Tetrahedron 71 (2015) 4581–4589

Contents lists available at ScienceDirect

Tetrahedron

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

Divergent total synthesis of crassalactones B and C and evaluation of their antiproliferative activity

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ARTICLE INFO

Article history:
Received 26 March 2015
Received in revised form 26 April 2015
Accepted 11 May 2015
Available online 15 May 2015

Keywords:
Styryl lactones
(+)-Crassalactone B
(+)-Crassalactone C
Polyalthia crassa
Antitumour agents
Protecting-group-free synthesis

ABSTRACT

A divergent total synthesis of cytotoxic natural products (+)-crassalactones B (**2**) and C (**3**) has been achieved by utilizing diacetone D-glucose (**4**) as a chiral precursor. The key steps of the synthesis of both targets **2** and **3** were a stereo-selective addition of phenyl magnesium bromide to a dialdose derivative, a regioselective introduction of the cinnamic acid residue, and a stereospecific furano-lactone ring formation by cyclocondensation of a suitable hemiacetal derivative with Meldrum's acid. No protection is necessary for the synthesis of the (+)-crassalactone C (**3**), except the diacetonide function that is already present in the commercially available starting material **4**. Preparation of (+)-crassalactone B (**2**) from the same starting material, requires the use of a single silyl ether protecting group throughout the synthesis. The synthesized natural products were evaluated for their in vitro antiproliferative activity against PC3, HT29 and A549 human tumour cell lines.

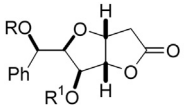
1. Introduction

The Annonaceae family consists of 120 genera and over 2000 species. *Polyalthia* is one of the Annonaceae genera with over 20 species distributed throughout tropical and subtropical region of Asia. Species from the *Polyalthia* genus have been widely used in traditional medicine in several Asian countries.[1] Chemical constituents of the *Polyalthia* species exhibit a variety of pharmacological activities, such as cytotoxic,[2] antimicrobial,[3] antimalarial,[4] and *anti*-HIV.[5] A number of styryl lactones have been recently isolated from the tropical plant *Polyalthia crassa*.[6] The bioassay-directed separation of the ethyl acetate extract of the leaves and twigs led to the isolation of the known antitumour agent (+)-goniofufurone (**1**, Fig. 1), along with several new compounds including the 5-*O*- and 7-*O*-cinnamoyl derivatives of **1**, as (+)-crassalactones B (**2**) and C (**3**), respectively. Their structures were elucidated on the basis of spectroscopic methods, while their absolute configurations were resolved by a (non-selective) chemical transformation of natural (+)-goniofufurone. We have recently reported a few approaches to (+)-crassalactone C (**3**) starting from D-xylose,[7] as well as a preliminary account related the first total synthesis of (+)-crassalactone B (**2**) starting from D-glucose.[8] Immediately thereafter a new total synthesis of (+)-crassalactones B and C starting from diacetone D-glucose,[9] as well as a novel total synthesis of (−)-crassalactone C from tartaric acid[10] has been reported by other groups. Finally, an efficient semi-synthesis of both **2** and **3** starting from (+)-goniofufurone has been very recently completed in our laboratory.[11] Herein, we disclose a new and concise total synthesis of (+)-crassalactones B (**2**) and C (**3**) starting from D-glucose. A brief study of their antitumour activity against several human cancer cell lines is an additional objective of this work.

2. Results and discussion

2.1. Chemical synthesis

As both natural products **2** and **3** display a clear structural similarity we wanted to design a divergent approach to **2** and **3**



1 R = R^1 = H
2 R = H; R^1 = *trans*-cinnamoyl
3 R = *trans*-cinnamoyl; R^1 = H

Fig. 1. Styryl lactones from *Polyalthia crassa*: (+)-goniofufurone (**1**) and (+)-crassalactones B (**2**) and C (**3**).

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http://dx.doi.org/10.1016/j.tet.2015.05.040
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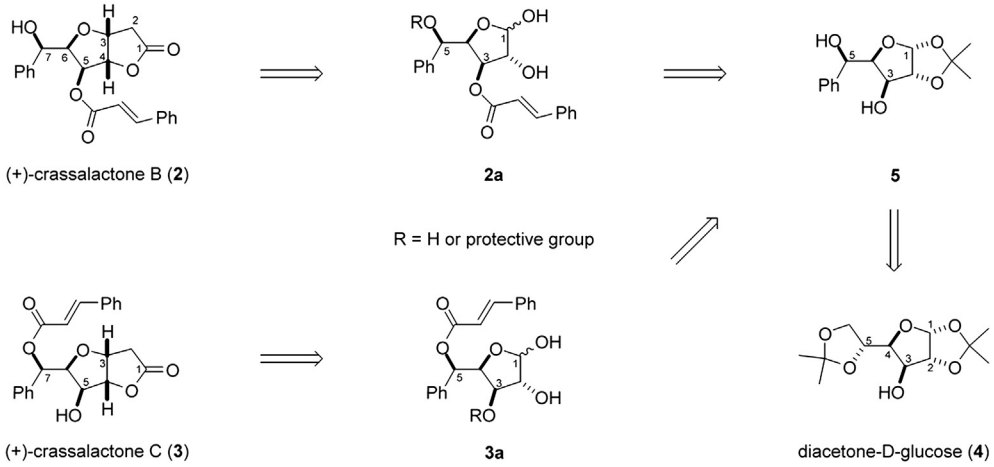
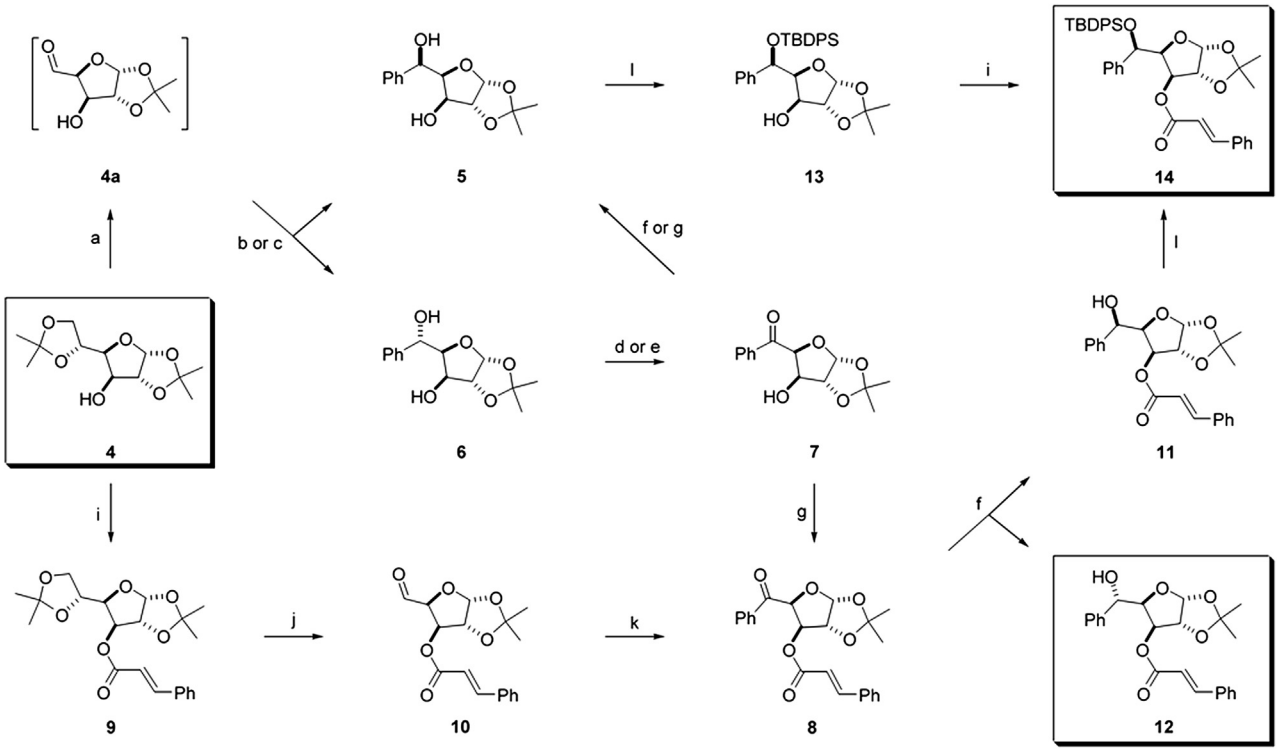
from a common intermediate. It was assumed that the required [3.3.0] bicyclic lactone core may be built up through the *cyclo*-condensation of Meldrum's acid with a protected sugar lactol derivative.[12–14] Accordingly, we designed the retrosynthetic concept shown in Scheme 1. For both natural products **2** and **3**, lactone ring-removal would give rise to the lactol derivatives **2a** and **3a**, respectively. These can be prepared by a number of selective chemical transformations of the benzylic alcohol **5**. Synthesis of divergent intermediate **5** is visualized from the commercially available diacetone-D-glucose (**4**) through the Grignard addition of PhMgBr to protected dialdose **4a** (Scheme 2).[12,15]

When diacetone-D-glucose (**4**) was treated with periodic acid in dry ethyl acetate, and the crude product **4a** was allowed to react with phenyl magnesium bromide in ether, the known[12,15] benzylic alcohols **5** and **6** were obtained (Scheme 2), with L-*ido*-derivative **6** as the major product (**6**/**5** ~3:1). Since only the minor diastereomer **5** has the correct configuration for a synthesis of both **2** and **3**, we were looking for a way to shift the stereomeric ratio towards **5**. Accordingly we have studied the influence of several solvents[16] and found that the best stereoselectivity in favour of D-*gluco*-isomer **5** (**5**/**6** ~3:1) was obtained when the reaction is performed in a mixture of anhydrous THF and toluene, in the presence of TMEDA



Scheme 1. Retrosynthetic analysis of (+)-crassalactones B and C.

