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1 Article

From a sequential chemo-enzymatic approach to a continuous process for HMF production from glucose

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15 Abstract: Notably available from the cellulose contained in lignocellulosic biomass, glucose is a 16 highly attractive substrate for eco-efficient processes towards high-value chemicals. A recent 17 strategy for biomass valorization consists on combining biocatalysis and chemocatalysis to realise 18 the so-called chemo-enzymatic or hybrid catalysis. Optimisation of the glucose conversion to 19 5-hydroxymethylfurfural (HMF) is the object of many research efforts. HMF can be produced by 20 chemo-catalyzed fructose dehydration, while fructose can be selectively obtained from enzymatic 21 glucose isomerization. Despite recent advances in HMF production, a fully integrated efficient 22 process remains to be demonstrated. Our innovative approach consists on a continuous process 23 involving enzymatic glucose isomerization, selective arylboronic-acid mediated fructose 24 complexation/transportation and chemical fructose dehydration to HMF. We designed a novel 25 reactor based on two aqueous phases dynamically connected via an organic liquid membrane, 26 which enabled substantial enhancement of glucose conversion (70%) while avoiding intermediate 27 separation steps. Further, in the as-combined steps, the use of an immobilized glucose isomerase 28 and an acidic resin facilitates catalyst recycling.

- 29 Keywords: (bio)catalysis; Biomass; Glucose; HMF; chemo-enzymatic catalysis
- 30

31 **1. Introduction**

- 32 In a context of fast depletion of fossil resources, lignocellulosic biomass possesses a high potential as
- a sustainable raw material for fuels [1] and fine chemicals [2, 3] production in industrially significant
- volumes. Upon specific physical, chemical and/or biological treatments of lignocellulose, access can
- 35 be given to monomeric sugars [4], like glucose as its main saccharidic component. Glucose is, as a
- 36 result, currently considered as one of the most promising starting materials for the conversion of
- 37 biomass into drop-in or new high-value platform chemicals [5].
- 38 Efficient and eco-responsible valorization routes for this new renewable carbon source are highly
- desirable, and every field of catalysis should be mobilized as the key to success for a selective
- 40 valorization of such a polyfunctional substrate. Biocatalytic processes are already or about to be
- 41 industrialized [6] and heterogeneous or homogeneous catalysis, alone or in combination, have
- 42 shown high efficiency for biomass valorization, but lack of selectivity still causes problems in the
- 43 products separation steps [7]. In the very last years, a new paradigm of catalysis has emerged to

- 44 answer the challenges of selectivity and productivity by combining biocatalysis and chemocatalysis
- 45 [8-10]. This combination is known as chemo-enzymatic catalysis or hybrid catalysis [11, 12].

46 In the field of sustainable and biosourced chemistry, glucose conversion to 5-hydroxymethylfurfural 47 (HMF) drives a specific attention as a platform molecule [13]. Its hydrogenation, for instance, can 48 lead to 2,5-dimethylfuran (2,5-DMF), a new fuel additive [14] and a precursor of terephthalic acid 49 after dehydrative Diels-Alder reaction with ethylene to p-xylene and subsequent oxidation [15, 16]. 50 HMF oxidation is also envisioned to obtain 2,5-furandicarboxylic acid (FDCA), a biosourced 51 alternative to terephthalic acid in the polyesters plastic production with a high market potential [17, 52 18]. Unfortunately, abundant glucose is not the substrate of choice for efficient dehydration to HMF 53 compared to its more expensive fructose isomer. The latter presents a fructofuranose mutamer more 54 prone to dehydration to a furanic ring with lower energetic barriers [19]. Isomerization of glucose to 55 fructose thus appears as a key step that can hardly be circumvented. A well-known transformation 56 of glucose to fructose is the enzymatic glucose isomerization, employed in the industrial production 57 of high-fructose corn syrup "HFCS" [20]. Despite the need for expensive high purity glucose, for 58 buffers and multiple ion-exchange resins to get rid of all the metallic residues in alimentary HFCS, 59 enzymatic isomerization remains the preferred process compared to chemical isomerization, even if 60 the latter receives a recent renewed interest [21-25]. The aforementioned enzymatic reaction reaches 61 a thermodynamic equilibrium (Keq ~ 1), which limits glucose conversion [26-28]. Whereas this 62 equilibrium is particularly well-adapted for HFCS production involving glucose/fructose mixtures, 63 HMF synthesis requires a pure fructose feed for the subsequent dehydration step, where 64 compatibility issues between catalysts arise: Huang & al indeed shown incompatibility between a 65 thermophilic glucose isomerase immobilized on aminopropyl-functionalized mesoporous silica 66 (FMS) and a heterogeneous Brønsted acid propylsulfonic-FMS-SO₃H in a THF:H₂O (4:1 v/v) mixture 67 [29]. Isomerization is carried out at 363 K to reach 61% fructose yield, and then the temperature has 68 to be increased to 403 K to reach a 30% HMF yield. In the meantime, glucose isomerase is fully 69 denatured.

70 Strategies have thus been set up to combine enzymatic isomerization of glucose to fructose and 71 chemical by separating bio- and chemo-catalysis involving fructose transportation between the 72 isomerization aqueous phase and an organic phase for subsequent reactivity. Huang et al [30] 73 describe this concept for the first time by adding sodium tetraborate in the aqueous isomerization 74 medium to form a fructoborate compound by complexation with fructose. Transportation of this 75 complex to a separated organic phase is assisted by a cationic quaternary ammonium and enables 76 enhanced glucose conversion by shifting the isomerization equilibrium towards fructose. The 77 separate dehydration of fructose in the organic phase gives an increase in HMF yield up to 63% 78 compared to a yield of 28% HMF without borate addition, associated to a glucose conversion of 88% 79 instead of 53%. However, in this work, the complexation selectivity between glucose and fructose 80 was not satisfactory and was thus further improved by Palkovits & al, who optimized the chemical 81 nature of the complexing boronate species [31]. A global process exploiting this concept for glucose 82 to HMF production has concomitantly been proposed by Alipour et al [32] Therein, fructose is 83 complexed with phenylboronic acid and transferred to an organic phase, which is separated and 84 contacted with an acidic ionic liquid phase to promote the release of free fructose. This fructose-rich 85 ionic liquid is further used for dehydration to HMF in a biphasic medium. The produced HMF is 86 then back-extracted to a last low-boiling point organic phase. This method involves the use of four 87 different media with intermediate phase separations. Inspired by these sequential processes, we 88 propose here to circumvent this drawback by setting up an innovative integrated implementation 89 minimizing the number of steps and preventing among others the use of costly and hard to recover 90 ionic liquids.

