Indian Journal of Chemistry
Vol. 46B, December 2007, pp. 2026-2044

Enzymatic syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates using *Candida rugosa* lipase

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Received 29 January 2007; accepted (revised) 20 August 2007

L-Valyl **1** L-leucyl **2** and L-isoleucyl **3** esters of carbohydrates **4-14** have been prepared through lipase catalysis using *Candida rugosa* lipase in organic media. This enzymatic esterification involving unprotected and unactivated L-amino acids **1-3** and carbohydrates, comprising, aldohexoses **4-6**, ketohexose **7**, pentoses **8-9**, disaccharides **10-12** and carbohydrate alcohols **13-14**, results in the formation of both monoesters (1-*O*-, 2-*O*-, 3-*O*-, 4-*O*, 5-*O*-, 6-*O*- and 6′-*O*-) and diesters (1,6-di-*O*-, 2,5-di-*O*-, 2,6-di-*O*-, 3,6-di-*O*-, 4,6-di-*O*- and 6,6'-di-*O*-) with conversion yields in the range 21-68%. Among 70 esters synthesized on the whole, 65 esters are reported here for the first time.

Keywords: *Candida rugosa* lipase, diastereomeric, esters, enzymatic, esterification, amino acids, carbohydrates, L-valyl, L-leucyl, L-isoleucyl

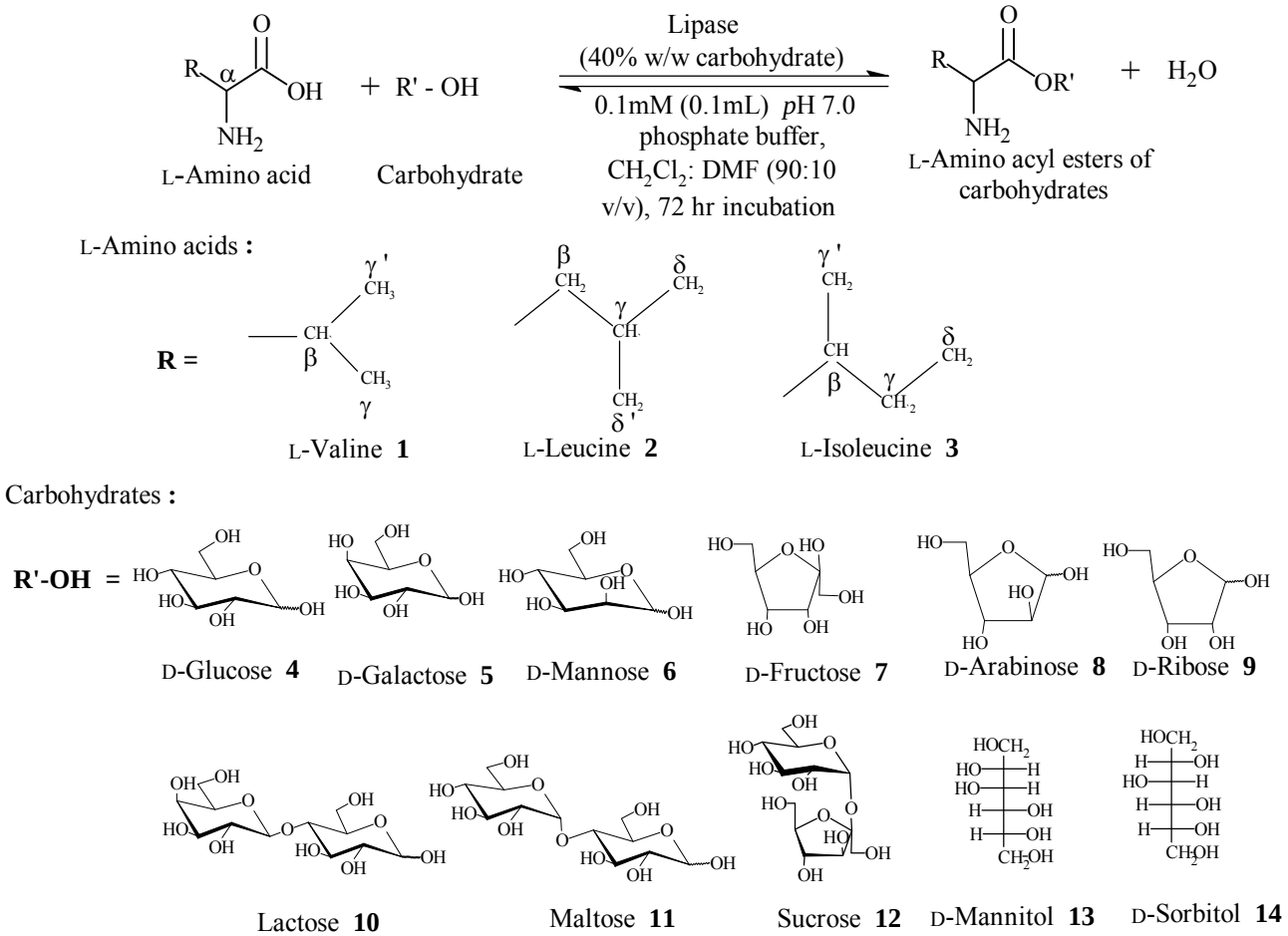
Amino acyl esters of carbohydrates have a number of potential applications in industry as detergents, microcapsules in pharmaceutical preparations, in the delivery of biologically active agents, antibiotics, sweetening agents, emulsifying agents, active nucleoside amino acid esters, antitumor agents, plant growth inhibitors and peptide prodrugs[1-5]. So far, attempts to synthesize amino acyl esters of carbohydrates, chemically, have proven to be difficult due to expensive protection and deprotection procedures involved[6-9], as can be inferred from the reports on the chemical synthesis of monoesters: methyl-2-*O*-[N-*t*-boc]-L-valyl-glucose and methyl-3-*O*-[N-*t*-boc]-L-valyl-glucose and diesters: methyl 2,3-di-*O*-[N-*t*-boc]-L-valyl-D-glucose, methyl-2,3-di-*O*-L-valyl-D-glucose, methyl-4,6-di-*O*-L-valyl-D-glucose methyl-2,3-di-*O*-L-leucyl-α-D-glucose and methyl-2,3-di-*O*-L-isoleucyl-α-D-glucose.

Enzymatically, Park *et al*. carried out transesterification in presence of Optimase M 440 to synthesize *t*-boc-leucyl-sucrose using *t*-butoxycarbonyl-L-leucyl-cyanomethyl ester/*t*-butoxycarbonyl-L-leucyl-trifluroethyl ester and sucrose[6]. Maruyama *et al*. investigated the synthesis of N-acetyl-L-leucyl-D-glucose in *t*-butanol containing 10% dimethyl sulfoxide, through transesterification between N-acetyl-L-leucyl-cyanomethyl ester and D-glucose using a lipase-surfactant complex[10]. There were reports that few of the lipases exhibited very little activity in dimethylformamide[2]. In brief, very few reports are available on the enzymatic synthesis of amino acyl esters of carbohydrates.

In a previous study, the lipase catalyzed esterification of unprotected and unactivated L-alanine with D-glucose[11] and L-alanine with different carbohydrates[12] have been investigated in organic media. In the present investigation, lipases from *Candida rugosa* lipase (CRL) was employed to esterify L-valine **1**, L-leucine **2** and L-isoleucine **3** with carbohydrates which include aldohexoses (D-glucose **4**, D-galactose **5** and D-mannose **6**), ketohexose (D-fructose **7**), pentoses (D-arabinose **8** and D-ribose **9**), disaccharides (lactose **10**, maltose **11** and sucrose **12**) and carbohydrate alcohols (D-mannitol **13** and D-sorbitol **14**) using unprotected and unactivated amino acids and carbohydrates (**Scheme I**). The results are now being presented.

Results and Discussion

L-Valine **1**, L-leucine **2** and L-isoleucine **3** are amino acids containing alkyl side chains – isopropyl, 2-methyl propyl and 3-methyl propyl groups, respectively, attached to the α-CH group. The alkyl side chain imparts hydrophobicity to the amino acids employed. However, this did not deter them from being efficient acyl donors as far as *Candida rugosa*



Scheme I

lipase is concerned. The conversion yields in this esterification reaction is quite good, ranging from 21-68%. Unprotected and unactivated amino acids were reacted with about 11 carbohydrates – aldohexoses (D-glucose **4**, D-galactose **5** and D-mannose **6**), ketohexose (D-fructose **7**), pentoses (D-arabinose **8** and D-ribose **9**), disaccharides (lactose **10**, maltose **11** and sucrose **12**) and carbohydrate alcohols (D-mannitol **13** and D-sorbitol **14**). With each amino acid, only few carbohydrates did not show esterification.

The isolated esters were characterized by UV-Vis, IR, MS and Two-dimensional Heteronuclear Single Quantum Coherence Transfer (2D-HSQCT) NMR spectroscopic techniques. Two-dimensional HSQCT NMR spectroscopy of all the three amino acyl esters of carbohydrates gave good information on the nature and proportion of the esters formed (**Table I**). Both mono- and di- esters were found to be formed. Two-dimensional HSQCT NMR data showed that the upfield chemical shift values for αCH from L-valyl, L-leucyl and L-isoleucyl units indicated that the respective amino acids reacted with the carbohydrates (**15a-e** to **43 a, b**) and multiple cross peaks indicated that the reaction occurred at more than one hydroxyl group of the carbohydrate molecules employed.

L-Valyl esters of carbohydrates 15a-e to 23

UV-Vis spectra of L-valyl esters of carbohydrates showed shifts in the $\sigma \rightarrow \sigma^*$ band in the 200-230 nm range (195 nm for L-valine **1**) and IR carbonyl stretching frequencies in the 1588-1644 cm^{-1} range (1605 cm^{-1} for L-valine **1**) indicating that L-amino acid carboxylic group had been converted into their corresponding carbohydrate esters. In the 2D HSQCT spectra of the L-valyl esters of carbohydrates, the following ester formation were confirmed from their respective chemical shift values: from D-glucose **4**, 2-*O*- ester **15a** to C2α at 77.4 ppm and the corresponding H-2α cross peak at 3.75 ppm, 3-*O*-ester **15b** to C3α at 82.6 ppm (H-3α at 3.96 ppm) and

Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

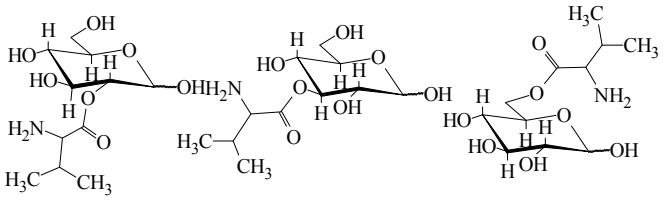
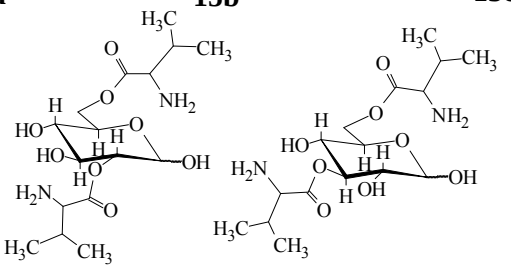
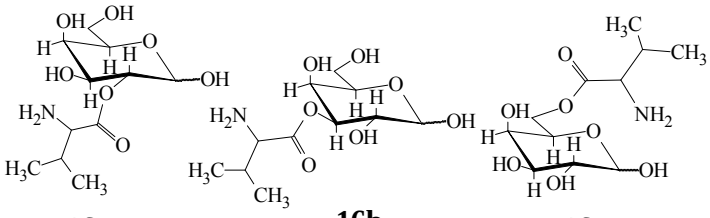
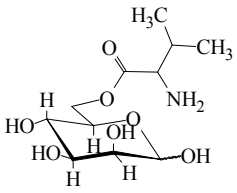
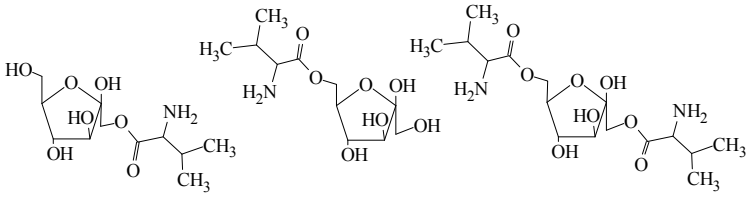
L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **15a** **15b** **15c**  **15d** **15e**	68 (mono esters- 45, di esters-23)	**15a**: 2-*O*-L-valyl-D-glucose (10) **15b**: 3-*O*-L-valyl-D-glucose (12) **15c**: 6-*O*-L-valyl-D-glucose (31) **15d**: 2,6-di-*O*-L-valyl-D-glucose (23) **15e**: 3,6-di-*O*-L-valyl-D-glucose (24)
 **16a** **16b** **16c**	30 (only mono esters)	**16a**: 2-*O*-L-valyl -D-galactose (48) **16b**: 3-*O*-L-valyl-D-galactose (26) **16c**: 6-*O*-L-valyl-D-galactose (26)
 **17**	51 (only mono ester)	**17**: 6-*O*-L-valyl-D-mannose
 **18a** **18b** **18c**	34 (mono esters-24, diester-10)	**18a**: 1-*O*-L-valyl-D-fructose 29) **18b**: 6-*O*-L-valyl-D-fructose (34) **18c**: 1,6-di-*O*-L-valyl-D-fructose (37)

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Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

