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ARTICLE

Synthesis and antibacterial activity of ricinoleic acid glycosides

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The antibacterial properties of twenty-eight novel ricinoleic acid glycosides synthesized by Koenigs-Knorr glycosylation are reported. Seven of them were found to show promising wide spectrum antibacterial activity against Gram positive bacteria of which two compounds, the mannopyranosyl- and the arabinofuranosyl derivatives, were proven effective against various non-clinical/clinical/NorA-overexpressed/resistant strains of *Staphylococcus aureus* as well as other Gram +ve bacteria such as *Bacillus subtilis* ATCC 6051 and *Micrococcus luteus* MTCC 2470. It was found that both the presence of the sugar and its structure are necessary and important for the compounds to be bioactive. The methyl ester protection of the carboxylic acid moiety of the ricinoleic acid unit was also found important for imparting good bioactivity to the molecule. Based on the membrane permeability and cell disintegration studies, these compounds are found to be increasing the bacterial cell membrane permeability and subsequently causing the cell death.

Introduction

Traditionally, man has relied heavily on natural products for alleviating their problems associated with diseases and infections caused by various microbes. It is therefore not surprising that many of the antibiotics currently in use are natural products or natural products-based/inspired. Although, following a surge in the development and marketing of antibiotics that was witnessed during the first three quarters of last century, it was suggested during the fourth quarter of the century that there was perhaps no unmet needs in the antibiotic therapeutics,[1] a renewed search for new antibiotic molecules returned by the turn of the century, largely due to the increasing emergence of instances of drug resistance.[2,3,4]In fact antibiotic-resistant infections has now become a major health concern globally. Thus, the incidents of drug resistant cases of methicillin resistant *Staphylococcus aureus* (MRSA),[5,6]as well as multidrug drug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB)[7] are being frequented worldwide and hence the demand for identifying drug candidates with novel modes of action has been growing.[7,8]Many of the drugs used for the treatment of bacteria including mycobacterial infection are cell wall biosynthesis inhibitors.[9] The bacterial cell wall possesses a complex architecture made up of, besides others, glycans constituted of sugars such as *N*-acetylglucosamine, mannose, galactopyranose (Gal*p*), galactofuranose (Gal*f*), arabinofuranose, ribose and rhamnose,[10] and use different biosynthetic pathways, many of which are validated drug targets, for the construction of these glycan structures.

The antimicrobial activity of unsaturated fatty acids such as oleic/linoleic/linolenic/arachidonic acid, etc, as for example evident from their use as effective antimicrobial food additives, is well known.[11,12]Likewise, ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid, **1**, Fig 1), found in castor oil is also well-known for its medicinal values and commercial importance. The first report of ricinoleic acid derivative, for example, sodium ricinoleate (**2a**, Fig 1) was as a surface tension depressant on the growth of bacterial culture media.[13,14] Its ability to act as an antibacterial agent against organisms such as *Corynebacterium diphtheria*, *Gonococci*,etc has also been demonstrated.[15,16,17] Potassium and zinc ricinoleates (**2b**and **4**, Fig 1) were also shown to be antibacterial in nature.[15] Presently ricinoleic acid and its various salts/derivatives have reportedly been used in more than 750 commercial products[18] in which they also fulfill their role as emulsion stabilizers, cosmetics, skin-conditioning agents, etc. Also, mono- and diglycerides of ricinoleic acid have been reported as potential antimycobacterial agents.[19]D'Oca*et al.* reported different fatty acid amides and their structure activity relationship in the context of anti-TB activity and ricinoleic acid amide (**5**, Fig 1) was among those exhibited promising anti-TB activity against *M. tb* $H_{37}Rv$.[20] More recently, during the progress of this work, certain Schiff base analogues of methyl ricinoleate were shown to be good antimicrobials against *S. aureus.*[21]

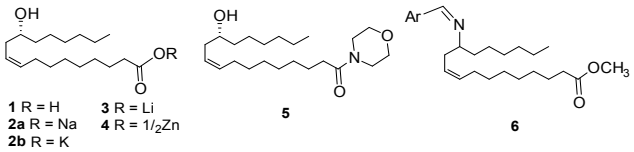


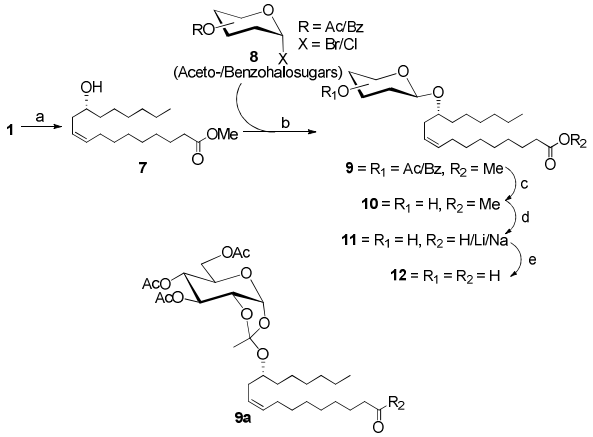
Figure 1: Ricinoleic acid-derived compounds known in the literature as anti-bacterial and antimycobacterial agents.

The present work was aimed at synthesizing a library of various glycosides of ricinoleic acid with a view to evaluating their potential as antibacterial agents. D-Glucose, D-galactopyranose, D-mannose, *N*-acetyl-D-glucosamine, L-rhamnose, D-arabinose, D-galactofuranose and D-lactose were chosen as the sugar substrates to be linked glycosidically to methyl ricinoleate.

Results and discussion

Methyl ricinoleate (**7**), prepared by reacting **1** with MeOH in the presence of anhydrous HCl,[22] was chosen as the acceptor alcohol for glycosylation with various acetohalosugars (**8**) essentially under the traditional Ag_2CO_3-$AgClO_4$-mediated Koenigs-Knorr conditions to afford the desired 1,2-*trans*-linked glycosides (**9**) in good yields (Scheme 1). The structure of these compounds were confirmed by

NMR spectroscopy in which the $J_{1,2}$value of 7.9-8.0 Hz was typical, for example, of the 1,2-*trans*-linked H-1 and H-2 for the *galacto*- and *gluco*-configured pyranosides. Likewise, the *manno*-, *arabino*-, *rhamno*- and the *galactofurano*-compounds also gave the $J_{1,2}$ values characteristic of them (1.5-1.7 Hz, see electronic SI for details). It was observed that among the three hexose-derived acetobromosugars (**13**-**15**) the comparatively less reactive *gluco*-compound (**14**), not surprisingly though, did also yield some orthoester (**9a**) as a by-product (see electronic SI for details). The acyl group deprotection of these glycoside-derivatives was done by *trans*-esterification using methanolic NaOMe to afford **10**. Compound **10** upon aqueous alkaline hydrolysis yielded the desired glycosylated ricinoleic acids **11** in the form of their metal salts corresponding to the alkali employed for the hydrolysis.



Reagents and conditions: (i) HCl/MeOH, rt, 4 h, 90%; (ii) Ag_2CO_3, $AgClO_4$, powdered molecular sieves (4Å), CH_2Cl_2 rt, 10-12 h, 53-68%; (iii) NaOMe/MeOH, 20-40 min, rt, 90-94%; (iv) LiOH/aq THF, 2-4 h, rt, 80-87%; (v) Amberlite IR 120 (H^+), rt, 80-88%

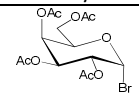
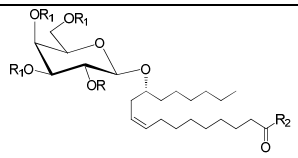
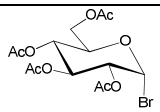
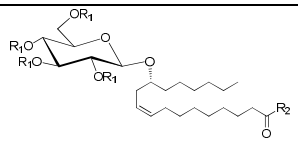
Scheme1. Synthesis of ricinoleic acid glycosides in their partially/fully deprotected form and/as their sodium/lithium salts.

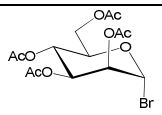
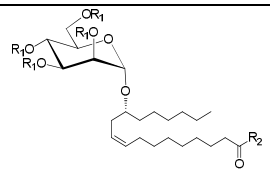
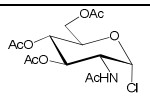
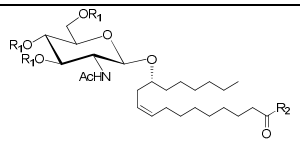
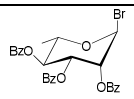
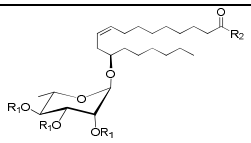
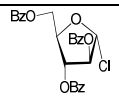
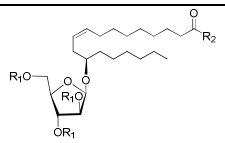
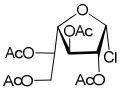
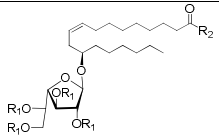
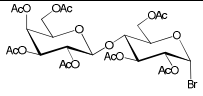
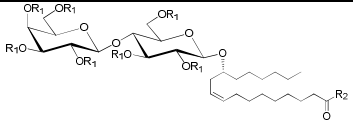
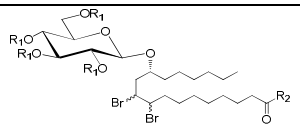
When the free acid was required, the salts obtained were deionized using Amberlite IR 120 H^+ resin (Scheme 1). The compounds synthesized (**21**-**48**) are shown in Table 1.

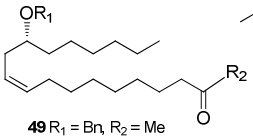
While acetobromosugars (**13**-**15** and **20**) were used as starting materials for the synthesis of the hexopyranose-based **21**, **24**, **28** and **44** (mono- and disaccharide derivatives), the acetochlorosugar donors **16** and **19** were found more convenient for the preparation of the corresponding glucosaminide and the galactofuranoside derivatives **32** and **41** as shown in Table 1. On the other hand for the rhamnopyranosyl and the arabinofuranosyl ricinoleates**35** and **38**, the easily accessible benzobromo- and benzochlorosugar donors **17** and **18**, respectively, were utilized for the crucial glycosylation reaction.

In view of the presence of the double bond in the compounds described above, choosing **24** as a model compound, the easily obtainable dibromo derivative **47** (and **48** derived therefrom) was also synthesized from **24** (Table 1, last entry). As expected **47** obtained on addition of molecular bromine to the double bond in **24** was in the form of a pair of enantiomers (as also the derived **48**) as evident from the NMR spectra of **47**. Further, in order to establish the importance (or otherwise) of the sugar moiety in the biological activity to be studied, a set of ether analogues **49** (benzyl ether) and **50** (methyl ether) were also synthesized from methyl ricinoleate **7**. It must be pointed out that for the synthesis of the 12-*O*-benzyl ricinoleate **49** from **7** and BnBr neither the method using Ag_2O [23] nor the one using Cs_2CO_3 [24] was successful. However, the reaction of the two (**7** and BnBr) promoted by NaH yielded the desired product **49** in admixture with **49a** as an inseparable mixture in a ratio of 1:0.5 as determined by the ^{1}H NMR spectroscopy of the chromatographically isolated mixture (see electronic SI for details). The mixture was therefore subjected to the conditions of Zemplen transesterification in MeOH whereby the benzyl ester **49a** was transformed into the methyl ester analogue **49** thus affording the latter overall neatly.

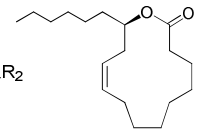
Table 1. Different ricinoleic acid glycosides and their partially or fully deprotected and sodium/lithium salt derivatives

Acetohalosugar used for synthesis	Glycosylated ricinoleates synthesized		Yield (%)
	Structure	Compound number	
 **13**		**21**, R_1 = Ac, R_2 = OCH_3	68
		22, R_1 = H, R_2 = OCH_3	92
		23, R_1 = H, R_2 = OH	87
 **14**		**24**, R_1 = Ac, R_2 = OCH_3 [25]	58
		25, R_1 = H, R_2 = OCH_3	90
		26, R_1 = H, R_2 = OH	81
		27, R_1 = H, R_2 = O^-Li^+	83

 **15**		**28**, R_1 = Ac, R_2 = OCH_3 **29**, R_1 = H, R_2 = OCH_3 **30**, R_1 = H, R_2 = OH **31**, R_1 = H, R_2 = O^-Na^+	60 94 85 80
 **16**		**32**, R_1 = Ac, R_2 = OCH_3 **33**, R_1 = H, R_2 = OCH_3 **34**, R_1 = H, R_2 = OH	52 92 83
 **17**		**35**, R_1 = Bz, R_2 = OCH_3 **36**, R_1 = H, R_2 = OCH_3 **37**, R_1 = H, R_2 = OH	66 91 87
 **18**		**38**, R_1 = Bz, R_2 = OCH_3 **39**, R_1 = H, R_2 = OCH_3 **40**, R_1 = H, R_2 = OH	57 92 79
 **19**		**41**, R_1 = Ac, R_2 = OCH_3 **42**, R_1 = H, R_2 = OCH_3 **43**, R_1 = H, R_2 = OH	60 94 85
 **20**		**44**, R_1 = Ac, R_2 = OCH_3 **45**, R_1 = H, R_2 = OCH_3 **46**, R_1 = H, R_2 = OH	53 91 84
24 (For the structure, see the entry at row 2 above)		**47**, R_1 = Ac, R_2 = OCH_3 **48**, R_1 = H, R_2 = OCH_3	80 87



49 R_1 = Bn, R_2 = Me
49a R_1 = R_2 = Bn
50 R_1 = R_2 = Me



50a

Likewise, the reaction of **7** with MeI (promoted by NaH) was also successful in yielding the methyl ether analogue **50** but, again, along with an inseparable by-product. But unlike **49a**, the by-product in the latter case was a mixture of **50**[26] and the lactone **50a** (in a ratio of 1:0.5) as characterized by ^{1}H NMR spectroscopy (see electronic SI for details).The separation of the lactone (**50a**) from the ester (**50**)could however be very successfully facilitated by methanolysis of the lactone (to yield **7**) using anhydrous methanolic hydrogen chloride followed by purification by column chromatography.