91 Herein, we highlight an efficient and fully-integrated cascade reactions process to convert glucose to

- 92 HMF using an organic liquid membrane separating two aqueous phases for fructose transportation.
- 93 This approach that we first proposed as a concept exemplifying the potential of hybrid catalysis [33]

94 is based on continuous aqueous glucose isomerization, fructose complexation, extraction and 95 transportation towards an intermediate organic phase. Fructose is then released at the interface with 96 the second aqueous phase (aqueous receiving phase), where it is subsequently dehydrated to HMF. 97 This integrated process without intermediate phase recovery, separation and recycling is presented 98 in Figure 1. Before fully designing the entire process, where all the reactions are carried out 99 simultaneously, we separately investigated each reaction to notably determine their respective 100 optimal ranges of conditions before combination: (i) Glucose isomerization to fructose occurs in a 101 primary aqueous feed phase; (2) The as-formed fructose transportation is then made possible 102 through selective complexation with phenylboronic acid as a carrier associated to a quaternary 103 ammonium into an organic solvent (4-methyl-2-pentanone "MIBK"); (3) Eventually, the 104 fructoboronate complex is hydrolyzed by acidic conditions in a receiving aqueous phase containing 105 an acidic resin, which also promotes dehydration of fructose to HMF. Our approach is not only 106 focused on the compatibility issues of bio- and chemocatalytic reactions with the aim of overcoming 107 the isomerization equilibrium limitation, but also pays a particular attention in integrating all the 108 steps to minimize separation and recycling burdens, which can be detrimental for the overall 109 economics and efficiency of the process. The methodology envisioned to move from a sequential

110 approach towards an integrated continuous process is schematized in Figure 2.



- 112 Figure 1. Hybrid catalysis simultaneous process applied to the transformation of glucose to HMF
- 113 (D-Glc = D-glucose, D-Fru = D-Fructose, HMF = 5-hydroxymethylfurfural)
- 114 **Figure 2.** Methodology from a sequential approach towards an integrated continuous process.



115

116 2. Results and Discussion

117 2.1. Study of D-glucose enzymatic isomerization in the aqueous feed phase

118 The optimal temperature of use ranges from 333 to 353 K and the optimum pH ranges from pH 7.0 to 119 9.0 [34]. We first checked the influence of pH and temperature using the lot of IGI received from our 120 supplier. A glucose solution at pH 7.5 (Tris-HCl buffer, 100 mM) was prepared and isomerized from 121 323 K to 363 K. The relative enzymatic activity is presented in Figure 3, which highlights an optimal 122 enzymatic activity at 343 K. A glucose solution of a pH from 4.5 to 9 (Tris-HCl buffer, 100 mM from 123 pH 7 to 9; sodium phosphate buffer, 100 mM pH 6.5; sodium citrate buffer, 100 mM pH 4.5) was thus 124 prepared and isomerized at 343 K. The relative enzymatic activity presented in Figure 4 shows an 125 optimal IGI activity at a pH around 7.5. It is noteworthy that the IGI maintained more than 80% of its 126 optimal activity between pH 7 and 9. Below a pH of 6.5, the IGI lost more than 50% of its activity. 127 The selected pH in the aqueous feed phase for the enzymatic isomerization reaction was 8.5. A 128 D-glucose enzymatic isomerization was then performed in the selected conditions (Tris-HCl 100 129 mM, pH 8.5, 343 K). The results are presented in Figure 5 as a function of time. A blank reaction 130 (grey dots) was ran in the absence of IGI and showed the absence of D-glucose isomerization or 131 degradation in the chosen experimental conditions. When IGI was present in the system, the 132 D-fructose amount increased from 0% to 42% during the first 30 min. From 30 to 60 min, the 133 D-glucose conversion was slower, while the D-Fructose amount increased from 42% to 55%. A 134 maximum D-glucose conversion of 55% was finally reached in agreement with the glucose-fructose 135 thermodynamic equilibrium expected at this temperature (Keq = 1,23), as already observed by



136 McKay et al [35].

Figure 3. Relative enzymatic activity of IGI in D-glucose isomerization at pH 7.5 as a function oftemperature.

139 Figure 4. Relative enzymatic activity of IGI in *D*-glucose isomerization at 343 K as a function of pH

- 140
- 141 **Figure 5.** D-glucose consumption during the isomerization step as a function of time; (Δ), D-fructose



% of C6



90

120

142 formation (□), D-glucose amount in the blank experiment at pH 8.5 (○). Initial conditions: D-Glucose

60 Time (min)



30

144 2.2. Organic liquid membrane for *D*-fructose complexation/transportation

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145 In order to enhance the isomerization yield, simultaneous isomerization and 146 complexation/transportation of D-fructose in a liquid/liquid aqueous/organic biphasic system was 147 further studied. The temperature was set at 343 K and the pH was chosen as a trade-off to 148 simultaneously maximize the enzymatic activity (7 < pH < 9), to avoid monosaccharide degradation, 149 and to enhance the extraction process as reported previously [36]: the pH was thus kept at a value of 150 8.5 by using a Tris-HCl 100 mM buffer. The extraction was conducted in an organic solvent with low 151 water miscibility (MIBK) containing a lipophilic arylboronic acid (carrier) and a quaternary 152 ammonium salt (Aliquat336®) as a phase transfer agent [37]. At pH 8.5, aryl boronic acid ArB(OH)2 153 was actually present under its hydroxylated anionic form, as a tetrahedral aryltrihydroxyborate 154 ArB(OH)3⁻ [38]. At the interface between the aqueous and organic phases, D-fructose further reacts 155 with the arylborate to form a tetrahedral fructoboronate ester (Figure 6) [38]. The fructoboronate 156 complex then forms an intimate ion pair with Aliquat336®, which enables its transportation to the 157 organic phase [39].



158 Figure 6. Fructoboronate complex formation at the interphase between the first aqueous phase and159 the organic liquid membrane.

