2009-01-01

The Antimicrobial Efficacy and Structure Activity Relationship of Novel Carbohydrate Fatty Acid Derivatives Against Listera spp. and Food Spoilage Microorganisms

Patricia Nobmann
Technological University Dublin, Patricia.Nobmann@tudublin.ie

Aoife Smith
Technological University Dublin, Aoife.Smith@tudublin.ie

Julie Dunne
Technological University Dublin, Julie.Dunne@tudublin.ie

Gary Henehan
Technological University Dublin, gary.henehan@tudublin.ie

Paula Bourke
Technological University Dublin, paula.bourke@tudublin.ie

Title: The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against *Listeria* spp. and food spoilage microorganisms

Patricia Nobmann, Aoife Smith, Julie Dunne, Gary Henehan and Paula Bourke*

School of Food Science and Environmental Health, Dublin Institute of Technology,
Cathal Brugha Street, Dublin 1, Ireland

* Corresponding author. Tel: +353-14027594; Fax: +353-14024495; E-mail: paula.bourke@dit.ie

Running Title: Antimicrobial efficacy of novel carbohydrate fatty acid derivatives

Abstract

Novel mono-substituted carbohydrate fatty acid (CFA) esters and ethers were investigated for their antibacterial activity against a range of pathogenic and spoilage bacteria focussing on *Listeria monocytogenes*. Carbohydrate derivatives with structural differences enable comparative studies on the structure/activity relationship for antimicrobial efficacy and mechanism of action. The antimicrobial efficacy of the synthesized compounds was compared with commercially available compounds such as monolaurin and monocaprylin, as well as the pure free fatty acids, lauric acid and caprylic acid, which have proven antimicrobial activity. Compound efficacy was compared using an absorbance based broth microdilution assay to determine the minimum inhibitory concentration (MIC), increase in lag phase and decrease in maximum growth rate.

Among the carbohydrate derivatives synthesized, lauric ether of methyl α-D-glucopyranoside and lauric ester of methyl α-D-mannopyranoside showed the highest growth-inhibitory effect with MIC values of 0.04mM, comparable to monolaurin. CFA derivatives were generally more active against Gram positive bacteria than Gram negative bacteria. The analysis of both ester and ether fatty acid derivatives of the same carbohydrate, in tandem with alpha and beta configuration of the carbohydrate moiety suggest that the carbohydrate moiety is involved in the antimicrobial activity of the fatty acid derivatives and that the nature of the bond also has a significant effect on efficacy, which requires further investigation. This class of CFA derivatives has great potential for developing antibacterial agents relevant to the food industry, particularly for control of *Listeria* or other Gram-positive pathogens.

Keywords: *Listeria monocytogenes*; Carbohydrate fatty acid derivatives; Monolaurin; Lauric acid; Caprylic acid; Antimicrobial activity

1. Introduction

Consumer demand for fresh, minimally processed and "natural" foods, along with the requirement for maintenance and enhancement of safety, quality and shelf-life characteristics has fuelled research for alternative antimicrobials. *Listeria monocytogenes* has emerged as one of the most important food pathogens in ready-to-eat processed meals and dairy foods (EFSA, 2007), given that it can adapt to a wide range of food processes and storage conditions including refrigeration temperatures, and acidic or high salt foods. Moreover, *Listeria* has one of the highest case fatality rates of all the foodborne infections: 20-30% (de Valk, *et al*., 2005). Therefore, there is a need for investigation of new approaches for the control or elimination of this pathogen in foods whilst also addressing food spoilage concerns.

Fatty acids (FA) and their corresponding esters are one group of chemicals found in nature considered to have little or no toxicity, with proven antimicrobial activity. Kabara *et al*., (1972) showed that while fatty acids esterified with monohydric alcohols were inactive against microorganisms, those esterified with certain polyhydric alcohols yielded antimicrobial derivatives (Conley and Kabara, 1973). Monoglycerides (MG) are commonly employed in the food industry as flavoring and emulsifying agents and Monolaurin (ML), a food-grade glycerol monoester of lauric acid, is approved in the US as a food emulsifier (21 CFR GRAS 182.4505). The anti-listerial activity of fatty acids and monoglycerides has been previously documented (Oh and Marshall, 1993; Wang and Johnson, 1997; Sprong *et al*., 2001). Their antimicrobial activity against spoilage microorganisms has also been reported (Ouattara *et al*., 1997; Blaszyk and Holley, 1998).

