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Urease-catalyzed synthesis of aminocyanopyridines from urea under fully green conditions

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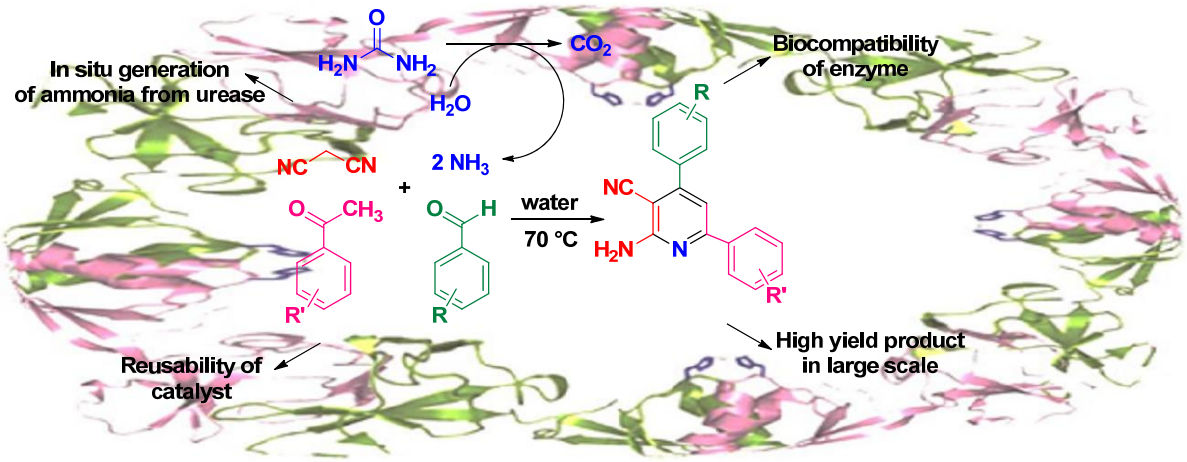
GRAPHICAL ABSTRACT



Highlights

- This work supports the catalytic performance of urease in organic synthesis.
- This specific urease-catalyzed reaction inhibits by trace amount of Pb^{2+}, Hg^{2+}, or Ag^{+}.
- The authority of urease is bio-production of ammonia from urea.

Abstract

This is an original work on catalytic performance of urease in organic synthesis which in one-pot dissociation of urea and condensation of the *in situ* generated ammonia with aldehydes, acetophenones, and malononitrile occurs in water to give 2-amino-3-cyanopyridines. Comparative experiments with ammonium salts supported the enzymatic specify of 0.01 g/mL (50 U/mg) of urease for bio-production of ammonia, while trace amount of heavy metal ions such as Pb^{2+}, Hg^{2+}, and Ag^{+} inhibit these specific reactions. The scalability and promiscuity of urease facilitate the applicability of the process for biotechnological organic reactions based on ammonia.

Keywords: Urease, Enzymatic reactions, *In situ* generation of ammonia, Biocompatible, Pyridines

1. Introduction

Replacement of hazardous procedures with eco-environmentally benign alternatives is one of the essential challenges of green chemistry [1,2], though the enzymatic water-based organic reactions are of the most attractive processes [3-7] due to the biocompatibility, non-toxicity, and easier workup. Hydrolases are known as substrate specify biocatalysts in organic reactions with high enzymatic promiscuity [8] that categorized as condition promiscuity, substrate promiscuity, and catalytic promiscuity. These promiscuities define as preservation of enzymatic activity under different conditions [9-11], broad range of substrate specify [12,13], and the ability of the active site of a single enzyme to catalyze various chemical transformations [14]. While immobilization of enzymes improves their reusability, purity, stability, activity, specificity, selectivity, and inhibitions [15], hydrolase enzymes illustrated the catalytic promiscuity in water-based enzymatic organic synthesis [16-18]. Urease is a binuclear Ni-containing hydrolase enzyme with high substrate specify for dissociation of urea to ammonia which in conjugation with urea can be considered as a bio source of nitrogen instead of the risky odorous ones.