- 160
- 161 2.2.1. Influence of the boronic acid structure

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162 The influence of the boronic acid structure was investigated in order to optimize kinetics and 163 maximize selectivity of D-fructose complexation/transportation. Seven arylboronic acids differing 164 by the electronic properties of their substituents and thus by their pKa were screened in the 165 complexation reaction with D-fructose: 2,3-DCPBA, 2,4-DCPBA, 3,4-DCPBA, 3,5-DCPBA, PBA, 166 4-TBPBA, 4-TFMPBA. The complexation/transportation, which will be referred to as "extraction" in 167 the following sections, was carried out in a biphasic system, as described before. The pKa of the 168 different boronic acids and the relative extraction yields are summarized in Table 1 and reported in 169 Figure 7.

170

171 **Table 1**. Influence of the boronic acid structure on the extraction yield and rate.



187

Figure 7. Influence of the arylboronic acid structure on the complexation/transportation process
 after 45 min. [D-Fructose]I = 100 mM, [Boronic acid] = 100 mM, [Aliquat336[®]] = 200 mM, Tris HCl 100

190 mM pH 8.5, MIBK, 343 K, 750 rpm.

192 A group composed by 2,4-DCPBA, 3,4-DCPBA, 2,3-DCPBA, 4TFMPBA and 3,5-DCPBA showed the 193 best results with extraction yields between 43% and 55%, while with 4-TBPBA and PBA only 8% and 194 32% were achieved, respectively. These results also show that the D-fructose extraction yield 195 increases when the pKa of the boronic acid used decreases. Better extraction yields were observed 196 when the pKa of the boronic acid was lower than (or close to) the pH of the aqueous feed phase (8.5). 197 This analysis supports the hypothesis from Morin et al. [36] and Karpa et al [37] indicating that 198 higher extraction yields are obtained when $pH \ge pKa$, favoring a predominant tetrahedral anionic 199 borate form of the complex, which enables its transportation by association with the ammonium 200 carrier. An exception was however observed with 4-TFMPBA with a pKa of 9.1. This might be due to 201 the fact that 4-TFMPBA has a higher dipolar moment (2.90 D, against 0.43 D to 1.86 D for the other 202 used boronic acids) that could increase the reactivity. The extraction rates and the pKas (Table 1) 203 seem to be correlated. A higher pKa of the boronic acid was linked with a lower initial extraction 204 rate. Among the most effective boronic acids, 3,4-DCPBA was selected for further studies.

205

206 2.2.2. Influence of the boronic acid:Aliquat336[®] molar ratio

207 With 3,4-DCPBA as the selected boronic acid, the influence of the molar ratio between the boronic 208 acid and the ammonium salt Aliquat336[®] in the organic solvent was investigated. Figure 8 shows the 209 evolution of D-Fructose concentration in the aqueous phase as a function of time for different 210 Aliquat336[®] concentrations (from 0 to 400 mM). D-Fructose and 3,4-DCPBA were initially 211 introduced at concentrations of 100 mM. In the first 15 minutes of reaction, the concentration of 212 D-Fructose decreased irrespective of the Aliquat336® concentration with various initial rates. An 213 equilibrium seemed to be progressively reached between 15 and 45 minutes, as no more evolution of 214 the D-Fructose concentration was then observed. A blank reaction (blue dots) without Aliquat336® 215 nor 3,4-DCPBA was also tested, which showed no evolution. From these observations, it can be 216 considered that the molar ratio 3,4-DCPBA:Aliquat336® is a relevant parameter to control the initial 217 D-fructose extraction rate. Figure 9 shows the evolution of the initial extraction rate and of the 218 D-fructose extraction yield for different 3,4-DCPBA:Aliguat336® molar ratios (from 1:0.5 to 1:4).The 219 initial extraction rate continuously increased when the 3,4-DCPBA:Aliquat336® molar ratio 220 increased from 1:0.5 to 1:2 with respectively 0.37 µmol/min and 1.50 µmol/min. Above a 1:2 molar 221 ratio, no further kinetic benefits could be clearly observed from such an excess of Aliquat336[®] in the 222 reaction medium. This result is consistent with an ion-pairing phenomenon between the 223 fructoboronate complex and the Aliquat336® occurring at the interphase enabling D-fructose 224 transportation. The D-fructose extraction yield increased when the 3,4-DCPBA:Aliquat336® molar 225 ratio increased from 1:0.5 to 1:2 with respectively 21.5% and 50.0%. Like in the case of the initial 226 extraction rate, above a 1:2 molar ratio no further benefits on the extraction yield were displayed by 227 using an excess of Aliquat336®. Therefore, in order to maximize the initial extraction yield and the 228 initial rate while optimizing the quantity of ammonium salt added, an arylboronic acid:Aliquat336® 229 ratio of 1:2 was selected for the following studies.



Figure 8. Evolution of [D-fructose] in the aqueous phase as a function of time for different
3,4-DCPBA/Aliquat336[®] ratios. [D-fructose]i = 100 mM, [3,4-DCPBA] = 100 mM, [Aliquat336[®]] = X
mM (X varies from 0 to 400 mM), Tris-HCl 100 mM pH 8.5, MIBK, 343 K, 750 rpm.

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Figure 9. Initial extraction rate in aqueous phase (blue rods) and extraction yield (red dots) in
function of 3,4-DCPBA:Aliquat336[®] molar ratio. [D-Fructose]I = 100 mM, [3,4-DCPBA] = 100 mM,
[Aliquat336[®]] = X mM (X varies from 0 to 400 mM), Tris-HCl

239 2.2.3. Influence of boronic acid and Aliquat336[®] concentrations

240 Keeping a molar ratio of arylboronic acid:Aliquat336[®] equal to 1:2 and at fixed initial D-Fructose 241 amount, both 3,4-DCPBA and Aliquat336® concentrations were varied, considering the best 242 compromise between component concentrations and costs (Figure 10). From 0 to 15 min, the 243 D-Fructose extraction yield increases rapidly and then stabilizes for lowest 3,4-DCPBA 244 concentrations less than 100 mM. The best extraction yields are thus obtained for higher 3,4-DCPBA 245 concentrations, the final yield being the highest at 300 mM. The slight difference in extraction yields 246 between 100 mM and 300 mM 3,4-DCPBA concentration does not justify the use of such a 247 concentrated solution. From Figure 11, the initial extraction rate values for different 248 3,4-DCPBA:Aliquat336[®] concentrations have been calculated (Table 2). Two behaviors can be 249 observed, the first one for the 25:50 ratio and the second one for all the other ratios, namely from 250 50:100 to 300:600. We observed that the larger the 3,4-DCPBA amount, the higher the initial 251 extraction rate. However, after a certain amount of 3,4-DCPBA, the interface seems to be saturated 252 by 3,4-DCPBA:Aliquat336[®] complexes and then the initial rate does not increase anymore. 253 Therefore, further optimization steps considered a concentration of 100 mM while keeping a molar 254 ratio of 1:2 with Aliquat 336[®].

