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*Article*

# Nano-Structured $Na_2CaP_2O_7$: a New and Efficient Catalyst for One-Pot Synthesis of 2-Amino-3-Cyanopyridine Derivatives and Evaluation of Their Antibacterial Activity

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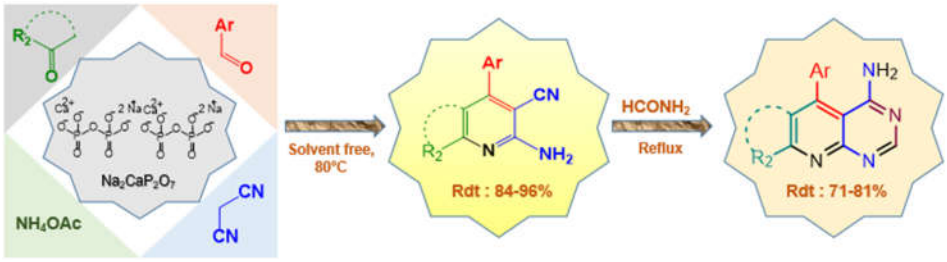
**Abstract:** A facile and novel synthesis of thirteen 2-amino-3-cyanopyridine derivatives 5(a-m), by a one-pot multicomponent reaction (MCRs), is described for the first time, starting from aromatic aldehydes, malononitrile, methyl ketones, or cyclohexanone and ammonium acetate in the presence of the nanostructured diphosphate Na2CaP2O7 (DIPH) at 80 °C, under solvent-free conditions. These compounds were synthesized in short reaction times with good to excellent yields (84-94%). The diphosphate Na2CaP2O7 is used as an efficient catalyst, environmentally, easy handling, non-toxic, stable, and reusable. Our study was strengthened by the synthesis of five new pyrido[2,3-d]pyrimidine derivatives **6(b, c, g, h, j)** by intramolecular cyclization of 2-amino-3-cyanopyridines **5(b, c, g, h, j)**, with formamide. The synthesized products were characterized by FT-IR, SEM, XRD, TEM, $^1$H NMR, $^{13}$C NMR, TLC, and BET. The operating conditions were optimized using a model reaction in which the catalyst amount, temperature, time, and solvent effect were evaluated. The antibacterial activity was tested against Gram-positive and Gram-negative strains for the synthesized compounds.




**Keywords:** Catalyst; antibacterial activity; solvent-free conditions; heterogeneous catalysis; Synthesis; cyanopyridines; pyrimidines; nano-structured $Na_2CaP_2O_7$; catalyst recovery.

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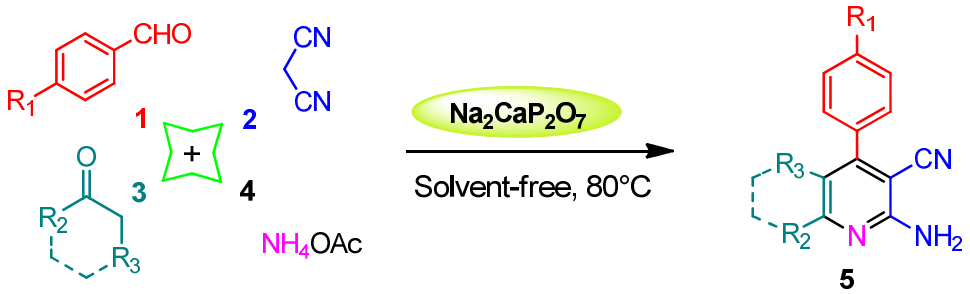
## 1. Introduction

Pyridine and its derivatives are known to be the important chemical compounds in medicinal chemistry [1–3]. Indeed, they are key scaffolds in biologically active and naturally occurring substances. Many pharmacological properties of pyridine and its derivatives have been reported including antimicrobial [4], anticancer [5], anti-inflammatory [6], anti-viral [7], anti-diabetic [8], and anti-malarial activities [2]. On the other hand, heterocyclic systems containing the b-enaminonitrile moiety represent a class of intermediates known to be highly reactive and used as precursors for synthesizing newly heterocyclic compounds [9–11]. The literature mentioned that different and several pyridine derivatives, particularly, 2-amino-2-cyanopyridines had been prepared as target structures using sustainable catalyst materials [12] coupling with environmentally benign protocols. Moreover, it is interesting to note that multicomponent reactions (MCRs) have attracted more attention of many researchers due to their efficiency and simplicity in the last decade. MCRs are used for the preparation of biologically active compounds from readily available commercial reagents in a single step [13]. Further, in our case, the combination of this process with a solvent-free medium for the preparation of these heterocyclic derivatives makes the use of MCRs comply with the principles of green chemistry.