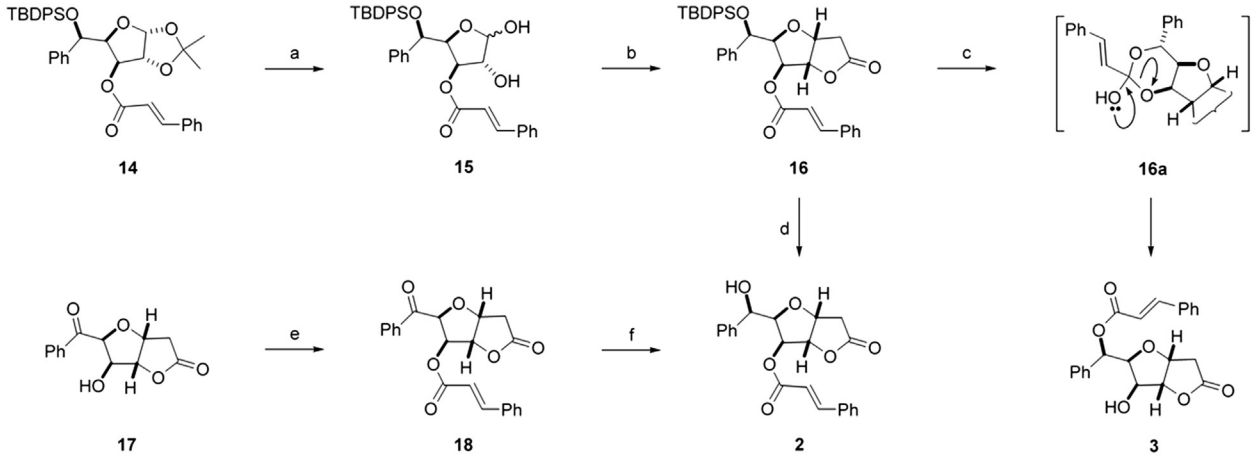


Scheme 2. Reagents and conditions: (a) H_5IO_6, EtOAc, rt, 24 h; (b) PhMgBr, Et_2O, 0 °C, 4 h, then rt 20 h, 60% of **6**, 19% of **5** (both from **4**); (c) PhMgBr, 1:1 THF/PhMe, TMEDA, 0 °C, 4 h, rt, 72 h, 49% of **5**, 15% of **6** (both from **4**); (d) PCC, H_5IO_6, EtOAc, 0 °C, 2 h, 57%; (e) DDQ, 10:1 CH_2Cl_2/H_2O, rt, 72 h, 79%; (f) $NaBH_4$, L-tartaric acid, THF, −7 °C→rt, 24 h for **7**, 78% of **5**, 7% of **6**, 4 h for **8**, 92% of **11**, 2% of **12**; (g) $NaBH_4$, silica gel, hexane, rt, 26 h, 86% of **5**, 9% of **6**; (h) cinnamoyl chloride, DMAP, CH_2Cl_2, 0 °C, 45 min, 75%; (i) cinnamic acid, DMAP, DCC, CH_2Cl_2, rt, 20 h for **4**, 98% of **9**, 50 h for **13**, 96% of **14**; (j) H_5IO_6, EtOAc, rt, 1.5 h, 93%; (k) (*i*) PhMgBr, THF, PhMe, 0 °C, 3 h; (*ii*) PCC, CH_2Cl_2, reflux, 4 h, 34% from 2 steps; (l) $BuPh_2SiCl$, imdazole, CH_2Cl_2, rt, 48 h, 97% from **5**, 90% from **11**.

as a *N*-donor ligand.[17] The combined yield of stereoisomers **5** and **6** was 64%. Somewhat lower selectivity (**5**/**6** ~2:1), but a higher combined yield of **5** and **6** (80%) was achieved when the Grignard reaction was performed in THF as a solvent. Additional efforts to improve the epimeric ratio in favour of **5** possessing the correct stereochemistry for **2** and **3** were unsuccessful. However, a more efficient access to **5** was possible by using an alternative two-step sequence that involved selective oxidation of the benzylic hydroxyl function in **6** followed by stereoselective reduction of the resulting prochiral ketone **7**. Thus, oxidation of **6** under the Hunsen conditions[18] gave the corresponding ketone **7** that was obtained in 57% yield. However, a DDQ-oxidation of **6** gave ketone **7** in 79% yield. Stereocontrolled reduction of the ketone functionality in **7** was achieved using the method of Yatagai and Ohnuki for prochiral ketones.[19] Thus, the reduction of **7** with $NaBH_4$ in the presence of L-tartaric acid gave a mixture of the required alcohol **5** and the alternative stereoisomer **6** in 78 and 7% yields, respectively. Slightly better yield of desired stereoisomer **5** is obtained if the reduction of ketone **7** was carried out with $NaBH_4$ in dry hexane in the presence of silica gel.[20] This procedure provided the requisite intermediate **5** in 89% yield (61% from **4**), accompanied with a minor amount of epimer **6** (9%). Apart from the better yield, the main advantage of this procedure in relation to the previous one is the usage of a simple experimental technique and inexpensive reagents. Treatment of ketone **7** with cinnamoyl chloride, gave the corresponding 3-*O*-cinnamoyl derivative **8** in 75% yield (36% from **4**). An alternative but slightly less efficient synthesis of **8** is accomplished through a three-step sequence that commenced with esterification of diacetonide **4** with *trans*-cinnamic acid under the Neises-Steglich conditions,[21] to provide an almost quantitative yield of the expected cinnamic ester **9**. The terminal isopropylidene function in **9** was directly converted into an aldehyde group, in a single step, by treatment with periodic acid in dry ethyl acetate,[22] whereupon the expected aldehyde **10** was obtained in 93% yield. Addition of phenylmagnesium bromide in THF, to a solution of **10** in toluene, led to a mixture of two epimeric alcohols. The mixture was not separated but was oxidised with PCC in dichloromethane to give the corresponding ketone **8** in 34% yield (31% from **4**). The final reduction of prochiral ketone **7** with $NaBH_4$/L-tartaric acid took place stereoselectively to afford **11** and **12** in 92 and 2% respective yields. The intermediate **11** was thus obtained in 28% overall yield with respect to the starting compound **4**. However, when this four-step sequence was carried out without purification of the intermediates **8** and **10** the desired product **11** was obtained in a slightly higher overall yield (34% from **4**). To prevent the benzylic hydroxyl group in **11** from taking part in ensuing reactions, it was blocked as a silyl ether by treatment of **11** with *tert*-butyldiphenylsilyl chloride to give the key intermediate **14** in 90% yield (31% overall yield from **4**). An alternative and more efficient route to the intermediate **14** started with selective silylation of the benzylic hydroxyl group in **5** to give the corresponding silyl ether **13** in 97% yield. Further esterification of **13** with cinnamic acid (DCC, DMAP) gave the key chiral intermediate **14** in 96% yield. Thus, the six-step sequence based on **13** as an intermediate represents a more convenient route to the key intermediate **14**, since it provided a considerably higher overall yield (64% from **4**) compared to the six-step sequence carried out via the cinnamoate **9** as an intermediate (31% from **4**).