Sugar esters are biodegradable, nontoxic and nonionic surfactants, currently employed in the food, pharmaceutical, cosmetics and detergent industries (Hill and Rhode, 1999; Piccicuto *et al*., 2001). Furthermore, their antimicrobial activities have been reported (Monk *et al.*, 1996; Devulapalle *et al*., 2004; Ferrer *et al.,* 2005).

Carbohydrate fatty acid (CFA) esters have been synthesized chemically and enzymatically by interesterification, transesterification and direct esterification. An issue regarding the synthesis of commercial sucrose esters is related to the high functionality of the carbohydrate molecule with many hydroxyl groups, which compete during the derivatization step, leading to product mixtures of mono-, di- and polyesters (Hill and Rhode, 1999). Enzymatic synthesis of novel sugar fatty acid esters has been widely employed and can be highly regioselective, although for some carbohydrates minor regiomeric isomers may be obtained.

The exact mode of action of fatty acid esters has not yet been elucidated, but the cytoplasmic membrane is thought to be the primary site of action for fatty acid esters, affecting respiratory activity through inhibition of enzymes involved in oxygen uptake (Kabara, 1993). Ruzin and Novick, (2000) reported a monolaurin esterase activity in association with the *S. aureus* cell membrane and cytoplasm. It was shown that the half life of monolaurin in cultures of *S. aureus* was *ca*. 5 minutes due to its cleavage by cellular esterases. These studies raise the question as to whether the ester, or free fatty acid derived from hydrolysis of the ester, was responsible for antimicrobial activity.

Recently, a number of novel fatty acid derivatives of carbohydrates have been synthesized and their antimicrobial activity assessed (Devulapalle *et al.*, 2004; Ferrer *et al.*, 2005). These workers have pointed out that a complication of some earlier studies was that they

were carried out using commercial preparations that contained a mixture of compounds. Thus, it was difficult to correlate antimicrobial activity with chemical structure. It is clear that future studies in this area will require the use of pure compounds. Moreover, there is a need to standardize antimicrobial activity of novel compounds by the use of reference compounds. Finally, quantification of antimicrobial activity is desirable to allow comparison between different studies.

The objectives of this study were to compare the *in vitro* antimicrobial activity of a range of pure, novel, fatty acid esters with the corresponding fatty acid ethers and commercial fatty acids and monoglycerides to ascertain the role of the free fatty acid in the antimicrobial efficacy. These compounds were compared quantitatively to allow an estimation of the enhancement of the efficacy over the free fatty acids. This work has used a synthesis designed to allow the production of pure, novel regiochemically defined monosaccharide mono-fatty acid esters, and their corresponding ethers. The effect of different carbohydrate scaffolds as well as a non-carbohydrate (pentaerythritol) on antimicrobial efficacy was also examined. The effect of fatty acid chain length and anomeric configuration of the carbohydrate was also explored.

The activity of eight CFA derivatives and three non-carbohydrate polyhydroxylated ester derivatives, together with their corresponding monosaccharide, fatty acids and monoglycerides as controls, were assessed against a range of Gram-positive and negative bacteria of interest to the food industry. Efficacy and structure-activity relationships were assessed by comparing MIC values, the increase in Lag phase and maximum specific growth rate.

2. Materials and methods

2.1 Bacteria and growth conditions

Bacterial strains used in this study are listed in Table 1. Stock cultures were maintained in tryptic soy broth (TSB, Sharlau Chemie, Spain) supplemented with 20% glycerol at -70°C. Cultures were routinely grown by subculturing one hundred microliters of stock culture into 9 mL TSB and incubating at 35°C for 18 h, except for *Pseudomonas* spp. which were incubated at 30°C. All cultures were then maintained on tryptic soy agar (TSA, Sharlau Chemie, Spain) plates at 4°C. Working cultures were prepared by inoculating a loop of pure culture into TSB and incubating at the optimum temperature for each strain for 18 h. A bacterial suspension was prepared in saline solution (NaCl 0.85%, BioMérieux, France) equivalent to a McFarland standard of 0.5, using the Densimat photometer (BioMérieux, SA, France), to obtain a concentration of $1x10^8$ cfu/mL. This suspension was then serially diluted in TSB to obtain a working concentration of $1x10^6$ cfu/mL.