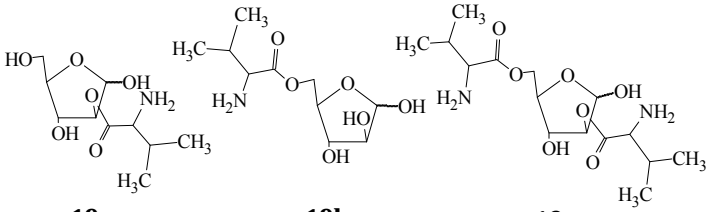
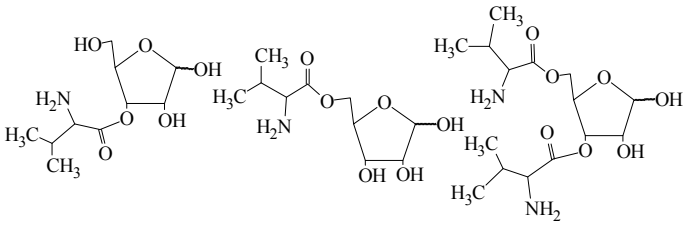
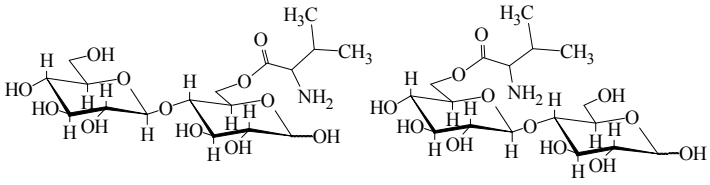
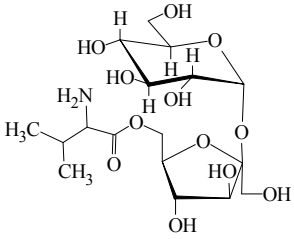
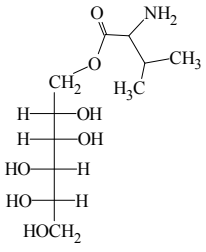
L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **19a** **19b** **19c**	25 [c] (mono esters-14, diester-11)	**19a**: 2-*O*-L-valyl-D-arabinose (32) **19b**: 5-*O*-L-valyl-D-arabinose (25) **19c**: 2,5-di-*O*-L-valyl-D-arabinose (43)
 **20a** **20b** **20c**	33 [c] (mono esters-17, diester-16)	**20a**: 3-*O*-L- valyl-D-ribose (26) **20b**: 5-*O*-L-valyl -D-ribose (26) **20c**: 3,5-di-*O*-L-valyl-D-ribose (48)
 **21a** **21b**	47 (only mono esters)	**21a**: 6-*O*-L-valyl - maltose (49) **21b**: 6′-di-*O*-L-valyl-maltose (51)
 **22**	60 (only mono ester)	**22**: 6-*O*-L-valyl- sucrose
 **23**	52 (only mono ester)	**23**: 1-*O*-L-valyl-D-mannitol

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Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

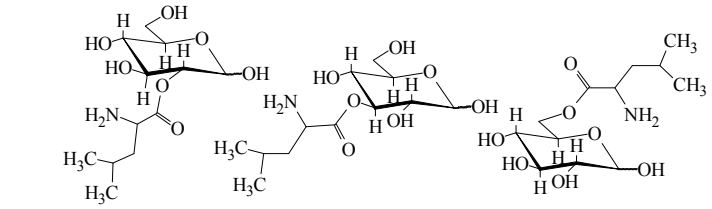
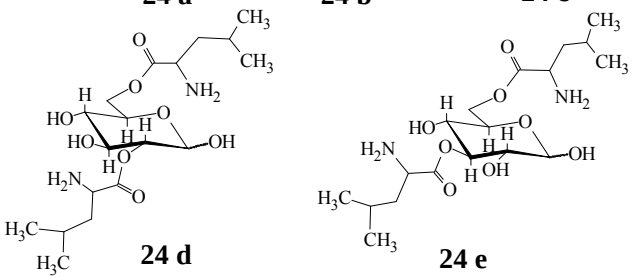
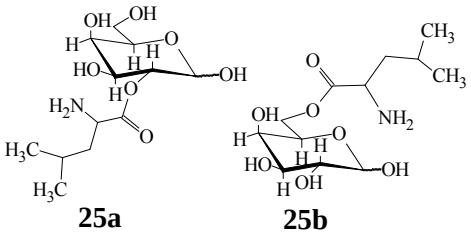
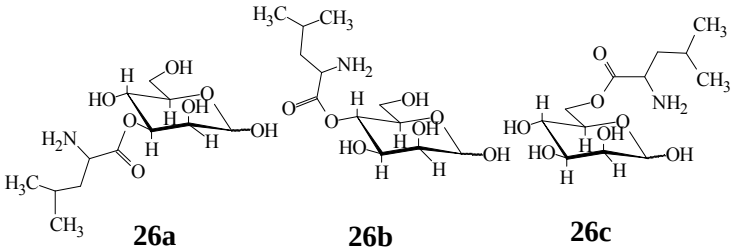
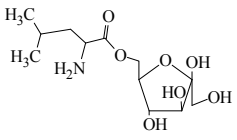
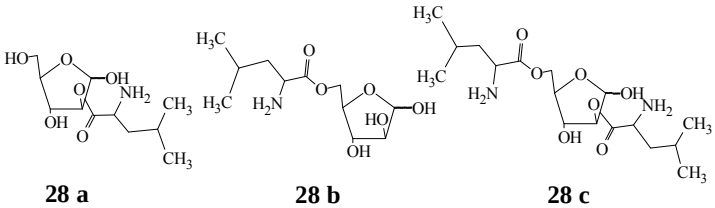
L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **24 a** **24 b** **24 c**	43 (mono esters- 34, di-esters-9)	**24a**: 2-*O*-L-leucyl -D-glucose (17) **24b**: 3-*O*-L-leucyl-D-glucose (20) **24c**: 6-*O*-L-leucyl-D-glucose (42) **24d**: 2,6-di-*O*-L-leucyl-D-glucose (10) **24e**: 3,6-di-*O*-L-leucyl-D-glucose (12)
 **24 d** **24 e**		
 **25a** **25b**	21 (only mono esters)	**25a**: 2-O-L-leucyl-D-galactose (48) **25b**: 6-O-L-leucyl-D-galactose (52)
 **26a** **26b** **26c**	31 (only mono esters)	**26a**: 3-*O*-L-leucyl-D-mannose (28) **26b**: 4-*O*-L-leucyl-D-mannose (30) **26c**: 6-*O*-L-leucyl-D-mannose (42)
 **27**	48 (only mono ester)	**27**: 6-*O*-L-leucyl-D-fructose
 **28 a** **28 b** **28 c**	42 [c] (mono esters-24, diester-18)	**28a**: 2-*O*-L-leucyl-D-arabinose (24) **28b**: 5-*O*-L-leucyl-D-arabinose (33) **28c**: 3,5-di-*O*-L-leucyl-D-arabinose (43)

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Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

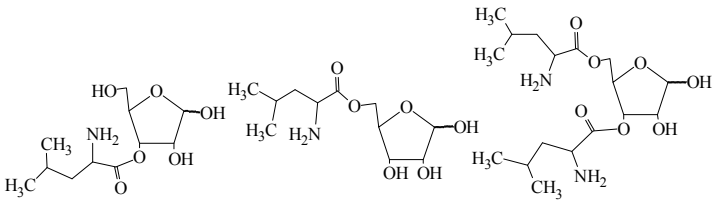
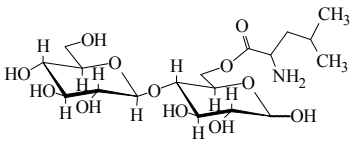
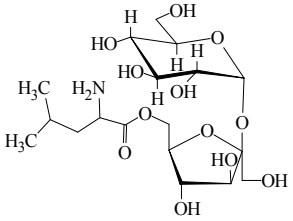
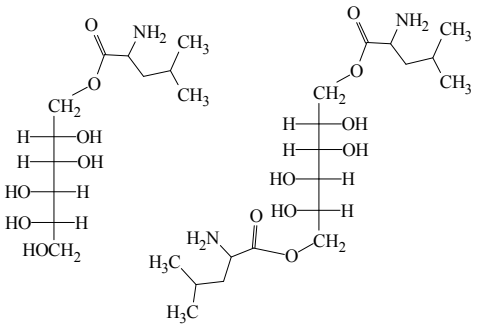
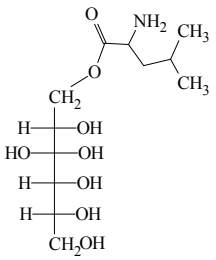
L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **29a** **29b** **29c**	38 [c] (mono esters-23, diester-15)	**29a**: 3-*O*-L-leucyl-D-ribose (16) **29b**: 5-*O*-L-leucyl-D-ribose (32) **29c**: 3,5-di-*O*-L-leucyl-D-ribose (52)
 **30**	44 (only mono ester)	**30**: 6-*O*-L-leucyl-maltose
 **31**	38 (only mono ester)	**31**: 6-*O*-L- leucyl-sucrose
 **32a** **32b**	45 (mono ester-25, diester-20)	**32a**: 1-*O*-L-leucyl-D-mannitol (56) **32b**: 1,6- di-*O*-L-leucyl-D-mannitol (44)
 **33**	25 (only mono ester)	**33**: 1-*O*-L-leucyl-D-sorbitol

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Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

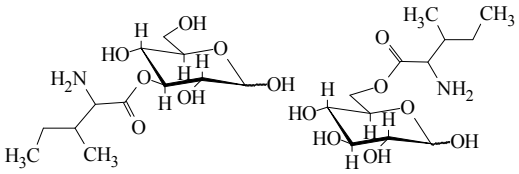
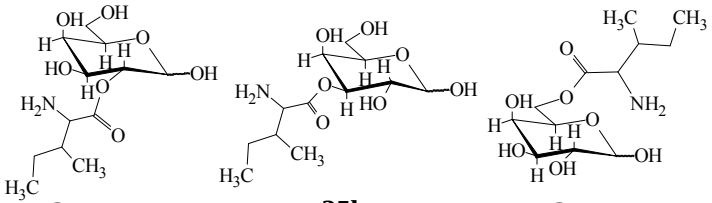
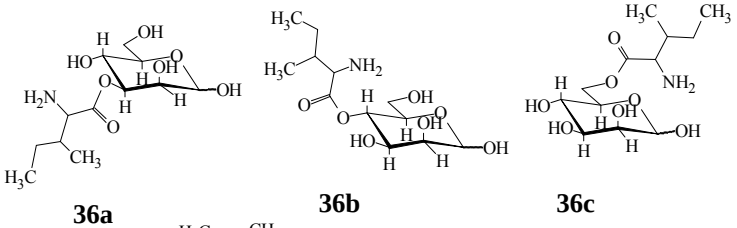
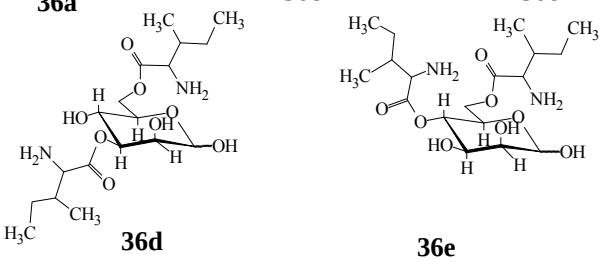
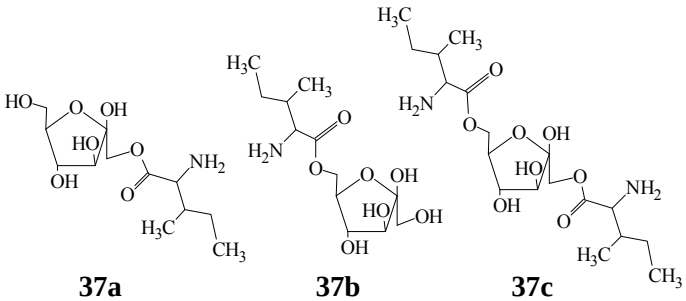
L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **34a** **34b**	47 (only mono esters)	**34a**: 3-*O*-L-isoleucyl -D-glucose (42) **34b**: 6-*O*-L-isoleucyl -D-glucose (58)
 **35a** **35b** **35c**	46 (only mono esters)	**35a**: 2-*O*-L-isoleucyl -D-galactose (78) **35b**: 3-*O*-L-isoleucyl -D-galactose (10) **35c**: 6-*O*-L-isoleucyl -D-galactose (12)
 **36a** **36b** **36c**	55 (mono esters-25, diesters-30)	**36a**: 3-*O*-L-isoleucyl-D-mannose (19) **36b**: 4-*O*-L- isoleucyl -D-mannose (13) **36c**: 6-*O*-L-isoleucyl-D-mannose (13) **36d**: 3,6-di-*O*-L-isoleucyl -D-mannose (27) **36e**: 4,6-di-*O*-L-isoleucyl-D-mannose (28)
 **36d** **36e**		
 **37a** **37b** **37c**	43 (mono esters-28, diester-15)	**37a**: 1-*O*-L-isoleucyl -D-fructose (36) **37b**: 6-*O*-L-isoleucyl -D-fructose (30) **37c**: 1,6-di-*O*-L-isoleucyl-D-fructose (34)