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- 260 Table 2. Initial extraction rates for various concentrations in the system. [D-Fructose]I = 100 mM,
- 261 [3,4-DCPBA] = Y mM (Y varies from 25 to 300 mM), [Aliquat336[®]] = Yx2 mM, Tris-HCl 100 mM, pH
- 262 8.5, MIBK, 343 K, 750 rpm.

[3,4-DCPBA]	Extraction 63te			
(mM)	(µmol/min)			
25	0.89	$\pm 0.15^{+264}$		
50	1.37	±024615		
150	1.45	±0.05		
100	1.22	$\pm 0.15^{+0.156}$		
200	1.40	± 0.2217		
300	1.33	±0.14		



268

- Figure 10. Evolution of the D-Fructose extraction yield for different 3,4-DCPBA and Aliquat336[®]
 concentrations for a 1:2 molar ratio. [D-Fructose]I = 100 mM, [3,4-DCPBA] = Y mM (Y varies from 25)
- 271 to 300 mM), [Aliquat336[®]] = Y x 2 mM, Tris-HCl 100 mM, pH 8.5, MIBK, 343 K, 750 rpm.
- 272 2.2.4. Influence of the D-fructose:boronic acid molar ratio

The influence of the D-Fructose:3,4-DCPBA molar ratio was further investigated. Figure 11 shows the D-fructose extraction yield as a function of this molar ratio. The yield ranged between 40% and 60%, for ratios from 0.25:1 to 1:1 and between 18% and 28% for ratios from 2:1 to 10:1. When the 3,4-DCPBA was introduced in excess or stoechiometrically compared to D-Fructose (red rods), the

extraction yield was larger than 40%, reaching a maximum of 60% for a two-fold excess of boronic

acid. However, when it was introduced in default (blue rods), the extraction yield did not exceed
35% without any significant further evolution between a molar ratio of 2:1 to 10:1.



280

Figure 11. Influence of the initial D-Fructose:3,4-DCPBA molar ratio on the the extraction yield. [D-Fructose]I = X mM, [3,4-DCPBA] = 100 mM, [Aliquat336[®]] = 200 mM, Tris-HCl 100 mM, pH 8.5,

283 MIBK, 343 K, 750 rpm.

284 2.2.5. Influence of D-fructose and boronic acid concentrations

285 The evolution of the extracted D-fructose concentration as a function of the initial D-fructose 286 concentration, for the same 3,4-DCPBA concentration, is shown in Figure 12. Logically, when the 287 initial concentration of D-fructose increased from 25 mM to 1000 mM, the extracted concentration 288 linearly increased, according to a straight correlation between the initial amount of D-fructose and 289 the amount of extracted D-fructose. Keeping a molar D-fructose:boronic acid ratio equal to 1:1 and a 290 molar ratio of arylboronic acid:Aliquat336[®] equal to 1:2, a benchmark experiment was set with the 291 following parameters: T = 34 K, [D-Fructose] = 100 mM, [3,4-DCPBA] = 100 mM, [Aliquat 336[®]] = 200 292 mM. The other experiments have been set up by doubling all concentrations. Table 3 presents the 293 extraction yields and initial extraction rates depending on the variation of the initial D-fructose 294 concentration while keeping all the molar ratios relative to 3,4-DCPBA and Aliquat336[®] as constant. 295 An increase in the concentrations (D-Fructose, 3,4-DCPBA and Aliquat336®) led to a decrease in the 296 D-Fructose extraction yield. Indeed, at 100 mM of D-Fructose, 43.3% of the fructoboronate complex 297 were extracted whereas only 32.7% and 27.3% were respectively extracted at 200 mM and 300 mM of 298 D-fructose. Therefore, the optimal conditions ([D-Fru]/[3,4-DCPBA]/[Aliquat336] ratio of 299 100/100/200 mM) were kept for the following studies.



301 **Figure 12.** Influence of the initial D-Fructose concentration on the amount of D-fructose extracted.

Table 3. D-fructose extraction yield and initial extraction rate for different concentrations.

[D-Fru]	[3,4-DCPBA]	[Aliquat336®]	Extraction yield	
mM	mM	mM	%	
100	100	200	43.34	± 4.22
200	200	400	32.61	± 1.22
300	300	600	27.31	± 4.6

306 2.2.6. Hydrolysis of the fructoboronate complex for D-fructose release in the second aqueous phase

307 The further stage of the D-fructose transportation is the fructoboronate complex hydrolysis for 308 D-fructose release in the second aqueous phase (the aqueous receiving phase). Therefore, the 309 influence of the pH of the second aqueous phase on the release yield was investigated. pHs of 1, 3, 5 310 and 8 were tested by using with H₂SO₄ for pH 1, citrate buffer for pH 3 and 5 and Tris HCl buffer for 311 pH 8. Prior to these experiments, a D-fructose extraction experiment was performed 312 complexation/transportation) in the optimized conditions (100 mM D-fructose and a 313 D-fructose:3,4-DCPBA:Aliquat336® molar ratio of 1:1:2). The organic phase was then recovered and 314 contacting with the second aqueous phase for the release step. As observed by Paugam et al [40] and 315 keeping in mind the final objective of this work that is the obtaining of a simultaneous process, the 316 saccharide flow from the basic aqueous donor phase to the acidic receiving phase through an 317 organic phase is favored when a pH gradient is applied between the two phases. Figure 13 shows 318 the evolution of the D-fructose release yield as a function of time for different pH of the aqueous 319 receiving phase. In the cases of pH 8 and pH 1, an increase was observed until 30 minutes (with an 320 initial rate of about 0.6 and 1.8 µmol/min, respectively, Table 4) and a steady-state was reached for a 321 yield of 22.7% and 54.5% for pH 8 and pH 1, respectively. Concerning pH 3 and pH 5, an increase 322 was observed up to 60 minutes (with an initial rate of 1.5 µmol/min, Figure 13) to obtain respectively 323 91% and 100% of release. Considering that the pKa of 3,4-DCPBA is around 7.4, when the pH of the 324 receiving aqueous phase is 8, the pH is then superior to pKa. Then, the release mechanism is 325 unfavorable [41]. With pH 3 and 5, more than 90% of the extracted D-Fructose were released. 326 Moreover, the experiment with H₂SO₄ at pH 1 shows full hydrolysis of the fructoboronate complex 327 followed by conversion of D-Fructose to HMF, levulinic and formic acid, which explains the high 328 release rate and the decrease in the actual D-fructose concentration after 15 minutes.

