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Development of a Robust and Sustainable Process for Nucleoside Formation

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ABSTRACT: A practical and robust process for the synthesis of an Isatoribine pro-drug was demonstrated. The process relies on a streamlined glycosylation carried out in xylene and an effective regioselective enzymatic hydrolysis that can be run in a semicontinuous way. Analysis of the process mass intensity established the high impact from an environmental standpoint of our process improvement.

In the course of one of our development projects, we became interested in an oral pro-drug (**1**) of Isatoribine (**2**), a nucleoside analogue potentially useful for the treatment of patients with chronic hepatitis C and potentially other viral infections (see Figure 1). Delivered intravenously, it is a specific

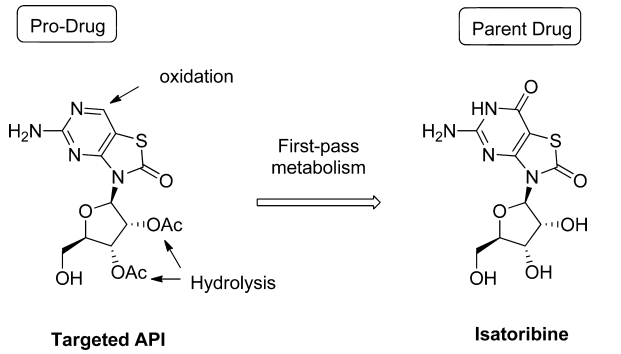

Figure 1. Targeted API (**1**).

Toll-like receptor agonist that induces the production of cytokines such as α interferon. The parent drug, Isatorabine (**2**), is obtained *in vivo* after full hydrolysis of the esters and oxidation of the base moiety.[1,2]

The original synthesis of **1** relied on the stepwise preparation of the base (**5**) followed by glycosylation and selective hydrolysis steps to the desired active pharmaceutical ingredient (API) (see Scheme 1). Key glycosylation, by coupling of 5-Aminothiazolo[4.5-*d*]pyrimidine-2,7-(3H,6H)-dione (**6**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**7**) using *N*,*N*-bis-(trimethylsilyl)acetamide (BSA) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in acetonitrile provided the product after direct isolation by precipitation. Further purification by recrystallization from acetonitrile gave 5-amino-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-3H-thiazolo-[4,5-*d*]pyrimidin-2,7-dione (**8**) in 70% yield. It was then selectively hydrolyzed with Novozyme 435 (*Candida antarctica* lipase) in a mixture of acetone and sodium phosphate buffer at pH 7 to give the desired API, 5-amino-3-(2′,3′-di-*O*-acetyl-β-D-ribofuranosyl)-3H-thiazolo[4,5-*d*]pyrimidin-2-one (**1**). The product was further purified by crystallization from a mixture of ethanol and heptane to give 87% of a white powder.

While the synthesis of the base moiety (**6**) was quite expeditive, the final glycosylation and selective ester hydrolysis presented numerous challenges. The hygroscopicity and poor solubility of the base led, for example, to a tedious, low yielding, and unreliable process in the glycosylation step. As for the final selective deprotection step, very low throughput, poor productivity, and yield, as well as high cost due to a high catalyst loading, were observed. All these aspects contributed to a very low overall performance of the initial process and a resulting high drug substance cost, both from the raw material end (large amount of solvent, high loading of enzyme required) and the processing cost. In addition, the API and all intermediates also suffered from poor physical properties that rendered all purifications by crystallization difficult. Our efforts therefore focused on these last two transformations, with the goal to rapidly come up with a practical solution for an improved and scalable process.

GLYCOSYLATION

We started our investigations on the glycosylation step, as it appeared pivotal to the overall synthetic approach. We were indeed hoping we could gain not only a better understanding of the handling of the base, but also use this know-how for its purification in the previous step. Earlier investigations had showed the Vorbrueggen protocol or variants thereof were optimal for the formation of the desired bond.[3−7] Preliminary requirement was a complete persilylation of the base, typically reported in chlorinated solvents, or acetonitrile. In our case, a combination of the very strong silylating agent, BSA and acetonitrile seemed necessary due to the overall low reactivity of our substrate. This led however to numerous side-reactions such as the decomposition *via* the Ritter manifold in the glycosylation step, or hydrolysis of the sensitive acetate protecting groups. Initial monitoring of this persilylation step *via in situ* IR revealed that the double silylation occurred quite readily and could be easily monitored using this technique. The impact of residual water was rapidly demonstrated to be critical (the presence of 0.5% water could severely impede the silylation). A solvent screening was conducted first. BSA

