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Biphasic Catalysis with Disaccharide Phosphorylases: Chemoenzymatic Synthesis of α-D-Glucosides Using Sucrose Phosphorylase

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Supporting Information

ABSTRACT: Thanks to its broad acceptor specificity, sucrose phosphorylase (SP) has been exploited for the transfer of glucose to a wide variety of acceptor molecules. Unfortunately, the low affinity (K_m > 1 M) of SP towards these acceptors typically urges the addition of cosolvents, which often either fail to dissolve sufficient substrate or progressively give rise to enzyme inhibition and denaturation. In this work, a buffer/ethyl acetate ratio of 5:3 was identified to be the optimal solvent system, allowing the use of SP in biphasic systems. Careful optimization of the reaction conditions enabled the synthesis of a range of α-D-glucosides, such as cinnamyl α-D-glucopyranoside, geranyl α-D-glucopyranoside, 2-*O*-α-D-glucopyranosyl pyrogallol, and series of alkyl gallyl 4-*O*-α-D-glucopyranosides. The usefulness of biphasic catalysis was further illustrated by comparing the glucosylation of pyrogallol in a cosolvent and biphasic reaction system. The acceptor yield for the former reached only 17.4%, whereas roughly 60% of the initial pyrogallol was converted when using biphasic catalysis.

1. INTRODUCTION

Numerous biologically active molecules exist as glycosides in nature.[1] Glycosylation can expand structural diversity,[2] induce targeting of drugs to specific organs and tissues,[3] and improve the solubility of hydrophobic compounds.[4] Furthermore, glycosylation is known to drastically extend the stability of labile molecules[5] and mediate the controlled release of flavors and fragrances.[6] Unfortunately, large-scale chemical synthesis of these molecules is limited by low yields, the use of toxic catalysts, and the generation of waste.[7,8] Biocatalytic approaches, allowing one-step reactions with high regio- and stereoselectivity, have therefore attracted much attention over the past decade.[9]

Enzymatic glycosylation is typically performed with glycosyltransferases (GTs) or glycoside hydrolases (GH). However, the former requires relatively expensive nucleotide-activated sugars,[10] while the latter suffers from low yields when used in the synthetic direction.[11] Less research has been done with disaccharide phosphorylases, although they show potential as biocatalysts for glycoside synthesis.[12−14] Indeed, these enzymes use a glycosyl phosphate donor, which is much cheaper than the activated sugar nucleotides required by GTs and can be synthesized by phosphorolysis of their natural substrates.[14]

Sucrose phosphorylase (SP) catalyzes the reversible phosphorolysis of sucrose into α-D-glucose 1-phosphate (αG1P) and D-fructose. Its broad acceptor specificity has been exploited for the transfer of glucose to a wide variety of acceptor molecules, such as polyols,[15] phenolics,[16,17] hydroxyfuranones,[18] and stilbenoids.[19] Unfortunately, the low affinity (K_m > 1 M) of SP towards these acceptors typically urges the addition of cosolvents such as DMSO or methanol. However, low concentrations of these cosolvents often fail to dissolve sufficient substrate, whereas high concentrations progressively give rise to enzyme inhibition and denaturation.[20,21]

The use of liquid−liquid biphasic systems containing water and a water-immiscible organic solvent, however, provides an interesting alternative. The aqueous phase contains the enzymes and water-soluble substrates, while hydrophobic substrates are dissolved in the organic phase. Stirring or shaking will transfer these substrates from the organic to the aqueous phase, where they can be converted by the enzymes. Improved enzyme stability and ease of product recovery, while avoiding substrate and product inhibition, are among the major advantages of biphasic systems.[22,23] The usefulness of this type of catalysis has been widely illustrated for numerous applications, including the synthesis of oligosaccharides[24] and glycosides[25,26] with glycosidases.

Although SP has recently been successfully applied for glycosylation reactions in ionic liquids[19] and supercritical carbon dioxide,[27] no reports on its use in biphasic reaction systems are available to date. In this work, we describe the glycosylation of various acceptors with the SP from *Bifidobacterium adolescentis* in biphasic systems.

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2. RESULTS AND DISCUSSION

The β-D-glucoside of cinnamyl alcohol has recently gained attention from the pharmaceutical industry due to its claimed antifatigue, antiaging, antioxidant, and immune enhancing properties.[28−30] The enzymatic glucosylation of cinnamyl alcohol has already been reported in an IL based cosolvent system using SP from *B. adolescentis*.[19] Unfortunately, the latter synthesis suffered from low yields and labor-intensive product recovery. Consequently, the use of a cosolvent was avoided by reacting 250 μL of the alcohol with 250 μL MES buffer at pH 6.5 supplemented with 2 M sucrose and 50 U/mL SP. TLC analysis of the reaction mixture after 48 h incubation at 60 °C revealed a clear new spot having R_f higher than that of sucrose and lower than that of cinnamyl alcohol (Figure S.1 of the Supporting Information [SI]). The product was purified by silica gel chromatography and identified to be cinnamyl α-D-glucopyranoside by NMR spectroscopy.