Several studies have reported that the usefulness and importance of these processes were exploited for the synthesis of 2-amino-3-cyanopyridine in the presence of various catalysts such as the ytterbium perfluorooctanoate [Yb(PFO)$_3$] [14], $Bu_4N^+Br^-$ [15], Cu@imineZCMNPs [16], Cellulose-$SO_3H$ [17], MgO [18], $HBF_4$ [19], $Fe_3O_4$@$SiO_2$@$(CH_2)$Im}C$(CN)_3$ [20], $FePO_4$ [21] and poly(ethylene glycol) (PEG-400) [22]. However, these procedures have disadvantages such as long reaction time, harsh reaction conditions, the need for excess amounts of reagents, the use of organic solvents and toxic reagents, and the non-recoverability of the catalyst. Thus, the development of a new efficient and environmentally friendly procedure for the synthesis of 2-amino-3-cyanopyridines is of significant interest. The object of this work is to study and to examine particularly $Na_2CaP_2O_7$ as an alternative catalyst since it has received increased attention recently, mainly in the environmental field [23–25].

In continuation of our investigation and following our results obtained in a previous work based on the use of $Na_2CaP_2O_7$ as a catalyst in organic synthesis [26–28], particularly in the synthesis of heterocyclic compounds via multi-component reactions in an eco-friendly medium [29,30]. Herein, we report here an efficient and rapid one-pot synthesis of thirteen 2-amino-3-cyanopyridine derivatives by condensation of aromatic aldehydes, malononitrile, methyl ketone or cyclohexanone and ammonium acetate, using a nano-structured diphosphate $Na_2CaP_2O_7$ as a heterogeneous catalyst under solvent-free reaction conditions at 80°C (Scheme 1). Five prepared 2-amino-3-cyanopyridine were converted to pyrido[2,3-*d*]pyrimidines, the evaluation of the antibacterial activity of all prepared compounds was studied.




**Scheme 1.** The synthesis of 2-amino-3-cyanopyridine derivatives catalyzed by $Na_2CaP_2O_7$.

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## 2. Results and Discussion

### *2. 1. Synthesis and characterization of $Na_2CaP_2O_7$ nanoparticles*

The $Na_2CaP_2O_7$ nanoparticles are synthesized according to literature procedures [31]. Nanostructured pyrophosphate was synthesized using the dry method. The stoichiometric amounts of sodium carbonate ($Na_2CO_3$), calcium carbonate ($CaCO_3$), and ammonium dihydrogen phosphate ($NH_4.H_2PO_4$), respectively, with a molar ratio of 1:1:2, were mixed in an agate mortar. The mixture was transferred into a porcelain crucible and heated progressively from 100 to 600 °C (Figure 1). Then, the obtained powder was characterized by X-ray diffraction, Fourier transform infrared spectroscopy, scanning, and transmission electron microscopy.

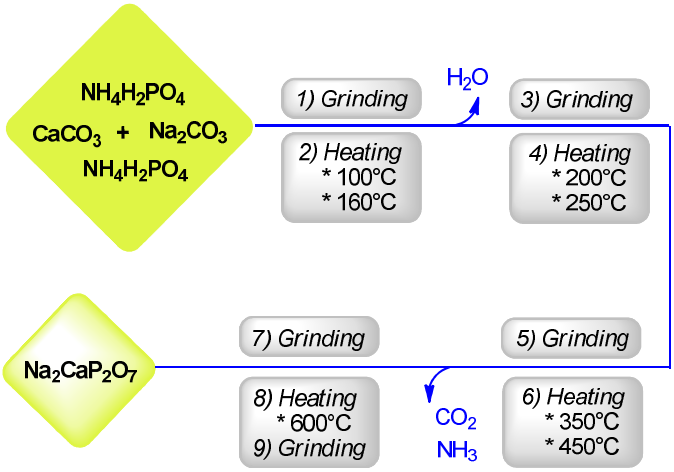


**Figure 1.** Schematic drawing describing the preparation of $Na_2CaP_2O_7$ nanoparticles.

### *2. 2. Characterization of the diphosphate $Na_2CaP_2O_7$*

The X-Ray Diffraction pattern of the diphosphate $Na_2CaP_2O_7$ is shown in Figure 2. It is observed that all diffraction peaks are consistent with the standard data of the ICSD collection code: 89468. Crystals of diphosphate $Na_2CaP_2O_7$ are triclinic structure, space group P1bar and crystal parameters a = 5.361 Å, b = 7.029 Å and c = 8.743 Å, V = 308.31 $Å^3$ and Z = 2.