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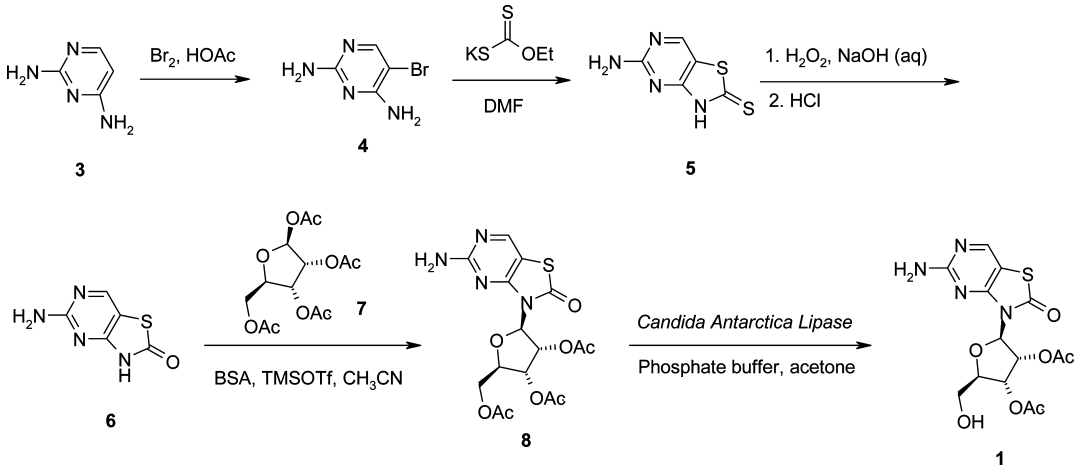
Scheme 1. First Generation Process



could not be used as a solvent as it would lead not only to extensive decomposition of the product of the glycosylation, but also to an increase of the overall process cost. Acetonitrile allowed for sufficient solubility but raised other challenges (byproducts resulting from Ritter manifold). Dichloromethane and dicholoroethane were also known solvents for such transformation but were clear undesirable options for environmental reasons. We therefore turned our attention to aromatic solvents. Toluene has been reported to allow efficient glycosylation in certain cases[8] but did not allow solubilization of our specific base, resulting in almost no reactivity. We therefore switched to the more polar aromatic and higher boiling point solvents xylene,[9] cymene and anisole. To our delight, we observed that xylene already displayed sufficient desirable solubilization properties and reactivity to allow for the persilylation in a few minutes at 60 °C (entries 1−5, Table 1).

Table 1. Solvent Effect in the Glycosylation Step[a]

entry	solvent	assay yield[b]	isolated yield[c]	purity[d]
1	CH_3CN	80%		
2	toluene	45%		
3	xylene	*ca.* 85%	67%	>99%
4	cymene	*ca.* 85%	50−55%	>99%
5	anisole	*ca.* 85%	48−50%	>99%

[a]Reaction conditions: BSA (5 equiv), TMSOTf (1.1 equiv), tetraacetylated D-ribose (1.1 eq, added in 1 h as a solid), 40 °C, 16 h. [b]Assay yield determined by HPLC by comparison with reference material. [c]Isolated yield after crystallization from EtOAc/Solvent of reaction. [d]Purity determined by HPLC at 210 nm.

We therefore focused on this option as other high boiling point solvents would eventually be problematic. Although the mixture was heterogeneous at the beginning of the reaction, xylene offered the opportunity to reduce the water content from the hygroscopic base prior to the persilylation thanks to a very efficient azeotropic drying. In addition, at the end of the persilylation, the excess BSA and its resulting byproducts could be removed by distillation to minimize the decomposition of the product to overhydrolysis products **A**, diols **B**, **C** and triol **D** (Figure 2) and bis-sugar byproducts which could not be isolated and characterized. An interesting feature of the process was the visual transition from an heterogeneous mixture to a clear solution once the persilylation had completed. The distillation besides allowed for maintaining the overall throughput attractive as the volume would remain constant despite the successive addition of the various components. The additional benefit was the opportunity to recycle xylene and silyl acetamide, which was achieved by treatment with hexamethyldisilane.

