Simultaneous isolation of gallotannins and a related phenolic from *Mangifera indica* kernels and assessment of their anti-*Trichomonas vaginalis* activities

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**ABSTRACT**

Therapeutic alternatives are being searched for trichomoniasis as a result of the increased prevalence of metronidazole-resistant infections. *Mangifera indica* (Anacardiaceae) is an important tree with a long history in medicine. Traditionally, it has been used as an anti-diarrheal and anti-diabetic, and recently, its gallotannin-rich leaves and stem bark extracts have shown antiparasitic activities against various parasites. Aiming at exploring the anti-*Trichomonas vaginalis* activity of mango’s gallotannins, an aqueous ethanol extract of fresh kernels of *M. indica* was phytochemically investigated. Based on a simple gel chromatographic procedure, ethyl gallate (2), a group of five isomeric tetragalloyl-glucoses (3–7), and a pentagalloyl-glucose (8) were simultaneously isolated from a single fraction by a preparative Reversed-phase-high performance liquid chromatography. The isolates were identified based on spectroscopic analyses and comparison with reported data. They showed structural-dependent inhibitory effects on the growth of *T. vaginalis* trophozoites in an *in vitro* investigation. Ethyl gallate and 1,2,4,6-tetra-O-galloyl-β-D-glucose (7) exhibited elevated anti-*T. vaginalis* activity (IC₅₀ = 1.3, 2.4 μg/ml, respectively). This is the first report exploring the potential of gallotannins as trichomonacidal agents.

**INTRODUCTION**

*Trichomonas vaginalis* (*T. vaginalis*) is the most common sexually transmitted parasite. It affects the external genitalia of men and women leading to a genitourinary disease trichomoniasis. The infections are sometimes associated with preterm birth of pregnant women (Silver et al., 2014) and prostatic tumour of men (Sutcliffe et al., 2012). The development of resistance, allergy, and other side effects due to repeated treatment with imidazole derivatives urged exploring safe alternative anti-trichomoniasis (Cudmore et al., 2004). The gallotannins-rich leaves and stem bark of the tree *Mangifera indica* (Anacardiaceae) (Núñez Sellés et al., 2002) have shown antiparasitic activities against *Entamoeba histolytica* (Tona et al., 1998); *Histomonas meleagridis*, *Tetratrichomonas gallinarum*, and *Blastocystis* sp (Grabensteiner et al., 2008); *Plasmodium falciparum* (Awe et al., 1998; Ruiz et al., 2011; Zirhi et al., 2005); and *Giardia lamblia* (Amaral et al., 2006). The mango kernels largely produce gallotannins, gallic acid, and benzoic acid derivatives (Masibo and He, 2008). The kernels are a very rich source of gallotannins (15.5 mg/g dry kernel; Berardini et al., 2004). It was traditionally employed to expel tapeworms and as an anti-diarrheal agent (Sairam et al., 2003). Extracts of the kernels have also demonstrated antimicrobial activities against a wide range of the Gram-positive organisms which were ascribed to its gallotannins content (Ahmed et al., 2007; Ka buki et al., 2000). In our previous report, gallotannins, benzophenones, and xanthone C-glucosides from the mango stem bark were shown...
together with their antiviral and cytotoxic activities (Abdel-Mageed et al., 2014). To explore the anti-\textit{T. vaginalis} activity of the tannin constituents of the mango seed kernels, we conducted a phytochemical investigation focusing on the isolation of gallotannins. As a result, gallic acid (1), ethyl gallate (2), 2,3,4,6-tetra-O-galloyl-\textbeta-D-glucose (3), 1,2,3,4-tetra-O-galloyl-\textbeta-D-glucose (4), and a combination of almost equal proportions of 1,2,3,6-tetra-O-galloyl-\textbeta-D-glucose (5) and 1,3,4,6-tetra-O-galloyl-\textbeta-D-glucose (6), 1,2,4,6-tetra-O-galloyl-\textbeta-D-glucose (7), and 1,2,3,4,6-penta-O-galloyl-\textbeta-D-glucose (8) were purified. This study reports an easy chromatographic procedure for the simultaneous isolation of ethyl gallate (2), and the six gallotannins (3–8, Fig. 1) from seed kernels of \textit{M. indica} variety Tymor. The effect of these seven compounds on the viability of \textit{T. vaginalis} trophozoites is also reported here for the first time. A growth inhibitory mechanism of the trophozoite is suggested in view of the known protein binding and iron-binding affinity of the tannins.