2.2 Chemical synthesis

Chemical synthesis was performed according to Smith *et al*., (2008). An overview of the test compounds synthesized and used in the antimicrobial assay is given in Figure 1.

2.3 Test compounds preparation

The saturated free fatty acids, lauric acid (LA - C_{12}) and caprylic acid (CA - C_8), as well as their corresponding monoglycerides, monolaurin (ML) and monocaprylin (MC) (Sigma-Aldrich ~99% purity), were used as standards in this study.

Stock solutions (100 mM) of test compounds and standards were prepared in sterile hydroalcoholic diluent (ethanol-distilled water, 1:1) and stored at -20°C. Stock solutions were diluted in TSB to obtain initial working concentrations (10 or 20mM).

2.4 Antimicrobial activity assay

Solutions of the working test compounds and standards were serially diluted in sterile TSB to a final volume of 100 µL within the 96-well microtiter plate. 100 µL of freshly prepared inoculum of the organism under study was added to each appropriate well. The final concentration of each microorganism in each well was approximately $5x10^5$ cfu/mL and the concentration of chemical compounds ranged from 1:2 to 1:256. Each concentration was assayed in duplicate. The following controls were used in the microplate assay for each organism and test compound; blank: uninoculated media without test compound to account for changes in the media during the experiment; negative control: uninoculated media containing only the test compound; positive control 1: inoculated media without compound; positive control 2: inoculated media without compound but including the corresponding sugar to evaluate any effect of the sugar alone; and positive control 3: inoculated media without compound but with the equivalent concentration of ethanol used to dissolve the test compound thereby assessing any activity of the alcohol. The 96-well plates were incubated for 18 hours in a microtiterplate reader (PowerWave microplate Spectrophotometer, BioTek) at 35°C, except for *Pseudomonas* spp. which were incubated at 30°C, and effects were monitored by measuring the optical density (OD) at 600 nm for each well every 20 minutes with 20 seconds agitation before each OD measurement. Each experiment was replicated three times.

2.5 Data analysis

2.5.1 Minimum inhibitory concentration (MIC)

The MIC was defined as the lowest concentration of compound that showed no increase in OD values for all the replicates compared to the negative control after 18 hours. The

absorbance readings obtained from the kinetic data were plotted against time to obtain the growth curves of the test organisms. Subtraction of the absorbance of the negative control eliminated interferences due to possible variations in the media.

2.5.2 Lag time increase (λ)

The increase in Lag time was calculated using the Gen5^{TM} software. The increase in lag time was defined as the time required for the culture with test compound to record an increase in OD_{600} of 0.10 *minus* the time that the positive control 1 without test compound required to record the same increase in OD_{600}.

2.5.2 Maximum specific growth rate (μ_{max})

The maximum growth rate was also calculated using the Gen5^{TM} software. The μ_{max} was determined from the slope of the regression equation from the linear portion of the log plot during early exponential phase.

2.5.3 Statistical analysis

All experiments were performed in duplicate and replicated at least three times. Statistical differences between compound efficacies were determined using ANOVA followed by LSD testing at $p < 0.05$ level using SPSS software, Version 15.

3. Results

3.1 *Antimicrobial activity of carbohydrate fatty acid derivatives*

3.1.1 Minimum inhibitory concentrations

The MIC results are summarized in Table 2. The monoglycerides, ML and MC, had greater activity ($p<0.05$) against the Gram positive *Listeria* spp. compared to their corresponding free fatty acids (LA, CA), and comparable activity at the concentrations

tested against the Gram negative microorganisms. Of the monoglycerides and free fatty acids tested, ML had the lowest MIC values ($p<0.05$) and was particularly effective for inhibition of *Listeria* strains with MIC values of 0.04mM, by comparison with the range observed for LA with MIC values between 0.63mM to 1.25mM. A similar trend was observed for MC (MIC = 2.5mM, 5.0mM) compared to the free fatty acid CA (MIC ≥5mM).