Pyridine derivatives are among the interest heterocycles which constitute the back bone of many natural products and bioactive molecules [19] with anti-microbial, cardiotonic, anti-parkinsonism, anti HIV, antitumor, and anti-inflammatory activities. Multi-functionalized pyridines with amino- and cyano-substituents (AmCyPys) are of these biologically active and fluorescent molecules [20] that extra conversion of the cyano and amino into the other functional groups [21] make them favored for synthesis of bioorganic compounds such as vitamin B_3 [22]. There are various malononitrile-based multi-component reactions (MCRs) to synthesis of these potent pyridines [19, 23-25], although due to the benefits of the MCRs in water [26-30] and malononitrile-based synthesis of AmCyPys [31-35], their conjugation with advanced enzymatic

reactions is highly desirable. To the best of our knowledge this is the first report on the application of urease in organic synthesis that due to the benefits of the water-based enzymatic reactions have been designed to improve the four-component synthesis of AmCyPys under fully biocompatible conditions (Scheme 1).

<< Scheme 1 >>

2. Material and methods

2.1. Materials and analytical methods

All materials and urease with art no from jack beans were purchased from Merck. All reagents were obtained from commercial suppliers and were used without further purification unless otherwise noted. The NMR spectra were recorded on a Bruker 500 MHz instrument using DMSO-d_6 or $CDCl_3$ as solvents. Chemical shifts (δ) were expressed in ppm with TMS as internal standard, and coupling constants (*J*) were reported in Hz.

2.2. General procedure for the synthesis of 2-amino-3-cyanopyridines

A mixture of an aldehyde (1 mmol), substituted acetophenone (1 mmol), malononitrile (1 mmol), urea (1.5 mmol) and urease (0.01 g (50 U/mg)) in 0.25 mL water was stirred at 70 ^{o}C for appropriate times (Table 2). The progress of the reaction was monitored by TLC (70:30, *n*-hexane/acetone). The reaction mixture was cooled after completion and 95% cold EtOH (2 mL) was added. The precipitate was filtered off, washed with cold ethanol, and dried to give the pure product.

3. Results and discussion

Initially, to optimize the reaction conditions for access to the maximum activity and specify of enzyme, the catalytic performance of the commercially available urease was investigated in the reaction of benzaldehyde (**1**), acetophenone (**2**), malononitrile (**3**), and urea (**4**) screened at various temperatures and loading of ureases (Table 1).

<< **Table 1** >>

As results show, the maximum yield of 2-amino-4,6-diphenylnicotinonitrile **5** was obtained in water using 0.01 g of commercially available urease after 1.5 h at 70 °C (Table 1, entry 13). A control experiment without urease under similar conditions did not give the product **5** even after 24 h and confirmed the catalytic role of urease for dissociation of urea to ammonia.

To distinct the catalytic role of urease for the *in situ* generation of ammonia or further transformations, the model reaction was run with ammonium acetate instead of urea in the presence and absence of 0.01 g of urease. The similar yields and reaction times of both of reactions support the substrate specify of urease for the only *in situ* generation of ammonia from urea in the first step of the reaction. Another control experiment with thiourea supported the promiscuity of urease for exclusive dissociation of urea (entry 16).

To determine the details of this enzymatic reaction, the influences of enzyme loading (Fig 1.), molar ratio of starting materials (Fig 2.), and reaction temperature (Fig 3.) were finely investigated. In respect of the reaction time and yield, the optimal reaction temperature, enzyme loading, and molar ratios of components **1-4** were 70 °C, 0.01 g/mL of enzyme, and mole ratio of 1:1:1:4 for (**1**), (**2**), (**3**), and (**4**), respectively.

<< **Fig 1** >>

<< **Fig 2** >>

<< **Fig 3** >>

In order to investigate the influence of pH, the urease-catalyzed model reaction was performed in phosphate buffers with pH 6-8. As Table **2** shows, pH 7 is optimum for superior activity of urease in this enzymatic reaction, presumably due to the changes in active site of urease or denaturation of peptide residual of enzyme in acid or base conditions.

<< **Table 2** >>

Owing to the importance of the enzymatic methods in large scale, the model reaction was run with 50 mmol scale of substrates under optimized conditions and fortunately the product **5** was obtained in 85% yield. The reusability tests for this large scale reaction show only 3% decrease in the reaction yield with reused reaction medium after three consequence runs (Fig. 4).