Encouraged by these results, conditions enabling the efficient enzymatic glucosylation with *B. adolescentis* SP were further optimized. Indeed, a number of factors are known to influence the glycosylation efficiency, including the pH, the type and concentration of both donor and acceptor, and the reaction temperature.[31] A full factorial design experiment was performed to determine their relative importance on the glucosylation of cinnamyl alcohol. The cheap and readily available donor sucrose was used, and the temperature was fixed at 37 °C (Table 1).

Table 1. Optimization of SP-catalyzed synthesis of cinnamyl α-D-glucopyranoside in a biphasic system: 2^3 factorial design; the reaction mixtures were incubated during 48 h at 37 °C

run	pH	[sucrose] (M)	buffer/acceptor ratio	product spot intensity
1	6	0.5	5:1	++
2	6	0.5	1:1	+
3	6	2	5:1	+++
4	6	2	1:1	++++
5	7.5	0.5	5:1	++
6	7.5	0.5	1:1	+
7	7.5	2	5:1	+++++
8	7.5	2	1:1	++++

The glucosylation yield was found to be strongly influenced by the pH and buffer/acceptor (solvent) ratio, while high sucrose concentrations were generally required to obtain proper glucosylation. Indeed, a product concentration of 4.8 g/L was achieved when using 2 M sucrose at pH 7.5 with a 5:1 solvent ratio. Next, the pH (5−9) and solvent ratio (10:1 to 1:5) were varied to identify optima at pH 7.5 and a ratio of 5:3 (data not shown). Although higher sucrose concentrations were not feasible due to viscosity limitations, the glycosylation efficiency could be further improved by increasing the reaction temperature to 50 °C. Interestingly, using αG1P as donor resulted in significantly less product formation, confirming sucrose to be the preferred donor substrate[32] (data not shown). Finally, the optimal reaction conditions were found to be a solvent ratio of 5:3, 2 M sucrose, pH 7.5, and 50 °C, resulting in the production of 6.4 g/L cinnamyl α-D-glucopyranoside.

Although these results confirmed the SP-mediated transfer of a glucose moiety using biphasic catalysis, a suitable water-immiscible solvent is required to allow glycosylation of solid acceptors. The latter solvent should be able to dissolve high amounts of acceptor, without impairing the glucosylation reaction catalyzed by the enzyme. Various solvents were supplemented with 50 mg/mL cinnamyl alcohol and reacted under the optimal conditions. TLC analysis revealed the highest conversion when using ethyl acetate (EtOAc). However, SP-mediated glucosylation of cinnamyl alcohol was also observed when butyl acetate (BuOAc), methyl-*tert*-butylether (MTBE), octane, diethyl ether, cyclohexene, and pentane were used (Figure S.2 of SI).

Next, the solubility of different acceptors was evaluated in the most promising solvents (Table S.1 of SI). Although the solubility of all compounds was found to be very low in octane, the majority of the evaluated acceptors could be dissolved in BuOAc, MTBE, and EtOAc. The latter was found to be less deleterious for the glycosylation activity of SP and was therefore used in all further experiments (Figure S.2 of SI). Finally, the concentration of cinnamyl alcohol in the organic phase was varied to reveal optimal glucoside formation at 100 mg/mL (data not shown).

The glycosylation potential of SP was then evaluated by incubating various acceptors under optimal glucosylation conditions. Semiquantative data for a large number of acceptors was obtained trough TLC analysis (Table 2), while HPLC experiments allowed detailed analysis of a limited amount of reactions (Table 3).

These results illustrate the versatile applicability of biphasic catalysis for the production of glycosides with SP. The structure of numerous glucosides was confirmed by NMR spectroscopy. Aliphatic alcohols could be glycosylated up to octanol, but the obtained product concentrations decreased with increasing chain lengths. Surprisingly, secondary alcohols were found to

Table 2. Glycosylation potential of the SP from *B. adolescentis* in a biphasic reaction system; TLC analysis was performed after 48 h incubation at 50 °C

acceptor	product spot intensity	acceptor	product spot intensity
pentanol[a]	+++	cinnamyl alcohol[a]	+++
hexanol[a]	++	menthol[b]	−
heptanol[a]	+	saligenin[b]	++++
octanol[a]	+	*p*-nitrophenol[b]	+
nonanol[a]	−	phenol[b]	−
decanol[a]	−	hydroquinone[b]	+
dodecanol[a]	−	catechol[b]	+++
cyclohexanol[a]	++++	resorcinol[b]	+++
2-hexanol[a]	+++	pyrogallol[b]	+++++
linalool[a]	−	gallic acid methyl ester[b]	++++
eugenol[a]	++	gallic acid ethyl ester[b]	++++
nerolidol[a]	−	gallic acid propyl ester[b]	+++
β-citronellol[a]	+	gallic acid lauryl ester[b]	−
geraniol[a]	++	salicylic acid methyl ester[a]	+
2-phenylethanol[a]	+	curcumin[b]	−
1*R*-phenylethanol[a]	+++++	resveratrol[b]	+
1*S*-phenylethanol[a]	+++++	quercetin[b]	+
benzyl alcohol[a]	++	vanillin[b]	++
anisyl alcohol[a]	+	vanillyl alcohol[b]	+++

[a]Glycosylation was performed using the acceptor as the organic phase. [b]Glycosylation was performed using EtOAc supplemented with 100 mg/mL acceptor as the organic phase.

