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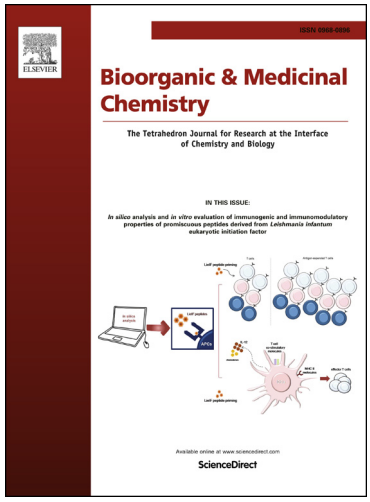

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One-step Synthesis of Carbohydrate Esters as Antibacterial and Antifungal Agents

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ABSTRACT. Carbohydrate esters are biodegradable, and the degraded adducts are naturally occurring carbohydrates and fatty acids which are environmentally friendly and non-toxic to human. A simple one-step regioselective acylation of mono-carbohydrates has been developed that leads to the synthesis of a wide range of carbohydrate esters. Screening of these acylated carbohydrates revealed that several compounds were active against a panel of bacteria and fungi, including *Staphylococcus aureus*. methicillin-resistant *S. aureus* (MRSA), *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus* and *Fusarium graminearum*. Unlike prior studies on carbohydrate esters that focus only on antibacterial applications, our compounds are found to be active against both bacteria and fungi. Furthermore, the synthetic methodology is suitable to scale-up production for a variety of acylated carbohydrates. The identified lead compound, **MAN014,** can be used as an antimicrobial in applications such as food processing and preservation and for treatment of bacterial and fungal diseases in animals and plants.

Keywords: carbohydrate esters, antibacterial, antifungal

1. Introduction

Fungal and bacterial pathogens cause significant economic losses in agriculture and food industry. For example, it is estimated that fungal pathogens destroy more than 125 million tons/yr of the top five food crops (rice, wheat, maize, potatoes and soybeans) that can feed more than 600 million people.[1] Food recalls due to pathogen contamination or illness from foodborne diseases cause significant economic loss.[2] Common disinfection practices in the food industry involve chemical disinfection and irradiation (e.g., γ-ray).[3, 4] While chemicals, such as hypochlorites, iodophors, peroxyacetic acid, and quaternary ammonium compounds, have longer lasting effectiveness than observed following

irradiation,[5] these chemicals pose health and environmental hazards to humans and animals.[6] Recently, there is a trend of using natural products (e.g., tea) in combating foodborne bacterial and fungal pathogens,[7, 8] however, the antimicrobial activities of most natural products are too low to enable their practical use. In the area of food preservatives, sodium benzoate and maleic acid are two common food preservatives. Nonetheless, sodium benzoate may undergo decarboxylation when used with acidic components, (such as vitamin C), leading to the formation of carcinogenic benzene, and maleic acid interrupts the cell energy metabolism.[9, 10] Potassium sorbate is a popular natural food preservatives that are generally regarded as safe (GRAS) by the FDA. Nevertheless, potassium sorbate has been reported to be toxic and mutagenic to human blood cells,[11] In short, there is still a great need for effective natural antimicrobial food additives that are GRAS.

Carbohydrate esters have attracted great interest due to their wide range of applications in industry and medicine.[12, 13] These compounds, mostly acylated at the primary hydroxyl group, are biodegradable and non-toxic while they act as antimicrobials.[14, 15] Carbohydrate esters are synthesized by two general methods: chemical and enzyme-catalyzed synthesis.[16] Both have advantages and disadvantages. Most of the syntheses of carbohydrate esters focus on enzyme-catalyzed esterification or transesterification. These latter methods have the advantage of selective incorporation of the acyl group at the primary hydroxyl group of carbohydrates without tedious protection and deprotection steps.[17-19] However, there are several shortcomings in this method. First, the enzyme-catalyzed reactions often employ chemically synthesized vinyl esters as the substrates or require the use of organic solvents.[20, 21] Therefore, the synthetic conditions are not amenable to “green” processes. Secondly, many enzymes, such as lipases, exert a significant degree of substrate specificity and the yields or conversion efficiency may vary drastically among different carbohydrates and fatty acids of various chainlength.[22] Third, many of these reported reactions were conducted at mg to gram scale .[23] The feasibility for scale-up production to kg scale remain to be demonstrated in most cases.

Chemical-based syntheses can generate a wide variety of carbohydrate esters without the limitation of substrate specificity. The main challenge in the chemical synthesis of carbohydrate esters is regioselectivity. It is difficult to incorporate the acyl group selectively to the desired hydroxyl groups on carbohydrates. To circumvent this problem, many reported methods rely on multiple protection and deprotection steps to achieve the regioselective incorporation of the acyl group at the primary hydroxyl group of carbohydrates.[24-26] This strategy not only increases the cost of synthesis but also reduces the prospects for green production processes and impedes the economical large scale production of carbohydrate esters. In short, the enzymatic process is regioselective but can be challenging for scaled-up production while the chemical process is not regionspecific but is more suitable for making libraries of carbohydrate esters allowing a facile identification of leads.

In light of the problems with traditional synthetic strategies of carbohydrate esters, our group began to explore a novel one-step chemical synthesis strategy for the regioselective production of carbohydrate esters. Our approach takes advantage of the fact that most monosaccharides have one primary hydroxyl group which will be more reactive than secondary hydroxyl groups.[27] By controlling the reaction condition, we promote regioselectively incorporation of the acyl group at the primary hydroxyl group similar to the enzymatic method. With this strategy, we gain easy access to diverse carbohydrate esters made from natural constituents and that are subject to detailed structure-activity relationship (SAR) studies. The lead compounds can be quickly identified and then synthesized in large scale. The lead carbohydrate esters can be purified to provide bioactive compounds consist of natural components.

2. Materials and Methods

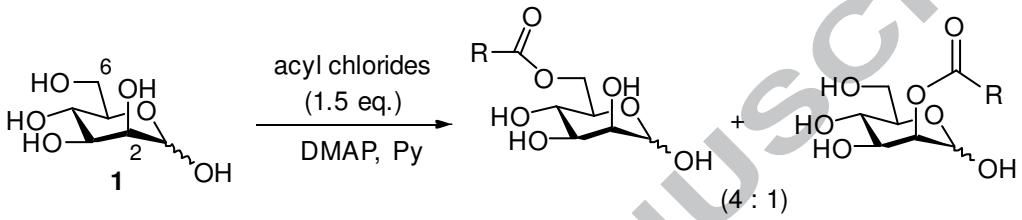
We selected four natural monosaccharides (mannose (**1**), glucose (**2**), *N*-acetylglucosamine (**3**) and galactose (**4**)) and ten acyl groups (acetyl (C2), butanoyl (C4), hexanoyl (C6), octanoyl (C8),

decanoyl (C10), dodecanoyl (C12), tridecanoly (C13), tetradecanoyl (C14), pentadecanoyl (C15) and hexadecanoyl (C16) for constructing libraries of carbohydrate esters. To accommodate the solubilities of the carbohydrates and acyl chlorides, pyridine was used as the solvent. The primary hydroxyl groups are more nucleophilic than the anomeric and the secondary hydroxyl groups. Therefore, the regioselective acylation is expected to be achieved by controlling the equivalent of acyl chloride and the reaction temperature. In most of the cases, 1.5 equivalents of acyl chloride were employed to optimize the production of mono- over di-acylated carbohydrate esters. In some cases, a 1.2 equivalents of the acyl chloride were used to obtain the mono acylated predominantly and 2.5 equivalents to yield predominantly the di-acylated products for comparison. All the reactions were conducted with 1-2 g of carbohydrates and *N,N*-dimethylaminopyridine (DMAP) was used as the catalyst.

