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Synthesis of anti-inflammatory 2,3-unsaturated *O*-glycosides using conventional and microwave heating techniques

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Abstract: The preparation of eight 2,3-unsaturated *O*-glycosides from D-glycals and alcohols, using montmorillonite K-10 as an acid catalyst, is described. The Ferrier rearrangement products were obtained in good yields using conventional heating and microwave irradiation but the reaction time was substantially reduced employing the latter procedure. The yields were slightly lower under microwave exposure. Five of the di-*O*-acetylated products were deacetylated to the glycosides in excellent yields. The acetylated products possess good anti-inflammatory property suggesting that the acetyl group plays an important role in reducing the inflammation. Among the compounds tested, glycosides containing thiophene as an aglycone present much better inflammation reducing characteristics than the analogues without this function.

Keywords: anti-inflammatory glycosides; Ferrier rearrangement; tri-*O*-acetyl-D-galactal; tri-*O*-acetyl-D-glucal; 2,3-unsaturated glycosides.

Introduction

The Ferrier rearrangement involves the reaction between a glycal and a nucleophile in the presence of a mild Lewis acid causing an allylic shift in the glycal [1–3]. This protocol furnishes a variety of glycosides as potential building blocks for the synthesis of natural products with promising biological activities [4]. In recent years, diverse conditions have been applied to facilitate this kind of synthesis with enhanced yields and often providing predominantly α-anomers of the glycosides. Examples are the use of montmorillonite K-10 [5], montmorillonite K-10 clay doped with $FeCl_3 \cdot 6H_2O$ [6], microwave-irradiation under solventless conditions [7], metal-free Ferrier reaction [8], $ZnCl_2$-alumina-impregnated catalysts [9], Mitsunobu reaction [10] and other reported protocols [11–13] furnishing the desired products with satisfactory results.

The literature cites the Ferrier type of strategy for the synthesis of a variety of sugar molecules for biological assays including glycosides containing phthalimido moiety as aglycones [14]. Such glycosides are interesting because phtalimides themselves are known to possess biological activities. For example, some phthalimide derivatives are antitumor [15], anticonvulsant [16] and anti-inflammatory agents [17]. A phthalimidomethyl-4,6-di-*O*-benzoyl-α-D-mannopyranoside shows significant reduction of plasma triglyceride concentration when tested in mice [14]. Recently, alkyl-substituted phthalimido-1,2,3-triazoles linked to 2,3-unsaturated *O*-glycoside have been synthesized and shown to decrease carrageenan-induced edema in mice [18]. In 2007, glycosides containing a phthalimido group were synthesized in an attempt to improve their biological activity [19].

In this work, we describe the synthesis of 9 new 2,3-unsaturated *O*-glycosides **3b,c**, **5b,c**, **6b,c** and **7a–c** from D-glucal and D-galactal and evaluation their anti-inflammatory activity.

Results and discussion

In order to synthesize 2,3-unsaturated-*O*-glycoside from glycals and alcohols, we decided to use montmorillonite K-10 clay as an acidic catalyst since it is inexpensive,

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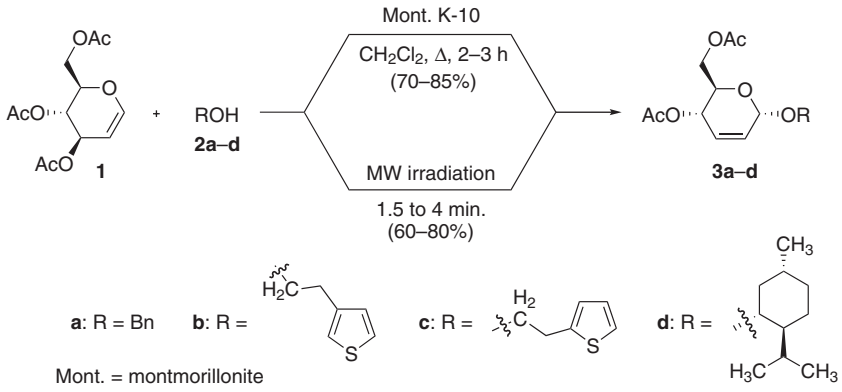
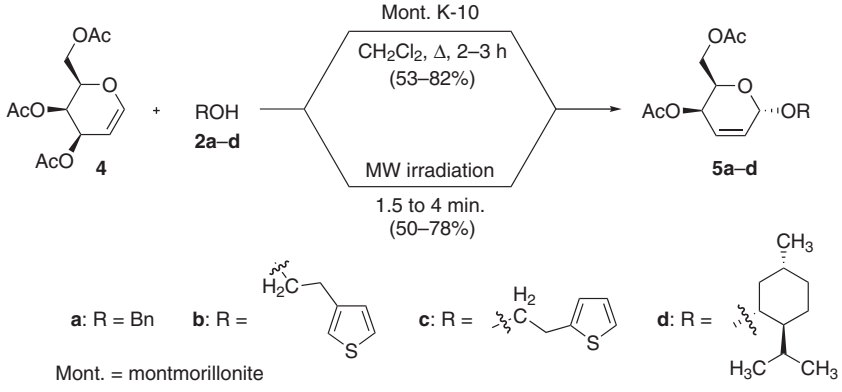


Scheme 1 Synthesis of 2,3-unsaturated *O*-glucosides **3a–d**.



