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# Mutagenicity of quaternary ammonium salts containing carbohydrate moieties

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ABSTRACT

Quaternary ammonium salts are widely used in industrial, agricultural, healthcare and domestic applications. They are believed to be safe compounds, with little or no health hazard to humans. However, in this report, we demonstrate that a series of newly synthesized quaternary ammonium salts containing carbohydrate moieties reveal potent mutagenic activities, as assessed by using the *Vibrio harveyi* bioluminescence mutagenicity test. D-Gluco- and D-galacto-derivatives were found to have a higher mutagenic potential than D-manno-derivatives. Among the former groups of compounds, the *N*-[2-(D-glycopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium salts were of the highest activity in the mutagenicity assay. These results suggest that the safety of quaternary ammonium salts may be lower than previously supposed, indicating a need for testing such compounds for their mutagenicity.



## 1. Introduction

Quaternary ammonium salts (QAS) are extensively used in various applications. They are present in fabric softeners and corrosion inhibitors [1,2], they act as fungicides, pesticides and insecticides [3], they reveal antibacterial and antifungal activities employed in antimicrobial drugs [4–6], they are ingredients of shampoos and hair conditioners [7–9] and they are used in organoclay preparations, which have diverse applications, including environmental remediation and synthesis of nano-materials [10–12]. Therefore, global use of QAS in industry, agriculture, healthcare and domestic purposes is doubtless.

Although toxicity of some QAS to bacteria, protists and animals has been reported [13–16], majority of these compounds were considered as non-toxic or of low toxicity [2,17–19]. Therefore, QAS are generally believed to be safe for humans. This is perhaps one of the most important reasons for the extensive use of QAS in recent years.

Apart from acute toxicity, one of the most carefully studied features of any compound newly introduced into common use should be its mutagenicity. This is because mutagenic chemicals can induce serious diseases, including cancer, due to their genotoxic activities [20–25]. The germ line of higher organisms, including humans, may also be affected by these compounds, which may lead to fertility problems and to harmful genetic changes in future generations [26]. One of the major problems with mutagens is that usually they reveal genotoxic effects at very low concentrations, and that they may accumulate in the organism. Thus, effects of their actions may be visible even after many years, with no symptoms occurring shortly after their introduction into organism. In this light, it seems very important to use highly sensitive tests when assessing mutagenic activities of compounds introduced into global usage [27–30].

In the course of our studies on development of new antifungal compounds, we have synthesized and characterized QAS containing carbohydrate moieties. Since safety is the crucial feature of any newly developed compound, which may potentially be introduced into the practical use, we aimed to test toxicity of these new QAS. In this work, we put particular attention on their mutagenicity. Widely well-known and used QAS contain four hydrocarbon residues at the nitrogen atom. For this work, we synthesized QAS in which, apart from hydrocarbon residues, one glycosidic moiety with configuration D-galacto, D-gluco and D-manno was introduced. 2-Bromoethyl D-glycopyranoside, in the reaction with two tertiary amines, trimethylamine and pyridine, gave quaternary ammonium salts according to the Menschutkin's procedure.

## 2. Experimental

### 2.1. Source chemicals and general chemical methods

Commercial D-galactose, D-glucose and D-mannose (Fluka) were used. All reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 $F_{254}$ Silica Gel plates (E. Merck, 0.20 mm thick-

*Abbreviation:* QAS, quaternary ammonium salts.

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ness). The spots were detected by spraying with 5% ethanolic $H_2SO_4$ and charring. $^1H$ NMR and $^{13}C$ NMR spectra were recorded at 25 °C with a Varian Mercury Spectrometer at 400 and 100 MHz, respectively, with $Me_4Si$ as internal standard. Assignments were based on homonuclear decoupling experiments, and homo- and heteronuclear correlation. Optical rotations were measured with a JASCO J-20 polarimeter. Positive-ion mode MALDI TOF mass spectra were obtained using a BRUKER BIFLEX III spectrometer with α-cyano-4-hydroxycinnamic matrix (CHCA).

