

Improved Enantioselective Enzymatic Synthesis of (S) - Pregabalin



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Abstract

The manuscript aims to throw light on the route of synthesis of (3S)-3-(Amino methyl)-5-methylhexanoic acid: (PREGABALIN), which is a cost effective and efficient route. Pregabalin is a drug that is used in the treatment of epilepsy, neuropathic pain, fibromyalgia, and generalized anxiety disorder. This process of synthesis makes use of a liquid enzyme to avoid cost hike and improves the overall chiral purity of the final product accompanied with improved higher yields and purity. The investigational studies performed in this article will help improve the existing processes in certain aspects as discussed above.

Introduction

Pregabalin is available in the market under the brand name of Lyrica among others. It is a drug of choice for the treatment of epilepsy, fibromyalgia, sweeping anxiety disorder and neuropathic pain. It effectively relieves neuropathic pain (pain from damaged nerves) that occurs in your arms, hands, fingers, legs, feet, or toes if you are diabetic or in the area of your rash if you once were affected with shingles (a rash that occurs due to infection with herpes zoster and is also painful) [1]. Pregabalin also finds use in the treatment of fibromyalgia (which is a long-lasting condition causing pain, fatigue, muscle stiffness, tenderness of muscles and difficulty in falling asleep or staying asleep [1]. Pregabalin is used in combination therapy with certain other medications for the treatment of various types of seizures in people who have epilepsy. Pregabalin comes under the class of medications that are known as anticonvulsants [2].

In 1990, Pregabalin was synthesized as an anticonvulsant and was invented by medicinal chemist Richard Bruce Silverman at Northwestern University of Chicago, Illinois [3]. The drug received approval in the European Union in the year of 2004. The US also received FDA approval for its use in treatment of epilepsy, post-therapeutic neuralgia and diabetic neuropathic pain in the December of 2004. It was launched in the US market as Lyrica in the fall of 2005 [4]. Pregabalin also received approval in the European Union and Russia (not in US) for treating generalized anxiety disorder [5].

Mechanism of action

Pregabalin's mechanism of action involves reducing the number of pain signals that are sent to the brain by the damaged nerves in the body by binding to certain areas in the brain, which helps reducing nerve pain, seizures, and anxiety (Figure 1) [6]. Pregabalin usually binds to the alpha-2-delta site in the neuron with great affinity and decreases the release of many neurotransmitters that are Ca-dependent (For ex: Glutamate). As it is classified as a GABA (gamma amino butyric acid) analogue, this leads to increase in the levels of GABA in the neurons and subsequent reduction in the associated neuropathic pain, due to its anxiolytic and anti-convulsive effects [7].

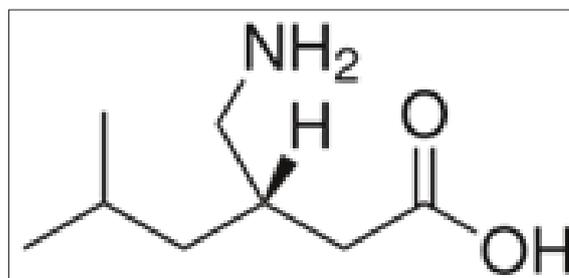


Figure 1: Pregabalin structure.

The common side effects of Pregabalin include [8]:

- i. Sleepiness or drowsiness

- ii. Confusion
- iii. Forgetfulness
- iv. Poor motor coordination
- v. Dry mouth
- vi. Vision related problems
- vii. Weight gain

There are also some potentially serious side effects of Pregabalin [8]:

- i. Angioedema
- ii. Drug misuse or addiction
- iii. Increased risk of suicide

Also, high doses of Pregabalin consumption over a longer period of time might lead to addiction. However, if prescribed dose is taken, the risk of addiction is low. (3S)-3-(Amino methyl)-5-methylhexanoic acid (PREGABALIN) is of great importance and the current procedure of its preparation had much scope of improvement and hence it was felt worthwhile to examine an alternate means to prepare this compound (Figure 1).

Material and Methods

Enzymes

TL lipase liquid was purchased from Advanced enzymes.

Chemical reagents

Isovaleraldehyde was purchased from Spectrochem

Analytical Methods

Gas chromatography: Gas chromatographic analysis during the preparation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester and 2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester was performed on Agilent Technologies Gas chromatography instrument using DB-1, (30 m × 0.53mm, 3.0 μm) capillary

column. The elution was carried out with nitrogen as carrier gas and the eluents were detected by Flame ionization detector. The retention time was 19.2 min and 20.18 min for the preparation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester and 2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester.