In the absence of silyl acetamide byproducts, a smaller amount of decomposition was observed (no more than 5−8% **A**+**B**+**C**+**D** observed) (see table 2, entries 2, 3, 4). However, the presence of other uncharacterized regioisomer and bis-sugar byproducts was still observed. Trimethylsilyl triflate had appeared as the optimal acid catalyst from the various Lewis acids evaluated ($SnCl_4$, $ZnCl_2$, $TiCl_4$, $BF_3.OEt_2$, $AlCl_3$). The peracetylated sugar starting material also displayed limited stability under the reaction conditions (*ca.* 50% decomposition after 24 h at 80 °C), as well as the product itself (*ca.* 20% decomposition after 24 h at 80 °C). These observations prompted us to evaluate the influence of the sugar addition (Table 3). Addition of the sugar in solution, as a slurry or as a solid had no impact on the outcome of the reaction (entries 1, 2, and 3). The solid addition was therefore preferred in order to

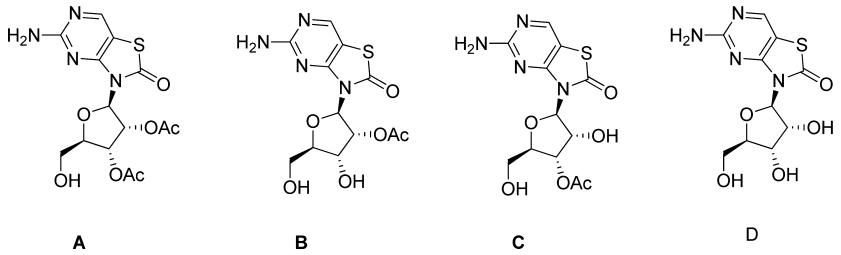


Figure 2. Main byproducts from glycolysation.

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Table 2. Influence of the Temperature and BSA and Its Byproducts' Effect on the Glycosylation Step[a]

entry	solvent	distillation after the persilylation	temp (°C)	assay yield[b]	isolated yield[c]	purity[d]
1	xylene	yes	20	51% (optimal after 10−12 h)		
2	xylene	no	60	67%		
				>15% **A** + **B** + **C** + **D**		
3	xylene	yes	60	85%	65%	>99%
				5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**
4	xylene	yes	80	85% (optimal after 2−3 h)	65%	>99%
				5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**

[a]Reaction conditions: BSA (5 equiv), TMSOTf (1.1 equiv), tetraacetylated D-ribose (1.1 equiv, added in 1 h as a solid), 4−5 h. [b]Assay yield determined by HPLC by comparison with reference material. [c]Isolated yield after crystallization from EtOAc/xylene of reaction. [d]Purity determined by HPLC at 210 nm.

Table 3. Influence of the Sugar Addition[a]

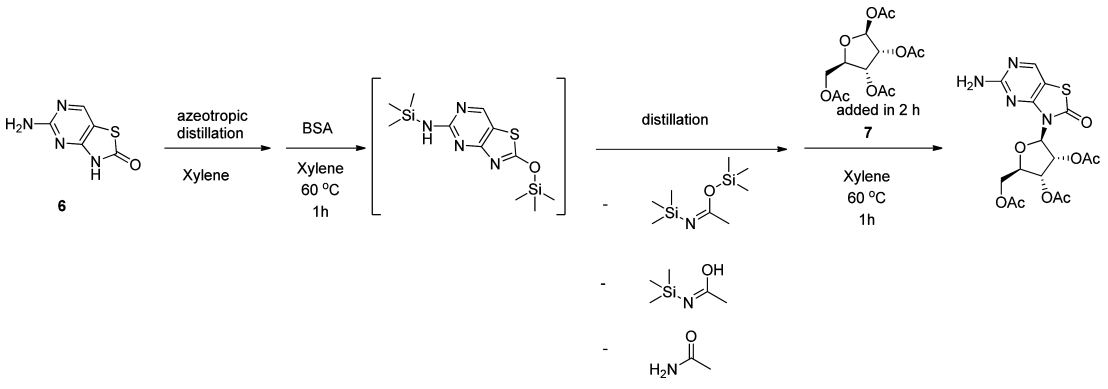
entry	sugar addition	assay yield[b]	isolated yield[c]	purity[d]
1	solid in 1 h	85%	65%	>99%
		5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**
2	solution in 1 h	85%	65%	>99%
		5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**
3	slurry in 1 h	85%	65%	>99%
		5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**
4	solid in single portion	75−80%	57%	>99%
		5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**
5	solid in 10 portions over 1 h	85%	65%	>99%
		5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**
6	solid in 10 portions over 4 h	85%	65%	>99%
		5−8% **A** + **B** + **C** + **D**		<0.6% **A** + **B** + **C** + **D**