### 2.2. Synthesis and analysis of new chemicals

- *N*-[2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**5a**) – 2-bromoethyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (**3**) (0.366 g, 0.80 mmol) was placed in a screw capped ampoule, dissolved in 33% EtOH solution of $NMe_3$ (0.8 mL) and heated at 70 °C. After 5 h the solution was evaporated to a dense oil, dissolved in $H_2O$ (2 mL) and extracted with $CHCl_3$ (2 mL). The aqueous layer was concentrated under reduced pressure at temperature below 40 °C, and the colorless oil was dried over $P_2O_5$ in a vacuum desiccator. The yield of compound **5a** was 0.335 g (96%); $R_f$ = 0.0 (ethyl acetate–petrol ether 1:1); $[\alpha]_D^{20}$ −8.4° (*c* 0.3, $H_2O$); $^1H$ NMR ($D_2O$): δ 5.37 (t, 1H, H-3, $J_{3,4}$ 9.6 Hz); 5.13 (t, 1H, H-4, $J_{4,5}$ 9.6 Hz); 5.03 (dd, 1H, H-2, $J_{2,3}$ 9.6 Hz); 4.90 (d, 1H, H-1, $J_{1,2}$ 8.4 Hz); 4.39 (dd, 1H, H-6′, $J_{5,6'}$ 3.6 Hz); 4.13 (dd, 1H, H-6, $J_{6',6}$ 12.8 Hz); 4.06 (m, 1H, H-5); 3.88–3.65 (m, 2H, O–$CH_2$); 3.14 (t, 2H, $CH_2$–N, *J* 4.4 Hz); 2.68 (s, 9H, 3 × $CH_3$ of $N(CH_3)_3$); 2.13–2.06 (4 s, 12H, 4 × OAc); $^{13}C$ NMR ($D_2O$): 173.34–172.69 (4C, 4 × OO*C*$CH_3$), 99.96 (C-1), 73.17 (C-3), 71.69 (C-2), 71.50 (C-5), 68.42 (C-4), 65.16 ($CH_2$–N), 63.58 (C-6), 61.89 (O–$CH_2$), 54.17 (3C, $N(CH_3)_3$), 20.40–20.28 (4C, COO*C*$H_3$); MALDI TOF-MS (CHCA): *m*/*z* 434.1 ([M−Br]$^+$).
- *N*-[2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)ethyl] pyridinium bromide (**5b**) – 2-bromoethyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (**3**) (0.382 g, 0.84 mmol) was dissolved in dry pyridine (0.5 mL). The reaction mixture was kept in a screw capped ampoule at 70 °C for 24 h. After cooling, the solution was evaporated to dryness, dissolved in $H_2O$ (2 mL) and extracted with $CHCl_3$ (2 mL). The aqueous layer, concentrated under reduced pressure and crystallised from water, gave title compound as a white solid: **5b** (0.374 g, 98% yield); m.p. 124.2–128.6 °C; $R_f$ = 0.0 (ethyl acetate–petrol ether 1:1); $[\alpha]_D^{20}$ −12.7 (*c* 0.23, $H_2O$); $^1H$ NMR ($D_2O$): δ 8.78–8.05 (m, 5H, Py); 5.23 (t, 1H, H-3, $J_{3,4}$ 9.6 Hz); 4.97 (t, 1H, H-4, $J_{4,5}$ 9.6 Hz); 4.84 (dd, 1H, H-2, $J_{2,3}$ 9.6 Hz); 4.77 (t, 2H, $CH_2$–N, *J* 4.8 Hz); 4.74 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 4.28–4.14 (dt, 2H, $OCH_2$, *J* 4.8 Hz); 4.23 (dd, 1H, H-6′, $J_{6,6'}$ 12.6 Hz, $J_{5,6'}$ 3.8 Hz); 4.04 (dd, 1H, H-6, $J_{5,6}$ 2.4 Hz); 3.90 (m, 1H, H-5); 2.06–1.92 (4 s, 12H, 4 × OAc); $^{13}C$ NMR ($D_2O$): 173.86–172.69 (4C, 4 × OO*C*$CH_3$), 146.42–145.15, 128.27 (5C, Py), 99.80 (C-1), 73.09 (C-3), 71.54 (C-2), 71.31 (C-5), 68.32 (C-4), 67.96 (O–$CH_2$), 61.88 (C-6), 61.34 ($CH_2$–N), 20.37–20.26 (4C, COO*C*$H_3$); MALDI TOF-MS (CHCA): *m*/*z* 454.1 ([M−Br]$^+$).
- *N*-[2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**6a**) – 2-bromoethyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranoside (**4**) (0.106 g, 0.23 mmol) was placed in a screw capped ampoule and dissolved in 33% EtOH solution of $NMe_3$ (1.0 mL) and heated at 70 °C. After 5 h the solution was evaporated to a dense oil, dissolved in $H_2O$ (2 mL) and extracted with $CHCl_3$ (2 mL). The aqueous layer was concentrated under reduced pressure at temperature below 40 °C, and the oil was dried over $P_2O_5$ what gave *N*-[2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**6a**) (10.0 mg; 99%): $R_f$ = 0.0 (ethyl acetate–petrol ether 1:1); $[\alpha]_D^{20}$ 32.5° (*c* 0.3, $H_2O$); $^1H$ NMR ($D_2O$): δ 5.44 (*b*d, 1H, H-4, $J_{4,5}$ 3.6 Hz); 5.21 (dd, 1H, H-3, $J_{3,4}$ 3.6 Hz); 5.07 (dd, 1H, H-2, $J_{2,3}$ 10.0 Hz); 4.84 (d, 1H, H-1, $J_{1,2}$ 7.6 Hz); 4.28–4.07 (m, 2H, O–$CH_2$); 4.22 (m, 3H, H-5, H-6, H-6′); 3.60 (t, 2H, $CH_2$–N, *J* 4.8 Hz); 3.13 (s, 9H, 3 × $CH_3$ of $N(CH_3)_3$); 2.17–1.98 (12H, 4 × OAc); $^{13}C$ NMR ($D_2O$): 173.77–172.92 (4C, 4 × OO*C*$CH_3$), 100.30 (C-1), 71.22 (C-3), 71.03 (C-5), 69.68 (C-2), 68.32 (C-4), 65.78 ($CH_2$–N), 63.59 (O–$CH_2$), 63.58 (C-6), 54.17 (3C, $N(CH_3)_3$), 20.40–20.20 (4C, COO*C*$H_3$); MALDI TOF-MS (CHCA): *m*/*z* 434.4 ([M−Br]$^+$).
- *N*-[2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl] pyridinium bromide (**6b**) – 2-bromoethyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranoside (**4**) (9.6 mg, 0.021 mmol) was dissolved in dry pyridine (0.07 mL). The solution was kept in a screw capped ampoule at 70 °C for 24 h. After this time the solvent was evaporated, dissolved in $H_2O$ (2 mL) extracted with $CHCl_3$ (2 mL). The aqueous layer was evaporated to dryness and dried over $P_2O_5$ gave the title compound **6b** as an oil (9.5 mg; 99%); $R_f$ = 0.0 (ethyl acetate–petrol ether 1:1), $[\alpha]_D^{20}$ 37.4 (*c* 0.23, $H_2O$); $^1H$ NMR ($D_2O$): δ 8.84–8.16 (m, 5H, Py); 5.41 (*b*d, 1H, H-4, $J_{4,5}$ 3.2 Hz); 5.16 (dd, 1H, H-3, $J_{3,4}$ 3.2 Hz); 4.99 (dd, 1H, H-2, $J_{2,3}$ 8.4 Hz); 4.85 (t, 2H, $CH_2$–N, *J* 4.8 Hz); 4.78 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 4.35–4.19 (2 × dt, 2H, O–$CH_2$, *J* 4.8 Hz); 4.13 (m, 2H, H-6, H-6′, $J_{6,6'}$ 10.8 Hz); 4.08 (m, 1H, H-5); 2.21–1.98 (4 s, 12H, 4 × OAc); $^{13}C$ NMR ($D_2O$): 173.39–172.87 (4C, 4 × OO*C*$CH_3$), 147.59–145.17, 128.29 (5C, Py), 99.90 (C-1), 71.13 (C-3), 70.72 (C-5), 69.55 (C-2), 68.16 (C-4), 67.66 (O–$CH_2$), 62.02 (C-6), 61.34 ($CH_2$–N), 20.39–20.18 (4C, COO*C*$H_3$); MALDI TOF-MS (CHCA): *m*/*z* 454.0 ([M−Br]$^+$).