<< **Fig 4** >>

Heavy metal ions are non-competitive inhibitors for urease, thus to check the enzyme inhibition, the model reaction was run with 0.5 mL of aqueous solution of 10^{-6} molar of heavy metal ions such as Pb^{2+}, Hg^{2+}, and Ag^{+} that resulted in the dramatic reduction of the reaction yield in all cases, even in the longer reaction times (Fig. 5).

<< **Fig 5** >>

A plausible mechanism for the urease-catalyzed model reaction is proposed in Scheme **2**, which begins with urease-catalyzed hydrolysis of urea to ammonia.

<< **Scheme 2** >>

Encouraged by results of the model reaction under optimal conditions, the generality of the urease-catalyzed one-pot synthesis of AmCyPys was investigated for substituted aldehydes and acetophenones in water that led to the high yield synthesis of the desired products under these biocompatible conditions (Table 3).

<< Table 3 >>

Table **4** shows the merit of this enzymatic reaction and catalytic performances of urease versus the previously reported methods for the model reaction with ammonium acetate [32,34].

<< Table 4 >>

4. Conclusion

In conclusion, an extremely efficient and green enzymatic process has been developed to synthesis of 2-amino-3-cyanopyridines *via* the one-pot condensation of aldehydes, acetophenones, and malononitrile with the *in situ* released ammonia from the urea without using any chemical catalyst, solvent, or additive. This method represents merits such as no use of any base, metal, or Lewis acid catalyst, simple isolation/purification of products by aqueous work-up, high selectivity, low cost, simplicity of handling, and eco-environmentally benign of the process. Scalability of this novel example of enzyme promiscuity provides the possible applications of urease in biotechnological processes.

Acknowledgements

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References

[1] T. Horvath, P.T. Anastas, Chem. Rev. 107 (2007) 2169-2173.

[2] Z. Guo, B. Liu, Q. Zhang, W. Deng, Y. Wang, Y. Yang, Chem. Soc. Rev. 43 (2014) 3480-3524.

[3] H. Abd-Elnabi, A.M. Abdel Hameed, R.A. Mekheimer, R.R. Awed, K. U. Sadek, Green Sustain. Chem. 3 (2013) 141-145.

[4] M. Heidary, M. Khoobi, S. Ghasemi, Z. Habibi, M.A. Faramarzi, Adv. Synth. Catal. 356 (2014) 1789-1794.

[5] R.A. Copeland, Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis, Second Edition. Wiley-VCH, Inc. 2000.

[6] K. Buchholz, V. Kasche, U.T. Bornscheuer, Biocatalysts and Enzyme Technology. Willy-VCH Verlag GmbH & Co. KGaA, Weinheim. 2005.

[7] Z. Guan, J. Song, Y. Xue, D.-C. Yang, Y.-H. He, J. Mol. Catal. B: Enzym. 111 (2015) 16-20.

[8] B.C.H. May, J. A. Zorn, J. Witkop, J. Sherrill, A.C. Wallace, G. Legname, S.B. Prudiner, F.E.J. Cohen, Med. Chem. 50 (2007) 65-73.

[9] E. Busto, V. Gotor-Fernandez, V. Gotor, Chem. Soc. Rev. 39 (2010) 4504-4523.

[10] M.S. Humble, P. Berglund, Eur. J. Org. Chem. (2011) 3391-3401.

[11] L.-H. Zhou, N. Wang, W. Zhang, Z.-B. Xie, X.-Q. Yu, J. Mol. Catal. B: Enzym. 91 (2013) 37-43.

[12] U.T. Bornscheuer, R.J. Kazlauskas, Angew. Chem. Int. Ed. 43 (2004) 6032-6040.

[13] Z.B. Xie, N. Wang, W. X. Wu, X. Le, G. ZH, X.Q. Yu, J. Biotechnol**.** 170 (2014) 1-5. http://dx.doi.org/10.1016/j.jbiotec.2013.10.031.

[14] M. Lei, L. Ma, L. Hu, Monatsh. Chem. 141 (2010) 1005-1008.

