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Syntheses of dopa glycosides using glucosidases

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Abstract Syntheses of L-dopa **1a** glucoside **10a,b** and DL-dopa **1b** glycosides **10**–**18** with D-glucose **2**, D-galactose **3**, D-mannose **4**, D-fructose **5**, D-arabinose **6**, lactose **7**, D-sorbitol **8** and D-mannitol **9** were carried out using amyloglucosidase from *Rhizopus* mold, β-glucosidase isolated from sweet almond and immobilized β-glucosidase. Invariably, L-dopa and DL-dopa gave low to good yields of glycosides **10–18** at 12–49% range and only mono glycosylated products were detected through glycosylation/arylation at the third or fourth OH positions of L-dopa **1a** and DL-dopa **1b**. Amyloglucosidase showed selectivity with D-mannose **4** to give 4-*O*-C1β and D-sorbitol **8** to give 4-*O*-C6-*O*-arylated product. β-Glucosidase exhibited selectivity with D-mannose **4** to give 4-*O*-C1β and lactose **7** to give 4-*O*-C1β product. Immobilized β-glucosidase did not show any selectivity. Antioxidant and angiotensin converting enzyme inhibition (ACE) activities of the glycosides were evaluated glycosides, out of which L-3-hydroxy-4-*O*-(β-D-galactopyranosyl-(1′→4)β-D-glucopyranosyl) phenylalanine **16** at 0.9± 0.05 mM and DL-3-hydroxy-4-*O*-(β-D-glucopyranosyl) phenylalanine **11b,c** at 0.98±0.05 mM showed the best IC_{50} values for antioxidant activity and DL-3-hydroxy-4-*O*-(6-D-sorbitol)phenylalanine **17** at 0.56±0.03 mM, L-dopa-D-glucoside **10a,b** at 1.1±0.06 mM and DL-3-hydroxy-4-*O*-(D-glucopyranosyl)phenylalanine **11a-d** at 1.2±0.06 mM exhibited the best IC_{50} values for ACE inhibition.

Keywords Arylation · L-dopa · L-Dopa Glucosides · DL-Dopa · DL-Dopa Glycosides · Glycosylation · Glucosidases · Regioselectivity

Introduction

DL-Dopa (DL-3,4-dihydroxy phenylalanine), an aromatic amino acid precursor of dopamine is the most effective drug for Parkinson's disease [1]. Parkinson's disease is characterized by a severe and progressive degeneration of nigrostriatal dopamine neurons [2] associated with the deficiency of catecholamine and dopamine [3]. It is generally accepted that after administration, L-dopa in Parkinson's disease is converted into dopamine by aromatic L-amino acid decarboxylase within the serotonergic (5-HT) fibers in the striatum and *substancia nigra pars reticulate* [4]. Glucose is the brain source of energy and this as well as other hexoses are also transferred across the blood brain barrier by the glucose carrier GLUT1 [5]. Such a transport will be facilitated if L-dopa is converted into the glycoside as the L-dopa converted product glucosyl-dopamine is able to interact with the glucose transporter (GLUT1) and absorbed into the central nervous system from the blood stream [6]. During chronic treatment with L-dopa, a variety of transport problems like involuntary movements occur, which can be overcome by the employment of glucosyl derivatives [7, 8]. Side effects of the drugs can be reduced and drug stability can be increased by modification of the aglycon molecule. For

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example, L-dopa and new dopaminergics can be modified to improve bioactivity properties [9, 10].

L-Dopa glucoside was first isolated from *Vicia faba* [11, 12] as a β-anomer [13, 14]. *V. faba* was incorporated into dietary strategies to manage Parkinsonian motor oscillations [15]. A crude extract from the petals of *Mirabilis jalapa* was mixed with *cyclo*-dopa and UDP-glucose to give *cyclo*-dopa-5-*O*-glucoside by the action of glucosyl transferases [16, 17]. However, no chemical or enzymatic methods have been reported yet for the synthesis of DL-dopa glycosides. Enzymatic method could be the best method as it does not require protection and deprotection steps [18, 19], and provide milder reaction conditions, easy workup, less pollution, higher yields and selectivity. It can also give rise to stable dopa derivatives with enhanced stability and pharmacological activity [20, 21].

The present work has been undertaken to synthesize L-dopa glucoside and DL-dopa glycosides using amyloglucosidase from *Rhizopus* mold and β-glucosidase (native/immobilized) from sweet almond in organic media. DL-Dopa glycosides were also tested for Angiotensin Converting Enzyme inhibition and antioxidant activities. The results of this investigation are presented in detail.

Materials and methods

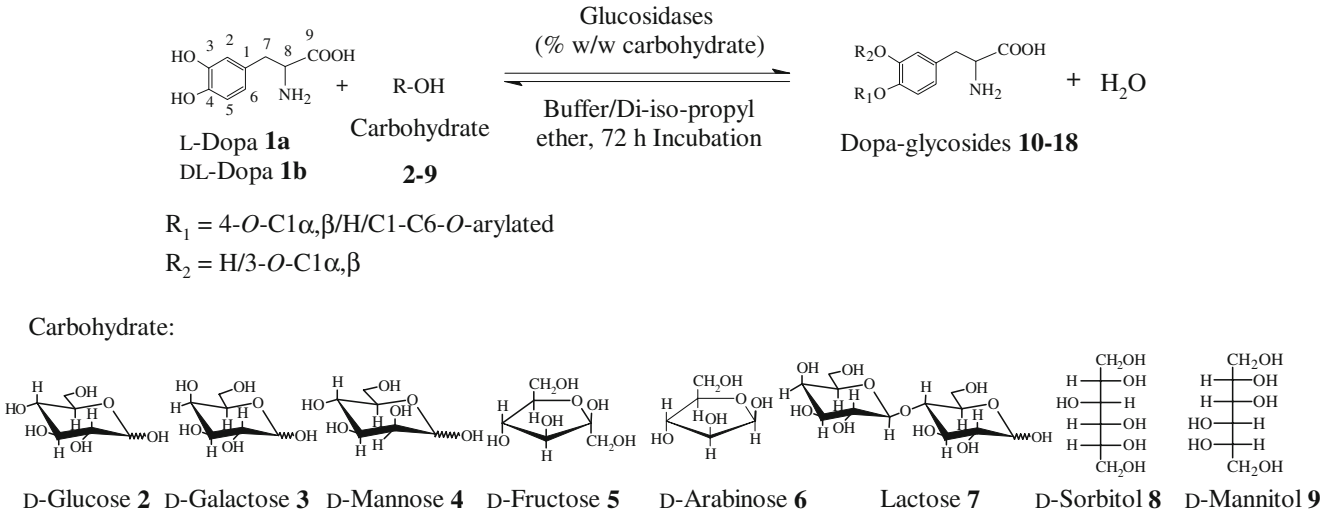
Enzymes and chemicals

Amyloglucosidase from *Rhizopus* sp. was purchased from Sigma, St. Louis, MO, USA and β-glucosidase was isolated from sweet almonds [22] and immobilized onto calcium alginate beads [23]. Amyloglucosidase activity [24] was found to be 11.2 AU (μmol/mg enzyme min), β-glucosidase 3.12 AU (mmol/mg enzyme min) and immobilized β-glucosidase 0.081 AU [25].