[a]Reaction conditions: xylene, BSA (5 equiv), TMSOTf (1.1 equiv), tetraacetylated D-ribose (1.1 equiv), 60 °C, 4−5 h at the end of the sugar addition. [b]Assay yield determined by HPLC by comparison with reference material. [c]Isolated yield after crystallization from EtOAc/xylene of reaction. [d]Purity determined by HPLC at 210 nm.

minimize the overall volume of the reaction. The rate of the sugar addition was also found to be critical. Decomposition of the sugar was found to take place as demonstrated by the formation of bis-sugar side-products and required controlled addition of the sugar to minimize formation of these side-products (entries 4, 5, 6).

Overall, the process appeared quite robust, as very similar yields and purity were observed from our multigram laboratory experiments. The following options were eventually selected as the most efficient and with the least amount of side-products (see Figure 2): the portionwise addition of the peracetylated sugar (1.5 equiv) in 2−3 h, a slightly longer addition time preferred in our production facilities, and reaction over 12 h at 60 °C (Scheme 2), resulting in a *ca.* 85% chemical yield. Our solvent screening had shown that a combination of aromatic solvent and an ester could result in crystalline material of very high purity. Therefore, we just had to proceed with an aqueous workup followed by distillation to a *ca.* 12−18% mixture in xylene (determined by GC in the laboratory and by weighing of the distillate in our plant), resuspension in isopropyl acetate, demonstrated to be the optimal ester for purification and throughput reasons, and slow cooling to 0 °C, resulting in highly crystalline material that could be isolated by simple filtration and drying to result in an overall 65% yield of product as a single isomer, in a purity >99%, with the hydrolysis products as the main byproducts. The critical content of xylene for the crystallization was at the time determined by the weight of the distillate, hence a rather large range to allow for a robust process. This process was repeated with various grades of base and turned once again extremely robust, in terms of both chemical yield and isolated purity, which was one of our major concerns to be able to produce on time. We had besides demonstrated a more than 4-fold improvement in our throughput, based on the development of an heterogeneous process, and the recycling of the various components of the reaction, which can be further illustrated by our overall performance in process mass intensity, the currently accepted metrics to express the greenness of a process.[10]

Scheme 2. Final Glycosylation Process



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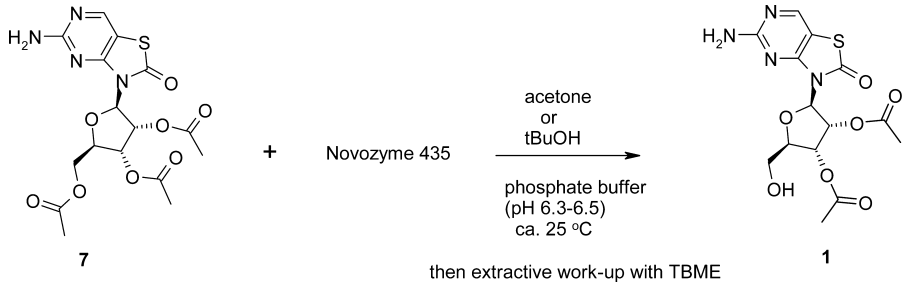
Scheme 3. Original Hydrolysis Step



This practical process to glycosylate a sugar with a nucleobase was later demonstrated to be general, operationally simple, highly practical, and robust. It is of particular interest for highly hygroscopic and poorly soluble nucleobases and is based on the use of a high boiling point, nonpolar solvent, which allows for the azeotropic drying of the nucleobase and the removal of silylating agent byproducts after the initial persilylation step.

HYDROLYSIS

The final step consisted in the regioselective hydrolysis of the acetate of the primary alcohol (see Scheme 3). Enzymes have long been demonstrated to be the best option in cases where such selectivity is expected from a transformation and in the presence of sensitive functional groups. In our case, *Candida antarctica* lipase had been identified as the lipase of choice from an earlier screening. Its polymer immobilized version Novozym 435[11] was the most attractive option from a long-term process standpoint, as we could envision enzyme recycling. Immobilized lipases are generally used to perform biotransformations of most interesting industrial applications that quite often take place in nonaqueous media.