Evaluation of antimicrobial activity and observations on the structure activity relationship (SAR)

Once in hand, the compounds described above were evaluated for their antimicrobial activity. In the initial screening they were tested for their activity against three Gram +ve bacteria, viz. *Micrococcus luteus* (MTCC-2470), *Staphylococcus aureus* (MTCC-96), *and Bacillus subtilis* (MTCC-121), three Gram –ve bacteria, viz. *E. coli* (MTCC-739), *Pseudomonas aeruginosa* (MTCC-2453), *Klebsiella planticola* (MTCC-530) and a fungus, *Candida albicans* (MTCC-3017). The evaluations were done on the basis of the zone of inhibition (results not shown here, see electronic **SI** for details) in the usual manner. The deacylated glycosides of methyl ricinoleate **22**, **25**, **29**, **36**, **39**, **42**, **45**, and **48** showed good inhibition of the Gram +ve bacteria evaluated. However, the analogous *N*-acetyl-glucosamine-linked methyl ricinoleate **33** was proved inactive. Also, the glycosides of methyl ricinoleate showed better inhibition as compared to the

simple ether analogues methyl 12-*O*-benzyl ricinoleate (**49**) and methyl 12-*O*-methyl ricinoleate (**50**). Neither methyl ricinoleate (**7**) nor ricinoleic acid/its lithium/sodium salt (**1**/**3**/**2a**) were also active against any of the strains tested above (see electronic SI for details). This showed the importance of the presence of sugar unit in the structure for the antimicrobial activity. Smith *et al* while evaluating the antimicrobial activity of 6-*O*-substituted fatty alkyl ethers/fatty acyl esters of methyl glucopyranosides similarly had observed the importance of the presence of the sugar residue (as well as its nature) in the molecule for significant antimicrobial activity.[27] Again, neither the acyl-protected glycosides of methyl ricinoleate **21**, **24**, **28**, **32**, **35**, **38** and **41** nor the fully deprotected ricinoleic acid glycosides (as free acids) **23**, **26**, **30**, **37**, **40**, **43** and **46** were active against either of the Gram +ve or the Gram –ve bacteria tested. However, compounds **37** (the rhamnosylated compound) and **44** (the lactose-linked compound) showed moderate inhibition against *Bacillus subtilis* (MTCC-121). This revealed the importance of ricinoleic acid present in the form of ester in imparting the inhibitory activity. In the work of Smith *et al* referred to above also, they had observed that the 6-*O*-acyl glucosides were more active than the corresponding ethers.[27] The deacylated 9,10-dibromo compound **48** (as racemic mixture) showed better inhibition against *M. luteus* (MTCC-2470) and *S. aureus* (MTCC-96) compared to the corresponding acylated compound **47** which was inactive. Moreover, the sodium/lithium salts of the deacylated ricinoleic acid glycosides **27** and **31** were also inactive as were the salts of the acid (**2** and **3**) themselves. Based on these initial results compounds **22**, **25**, **29**, **36**,**37**,**39**, **42**, **44, 45** and **48**were taken up for the determination of their MIC values against different *Staphylococcal* strains along with some of the standard drugs in use for comparison. Of these glycosides studied **37**, the L-rhamnosylated methyl ester, and **44** and **45**,the lactosylated methyl esters, showed high MIC values (>1000, results not shown) against the non-clinical *S. aureus* MTCC-96. This reiterated the fact that not only that the acid has to be in its methyl ester form but also that not all sugars are suitable as substituents on the ricinoleic acid derivatives for them to be sufficiently biologically active. The above experiments thus clearly elucidated the importance of the structure of the sugar moiety present in the molecule. The poorer activity of the free acid **37** is in line with the fact that ester form of the acid is necessary for the compound to be effectively active. Likewise, the bulky disaccharide units in **44** and **45** were also proven to be detrimental for good activity. Based on these results compounds **37, 44** and **45** were excluded from further *in vitro* screening experiments on the bacterial strains. Instead, for ascertaining the generality of application of these compounds to other Gram +ve bacterial strains, *Bacillus subtilis* and *Micrococcus luteus* strains were included in the study. The results obtained for the promising compounds chosen are summarized in Table 2 and clearly demonstrates the effectiveness of these compounds to act against all the Gram +ve bacteria tested.

Table 2 Antibacterial activity of methyl ricinoleate-glycosides against *Staphylococcal, Bacillus subtilis* and *Micrococcus luteus* strains

Entry	Test compounds/ Antibiotics	MIC (µg/ml)					
		S. aureus MTCC-96 ~ ATCC 9144 (non-clinical)	MRSA 831(clinical isolate)	*S. aureus* 1199 (wild type clinical isolate)	*S. aureus* 1199B (Nor A overproducing strain)	*Bacillus subtilis* (MTCC 121 ~ ATCC 6051)	*Micrococcus luteus* (MTCC 2470)
1	**22**	16	1000	8	125	16	16
2	**25**	8	1000	8	1000	2	2
3	**29**	**2**	1000	**2**	**32**	**0.25**	**0.5**
4	**36**	4	1000	250	1000	0.125	0.5
5	**39**	**4**	**32**	**8**	**4**	**0.5**	**1**
6	**42**	4	1000	2	8	1	2
7	**48**	32	1000	62.5	125	2	4
8	Erythromycin	0.78	25	0.39	6.12	0.5	1
9	Teicoplanin	1.56	12.5	0.19	3.125	0.25	0.5
10	Norfloxacin	0.39	100	0.78	50	0.5	2
11	Oxacillin	3.12	2000	0.48	1000	0.5	1
12	Ampicillin	32	250	1	4	4	16
13	Amoxicillin	16	250	0.5	2	2	4
14	Linezolid	1.56	3.9	1.95	7.8	1	2
15	Vancomycin	1.56	3.12	0.78	6.25	0.125	1

Possibly the glycosides of methyl ricinoleate would have permeabilized the bacterial cell membrane more effectively due to their better surfactant properties[11] and thus showing the antibacterial activity. As it has been reported in the past, another possible cause for the antibacterial properties of these compounds could be the inhibition of fatty acid biosynthesis (Fab1) enzyme which is an essential component of the fatty acid synthesis in Gram +ve bacteria.[11,28,29] Among the promisingly active compounds, **29** and **39** showed (entries 3 and 5, Table 2) a wide spectrum antibacterial activity against different *Staphylococcal* species. It is possible that the glycolipids **29** and **39** may be playing some specific role in the bacterial cell wall biosynthesis due to the presence of mannose and arabinose in them. They could also possibly act by inhibiting the transferases (or any other glycan-forming enzymes) involved in the bacterial cell wall biosynthesis. To determine the above mentioned possible mechanism of action of these glycosides of ricinoleic acid a set of experiments with compounds **29**, **39** and vancomycin (selectively active against Gram +ve bacteria, as the positive control) was carried out as described below.

The binding of vancomycin to D-Ala-D-Ala prevents cell wall synthesis of the long polymers of *N*-acetylmuramic acid and *N*-acetylglucosamine that form the backbone strands of the bacterial cell wall, and it prevents the backbone polymers that do manage to form cross-linking with each other. Since the MIC of vancomycin is more or less matching with **29** and **39**, we used these compounds for membrane permeability assay as well as cell membrane disintegration assay (see below). The cell permeability effect of **29** and **39** is comparable with vancomycin, a membrane active antibiotic. The uniform increase in the absorbance of *o*-nitrophenol suggests that the non-membrane permeable *o*-nitrophenyl-β-galactoside (ONPG) is slowly permeabilized inside the bacterial cells and subsequently is hydrolyzed by the intracellular β-galactosidase to *o*-nitrophenol that shows absorbance at 410 nm. As shown in Fig. 2 compounds **29** and **39** induced an increase in the permeability of *S. aureus* cytoplasmic membrane over a period of time in a manner comparable to that of vancomycin. Moreover, the test compounds as well as vancomycin possessed similar MIC values against *S. aureus*. This suggests that the cytoplasmic membrane would have been permeabilized by compounds **29** and **39**.

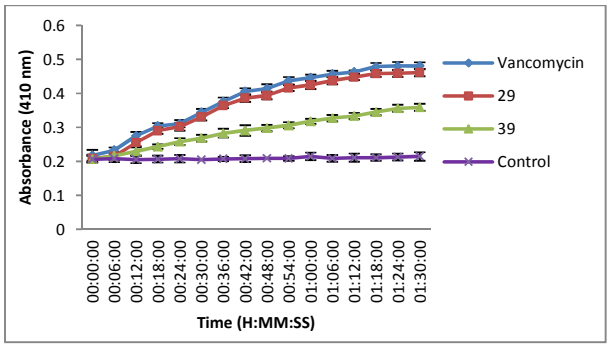


Figure 2: The cytoplasmic membrane permeabilization of *S. aureus* cells treated with compounds **29** (squares), **39** (triangles) and vancomycin (diamonds).The untreated *S. aureus* cells (crosses) were taken as control. (Compounds **29** and **39** induced an increase in the permeability of *S. aureus*. The experiment was carried out two times in triplicate. The results are presented as mean ± SD.)

To verify whether the cells are permeabilized or disintegrated, the propidium iodide (PI) uptake assay was carried out. PI is a viability fluorescent marker that can penetrate impaired cells and intercalate into nucleic acids. Thus, compounds **29**-, **39**- and vancomycin- induced membrane damage of *S. aureus* cells, was determined by treating the cells with PI at 37 °C for half an hour using Synergy reader (BioTek, USA). As shown in Fig. 3, in the absence of any antibacterial agent, the untreated control cells of *S. aureus* showed no PI fluorescence signal. However a significant increase in the PI fluorescence signal was observed in the case of **29**, **39** and vancomycin. These results indicate that the membrane integrity of *S. aureus* cells was affected by the treatment with the test compounds as comparable to that of vancomycin.

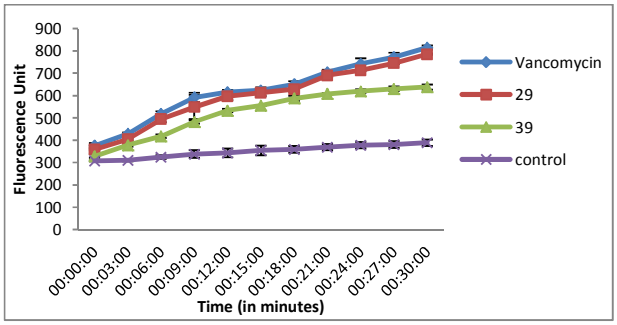


Figure 3: Effect of **29** (squares), **39** (triangles) and vancomycin (diamonds) on membrane integrity of *S. aureus* cells by propidium iodide uptake assay. The untreated *S. aureus* cells (crosses) were taken as control. For each sample 10^6 CFU ml^{-1} were analyzed. The membrane integrity of *S. aureus* cells was destroyed by **29** and **39**. The experiment was carried out two times in triplicate. The results were presented as mean ± SD.

The cytoplasmic membrane is the main barrier that limits the distribution and entry of antibiotics. In addition to the antimicrobial activities, some serve as an anti-resistance compounds and are able to interact with bacterial membranes, and create ion permeable channels leading to an increased cytoplasmic membrane permeability and hence, bacterial cell death.[30] In the case of **29** and **39**, they showed permeabilization effect on *S. aureus* membrane and increased the plasma membrane permeability for entry of ONPG into cells. Moreover, the gradual increase in the fluorescence due to the influx of PI into the cells, indicates that the cytoplasmic membrane could be the most probable target of action of ricinoleic acid glycosides.

Cytotoxicity

The compounds that showed good MIC values in the antibacterial activity evaluation were taken for the cell viability study with a view to assessing their toxicity characteristics. According to FDA castor oil is classified as a safe and effective stimulant laxative[18]and it was hoped that attaching a sugar molecule would not result in seriously altering its cytotoxic tolerance. It was therefore not surprising that the synthesized glycosides of methyl ricinoleate **22**, **25**, **29**, **33**, **36**, **39**, **42**, **45**, and **48** exhibited no significant cytotoxicity when tested up to 500 µg mL^{-1} (cell viability >75 %) on J774A.1 cells as shown in Fig 4. However, at higher concentrations of 1000 µg mL^{-1} compounds **25**, **42**, **45**, and **48**demonstrated cell viability of only 56.2, 68.4, 67.5 and 56.8 % respectively. Thus, the results are indicative of good potential for these compounds to be studied in detail further

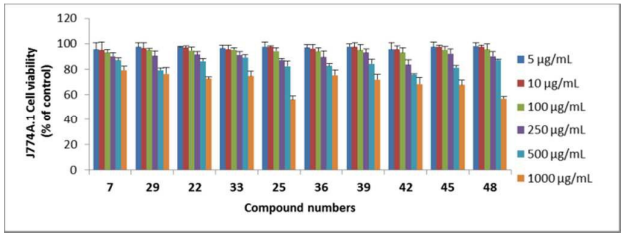


Figure 4: Cytotoxicity study of biologically active glycosides of methyl ricinoleate (Results are expressed as mean ± SD of three independent experiments performed in triplicates)

Experimental

Materials and methods

All reagents and chemicals were purchased from Sigma-Aldrich and were used without further purification. TLC analyses were performed on 0.2 mm Merck pre-coated silica gel 60 F_{254} aluminium sheets and the spots were visualized under UV lamp and/or by immersion in an ethanolic solution of sulphuric acid (5%, v/v) followed by heating. Final purifications were performed using silica gel 200-400 mesh size. Specific rotations were recorded on a Rudolph Autopol IV Polarimeter at 20 ˚C. NMR spectra were recorded on a Bruker Avance DPX (400 MHz) spectrometer. ^{1}H NMR and ^{13}C NMR spectra were referenced to the internal standard tetramethylsilane, in the respective deuterated solvents. Coupling constants (*J*) are reported in Hertz. All assignments were confirmed with the aid of two-dimensional ^{1}H-^{1}H (COSY) and/or ^{1}H-^{13}C (HSQC) experiments using standard pulse programs. Processing of the spectra was performed with MestReNova software. High resolution mass spectra (HRMS) were recorded on a Bruker Maxis spectrometer. Biosafety Cabinet (Clean Air, Chennai, India), CO_2 incubator (WTC Binder, Tuttlingen, Germany), Ultracentrifuge (Sigma, St. Louis, MO, USA), autopipettes, ELISA plate reader (Labsystems, Helsinki, Finland) and Neubauer chamber (HBG, Gießen, Germany) were used for the cell culture.