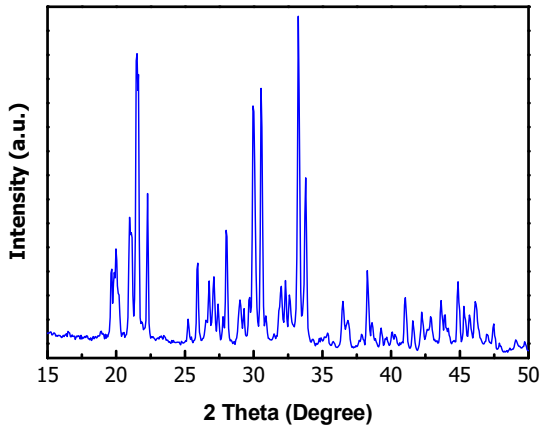


**Figure 2.** X-ray powder diffraction pattern of $Na_2CaP_2O_7$.

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The FT-IR spectrum of $Na_2CaP_2O_7$ is presented in Figure 3. The band at 720 $cm^{-1}$ and 888 $cm^{-1}$ may be assigned respectively to symmetrical and anti-symmetric vibration of P-O-P. These bands confirm the presence of pyrophosphate groups $P_2O_7$. Two fields shared the associated vibrations of the $PO_4$ groups: a field of symmetrical vibrations (997 $cm^{-1}$, 1031 $cm^{-1}$) and a field going from 1112 $cm^{-1}$ to 1278 $cm^{-1}$. The described bands confirm that $Na_2CaP_2O_7$ has been prepared.

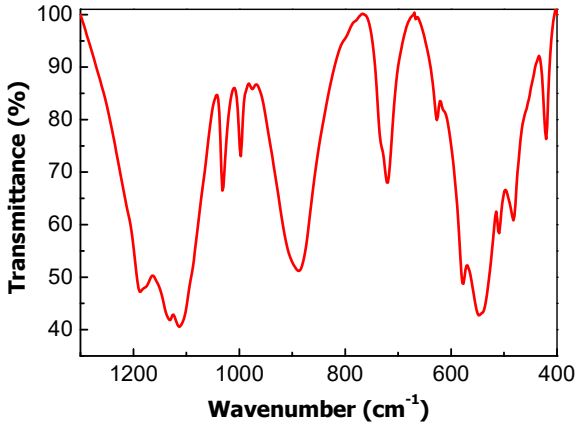


**Figure 3.** FT-IR spectrum of $Na_2CaP_2O_7$.

The morphology of the surface of $Na_2CaP_2O_7$ was observed by scanning electron microscopy (SEM, Figure 4). The $Na_2CaP_2O_7$ shows a homogeneous microstructure that consists of layers of various sizes and forms.

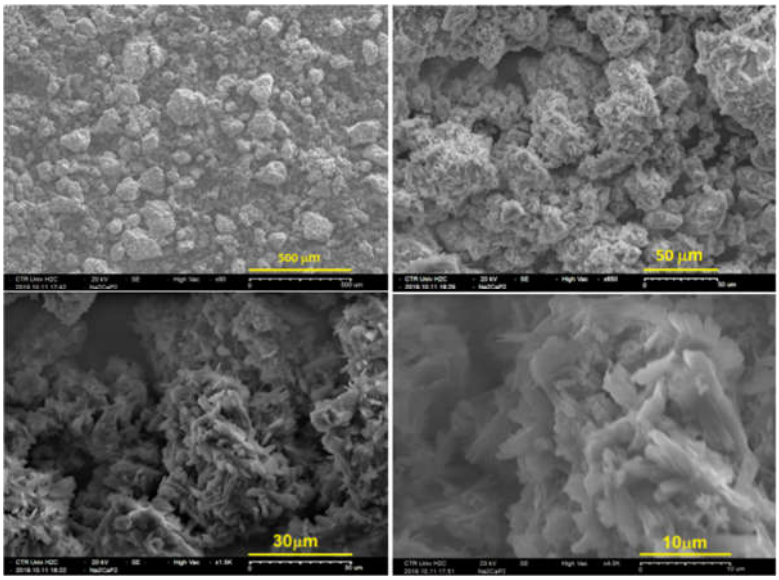


**Figure 4**. SEM images of $Na_2CaP_2O_7$ at different magnifications.

Transmission electron microscopy (TEM) was further used to study the morphology and microstructure of the $Na_2CaP_2O_7$. Figure 5 shows rod-like nanoparticles that agglomerate to form superstructures with different grain crystal aspect ratios. The specific surface of the $Na_2CaP_2O_7$ areas was determined by the Brunauer-Emmett-Teller

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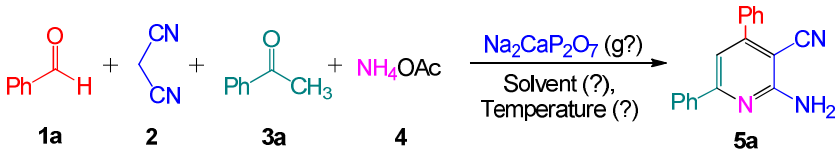
(BET) method from the adsorption-desorption isotherm of $N_2$ at 77 K. It was found to be 2.4 $m^2.g^{-1}$.


