Bioorganic & Medicinal Chemistry 26 (2018) 765–774

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

One-step synthesis of carbohydrate esters as antibacterial and antifungal agents

Madher N. AlFindee [a,c], Qian Zhang [a], Yagya Prasad Subedi [a], Jaya P. Shrestha [a], Yukie Kawasaki [b], Michelle Grilley [b], Jon Y. Takemoto [b], Cheng-Wei Tom Chang [a,*]

[a] Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322-0300, USA
[b] Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322-5305, USA
[c] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basra, Basra, Iraq

ARTICLE INFO

Article history:
Received 26 November 2017
Revised 22 December 2017
Accepted 24 December 2017
Available online 26 December 2017

Keywords:
Carbohydrate esters
Antibacterial
Antifungal

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.

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 preservative that is 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

* Corresponding author.
E-mail address: tom.chang@usu.edu (C.-W.T Chang).

https://doi.org/10.1016/j.bmc.2017.12.038
0968-0896/

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 remains to be demonstrated in most cases.

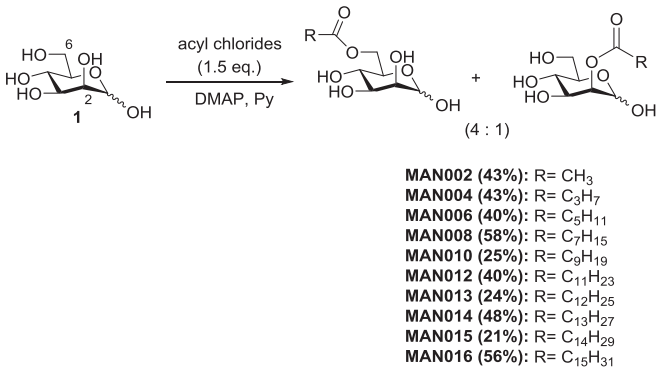
Chemical-based syntheses can generate a wide variety of carbohydrate esters without the limitation of substrate specificity. The main challenge in the chemical synthesis of carbohydrate esters is regioselectivity. It is difficult to incorporate the acyl group selectively to the desired hydroxyl groups 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 (*C*2), 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.

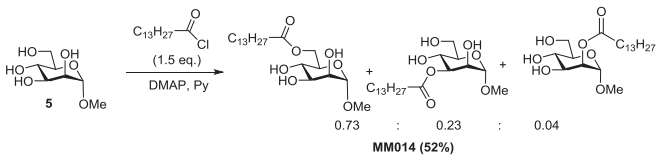


Scheme 1.

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. A similar ratio of 6-*O*-acylated mannose and 2-*O*-acylated mannose was observed for all the reactions.

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]

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 products, 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



Scheme 2.