Materials and methods for antimicrobial evaluation

Chemicals and reagents used for biological evaluation

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and standard antibiotics such as ampicillin, amoxicillin, amphoterecin B, norfloxacin, oxacillin, linezolid, vancomycin, teicoplanin and erythromycin were procured from Sigma-Aldrich, USA. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), antibiotic solution and other chemicals for the biological experiments were purchased from Hi-media Limited (Mumbai, India). Lipopolysaccharide (*Escherichia coli* 026:B6, LPS) and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Bacterial strains

Clinical isolate of *S. aureus* (MRSA-831) was obtained from Government Medical College and Hospital, Chandigarh. *S. aureus* 1199 (SA-1199) is a methicillin- and fluoroquinolone-susceptible clinical isolate. *S. aureus* 1199B (SA-1199B) is fluoroquinolone-resistant mutant of SA-1199 strain that was recovered from the blood and cardiac vegetation of rabbits that had experimental endocarditis with SA-1199 and had failed ciprofloxacin therapy given for the treatment of this infection.[31]Both the strains viz. SA-1199 and SA-1199B were obtained from CSIR-Indian Institute of Integrative Medicine, Jammu, India with kind permission of Dr G. W. Kaatz, Wayne State University School of Medicine, and Detroit, MI, USA. *S. aureus* MTCC 96 (SA-96), *Micrococcus luteus* MTCC 2470, *Bacillus subtilis* MTCC 121, *E. coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella planticola* 530 and *Candida albicans* MTCC 3017 were obtained from Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh.

Susceptibility study of *S. aureus* strains and MIC determination

For the initial screening of the antimicrobial activity exhibited by synthesized compounds they were tested by the agar diffusion method. We used *Candida albicans* MTCC 3017 and the battery of Gram's +ve and Gram -ve test bacterial strains, viz. *Micrococcus luteus* MTCC 2470, *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96, *E. coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella planticola* MTCC 530 with suitable positive control for antibacterial and antifungal. The susceptibility of MRSA-831, SA-1199, SA-1199B and SA-96 towards various antibiotics was studied. It comprised MIC determination of antibiotics in Ca^{2+} and Mg^{2+}adjusted Muller Hinton Broth (MHB) as described previously [32].Based on literature, the specific concentration range was used for individual antibiotic and serial dilutions were made accordingly. Similarly, the MIC of standards and shortlisted test compounds was determined in MHB. Briefly, the compounds were first dissolved in DMSO and then diluted in MHB, to give the starting concentration of 400 µg mL^{-1}which was diluted across a 96 well microtiter plate in two fold serial dilution to give the final concentration range from 400 to 0.195 µg mL^{-1}. Bacterial inocula equivalent to the 0.5 McFarland standards were prepared in normal saline and diluted to give the final density of 5×10^{5} cfu mL^{-1}. The inoculum (100 µL) was added to all the wells and the microtiter plate was incubated at 37 °C for 48 h. The MIC was recorded as the lowest concentration at which no bacterial growth was observed. This was facilitated by the addition of 20 µL of MTT at the concentration 10 mg mL^{-1} in methanol to each well and incubated at 37 °C for 20 min where bacterial growth was indicated by purple coloration adhered to cells. Appropriate DMSO, cell and sterile saline controls were carried out in the same set of experiments.

Cytoplasmic membrane permeability assay:

To demonstrate the membrane permeability, previously described method was adopted.[30,33] *Staphylococcus aureus* MTCC 96 cells were grown in M9 minimal medium with lactose as a sole carbon source from a single colony, overnight at 37˚C. After three washings in phosphate buffer saline (PBS, pH 7.4), the culture was diluted to 10^{6} CFU ml^{-1} in PBS and added to all wells in a non-culture-treated polystyrene microplate, together with compounds **29**, **39** and vancomycin (as a positive control) at MIC concentrations of 2µg ml^{-1}, 4 µg $ml^{-1}$and 1.56 µg ml^{-1},respectively. Each well also contained 1.5mM ONPG in PBS. The plates were incubated with gentle shaking at 37˚C. The hydrolysis of ONPG to o-nitrophenol over time was monitored at 410 nm with a microplate reader (BioTek, USA). Similar procedure was adopted for untreated cells, taken as control.

Propidium iodide uptake assay:

According to previously described method[34] *Staphylococcus aureus* MTCC 96 cells were collected in mid-log phase. This was followed by addition of compounds **29**, **39** and vancomycin (as a positive control) at MIC concentrations of 2µg ml^{-1}, 4µg ml^{-1} and 1.56µg ml^{-1}, respectively. Then the mixtures were incubated for 6 h at 3737˚C. The bacterial cells were washed 2-3 times with PBS (pH 7.4) and resuspended at a concentration of 10^{6} CFU ml^{-1} in PBS. Cells were treated with propidium iodide (PI) at 10µM concentration and aliquots of 200µl were transferred in triplicate to a 96-well plate. With excitation wavelength of 535 nm and emission wavelength of 625 nm, PI fluorescence was monitored for 30 min at 3 min interval using Synergy HT multi-mode microplate reader (BioTek, USA). Similar procedure was adopted with untreated cells, taken as control.

Cell viability assay

J774A.1 cells were obtained from the National Center for Cell Science (NCCS, Pune, India) and were cultured in 250 ml culture flasks containing DMEM media supplemented with heat-inactivated 10% FBS, 10,000 units ml^{-1} penicillin and 10 mg ml^{-1} streptomycin in 0.9% saline, in a CO_2 incubator (5% CO_2 in air) at 37 °C. The conventional MTT assay was carried out to assess the cell viability of J774A.1 cells using previously reported method.[35] All test samples dissolved in DMSO and 0.05% DMSO was used as the control group. The cell viability was calculated in respect to that of control (% of control).

General experimental procedures

General experimental procedure of Koenigs-Knorr glycosylation conditions for the synthesis of glycosides of ricinoleic acid

Methyl ricinoleate (**7**, 2.5 equiv.) was added to a mixture of the glycosyl halide (**13**[36]/**14**[36]/**15**[36]/**16**[37]/**17**[38]/**18**[39]/**19**[40]/**20**[36]), Ag_2CO_3 (1.1 equiv.), $AgClO_4$(1.1 equiv.) and powdered molecular sieves (4Å, weight equivalent to that of **7**) in anhydrous CH_2Cl_2 and was stirred at the ambient temperature (23-27 °C) for 14-18 h in the dark under an inert gas atmosphere. When the reaction was complete (from the completion of the consumption of **8** as judged by TLC) the mixture was diluted with CH_2Cl_2 and the insolubles were separated by filtration through a Celite-pad. The filtrate after concentration to dryness under reduced pressure was chromatographed on a column of silica gel using EtOAc:hexanes as the eluent to yield the respective glycosides (**21**/**24**/**28**/**32**/**35**/**38**/**41**/**44**, respectively) of methyl ricinoleate.

General experimental procedure for the de-acylation of acyl-protected glycosides 21/24/28/32/35/38/41/44 by Zemplen's transesterification

To a solution of the glycoside **21/24/28/32/35/38/41/44/47** in anhydrous MeOH was added a catalytic amount of NaOMe and it was stirred at room temperature until the reaction was complete (0.5-4 h, depending upon the sugar derivative used, as judged by TLC). The solution was neutralized with Amberlite IR 120 H^+ resin and, after the removal of the used resin by filtration, was concentrated to afford the respective deacylated product (**22/25/29/33/36/39/42/45/48**) in good yield. In all cases they were obtained in the form of syrup except **33** which was obtained as an amorphous fluffy powder.

General procedure for the saponification of methyl ricinoleate-glycosides 21/24/28/32/35/38/41/44

Freshly prepared aqueous LiOH (1M, 0.3 mL/100 mg ester, **21/24/28/32/35/38/41/44**) was added to the desired acyl-protected glycoside of the methyl ricinoleate dissolved in THF (2 mL/100 mg) and was allowed to stand with gentle stirring for 3-8 h at room temperature. The reaction mixture that turned turbid on the addition of the alkali became clear by the completion of the hydrolysis which was also confirmed by TLC. Addition of Amberlite IR 120 H^+ resin to a slightly acidic pH (approximately 6) followed by removal of the used resin by filtration and concentration of the filtrate under reduced pressure to dryness afforded the respective ricinoleic acids **23/26/30/34/37/40/43/46**in good yield.

General procedure for the preparation of ricinoleic acid-glycosides as their Li^+/Na^+ salt

Freshly prepared aqueous alkali (1M, LiOH or NaOH as desired; 0.3 mL/100 mg ester) was added to a solution of the methyl ricinoleate derivative **24/28** in THF and was stirred for 3-5 h at room temperature. The reaction mixture that turned turbid on the addition of the alkali became clear by the completion of the hydrolysis which was also confirmed by TLC. It was then concentrated to a small volume and was then diluted with EtOAc when the product got precipitated. Filtration and successive washing with a small volume of cold water and EtOAc gave the desired product **27/31** in good yields after drying.

Lithium ricinoleate (3)

The title compound **3** was prepared from **2** (500 mg, 1.6 mmol), THF (10 mL) and aqueous LiOH solution (1M, 1.5 mL) by the method (4 h) described above in a yield of 83% (153 mg) as a white fluffy solid. ^{1}H NMR (400 MHz, DMSO) δ 5.43-5.33 (m, 2H, H-9, H-10) 3.47-3.43 (m, 1H, H-12), 2.09-2.06 (m, 2H, 2×H-11), 1.97 (q, 2H, *J*= 6.4 Hz, *J*= 6.5 Hz, 2×H-8), 1.90 (t, *J*= 7.4 Hz, 2×H-2) 1.46-1.23 (m, 20H, 2×H-7, 2×H-13 -(CH_2)$_8$-), 0.87 (t, $J_{18,17}$ =6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO) δ 175.65 (C-1), 130.58 (C-9) 126.64 (C-10), 69.84 (C-12), 38.16 (C-2) 36.48 (C-11), 35.23 (C-8), 31.43, 29.45, 29.22, 29.11, 28.93, 28.84, 26.93, 26.30, 22.14, (m, -(CH_2)$_{10}$-), 14.02 (C-18); HR MS m/z Calculated for $C_{18}H_{33}LiO_3$ $[M+Na]^+$ = 327.2487, found 327.2480.

Methyl 12-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-ricinoleate (21)

The title compound was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**13**, 1.00 g, 2.4 mmol) by the general procedure described above. The reaction time was 14 h; and **21** (1.05 g) was obtained in 68% yield as a thick syrup. $[\alpha]_D^{23}$ = –1.5° (*c* = 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 – 5.38 (m, 1H, H-9'), 5.38 – 5.30 (m, 2H, H-4, H-10'), 5.19 (dd, $J_{2,3}$= 10.5 Hz, $J_{2,1}$ = 7.9 Hz, 1H, H-2), 5.00 (dd, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 4.50 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.16 (dd, $J_{6a,6b}$ = 11.2 Hz, $J_{6a,5}$ = 6.8 Hz, 1H, H-6a), 4.09 (dd, $J_{6b,6a}$ = 11.2 Hz, $J_{6b,5}$ = 6.8 Hz, 1H, H-6b), 3.88 (dt, $J_{5,6a}$ = $J_{5,6b}$ = 6.8 Hz, $J_{5,4}$ = 1.0 Hz, 1H, H-5), 3.65 (s, 3H, -OCH_3), 3.59 – 3.47 (m, 1H, H-12'), 2.43 – 2.24 (m, 4H, , 2×H-11', 2×H-2'), 2.13 (s, 3H, -OCOCH_3), 2.02 (s, 3H, -OCOCH_3), 2.02 (s, 3H, -OCOCH_3), 2.00 – 1.95 (m, 5H, 2×H-8', -OCOCH_3), 1.66 – 1.52 (m, 2H, 2×H-7'), 1.48 – 1.37 (m, 2H, 2×H-13'), 1.37 – 1.14 (m, 16H, -(CH_2)$_8$-), 0.86 (t, $J_{18',17'}$ = 6.9 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.37 (C-1'), 170.48 , 170.46, 170.31, 169.36 (4×O*C*OCH$_3$), 132.03 (C-9'), 125.08 (C-10'), 101.82 (C-1), 82.27 (C-12'), 71.21 (C-3), 70.61 (C-5), 69.34 (C-2), 67.20 (C-4), 61.46 (C-6), 51.54 (-O*C*H$_3$), 34.19 (C-13'), 34.02 (C-2'), 33.41 (C-11'), 31.98, 29.62, 29.54, 29.29, 29.26, 29.22, 27.56, 25.31, 25.04, 22.80, 22.75 (m, -(CH_2)$_{10}$-), 20.88, 20.81, 20.76, 20.72 (4×OCO*C*H$_3$), 14.18 (C-18'); HR MS m/z Calculated for $C_{33}H_{54}O_{12}$ $[M+Na]^+$ = 665.3513, found 665.3519.

Methyl 12-*O*-(β-D-galactopyranosyl)-ricinoleate (22)

The title compound was prepared from **21** (200 mg, 0.31 mmol) by the general procedure described above. The reaction time was 0.5 h; and **22** (135 mg) was obtained in 92% yield as a thick syrup. $[\alpha]_D^{23}$ = –9.8° (*c* = 1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.52 – 5.39 (m, 2H, H-9', H-10'), 4.31 (d, $J_{1,2}$ = 7.1 Hz, 1H, H-1), 3.87 (d, $J_{4,3}$ = $J_{4,5}$ = 2.8 Hz, 1H, H-4), 3.80 – 3.69 (m, 3H, H-6a, 6b, H-12'), 3.67 (s, 3H, OCH_3), 3.56 – 3.45 (m, 3H, H-2, H-3, H-5), 2.49 – 2.28 (m, 4H, 2×H-11', 2×H-

8′), 2.14 – 2.00 (m, 2H, 2×H-2′), 1.67 – 1.58 (m, 2H, H-7′), 1.58 – 1.50 (m, 2H, 2×H-13′), 1.50 – 1.27 (m, 16H, -(CH_2)$_8$-), 0.92 (t, $J_{18'\,17'}$ = 6.6 Hz 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 175.99 (C-1′), 132.27 (C-9′), 126.95 (C-10′), 104.81 (C-1), 81.10 (C-12′), 76.35 (C-3), 75.04 (C-2), 72.78 (C-5), 70.12 (C-4), 62.22 (C-6), 51.98 (-OCH_3), 34.80 (C-13′), 34.23 (C-8′), 33.02 (C-11′), 30.67, 30.29, 30.22, 30.17, 28.41, 26.14, 26.02, 23.72 (m, -(CH_2)$_{10}$-), 14.47 (C-18′); HR MS m/z Calculated for $C_{25}H_{46}O_8$ $[M+Na]^+$ = 497.3090, found 497.3110.