[15] C. Garcia-Galan, A. Berenguer-Murcia, R. Fernandez-Lafuente, R.C. Rodrigues, Adv. Synth. Catal. 353 (2011) 2885-2904.

[16] A. Gernot, H. Strohmeier, P. O. May, M. Gruber-Khadjawi, 111 (2011) 4141-4164.

[17] B. Yang, Z. Dai, Sh.-Y. Ding, Ch. E. Wyman, Biofuels. 2 (2011) 421-450.

[18] D. Monti, G. Ottolina, G. Carrea, S. Riva, Chem. Rev. 111 (2011) 4111-4140.

[19] C. Allais, J-M. Grassot, J. Rodriguez, T. Constantieux, Chem. Rev. 114 (2014) 10829-10868.

[20] S. R. Atla, N. R. Nagireddy, R.P. Yejella, Int. J. Pharm. Chem. Anal. 1 (2014) 50-60.

[21] K.L. Bogan, Ch. Brenner, Annu. Rev. Nutr. 28 (2008) 115-130.

[22] K. Maiese, Zh. Zhong Chong, J. Hou, Y. Chen Shang, Molecules 14 (2009) 3446-3485.

[23] R. Ghorbani-Vaghei, Z. Toghraei-Semiromi, R. Karimi-Nami, C. R. Chimie. 16 (2013) 1111-1117.

[24] F. Shi, S. Tu, F. Fang, T. Li, ARKIVOC (i) (2005) 137-142.

[25] N. Kumar, A. Chauhan, S. Drabu, Biomed. Pharmacotherapy 65 (2011) 375-380.

[26] F. Tamaddon, F. Amirpoor, Synlett 24 (2013) 1791-1794.

[27] F. Tamaddon, Z. Razmi, A.A. Jafari, Tetrahedron Lett. 51 (2010) 1187-1189.

[28] V. Estevez, M. Villacampa, J.C. Menendez, Chem. Soc. Rev. 39 (2010) 4402-4421.

[29] F. Tamaddon, M. Alizadeh, Synlett 26 (2015) 525-530.

[30] F. Tamaddon, M. Alizadeh, Tetrahedron Lett. 55 (2014) 3588-3591.

[31] S. Khaksar, M. Yaghoobi, J. Fluorine Chem. 142 (2012) 41-44.

[32] J. Tang, L. Wang, Y. Yao, L. Zhang, W. Wang, Tetrahedron Lett. 52 (2011) 509-511.

[33] K. Niknam, A. Jamali, M. Tajaddod, A. Deris, Chin. J. Catal. 33 (2012) 1312-1317.

[34] A.A. Yelwande, M.E. Navgire, D.T. Tayde, B.R. Arbad, M. K. Lande, S. Afr. J. Chem. 65 (2012) 131-137.

[35] Q. Wu, Y. Zhang, S. Cui, Org. Lett. 16 (2014) 1350-1353.

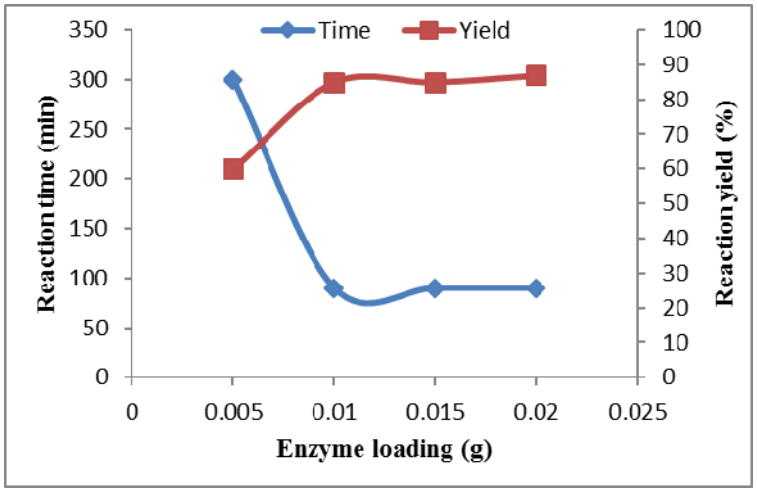


Fig 1. Effect of enzyme loading on the reaction time and yield.