 ^{302 [}D-Fructose]I = X mM (X varies from 25 to 1000), [3,4-DCPBA] = 100 mM, [Aliquat336[®]] = 200 mM,
 303 Tris-HCl 100 mM, pH 8.5, MIBK, 343 K, 750 rpm.



Figure 13. Influence of the pH of the aqueous receiving phase on the D-fructose complex

transportation. Extraction process: Tris-HCl 100 mM pH 8.5, [D-Fru]I = 100 mM, [3,4-DCPBA] = 100

332 mM, [Aliquat336[®]] = 200 mM, MIBK, 343 K. Receiving phase: Citrate buffer

Table 4. Transport yield and extraction rate of D-fructoboronate hydrolysis to D-fructose andD-fructose release in the aqueous phase as a function of its pH.

pН	Transp	ort Yield	Extract	ion rate
	(9	%)	(µmo	l/min)
5	100	± 4.2	1.58	± 0.14
3	91.5	± 6.3	1.53	±0.13
1	54.5	± 3.7	1.84	± 0.09
8	22.7 ± 5.2		0.63	± 0.14

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337 2.3. Study of D-Fructose dehydration in the receiving aqueous phase

D-Fructose dehydration to HMF was carried out in the receiving aqueous phase. We previously observed when studying fructoboronate complex transportation and hydrolysis in a highly acidic aqueous phase the formation of formic and levulinic acids. This confirmed that solutions to minimize HMF degradation by rehydration in the aqueous phase are requested. An alternative to strong homogeneous acid has been to use an acidic resin containing strong sulfonic groups, its supported character facilitating a potential regenerability and recyclability of the catalyst.

344

345 2.4. Study of sequential process

346 In the formerly optimized conditions, a sequential process was tested. The first step consisted on a 347 simultaneous D-glucose isomerization and D-fructose transportation through a fructoboronate 348 complex, followed by the second step characterized by the hydrolysis of the fructoboronate complex 349 to D-fructose and release of D-fructose in the aqueous receiving phase, and finally by the

dehydration of fructose to HMF by heterogeneous catalyst in the third step (Figure 14).

351 2.4.1. D-glucose isomerization and D-fructose complexation/transportation

352 The aqueous feed phase contained Tris-HCl buffer 100 mM at pH 8.5, D-Glucose and immobilized 353 glucose isomerase at the required amount. The organic layer contained the system determined by 354 the previous study: [3,4-DCPBA] = 100 mM, [Aliquat336[®]] = 200 mM. The initial aqueous D-glucose 355 concentration was 100 mM. The system was mixed in a reactor where the enzyme was placed in a 356 basket to avoid any contact with the organic layer. Moreover, the use of MIBK, with a low solubility 357 in water, as an organic solvent contributed to bring the contact between MIBK and enzyme 358 negligible. The system was heated at 343 K for 3 hours. The amount of D-glucose and D-fructose 359 were chromatographically determined in the aqueous phase during the process. Table 5 shows the 360 performances of each step. An interesting isomerization yield of 74.5% was obtained showing the 361 actual shifting of the equilibrium of the enzymatic catalysis (55%) by fructose elimination from the 362 media through complexation with the boronic acid. Moreover, according to the extraction yield of 363 D-fructose and D-glucose, 56.5% and 1%, respectively, a very high selectivity of the complexation 364 reaction with the chosen boronic acid towards the D-fructose was actually demonstrated. The partial 365 D-fructose extraction yield of 56.5% showed that the reaction conditions could be still improved.



366

Figure 14. Illustration of the sequential process with isomerization and complexation/transportationstep, hydrolysis/release step and dehydration step.

369

370 Table 5. Results obtained from sequential process. The D-Fructose released yield was calculated 371 using the amount of D-Fructose in the organic phase at the end of the 1st transport. The fructose 372 conversion yield was calculated using the amount of fructose in the aqueous receiving phase at the 373 end of the 2nd transport. The HMF total amount was calculated using the initial amount of glucose

in the aqueous donor phase.

		_	1 st step		2 nd step	3 rd step		_
-		isomerization	D-Fructose	D-Glucose	D-Fructose	Fructose	HMF	HMF
_		ISOITIETIZALIOIT	extracted	extracted	released	conversion yield		total
	%	74.5	56.5	1.56	57.4	52	21.9	5.3
_	time		3 h		3 h	35 h		41 h

375

376

377 2.4.2. Fructoboronate complex hydrolysis, release of D-fructose and dehydration to HMF

378 The basket of IGI was removed and the aqueous feed phase was discarded and replaced by the

379 receiving aqueous phase with a citrate buffer at 100 mM keeping a pH 3. The system was heated at

343 K for 3 hours. In these conditions, the hydrolysis step led to 63% of fructose release in the
aqueous phase. Then, after 3 hours at 343 K, the organic layer was discarded and the acidic resin was
introduced. The system was heated at 363 K. The dehydration step led to a HMF yield of 20% after
10 h. The total HMF yield was 5%.

384

385 2.5. Study of continuous process

386 In the optimal conditions previously determined, a continuous integrated process was tested [33]. 387 The reactor, phases and matter flows are presented in Figure 15. The "coaxial" reactor is composed 388 of two cylinders coaxially placed, a cover enabling introduction of the stirring system and a 389 refrigerant for temperature control. The inner cylinder contains the donor aqueous phase and has a 390 height less than that of the outer cylinder which contains the receiving aqueous phase, which allows 391 the organic phase to be in contact with both aqueous phases. The experiment was conducted with a 392 donor aqueous phase formed by 50 mL of 100 mM Tris-HCl pH 8.5 containing 100 mM of D-glucose, 393 200 mg IGI, 20 mM MgSO₄, 8 mM Na₂SO₃. The receiving aqueous phase was formed by 55 mL of 100 394 mM sodium citrate buffer, pH 3. The organic phase consisted of 100 mL of MIBK containing 100 mM 395 of 3,4-DCPBA and 200 mM of Aliquat336[®]. All the phases were maintained at 343 K. The stirring was 396 carried out at 180 rpm, which is the maximum rate to avoid the mixing of the phases, thanks to a 397 stirring blade immersed in the organic phase. This "coaxial" reactor was used to simultaneously 398 perform the isomerization, extraction, hydrolysis and dehydration steps. The monitoring of the 399 D-fructose concentration in the aqueous phases was carried out, making it possible to calculate the 400 efficiency of the continuous process for the simultaneous isomerization, fructose extraction and

401 dehydration. The results are shown in Table 6.