The conversion of the intermediate **14** to (+)-crassalactones B and C is outlined in Scheme 3. Hydrolytic removal of the isopropylidene protecting group in **14** gave the expected lactol **15** (92%), which upon treatment with Meldrum's acid in the presence of triethylamine gave the protected furano-lactone **16** in 65% yield. We initially carried out the TBDPS cleavage in **16** using TBAF, which surprisingly gave (+)-crassalactone C (**3**) as the major product (43%), accompanied by a minor amount (14%) of (+)-crassalactone B (**2**). Both the ^1H and ^{13}C NMR data of compound **3** were consistent with naturally occurring (+)-crassalactone C and its physical properties were in good agreement with those reported previously.[16] Product **3** was presumably formed by a competitive intramolecular cinnamate migration process in **2**, via the cyclic orthoester intermediate **16a** (Scheme 3). A similar intramolecular *O*-acyl rearrangement during the silyl deprotection with TBAF has already been described in the literature.[23] Moreover, treatment of **16** with $SOCl_2$ in MeOH gave a 56% yield of (+)-crassalactone B (**2**). Our optical rotation value is greater than the reported value for isolated (+)-crassalactone B {lit.[6] $[\alpha]_D^{20}$ +8.0 (*c* 0.5, EtOH); this work: $[\alpha]_D^{20}$ +45.7 (*c* 0.5, EtOH)}, but was in reasonable agreement with the reported data of synthetic **2** {lit.[9] $[\alpha]_D^{20}$ +31.6 (*c* 1.0, $CHCl_3$); this work: $[\alpha]_D^{20}$ +35.5 (*c* 1.0, $CHCl_3$)}. The melting point and NMR data of synthesized sample **2** were in full agreement with those reported previously.[6,9,16]

This synthesis of natural product **2** was successfully accomplished in nine steps with an overall yield of 19% starting from commercially available diacetone-D-glucose (**4**). However, the natural product **2** was also prepared starting from the keto-lactone **17**. Compound **17** is readily available from 7-*epi*-(+)-goniofufurone through a regioselective oxidation of the benzylic hydroxyl group at the C-7 position.[24] Treatment of **17** with cinnamoyl chloride and DMAP in dichloromethane, gave the corresponding 5-*O*-cinnamoyl derivative **18** in a yield of 84%. Stereocontrolled reduction of the



Scheme 3. Reagents and conditions: (a) 90% aq TFA, CH_2Cl_2, 10 °C, 0.5 h, 92%; (b) Meldrum's acid, Et_3N, DMF, 46–48 °C, 64 h, 65%; (c) TBAF, AcOH, THF, rt, 144 h, 43% of **3**, 14% of **2**; (d) $SOCl_2$, MeOH, rt, 6 h, 56%; (e) cinnamoyl chloride, DMAP, MeCN, 0.5 h at 0 °C, then 1 h at rt, 84%; (f) $NaBH_4$, L-tartaric acid, THF, 2 h at −7 °C, then 1 h at 0 °C, 74%.

ketone functionality in **18** under the Yatagai-Ohnuki conditions gave the natural product **2** as the only stereoisomer in 74% yield.

The new synthesis of crassalactone C (**3**) proceeded in nine linear steps, with 15% overall yield from the same starting compound **4**. The preceding preparation of **3** was accomplished starting from D-xylose in 8% overall yield over ten linear steps.[7]

An alternative and more efficient synthesis of **3** is presented in Scheme 4. Treatment of **5** with cinnamoyl chloride in anhydrous 1,2-dichloroethane gave the corresponding 5-*O*-cinnamoyl derivative **19** (55%) along with a minor amount of 3,5-di-*O*-cinnamoyl derivative **20** (12%). Hydrolytic removal of the isopropylidene protecting group in **19** gave the known[7] lactol **19a** (85%), which upon treatment with Meldrum's acid in the presence of triethylamine gave the natural product **3** in 61% yield (20% overall yield form **4**). It appears that this seven-step sequence represents the most efficient synthesis of natural product **3** so far. An important feature of this new approach is that no protection is necessary for the synthesis of the (+)-crassalactone C except the diacetonide function that is already present in the commercially available starting material **4**.[25] Finally, a simple semi-synthesis of (+)-crassalactone C (**3**) was also accomplished by treatment of (+)-goniofufurone (**1**) with cinnamoyl chloride in boiling acetonitrile, whereby the target **3** was obtained in 79% yield.

2.2. In vitro antitumour activity

We have previously found that both natural products **2** and **3** exhibited a potent cytotoxic activity against several cancer cell lines.[11] Hence, we wanted to explore their cytotoxic effects against the following additional human tumour cell lines: prostate cancer cells (PC3), colon adenocarcinoma (HT29) and lung adenocarcinoma epithelial cell line (A549). Cell growth inhibition was evaluated by using the standard MTT colorimetric assay after exposure of cells to the test compounds for 72 h (+)-Goniofufurone (**1**) was used as the positive control in this assay. The results are shown in Table 1.

As shown in Table 1, crassalactones B (**2**) and C (**3**) exhibited a moderate antiproliferative activity toward PC3 cancer cells with respective IC_{50} values 25 and 31 μM. However, the control compound **1** was completely inactive against the same cell line. In contrast, (+)-goniofufurone (**1**) showed submicromolar activity against HT-29 cell line (IC_{50} 0.59 μM). (+)-Crassalactone C (**3**) demonstrated a potent cytotoxicity (IC_{50} 2.5 μM), while (+)-crassalactone B (**2**) was completely inactive against this cell line. A549 cancer cells were sensitive to all three tested compounds.

Table 1
Antiproliferative activities of natural products **1**, **2**, and **3**

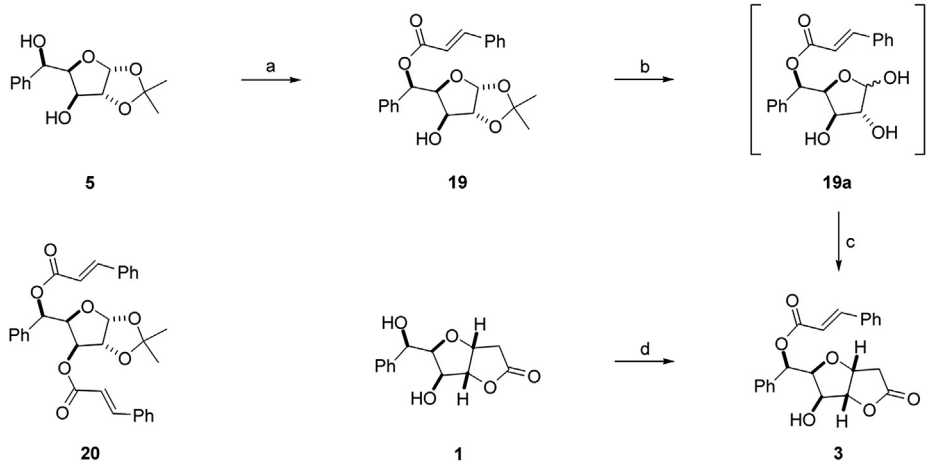
Compounds	IC_{50}, μM[a]		
	PC3	HT-29	A549
(+)-Goniofufurone (**1**)	>100	0.59	35
(+)-Crassalactone B (**2**)	25	>100	4.4
(+)-Crassalactone C (**3**)	31	2.5	2.6

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control. Values are means of three independent experiments done in quadruplicates. Coefficients of variation were <10%.

(+)-Goniofufurone (**1**) showed a moderate cytotoxicity against this cell line, while (+)-crassalactones B (**2**) and C (**3**) exhibited a potent activity with growth inhibitory concentrations in the range of low micromolar values (IC_{50} 4.4 and 2.6 μM, respectively). From Table 1 can be further seen that both natural products **2** and **3** demonstrated significantly higher potency with respect to control compound **1** (8- and 14-fold respectively).

3. Conclusion

In conclusion we have demonstrated an efficient four-step conversion of diacetone-D-glucose (**4**) into the benzylic alcohol **5**, the key divergent intermediate for a new total synthesis of (+)-crassalactones B (**2**) and C (**3**). Further, a series of selective chemical transformations were carried out to construct the advanced intermediate **14** containing the tetrahydrofuran core of **2**. Compound **14** was finally converted to the final precursor of (+)-crassalactones B and C (compound **16**), through a two-step sequence that involves hydrolytic removal of the isopropylidene protecting group, followed by a successive cyclocondensation of the resulting lactol **15** with Meldrum's acid. The intermediate **16** can be converted into (+)-crassalactone B (**2**) or C (**3**). Selective access to either **2** or **3** was accomplished by simply changing the conditions for TBDPS cleavage in the final intermediate **16**. The most efficient route to target **2** was achieved in 15% overall yield from **4**. This new synthesis of **2** requires the use of a single silyl ether protecting group throughout the synthesis. Finally, we have achieved a practical, step-economic, and protecting-group-free synthesis of (+)-crassalactone C (**3**) in 20% overall yield from diacetone-D-glucose (**4**). The synthesis of both **2** and **3** is highly efficient and competitive with previous reports in literature. In vitro antiproliferative activities of **2** and **3** against three human tumour cell lines were recorded and compared with those observed for



Scheme 4. Reagents and conditions: (a) cinnamoyl chloride, 1,2-dichloroethane, 70 °C, 20 h, 55% of **19**, 12% of **20**; (b) 50% aq TFA, rt, 18 h, 85%; (c) Meldrum's acid, Et_3N, DMF, 44–46 °C, 72 h, 61%; (d) cinnamoyl chloride, MeCN, reflux, 2 h, 79%.

(+)-goniofufurone (**1**). Against A549 cells natural products **2** and **3** showed 8- and 14 times greater potency compared to the parent compound **1**.