12-*O*-(β-D-Galactopyranosyl)-ricinoleic acid (23)

The title compound was prepared from **21** (300 mg, 0.46 mmol) by the general procedure described above. The reaction time was 0.5 h; and **23** (185 mg) was obtained in 87% yield as a thick syrup. $[\alpha]_D^{23}$ = –9.9° (*c* = 0.3, MeOH); ^{1}H NMR (400 MHz, DMSO) δ 5.37 (m, 2H, H-9′, H-10′), 4.11 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-1), 3.85 – 3.33 (m, 4H, H-4, H-12′, H-6a,b), 3.33 – 3.16 (m, 3H, H-5, H-3, H-2), 2.38 – 2.07 (m, 4H, 2×H-11′, 2×H-2′), 1.97 (m, 2H, 2×H-8′), 1.56 – 1.03 (m, 20H, -(CH_2)$_{10}$-), 0.84 (t, $J_{18',17'}$ = 6.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ 174.83 (C-1′), 131.11 (C-9′), 126.18 (C-10′), 103.72 (C-1), 78.92 (C-12′), 75.04 (C-5), 73.68 (C-2), 70.96 (C-3), 68.12 (C-4), 60.38 (C-6), 33.87 (C-2′), 33.51 (C-11′), 33.15 (C-13′), 31.51, 29.21, 29.15, 28.86, 28.77, 28.75, 27.04, 24.69, 24.54, 22.28 (m, -(CH_2)$_8$-) 14.19 (C-18′); HR MS m/z Calculated for $C_{24}H_{44}O_8$ $[M+Na]^+$ = 483.2934, found 483.2941.

Methyl 12-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-ricinoleate (24)

The title compound was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**14**, 500 mg, 1.2 mmol) by the general procedure described above. The reaction time was 14 h; and **24** (455 mg) was obtained in 58% yield as a thick syrup. $[\alpha]_D^{23}$ = +10.1° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 – 5.38 (m, 1H, H-9′), 5.35 (m, 1H, H-10′), 5.19 (t, $J_{3,2}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.06 (t, $J_{4,5}$ = $J_{4,3}$ = 9.7 Hz, 1H, H-4), 4.97 (dd, $J_{2,3}$ = 9.6 Hz, $J_{2,1}$ = 8.0 Hz, 1H, H-2), 4.55 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.24 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 5.2 Hz, 1H, H-6a), 4.11 (dd, $J_{6b,6a}$ = 12.1 Hz, $J_{6b,5}$ = 2.5 Hz, 1H, H-6b), 3.72 – 3.66 (m, 1H, H-5), 3.66 (s, 3H, -OCH_3), 3.59 – 3.49 (m, 1H, H-12′), 2.47 – 2.18 (m, 4H, 2×H-11′, 2×H-2′), 2.12 – 1.92 (m, 14H, 4×OCOCH_3, 2×H-8′), 1.67 – 1.53 (m, 2H, 2×H-7′), 1.51 – 1.36 (m, 2H, 2×H-13′), 1.38 – 1.11 (m, 16H, -(CH_2)$_8$-), 0.87 (t, $J_{18'\,17'}$= 6.9 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.44 (C-1′), 170.80, 170.53, 169.57, 169.34 (4×O*C*OCH$_3$), 132.09 (C-9′), 125.06 (C-10′), 101.22 (C-1′), 82.10 (C-12′), 73.11 (C-3), 71.78 (C-2), 71.72 (C-5), 68.76 (C-4), 62.34 (C-6), 51.59 (-OCH_3), 34.22 (C-13′), 34.03 (C-2′), 33.35 (C-11′), 32.00, 29.65, 29.54, 29.32, 29.28, 29.25, 27.57, 25.31, 25.07, 22.77 (m, -(CH_2)$_8$-), 20.86, 20.80, 20.79, 20.76 (4×OCO*C*H$_3$), 14.21 (C-18′); HR MS m/z Calculated for $C_{33}H_{54}O_{12}$ $[M+Na]^+$ = 665.3513, found 665.3519.

The orthoester **9a** formed as the by-product in the above reaction was also isolated (132 mg, 17 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59 – 5.45 (m, 1H, H-9′), 5.42 – 5.30 (m, 1H, H-10′), 5.18 (t, $J_{3,4}$ = $J_{3,2}$ = 9.7 Hz, 1H, H-3), 5.02 (d, $J_{1,2}$ =4.0 Hz, 1H, H-1), 4.99 (d, $J_{4,3}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.26 (dd, $J_{6a,6b}$= 12.3 Hz, $J_{6a,5}$ = 4.8 Hz, 1H H-6a), 4.11 – 4.00 (m, 2H, H-6b, H-5), 3.71 – 3.59 (m, 5H, H-2, H-12′, -OCH_3), 2.38 – 2.22 (m, 4H, 2×H-11′, 2×H-2′), 2.13 – 1.98 (m, 11H, 3×OCO*C*H$_3$, 2×H-8′), 1.70 – 1.47 (m, 7H, 2×H-7′, 2×H-13′, -CH_3), 1.44 – 1.14 (m, 16H, -(CH_2)$_8$-), 0.89 (t, $J_{18'\,17'}$ = 7.0 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.34 (C-1′), 170.97, 170.69, 169.67 (3×O*C*OCH$_3$), 133.28 (C-9′), 124.64 (C-10′), 97.84 (C-1), 79.95 (C-12′), 77.23 (O-*C*-O), 73.51 (C-3), 70.95 (C-2) , 68.06 (m, C-4, C-5), 62.11 (C-6), 51.48 (-O*C*H$_3$), 34.76 (-*C*H$_3$), 34.10 (C-13′), 31.79 (C-2′), 31.45 (C-11′), 29.42, 29.38, 29.13, 29.10, 27.39, 25.62, 24.94, 22.63 (m, -(*C*H$_2$)$_8$-), 20.93, 20.74, 20.69 (4×OCO*C*H$_3$), 14.09 (C-18′); MALDI MS m/z Calculated for $C_{33}H_{54}O_{12}$ $[M+K]^+$ = 681.873, found 681.747.

Methyl 12-*O*-(β-D-glucopyranosyl)-ricinoleate (25)

The title compound was prepared from **24** (150 mg, 0.23 mmol) following the general procedure described above. The reaction time was 0.5 h; and **25** (98 mg was obtained in 90% yield as a thick syrup. $[\alpha]_D^{23}$ = –21.4° (*c* = 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.51 – 5.40 (m, 2H, H-9′, H-10′), 4.34 (d, $J_{1,2}$= 7.8 Hz, 1H, H-1), 3.86 (dd, $J_{6a,6b}$= 11.8 Hz, $J_{6a,5}$ = 2.3 Hz, 1H, H-6a), 3.75 – 3.67 (m, 2H, H-12′, H-6b), 3.66 (s, 3H, -OCH_3), 3.39 -3.34 (m, 2H, H-3, H-4), 3.28 – 3.23 (m, 1H, H-5), 3.18 (t, $J_{1,2}$= $J_{2,3}$= 8.4 Hz, 1H, H-2), 2.49 – 2.26 (m, 4H, 2×H-11′, 2×H-2′), 2.13 – 2.02 (m, 2H, 2×H-8′), 1.67 – 1.58 (m, 2H, 2×H-7′), 1.57 – 1.44 (m, 2H, 2×H-13′), 1.44 – 1.22 (m, 16H, -(CH_2)$_8$-), 0.91 (t, $J_{18'\,17'}$ = 6.8Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 176.01 (C-1′), 132.35 (C-9′), 126.89 (C-10′), 104.20 (C-1), 81.14 (C-12′), 78.14 (C-3), 77.76 (C-5), 75.34 (C-2), 71.69 (C-4), 62.82 (C-6), 51.98 (-OCH_3), 34.80 (C-13′), 34.79 (C-2′), 34.20 (C-11′), 33.03, 30.69, 30.66, 30.30, 30.24, 30.18, 28.42, 26.14, 26.02, 23.72 (m, -(*C*H$_2$)$_{10}$-) 14.46 (C-18′); HR MS m/z Calculated for $C_{25}H_{46}O_8$ $[M+Na]^+$ = 497.3090, found 497.3098.

12-*O*-(β-D-Glucopyranosyl)-ricinoleic acid (26)

The title compound was prepared from **24** (250 mg, 0.39 mmol) following the general procedure described above. Reaction time was 3 h; and **26** (145 mg) was obtained in 81% yield as a thick syrup. $[\alpha]_D^{23}$ = –14.8° (*c* = 0.3, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 5.51 – 5.35 (m, 2H, H-9′, H-10′), 4.32 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 3.84 (dd, $J_{6a,6b}$= 11.8 Hz, $J_{6a,5}$ = 2.2 Hz, 1H, H-6a), 3.74 – 3.61 (m, 2H, H-12′, H-6b), 3.38 – 3.30 (m, 2H, H-3, H-4), 3.27 – 3.21 (m, 1H, H-5), 3.16 (t, $J_{1,2}$= $J_{2,3}$= 8.3 Hz, 1H, H-2), 2.47 – 2.22 (m, 4H, m, 4H, 2×H-11′, 2×H-2′), 2.10 – 1.99 (m, 2H, 2×H-8′), 1.64 – 1.55 (m, 2H, 2×H-7′), 1.55 – 1.47 (m, 2H, 2×H-13′), 1.48 – 1.24 (m, 16H, -(CH_2)$_8$-), 0.89 (t, $J_{18'\,17'}$ = 6.6 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 178.05 (C-1′), 132.37 (C-9′), 126.86 (C-10′), 104.17 (C-1), 81.13 (C-12′), 78.11 (C-3), 77.72 (C-5), 75.31 (C-2), 71.67 (C-4), 62.80 (C-6), 35.26 (C-13′), 34.77 (C-2′), 34.18 (C-11′), 32.92, 30.71, 30.65, 30.35, 30.27, 28.43, 26.20, 26.12, 23.71 (m, -(*C*H$_2$)$_{10}$-), 14.47 (C-18′); HR MS m/z Calculated for $C_{24}H_{44}O_8$ $[M+Na]^+$ = 483.2934, found 483.2934.

Lithium 12-*O*-(β-D-glucopyranosyl)-ricinoleate (27)

The title compound was prepared from **24** (250 mg, 0.39 mmol) by the general procedure described above using LiOH as the alkali. The reaction time was 3 h; and **27** was obtained as colourless fluffy solid (152 mg) in 83% yield. $[\alpha]_D^{23}$ = –8.4° (*c* = 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.56 – 5.32 (m, 2H, H-9′, H-10′), 4.37 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 3.86 – 3.72 (m, 2H, H-6a, 6b), 3.71 – 3.58 (m, 1H, H-12′), 3.51 – 3.39 (m, 2H, H-3, H-4), 3.34 – 3.18 (m, 2H, H-5, H-2), 2.53 – 2.20 (m, 2H, 2×H-11′), 2.14 (t, $J_{2',3'}$ = 7.5 Hz 2H, 2×H-2′), 2.08 – 1.95 (m, 2H, 2×H-8′), 1.59-1.45 (m, 4H, 2×H-7′, 2×H-13′), 1.41-1.18 (m, 16H, -(CH_2)$_8$-) 0.84 (t, $J_{18',17'}$ = 6.5 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, D_2O) δ 181.50 (C-1′), 132.55 (C-9′), 125.06 (C-10′), 102.55 (C-1), 80.89 (C-12′), 75.96 (C-3), 75.71 (C-5), 73.23 (C-2), 69.27 (C-4), 60.54 (C-6), 37.81 (C-2′), 33.31 (C-13′), 32.61 (C-11′), 31.60, 29.32, 29.28, 29.14, 29.01, 28.95, 27.23, 26.14, 24.84, 23.24, 22.48 (m, -(CH_2)$_{10}$-), 13.89(C-18′); HR MS m/z Calculated for $C_{24}H_{43}LiO_8$ $[M+Na]^+$ = 489.3016, found 489.3011.

Methyl 12-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-ricinoleate (28)

The title compound was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**15**, 1.00 g, 2.4 mmol) following the general procedure described above. The reaction time was 14 h; and **28** (0.92 mg) was obtained in 60% yield as a thick syrup. $[\alpha]_D^{23}$ = +51.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 – 5.41 (m, 1H, H-9′), 5.38 – 5.21 (m, 3H, H-3, H-10′ H-4), 5.17 (dd, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.8 Hz, 1H, H-2), 4.94 (d, $J_{1,2}$= 1.6 Hz, 1H, H-1), 4.27 (dd, $J_{6a,6b}$= 12.7 Hz, $J_{6a,5}$ = 5.8 Hz, 1H, H-6a), 4.11 – 4.02 (m, 2H, H-6b, H-5), 3.66 (s, 3H, -OCH_3), 3.65 – 3.60 (m, 1H, H-12′), 2.33 – 2.19 (m, 4H, 2×H-2′, 2×H-11′), 2.15 (s, 3H, -OCOCH_3), 2.09 (s, 3H, -OCOCH_3), 2.04 (s, 3H, -OCOCH_3), 2.02 – 1.97 (m, 5H, -OCOCH_3, 2×H-8′), 1.67 – 1.46 (m, 4H, 4H, 2×H-7′, 2×H-13′), 1.42 – 1.18 (m, 16H, -(CH_2)$_8$-), 0.88 (t, $J_{18',17'}$ = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.43 (C-1′), 170.77, 170.19, 170.00, 169.91 (4×*O*COCH$_3$), 132.85 (C-9′), 124.32 (C-10′), 96.48 (C-1), 78.81 (C-12′), 70.30 (C-2), 69.30 (C-3), 68.91 (C-5), 66.43 (C-4), 62.74 (C-6), 51.57 (-O*C*H$_3$), 34.52 (C-13′), 34.24 (C-8′), 31.91, 31.34, 29.61, 29.45, 29.29, 29.25, 27.52, 25.83, 25.08, 22.76, 21.08, 20.87, 20.85 (m, -(*C*H$_2$)$_{10}$-), 14.22 (C-18′); HR MS m/z Calculated for $C_{33}H_{54}O_{12}$ $[M+Na]^+$ = 665.3513, found 665.3550.