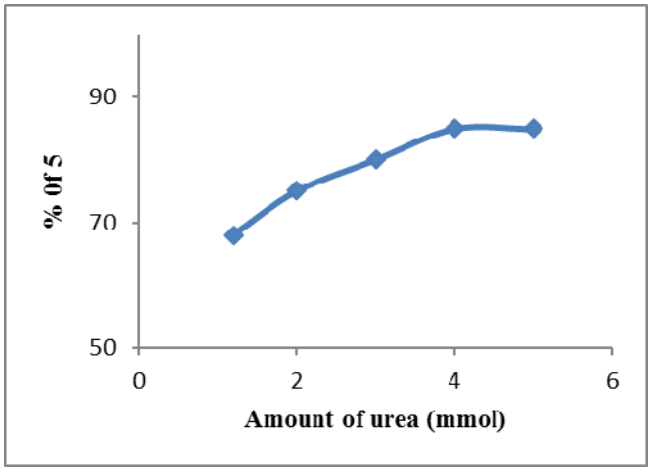


Fig 2. Effect of urea loading on the yield of **5**.

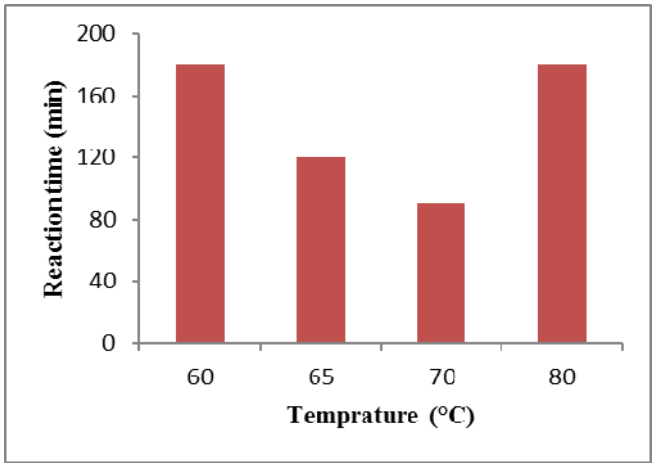


Fig 3. Effect of temperature on the rate of urease-catalyzed reaction.

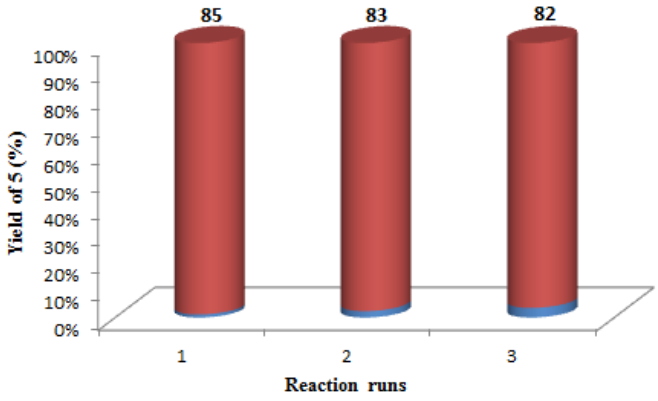


Fig 4. Reusability of the reaction medium.