Eight mannose esters with carbon chain from 2, 4, 6, 8, 10, 12, 14, and 16 were synthesized, and 1.5 eq. of acyl chloride was found to be optimal for generation of mono-acylated mannose (Scheme 1). Despite numerous attempts, the mono-acylated mannose was obtained as an inseparable mixture of 6-*O*-acylated mannose and 2-*O*-acylated mannose in 4:1 ratio (calculated from the ^{1}H NMR and the sites of acylation were confirmed by the characteristic downfield shift of the diastereotopic H-6 protons as well as the coupling constant of H-2). Chemical derivatization of 6-*O*-acylated mannose and 2-*O*-acylated mannose using trityl chloride also failed to achieve the separation of these two regioisomers. Therefore, these mono-acylated mannose derivatives were tested for their biological activity as a mixture. Following the initial testing of biological activity, we found that the mannose ester with fourteen carbon chain (**MAN014**) was the most active against bacteria and fungi. To optimize the carbon chain length and provide more detailed SAR, we decided to expand our library to include methylmannopyranoside, **5** with fourteen carbon chain ester (**MM014**) (Scheme 2). 6-*O*-acylated adduct was obtained as the major product as expected. However, 3-*O*-acylated adduct was observed as the main by-product according to the integral ratio calculated from the ^{1}H NMR.

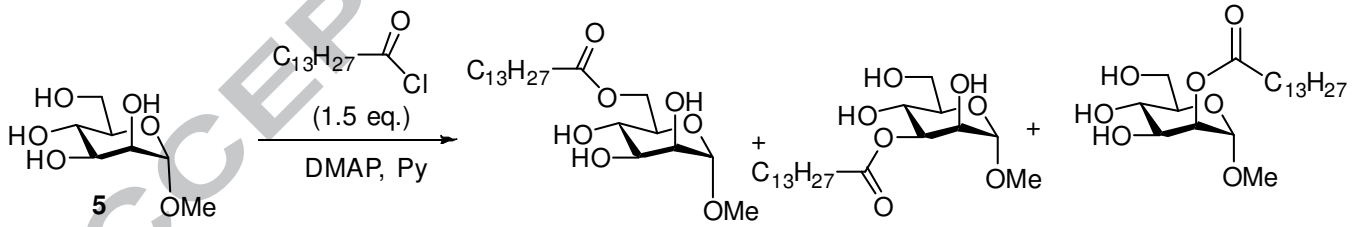
For the same reason, mannose esters with acetyl (C2, **MAN002**), butanoyl (C4, **MAN004**), hexanoyl (C6, **MAN006**), tridecanoyl (C13, **MAN013**) and pentadecanoyl (C15, **MAN015**) groups were also prepared. Similar ratio of 6-*O*-acylated mannose and 2-*O*-acylated mannose was observed for all the reactions.

Scheme 1.



MAN002 (43%): R= CH_3
MAN004 (43%): R= C_3H_7
MAN006 (40%): R= C_5H_{11}
MAN008 (58%): R= C_7H_{15}
MAN010 (25%): R= C_9H_{19}
MAN012 (40%): R= $C_{11}H_{23}$
MAN013 (24%): R= $C_{12}H_{25}$
MAN014 (48%): R= $C_{13}H_{27}$
MAN015 (21%): R= $C_{14}H_{29}$
MAN016 (56%): R= $C_{15}H_{31}$

Scheme 2.

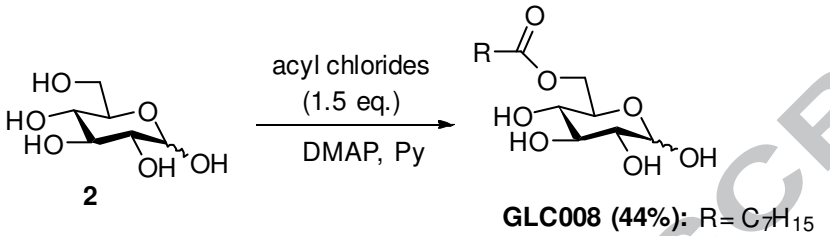


MM014 (52%)

Acylation of glucose with acyl chlorides of C8, C10, C12 and C14 was conducted in a similar fashion. The acylation of glucose is more regioselective than mannose. Five 6-acylated glucose derivatives,

GLC008, **GLC010**, **GLC012**, **GLC014**, and **GLC016** were synthesized, and site of acylation was confirmed again by the characteristic downfield shift of the diastereotopic H-6 protons (Scheme 3).[28]-[18],[29, 30]

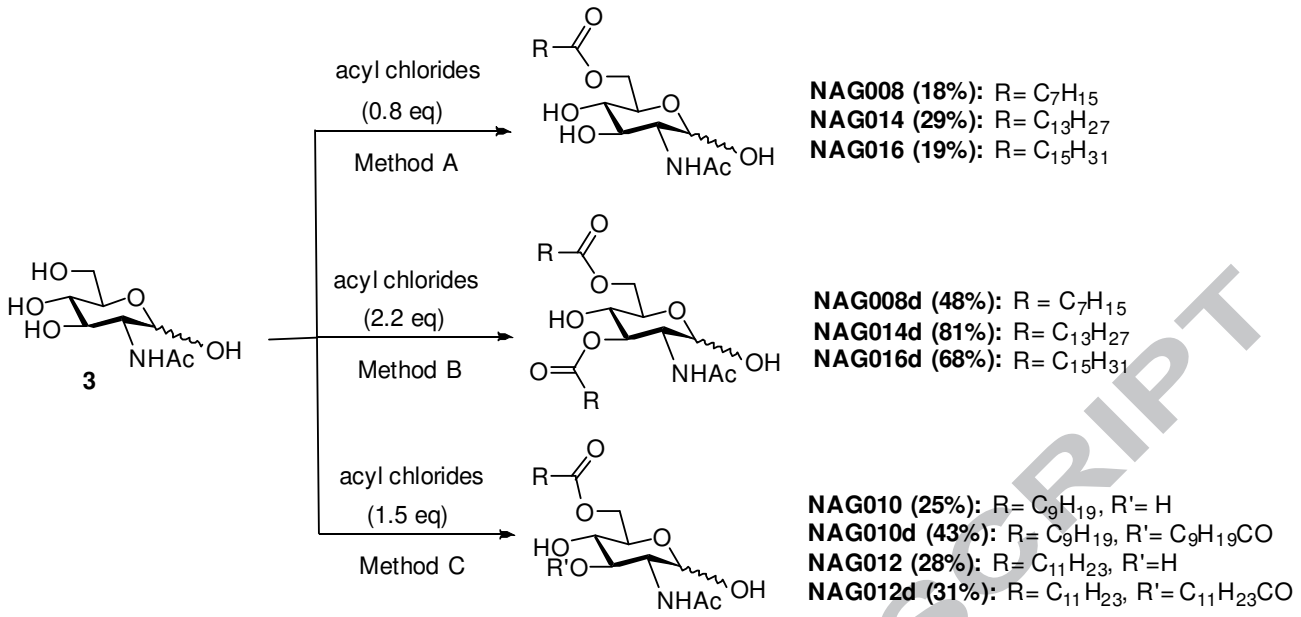
Scheme 3.



GLC008 (44%): R= C_7H_{15}
GLC010 (14%): R= C_9H_{19}
GLC012 (57%): R= $C_{11}H_{23}$
GLC014 (45%): R= $C_{13}H_{27}$
GLC016 (56%): R= $C_{15}H_{31}$

Acylation of *N*-acetylglucosamine with acyl chlorides of C8, C10, C12, C14, and C16 was more challenging and offered a mixture of mono and diacetylated adducts (Scheme 4). To optimize the yields of both mono and diacetylated *N*-acetylglucosamine for biological study, we developed three different methods (Methods A, B and C) for their synthesis. Method A involves the use of 0.8 eq. of acyl chlorides to optimize the production of mono-acylated *N*-acetylglucosamine (NAG). In method C, 2.2 eq. of acyl chlorides was used to favor the production of di-acylated NAG. Finally, we discovered that using 1.5 eq. acyl chlorides (Method C) can generate mono- and di-acylated *N*-acetylglucosamine (NAG) in one pot with satisfactory yields for both prodiuts, and these two products can be separated using column chromatography. The acylation took place at the *O*-6 position for the mono-acylated products. The sites of acylation for the diacetylated products occurred at *O*-6 and *O*-3. In all the cases the sites of acylation were confirmed by ^{1}H and ^{1}H-^{1}H COSY NMR.

Scheme 4.