Mass Spectrometry: Electron Spray Ionization-Mass spectra (ESI-MS) was measured using Agilent 1100 LC/MSD Trap SL instrument.

NMR spectra: ¹H NMR spectra was recorded on a Bruker Avance 300 MHz spectrometer. The spectra was recorded with CDCl₃ as solvent and trimethylsilane (TMS) as an internal standard for measuring chemical shifts. A region from 0 – 6 ppm was scanned for all the samples.

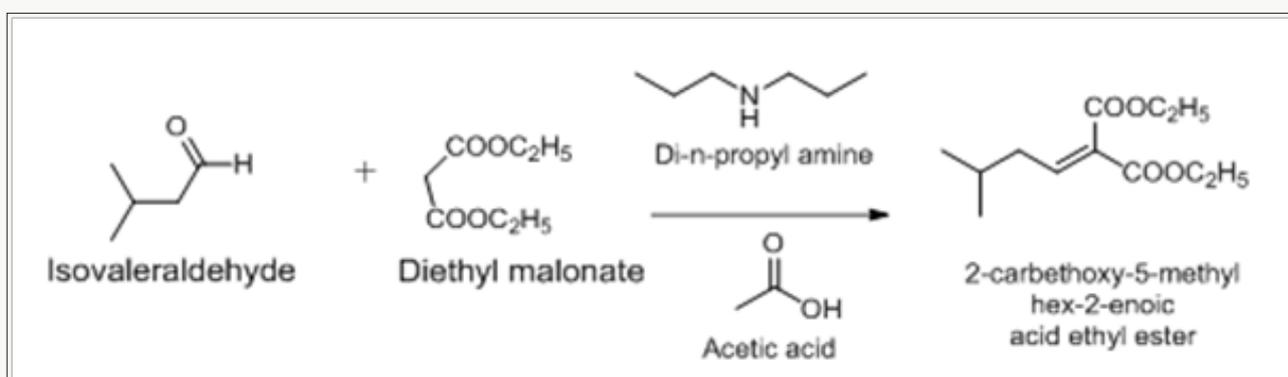
Specific Optical Rotation: Specific optical rotation was measured using Perkin-Elmer 243 polarimeter. The specific optical rotation of the compounds were measured at the sample concentration of 1.1% w/v in methanol at 25 °C.

Results and Discussion

The preparation of Pregabalin involves 4 reaction steps which will be studied in this manuscript and will be discussed in detail illustrating some noteworthy observations for an improved process.

Preparation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester

This reaction involves the well-known naming reaction Claisen condensation between isovaleraldehyde and diethyl malonate in the presence of di-n-propyl amine and acetic acid and refluxed azeotropically to obtain ene-diester. In the reported procedure, the reactants Di-n-propyl amine and acetic acid were added in two lots, one in the starting, and other after 4 hrs. In our process, we added acetic acid and di-n-propyl amine in the starting only and that too 0.05 equivalent of di-n-propyl amine and 0.13 equivalent of acetic acid, in catalytic amount, that results in cost reduction and increased HPLC purity, with no change in the overall yield (Scheme 1).

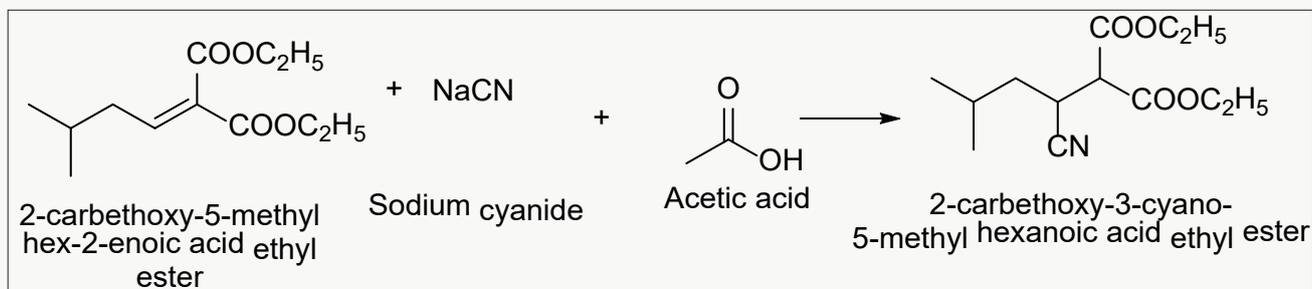


Scheme 1: Preparation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester.