**MATERIALS AND METHODS**

**General experimental procedures**

The one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA AS 600 instrument (Agilent, Santa Clara, CA, USA; 0.151 GHz for $^{13}$C and 0.6 GHz for $^1$H). The chemical shift values were shown in $\delta$ (ppm) relative to the solvent signals [(CD$_3$)$_2$CO-$d_6$ (δ$^H$ 2.04; δ$^C$ 29.8) on the tetramethylsilane (TMS) scale. Reversed-phase-high performance liquid chromatography (RP-HPLC) was done on a YMC-Pack ODS-A A-303 (product of YMC, Japan) column (4.6 i.d. x 250 mm) using acetonitrile – water (2:8, v/v) with 0.1% acetic acid. The flow rate was set at 1 ml/min and the oven temperature at 40°C. The eluates were monitored by a Ultraviolet (UV) detector at $\lambda_{max} = 280$ nm. Preparative RP-HPLC was carried out on a YMC-Pack ODS-A, A-324 column (10 i.d. × 300 mm) using the mobile phase acetonitrile – water (2:8, v/v) with 0.1% acetic acid. The flow rate of 2 ml/min at column oven temperature 40°C, and UV detection ($\lambda_{max} = 280$ nm) were applied. The gels, Dia-ion HP-20 and MCI-gel CHP-20P (products of Mitsubishi Chemical, Japan), were used for the chromatographic experiments.

**Plant material**

Fresh seed kernels of \textit{M. indica} variety Tymor were collected from mature ripe fruits which were purchased from a private farm in Assiut. The plant species was identified by Prof. Dr. Ayman Koth, Horticulture Department, Faculty of Agriculture, Assiut University. An authentic sample (No. 2012MT) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

**Extraction and isolation procedures**

\textit{Mangifera indica} fresh seed kernels (~2.5 kg) were extracted by maceration in EtOH/water [7:3, v/v, (3 L × 4)] at ambient temperature. The obtained extract was concentrated under vacuum at ~40°C. The obtained concentrate (~400 ml) was applied to a Diaion HP-20 column (5.5 i.d. x 63 cm), which was eluted with distilled water (6 L), methanol/water (2.5:7.5, v/v, 5 L), methanol/water (5:5, v/v, 5 L), methanol/water (7.5:2.5, v/v, 5 L), and methanol (5 L) successively. The respective eluates were dried under vacuum and yielded the respective water (54 g), methanol/water (2.5:7.5, v/v) (98.5 g), methanol/water (5:5, v/v) (55 g), methanol/water (7.5:2.5, v/v) (4.7 g), and methanol (4.4 g) fractions. A portion (3 g) of the methanol/water (2.5:7.5, v/v) fraction was subjected to chromatographic purification on MCI-gel CHP-20P (1.1 i.d. x 37 cm) with water, water/methanol (9:1 → 8.5:1.5 → 8:2 → 7.5:2.5 → 7.3 → 6.5:3.5 → 6:4 → 3:7 and 0:10, v/v). All the 700 drops of

![Figure 1. Structures of gallic acid (1) ethyl gallate (2), and the gallotannins 3–8.](image-url)
the column eluates were gathered separately in a test tube using a fraction collector. The fractions were visualized by monitoring the RP-HPLC. The water eluate furnished crude gallic acid [1 (519 mg)]. The water/methanol (8:2, 133 mg), (7.5:2.5, 222 mg), and (7:3, v/v, 221.4 mg) eluates demonstrated identical RP-HPLC profiles (Fig. 2), indicating the presence of the same compounds in these elutes. A preparative HPLC purification of part (300 mg) of the water/methanol (7.5:2.5, v/v) eluted fraction led to purification of 2,3,4,6-tetra-O-galloyl-β-D-glucose [3 (4.2 mg)], 1,2,3,4-tetra-O-galloyl-β-D-glucose [4 (3 mg)], mixture (13.4 mg) of almost equal proportions of 1,2,3,6-tetra-O-galloyl-β-D-glucose (5) and 1,3,4,6-tetra-O-galloyl-β-D-glucose (6), 1,2,4,6-tetra-O-galloyl-β-D-glucose (7), 1,2,3,4,6-penta-O-galloyl-β-D-glucose (8, 29 mg), and another pure sample of the ethyl gallate [2 (34.8 mg)]. In addition, the water/methanol (6:4, v/v) eluted fraction also afforded 1,2,3,4,6-penta-O-galloyl-β-D-glucose [8, (188.5 mg)].