DL-Dopa, D-galactose and D-fructose procured from HiMedia Pvt. Ltd, India; D-glucose from SD Fine Chemicals (Ind.) Ltd.; D-mannose, D-arabinose, D-sorbitol and D-mannitol from Loba Chemie Pvt. Ltd.; L-dopa from Rolex Chemical (Ind.), India were employed as such. Lactose, high performance liquid chromatography (HPLC) grade acetonitrile and di-isopropyl ether were from Sisco Research Laboratories Pvt. Ltd. India. Di-isopropyl ether was distilled once before use.

Glycosylation procedure

Synthesis of L-dopa glucoside and DL-dopa glycosides involved refluxing 0.2–2 mmol L-dopa **1a** /DL-dopa **1b** with 1 mmol carbohydrates—D-glucose **2**, D-galactose **3**, D-mannose **4**, D-fructose **5**, D-arabinose **6**, lactose **7**, D-sorbitol **8**, D-mannitol **9** in 100 mL di-isopropyl ether in presence of 10–75% (*w*/*w* carbohydrate) glucosidases and 0.03–0.22 mM (0.3–2.2 mL) of 0.01 M pH 4–8 buffer for an incubation period of 72 h at 68°C (Scheme 1). The solvent was evaporated and the enzyme denatured at 100°C by holding in boiling water bath for 5–10 min. The reaction mixture was evaporated to dryness. The dried residue material was subjected to HPLC analysis to determine the extent of conversion. Glycosides were separated through column chromatography using Sephadex G15 column (100× 1 cm), eluting with water at 1 mL/h rate. Although the glycosides were separated from unreacted carbohydrates,



Scheme 1 Syntheses of L-dopa and DL-dopa glycosides

individual glycosides could not be isolated in pure form, due to similar molecular weight of these molecules.

High performance liquid chromatography

Glycosides prepared were analyzed by HPLC on an aminopropyl column (250×4.6 mm) eluting with 70:30 (*v*/*v*) acetonitrile:water at a flow rate of 1 mL/h and monitored using a RI detector (Fig. 1). Conversion yields were determined from HPLC peak areas of the glycoside and free carbohydrate and expressed with respect to the free carbohydrate concentration employed. Error based on HPLC measurements are of the order of ±10%. HPLC retention times for the substrates and products are: L-dopa—8.4 min, L-dopa-D-glucoside—12.8 min, DL-dopa—8.5 min, D-glucose—6.2 min, DL-dopa-D-glucoside—13 min, D-galactose—7.1 min, DL-dopa-D-galactoside—11.9 min, D-mannose—6.7 min, DL-3-hydroxy-4-*O*-(β-D-mannopyranosyl) phenylalanine—11.7 min, D-fructose—6.8 min, DL-3-hydroxy-4-*O*-(D-fructofuranosyl) phenylalanine—12 min, D-arabinose—6.5 min, DL-3-hydroxy-4-*O*-(D-arabinofuranosyl) phenylalanine—11.7 min, lactose—9.3 min, DL-3-hydroxy-4-*O*-(β-D-galactopyranosyl-(1′→4)β-D-glucopyranosyl)phenylalanine—11.9 min, D-sorbitol—6.7 min, DL-3-hydroxy-4-*O*-(6-D-sorbitol) phenylalanine—7.9 min, D-mannitol—6.8 min and DL-dopa-D-mannitol—7.8 min.

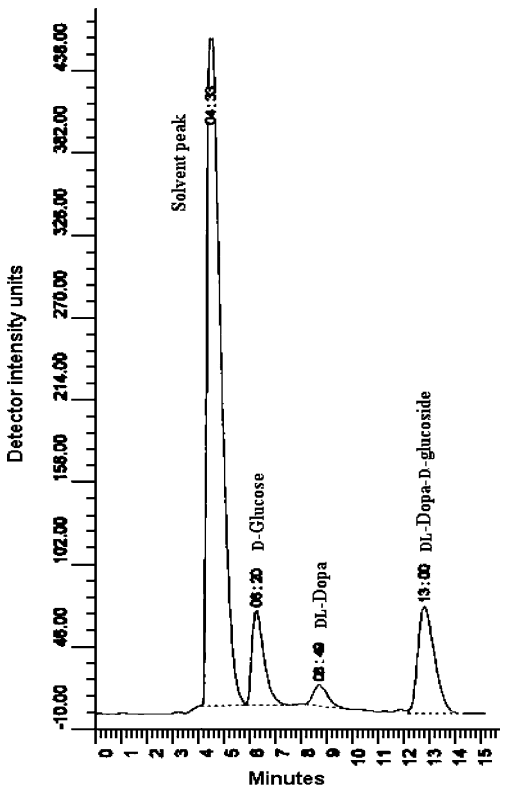


Fig 1 HPLC chromatogram for the reaction mixture of DL-dopa-D-glucoside. HPLC conditions: Aminopropyl column (10 μm, 250×4.6 mm), solvent-CH_3CN/H_2O (70:30 *v*/*v*), Flow rate—1 mL/min, RI detector. Retention times: D-glucose—6.2 min, DL-dopa—8.5 min and DL-dopa-D-glucoside—13 min

Antioxidant activity measurement

Antioxidant activity of DL-dopa and DL-dopa glycosides were determined by 2,2 diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method [26, 27]. Butylated hydroxy anisole (BHA—5.6 mM) was used as the positive control. IC_{50} value for the antioxidant activity was expressed as the concentration of the glycoside corresponding to 50% decrease in absorbance of DPPH at 517 nm from a plot of decrease in absorbance versus concentration of the glycoside. Error in activity measurements is ±5%.

Angiotensin converting enzyme inhibition assay

ACE inhibition assay for the DL-dopa glycosides were performed using Cushman and Cheung method [28, 29] by measuring hippuric acid released from hippuryl-L-histidyl-L-leucine by the action of ACE on the glycoside. Blanks were performed without the enzyme. Hippuric acid released was estimated from a calibration plot showing 0.011 Abs units/nmol hippuric acid. Percentage inhibition was expressed as the ratio of specific activity of ACE in presence of the inhibitor to that in its absence, the latter being considered as 100%. IC_{50} value was expressed as the concentration of the inhibitor required for 50% reduction in ACE specific activity. Error in measurements will be ±5%.

Spectral characterization

The isolated glycosides were subjected to measurement of melting point and optical rotation besides characterization by recording ultraviolet (UV), infrared (IR), mass and two-dimensional heteronuclear single quantum coherence transfer spectra (2D-HSQCT). In the nuclear magnetic resonance (NMR) data only resolvable signals are shown. Some of the assignments are interchangeable and non-reducing end carbohydrate units are primed. Glycosides are surfactant molecules and since the concentrations employed for 2D-HSQCT spectral measurements are very much higher than their respective critical micellar concentrations, the proton NMR signals were unusually broad to the extent that in spite of recording the spectra at 35°C, the individual coupling constant values could not be determined precisely for all the signals.