Preparation of 2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester

Sodium Cyanide was used instead of Potassium Cyanide that is recommended in the reported procedure. NaCN is much safer to handle when compared to KCN in industrial use, hence, the change

was made. The reported procedure also mentioned distillation of the reaction solvents at 70-100°C but this gave rise to decreased yield of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester. Hence, an alternative was taken of vacuum distillation, which corrected the yield in a positive way (Scheme 2).

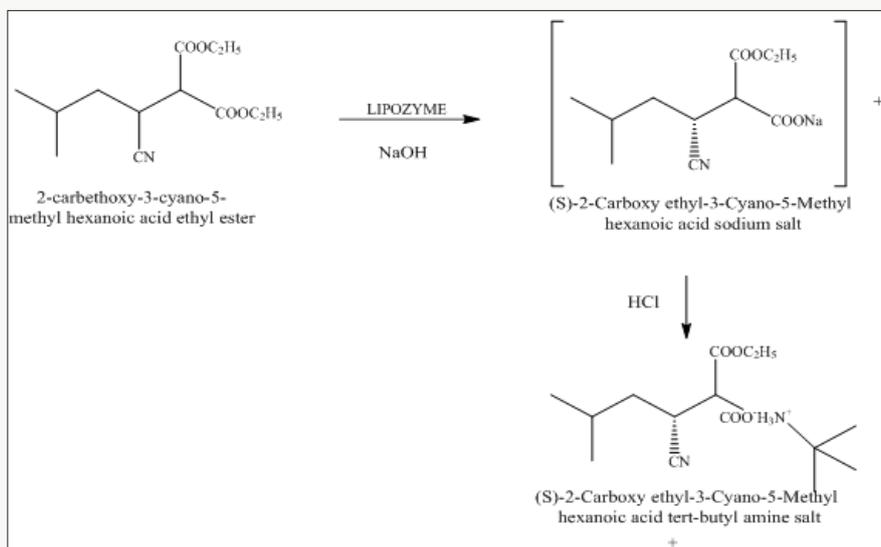


Scheme 2: Preparation of 2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester.

Preparation of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester salt

This reaction involves selective enzymatic hydrolysis using liquid enzyme TL lipase liquid to form sodium salt of 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid, which was then further

acidified with Hydrochloric acid to give 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid, which was then treated with *t*-butyl amine and *S* (-)- α methyl benzylamine to form its corresponding salt. These salts can be further converted to (S)-3-cyano-5-methyl-hexanoic acid ethyl ester and finally to Pregabalin (Scheme 3).



Scheme 3: Preparation of 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt.

Some of the other enzymes were screened in our lab, out of which reaction proceeded only with immobilized enzyme but the overall cost is affected because of the high market price of the immobilized enzyme over liquid enzyme. Since no significant change in the overall yield was found, we also proceeded with TL Lipase liquid to have more of an economical process (Table 1).=

The 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid when isolated was found to be unstable and hence, 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt was prepared

in-situ (Table 2). The chirality of the 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt was checked by its Specific Optical Rotation (SOR) which was found to be 33.480 (for the R-isomer) and -36.80 (for the S-isomer).

Preparation of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester

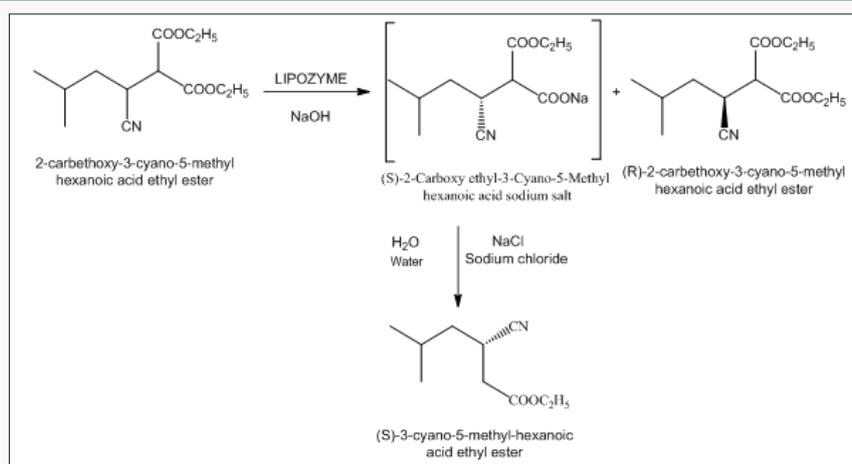
Preparation of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester was done in situ in this step, without isolation of 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt (Scheme 4).