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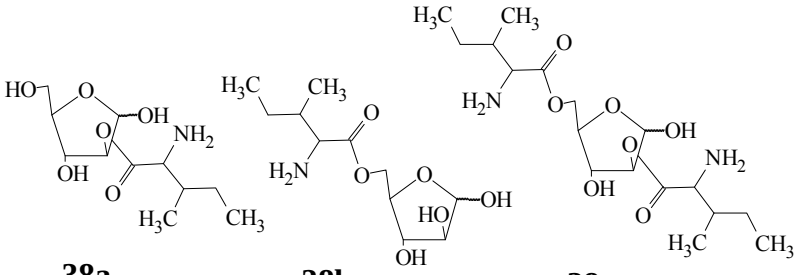
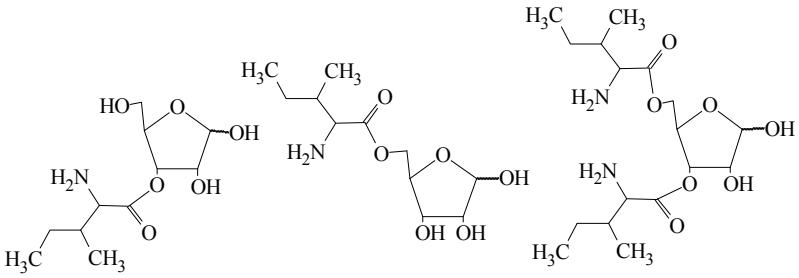
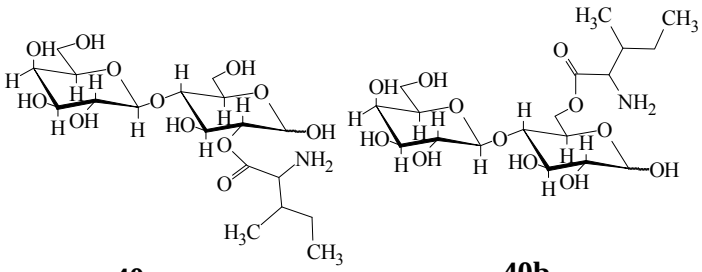
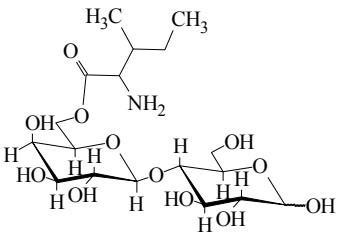
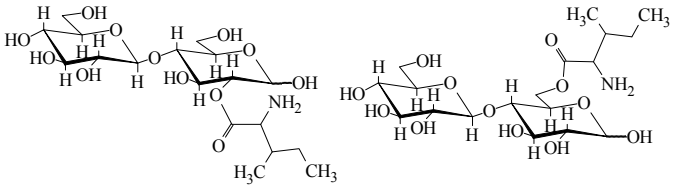
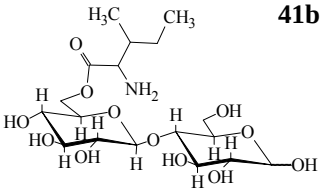
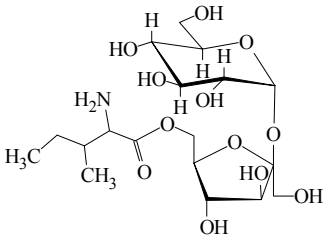
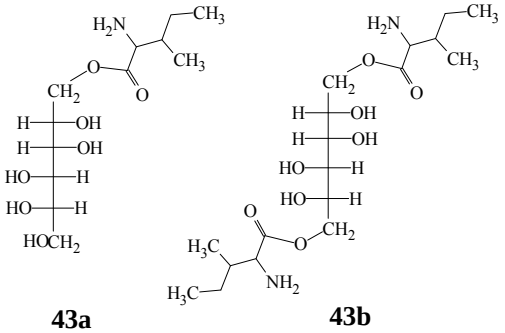
Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **38a** **38b** **38c**	55 [c] (mono esters-31, diester-24)	**38a**: 2-*O*-L-isoleucyl-D-arabinose (24) **38b**: 5-*O*-L-isoleucyl-D-arabinose (33) **38c**: 2,5-di-*O*-L-isoleucyl-D-arabinose (43)
 **39a** **39b** **39c**	53 [c] (mono esters-39, diester-14)	**39a**: 3-*O*-L-isoleucyl-D-ribose (52) **39b**: 5-*O*-L-isoleucyl-D-ribose (20) **39c**: 3,5-di-*O*-L-isoleucyl-D-ribose (28)
 **40a** **40b**	45 (only mono esters)	**40a**: 2-*O*-L- isoleucyl - lactose (39) **40b**: 6-*O*-L-isoleucyl -lactose (40) **40c**: 6′-*O*-L-isoleucyl -lactose (21)
 **40c**		

— Contd

Table I — Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates[a] — *Contd*

L-Amino acyl esters of carbohydrates	Yield (%)	Esters (% proportions)[b]
 **41a** **41b**  **41c**	54 (only mono esters)	**41a**: 2-*O*-L-isoleucyl-maltose (38) **41b**: 6-*O*-L-isoleucyl - maltose (40) **41c**: 6′-*O*-L-isoleucyl - maltose (22)
 **42**	22 (only mono ester)	**42**: 6-*O*-L-isoleucyl - sucrose
 **43a** **43b**	52 (mono esters-32, diester-20)	**43a**: 1-*O*-L-isoleucyl -D-mannitol (62) **43b**: 1,6-di-*O*-L-isoleucyl-D-mannitol (38)

[a] L-Amino acids (**1-3**) – 2 mmol, carbohydrates (**4-14**) – 1 mmol, CRL – 40% (w/w based on carbohydrate), buffer – 0.1 mM (0.1 mL of 0.1M *p*H 7.0) phosphate buffer, CH_2Cl_2: DMF (v/v, 90: 10) at 40°C, incubation period – 72 hr. Conversion yields were from HPLC with respect to L-amino acids concentration.

[b] Percentage proportions of individual esters determined from the peak areas of the ^{13}C C6 and C5 (in case of pentoses) signals or from cross peaks of the 2D HSQCT spectrum.

[c] Several cross peaks due to opening and/or degradation of the five membered ring during esterification.

C3β at 83.1 ppm (H-3β at 4.01 ppm), 6-*O*- ester **15c** to C6 at 63.4 ppm (H-6 at 3.15 ppm), 2,6-di-*O*- ester **15d** to C6α at 61.5 ppm (H-6 at 3.56 ppm) and C2α at 76.4 ppm (H-2α at 3.85 ppm) and 3,6-di-*O*- ester **15e** to C6α at 61.8 ppm (H-6 at 3.52 ppm) and C3α at 82.4 ppm (H-3α at 3.70 ppm); from D-galactose **5**, 2-*O*- ester **16a** to C2α at 76.4 ppm (H-2α at 3.83 ppm) and C2β at 77.6 ppm (H-2β at 3.65 ppm), 3-*O*- ester **16b** to C3α at 81.6 ppm (H-3α at 3.80 ppm) and C3β at 82.7 ppm (H-3β at 3.67 ppm) and 6-*O*- ester **16c** to C6α at 63.1 ppm (H-6 at 3.35 ppm); from D-mannose **6**, 6-*O*- ester **17** to C6α at 63.5 ppm (H-6 at 3.63

ppm); from D-fructose (**7**), 1-*O*- ester **18a** to C1α at 64.1 ppm (H-1α at 3.78 ppm), 6-*O*- ester **18b** to C6α at 64.0 ppm (H-6 at 3.18 ppm) and 1,6-di-*O*- ester **18c** to C1 and C6α at around 63.4 ppm (H-1 and H-6 at around 3.28 ppm); from D-arabinose **8**, 2-*O*- ester **19a** to C2α at 77.0 ppm (H-2α at 3.65 ppm), 5-*O*- ester **19b** to C5α at 65.0 ppm (H-5 at 3.64 ppm) and 2,5-di-*O*- ester **19c** to C2α at 75.6 ppm (H-2α at 3.45 ppm) and C5α and C5β at 63.3 ppm and 65.0 ppm (H-5α at 3.35 ppm and H-5β at 3.35) respectively; from D-ribose **9**, 3-*O*- ester **20a** to C3α at 77.1 ppm (H-3α at 3.98 ppm), 5-*O*- ester **20b** to C5α at 63.9 ppm (H-5 at 3.62 ppm) and 3,5-di-*O*- ester **20c** to C3α at 74.6 ppm (H-3α at 3.67ppm) and C5α at 65.4 ppm (H-5 at 3.34 ppm); from maltose **11**, 6-*O*- ester **21a** to C6α,β at 66.5 ppm (H-6 at 3.93 ppm) of reducing end glucose moiety, 6′-*O*- ester **21b** to C6′ at 68.0 ppm (H-6′ at 3.83 ppm) of the non reducing end glucose moiety; from sucrose **12**, 6-*O*-ester **22** to C6β at 68.2 ppm (H-6β at 3.64 ppm) of the fructose moiety; and from D-mannitol **13**, 1-*O*- ester **23** to C1 at 60.6 ppm and the corresponding H-1 cross peak at 3.53 ppm. Mass spectra also confirmed the formation of the above mentioned esters.

L-Leucyl esters of carbohydrates 24a-e to 33

UV-Vis spectra of L-leucyl esters of carbohydrates showed shifts in the σ→σ* band in the 203-230 nm range (190 nm for L-leucine **2**) and IR carbonyl stretching frequencies in the 1580 -1657 cm^{-1} range (1605 cm^{-1} for L-leucine **2**) indicating that L-amino acid carboxylic group had been converted into their corresponding carbohydrate esters. In the 2D HSQCT spectra of the L-leucyl esters of carbohydrates, the following ester formation were confirmed from their respective chemical shift values: from D-glucose **4**, 2-*O*- ester **24a** to C2α at 75.5 ppm and the corresponding H-2α cross peak at 3.85 ppm, 3-*O*- ester **24b** to C3α at 83.5 ppm (H-3α at 3.85 ppm) and C3β at 83.6 ppm (H-3β at 3.96 ppm), 6-*O*- ester **24c** to C6α at 65.0 ppm (H-6 at 3.80 ppm), 2,6-di-*O*- ester **24d** to C6α at 65.0 ppm (H-6 at 3.59 ppm) and C2α at 76.3 ppm (H-2α at 3.21 ppm) and 3,6-di-*O*- ester **24e** to C6α at 65.0 ppm (H-6 at 3.59 ppm) and C3α at 81.5 ppm (H-3α at 3.68 ppm); from D-galactose **5**, 2-*O*- ester **25a** to C2α at 72.5 ppm (H-2α at 3.74 ppm), 6-*O*- ester **25b** to C6α at 62.5 ppm (H-6 at 3.65 ppm); from D-mannose **6**, 3-*O*- ester **26a** to C3α at 81.4 ppm (H-3α at 3.86 ppm) and C3β at 82.5 ppm (H-3β at 3.97 ppm), 4-*O*- ester **26b** to C4α at 73.8 ppm (H-4α at 3.86 ppm) and C4β at 75.0 ppm (H-4β at 3.78 ppm) and 6-*O*- ester **26c** to C6α at 63.2 ppm (H-6 at 3.80 ppm); from D-fructose **7**, 6-*O*- ester **27** to C1α at 63.5 ppm (H-1α at 3.83 ppm); from D-arabinose **8**, 2-*O*- ester **28a** to C2α at 75.0 ppm (H-2α at 3.76 ppm) C2β at 75.2 ppm (H-2β at 3.45 ppm) and 5-*O*- ester **28b** to C5α at 64.8 ppm (H-5 at 3.64 ppm), 2,5-di-*O*- ester **28c** to C2α at 77.2 ppm (H-2α at 3.66 ppm) and C5α at 64.8 ppm (H-5 at 3.35); from D-ribose **9**, 3-*O*- ester **29a** to C3α at 76.0 ppm (H-3α at 3.60 ppm), 5-*O*- ester **29b** to C5α at 63.0 ppm (H-5a at 3.59 ppm and H-5b at 3.68 ppm), 3,5-di-*O*- ester **29c** to C3α at 76.2 ppm (H-3α at 3.77 ppm) and C5α at 64.8 ppm (H-5a at 3.34 ppm and H-5b at 3.37 ppm); from maltose **11**, 6-*O*- ester **30** to C6 at 62.9 ppm (H-6 at 3.33 ppm) of reducing end glucose moiety; from sucrose **12**, 6-*O*- ester **31** to C6β at 68.2 ppm (H-6 at 3.64 ppm) of the fructose moiety; from D-mannitol (**13**), 1-*O*- ester **32a** to C1 at 60.4 ppm (H-1 at 3.51 ppm), 1,6-di-*O*- ester **32b** to C1 at 60.4 ppm (H-1 at 3.61 ppm) and C6 at 68.2 ppm (H-6 at 3.64 ppm); and from D-sorbitol **14**, 1-*O*- ester **33** to C1 at 60.5 ppm (H-1 at 3.51 ppm). Mass spectra also confirmed the formation of the above mentioned esters.