**Figure 5.** TEM micrographs of $Na_2CaP_2O_7$ nanopowder.

*2. 3. Optimization of reaction conditions*

In order to establish the optimum condition synthesis of substituted 2-amino-3-cyanopyridines, the reaction of benzaldehyde **1a** (1 mmol), malononitrile **2** (1.1 mmol), acetophenone **3a** (1 mmol), and ammonium acetate **4** (1.5 mmol) was chosen as a model, and performed various conditions, $Na_2CaP_2O_7$ was used as a catalyst (Scheme 2).




**Scheme 2.** Synthesis of 2-amino-3-cyanopyridine **5a**.

*2. 4. Influence of the amount of the catalyst*

To optimize the catalyst amount, the model reaction was performed with different quantities of the catalyst and according to obtained results (Table 1, entries 2-8). 0.05 g (20%) of the nano-structured diphosphate $Na_2CaP_2O_7$ was chosen as the best catalyst amount, with that the reaction can be achieved in 30 min affording 94% yield of 5a (Figure 6). On increasing the amount of $Na_2CaP_2O_7$, there was no improvement in the product yields (Table 1, entries 7-8).

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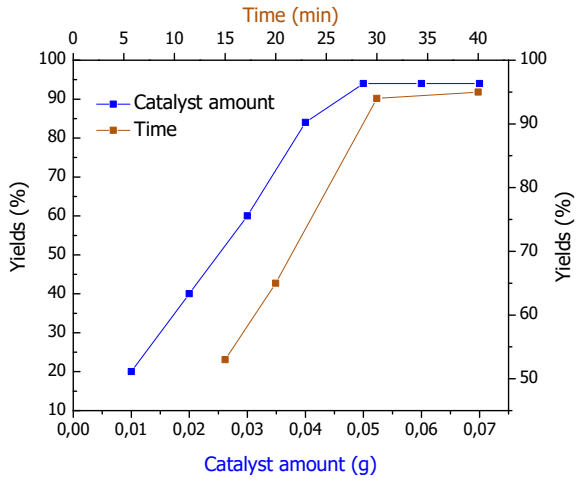


**Figure 6.** Influence of the amount of the catalyst $Na_2CaP_2O_7$ and réaction time in the synthesis of 2-amino-3-cyanopyridine **5a.**

This may be because of the attainment of the maximum conversion efficiency of the catalyst. In the absence of the catalyst, no new product was detected.This result anticipates that the catalyst plays an important role in this transformation (Table 1, Entry 1).

*2. 5. Influence of the reaction time*

Temperature and time also play a significant role in reaction kinetics. In order to study the effect of these two parameters, a varied range of temperature (40-100°C) is used to carry out the model reaction for different time periods (15-120 min) and by using 0,05 g of $Na_2CaP_2O_7$ (Table 1, entries 9-14). The first period, time ranges from 15 to 30 min, is characterized by significant changes in the yield of the product. During this period, the product yield increases by 12% for 5 min (from 15 to 20 min) and 29% during the following 10 min (from 20 to 30 min). At 80°C for a reaction time of 30 min, the percentage yield (94%) was found as maximum. The yield of **5a** remained unchanged after prolonged reaction time and increased temperature (Table 1, entries 11, 13, 14).

**Table 1.** Optimization of the amount of the catalyst, the temperature, and the reaction time in the synthesis of 2-amino-3-cyanopyridine **5a.**

| | Entry | Amount of catalyst (g) | Temperature (°C) | Time (min.) | Yields (%) |
|---|---|---|---|---|---|
| **absence of a catalyst** | 1 | 0 | 80 | 120 | - |
| **Influence of the amount of the catalyst** | 2 | 0.01 | 80 | 30 | 20 |
| | 3 | 0.02 | 80 | 30 | 40 |
| | 4 | 0.03 | 80 | 30 | 60 |
| | 5 | 0.04 | 80 | 30 | 84 |
| | **6** | **0.05** | **80** | **30** | **94** |
| | 7 | 0.06 | 80 | 30 | 94 |
| | 8 | 0.07 | 80 | 30 | 94 |
| **Influence of the temperature and reaction time** | 9 | 0.05 | 80 | 20 | 65 |
| | 10 | 0.05 | 80 | 15 | 53 |
| | 11 | 0.05 | 80 | 40 | 95 |
| | 12 | 0.05 | 40 | 30 | 75 |

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| | | | | |
|---|---|---|---|---|
| 13 | 0.05 | 60 | 30 | 85 |
| 14 | 0.05 | 100 | 30 | 94 |

*2. 6. Influence of the solvent*

The effect of solvent on the rate of reaction was also investigated by performing the model reaction in the presence of 0.05 g of $Na_2CaP_2O_7$ for a reaction time of 30 min in various solvents (1 mL) such as water, ethanol, dichloromethane (DCM), ethyl acetate (EtOAc), *n*-hexane and acetonitrile (MeCN). Figure 7 summarizes the result of various solvents on the percentage yield of 2-amino-3-cyanopyridine **5a**. It was observed that when solvents were used, the yield decreased. However, a high yield of the target product was obtained when the reaction was performed under solvent-free conditions (Figure 7).