Unfortunately, acylation of galactose provided a complex inseparable mixture with no distinct major products.

3. Results and Discussion

3.1 Antibacterial activity

All of the carbohydrate esters were tested against Gram-positive (G+) *S. aureus* (ATCC25923) and Gram-negative (G-) *Escherichia coli* (ATCC25922) using vancomycin, kanamycin A, neomycin as the controls. The minimum inhibitory concentrations (MICs) for these compounds are summarized in Table 1. **MAN014** is the most active compound with MICs of 16 - 32 μg/mL and 128 μg/mL against *S. aureus* and *E. coli*, respectively. **MAN012**, **MAN014**, and **MM014** were chosen to be further tested against methicillin-resistant *S. aureus* (MRSA, ATCC33591 and ATCC43300), *Pseudomonas aeruginosa* (G-, ATCC 27853), *Klebsiella pneumoniae* (G-, ATCC 13883). **MAN014** is the most active compound against all of these bacterial strains with MICs of 16 - 32 μg/mL and 128 μg/mL against MRSA and G- bacteria, respectively. Sodium myristate (C14) was tested and no antibacterial activity was noted. Overall, the lead carbohydrate esters are more active against G+ than G- bacteria. In conclusion,

MAN014 has superior antibacterial activity than aminoglycosides (kanamycin and neomycin) against MRSA, and better activity against G- bacteria than vancomycin.

Table 1. MIC values of carbohydrate esters against bacteria[a]

Entry	Compound	*S. aureus* (ATCC25923)	*E. coli* (ATCC25922)	*S. aureus* (ATCC33591) (MRSA)
1	**MAN012**	**128**	>256	128 - 256
2	**MAN013**	**64 - 128**	>256	ND
3	**MAN014**	**16 - 32**	128	**16 - 32**
4	**MM014**	128	>256	64 -128
5	**MAN015**	**64**	>256	ND
6	Vancomycin	2	>256	2
7	Kanamycin	1 - 2	2 - 4	256
8	Neomycin	2	2 - 4	>256

[a] Unit: (μg/mL), [b] ND: Not determined.

3.2 Antifungal activity

The synthesized compounds were tested against a panel of human and plant fungal pathogens using voriconazole (an azole class antifungal agent) and **K20** [31] as controls (Table 2). In general, mannose esters, **MAN015**, **MAN014**, and **MAN013** are relatively active against all the tested strains. Other compounds have MIC values >256 μg/mL against the tested fungi. *Candida albicans 64124* is a human pathogen that is resistant to many clinically used azole-based antifungal agents. *Aspergillus* species are typically recalcitrant to most recognized antifungal drugs. Thus, infectious mycoses of humans and animals caused by pathogenic *Aspergillus* species are often untreatable and serious and have high mortality rates. It is noted that all three mannose esters are more active than the controls against *C. albicans* 64124 and *Aspergillus flavus*. Based on the results of antibacterial and antifungal activities, we conclude that the mannose plays an essential role in the biological activity of carbohydrates esters.

Table 2. MIC of carbohydrate esters against fungi[a]

Entry	Compound	*Aspergillus flavus*	*Fusarium graminearum* B4-5A	*Candida albicans 64124* (azole-resistant)	*Candida albicans MYA2876* (azole susceptible)	*Cryptococcus neoformans* H99	*Rhodotorula pilimanae*
1	**MAN012**	128	128	256	256	256	128
2	**MAN013**	**32 - 64**	**16 - 32**	**64**	**64 - 128**	**32 - 64**	**32 - 64**
3	**MAN014**	**32**	**32**	**64**	**64**	**8**	**8**
4	**MM014**	> 256	**4 - 16**	> 256	> 256	256	> 256
5	**MAN015**	**4 - 16**	**8**	**16 - 32**	64	**8 - 16**	16
6	**K20**	> 256	8	256	64	4	4
7	Voriconazole	1	32	>256	0.125	0.125	8

[a] Unit: (μg/mL).

We have reported that amphiphilic antifungal kanamycin K20 can exert strong synergism with azole-based antifungal agents.[32] Thus, we also conducted in vitro synergistic studies using **MAN014** and several azole class antifungal agents using the checkerboard method. The fractional inhibitory concentration index (FICI) is summarized in Table 3. In contrast to **K20** that shows strong synergism with most azoles, **MAN014** showed good synergy (FIC indices > 0.5) with all tested crop fungicides against *A. flavus*. On the other hand, **MAN014** did not show synergy with all azoles tested against other fungal strains (SI table1). **K20** is active against fungi but inactive against most bacteria while **MAN014** displays a broad-spectrum activity against both fungi and bacteria.

Table 3. *A. flavus* Antifungal synergistic activities against of azoles combined with **MAN014**[a]

Azole	MIC or inhibitory concentration (IC) (μg/mL)			FICI (**MAN014** with azole)
	alone	IC of azole in the presence of **MAN014**	IC of **MAN014** in the presence of azole	
MAN014	31.25			
Caramba (met)	0.781	0.195	7.810	0.499
Headline (pyra)	6.25	0.195	3.910	0.156
Proline (prothio)	6.25	0.781	7.810	0.375
Propicon (propio)	3.13	0.391	7.810	0.375
Prosaro (prothio&tebu)	0.781	0.095	7.810	0.375

[a] Combination interactions are classified as synergistically inhibitory if the FICI is ≤0.5, indifferent if >0.5 - 4, and antagonistic if >4

3.3 Mode of growth inhibitory action by MAN014

Carbohydrate esters are amphiphilic compounds and consequently are predicted to perturb cell membranes as their mode of action.[33-35] Based on this assumption, Sytox Green dye was used to examine **MAN014** effects on cell membrane permeability.[36] Normally non-fluorescent, Sytox Green becomes fluorescent when it enters cells through pores of permeable membranes and binds to nucleic acids. The capability of **MAN014** to form membrane pores was tested with bacterial strain *S. aureus* (ATCC25923) and the filamentous fungal strain, *Fusarium graminearum* B-4-5A using the protocols described by Weerden et al.[34, 37] Strains were incubated with and without **MAN014** for two hours. Strains were incubated with **MAN014** at 1xMIC, 2xMIC, and 4x MIC. **MAN014** at 1xMIC caused increases of green fluorescence by Sytox Green in cells of both *S. aureus* (ATCC25923) and *F. graminearum* B-4-5A (Figures 1 and 2). Similar results were obtained with 2xMIC and 4xMIC of **MAN014**. These observations support membrane pore formation as the mode of growth inhibitory action of **MAN014** against bacteria and fungi.

Figure 1. *S. aureus* (ATCC25923) single hypha experiment, blank control (top row), and bacteria incubated with **MAN014** at 1X MIC for 2h (bottom row).