4. Experimental section

4.1. General

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on P 3002 (Krüss) and Autopol IV (Rudolph Research) polarimeters at 20 °C. ^{1}H (250 MHz) and ^{13}C (62.9 MHz) NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in parts per million downfield from TMS. IR spectra were recorded with an FTIR Nexus 670 spectrophotometer (Thermo-Nicolet). High resolution mass spectra (ESI) of synthesized compounds were acquired on a Agilent Technologies 1200 series instrument equipped with Zorbax Eclipse Plus C18 (100 mm×2.1 mm i.d. 1.8 μm) column and DAD detector (190–450 nm) in combination with a 6210 time-of-flight LC/MS instrument (ESI) in the positive ion mode. Flash column chromatography was performed using Kieselgel 60 (0.040–0.063, E. Merck). All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under reduced pressure at a bath temperature below 35 °C.

4.2. 1,2-*O*-Isopropylidene-5-*C*-phenyl-α-L-*ido*-pentofuranose (6)

To a solution of **4** (0.50 g, 1.92 mmol) in dry EtOAc (50 mL), was added H_5IO_6 (0.394 g, 1.73 mmol) and the mixture was stirred at room temperature for 20 h. The suspension was first filtered, and then concentrated to syrup. The residue was dried in vacuum (10 mm Hg) for 1 h. The remaining crude aldehyde **4a** (0.42 g) was dissolved in dry Et_2O (50 mL) and the solution cooled to 0 °C under atmosphere of nitrogen. To the mixture was added a commercial 3 M solution of PhMgBr in Et_2O (6.50 mL, 19.5 mmol) previously cooled to 0 °C. The reaction was stirred at 0 °C for 4 h, then at room temperature for 20 h. The mixture was poured into cold (+5 °C) saturated aqueous NH_4Cl (300 mL) and extracted first with Et_2O (2×50 mL) and then with CH_2Cl_2 (2×50 mL). The combined extracts were dried and evaporated, and the residue purified by flash column chromatography (2:1 Et_2O/light petroleum→Et_2O). The minor stereoisomer **5** (0.097 g, 19%) was first isolated as a colourless solid. Eluted second was pure **6** (0.307 g, 60%), isolated as a colourless solid, R_f=0.25 (1:1 toluene/EtOAc), mp 166–168 °C (EtOH), $[\alpha]_D^{25}$ +22.9 (*c* 1, MeOH), $[\alpha]_D^{25}$ +7.3 (*c* 1, $CHCl_3$); lit.[12] mp 162–165 °C (EtOH), $[\alpha]_D^{25}$ +32.1 (*c* 1.34, MeOH), lit.[15] mp 159–160 °C (EtOH), $[\alpha]_D^{25}$ +25.0 (*c* 1.1, $CHCl_3$). NMR data for **5** and **6** matched those previously reported.[16]

4.3. 1,2-*O*-Isopropylidene-5-*C*-phenyl-α-D-*xylo*-pentofurano-5-ulose (7)

4.3.1. Procedure A. To a cooled (0 °C) and stirred solution of **6** (0.210 g, 0.79 mmol) in dry CH_3CN (27 mL) was added H_5IO_6 (0.234 g, 1.02 mmol). A solution of PCC (0.010 g; 0.056 mmol) in dry CH_3CN (5 mL) was added during 1 min and the resulting reaction mixture was stirred vigorously at 0 °C for 2 h. The mixture was poured to 10% aq NaCl (150 mL) extracted with EtOAc (3×50 mL). The combined organic solutions were dried and evaporated. Flash column chromatography (1:1 Et_2O/PE). of the residue gave pure **7** (0.119 g; 57%) as a colourless syrup Crystallization from $CHCl_3$ gave colourless needles, mp 97 °C, $[\alpha]_D^{25}$ −61.8 (*c* 0.5, $CHCl_3$), R_f=0.27 (3:2 toluene/Et_2O). IR ($CHCl_3$): 3444 (br), 1695, 1597 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.35 and 1.57 (2×s, 3H each, C**Me**$_2$), 3.23 (br s, 1H, OH), 4.60 (d, 1H, $J_{3,4}$=3.6 Hz, H-2), 4.63 (d, 1H, $J_{3,4}$=2.6 Hz, H-3), 5.40 (d, 1H, $J_{3,4}$=2.6 Hz, H-4), 6.09 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 7.50–8.11 (m, 5H, Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.1 and 26.9 (C**Me**$_2$), 76.5 (C-3), 82.1 (C-2), 84.4 (C-4), 105.2 (C-1), 112.1 (**C**Me$_2$), 128.7, 129.0, 134.1, 135.2 (Ph), 196.4 (C-7). HRMS (ESI−) calcd for $C_{14}H_{15}O_5$ 263.0925, found 263.0923 (M−H). Eluted second was unreacted starting material **6** (0.020 g, 9%).

4.3.2. Procedure B. To a solution of **6** (0.08 g, 0.30 mmol) in a 10:1 mixture CH_2Cl_2/H_2O (5 mL), was added DDQ (0.136 g, 0.60 mmol) and the mixture was stirred at room temperature for 72 h. The mixture was poured in 1% aqueous $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3×20 mL) and then with EtOAc (20 mL). The combined extracts were dried and evaporated, and the residue purified by flash column chromatography (2:1 Et_2O/light petroleum→Et_2O). Pure ketone **7** (0.063 g, 79%) was first isolated as a colourless syrup. Eluted second was unreacted starting material **6** (0.014 g, 18%). R_f=0.27 (3:2 toluene/Et_2O).

4.4. 1,2-*O*-Isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (5)

4.4.1. Procedure A. To a solution of **4** (1.00 g, 3.84 mmol) in dry EtOAc (100 mL), was added H_5IO_6 (0.788 g, 3.46 mmol) and the mixture was stirred at room temperature for 20 h. The suspension was filtered, then concentrated to syrup (crude **4a**) and dried in vacuum (10 mm Hg) for 1 h. To the stirred 2 M solution of PhMgBr in THF (14.4 mL, 28.80 mmol) was added TMEDA (5.18 mL, 34.56 mmol). After being stirred at room temperature for 1 h the solution was cooled to 0 °C under atmosphere of nitrogen. To the mixture was added a solution of crude **4a** in dry toluene (14 mL) during 10 min and the resulting solution was stirred at 0 °C for 4 h, then at room temperature for 72 h. The mixture was poured into cold (+4 °C) 10% aqueous HCl (150 mL) and extracted first with CH_2Cl_2 (3×35 mL) and then with EtOAc (1×30 mL). The combined extracts were washed with 2% aqueous NaOH (100 mL), dried and evaporated, and the residue purified by flash column chromatography (2:1→4:1 Et_2O/light petroleum). The major stereoisomer **5** (0.497 g, 49%) was first isolated as a colourless solid, R_f=0.32 (1:1 toluene/EtOAc), mp 106–107 °C (Me_2CO/light petroleum), $[\alpha]_D^{25}$ −12.2 (*c* 1, MeOH), $[\alpha]_D^{25}$ −22.9 (*c* 0.5, $CHCl_3$), lit.[12] mp 104–106 °C (EtOH), $[\alpha]_D^{25}$ −43.4 (*c* 1.3, MeOH); lit.[15] mp 102–103 °C (^{i}Pr$_2$O/light petroleum), $[\alpha]_D^{25}$ −26.0 (*c* 0.8, $CHCl_3$). Eluted second was pure **6** (0.155 g, 15%), isolated as a colourless solid. NMR data matched what was previously reported for both **5** and **6**.[16]

4.4.2. Procedure B. To a solution of L-tartaric acid (0.199 g, 1.32 mmol) in dry THF (3 mL) was added $NaBH_4$ (0.032 g, 1.32 mmol) portionwise and the suspension was heated at reflux for 2 h before being cooled to −7 °C. A solution of **7** (0.07 g, 0.26 mmol) in dry THF (2 mL) was added dropwise and the mixture was stirred at −7 °C for 0.5 h, then at 0 °C for 1 h, and finally at room temperature for 24 h. The mixture was evaporated with silica gel (1 g) and the residue purified by flash column chromatography (7:3 light petroleum/Et_2O). The major stereoisomer **5** (0.055 g, 78%) was first eluted, followed by a minor amount of **6** (0.005 g, 7%). Compound **5**: R_f=0.32 (1:1 toluene/EtOAc).

4.4.3. Procedure C. To a suspension of ketone **7** (0.174 g, 0.66 mmol) in dry hexane (15 mL) was added $NaBH_4$ (0.013 g, 0.34 mmol) and silica gel (0.75 g). After the reaction was stirred at 22° C for 20 h, after the next two hours an additional amount of $NaBH_4$ (0.013 g, 0.34 mmol) was added in two equal portions and the stirring at room temperature was continued for the next 6 h. The mixture was evaporated and the solid residue was purified by flash column chromatography (2:1 Et_2O/light petroleum→Et_2O). The major

stereoisomer **5** (0.155 g, 89%) was first eluted, followed by a minor amount of **6** (0.015 g, 9%). Compound **5**: R_f=0.32 (1:1 toluene/EtOAc).

