Mutagenic activity of quaternary ammonium salt derivatives of carbohydrates

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Full Research Paper

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Keywords:
6-bromohexyl D-glucopyranoside; microbiological mutagenicity test; quaternary ammonium salt

Abstract
This paper presents a study on a series of quaternary ammonium salt (QAS) derivatives of glucopyranosides with an elongated hydrophobic hydrocarbon chain. The new N-[6-(β-D-glucopyranosyloxy)hexyl]ammonium bromides and their O-acetyl derivatives were analyzed via 1H and 13C NMR spectroscopy. The mutagenic activity of the newly synthesized QAS was investigated using two different techniques: The Vibrio harveyi luminescence assay and the Ames test. The obtained results support previous findings contesting QAS safety and indicate that QAS, specifically pyridinium derivatives, might be mutagenic.

Introduction
Carbohydrates and alditols occur broadly in nature and possess many biological functions essential to living organisms. Sugars not only contribute as energetic substances, but also serve as building materials for fungi, microbes, plants, and animals. The polymers (oligosaccharides) of D-glucose found, for example, in wood (cellulose) and D-glucosamine present in shells of crabs and insects (chitin), are the widely known ones [1,2]. Another class of carbohydrate biopolymer derivatives – D-ribose and 2-deoxy-D-ribose – constitutes the backbone of RNA and DNA, respectively.

The mechanisms of molecular recognition and cell interaction are mostly explained by the interaction of carbohydrates with proteins, called lectins, exposed at the cell surface. This process, on the one hand, allows bacteria to interact with other cells during infection, but the same recognition pattern is used
to fight bacterial infections. Dendritic cells from mammalian
immune system express a variety of sugar-binding proteins
(lectins) at their surface. They capture, process, and display
antigens to native T-cells and trigger the adaptive immune
system [3]. Proteins located at the surface of the cell serve as
potential targets for new drugs containing sugars [1,2,4].

Quaternary ammonium salts (QASs) constitute a class of
organic compounds with a broad range of applications. A
typical QAS consists of a positively charged nitrogen atom with
four residues (aliphatic or aromatic). Mostly, one of the aliphat-
ic or aromatic residues possesses hydrophobic properties,
whereas the nitrogen group is hydrophilic. The QAS molecules
have a typical head/tail structure determining their amphiphilic
character [5].

QASs find application in many fields of everyday life, and their
usage reaches hundreds of thousands of tons every year – in
2004 that number reached 0.5 million tons [6]. Antibacterial
and antifungal action is the significant property of QAS. There-
fore, they are being used as disinfectants, starting from hospital
services to wood protection and house construction [7–9]. More-
over, QASs are applied in industry, agriculture (as pesticides
and herbicides) [10], and chemistry (as catalysts and solvents)
[11,12]. QASs are also used as ingredients in hair conditioners,
shampoos, and toothpastes [13].

Ionic liquids (IL) are recognized as a particularly interesting
group of QASs. These are extensively explored worldwide and
find many applications. Because of their unique properties, in-
cluding low melting point (by definition below 100 °C), high
conductivity, high thermal stability, low flammability, and very
low (if any) volatility [14], they are called “green chemistry”
solvents, and are considered as a good alternative for classical
organic solvents. Among many applications, dissolving cellu-
lose (which was impossible for common solvents), depolymer-
izing nonnatural polymers, and capturing CO₂ appear particu-
larly interesting [15].

Despite their unique properties, QASs have many drawbacks.
Their rapid spread in society and the increasing resistance of
pathogenic microorganisms [16] require new chemicals, includ-
ing QASs, posing new risks for the environment and the living
organisms. However, the long-term effects of these new com-
pounds with regard to their possible toxicity toward human cells
and aquatic organisms are still unknown [17,18]. Additionally,
the impact of these chemicals on the environment is raised;
many reports describe accumulation of QASs in sludge, soils,
and water [6,10]. It is for this reason that the biodegradation
pathways of the newly introduced chemicals need to be thor-
oughly investigated [19–21]. One of the issues of greatest

concern regarding the introduction of newly synthesized chemi-
cals is their possible long-term effect on the living organisms.
Many of these chemicals can potentially accumulate in organ-
isms, thus rendering their overall long-term effect difficult to
assess. Hence, screening of newly synthesized chemicals for
their possible genotoxic activity seems to be a matter of particu-
lar importance, as long-term exposure to even low doses of
genotoxic compounds can induce mutations, which might lead
to cancer [22–24].