L-Isoleucyl esters of carbohydrates 34a,b-43a,b

UV-Vis spectra of L-leucyl esters of carbohydrates showed shifts in the σ→σ* band in the 214-227 nm range (195 nm for L-isoleucine **3**) and IR carbonyl stretching frequencies in the 1616 -1646 cm^{-1} range (1584 cm^{-1} for L-isoleucine **3**) indicating that L-amino acid carboxylic group had been converted into their corresponding carbohydrate esters. In the 2D HSQCT spectra of the L-isoleucyl esters of carbohydrates, the following ester formation were confirmed from their respective chemical shift values: from D-glucose **4**, 3-*O*- ester **34a** to C3α at 82.0 ppm (H-3α at 4.01 ppm) and C3β at 81.9 ppm (H-3β at 3.88 ppm), 6-*O*- ester **34b** to C6α at 63.6 ppm (H-6 at 3.82 ppm); from D-galactose **5**, 2-*O*- ester **35a** to C2α at 76.2 ppm (H-2α at 3.81 ppm), 3-*O*- ester **35b** to C3α at 81.3 ppm (H-3α at 3.58 ppm) and C3β at 82.2 ppm (H-3β at 3.66 ppm), 6-*O*- ester **35c** to C6α at 62.8 ppm (H-6 at 3.40 ppm); from D-mannose **6**, 3-*O*- ester **36a** to C3α at 80.5 ppm (H-3α at 3.72 ppm) and C3β at 83.1 ppm (H-3β at 3.85 ppm), 4-*O*- ester **36b** to C4α at 75.0 ppm (H-4α at 3.80 ppm) and C4β at 77.1 ppm (H-4β at 3.90 ppm), 6-*O*- ester **36c** to C6α at 63.1 ppm (H-6

at 3.79 ppm), 3,6-di-*O*- ester **36d** to C6α at 62.9 ppm (H-6 at 3.41 ppm) and C3α at 82.1 ppm (H-3α at 3.55 ppm) and 4,6-di-*O*- ester **36e** to C6α at 62.9 ppm (H-6 at 3.52 ppm) and C4α at 76.0 ppm (H-4α at 3.75 ppm); from D-fructose **7**, 1-*O*- ester **37a** to C1α at 63.7 ppm (H-1α at 3.25 ppm), 6-*O*- ester **37b** to C6α at 63.1 ppm (H-6 at 3.78 ppm) and 1,6-di-*O*- ester **37c** to C1α and C6α at around 64.4 ppm (H-1 and H-6 at around 3.28 ppm); from D-arabinose **8**, 2-*O*- ester **38a** to C2α at 75.0 ppm (H-2α at 3.76 ppm) C2β at 75.2 ppm (H-2β at 3.45 ppm), 5-*O*- ester **38b** to C5α at 64.8 ppm (H-5 at 3.64 ppm) and 2,5-di-*O*- ester **38c** to C2α at 77.2 ppm (H-2α at 3.66 ppm) and C5α at 64.8 ppm (H-5 at 3.35 ppm); from D-ribose **9**, 3-*O*- ester **39a** to C3α at 76.5 ppm (H-3α at 3.98 ppm) and C3β at 77.7 ppm (H-3β at 3.92 ppm), 5-*O*- ester **39b** to C5α at 63.2 ppm (H-5 at 3.58 ppm) and 3,5-di-*O*- ester **39c** to C3α at 75.5 ppm (H-3α at 3.60 ppm) and C5α at 63.3 ppm (H-5 at 3.52 ppm); from lactose **10**, 2-*O*- ester **40a** to C2α at 84.5 ppm (H-2α at 3.40 ppm), C2β at 85.0 ppm (H-2β at 3.82 ppm) of glucose moiety, 6-*O*- ester **40b** to C6 at 66.5 ppm (H-6 at 3.85 ppm) of glucose moiety and 6′-*O*- ester **40c** to C-6′ at 66.9 ppm (H-6′ at 3.85 ppm) of galactose moiety; from maltose **11**, 2-*O*- ester **41a** to C2α at 81.2 ppm (H-2α at 4.05 ppm) C2β at 81.4 ppm (H-2β at 3.96 ppm) of reducing end glucose moiety, 6-*O*- ester **41b** to C6 at 68.0 ppm (H-6 at 3.62 ppm) of reducing end glucose moiety and 6′-*O*- ester **41c** to C6′ at 67.2 ppm (H-6′ at 3.62 ppm) of the non reducing end glucose moiety; from sucrose **12**, 6-*O*- ester **42** to C6β at 67.2 ppm (H-6 at 3.63 ppm) of the fructose moiety; and from D-mannitol **13**, 1-*O*- ester **43a** to C1 at 60.8 ppm (H-1 at 3.51 ppm) and 1,6-di-*O*- ester **43b** to C1 at 60.8 ppm (H-1 at 3.63 ppm) and C6 at 63.5 ppm (H-6 at 3.29 ppm). In this case also, mass spectra confirmed the formation of the above mentioned esters.

Multiple hydroxyl groups in carbohydrates like aldohexoses, ketohexose, aldopentoses, alcohols and disaccharides can give a lot of diastereomeric esters (mono, di, tri, tetra and penta from both the anomers). Since the reactions were carried out at a relatively low temperature of 40°C, the formation of peptides were less (< 3%), even though unprotected L-amino acids were employed for the reaction. Maillard and Pictet Spengler phenolic condensation products have been reported in such reactions[13,14]. However, no such products were observed in the present work, as, such reactions require higher temperature in the order of 90°C. MS as well as NMR analyses also detected no Maillard reaction products.

Candida rugosa lipase showed broad substrate specificity towards amino acids as well as carbohydrates (**Table I**). The present work describes preparation of 70 L-amino acyl esters of carbohydrates of which 65 esters are being reported for the first time. So far unreported esters are L-valyl-D-glucose **15a-e**, L-valyl-D-galactose **16a-c**, L-valyl-D-mannose **17**, L-valyl-D-fructose **18a-c**, L-valyl-D-arabinose **19a-c**, L-valyl-D-ribose **20a-c**, L-valyl-maltose **21a, b**, L-valyl-sucrose **22**, L-valyl-D-mannitol **23**, L-leucyl-D-galactose **25a, b**, L-leucyl-D-mannose **26a-c**, L-leucyl-D-fructose **27**, L-leucyl-D-arabinose **28a-c**, L-leucyl-D-ribose **29a-c**, L-leucyl-maltose **30**, L-leucyl-sucrose **31**, L-leucyl-D-mannitol **32a, b**, L-leucyl-D-sorbitol **33**, L-isoleucyl-D-glucose **34a, b,** L-isoleucyl-D-galactose **35a-c,** L-isoleucyl-D-mannose **36a-c**, L-isoleucyl-D-fructose **37a-c**, L-isoleucyl-D-arabinose **38a-c**, L-isoleucyl-D-ribose **39a-c**, L-isoleucyl-lactose **40a-c**, L-isoleucyl-maltose **41a-c**, L-isoleucyl-sucrose **42** and L-leucyl-D-mannitol **43a, b**. Except lactose **10** and D-sorbitol **14**, all the carbohydrates employed have reacted with all the three amino acids (**1-3**). L-Valine **1**, L-leucine **2** with D-glucose **4** and L-isoleucine **3** with D-mannose **6** gave five diastereomeric esters. Both D-arabinose and D-ribose have shown three diastereomeric esters with all the three amino acids (**1-3**). Lactose with L-valine and L-leucine and D-sorbitol with L-valine and L-isoleucine did not undergo any reaction. L-Valyl-D-mannose **17**, L-valyl- sucrose **22**, L-leucyl- maltose **30**, L-leucyl- sucrose **31** and L-isoleucyl- sucrose **42**, formed only 6-*O*- ester.

Nature of the products clearly indicated that primary hydroxyl groups of the carbohydrates (1-*O*-, 5-*O*-, 6-*O*- and 6′-*O*-) were esterified preferencially over secondary hydroxyl groups (2-*O*-, 3-*O*- and 4-*O*-). Among the secondary hydroxyl groups, 4-*O*-ester was formed only in case of D-mannose (**26b** and **36b**). Carbohydrates containing axial hydroxyl groups in axial position like C2 in D-mannose and D-ribose and C4 in D-galactose, have not reacted indicating that esterification with axial secondary hydroxyl groups are difficult, especially with alkyl amino acyl donors.

The anomeric hydroxyl group of the carbohydrate molecules did not react because of rapid glycosidic ring opening and closing process. Loss of specificity could be due to use of larger amount of enzymes

(about 40 % w/w of carbohydrate) and substrates, which gave a large number of esters.

Carbohydrates like lactose and D-sorbitol reacted selectively depending on the amino acid indicating that they may not be good nucleophiles. This could be due to greater hydrogen bonding propensity for D-sorbitol and steric hindrance in case of lactose. In case of aldopentoses (D-arabinose and D-ribose), NMR spectrum clearly indicated degradation and/or ring opening during the reaction. In case of L-valyl-D-ribose, opening of the five membered ring during esterification was noticed by observation of a large number of signals in the 3.0-5.0 ppm (^{1}H) and 63-75 ppm (^{13}C) region which could be due to excess strain on the ring due to introduction of bulky amino acid groups to D-ribose OH groups.

Thus, this study has shown that unprotected and unactivated amino acids containing hydrophobic alkyl side chains can serve as good acyl donors in the esterification reaction catalyzed by *Candida rugosa* lipase.

Experimental Section

Lipases

Lipase from *Candida rugosa*, Type VII (CRL) was purchased from Sigma Chemical Co., USA. Esterification activity of CRL was found to be of 0.03 μmol/min/mg enzyme preparation[15].

Chemicals and reagents

D-Galactose, D-fructose, L-leucine and L-isoleucine were purchased from Himedia Pvt. Ltd. India; maltose and Sephadex G-10 from Sigma Chemical Co., USA; D-mannose, D-arabinose, D-ribose, D-mannitol, ninhydrin and 1-naphthol from LOBA Chemie Pvt. Ltd. India; D-glucose, sucrose, sulfuric acid silica gel from SD Fine Chemicals (India) Ltd.; lactose and potassium bromide (IR grade) from SISCO Research Laboratories Pvt. Ltd. India; Karl Fischer reagent from Qualigens Fine Chemicals Pvt. Ltd. India; D-sorbitol from Rolex Laboratory Reagent India Ltd. and Bio Gel P-2 obtained from Bio-Rad Laboratories, USA, were used as received. Solvents - CH_2Cl_2, HPLC grade acetonitrile and DMF from SD Fine Chemicals (India) Ltd., were used after distilling once.

Syntheses of L-valyl, L-leucyl and L-isoleucyl esters of carbohydrates

L-Amino acyl esters of carbohydrates (**15a-e** to **43a,b**) were synthesized by reacting L-amino acids (2 mmol) and carbohydrates **4-14** (1mmol) in presence of 0.060-0.14 g of CRL (40 % w/w of **4-14**) in 100 mL CH_2Cl_2 : DMF (90:10 v/v, 40°C) containing 0.1 mM phosphate buffer (0.1mL of 0.1 M, *p*H 7.0) in a flat bottomed two necked flask under reflux with stirring for a period of 72 hr. The condensed solvent vapour forming azeotrope with water was passed through a desiccant in a Soxhelet set up before being returned into the reaction mixture. This results in complete removal of water of reaction[16] and maintained a very low water activity of a_w= 0.0054 throughout the reaction period. The water content of the reaction mixture was determined by Karl Fischer titration of the reaction mixture against Karl Fischer reagent by removing aliquots of the reaction mixture during the course of the reaction. The enzyme was '*p*H tuned' by adding known volumes of 0.1 M buffer solutions of specified *p*H to 100 mL (solvent) of the reaction mixture[17,18]. After completion of the reaction, the solvent was distilled off, the enzyme was heat denatured and treated with 30-50 mL of water, stirred and filtered to remove the CRL. The filtrate was evaporated to get a mixture of the unreacted L-amino acid (**1-3),** unreacted carbohydrate (**4-14)** and the product esters (**15a-e** to **43a, b)** which were then subjected to HPLC analysis.

The product ester formation was monitored by thin layer chromatography (TLC) using *n*-butanol: acetic acid: water (70:20:10 v/v/v) mixture as eluent and the resolved spots were identified by ninhydrin (for NH_2 group detection) and 1-naphthol (reducing sugar detection). The reaction mixture was also analyzed by HPLC using a Shimadzu LC10AT, Kyoto, Japan instrument employing a LiChrosorb RP-18 reverse phase column (5 μm particle size, 4.6 × 150 mm length) with acetonitrile: water (20:80 v/v) as a mobile phase at a flow rate of 1 mL/ min and monitoring by UV-Vis detector at 210 nm. The conversion yields were determined from the peak areas of the ester and the free amino acid and the conversion yields were expressed with respect to the L-amino acid employed. Errors in HPLC yields were within ± 10 %. The esters formed were separated by size exclusion chromatography using Sephadex G-10 / Bio Gel P-2 as column materials and eluted with water and subjected to spectral characterization.

Spectral characterization

A Shimadzu UV–1601, Kyoto, Japan, was used for recording the UV-Vis spectra of the isolated esters in aqueous solutions at 0.1-0.5 mM concentration. A

Nicolet 5700 FTIR Madison, USA, instrument was used for recording the IR spectra with 2.0-3.0 mg of the ester sample as KBr pellet. Specific rotations of the isolated esters were measured at 25 °C using Perkin-Elmer 243 polarimeter, West Germany, with a 0.2 -1.5% aqueous solution of the esters. Mass spectra of the isolated esters were recorded using a Q-TOF Waters Ultima instrument (No.Q-Tof GAA 082, Waters Corporation, Manchester, UK) fitted with an electron spray ionization (ESI) source.