4.5. 3-*O*-Cinnamoyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (9)

A solution of **4** (1.0 g, 3.84 mmol), cinnamic acid (1.253 g, 8.46 mmol), DCC (1.903 g, 9.22 mmol) and DMAP (1.878 g, 15.37 mmol) in dry CH_2Cl_2 (100 mL) was stirred at room temperature for 20 h. The mixture was poured in water (300 mL) and extracted with CH_2Cl_2 (2×50 mL), the combined organic solutions were washed with 10% aqueous NaCl (250 mL), dried and evaporated. Flash column chromatography of the residue (3:1 light petroleum/Et_2O) gave pure **9** (1.476 g, 98%) as a colourless syrup, $[\alpha]_D^{25}$ −48.7 (*c* 1, $CHCl_3$), R_f=0.32 (3:1 light petroleum/Et_2O). IR (neat): 1720, 1637, 1578 1162 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.31, 1.32, 1.42, 1.54 (4×s, 3H each, 2×C**Me**$_2$), 4.03–4.05 (m, 2H, 2×H-6), 4.26–4.37 (m, 2H, H-4 and H-5), 4.58 (d, 1H, $J_{1,2}$=3.7 Hz, H-2), 5.39 (d, 1H, $J_{3,4}$=2.2 Hz, H-3), 5.93 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 6.44 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.35–7.59 (m, 5H, Ph), 7.72 (d, 1H, $J_{2',3'}$=16.0 Hz, H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 25.2, 26.1, 26.6, 26.8 (2×C**Me**$_2$), 67.0 (C-6), 72.4 (C-5), 76.1 (C-3), 79.7 (C-4), 83.3 (C-2), 105.0 (C-1), 109.2 and 112.2 (2×**C**Me$_2$), 117.0 (C-2′), 128.1, 128.9, 130.6, 133.9 (Ph), 146.0 (C-3′), 165.4 (C-1′). HRMS (ESI) calcd for $C_{21}H_{26}NaO_7$ 413.1571, found 413.1577 (M^++Na).

4.6. 3-*O*-Cinnamoyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (10)

To a solution of **9** (0.446 g, 1.14 mmol) in dry EtOAc (45 mL) was added H_5IO_6 (0.338 g, 1.48 mmol). The mixture was stirred vigorously at room temperature for 1.5 h. The suspension was filtered, then concentrated under vacuum. The residue was purified by flash column chromatography (3:2 light petroleum/Et_2O) to give pure **10** (0.337 g, 93%) as a colourless syrup, $[\alpha]_D^{25}$ −11.2 (*c* 0.5, $CHCl_3$), R_f=0.26 (3:2 light petroleum/Et_2O). IR (neat): 1720 (br s), 1636, 1578, 1161 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.34 and 1.53 (2×s, 3H each, C**Me**$_2$), 4.67 (d, 1H, $J_{1,2}$=3.6 Hz, H-2), 4.81 (d, 1H, $J_{3,4}$=3.4 Hz, H-3), 5.64 (d, 1H, $J_{3,4}$=3.4 Hz, H-4), 6.13 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 6.34 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.33–7.56 (m, 5H, Ph), 7.67 (d, 1H, $J_{2',3'}$=16.0 Hz, H-3′), 9.70 (s, 1H, H-5). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.2 and 26.8 (C**Me**$_2$), 77.1 (C-4), 83.0 (C-2), 83.4 (C-3), 105.6 (C-1), 112.9 (**C**Me$_2$), 116.0 (C-2′), 128.2, 128.9, 130.8, 133.7 (Ph), 146.8 (C-3′), 165.3 (C-1′), 196.8 (C-5). HRMS (ESI) calcd for $C_{17}H_{19}O_6$ 319.1176, found 319.1161 (M^++H).

4.7. 3-*O*-Cinnamoyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*xylo*-pentofuran-5-ulose (8)

4.7.1. Procedure A. To a cooled (0 °C) and stirred solution of **10** (0.333 g, 1.05 mmol) in dry toluene (25 mL) was added 1 M solution of PhMgBr in THF (2.1 mL, 2.1 mmol). The mixture was stirred at 0 °C under atmosphere of nitrogen for 3 h, then neutralized with glacial AcOH (1 mL) and poured in 15% aqueous NH_4Cl (200 mL). The mixture was extracted with CH_2Cl_2 (3×60 mL), the organic solutions were combined, dried and evaporated. The residue was taken up in dry CH_2Cl_2 (30 mL) and to the solution was added PCC (0.76 g, 3.68 mmol). After the mixture heated to reflux for 4 h, it was concentrated with silica gel (1 g) and the residue purified by flash chromatography (CH_2Cl_2). Pure **8** (0.14 g, 34%) was obtained as a colourless syrup, $[\alpha]_D^{25}$ −108.1 (*c* 1, $CHCl_3$), R_f=0.25 (CH_2Cl_2). IR (neat): 1718, 1635, 1597, 1156 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.39 and 1.63 (2×s, 3H each, C**Me**$_2$), 4.70 (d, 1H, $J_{1,2}$=3.6 Hz, H-2), 5.71–5.80 (m, 2H, H-3 and H-4), 6.22 (d, 1H $J_{1,2}$=3.6 Hz, H-1), 6.23 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.33–8.01 (m, 11H, 2×Ph and H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.2 and 26.9 (C**Me**$_2$), 77.6 (C-3), 81.7 (C-4), 83.2 (C-2), 105.1 (C-1), 112.7 (**C**Me$_2$), 116.2 (C-2′), 128.1, 128.2, 128.7, 128.9, 130.7, 133.7, 135.3 (Ph), 146.4 (C-3′), 165.1 (C-1′), 192.8 (C-5). HRMS (ESI) calcd for $C_{23}H_{22}NaO_6$ 417.1309, found 417.1310 (M^++Na).

4.7.2. Procedure B. A solution of **7** (0.225 g, 0.85 mmol), cinnamoyl chloride (0.181 g, 1.09 mmol) and DMAP (0.160 g, 1.31 mmol) in dry CH_2Cl_2 (20 mL) was stirred vigorously at 0 °C for 45 min. The mixture was partitioned between H_2O (150 mL) and CH_2Cl_2 (60 mL), the organic layer was separated and the aqueous phase extracted with EtOAc (40 mL). The combined organic solutions were washed with 10% aq NaCl (100 mL), dried and evaporated. Flash column chromatography (CH_2Cl_2) of the residue gave pure **8** (0.253 g, 75%) as a colourless syrup.

4.8. 3-*O*-Cinnamoyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (11)

4.8.1. Procedure A. To a solution of L-tartaric acid (0.19 g, 1.27 mmol) in dry THF (3 mL) was added $NaBH_4$ (0.048 g, 1.27 mmol) portionwise and the suspension was heated at reflux for 2 h before being cooled to −7 °C. A solution of **8** (0.12 g, 0.30 mmol) in dry THF (3 mL) was added dropwise and the temperature maintained at −7 °C for 4 h. The solution was allowed to warm to room temperature and evaporated with silica gel (1 g). The residue was purified by flash chromatography (2:1→1:2 light petroleum/Et_2O) to give pure **11** (0.111 g, 92%), as a colourless syrup, $[\alpha]_D^{25}$ +18.0 (*c* 0.5, $CHCl_3$), R_f=0.6 (1:1 light petroleum/Et_2O). IR (neat): 3483 (br), 1716, 1636, 1579, 1162 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.32 and 1.52 (2×s, 3H each, C**Me**$_2$), 4.46 (dd, 1H, $J_{3,4}$=2.5, $J_{4,5}$=8.6 Hz, H-4), 4.67 (d, 1H, $J_{1,2}$=3.7 Hz, H-2), 4.74 (d, 1H, $J_{4,5}$=8.7 Hz, H-5), 5.51 (d, 1H, $J_{3,4}$=2.5 Hz, H-3), 5.97 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 6.53 (d, 1H, $J_{2',3'}$=15.9 Hz, H-2′), 7.29–7.64 (m, 10H, 2×Ph), 7.82 (d, 1H, $J_{2',3'}$=16.0 Hz, H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.2 and 26.6 (C**Me**$_2$), 70.6 (C-5), 76.7 (C-3), 82.3 (C-4), 83.2 (C-2), 104.9 (C-1), 112.2 (**C**Me$_2$), 116.4 (C-2′), 126.8, 128.1, 128.3, 128.5, 129.0, 130.9, 133.8, 140.3 (Ph), 147.1 (C-3′), 166.8 (C-1′). HRMS (ESI) calcd for $C_{23}H_{24}NaO_6$ 419.1465, found 419.1460 (M^++Na). Eluted second was pure **12** (0.002 g, 2%), isolated as a colourless oil, $[\alpha]_D^{20}$ −87.4 (*c* 0.5, $CHCl_3$); R_f=0.25 (1:1 light petroleum/Et_2O). IR (neat): ν_{max} 3474, 1716, 1636, 1578, 1496, 1161. ^1H NMR ($CDCl_3$): δ 1.32 and 1.56 (2×s, H each, C**Me**$_2$), 4.48 (dd, 1H, $J_{3,4}$=2.9, $J_{4,5}$=8.5 Hz, H-4), 4.56 (d, 1H, $J_{1,2}$=3.6 Hz, H-2), 4.87 (d, 1H, $J_{3,4}$=2.9 Hz, H-3), 5.07 (d, 1H, $J_{4,5}$=8.5 Hz, H-5), 6.04 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 6.51 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.29–7.65 (m, 10H, Ph), 7.76 (d, 1H, $J_{2',3'}$=16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 26.2 and 26.7 (C**Me**$_2$), 72.6 (C-5), 75.9 (C-3), 83.6 (C-4), 83.7 (C-2), 104.9 (C-1), 112.4 (**C**Me$_2$), 116.6 (C-2′), 126.7, 128.3, 128.7, 128.8, 129.0, 130.9, 133.9, 138.7 (Ph), 146.6 (C-3′), 165.3 (C-1′). HRMS (ESI) calcd for $C_{23}H_{24}NaO_6$: 419.1465, found: 419.1444 (M^++Na).