Table 1. 1H NMR data (δ in ppm, J in Hz) of glucose moiety of the galloylglycloses 3–8 (0.6 GHz, (CD3)2CO-d9/D2O, 9:1, 27°C).

| glc. | 3u  | 3p  | 4    | 5    | 6    | 7    | 8    |
|------|-----|-----|------|------|------|------|------|
| 1    | 5.55 (d, 3.6) | 5.16 (d, 7.8) | 6.19 (d, 8.4) | 6.10 (d, 8.4) | 5.96 (d, 8.4) | 6.03 (d, 8.4) | 6.27 (d, 8.4) |
| 2    | 5.11 (dd, 3.6, 10.2) | 5.26 (dd, 7.8, 10.2) | 5.55 (dd, 8.4, 9.6) | 5.45 (dd, 8.4, 9.6) | 4.023 (dd, 8.4, 9.6) | 5.37 (dd, 8.4, 9.6) | 5.62 (d, 8.4, 9.6) |
| 3    | 6.04 (t, 10.2) | 5.75 (t) | 5.92 (t) | 5.64 (t) | 5.65 (t) | 4.35 (t) | 6.00 (t) |
| 4    | 5.55 (t, 10.2) | 5.47 (t, 10.2) | 5.46 (t, 9.6) | 4.04 (t, 9.6) | 4.04 (t, 9.6) | 5.38 (t, 9.6) | 5.65 (t, 9.6) |
| 5    | 4.58 (dd, 1.8, 6.6, 10.2) | 4.24 (dd, 1.8, 6.6, 10.2) | 4.16 (dd, 2.4, 5.4, 9.6) | 4.14 (dd, 2.4, 5.4, 9.6) | 4.14 (dd, 2.4, 5.4, 9.6) | 4.29 (dd, 2.4, 5.4, 9.6) | 4.54 (dd, 2.4, 5.4, 9.6) |
| 6    | 4.24 (dd, 4.8, 12) | 4.24 (dd, 4.8, 12) | 3.73 (dd, 4.8, 12) | 4.63 (dd, 4.8, 12) | 4.51 (dd, 4.8, 12) | 4.52 (dd, 4.8, 12) | 4.57 (dd, 4.8, 12) |
| 7    | 4.50 (dd, 4.8, 12) | 4.50 (dd, 4.8, 12) | 3.65 (dd, 4.8, 12) | 4.46 (dd, 4.8, 12) | 4.21 (dd, 4.8, 12) | 4.19 (dd, 4.8, 12) | 4.3 (dd, 4.8, 12) |

Figure 2. RP-HPLC chromatogram (at λmax = 280 nm) of the main compounds in the water/methanol (8:2–6:5:3.5, v/v) elutes from an MCI-gel CHP-20P column, from the water/methanol (8:2, v/v) eluate of the dia-ion HP-20 chromatographic fractionation for the aqu. EtOH extract of mango seed kernels.

Table 1. 1H NMR data (δ in ppm, J in Hz) of glucose moiety of the galloylglycloses 3–8 (0.6 GHz, (CD3)2CO-d9/D2O, 9:1, 27°C).

Gallic acid (1): White, non-crystalline powder; RP-HPLC analysis and co-chromatography with authentic sample (tR = 3.80 min); 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.08 (2H, s, H-2,6); Electrospray ionization mass spectrometry (ESIMS) m/z 171 [M + H]+ (C7H4O4).

Ethyl gallate (2): Off-white, non-crystalline powder; 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.13, 7.02, 7.00, 6.92 [each s, 2H, galloyl H-2,6 × 4], galloyls of the β-anomer of the D-glucose, 7.15, 7.03, 7.028, 6.98 [each s, 2H, galloyl H-2,6 × 4], galloyls of the α-anomer of the D-glucose, glucose protons (Table 1); ESIMS m/z 789 [M + H]+ (C16H12O5).