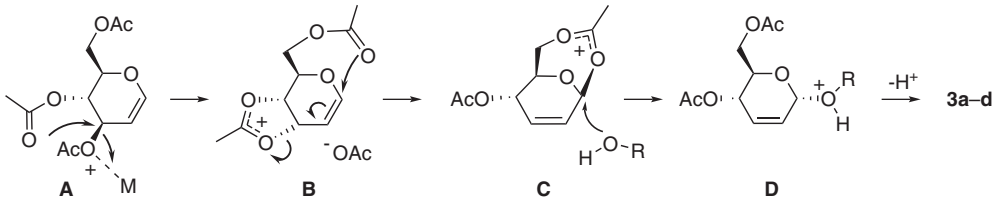
Scheme 2 Synthesis of 2,3-unsaturated *O*-galactosides **5a–d**.

reusable and eco-friendly. In this context, tri-*O*-acetyl-D-glucal **1** was initially used to react with some alcohols, namely benzyl alcohol (**2a**), 2-(thiophene-3-yl)ethanol (**2b**), 2-(thiophene-2-yl)ethanol (**2c**) and (1R,2S,5R)-(–)-menthol (**2d**) using two different methods. In method A, a mixture of montmorillonite K-10 and the reactants was heated under reflux in CH_2Cl_2 [5] and in method B, montmorillonite K-10 was mixed with the reactants and irradiated in a microwave oven [7]. These two methodologies [5, 7] were revisited in this work to afford *O*-glycosides **3a–d** from D-glucal and alcohols. Procedure A yielded 70–85% of the desired glucosides after 2–3 h of heating, while in procedure B the microwave irradiation was completed in 1.5–4.0 min with 60–80% yields (Scheme 1). A similar synthesis with D-galactal gave 2,3-unsaturated *O*-galactosides **5a–d** in moderate to good yields (Scheme 2).

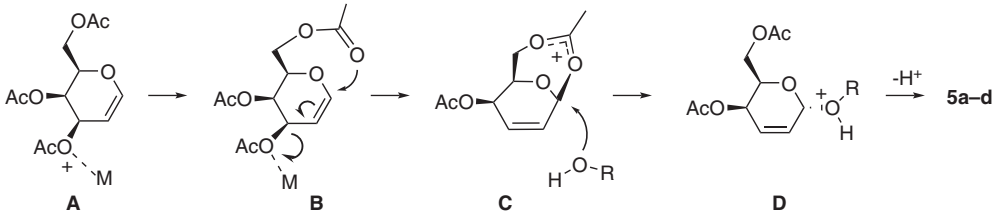
Two main features were observed. First, 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosides **3a–d** were obtained in better yields compared to 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosides **5a–d** under similar conditions. Second, only the α-anomers were isolated in all cases, except for **3b** where the α : β ratio was 85 : 15 in both A and B methods as determined by ^{1}HNMR spectroscopy. Initially, the difference in yields in the two sets of reactions appeared difficult to explain, but eventually the following reasonable interpretation emerged.

When tri-*O*-acetyl-D-glucal is treated with K-10, it is logical to assume that the metal combines with the oxygen atom of the C-3 *O*-acetyl group forming a metal-oxygen complex as shown in Scheme 3. This causes the weakening of C_3-OAc bond accompanied with anchimeric assistance from C_4-OAc group facilitating the elimination of the OAc group from C-3 and forming an intermediate product B. The restoration of the C-4-OAc moiety occurs when the carbonyl oxygen of C-6 approaches C-1 shifting the double bond to C_2–C_3 as depicted by C (Scheme 3). In this intermediate, the positive charge at carbon atom is stabilized by two oxygen atoms. In this case, the alcohol can attack C-1 only from below giving the protonated form of the molecule represented by D which after losing proton is transformed to **3a–d**.

In the case of 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosides **5a–d**, the lack of anchimeric



Scheme 3 Proposed mechanism to obtain glucosides **3a–d**.



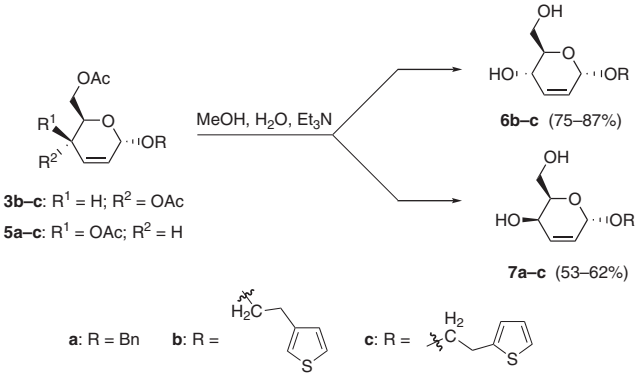
Scheme 4 Proposed mechanism to obtain galactosides **5a–d**.

assistance from C_4-acetoxy function would account for the slower removal of C_3-OAc group, thus furnishing the products in lower yields under similar conditions. The suggested mechanism of formation of α-anomers only is shown in Scheme 4. The attack of carbonyl oxygen of C_6-OAc at C-1 (structure B) causes the allylic rearrangement to generate C.

Since there is no anchimeric assistance of C_4-acetyl group in galactal **4**, the exit of C_3-OAc becomes slower in these compounds, hence smaller yields under similar reaction conditions. Again, only α-anomers were isolated. Subsequently, five of the unsaturated glycosides, **3b,c** and **5a–c**, were hydrolyzed under Fraser Reid's protocol [20] with good to excellent yields (Scheme 5).