L-Dopa-D-glucoside **10a,b:** Isolated yield (β-glucosidase)—0.052 g (28.8%): Solid, UV (H_2O, λ_{max}): 198.5 nm (σ→σ*, $\varepsilon_{198.5}$—7,668 M^{-1}), 251 nm (π→π*,

ε_{251}—2,988 M^{-1}), 267.5 nm ($\pi\rightarrow\pi^*$, $\varepsilon_{267.5}$—3,046 M^{-1}), 397.5 nm ($n\rightarrow\pi^*$, $\varepsilon_{397.5}$–2,766 M^{-1}), IR (stretching frequency, cm^{-1}): 3,484 (OH), 1,289 (glycosidic aryl alkyl C–O–C asymmetrical), 1,036 (glycosidic aryl alkyl C–O–C symmetrical), 1,651 (CO), MS (*m/z*)–360.1 $[M+1]^+$, 2D-HSQCT (DMSO-d_6) L-3-Hydroxy-4-*O*-(D-gluco pyranosyl) phenylalanine: 4-*O*-C1β-glucoside **10a**: ^{1}H NMR Glu: 4.21 (H-1β, d, J = 6.1 Hz), 2.89 (H-2β), 3.10 (H-3β), 3.67 (H-4β), 3.45 (H-5β), 3.58 (H-6a), L-Dopa: 6.66 (H-2), 6.52 (H-6), 3.09 (βCH_{2a}-7), 2.73 (βCH_{2b}-7), 3.45 (αCH-8), ^{13}C NMR δ_{ppm} Glu: 103.4 (C1β), 73.4 (C2β), 77.1 (C3β), 72.1 (C4β), 62.9 (C6β), L-Dopa: 127.9 (C1), 115.8 (C2), 144.9 (C3), 144.1 (C4), 120.1 (C5), 114.1 (C6), 36.5 (C7), 55.8 (C8), 171.1 (C9); L-4-Hydroxy-3-*O*-(β-D-glucopyranosyl) phenylalanine: 3-*O*-C1β-glucoside **10b**: ^{1}H NMR Glu: 4.57 (H-1β, d, J = 6.2 Hz), L-Dopa: 3.45 (αCH-8), ^{13}C NMR Glu: 103 (C1β), L-Dopa: 53.2 (C8), 174.2 (C9).

DL-Dopa-D-glucoside **11a**–**d**: Isolated yield (amyloglucosidase)—0.1 g (55%): Solid, UV (H_2O, λ_{max}): 192 nm ($\sigma\rightarrow\sigma^*$, ε_{192}—3,319 M^{-1}), 226 nm ($\sigma\rightarrow\pi^*$, ε_{226}—959 M^{-1}), 295.5 nm ($n\rightarrow\pi^*$, $\varepsilon_{295.5}$—571 M^{-1}), IR (stretching frequency, cm^{-1}): 3358 (OH), 1328 (glycosidic aryl alkyl C–O–C asymmetrical), 1038 (glycosidic aryl alkyl C–O–C symmetrical), 1663 (CO), MS (*m/z*) – 359.1 $[M]^+$, 2D-HSQCT (DMSO-d_6) DL-3-Hydroxy-4-*O*-(D-glucopyranosyl)phenylalanine: 4-*O*-C1α-glucoside **11a**: ^{1}H NMR δ_{ppm} (500.13) Glu: 5.04 (H-1α, d, J = 3.4 Hz), 3.21 (H-2α), 3.47 (H-3α), 3.05 (H-4α), 3.58 (H-5α), 3.44 (H-6a), DL-Dopa: 6.81 (H-2), 6.80 (H-6), 3.02 (βCH_{2a}-7), 2.70 (βCH_{2b}-7), 3.48 (αCH-8), ^{13}C NMR δ_{ppm} (125 MHz) Glu: 96.1 (C1α), 72 (C2α), 71.5 (C3α), 70.5 (C4α), 72 (C5α), 61.1 (C6α), DL-Dopa: 130.2 (C1), 110 (C2), 116 (C6), 57.4 (C8), 172.1 (C9); 4-*O*-C1β-glucoside **11b**: Solid, UV (H_2O, λ_{max}): 192.5 nm ($\sigma\rightarrow\sigma^*$, $\varepsilon_{192.5}$—3,012 M^{-1}), 225 nm ($\sigma\rightarrow\pi^*$, ε_{225}—842 M^{-1}), 291.5 nm ($n\rightarrow\pi^*$, $\varepsilon_{291.5}$—356 M^{-1}), IR (stretching frequency, cm^{-1}): 3325 (OH), 1330 (glycosidic aryl alkyl C–O–C asymmetrical), 1026 (glycosidic aryl alkyl C–O–C symmetrical), 1650 (CO), MS (*m/z*)—360.2 $[M+1]^+$, ^{1}H NMR Glu: 4.20 (H-1β, d, J = 6.3 Hz), 2.94 (H-2β), 3.13 (H-3β), 3.68 (H-4β), 3.37 (H-5β), 3.55 (H-6a), DL-Dopa: 6.71 (H-2), 6.40 (H-5), 6.60 (H-6), 3.02 (βCH_{2a}-7), 2.70 (βCH_{2b}-7), 3.47 (αCH-8), ^{13}C NMR δ_{ppm} Glu: 103.4 (C1β), 73.5 (C2β), 77 (C3β), 72 (C4β), 62.8 (C6β), DL-Dopa: 127.9 (C1), 116.9 (C2), 145.2 (C3), 144.1 (C4), 120.2 (C5), 115.8 (C6), 36.6 (C7), 55.9 (C8), 172 (C9); 4-*O*-C6-*O*-arylated **11c**: ^{1}H NMR Glu: 3.51 (H-6a), DL-Dopa: 3.55 (αCH-8), ^{13}C NMR Glu: 66.9 (C6α), DL-Dopa: 55.9 (C8), DL-4-Hydroxy-3-*O*-(β-D-glucopyranosyl)phenylalanine **11d**: ^{1}H NMR Glu: 4.55 (H-1β, d, J = 5.9 Hz), DL-Dopa: 3.58 (αCH-8), ^{13}C NMR Glu: 98.5 (C1β), DL-Dopa: 52.4 (C8).

DL-Dopa-D-galactoside **12a**-**e**: Isolated yield (amyloglucosidase)—0.095 g (53%): Solid, UV (H_2O, λ_{max}): 191.5 nm ($\sigma\rightarrow\sigma^*$, $\varepsilon_{191.5}$—2810 M^{-1}), 225.5 nm ($\sigma\rightarrow\pi^*$, $\varepsilon_{225.5}$—876 M^{-1}), 296 nm ($n\rightarrow\pi^*$, ε_{296}—624 M^{-1}), IR (stretching frequency, cm^{-1}): 3422 (OH), 1307 (glycosidic aryl alkyl C–O–C asymmetrical), 1039 (glycosidic aryl alkyl C–O–C symmetrical), 1407 (C=C), 1641 (CO), 2940 (CH), MS (*m/z*)—360.2 $[M+1]^+$, 2D-HSQCT (DMSO-d_6) DL-3-Hydroxy-4-*O*-(D-galactopyranosyl)phenylalanine: 4-*O*-C1α-galactoside **12a**: ^{1}H NMR δ_{ppm} (500.13) Gal: 4.98 (H-1α, d, J = 3.9 Hz), 3.55 (H-2α), 3.57 (H-3α), 3.67 (H-4α), 3.35 (H-5α), 3.44 (H-6a), DL-Dopa: 6.76 (H-2), 6.66 (H-6), 2.90 (βCH_{2a}-7), 2.65 (βCH_{2b}-7), 3.35 (αCH-8), ^{13}C NMR δ_{ppm} (125 MHz): Gal: 96.3 (C1α), 64 (C2α), 68.5 (C3α), 68 (C4α), 70.7 (C5α), 63 (C6α), DL-Dopa: 124.4 (C1), 114.5 (C2), 145.4 (C3), 144.4 (C4), 122.3 (C5), 116.8 (C6), 36.8 (C7), 57.1 (C8), 173 (C9); 4-*O*-C1β-galactoside **12b**: ^{1}H NMR Gal: 4.90 (H-1β, d, J = 7.1 Hz), 3.56 (H-3β), 3.04 (H-4β), 3.28 (H-5β), 3.46 (H-6a), DL-Dopa: 6.72 (H-2), 2.68 (βCH_{2b}-7), 3.45 (αCH-8),^{13}C NMR δ_{ppm} Gal: 102.1 (C1β), 69.7 (C3β), 72.6 (C4β), 74.2 (C5β), 63.5 (C6β), DL-Dopa: 115.6 (C2), 144.8 (C4), 36.6 (C7), 53.2 (C8); 4-*O*-C6-*O*-arylated **12c**: ^{1}H NMR Gal: 3.68 (H-6a), ^{13}C NMR Gal: 66.5 (C6α), DL-4-Hydroxy-3-*O*-(D-galactopyranosyl)phenylalanine: 3-*O*-C1α-galactoside **12d**: ^{1}H NMR Gal: 5.07 (H-1α, d, J = 3.7 Hz), 3.46 (H-6a), DL-Dopa: 6.80 (H-2), 6.70 (H-6), 2.70 (βCH_{2b}-7), 3.45 (αCH-8), ^{13}C NMR Gal: 95.7 (C1α), 63.2 (C6α), DL-Dopa: 125.5 (C1), 114.9 (C2), 117 (C6), 29.3 (C7), 56.2 (C8); 3-*O*-C1β-galactoside **12e**: ^{1}H NMR Gal: 4.65 (H-1β, d, J = 6.1 Hz),^{13}C NMR δ_{ppm} Gal: 103.5 (C1β).