### 2.3. General procedure of de-O-acetylation

To a solution of **5a** or **6a** or **5b** or **6b** (0.2 mmol) in MeOH (0.90 mL), MeONa was added (0.15 mL, *c* = 0.22 M) at room temperature. The mixtures were stirred for 4 h and then neutralized with Dowex 50 WX 8 [$H^+$]. The solutions were filtered and concentrated under reduced pressure to a thick syrup. One received **7a**, **7b**, **8a**, and **8b**.

- *N*-[2-(β-D-Glucopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**7a**): deacetylation of **5a** yielded **7a** (63%); $R_f$ = 0 ($CHCl_3$:MeOH 3:1); $[\alpha]_D^{20}$ −28.0° (*c* 0.2, $H_2O$); $^1H$ NMR ($D_2O$): δ 4.46 (d, 1H, H-1, $J_{1,2}$ 8.4 Hz); 4.04–4.08 (m, 2H, O–$CH_2$); 3.87 (dd, 1H, H-6, $J_{5,6}$ 2.4 Hz); 3.68 (dd, 1H, H-6′, $J_{6',6}$ 12.0 Hz); 3.61 (t, 2H, $CH_2$–N, *J* 4.6 H); 3.44 (q, 1H, H-3, $J_{3,4}$ 9.6 Hz); 3.42 (dt, 1H, H-5, $J_{5,6'}$ 5.6 Hz); 3.34 (dd, 1H, H-4, $J_{4,5}$ 9.6 Hz); 3.26 (t, 1H, H-2, $J_{2,3}$ 9.2 Hz); 3.16 (s, 9H, 3 × $CH_3$ of $N(CH_3)_3$); $^{13}C$ NMR ($D_2O$): 102.47 (C-1), 76.27 (C-3), 75.96 (C-5), 73.20 (C-4), 69.85 (C-2), 65.84 ($CH_2$–N), 63.58 (O–$CH_2$), 60.93 (C-6), 54.21–54.29 (3C, $N(CH_3)_3$); MALDI TOF-MS (CHCA): *m*/*z* 265.9 ([M−Br]$^+$).
- *N*-[2-(β-D-Glucopyranosyloxy)ethyl]pyridinium bromide (**7b**) – (69%); $R_f$ = 0 ($CHCl_3$:MeOH 3:1); $[\alpha]_D^{20}$ −35.0 (*c* 0.2, $H_2O$); $^1H$ NMR ($D_2O$): δ 8.84–8.04 (m, 5H, Py); 4.38 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 4.32–4.17 (m, 2H, O–$CH_2$); 4.82 (q, 2H, $CH_2$–N, *J* 5.2 Hz); 3.80 (dd, 1H, H-6, $J_{5,6}$ 2.4 Hz); 3.58 (q, 1H, H-6′, $J_{6,6'}$ 12.0 Hz); 3.35 (m, 1H, H-5, $J_{5,6'}$ 6.0 Hz); 3.37 (m, 1H, H-4, $J_{4,5}$ 9.6 Hz); 3.27 (dd, 1H, H-3, $J_{3,4}$ 9.6 Hz); 3.18 (t, 1H, H-2, $J_{2,3}$ 9.2 Hz); $^{13}C$ NMR ($D_2O$): 146.31–145.15, 128.30 (5C, Py), 102.55 (C-1), 76.19 (C-5), 75.87 (C-4), 73.13 (C-2), 69.77 (C-3), 68.24 (O–$CH_2$), 61.53 ($CH_2$–N), 60.88 (C-6); MALDI TOF-MS (CHCA): *m*/*z* 286.1([M−Br]$^+$).
- *N*-[2-(β-D-Galactopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**8a**) – (80%); $R_f$ = 0 ($CHCl_3$:MeOH 3:1); $[\alpha]_D^{20}$ 12.0° (*c* 0.2, $H_2O$); $^1H$ NMR ($D_2O$): δ 4.34–4.06 (m, 2H, O–$CH_2$); 4.40 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.90 (*b*d, 1H, H-4, $J_{4,5}$ 4.0 Hz); 3.75–3.61 (m, 3H, H-5, H-6, H-6′); 3.74 (d, 2H, $CH_2$–N, *J* 1.6 Hz); 3.63 (dd, 1H, H-3, $J_{3,4}$ 4.0 Hz); 3.49 (dd, 1H, H-2, $J_{2,3}$ 9.6 Hz); 3.17 (s, 9H, 3 × $CH_3$ of $N(CH_3)_3$); $^{13}C$ NMR ($D_2O$): 103.04 (C-1), 75.55 (C-5), 72.96 (C-3), 70.92 (C-2), 68.89 (C-4), 65.92

(C-6), 63.55 (O–$CH_2$), 61.33 ($CH_2$–N), 54.36–54.29 (3C, N($CH_3$)$_3$); MALDI TOF-MS (CHCA): *m*/*z* 266.1 ([M−Br]$^+$).