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Table 3. Glycosylation of various acceptors with the SP from *B. adolescentis*; HPLC analysis was performed after 48 h incubation at 50 °C

acceptor	[glucoside] (mM)	[glucoside] (g/L)	glucoside[d] (g)	yield acceptor (%)	yield donor (%)
geraniol[a]	20.4	6.5	0.65	0.9	1.6
1*R*-phenylethanol[a]	133.2	37.9	3.79	4.3	10.7
1*S*-phenylethanol[a]	126.7	36.0	3.60	4.1	10.1
cinnamyl alcohol[a]	21.5	6.4	0.64	0.8	1.7
cinnamyl alcohol[b]	16.2	4.8	0.48	5.8	1.3
hydroquinone[b]	2.6	0.7	0.07	0.7	0.2
resorcinol[b]	27.1	7.4	0.74	7.9	2.2
pyrogallol[b]	175.8	50.6	5.06	59.1	14.1
pyrogallol[c]	112.6	32.4	3.24	75.7	9.0
gallic acid methyl ester[b]	87.4	30.2	3.02	42.9	7.0
gallic acid ethyl ester[b]	50.9	18.3	1.83	26.9	4.0
gallic acid propyl ester[b]	24.7	9.2	0.92	14.0	1.9

[a]Glycosylation was performed using the acceptor as the organic phase. [b]Glycosylation was performed using EtOAc supplemented with 100 mg/mL acceptor as the organic phase. [c]Glycosylation was performed using EtOAc supplemented with 50 mg/mL acceptor as the organic phase. [d]Glucoside produced in a 100 mL reaction.

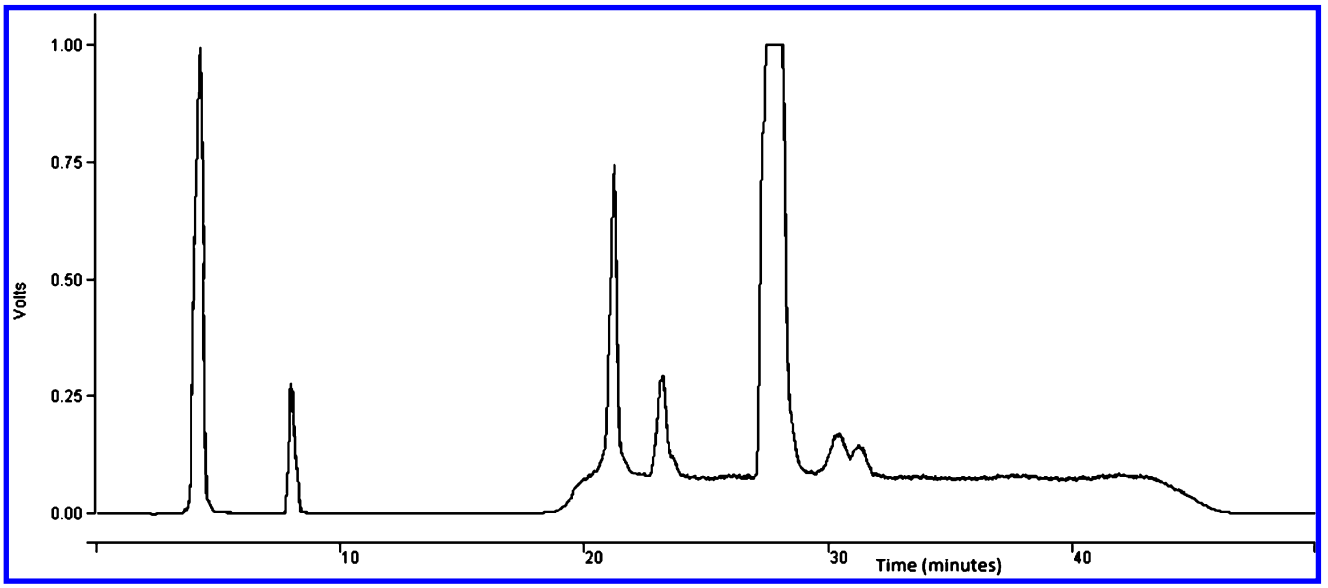

Figure 1. HPLC chromatogram showing the glucosylation of gallic acid propyl ester and formation of glucobiose products with SP. The sample was analyzed after 24 h incubation under optimal biphasic reaction conditions at 50 °C: gallic acid propyl ester (4.9 min), propyl gallyl 4-*O*-*α*-D-glucopyranoside (8.2 min), D-fructose (21.7 min), D-glucose (23.2 min), sucrose (28.3 min), and glucobiose products (30.2−31.9 min).

be more easily glycosylated compared to primary alcohols. Indeed, 48 h incubation under optimal reaction conditions resulted in glucoside concentrations of 37.9 and 36.0 g/L for 1*R*- and 1*S*-phenylethanol, respectively, while only traces of product were detected when reacting 2-phenylethanol. The structure of *R*- and *S*-1-phenylethyl *α*-D-glucopyranoside was confirmed by NMR spectroscopy. Also, SP from *B. adolescentis* was found to glycosylate a range of structurally diverse compounds with olfactory properties. Examples include the monoterpenoids geraniol and *β*-citronellol, aromatic alcohols such as benzyl alcohol, anisyl alcohol, cinnamyl alcohol and vanillyl alcohol, as well as the phenylpropanoid eugenol, and the phenolic aldehyde vanillin. Although these glycosides were generally produced in significantly lower concentrations compared to secondary alcohols (Table 3), we were able to confirm the formation of geranyl *α*-D-glucopyranoside and cinnamyl *α*-D-glucopyranoside by NMR spectroscopy.