When tested against the Gram negative bacteria, LA and ML had no activity at concentrations up to 20mM (Table 2). An exception to this was recorded for *E. coli* NCTC12900 with a MIC value of 12.5mM for LA and ML. *P. fluorescens* was susceptible to CA and MC at a concentration of 5 mM for both compounds, whereas for *E. coli* strains, MIC values were 10 mM and 5 mM respectively. Minimum inhibitory concentrations of CA were ≥ 20 mM for the other Gram negative bacteria (Table 2).

All CFA derivatives showed greater antimicrobial activity against Gram positive microorganisms than Gram negative ($p<0.05$). For *Listeria* spp., compounds **2** and **6** were the most active derivatives with MIC values of 0.04 mM, comparable to ML (Table 2). The next in order of overall efficacy was compound **3** with MIC values between 0.08 mM and 0.16 mM for *Listeria* spp. Compound **1** recorded an MIC range of 0.08 mM to 0.31 mM. The antimicrobial activity of compound **4** was significantly lower than that observed with the corresponding α-ether (Table 2). Compound **9** (a non-carbohydrate mono-ester) was evaluated, but its antimicrobial activity was negligible (results not shown). Compounds **7**, **8**, **10** and **11** could not be accurately tested for antimicrobial efficacy due to poor solubility in water. Compound **5** had a greater activity ($p<0.05$) compared with MC against all *Listeria* strains (Table 2). Compound **5** was more active than the lauric acid derivatives

against *E. coli* ATCC 25922 and *P. fluorescens,* with MIC values of 12.5 mM and 5 mM respectively (Table 2).

In each antimicrobial efficacy assay, the corresponding carbohydrates for the fatty acid derivatives were included as a control, but had no antimicrobial or growth promoting effect on the microorganisms under investigation. Although the concentrations of ethanol corresponding to that within the wells with the highest concentrations of compound used (10mM for the Gram positive and 20mM for the Gram negative bacteria) had a minor effect on bacteria viability, there was no anti-microbial effect observed at the concentrations used when incorporated with the compounds at MIC levels.

3.1.2 Increase in Lag time and decrease of maximum specific growth rate

The increase in lag time and decrease in maximum specific growth rate was estimated for *L. monocytogenes* ATCC 7644 to allow further comparison between compound efficacies. Results were found to be concentration and compound dependent (Table 3) ($p < 0.05$). Generally, the increase in lag time between concentrations of a compound was observed to be more marked than the decrease in growth rate which was more gradual. For example, at sub-MIC concentrations, compound **3** had an increase in lag time from 0.5h to 5.3h associated with a small increase in concentration from 0.02mM to 0.04mM. This trend was also true for LA, CA, MC and compound **4** (Table 3). With respect to μ-max, different patterns were observed, there was a gradual decrease noted with LA, CA, MC and compound **4**, associated with the higher MIC values for these compounds. Whereas, for ML and compound **3**, there was a non-linear association of μ-max reduction with concentration, associated with the very low MIC values determined for these compounds.

4. Discussion

The antimicrobial potential of carbohydrate fatty acid derivatives has received less attention than their other functional properties as emulsifiers or non-ionic surfactants. In contrast to the extensive literature for the antimicrobial properties of monoglycerides, there is limited information about the use of CFA derivatives as food preservatives. Previous studies on antimicrobial properties of sugar esters mainly involved sucrose or other disaccharides esters (Hathcox and Beuchat, 1996; Devulapalle *et al*., 2004). Many of the studies were not carried out using regiochemically pure compounds, were not quantitative and did not include controls to compare activity of free fatty acids with fatty acid derivatives. As a result correlation of chemical structure with efficacy and/or mechanism of action has been difficult.