- *N*-[2-(β-D-Galactopyranosyloxy)ethyl]pyridinium bromide (**8b**) – (66%); $R_f$ = 0 ($CHCl_3$:MeOH 3:1); $[\alpha]_D^{20}$ 10.0 (*c* 0.2, $H_2O$); $^1$H-NMR ($D_2O$): $\delta$ 8.87–8.05 (m, 5H, Py); 4.84 (t, 2H, $CH_2$–N, *J* 4.4 Hz); 4.36–4.18 (m, 2H, O–$CH_2$); 4.34 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.86 (*b*d, 1H, H-4, $J_{4,5}$ 3.6 Hz); 3.61 (m, 3H, H-5, H-6, H-6′); 3.56 (dd, 1H, H-3, $J_{3,4}$ 3.6 Hz); 3.43 (dd, 1H, H-2, $J_{2,3}$ 9.8 Hz); $^{13}$C NMR ($D_2O$): 147.58–145.26, 128.36 (5C, Py), 103.26 (C-1), 75.47 (C-5), 72.93 (C-3), 70.87 (C-2), 68.83 (C-4), 68.36 (O–$CH_2$), 61.66 ($CH_2$–N), 61.24 (C-6); MALDI TOF-MS (CHCA): *m*/*z* 285.9 ([M−Br]$^+$).
- *N*-[2-(α-D-Mannopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**10a**): 68.0 mg (0.24 mmol) **9** and 0.25 mL 33% trimethylamine in ethanol were placed in a screw capped ampoule and heated at 70 °C for 5 h. After this time, the solution was concentrated, dissolved in $H_2O$ (2 mL) and extracted with $CH_3Cl$ (2 mL). Water solution containing product **10a** was concentrated under reduced pressure to give 82.2 mg (97%) as a thick oil $R_f$ = 0.0 ($CH_2Cl_2$:$CH_3OH$ 9:1), TLC-RP $R_f$ = 0.08 ($CH_3CN$:$H_2O$ 14:1); $[\alpha]_D^{20}$ 35.8° (*c* 1; $H_2O$); $^1$H NMR ($D_2O$) $\delta$: 4.86 (*b*s, 1H, H-1); 4.09 (m, 1H, O–$CH_a$); 3.92 (m, 2H, O–$CH_b$, H-2, $J_{2,3}$ 3.2 Hz); 3.84 (dd, 1H, H-6, $J_{5,6}$ 1.6 Hz, $J_{6,6'}$ 12.4 Hz); 3.71 (m, 2H, H-3, H-6′, $J_{3,4}$ 9.2 Hz, $J_{5,6'}$ 5.6 Hz); 3.57 (m, 4H, H-4, H-5, $CH_2$–N, $J_{4,5}$ 9.2 Hz, $J_{CH2-CH2}$ 6.4 Hz); 3.14 (s, 9 H, 3 × $CH_3$ of N($CH_3$)$_3$); $^{13}$C NMR ($D_2O$): 100.17 (C-1), 73.52 (C-5), 70.73 (C-3), 70.07 (C-2), 66.90 (C-4), 65.83 ($CH_2$–N), 61.53 (O–$CH_2$), 61.19 (C-6), 54.19, 54.23, 54.27 (3C, N($CH_3$)$_3$); MALDI TOF-MS (CHCA): *m*/*z* 266.2 ([M−Br]$^+$).
- *N*-[2-(α-D-Mannopyranosyloxy)ethyl]pyridinium bromide (**10b**). A screwed capped ampoule with 65.0 mg (0.23 mmol) **9** and 0.25 mL of dry pyridine was heated at temperature 70 °C. After 24 h the solution was concentrated to dryness, dissolved in $H_2O$ (2 mL) and extracted with $CH_3Cl$ (2 mL). The aqueous layer was concentrated under reduced pressure at temperature below 40 °C, and the colorless oil was dried over $P_2O_5$ to give 82.2 mg (95%) of **10b** as an yellow oil, $R_f$ = 0.0 ($CH_2Cl_2$:$CH_3OH$ 9:1), TLC-RP $R_f$ = 0.1 ($CH_3CN$:$H_2O$ 14:1); $[\alpha]_D^{20}$ 30.3° (*c* 1; $H_2O$); $^1$H NMR ($D_2O$) $\delta$: 8.84–8.05 (m, 5H, Py); 4.81 (t, 2H, $CH_2$–N, $J_{CH2-CH2}$ 5.0 Hz); 4.75 (d, 1H, H-1, $J_{1,2}$ 1.6 Hz); 4.14 (m, 1H, O–$CH_a$); 3.94 (m, 1H, O–$CH_b$, $J_{H-C-H}$ 11.2 Hz); 3.79 (m, 1H, H-2, $J_{2,3}$ 3.2 Hz); 3.68 (dd, 1H, H-6, $J_{6,6'}$ 12.0 Hz); 3.56 (m, 3H, H-3, H-4, H-6′, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.2 Hz); 3.03 (m, 1 H, H-5, $J_{5,6'}$ 6.0 Hz, $J_{5,6}$ 2.0 Hz); $^{13}$C NMR ($D_2O$): 146.53–145.01, 128.4 (5C, Py), 99.88 (C-1), 73.37 (C-5), 70.70 (C-3), 69.83 (C-2), 66.66 (C-4), 65.75 (O–$CH_2$), 61.26 ($CH_2$–N), 60.98 (C-6); MALDI TOF-MS (CHCA): *m*/*z* 286.2 ([M−Br]$^+$).