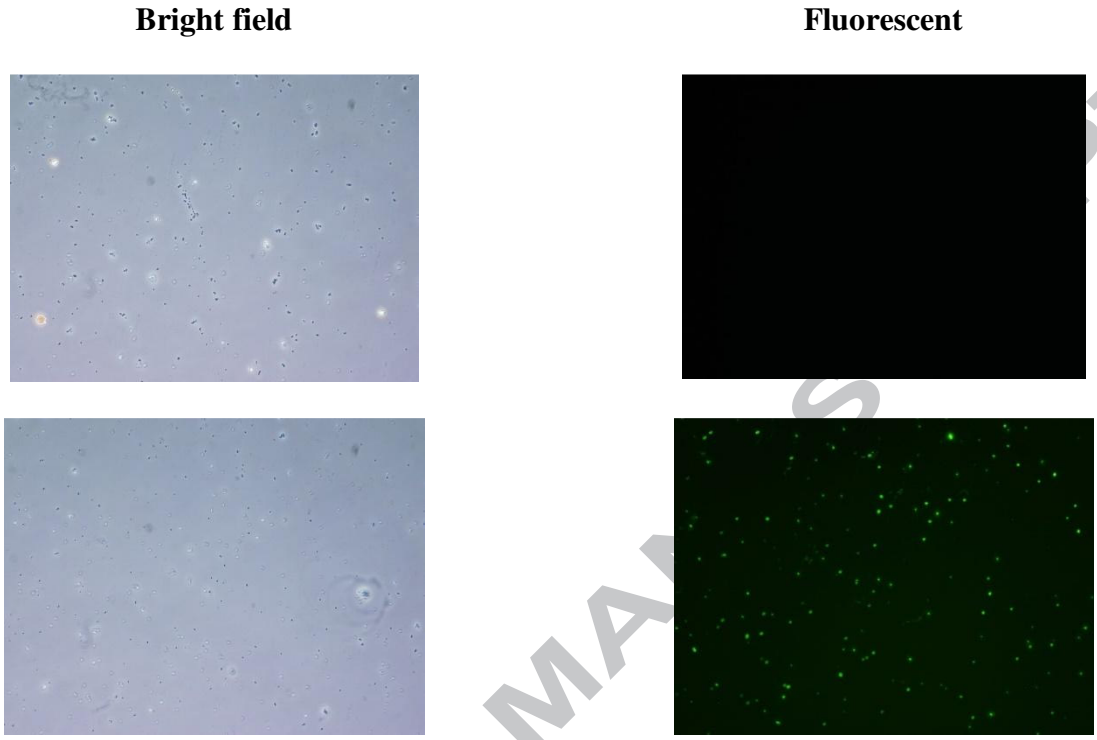
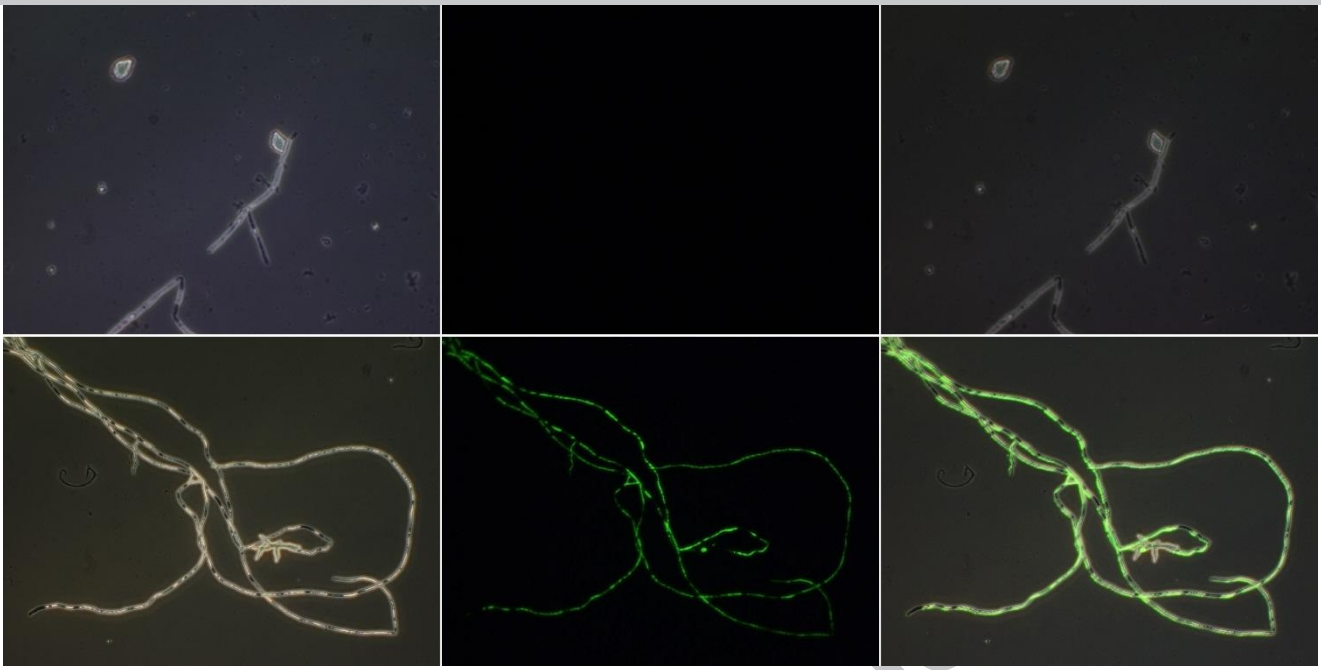


Figure 2. *Fusarium graminearum* single hypha experiment, blank control (top row), and fungi incubated with **MAN014** at 1X MIC for 2h (bottom row).

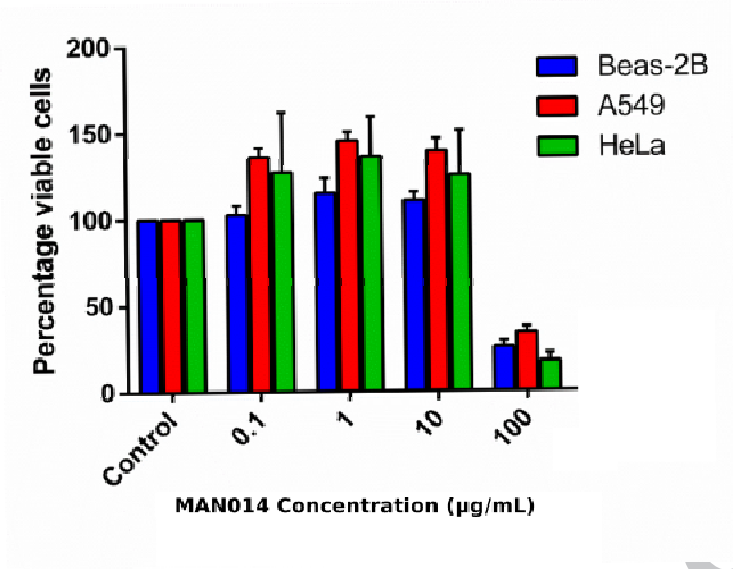
Bright field **Fluorescent** **Merge**



3.4 Mammalian cell cytotoxicity

A primary concern of using amphiphilic compounds in food, human, animal or other applications, is toxicity toward mammalian cells. The cytotoxicity of **MAN014** were evaluated using three mammalian cells, including A549 (cancer epithelial lung), Beas-2B (normal, epithelial lung) and HeLa (adenocarcinoma, epithelial cervix), and MTT assay (Figure 3). The IC_{50} values for **MAN014** were determined to be 107.65 ± 1.98, 77.35 ± 6.14 and 71.76 ± 4.69 μg/mL for A549, Beas-2B and HeLa, respectively. At low concentrations (up to 10 μg/mL), **MAN014** appeared to have no cytotoxicity or even slightly promoted the cell growth. Significant cytotoxicity of **MAN014** was observed with a concentration at 100 μg/mL, which is 2-8 fold higher than the MICs.

Figure 3. Cytotoxicity of **MAN014**



4. Large scale synthesis of MAN014

In an effort to demonstrate the feasible of large scale synthesis of lead compound. We carried out a 30g scale synthesis of **MAN014 u**sing 1.5 eq. myristoyl chloride. The reaction mixture was quenched with 1 N HCl and the products, which contained both mono- and di-acylated mannose, were collected by filtration. Further purification using flash column chromatography provided **MAN014** in 48% yield along with 28% of the di-acylated adduct. The purity of **MAN014** was examined by ^{1}H NMR and no residual pyridine or DMAP can be observed. In contrast to the smaller scale synthesis, two types of di-acylated mannose derivatives were isolated as an inseparable mixture, which consist of about 1/1 ratio of 2,6-di-*O*-acylated (α/β = 85/15, confirmed by gated ^{13}C NMR and COSY) and 1,3 di-*O*-acylated (α/β = 60/40) adducts. These di-acylated products are difficult to assay for biological activity due to poor solubility in aqueous media.

5. Conclusions

We have successfully established a one-step synthesis of a library of carbohydrate esters. In contrast to most of studies in this area that focus on antibacterial activity, several of the currently described synthesized carbohydrate esters are active against both fungi and bacteria. A detailed SAR was conducted allowing the identification of a lead compound, **MAN014**. **MAN014** shows a broad-spectrum activity against bacteria and fungi while having relatively low cytotoxicity toward animal cells. Mode of action studies confirm that **MAN014** exerts its bioactivity via pore formation of the cell membrane. Finally, we have demonstrated that **MAN014** can be synthesized in large scale with high purity. All of these features combined make **MAN014** a prominent and environment-friendly antibacterial and antifungal natural compound that can be used for applications in medicine, public health, and agriculture.