4.8.2. Procedure B. To a solution of **9** (0.72 g, 1.84 mmol) in dry EtOAc (70 mL), $NaIO_4$ (0.395 g, 1.84 mmol) and H_5IO_6 (0.378 g, 1.66 mmol) were consecutively added and the mixture was stirred at room temperature for 3 h. The suspension was filtered, then concentrated under vacuum. The remaining crude aldehyde **10** (0.63 g) was taken up in dry toluene (60 mL) and the solution cooled to 0 °C under atmosphere of nitrogen. To the solution was added 1 M solution of PhMgBr in THF (5 mL, 5 mmol) and the mixture was stirred at 0 °C for 3 h, then neutralized with glacial AcOH and poured in 15% aqueous NH_4Cl (250 mL). The mixture was extracted with CH_2Cl_2 (3×80 mL), the extracts were combined, dried and evaporated. The residue (0.85 g) was taken up in dry DMSO (10 mL) and treated with Ac_2O (3.5 mL, 37.03 mmol) while stirring at room temperature for 20 h. The mixture was poured in

10% aqueous NaCl (200 mL) and extracted with CH_2Cl_2 (3×70 mL). The combined organic solutions were washed with water (200 mL), dried and evaporated. The remaining crude ketone **8** (0.95 g) was dissolved in dry THF (6 mL) and treated with $NaBH_4$/L-tartaric acid reagent system as described above (Procedure A). The reduction was carried out at −7 °C for 1 h, then at 0 °C for 1 h and finally at room temperature for 18 h. The mixture was evaporated with silica gel (1 g) and the residue was purified by flash chromatography (19:1 toluene/Et_2O) to give slightly contaminated product **11** (0.40 g). Repeated chromatographic purification (7:3 light petroleum/Et_2O) gave pure **11** (0.255 g, 35% from **9**). R_f=0.6 (1:1 light petroleum/Et_2O).

4.9. 5-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (13)

To a solution of **5** (0.5 g, 1.88 mmol) and imidazole (0.32 g, 4.69 mmol) in dry CH_2Cl_2 (30 mL) was slowly added TBDPSCl (0.98 mL, 3.76 mmol). After being stirred at room temperature for 48 h, the mixture was poured in water (300 mL) and extracted with CH_2Cl_2 (3×50 mL). The organic layers were combined, dried and evaporated, and the residue purified by flash column chromatography (5:1 light petroleum/Et_2O). Pure **13** (0.92 g, 97%) was obtained as a colourless oil, $[\alpha]_D^{25}$ −43 (*c* 2, $CHCl_3$), R_f=0.38 (CH_2Cl_2). IR (neat): 3448 (br), 1590 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.09 (s, 9H, C**Me**$_3$), 1.31 and 1.41 (2×s, 3H each, C**Me**$_2$), 4.00 (dd, 1H, $J_{3,4}$=1.8, $J_{4,5}$=2.7 Hz, H-4), 4.29 (br d, 1H, $J_{3,4}$=1.8 Hz, H-3), 4.57 (d, 1H, $J_{1,2}$=3.6 Hz, H-2), 5.26 (d, 1H, $J_{4,5}$=2.7 Hz, H-5), 5.36 (br s, 1H, O**H**), 6.07 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 7.15–8.75 (m, 15H, 3×Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 19.2 (**C**Me$_3$), 26.0 and 26.6 (C**Me**$_2$), 26.8 (C**Me**$_3$), 75.2 (C-3), 77.2 (C-5), 81.7 (C-4), 85.1 (C-2), 104.7 (C-1), 111.3 (**C**Me$_2$), 126.8, 127.3, 127.5, 127.9, 128.3, 129.7, 130.0, 131.8, 132.0, 135.9, 136.2, 139.4 (Ph). HRMS (ESI) calcd for $C_{30}H_{36}NaO_5Si$ 527.2224, found 527.2222 (M^++Na).

4.10. 5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-cinnamoyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (14)

4.10.1. Procedure A. To a solution of **11** (0.25 g, 0.63 mmol) and imidazole (0.06 g, 0.82 mmol) in dry CH_2Cl_2 (10 mL) was added TBDPSCl (0.17 mL, 0.63 mmol) After being stirred for 20 h at room temperature an additional amount of imidazole (0.116 g, 1.7 mmol) and TBDPSCl (0.17 mL, 0.63 mmol) was added and the mixture was stirred at room temperature for 20 h. After 40 h, a new portion of imidazole (0.044 g, 0.63 mmol) and TBDPSCl (0.085 mL, 0.32 mmol) was added and the stirring was continued at for the next 7 h. The mixture was poured in water (250 mL), extracted with CH_2Cl_2 (2×40 mL) and EtOAc (40 mL) successively, and dried. After evaporation of the solvent, the crude product was subjected to flash column chromatography (9:1→1:1 light petroleum/Et_2O) to give pure **14** (0.36 g, 90%) as a colourless oil, $[\alpha]_D^{25}$ +18.6 (*c* 2, $CHCl_3$), R_f=0.23 (4:1 light petroleum/Et_2O). IR (neat): 1718, 1638, 1160 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 0.93 (s, 9H, C**Me**$_3$), 1.30 and 1.58 (2×s, 3H each, C**Me**$_2$), 4.57 (d, 1H, $J_{1,2}$=3.7 Hz, H-2), 4.70 (dd, 1H, $J_{3,4}$=2.6, $J_{4,5}$=8.6 Hz, H-4), 4.95 (d, 1H, $J_{4,5}$=8.6 Hz, H-5), 5.47 (d, 1H, $J_{3,4}$=2.6 Hz, H-3), 5.77 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 6.15 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.08–8.65 (m, 21H, 4×Ph and H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 19.2 (**C**Me$_3$), 26.2 and 26.7 (C**Me**$_2$), 26.74 (C**Me**$_3$), 72.6 (C-5), 76.4 (C-3), 82.1 and 82.9 (C-2 and C-4), 104.4 (C-1), 112.0 (**C**Me$_2$), 117.3 (C-2′), 127.0, 127.8, 128.0, 128.1, 128.13, 128.9, 129.2, 129.4, 130.5, 132.9, 133.1, 134.0, 135.8, 136.0, 140.6 (Ph), 145.2 (C-3′), 165.7 (C-1′). HRMS (ESI) calcd for $C_{39}H_{42}NaO_6Si$ 657.2643, found 657.2651 (M^++Na).

4.10.2. Procedure B. A solution of **13** (0.28 g, 0.55 mmol), cinnamic acid (0.18 g, 1.22 mmol), DCC (0.275 g, 1.33 mmol) and DMAP (0.27 g, 2.22 mmol) in dry CH_2Cl_2 (30 mL) was stirred at room temperature for 50 h. The mixture was poured in water (150 mL) and extracted with CH_2Cl_2 (3×30 mL). The combined organic solutions were washed with 10% aqueous NaCl (100 mL), dried and evaporated. Flash column chromatography of the residue (5:1 light petroleum/Et_2O) gave pure **14** (0.337 g, 96%) as a colourless oil. R_f=0.23 (4:1 light petroleum/Et_2O).