### 2.4. General procedure for exhaustive O-acetylation

70 mg (0.20 mmol) of *N*-[2-(α-D-mannopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**10a**) or 70 mg (0.19 mmol) of *N*-[2-(α-D-mannopyranosyloxy)ethyl]pyridinium bromide (**10b**) was dissolved in Py (3 mL) and $Ac_2O$ (3 mL). After 24 h the solutions were evaporated to a dense oil, dissolved in $H_2O$ (2 mL) and extracted with $CHCl_3$ (2 mL). The aqueous layer was concentrated under reduced pressure at temperature below 40 °C, and the oil was dried over $P_2O_5$. One received **11a** and **11b**.

- *N*-[2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium bromide (**11a**) mp: 255.1–260.3; $R_f$ = 0.0 ($CH_2Cl_2$:$CH_3OH$ 9:1); $[\alpha]_D^{20}$ 33.2° (*c* 1; $H_2O$); $^1$H NMR ($D_2O$) $\delta$: 5.31 (m, 1H, H-2); 5.27 (m, 1H, H-3); 5.02 (d, 1H, H-1, $J_{1,2}$ 1.6 Hz); 4.16 (m, 4H, O–$CH_a$, H-4, H-6, H-6′, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.4 Hz); 4.03 (m, 1H, O–$CH_b$); 3.64 (m, 3H, $CH_2$N, H-5, $J_{5,6'}$ 3.6 Hz); 3.17 (s, 9H, 3 × $CH_3$ of N($CH_3$)$_3$); 2.16–1.98 (4 s, 12H, 4 × OAc); $^{13}$C NMR ($D_2O$): 176.51–175.54 (4C, 4 × OO*C*$CH_3$), 100.01 (C-1), 72.42 (C-3), 71.97 (C-2), 71.41 (C-4), 68.29 (C-5, $CH_2$–N), 64.77 (O–$CH_2$), 64.68 (C-6), 56.82 (3C, N($CH_3$)$_3$), 22.93–22.90 (4C, COO*C*$H_3$); MALDI TOF-MS (CHCA): *m*/*z* 434.2 ([M−Br]$^+$).
- *N*-[2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl] pyridinium bromide (**11b**): 75.8 mg (88%) of **3** as yellow oil; $R_f$ = 0.0 ($CH_2Cl_2$:$CH_3OH$ 9:1); $[\alpha]_D^{20}$ 14.8° (*c* 1; $H_2O$); $^1$H NMR ($D_2O$) $\delta$: 8.91–8.10 (m, 5H, Py); 5.12 (m, 3H, H-2, H-3, H-4); 4.87 (m, 3H, H-1, $CH_2$–N); 4.25 (dd, 1H, H-6, $J_{6,6'}$ 12.4 Hz); 4.19 (m, 1H, O–$CH_a$); 4.03 (m, 1H, O–$CH_b$); 4.02 (m, 1H, H-6′); 3.56 (m, 1H, H-5, $J_{5,6'}$ 4 Hz, $J_{5,6}$ 2.4 Hz,); 2.11–1.98 (4 s, 12H, 4 × OAc); $^{13}$C NMR ($D_2O$): 173.81–172.89 (4C, 4 × OO*C*$CH_3$), 146.69–145.13, 128.53 (5 C, Py), 97.09 (C-1), 65.63, 69.16, 69.72 (C-2, C-3, C-4), 68.67 (C-5), 66.25 (O–$CH_2$), 62.12 (C-6), 61.05 ($CH_2$–N), 20.29–20.26 (4C, COO*C*$H_3$); MALDI TOF-MS (CHCA): *m*/*z* 454.2 ([M−Br]$^+$).

### 2.5. Vibrio harveyi luminescence mutagenicity test

The *V. harveyi* luminescence mutagenicity test was performed according to previously described procedure [27] with modifications introduced recently [28]. The *V. harveyi* A16 strain (a dim *luxE* mutant) was used. Briefly, bacteria were cultivated in the liquid BOSS medium [31], containing 1% bacto-peptone, 0.3% beef extract, 0.1% glycerol and 3% NaCl (pH 7.3), at 30 °C and 200 rpm. Dilutions of tested chemicals were added to exponentially growing cultures and the cultivation was continued for 3 h. Following transfer of 250 μL of each culture on the 96-well plate (Nunc 96 Well Optical Bottom White Plate; Thermo Fischer Scientific, Roskilde, Denmark), absorbance at 575 nm and luminescence were measured using Wallac 1420 Victor$^2$ plate spectrometer (PerkinElmer). The luminescence of each culture was corrected for its absorbance and the results were calculated as relative light units (RLU). The results are presented as percentage of control.

## 3. Results

### 3.1. New quaternary ammonium salts containing carbohydrate moieties

The salts were synthesized according to the methods described earlier [32,33], and quaternization reaction was achieved according to Menschutkin's procedure (Figs. 1 and 2). Satisfying yields of the synthesis performed according to the scheme presented in Fig. 1 were obtained for derivatives with the D-gluco and D-galacto configurations. A very poor yield of the first stage of the synthesis in the case of D-mannose (Fig. 2, step **b**) prompted us to propose another way of the reaction (Fig. 2, step **i**). Routine conditions for the quaternization reaction did not need large modifications in particular cases to obtain totally satisfying yields (Section 2.4).

Ultimately, we synthesized six salts: **5a**, **5b**, **6a**, **6b** and **10a**, **10b** (for structures and names see Figs. 1 and 2 and Sections 2.2–2.4). They contain six-membered sugar ring with D-galacto, D-gluco or D-manno configuration as well as trimetylhylaminium and pyridinium moieties.