Methyl 12-*O*-(α-D-mannopyranosyl)-ricinoleate (29)

The title compound was prepared from **28** (0.5 g, 0.78 mmol) by the general procedure described above. The reaction time was 0.5 h; and compound **29** (0.35 g) was obtained in 94% yield as a thick syrup. $[\alpha]_D^{23}$ = +49.8° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.56 – 5.45 (m, 1H, H-9′), 5.45 – 5.36 (m, 1H, H-10′), 4.92 (s, 1H, H-1) 3.84 – 3.59 (m, 10H, H-2, H-6a,b, H-12′ H-3, H-4, H-5, -OCH_3), 2.39 – 2.23 (m, 4H, 2×H-2′, 2×H-11′), 2.13 – 2.03 (m, 2H, 2×H-8′), 1.68 – 1.57 (m, 2H, 2×H-7′), 1.58 – 1.49 (m, 2H, H-13′), 1.47 – 1.26 (m, 16H), 0.92 (t, $J_{17',18'}$ = 6.5 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 175.98 (C-1′), 133.03 (C-9′), 126.07 (C-10′), 99.91 (C-1), 77.59 (C-12′), 74.84 (C-4), 72.67 (C-2), 72.64 (C-3), 68.45 (C-5), 62.79 (C-6), 51.98 (-O*C*H$_3$), 35.68 (C-13′), 34.79 (C-11′), 32.98, 31.82, 30.64, 30.54, 30.26, 30.21, 30.15, 28.37, 26.69, 26.01, 23.73 (m, -(*C*H$_2$)$_{10}$-), 14.48 (C-18′); HR MS m/z Calculated for $C_{25}H_{46}O_8$ $[M+Na]^+$ = 497.3091, found 497.3091.

12-*O*-(α-D-Mannopyranosyl)-ricinoleic acid (30)

The title compound was prepared from **28** (300 mg, 0.46 mmol) by the general procedure described above. The reaction time was 3 h; and compound **30** (180 mg) was obtained as a thick syrup in a yield of 85%). $[\alpha]_D^{23}$ = +68.4° (*c* = 0.5, MeOH); ^{1}H NMR (400 MHz, DMSO) δ 5.50 – 5.21 (m, 2H, H-9′, H-10′), 4.73 (s, 1H, H-1), 3.85 – 3.00 (m, 7H, H-2, H-3, H-4, H-5, H-6a,b, H-12′), 2.18 (m, 4H, 2×H-11′, 2×H-2′), 1.99 (m, 2H, 2×H-8′), 1.58 – 1.01 (m, 20H, -(CH_2)$_{10}$-), 0.85 (t, $J_{18',17'}$ = 6.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ 174.63 (C-1′), 131.61 (C-9′), 125.18 (C-10′), 97.89 (C-1), 74.38 (C-12′), 74.31 (C-4), 71.04 (C-2), 70.89 (C-3), 66.85 (C-5), 61.23 (C-6), 33.99 (C-2′), 33.73 (C-11′), 31.36 (C-13′), 30.22, 29.08, 28.87, 28.74, 28.66, 28.62, 26.90, 25.06, 24.57, 22.18 (m, -(*C*H$_2$)$_{10}$-) 14.06 (C-18′); HR MS m/z Calculated for $C_{24}H_{44}O_8$ $[M+Na]^+$ = 483.2934, found 483.2938.

Sodium 12-*O*-(α-D-mannopyranosyl)-ricinoleate (31)

The title compound was prepared from **28** (200 mg, 0.31 mmol) by the general procedure described above using NaOH as the alkali. The reaction time was 3 h; and compound **31** (120 mg) was obtained in 80% yield as a colourless fluffy solid. $[\alpha]_D^{23}$ = +45.9° (*c* = 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.62 – 5.46 (m, 1H, H-9′), 5.46 – 5.28 (m, 1H, H-10′), 4.98 (s, 1H, H-1), 3.90 – 3.82 (m, 2H, H-2, H-6a), 3.82 – 3.57 (m, 5H, H-3, H-6a, H-12′, H-5, H-4), 2.37 – 2.12 (m, 4H, 2×H-11′, 2×H-2′), 2.12 – 1.99 (m, 2H, 2×H-8′), 1.69 – 1.46 (m, 4H, 2×H-7′, 2×H-13′), 1.46 – 1.14 (m, 16H, -(CH_2)$_8$-), 0.87 (t, $J_{17',18'}$ = 6.3 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, D_2O) δ 183.42 (C-1′), 132.82 (C-9′), 124.39 (C-10′), 98.36 (C-1), 76.54 (C-12′), 72.95 (C-4), 70.81 (C-2), 70.76 (C-3), 66.10 (C-5), 60.43 (C-6), 37.71 (C-2′), 34.14 (C-13′), 31.54, 30.43, 29.31, 29.23, 29.14, 28.99, 28.91, 27.20, 26.11, 25.38, 22.49 (m, -(CH_2)$_{11}$-), 13.85 (C-18′); HR MS m/z Calculated for $C_{24}H_{43}NaO_8$ $[M+Na]^+$ = 505.2753, found 505.2751.

Methyl 12-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-ricinoleate (32)

The title compound was prepared from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**16**, 1.00 g, 2.74 mmol) following the general procedure described above. The reaction time was 16 h; and **32** (930 mg) was obtained as a colorless glassy solid in 53%). $[\alpha]_D^{23}$ = –15.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (brs, 1H, -N*H*), 5.47 – 5.28 (m, 3H, H-9′, H-10′, H-3), 5.02 (t, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4), 4.78 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1), 4.23 (dd, $J_{6a,6b}$= 12.1 Hz, $J_{6a,5}$= 5.2 Hz, 1H, H-6a), 4.10 (dd, $J_{6b,6a}$= 12.1 Hz, $J_{6b,5}$= 1.9 Hz, 1H), 3.78 – 3.67 (m, 2H, H-2, H-5), 3.65 (s, 3H, -OCH_3), 3.57 – 3.46 (m, 1H, H-12′), 2.43-2.26 (m, 4H, 2×H-11′, 2×H-2′), 2.07- 1.89 (m, 14H, 4×OCOCH_3, 2×H-8′), 1.67 – 1.53 (m, 2H, 2×H-7′), 1.48 – 1.37 (m, 2H, 2×H-13′), 1.26 (m, 16H, -(CH_2)$_8$-) 0.86 (t, $J_{18',17'}$ = 6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.53 (C-1′), 170.99, 170.84, 170.14, 169.64 (4×*O*COCH$_3$), 132.03 (C-9′), 125.22 (C-10′), 101.15 (C-1), 82.11 (C-12′), 72.42 (C-3), 71.69 (C-5), 69.08 (C-4), 62.56 (C-6), 55.66 (C-2), 51.61 (-O*C*H$_3$), 34.22 (C-13′), 34.05 (C-2′), 33.35 (C-11′), 32.05, 29.61, 29.56, 29.29, 29.23 (m, -(*C*H$_2$)$_8$-), 27.54 (C-8′), 25.50 (-(*C*H$_2$)$_8$-), 25.06 (C-7′), 23.46 (NHCO*C*H$_3$), 22.79 , 20.87, 20.81 (3×OCO*C*H$_3$), 14.22 (C-18′); HR MS m/z Calculated for $C_{33}H_{55}NO_{12}$ $[M+Na]^+$ = 664.3673, found 664.3694.

Methyl 12-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-ricinoleate (33)

The title compound was prepared from **32** (0.5 g, 0.78 mmol) following the general procedure described above. The reaction time was 0.5 h; 16 h; and **33** (0.46 g) was obtained as a light brown glassy solid in 92% yield. $[\alpha]_D^{23}$ = –40.8° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.41 (m, 2H, H-9′, H-10′), 4.49 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 3.86 (d, $J_{6a,6b}$= $J_{6a,5}$= 11.8 Hz, 1H, H-6a), 3.70 (dd, $J_{6b,6a}$= 11.8 Hz, $J_{6b,5}$= 5.1 Hz, 1H, H-6b), 3.65 (s, 3H, -OCH_3), 3.60 (m, 2H, H-12′, H-2), 3.49 (t, $J_{3,2}$ = $J_{3,4}$ = 9.3 Hz, 1H, H-3), 3.38 – 3.22 (m, 2H, H-4, H-5), 2.56-2.20 (m, 4H, 2×H-11′, 2×H-2′), 2.05 (m, 2H, 2×H-8′), 1.97 (s, 3H, NHCOCH_3), 1.66 – 1.54 (m, 2H, 2×H-7′), 1.51 – 1.39 (m, 2H, 2×H-13′), 1.41-1.29 (m, 16H, -(CH_2)$_8$-), 0.90 (t, $J_{18',17'}$ = 6.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 176.17 (NH*C*OCH$_3$), 173.53 (C-1′), 132.52 (C-9′), 126.70 (C-10′), 103.01 (C-1), 82.31 (C-12′), 77.71 (C-5), 75.83 (C-3), 72.00 (C-4), 62.74 (C-6), 57.85 (C-2), 52.06 (-O*C*H$_3$), 34.81 (C-2′), 34.21 (C-13′), 33.10 (C-11′), 30.72, 30.67, 30.28, 30.22, 30.16, 28.40, 26.17, 26.01, 23.76 (m, -(*C*H$_2$)$_8$-) 23.16 (NHCO*C*H$_3$), 14.48 (C-18′); HR MS m/z Calculated for $C_{27}H_{49}NO_8$ $[M+Na]^+$ = 538.3356, found 538.3375.

12-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-ricinoleic acid (34)

The title compound was prepared from **32** (200 mg, 0.31 mmol) by the general procedure described above. The reaction time was 3 h; and compound **34** (130 mg) was obtained as a light brown fluffy solid in 83% yield. $[\alpha]_D^{23}$ = +4.8° (*c* = 0.25, MeOH); ^{1}H NMR (400 MHz, DMSO) δ 7.86 (d, $J_{NH,2}$ = 7.2 Hz, 1H, -N*H*), 5.43 – 5.26 (m, 2H, H-9′, H-10′), 4.31 (d, $J_{1,2}$ = 6.9 Hz, 1H, H-1), 3.72 – 3.51 (m, 2H, H-6a,b), 3.46 – 3.19 (m, 3H, H-12′, H-2, H-5), 3.17 – 2.83 (m, 2H, H-3, H-4), 2.46 –

2.01 (m, 2H, 2×H-11'), 2.03 – 1.80 (m, 4H, 2×H-8', 2×H-2'), 1.76 (s, 3H, NHCOCH_3), 1.43 – 1.00 (m, 20H, -(CH_2)$_{10}$-), 0.84 (t, $J_{18',17'}$ = 6.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ 168.97 (C-1'), 131.24 (C-9'), 125.90 (C-10'), 102.13 (C-1), 80.25 (C-12'), 76.95 (C-4), 74.04 (C-5), 70.81 (C-3), 61.24 (C-6), 55.91 (C-2), 33.44, 33.11, 31.45, 29.45, 29.22, 29.09, 29.07, 28.86, 26.97, 24.42, 23.10, 22.20 (m, -(CH_2)$_8$-), 14.08 (C-18'); HR MS m/z Calculated for $C_{26}H_{47}NO_8$ $[M+Na]^+$ = 524.3200, found 524.3193.

Methyl 12-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-ricinoleate (35)

The title compound was prepared from 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (**17,** 1.00 g, 2.0 mmol) following the general procedure described above. The reaction time was 18 h; and compound **35** (1.016 g) was obtained in 66% yield as a thick syrup. $[\alpha]_D^{23}$ = +84.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, $J_{A,B}$ = 8.0 Hz, $J_{A,B}$ = 1.0 Hz, 2H, Ph-C*H*), 7.98 (dd, $J_{A,B}$ = 8.1, $J_{A,B}$ = 1.0 Hz, 2H, Ph-C*H*), 7.83 (dd, $J_{A,B}$ = 8.2 Hz, $J_{A,B}$ = 1.0 Hz, 2H, Ph-C*H*), 7.65 – 7.55 (m, 1H, Ph-C*H*), 7.55 – 7.35 (m, 6H, Ph-C*H*), 7.26 (dd, $J_{A,B}$ = 9.0 Hz, $J_{A,B}$ = 6.6 Hz, 2H, Ph-C*H*), 5.83 (dd, $J_{3,4}$ = 10.1 Hz, $J_{3,2}$ = 3.4 Hz, 1H, H-3), 5.67 (t, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.60 (dd, $J_{2,3}$ = 3.2, $J_{2,1}$ = 1.7 Hz, 1H), 5.58 – 5.44 (m, 2H, H-9', H-10'), 5.10 (s, 1H, H-1), 4.33 (dq, $J_{5,CH3}$ = 12.4 Hz, $J_{5,4}$ = 6.2 Hz, 1H, H-5), 3.76 – 3.68 (m, 1H, H-12'), 3.65 (s, 3H, -OCH_3), 2.51 – 2.23 (m, 4H, 2×H-11', 2×H-2'), 2.17 – 2.01 (m, 2H, 2×H-8'), 1.71 – 1.50 (m, 4H, 2×H-7', 2×H-13'), 1.45 – 1.24 (m, 19H, -(CH_2)$_8$-, -CH_3), 0.89 (t, $J_{17',18'}$ = 6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.26 (C-1'), 165.83, 165.64, 165.49 (3×Ph-*C*O), 133.39, 133.26 133.03 (3×Ph-*C*), 132.25 (C-9'), 129.90, 129.72, 129.68, 129.57, 129.41, 129.32, 128.56, 128.40, 128.25 (m, Ph-*C*) 125.48 (C-10'), 96.81 (C-1), 79.46 (C-12'), 72.03 (C-4), 71.47 (C-2), 70.13 (C-3), 66.78 (C-5), 51.42 (-OCH_3), 34.08 (C-2'), 33.58 (C-13'), 32.73 (C-11'), 31.79, 29.62, 29.45, 29.21, 29.14, 27.51, 25.14, 24.95, 22.65, 22.58, 17.58 (m, -(CH_2)$_{10}$-), 14.11(C-18'); HR MS m/z Calculated for $C_{46}H_{58}O_{10}$ $[M+Na]^+$ = 793.3928, found 793.3933.