The current study evaluated the antimicrobial properties of pure fatty acid esters and their corresponding ethers to provide insights into structure/activity relationships for these compounds. The CFA derivatives synthesized in this study were shown to be more effective against Gram positive than Gram negative bacteria ($p<0.05$). This trend was also observed for the fatty acid and monoglyceride controls, in accordance with previous studies (Conley and Kabara 1973; Ruzicka *et al*., 2003). We obtained similar MIC values of 10 μg/ml for monolaurin against *L. monocytogenes* as those reported by Wang and Johnson (1992), and Oh and Marshall (1993). The activity of lauric derivatives **2** and **6** against *Listeria monocytogenes* was found to be equivalent to that of monolaurin and in excess of that reported by Monk *et al.,* (1996), for a lauroyl-sucrose ester.

With respect to the effect of chain length on antimicrobial efficacy of the CFA's, there was a difference in efficacy between Gram positive and Gram negative bacteria. Lauric acid and

derivatives had higher activity against Gram positive bacteria, whereas caprylic acid and its derivative **5** were more active than lauric acid derivatives against *E. coli* ATCC 25922 and *P. fluorescens.* Our data are similar to that of Nair *et al.* (2004a), where populations of *L. monocytogenes* and *E. coli* O157:H7 were shown to decrease below detection levels using 50mM of MC or CA in bovine milk. The same authors, Nair *et al.* (2005), described antimicrobial activity for both CA and MC and found that *Streptococcus* spp. were the most sensitive, and *E. coli* the most tolerant. Whilst both lauric and caprylic fatty acid derivatives retained good activity against Gram positive bacteria, only the caprylic acid derivative displayed useful efficacy against Gram negative bacteria. These trends were also observed with the free FAs and MGs. The enhanced efficacy of the shorter chain fatty acid over the medium chain fatty acid could be attributed to the differences in the outer membrane structure and permeability between Gram-negative and Gram-positive bacteria.

This study also looked at fatty acids conjugated to sugars by ether bonds. Such bonds are not as readily hydrolyzed in biological systems as their ester equivalents. It was interesting to note that these compounds still retained antimicrobial activity indicating that hydrolysis of the ester bond is not necessary for antimicrobial activity. Compound **4** (β ether) was less inhibitory than the free fatty acid (LA) and monoglyceride (ML) against *Listeria* spp. In some cases, compound **2** (α ether) had an enhanced activity by comparison with compound **1** (α ester) and **3** (β ester), particularly for the *Listeria* spp. This may be due to the greater stability of ether bonds over esters (Ved *et al*., 1984), since ether bonds are not subject to cleavage by cellular esterases. Reporting on the antimicrobial efficacy of ether and ester glyceride compounds, Isaacs *et al*., (1995), suggested that ether lipids should remain antimicrobial for a longer period of time than monoglycerides with ester linkages, which

assumes that the fatty acid component does not require release, for example, by esterases for activity. Ruzin and Novik, (2000) showed that monolaurin was rapidly hydrolyzed ($t_{1/2}$ of ~5 min) by esterases in *S. aureus* suggesting that inhibitory activity could be due to free fatty acid liberated from monolaurin by hydrolysis. The differences observed in this study between the ester and ether bonds of the same carbohydrate fatty acid (compounds **1** and **2** and compounds **3** and **4**) show that the nature of the bond between the fatty acid and the sugar has an influence on antimicrobial activity.

The focus of many studies on the mechanism of action of monoglycerides is on cellular membranes. Ruzin and Novik, (2000) reported a monolaurin esterase activity in association with the cell membrane and also in the cytoplasm and the Geh lipase was responsible for approximately 80% of the monolaurin hydrolysing activity. The same authors reported increased lipolytic activity in membrane fractions of *S. aureus* and concluded that *S. aureus* had a membrane bound esterase that participated in the hydrolysis of monolaurin and release of lauric acid. However, the current work suggests that while membrane bound or free esterases may cleave ester bonds of a glycerol or a carbohydrate fatty acid derivative, the ether carbohydrate fatty acid derivatives retained higher activity than the ester derivatives and that the release of a free fatty acid may not be required for potent antimicrobial activity.