Pyranose rings of all salts adopt the $^4C_1$ conformation, what was confirmed by values of the coupling constant $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ (Table 1). In turn, values of the coupling constant $J_{1,2}$ (Table 2) confirm the β-D configuration of anomeric carbon atom for derivatives with the gluco and galacto configurations and the α-D configuration of anomeric carbon atom for derivatives with manno configuration.

### 3.2. Mutagenicity of quaternary ammonium salts containing carbohydrate moieties

Quaternary ammonium salts are generally recognized as safe, soft compounds [17]. Nevertheless, we aimed to test mutagenicity

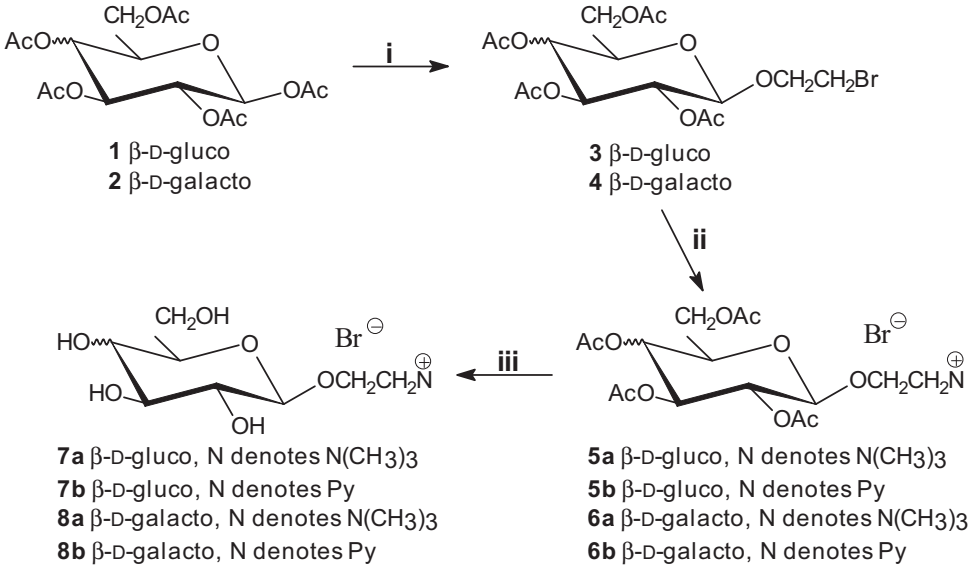


**Fig. 1.** Synthesis of D-gluco- and D-galacto-derivatives of QAS. Reagents: (i) $HO(CH_2)_2Br$, $BF_3{\cdot}Et_2O/CH_2Cl_2$; (ii) 33% ethanolic solution of $NMe_3$ (compounds **5a** and **6a**), Py (compounds **5b** and **6b**); (iii) MeONa/MeOH.

**Table 1**
The $^1H$–$^1H$ coupling constants (Hz) for compounds **5a**–**8b** and **10a**–**11b**.

| Compound | Configuration | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $J_{5,6}$ | $J_{5,6'}$ | $J_{6',6}$ |
|---|---|---|---|---|---|---|---|
| **5a** | β-D-Gluco | 9.6 | 9.6 | 9.6 | – | 3.6 | 12.8 |
| **5b** | β-D-Gluco | 9.6 | 9.6 | 9.6 | 2.4 | 3.8 | 12.6 |
| **6a** | β-D-Galacto | 10.0 | 3.6 | 3.6 | – | – | – |
| **6b** | β-D-Galacto | 8.4 | 3.2 | 3.2 | – | – | 10.8 |
| **7a** | β-D-Gluco | 9.2 | 9.6 | 9.6 | 2.4 | 5.6 | 12.0 |
| **7b** | β-D-Gluco | 9.2 | 9.6 | 9.6 | 2.4 | 6.0 | 12.0 |
| **8a** | β-D-Galacto | 9.6 | 4.0 | 4.0 | – | – | – |
| **8b** | β-D-Galacto | 9.8 | 3.6 | 3.6 | – | – | – |
| **10a** | α-D-Manno | 3.2 | 9.2 | 9.2 | 1.6 | 5.6 | 12.4 |
| **10b** | α-D-Manno | 3.2 | 9.6 | 9.2 | 2.0 | 6.0 | 12.0 |
| **11a** | α-D-Manno | nd | nd | nd | 2.4 | 3.6 | 12.4 |
| **11b** | α-D-Manno | nd | nd | nd | 2.4 | 4.0 | 12.4 |

nd, not determined.

**Table 2**
The $^1H$–$^1H$ coupling constant $J_{1,2}$ (Hz) for compounds **5a**–**8b** and **10a**–**11b**.

| Compound | Configuration | $J_{1,2}$ |
|---|---|---|
| **5a** | β-D-Gluco | 8.4 |
| **5b** | β-D-Gluco | 8.0 |
| **6a** | β-D-Galacto | 7.6 |
| **6b** | β-D-Galacto | 8.0 |
| **7a** | β-D-Gluco | 8.4 |
| **7b** | β-D-Gluco | 8.0 |
| **8a** | β-D-Galacto | 8.0 |
| **8b** | β-D-Galacto | 8.0 |
| **10a** | α-D-Manno | ~0 (*bs*) |
| **10b** | α-D-Manno | 1.6 |
| **11a** | α-D-Manno | 1.6 |
| **11b** | α-D-Manno | ~0 (*bs*) |