Methyl 12-*O*-(α-L-rhamnopyranosyl)-ricinoleate (36)

The title compound was prepared from **35** (500 mg, 0.65 mmol) by the general procedure described above. The reaction time was 4 h; and **36** (270 mg) was obtained in 91% yield as a thick syrup. $[\alpha]_D^{23}$ = –57.8° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.53 – 5.34 (m, 2H, H-9', H-10'), 4.77 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 3.78 (dd, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.6 Hz, 1H, H-2), 3.76 – 3.67 (m, 1H, H-5), 3.67 – 3.57 (m, 5H, -OCH_3, H-3, H-12'), 3.38 (t, $J_{4,3}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 2.40 – 2.21 (m, 4H, 2×H-11', 2×H-2'), 2.11– 2.01 (m, 2H, 2×H-8'), 1.67 – 1.44 (m, 4H, 2×H-7', 2×H-13'), 1.44 – 1.26 (m, 16H, -(CH_2)$_8$-), 1.24 (d, $J_{5,CH3}$ = 6.3 Hz, 3H, -CH_3), 0.91 (t, $J_{17',18'}$ = 6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 174.72 (C-1'), 131.42 (C-9'), 125.34 (C-10'), 99.72 (C-1), 78.02 (C-12'), 72.53 (C-4), 71.30 (C-2), 71.06 (C-3), 68.65 (C-5), 50.65 (-OCH_3), 33.43 (C-2'), 33.01 (C-13'), 32.35 (C-11'), 31.59, 29.24, 29.16, 28.85, 28.81, 28.76, 26.97, 24.76, 24.62, 22.28 (m, -(CH_2)$_{10}$), 16.55 (-CH_3), 13.06 (C-18'); HR MS m/z Calculated for $C_{24}H_{46}O_7$ $[M+Na]^+$ = 481.3142, found 481.3154.

12-*O*-(α-L-Rhamnopyranosyl)-ricinoleic acid (37)

The title compound was prepared from **35** (2.00 g, 2.6 mmol) by the general procedure described above. The reaction time was 8 h; and **37** was obtained (1.00 g) in a yield of 87%) as a thick syrup. $[\alpha]_D^{23}$ = –14.7° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.51 – 5.38 (m, 2H, H-9', H-10'), 4.77 (d, $J_{1,2}$ = 1.1 Hz, 1H, H-1), 3.78 (dd, $J_{2,3}$ = 3.1 Hz, $J_{2,1}$ = 1.5 Hz, 1H, H-2), 3.76 – 3.69 (m, 1H, H-5), 3.68 – 3.57 (m, 2H, , H-3, H-12'), 3.38 (t, $J_{4,3}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 2.41 – 2.20 (m, 4H, 2×H-11', 2×H-2'), 2.14 – 1.98 (m, 2H, 2×H-8'), 1.65 – 1.45 (m, 4H, 2×H-7', 2×H-13'), 1.45 – 1.26 (m, 16H, -(CH_2)$_8$-), 1.24 (d, $J_{5,CH3}$ = 6.2 Hz, 3H, -CH_3), 0.90 (t, $J_{17',18'}$ = 6.7 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 177.78 (C-1'), 132.80 (C-9'), 126.69 (C-10'), 101.09 (C-1), 79.40 (C-12'), 73.92 (C-4), 72.69 (C-2), 72.44 (C-3), 70.02 (C-5), 34.98 (C-2'), 34.38 (C-13'), 33.72 (C-11'), 32.97, 30.63, 30.53, 30.28, 30.22, 30.19, 28.36, 26.14, 26.08, 23.65 (m, -(CH_2)$_{10}$), 17.92 (-CH_3), 14.44 (C-18'): HR MS m/z Calculated for $C_{24}H_{44}O_7$ $[M+Na]^+$ = 467.2985, found 467.2985.

Methyl 12-*O*-(2,3,5-tri-*O*-benzoyl-β-D-arabinofuranosyl)-ricinoleate (38)

The title compound was prepared from 2,3,5-tetra-*O*-benzoyl-α-D-arabinofuranosyl chloride (**18**,2.18 g, 4.5 mmol) by the general procedure described above. The reaction time was 16 h; and **38** (1.95 g) was obtained in a yield of 57% as a thick syrup. $[\alpha]_D^{23}$ = –14.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 7.94 (m, 6H, Ph-C*H*), 7.63 – 7.27 (m, 9H, Ph-C*H*), 5.62 – 5.26 (m, 5H, H-3, H-2, H-9', H-10', H-1), 4.80 (d, $J_{5a,5b}$= $J_{5a,4}$= 10.8 Hz, 1H, H-5a), 4.73 – 4.53 (m, 2H, H-5b, H-4), 3.83 – 3.67 (m, 1H, H-12'), 3.66 (s, 3H, -OCH_3), 2.42 – 2.17 (m, 4H, 2×H-11', 2×H-2'), 2.01-2.00 (m, 2H, H-8'), 1.68 – 1.16 (20H, -(CH_2)$_{10}$-), 0.86 (t, $J_{17',18'}$ = 5.6 Hz, 3H, -CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.44 (C-1'), 166.37, 165.97, 165.58 (3×C=O), 133.62, 133.57, 133.16, 132.66 (Ph-*C*), 131.88 (C-9'), 130.09, 130.00, 129.90, 129.34, 129.32, 128.62, 128.59, 128.44 (Ph-*C*), 125.83 (C-10'), 104.92 (C-1), 82.55 (C-2), 80.94 (C-4), 78.07 (C-3), 77.77 (C-12'), 64.07 (C-5), 51.58 (-OCH_3), 34.22 (C-2'), 33.79 (C-11'), 32.85 (C-13'), 31.93, 29.67, 29.59, 29.29, 29.24, 27.52, 25.31, 25.06, 22.75 (-(CH_2)$_{10}$-), 14.23 (C-18'); HR MS m/z Calculated for $C_{45}H_{56}O_{11}$ $[M+Na]^+$ = 779.3371, found 779.3372.

Methyl 12-*O*-(β-D-arabinofuranosyl)-ricinoleate (39)

The title compound was prepared from **38** (1.00 g, 1.32 mmol) by the general procedure described above. The reaction time was 3 h; and **39** (540 mg) was obtained in 92% yield as a thick syrup. $[\alpha]_D^{23}$ = –74.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.50 – 5.37 (m, 2H, H-9', H-10'), 4.97 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.00 – 3.92 (m, 2H, H-4, H-2), 3.84 (dd, $J_{3,2}$ = 6.4 Hz, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 3.74 (dd, $J_{5a,5b}$= 11.9 Hz, $J_{5a,4}$ = 3.2 Hz, 1H, H-5a), 3.69 – 3.59 (m, 5H, -OCH_3, H-5b, H-12'), 2.38 – 2.25 (m, 4H, 2×H-2', 2×H-11'), 2.10 – 2.00 (m, 2H, 2×H-8'), 1.66 – 1.44 (m, 4H, 2×H-7', 2×H-13'), 1.44 – 1.25 (m, 16H, -(CH_2)$_8$-), 0.90 (t, $J_{17',18'}$ = 6.7 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 176.18 (C-1'), 132.46 (C-9'), 127.00 (C-10'), 108.80 (C-1), 85.22 (C-4), 83.66 (C-2), 78.99 (C-3), 78.65 (C-12'), 62.85 (C-5), 52.05 (-OCH_3), 34.81 (C-2'), 34.68 (C-13'), 34.04 (C-11'), 32.97, 30.62, 30.56, 30.22, 30.16, 30.12, 28.34, 26.26, 25.99, 23.67 (m, -(CH_2)$_{10}$-), 14.44 (C-18'); HR MS m/z Calculated for $C_{24}H_{44}O_7$ $[M+Na]^+$ = 467.2985, found 467.3003.

12-*O*-(β-D-Arabinofuranosyl)-ricinoleic acid (40)

The title compound was prepared from **38** (1.00 g, 1.32 mmol) by the general procedure described above. The reaction time was 8 h and compound **40** (0.45 g) was obtained in 79% yield as a thick syrup. $[\alpha]_D^{23}$ = –67.0° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.49 – 5.39 (m, 2H, H-9', H-10'), 4.97 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 4.00 – 3.94 (m, 2H, H-4, H-2), 3.84 (dd, $J_{3,2}$ = 6.3 Hz, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 3.74 (dd, $J_{5a,5b}$= 11.9 Hz, $J_{5a,4}$ = 3.3 Hz, 1H, H-5a), 3.68 – 3.59 (m, 2H, H-5b, H-12'), 2.41 – 2.24 (m, 4H, 2×H-11', 2×H-2'), 2.11 – 1.98 (m, 2H, 2×H-8'), 1.67 – 1.45 (m, 4H, 2×H-7', 2×H-13'), 1.45 – 1.22 (m, 16H, -(CH_2)$_8$-), 0.90 (t, $J_{17',18'}$ = 6.7 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 177.89 (C-1'), 132.49 (C-9'), 126.96 (C-10'), 108.75 (C-1),

85.19 (C-4), 83.64 (C-2), 79.01 (C-3), 78.62 (C-12′), 62.81 (C-5), 34.98 (C-2′), 34.65 (C-13′), 34.01 (C-11′), 32.95, 30.62, 30.54, 30.25, 30.18, 30.16, 28.34, 26.24, 26.05, 23.65 (m, -(CH_2)$_{10}$-), 14.44 (C-18′); HR MS m/z Calculated for $C_{23}H_{42}O_7$ $[M+Na]^+$ = 453.2829, found 453.2838.

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Methyl 12-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-ricinoleate (41)

The title compound was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-galactofuranosyl chloride (**19**, 2.00 g, 5.5 mmol) by the general procedure described above. The reaction time was 14 h; and **41** (2.12 g) was obtained in 60% yield as a thick syrup. $[\alpha]_D^{23}$ = –24.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 – 5.32 (m, 3H, H-9′, H-10′, H-5), 5.08 (s, 1H, H-1), 5.00 (dd, $J_{2,3}$ = 2.0 Hz, $J_{1,2}$ = 0.5 Hz, 1H, H-2), 4.96 (dd, $J_{3,4}$ = 6.1 Hz, $J_{3,2}$ = 1.7 Hz, 1H, H-3), 4.33 – 4.25 (m, 2H, H-6a, H-4), 4.18 (dd, $J_{6a,6b}$ = 11.7 Hz, $J_{6b,5}$ = 7.3 Hz, 1H, H-6b), 3.65 (s, 3H, -OCH_3), 3.63 – 3.53 (m, 1H, H-12′), 2.32 – 2.18 (m, 4H, 2×H-2′, 2×H-11′), 2.12, 2.09, 2.06, 2.03 (4×s, 12H, 4×OCOCH_3), 2.03 – 1.92 (m, 2H, 2×H-8′), 1.66 – 1.54 (m, 2H, 2×H-7′), 1.52 – 1.40 (m, 2H, 2×H-13′), 1.39 – 1.17 (m, 16H, -(CH_2)$_8$-), 0.86 (t, $J_{17',18'}$ = 6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.38 (C-1′), 170.61, 170.17, 170.16, 169.79 (4×O*C*OCH$_3$), 131.96 (C-9′), 125.70 (C-10′), 104.87 (C-1), 81.89 (C-2), 79.73 (C-4), 78.21 (C-12′), 76.64 (C-3), 69.33 (C-5), 62.85 (C-6), 51.56 (-O*C*H$_3$), 34.20 (C-2′), 33.74 (C-13′), 32.87 (C-11′), 31.90, 29.67, 29.49, 29.30, 29.29, 29.23, 27.54, 25.23, 25.05, 22.72, 20.94, 20.92, 20.81 (m, -(*C*H$_2$)$_{10}$-), 14.20 (C-18′); HR MS m/z Calculated for $C_{33}H_{54}O_{12}$ $[M+Na]^+$ = 665.3513, found 665.3547.

Methyl 12-*O*-(β-D-galactofuranosyl)-ricinoleate (42)

The title compound was prepared from **41** (250 mg, 0.38 mmol) following the general procedure described above. The reaction time was 0.5 h; and **42** (170 mg) was obtained in 94% yield as a thick syrup. $[\alpha]_D^{23}$ = –36.6° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 5.49 – 5.39 (m, 2H, H-9′, H-10′), 4.95 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.00 (dd, $J_{3,4}$ = 6.5 Hz, $J_{4,5}$ = 4.1 Hz, 1H, H-4), 3.95 –3.93 (m, 2H, H-2, H-3), 3.72 (m, 1H, H-5), 3.64 – 3.56 (m, 6H, -OCH_3, H-12′, H-6a,b), 2.41 – 2.23 (m, 4H, 2×H-2′, 2×H-11′), 2.12 – 1.97 (m, 2H, 2×H-8′), 1.67 – 1.52 (m, 2H, 2×H-7′), 1.55 – 1.42 (m, 2H, 2×H-13′), 1.42 – 1.22 (m, 16H, -(CH_2)$_8$-), 0.90 (t, $J_{17',18'}$ = 6.7 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 176.13 (C-1′), 132.45 (C-9′), 127.06 (C-10′), 108.90 (C-1), 84.11 (C-2), 83.62 (C-3) 79.26 (C-6), 78.63 (C-4), 72.25 (C-5), 64.89 (C-12′), 52.03 (OCH_3), 34.81 (C-2′), 34.07 (C-13′), 32.98 (C-11′), 30.65, 30.57, 30.25, 30.18, 30.14, 28.37, 26.29, 26.00, 23.68(m, -(*C*H$_2$)$_{10}$-), 14.45 (C-18′); HR MS m/z Calculated for $C_{25}H_{46}O_8$ $[M+Na]^+$ = 497.3091, found 497.3111.

12-*O*-(β-D-Galactofuranosyl) ricinoleic acid (43)

The title compound was prepared from **41** (300 mg, 0.46 mmol) following the general procedure described above. The reaction time was 3 h; and **43** (181 mg was obtained in 85% yield as a thick syrup. $[\alpha]_D^{23}$ = –31.2° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO) δ 5.37 (m, 2H, H-9′, H-10′), 4.74 (s, 1H, H-1), 3.91 – 3.24 (m, 7H, H-4, H-2, H-3, H-5, H-12′ H-6a,b), 2.34 – 2.07 (m, 2H, H-11′), 2.05 – 1.84 (m, 4H, 2×H-2′, 2×H-8′), 1.58 – 1.09 (m, 20H, -(CH_2)$_{10}$-), 0.84 (t, $J_{18',17'}$ = 6.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ 174.46 (C-1′), 131.07 (C-9′), 126.18 (C-10′), 107.56 (C-1), 82.56 (C-2), 82.06 (C-3), 77.00 (C-12′), 76.74 (C-4), 70.48 (C-5), 63.36 (C-6), 36.84 (C-2′), 33.53 (C-13′), 32.89 (C-11′), 31.43, 29.23, 29.03, 28.82, 27.01, 25.82, 24.80, 22.23 (m, -(*C*H$_2$)$_{10}$-) 14.12 (C-18′); HR MS m/z Calculated for $C_{24}H_{44}O_8$ $[M+Na]^+$ = 483.2934, found 483.2933.