402

403 **Figure 15.** Illustration of the continuous process.

404

405 **Table 6.** Results obtained by continuous process.

		Isomerizatio n	Fructose extraction	HMF	Selectivit Y
Yie (%	ld 5)	70.1	50.2	4.1	70.4

406

The D-glucose isomerization yield was 70%, with 50% of the formed D-fructose transported and a global HMF yield of 4% (table 8). Those results are comparable to those obtained by the sequential process. Therefore, this process demonstrates the efficiency of using a cascade chemo-enzymatic catalysis to continuously obtain HMF from D-glucose. However, insufficient agitation of each phase seems to be the main limitation of this reactor configuration. Further improvements of this proof of concept towards enhanced HMF yields and global performances will be reported in due course.

414 3. Materials and Methods

415 *3.1. Chemicals and reagents*

416 D-Glucose (≥99.5%), D-Fructose (≥99%), N-methyl-N,N,N-trioctylammonium chloride 417 (Aliquat336[®]), 3,4-dichlorophenylboronic acid (3,4-DCPBA), 3,5-dichlorophenylboronic acid 418 (3,5-DCPBA) (98%), 2,4-dichlorophenylboronic acid (2,4-DCPBA), 2,3-dichlorophenylboronic acid 419 (2,3-DCPBA), 4-tert-butylphenylboronic acid (4-TBPBA) (≥ 95%), 4-(trifluoromethyl)phenylboronic 420 acid (4-TFMPBA), Trizma base (≥ 99.9%), hydrochloric acid (36.8-38%), sulfuric acid (95-97%), 421 Sweetzyme IT[®]Extra (IGI) (\geq 350 U/g), Dowex monosphere 650C, levulinic acid (LA) (98%), formic 422 acid (FA) (95%), trisodium citrate (≥ 99%), 5-hydroxymethylfurfural (HMF) (99%) were purchased 423 from Sigma Aldrich Co. (St. Louis, MO). Phenylboronic acid (PBA) (95%) and 4-methyl-2-pentanone 424 (MIBK) (\geq 99%) were purchased from Sigma-Fluka. Citric acid (\geq 99%) was purchased from Merck. 425 Distilled-deionized water (Mili-Q) grad was used whenever necessary, obtained from a MilliQ water 426 purification system (Millipore, Academic, SA, France).

427

428 3.2. Characterization

429 In the conversion/complexation experiments, the determination of reactants, intermediates and 430 products quantities (Table 7) was carried out using an HPLC Shimadzu equipped with a 431 LC-20ADXR pump, a DGU-20A5R degasser, a SIL-20ACXR autosampler, a SPD-MD20A diode array 432 detector, a CTO-20AC oven and a CBM-20A communicator module piloted by a LabSolution 433 software The column was an Aminex HPX-87H (300 mm x 7.8 mm, 9 µm; Bio-Rad). The mobile 434 phase was 5 mM H2SO4, using isocratic mode with 0.4 mL.min-1. The chosen wavelength for HMF 435 and levulinic acid detection were 284 nm and 266 nm, respectively. The chosen wavelength for 436 detection of formic acid, D-Glucose and D-Fructose was 195 nm. In the case of monosaccharides, a 437 Refractive Index Detector was also used (analytical standards are listed in Table 7).

438

439 **Table 7.** Concentration range of products used for analytical standards.

	Analytical method	RID				UV 284 nm		UV 195 nm	
	Product	HMF	D-Glc	D-Fru	LA	HMF	D-Glc	D-Fru	FA
	Concentration range (mM)	0.14-30	0.5-25	0.5-25	0.5-25	0.007-0.5	0.5-25	0.5-25	0.5-35

- 440
- 441

442 3.3. Aqueous phase D-glucose isomerization (aqueous feed phase) and D-fructose complexation/transportation

443 3.3.1. D-glucose isomerization in aqueous feed phase

We used one of commercial enzymes the most largely used for the conversion of starches to high fructose syrup, namely immobilized glucose isomerase (IGI, Sweetzyme®IT extra).31 1 mL of a D-glucose solution was prepared by mixing D-glucose (100 mM), Na₂SO₃ (8 mM) and MgSO₄ (20 mM) in a buffer solution (100 mM) at a selected pH (Tris-HCl buffer for pH 7, 7.5, 8, 8.5, 9, phosphate buffer for pH 6.5, or citrate buffer for pH 4.5) and then introduced in an Eppendorf tube. The

solution was incubated at the desired temperature (323 to 363 K) in a Mixing Block (BIOER-102

- Thermocell) at 900 rpm. After rehydration in water for 12 h, IGI (10 mg) was put in contact with the aforementioned prepared D-glucose solution. The reaction time was set at 90 min.
- 452 3.3.2. D-fructose transport in the organic phase

First, a D-fructose/D-glucose (25 to 600 mM) solution was prepared in Tris-HCl buffer (100 mM). 600 μ L of this solution were introduced in an Eppendorf tube (solution 1). Aliquat336[®] (25-300 mM), and arylboronic acid (25-300 mM) were dissolved in MIBK as a solvent to prepare solution 2. 600 μ L of solution 2 were then introduced in a second Eppendorf tube. Solutions 1 and 2 were incubated at the desired temperature in a Mixing Block (BIOER-102 Thermocell) at 750 rpm. After 5 min, both solutions were mixed in an Eppendorf tube placed in the Mixing Block in the same conditions. After

- 459 D-fructose transport, the aqueous and organic phases were separated by pipetting.
- 460
- 461
- 462 3.3.3. Aqueous phase D-fructose complex hydrolysis in receiving aqueous phase
- 463 600 μL of citrate buffer were introduced in an Eppendorf tube and incubated at the required

464 temperature (343 K) in a Mixing Block at 750 rpm. After 5 min, 600 μL of the organic phase obtained

465 after the extraction procedure and containing the fructoboronate complex were introduced in the

466 tube. After a reaction time of 45 min, the aqueous and organic phases were separated by pipetting.