Two-dimensional Heteronuclear Single Quantum Coherence Transfer spectra (2DHSQCT) and ^{1}H and ^{13}C NMR spectra were recorded on a Brüker DRX-500 MHz spectrometer, Switzerland (500.13 MHz for ^{1}H and 125 MHz for ^{13}C). Proton and carbon 90° pulse widths were 10.5 and 12.25 μs respectively. About 40 mg of the sample dissolved in DMSO-d_6 and D_2O was used for recording the spectra at 35°C. Chemical shift values were expressed in ppm relative to internal tetramethylsilane standard to within ± 0.01 ppm. In the NMR data, only resolvable signals are shown. Some assignments are interchangeable. Non-reducing end carbohydrate atoms are primed. Since, the esters are surfactant molecules, they aggregate in the solvent and usually give broad signals, thus making it difficult to resolve the coupling constant values accurately. Mass data for the monoesters are shown. Although, the esters were separated from unreacted amino acids and carbohydrates through column chromatography on Sephadex, the individual esters could not be resolved due to the similar polarity of these ester molecules. HPLC also showed only a single peak for all esters formed. However, 2D NMR connectivities clearly indicated signals from individual esters.

L-Valyl esters of carbohydrates (15a-e to 23)

L-Valine (1): Solid; m.p.: 298°C; HPLC t_{ret}: 2.7 min; R_f: 0.32; UV-Vis (H_2O, λ_{max}): 195.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{195.0}$ - 116 M^{-1}); IR (KBr): 3415 (OH), 2945 (CH), 1605 cm^{-1} (CO); $[\alpha]_D^{25}$ = +3.33° (C 1.0, H_2O); 2D-HSQCT (DMSO-d_6): ^{1}H NMR (500.13 MHz): δ 3.30 (αCH), 1.90 (βCH), 0.98 (γ, γ′CH_3); ^{13}C NMR (125 MHz): δ 53.2 (αCH), 29.2(βCH), 18.1 (γ, γ′CH_3), 170.5 (CO).

L-Valyl-D-glucose (15a-e): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 200.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{200.0}$ – 2630 M^{-1}), 295.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{295.0}$ - 2089 M^{-1}); IR (KBr): 2889 (NH), 3407 (OH), 2950 (CH), 1622 cm^{-1} (CO); MS: *m/z* 302 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester (15a)**: ^{1}H NMR (500.13 MHz): δ 3.08 (αCH), 1.35 (γCH_3), 3.75 (H-2α), 3.67 (H-3α), 3.78 (H-4α), 4.22 (H-5α), 3.50 (H-6); ^{13}C NMR (125 MHz): δ 51.5 (αCH), 19.4 (γCH_3), 77.4 (C2α), 74.6 (C3α), 71.3 (C4α), 71.3 (C5α), 60.0 (C6α); **3-*O*-ester (15b)**: ^{1}H NMR: δ 3.10 (αCH), 1.15 (βCH_3), 3.85 (H-2α), 3.90 (H-3α), 4.01 (H-3β), 3.34 (H-6); ^{13}C NMR: δ 52.3 (αCH), 18.3 (γCH_3), 71.3 (C2α), 82.6 (C3α), 83.1 (C3β), 60.4 (C6α); **6-*O*-ester (15c)**: ^{1}H NMR: δ 3.20 (αCH), 3.35 (βCH), 1.55 (γCH_3), 4.95 (H-1α), 4.24 (H-1β), 3.90 (H-3α), 3.74 (H-4α), 3.41 (H-5α), 3.15 (H-6); ^{13}C NMR: δ 53.4 (αCH), 29.9 (βCH), 19.8 (γCH_3), 94.9 (C1α), 103.4 (C1β), 76.2 (C3α), 69.5 (C4α), 69.0 (C5β), 63.4 (C6α); **2,6-di-*O*-ester (15d)**: ^{1}H NMR: δ 3.20 (αCH), 3.85 (H-2α), 3.56 (H-6); ^{13}C NMR δ: 51.4 (αCH), 76.4 (C2α), 61.5 (C6α) ; **3,6-di-*O*-ester (15e)**: ^{1}H NMR: δ 3.15 (αCH), 1.45 (γCH_3), 3.70 (H-3α), 3.52 (H-6); ^{13}C NMR: δ 53.4 (αCH), 19.5 (γCH_3), 82.4 (C3α), 61.8 (C6α).

L-Valyl-D-galactose (16a-c): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.21; UV-Vis (H_2O, λ_{max}): 225.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{225.0}$ –871 M^{-1}), 294.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{294.0}$ – 407 M^{-1}), IR (KBr): 3309 (NH), 2944 (CH), 1621 cm^{-1} (CO); MS: *m/z* 302 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester (16a)**: ^{1}H NMR (500.13 MHz): δ 3.30 (αCH), 1.90 (βCH), 0.98 (γ, γ′CH_3), 3.83 (H-2α), 3.65 (H-2β), 3.54 (H-6); ^{13}C NMR (125 MHz): δ 53.2 (αCH), 29.2 (βCH), 18.1 (γ, γ′CH_3), 171.2 (CO), 76.4 (C2α), 77.6 (C2β), 60.6 (C6α); **3-*O*-ester (16b)**: ^{1}H NMR: δ 3.80 (H-3α), 3.67 (H-3β), 3.54 (H-6); ^{13}C NMR: δ 81.6 (C3α), 82.7 (C3β), 60.6 (C6α); **6-*O*-ester (16c)**: ^{1}H NMR: δ 4.98 (H-1α), 4.89 (H-1β), 3.56 (H-2α), 3.71 (H-3α), 3.31 (H-3β), 3.78 (H-4α), 3.23 (H-4β), 3.44 (H-5α), 3.35 (H-6); ^{13}C NMR: δ 95.5 (C1α), 101.9 (C1β), 69.6 (C2α), 69.1 (C3α), 73.7 (C3β), 70.5 (C4α), 72.3 (C4β), 70.5 (C5α), 63.1 (C6α).

L-Valyl-D-mannose (17): Solid; m.p.: 98°C; HPLC t_{ret}: 2.0 min; R_f: 0.59; UV-Vis (H_2O, λ_{max}): 227.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{227.0}$ - 447 M^{-1}), 290.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{290.0}$ - 603 M^{-1}); IR (KBr): 3410 (NH), 3354 (OH), 2932 (CH), 1644 cm^{-1} (CO); $[\alpha]_D^{25}$ = -20° (C 0.5, H_2O); MS: *m/z* 302 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **6-*O*-ester (17)**: ^{1}H NMR (500.13 MHz): δ 3.90 (αCH), 2.16 (βCH), 1.02 (γ, γ′CH_3), 4.31 (H-1α), 3.63 (H-2α), 3.32 (H-3α), 3.51 (H-4α), 3.45 (H-5α), 3.63 (H-6); ^{13}C NMR (125 MHz): δ 53.0 (αCH), 29.2 (βCH),

18.2 (γ, γ′CH_3), 171.0 (CO), 96.8 (C1α), 67.5 (C2α), 73.5 (C3α), 67.5 (C4α), 75.5 (C5α), 63.5 (C6α).

L-Valyl-D-fructose (**18a-c**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.20; UV-Vis (H_2O, λ_{max}): 223.0 nm (σ→σ* $\lambda_{223.0}$ - 53 M^{-1}), 288.0 nm (n→π* $\varepsilon_{288.0}$ - 23 M^{-1}); IR (KBr): 3352 (NH), 3290 (OH), 2946 (CH), 1623 cm^{-1} (CO); MS: *m/z* 304 [M^{+2}+Na^+]; 2D-HSQCT (DMSO-d_6): **1-*O*-ester** (**18a**): ^{1}H NMR (500.13 MHz): δ 3.38 (αCH), 2.14 (βCH), 1.06 (γ, γ′CH_3), 3.78 (H-1α), 3.32 (H-3α), 3.55 (H-3β), 3.78 (H-4α), 3.45 (H-4β), 3.22 (H-5α), 3.32 (H-6); ^{13}C NMR (125 MHz): δ 52.5 (αCH), 29.2 (βCH), 18.4 (γ, γ′CH_3), 171.2 (CO), 64.1 (C1α), 104.3 (C2β), 70.8 (C3α), 83.9 (C3β), 72.5 (C4α), 76.0 (C4β), 73.2 (C5α), 64.0 (C6α); **6-*O*-ester** (**18b**): ^{1}H NMR: δ 3.63 (H-1α), 3.73 (H-4β), 3.72 (H-5β), 3.18 (H-6); ^{13}C NMR: δ 63.0 (C1α), 75.9 (C4β), 81.2 (C5β), 64.0 (C6α); **1,6-di-*O*-ester** (**18c**): ^{1}H NMR: δ 3.83 (H-4β), 3.28 (H-6α); ^{13}C NMR: δ 76.1 (C4β), 63.4 (C6α).

L-Valyl-D-arabinose (**19a-c**): Solid; HPLC t_{ret}: 3.3 min; R_f: 0.23; UV-Vis (H_2O, λ_{max}): 224.0 nm (σ→σ* $\varepsilon_{224.0}$ - 1585 M^{-1}), 281.0 nm (n→π* $\varepsilon_{281.0}$ - 933 M^{-1}); IR (KBr): 3339 (NH), 3290 (OH), 2963 (CH), 1598 cm^{-1} (CO); MS: *m/z* 273 [M^{+1}+Na^+]; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**19a**): ^{1}H NMR (500.13 MHz): δ 1.05 (αCH), 4.99 (H-1α), 4.91 (H-1β), 3.65 (H-2α), 3.19 (H-3α), 3.72 (H-5); ^{13}C NMR (125 MHz): δ 15.5 (αCH), 95.9 (C1α), 102.1 (C1β), 77.0 (C2α), 71.9 (C3α), 60.5 (C5α); **5-*O*-ester (19b)**: ^{1}H NMR: δ 3.39 (αCH), 2.08 (βCH), 1.02 (γ, γ′CH_3), 4.32 (H-1α), 4.21 (H-1β); 3.28 (H-2α), 3.33 (H-3α), 3.69 (H-4α), 3.64 (H-5); ^{13}C NMR: δ 53.0 (αCH), 29.5 (βCH), 19.3 (γ, γ′CH_3), 172.0 (CO), 96.8 (C1α), 103.9 (C1β), 74.8 (C2α), 70.8 (C3α), 67.6 (C4α), 65.0 (C5α); **2,5-di-*O*-ester** (**19c**): ^{1}H NMR: δ 3.45 (H-2α), 3.26 (H-3α), 3.68 (H-5α), 3.35 (H-5β); ^{13}C NMR δ : 75.6 (C2α), 73.4 (C3α), 63.3 (C5α), 65.0 (C5β).

L-Valyl-D-ribose (**20a-c**): Solid; HPLC t_{ret}: 3.4 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 230.0 nm (σ→σ* $\varepsilon_{230.0}$ - 1412 M^{-1}), 297.0 nm (n→π* $\varepsilon_{297.0}$-1072 M^{-1}); IR (KBr): 3349 (NH), 3137 (OH), 2934 (CH), 1588 cm^{-1} (CO); MS: *m/z* 273 [M^{+1}+Na^+]; 2D-HSQCT (DMSO-d_6): **3-*O*-ester** (**20a**): ^{1}H NMR (500.13 MHz): δ 3.38 (αCH), 1.82 (βCH), 0.68 (γ, γ′CH_3), 3.98 (H-3α), 3.72 (H-5); ^{13}C NMR (125 MHz): δ 53.2 (αCH), 30.5 (βCH), 18.4 (γ, γ′CH_3), 174.3 (CO), 77.1 (C3α), 61.2 (C5α); **5-*O*-ester** (**20b**): ^{1}H NMR: δ 2.08 (βCH), 1.03 (γCH_3), 4.20 (H-1α), 4.93 (H-1β), 3.22 (H-2α), 3.31 (H-2β), 3.31 (H-3α), 3.54 (H-3β), 3.81 (H-4α), 3.62 (H-5); ^{13}C NMR: δ 29.7 (αCH), 18.3 (γCH_3), 173.5 (CO), 101.5 (C1α), 93.8 (C1β), 72.1 (C2α), 73.4 (C2β), 70.4 (C3α), 68.6 (C3β), 65.1 (C4α), 63.9 (C5α); **3,5-di-*O*-ester** (**20c**): ^{1}H NMR: δ 4.42 (H-1α), 4.62 (H-1β), 3.67 (H-3α), 3.34 (H-5); ^{13}C NMR δ : 97.0 (C1α), 94.0 (C1β), 74.6 (C3α), 65.4 (C5α).

L-Valyl-maltose (**21a, b**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.13; UV-Vis (H_2O, λ_{max}): 221.0 nm (σ→σ* $\varepsilon_{221.0}$ - 1622 M^{-1}), 291.0 nm (n→π* $\varepsilon_{291.0}$ - 776 M^{-1}); IR (KBr): 3419 (NH), 3267 (OH), 2936 (CH), 1634 cm^{-1} (CO); MS: *m/z* 464 [M+Na]$^+$; 2D-HSQCT (DMSO-d_6): **6-*O*-ester** (**21a**): ^{1}H NMR (500.13 MHz): δ 3.12 (αCH), 2.25 (βCH), 1.05 (γ, γ′CH_3), 4.92 (H-1α), 5.0 (H-1β), 3.32 (H-2α), 3.41 (H-3α), 4.14 (H-4α), 3.39 (H-5α), 3.93 (H-6), 4.90 (H-1′α), 3.27 (H-2′), 3.35 (H-3′), 3.63 (H-4′), 3.68 (H-5′), 3.67 (H-6′); ^{13}C NMR (125 MHz): δ 51.0 (αCH), 31.1 (βCH), 18.8 (γCH_3), 175.5 (CO), 97.0 (C1α), 100.5 (C1β), 73.0 (C2α), 76.3 (C3α), 80.5 (C4α), 78.8 (C5α), 66.5 (C6), 100.7 (C1′α), 67.5 (C2′), 67.0 (C3′), 70.2 (C4′), 72.3 (C5′), 60.5 (C6′); **6′-*O*-ester** (**21b**): ^{1}H NMR: δ 2.90 (αCH), 3.98 (H-4α), 3.07 (H-2′), 3.32 (H-3′), 3.83 (H-6′); ^{13}C NMR: δ 52.0 (αCH), 80.6 (C4α), 71.5 (C2′), 70.5 (C3′), 68.0 (C6′).