Methyl 12-*O*-(2,3,6, 2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)-ricinoleate (44)

The title compound was prepared from acetobromolactose (**20**, 5.00 g, 7.15 mmol following the general procedure described above. The reaction time was 16 h; and **44** (3.52 g) was obtained in 53% yield as a thick syrup. $[\alpha]_D^{23}$ = –15.6° (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 – 5.36 (m, 1H, H-9′′), 5.36 – 5.26 (m, 2H, H-10′′, H-4′), 5.17 (t, $J_{3,4}$ = $J_{3,2}$ = 9.3 Hz, 1H, H-3), 5.09 (dd, $J_{2',3'}$ = 10.4 Hz, $J_{2',1'}$ = 7.9 Hz, 1H, H-2′), 4.94 (dd, $J_{3',2'}$ = 10.4 Hz, $J_{3',4'}$ = 3.4 Hz, 1H, H-3′), 4.87 (dd, $J_{2,3}$ = 9.6 Hz, $J_{2,1}$ = 8.0 Hz, 1H, H-2), 4.50 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.47 (m, ($J_{1',2'}$ = 8.0 Hz, H-1′), 2H, H-1′, H-6′a), 4.08 (m, 3H, H-6a,b, H-6′b), 3.86 (m, 1H, H-5′), 3.75 (t, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, 1H, H-4), 3.65 (s, 3H, OCH_3), 3.58 (m, 1H, H-5), 3.54 – 3.44 (m, 1H, H-12′′), 2.36 – 2.18 (m, 4H, 2×H-2′′, 2×H-11′′), 2.15 – 1.92 (m, 23H, 7×-OCOCH_3, 2×H-8′′), 1.59 (m, 2H, 2×H-7′′), 1.38 (m, 2H, 2×H-13′′), 1.33 – 1.15 (m, 16H, -(CH_2)$_8$-), 0.86 (t, $J_{18'',17''}$= 6.9 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.30 (C-1′′), 170.37, 170.34, 170.17, 170.08, 169.89, 169.52, 169.11 (7×O*C*OMe), 131.86 (C-9′′), 124.98 (C-10′′), 101.09 (C-1), 100.85 (C-1′), 81.89, (C-12′′) 76.56 (C-4), 73.03 (C-3), 72.44 (C-5), 72.01 (C-2), 70.99 (C-3′), 70.66 (C-5′), 69.11 (C-2′), 66.62 (C-4′), 62.15 (C-6′), 60.82 (C-6), 51.46 (COO*C*H$_3$), 34.08 (C-13′′), 33.94 (C-8′′), 33.18 (C-11′′), 31.86, 29.50, 29.39, 29.18, 29.14, 29.11, 27.42, 25.19, 24.93, 22.63 (m, -(*C*H$_2$)$_8$-), 20.83, 20.81, 20.70, 20.64, 20.52 (m, 7×OCO*C*H$_3$), 14.07 (C-18′′); HR MS m/z Calculated for $C_{45}H_{70}O_{20}$ $[M+Na]^+$ = 953.4358, found 953.4364.

Methyl 12-*O*-(β-D-lactosyl)-ricinoleate (45)

The title compound was prepared from **44** (500 mg, 0.54 mmol) following the general procedure described above. The reaction time was 1 h; and **45** (310 mg) was obtained in 91% as a thick syrup. $[\alpha]_D^{23}$ = +68.4° (*c* = 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 5.51 – 5.37 (m, 2H, H-9′′, H-10′′), 4.38 (d, $J_{1,2}$= 7.4 Hz, 1H, H-1), 4.37 (d, $J_{1',2'}$ = 7.8 Hz, H-1′) 3.89 – 3.36 (m,15H, H-3′, H-4′, H-5′, H-6′a,b, H-2, H-3, H-4, H-5, H-6a,b, H-12′′, -OCH_3), 3.24 (dd, $J_{2',3'}$ = 8.9Hz, $J_{2',1'}$ = 8.0 Hz, 1H, H-2′), 2.32 (m, 4H, 2×H-2′′, 2×H-11′′), 2.06 (m, 2H, H-8′′), 1.63 – 1.56 (m, 2H, 2xH-7′′), 1.56 – 1.47 (m, 2H, 2xH-13′′), 1.42 – 1.20 (m, 16H, -(CH_2)$_8$-), 0.90 (t, $J_{18'',17''}$= 6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 176.12 (C-1′′), 132.47 (C-9′′), 126.86 (C-10′′), 105.09 (C-1), 104.16 (C-1′), 81.43, 80.78, 77.11, 76.49, 76.33, 74.94, 74.78, 72.60, 70.32, 62.51, 62.09 (m), 52.07 (-OCH_3), 34.84, 34.23, 33.05, 30.71, 30.68, 30.32, 30.3, 30.20, 28.45 26.15, 26.05, 23.75 (m, -(*C*H$_2$)$_8$-), 14.52 (C-18′′); HR MS m/z Calculated for $C_{31}H_{56}O_{13}$ $[M+Na]^+$ = 659.3619, found 659.3622.

12-*O*-(β-D-Lactosyl)-ricinoleic acid (46)

The title compound was prepared from **44** (200 mg, 0.21 mmol) following the general procedure described above. The reaction time was 4 h; and **46** (110 mg) was obtained in 84% yield as a thick syrup. $[\alpha]_D^{23}$ = +39.2° (*c* = 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 5.49 – 5.41 (m, 2H, H-9′′, H-10′′) 4.41 (d, $J_{1,2}$ = 7.0 Hz, 1H, H-1), 4.39 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1′), 3.92 – 3.77 (m, 4H, H-6a′, H-6b′, H-4′, H-6a), 3.76 – 3.67 (m, 2H, H-6b, H-12′′), 3.66 – 3.49 (m, 5H, H-4, H-5, H-3′, H-2, H-3), 3.47 – 3.39 (m, 1H, H-5′), 3.27 (t, $J_{2',3'}$ = $J_{2',1'}$ = 8.0 Hz, 1H, H-2′), 2.50 – 2.19 (m, 4H, 2×H-11′′, 2×H-2′′), 2.13 – 2.00 (m, 2H, H-8′′), 1.68 – 1.43 (m, 4H, 2×H-7′′, 2×H-13′′), 1.44 – 1.23 (m, 16H, -(CH_2)$_8$-), 0.91 (t, $J_{18'',17''}$ =6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 173.99 (C-1′′), 131.23 (C-9′′), 125.28 (C-10′′), 103.61 (C-1), 102.70 (C-1′), 80.15 (C-12′), 79.28 (C-4), 75.65 (C-5), 75.06 (C-2′), 74.90 (C-5′), 73.51 (C-2), 73.36 (C-3), 71.20 (C-3′), 68.89 (C-4′), 61.09 (C-6), 60.62 (C-6′), 36.05 (C-2′′), 33.37 (C-13′′), 32.76 (C-11′′), 31.58, 29.34, 29.20, 29.06, 28.96, 27.07, 25.66, 24.70, 22.30 (m, -(*C*H$_2$)$_8$-), 13.10

(C-18''); HR MS m/z Calculated for $C_{30}H_{54}O_{13}$ $[M+Na]^+$ = 645.3462, found 645.3470.

Methyl 9,10-dibromo-12-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-ricinoleate (47)

Bromine (0.05 mL, 0.87 mmol) was added to a stirred solution of **24** (370 mg, 0.58 mmol) in chloroform (5 mL) at room temperature and the stirring was continued for 5 h at which time TLC showed complete consumption of **24**. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and was washed successively water, aqueous $NaHCO_3$, and brine solutions in the cold. The organic layer was then dried over anhydrous Na_2SO_4 and was concentrated under reduced pressure to yield, after chromatography on a column of silica gel (eluent, EtOAc: hexane), the title compound **47** (370 mg, 80%) (as a pair of enantiomers) in the form of a thick syrup. $[\alpha]_D^{23}$ = + 41.6° (*c* = 0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) (The two enantiomers are marked A & B) δ 5.45 (dd, 2H, $J_{3,4}$ = 9.5 Hz, $J_{4,3}$ = 9.9 Hz H-3A, H-3B), 5.17-5.13 (2×d, $J_{1,2}$ = 5.1 Hz, 2H, H-1A, H-1B), 5.06 (2×t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 2H, H-4A, H-4B), 4.97-4.87 (2×d, $J_{2,3}$ = 10.4 Hz, $J_{2,1}$ = 3.7 Hz, 2H, H-2A, H-2B), 4.29-4.03 (m, 10H, 2×H-6a, 2×H-5, 2×H-9′, 2×H-10′, 2×H-6b), 3.85 -3.76 (m, 2×H-12′), 3.66 (s, 6H, 2×-OCH_3), 2.30 (t, 4H, $J_{2',3'}$ = 7.5 Hz, 4×H-2′), 2.1-2.0 (7×s, 24H, 8×OCOCH_3), 1.67-1.57 (m, 48H, 2× -(CH_2)$_{12}$), 0.88 (t, $J_{18',17'}$ = 6.8 Hz, 6H, 2×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.38 (2×C-1′), 170.80, 170.35, 170.29, 170.18, 170.10, 169.73, 169.71 (m, 8×OCOCH$_3$), 98.23 (C-1A), 95.17 (C-1B), 81.01 (C-12′A), 78.38 (C-12′B), 70.98 (C-2), 70.25 (C-3), 68.80 (C-4A), 68.63 (C-4B), 67.94 (C-5), 62.21 (C-6A), 62.08 (C-6B), 59.86 (C-9′A), 59.14 (C-9′B), 56.69 (C-10′A), 54.32 (C-10′B), 51.61 (-OCH$_3$), 40.53, 35.67, 35.37, 34.04, 33.70, 31.75, 29.50, 29.24, 29.00, 28.66, 28.63, 27.69, 25.08, 24.87, 22.62, 22.56, 21.00, 20.82, 20.71, 20.63 (m, 2×(CH$_2$)$_{13}$), 14.21 (C-18′A); 14.17 (C-18′B); HR MS m/z Calculated for $C_{35}H_{54}Br_2O_{12}$ $[M+Na]^+$ = 823.1880, found 823.1880, 825.1863 $[M+2+Na]^+$.

Methyl 9,10-dibromo-12-*O*-(β-D-glucopyranosyl)-ricinoleate (48)

The title compound was prepared from **47** (45 mg, 0.06 mmol) following the general procedure described above. The reaction time was 0.5 h; and **48** (31 mg, 87%) was obtained as a thick syrup. $[\alpha]_D^{23}$ = +37.1° (*c* = 0.75, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) (The two enantiomers are marked A & B) δ, 4.89- 4.78 (2×d, $J_{1,2}$ = 4.0 Hz, 2H, H-1A, H-1B), 4.39-4.35 (m, 2H, H-4A, H-4B), 4.26-4.22 (m, 2H, H-3A, H-3B), 4.14-4.10 (m, 2H, H-5A, H-5B),), 3.81-3.48 (m, 16H, 2×H-12′, 2×H-6a, 2×H-6b, 2×-OCH_3, 2×H-9′, 2×H-10′), 3.30-3.24 (m, 2H, H-2A, H-2B), 2.24-1.22 (m, 52H, 2× -(CH_2)$_{13}$), 0.88 (t, $J_{18',17'}$ = 6.9 Hz, 6H, 2×CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 174.66 (2×C-1′), 101.14 (C-1A), 98.61 (C-1B), 78.72 (C-12′A), 76.99 (C-12′B), 73.59, 73.52, 72.69, 72.47, 72.42, 72.13 (m, 2×C-6, 2×C-9′, 2×C-10′), 70.36 (C-2A), 70.22 (C-2B), 61.15, 61.03, 60.92, 59.58, 57.42, 55.43, 53.32 (m, 2×C-5, 2×C-3, 2×C-4) 50.61 (2×OCH$_3$), 43.26, 40.88, 37.34, 36.55, 35.53, 34.23, 33.44, 31.54, 29.15, 29.01, 28.71, 28.68, 28.65, 28.41, 28.39, 27.18, 27.07, 24.84, 24.71, 24.56, 22.30, 22.24 (m, 2×(CH$_2$)$_{13}$), 13.01 (C-18′A); 12.99 (C-18′B); HRMS m/z Calculated for $C_{25}H_{46}Br_2O_8$ $[M+Na]^+$ = 655.1457, found 657.1448 $[M+2+Na]^+$.