6. Experimental Section

6.1 General Procedures.

All chemicals were purchased from the commercially available resources without any further purification. Dry pyridine was dried over calcium hydride. Mass spectrometry was taken by high resolution mass spectrometry (HRMS) using a TOF mass spectrometer. Two NMR instruments were used 300 or 500 MHz for the ^{1}H and 75 or 125 MHz for the ^{13}C Nuclei. $CDCl_3$ and CD_3OD, were used as solvents. The parts per million (ppm) were used to express the chemical shifts on scale. The peaks splitting pattern were expressed as (s; for the singlet), (d; doublet), (t; triplet), (q; quadrate), (m; multiplet), and (ddd; doublet of doublets of doublets). Coupling constants J were measured in Herts (Hz).

6.2 General procedure for synthesis of acylated mannose and glucose.

Synthesis of mono-acylated sugars, 0.5 g (1 equiv.) of sugar (glucose or mannose) and 1.5 equiv. of corresponding acyl chloride was stirred in 20 mL of pyridine for overnight. The formation of product

was confirmed by TLC (EtOAc/MeOH = 9/1). The reaction solution was concentrated under reduced pressure and the crude oily reaction mixture was loaded to silica gel column. The column chromatography was eluted by a gradient from pure EtOAc to EtOAc/MeOH = 9/1. The product was collected and dried under reduced pressure to offer slight yellowish solid. The purity of the carbohydrates was confirmed by H NMR (the final product is mainly sugar mixed with small amount of sugar due to the natural constitution of the corresponding sugar).

6.3 General procedure for synthesis of acylated *N*-acetylglucosamine.

Method A: 0.5 g (1 equiv.) of *N*-acetylglucosamine and 0.8 equiv. of corresponding acyl chloride was stirred in 20 mL of pyridine for overnight. The formation of product was confirmed by TLC (Hexane: EtOAc = 7.5:2.5). The reaction solution was concentrated under reduced pressure and the crude oily reaction mixture was loaded to silica gel column. The column chromatography was eluted by a gradient from pure Hexans to Hexans: EtOAc = 7.5:2.5. The product was collected and dried under reduced pressure to offer slight yellowish solid

Method B: 0.5 g (1 equiv.) of *N*-acetylglucosamine and 2.2 equiv. of corresponding acyl chloride was stirred in 20 mL of pyridine for overnight. The formation of product was confirmed by TLC (Hexans:EtOAc = 7.5:2.5). The reaction solution was concentrated under reduced pressure and the crude oily reaction mixture was loaded to silica gel column. The column chromatography was eluted by a gradient from pure Hexans to Hexans:EtOAc = 7.5:2.5. The product was collected and dried under reduced pressure to offer slight yellowish solid.

Method C: 0.5 g (1 equiv.) of *N*-acetylglucosamine and 1.5 equiv. of corresponding acyl chloride was stirred in 20 mL of pyridine for overnight. The formation of product was confirmed by TLC (Hexane:EtOAc = 7.5:2.5). The reaction solution was concentrated under reduced pressure and the crude oily reaction mixture was loaded to silica gel column. The column chromatography was eluted by

a gradient from pure Hexaneto Hexane:EtOAc = 7.5:2.5. The product was collected and dried under reduced pressure to offer slight yellowish solid.

6.4 6-*O*-Acetyl-D-mannopyranose (MAN002). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN002** was obtained as a light yellowish oil (0.27 g, 1.19 mmole, 43%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.06 (s, 1H), 4.38 (dd, *J* = 11.8, 2.0 Hz, 1H), 4.22 (dd, *J* = 11.9, 6.2 Hz, 1H), 3.6 – 4.0 (m, 4H), 2.07 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 171.61, 94.51, 71.38, 70.78, 70.22, 67.43, 63.94, 19.37; ESI/APCI Calcd. for $C_8H_{13}O_6$ $[M - OH]^+$: 205.0712; Measured m/e : 205.0718.

6.5 6-*O*-Butanoyl-D-mannopyranose (MAN004). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN004** was obtained as a light yellowish oil (0.30 g, 1.19 mmole, 43%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 4.38 (dd, *J* = 11.8, 2.0 Hz, 1H), 4.22 (dd, *J* = 11.8, 6.2 Hz, 1H), 3.6 – 4.0 (m, 4H), 2.34 (t, *J* = 7.3 Hz, 2H), 1.6 – 1.7 (m, 2H), 0.9 – 1.0 (m, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.12, 94.84, 71.38, 70.80, 70.30, 67.46, 63.75, 35.48, 18.00, 12.59; ESI/APCI Calcd. for $C_{10}H_{17}O_6$ $[M-OH]^+$: 233.1025; Measured m/e : 233.1023.

6.6 6-*O*-Hexanoyl-D-mannopyranose (MAN006). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN006** was obtained as a light yellowish oil (0.31 g, 1.11 mmole, 40%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.06 (s, 1H), 4.38 (dd, *J* = 11.8, 2.0 Hz, 1H), 4.22 (dd, *J* = 11.8, 6.2 Hz, 1H), 3.6 – 4.0 (m, 4H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.6 – 1.78 (m, 2H), 1.3 – 1.4 (m, 4H), 0.9 – 1.0 (m, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.29, 94.49, 71.38, 70.80, 70.30, 67.46, 63.78, 33.55, 31.04, 24.27, 21.99, 12.59; ESI/APCI Calcd. for $C_{12}H_{21}O_6$ $[M - OH]^+$: 261.1338; Measured m/e : 261.1360.

6.7 6-*O*-Octanoyl-D-mannopyranose (MAN008). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN008** was obtained as a light yellowish oil (0.49 g, 1.61 mmole, 58%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.05 (s, 1H), 4.35 (dd, *J* = 11.3, 2.0 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.4 – 4.0 (m, 4H), 2.3 – 2.4 (m, 2H), 1.5 – 1.7 (m, 2H), 1.2 - 1.4 (m, 8H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.28, 94.49, 71.38, 70.81, 69.41, 67.47, 61.38, 33.59, 31.48, 28.81, 28.71, 24.60, 22.28, 13.05; ESI/APCI Calcd. for $C_{14}H_{25}O_6$ $[M\text{-}OH]^+$: 289.1651; Measured m/e : 289.1680.

6.8 6-*O*-Decanoyl-D-mannopyranose (MAN010). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN010** was obtained as a light yellowish oil (0.23 g, 0.69 mmole, 25%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.03 (s, 1H), 4.35 (dd, *J* = 11.5, 2.0 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.4 – 4.0 (m, 4H), 2.3 – 2.4 (m, 2H), 1.5 – 1.7 (m, 2H), 1.2 - 1.4 (m, 12H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.28, 94.49, 71.37, 70.81, 70.30, 67.77, 63.81, 33.60, 31.67, 29.21, 29.06, 29.04, 28.87, 24.61, 22.35, 13.11; ESI/APCI Calcd. for $C_{16}H_{29}O_6$ $[M - OH]^+$: 317.1964; Measured m/e : 317.1990.

6.9 6-*O*-Dodecanoyl-D-mannopyranose (MAN012). This compound has been reported previously.[38, 39] Please refer to the general procedure for synthesis of carbohydrates esters**. MAN012** was obtained as a light yellowish oil (0.40 g, 1.11 mmole, 40%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 300 MHz) 5.0 – 5.1 (m, 1H), 4.35 (dd, *J* = 11.6, 2.0 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.4 – 4.0 (m, 4H), 2.3 – 2.4 (m, 2H), 1.5 – 1.7 (m, 2H), 1.2 - 1.4 (m, 16H), 0.88 (t, J = 6.5 Hz, 3H).