In contrast to earlier work with the SP from *L. mesenteroides*,[16] we failed to obtain any glycosides of phenol. However, we were able to couple a glucose moiety to hydroquinone, catechol, resorcinol, and pyrogallol. Indeed, the formation of 1-*O*-*α*-D-glucopyranosyl hydroquinone (0.7 g/L), 1-*O*-*α*-D-glucopyranosyl resorcinol (7.4 g/L) and 2-*O*-*α*-D-glucopyranosyl pyrogallol (50.6 g/L) was confirmed by NMR spectroscopy. In addition, glucosylation of a series of gallic acid esters was observed, as confirmed by NMR spectroscopy. These potent antioxidants, including ethyl gallate (E313) and propyl gallate (E310), are commonly applied in foods, cosmetics, and hair products.[33,34] The methyl ester glucoside could be produced up to 30 g/L, while longer alkyl chains were found to result in lower glucoside concentrations (Table 3).

In conclusion, a wide variety of glucosides could be synthesized using the SP from *B. adolescentis*. Industrially relevant glucoside concentrations of several g/L were obtained for a number of acceptors.[35,36] Not surprisingly, the acceptor yields were much lower when using the pure acceptor as organic phase (Table 3). The latter, however, could be increased from 0.8 to 5.8% for cinnamyl alcohol by dissolving

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100 mg/mL of the acceptor in EtOAc rather than using the alcohol in its undiluted form. Moreover, the acceptor yield for pyrogallol could be improved from 59.1 to 75.7% by decreasing the acceptor concentration from 100 to 50 mg/mL. However, these increased yields were achieved at the expense of lower product concentrations (Table 3).

The donor yields, on the contrary, were less than 20% for all acceptors (Table 3). Indeed, higher concentrations of sucrose were added to stabilize the enzyme,[19] and push the conversion in the synthesis direction. Therefore, considerable amounts of sucrose remained present at the end of the reaction (Figure 1). Moreover, SP is known to exhibit an undesirable hydrolytic side reaction, yielding D-glucose and D-fructose as products. The liberated glucose can then be glucosylated by SP, resulting in the formation of 'glucobiose' products.[32,37] Unfortunately, this competition between D-glucose and the acceptor compound was found to further decrease the donor yield (Figure 1).

Finally, the performance of biphasic catalysis was compared with a recently reported cosolvent system,[19] using the glycosylation of pyrogallol as a case study. Therefore, the water-immiscible EtOAc was replaced by the IL AMMOENG 101, which has been successfully applied for the SP-mediated synthesis of 3-*O*-α-D-glucopyranosyl-(*E*)-resveratrol.[19] All other parameters were identical for both reactions (Figure 2).

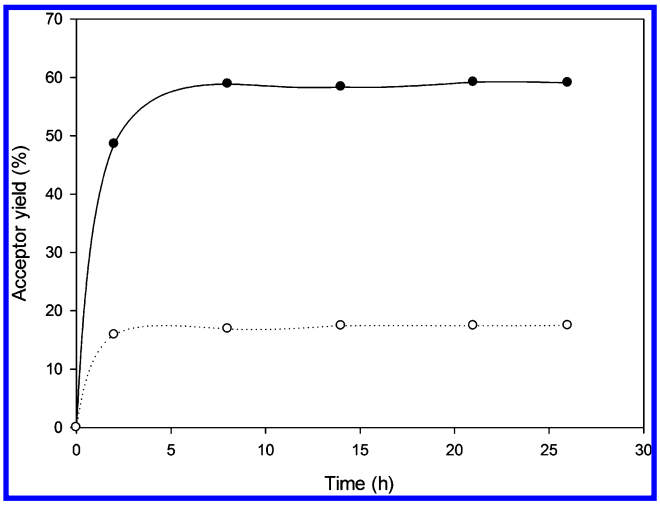

Figure 2. Synthesis of 2-*O*-α-D-glucopyranosyl pyrogallol in a cosolvent (○) and biphasic (●) reaction system. The reaction mixture consisted of 62.5% aqueous MOPS buffer at pH 7.5 containing 2 M sucrose and 50 U/mL SP, and 37.5% EtOAc supplemented with 100 mg/mL pyrogallol. For the cosolvent system, EtOAc was replaced by the IL AMMOENG 101.

Interestingly, the acceptor yield for the cosolvent system reached only 17.4%, while roughly 60% of the initial pyrogallol was converted when using a biphasic system. This significant difference can be explained by the transfer ratio of the enzyme under the operational conditions. Indeed, a ratio of 5.7 for the biphasic system, compared to 1.1 for the cosolvent system, indicates that less hydrolysis (and synthesis of glucobioses) occurs upon application of biphasic catalysis. In addition, the course of product formation reveals the absence of secondary hydrolysis (i.e., the degradation of 2-*O*-α-D-glucopyranosyl pyrogallol), as previously reported by the Nidetzky group for α-glucosyl glycerol[15] (Figure 2).