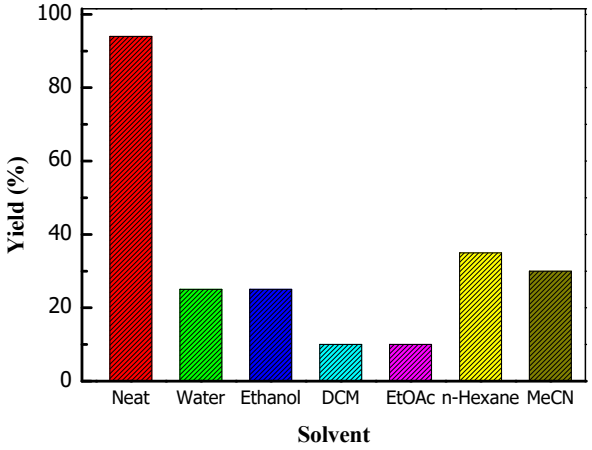
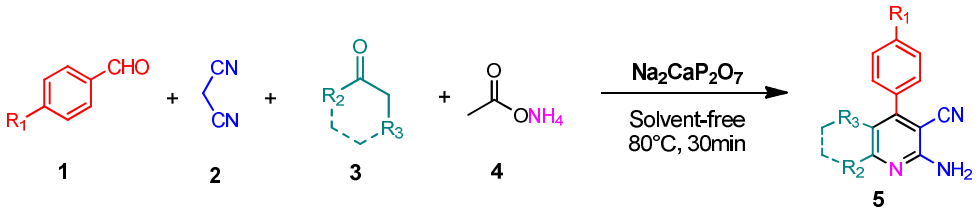



**Figure 7**. Influence of the solvent in the catalytic synthesis of 2-amino-3-cyanopyridine **5a.**

After determining the optimal conditions for the synthesis of 2-amino-3-cyanopyridine **5a**, the reactions of different aromatic aldehydes containing substituents in the aromatic ring such as Me, OMe, Cl, and $NO_2$ with malononitrile **2**, acetophenone derivatives or cyclohexanone **3** and ammonium acetate **4** were performed under identical reaction conditions. The thirteen desired 2-amino-3-cyanopyridine derivatives **5(a-m)** were obtained in good to excellent yields (84-94%), as shown in Table 2. The nature of the substituents on the aromatic ring showed no noticeable effect on the yields of synthesized 2-amino-3-cyanopyridines **5**.

**Table 2.** Synthesis of 2-amino-3-cyanopyridine derivatives **5**.



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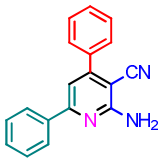
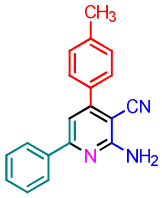
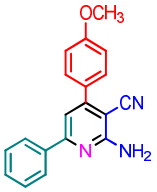
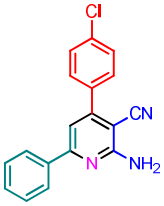
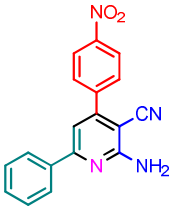
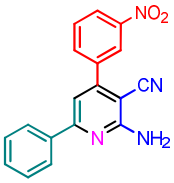
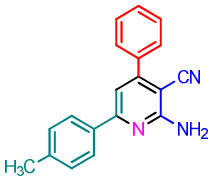
| Entry | $R_1$ | $R_2$ | $R_3$ | Product [a] | | Yield [b] (%) |
|---|---|---|---|---|---|---|
| **1** | H | Ph | H | **5a** |  | **94** |
| **2** | $CH_3$ | Ph | H | **5b** |  | **85** |
| **3** | $CH_3O$ | Ph | H | **5c** |  | **84** |
| **4** | Cl | Ph | H | **5d** |  | **95** |
| **5** | $NO_2$ | Ph | H | **5e** |  | **86** |
| **6** | $NO_2$ | Ph | H | **5f** |  | **93** |
| **7** | H | 4-$CH_3C_6H_4$ | H | **5g** |  | **92** |