- 467 3.3.4. Study of sequential process from glucose isomerization to fructose dehydration
- 468 A 250 mL vessel (Reactor-ReadyTM Lab Reactor, Radley, Figure 16) equipped with a temperature
- 469 control system (Ministat, Huber temperature control system, HUBER, UK) was used for these470 experiments.
- 471 Simultaneous D-glucose isomerization by enzymatic catalysis in the aqueous feed phase and
 472 D-fructose complexation/transportation in the organic phase
- IGI (0.5 g) was loaded in a stirring basket introduced in a first reactor (Figure 16 Left) with 100 mL
 of a aqueous solution containing D-Glucose (100 mM), Na₂SO₄ (8 mM), MgCl₂ (20 mM), in Tris-HCl
 buffer (100 mM, pH 8.5) at 343 K. Then, 100 mL of MIBK containing 3,4-DCPBA (100 mM) and
 Aliquat336[®] (200 mM) were introduced in the reactor. The mixture was stirred via the stirring basket
 at 200 rpm, with the temperature kept at 343 K for 180 min, before discarding the aqueous feed
 phase.
- D-fructose complex hydrolysis in the aqueous receiving phase
- 480 100 mL of the organic phase issued from the first reactor and containing the fructoboronate complex
 481 were transferred in a second reactor (Figure 16 Middle) and heated at 343 K. Then, 100 mL of a
 482 citrate buffer solution (100 mM, pH 3) were introduced. The mixture was stirred with a turbine
- 483 stirring shaft at 200 rpm and set up at 343 K for 180 min.
- 484

485 4. Conclusion

486 In this work, we have first provided the conditions of the key parameters (temperature, pH,

- 487 arylboronic acid structure and concentrations, Aliquat336[®] concentrations) applied for a sequential
- 488 process from glucose to HMF through fructose transportation in an organic liquid membrane. The

489 optimized conditions have been then applied to an unprecedented integrated process in a 490 specifically designed reactor. Therein, enzymatic glucose isomerization and fructose dehydration to 491 HMF, involving an intermediate fructose transportation by complexation with an aryboronic 492 acid-Aliquat336[®] complex, could be carried out simultaneously in independent aqueous phases. 493 These phases were both in contact with a single organic phase enabling the transportation of fructose 494 from the first aqueous phase to the second. In this process, we thus succeeded in simultaneously 495 enzymatically isomerizing glucose to fructose and dehydrating fructose to HMF by minimizing the 496 number of reaction media and separation steps. Indeed, no intermediate product or phase isolation 497 was necessary. The use of numerous organic solvents or ionic liquid, as previously described in the 498 literature, is limited to a unique organic phase used for fructose transportation. Improvement of 499 performances in terms of HMF yields implying better diffusion of species between the different 500 phases by improving the conception of new reactor systems will be reported in due course.

501

502

503

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510

511 References

- Nanda, S.; Mohammad, J.; Reddy, S. N.; Kozinski, J. A.; Dalai, A. K. Pathways of lignocellulosic biomass
 conversion to renewable fuels. *Biomass Conv. Bioref.* 2014, 4, 157–191.
- 514 2. Besson, M.; Gallezot, P.; Pinel, C. Conversion of Biomass into Chemicals over Metal Catalysts. *Chem. Rev.*515 2014, 114, 1827–1870.
- Aresta, M.; Dibenedetto, A.; Dumeignil, F. *Biorefineries: From Biomass to Chemicals and Fuels*, 3rd ed.; De
 Gruyter: Berlin, Boston, 2012; ISBN 978-3-11-026023-6
- 4. Agbor, V.B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D.B. Biomass pretreatment: Fundamentals toward
 application. *Biotechnol. Adv.* 2011, 29, 675-682.
- 5205.Bozell, J. J., Petersen, G. R. Technology development for the production of biobased products from
biorefinery carbohydrates—the US Department of Energy's 'Top 10' revisited. Green Chem. 2010, 12,
539-545.
- 523 6. Van Heerden, C.D., Nicol, W. Continuous succinic acid fermentation by Actinobacillus succinogenes.
 524 *Biochem. Eng. J.* 2013, *73*, 5-11.
- 525 7. Girard, E.; Delcroix, D.; Cabiac, A. Catalytic conversion of cellulose to C2–C3 glycols by dual association of
 526 a homogeneous metallic salt and a perovskite-supported platinum catalyst. *Catal. Sci.* 2016, *6*, 5534-5542.
- Shanks, B. H. Unleashing biocatalysis/chemical catalysis synergies for efficient biomass conversion. ACS
 Chem. Biol. 2007, *2*, 533–535.
- Vennestrøm, P. N. R., Taarning, E., Christensen, C.H., Pedersen, S., Grunwaldt, J.D., Woodley, J.M.
 Chemoenzymatic Combination of Glucose Oxidase with Titanium Silicalite-1. *ChemCatChem* 2010, *2*, 943–945.
- 532 10. Denard, C. A., Hartwig, J. F., Zhao, H. Multistep One-Pot Reactions Combining Biocatalysts and Chemical
 533 Catalysts for Asymmetric Synthesis. *ACS Catal.* 2013, *3*, 2856–2864.
- 534 11. Dumeignil, F. Public Serv. Rev. Eur. Union 2011, 22, 528.
- 535 12. F. Dumeignil, Chemie Ing. Tech., 2014, 86, 1496.