6.10 6-*O*-Tridecanoyl-D-mannopyranose (MAN013). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN013** was obtained as a light yellowish oil (0.23 g, 0.61 mmole, 24%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.03 (s, 1H), 4.35 (dd, *J* = 11.6, 2.0 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.4 – 4.0 (m, 4H),

2.3 – 2.4 (m, 2H), 1.5 – 1.7 (m, 2H), 1.2 - 1.4 (m, 18H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.26, 94.51, 71.40, 70.81, 71.31, 67.47, 63.78, 33.58, 31.68, 29.39 (2 Carbons), 29.36 (2 Carbons), 29.34, 29.22, 29.08, 28.85, 22.33, 13.03; ESI/APCI Calcd. for $C_{19}H_{36}O_7Na$ $[M + Na]^+$: 399.2353; Measured m/e : 399.2339.

6.11 6-*O*-Tetradecanoyl-D-mannopyranose (MAN014). This compound has been reported previously.[40] Please refer to the general procedure for synthesis of carbohydrates esters**. MAN014** was obtained as a light yellowish solid (0.52 g, 1.33 mmole, 48%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.03 (d, *J* = 1.6 Hz, 1H), 4.39 (dd, *J* = 11.7, 2.0 Hz, 1H), 4.22 (dd, *J* = 11.7, 6.1 Hz, 1H), 3.7 – 4.0 (m, 3H), 3.63 (t, *J* = 9.6 Hz, 1H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.6 – 1.7 (m, 2H), 1.2 - 1.4 (m, 20H), 0.91 (t, *J* = 6.9 Hz, 3H).

6.12 2,6-Di-*O*-tetradecanoyl-D-mannopyranose (MAN014d). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN014d** was obtained as a white solid: mp 88.7 - 94.6 °C (0.38 g, 0.63 mmole, 28%) in a mixture of / anomers in a 8.5/1.5 ratio. The reported NMR is for the anomer. ^{1}H NMR ($CDCl_3$, 500 MHz) 5.26 (s, H1), 5.17 (dd, *J* = 3.4, 1.6 Hz, H2), 4.57 (dd, *J* = 12.1, 4.1 Hz, H6eq), 4.29 (dd, *J* = 12.1, 2.1 Hz, H6ax), 4.11 (dd, *J* = 9.5, 3.4 Hz, H3), 4.0 – 4.1 (m, H5), 3.64 (t, *J* = 9.7 Hz, H4), 3.4 – 4.0 (m, 4H), 2.3 – 2.4 (m, 4H), 1.6 – 1.7 (m, 4H), 1.2 - 1.4 (m, 40H), 0.90 (t, *J* = 7.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 174.86, 173.62, 93.78, 71.85, 70.71, 69.31, 67.85, 63.19, 34.34, 34.24, 31.92 (2 Carbons), 29.69 (2 Carbons), 29.66 (7 Carbons), 29.61, 29.51, 29.46, 29.36 (2 Carbons), 29.30, 29.28, 29.15, 29.10, 25.01, 24.93, 22.69 (2 Carbons); ESI/APCI Calcd. for $C_{34}H_{64}O_8Na$ $[M + Na]^+$: 600.4601; Measured m/e : 623.4494.

6.13 3,6-Di-*O*-tetradecanoyl-D-mannopyranose (MAN014d). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN014d** was obtained as a white solid: mp 88.7 - 94.6 °C (0.38 g, 0.63 mmole, 28%) in a mixture of / anomers in a 6/4 ratio. The reported NMR is for the anomer. ^{1}H NMR ($CDCl_3$, 500 MHz) 5.28 (s, H1), 5.21 (dd, *J* = 9.8, 3.1 Hz, H3), 4.57 (dd, *J* = 12.2, 4.5 Hz,

H6eq), 4.37 (dd, *J* = 12.3, 2.2 Hz, H6ax), 4.0 – 4.1 (m, H2, H5), 3.87 (t, *J* = 9.8 Hz, H4), 3.4 – 4.0 (m, 4H), 2.3 – 2.4 (m, 4H), 1.6 – 1.7 (m, 4H), 1.2 - 1.4 (m, 40H), 0.90 (t, *J* = 7.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 174.86, 173.62, 92.54, 71.17, 69.68, 68.85, 65.89, 63.14, 34.23, 34.17, 31.92 (2 Carbons), 29.69 (2 Carbons), 29.66 (8 Carbons), 29.51, 29.45, 29.36 (2 Carbons), 29.30, 29.26, 29.20, 29.10, 24.95, 24.87, 22.69 (2 Carbons); ESI/APCI Calcd. for $C_{34}H_{64}O_8Na$ $[M + Na]^+$: 600.4601; Measured m/e : 623.4494.

6.14 Methyl 6-*O*-tridecanoyl-α-D-mannopyranose (MM014). Please refer to the general procedure for synthesis of carbohydrates esters**. MM014** was obtained as a light yellowish oil (0.58 g, 1.44 mmole, 52%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 4.62 (d, *J* = 1.5 Hz, 1H), 4.42 (dd, *J* = 11.7, 1.7 Hz, 1H), 4.22 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.6 – 3.9 (m, 4H), 3.38 (s, 3H), 2.73 (t, *J* = 7.4 Hz, 2H), 1.6 – 1.7 (m, 2H), 1.3 - 1.4 (m, 20H), 0.92 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.08, 101.38, 71.13, 70.63, 70.49, 67.30, 63.69, 53.81, 33.63, 31.69, 29.41, 29.38 (2 Carbons), 29.35, 29.23, 29.10, 29.04, 28.84, 24.66, 22.35, 13.08; ESI/APCI Calcd. for $C_{21}H_{40}O_6$ $[M\text{-}OH]^+$: 387.2747; Measured m/e : 387.2782.

6.15 6-*O*-Pentadecanoyl-D-mannopyranose (MAN015). Please refer to the general procedure for synthesis of carbohydrates esters**. MAN015** was obtained as a light yellowish oil (0.19 g, 0.47 mmole, 21%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 500 MHz) 5.05 (d, *J* = 1.75 Hz, 1H), 4.39 (dd, *J* = 11.8, 2.0 Hz, 1H), 4.22 (dd, *J* = 11.8, 6.1 Hz, 1H), 3.7 – 4.0 (m, 3H), 3.6 – 3.7 (m, 1H), 2.3 – 2.4 (m, 2H), 1.6 – 1.7 (m, 2H), 1.2 - 1.4 (m, 22H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.26, 94.51, 71.40, 70.80, 71.31, 67.47, 63.78, 33.58, 31.68, 29.39 (2 Carbons), 29.36 (2 Carbons), 29.34, 29.22, 29.08 (2 Carbons), 28.85, 24.58, 22.33, 13.03; ESI/APCI Calcd. for $C_{21}H_{40}O_6Na$ $[M + Na]^+$: 427.2666; Measured m/e : 427.2652.

6.16 6-*O*-Hexadecanoyl-D-mannopyranose (MAN016). This compound has been reported previously.[39] Please refer to the general procedure for synthesis of carbohydrates esters**. MAN016** was obtained as a light yellowish solid (0.53 g, 1.26 mmole, 56%) in a mixture of / anomers in a 3/1 ratio. The reported NMR is for the anomer. ^{1}H NMR (CD_3OD, 300 MHz) 5.05 (s, 1H), 4.35 (dd, *J* = 11.7, 2.0 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.4 – 3.9 (m, 4H), 2.3 – 2.4 (m, 2H), 1.6 – 1.7 (m, 2H), 1.2 - 1.4 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H).

6.17 2-Acetamido-2-deoxy-6-*O*-octanoyl-α-D-glucopyranose (NAG008). Please refer to the method (**A**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG008** was obtained as a light yellowish oil (0.44 g, 1.27 mmole, 58%). ^{1}H NMR (CD_3OD, 500 MHz) 5.04 (d, *J* = 3.4 Hz, 1H), 4.35 (dd, *J* = 11.8, 2.0 Hz, 1H), 4.18 (dd, *J* = 11.8, 5.1 Hz, 1H), 3.95 (ddd, *J* = 9.9, 5.1, 2.0 Hz, 1H), 3.83 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.67 (dd, *J* = 10.6, 8.9 Hz, 1H), 3.33 (dd, *J* = 9.9, 8.9 Hz, 1H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.96 (s, 3H), 1.5 – 1.7 (m, 2H), 1.2 - 1.4 (m, 8H), 0.88 (t, *J* = 6.1 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 175.64, 173.79, 92.70, 72.64, 70.86, 70.01, 64.89, 55.94, 35.35, 32.99, 30.29, 30.22, 26.15, 23.80, 22.76, 14.55; ESI/APCI Calcd. for $C_{16}H_{30}NO_7$ $[MH]^+$: 348.2017; Measured m/e : 348.2025.