DL-3-Hydroxy-4-*O*-(β-D-mannopyranosyl)phenylalanine **13**: Isolated yield (amyloglucosidase)—0.105 g (58%): Solid, mp: 108°C; UV (H_2O, λ_{max}): 192 nm ($\sigma\rightarrow\sigma^*$, ε_{192}—2433 M^{-1}), 224.5 nm ($\sigma\rightarrow\pi^*$, $\varepsilon_{224.5}$—931 M^{-1}), 295.5 nm ($n\rightarrow\pi^*$, $\varepsilon_{295.5}$—583 M^{-1}), IR (stretching frequency, cm^{-1}): 3385 (OH), 1304 (glycosidic aryl alkyl C–O–C asymmetrical), 1040 (glycosidic aryl alkyl C–O–C symmetrical), 1406 (C=C), 1641 (CO), 2946 (CH), optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25°C=−29.7, MS (*m/z*)—360.2 $[M+1]^+$, 2D-HSQCT (DMSO-d_6) 4-*O*-C1β-mannoside: ^{1}H NMR Man: 4.69 (H-1β, d, J = 3.4 Hz), 3.40 (H-2β), 3.50 (H-3β), 3.55 (H-4β), 3.05 (H-5β), 3.45 (H-6a), DL-Dopa: 6.77 (H-2), 6.40 (H-5), 6.57 (H-6), 2.95 (βCH_{2a}-7), 2.60 (βCH_{2b}-7), 3.44 (αCH-8), ^{13}C NMR δ_{ppm} Man: 102.8 (C1β), 70.5 (C2β), 73.1 (C3β), 67 (C4β), 73.1 (C5β), 63.9 (C6β), DL-Dopa: 128.7 (C1), 115.5 (C2), 145 (C3), 144 (C4), 120.2 (C5), 116.8 (C6), 36.4 (C7), 56 (C8), 172 (C9).

3-Hydroxy-4-*O*-(1-D-fructofuranosyl)phenylalanine **14a, b**: Isolated yield (Imm. β-glucosidase)—0.085 g (48%): Solid, UV (λ_{max}): 191.5 nm ($\sigma\rightarrow\sigma^*$, $\varepsilon_{191.5}$—3554 M^{-1}), 221.5 nm ($\sigma\rightarrow\sigma^*$, $\varepsilon_{221.5}$—1854 M^{-1}), 259.0 nm ($\pi\rightarrow\pi^*$, $\varepsilon_{259.0}$—438 M^{-1}), 284.0 nm ($n\rightarrow\pi^*$, $\varepsilon_{284.0}$—615 M^{-1}); IR (stretching frequency, cm^{-1}): 3375 (OH), 1287 (glycosidic

aryl alkyl C–O–C asymmetrical), 1050 (glycosidic aryl alkyl C–O–C symmetrical), 1402 (C=C), 1655 (CO), 2920 (CH); MS (*m/z*) – 360.8 $[M+1]^+$, 2D-HSQCT (DMSO-d_6) 4-*O*-C1-*O*-fructoside **14a**: ^{1}H NMR Fruc: 3.83 (H-1β), 3.57 (H-3β), 3.59 (H-5β), 3.51 (H-6β), DL-Dopa: 6.65 (H-5), 6.51(H-6), 3.32 (αCH-8), ^{13}C NMR δ_{ppm} Fruc: 64.6 (C1β), 102.1 (C2β), 81.2 (C3β), 83.1 (C4β), 82.0 (C5β), DL-Dopa: 61.3 (C8), 4-Hydroxy-3-*O*-(1-D-fructofuranosyl) phenylalanine: 3-*O*-C1-*O*-fructoside **14b**: ^{1}H NMR Fruc: 3.83 (H-1β), 3.57 (H-3β), 3.59 (H-5β), 3.51 (H-6β), DL-Dopa: 6.65 (H-5), 6.51(H-6), 3.32 (αCH-8), ^{13}C NMR δ_{ppm} Fruc: 63.9 (C1β), 104.3 (C2β), 75.5 (C3β), 76.2 (C4β), 76.0 (C5β), DL-Dopa: 61.3 (C8).

3-Hydroxy-4-*O*-(D-arabinofuranosyl)phenylalanine **15a, b**: Isolated yield (Imm. β-glucosidase)—0.1 g (60%): Solid, UV (λ_{max}): 191.5 nm (σ→σ*, $\varepsilon_{191.5}$—7412 M^{-1}), 214.0 nm (σ→σ*, $\varepsilon_{214.0}$—4547 M^{-1}), 259.0 nm (π→π*, $\varepsilon_{259.0}$—1859 M^{-1}), 283.0 nm (n→π*, $\varepsilon_{283.0}$—2282 M^{-1}); IR (stretching frequency, cm^{-1}): 3574 (OH), 1280 (glycosidic aryl alkyl C–O–C asymmetrical), 1061 (glycosidic aryl alkyl C–O–C symmetrical), 1536 (C=C), 1635 (CO), 2940 (CH); MS (*m/z*)—330.7 $[M+1]^+$, 2D-HSQCT (DMSO-d_6) 4-*O*-C1α-arabinoside **15a**: ^{1}H NMR δ_{ppm} (500.13) Arab: 4.95 (H-1α), 5.04 (H-3α), 3.73 (H-5α), DL-Dopa: 6.71 (H-2), 6.62 (H-5), 6.56 (H-6), ^{13}C NMR δ_{ppm} (125 MHz): Arab: 96.0 (C1α), 71.9 (C2α), 72.2 (C3α), 71.1 (C4α), 62.3 (C5α), DL-Dopa: 114.8 (C2), 144.2 (C3), 116.5 (C5); 4-*O*-C1β-arabinoside **15b**: ^{1}H NMR Arab: 4.30 (H-1β), 3.71 (H-2β), 5.05 (H-3β), 3.39 (H-4β), 3.69 (H-5β), ^{13}C NMR δ_{ppm} Arab: 102.3 (C1β), 69.5 (C2β), 69.9 (C3β), 71.3 (C4β), 63.2 (C5β).

