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Synthesis of 4-Iodoimidazole Nucleosides: A Biocatalyzed Whole Cell Approach

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Abstract:
Nucleoside analogues are biologically active drugs that are well known for their therapeutic properties. Chemical synthesis of these compounds is complex due to time constraints, contaminant methodologies, and numerous steps involved. In contrast, biocatalyzed synthesis, particularly using microorganisms, offers many advantages. In this work, we introduce the synthesis of 4-iodoimidazole-ribonucleoside, through a microbial transglycosylation biocatalyzed by *Erwinia amylovora* whole cells, selected as the optimal catalyst for this biotransformation upon evaluating our microbial collection. Parameters were analyzed to optimize the reaction, and the use of this biocatalyst in transglycosylation with other natural and non-natural bases and different sugar donor nucleosides was also verified.

Introduction

As fundamental biomolecules, nucleosides play key roles in diverse cellular processes, ranging from cell signaling to metabolism.[1] Not surprisingly, synthetic nucleoside analogs (NAs), which are designed to mimic their natural counterparts, are widely exploited in medicinal chemistry, and used as tool compounds in synthetic biology.[2] Over the years, many NAs have proven to be effective antiviral or anticancer agents in clinical settings.[3] Traditionally, nucleosides are synthesized using a variety of chemical techniques that often include challenging, inefficient, and time-consuming multi-step processes.[4] The standard approach to nucleoside synthesis involves coupling the nucleobase (or its derivatives) to the carbohydrate moiety. Synthetic procedures usually require glycosyl activation and the use of protecting groups on the heterocyclic base and sugar portion, along with precise control of anomeric configurations.[5]

A chemo-enzymatic approach is one effective method for overcoming the limitations of multi-step chemical synthesis.[6] Biocatalytic pathways (using whole cells or isolated enzymes) are usually enantio-, chemo-, and regioselective, which is an important benefit for the synthesis of nucleoside analogs. In this case, the reduction of side reactions and the absence of protection and deprotection steps reduce the reaction time and improve the downstream process. Furthermore, biocatalyzed reactions are carried out under mild conditions, which is a powerful tool for the development of green chemistry.[7] As a result, the pursuit of new biocatalysts is particularly appealing. In this context, microorganisms provide a rich source of enzymatic diversity and can be utilized as whole-cell biocatalysts, offering a potentially cost-effective and suitable option for industrial use.[8]
In particular, microbial transglycosylation catalyzed by enzymes from the *N*-glycosyltransferase family is a valuable method for converting pyrimidine nucleoside analogues into purine ones. (Scheme 1, **A**).[9] This method relies on transferring a sugar moiety from a pyrimidine donor nucleoside to a purine acceptor base via a one-pot cascade biotransformation process facilitated by nucleoside phosphorylases (NPs).[10] These enzymes enable the reversible phosphorolysis of the nucleosidic bond in the presence of inorganic phosphate. Depending on the enzyme involved, biotransformation equilibrium shifts towards synthesis or phosphorolysis reaction, being the constant (keq) for the phosphorolysis of pyrimidine nucleosides approximately 20 times greater than the one for purine nucleoside phosphorolysis. The first step of the transglycosylation is the phosphorolysis of the nucleosidic bond of a pyrimidine nucleoside to produce α-D-ribose-1-phosphate and its respective nitrogenous base, which is catalyzed by pyrimidine phosphorylase enzymes (PyNs). These PyNs can be classified according to the nature of the pyrimidine nucleoside as thymidine phosphorylase (TP) (EC 2.4.2.4), uridine phosphorylase (UP) (EC 2.4.2.3) and pyrimidine nucleoside phosphorylase (PyNP) (EC 2.4.2.2). In a second step, the stereoselective formation of the β *N*-glycosidic bond of the new nucleoside is catalyzed by the purine nucleoside phosphorylase (PNP) (EC 2.4.2.1).