4.11. 5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-cinnamoyl-5-*C*-phenyl-D-*gluco*-pentofuranose (15)

To a cooled (0 °C) and stirred solution **14** (0.53 g, 0.84 mmol) in CH_2Cl_2 (0.5 mL) was added a cooled (10 °C) solution of 90% aqueous TFA (14 mL). After stirring for 0.5 h, the mixture was concentrated by co-distillation with toluene and the residue purified by flash chromatography (49:1 CH_2Cl_2/MeOH). Pure **15** (0.459 g, 92%) was obtained as a colourless solid. The product **15** was isolated as a 5:1 mixture of α- and β-anomers. Data for mixture of isomers: mp 58–60 °C, R_f=0.28 (1:1 light petroleum/Et_2O), $[\alpha]_D^{25}$ +57.7 (*c* 2, H_2O). IR (neat): 3438, 1789, 1713, 1638, 1169 cm^{-1}. HRMS (ESI) calcd for $C_{36}H_{38}NaO_6Si$ 617.2330, found 617.2332 (M^++Na). Data for the major α-anomer: ^1H NMR (250 MHz, $CDCl_3$): δ 0.95 (s, 9H, C**Me**$_3$), 3.89 (br s, 2H, 2×O**H**), 4.03 (dd, 1H, $J_{1,2}$=4.2, $J_{2,3}$=1.6 Hz, H-2), 4.77 (dd, 1H $J_{3,4}$=3.4, $J_{4,5}$=8.1 Hz, H-4), 4.91 (d, 1H, $J_{4,5}$=8.2 Hz, H-5), 5.28 (d, 1H, $J_{1,2}$=4.4 Hz, H-1), 5.37 (dd, 1H, $J_{2,3}$=1.5, $J_{3,4}$=3.2 Hz, H-3), 6.15 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.12–7.70 (m, 21H, Ph and H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 19.2 (**C**Me$_3$), 26.7 (C**Me**$_3$), 73.0 (C-5), 75.0 (C-2), 79.0 (C-3), 80.9 (C-4), 95.9 (C-1), 117.1 (C-2′), 127.1, 128.0, 128.1, 128.2, 128.9, 129.3, 129.4, 129.6, 130.6, 132.8, 133.3, 134.0, 135.7, 140.8 (Ph), 145.5 (C-3′), 166.5 (C-1′). Data for the minor β-anomer: ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 103.0 (C-1).

4.12. 3,6-Anhydro-7-*O*-(*tert*-Butyldiphenylsilyl)-5-*O*-cinnamoyl-2-deoxy-7-*C*-phenyl-D-*glycero*-D-*ido*-heptono-1,4-lactone (16)

To a solution of **15** (0.375 g, 0.63 mmol) in dry DMF (8 mL), was added Meldrum's acid (0.091 g, 0.63 mmol) and dry Et_3N (0.1 mL, 0.72 mmol). The mixture was stirred for 64 h at 46–48 °C and then evaporated. The residue was purified by flash chromatography (1:1 light petroleum/Et_2O) to afford pure **16** (0.254 g, 65%) as a colourless oil, $[\alpha]_D^{25}$ +41 (*c* 1, $CHCl_3$), R_f=0.27 (1:1 light petroleum/Et_2O). IR (neat): 1780, 1753, 1637, 1153 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 0.91 (s, 9H, C**Me**$_3$), 2.52 (br d, 1H, $J_{2a,2b}$=18.9 Hz, H-2a), 2.65 (dd, 1H, $J_{2a,2b}$=18.9, $J_{2b,3}$=6.1 Hz, H-2b), 4.51 (dd, 1H, $J_{5,6}$=2.6, $J_{6,7}$=8.6 Hz, H-6), 4.80 (br t, 1H, $J_{3,4}$=$J_{2b,3}$=4.6 Hz, H-3), 4.89 (d, 1H, $J_{6,7}$=8.6 Hz, H-7), 4.97 (d, 1H, $J_{3,4}$=4.6 Hz, H-4), 5.66 (d, 1H, $J_{5,6}$=2.6 Hz, H-5), 6.13 (d, 1H, $J_{2',3'}$=16.1 Hz, H-2′), 7.08–8.62 (m, 21H, Ph and H-3′). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 19.2 (**C**Me$_3$), 26.8 (C**Me**$_3$), 35.7 (C-2), 72.6 (C-7), 75.2 (C-5), 76.5 (C-3), 82.7 (C-6), 84.9 (C-4), 116.8 (C-2′), 127.1, 127.8, 128.2, 128.23, 128.3, 129.0, 129.4, 129.5, 130.8, 132.8, 133.1, 134.0, 135.8, 136.0, 140.6 (Ph), 145.8 (C-3′), 165.4 (C-1′), 175.1 (C-1). HRMS (ESI) calcd for $C_{38}H_{38}NaO_6Si$ 641.2330, found 641.2338 (M^++Na).

4.13. 3,6-Anhydro-5-*O*-cinnamoyl-2-deoxy-7-*C*-phenyl-L-*ido*-hept-7-ulosono-1,4-lactone (18)

A solution of **17** (0.040 g, 0.16 mmol), *trans*-cinnamoyl chloride (0.035 g, 0.21 mmol) and DMAP (0.030 g, 0.24 mmol) in dry MeCN (10 mL) was stirred at 0 °C for 0.5 h and then at room temperature for 1 h. The mixture was poured to water (150 mL) and extracted with EtOAc (3×30 mL). The combined organic phases were washed with 10% aq NaCl (100 mL), dried and evaporated, and the residue purified by flash column chromatography (2:1 Et_2O/light petroleum), to give pure **18** (0.051 g, 84%) as a solid. Recrystallization

from Et_2O gave colourless needles, mp 108 °C, $[\alpha]_D^{20}$ −92.2 (*c* 0.5, $CHCl_3$); R_f=0.30 (2:1 Et_2O/light petroleum). IR ($CHCl_3$): ν_{max} 1791 (C=O, lactone), 1716 (C=O, ester), 1635 (C=C, cinnamate), 1497 (Ph) and 1147 (OC=O, ester). ^{1}H NMR ($CDCl_3$): 2.83 (d, 1H, $J_{2a,2b}$=18.9 Hz, H-2a), 2.92 (dd, 1H, $J_{2a,2b}$=18.6, $J_{2b,3}$=2.1 Hz, H-2b), 5.04 (d, 1H, $J_{5,6}$=4.0 Hz, H-4), 5.19–5.31 (m, 1H, H-3), 5.78 (d, 1H, $J_{5,6}$=4.9 Hz, H-6), 6.04 (d, 1H, $J_{5,6}$=4.9 Hz, H-5), 6.13 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.32–7.95 (m, 6H, Ph and H-3′). ^{13}C NMR ($CDCl_3$): 35.9 (C-2), 76.5 (C-5), 78.3 (C-3), 81.8 (C-6), 85.7 (C-4), 115.5 (C-2′), 127.9, 128.2, 128.8, 130.8, 133.6, 133.9, 135.0 (2×Ph), 146.8 (C-3′), 164.6 (C-1′), 174.2 (C-1), 193.4 (C-7). HRMS (ESI): *m*/*z* 379.1193 (M^++H), calcd for $C_{22}H_{19}O_6$: 379.1176.

4.14. (+)-Crassalactone B (2)

4.14.1. Procedure A. To a solution of **16** (0.09 g, 0.14 mmol) in dry MeOH (5 mL) was added $SOCl_2$ (0.1 mL, 1.38 mmol). After being stirred at room temperature for 6 h, the mixture was poured in water (50 mL), then neutralized with 10% aqueous $NaHCO_3$ (2.5 mL), and extracted with CH_2Cl_2 (3×30 mL). The organic extracts were combined, dried and evaporated, and the residue purified by flash column chromatography (97:3 CH_2Cl_2/Me_2CO) to afford slightly impure (+)-crassalactone B (**2**). Repeated chromatography using the same solvent system gave pure **2** (0.031 g, 56%) as a colourless solid. Recrystallization from Et_2O gave colourless needles of pure **2**, mp 173–174 °C, $[\alpha]_D^{20}$ +35.5 (*c* 1.0, $CHCl_3$), lit.[9] 168–171 °C (EtOH), $[\alpha]_D^{20}$ +31.6 (*c* 1.0, $CHCl_3$), $[\alpha]_D^{20}$ +45.7 (*c* 0.5, EtOH), lit.[6] 171–173 °C, $[\alpha]_D^{20}$ +8.0 (*c* 0.5, EtOH); R_f=0.38 (97:3 CH_2Cl_2/Me_2CO). IR (KBr): 3444 (br), 1789, 1717, 1636 cm^{-1}. ^{1}H and ^{13}C NMR spectral data for **2** were in full agreement with those reported previously.[16] HRMS (ESI): *m*/*z* 403.1143 (M^++Na), calcd for $C_{22}H_{20}NaO_6$: 403.1152.

4.14.2. Procedure B. To a stirred suspension of L-tartaric acid (0.065 g, 0.43 mmol) in dry THF (2.5 mL), was added $NaBH_4$ (0.017 g, 0.45 mmol) in two equal portions and the suspension was heated at reflux for 2 h before being cooled to −7 °C. A solution of **18** (0.035 g, 0.09 mmol) in dry THF (1.5 mL) was added dropwise and the temperature maintained at −7 °C for 2 h and then at 0 °C for the next 1 h. The mixture was evaporated with silica gel (1 g) and the residue was purified on a column of flash silica (49:1 →97:3 CH_2Cl_2/Me_2CO). Eluted first was unreacted starting compound **18** (0.008 g, 23%). Eluted second was pure natural product **2** (0.026 g, 74%), isolated as a colourless syrup. R_f=0.38 (97:3 CH_2Cl_2/Me_2CO).