Table 1: List of enzymes screened.

Enzyme used	Yield of (S)-3-cyano-5-methyl Hexanoic acid ethyl ester	Reaction time
TL Lipase Immobilized	0.27w/w	24hrs
Novozym 435	No reaction occurred	14 days
TL Lipase liquid	0.27w/w	24hrs

Table 2: Comparison data.

Intermediate name	Percentage purity
2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid	63%
2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt	99%

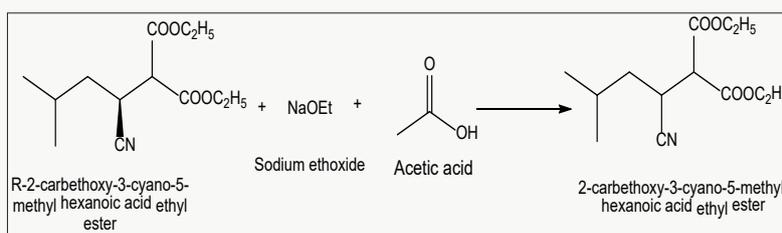
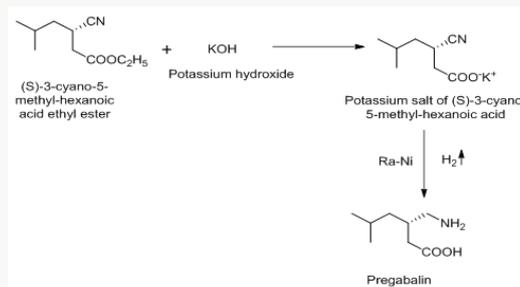
**Scheme 4:** Preparation of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester.

Preparation of rac-3-cyano-5-methyl-hexanoic acid ethyl ester

Describes the reaction wherein the unreacted R-2-carboxy-3-cyano-5-methyl hexanoic acid ethyl ester is reversed to form racemic 2-carboxy-3-cyano-5-methyl hexanoic acid ethyl ester using sodium ethoxide (Scheme 5).

Preparation of (3S)-3-(Amino methyl)-5-methylhexanoic acid or PREGABALIN

This reaction involves the hydrolysis of ethyl ester, followed by its hydrogenation with Raney Nickel to yield the final product i.e. Pregabalin (Chiral purity >99.9% and purity > 99.8%) (Scheme 6).

**Scheme 5:** Preparation of racemic 2-carboxy-3-cyano-5-methyl hexanoic acid ethyl ester.**Scheme 6:** Preparation of (3S)-3-(Amino methyl)-5-methylhexanoic acid or PREGABALIN.

Characterization of the 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt: Detailed characterization was conducted to confirm the structure of 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt intermediate of Pregabalin.

a) IR spectroscopy:

Instrument used: Perkin Elmer Spectrum one FTIR spectrometer

Method used: 1% KBr method (Table 3).

Table 3: IR data of the 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt.

Wave number (cm ⁻¹)	Assignment	Mode of vibration
3437, 3049	N-H	Stretching
2959, 2917, 2881, 2836	Aliphatic C-H	Stretching
2240	C≡N	Stretching
1734	C=O (Ester)	Stretching
1629	C=O (Acid)	Stretching
1577	N-H	Bending
1405, 1384, 1365	Aliphatic C-H	Bending
1221, 1185, 1149, 1081, 1022	C-O/C-N	Stretching

b) NMR: (Table 4)

Table 4: NMR data of the 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt.

Position	1H	d(ppm)	J(Hz)	¹³ C	DEPT
1,1'	6H	0.96	t(6.0)	23.21, 24.99	CH ₃
2	1H	1.78-1.92	m	32.57	CH
3Ha	1H	1.34-1.41	m	50.00	CH ₂
3Hb	1H	1.61-1.71	m		
4	1H	3.55	d(7.8)	28.58	CH
5	1H	3.3-3.38	m	59.62	CH
6	-	-	-	173.67	-
7	2H	4.23-4.30	m	65.04	CH ₂
8	3H	1.26	t(6.0)	16.09	CH ₃
9	-	-	-	175.07	-
10	-	-	-	54.76	-
11,11',11''	9H	1.37	s	29.45	CH ₃
12	-	-	-	125.25	-

c) Mass spectroscopy: (Table 5)

Table 5: Describes the mass number of the 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt.