3. CONCLUSIONS

Following the work on SP-mediated glycoside synthesis in conventional solvents,[32] ILs,[19] and supercritical carbon dioxide,[27] this is the first report on the use of disaccharide phosphorylases in biphasic reaction systems. Careful optimization allowed the glucosylation of aliphatic alcohols, monoterpenoids, aromatic alcohols and phenolics. In addition a series of alkyl gallyl 4-*O*-α-D-glucopyranosides were successfully synthesized up to 30 g/L. These glucosylations were achieved by reacting 62.5% aqueous MOPS buffer at pH 7.5 containing 2 M sucrose and 50 U/mL SP, and 37.5% EtOAc supplemented with 100 mg/mL acceptor. Also, the production of 2-*O*-α-D-glucopyranosyl pyrogallol was compared in cosolvent and biphasic systems. The transfer ratio was found to be 5 times higher when using the latter system, resulting in less hydrolysis and formation of glucobiose side product. Consequently, the use of biphasic catalysis was identified to be a valuable alternative for glycoside synthesis with disaccharide phosphorylases.

4. EXPERIMENTAL SECTION

4.1. Materials, Enzymes, and Instruments. The IL AMMOENG 101 was kindly provided by Evonik Industries AG, and ethyl acetate was bought from Fiers. All other chemicals were analytical grade and purchased from Sigma-Aldrich. The recombinant SP from *B. adolescentis* was produced and partially purified by heat treatment as described recently.[38] The activity of the enzyme was determined as published earlier,[19] and one unit of SP was defined as the activity that corresponds to the release of 1 μmol fructose from 100 mM sucrose in 100 mM phosphate buffer at pH 7 and 37 °C. NMR spectra were measured on a Bruker AVANCE III 600 MHz spectrometer (600.23 MHz for ^{1}H, and 150.94 MHz for ^{13}C) in CD_3OD at 25 °C. HPLC measurements were performed on a Varian Prostar.

4.2. Glycosylation Reactions with SP. Small-scale biphasic glycosylation reactions were performed in a thermoshaker (Eppendorf) at 1400 rpm. Varying amounts of different water-immiscible acceptors were added to 500 μL aqueous buffer in a 1.5 mL Eppendorf. Alternatively, water-immiscible organic solvents containing 100 mg/mL acceptor were used. The acidity of the aqueous phase was varied by using a citrate-phosphate buffer (pH 5−5.4), MES buffer (pH 5.5−6.5), MOPS buffer (pH 6.6−7.5), or tricine buffer (pH 7.6−9). Unless stated otherwise, the reactions were incubated at 50 °C in the presence of 2 M sucrose and 50 U/mL SP. The acceptor ($Yield_{Acceptor}$) and donor yield ($Yield_{Donor}$) are defined as the ratio of the amount of product formed to the amount of acceptor and donor added to the reaction, respectively (mol/mol).

4.3. Solubility Measurements. The dissolution of various acceptor molecules in different water-immiscible solvents was performed in a water bath at 50 °C with ±0.1 °C accuracy. Varying amounts of acceptor were added to a 2 mL Eppendorf, and solvent was added to 1 mL. Next, the samples were vortexed multiple times and allowed to equilibrate for 48 h, after which the samples were checked for remaining particles.

4.4. TLC Analysis. TLC analysis was performed on Merck Silica gel 60 F_{254} precoated plates. The eluens was a mixture of EtOAc/methanol/water (30:5:4 by volume), and spots were visualized by UV detection at 254 nm, or charring with 10% (v/v) H_2SO_4. All TLC plates of a single experiment were

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compared, thereby rating the intensity of product spots between +++++ and + for the highest intensity and a barely visible spot, respectively. Both the aqueous and the solvent phase were spotted on TLC. The intensity of both product spots was combined to yield a single rating.

4.5. HPLC Analysis. HPLC analysis was performed on an X-bridge amide column (250 mm × 4.6 mm, 3.5 μm, Waters, U.S.A.) with Milli-Q water (solvent A) and acetonitrile (solvent B), both containing 0.2% triethylamine, as the mobile phase. The flow rate and temperature were set at 1.0 mL/min and 30 °C, respectively. The gradient elution was as follows: 95% of solvent A (0−12 min), 5− 25% solvent B (12−15 min), 25% solvent B (15−40 min), 25 to 5% solvent B (40−41 min) and 95% solvent A (41−50 min). Adequate detection was obtained with an Alltech 2000ES evaporative light-scattering detector (ELSD). The tube temperature, gas flow, and gain were set at 30 °C, 1.5 L/min, and 1, respectively. Homogeneous samples containing both phases were obtained after intensive mixing. These samples were then diluted in DMSO, and subjected to HPLC analysis. The resulting concentrations thus refer to the amount of product present in the total reaction volume. The obtained peaks were calibrated using standard curves prepared in Milli-Q water or methanol. All HPLC analyses were performed in triplicate.