In an effort to probe the importance of the carbohydrate moiety, ester and ether fatty acid derivatives based on the following carbohydrates were synthesized and tested: α-glucose, β-glucose, α-mannose and α-galactose. Of these, differences in efficacy were measured for compounds which have the same glycoconjugate bond and alkyl chain length (see entries in Table 2 for compounds **1**, **3**, **6**, **7**). Therefore we conclude that the sugar itself can be a

determining factor on efficacy. This is in accordance with the findings of Watanabe *et al.,* (2000) who also concluded that the configuration of the carbohydrate moiety in similar compounds markedly affected antibacterial activity. In addition, we found that a minor structural change in the carbohydrate can have a major influence on the solubility of the compound. For example, compounds **1**, **3**, and **6** are soluble, whereas the structurally similar compound **7** is insoluble. This further highlights the importance of the choice of carbohydrate.

We found that not only were free single or multiple hydrophilic groups necessary for biological activity, as observed by Conley and Kabara (1973), but that the nature of the hydrophilic group *per se* is also important for the antibacterial activity, as antimicrobial activity associated with the lauroyl pentaerythritol monoester **9** with three free hydroxyl groups was negligible compared to compounds **1**, **3** and **6** which also had the same number of free hydroxyl groups.

Results for compound **8** demonstrates that there is a limit to the number of fatty acids which can be esterified to a monosaccharide and this appears to be one, whereas for the sucrose it has been demonstrated that it is two (Kato and Shibasaki, 1975). Due to the poor solubility in water of compounds **7, 8, 10** and **11**, their potential for application in food systems is limited.

The data obtained from the increase in λ and decrease in μ-max studies showed that sub-MIC concentrations can modify bacterial growth significantly. Nair *et al*., (2004b) also observed this behaviour using MC (50 mM) which reduced *Enterobacter sakazakii* in reconstituted infant formula by >5 log CFU/ml at 37°C, whereas approximately 1.5 log CFU/ml of the pathogen survived after 24 h of incubation using half the concentration of

antimicrobial. This is important towards possible combinations with other antimicrobials or alternative preservation strategies for optimization of practical application of CFA derivatives to microbiological issues within the food and other industries. Combinations of sub-MIC preservatives with other minimal 'hurdles' may contribute to the control of microbiological issues in food systems while minimizing sensory and quality impacts on a food. Combinations of LA or a derivative and other antimicrobials have shown additive or synergistic effects against pathogenic or spoilage bacteria in several matrices (Bell and De Lacy, 1987, Wang and Johnson, 1997; Blaszyck and Holley, 1998; Yamazaki *et al.*, 2004). Lauric esters of methyl glucopyranoside (**1** and **3**) had comparable activity ($p>0.05$) against all Gram positive bacteria tested, regardless of the anomeric configuration of the sugar. With regard to the lauric ethers, compound **2** showed lower MIC values (0.04 mM) against the Gram positive microorganisms compared to compound **4** (2.5 mM to 5 mM, $p<0.05$). This suggests that the alpha or beta configuration of the ether derivative has a considerable effect on the anti-microbial efficacy. In general, the alpha configuration of the carbohydrate moiety of the synthesized compounds was more effective than the beta, for both ester and ether derivatives of the same carbohydrate. This further supports the observation that the carbohydrate moiety has a role in the antimicrobial efficacy of the carbohydrate fatty acid derivative. This finding suggests that there is potential to develop carbohydrate fatty acid derivatives with an efficacy comparable to that of glycerol fatty acid derivatives such as monolaurin.

5. Conclusions

A series of pure, regiochemically defined monosaccharide mono-fatty acid esters and their corresponding ethers were evaluated for antimicrobial activity. The CFA derivatives were found to be significantly more active against Gram positive bacteria than Gram negative bacteria, and lauric esters of methyl glucopyranoside and mannopyranoside as well as the lauric ether of methyl glucopyranoside were comparable to Monolaurin for antimicrobial efficacy. The analysis of both ester and ether fatty acid derivatives of the same carbohydrate, in tandem with alpha and beta configuration of the carbohydrate moiety suggest that the carbohydrate moiety is involved in the antimicrobial activity of the fatty acid derivatives and that the nature of the bond also has a significant effect on efficacy, which requires further investigation. No significant variability in the efficacy of the compounds was observed between *Listeria* strains. The use of a synthetic route to control production of regiochemically defined compounds allows the optimization of the carbohydrate moiety configuration and bond with regard to anti-microbial efficacy, highlighting compounds suitable for regioselective enzymatic synthesis. Carbohydrate fatty acid derivatives have potential as effective antimicrobial compounds for use as preservatives to address a range of microbiological stability and safety issues. Additional knowledge on the mode of action of such compounds in combination with data on their MICs would allow for effective applications.