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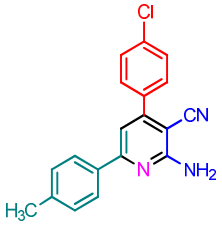
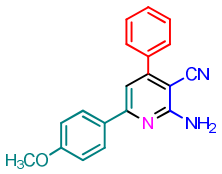
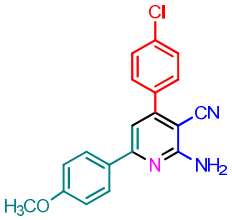
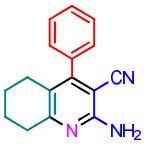
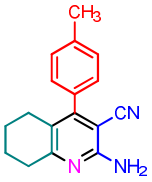
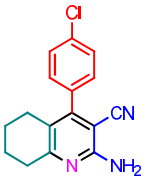
| | | | | | | |
|---|---|---|---|---|---|---|
| **8** | Cl | 4- $CH_3C_6H_4$ | H | **5h** |  | **90** |
| **9** | H | 4- $CH_3OC_6H_4$ | H | **5i** |  | **91** |
| **10** | Cl | 4- $CH_3OC_6H_4$ | H | **5j** |  | **89** |
| **11** | H | -$(CH_2)_4$- | | **5k** |  | **94** |
| **12** | $CH_3$ | -$(CH_2)_4$- | | **5l** |  | **88** |
| **13** | Cl | -$(CH_2)_4$- | | **5m** |  | **94** |

[a] All products were characterized by $^1H$, $^{13}C$ NMR, and IR spectral data. [b] Isolated yields.

In order to explain the formation of 2-amino-3-cyanopyridines **5**, we have proposed a plausible reaction mechanism, which is shown in Scheme 3.

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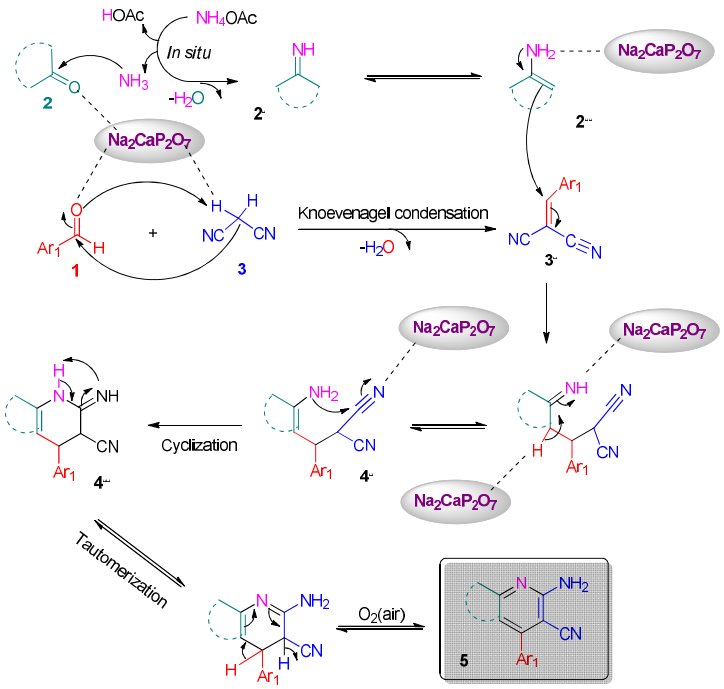


**Scheme 3.** Proposed mechanism for $Na_2CaP_2O_7$-catalyzed synthesis of 2-amino-3-cyanopyridine derivatives.

$Na_2CaP_2O_7$ catalyzes the synthesis of 2-amino-3-cyanopyridine derivatives **5** by activating the carbonyl group of aromatic aldehyde **1,** making it more susceptible to nucleophilic attack by malononitrile to form the arylidenemalononitrile derivative **3′**, which reacted with imino derivative **2′** formed by reaction between ammonium acetate and ketone **2**, via Michael addition to form adduct **4′**. The intermediate **4′** cyclized to dihydropyridine **4″**, followed by tautomerization aromatization to afford the 2-amino-3-cyanopyridine derivatives **5**.

### **2. 7.** *Recyclability of $Na_2CaP_2O_7$ catalyst*

To investigate the recyclability and regeneration of the catalyst, the $Na_2CaP_2O_7$ was regenerated by two procedures. The first method, the catalyst was washed with acetone and dried at 100°C for 1 h after each experiment. The second method employed for regeneration was carried out by calcination at 500°C for 1 h after washing with acetone and drying at 100°C. Figure 8 summarizes the reusability and regeneration research of $Na_2CaP_2O_7$. The recycled $Na_2CaP_2O_7$ showed almost the same catalytic performance compared with the first run (Figure 8).