1,2,3,4-Tetra-O-galloyl-β-D-glucose (3): Off-white, non-crystalline powder; 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.07, 7.025, 6.98, 6.95 [each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS m/z 789 [M + H]+ (C16H12O5).

1,2,3,6-Tetra-O-galloyl-β-D-glucose (4): Off-white, non-crystalline powder; 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.13, 7.06, 7.04, 6.98 [each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS m/z 789 [M + H]+ (C16H12O5).

1,3,4,6-Tetra-O-galloyl-β-D-glucose (5): Off-white, non-crystalline powder; 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.14, 7.11, 7.08, 7.05 [each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS m/z 789 [M + H]+ (C16H12O5).

1,2,4,6-Tetra-O-galloyl-β-D-glucose (6): Off-white, non-crystalline powder; 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.16, 7.10, 7.05, 7.02 [each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS m/z 789 [M + H]+ (C16H12O5).

1,2,3,4,6-Penta-O-galloyl-β-D-glucose (8): Off-white, non-crystalline powder; 1H NMR [0.6 GHz; (CD3)2CO-d9/D2O, 9:1] δH: 7.07, 7.025, 6.98, 6.95 [each s, 2H, galloyl H-2,6 × 4], glucose protons (Table 1); ESIMS m/z 789 [M + H]+ (C16H12O5).
RESULTS AND DISCUSSION

Simultaneous isolation of galloittannins

The development of analytical technique Liquid chromatography mass spectrometry/mass spectrometry (LC/MS) made profiling and identification of multi-components of plant extracts more rapid. Identification of a series of galloittannins in extracts of morphological parts of mango (peel, pulp, and kernel) by such technique has recently been reported (Berardini et al., 2004). However, estimation of the activity of the individual galloittannins, as well as its pharmacokinetic and pharmacodynamic parameters, are largely dependent on the tannin structure (Berardini et al., 2004, Gan et al., 2018), which necessitate obtaining the tannin in pure form. We herein succeeded to simultaneously purify a group of five isomeric tetragalloyl glucoses (3–7), a pentagalloyl glucose (8), and a tannin related compound, ethyl gallate (2), based on a preparative RP-HPLC purification of a crude tannin mixture obtained from gel chromatography. Briefly, a concentrated aqueous EtOH extract of fresh mango kernels was fractionated on a Diaion HP-20 column with methanol/water gradients (0:10 → 2.5:7.5 → 5:5 → 10:0, v/v). The methanol/water (2.5:7.5, v/v) eluted fraction was further fractionated on an MCI-gel CHP-20P with water, methanol/water, and water gradients (9:1 → 8.5:1.5 → 8:2 → 7:5:2.5 → 7:3 → 6.5:3.5 → 6:4 → 3:7), and methanol. Then, simultaneous isolation of the compounds 2–8 (Fig. 1) was attained by RP-HPLC purification of the water/methanol (7:5:2.5, v/v) eluate on A-324 (10 i.d. × 300 mm) ODS-A column (YMC-Pack) using CH₃CN–water (2:8, v/v) acidulated with 1 ml L acetic acid. A 2 ml/min flow rate at column oven 40°C was used, and the eluates were monitored by a UV detector set at λₘ₉ₐₓ 280 nm.

The easy and fast isolation procedures described here for galloittannins could be useful for the rapid purification of such compounds from other galloittannins-rich plants including other morphological parts of M. indica.

Identification of the isolated compounds

Analysis of the data obtained from ESIMS and NMR spectroscopic experiments, 'H, 13C 'H–'H correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC), and comparison of the found data with literature ones, allowed identification of compounds 1 and 2 as gallic acid (Lee et al., 2005) and ethyl gallate (Cui et al., 2002), and galloylglucoses (3–8) as 2,3,4,6-tetra-O-galloyl-β-D-glucose (3) (Haddock et al., 1982), 1,2,3,4-tetra-O-galloyl-β-D-glucose (4) (Berardini et al., 2004), mixture of almost equal proportions of 1,2,3,6-tetra-O-galloyl-β-D-glucose (5) (Hagenah and Gross, 1993) and 1,3,4,6-tetra-O-galloyl-β-D-glucose (6) (Haddock et al., 1982), 1,2,4,6-tetra-O-galloyl-β-D-glucose (7) (Haddock et al., 1982), and 1,2,3,4,6-penta-O-galloyl-β-D-glucose (8) (Haddock et al., 1982). Worthy, gallic acid (1), ethyl gallate (2), and the pentagalloylglucose (8) are the most abundant isolates.