The structures of all compounds **3a–d**, **5a–d**, **6b,c** and **7a–c** were assigned by ^{1}H and ^{13}C NMR spectral and elemental analyses. The configuration at C-4 in 2,3-unsaturated compounds was assigned by analysis of the coupling constant between H-4 and H-5. The vicinal constant $^3J_{4,5}$ is approximately 9.6 Hz for **3a–d** and **6b,c** indicative of *erythro* structure. The coupling constant in compounds **5a–d** and **7a–c** is in a range between 4.2 Hz and 6.6 Hz indicative of *threo* configuration. A 1D NOE-DIFF spectral investigation for compounds **3b** and **5b** showed the correct spatial interactions between the protons in the saccharide portion, demonstrating the correct relationships. During the preparation of this manuscript, we came across a very recent publication [21] where the authors described the preparation of (1*R*,2*S*,5*R*)-menthyl 4,6-di-*O*-acetyl-2,3-didesoxy-α-D-*threo*-hex-2-enopyranoside **5d**, but did not provide adequate characterization of this compound. Full characterization of **5d** is given in the Experimental section.

There are reports in the literature that 2,3-unsaturated glycosides possess anti-inflammatory property [19]. In particular, thiophene derivatives containing acetylenic subsituents are effective in reducing inflammation [22]. More recently, another thiophene-comprising product has been found to exhibit inflammation-reducing action [23]. Therefore, it was decided to explore the possible anti-inflammatory activity of compounds **3a**, **5a** and **6b**. Compound **3a** has a significant anti-inflammatory activity while **5a** with *threo* configuration and **6b** (deacetylated glycoside) have no anti-inflammatory activity at all. On the basis of on these results, it was decided to assay the activity of other 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosides **3b–d**. These products show moderate to good inflammation-reducing property ranging from 41% to 62% at a dose of 100 mg/kg of the animal weight (Table 1). Compound **3c** is the most active in reducing the inflammation by 62% at the dose of 100 mg/kg, while



Scheme 5 Synthesis of compounds **6b–c** and **7a–c**.

Table 1 Anti-inflammatory activity of compounds **3a–d** and their comparison to indomethacin and ASA as references.

Treated	Dose (mg/kg)	No. of PMNL/mL ($\times 10^6$) ($n=6$, after 6 h)[a]	Inhibition (%)
Carregenan control		4.13 ± 0.07	–
3a	100	1.96 ± 0.16[a]	52.0
3b	100	2.40 ± 0.20[a]	41.3
3c	100	1.57 ± 0.20[a]	62.0
3d	100	2.17 ± 0.30[a]	46.7
Indomethacin	10	1.75 ± 0.20[a]	55.5
ASA	200	1.32 ± 0.06[a]	73.7

All data are expressed as mean ± SD ($n=6$). Statistically significant [a]$p<0.05$ vs. control. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with carrageenan control group. PMNL, polymorphonuclear leukocyte; ASA, acetylsalycilic acid. N = 6 animals per group.

indomethacin shows 56% decrease in the cellular migration at a dose of 10 mg/kg, and acetylsalisylic acid, the most common anti-inflammatory agent in the market, exhibits 74% inflammation reduction at a dose of 200 mg/kg of the body weight. It is obvious that the acetyl group plays an important role in these unsaturated sugars to reduce the inflammation. The well known example of aspirin is instructive [24].

Conclusions

Eight 4,6-di-*O*-acetyl-2,3-unsaturated glycosides were synthesized by Ferrier's rearrangement from D-glucal or D-galactal under ordinary heating and microwave irradiation. Four thiophene derivatives **3b,c** and **5b,c** exhibit potent anti-inflammatory activity. Their deacylated analogues are devoid of anti-inflamatory activity.

Experimental

Melting points were determined using a MEL-TEMP Electro-Thermal instrument, model 1002D and are uncorrected. All commercially available reagents were used without purification (Sigma-Aldrich or Acros). All organic solvents used for the synthesis were of analytical grade (Vetec). Column chromatography was performed on Merck silica gel 60 (70–230 mesh). The reactions were monitored by TLC analysis with the plates containing GF_{254} from Merck and using UV fluorescence, or 5% H_2SO_4/EtOH for visualization. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian unity plus-300 spectrometer. Optical rotations were measured using a Perkin-Elmer 241 at 20°C. Elemental analyses were carried out on an EA1110 CHNS-O analyzer. Microwave irradiation was performed in the domestic multimode oven SANYO, EM-804 TGR (2450 MHz, 700W). Tri-*O*-acetyl-D-glucal **1** and D-galactal **4** were prepared according to the literature procedures [25].

General procedure for the synthesis of 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro* and *threo*-hex-2 enopyranosides

Method A (conventional heating) A mixture of tri-*O*-acetyl-D-glucal **1** or tri-*O*-acetyl-D-galactal **4** (1 mmol), an alcohol **2a–d** (1.5 mmol) and dried CH_2Cl_2 (30 mL) was heated under reflux for 120 min (for **3d** and **5d**), 150 min (for **3b**), 180 min (**3a**, **3c** and **5c**) or 240 min (for **5a** and **5b**). Then the mixture was cooled at 0–5°C, treated with montmorillonite K-10 (50% w/w of **1**) or (100% w/w of **4**) and heating was continued for several hours. Removal of montmorillonite K-10 by filtration followed by concentration under reduced pressure afforded the desired product which was purified by column chromatography on silica gel using hexane/ethyl acetate (6 : 4) as eluent.

Method B (microwave irradiation) A mixture of tri-*O*-acetyl-D-glycal **1** or **4** (1 mmol), an alcohol **2a–d** (1.5 mmol), and montmorillonite K-10 (50% w/w of **1**) or (100% w/w of **4**) in an open glass test tube was irradiated in the domestic microwave oven for 1 min (for **3b**,**c** and **5b**,**c**), 1.5 min (for **3d** and **5d**) or 4 min (for **3a** and **5a**). After cooling, the mixture was treated with dichloromethane. Filtration followed by concentration under reduced pressure furnished an oil that was subjected to silica gel column chromatography eluting with a mixture of 5% ethyl acetate in hexane (v/v) as eluent.

Benzyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (3a) Yield 85% (method A); Yield 80% (method B); colorless oil; $[\alpha]_D^{20}+125°$ (*c* 1, $CHCl_3$); $[\alpha]_D^{20}+75°$ (*c* 0.44, $CHCl_3$) [12]. The ^{1}H NMR spectrum of this compound is virtually identical with the previously reported data [12].

2-(Thiophene-3-yl)ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (3b) Yield 70% (method A, α : β = 85 : 15); yield 60% (method B, α : β = 85 : 15); colorless oil. R_f 0.6 (5% EtOAc/CH_2Cl_2); $[\alpha]_D^{20}+79°$ (*c* 0.85, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 2.01 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.97 (t, 2H, $J=6.9$ Hz, CH_2-Het), 3.76 (dt, 1H, $J=9.6$ Hz and 6.6 Hz, OCH_2), 3.97–4.05 (m, 2H, H-5, OCH_2), 4.12 (s, 1H, H-6 or H-6′), 4.13 (d, 1H, $J=1.8$ Hz, H-6 or H-6′), 5.07 (br s, 1H, H-1), 5.25 (dd, 1H, $J=9.6$ Hz and 1.2 Hz, H-4), 5.87 (s, 2H, H-2, H-3), 7.07 (dd, 1H, $J=4.8$ Hz and 1.5 Hz, Ar-H_a), 7.18–7.20 (m, 1H, Ar-H_c), 7.37 (dd, 1H, $J=4.8$ Hz and 3.0 Hz, Ar-H_b); ^{13}C NMR (acetone-d_6): δ 21.3, 21.5, 32.0, 64.4, 66.7, 68.5, 70.0, 95.7, 122.6, 126.7, 129.8, 130.0, 130.2, 141.0, 171.2, 171.4. Anal. Calcd for $C_{16}H_{20}O_6S$: C, 56.46; H, 5.92. Found: C, 56.66; H, 5.77.

2-(Thiophene-2-yl)ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (3c) Yield 85% (method A); yield 70% (method B); colorless oil. $R_f=0.7$ (5% EtOAc/CH_2Cl_2). $[\alpha]_D^{20}+44°$ (*c* 0.5, $CHCl_3$). ^{1}H NMR (acetone-d_6): δ 1.99 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 3.12 (dd, 2H, $J=13.2$ Hz and 6.8 Hz, CH_2-Het), 3.70–3.79 (dt, 1H, $J=9.9$ Hz and 6.6 Hz, OCH_2), 3.96–4.07 (m, 2H, H-5, OCH_2), 4.14 (d, 1H, $J=4.2$ Hz, H-6 or H-6′), 4.20 (d, 1H, $J=5.4$ Hz, H-6 or H-6′), 5.08 (br s, 1H, H-1), 5.20 (dd, 1H, $J=9.6$ Hz and 1.2 Hz, H-4), 5.87, 5.96 (2s, 2H, H-2 and H-3), 6.90–6.95 (m, 2H, Ar-H_a and Ar-H_b), 7.26 (ddd, 1H, $J=5.1$ Hz, 5.1 Hz and 1.2 Hz, Ar-H_c); ^{13}C NMR (acetone-d_6): δ 21.3,

21.5, 30.8, 64.3, 66.6, 68.6, 70.6, 95.8, 125.2, 126.7, 128.1, 129.6, 130.4, 142.9, 171.3, 171.5. Anal. Calcd for $C_{16}H_{20}O_6S$: C, 56.46; H, 5.92. Found: C, 56.57; H, 6.01.

(1R,2S,5R)-Menthyl 4,6-di-*O*-acetyl-2,3-didesoxy-α-D-*erythro*-hex-2-enopyranoside (3d) Yield 82% (method A); yield 71% (method B); colorless oil; R_f 0.6 (5% EtOAc/CH_2Cl_2); $[\alpha]_D^{20}$ +44° (*c* 1, CH_2Cl_2); $[\alpha]_D^{20}$ +46° (*c* 0.75, CH_2Cl_2) [23]. The ^{1}H NMR spectrum is virtually identical with previously reported data [26].

Benzyl 4,6-di-*O*-acetyl-2,3-didesoxy-α-D-*threo*-hex-2-enopyranoside (5a) Yield 82% (method A); yield 78% (method B); colorless oil. R_f 0.8 (10% EtOAc/CH_2Cl_2); $[\alpha]_D^{20}$ −134° (*c* 2, $CHCl_3$); $[\alpha]_D^{20}$ −83° (*c* 0.27, $CHCl_3$). The ^{1}H NMR spectrum is virtually identical with previously reported details [12].