Furthermore, NPs accept diverse bases and modified or non-natural sugars as substrates.[11] This characteristic expands the range of applications of these enzymes, making them useful in the preparation of modified nucleosides with great therapeutic value such as fludarabine,[12] vidarabine,[13] ribavirin[14] and didanosine.[15] The employment of whole bacterial cells as biocatalysts in transglycosylation reactions offers additional advantages, such as avoiding the necessity for purified enzymes, which are often scarce and not widely available commercially.[16]

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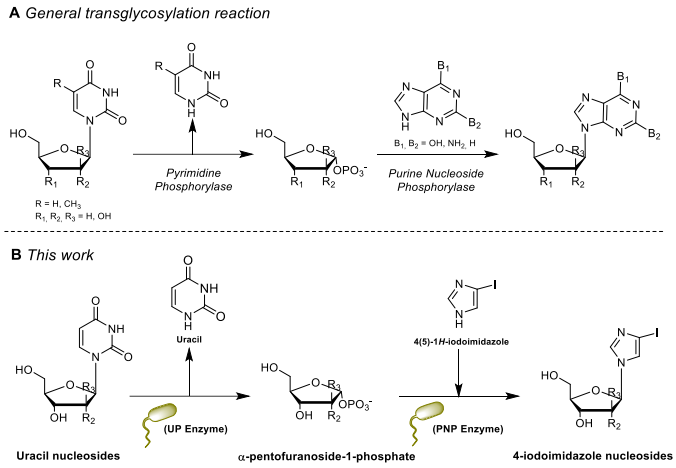

Scheme 1. Enzyme mediated transglycosylation reaction

With the aim of synthesizing new pharmacologically active NAs, among the possible modifications of its structure, the replacement of the nitrogenous base with non-natural bases can be considered.[17] Specifically, we are focused on imidazole-based nucleosides, since the imidazole ring is a widespread heterocycle that is present in natural products and biologically active compounds.[18] Imidazole nucleosides hold significant promise as potential antiviral candidates, largely attributed to their remarkable resilience against specific enzymes responsible for nucleoside deactivation, such as adenosine deaminase (ADA).[19] A major example of this nucleoside analog family with biological activity is the one developed by Okano et al.[20] In their report, they developed the synthesis of effective and safe compounds with potent DENV RdRp inhibitory activity. Moreover, the Seley-Radtke group used modified imidazole nucleosides as potential ligands to recognize CG base pairs.[21] They designed nucleosides containing imidazoles substituted with different aromatic groups, linked by a carbon-carbon bond. This molecular arrangement provides molecules with extra conformational and torsional flexibility, while still preserving the essential hydrogen bonding and aromatic characteristics, crucial for base pairing and molecular recognition.[22]

The aim of this work was to synthesize 4(5)-iodo-1*H*-imidazole nucleosides through microbial transglycosylation (scheme 1, **B**), and compare the process with a conventional multi-step chemical synthesis. The choice of this substituted imidazole was not random, as this halogenated derivative will be further used as a precursor of new substituted imidazole nucleosides.

Results and Discussion

Biocatalyst selection

For years, our laboratory has utilized whole cell biocatalysts to prepare several modified nucleosides.[23] We screen microorganisms to identify the most efficient biocatalyst for each biotransformation, using an optimized methodology we have developed.[24]

In this way, the screening of 71 bacteria (Table S1) from different genera and species from our cell collection was conducted to find the optimal biocatalyst for the transglycosylation reaction between uridine and 4(5)-iodo-1*H*-imidazole. The reaction conditions used to test the biocatalytic activity of each microorganism were considered as standard conditions (Table 1). A common challenge in enzymatic reactions is balancing the low solubility of substrates in water with the stability of biocatalysts in organic solvents. However, in this case all reagents were soluble in phosphate buffer. As summarized in Table 1, 17 bacteria capable of carrying out this biotransformation were identified. Among them, 4 bacteria showed a conversion rate exceeding 50%. *Erwinia amylovora* (CECT 222), which exhibited a conversion rate of 95.3% (entry 9, Table 1), was selected as the best biocatalyst for this transglycosylation reaction.

Table 1. Biocatalyst screening for the synthesis of 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**) under standard conditions.[a]

Entry	Microorganism	N° CECT	Conversion[b]	Time[c]
1	*Aeromonas hydrophila*	839	<10	24
2	*Aeromonas hydrophila*	4226	69.1	72
3	*Cellulomonas celulans*	3050	<10	24
4	*Chromobacterium violaceum*		<10	24
5	*Citrobacter freundii*	401	55.7	72
6	*Enterobacter aerogenes*	684	<10	24
7	*Enterobacter cloacae*	4214	<10	24
8	*Enterobacter cloacae*	4502	<10	24
9	*Erwinia amylovora*	222	95.3	48
10	*Escherichia coli*	731	<10	24
11	*Escherichia coli*	100	29.2	24
12	*Klebsiella sp*	367	15.6	24
13	*Proteus rettgeri*	4557	69.9	48
14	*Serratia macescens*	159	<10	24
15	*Serratia rubidaea*	868	<10	24
16	*Streptomyces badius*	3275	21.7	48
17	*Streptomyces baldaccii*	3249	18.5	48

[a] Standard conditions: biotransformations were carried out at 60°C in 30mM phosphate buffer pH 7 using 30 mM uridine, 10 mM 4(5)-iodo-1*H*-imidazole and $1.5x10^{10}$ cells/mL of biocatalyst. [b] Conversions were determined by a standard calibration curve of 4(5)-iodo-1*H*-imidazole absorbance (λ:230nm). [c] Reactions were analyzed by HPLC until no further conversion was detected.