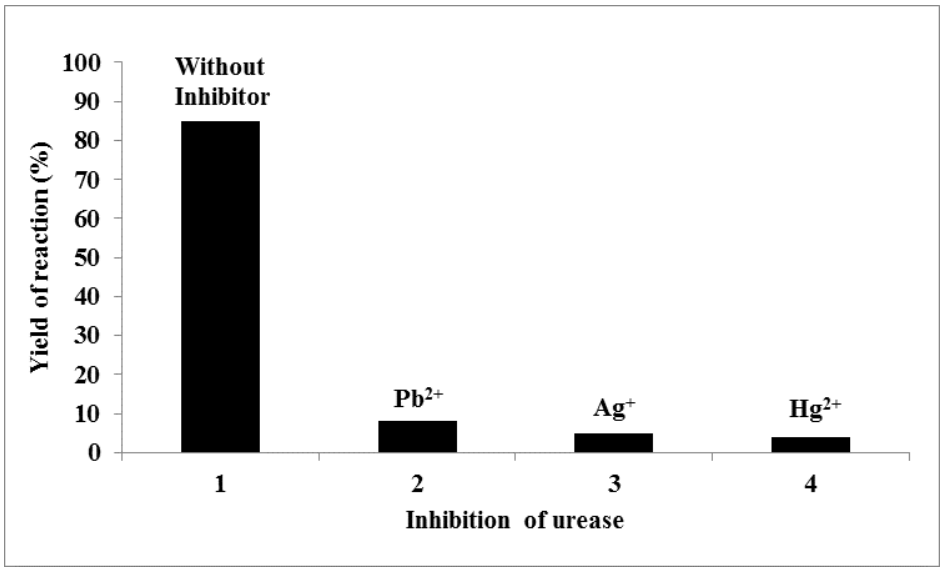
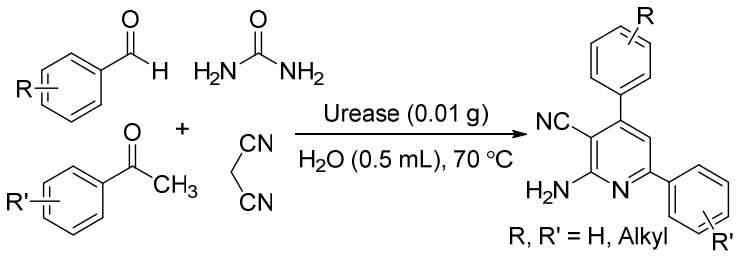
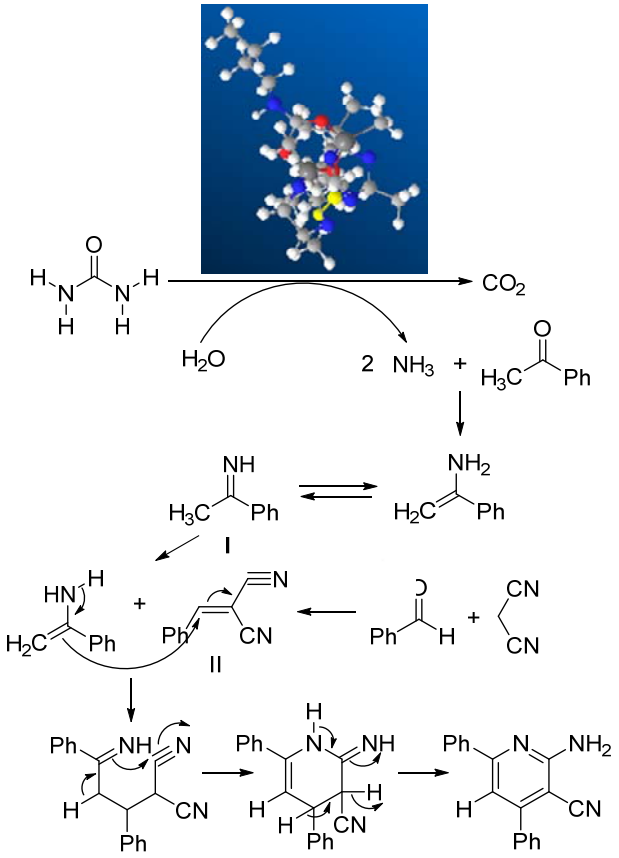


Fig 5. Inhibition of the urease-catalyzed reaction.



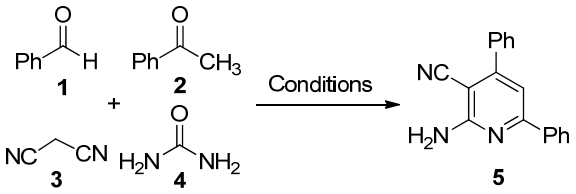
Scheme 1. Biocompatible synthesis of AmCyPys in water



Scheme 2. Plausible mechanism for urease-catalyzed synthesis of AmCyPy.