Methyl 12-*O*-benzyl-ricinoleate (49)

NaH (46 mg, 0.96 mmol) was added to a solution of methyl ricinoleate (**7**, 200 mg, 0.6 mmol) in DMF (5 mL) at 0 °C and the mixture was stirred for 15 min. BnBr (0.1 mL, 0.76 mmol) was then added to the mixture and the temperature was allowed to be brought to 10 °C, and the stirring was continued for 10 h. At this time TLC showed complete disappearance of **7**. MeOH (1 mL) followed by triethyl amine (1 mL) were added to the mixture. After stirring for a few minutes the reaction mixture was diluted with diethyl ether and was washed successively with water (x3) and brine in a separatory funnel in the cold. The ether layer was then dried over anhydrous Na_2SO_4 and was concentrated to dryness under reduced pressure. Purification of the product by chromatography on silica (eluent, EtOAc: hexane) yielded **49** and **49a** (140 mg; 1: 0.5 by ^{1}H NMR) as a mixture. The mixture (30 mg) was therefore dissolved in anhydrous MeOH and was treated with NaOMe (catalytic) for 2 h at room temperature. Amberlite IR 120 H^+ resin was then added to it to acidic pH and the solution on concentration to dryness and filtration through a short column of silica afforded the title compound neat (20 mg, 72 %). $[\alpha]_D^{23}$ = +19.3° (*c* = 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.22 (m, 5H, Ph-C*H*), 5.48-5.35 (m, 2H, H-9, H-10), 4.57 (d, $J_{A,B}$= 11.7 Hz, 1H, Ph-C*H*H),), 4.49 (d, $J_{A,B}$ = 11.7 Hz, 1H, Ph-CH*H*), 3.66 (s, 3H, -COOCH_3), 3.40 (p, *J* = 6.0 Hz, 1H, H-12), 2.36-2.23 (m, 4H, 2×H-2, 2×H-11), 2.03 (q, 2H, *J*= 6.4 Hz, *J*= 6.7 Hz, 2×H-8), 1.65-1.22 (m, 20 H -(CH_2)$_{10}$), 0.88 (t, $J_{18,17}$ =6.8 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.43 (C-1), 139.20 (Ph-*C*), 131.85 (C-9), 128.40, 127.86, 127.52 (Ph-*C*), 125.69 (C-10), 79.14 (C-12), 71.07 (Ph-*C*H$_2$), 51.57 (-O*C*H$_3$), 34.25 (C-2), 34.13 (C-13), 32.02 (C-11), 31.80, 29.71, 29.59, 29.32, 29.29, 29.27, 27.57, 25.59, 25.10, 22.79 (m, -(*C*H$_2$)$_9$-), 14.09 (C-18); HR MS m/z Calculated for $C_{26}H_{42}O_3$ $[M+Na]^+$ = 425.3032, found 425.3035.

Methyl 12-*O*-methyl-ricinoleate (50)

The title compound was prepared from **7** (313 mg, 1 mmol) using NaH(70 mg, 1.5 mmol) and MeI (0.09 mL, 1.5 mmol) in DMF (5.0 mL) by the same procedure as described for **49**. The reaction time was 4 h and product obtained (210 mg) was a mixture (1:0.5 by ^{1}H NMR) of the ester **50** and the lactone **50a**. Treatment of the mixture (60 mg) with a solution of anhydrous HCl in MeOH (prepared by reacting 0.02 mL thionyl chloride with anhydrous MeOH, 3 mL, at -5 °C) under the nitrogen atmosphere for 8 h at room temperature, followed by concentration of the reaction mixture to dryness and purification of the residue by filtration through a column of silica (eluent, EtOAc: hexanes) gave **50** as a neat product (32 mg, 80% based on **50** weight). $[\alpha]_D^{23}$ = +17.2° (*c* = 0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46-5.33 (m, 2H, H-9, H-10) 3.65 (s, 3H, -COOCH_3), 3.32 (s, 3H, -OCH_3), 3.17 (p, *J*= 5.8 Hz, 1H, H-12), 2.31-2.15 (m, 4H, , 2×H-2, 2×H-11), 2.01 (q, 2H, *J*= 6.5 Hz, *J*= 6.7 Hz, 2×H-8), 1.65 – 1.53 (m, 2H, 2×H-7), 1.48 – 1.37 (m, 2H, 2×H-13),1.37-1.21 (m, 16 H -(CH_2)$_8$-), 0.86 (t, $J_{18,17}$ =6.6 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.23 (C-1), 131.67 (C-9) 125.42 (C-10), 80.97 (C-12), 56.54 (-O*C*H$_3$), 51.39 (-COO*C*H$_3$), 34.07 (C-2), 33.57 (C-13), 31.86 (C-11), 31.86, 31.06, 29.54, 29.47, 29.11, 27.38, 25.34, 24.93, 22.61, (m, -(*C*H$_2$)$_9$-), 14.06 (C-18); HR MS m/z Calculated for $C_{20}H_{38}O_3$ $[M+Na]^+$ = 349.2719, found 349.2714.

Conclusions

In conclusion, 28 novel glycosides of ricinoleic acid were synthesized and their antimicrobial activity was evaluated, seven of them showing promising wide spectrum antibacterial activity against Gram +ve bacteria. Two compounds, **29** (NP-2672) and **39** (NP-2689), showed good to excellent activity against various non-clinical/clinical/NorA overexpressed/resistant strains of *S. aureus* as well as other Gram +ve bacteria. These results suggest that carbohydrate moiety in the glycosides of the ricinoleic acid played an important role for imparting the antibacterial activity by increasing the membrane permeability. It is presumably because of binding of these molecules with D-Ala-D-Ala, preventing the synthesis of the long polymers of *N*-acetylmuramic acid and *N*-acetylglucosamine that form the backbone strands of the bacterial

cell wall. In this sense, these compounds could be potential candidates for therapeutics as they may not be targeting the cell components such as nucleic acids and proteins. The future studies in this context shall therefore be for answering these questions.

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Author Contributions

All authors have given approval to the final version of the manuscript.
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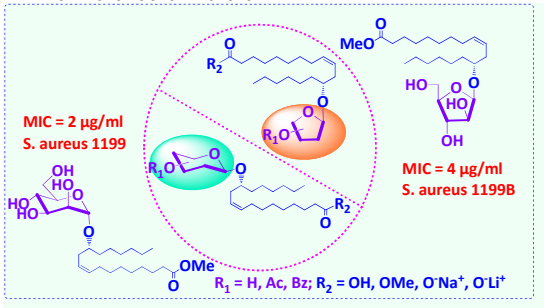
Synthesis and antibacterial activity of ricinoleic acid glycosides

Ramakrishna Kuppala, Mugunthan Govindarajan., Rushikesh Tambat, Neeraj Patel Hemraj Nandanwar, Kamlesh K. Bhutani and K. P. Ravindranathan Kartha



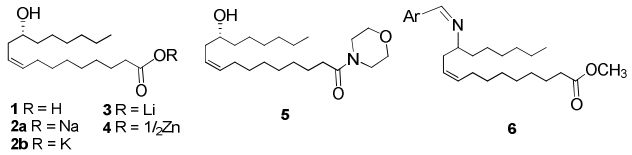
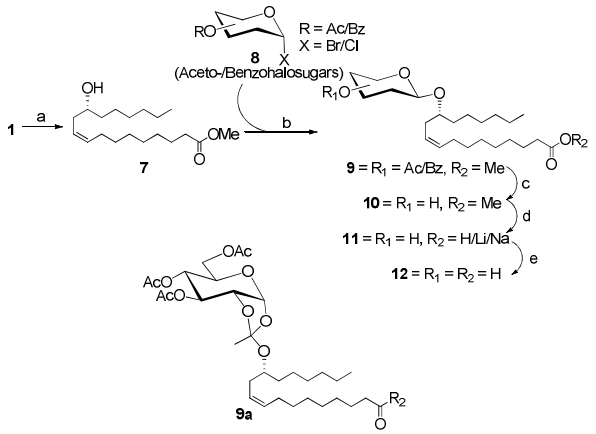


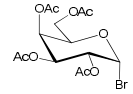
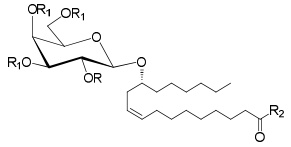
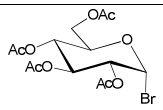
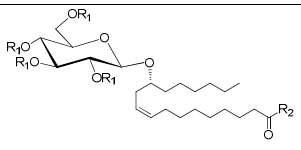
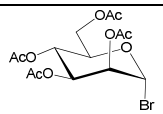
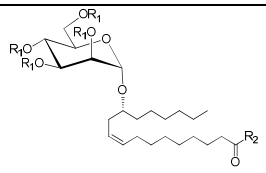
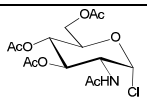
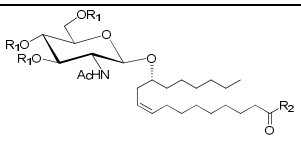
Figure 1: Ricinoleic acid-derived compounds known in the literature as anti-bacterial and antimycobacterial agents.

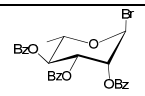
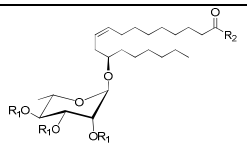
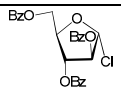
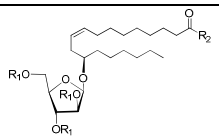
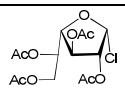
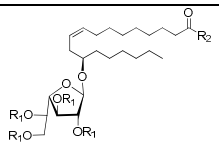
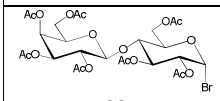
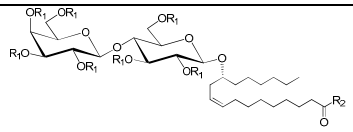
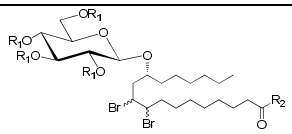


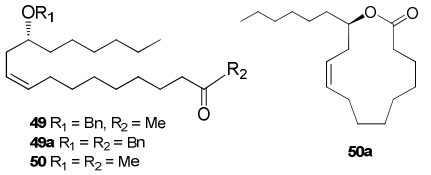
Reagents and conditions: (i) HCl/MeOH, rt, 4 h, 90%; (ii) Ag_2CO_3, $AgClO_4$, powdered molecular sieves (4Å), CH_2Cl_2 rt, 10-12 h, 53-68%; (iii) NaOMe/MeOH, 20-40 min, rt, 90-94%; (iv) LiOH/aq THF, 2-4 h, rt, 80-87%; (v) Amberlite IR 120 (H^+), rt, 80-88%

Scheme1. Synthesis of ricinoleic acid glycosides in their partially/fully deprotected form and/as their sodium/lithium salts.

Table 1. Different ricinoleic acid glycosides and their partially or fully deprotected and sodium/lithium salt derivatives

Acetohalosugar used for synthesis	Glycosylated ricinoleates synthesized		Yield (%)
	Structure	Compound number	
 **13**		**21**, R_1 = Ac, R_2 = OCH_3	68
		22, R_1 = H, R_2 = OCH_3	92
		23, R_1 = H, R_2 = OH	87
 **14**		**24**, R_1 = Ac, R_2 = OCH_3 [25]	58
		25, R_1 = H, R_2 = OCH_3	90
		26, R_1 = H, R_2 = OH	81
		27, R_1 = H, R_2 = O^-Li^+	83
 **15**		**28**, R_1 = Ac, R_2 = OCH_3	60
		29, R_1 = H, R_2 = OCH_3	94
		30, R_1 = H, R_2 = OH	85
		31, R_1 = H, R_2 = O^-Na^+	80
 **16**		**32**, R_1 = Ac, R_2 = OCH_3	52
		33, R_1 = H, R_2 = OCH_3	92
		34, R_1 = H, R_2 = OH	83

 **17**		**35**, R_1 = Bz, R_2 = OCH_3 **36**, R_1 = H, R_2 = OCH_3 **37**, R_1 = H, R_2 = OH	66 91 87
 **18**		**38**, R_1 = Bz, R_2 = OCH_3 **39**, R_1 = H, R_2 = OCH_3 **40**, R_1 = H, R_2 = OH	57 92 79
 **19**		**41**, R_1 = Ac, R_2 = OCH_3 **42**, R_1 = H, R_2 = OCH_3 **43**, R_1 = H, R_2 = OH	60 94 85
 **20**		**44**, R_1 = Ac, R_2 = OCH_3 **45**, R_1 = H, R_2 = OCH_3 **46**, R_1 = H, R_2 = OH	53 91 84
24 (For the structure, see the entry at row 2 above)		**47**, R_1 = Ac, R_2 = OCH_3 **48**, R_1 = H, R_2 = OCH_3	80 87



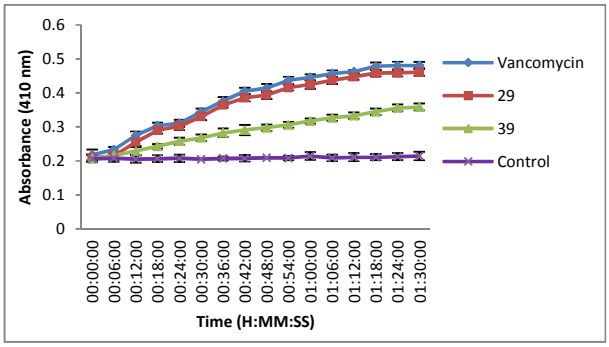


Figure 2: The cytoplasmic membrane permeabilization of *S. aureus* cells treated with compounds **29** (squares), **39** (triangles) and vancomycin (diamonds).The untreated *S. aureus* cells (crosses) were taken as control. (Compounds **29** and **39** induced an increase in the permeability of *S. aureus*. The experiment was carried out two times in triplicate. The results are presented as mean ± SD.)

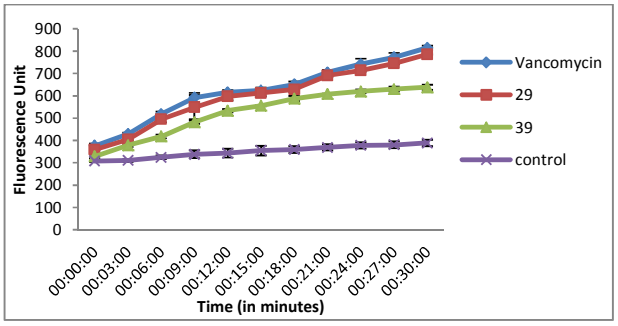


Figure 3: Effect of **29** (squares), **39** (triangles) and vancomycin (diamonds) on membrane integrity of *S. aureus* cells by propidium iodide uptake assay. The untreated *S. aureus* cells (crosses) were taken as control. For each sample 10^6 CFU ml^{-1} were analyzed. The membrane integrity of *S. aureus* cells was destroyed by **29** and **39**. The experiment was carried out two times in triplicate. The results were presented as mean ± SD.

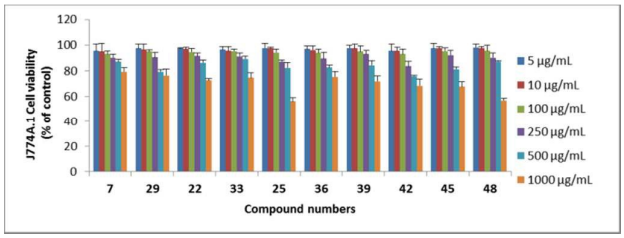


Figure 4: Cytotoxicity study of biologically active glycosides of methyl ricinoleate (Results are expressed as mean ± SD of three independent experiments performed in triplicates)