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Process development and optimized reaction conditions

The best culture growth time for *E. amylorova* strain was evaluated by taking aliquots containing 1.5x10^{10} cells/mL at different growth stages and performing the biotransformation in standard conditions (Figure 1). In most bacteria, the NPs appear in the late exponential phase since they are involved in salvage metabolic pathways.[25] Since *E. amylorova* showed activity from the early stages of bacterial growth, this would suggest that the NPs are constitutive enzymes in this bacterium, meaning that they are present from the first stage of growth. Furthermore, taking into account the biomass obtained at each stage, it was determined that the optimal growth time to carry out the transglycosylation reaction and which also requires the smallest amount of culture to obtain the necessary number of cells, is 24 hours.

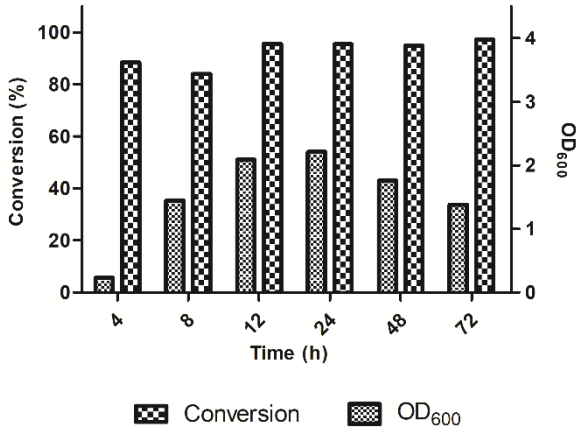


Figure 1. Influence of the culture stage of *E. amylorova* on the production of 4-iodo-1*H*-imidazole riboside (**1**) and the amount of biomass (equivalence: 0.4 OD units: 2x10^{8} cells/ml). The assays were carried out under standard conditions, using in all cases 1.5x1010 cells/mL of reaction mixture.

Figure 2 shows the time course reaction using the biocatalyst grown for 24 hours and the standard reaction conditions. The substrate conversion and product yield were quantified using calibration curves of 4(5)-iodo-1*H*-imidazole and 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**) standards respectively. It was found that maximum conversion occurs at 48 hours and then stagnates. In general, in transglycosylation reactions reported using other microorganisms and natural bases, the time to obtain maximum yields rarely exceed 8 h and the disappearance of the products is observed around 24 h given the reversibility of the activity in NPs. Therefore, it is not surprising that, since 4(5)-iodo-1*H*-imidazole is a non-natural substrate, the time to reach maximum yield was longer. In the same sense, no reversal occurred in this biotransformation, so it was presumed that the riboside formed was a poor substrate for the *E. amylorova* PNP.[26]

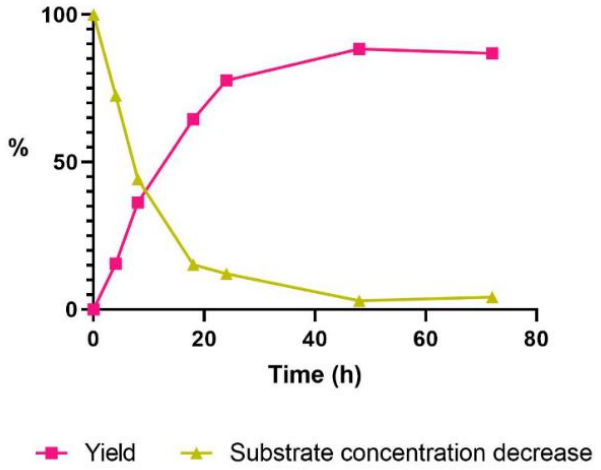
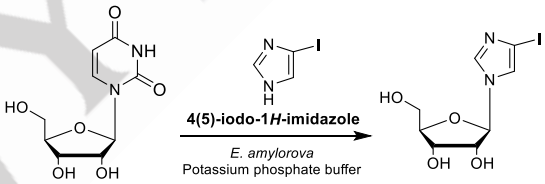


Figure 2. Conversion of 4(5)-iodo-1*H*-imidazole and yield of 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**) over biotransformation time using *E. amylovora* grown for 24 hours (1.5x10^{10} cells/mL).