Anti-T. vaginalis activities of the isolated compounds

Secondary metabolites from natural sources have played a unique role in the discovery of anti-infectious compounds. Studies on the inhibitory effects of plant extracts on the growth of T. vaginalis trophozoites evidenced that extracts from the volatile oils producing plants Mentha piperita, Salvia officinalis,
and *Tanacetum parthenium* demonstrate anti-*T. vaginalis* activity identical to MTZ (Ezz Eldin and Badawy, 2015; Sharafi et al., 2013). A commercial garlic-based product (Tomex®) has been also shown to significantly reduce the multiplication and motility of the *T. vaginalis* trophozoites (Ali, 2007). In another study, the hydrolyzable tannins containing ethyl acetate fraction of a *Eucalyptus* extract exhibited MTZ-like activity on the growth of the *Trichomonas* trophozoites (Hassani et al., 2013). Meanwhile, limited research on the effects of pure compounds from natural sources, such as berberine alkaloid (de Brum Vieira et al., 2015; Sharafi et al., 2013) and piperazinyl derivatives of betulinic acid (Innocente et al., 2014), have been reported.

Herein, the effect of a group of gallotannins and ethyl gallate from kernels of *M. indica* on the viability of *T. vaginalis* clinical isolates was estimated (see experimental section). Although the common chemical nature of the investigated compounds, galloyl esters of a glucose core (Fig. 1), their antiprotozoal activities were varied based on the molecular structure (Fig. 3). All the examined compounds (2–8) exhibited a remarkable dose-dependent and time-dependent decrease in the percentage of living trophozoites (Fig. 3). After 24 hours of incubation, ethyl gallate, a tannin-related compound, showed the highest inhibitory effect on the *T. vaginalis* trophozoites viability (IC₅₀ 1.3 μg/ml, Fig. 4). The gallotannin with an unacylated O-3 position of the glucose core, 1,2,4,6-tetra-O-galloyl-β-D-glucose (7), exerted a potent effect (IC₅₀ 2.4 μg/ml), while the inhibitory effect of ~1:1 mixture of 5 and 6 on the *T. vaginalis* trophozoites was relatively low (IC₅₀ 36.1 μg/ml). The other gallotannins (3, 4 and 8) exhibited

![Figure 3. In vitro inhibitory effects of the compounds 2–8 on viability of *T. vaginalis* trophozoites. The bars represent standard division (SD) of the means.](image-url)
noticeable anti-*T. vaginalis* activities with comparable potencies (IC₅₀ 3.9 – 9.9 μg/ml). Due to the presence of some differences in the activity of the examined gallotannins, a structural activity relationship can’t be generated because of the limited number of the investigated compounds.

The broad-spectrum antiprotozoal activities of the mango leaf and stem bark extracts (Núñez Sellés et al., 2002) against Entamoeba histolytica (Tona et al., 1998), Histomonas meleagridis, Tetrahymena gallinarum and Blastocystis sp (Grabensteiner et al., 2008), Plasmodium falciparum (Awe et al., 1998; Ruiz et al., 2011; Zirihi et al., 2005), and Giardia lamblia (Amaral et al., 2006) agree with our herein obtained results. In addition, the reported leishmanicidal activity of several tannins against amastigotes of Leishmania donovani (Kolodziej et al., 2001) also supports our results. Likewise, the antibacterial activity of gallotannins and/or extracts of mango kernels on different bacterial species have been also shown in several reports (El-Gied et al., 2012; Engels et al., 2010; Ka buki et al., 2000; Rajan et al., 2011; Rakholtiya et al., 2015; Shabani and Sayadi, 2014; Subbija et al., 2013). The antimicrobial properties of the kernel gallotannins were ascribed to its ability to intermingle with proteins, hinder the enzyme activity (Rajan et al., 2011), and/or its ability to make a complex with metal ions such as iron (Engels et al., 2009). Altogether, the anti-*T. vaginalis* activity of the isolated gallotannins may be attributed to either or all of the aforementioned mechanisms. *T. vaginalis* uses the iron-containing proteins lactoferrin and hemoglobin (Sehgal et al., 2012 delivered by the menstruation blood (Figueroa-Angulo et al., 2012). A study on the effect of iron deficiency in the host on *T. vaginalis* demonstrated changes in the parasite propagation, cytotoxicity, and immune evasion (Alvarez-Sánchez et al., 2007). Therefore, iron deficiency by complexation with gallotannins may be the cause of the trophozoite cellular damage and the parasite survival inhabitation at the experimental conditions.