2-(Thiophene-3-yl)ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (5b) Yield 60% (method A); yield 54% (method B); colorless oil. R_f 0.7 (5% EtOAc/CH_2Cl_2); $[\alpha]_D^{20}$ −101° (*c* 2, $CHCl_3$). ^{1}H NMR (acetone-d_6): δ 1.98 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.94 (t, 2H, *J* = 6.9 Hz, CH_2-Het), 3.74 (dt, 1H, *J* = 9.6, 6.9 Hz, OCH_2), 3.99 (dt, 1H, *J* = 9.6, 6.9 Hz, OCH_2), 4.13 (dd, 1H, *J* = 11.4 Hz and 7.8 Hz, H-6 or H6′), 4.20 (dd, 1H, *J* = 11.4 Hz and 4.8 Hz, H-6 or H6′), 4.29 (ddd, 1H, *J* = 7.8 Hz, 4.8 Hz and 2.4 Hz, H-5), 5.00 (dd, 1H, *J* = 4.8 Hz and 2.4 Hz, H-4), 5.08 (brd, 1H, *J* = 1.5 Hz, H-1), 6.04 (dd, 1H, *J* = 11.1 Hz and 0.9 Hz, H-2 or H-3), 6.05 (d, *J* = 11.1, 1H, H-2 or H-3), 7.05 (dd, 1H, *J* = 5.1 Hz and 1.2 Hz, Ar-H_a), 7.17–7.19 (m, 1H, Ar-H_c), 7.38 (dd, 1H, *J* = 5.1 Hz and 2.7 Hz, Ar-H_c); ^{13}C NMR (acetone-d_6): δ 21.3 (2C), 32.0, 64.1 (2C), 68.4, 69.6, 95.4, 122.6, 126.4, 126.7, 130.0, 132.3, 141.0, 171.2, 171.4. Anal. Calcd for $C_{16}H_{20}O_6S$: C, 56.46; H, 5.92. Found: C, 56.36; H, 5.73.

2-(Thiophene-2-yl)ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (5c) Yield 53% (method A); yield 50% (method B); colorless oil. R_f 0.7 (5% EtOAc/CH_2Cl_2); $[\alpha]_D^{20}$ −121° (*c* 1, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 1.98 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.14 (t, 2H, *J* = 6.9 Hz, CH_2-Het), 3.74 (dt, 1H, *J* = 9.9 Hz and 6.9 Hz, OCH_2), 4.00 (dt, 1H, *J* = 9.9 Hz and 6.9 Hz, OCH_2), 4.15 (dd, 1H, *J* = 11.4 Hz and 7.8 Hz, H-6 or H-6′), 4.21 (dd, 1H, *J* = 11.4 Hz and 4.8 Hz, H-6 or H-6′), 4.32 (ddd, 1H, *J* = 7.8 Hz, 4.8 Hz and 2.7 Hz, H-5), 5.02 (brdd, 1H, *J* = 4.4 Hz and 2.7 Hz, H-4), 5.10 (br d, 1H, *J* = 1.5 Hz, H-1)), 6.06 (s, 1H, H-2 or H-3), 6.07 (d, *J* = 1.2, 1H, H-2 or H-3), 6.91–6.95 (m, 2H, Ar-H_a and Ar-H_b), 7.25 (dd, 1H, *J* = 5.1 Hz and 1.2 Hz, Ar-H_c); ^{13}C NMR (acetone-d_6): δ 20.6, 20.7, 31.1, 63.4, 63.5, 67.8, 69.5, 94.8, 124.5, 125.8, 126.0, 127.5, 131.5, 142.1, 170.6, 170.7. Anal. Calcd for $C_{16}H_{20}O_6S$: C, 56.46; H, 5.92. Found: C, 56.49; H, 6.18.

(1R,2S,5R)-Menthyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (5d) Yield 72% (method A); yield 65% (method B); colorless solid from EtOAc/hexane; mp 65–67°C; $[\alpha]_D^{25}$ −200° (*c* 1, CH_2Cl_2); $[\alpha]_D^{20}$ −159° (*c* 0.16, CH_2Cl_2) [21]; ^{1}H NMR ($CDCl_3$): δ 0.77 (d, 3H, *J* = 7.1 Hz, CH_3, menthyl), 0.90 (2d, 6H, *J* = 7.0 and 6.5 Hz, $2CH_3$, menthyl), 0.80–1.65 (m, 8H, menthyl), 2.06 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.20 (bd, 1H, *J* = 12.3 Hz, menthyl), 3.43 (ddd, pseudo-td, 1H, *J* = 10.6 Hz, 10.6 Hz and 4.1 Hz, menthyl), 4.18 (dd, 1H, *J* = 11.5 Hz and 7.6 Hz, H-6 or H-6′), 4.23 (dd, 1H, *J* = 11.5 Hz and 5.0 Hz, H-6 or H-6′), 4.40 (ddd, 1H, *J* = 7.6 Hz, 5.0 Hz and 2.6 Hz, H-5), 5.00 (dd, 1H, *J* = 4.7 Hz and 2.6 Hz, H-4), 5.13 (br d, 1H, *J* = 2.4 Hz, H-1), 6.03 (dd, 1H, *J* = 10.0 Hz and 2.4 Hz, H-2), 6.09 (dd, 1H, *J* = 10.0 Hz and 4.7 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 16.2, 20.8, 21.1, 22.4, 23.1, 25.6, 31.7, 34.3, 43.2, 48.9, 63.0, 63.2, 66.6, 80.7, 95.6, 124.7, 130.7, 170.3, 170.6.

General procedure for synthesis of 2,3-dideoxy-hex-2-enopyranosides

A mixture of the acetylated compound **3b,c** or **5b,c** in a mixed solvent of MeOH/H_2O/Et_3N (9 : 6 : 1) was stirred at room temperature for 2 h (for **6b**,**c**) or 3 h (for **7b**) or 4 h (for **7a** and **7c**). TLC plates were developed using methanol/$CHCl_3$ (1 : 9). The solvent was removed under reduced pressure and the residue was subjected to silica gel column chromatography eluting with a mixed solvent of ethyl acetate/hexane (4 : 5).