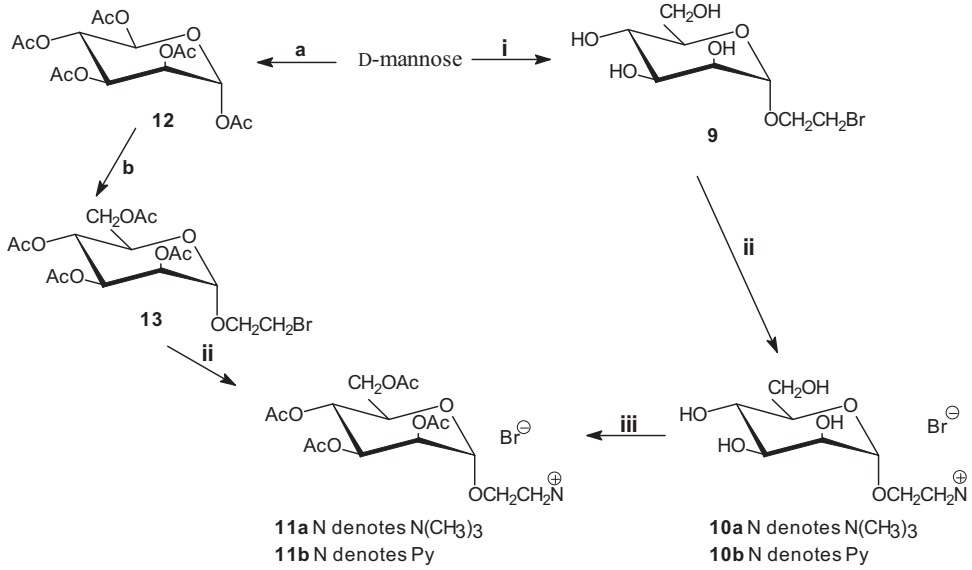


**Fig. 2.** Synthesis of D-manno-derivatives of QAS. Reagents: (i) $HO(CH_2)_2Br$, AcCl; (ii) 33% ethanolic solution of $NMe_3$ (compound **10a**/**11a**), Py (compound **10b**/**11b**); (iii) $Ac_2O$, Py; (a) $Ac_2O$, $ZnCl_2$; (b) $HO(CH_2)_2Br$, $BF_3{\cdot}Et_2O/CH_2Cl_2$.

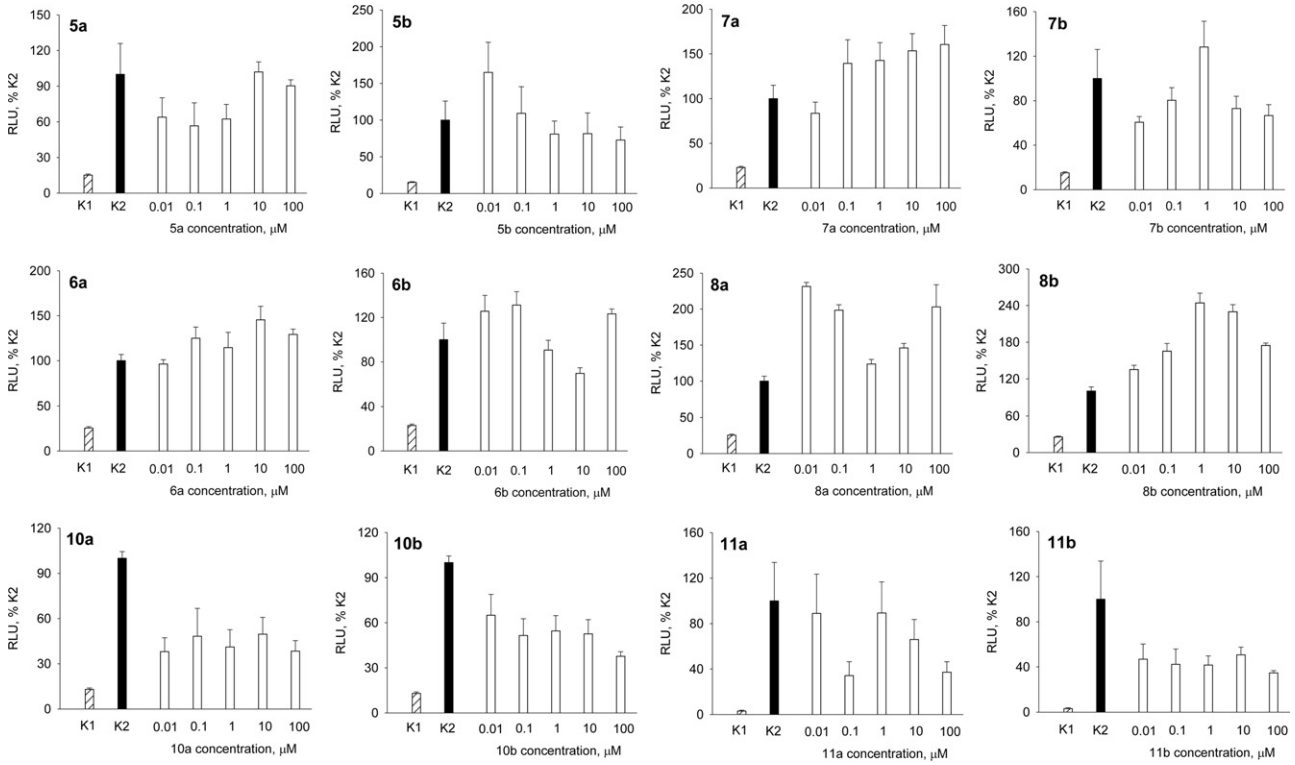


**Fig. 3.** Mutagenicity of D-gluco- (upper panels: **5a**, **5b**, **7a**, **7b**), D-galacto- (middle panels: **6a**, **6b**, **8a**, **8b**) and D-manno- (lower panels: **10a**, **10b**, **11a**, **11b**) derivatives of QAS as assessed by the *V. harveyi* bioluminescence mutagenicity assay. Exponentially growing cultures of *V. harveyi* A16 strain were treated with water (K1, negative control), 0.01 μM ICR-191 (K2, positive control) or indicated concentrations of QAS derivatives, and incubated for 3 h. Bars indicate means ± SD of bacterial luminescence, expressed as relative light units (RLU) per $A_{575}$ of bacterial culture.

of QAS containing carbohydrate moieties, which were synthesized in this work. Majority of previous studies on mutagenic activities of newly obtained compounds were performed by using the Ames test [29]. However, recent works suggested that a newly developed *V. harvey* luminescence mutagenicity assay is more sensitive than the Ames test [27,28]. Therefore, we have used the *V. harveyi* assay (based on reappearance of luminescence in a dim mutant) in testing mutagenic activities of D-gluco-, D-galacto-, and D-manno-derivatives of QAS, obtained in this work.