Table 2. Influence of experimental variables in the transglycosylation reaction to obtain 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**) by *E. amylorova* whole cells.[a]



Entry	Buffer (mM)	[U]:[I][b]	Temperature (°C)	Yield (%)[c]	Time (h)[d]
1	30	30:30	60	70.5	72
2	30	30:10	60	88.3	48
3	30	20:10	60	75.7	48
4	30	10:10	60	77.2	48
5	20	30:10	60	75.2	48
6	10	30:10	60	67.3	24
7	30	30:10	45	40.1	48

[a]Standard conditions: reactions were carried out using 1.5x10^{10} cells *E. amylorova* mL^{-1} in phosphate buffer pH7. [b] Uridine:4-iodo-1*H*-imidazole concentration ratio. [c]Yields were determined by HPLC using a standard calibration curve of the product (**1**) (λ:230nm). [d] Reactions were analyzed by HPLC until no further conversion was detected.

Traditionally, microbial transglycosylation screenings have been conducted in 30 mM phosphate buffer pH7 (Table 2 entry 2)[23-25]. As noted above, this biotransformation is a two-step enzymatic process where the product of the first step, α-pentose-

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1-phosphate, is used as a substrate for the second step to generate the target purine nucleoside. Phosphate is crucial in this mechanism as it affects the rate of both reversible reactions, albeit in opposing directions.[11] Since higher phosphate buffer concentrations make it more challenging to purify the products, we performed the reaction with lower phosphate concentrations. The reaction was carried out using phosphate buffer concentrations of 10 mM and 20 mM, but the yields obtained, 67.3% and 75.2% respectively (Table 2, entries 5-6), were lower, so the use of 30 mM phosphate buffer was continued.

To achieve maximum yield, it is essential to study the concentration and ratio of substrates. Conversions were determined after 24 hours of reaction using ratios of 20:10 and 10:10 of uridine and 4(5)-iodo-1*H*-imidazole (Table 2, entries 3-4), respectively, in addition to the 30:10 concentration ratio used in the standard reaction. The highest conversion obtained was obtained with 30:10 ratio, which aligns with previous reports (88.3%, Table 2, entry 2). It has been shown that a concentration of 30 mM of uridine is necessary to produce enough phosphorylated substrate to promote the activity of the PNP enzyme involved in the second step of biotransformation.[26] Considering the possibility of carrying out this biotransformation in a productive process, where a large volumetric amount of product is required, the reaction was carried out using a donor:acceptor ratio of 30:30. Although 70.5 % yield was achieved in 72 hours (Table 2, entry 1), it would be a good option in terms of mass for a scale-up.