2-(Thiophene-3-yl)ethyl 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (6b) Yield 87%; colorless oil; R_f 0.5 (10% EtOAc/$CHCl_3$); $[\alpha]_D^{20}$ +29° (*c* 0.7, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 2.91 (t, 2H, *J* = 6.9 Hz, CH_2-Het), 3.59–3.80 (m, 5H, 2xOH, OCH_2, H-4, H-5), 3.94–4.05 (m, 3H, OCH_2, H-6, H-6′), 4.96 (br dd, 1H, *J* = 2.7 Hz and 1.2 Hz, H-1), 5.67 (ddd, 1H, *J* = 10.2 Hz, 2.7 Hz and 1.8 Hz, H-2), 5.88 (ddd, 1H, *J* = 10.2 Hz, 1.8 Hz and 1.8 Hz, H-3), 7.04 (dd, 1H, *J* = 5.1 Hz and 1.2 Hz, Ar-H_a), 7.15–7.18 (m, 1H, Ar-H_c), 7.54 (dd, 1H, *J* = 5.1 Hz and 3.0 Hz, Ar-H_b); ^{13}C NMR (acetone-d_6): δ 30.8, 61.9, 63.4, 68.5, 72.8, 94.5, 121.4, 125.5, 125.9, 129.0, 134.4, 139.9. Anal. Calcd for $C_{12}H_{16}O_4S$ (1/4H_2O): C, 55.26; H, 6.38. Found: C, 55.25; H, 6.56.

2-(Thiophene-2-yl)ethyl 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (6c) Yield 75%; colorless solid; mp 68–69°C. R_f 0.5 (EtOAc/$CHCl_3$, 1;9); $[\alpha]_D^{20}$ +51° (*c* 1, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 3.10 (td, 2H, *J* = 6.6 Hz and 1.2 Hz, CH_2-Het), 3.62–3.73 (m, 4H, 2xOH, OCH_2, H-4), 3.80 (ddd, 1H, *J* = 11.4 Hz, 4.8 Hz and 2.1 Hz, H-5), 3.97–4.08 (m, 2H, OCH_2, H-6 or H-6′), 4.98 (br d, 1H, *J* = 2.7 Hz, H-1), 5.68 (ddd, 1H, *J* = 10.2 Hz, 4.8 Hz and 2.7 Hz, H-2), 5.90 (dd, 1H, *J* = 10.2 Hz and 2.7 Hz, H-3), 6.89–6.94 (m, 2H, Ar-H_a and H_b), 7.23 (dd, 1H, *J* = 5.4 Hz and 1.5 Hz, Ar-H_c); ^{13}C NMR (acetone-d_6): δ 31.2, 63.0, 64.5, 69.5, 73.4, 95.2, 124.4, 126.0, 126.5, 127.4, 134.9, 142.4. Anal. Calcd for $C_{12}H_{16}O_4S$: C, 56.23; H, 6.29. Found: C, 56.03; H, 5.92.

Benzyl 2,3-didesoxy-α-D-*threo*-hex-2-enopyranoside (7a) Yield 60%; colorless crystals; mp 102–103°C. R_f 0.5 (EtOAc/$CHCl_3$, 1:9); $[\alpha]_D^{20}$ −106° (*c* 1, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 2.85 (brs, 2H, OH), 3.68–3.81 (m, 2H, H-6 and H-6′), 3.85 (ddd, 1H, *J* = 5.4 Hz, 2.4 Hz and 2.4 Hz, H-5), 4.04 (dd, 1H, *J* = 6.0 Hz and 2.4 Hz, H-4), 4.55 (d, 1H, *J* = 11.8 Hz, OCH_2Ph), 4.82 (d, 1H, *J* = 11.8 Hz, OCH_2Ph), 5.07 (brd, 1H, *J* = 3.0 Hz, H-1), 5.86 (dd, 1H, *J* = 9.9 Hz and 3.0 Hz, H-2), 6.09 (dd, 1H, *J* = 9.9 Hz and 6.0 Hz, H-3), 7.25–7.40 (m, 5H, Ar-H); ^{13} C NMR (acetone-d_6): δ 62.1, 62.6; 69.7, 72.6, 94.2, 128.2, 128.7, 128.8 (2C), 129.0 (2C), 130.8, 139.5. Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 65.56; H, 6.76.

2-(Thiophene-3-yl)ethyl 2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (7b) Yield 62%; colorless oil; R_f 0.5 (10% EtOAc/$CHCl_3$); $[\alpha]_D^{20}$ −91° (*c* 0.7, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 2.89 (t, 2H, *J* = 6.9 Hz, CH_2-Het), 3.63–3.83 (m, 6H, 2xOH, OCH_2, H-5, H-6, H-6′), 3.91–4.04 (m, 2H, OCH_2, H-4), 4.97 (br d, 1H, *J* = 3.0 Hz, H-1), 5.81 (ddd, 1H, *J* = 10.2 Hz, 3.0 Hz and 0.6 Hz, H-2), 6.05 (ddd, 1H, *J* = 10.2 Hz, 5.4 Hz and 1.2 Hz, H-3), 7.04 (dd, 1H, *J* = 4.8 Hz and 1.2 Hz, Ar-H_a), 7.17 (m, 1H, Ar-H_c), 7.35 (dd, 1H, *J* = 4.8 Hz and 3.0 Hz, Ar-H_b); ^{13}C NMR ($CDCl_3$): δ 32.1, 64.5, 64.7, 68.2, 73.2, 95.8, 122.5, 126.9, 129.5, 130.0, 131.5, 141.1. Anal. Calcd for $C_{12}H_{16}O_4S$: C, 56.23; H, 6.29. Found: C, 55.77; H, 6.02.