To overcome many problems associated with QASs, they were
combined with sugars. We intended to obtain biologically
active compounds – especially antibacterial and antifungal –
with high biocompatibility and good biodegradation properties.
Some examples of fused molecules containing QASs and sugar
moieties were described before [22,25,26]. It was shown that
quaternization of chitosan increases its antimicrobial activity;
such polymer is proposed to be used as a wound dressing after
surgeries to eliminate infections and to improve the healing
process [27–29]. Moreover, the introduction of a sugar moiety
to anticancer drug molecules enhanced their activity and select-
ivity [30,31].

In this paper, we describe process of synthesis, structural char-
acteristics, and mutagenic activity profile of eight new QAS de-
rivatives of 6-aminohexyl-D-glucopyranosides. These com-
pounds correspond to previously described derivatives with two
carbon atom spacers dividing the sugar moiety and the ammoni-
um group [22]. Here, we synthesized compounds with a long
hydrocarbon chain (containing six carbon atoms), which will
enable us to analyze the influence of the distance between
QASs and the sugar groups on the efficiency of the synthesis
process and the influence on their biological activity.

Results and Discussion

Chemistry

We synthesized N-[6-(β-D-glucopyranosyloxy)hexyl]ammoni-
um salts to determine the effect of the linker length in QASs on
their mutagenic potential (Scheme 1). The main product of the
first step of the synthesis was the 1,2-trans-glucoside, i.e.,
6-bromohexyl 2',3',4',6'-tetra-O-acetyl-β-D-glucopyranoside (2)
in 36% yield [32]. By using a Lewis acid (BF₃·Et₂O) as an acti-
vator and by extending the reaction time to 72 h, the product
with configuration α-D-gluco, i.e., 6-bromohexyl 2',3',4',6'-
tetra-O-acetyl-α-D-glucopyranoside (3) in 24% yield and 6-
bromohexyl 2',3',4',6'-tetra-O-acetyl-β-D-glucopyranoside (2)
in 17% yield were obtained. 6-Bromohexyl 2',3',4',6'-tetra-O-
acetyl-β-D-glucopyranoside (2) and 6-bromohexyl 2',3',4',6'-
tetra-O-acetyl-α-D-glucopyranoside (3) were then reacted with
trimethylamine in ethanol and with pyridine to assess the effect
of the sugar substituent on the course of quaternization.
pounds 4a, 4b, 6a, 6b were obtained in almost quantitative yields (92–99%). Since de-O-acetylated salts could not be obtained using sodium methanolate in methanol, an alternative route involving the reaction of 6-bromohexyl D-glucopyranoside (2′ [32] or 3′) with tertiary amines (trimethylamine in ethanol and with pyridine) was applied; the yields were of 90–94%. Shifting the pyranose leaving group (halogen) by six carbon atoms from C1 makes it easier to design the QAS. All the newly synthesized N-[6-(β-D-glucopyranosyloxy)hexyl]ammonium bromides were water soluble. The identities of all compounds were confirmed by 1H and 13C NMR.

**Mutagenic activity of QAS**

QASs are generally recognized as safe compounds. Nevertheless, considering our previous observations on the pronounced mutagenic activity of QAS derivatives with two carbon atom spacers dividing the sugar moiety and the ammonium group [22], we investigated the mutagenic activity of newly synthesized QAS with a carbohydrate spacer containing six carbon atoms.