Acknowledgements

Funding for this project was provided by TSR Strand I funding from the Irish Government under the National Development Plan.

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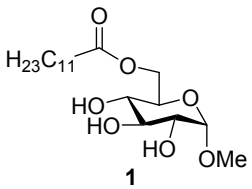
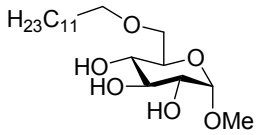


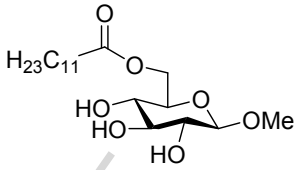
1

Methyl 6-O-lauroyl-α-D-glucopyranoside



2

Methyl 6-O-dodecanyl-α-D-glucopyranoside



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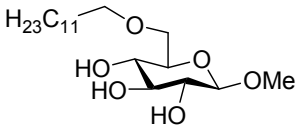

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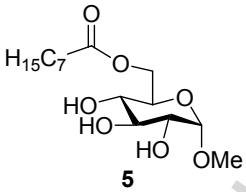
Methyl 6-O-dodecanyl-β-D-glucopyranoside



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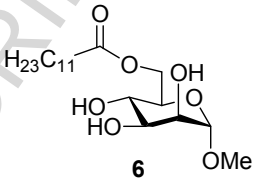
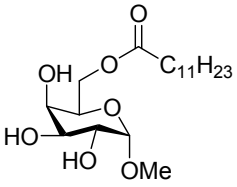
Methyl 6-O-octanoyl-α-D-glucopyranoside



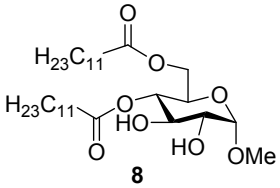
6

Methyl 6-O-lauroyl-α-D-mannopyranoside



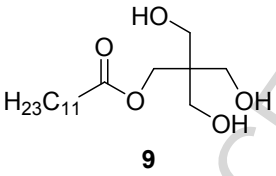
7

Methyl 6-O-lauroyl-α-D-galactopyranoside



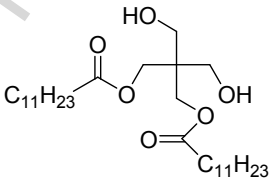
8

Methyl 4,6-di-O-lauroyl-α-D-glucopyranoside



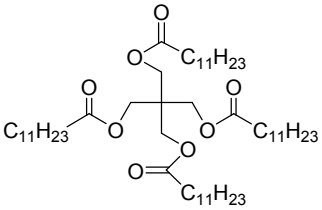
9

Mono-lauroyl pentaerithrytol



10

Di-lauroyl pentaerithrytol



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Tetra-lauroyl pentaerithrytol

Figure 1

Figure Captions

Fig 1. Structures of the novel carbohydrate fatty acid derivatives and non-carbohydrate polyhydroxylated esters synthesized and investigated.

Table 1. Microorganisms used in this study

Strain	Reference [a]	Source
Gram-positive bacteria		
Listeria innocua	NCTC 11288	Cow brain, serotype 6a
Listeria monocytogenes	ATCC 7644	Human
Listeria monocytogenes	NCTC 11994	Cheese, serotype 4b
Listeria monocytogenes	NCTC 7973	Pig mesenteric lymph node
Gram-negative bacteria		
Escherichia coli	ATCC 25922	Clinical isolate
Escherichia coli	NCTC 12900	Human, serotype O157:H7 nontoxigenic
Salmonella enterica (serovar Typhimurium)	ATCC 14028	Animal tissue
Enterobacter aerogenes	ATCC 13048	Sputum
Pseudomonas fluorescens	*	Lettuce

[a] Strains indicated with an asterisk were provided by the Department of Life Sciences, University of Limerick, Ireland

Table 2. Minimum Inhibitory Concentration (MIC; mM) values of Carbohydrate Fatty Acid derivatives and Standards in tryptic soy broth at 37°C after 18 hours.