L-Valyl-sucrose (**22**): Solid; m.p.: 184°C; HPLC t_{ret}: 3.4 min; R_f: 0.13; UV-Vis (H_2O, λ_{max}): 225.0nm (σ→σ* $\varepsilon_{225.0}$ - 195 M^{-1}), 275.0 nm (n→π* $\varepsilon_{275.0}$ - 91 M^{-1}); IR (KBr): 3520 (NH), 3411 (OH), 2940 (CH), 1588 cm^{-1} (CO); $[\alpha]_D^{25}$ = +36° (C 0.5, H_2O); MS: *m/z* 464 [M+Na]$^+$; 2D-HSQCT (DMSO-d_6): **6-*O*-ester** (**22**): ^{1}H NMR (500.13 MHz): δ 3.13 (αCH), 2.42 (βCH), 1.05 (γ, γ′CH_3), 3.26 (H-1α), 3.79 (H-3β), 3.33 (H-4β), 3.90 (H-5β), 3.64(H-6), 4.92 (H-1′α), 3.34 (H-2′), 3.46 (H-3′), 3.34 (H-4′), 3.62 (H-5′), 3.52 (H-6′); ^{13}C NMR (125 MHz): δ 53.5 (αCH), 28.8 (βCH), 18.5 (γ, γ′CH_3), 172.0 (CO), 59.5 (C1β), 103.3 (C2β), 74.1 (C3β), 80.0 (C4β), 74.3 (C5β), 68.2 (C6β), 91.8 (C1′α), 72.9 (C2′), 75.2 (C3′), 70.6 (C4′), 72.6 (C5′), 60.3 (C6′).

L-Valyl-D-mannitol (**23**)**:** Solid; m.p.: 132°C; HPLC t_{ret}: 3.5 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 225.0nm (σ→σ* $\varepsilon_{225.0}$ -372 M^{-1}), 270.0 nm (n→π* $\varepsilon_{270.0}$ - 178 M^{-1}); IR (KBr): 3294 (OH), 2957 (CH), 1630 cm^{-1} (CO); $[\alpha]_D^{25}$ = +6.6° (C 0.5, H_2O); MS: *m/z* 464 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **1-*O*-ester**

(**23**): ^{1}H NMR (500.13 MHz): δ 3.12 (αCH), 2.05 (βCH), 0.92 (γ, γ′CH_3), 3.53 (H-1), 3.46 (H-2), 3.53 (H-3), 3.56 (H-4), 3.41 (H-5), 3.46 (H-6); ^{13}C NMR (125 MHz): δ 55.8 (αCH), 29.8 (βCH_2), 19.1 (γ,γ′CH_3), 60.6 (C1), 69.5 (C2), 67.5 (C3), 71.2 (C4), 71.0 (C5) 63.6 (C6).

L-Leucyl esters of carbohydrates (**24a-e to 33**)

L-Leucine (**2**): Solid; m.p.: 293-95°C; HPLC t_{ret}: 2.6 min; R_f: 0.32; UV-Vis (H_2O, λ_{max}): 190.0 nm (σ→σ* $\varepsilon_{190.0}$ - 132 M^{-1}), IR (KBr): 3415 (OH), 2945 (CH), 1595 cm^{-1} (CO); $[\alpha]_D^{25}$ = -15.2° (C 1.0, H_2O); 2D-HSQCT (DMSO-d_6): ^{1}H NMR (500.13 MHz): δ 3.41 (αCH), 1.56 (βCH_2), 1.64 (γCH), 0.85 (δ, δ′ CH_3); ^{13}C NMR (125 MHz): δ 53.2 (αCH), 39.4 (βCH_2), 24.0 (γCH), 21.8 (δ, δ′ CH_3), 173.0 (CO).

L-Leucyl-D-glucose (**24a-e**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 230.0 nm (σ→σ* $\varepsilon_{230.0}$ - 724 M^{-1}), 297.0 nm (n→π* $\varepsilon_{297.0}$ - 363 M^{-1}); IR (KBr): 3383 (NH), 3360 (OH), 2240 (CH), 1657 cm^{-1} (CO); MS: *m/z* 316 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**24a**): ^{1}H NMR (500.13 MHz): δ 3.85 (H-2α); ^{13}C NMR (125 MHz): δ 75.5 (C2α); **3-*O*-ester** (**24b**): ^{1}H NMR: δ 3.15 (αCH), 3.85 (H-3α), 3.96 (H-3β); ^{13}C NMR: δ 50.0 (αCH), 83.5 (C3α), 83.6 (C3β); **6-*O*-ester** (**24c**): ^{1}H NMR: δ 3.10 (α CH), 1.56 (βCH_2), 1.82 (γCH), 0.82 (δ, δ′ CH_3), 4.99 (H-1α), 3.81 (H-2α), 3.45 (H-3α), 3.68 (H-4α), 3.55 (H-5α), 3.80 (H-6); ^{13}C NMR: δ 53.2 (αCH), 40.5 (βCH_2), 23.5 (γCH), 25.0 (δ, δ′ CH_3), 173.6 (CO), 92.5 (C1α), 70.3 (C2α), 75.8 (C3α), 70.2 (C4α), 71.0 (C5α), 65.0 (C6α); **2,6-di-*O*-ester** (**24d**): ^{1}H NMR: δ 3.21 (H-2α), 3.59 (H-6); ^{13}C NMR: δ 76.3 (C2α), 65.0 (C6α). **3,6-di-*O*-ester (24e)**: ^{1}H NMR: δ 3.68 (H-3α), 3.59 (H-6); ^{13}C NMR: δ 81.5 (C3α), 65.0 (C6α).

L-Leucyl-D-galactose (**25a, b**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 226.0 nm (σ→σ* $\varepsilon_{226.0}$ - 2291 M^{-1}), 295.0 nm (n→π* $\varepsilon_{308.0}$ - 1349 M^{-1}); IR (KBr): 3426 (NH), 3317 (OH), 2815 (CH), 1589 cm^{-1} (CO); MS: *m/z* 316 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**25a**): ^{1}H NMR: δ 3.74 (H-2α), 3.70 (H-6); ^{13}C NMR: δ 171.4 (CO), 72.5 (C2α), 59.5 (C6α); **6-*O*-ester** (**25b**): ^{1}H NMR: δ 3.50 (αCH), 1.54 (βCH_2), 1.44 (γCH), 0.95 (δ, δ′CH_3), 5.0 (H-1α), 4.49 (H-1β), 3.66 (H-2α), 3.68 (H-3α), 3.74 (H-4α), 3.42 (H-4β), 3.62 (H-5α), 3.48 (H-5β), 3.65 (H-6); ^{13}C NMR: δ 54.0 (αCH), 39.2 (βCH_2), 24.0 (γCH), 21.6 (δ, δ′ CH_3), 172.1 (CO), 92.0 (C1α), 96.1 (C1β), 62.5 (C2α), 68.0 (C3α), 68.2 (C4α), 72.5 (C4β), 70.0 (C5α), 74.5 (C5β), 62.5 (C6α).

L-Leucyl-D-mannose (**26a-c**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 230.0 nm (σ→σ* $\varepsilon_{230.0}$ - 1122 M^{-1}), 292.0 nm (n→π* $\varepsilon_{292.0}$ - 120.2 M^{-1}); IR (KBr): 3425 (NH), 3329 (OH), 2950 (CH), 1591 cm^{-1} (CO); MS: *m/z* 316 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **3-*O*-ester** (**26a**): ^{1}H NMR (500.13 MHz): δ 3.05 (αCH), 3.86 (H-3α), 3.97 (H-3β), 3.55 (H-6); ^{13}C NMR (125 MHz): δ 51.5 (αCH), 81.4 (C3α), 82.5 (C3β), 61.7 (C6α); **4-*O*-ester** (**26b**): ^{1}H NMR: δ 3.86 (H-4α), 3.78 (H-4β), 3.61 (H-6); ^{13}C NMR: δ 73.8 (C4α), 75.0 (C4β), 61.6 (C6α); **6-*O*-ester** (**26c**): ^{1}H NMR: δ 3.47 (αCH), 1.59 (βCH_2), 1.64 (γCH), 1.0 (δ, δ′CH_3), 4.91 (H-1α), 4.75 (H-2α), 3.65 (H-3α), 3.43 (H-4α), 3.63 (H-5α), 3.80 (H-6); ^{13}C NMR: δ 53.9 (αCH), 39.4 (βCH_2), 24.0 (γCH), 21.1 (δ, δ′CH_3), 172.6 (CO), 95.3 (C1α), 68.0 (C2α), 68.1 (C3α), 66.3 (C4α), 72.3 (C4β), 70.5 (C5α), 63.2 (C6α).

L-Leucyl-D-fructose (**27**): Solid; m.p.: 128°C; HPLC t_{ret}: 3.5 min; R_f: 0.22; UV-Vis (H_2O, λ_{max}): 223.0 nm (σ→σ* $\varepsilon_{223.0}$ -1023 M^{-1}), 284.0 nm (n→π* $\varepsilon_{284.0}$ - 489.8 M^{-1}); IR (KBr): 3266 (NH), 3219 (OH), 2951 (CH), 1630 cm^{-1} (CO); $[\alpha]_D^{25}$ = -6.7° (C 0.5, H_2O); MS: *m/z* 332 [M+K]$^+$; 2D-HSQCT (DMSO-d_6): **6-*O*-ester** (**27**): ^{1}H NMR (500.13 MHz): δ 3.47 (αCH), 1.56 (βCH_2), 1.64 (γCH), 1.0 (δ, δ′CH_3), 3.58 (H-1α), 3.61 (H-3α), 3.87 (H-3β), 3.77 (H-4β), 3.65 (H-5α), 3.83 (H-6); ^{13}C NMR (125 MHz): δ 52.8 (αCH), 39.4 (βCH_2), 24.0 (γCH), 22.2 (δ, δ′CH_3), 173.1 (CO), 61.5 (C1α), 104.2 (C2α), 69.0 (C3α), 82.0 (C3β), 75.4 (C4β), 66.8 (C5α), 63.5 (C6α).

L-Leucyl-D-arabinose (**28a-c**): Solid; HPLC t_{ret}: 3.3 min; R_f: 0.24; UV-Vis (H_2O, λ_{max}): 210.0 nm (σ→σ* $\varepsilon_{210.0}$ - 7080 M^{-1}), 295.0 nm (n→π* $\varepsilon_{295.0}$ - 3236 M^{-1}); IR (KBr): 3275 (NH), 3094 (OH), 2958 (CH), 1609 cm^{-1} (CO); MS: *m/z* 287 [M^{+1}+Na^+]; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**28a**): ^{1}H NMR (500.13 MHz): δ 3.41 (αCH), 2.51 (βCH_2), 1.64 (γCH), 0.85 (δ, δ′ CH_3), 4.32 (H-1α), 4.89 (H-1β), 3.76 (H-2α), 3.45 (H-2β), 3.70 (H-3α), 3.68 (H-5); ^{13}C NMR (125 MHz): δ 53.0 (αCH), 39.4 (βCH_2), 24.0 (γCH), 22.2 (δ, δ′ CH_3), 173.0 (CO), 96.6 (C1α), 92.0 (C1β), 75.0 (C2α), 75.2 (C2β), 69.5 (C3α), 62.6 (C5α); **5-*O*-ester** (**28b**): ^{1}H NMR: δ 4.91 (H-1α), 4.99 (H-1β), 3.47 (H-2α), 3.60 (H-3α), 3.67 (H-4α),

3.64 (H-5); ^{13}C NMR: δ 101.6 (C1α), 94.2 (C1β), 74.2 (C2α), 69.5 (C3α), 67.3 (C4α), 64.8 (C5α); **2,5-di-*O*-ester** (**28c**): ^{1}H NMR: δ 4.18 (H-1α), 3.66 (H-2α), 2.96 (H-3α), 3.35 (H-5); ^{13}C NMR: δ 103.5 (C1α), 77.2 (C2α), 74.2 (C3α), 64.8 (C5α).

L-Leucyl-D-ribose (**29a-c**): Solid; HPLC t_{ret}: 3.3 min; R_f: 0.23; UV-Vis (H_2O, λ_{max}): 226.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{226.0}$ - 170 M^{-1}), 295.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{295.0}$ - 891 M^{-1}); IR (KBr): 3332 (NH), 3120 (OH), 2960 (CH), 1585 cm^{-1} (CO); MS: *m/z* 286 $[M+Na]^+$ and 400 (diester) $[M^{+1}+Na^+]$; 2D-HSQCT (DMSO-d_6): **3-*O*-ester** (**29a**): ^{1}H NMR (500.13 MHz): δ 3.41 (αCH), 1.56 (βCH_2), 1.64 (γCH), 0.85 (δ, δ′CH_3), 4.89 (H-1α), 4.85 (H-1β), 3.18 (H-2α), 3.60 (H-3α), 3.68 (H-4α), 3.64 (H-5); ^{13}C NMR (125 MHz): δ 53.0 (αCH), 39.4 (βCH_2), 24.0 (γCH), 22.2 (δ, δ′CH_3), 173.0 (CO), 92.3 (C1α), 92.6 (C1β), 71.8 (C2α), 76.0 (C3α), 67.2 (C4α), 60.8 (C5α); **5-*O*-ester** (**29b**): ^{1}H NMR: δ 4.92 (H-1α), 4.22 (H-1β), 3.45 (H-2α), 3.48 (H-3α), 3.72 (H-4α), 3.59 (H-5a) and 3.68 (H-5b); ^{13}C NMR: δ 101.5 (C1α), 97.0 (C1β), 75.8 (C2α), 69.0 (C3α), 70.8 (C4α), 63.0 (C5α); **3,5-di-*O*-ester** (**29c**): ^{1}H NMR: δ 3.27 (H-2α), 3.77 (H-3α), 3.77 (H-4α), 3.34 (H-5a), 3.37 (H-5b); ^{13}C NMR: δ 74.7 (C2α), 76.2 (C3α), 71.0 (C4α), 64.8 (C5α).