Two different bacterial mutagenicity assays were applied. In the first approach, a recently developed *Vibrio harveyi* bioluminescence assay was used. This assay has been reported as being highly sensitive and therefore capable for antimutagenicity screening [33,34]. Moreover, all QASs synthesized in this work were tested for their mutagenic activity using the *Salmonella typhimurium* TA98 strain in the Ames test. The Ames test is a well-established and routinely used bacterial mutagenicity assay for examining the safety of newly obtained compounds before they are commercially available.

In this work, five out of eight tested QASs, namely, 4b, 5a, 5b, 6b, and 7b exhibited a substantial mutagenic activity in the *Vibrio* bioluminescence assay (Figure 1). The extent of their mutagenic activity was comparable to that of a model acridine mutagen, ICR191, used as a positive control. Compounds 4a, 6a, and 7a did not display mutagenic activity in the *Vibrio* luminescence assay at all. Apart from N-[6-(β-D-glucopyranosyl-oxy)hexyl]-N,N,N-trimethylammonium bromide (5a), pyridinium salts tend to be more mutagenic than their trimethylam-
Figure 1: Mutagenic activity of the QASs in the *Vibrio harveyi* A16 strain bioluminescence assay. A, 4a; B, 4b; C, 5a, D, 5b; E, 6a; F, 6b; G, 7a; H, 7b. C1 (marked in white), negative (water) control; C2 (marked in black), positive control (6-chloro-9-[3-(2-chloroethylamino)propylamino]-2-methoxyacridine dihydrochloride /ICR191/, 100 nM). Bars indicate mean values (± standard deviation) of bacterial luminescence, expressed as relative light units (RLU) per A575 of bacterial culture. Luminescence of the positive control (C2) was considered 100%.

Monium counterparts. Moreover, the position of the hexyl chain occurs to be important for mutagenicity of trimethylammonium salts; compounds with an hexyl chain in equatorial position (4a, 5a) are more mutagenic than those with an hexyl chain in axial position (6a, 7a). By contrast, in the corresponding analyses conducted with *Salmonella typhimurium* TA98, no mutagenic effects of the eight tested QASs were observed (Figure 2). Such results are consistent with previously published data indicating that the *Vibrio harveyi* assay can detect very weak mutagenic activity [22,34].

Despite the existing assumption about the general safety of QASs [35-37], our findings suggest that at least some of the QASs might exhibit genotoxic potential. Our observations are in agreement with the previous reports demonstrating the genotoxic potential of QASs. In 2007, Ferk et al. [38] demonstrated genotoxic activity of two commonly used QASs, benzalkonium chloride and dimethyldioctadecylammonium bromide. Although tested compounds were nonmutagenic in bacteria, further analysis on eukaryotic cells revealed their significant genotoxic effects. Moreover, described genotoxicity of QASs toward plant tissues indicates their significance as environmental genotoxins [38]. In the 2011 paper [22], we described a pronounced mutagenic activity of QASs containing a carbohydrate moiety with a two-carbon atom linker in the *Vibrio harveyi* bioluminescence assay. Here, we provide evidence for the mutagenic potential of corresponding QAS–carbohydrate derivatives with a longer, six-carbon atom linker. This suggests that the introduction of a longer hydrocarbon chain does not affect the genotoxic potential of the QASs, indicating the need for other structural modifications that might minimize genotoxic activity of the QAS–carbohydrate derivatives.

**Conclusion**

Most of the newly-synthesized QAS were proven to be mutagenic in the *Vibrio harveyi* bioluminescence assay. Obtained results suggest that at least some of QASs can be genotoxic. Moreover, we observed that pyridinium salts tend to be more mutagenic than trimethylammonium derivatives, whereas the position of the hexyl chain seems to be important for the extent of the mutagenic activity of trimethylammonium salts. Regarding extensive usage of QASs, not only in the industry [6-9], but also as constituents of cosmetics and drugs [10,12,13], further research is needed to assess possible genotoxic activity of both newly developed and commercially available compounds as well as to further develop structural modifications that could increase the safety of QASs, particularly the ones intended for medical usage.
Figure 2: Mutagenic activity of the QASs in the Ames test with histidine-dependent Salmonella typhimurium TA98 strain. A, 4a; B, 4b; C, 5a; D, 5b; E, 6a; F, 6b; G, 7a; H, 7b. C1 (marked in white), negative (water) control; C2 (marked in black), positive control (2-amino-3-methylimidazo[4,5-f]quinoline [IQ], 50 nmol/plate). Results are reported as percentage number of revertants observed for the positive control (C2). Bars indicate mean values from three plates ± standard deviation.