6.18 2-Acetamido-3,6-di-*O*-octanoyl-2-deoxy-α-D-glucopyranose (NAG008d). Please refer to the method (**B**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG008d** was obtained as a light yellowish solid: mp 111.9 – 116.5 °C (0.51 g, 1.08 mmole, 48%). ^{1}H NMR ($CDCl_3$, 500 MHz) 6.37 (d, *J* = 9.2 Hz, 1H), 5.2 – 5.3 (m, 2H), 4.72 (d, *J* = 3.4 Hz, 1H), 4.43 (dd, *J* = 12.2, 3.6 Hz, 1H), 4.27 (dd, *J* = 12.0, 1.7 Hz, 1H), 4.0 - 4.2 (m, 2H), 3.5 – 3.6 (m, 1H), 3.24 (d, *J* = 6.1 Hz, 1H), 2.3 – 2.4 (m, 4H), 1.93 (s, 3H), 1.5 – 1.7 (m, 4H), 1.2 - 1.4 (m, 16H), 0.8 – 0.9 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 175.55, 175.03, 170.91, 91.92, 73.17, 70.06, 68.95, 63.04, 52.35, 34.53, 34.34, 31.84 (2 Carbons), 29.29 (2 Carbons), 29.20, 29.10, 25.13, 25.06, 23.18, 22.78 (2 Carbons), 14.24 (2 Carbons); ESI/APCI Calcd. for $C_{24}H_{44}NO_8$ $[MH]^+$: 474.3061; Measured m/e : 474.3081.

6.19 2-Acetamido-2-deoxy-6-*O*-decanoyl-α-D-glucopyranose (NAG010). Please refer to the method (**C**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG010** was obtained as a light yellowish oil (0.21 g, 0.57 mmole, 25%). ^{1}H NMR (CD_3OD, 500 MHz) 5.08 (d, J = 3.4 Hz, 1H), 4.40 (dd, J = 11.8, 2.1 Hz, 1H), 4.23 (dd, J = 11.8, 5.3 Hz, 1H), 3.9 – 4.0 (m, 1H), 3.87 (dd, J = 10.7, 3.5 Hz, 1H), 3.72 (dd, J = 10.6, 8.8 Hz, 1H), 3.38 (dd, J = 9.9, 8.9 Hz, 1H), 2.36 (t, J = 7.5 Hz, 2H), 2.00 (s, 3H), 1.5 – 1.6 (m, 2H), 1.2 - 1.4 (m, 12H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.14, 172.27, 91.19, 71.15, 71.13, 69.35, 63.37, 54.44, 33.59, 31.63, 29.17, 29.01 (2 Carbons), 28.80, 24.62, 22.31, 21.20, 13.02; ESI/APCI Calcd. for $C_{18}H_{34}NO_7$ $[MH]^+$: 376.2330; Measured m/e : 376.2331.

6.20 2-Acetamido-3,6-di-*O*-decanoyl-2-deoxy-α-D-glucopyranose (NAG010d). Please refer to the method (**C**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG010d** was obtained as a white solid: mp 107.1 – 115.9 °C (0.51 g, 0.97 mmole, 43%). ^{1}H NMR ($CDCl_3$, 500 MHz) 6.37 (d, J = 9.2 Hz, 1H), 5.2 – 5.3 (m, 2H), 4.72 (d, J = 3.4 Hz, 1H), 4.43 (dd, J = 12.2, 3.6 Hz, 1H), 4.27 (dd, J = 12.0, 1.7 Hz, 1H), 4.0 - 4.2 (m, 2H), 3.5 – 3.6 (m, 1H), 3.24 (d, J = 6.1 Hz, 1H), 2.3 – 2.4 (m, 4H), 1.93 (s, 3H), 1.5 – 1.7 (m, 4H), 1.2 - 1.4 (m, 16H), 0.8 – 0.9 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 175.55, 175.03, 170.91, 91.92, 73.17, 70.06, 68.95, 63.04, 52.35, 34.53, 34.34, 31.84 (2 Carbons), 29.29 (2 Carbons), 29.20, 29.10, 25.13, 25.06, 23.18, 22.78 (2 Carbons), 14.24 (2 Carbons); ESI/APCI Calcd. for $C_{28}H_{52}NO_8$ $[MH]^+$: 530.3687; Measured m/e : 530.3674.

6.21 2-Acetamido-2-deoxy-6-*O*-dodecanoyl-α-D-glucopyranose (NAG012). Please refer to the method (**C**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG012** was obtained as a white solid (0.26 g, 0.63 mmole, 28%). ^{1}H NMR (CD_3OD, 500 MHz) 5.08 (d, J = 3.5 Hz, 1H), 4.39 (dd, J = 11.8, 2.0 Hz, 1H), 4.22 (dd, J = 11.8, 5.2 Hz, 1H), 3.9 – 4.0 (m, 1H), 3.86 (dd, J = 10.6, 3.4 Hz, 1H), 3.72 (dd, J = 10.6, 8.9 Hz, 1H), 3.37 (dd, J = 9.3, 9.3 Hz, 1H), 2.35 (t, J = 7.5 Hz, 2H), 2.00 (s, 3H), 1.5 – 1.6 (m, 2H), 1.2 - 1.4 (m, 16H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.12, 172.26, 91.19, 71.15, 71.13, 69.35, 63.37, 54.44, 33.59, 31.67, 29.33 (2 Carbons), 29.21, 29.06,

29.01, 28.81, 24.62, 22.33, 21.21, 13.03; ESI/APCI Calcd. for $C_{20}H_{37}NO_7$ $[MH]^+$: 403.2570; Measured m/e : 403.2649.

6.22 2-Acetamido-3,6-di-*O*-dodecanoyl-2-deoxy-α-D-glucopyranose (NAG012d). Please refer to the method (**C**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG012d** was obtained as a white solid: mp 72.3 – 75.8 °C (0.51 g, 0.97 mmole, 43%). ^{1}H NMR ($CDCl_3$, 500 MHz) 6.42 (d, *J* = 9.2 Hz, 1H), 5.2 – 5.3 (m, 2H), 4.44 (dd, *J* = 12.2, 3.6 Hz, 1H), 4.32 (dd, *J* = 12.1, 1.7 Hz, 1H), 4.1 - 4.2 (m, 1H), 4.0 - 4.1 (m, 1H), 3.59 (d, *J* = 9.4 Hz, 1H), 2.3 – 2.4 (m, 4H), 1.97 (s, 3H), 1.5 – 1.7 (m, 4H), 1.2 - 1.4 (m, 32H), 0.88 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 175.35, 174.80, 170.86, 91.69, 72.97, 69.90, 68.78, 62.85, 52.23, 34.35, 34.15, 31.90 (2 Carbons), 29.62 (2 Carbons), 29.61 (2 Carbons), 29.50 (2 Carbons), 29.33 (2 Carbons), 29.29 (2 Carbons), 29.09, 29.06, 24.96, 24.88, 22.95, 22.67 (2 Carbons), 14.09 (2 Carbons); ESI/APCI Calcd. for $C_{32}H_{60}NO_8$ $[MH]^+$: 586.4313; Measured m/e : 586.4336.

6.23 2-Acetamido-2-deoxy-6-*O*-tetradecanoyl-α-D-glucopyranose (NAG014). Please refer to the method (**A**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG014** was obtained as a light yellowish solid: mp 149.1 – 153.2 °C (0.58 g, 1.33 mmole, 59%). ^{1}H NMR (CD_3OD, 500 MHz) 5.04 (d, *J* = 3.4 Hz, 1H), 4.37 (dd, *J* = 11.8, 2.2 Hz, 1H), 4.20 (dd, *J* = 11.7, 5.4 Hz, 1H), 3.9 – 4.0 (m, 1H), 3.84 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.69 (dd, *J* = 10.4, 8.7 Hz, 1H), 3.35 (dd, *J* = 9.6, 8.5 Hz, 1H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.96 (s, 3H), 1.5 – 1.6 (m, 2H), 1.2 - 1.4 (m, 20H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 175.50, 175.02, 170.71, 92.01, 73.13, 70.20, 68.96, 63.03, 52.31, 34.55, 32.12, 29.86, 29.71, 29.57, 29.50, 29.37, 29.31, 25.17, 25.10, 24.97, 23.28, 22.89, 14.32; ESI/APCI Calcd. for $C_{22}H_{42}NO_7$ $[MH]^+$: 432.2956; Measured m/e : 432.2945.