L-Leucyl-maltose (**30**): Solid; m.p.: 157°C; HPLC t_{ret}: 3.3 min; R_f: 0.13; UV-Vis (H_2O, λ_{max}): 224.0 nm ($\sigma\rightarrow\sigma^*_{224.0}$ - 617 M^{-1}), 281.0 nm ($n\rightarrow\pi^*_{281.0}$ – 191 M^{-1}); IR (KBr): 3419 (NH), 3300 (OH), 2956 (CH), 1642 cm^{-1} (CO); $[\alpha]_D^{25}$ = +54.6° (C 0.5, H_2O); MS: *m/z* 478 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **6-*O*-ester** (**30**): ^{1}H NMR (500.13 MHz): δ 3.60 (αCH), 1.58 (βCH_2), 1.69 (γCH), 0.87 (δ, δ′CH_3), 4.91 (H-1α), 4.19 (H-1β), 2.97 (H-2α), 3.23 (H-2β), 3.40 (H-3α), 3.23 (H-4β), 3.32 (H-5α), 3.33 (H-6), 5.0 (H-1′α), 3.06 (H-2′), 3.23 (H-3′), 3.63 (H-4′), 3.67 (H-5′), 3.59 (H-6′); ^{13}C NMR (125 MHz): δ 51.0 (αCH), 39.5 (βCH_2), 24.0 (γCH), 22.4 (δ, δ′ CH_3), 173.5 (CO), 94.0 (C1α), 103.5 (C1β), 73.2 (C2α), 75.4 (C2β), 76.3 (C3α), 79.9 (C4β), 70.5 (C5α), 62.9 (C6α), 102.2 (C1′α), 70.2 (C2′), 71.3 (C3′), 69.8 (C4′), 72.3 (C5′), 62.5 (C6′).

L-Leucyl-sucrose (**31**) Solid; m.p.: 125°C; HPLC t_{ret}: 3.3 min; R_f: 0.13; UV-Vis (H_2O, λ_{max}): 223.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{223.0}$ - 324 M^{-1}), 275.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{275.0}$ - 174 M^{-1}); IR (KBr): 3598 (NH), 3455 (OH), 3105 (CH), 1616 cm^{-1} (CO); $[\alpha]_D^{25}$ = -27.° (C 0.5, H_2O); MS: *m/z* 478 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **6-*O*-ester** (**31**): ^{1}H NMR (500.13 MHz): δ 3.45 (αCH), 1.55 (βCH_2), 1.68 (γCH), 0.50 (δ, δ′ CH_3), 3.39 (H-1β), 3.76 (H-3β), 3.58 (H-4β), 3.89 (H-5β), 3.64 (H-6), 5.16 (H-1′α), 3.23 (H-2′), 3.41 (H-3′), 3.49 (H-4′), 3.59 (H-5′), 3.51 (H-6′); ^{13}C NMR (125 MHz): δ 53.0 (αCH), 39.5 (βCH_2), 24.1 (γCH), 22.0 (δ, δ′CH_3), 172.0 (CO), 64.5 (C1β), 103.4 (C2β), 74.0 (C3β), 84.1 (C4β), 79.0 (C5β), 68.2 (C6β), 92.1 (C1′α), 71.3 (C2′), 71.6 (C3′), 74.5 (C4′), 72.8 (C5′), 64.5 (C6′).

L-Leucyl-D-mannitol (**32a, b**) Solid; HPLC t_{ret}: 3.3 min; R_f: 0.19; UV-Vis (H_2O, λ_{max}): 224.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{224.0}$ - 2344 M^{-1}), 294.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{294.0}$ - 1288 M^{-1}); IR (KBr): 3294 (NH), 3068 (OH), 2957 (CH), 1630 cm^{-1} (CO); MS: *m/z* 320 $[M^{+2}+Na^+]$; 2D-HSQCT (DMSO-d_6): **1-*O*-ester** (**32a**): ^{1}H NMR (500.13 MHz): δ 3.58 (αCH), 1.57 (βCH_2), 1.67 (γCH), 0.71 (δ, δ′CH_3), 3.51 (H-1), 3.45 (H-2), 3.54 (H-3), 3.56 (H-4), 3.39 (H-5), 3.46 (H-6); ^{13}C NMR (125 MHz): δ 51.5 (αCH), 39.5 (βCH_2), 24.0 (γCH), 21.8 (δ,δ′ CH_3), 171.5 (CO), 60.4 (C1), 75.4 (C2), 72.6 (C3), 71.3 (C4), 71.2(C5), 63.5 (C6); **1,6-di-*O*-ester** (**32b**): ^{1}H NMR: δ 1.02 (δ,δ′ CH_3), 3.61 (H-1), 3.32 (H-2), 3.32 (H-5), 3.64 (H-6); ^{13}C NMR: δ 21.8 (δ,δ′ CH_3), 60.4 (C1), 72.8 (C2), 70.5 (C5), 68.2 (C6).

L-Leucyl-D-sorbitol (**33**): Solid; m.p.: 155°C; HPLC t_{ret}: 3.3 min; R_f: 0.19; UV-Vis (H_2O, λ_{max}): 203.0 nm ($\sigma\rightarrow\sigma$ $\varepsilon_{203.0}$ - 4677 M^{-1}), 285.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{285.0}$ - 182 M^{-1}); IR (KBr): 3250 (NH), 3150 (OH), 2917 (CH), 1606 cm^{-1} (CO); $[\alpha]_D^{25}$ = -5.3° (C 0.5, H_2O); MS: *m/z* 316 $[M^{-2}+Na^+]$; 2D-HSQCT (DMSO-d_6): **1-*O*-ester** (**33**): ^{1}H NMR (500.13 MHz): δ 3.68 (αCH), 1.59 (βCH_2), 1.72 (γCH), 1.04 (δ, δ′CH_3), 3.51 (H-1), 3.66 (H-2), 3.56 (H-3), 3.32 (H-4), 3.32 (H-5), 3.57 (H-6); ^{13}C NMR (125 MHz): δ 51.3 (αCH), 35.9 (βCH_2), 24.0 (γCH), 22.0 (δ,δ′ CH_3), 171.6 (CO), 60.5 (C1), 73.8 (C2), 68.8 (C3), 74.2 (C4), 70.5 (C5), 63.4 (C6).

L-Isoleucyl esters of carbohydrates (34a, b to 43a, b)

L-Isoleucine (**3**): Solid; m.p.: 284°C; HPLC t_{ret}: 2.7 min; R_f: 0.33; UV-Vis (H_2O, λ_{max}): 195.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{195.0}$ - 126 M^{-1}), IR (KBr): 3415 (OH), 2945 (CH), 1584 cm^{-1} (CO); $[\alpha]_D^{25}$ = +8.6° (C 1.0, H_2O); 2D-HSQCT (DMSO-d_6): ^{1}H NMR (500.13 MHz): δ 3.03 (αCH), 1.72 (βCH), 0.84 (γCH_2), 1.38 ($\gamma' CH_3$), 0.84 (δCH_3); ^{13}C NMR (125 MHz): δ 52.5 (αCH), 36.8 (βCH), 15.1 (γCH_2), 25.0 ($\gamma' CH_3$), 11.2 (δCH_3), 171.4 (CO).

L-Isoleucyl-D-glucose (**34a, b**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.23; UV-Vis (H_2O, λ_{max}): 227.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{227.0}$ - 1862 M^{-1}), 278.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{278.0}$ - 1047 M^{-1}); IR (KBr): 3420 (NH), 3350 (OH), 2966 (CH), 1631 cm^{-1} (CO); MS: *m/z* 316 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **3-*O*-ester** (**34a**): ^1H NMR (500.13 MHz): δ 3.13 (αCH), 1.61 (βCH), 0.61 (γCH_2), 1.08 ($\gamma' CH_3$), 0.59 (δCH_3), 5.0 (H-1α), 4.4 (H-1β), 4.01 (H-3α), 3.88 (H-3β), 3.46 (H-4α), 3.58 (H-6); ^{13}C NMR (125 MHz): δ 51.0 (αCH), 35.3 (βCH), 14.0 (γCH_2), 25.6 ($\gamma' CH_3$), 11.2 (δCH_3), 171.4 (CO), 91.8 (C1α), 95.8 (C1β). 82.0 (C3α), 81.9 (C3β), 67.5 (C4α), 63.0 (C6α); **6-*O*-ester** (**34b**): ^1H NMR: δ 3.11 (αCH), 1.61 (βCH), 1.08 ($\gamma' CH_3$), 0.61 (γCH_2), 0.59 (δCH_3), 5.0 (H-1α), 4.4 (H-1β), 3.59 (H-2α), 3.48 (H-3α), 3.64 (H-4α), 3.63 (H-5α), 3.82 (H-6); ^{13}C NMR: δ 53.1 (αCH), 35.3 (βCH), 25.2 ($\gamma' CH_3$), 14.0 (γCH_2), 11.2 (δCH_3), 91.8 (C1α), 95.8 (C1β), 70.0 (C2α), 72.2 (C3α), 69.0 (C4α), 69.2 (C4α), 63.6 (C6α).

L-Isoleucyl-D-galactose (**35a-c**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.23; UV-Vis (H_2O, λ_{max}): 224.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{224.0}$ - 1622 M^{-1}), 290.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{290.0}$ - 776 M^{-1}), IR (KBr): 3309 (NH), 2944 (CH), 1621cm^{-1} (CO); MS: *m/z* 316 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**35a**): ^1H NMR (500.13 MHz): δ 3.81 (H-2α), 3.45 (H-5α), 3.40 (H-6); ^{13}C NMR (125 MHz): δ 76.2 (C2α), 70.5 (C5α), 60.4 (C6α); **3-*O*-ester** (**35b**): ^1H NMR: δ 3.58 (H-3α), 3.58 (H-3α), 3.66 (H-3β), 3.64 (H-6); ^{13}C NMR: δ 66.3 (C2α), 81.3 (C3α), 82.2 (C3β), 60.4 (C6α); **6-*O*-ester** (**35c**): ^1H NMR: δ 2.79 (αCH), 1.78 (αCH), 0.94 (γCH_2), 1.35 ($\gamma' CH_3$), 0.93 (δCH_3), 4.97 (H-1α), 4.87 (H-1β), 3.51 (H-2α), 3.69 (H-3α), 3.76 (H-4α), 3.22 (H-4β), 3.59 (H-5α), 3.30 (H-5β), 3.40 (H-6); ^{13}C NMR: δ 53.5 (αCH), 36.5 (βCH), 15.2 (γCH_2), 25.0 ($\gamma' CH_3$), 11.2 (δCH_3), 95.0 (C1α), 101.6 (C1β), 68.8 (C2α), 68.5 (C3α), 70.5 (C4α), 72.6 (C4β),75.0 (C5α), 62.8 (C6α).

L-Isoleucyl-D-mannose (**36a-e**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.23; UV-Vis (H_2O, λ_{max}): 226.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{226.0}$ - 1737.8 M^{-1}), 281.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{281.0}$ - 891 M^{-1}); IR (KBr): 3385 (NH), 3267 (OH), 2966 (CH), 1641 cm^{-1} (CO); MS: *m/z* 316 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **3-*O*-ester** (**36a**): ^1H NMR (500.13 MHz): δ 3.72 (H-3α), 3.85 (H-3β), 2.92 (H-4α), 3.41 (H-6); ^{13}C NMR (125 MHz): δ 80.5 (C3α), 83.1 (C3β), 65.9 (C4α), 60.5 (C6α); **4-*O*-ester** (**36b**): ^1H NMR: δ 2.72 (αCH), 3.80 (H-4α), 3.90 (H-4β), 3.41 (H-6); ^{13}C NMR δ : 52.7 (αCH), 75.0 (C4α), 77.1 (C4β), 60.5 (C6α); **6-*O*-ester** (**36c**): ^1H NMR: δ 2.68 (αCH), 1.72 (βCH), 0.77 (γCH_2), 1.38 ($\gamma' CH_3$), 0.79 (δCH_3), 4.31 (H-1α), 3.64 (H-2α), 3.58 (H-3α), 3.06 (H-4α), 3.58 (H-5α), 3.79 (H-6); ^{13}C NMR: δ 52.4 (αCH), 36.8 (αCH), 25.0 ($\gamma' CH_3$), 15.0 (γCH_2), 11.3 (δCH_3), 103.0 (C1α), 69.0 (C2α), 69.8 (C3α), 65.8 (C4α), 69.9 (C5α), 63.1 (C6α); **3,6-di-*O*-ester** (**36d**): ^1H NMR: δ 3.55 (H-3α), 3.41 (H-6); ^{13}C NMR: δ 82.1 (C3α), 62.9 (C6α); **4,6-di-*O*-ester** (**36e**): ^1H NMR: δ 3.75 (H-4α), 3.52 (H-6); ^{13}C NMR δ : 76.0 (C4α), 62.9 (C6α).