- 536 13. Van Putten, R., Van der Waal, J.C., de Jong, E., Rasrendra, C.B., Heeres, H.G., de Vries, J.G.
 537 Hydroxymethylfurfural, A versatile platform chemical made from renewable resources. *Chem. Rev.* 2013, 113, 1499–1597.
- Moliner, M.; Román-leshkov, Y., Davis, M. E. Tin-containing zeolites are highly active catalysts for the isomerization of glucose in water. *Proc Natl Acad Sci U S A.* 2010, 107, 6164–6168.
- 541 15. Pacheco, J. J., Davis, M. E. Synthesis of terephthalic acid via Diels-Alder reactions with ethylene and oxidized variants of 5-hydroxymethylfurfural. *Proc Natl Acad Sci U S A.* 2014, *111*, 8363-8367.
- 543 16. Lin, Z., Nikolakis, V., Ierapetritou, M. Alternative Approaches for p-Xylene Production from Starch:
 544 Techno-Economic Analysis. *Ind. Eng. Chem. Res.* 2014, 53, 10688–10699.
- 545 17. E. Jong, M.A. Dam, L. Sipos, and G. Gruter. Furandicarboxylic Acid (FDCA), A Versatile Building Block
 546 for a Very Interesting Class of Polyesters *Am. Chem. Soc.* 2012, *1*, 1-13.
- 547 18. Boisen, A., Christensen, T.B., Fu, W., Gorbanev, Y.Y., Hansen, T.S., Jensen, J.S., Klitgaard, S.K., Pedersen,
 548 S., Riisager, A., Stahlberg, T., Woodley, J.M. *Chem. Eng. Res. Des.* 2009, *87*, 1318-1325.
- 549 19. Akien, G.R., Qi, L., Horvath, I.T. Molecular mapping of the acid catalysed dehydration of fructose. *Chem.* 550 *Commun.* 2012, 48, 5850-5852.
- 551 20. Parker, K., Salas, M., Nwosu, V.C. High fructose corn syrup: Production, uses and public health concerns
 552 *Biotechnol. Mol. Biol. Rev.* 2010, *5*, 71-78.
- Li, H., Yang, S., Saravanamurugan, S., Riisager, A. Glucose Isomerization by Enzymes and Chemo-catalysts: Status and Current Advances. *ACS Catal* 2017, *7*, 3010-3029.
- 555 22. Delidovich, I., Palkovits, R. Catalytic Isomerization of Biomass-Derived Aldoses: A Review. *ChemSusChem* 556 2016, 9, 547–561.
- 557 23. Choudhary, V., Pinar, A.B., Lobo, Vlachos, D.G., Sandler, S.I. Comparison of homogeneous and heterogeneous catalysts for glucose-to-fructose isomerization in aqueous media. *ChemSusChem* 2013, 6, 2369-2376.
- Marianou, A.A., Michailof, C.M., Pineda, A., Iliopoulou, E.F., Triantafyllidis, K.S., Lappas, A.A. Glucose to
 Fructose Isomerization in Aqueous Media over Homogeneous and Heterogeneous Catalysts.
 ChemCatChem 2016, *8*, 1100-1110.
- 563 25. Zhao, S., Guo, X., Bai, P., Lv, L. Catalytic Isomerization of Biomass-Derived Aldoses: A Review. Asian J.
 564 *Chem.* 2014, 26, 4537-4542.
- 565 26. Demerdash, M., Attia, R. M. Equilibrium kinetics of D-glucose to D-fructose isomerization catalyzed by glucose isomerase enzyme from Streptomyces phaeochromogenus. *Zentralbl. Mikrobiol.* **1992**, 147, 297–303.
- 567 27. Gaily, M.H., Elhassan, B.M., Abasaeed A.E., Al-shrhan, M. Kinetics of a Three-Step Isomerization of
 568 Glucose to Fructose Using Immobilized Enzyme. *Int. J. Eng. Technol.* 2010, 10, 1-6.
- 569 28. Takasaki, Y. Kinetic and Equilibrium Studies on D-Glucose-D-Fructose Isomerization Catalyzed by
 570 Glucose Isomerase from Streptomyces sp. *Agric. Biol. Chem.* 1967, *31*, 309–313.
- 571 29. Huang, H., Denard, C.A., Alamillo, R., Crisci, A.J., Miao, Y., Dumesic, J.A., Scott, S.L., Zhao, H. Tandem
 572 catalytic conversion of glucose to 5-hydroxymethylfurfural with an immobilized enzyme and a solid acid.
 573 ACS Catal. 2014, 4, 2165-2172.
- 574 30. Huang, R., Qi, W., Su, R., He, Z. Integrating enzymatic and acid catalysis to convert glucose into 575 5-hydroxymethylfurfural. *Chem. Commun. (Camb).* **2010**, *46*, 1115-1125.
- 576 31. Delidovich, I., Palkovits, P. Catalytic Isomerization of Biomass-Derived Aldoses: A Review. *Green Chem.*577 2016, 18, 5822-5830.
- Alipour, S., Relue, P.A., Viamajala, S., Varanasi, S. High yield 5-(hydroxylmethyl)furfural production from
 biomass sugars at facile reaction conditions: a hybrid enzyme- and chemo-catalytic technology. *Green Chem.* 2016, *18*, 4990-5002.
- 581 33. Gimbernat, A., Guehl, M., Capron, M., Lopes-Ferreira, N., Froidevaux, R., Girardon, J.S., Dhulster, P.,
 582 Delcroix, D., Dumegnil, F. Hybrid Catalysis: A Suitable Concept for the Valorization of Biosourced
 583 Saccharides to Value-Added Chemicals. *ChemCat Chem* 2017, *9*, 2080-2888.
- 584 34. Bhosale, S.H., Rao, M.B., Deshpande, V.V. Molecular and industrial aspects of glucose isomerase.
 585 *Microbiol. Rev.* 1996, 60, 280-290.
- 586 35. McKay, G., Tavlarides, T. Enzymatic isomerization kinetics of D-Glucose to D-Fructose. J. Mol. Catal. 1979,
 587 6, 57-65.

- 588 36. Morin, G.T., Paugam, M.F., Hughes, M.P., Smith, B.D. Boronic Acids Mediate Glycoside Transport through a Liquid Organic Membrane via Reversible Formation of Trigonal Boronate Esters. *J. Org. Chem.*590 1994, 59, 2724-2730.
- 591 37. Karpa, M.J., Duggan, P.J., Griffin, G.J., Freudigmann, S.J. Competitive transport of reducing sugars
 592 through a lipophilic membrane facilitated by aryl boron acids. *Tetrahedron* 1997, *53*, 3669-3676.
- 38. Westmark, P.R., Gardiner, S.J., Smith, B.D. Selective monosaccharide transport through lipid bilayers
 using boronic acid carriers. J. Am. Chem. Soc. 1996, 118, 11093-11100.
- 595 39. Shinbo, T., Nishimura, K., Yamaguchi, T., Sugiura, M. Transport of monosaccharides across an organic
 596 liquid membrane. *J. Chem. Soc. Chem. Commun.* 1986, 349-358.
- 40. Paugam, M.; Riggs, J.A.; Smith, B.D. High fructose syrup production using fructose-selective liquid
 membranes. *ChemComm.* 1996, 22, 2539-2540.
- 599 600