DL-3-Hydroxy-4-*O*-(β-D-galactopyranosyl-(1′–4)β-D-glucopyranosyl)phenyl alanine **16**: Isolated yield (β-glucosidase)—0.071 g (28%):Solid, mp: 140°C, UV (H_2O, λ_{max}): 198.5 nm (σ→σ*, $\varepsilon_{198.5}$—4437 M^{-1}), 221.5 nm (σ→π*, $\varepsilon_{221.5}$—1208 M^{-1}), 279.5 nm (π→π*, $\varepsilon_{279.5}$—562 M^{-1}); IR (stretching frequency, cm^{-1}): 3385 (OH), 1311 (glycosidic aryl alkyl C–O–C asymmetrical), 1039 (glycosidic aryl alkyl C–O–C symmetrical), 1372 (C=C), 1659 (CO), 2895 (CH), optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25°C = +32.1, MS (*m/z*)—522.3 $[M+1]^+$, 2D-HSQCT (DMSO-d_6) 4-*O*-C1β-lactoside: ^{1}H NMR Lact: 4.92 (H-1β, d, J = 7.2 Hz), 3.28 (H-2β), 3.55 (H-3β), 3.38 (H-5β), 3.52 (H-6a), 4.21 (H-1′β), 3.40 (H-2′), 3.22 (H-3′), 3.62 (H-4′), 2.82 (H-5′), 3.52 (H-6′a), DL-Dopa: 6.64 (H-2), 6.45 (H-5), 6.50 (H-6), 2.90 (βCH_{2a}-7), 2.60 (βCH_{2b}-7), 3.43 (αCH-8), ^{13}C NMR δ_{ppm} Lact: 102 (C1β), 76 (C2β), 75 (C3β), 76.5 (C5β), 61.4 (C6β), 103.9 (C1′β), 70.5 -(C2′), 73 (C3′), 64 (C4′), 73 (C5′), 61 (C6′), DL-Dopa: 115.7 (C2), 144.3 (C4), 120.2 (C5), 116.9 (C6), 36.2 (C7), 55.9 (C8), 172 (C9).

DL-3-Hydroxy-4-*O*-(6-D-sorbitol)phenylalanine **17**: Isolated yield (amylo glucosidase)—0.034 g (19%): Solid, mp: 88°C, UV (H_2O, λ_{max}): 192.5 nm (σ→σ*, $\varepsilon_{192.5}$—1,204 M^{-1}), 224 nm (σ→π*, ε_{224}—358 M^{-1}), 275 nm (n→π*, ε_{275}—158 M^{-1}), IR (stretching frequency, cm^{-1}): 3050 (OH), 1301 (glycosidic aryl alkyl C–O–C asymmetrical), 1034 (glycosidic aryl alkyl C–O–C symmetrical), 1406 (C=C), 1644 (CO), 2946 (CH), optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25°C = −2.6, MS (*m/z*)—360.2 $[M-1]^+$, 2D-HSQCT (DMSO-d_6) 4-*O*-C6-*O*-sorbitol: ^{1}H NMR Sorb: 3.48 (H-1), 3.62 (H-2), 3.62 (H-5), 3.72 (H-6), DL-Dopa: 6.70 (H-2), 6.52 (H-5), 6.54 (H-6), 2.51 (βCH_{2b}-7), 3.46 (αCH-8), ^{13}C NMR δ_{ppm} Sorb: 63.5 (C1), 72.5 (C2), 72 (C5), 63.5 (C6), DL-Dopa: 128 (C1), 115.9 (C2), 144.3 (C4), 120.2 (C5), 116 (C6), 29 (C7).

DL-3-Hydroxy-4-*O*-(1-D-mannitol)phenylalanine **18a**,**b**: Isolated yield (amylo glucosidase)—0.11 g (61%):Solid, UV (H_2O, λ_{max}): 198.5 nm (σ→σ*, $\varepsilon_{198.5}$—6420 M^{-1}), 221.5 nm (σ→π*, $\varepsilon_{221.5}$—1484 M^{-1}), 287.5 nm (n→π*, $\varepsilon_{287.5}$—368 M^{-1}), IR (stretching frequency, cm^{-1}): 3281 (OH), 1320 (glycosidic aryl alkyl C–O–C asymmetrical), 1020 (glycosidic aryl alkyl C–O–C symmetrical), 1459 (C=C), 1644 (CO), 2939 (CH), MS (*m/z*)—360.2 $[M-1]^+$, 2D-HSQCT (DMSO-d_6) 4-*O*-C1-*O*-mannitol **18a**: ^{1}H NMR Mann: 3.52 (H-1), 3.48 (H-2), 3.61 (H-3), 3.61 (H-4), 3.48 (H-5), 3.60 (H-6), DL-Dopa: 6.71 (H-2), 6.64 (H-5), 6.51 (H-6), 3.01 (βCH_{2a}-7), 2.71 (βCH_{2a}-7), 3.45 (αCH-8), ^{13}C NMR δ_{ppm} Mann: 65 (C1), 70.5 (C2), 72 (C3), 72 (C4), 70.5 (C5), 64.5 (C6), DL-Dopa: 118 (C2), 144.3 (C4), 121 (C5), 115 (C6), 36.5 (C7), 1,6-*O*-(Bis DL-3-hydroxy-4-*O*-phenylalanine) D-mannitol **18b**: ^{1}H NMR Mann: 3.52 (H-1), 3.63 (H-6), ^{13}C NMR δ_{ppm} Mann: 65 (C1), 64.5 (C6).

Results and discussion

DL-Dopa-D-glucoside

Glucosylation reaction between DL-dopa **1b** and D-glucose **2** catalysed by amyloglucosidase from *Rhizopus* mold and immobilized β-glucosidase were optimized in terms of incubation period, pH, buffer, enzyme and DL-dopa concentrations.

Effect of incubation period

Effect of incubation period on the synthesis of DL-dopa-D-glucoside showed that there was no significant change in the glucosylation between 12 to 72 h (yield 45–49%) for amyloglucosidase catalysis (Table 1). Even shorter incubation periods showed significant glucosylation at 3 h–42% yield and 6 h–43% yield. Beyond 72 h the conversion yield decreased gradually (120 h–39% yield). Immobilized β-glucosidase showed a gradual increase in conversion from 3

Table 1 Optimization of reaction conditions for the synthesis of DL-dopa-D-glucoside using amyloglucosidase