4.6. Comparison of Biphasic and Cosolvent Glycosylation Reactions Catalyzed by SP. The glycosylation of pyrogallol was carried out at 10 mL scale. The reaction mixture consisted of 62.5% aqueous MOPS buffer at pH 7.5 containing 2 M sucrose and 50 U/mL SP, and 37.5% EtOAc supplemented with 100 mg/mL pyrogallol. Alternatively, EtOAc was replaced by the IL AMMOENG 101. Reactions mixtures were incubated in a thermoshaker (Eppendorf) at 750 rpm and 50 °C. The concentrations of 2-*O*-α-D-glucopyranosyl pyrogallol and fructose were determined by means of HPLC analysis. The transfer ratio is defined as the ratio of the amount of glucose transferred to the acceptor over the sum of the free glucose and the glucose incorporated in glucobioses. The former was calculated on the basis of the glucoside concentration, while the latter was obtained by subtracting the amount of glucoside from the obtained fructose concentration.

4.7. Production and Purification of Glucosides. The glycosylations of hydroquinone, resorcinol, pyrogallol, methyl gallate, ethyl gallate, and propyl gallate were carried out at 100 mL scale in magnetically stirred flasks. Biphasic reaction mixtures were created by adding 62.5 mL aqueous buffer to 37.5 mL organic solvent. The aqueous buffer consisted of 50 mM MOPS at pH 7.5 containing 2 M sucrose and 50 U/mL SP. EtOAc supplemented with 100 mg/mL hydroquinone, resorcinol, pyrogallol, methyl gallate, ethyl gallate, or propyl gallate was used as organic phase. Alternatively, glycosylation of 1-phenylethanol, geraniol, and cinnamyl alcohol was performed by substituting the EtOAc by the pure acceptor. Reactions were terminated after 48 h incubation at 50 °C, after which the reaction mixtures were heated (10 min at 95 °C) and centrifuged (12000*g*, 4 °C, 15 min) to remove debris. The samples were then evaporated *in vacuo*, and the residue was purified by column chromatography (silicagel, EtOAc-methanol−water (30:5:4 by volume)).

4.8. Structure Elucidation of Glucosides. The structures of the newly formed glucosides were determined by a combination of 1D NMR (^{1}H NMR and ^{13}C NMR) and 2D NMR (gCOSY, gHSQC and gHMBC) spectroscopy. Residual signals of solvent were used as internal standards (δ_H 3.330 ppm, δ_C 49.30 ppm), and digital resolution enabled us to report δ_H to three and δ_C to two decimal places. The proton spin systems were assigned by COSY, and then the assignment was transferred to carbons by HSQC. HMBC experiments enabled assigning quaternary carbons and joining individual spin systems together. Chemical shifts are given in δ-scale [ppm], and coupling constants in Hz.

4.8.1. 1-O-α-D-Glucopyranosyl hydroquinone: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 3.427 (1H, dd, J = 9.7, 8.9 Hz, H-4), 3.543 (1H, dd, J = 9.8, 3.7 Hz, H-2), 3.722 (1H, dd, J = 11.3, 4.9 Hz, H-6u), 3.752 (1H, ddd, J = 9.7, 4.9, 2.0 Hz, H-5), 3.791 (1H, dd, J = 11.3, 2.0 Hz, H-6d), 3. 863 (1H, dd, J = 9.8, 8.9 Hz, H-3), 5.310 (1H, d, J = 3.7 Hz, H-1), 6.720 (2H, m, ΣJ = 8.9 Hz, H-*meta*), 7.026 (2H, m, ΣJ = 8.9 Hz, H-*ortho*).
^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 62.73 (C-6), 71.92 (C-4), 73.73 (C-2), 74.50 (C-5), 75.28 (C-3), 100.85 (C-1), 116.99 (C-*meta*), 120.17 (C-*ortho*), 152.22 (C-*ipso*), 154.14 (C-*para*).

4.8.2. 1-O-α-D-Glucopyranosyl resorcinol: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 3.462 (1H, dd, J = 9.9, 8.9 Hz, H-4), 3.569 (1H, dd, J = 9.8, 3.7 Hz, H-2), 3.671 (1H, ddd, J = 9.9, 4.4, 2.7 Hz, H-5), 3.728 (1H, dd, J = 12.0, 4.4 Hz, H-6u), 3.762 (1H, dd, J = 12.0, 2.7 Hz, H-6d), 5.457 (1H, d, J = 3.7 Hz, H-1), 6.548 (1H, ddd, J = 8.1, 2.3, 0.8 Hz, H-6′), 6.636 (1H, dd, J = 2.3, 2.3 Hz, H-2′), 6.662 (1H, ddd, J = 8.2, 2.3, 0.8 Hz, H-4′), 7.087 (1H, dd, J = 8.2, 8.1 Hz, H-5′).
^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 62.58 (C-6), 71.75 (C-4), 73.63 (C-2), 74.58 (C-5), 75.26 (C-3), 99.52 (C-1), 105.85 (C-2′), 109.57 (C-4′), 110.83 (C-6′), 131.14 (C-5′), 159.90 (C-3′), 160.09 (C-1′).