The process was originally conducted in acetone or *tert*-butanol, under phosphate buffer conditions to allow for the hydrolysis and avoid further hydrolysis of the byproducts **B**, **C**, and **D** (Figure 2). Under these conditions, completion was observed after up to 2 days, and substantial amounts of mono- and diacetates were observed, as a consequence of the long reaction time. Furthermore, an overall very low volume efficiency was required (>50 L total organic solvent/kg product recovered), which rendered the process all the more unsuitable for large scale, along with a high catalyst loading.

We first screened a variety of mono- and biphasic solvent systems. Solvent selection is known to be a critical factor for biocatalysis,[12] as organic solvents are known to influence the enzyme activity and selectivity.[13−15] Solvents are indeed reported to impact an enzyme conformation by interacting with the hydration layer essential for catalysis and by altering hydrophobic or H-bonding in the core of the protein as well as protein solvation sites.[16]

Our screening confirmed that *tert*-butanol and acetone were some of the optimal solvents from a reactivity standpoint. However, alternatives were identified such as isopropanol, 2-butanol, and 2-MeTHF (see Figure 3).

The latter especially attracted our attention, as solubility studies had revealed the product to be more than 50 times more soluble in 2-MeTHF than in TBME, the extraction solvent used in the first generation process. Together, this meant a dramatic reduction of the solvent requirements for the workup in particular, as no additional water immiscible solvent

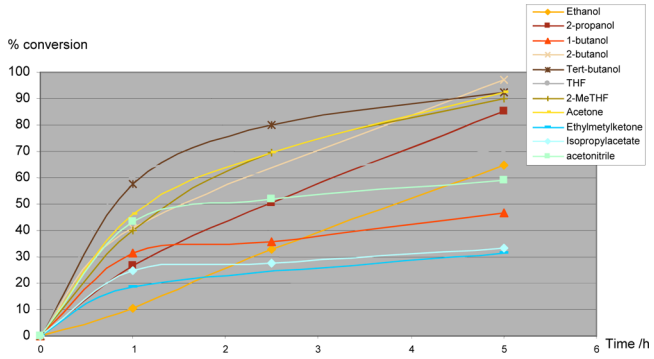


Figure 3. Solvent screening for the enzymatic hydrolysis.

was required. This resulted in an immediate improvement in the volume efficiency by *ca.* 30%. In practice, this meant that the same given reactor could allow us to produce 30% extra product within the same time frame.

We continued our optimization of the volume efficiency and demonstrated that the enzyme was actually very robust in this solvent system. No deactivation was observed even with relatively high concentration of starting material (see Table 4), and a robust 85% isolated yield was obtained after crystallization from MTBE/2-MeTHF.

Table 4. Optimization of the Concentration[a]

conc (mol/L)	0.1	0.12	0.135	0.15
isolated yield[b]	*ca.* 85%	*ca.* 85%	*ca.* 85%	*ca.* 85%
HPLC purity[c]	94.3%	94.6%	95.3%	94.5%
reaction volume efficiency (overall volume/kg product (**1**)	*ca.* 26	*ca.* 21	*ca.* 17	*ca.* 13

[a]Reaction conditions: Novozym 435, 2-MeTHF, pH 6.3−6.5, 25−27 °C, 16 h. [b]Isolation by crystallization from TBME/2-MeTHF. [c]Purity determined by HPLC at 210 nm.

Ultimately, an improvement of the throughput by a factor >2 was achieved for this batch process. The purity obtained at this stage was sufficient to meet the higher specifications expected at the selected salt form of the API.

We then turned our attention to the high catalyst loading required. The long-term goal was to accelerate the enzymatic process in such a way that could minimize the reaction time and avoid further hydrolysis of the diacetate product to the monohydrolyzed and fully hydrolyzed byproducts. Such a continuous or semicontinuous process could allow for recycling of the immobilized enzyme. In order to accelerate the process, we screened a series of additives, such as various Lewis acids ($FeCl_3$, for example[17]), the inorganic salts NaCl and LiCl[18]), or other additives (PEG[19]). An interesting but moderate effect

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with LiCl and PEG was observed (5−10% acceleration) so that the added benefit did not appear significant enough to justify such a change (see Figure 4).

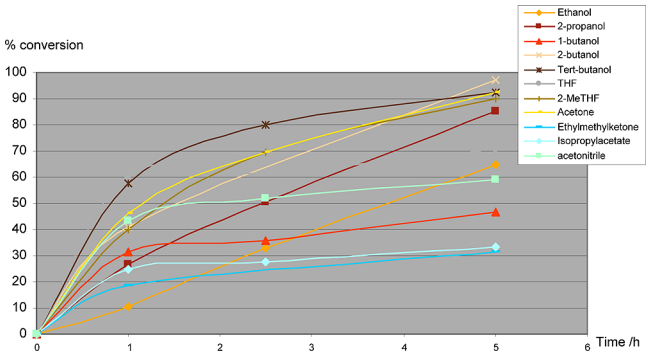

Figure 4. Additive effect.