Reaction conditions	Variable parameter[a]	Conversion yields (%)[b]
	Incubation period (h)	
DL-Dopa—0.5 mmol[c]	3	42
D-Glucose—1 mmol	6	43
pH—6	12	45
Buffer concentration—0.1 mM (1 mL)	24	45
Amyloglucosidase—10% *w/w* D-glucose	48	47
	72	49
	96	46
	120	39
	pH (0.01 M)	
DL-Dopa—0.5 mmol	4	16
D-Glucose—1 mmol	5	38
Amyloglucosidase—30% *w/w* D-glucose	6	49
Buffer concentration—0.1 mM (1 mL)	7	48
Incubation period—72 h	8	22
	Buffer concentration (mM)	
DL-Dopa—0.5 mmol	0.03	41
D-Glucose—1 mmol	0.06	42
Amyloglucosidase—30% *w/w* D-glucose	0.1	49
pH—6	0.14	46
Incubation period—72 h	0.18	45
	0.22	41
	Amyloglucosidase (% *w/w* D-glucose)	
DL-Dopa—0.5 mmol	10	48
D-Glucose—1 mmol	20	49
pH—6	30	46
Buffer concentration—0.1 mM (1 mL)	40	48
Incubation period—72 h	50	37
	75	34
	DL-Dopa (mmol)	
pH—6	0.2	22
Buffer concentration—0.1 mM (1 mL)	0.4	41
D-Glucose—1 mmol	0.5	49
Amyloglucosidase—10% *w/w* D-glucose	0.8	44
Incubation period—72 h	1.2	36
	1.6	35
	2	33

[a] Other variables are the same as under reaction conditions, except the specified ones
[b] HPLC yields expressed with respect to 1 mmol D-glucose employed.
[c] Initial reaction conditions

Table 2 Optimization of reaction conditions for the synthesis of DL-dopa-D-glucoside using immobilized β-glucosidase

Reaction conditions	Variable parameter[a]	Conversion yields (%)[b]
	Incubation (h)	
DL-Dopa—0.5 mmol[c]	3	14
D-Glucose—1 mmol	6	16
pH—5	12	20
Buffer concentration—0.12 mM (1.2 mL)	24	22
Imm. β-Glucosidase—10% *w/w* D-glucose	48	28
	72	33
	96	23
	pH (0.01 M)	
DL-Dopa—0.5 mmol	4	19
D-Glucose—1 mmol	5	22
Imm. β-Glucosidase—10% *w/w* D-glucose	6	7
Buffer concentration—0.1 mM (1 mL)	7	14
Incubation period—72 h	8	13
	Buffer concentration (mM)	
DL-Dopa—0.5 mmol	0.04	21
D-Glucose—1 mmol	0.08	25
Imm. β-Glucosidase—10% *w/w* D-glucose	0.1	22
pH—5	0.12	48
Incubation period—72 h	0.18	35
	0.25	16
	Imm. β-Glucosidase concentration (% *w/w* D-glucose)	
DL-Dopa—0.5 mmol	10	48
D-Glucose—1 mmol	20	37
pH—5	30	43
Buffer concentration—0.12 mM (1.2 mL)	40	32
Incubation period—72 h	50	30
	75	22
	DL-Dopa (mmol)	
pH—5	0.25	17
Buffer concentration—0.12 mM (1.2 mL)	0.5	48
D-Glucose—1 mmol	1	21
Imm. β-Glucosidase—10% *w/w* D-glucose	1.5	13
Incubation period—72 h	2	7

[a] Other variables are the same as under reaction conditions, except the specified ones
[c] HPLC yields expressed with respect to 1 mmol D-glucose employed.
[c] Initial reaction conditions

to 72 h with the highest yield of 33% at 72 h (Table 2). In both the cases, incubation period resulted in attaining a maximum equilibrium conversion at 72 h.

Effect of pH

In presence of 0.1 mM (1 mL) buffer, amyloglucosidase showed increase in the glucosylation yield from pH 4 to 6, which decreased thereafter (Table 1). The conversion yields were 16% at pH 4, 38% at pH 5, 49% at pH 6, 48% at pH 7 and 22% at pH 8. With immobilized β-glucosidase, the highest glucosylation yield of 22% was obtained at pH 5 (Table 2).

Effect of buffer concentration

Since pH 6 gave the highest glucosylation yield for amyloglucosidase catalysis, varying buffer concentration from 0.03–0.22 mM (0.3–2.2 mL) at pH 6 showed that between 0.03–0.14 mM (0.3–1.4 mL) buffer concentrations, the conversion yield did not show any significant change (41% to 49%). In case of immobilized β-glucosidase, varying the buffer concentration from 0.04 mM (0.4 mL) to 0.25 mM (2.5 mL) at pH 5, led to an optimum buffer concentration of 0.12 mM (1.2 mL), giving rise to 48% yield (Table 2).

Effect of enzyme concentration

In presence of 0.5 mmol DL-dopa and 1 mmol D-glucose, amyloglucosidase variation from 10–75% (*w*/*w* D-glucose) gave more or less similar conversion yields (46–49%) up to 40% (*w*/*w* D-glucose) enzyme concentration (Table 1). Further increase in enzyme concentrations led to a decrease (75% enzyme–34% yield) in the conversion yield (Table 1). However, immobilized β-glucosidase at 0.12 mM (1.2 mL) pH 5 buffer, gave the highest yield of 48% at 10% (*w*/*w* D-glucose) enzyme (Table 2).

Effect of DL-dopa concentration

Variation of DL-dopa concentration under the above determined optimum conditions showed that amyloglucosidase did not influence the conversion at higher concentrations of DL-dopa (Table 1). A maximum conversion of 49% was obtained at 0.5 mmol DL-dopa (Table 1). In case of immobilized β-glucosidase, the highest conversion yield of 48% was obtained for 0.5 mmol DL-dopa (Table 2).

Syntheses of L-dopa and DL-dopa glycosides

Syntheses of L-dopa glucoside **10a,b** was carried out under the optimum conditions described above. Similarly, DL-dopa glycosides with various carbohydrates: aldohexoses—D-glucose **2**, D-galactose **3** and D-mannose **4**; ketohexose—D-fructose **5**; pentoses—D-ribose and D-arabinose **6**; disaccharides—maltose, sucrose and lactose **7** and carbohydrate alcohols—D-sorbitol **8** and D-mannitol **9** were attempted with amyloglucosidase, β-glucosidase and immobilized β-glucosidase. Optimum conditions employed for amyloglucosidase/β-glucosidase catalyses are: L-dopa **1a**/DL-dopa **1b**—0.5 mmol, carbohydrates—1 mmol, amyloglucosidase/β-glucosidase—10% *w*/*w* of carbohydrate, 0.1 mM (1 mL) of 0.01 M pH 6 phosphate buffer for an incubation period of 72 h in 100 mL di-isopropyl ether (Scheme 1). Similarly, for immobilized β-glucosidase catalyses: the conditions are DL-dopa **1a**—0.5 mmol, carbohydrates—1 mmol, immobilized β-glucosidase—10% *w*/*w* of carbohydrate, 0.12 mM (1.2 mL) of 0.01 M pH 5 acetate buffer for an incubation period of 72 h in 100 mL di-isopropyl ether (Scheme 1).

UV spectra of L-dopa glycosides, showed shift in the $\sigma\rightarrow\sigma^*$ band at 198.5 nm (212.5 nm for L-dopa), shift in the $\pi\rightarrow\pi^*$ band at 267.5 nm (281 nm for L-dopa) and shift in the $n\rightarrow\pi^*$ band at 397.5 nm (390 nm for L-dopa). UV spectra of DL-dopa glycosides, showed shifts in the $\sigma\rightarrow\sigma^*$ band ranging from 191.5 to 214 nm (199.5 nm for DL-dopa), shifts in the $\sigma\rightarrow\pi^*$ band ranging from 221.5 to 226 nm (221 nm for DL-dopa) and shifts in the $n\rightarrow\pi^*$ band from 275 to 287.5 nm (285 nm for DL-dopa). IR spectra showed 1,019–1,061 cm^{-1} band for the glycosidic C–O–C aryl alkyl symmetrical stretching and 1,280–1,320 cm^{-1} band for the asymmetrical stretching frequencies. In the 2D HSQCT spectra, the respective C1α or β and C6 signals registered shifts, due to glycosylation at the specified carbons (vide spectral data in "Materials and methods"). Mass spectral data also confirmed product formation.

