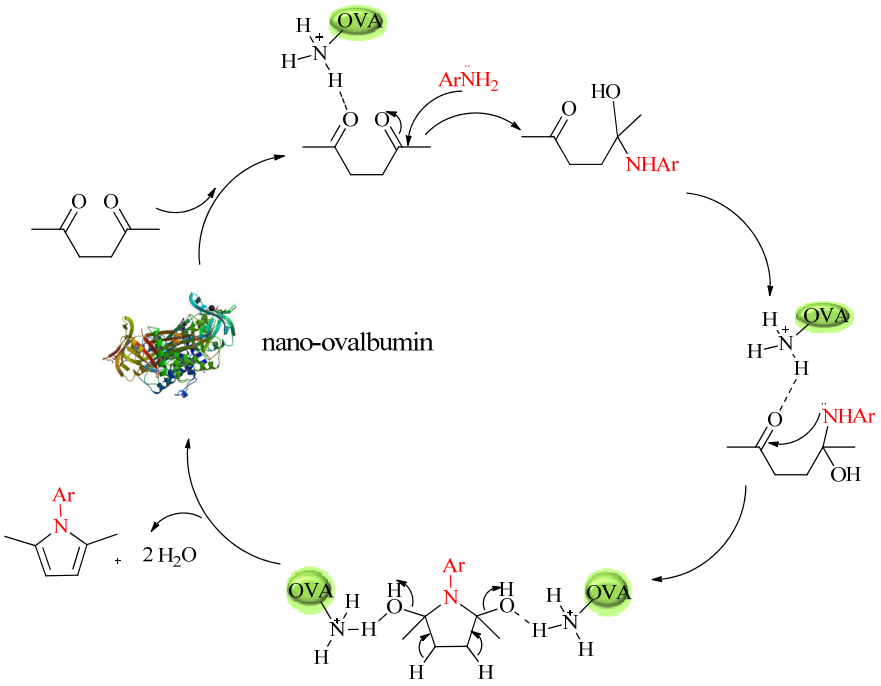
The Research Council of Yazd University was gratefully acknowledged for the financial support for this work.

Table 2. Egg white nano-ovalbumin catalyzed synthesis of *N*-aryl-2,5-dimethyl pyrroles.[a]



Entry	Amine	Time (min)	Yield[b] (%)	m.p. (°C)		Ref.
				Found	Reported	
1	$C_6H_5NH_2$	15	94	49-51	49-50	[38]
2	*m*-$(CH_3)C_6H_4NH_2$	20	90	50-51	45-46	[38]
3	*p*-$(CH_3)C_6H_4NH_2$	15	92	45-47	45-46	[38]
4	*o*-$(OCH_3)C_6H_4NH_2$	45	80	62-64	62-63	[38]
5	*p*-$(OCH_3)C_6H_4NH_2$	9	93	57-58	55-57	[38]
6	*p*-$(Et)C_6H_4NH_2$	10	94	58-59	57.5-58	[39]
7	*m*-$(Cl)C_6H_4NH_2$	15	92	48-50	47-49	[33]
8	*p*-$(Cl)C_6H_4NH_2$	15	94	47-48	47-48	[33]
9	*p*-$(Br)C_6H_4NH_2$	20	88	72-74	74-75	[38]
10	*p*-$(NO_2)C_6H_4NH_2$	90	60	143-144	144-146	[40]
11	*o*-$(OH)C_6H_4NH_2$	25	67	94-96	95-97	[33]
12	1-naphtyl	70	65	118-120	120-122	[40]
13	*p*-$H_2NC_6H_4NH_2$	20	90[c]	95-97	93-95	[33]
14	$H_2NC_6H_4CH_2C_6H_4NH_2$	70	60[d]	119-120	117-119	[33]

[a]Reaction conditions: Amine (1 mmol) and 2,5-hexanedione (1.2 mmol), nano-ovalbumin (0.07 g).
[b]Isolated yield.
[c]Mono-pyrrole has been produced as major product.
[d]Bis-pyrrole has been produced with excess of the diketone.



Scheme 2. Plausible mechanism for egg white ovalbumin catalyzed Paal-Knorr reaction.

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