Fortunately, the high stability of the enzyme provided an even simpler handle for acceleration, while maintaining the desired selectivity. A *ca.* 20% reaction acceleration was indeed observed when increasing the temperature to 35 °C but unfortunately only in acetone, thus requiring a second immiscible solvent for the isolation, most probably 2-MeTHF as previously identified.

With these considerations, a system was designed using a column packed with Novozym 435 and swollen with acetone. At that stage, a feed of the starting material in acetone at a concentration of 1.6 mol/L and 35 °C would be circulated at various flow rates in a loop mode until full conversion was observed, as indicated by the plateau observed during the *in situ* pH measurement. The pH was constantly adjusted to 6.3−6.5 with a phosphate buffer. It was rapidly discovered that the reaction could be >98% complete within a few hours with this simple setup, with *ca.* 1.3% overhydrolysis byproduct. This was achieved at this stage with a relatively high enzyme loading of 3 equivalent weight ratio of enzyme to substrate and a 1.6 mL/L flow rate (see Table 5 for reaction monitoring). By comparison,

Table 5. Head-to-Head Comparison of Batch/Semibatch Processes[a]

	batch process	semibatch process
isolated yield	85%	90%
purity	94.3% to 95.3%	95.4% to 98.2%
overhydrolysis	3%−5% total **B**, **C**, **D**	1% total **B**, **C**, **D**

a batch mode hydrolysis would result in the same conversion within 16 h and would display 3−5% overhydrolysis, with the same catalyst loading.

Encouraged by these preliminary results, we then studied the impact of the contact time. It was then demonstrated that the fastest flow rate and residence time would minimize overhydrolysis to the di- and monoacetate byproducts **B**, **C**, and **D** from 1.3% to <1.0% when going from 1.6 mL/min to 6.4 mL/min. The next obvious development was to reuse the enzyme. We were here delighted to find out that, after up to 15 uses, no significant loss of activity was observed with yields of the desired monohydrolyzed product oscillating between 95.4% and 98.2%, and less than 1.6% overhydrolysis in all cases (Figure 5). Only when the flow was interrupted and the column stored overnight could a drop in reactivity and slight

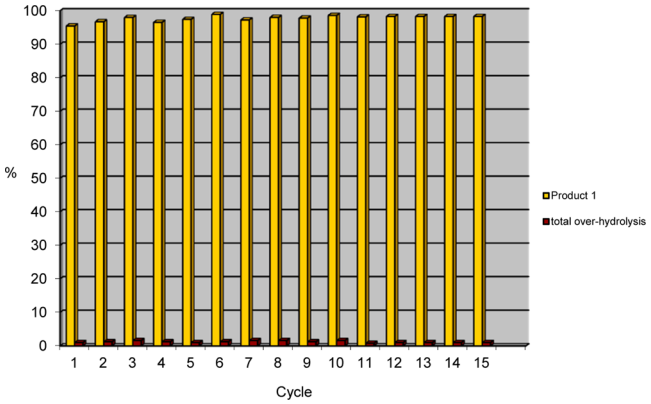

Figure 5. Enzyme activity over 15 cycles. The reaction was conducted as described in the Experimental Section. The percentages indicated are HPLC area at 210 nm. The overhydrolysis side-products and the desired product have the same extinction coefficient at that wavelength.

overhydrolysis be observed (yield down to 88.6% after the 10th cycle and up to 2% overhydrolysis byproducts), presumably due to a much longer contact time of substrate with the enzyme. Nevertheless, we had demonstrated that the enzyme could be recycled very efficiently to result in an optimal process.[20]

GREEN CHEMISTRY METRICS

To illustrate our process improvements, we wanted to measure some of the well-accepted green metrics such as the process mass intensity. The process mass intensity (PMI) is a well accepted metric of greenness currently recommended by the ACS Green Chemical Pharmaceutical Roundtable. It is defined as the quantity of raw material input in kilograms per the quantity of bulk API in kilograms. For the latter, several PMIs were calculated on solvents, water, and reagents (see Table 6).[21]