4.15. 5-*O*-Cinnamoyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (19)

A solution of **5** (0.125 g, 0.47 mmol) and *trans*-cinnamoyl chloride (0.090 g, 0.54 mmol) in dry 1,2-dichloroethane (12.5 mL) was stirred at 70 °C for 20 h. The mixture was evaporated and the residue was purified by flash column chromatography (2:3 →1:1 Et_2O/light petroleum). Dicinnamoate **20** (0.030 g, 12%) was first eluted. After crystallization from CH_2Cl_2/hexane pure **20** was obtained as a white powder, mp 137 °C, $[\alpha]_D^{20}$ −138.8 (*c* 0.25, $CHCl_3$), lit.[9] 125–126 °C, lit.[9] $[\alpha]_D^{20}$ −297.1 (*c* 0.23, $CHCl_3$); R_f=0.28 (1:5 Et_2O/light petroleum). IR (KBr): ν_{max} 1719 (C=O, ester), 1635 (C=C, cinnamate), 1497 (Ph) and 1161 (OC=O, ester). ^{1}H NMR ($CDCl_3$): δ 1.34 and 1.56 (2×s, 3H each, C**Me**$_2$), 4.66 (d, 1H, $J_{1,2}$=3.7 Hz, H-2), 4.80 (dd, 1H, $J_{3,4}$=3.0, $J_{4,5}$=9.3 Hz, H-4), 5.61 (d, 1H, $J_{3,4}$=3.0 Hz, H-3), 6.02 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 6.15 (d, 1H, $J_{4,5}$=9.3 Hz, H-5), 6.48 (d, 1H, $J_{2',3'}$=16.1 Hz, H-2′), 7.32–7.68 (m, 15H, 3×Ph), 7.71 (d, 1H, $J_{2',3'}$=16.1 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 26.2 and 26.7 (C**Me**$_2$) 72.1 (C-5), 75.8 (C-3), 80.6 (C-4), 83.2 (C-2), 105.1 (C-1), 112.2 (**C**Me_2), 116.7 and 117.3 (2×C-2′), 127.3, 127.4, 128.1, 128.3, 128.4, 128.5, 128.7, 128.8, 130.3, 130.5, 134.0, 134.1, 137.8 (3×Ph), 145.5 and 146.2 (2×C-3′), 165.0 and 167.1 (2×C-1′). HRMS (ESI): *m*/*z* 549.1886 (M^++Na), calcd for $C_{32}H_{30}NaO_7$: 549.1884. Eluted next was pure **19** (0.103 g, 55%; 63% when calculated to reacted **5**), as a colourless oil, $[\alpha]_D^{20}$ +43.1 (*c* 1.0, $CHCl_3$); R_f=0.25 (1:1 hexane/Et_2O). IR (film): ν_{max} 3453 (OH), 1712 (C=O, ester), 1636 (C=C, cinnamate) and 1164 (OC=O, ester). ^{1}H NMR ($CDCl_3$): δ 1.32 and 1.52 (2×s, 3H each, C**Me**$_2$), 4.28 (d, 1H, $J_{3,4}$=2.0 Hz, H-3), 4.48 (dd, 1H, $J_{3,4}$=2.1, $J_{4,5}$=9.3 Hz, H-4), 4.62 (d, 1H, $J_{1,2}$=3.6 Hz, H-2), 5.96 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 6.12 (d, 1H, $J_{4,5}$=9.2 Hz, H-5), 6.49 (d, 1H, $J_{2',3'}$=16.0 Hz, H-2′), 7.29–7.61 (m, 10H, 2×Ph), 7.77 (d, 1H, $J_{2',3'}$=16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 26.1 and 26.7 (C**Me**$_2$) 72.8 (C-5), 73.9 (C-3), 81.6 (C-4), 84.5 (C-2), 104.9 (C-1), 111.6 (**C**Me_2), 116.8 (C-2′), 126.7, 127.6, 128.2, 128.5, 128.6, 128.9, 128.9, 133.8, 136.9 (2×Ph), 146.8 (C-3′), 167.1 (C-1′). HRMS (ESI): *m*/*z* 419.1450 (M^++Na), calcd for $C_{23}H_{24}NaO_6$: 419.1465. The unreacted starting material **5** (0.015 g, 12%) was eluted last.

4.16. (+)-Crassalactone C (3)

4.16.1. Procedure A. To a stirred solution of **16** (0.079 g, 0.13 mmol) in a mixture of THF (2 mL) and AcOH (0.15 mL, 2.5 mmol) was added TBAF (1.53 mL, 1.53 mmol) in three equal portions over 48 h. After stirring at room temperature for additional 48 h, a new portion of TBAF (0.51 mL, 0.51 mmol) was added and the mixture stirred at room temperature for the next 24 h. An additional amount of TBAF (0.46 mL, 0.46 mmol) was added to the solution and the stirring continued for additional 24 h (total reaction time: 144 h). The mixture was evaporated and the residue purified by flash column chromatography (97:3→ 19:1 CH_2Cl_2/Me_2CO). Eluted first was pure **2** (0.007 g, 14%), isolated as a colourless solid. Data for **2** reported above. Eluted second was impure (+)-crassalactone C (**3**). The sample **3** was partitioned between CH_2Cl_2 (30 mL) and 1% aqueous $NaHCO_3$ (50 mL), the organic layer was separated, and the aqueous phase extracted with CH_2Cl_2 (2×30 mL). The combined organic extracts were dried and evaporated, and the residue purified by flash chromatography (Et_2O). Pure **3** (0.021 g, 43%) was obtained as a colourless solid. Recrystallization from diethyl ether gave colourless crystals, mp 153 °C, $[\alpha]_D^{25}$ +111.6 (*c* 0.5, EtOH), R_f=0.46 (1:1 light petroleum/EtOAc); lit.[6] mp 147–150 °C (EtOH), $[\alpha]_D^{30}$ +98.4 (*c* 0.5, EtOH), lit.[9] mp 145–148 °C; $[\alpha]_D$ +108.0 (*c* 0.37, $CHCl_3$). ^{1}H and ^{13}C NMR spectral data for **3** were in full agreement with those reported previously.[16]

4.16.2. Procedure B. A solution of **19** (0.145 g, 0.37 mmol) in 50% aq TFA (4 mL) was stirred at room temperature for 18 h. The volatiles were removed by co-distillation with toluene and the residue purified by flash column chromatography (7:3 EtOAc/hexane). Pure **19a** (0.111 g, 85%) was obtained a colourless solid. HRMS (ESI): *m*/*z* 379.1150 (M^++Na), calcd for $C_{20}H_{20}NaO_6$: 379.1152. To a solution of **19a** (0.034 g, 0.10 mmol) in dry DMF (1 mL) was added dry Et_3N (0.027 mL, 0.19 mmol) and Meldrum's acid (0.028 g, 0.19 mmol). The mixture was stirred for 72 h at 44–46 °C and then evaporated. The residue was purified by flash column chromatography (17:3 Et_2O/hexane) to afford pure **3** (0.022 g, 61%). R_f=0.46 (1:1 light petroleum/EtOAc).

4.16.3. Procedure C. A solution of **1** (0.025 g, 0.10 mmol) and *trans*-cinnamoyl chloride (0.083 g, 0.50 mmol) in dry MeCN (4 mL) was vigorously stirred under reflux for 2 h. The mixture was poured to 1% aq $NaHCO_3$ (75 mL) and extracted with EtOAc (3×25 mL). The combined organic solutions were washed with 10% aq NaCl (50 mL) dried and evaporated and the residue purified on a column of flash

silica (Et_2O) to give pure **3** (0.030 g, 79%). R_f=0.46 (1:1 light petroleum/EtOAc).

4.17. Cell lines

Prostate cancer cells (PC3), colon adenocarcinoma (HT29) and lung adenocarcinoma epithelial cell line (A549) were grown in DMEM medium. The media was supplemented with 10% of foetal calf serum (FTS, Sigma) and antibiotics (100 IU/mL of penicillin and 100 mg/mg of streptomycin). Cell lines were cultured in flasks (Costar, 25 mL) at 37 °C in the atmosphere of 100% humidity and 5% of CO_2 (Heraeus). Exponentially growing viable cells were used throughout the assay.

4.18. MTT assay

The colorimetric MTT assay was carried out following the reported procedure.[26]

Acknowledgements

The work was supported by a research grant from the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 172006).

Supplementary data

Supplementary data (General experimental procedures for preparation of known intermediates and copies of ^1H, and ^{13}C NMR spectra of key compounds.) related to this article can be found at http://dx.doi.org/10.1016/j.tet.2015.05.040. These data include MOL files and InChiKeys of the most important compounds described in this article.

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