Supporting Information
Supporting Information File 1
General procedures, analytical data and spectra of all new compounds.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-138-S1.pdf]

Acknowledgments
This work was financially supported by grant DS/ 530-8455-D501-15.

References
1. Dwek, R. A. Chem. Rev. 1996, 96, 683–720. doi:10.1021/cr940283b
2. Varki, A. Glycobiology 1993, 3, 97–130. doi:10.1093/glycob/3.2.97
3. Rieger, J.; Stoffebach, F.; Cui, D.; Imbery, A.; Lameignere, E.; Putaux, J.-L.; Jérôme, C.; Jérôme, R.; Auzély-Velty, R. Biomacromolecules 2007, 8, 2717–2725. doi:10.1021/bm070342y
4. Rieger, J.; Freicheis, H.; Imbery, A.; Putaux, J.-L.; Delair, T.; Jérôme, C.; Auzély-Velty, R. Biomacromolecules 2009, 10, 651–657. doi:10.1021/bm801492c
5. Pernak, J.; Borucka, N.; Wakiwicz, F.; Markiewicz, B.; Fochtman, P.; Stolle, S.; Steudt, S.; Stepnowski, P. Green Chem. 2011, 13, 2901–2910. doi:10.1039/c1gc15468k
6. Ismail, Z. Z.; Tezel, U.; Pavlou, G.; Strehlitz, S. G. Water Res. 2010, 44, 2303–2313. doi:10.1016/j.watres.2009.12.029
7. Cheng, G.; Zhang, Z.; Chen, S.; Bryers, J. D.; Jiang, S. Biomaterials 2007, 28, 4192–4199. doi:10.1016/j.biomaterials.2007.05.041
8. Ward, M.; Sanchez, M.; Elsner, M. O.; Lowe, A. B. J. Appl. Polym. Sci. 2006, 101, 1036–1041. doi:10.1002/app.23269
9. Olešek, J.; Plechcová, A.; Krásiková, A.; Leczyński, J. Microbiol. Res. 2013, 168, 630–636. doi:10.1016/j.micres.2013.06.001
10. Pateiro-Moure, M.; Arias-Estévez, M.; López-López, E.; Martinez-Carbajal, E.; Simal-Gándara, J. Bull. Environ. Contam. Toxicol. 2008, 80, 407–411. doi:10.1007/s00128-008-9403-z
11. Zhao, D.; Wu, M.; Kou, Y.; Min, E. Catal. Today 2002, 74, 157–189. doi:10.1016/S0920-5861(01)00541-7
12. Nguyen Van Bui, O.; Aupoix, A.; Doan Thi Hong, N.; Vo Thanh, G. New J. Chem. 2009, 33, 2060–2072. doi:10.1039/b902956g
13. Levinson, M. I. J. Surfactants Deterg. 1999, 2, 223–235. doi:10.1007/s11743-999-0077-4
14. Coleman, D.; Špulák, M.; Garcia, M. T.; Gathergood, N. Green Chem. 2012, 14, 1350–1356. doi:10.1039/c2gc16090k
15. Cevasco, G.; Chiappe, C. Green Chem. 2014, 16, 2375–2385. doi:10.1039/c3gc42096e
16. Sundheim, G.; Langsrud, S.; Heir, E.; Holck, A. L. Int. Biodeterior. Biodegrad. 1998, 41, 235–239. doi:10.1016/S0964-8305(98)00027-4
17. Nagamune, H.; Maeda, T.; Ohkura, K.; Yamamoto, K.; Nakajima, N.; Kouh, H. Toxicol. In Vitro 2000, 14, 139–147. doi:10.1016/S0887-2333(00)00003-5