4.8.3. 2-O-α-D-Glucopyranosyl pyrogallol: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 3.441 (1H, dd, J = 10.1, 8.9 Hz, H-4), 3.604 (1H, dd, J = 9.6, 3.9 Hz, H-2), 3.771 (1H, dd, J = 11.8, 5.5 Hz, H-6u), 3.881 (1H, dd, J = 9.6, 8.9 Hz, H-3), 3.902 (1H, dd, J = 11.8, 2.4 Hz, H-6d), 4.234 (1H, ddd, J = 10.1, 5.5, 2.4 Hz, H-5), 5.024 (1H, d, J = 3.9 Hz, H-1) 6.385 (2H, d, J = 8.2 Hz, H-*meta*), 6.813 (1Ht, J = 8.2 Hz, H-*para*).
^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 62.60 (C-6), 71.41 (C-4), 73.66 (C-2), 75.30 (C-3), 75.56 (C-5), 106.01 (C-1), 108.95 (C-*meta*), 126.47 (C-*para*), 136.12 (C-*ipso*), 152.27 (C-*ortho*).

4.8.4. Methyl gallyl 4-O-α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 3.482 (1H, dd, J = 10.1, 9.2 Hz, H-4), 3.619 (1H, dd, J = 9.6, 3.8 Hz, H-2), 3.796 (1H, dd, J = 11.9, 4.9 Hz, H-6u), 3.860 (3H, s, H-1′), 3.868 (1H, dd, J = 11.9, 2.5 Hz, H-6d), 3.902 (1H, dd, J = 9.6, 9.2 Hz, H-3), 4.243 (1H, ddd, J = 10.1, 4.9, 2.5 Hz, H-5), 5.149 (1H, d, J = 3.8 Hz, H-1), 7.063 (2H, s, H-*meta*).
^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 52.88 (C-1′), 62.41 (C-6), 71.18 (C-4), 73.66 (C-2), 75.22 (C-3), 75.46 (C-5), 105.45 (C-1), 110.34 (C-*meta*), 128.10 (C-*para*), 139.75 (C-*ipso*), 152.31 (C-*ortho*), 168.60 (CO).

4.8.5. Ethyl gallyl 4-O-α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 1.372 (3H, t, J = 7.1 Hz, H-2′), 3.479 (1H, dd, J = 10.1, 9.2 Hz, H-4), 3.619 (1H, dd, J = 9.6, 3.8 Hz, H-2), 3.793 (1H, dd, J = 11.8, 5.0 Hz, H-6u), 3.872 (1H, dd, J = 11.8, 2.5 Hz, H-6d), 3.901 (1H, dd, J = 9.6, 9.2 Hz, H-3), 4.242 (1H, ddd, J = 10.1, 5.0, 2.5 Hz, H-5), 4.317 (1H, q, J = 7.1 Hz, H-1′), 5.146 (1H, d, J = 3.8 Hz, H-1), 7.068 (2H, s, H-*meta*).
^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 14.85 (C-2′), 62.36 (C-1′), 62.45 (C-6), 71.22 (C-4), 73.67 (C-2), 75.24 (C-

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3), 75.50 (C-5), 105.51 (C-1), 110.31 (C-*meta*), 128.47 (C-*para*), 139.72 (C-*ipso*), 152.29 (C-*ortho*), 168.13 (CO).

4.8.6. Propyl gallyl 4-O-α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 1.038 (3H, t, *J* = 7.4 Hz, H-3′), 1.778 (2H, tq, *J* = 7.4, 6.6 Hz, H-2′), 3.485 (1H, dd, *J* = 10.0, 9.2 Hz, H-4), 3.622 (1H, dd, *J* = 9.6, 3.8 Hz, H-2), 3.798 (1H, dd, *J* = 11.9, 4.9 Hz, H-6u), 3.872 (1H, dd, *J* = 11.9, 2.5 Hz, H-6d), 3.906 (1H, dd, *J* = 9.6, 9.2 Hz, H-3),4.223 (2H, t, *J* = 6.6 Hz, H-1′), 4.246 (1H, ddd, *J* = 10.0, 4.9, 2.5 Hz, H-5), 5.153 (1H, d, *J* = 3.8 Hz, H-1), 7.070 (2H, s, H-*meta*).

^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 11.07 (C-3′), 23.40 (C-2′), 62.41 (C-6), 67.92 (C-1′), 71.17 (C-4), 73.64 (C-2), 75.21 (C-3), 75.45 (C-5), 105.44 (C-1), 110.28 (C-*meta*), 128.39 (C-*para*), 139.69 (C-*ipso*), 152.28 (C-*ortho*), 168.16 (CO).

4.8.7. 1R-Phenylethyl α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 1.493 (3H, d, *J* = 6.6 Hz, H-2′), 3.304 (1H, dd, *J* = 9.8, 8.9 Hz, H-4), 3.333 (1H, dd, *J* = 9.8, 3.8 Hz, H-2), 3.709 (1H, m, H-6u), 3.735 (1H, m, H-5), 3.775 (1H, dd, *J* = 9.7, 8.9 Hz, H-3), 3.886 (1H, m, H-6d), 4.626 (1H, d, *J* = 3.8 Hz, H-1), 4.909 (1H, q, *J* = 6.6 Hz, H-1′),7.272 (1H, m, H-*para*), 7.345 (2H, m, H-*meta*), 7.455 (2H, m, H-*ortho*).