L-Isoleucyl-D-fructose (**37a-c**): Solid; HPLC t_{ret}: 3.5 min; R_f: 0.23; UV-Vis (H_2O, λ_{max}): 226.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{226.0}$ – 309 M^{-1}), 302.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{302.0}$ -110 M^{-1}); IR (KBr): 3378 (NH), 3217 (OH), 2900 (CH), 1632 cm^{-1} (CO); MS: *m/z* 316 $[M+ Na]^+$; 2D-HSQCT (DMSO-d_6): **1-*O*-ester** (**37a**): ^1H NMR (500.13 MHz): δ 3.15 (αCH), 1.65 (βCH), 0.87 (γCH_2), 1.35 ($\gamma' CH_3$), 0.83 (δCH_3), 3.25 (H-1α), 3.32 (H-3α), 3.32 (H-4α), 3.73 (H-4β), 3.72 (H-5β), 3.50 (H-6); ^{13}C NMR (125 MHz): δ 57.0 (αCH), 36.9 (βCH), 14.8 (γCH_2), 24.8 ($\gamma' CH_3$), 11.4 (δCH_3), 63.7 (C1α), 104.0 (C2α), 72.8 (C3α), 70.5 (C4α), 75.8 (C4β), 81.0 (C5β), 62.4 (C6α); **6-*O*-ester** (**37b**): ^1H NMR: δ 3.77 (H-5α), 3.78 (H-6); ^{13}C NMR: δ 71.5 (C5α), 63.1 (C6); **1,6-di-*O*-ester** (**37c**): ^1H NMR: δ 3.28 (H-1α); ^{13}C NMR: δ 64.4 (C1α).

L-Isoleucyl-D-arabinose (**38a-c**): Solid; HPLC t_{ret}: 3.3 min; R_f: 0.21; UV-Vis (H_2O, λ_{max}): 225.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{225.0}$ - 661 M^{-1}), 297.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{297.0}$ - 363.1 M^{-1}); IR (KBr): 3320 (NH), 3168 (OH), 2929 (CH), 1629 cm^{-1} (CO); 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**38a**): ^1H NMR (500.13 MHz): δ 3.70 (αCH), 1.65 (βCH), 1.35 (γCH_2), 0.87 ($\gamma' CH_3$), 0.83 (δCH_3), 4.32 (H-1α), 4.89 (H-1β), 3.76 (H-2α), 3.45 (H-2β), 3.70 (H-3α), 3.68 (H-5); ^{13}C NMR (125 MHz): δ 54.8 (αCH), 36.5 (βCH), 14.8 (γCH_2), 24.8 ($\gamma' CH_3$), 11.4 (δCH_3), 96.6 (C1α), 92.0 (C1β), 75.0 (C2α), 75.2 (C2β), 69.5 (C3α), 62.6 (C5α); **5-*O*-ester** (**38b**): ^1H NMR: δ 4.91 (H-1α), 4.99 (H-1β), 3.27 (H-2α), 3.60 (H-3α), 3.67 (H-4α), 3.64 (H-5); ^{13}C NMR: δ 101.6 (C1α), 94.2 (C1β), 74.2 (C2α), 69.5 (C3α), 67.3 (C4α), 64.8 (C5α); **2,5-di-*O*-ester** (**38c**): ^1H NMR: δ 4.18 (H-1α), 3.66 (H-2α), 2.96 (H-3α), 3.35 (H-5); ^{13}C NMR: δ 103.5 (C1α), 77.2 (C2α), 74.2 (C3α), 64.8 (C5α).

L-Isoleucyl-D-ribose (**39a-c**): Solid; HPLC t_{ret}: 3.3 min; R_f: 0.21; UV-Vis (H_2O, λ_{max}): 225.0 nm ($\sigma\rightarrow\sigma^*$ $\lambda_{225.0}$ - 1585 M^{-1}), 284.0 nm ($n\rightarrow\pi^*$ $\lambda_{284.0}$ - 871 M^{-1}); IR (KBr): 3384 (NH), 3233 (OH), 2936 (CH), 1631 cm^{-1} (CO); MS: *m/z* 283 [M^{-2}+Na^+]; 2D-HSQCT (DMSO-d_6): **3-*O*-ester** (**39a**): ^{1}H NMR (500.13 MHz): δ 2.86 (αCH), 1.65 (βCH), 0.87 (γCH_2), 1.35 ($\gamma' CH_3$), 0.83 (δ CH_3), 4.90 (H-1β), 3.98 (H-3α), 3.92 (H-3β), 3.64 (H-5); ^{13}C NMR (125 MHz): δ 51.1 (αCH), 36.5 (βCH), 14.8 (γCH_2), 24.8 ($\gamma' CH_3$), 11.4 (δ CH_3), 91.9 (C1α), 76.5 (C3α), 77.7 (C3β), 60.4 (C5α); **5-*O*-ester** (**39b**): ^{1}H NMR: δ 5.05 (H-1α), 3.65 (H-2α), 3.50 (H-3β), 3.87 (H-4α), 3.72 (H-4β), 3.58 (H-5); ^{13}C NMR: δ 95.9 (C1α), 73.1 (C2α), 66.5 (C3β), 70.5 (C4α), 70.7 (C4β), 63.2 (C5α); **3,5-di-*O*-ester** (**39c**): ^{1}H NMR: δ 4.64 (H-1α), 3.60 (H-3α), 3.76 (H-4α), 3.52 (H-5); ^{13}C NMR: δ 93.5 (C1α), 75.5 (C3α), 70.3 (C4α), 63.3 (C5α).

L-Isoleucyl-lactose (**40a-c**): Solid; HPLC t_{ret}: 3.4 min; R_f: 0.12; UV-Vis (H_2O, λ_{max}): 227.0nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{227.0}$ – 81 M^{-1}), 279.0nm ($n\rightarrow\pi^*$ $\varepsilon_{279.0}$ - 37 M^{-1}); IR (KBr): 3520 (NH), 3344 (OH), 2900 (CH), 1640 cm^{-1} (CO); MS: *m/z* 478 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**40a**): ^{1}H NMR (500.13 MHz): δ 4.28 (αCH), 1.78 (βCH), 0.84 (γCH_2), 1.30 ($\gamma' CH_3$), 0.81 (δ CH_3), 4.96 (H-1α), 4.32 (H-1β), 4.0 (H-2α), 3.82 (H-2β), 3.56 (H-3α), 2.96 (H-3β), 3.27 (H-4α), 3.17 (H-5α), 3.28 (H-5β), 3.49 (H-6), 5.0 (H-1′α), 3.06 (H-2′), 3.23 (H-3′), 3.63 (H-4′), 3.67 (H-5′), 3.64 (H-6′); ^{13}C NMR (125 MHz): δ 53.8 (αCH), 36.5 (βCH), 15.3 (γCH_2), 24.5 ($\gamma' CH_3$), 11.2 (δ CH_3), 172.5 (CO), 92.0 (C1α), 96.5 (C1β), 84.5 (C2α), 85.0 (C2β), 71.5 (C3α), 74.8 (C3β), 81.4 (C4α), 81.1 (C4β), 72.3 (C5α), 75.1 (C5β), 63.0 (C6α), 100.7 (C1′α), 70.3 (C2′), 71.8 (C3′), 69.9 (C4′), 72.4 (C5′), 60.6 (C6′); **6-*O*-ester** (**40b**): ^{1}H NMR: δ 3.85 (H-6), 3.50 (H-6′); ^{13}C NMR: δ 66.5 (C6α), 68.0 (C6′); **6′-*O*-ester** (**40c**): ^{1}H NMR: δ 3.50 (H-6), 3.54 (H-4′), 3.85 (H-6′); ^{13}C NMR (125 MHz): δ 61.5 (C6α), 70.0 (C4′), 66.9 (C6′).

L-Isoleucyl-maltose (41a-c): Solid; HPLC t_{ret}: 3.4 min; R_f: 0.12; UV-Vis (H_2O, λ_{max}): 224.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{224.0}$ - 525 M^{-1}), 276.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{276.0}$ - 234.4 M^{-1}); IR (KBr): 3450 (NH), 3386 (OH), 2937 (CH), 1646 cm^{-1} (CO); MS: *m/z* 478 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **2-*O*-ester** (**41a**): ^{1}H NMR (500.13 MHz): δ 4.25 (αCH), 1.78 (βCH), 0.84 (γCH_2), 1.30 ($\gamma' CH_3$), 0.81 (δCH_3), 4.91 (H-1α), 4.32 (H-1β), 4.05 (H-2α), 3.96 (H-2β), 3.40 (H-3α), 3.29 (H-4β), 3.42 (H-5α), 3.38 (H-5β), 3.48 (H-6), 5.0 (H-1′α), 3.06 (H-2′), 3.23 (H-3′), 3.63 (H-4′), 3.67 (H-5′), 3.64 (H-6′); ^{13}C NMR (125 MHz): δ 53.8 (αCH), 36.5 (βCH), 15.3 (γCH_2), 24.5 ($\gamma' CH_3$), 11.2 (δCH_3), 172.5 (CO), 92.0 (C1α), 96.5 (C1β), 81.2 (C2α), 81.4 (C2β), 76.3 (C3α), 79.9 (C4β), 72.4 (C5α), 72.8 (C5β), 63.0 (C6α), 100.7 (C1′α), 70.3 (C2′), 71.8 (C3′), 69.9 (C4′), 72.4 (C5′), 60.6 (C6′); **6-*O*-ester** (**41b**): ^{1}H NMR: δ 3.21 (H-4β), 3.32 (H-5α), 3.62 (H-6), 3.70 (H-4′); ^{13}C NMR: δ 79.5 (C4β), 70.5 (C5α), 68.0 (C6α), 69.5 (C4′); **6′-*O*-ester** (**41c**): ^{1}H NMR: δ 3.55 (H-6), 3.62 (H-6′);^{13}C NMR (125 MHz): δ 63.0 (C6α), 67.2 (C6′).

L-Isoleucyl-sucrose (**42**): Solid; m.p.: 162°C; HPLC t_{ret}: 3.4 min; R_f: 0.12; UV-Vis (H_2O, λ_{max}): 226.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{226.0}$ - 324 M^{-1}), 275.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{275.0}$ - 174 M^{-1}); IR (KBr): 3598 (NH), 3455 (OH), 3105 (CH), 1616 cm^{-1} (CO); MS: *m/z* 478 [M+ Na]$^+$; 2D-HSQCT (DMSO-d_6): **6-*O*- ester** (**42**): ^{1}H NMR (500.13 MHz): δ 4.25 (αCH), 1.78 (βCH), 0.84 (γCH_2), 1.30 ($\gamma' CH_3$), 0.81 (δ CH_3), 3.41 (H-1β), 3.31 (H-3β), 3.27 (H-4β), 3.87 (H-5β), 3.63 (H-6), 4.89 (H-1′α), 3.19 (H-2′), 3.44 (H-3′), 3.32 (H-4′), 3.70 (H-5′), 3.63 (H-6′a) and 3.71 (H-6′b); ^{13}C NMR (125 MHz): δ 54.8 (αCH), 36.9 (βCH), 24.5 (γCH_2), 15.3 ($\gamma' CH_3$), 11.2 (δCH_3), 172.5 (CO), 62.3 (C1β), 103.5 (C2β), 73.0 (C3β, 81.0 (C4β), 74.5 (C5β), 67.2 (C6), 92.0 (C1′α), 71.8 (C2′), 75.0 (C3′), 70.5 (C4′), 69.5 (C5′), 60.7 (C6′).

L-Isoleucyl-D-mannitol (**43a, b**): Solid; HPLC t_{ret}: 3.3 min; R_f: 0.15; UV-Vis (H_2O, λ_{max}): 214.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{214.0}$ - 87 M^{-1}), 281.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{281.0}$ - 22 M^{-1}); IR (KBr): 3425 (NH), 3068 (OH), 2957 (CH), 1630 cm^{-1} (CO); MS: *m/z* 319 [M^{+1}+ Na^+]; 2D-HSQCT (DMSO-d_6): **1-*O*-ester** (**43a**): ^{1}H NMR (500.13 MHz): δ 3.15 (αCH), 1.78 (βCH), 1.30 (γCH_2), 0.84 ($\gamma' CH_3$), 0.81 (δCH_3), 3.49 (H-1a), 3.53 (H-1b), 3.41 (H-2), 3.48 (H-3), 3.54 (H-4), 3.32 (H-5), 3.45 (H-6); ^{13}C NMR (125 MHz): δ 58.2 (αCH), 39.5 (βCH), 24.5 (γCH_2), 15.3 ($\delta' CH_3$), 11.2 (δCH_3), 172.5 (CO), 60.8 (C1), 71.2 (C2), 69.0 (C3), 71.0 (C4), 70.8 (C5), 63.8 (C6); **1,6-di-*O*-ester** (**43b**): ^{1}H NMR: δ 3.63 (H-1), 3.45 (H-2), 3.63 (H-3), 3.32 (H-5), 3.29 (H-6); ^{13}C NMR: δ 60.8 (C1), 75.2 (C2), 68.0 (C3), 73.0 (C5), 63.5 (C6).

Acknowledgements

Department of Science and Technology, India, is gratefully acknowledged for funding this project. BRS acknowledges the DST, India, for providing the

Junior Research Fellowship. Authors also acknowledge Sophisticated Instruments Facility, Indian Institute of Science, Bangalore for recording the NMR spectra.

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