Table 6. Process Mass Intensity for the Old and New Processes on the Glycosylation and Hydrolysis Steps[a]

hydrolysis step	old process	new process in w/o recycling	new process w/ recycling
step PMI substrate, reagents, solvents	37.9	25.0	16.0
step PMI solvents	32.6	19.6	12.3
step PMI aqueous	37.2	9.6	9.6
step PMI	75.1	34.5	25.5

hydrolysis step		new process in 2-MeTHF	new process in 2-MeTHF w/ recycling
step PMI substrate, reagents, solvents	280.2	23.5	11.9
step PMI solvents	277.6	21.5	11.2
step PMI aqueous	51.8	43.6	43.6
step PMI	332.0	67.1	55.5

For the glycosylation step, a 2- and even 3-fold improvement of the overall mass intensity was achieved without or with recycling, respectively, due in part to a better choice of organic solvent to promote the reaction and to the superior physical properties of our selected solvents that did not require an additional water-immiscible solvent for the extraction. Besides, the product of the glycosylation, **7**, could be isolated and purified by crystallization without extensive operations and

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water washings in the workup, hence giving a *ca.* 4-fold improvement in the mass intensity on water.

For the enzymatic hydrolysis step, a very interesting >6-fold improvement in mass intensification was observed in batch using 2-MeTHF as the organic solvent. For the semicontinuous process, the benefits were not as obvious, as it could only perform comparably in acetone, which subsequently required a water-immiscible organic solvent.

In conclusion, a pratical and robust process for the synthesis of an Isatoribine pro-drug was demonstrated. The process relies on a streamlined glycosylation process in xylene and an effective and regioselective enzymatic hydrolysis with a much improved environmental impact. The catalytic activity of the immobilized lipase was demonstrated to be very robust, as the enzyme displayed an excellent behavior as catalyst in both aspects of high levels of activity, selectivity, and excellent operational stability. This process was further developed in a semicontinuous mode and showed to proceed in an even more efficient manner from a throughput standpoint.

EXPERIMENTAL SECTION

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(5-amino-2-oxothiazolo[4,5-*d*]pyrimidin-3(2H)-yl)tetrahydrofuran-3,4-diyl Diacetate (8). A solution of the 5-aminothiazolo[4,5-*d*]pyrimidin-2(3H)-one (**6**) (0.85 kg, 5.1 mol) in xylene (12 L) was distilled at 70−80 °C (100−150 mbar), removing *ca.* 1 L of solvent. The solution was cooled to 60 °C, and BSA (2.62 kg, 12.9 mol) was added dropwise and stirred for 1 h. The reaction mixture was distilled again, removing *ca.* 1 L of solvent, excess BSA, and byproducts. The reaction was cooled down to 60 °C, and TMSOTf (1.2 kg, 5.4 mol) was added dropwise, followed by the tetraacetylated D-ribose (**7**) (1.7 kg, 5.4 mol), which was added continuously over the course of 90 min. The reaction mixture was stirred at 65 °C for 16 h. At completion, the temperature was reduced to 35−40 °C and isopropylacetate (6 L) was added. The resulting mixture was stirred at that temperature for 10 min, and 8% sodium bicarbonate solution was added at a rate such that the temperature remained below 45−50 °C. The resulting mixture was stirred at 35−40 °C for 15 min. Stirring was stopped, and the phases were separated. The desired product is in the organic phase. The aqueous extract was extracted one more time. 20% sodium chloride solution was added, and the resulting mixture was stirred for 15 min at 20−25 °C. Stirring was stopped, and the phases were separated. The desired product is in the organic phase. Isopropylacetate was charged, and the resulting suspension was refluxed for 0.5 h (internal temperature 85−90 °C, jacket temperature 95 °C) to collect the solid on the side of the reactor. When a clear solution was obtained, with no more solid on the vessel, the temperature was reduced to 50−55 °C (jacket temperature 55 °C) and some seed product was added. The seeds remained suspended, and the resulting mixture was stirred at that temperature for 0.5 h until a fine suspension was observed. The temperature was decreased to 20−25 °C in 2 h and to 0−5 °C in 1 h. The suspension was held at that temperature for 2 h, and the solid was filtered through a glass sintered funnel (diameter 14 cm) under vacuum. The wet cake was rinsed with cold isopropylacetate (ca. twice the cake volume). The solid was dried for 16 h in a vacuum oven at 50 °C and 50 mbar to provide 1.4 kg of a white solid (single isomer observed by ^{1}H NMR). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, 1H), 6.15 (d, 1H, J = 3.2 Hz), 6.06 (dd, 1H, J = 5.6, 2.8 Hz), 6.01 (dd, 1H, J = 7.2, 6.0 Hz), 5.29 (bs, 2H), 4.51 (dd, 1H, J = 12.0, 3.6 Hz), 4.36−4.31 (m, 1H), 4.23 (dd, 1H, J = 12.0, 5.6 Hz), 2.13 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 169.7, 169.0, 160.9, 155.3, 150.5, 104.3, 85.6, 78.5, 72.0, 70.0, 62.7, 20.7, 20.6, 20.5 ppm. LR-MS (EI): *m/z*: calcd for $C_{16}H_{18}N_4O_8SH$ [M + H$^+$]: 427.1, found: 427.1. HPLC purity: 99.2% with major byproduct **A** observed in 0.19%; HPLC assay: 99.4%; titration: 98.8%; water content: 0.2%.