2-(Thiophene-2-yl)ethyl 2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (7c) Yield 53%; colorless oil; R_f 0.5 (10% EtOAc/$CHCl_3$); $[\alpha]_D^{20}$ −144° (*c* 0.8, $CHCl_3$); ^{1}H NMR (acetone-d_6): δ 3.10 (td, 2H, *J* = 6.6,

2.1 Hz, CH_2-Het), 3.63–3.84 (m, 6H, 2×OH, OCH_2, H-5, H-6, H-6′), 3.94–4.04 (m, 2H, OCH_2, H-4), 4.99 (br d, 1H, J = 2.7 Hz, H-1), 5.81 (dd, 1H, J = 10.1 Hz and 2.7 Hz, H-2), 6.06 (dd, 1H, J = 10.2 Hz and 5.3 Hz, H-3), 6.89–6.94 (m, 2H, Ar-H_a and Ar-H_b), 7.23 (dd, 1H, J = 5.1 Hz and 1.5 Hz, Ar-H_c); ^{13}C NMR ($CDCl_3$): δ 31.5, 62.8, 63.4, 69.9, 73.2, 95.9, 125.2, 126.8, 128.2, 129.4, 131.4, 143.2. Anal. Calcd for $C_{12}H_{16}O_4S$: C, 56.23; H, 6.29. Found: C, 56.62; H, 6.37.

Determination of anti-inflammatory activity

Experimental animals Male *Mus musculus mice* (N = 6), provided by the animal facilities of UFPE, Recife, Brazil, were used for the evaluation of anti-inflammatory activity of the compounds. The mice weighed between 25 g and 30 g and were kept in a room with controlled temperature (22 ± 2°C) and humidity (50–60%) under a 12 h/12 h light/dark cycle. Water and food were made available to the animals without restriction. Before beginning the experiments, animals were acclimated to the laboratory environment for at least 30 min. All animals were fasted for 8 h prior to experimentation.

The Animal Studies Committee of the Federal University of Pernambuco approved the experimental protocols. The animals were treated according to the ethical principles of animal experimentation of COBEA (Brazilian College of Animal Experiments) and the norms of the National Institute of Health Guide for Care and Use of Laboratory Animals.

Carrageenan-induced air pouch This assay was performed according to the methodology described previously [27, 28]. The anti-inflammatory activities of the compounds were tested by the formation of air pouches on the dorsal cervical region of mice via a subcutaneous injection of 2.5 mL of sterile air on day 0, followed by a second injection of 2.5 mL of sterile air 3 days later. On day six, the mice received vehicle, (saline solution 0.9%) indomethacin (10 mg/kg), acetylsalicylic acid (ASA, 200 mg/kg) or the compounds test (100 mg/kg) orally. The doses were chosen according to the results of a pilot experiment. One hour after drug administration, inflammation was induced by injecting 1 mL of carrageenan suspension (1% in saline solution) into the air pouch. After 6 h, the mice were euthanized in a CO_2 chamber and the pouches were flushed with 3 mL of phosphate-buffered solution (PBS) with heparin (10 IU/mL). Samples were diluted with Turk's solution and leucocytes were counted in a Neubauer Chamber under a microscope. The average numbers of leucocytes from treated groups were compared with the number of leucocytes of the control group, assumed to be 100%. Statistical analysis between the groups was performed by one-way analysis of variance (ANOVA), followed by the Bonferroni's test for a confidence interval of 95%, P values less than 0.05 ($P < 0.05$) determined using Origin 7.0 graphical sofware.

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References

[1] Ferrier, R. J.; Prasad, N. Unsaturated carbohydrates. Part IX. Synthesis of 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosides from tri-*O*-acetyl-D-glucal. *J. Chem. Soc. Sect. C* **1969**, *4*, 570–575.

[2] Ferrier, R. J. Substitution with Allylic Rearrangement Reactions of Glycal Derivatives. *Top. Curr. Chem*. **2001**, *215*, 153–175.

[3] Gómez, A. M.; Lobo, F.; Uriel, C.; López, J. C. Recent Developments in the Ferrier Rearrangement. *Eur. J. Org. Chem.* **2013**, 7221–7267.

[4] Panarese, J. D.; Waters, S. P. Enantioselective formal total synthesis of (+)-aspergillide. *Org. Lett*. **2009**, *11*, 5086–5088.

[5] Toshima, K.; Ishizuka, T.; Matsuo, G.; Nakata, M. Practical glycosidation method of glycals using montmorillonite K-10 as an environmentally acceptable and inexpensive industrial material. *Synlett* **1995**, *4*, 306–308.

[6] Melo, V. N.; Dantas, W. M.; Camara, C. A.; de Oliveira, R. N. Synthesis of 2,3-unsaturated alkynyl *O*-glucosides from tri-*O*-acetyl-D-glucal by using montmorillonite K-10/iron(III) chloride hexahydrate with subsequent copper(I)-catalyzed 1,3-dipolar cycloaddition. *Synthesis* **2015**, *47*, 3529–3541.

[7] De Oliveira, R. N.; De Freitas Filho, J. R.; Srivastava, R. M. Microwave-induced synthesis of 2,3-unsaturated *O*-glycosides under solvent-free conditions. *Tetrahedron Lett.* **2002**, *64*, 2141–2143.

[8] De, K.; Legros, J.; Crousse, B.; Bonnet-Delpon, D. Synthesis of 2,3-unsaturated glycosides via metal-free Ferrier reaction. *Tetrahedron* **2008**, *64*, 10497–10500.

[9] Gorityala, B. K.; Lorpitthaya, R.; Bai, Y.; Liu, X-W. ZnCl2/alumina impregnation Catalyzed Ferrier rearrangement: an expedient synthesis of pseudoglycosides. *Tetrahedron* **2009**, *65*, 5844–5848.