^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 24.92 (C-2′), 63.13 (C-6), 72.34 (C-4), 73.78 (C-2), 74.19 (C-5), 74.59 (C-1′), 75.40 (C-3), 97.14 (C-1), 128.19 (C-*ortho*), 128.90 (C-*para*), 129.71 (C-*meta*), 144.30 (C-*ipso*).

4.8.8. 1S-Phenylethyl α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 1.499 (3H, d, *J* = 6.5 Hz, H-2′), 3.323 (1H, dd, *J* = 10.0, 8.5 Hz, H-4), 3.362 (1H, ddd, *J* = 10.0, 4.3, 2.3 Hz, H-5), 3.429 (1H, dd, *J* = 9.7, 3.8 Hz, H-2), 3.435 (1H, dd, *J* = 11.8, 2.3 Hz, H-6u), 3.543 (1H, dd, *J* = 11.9, 4.3 Hz, H-6d), 3.664 (1H, dd, *J* = 9.7, 8.5 Hz, H-3), 4.808 (1H, q, *J* = 6.5 Hz, H-1′), 5.058 (1H, d, *J* = 3.8 Hz, H-1), 7.254 (1H, m, H-*para*), 7.326 (2H, m, H-*meta*), 7.427 (2H, m, H-*ortho*).

^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 22.44 (C-2′), 62.33 (C-6), 71.81 (C-4), 73.88 (C-2), 75.38 (C-3), 77.17 (C-1′), 78.42 (C-5), 99.34 (C-1), 127.73 (C-*ortho*), 128.65 (C-*para*), 129.54 (C-*meta*), 145.60 (C-*ipso*).

4.8.9. Cinnamyl α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 3.325 (1H, dd, *J* = 10.0, 8.9 Hz, H-4), 3.446 (1H, dd, *J* = 9.7, 3.7 Hz, H-2), 3.661 (1H, ddd, *J* = 10.0, 5.7, 2.3 Hz, H-5), 3.707 (1H, dd, *J* = 11.7, 5.7 Hz, H-6u), 3.714 (1H, dd, *J* = 9.7, 8.9 Hz, H-3), 3.851 (1H, dd, *J* = 11.7, 2.3 Hz, H-6d), 4.240 (1H, ddd, *J* = 12.8, 6.6, 1.4 Hz, H-1′u), 4.407 (1H, ddd, *J* = 12.8, 5.8, 1.6 Hz, H-1′d), 4.922 (1H, d, *J* = 3.7 Hz, H-1), 6.410 (1H, ddd, *J* = 16.0, 6.6, 5.8 Hz, H-2′), 6.714 (1H, ddd, *J* = 16.0, 1.6, 1.4 Hz, H-3′), 7.242 (1H, m, H-*para*), 7.321 (2H, m, H-*meta*), 7.439 (2H, m, H-*ortho*).

^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 63.03 (C-6), 69.44 (C-1′), 72.18 (C-4), 73.87 (C-2), 74.15 (C-5), 75.44 (C-3), 99.61 (C-1), 126.89 (C-2′), 127.82 (C-*ortho*), 129.01 (C-*para*), 129.89 (C-*meta*), 134.21 (C-3′), 138.52 (C-*ipso*).

4.8.10. Geranyl α-D-glucopyranoside: ^{1}H NMR (600.23 MHz, CD_3OD, 25 °C): δ 1.630 (3H, s, H-9′), 1.695 (3H, q, *J* = 1.0 Hz, H-8′), 1.715 (3H, s, 3′-Me), 2.067 (2H, m, H-4′), 2.141 (2H, m, H-5′), 3.316(1H, dd, *J* = 9.9, 8.8 Hz, H-4), 3.403 (1H, dd, *J* = 9.7, 3.8 Hz, H-2), 3.608 (1H, ddd, *J* = 9.9, 5.5, 2.4 Hz, H-5), 3.658 (1H, dd, *J* = 9.7, 8.8 Hz, H-3), 3.704 (1H, ddd, *J* = 11.8, 5.5 Hz, H-6u), 3.824 (1H, dd, *J* = 11.8, 2.4 Hz, H-6d), 4.121 (1H, dd, *J* = 12.1, 7.5 Hz, H-1′u), 4.242 (1H, dd, *J* = 12.1, 6.4 Hz, H-1′d), 4.838 (1H, d, *J* = 3.8 Hz, H-1), 5.128 (1H, m, H-6′), 5.409 (1H, m, H-2′).

^{13}C NMR (150.94 MHz, CD_3OD, 25 °C): δ 16.79 (3′-Me), 18.04 (C-9′), 26.17 (C-8′), 27.74 (C-5′), 41.01 (C-4′), 62.96 (C-6), 65.11 (C-1′), 72.12 (C-4), 73.85 (C-2), 73.97 (C-5), 75.49 (C-3), 99.17 (C-1), 121.95 (C-2′), 125.36 (C-6′), 132.82 (C-7′), 141.86 (C-3′).

■ ASSOCIATED CONTENT

Supporting Information

TLC plates showing the biphasic glycosylation of cinnamyl alcohol (Figure S.1 and S.2) and the solubility of various acceptors in different water-immiscible solvents (Table S.1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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