Interestingly, we found a substantial mutagenicity of all tested QAS derivatives in the *V. harveyi* assay (Fig. 3). The mutagenic activities of the D-gluco- (**5a**, **5b**, **7a**, **7b**) and D-galacto- (**6a**, **6b**, **8a**, **8b**) derivatives were significantly higher than those of D-manno-derivatives (**10a**, **10b**, **11a**, **11b**), and in fact, they were comparable to, and in some cases even higher than, that of the positive control, a potent mutagen, ICR-191 (2-methoxy-6-chloro-9-(3-(2-chloroethyl)aminopropylamino)acridine × 2HCl). Among the D-gluco- and D-galacto-derivatives, the most potent mutagens were *N*-[2-(D-glycopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium salts (**7a**, **8a**).

## 4. Discussion

Quaternary ammonium salts are generally recognized as safe compounds, and because of variety of their activities they are commonly used in various applications connected to human life (see Section 1). Unexpectedly, by using the *V. harveyi* luminescence mutagenicity assay, we have detected considerable mutagenic activities of all tested QAS derivatives. An intriguing question is: why are reports on genotoxicity of other QAS absent in the literature published to date? One possible explanation might be that the tests used previously to assess mutagenicity of QAS were of too low sensitivity to detect such a biological activity of these compounds. In fact, it was suggested recently that the *V. harveyi* luminescence mutagenicity assay is more sensitive than some other commonly used mutagenicity tests, including the Ames test [28]. Therefore, we have repeated the assessment of mutagenicity of D-gluco- (**5a**, **5b**, **7a**, **7b**) and D-galacto- (**6a**, **6b**, **8a**, **8b**) derivatives of QAS using a simplified Ames test (with the *Salmonella typhimurium* TA98 strain). However, in this test, we were able to detect only weak mutagenic signals (as assessed by the number of mutant colonies, which was only 1.5–2 times higher than that observed in control experiments) of only two D-galacto-derivatives (**6a** and **6b**), and no mutagenicity could be determined for other tested compounds (results not shown). These results may support the hypothesis suggested above. Thus, we speculate that re-assessment of mutagenic activities of many chemicals, previously recognized as non-genotoxic (including QAS), might be necessary with the use of highly sensitive mutagenicity assay(s).

The *V. harveyi* assay is based on the use of the mutant in the *luxE* gene, which is dim, but upon contact with mutagens fully luminescent revertants or pseudorevertants appear, thus, luminescence of a bacterial culture is significantly increased [27]. This assay has been demonstrated to be suitable for testing samples of marine water [34], marine sediments [35], plant tissue extracts [36] and animal tissue extracts [37]. Recent studies indicated that it is also suitable for testing mutagenicity of various individual chemicals or their mixtures [28]. Obviously, the *V. harveyi* mutagenicity assay is not the only test which reveals higher sensitivity than the Ames test, therefore, some other assays (for reviews see [38,39]) can also be considered when re-assessment of safety of particular compounds is planned.

In our studies, significant differences in mutagenic potency between D-gluco- or D-galacto-derivatives and D-manno-derivatives of QAS were found. Moreover, among the compounds from the former groups, *N*-[2-(D-glycopyranosyloxy)ethyl]-*N*,*N*,*N*-

trimethylaminium salts revealed the highest mutagenicity. In addition, the dose–response effects of various tested chemicals indicated occurrence of a maximum, with higher concentrations giving less pronounced effects in the mutagenicity assay. While the dose–response effects can be explained by high sensitivity of the assay and toxicity of mutagenic compounds due to accumulation of too many mutations in cells of the tester bacterial strain, as proposed previously [27], the reason(s) of higher mutagenicity of D-gluco and D-galacto-QAS derivatives relative to D-manno-derivatives, as well as the reason(s) of the highest mutagenic activities of *N*-[2-(D-glycopyranosyloxy)ethyl]-*N*,*N*,*N*-trimethylaminium salts among tested QAS, remain to be elucidated. One may speculate that if mutagenic activities of D-gluco and D-galacto-derivatives of QAS result from their direct interactions with DNA, such molecular conformations might be more likely to contact base pairs or other part(s) of the nucleic acid than the D-manno-conformation. Determination of specific kinds of mutations caused by particular QAS derivatives should be helpful in understanding the molecular mechanism(s) of their genotoxicity.

Irrespective of the mechanism(s) of mutagenicity of QAS, detection of such a biological activity of these compounds implies that their levels in environment should be taken into consideration when estimating food safety and environmental quality, especially in a long term perspective. In fact, there are reports indicating that in industrially developed countries, QAS can occur at relatively high concentrations not only in soil [40–47], river sediments [48] and groundwater resources [49], but also in drinking waters [50] and some vegetables, particularly leafy ones [51]. In this light, one might postulate that it is reasonable not only to monitor the fate of QAS during the food production processes, as proposed recently [52], but also to consider their mutagenicity, which may affect human health and quality of natural environment.

## 5. Conclusions

QAS containing carbohydrate moieties reveal potent mutagenic activities. Therefore, the safety of QAS may be lower than previously supposed, indicating a need for testing such compounds for their mutagenicity.

## Acknowledgments

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