Microorganism	FA		MG		Carbohydrate fatty acid derivatives					
	LA	CA	ML	MC	1	2	3	4	5	6
Listeria innocua NCTC 11288	0.63	5	0.04	2.5	0.08	0.04	0.08	5	0.63	0.04
Listeria monocytogenes ATCC 7644	0.63	> 5	0.04	5	0.08	0.04	0.08	2.5	2.5	0.04
Listeria monocytogenes NCTC 11994	1.25	> 5	0.04	2.5	0.31	0.04	0.16	> 2.5	1.25	0.04
Listeria monocytogenes NCTC 7973	1.25	5	0.04	2.5	0.08	0.04	0.16	> 2.5	0.31	0.04
Escherichia coli ATCC 25922	> 20	10	20	5	20	20	20	20	12.5	≥ 20
Escherichia coli NCTC 12900	12.5	10	12.5	5	12.5	10	12.5	10	12.5	N.D
Salmonella Typhimurium ATCC 14028	> 20	> 20	20	> 20	20	> 20	> 20	20	> 20	N.D
Enterobacter aerogenes ATCC 13048	> 20	20	20	10	20	> 20	> 20	> 20	> 20	N.D
Pseudomonas fluorescens	> 20	5	20	5	> 20	> 20	> 20	> 20	5	N.D

For each analysis the MIC was recorded as the concentration (mM) that resulted in total inhibition of all replicates. N.D: Not determined
1. Methyl 6-O-lauroyl-α-D-glucopyranoside; 2. Methyl 6-O-dodecanyl-α-D-glucopyranoside; 3. Methyl 6-O-lauroyl-β-D-glucopyranoside;
4. Methyl 6-O-dodecanyl-β-D-glucopyranoside; 5. Methyl 6-O-octanoyl-α-D-glucopyranoside; 6. Methyl 6-O-lauroyl-α-D-mannopyranoside

Table 3. Effect of FA, MG and CFA derivatives on the Lag time (λ) and Maximum specific growth rate (μ_{max}) of *L. monocytogenes* ATCC 7644

Compound (mM)		λ (h)	St.Dev.	μ_{max} (h^{-1})	St.Dev.
LA	0	-		0.30	± 0.034
	0.04	0.0	± 0.06	0.22	± 0.049
	0.08	0.2	± 0.26	0.17	± 0.041
	0.16	2.0	± 1.00	0.10	± 0.017
	0.31	4.8	± 1.73	0.07	± 0.037
	0.63	no growth		0	
ML	0	-		0.30	± 0.034
	0.02	2.3	± 1.09	0.25	± 0.040
	0.04	no growth		0	
1	0.08	no growth		0	
2	0.04	no growth		0	
3	0	-		0.30	± 0.034
	0.02	0.5	± 0.07	0.31	± 0.003
	0.04	5.3	± 0.67	0.27	± 0.006
	0.08	no growth		0	
4	0	-		0.30	± 0.034
	0.16	0.2	± 0.18	0.30	± 0.013
	0.31	0.5	± 0.25	0.27	± 0.009
	0.63	5.0	± 0.55	0.12	± 0.059
	1.25	no growth		0	
CA	0	-		0.30	± 0.034
	0.31	0		0.26	± 0.027
	0.63	0	± 0.04	0.24	± 0.037
	1.25	0.1	± 0.17	0.26	± 0.044
	2.5	0.8	± 0.19	0.21	± 0.034
	5	3.1	± 1.62	0.18	± 0.097
	10	no growth		0	
MC	0	-		0.30	± 0.034
	0.31	0.2	± 0.29	0.26	± 0.029
	0.63	0.3	± 0.40	0.25	± 0.043
	1.25	1.1	± 0.41	0.19	± 0.046
	2.5	5.6	± 1.35	0.01	± 0.034
	5	no growth		0	
5	0	-		0.30	± 0.034
	0.31	0.4	± 0.47	0.24	± 0.035
	0.63	1.6	± 0.94	0.22	± 0.008
	1.25	1.0	± 0.27	0.12	± 0.008
	2.5	1.9	± 0.34	0.08	± 0.001
	5	no growth		0	