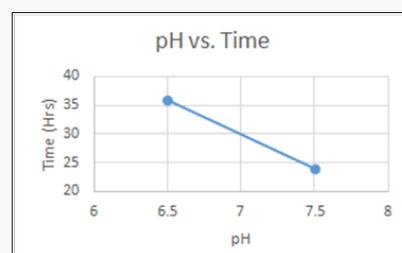
m/z	ions
226	(M-H)-

Optimization of parameters during the preparation of 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid tert-butyl amine salt:

Table 6: pH variations.

pH	Reaction time (h)	Yield (w/w)
6-7	36	0.49
7-8	24	0.5
8-9	24	0.4

i. pH variations that were studied are explained in the table below: (Table 6) (Graph 1)

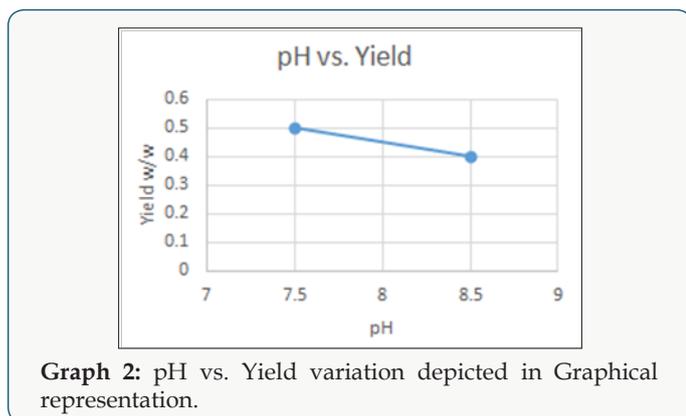


Graph 1: pH vs. Time variation depicted in Graphical representation.

Table 7: Temperature variations.

Temperature (oC)	Reaction time (h)	Yield (w/w)
20-25	36	0.49
25-30	24	0.5

ii. Temperature variations that were studied are explained in the table below: (Table 7) (Graph 2)



iii. Distillation variations

In the reported procedure, distillation was done at 70-1000C and this led to lesser yield of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester that was prepared in the further reactions. On the other hand, when vacuum distillation was performed, the yield obtained was significantly higher (Table 8).

High vacuum distillation was performed at the end of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester since traces of the diethyl malonate impurity would retard the reaction of preparation of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester (Table 9).

Table 8: Purity comparison of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester before and after vacuum distillation.

S.No.	Type	GC purity	Isomer	Diethyl malonate
1.	Before High vacuum distillation	76.88%	11.84	3.68
2.	After High vacuum distillation	88.23%	10.54	0.27

Table 9: Yield differences sighted for simple distillation vs. vacuum distillation.

Name of the intermediate	Yield (w/w)	
	Atmos. distillation	Vacuum distillation
2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester	2.52	2.4
2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester	0.74	1.4
(S)-3-cyano-5-methyl-hexanoic acid ethyl ester	0.1	0.27

This difference shown in the above table was due to degradation of the compound at higher temperatures during distillation that was performed at 70-1000C. Thus, the yield of the subsequent reactions was also affected. So, it was clear that if vacuum distillation was performed in during the preparation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester and 2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester, yield was higher when compared to distillation at temperature 70-1000C.

Purification techniques employed: At certain stages of the Pregabalin synthesis route, purification techniques were employed to increase the yield of the intermediate products of the subsequent stages.

- Fractional distillation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester was done that resulted in the increase in yield of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester.
- Column purification of 2-carbethoxy-3-cyano-5-methyl hexanoic acid ethyl ester was also done which in turn increased the purity of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester and 2-Carboxy ethyl-3-Cyano-5-Methyl hexanoic acid ter-butyl amine salt.

Conclusion

Modifications done in the preparation of 2-carbethoxy-5-methyl hex-2-enoic acid ethyl ester indirectly helped improve the

yield of (S)-3-cyano-5-methyl-hexanoic acid ethyl ester, during the synthesis of (3S)-3-(Amino methyl)-5-methylhexanoic acid. Higher purity and cheaper cost of the product, are the primary benefits that can be drawn from this improved synthetic procedure of (3S)-3-(Amino methyl)-5-methylhexanoic acid. Time saving is yet another advantage of the proposed method of synthesis. Hence, it can be concluded by saying that the proposed method in this manuscript will help modify the existing synthesis of (3S)-3-(Amino methyl)-5-methylhexanoic acid and give way to better process.

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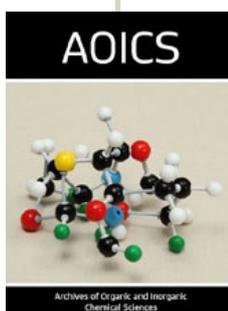
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