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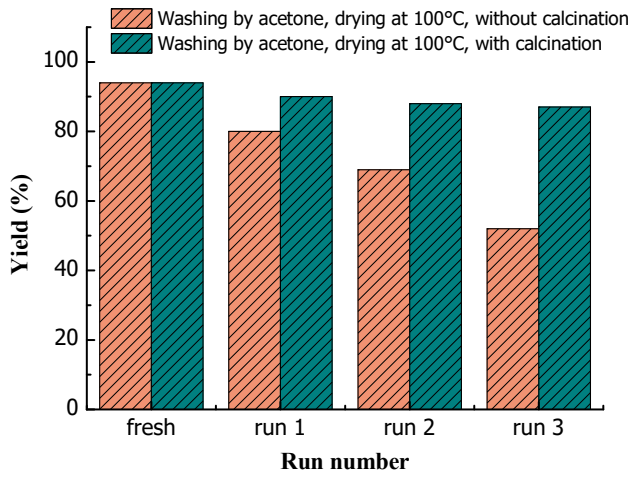
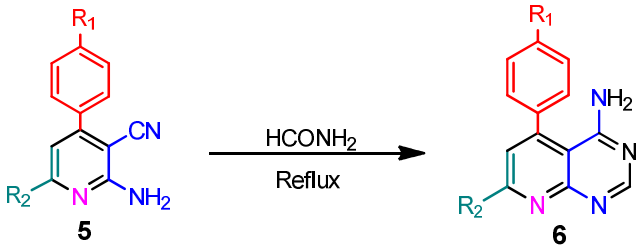



**Figure 8.** The recyclability and regeneration study of $Na_2CaP_2O_7$ in the synthesis of **5a**.

The importance of the 2-amino-3-cyanopyridines prepared can be seen through their reactions with formamide to produce the corresponding pyrido[2,3-*d*]pyrimidines, which have received considerable attention in recent years due to their diverse biological and pharmacological activities, such as antibacterial [32], antiallergic [33], anti-inflammatory [34], anti-HIV [35], antihypertensive [36], and antitumor activity [37]. The pyrido[2,3-d] pyrimidine **6** were synthesized by reaction of the 2-amino-3-cyanopyridines **5** with formamide (Scheme 2). Our study was focalized on the synthesis of the pyrido[2,3-*d*]pyrimidine derivatives **6(b, c, g, h, j)** by the condensation of the 2-amino-3-cyanopyridines **5(b, c, g, h, j)** with formamide.




**Scheme 2.** Synthesis of pyrido[2,3-*d*]pyrimidine derivatives **6**.

As mentioned below, the five pyrido[2,3-*d*]pyrimidine derivatives **6(b, c, g, h, j)** were obtained in moderate yields (71-81%), as shown in Table 3.

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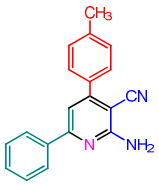
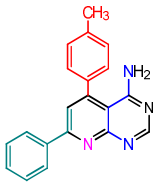
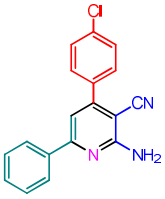
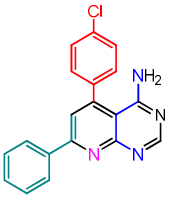
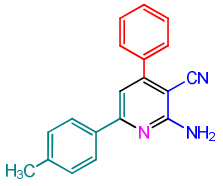
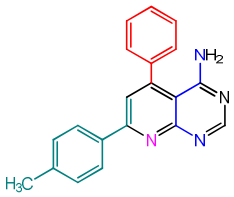
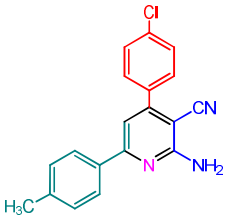
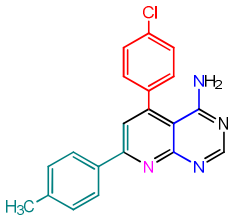
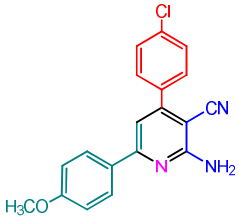
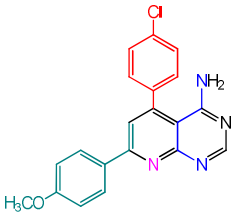
**Table 3.** Synthesis of pyrido[2,3-*d*]pyrimidine derivatives **6**.

| Entry | 2-amino-3-cyanopyridine | pyrido[2,3-*d*]pyrimidine [a] | Yield [b] (%) |
|---|---|---|---|
| **1** |  | **6b**  | **74** |
| **2** |  | **6c**  | **71** |
| **3** |  | **6g**  | **81** |
| **4** |  | **6h**  | **79** |
| **5** |  | **6j**  | **71** |

[a] All products were characterized by $^{1}H$, $^{13}C$ NMR, and IR spectral data. [b] Isolated yields.