Table 1

Optimization of urease-catalyzed reaction[a]



Entry	Conditions				Yield [b] (%)
	Urea (mmol)	Temp. (°C)	Urease (g)	Time (h)	
1	1.2	60	0.01	8	50
2	1.2	70	0.005	8	40
3	1.2	70	0.01	4.5	68
4	1.2	70	0.02	4.5	70
5	1.2	80	0.01	6	50
6	2	70	0.01	3.5	75
7	3	70	0.01	2.5	80
8	4	60	0.01	3	60
9	4	65	0.01	2	70
10	4	70	0.005	5	60
11	4	70	0.01	1.5	85
12	4	70	0.015	1.5	85
13	4	70	0.02	1.4	87
14	5	70	0.02	1.5	85
15	4	80	0.01	3	60
16	4[c]	70	0.01	12	-

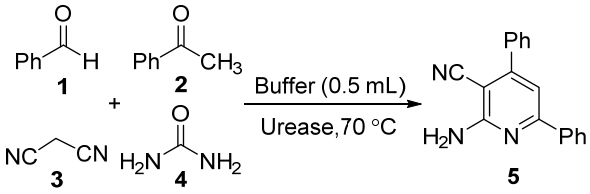
[a] Reaction was performed with benzaldehyde, acetophenone, malononitrile, and urea (in ratio of 1:1:1:1.2-4 mmol) in 0.5 mL of water and 0.005-0.02 g of urease.

[b] Isolated yield based on **3**.

[c] Reaction was run with thiourea.

Table 2

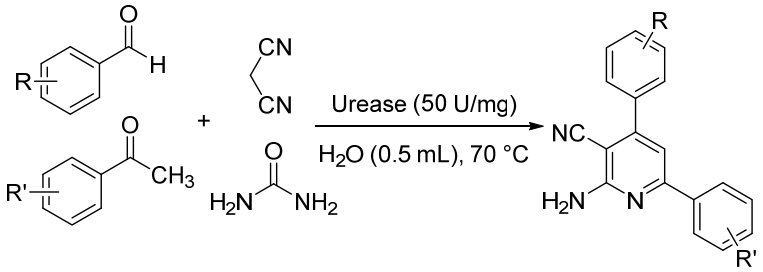
Effect of pH



Entry	pH	Time (min)	[a] Isolated yield (%)
1	6	140	58
2	7	100	86
3	8	80	83
4	7	90	87 (This work)

Table 3

Urease catalyzed synthesis of 2-amino-3-cyanopyridines.



Entry	R	R[1]	Time (min)	Yield[b] (%)	Mp (°C)	Ref.
1	H	H	90	85	(187-189)	[25]
2	4-CH_3	H	100	80	(175-177)	[31]
3	3-OMe	H	90	70	(201-204)	-
4	4-OMe	H	85	75	(189-192)	[24]
5	4-NO_2	H	150	80	(173-177)	[31]
6	2-Cl	H	60	75	(180-183)	[31]
7	4-Cl	H	20	88	(234-236)	[31]
8	4-F	H	20	85	(217-218)	[34]
9	3-pyridyl	H	60	70	(206-210)	[35]
10	2-furyl	H	110	80	(190-194)	[35]
11	H	4-NO_2	50	89	(123-125)	[25]
12	H	4-CH_3	90	86	(182-183)	[25]
13	4-NO_2	4-CH_3	95	80	(201-203)	[33]
14	2-furyl	4-CH_3	120	78	(150-153)	-
15	H	4-Cl	90	80	(238-241)	[25]
16	4-Cl	4-Cl	70	85	(245-247)	[33]
17	4-OMe	4-Cl	80	87	(205-207)	[25]

[a] Reaction was performed with, malononitrile (1 mmol), aldehyde (1 mmol), acetophenone (1 mmol) and urea (4 mmol) in 0.5 mL of water using 0.01 g (50 U/mg) of urease.

[b] Isolated yield.

Table 4

Catalytic performance of urease in synthesis of 2-amino-3-cyanopyridines

Entry	Catalyst/Nitrogen source/Solvent/Temp./Time/Yield (mol% or g)/-/-/(°C)/(h)/(% Isolated)	Ref.
1	SnO_2/SiO_2 (0.1 g)/NH_4OAc/EtOH/Reflux/24/91	[34]
2	$Yb(PFO)_3$ (1 mol%)/NH_4OAc/EtOH/r.t/24/90	[32]
3	Urease (0.01 g)/Urea/H_2O/70/1.5/85	This work