(2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(5-amino-2-oxothiazolo[4,5-*d*]pyrimidin-3(2H)-yl)tetrahydrofuran-3,4-diyl Diacetate (1). *Batch Process.* Peracetylated product (**8**; *ca.* 1.0 kg) was charged to an inert reactor. 2-MeTHF (2.2 L) was added at room temperature, and the suspension was stirred at room temperature. Phosphate buffer (15.8 L) was added at room temperature. (The phosphate buffer was freshly prepared according to the following recipe: 450 g of disodiumhydrogenophosphate was dissolved in 32 L of water, and *ca.* 67 mL of phosphoric acid (*ca.* 85%, Fluka 79617) was added to adjust the pH to 6.95−7.05. The density was measured to be 1.00027.) The pH was adjusted to 6.50 with 0.25 mol/L phosphoric acid. Novozym 435 (370 g) was added, and the suspension was stirred at IT 25−27 °C at *ca.* 150 rpm until completion. The pH was adjusted to 6.3−6.5 with a 0.5 mol/L disodium hydrogenophosphate solution about every 4 h. At completion, the reaction mixture was filtered through a Nutsche filter to remove the enzyme, and the cake was rinsed with 2-methyltetrahydrofuran (1 L). 2-Methyltetrahydrofuran was added to the filtrate, and the resulting biphasic mixture was stirred at IT 35−40 °C for 15 min. Stirring was stopped, and the phases were separated. The aqueous phase was extracted a second time with 2-methyltetrahydrofuran (3 L) at IT 35−40 °C, and the organic extracts were combined and washed with deionized water (9 L) at IT 20−25 °C. To the organic phase was added activated charcoal (8.8 g), and the resulting suspension was stirred at IT 20−25 °C for 2 h and filtered through a pad of Celite. The cake was rinsed with 2-methyltetrahydrofuran (3 L). The resulting yellowish solution was concentrated at IT 40−45 °C (IT < 50 °C) and *ca.* 180 mbar to about one-fourth of the volume and crystallized from TBME/2-MeTHF to result in 0.78 kg of desired product in 96.1% purity. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (s, 1H), 6.28 (d, 1H, J = 3.2 Hz), 6.08 (dd, 1H, J = 5.6, 2.8 Hz), 5.72 (dd, 1H, J = 7.2, 6.0 Hz), 5.50 (bs, 2H), 4.32 (bs, 1H), 3.98 (dd, 1H, J = 12.0, 2.8 Hz), 3.85 (bs, 1H), 2.19 (s, 3H), 2.09 (s, 3H) ppm. LRMS (EI): *m/z*: calcd for $C_{14}H_{16}N_4O_7SH$ [M + H$^+$]: 385.3, found: 385.3.

Semicontinuous Process. A 1 cm diameter Nutsche filter was charged with *ca.* 10 g of *Candida antarctica* lipase Novozym 435. A solution of adduct (*ca.* 10 g dissolved in 9 mL of acetone and 16 mL of pH 7.0 phosphate buffer) was passed through the filter at *ca.* 1.6 mL/min (*ca.* 0.2 bar pressure) until completion. The pH of the filtered mixture was continuously maintained between 6.3 and 6.5 with a Na_2HPO_4 solution. The reaction was complete after *ca.* 2 h. The process was repeated 10 times. The phases were then separated, and the aqueous phase was extracted one time with *ca.* 200 mL of 2-methyltetrahydrofuran. The combined organic phases were washed once with water and concentrated under reduced pressure to give the crude product in >90% yield and with <1% overhydrolysis byproduct.

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Notes

The authors declare no competing financial interest.

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