Optimization of DL-dopa-D-glucoside synthesis has been studied in detail. Amyloglucosidase gave much higher conversion yields than immobilized β-glucosidase and the optimum pH for amyloglucosidase and immobilized β-glucosidase catalyses were found to be 6 and 5, respectively. At this pH, the enzyme could attain a highly active conformation. Further increase in the pH was found to lower the yield. Glycosylation reactions using glucosidases, require certain amount of water [30, 31]. The amount of water added as buffer was found to be critical [32]. In both the enzyme systems, the yields decreased at increasing buffer concentrations beyond certain concentrations indicating onset of the backward reaction (hydrolysis).

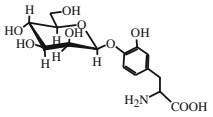
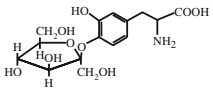
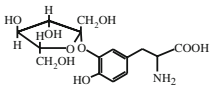
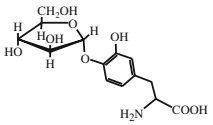
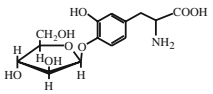
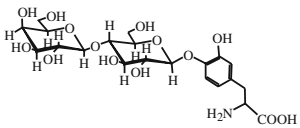
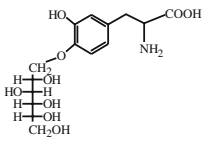
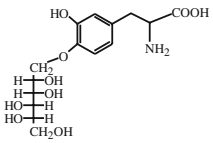
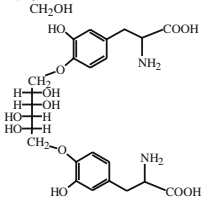
Effect of enzyme concentration at a fixed D-glucose concentration, showed that at certain higher concentrations of amyloglucosidase/immobilized β-glucosidase, conversion yields decreased. For a fixed D-glucose (1 mmol) and DL-dopa concentration (0.5 mmol), increase in enzyme concentrations affected the enzyme/substrate

ratio drastically due to competition in binding between D-glucose and DL-dopa. Further increase in the enzyme concentration led to a drop in the yield, which could be due to larger concentrations of enzymes being available for fixed substrate (D-glucose and DL-dopa) concentrations and the substrates bound differentially to the enzyme, the tightly bound DL-dopa (compared to D-glucose) could be unavailable for facile transfer to D-glucose molecule. This could lead to a decrease in the glycosylation yield at higher enzyme concentrations. Similarly, the yield increased up to a time period of 72 h and then showed a significant drop at longer incubation periods, which could

Table 3 Syntheses of L-dopa and DL-dopa glycosides using amyloglucosidase, β-glucosidase and immobilized β-glucosidase

S. No	Glycosides	Amyloglucosidase[a]		β-Glucosidase[a]		Imm. β-Glucosidase[b]	
		Yield[c] (%)	Proportions[d] (%)	Yield[c] (%)	Proportions[d] (%)	Yield[c] (%)	Proportions[d] (%)
1.	**10a** L-Dopa-D-glucoside 4-*O*-C1β	-	-	42	54	-	-
	10b L-Dopa-D-glucoside 3-*O*-C1β		-		46		-
2.	**11a** DL-Dopa-D-glucoside 4-*O*-C1α	49	24	33	-	48	-
	11b DL-Dopa-D-glucoside 4-*O*-C1β		48		28		11
	11c DL-Dopa-D-glucoside 4-*O*-C6-*O*		6		72		89
	11d DL-Dopa-D-glucoside 3-*O*-C1β		22		-		-
3.	**12a** DL-Dopa-D-galactoside 4-*O*-C1α	46	27	31	17	48	20
	12b DL-Dopa-D-galactoside 4-*O*-C1β		25		35		41
	12c DL-Dopa-D-galactoside 4-*O*-C6-*O*		29		23		29
	12d DL-Dopa-D-galactoside 3-*O*-C1α		10		21		10
	12e DL-Dopa-D-galactoside 3-*O*-C1β		9		4		-

Table 3 (continued)

4.	**13** DL-Dopa-D-mannoside 4-*O*-C1β		46	-	32	-	-	-
5.	**14a** DL-Dopa-D-fructoside 4-*O*-C1-*O*			-		-		62
			-		-		38	
	14b DL-Dopa-D-fructoside 3-*O*-C1-*O*			-		-		38
6.	**15a** DL-Dopa-D-arabinoside 4-*O*-C1α			-		-		42
			-		-		46	
	15b DL-Dopa-D-arabinoside 4-*O*-C1β			-		-		58
7.	**16** DL-Dopa-D-lactoside 4-*O*-C1β				17	-	-	-
8.	**17** DL-Dopa-D-sorbitol 4-*O*-C1-*O*		12	-	-	-	-	-
9.	**18a** DL-Dopa-D-mannitol 4-*O*-C1-*O*			70		-		-
			20		-		-	
	18b DL-Dopa-D-mannitol 4-*O*-C1/C6-*O*			30		-		-

[a]L-3,4-Dihydroxyphenylalanine/DL-3,4-Dihydroxyphenylalanine—0.5 mmol and carbohydrate—1 mmol; amyloglucosidase/β-glucosidase concentration 10% *w*/*w* of carbohydrate; solvent—di-isopropyl ether; buffer—0.1 mM (1 mL) pH 6 phosphate buffer; incubation period—72 h
[b]DL-3,4-Dihydroxyphenylalanine—0.5 mmol and carbohydrate—1 mmol; immobilized β-glucosidase concentration 10% *w*/*w* of carbohydrate; solvent—di-isopropyl ether; buffer—0.12 mM (1.2 mL) pH 5 acetate buffer; incubation period—72 h
[c]Conversion yields were from HPLC with respect to free carbohydrate. Error in yield measurements is ±10%
[d]The product proportions were calculated from the area of respective $^{1}H/^{13}C$ signals.

be due to prolonged heating at 68°C inactivating the enzymes at longer incubation periods.

The nature and proportion of the DL-dopa glycosides are shown in Table 3. Native β-glucosidase gave rise to L-dopa-D-glucoside **10a,b**. Amyloglucosidase catalysis gave rise to five glycosides: DL-dopa-D-glucoside **11a**–**d**, DL-dopa-D-galactoside **12a**–**e**, DL-3-hydroxy-4-*O*-(β-D-mannopyranosyl)phenyl alanine **13**, DL-3-hydroxy-4-*O*-(6-D-sorbitol)phenylalanine **17** and DL-dopa-D-mannitol **18a**,**b**. β-Glucosidase gave rise to four glycosides: DL-3-hydroxy-4-*O*-(D-glucopyranosyl) phenylalanine **11b**,**c**, DL-dopa-D-galactoside **12a**–**e**, DL-3-hydroxy-4-*O*-(β-D-mannopyranosyl)phenylalanine **13** and DL-3-hydroxy-4-*O*-(β-D-galacto pyranosyl-(1′→4)β-D-glucopyranosyl)phenylalanine **16**. Immobilized β-glucosidase gave rise to four glycosides: DL-3-hydroxy-4-*O*-(D-glucopyranosyl)phenylalanine **11b,c**, DL-dopa-D-galactoside **12a**–**d**, DL-dopa-D-fructoside **14a,b** and DL-3-hydroxy-4-*O*-(D-arabinofuranosyl)phenylalanine **15a,b**.