[10] Michigami, K.; Hayashi, M. *O*- and *N*-Glycosidation of D-glycals using Ferrier rearrangement under Mitsunobu reaction conditions. *Tetrahedron*. **2012**, *68*, 1092–1096.

[11] Zhang, G.; Liu, Q.; Shi, L.; Wang, J. Ferric sulfate hydrate-catalyzed O-glycosylation using glycals with or without microwave irradiation. *Tetrahedron.* **2008**, *64*, 339–344.

[12] Mukherjee, A.; Jayaraman, N. Reactivity switching and selective activation of C-1 or C-3 in 2,3-unsaturated thioglycosides. *Carbohydr. Res*. **2011**, *346*, 1569–1575.

[13] Drew, M. D.; Wall, M. C.; Kim, J. T. Stereoselective propargylation of glycals with allenyltributyltin(IV) via a Ferrier type reaction. *Tetrahedron Lett*. **2012**, *53*, 2833–2836.

[14] Srivastava, R. M.; Oliveira, F. J. S.; da Silva, L. P.; de Freitas-Filho, J. R.; Oliveira, S. P.; Lima, V. L. M. Synthesis and hypolipidemic activity of N-phthalimidomethyl tetra-*O*-acyl-α-D-mannopyranosides. *Carbohydr. Res*. **2001**, *332*, 335–340.

[15] Chan, C. L.; Lien, E. J.; Tokes, Z. A. Synthesis, biological evaluation, and quantitative structure-activity relationship analysis of 2-hydroxy-1H-isoindolediones as new cytostatic agents *J. Med. Chem*. **1987**, *30*, 509–514.

[16] Bailleux, V.; Vallee, L.; Nuyts, J. P.; Vamecq, J. Anticonvulsant activity of some 4-amino-*N*-phenylphthalimydes and

N-(3-amino-2-methylphenyl)phthalimides. *Biomed. Pharmacother.* **1994**, *48*, 95–101.

[17] Sharma, U.; Kumar, P.; Kumar, N.; Singh, B. Recent advances in the chemistry of phthalimide analogues and their therapeutic potential. *Mini-Rev. Med. Chem*. **2010**, *10*, 678–704.

[18] Assis, S. P. O.; Silva, M. T.; de Oliveira, R. N.; Lima, V. L. M. Synthesis and anti-inflammatory activity of New alkyl-substituted phthalimide 1*H*-1,2,3-triazole derivatives. *Sci. World J.* **2012**, Article ID 925925, Doi: 10.1100/2012/925925.

[19] Meng, X. B.; Han, D.; Zhang, S. N.; Guo, W.; Cui, J. R.; Li, Z. J. Synthesis and anti-inflammatory activity of *N*-phthalimidomethyl 2,3-dideoxy- and 2,3-unsaturated glycosides. *Carbohydr. Res.* **2007**, *342*, 1169–1174.

[20] Fraser-Reid, B.; Mclean, A.; Usherwood, E.W.; Yunker, M. Pyranosiduloses. II. The synthesis and properties of some alkyl 2,3-dideoxy-2-enopyranosid-4-uloses. *Can. J. Chem*. **1970**, *48*, 2877–2884.

[21] Zhou, J.; Chen, H.; Shan, J.; Li, J; Yang, G.; Chen, X.; Xin, K.; Zhang, J.; Tang. J. FeCl3 · 6H2O/C: An Efficient and Recyclable Catalyst for the Synthesis of 2,3-Unsaturated *O*- and *S*-Glycosides. *J. Carbohydr. Chem.* **2014**, *33*, 313–325.

[22] Zeni, G.; Nogueira, C. W.; Panatieri, R. B.; Silva, D. O.; Menezes, P. H.; Braga, A. L.; Silveira, C. C.; Stefani, H. A.; Rocha, J. B. T. Synthesis and anti-inflammatory activity of acetylenic thiophenes. *Tetrahedron Lett.* **2001**, *42*, 7921–7923.

[23] Mishra, R.; Jha, K. K.; Kumar, S.; Tomer, I. Synthesis, properties and biological activity of thiophene: a review. *Der Pharma Chim.* **2011**, *3*, 38–54.

[24] Vane, J. R.; Botting, R. M. The mechanism of action of aspirin. *Thromb. Res.* **2003**, *110*, 255–258.

[25] Shull, B. K.; Wu, Z.; Koreeda, M. A convenient, highly efficient one-pot preparation of peracetylated glycals from reducing sugars. *J. Carbohydr. Chem*. **1996**, *15*, 955–964.

[26] de Oliveira, R. N.; de Melo, A. C. N.; Srivastava, R. M.; Sinou, D. Exclusive formation of α-anomers in $NbCl_5$ promoted Ferrier rearrangement for the synthesis of 2,3 unsaturated glycosides. *Heterocycles* **2006**, *68*, 2607–2613.

[27] Romano, M.; Faggioni, R.; Sironi, M.; Sacco, S.; Echtenacher, B.; Di Santo, E.; Salmona, M.; Ghezzi, P. Carrageenan-induced acute inflammation in the mouse air pouch synovial model. Role of tumour necrosis factor. *Mediators Inflamm*. **1997**, *6*, 32–38.

[28] Guerra, A. S. H. S.; Malta, D. J. N.; Laranjeira, L. P. M.; Maia, M. B. S.; Colaço, N. C.; Lima, M. C. A.; Galdino, S. L.; Pitta, I. R.; Gonçalves-Silva, T. Anti-inflammatory and antinociceptive activities of indole–imidazolidine derivatives. *Int. Immunopharmacol*. **2011**, *11*, 1816–1822.