6.24 2-Acetamido-3,6-di-*O*-tetradecanoyl-2-deoxy-α-D-glucopyranose (NAG014d). Please refer to the method (**B**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG014d** was obtained as a light yellowish solid: mp 80.4 – 86.5 °C (1.17 g, 1.83 mmole, 81%). ^{1}H NMR ($CDCl_3$, 500 MHz) 6.23 (d, *J* = 9.2 Hz, 1H), 5.2 – 5.3 (m, 2H), 4.46 (dd, *J* = 12.2, 3.6 Hz, 1H), 4.2 – 4.3 (m, 1H), 4.1 - 4.2

(m, 1H), 4.0 - 4.1 (m, 1H), 3.58 (dd, *J* = 9.6 Hz, 1H), 2.3 – 2.4 (m, 4H), 1.96 (s, 3H), 1.6 – 1.7 (m, 4H), 1.2 - 1.4 (m, 40H), 0.86 (t, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 175.68, 173.81, 92.01, 73.13, 72.20, 68.96, 63.03, 52.31, 34.55, 34.36, 32.12, 29.86 (8 Carbons), 29.71 (2 Carbons), 29.57 (2 Carbons), 29.50 (2 Carbons), 29.37, 29.31, 25.17, 25.10, 24.97, 23.28, 22.89 (2 Carbons), 14.32 (2 Carbons); ESI/APCI Calcd. for $C_{36}H_{68}NO_8$ $[MH]^+$: 642.4939; Measured m/e : 642.4844.

6.25 2-Acetamido-2-deoxy-6-*O*-hexaadecanoyl-α-D-glucopyranose (NAG016). Please refer to the method (**A**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG016** was obtained as a light yellowish solid: mp 124.8 – 129.4 °C (0.59 g, 1.29 mmole, 57%). ^{1}H NMR (CD_3OD, 500 MHz) 5.08 (d, *J* = 3.5 Hz, 1H), 4.40 (dd, *J* = 11.8. 2.0 Hz, 1H), 4.23 (dd, *J* = 11.8, 5.3 Hz, 1H), 3.9 – 4.0 (m, 1H), 3.87 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.72 (dd, *J* = 10.5, 8.9 Hz, 1H), 3.35 (dd, *J* = 9.6, 8.5 Hz, 1H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.00 (s, 3H), 1.5 – 1.6 (m, 2H), 1.2 - 1.4 (m, 24H), 0.92 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.13, 172.26, 91.20, 71.15, 71.13, 69.35, 63.36, 54.44, 33.59, 31.67, 29.38 (3 Carbons), 29.36 (2 Carbons), 29.33, 29.21, 29.07, 29.02, 28.82, 24.63, 22.33, 21.20, 13.03; ESI/APCI Calcd. for $C_{24}H_{46}NO_7$ $[MH]^+$: 460.3269; Measured m/e : 460.3259.

6.26 2-Acetamido-3,6-di-*O*-tetradecanoyl-2-deoxy-α-D-glucopyranose (NAG016d). Please refer to the method (**B**) for synthesis of mono and di-acylated *N*-acetylglucosamine**. NAG016d** was obtained as a white solid: mp 101.4 – 109.5 °C (1.07 g, 1.54 mmole, 68%). ^{1}H NMR ($CDCl_3$, 500 MHz) 5.88 (d, *J* = 9.2 Hz, 1H), 5.2 – 5.3 (m, 2H), 4.52 (dd, *J* = 12.3, 3.7 Hz, 1H), 4.1 – 4.3 (m, 3H), 4.0 - 4.1 (m, 1H), 3.5 – 3.6 (m, 1H), 3.11 (s, 1H), 2.82 (s, 1H), 2.3 – 2.4 (m, 4H), 1.94 (s, 3H), 1.6 – 1.7 (m, 4H), 1.2 - 1.4 (m, 48H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 175.15 (2 Carbons), 174.71, 91.91, 72.85, 70.24, 68.70, 62.73, 52.11, 34.35, 34.16, 34.14, 31.93 (2 Carbons), 29.71 (7 Carbons), 29.67 (5 Carbons), 29.63, 29.50, 29.37 (2 Carbons), 29.28, 29.17, 29.11, 25.00, 24.92, 23.17, 22.70 (2 Carbons), 14.13 (2 Carbons); ESI/APCI Calcd. for $C_{40}H_{76}NO_8$ $[MH]^+$: 698.5565; Measured m/e : 698.5550.

6.27 Antibacterial MIC Determination. A liquid culture of a selected bacterial strain was inoculated into Trypticase Soy broth at 35°C for 1-2 h. The bacteria cell concentration (no. of cells/mL and absorbance at 600 nm) was determined and diluted with broth, if necessary, to an absorbance value of 0.08 to 0.1 at 600 nm. The adjusted inoculated medium (100 μL) was diluted with 10 mL of broth and then applied to a 96-well microtiter plate (50 μL). A series of solutions (50 μL each in 2-fold dilution) of the tested compounds was added to the testing wells. The 96-well plate was incubated at 35°C for 12 to18 h. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to completely inhibit the growth of bacteria. The MIC determinations for each experiment were repeated at least three times.

6.28 Antifungal MIC and FIC Index Determinations. *In vitro* growth inhibition of yeast strains by carbohydrate ester compounds were determined using MIC microbroth dilution assays in 96-well uncoated polystyrene microtiter plates (Corning Costar, Corning, NY, USA) as described in the M27-A2 reference methods of the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Standards Laboratory Standards) (NCCLS).[41] Serial dilutions of compounds were made in uncoated polystyrene 96-well plates in the range of 0.25 to 512 μg/mL. For MIC determinations with filamentous fungi, previously described methods were used.[42] FIC index determinations were carried out according to previously described methods by checkerboard analysis.[43] Viable yeast cell or fungal spore concentrations were determined by agar medium plate colony counting. The final concentration was adjusted to 1×10^5 colony forming units/mL. The range of concentrations of drugs used for FIC index determinations were based on their corresponding MIC values. Fungal growth was examined visually. All experiments were done in duplicate.

6.29 Cytotoxicity assay:

Mammalian cells were grown in DMEM 1X (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin. Two-hundred µL of cells (25,000 cells/mL) were added to 96-well cell culture treated plates (Corning^{TM}) and incubated for 24 h at 37 °C with 5% CO_2. Different concentrations of **MAN014** (1.0, 10.0, 100.0 µg/mL) were added and incubated for another 48 h. Triton X100 was added to a separate well as a positive control. For cell viability assay, 20 µL of MTT stock solution (5 mg/mL) was added to each well and incubated for 4 h. Upon completion of incubation, the media was carefully removed and washed twice with 100 mL of PBS buffer. The live cells will reduce MTT reagent to purple crystals, which was dissolved in 200 µL of DMSO, and the absorbance was measured at 570 nm and 670 nm with a microplate reader (Synergy H4). The results are expressed as percentage viability compared with that of control. For each type of mammalian cell, the assay was repeated 3 times and each assay was performed in triplicate. Standard deviations were determined from data sets of three independent experiments

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Supporting Information Available ^{1}H and ^{13}C NMR spectra of the synthesized compounds.

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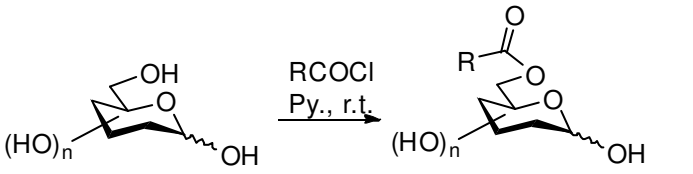
Graphic Abstract



antibacterial and antifungal