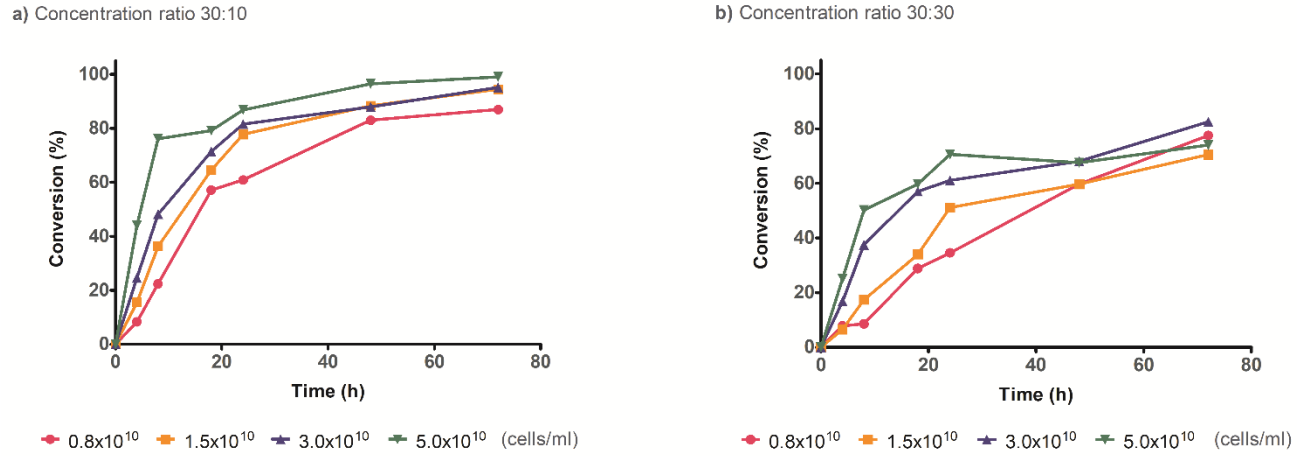

Figure 3. The effect of the amount of biocatalyst in the transglycosylation reaction of 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**) performed by *E. amylorova* whole cells using standard reaction conditions and a) 30mM uridine, 10 mM 4(5)-iodo-1*H*-imidazole; b) 30mM uridine, 30 mM 4(5)-iodo-1*H*-imidazole

The effect of the temperature in the biotransformation was also tested, keeping all other variables at standard conditions. While mesophilic microorganisms retain their enzymatic activity, specifically UP at temperatures up to 75°C and PNP at to 65°C, the activity of TP, the enzyme preferably involved in pyrimidine deoxynucleoside phosphorolysis, decreases significantly at 50°C.[27] In addition, the use of high temperatures helps to overcome the low solubility of the substrate in aqueous solution and prevents the deleterious action of endogenous deaminases present in microbial cells.[28] As a result, temperatures of either 45 or 60ºC were selected based on the thermal stability of the pyrimidine phosphorylase relevant to the biotransformation process. In this regard, a notable decrease in conversion (40%, Table 2, entry 7) was observed compared to the standard temperature of 60°C. Since it was shown that 4(5)-1*H*-iodo-imidazole was not a substrate of deaminases at 45ºC (Figures S13 and S14 in supporting information), this decrease in activity could indicate that the first step of transglycosylation would be carried out preferentially by UP.

To identify the best biocatalyst load required to obtain high yields of 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**), time-conversion-curves were recorded varying the biomass from $0.8x10^{10}$ to $5.0x10^{10}$ cell/ml (Figure 3). As expected, reaction time was dependent on the amount of biocatalyst. When small amounts of biocatalyst were used, long reaction times were needed to achieve high yields. Carrying out the biotransformation with 30:10 substrate ratio, when using $1.5x10^{10}$ and $3.0x10^{10}$ cells as biocatalyst, a minimal difference in conversion was observed at 48 hours (88-90%) meanwhile, a conversion greater than 96% was obtained in 48 hours using more than 3 times the amount of standard cells in the reaction (Figure 3a). However, although the yield was higher, the amount of culture broth needed to obtain $5.0x10^{10}$ cells/ml is excessive, which leads to the process being unproductive. Therefore, the amount of biocatalyst selected was $1.5x10^{10}$ cells/ml. The amount of catalyst was also varied using a substrate ratio of 30:30 mM (Figure 3b). Similar to the previous case, the reaction rate increased along with the increase in biocatalyst amount. The maximum conversion (83%) was observed at 72 hours using 3.0 $x10^{10}$ cells. According to these experiments, it is verified that in the proposed pathway, the production of phosphorylated ribose is the limiting step. Using the optimized conditions, the reaction was scaled up to 3 mL to facilitate the purification of the product for structural determination. This purification was carried out following our laboratory's standard methodology. In brief, the reaction mixture was

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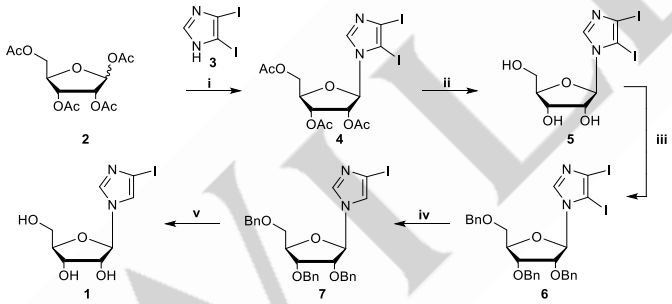
subjected to reverse phase flash chromatography, where the produced pyrimidine and residual purine bases remained adsorbed on the support, while nucleosides were selectively eluted using methanol or acetonitrile and water mixtures, resulting in high purification yields. This methodology is well-suited for scale-up processes due to the reusability of the stationary phase.[25]

Chemical synthesis of 4-iodoimidazole riboside (1)