### *2. 8. Antimicrobial activity*

Three derivatives, namely cyanopyridine (**5a** and **5b**) and pyrimidine (**6b**), revealed that they possess a very potent antibacterial activity against Gram-positive and Gram-negative bacteria tested with MIC and MBC values ranging from 64.5 to 250 μg/mL. Table 4 reports the IZDs, MICs, and MBCs values. In general, pyrimidine (**6b**) was the most active in comparison with the other components. It showed a strong effect against S. aureus and B. subtillis with IZDs values of 21-20.5 mm, respectively. Cyanopyridine (**5a** and **5b**) were less active against S. aureus and slightly less active against B. subtillis with an IZD of 18.5 and 17 mm, respectively. Moreover, The MBC to MIC ratios calculated for the derivatives indicates that they are bactericidal rather than

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bacteriostatic molecules. Hence, the derivatives possessing the methyl group exhibited good antibacterial activity.

**Table 4.** Determination of the inhibition zone diameter of the synthesis of cyanopyridine derivatives (**5a, 5b**) and pyrimidine (**6b**).

| | Cyanopyridine 5a | | | Cyanopyridine 5b | | | Pyrimidine 6b | | |
|---|---|---|---|---|---|---|---|---|---|
| | IZD (mm) | MIC (µl/ml) | MBC (µl/ml) | IZD (mm) | MIC (µl/ml) | MBC (µl/ml) | IZD (mm) | MIC (µl/ml) | MBC (µl/ml) |
| *P. aeruginosa (-)* | NS | - | - | NS | - | - | NS | - | - |
| *S. aureus (+)* | NS | - | - | NS | - | - | 21 | 125 | 125 |
| *S.epidermidis (+)* | NS | - | - | NS | - | - | NS | - | - |
| *K.pneumonaie (–)* | NS | - | - | NS | - | - | NS | - | - |
| *B.subtillis (+)* | 18.5 | 64.5 | 64.5 | 17 | 64.5 | 125 | 20.5 | 64.5 | 64.5 |
| *E. coli (-)* | 13 | 125 | 125 | 12 | 125 | 250 | 12 | 125 | 125 |
| *E.feacalis (+)* | NS | - | - | NS | - | - | NS | - | - |
| *C.albicans* | NS | - | - | NS | - | - | NS | - | - |

NS: Not susceptible; IZD : Inhibition zones diameter; MIC : Minimum inhibitory concentration; MBC : Minimum Bactericidal Concentration.

### *2. 9. Discussion*

In this study, we synthesized thirteen cyanopyridines and five Pyrimidine and screened their antibacterial activity in eight strains. We found that cyanopyridine derivatives (**5a** and **5b**) have an antibacterial effect against E. coli and B. subtilis. However, other synthesized molecules of the same family did not show any antimicrobial effect against both bacteria and fungi at the tested concentrations [38,39]**.**

A single pyrimidine derivative (**6b**) showed an antibacterial, probably due to the nature of the heterocycle. Our results were in agreement with other scientific findings [40], which carried out research on the antibacterial and antifungal effect of new pyrimidine derivatives based on benzothiazole, by testing them on bacterial strains: *S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*,and on the fungal agent *C. albicans*. They revealed that these derivatives exhibit an antibacterial and antifungal effect which varies from one molecule to another, some of these derivatives had antibacterial effects on all the strains tested and also an antifungal effect against *C. albicans*. This effect can be influenced by aromatic substituents, in particular, those with electron-donating properties**.**

There are several targets described for antibacterial agents, such as disrupting cell walls, and membrane-permeabilization, targeting drug efflux pumps, targeting R-plasmids, and targeting the quorum sensing, which plays an important role in regulating the biofilm. Several studies showed that antibacterial agents tend to act more strongly on Gram-positive than on Gram-negative bacteria. It is probably due to the differences in cell wall composition and structure since Gram-negative bacteria possess an outer membrane[41].

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## 3. Conclusion

In this work, we synthesized a series of 2-amino-3-cyanopyridine derivatives with $Na_2CaP_2O_7$ as a green and recoverable catalyst. The absence of solvent, ecology, simplicity of preparation, and a green catalyst are some of the significant advantages of this procedure.

The 2-amino-3-cyanopyridine derivatives were developed by a reaction with formamide to arrive at new pyrido[2,3-d]pyrimidine derivatives.

These synthesized products have a significant antibacterial effect.

Therefore, we suggest that $Na_2CaP_2O_7$ will receive increased attention in the future as an alternative catalyst for the one-pot synthesis of molecules known for their various biological and pharmacological activities.

## Supporting Information Summary

The supporting information includes the experimental procedures, the materials used in this research work and full characterization data for organic products.

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All authors have read and agreed to the published version of the manuscript.

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