**CONCLUSION**

We are reporting here on the accumulation of gallotannins (galloylglucoses), gallic acid, and gallate derivatives in mango kernels. The procedure as described here is an easy and fast isolation for gallotannins that could be useful for the preparation of these compounds either as a crude fraction or single pure sample from the kernels and from gallotannins-rich plant extracts including other morphological parts of *M. indica*. The present study demonstrated, to the first time, that the mango kernels along with its isolated gallotannins and ethyl gallate could be used for further studies on the development of novel preventive or therapeutic agents for the treatment of trichomoniasis. However, it is still required to achieve animal lab-work and more mechanistic studies to approve our *in vitro* finding.

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The authors declare that they have no conflict of interest.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflict of interest.

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SUPPLEMENTARY MATERIAL

S1. 1H NMR spectrum of 2 [600 MHz, (acetone-d$_6$ – D$_2$O, 9:1), 27 °C].

S2. 1H–1H COSY spectrum of 2 [600 MHz, (acetone-d$_6$ – D$_2$O, 9:1), 27 °C].
S3. $^{13}$C NMR spectrum of 2 [151 MHz, (acetone-$d_6$ – D$_2$O, 9:1), 27 °C].

S4. HSQC spectrum of 2 [600 MHz, (acetone-$d_6$ – D$_2$O, 9:1), 27 °C].
S5. HMBC spectrum of 2 [600 MHz, (acetone-d₆ – D₂O, 9:1), 27 °C].

S6. ¹H NMR spectrum of 3 [600 MHz, (acetone-d₆ – D₂O, 9:1), 27 °C].
S7. $^1\text{H} - ^1\text{H}$ COSY spectrum of 4 [600 MHz, (acetone-$d_6$ - D$_2$O, 9:1), 27 °C].

S8. $^1\text{H}$ NMR spectrum of 4 [600 MHz, (acetone-$d_6$ - D$_2$O, 9:1), 27 °C].
S9. $^1$H–$^1$H COSY spectrum of 4 [600 MHz, (acetone-$d_6$–D$_2$O, 9:1), 27 °C].

S10. $^1$H NMR spectrum of mixture of 5 and 6 [600 MHz, (acetone-$d_6$–D$_2$O, 9:1), 27 °C].
S11. Expanded sugar protons region of $^1$H NMR spectrum of mixture of 5 and 6 [600 MHz, (acetone-d$_6$–D$_2$O, 9:1), 27 °C].

S12. $^1$H–$^1$H COSY spectrum of mixture of 5 and 6 [600 MHz, (acetone-d$_6$, D$_2$O, 9:1), 27 °C].
S13. $^1$H NMR spectrum of 7 [600 MHz, (acetone-$d_6$ – D$_2$O, 9:1), 27 °C].

S14. $^1$H – $^1$H COSY spectrum of 7 [600 MHz, (acetone-$d_6$ – D$_2$O, 9:1), 27 °C].
S15. $^1$H NMR spectrum of 8 [600 MHz, (acetone-$d_6$–D$_2$O, 9:1), 27 °C].

S16. $^{13}$C NMR spectrum of 8 [151 MHz, (acetone-$d_6$–D$_2$O, 9:1), 27 °C].
S17. $^1$H–$^1$H COSY spectrum of 8 [600 MHz, (acetone-$d_6$–D$_2$O, 9:1), 27 °C].

S18. HSQC spectrum of 8 [600 MHz, (acetone-$d_6$–D$_2$O, 9:1), 27 °C].
S19. HMBC spectrum of 8 [600 MHz, (acetone-\text{\textit{d}}_6-D_2O, 9:1), 27 °C].