The chemical synthesis of **1** was conducted to serve as a template for monitoring biocatalyzed reactions and to compare the reaction regioselectivity, times, and yields. Additionally, it also provides a benchmark for the evaluation of the efficiency and specificity of biocatalytic processes. As shown in Scheme 2, the synthesis pathway unfolds in five steps:[29] first, a Vorbrüggen glycosylation reaction was carried out between tetra-*O*-acetyl riboside (**2**) and 4(5)- diiodoimidazole (**3**). In the second step, the imidazole glycoside (**4**) was deprotected and the sugar was protected with non-labile groups like benzyls to perform the regioselective deiodination reaction of the imidazole riboside. Finally, the deprotection of the imidazole nucleoside was performed to obtain the corresponding nucleoside (**1**), in an overall yield of 27%. The complete synthesis takes approximately 3 to 4 days since each step requires purification of the product due to the formation of stereoisomers and other by-products. In contrast, biocatalyzed transglycosylation only requires 24 hours to obtain a yield of 88% after purification of the product, which highlights the aforementioned advantages of using biocatalysts.



Scheme 2. Chemical synthesis of 4-iodo-1-(β-D-ribofuranosyl)imidazole (1) i. a) **2** (10 mmol), **3** (1.1 equiv.), ACN, bis(trimetilsilyl)acetamide (1.1 equiv.), 1h, rT, N_2. b) TMSOtf (1.1 equiv.), 0 to 60°C, 18h, 86% (crude yield) ii. **4**, NH_4OH (28%), EtOH, rT, 18h. 75% (crude yield). iii. a) **5**, NaH (90%), THF, rT, 3 h. b) BrBn (1.1 equiv./OH), N_2, TBAI, rT, 18h, 72%. iv. **6**, EtMgBr, Et_2O, rT, 3h, 82%. ▪ Deprotection reaction: v. **7**, $BF_3.Et_2O$ (5 equiv/OBn), EtSH, DCM, rT, 72h. 70%. Overall yield: 27%.

Substrate specificity

A data collection was conducted on the use of *E. amylovora* as biocatalyst in the synthesis of several nucleoside analogs of pharmaceutical interest, as shown in Table 3. Starting from uridine as nucleoside donor (Table 3, entries 1-6), the promiscuity of the *E. amylovora* enzymes is high enough to accept different non-natural bases. However, with non-natural bases, the reactions were either slower or less efficient. The best conversion/time ratio was obtained when amino-substituted bases were used as acceptors (Table 3, entries 2 and 6). In the presence of halogen as a substituent, good conversions were obtained, but longer reaction times were required (Table 3, entries 1 and 3). In addition, *E. amylorova* NPs tolerated the absence of the OH group on C-2 of the sugar since both reactions using 2'-deoxyuridine as sugar donor were successful with moderate yields (Table 3, entries 7-8). Finally, uracilarabinoside was employed as the sugar donor but unfortunately, *E. amylorova* was unable to produce arabino nucleosides properly, being the best yields obtained when 4-iodoimidazole was used as the base (Table 3, entry 9).

Conclusions

Transglycosylation between 4(5)-iodo-1*H*-imidazole and uridine was carried out to obtain the corresponding ribonucleoside through a microbial whole cell biocatalyzed reaction. For this purpose, a screening was conducted on 71 bacteria from our cell collection. Among these bacteria, the highest conversion was achieved using *Erwinia amylovora* as biocatalyst. Additionally, the influence of variables such as culture time, buffer concentration, biocatalyst load and substrates concentration and ratio have been analyzed. Under optimal operating conditions: 30mM uridine, 10 mM 4(5)-iodo-1*H*-imidazole, $1.5x10^{10}$ cells/mL (24h culture growth) in 30 mM phosphate buffer pH7 at 60°C, after 48 h 4-iodo-1-(β-D-ribofuranosyl)imidazole (**1**) was obtained in 88.3% yield after purification. Furthermore, the biocatalyst was able to accept different acceptor bases as substrates to obtain ribo- and deoxynucleosides. The synthesis was also carried out using traditional chemical methodologies, obtaining 1 after a 5-step synthetic route with a total yield of 27%. The biocatalysis methodology using whole cells is an excellent choice for the synthesis of nucleosides due to its environmental advantages but also in terms of production costs. In order to scale up the synthesis of 4-iodoimidazole nucleosides using *E. amylovora* as whole cell biocatalyst, immobilization of this bacteria is in progress.