L-Dopa **1a** is an optically active compound where DL-dopa **1b** is racemic. β-Glucosidase gave only 4-*O*-C1β and

3-*O*-C1β products. No 4-*O*-C6-*O* arylated product was detected with β-glucosidase unlike DL-dopa **1b**. Under the reaction conditions employed, DL-dopa **1b** gave a mixture of 3-*O*- and 4-*O*-glycosylated/arylated products with many of the carbohydrates employed. Glucosidases employed did not catalyse the reaction with D-ribose, maltose and sucrose, which could be due to stronger binding of DL-dopa **1b** to the enzymes compared to these carbohydrate molecules, thereby preventing their facile transfer to the nucleophilic phenolic OH of DL-dopa **1b**. Amyloglucosidase exhibited 'inverting' potentiality in reacting with D-galactose **3**. D-Galactose **3** showed 37% α and 34% β compared to the 92:8 α/β anomeric composition of free D-galactose employed. Immobilized β-glucosidase with D-arabinose **6** showed 42% α and 58% β compared to the 95:5 α/β anomeric composition of the D-arabinose employed.

However, only mono glycosylated/arylated products were formed. No bis products with both the OH groups at third and fourth positions glycosylated/arylated could be detected. This indicated that steric effects are responsible for the formation of only monoglycosylated products. Amyloglucosidase gave selectivity with D-mannose **4** to give 4-*O*-C1β and D-sorbitol **8** to give 4-*O*-C6-*O*-arylated product. β-Glucosidase gave selectivity with D-mannose **4** to give 4-*O*-C1β and lactose **7** to give 4-*O*-C1β product. Immobilized β-glucosidase did not show any selectivity. 4-*O*- and 3-*O*-Glycosylated/arylated products were detected from D-glucose **2**, D-galactose **3**, D-fructose **5** in the catalysis with all the three enzymes. No other carbohydrate molecule formed products at the third OH position. Hydrolysis of lactose **7** was observed during the course of reaction and the resultant carbohydrate did not show any transglycosylated product.

A few biological activities of the prepared DL-dopa glycosides were tested by evaluating antioxidant and ACE inhibition activities. L-Dopa glucoside and DL-dopa glycosides still possessed antioxidant activity in spite of conversion of the phenolic nucleophilic OH to -*O*-glycoside. IC_{50} Values for antioxidant avtivities observed were: L-dopa-D-glucoside **10a,b** at 1.17±0.06 mM, DL-3-hydroxy-4-*O*-(D-glucopyranosyl)phenylalanine **11a–d** at 1.11±0.05 mM, DL-3-hydroxy-4-*O*-(β-D-glucopyranosyl)phenylalanine **11b,c** at 0.98±0.05 mM, DL-3-hydroxy-4-*O*-(D-galactopyranosyl)phenylalanine **12a–e** at 2.26±0.11 mM, DL-3-hydroxy-4-*O*-(β-D-mannopyranosyl)phenylalanine **13** at 1.13±0.06 mM, DL-3-hydroxy-4-*O*-(D-fructofuranosyl)phenylalanine **14a,b** at 1.78±0.09 mM, DL-3-hydroxy-4-*O*-(D-arabinofuranosyl) phenylalanine **15a,b** at 1.75±0.08 mM, DL-3-hydroxy-4-*O*-(β-D-galactopyranosyl-(1′→4)β-D-glucopyranosyl)phenylalanine **16** at 0.90±0.05 mM, DL-3-hydroxy-4-*O*-(6-D-sorbitol) phenylalanine **17** at 1.86±0.09 mM and DL-3-hydroxy-4-*O*-(D-mannitol)phenylalanine **18a,b** at 1.90±0.09 mM. L-Dopa **1a** itself showed IC_{50} value of 0.04±0.002 mM, DL-dopa **1b** at 0.045±0.002 mM and BHA at 0.046±0.002 mM. Modification of the free nucleophilic phenolic OH group only resulted in reduction in antioxidant activity of the DL-dopa.

DL-Dopa glycosides showed significant IC_{50} values for ACE inhibition. IC_{50} values for ACE inhibition observed were: L-dopa-D-glucoside **10a,b** at 1.14±0.06 mM, DL-3-Hydroxy-4-*O*-(D-glucopyranosyl)phenylalanine **11a–d** at 1.2±0.06 mM, DL-3-hydroxy-4-*O*-(β-D-glucopyranosyl)phenylalanine **11b,c** at 1.26±0.06 mM, DL-3-hydroxy-4-*O*-(D-galactopyranosyl)phenylalanine **12a–e** at 1.71±0.09 mM, DL-3-hydroxy-4-*O*-(β-D-mannopyranosyl)phenylalanine **13** at 3.46±0.17 mM, DL-3-hydroxy-4-*O*-(D-fructofuranosyl) phenylalanine **14a,b** at 2±0.1 mM, DL-3-hydroxy-4-*O*-(D-arabinofuranosyl)phenylalanine **15a,b** at 2.44±0.12 mM, DL-3-hydroxy-4-*O*-(β-D-galactopyranosyl-(1′→4)β-D-glucopyranosyl)phenylalanine **16** at 3.33±0.16 mM, DL-3-hydroxy-4-*O*-(6-D-sorbitol)phenylalanine **17** at 0.56± 0.03 mM and DL-3-hydroxy-4-*O*-(D-mannitol)phenylalanine **18a,b** at 1.58±0.08 mM. L-Dopa **1a** showed an IC_{50} value of 0.74±0.04 mM, DL-dopa **1b** at 0.60±0.03 mM and enalapril at 0.071±0.004 mM for ACE inhibition. Introducing the carbohydrate molecule into the nucleophilic phenolic OH of DL-dopa **1b** did not alter inhibitory activity significantly. Since, the ACE inhibitory peptide drugs contain prolyl units, DL-dopa and its glycosides could not mimic their activities efficiently. Among the glycosides, DL-3-hydroxy-4-*O*-(6-D-sorbitol)phenylalanine **17** showed the best IC_{50} value of 0.56±0.03 mM.

About 20 individual glycosides were synthesized enzymatically using the glucosidases, of which 16 are being reported for the first time. The new glycosides reported are: L-dopa-D-glucoside **10a,b** DL-dopa-D-galactoside **12a-e**, DL-3-hydroxy-4-*O*-(β-D-mannopyranosyl)phenylalanine **13**, DL-dopa-D-fructoside **14a,b**, DL-3-hydroxy-4-*O*-(D-arabinofuranosyl)phenylalanine **15a,b**, DL-3-hydroxy-4-*O*-(β-D-galactopyranosyl-(1′→4)β-D-glucopyranosyl)phenylalanine **16**, DL-3-hydroxy-4-*O*-(6-D-sorbitol)phenylalanine **17** and DL-dopa-D-mannitol **18a**,**b**.

Thus, this investigation shows that DL-dopa glycosides could be synthesized enzymatically using glucosidases in a facile manner under milder reaction conditions to produce more stable L-dopa and DL-dopa derivatives with diverse carbohydrate molecules.

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