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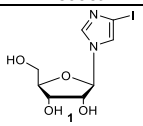
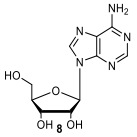
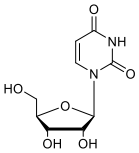
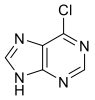
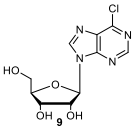
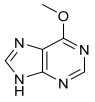
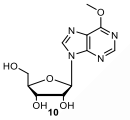
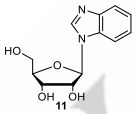
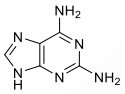
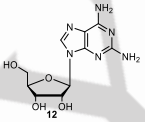
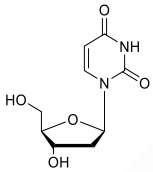
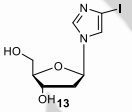
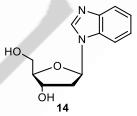
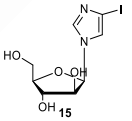
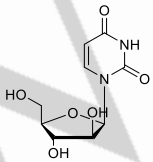
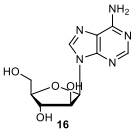
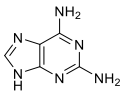
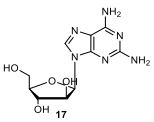
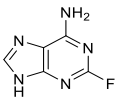
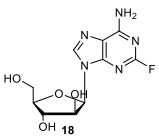
Tabla 3. Data collection of natural and modified nuclesides biocatalyzed synthesis by *E. Amilovora* [a]

Entry	Sugar Donor	Acceptor Base	Product	Conversion(%)	Time (h)	Ref
1				95.3[a]	48	*This work*
2				78	1	[30]
3				74	72	[30]
4				34	72	[30]
5				15	3	[31]
6				84	4	[32]
7				73[a]	24	*This work*
8				74	24	[31]
9				11[a]	48	*This work*
10				NC	72	[33]
11				5	24	[32]
12				NC	72	[33]

[a] Standard conditions for 4-iodoimidazole nucleosides synthesis: reactions were carried out at 60°C, using $1.5x10^{10}$ cells/mL in 30 mM phosphate buffer pH7 with a donor:acceptor ratio of 30:10 mM. Conversions were determined by HPLC (see Analytical Data in Supporting Information); NC: No conversion was detected.

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Experimental Section

General remarks: Microorganisms were supplied by the *Colección Española de Cultivos Tipo* (CECT), Universidad de Valencia (Spain). 4(5)-iodo-1*H*-imidazole was synthesized from imidazole using a reported procedure.[34] All the others chemicals were commercial products of analytical grade. TLC was performed by using Kieselgel 60 F_{254} aluminium sheets (Merck). For the determination of product yields and substrate conversions, samples dissolved in water and HPLC analyses were performed in a modular Gilson instrument (321 Pump, 156 UV/VIS detector and 234 Autoinjector Series) (Middleton, WI, USA), with a SiliaChrom SB RP18 column (150 × 4.6 mm, 5µm) (Quebec City, Quebec, Canada). All analytical data is in Supporting Information. NMR spectra were recorded at 500 MHz for ^{1}H and 126 MHz for ^{13}C (Bruker Avance Neo). The chemical shifts (δ) for ^{1}H and ^{13}C are expressed in parts per million (ppm) by using TMS as an internal standard and relative to residual signals of the solvents. Mass spectrometry was performed on a Shimadzu QP2010 mass spectrometer using electrospray ionization (ESI).

Biocatalysts preparation: Bacterial strains were inoculated in appropriate culture medium (supported by CECT) under sterile conditions and cultured in orbital shakers at 200 rpm in the optimal temperature for each strain. Growth was measured by determining the optical density at 600 nm (equivalence: 0.4 OD units: $2x10^8$ cells/ml). The resulting cultures were centrifuged at 10000 rpm for 15 min and the cell pellets were washed with reaction buffer and then used as biocatalysts.

General procedure for the synthesis 4-iodoimidazole nucleosides using *E. amylovora* biocatalyst

Analytical scale: In a 3.0 mL tube a mixture composed of wet cell paste containing $1.5x10^{10}$ cells/mL of *E. amylovora*, 0.01 mmol (2.0 mg) of 4(5)-iodo-1*H*-imidazole, 0.03 mmol of sugar donor and 30 mM potassium phosphate buffer pH 7 (final volume 1 mL), was shaken at 200 rpm and 60°C. Aliquots were taken at 0, 1, 4, 24, 48 and 72 hours and then centrifuged at 10000 rpm for 5 min. Samples were analyzed by HPLC using the following conditions: (1) 2.5 min water/methanol (95:5, v/v), (2) 5 min gradient to water/methanol (50:50, v/v) and (3) 9 min water/methanol (50:50, v/v), 1mL min^{-1} flow rate and setting the detector at λ:230nm. Retention times: uracil = 3.1 min; uridine = 5 min; **1** = 8.8 min; **13** =9.8min; 4(5)-iodo-1*H*-imidazole = 10.1 min).

Semi-preparative scale: A reaction mixture containing $1.5x10^{10}$ cells/mL of *E. amylovora*, 0.03 mmol (6.0 mg) of 4(5)-iodo-1*H*-imidazole, 0.09 mmol of sugar donor and 30 mM potassium phosphate buffer pH 7 (final volume 3 mL), was shaken at 200 rpm and 60°C. Once the reaction was completed, it was centrifuged at 10,000 rpm for 5 minutes. Nucleosides were purified from the supernatant using a variable volume column (10 mm × 200 mm, Kontes Flex-Column, Vineland, NJ, USA), containing C18 silica gel (Phenomenex, Torrance, CA, USA) eluting successively with a variable mixture of H_2O/MeOH as eluent.

4-Iodo-1-(β-D-ribofuranosyl)imidazole (1):
Yield: 88.3% (2.7 mg). White solid**,** R_f (CH_2Cl_2:MeOH, 8:2): 0.25. ^{1}H NMR (500 MHz, Methanol-d_3): δ [ppm] = 7.85 (d, *J* = 1.5 Hz, 1H), 7.51 (d, *J* = 1.5 Hz, 1H), 5.61 (d, *J* = 5.5 Hz, 1H), 4.24 – 4.13 (m, 2H), 4.03 (q, *J* = 3.4 Hz, 1H), 3.77 (dd, *J* = 12.2, 3.1 Hz, 1H), 3.68 (dd, *J* = 12.1, 3.5 Hz, 1H), 3.33 (d, *J* = 0.8 Hz, 1H), 1.92 (d, *J* = 0.8 Hz, 1H). ^{13}C (126 MHz, Methanol-d_3): 138.40, 123.10, 90.57, 85.94, 80.30, 76.19, 70.76, 61.44, 48.54, 48.33, 48.12, 47.90, 47.69, 47.48, 47.27, 47.05, 20.74. HRMS (ESI) *m/z* calcd for [$C_8H_{11}IN_2O_4$ + H]: 326.9909 found 327.0809.

1-(2-Deoxy-β-D-ribofuranosyl)-4-iodoimidazole (13):
Yield: 70.2% (1.6 mg). White solid, R_f (CH_2Cl_2:MeOH, 8:2): 0.40. ^{1}H-NMR (Methanol-d_3, 500 MHz) δ 7.76 (1H, s), 7.50 (1H, s), 6.11 (1H, dd, *J* = 6.10, 7.05 Hz), 4.55 (2H, m), 4.18 (1H, br), 3.98 (1H, dd, *J* = 3.91, 6.84 Hz), 3.72 (2H, m), 2.10 (1H, m), 2.01 (1H, m); ^{13}C-NMR (Methanol-d_3, 126 MHz) δ 138.9, 123.6, 89.1, 87.3, 82.7, 72.3, 63.1, 42.6. HRMS (ESI): *m/z* calcd for [$C_8H_{11}IN_2O_3$ + H]: 310.9893, found 310.9898.

Supporting Information

The authors have cited additional references within the Supporting Information. [35-39]

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Keywords: Whole cell • Biocatalysis • Nucleosides • 4(5)-1*H*-imidazole

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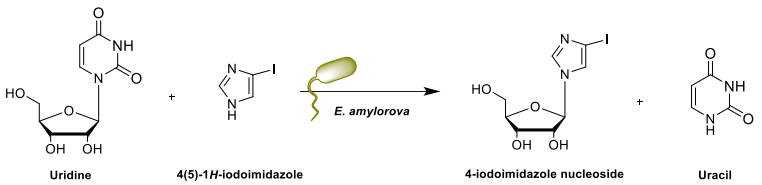
RESEARCH ARTICLE



We present the synthesis of 4-iodoimidazole-ribonucleoside via microbial transglycosylation using *Erwinia amylovora* as the whole cells biocatalyst, selected after screening our microbial collection. After all reaction parameters were optimized, the effectiveness of this biocatalyst in transglycosylation reactions with various natural and synthetic bases, as well as different sugar donor nucleosides, was confirmed.