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Original article

Design, synthesis and antiproliferative activity of styryl lactones related to (+)-goniofufurone

Velimir Popsavin [a,*], Bojana Srećo [a], Goran Benedeković [a], Jovana Francuz [a], Mirjana Popsavin [a], Vesna Kojić [b], Gordana Bogdanović [b]

[a] Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad, Serbia
[b] Oncology Institute of Vojvodina, Institutski put 4, 21204 Sremska Kamenica, Serbia

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ABSTRACT

This paper describes a straightforward divergent synthesis of (+)-goniofufurone mimics (**4**, **5** and **6**) starting from D-xylose. In a preliminary bioassay, analogues **4** and **5** exhibited a submicromolar antiproliferative activity towards HL-60 cells, while the corresponding parent compound **1** was completely inactive against this cell line. At the same time, these molecules showed approximately 10-fold stronger cytotoxicity in the same cell line when compared to the standard anticancer drug doxorubicin (DOX). Analogue **6** displayed 18- and 3-fold higher potency in Raji cell line when compared to control compounds **1** and DOX, respectively. A new divergent route for the preparation of (+)-goniofufurone (**1**) and (+)-crassalactone C (**3**) from D-xylose is also disclosed.

1. Introduction

Phytochemical studies of the genus *Goniothalamus* have resulted in the isolation and characterization of many compounds with a variety of biological activity [1,2]. Due to their proven use in folk medicine in Taiwan, Malaysia, and India to treat rheumatism, edema, as abortifacients, and as mosquito repellents, there has been an interest in the active ingredients as potential therapeutic targets. This resulted in the isolation by McLaughlin et al. [3–5] of a series of styryl lactones which were reported to show antitumour, pesticidal, and embryotoxic activities. Amongst these, (+)-goniofufurone (**1**, Fig. 1) containing a furano-furone bicyclic core has shown significant cytotoxic activities against several human tumour cell lines [6,7]. Due to its unique and intriguing structure, as well as its promising antitumour activity this natural product along with a number of its analogues has attracted the attention of many synthetic groups [8–18]. We have been involved in the synthesis of bio-active natural products and analogues having γ-lactone rings and have recently accomplished the total synthesis of a number of styryl lactones by chirality transfer from D-xylose [19–21]. In continuation of this strategy, we report herein on the synthesis and preliminary in vitro antitumour screening of new styryl lactones **4**, **5** and **6** as possible (+)-goniofufurone mimics. Compound **4** was designed as a conformationally flexible analogue of (+)-goniofufurone (**1**) and may be formally derived from **1** through a cleavage of the C_3–O_6 bond. In the same time, molecule **4** represents a 3-deoxy derivative of (+)-cardiobutanolide (**2**), a naturally occurring styryl lactone that was recently isolated from the stem bark of *Goniothalamus cardiopetalus* [22]. Both furano-lactones **5** and **6** were designed as less polar analogues of (+)-goniofufurone (**1**). Enhanced lipophilic character of **5** and **6** should improve their cell membrane permeation that may be beneficial for their antitumour activity. On the other hand, molecule **5** might be considered as a non-vinylogous analogue of (+)-crassalactone C (**3**), the naturally occurring styryl lactone that was very recently isolated from the leaves and twigs of *Polyalthia crassa* [23]. Apart from the synthesis of **4**–**6**, a novel divergent route to **1** and **3** was also developed in order to provide samples of the leads that would serve as positive controls in antitumour assays.

* Corresponding author. Tel.: +381 21 485 27 68; fax: +381 21 454 065.
E-mail address: velimir.popsavin@dh.uns.ac.rs (V. Popsavin).

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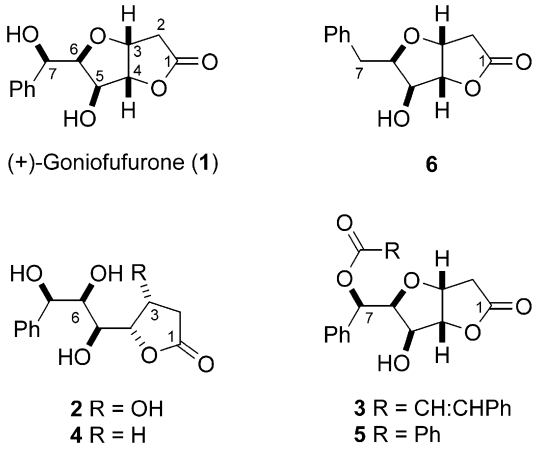


Fig. 1. Naturally occuring styryl lactones and analogues.

2. Results and discussion

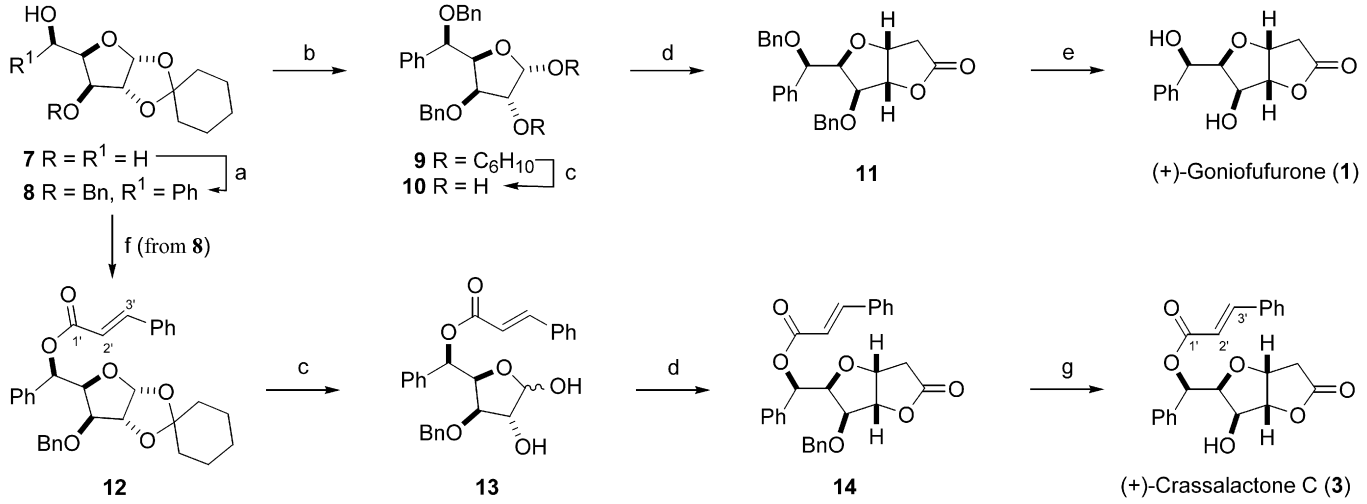
2.1. Chemistry

The divergent synthesis of natural products **1** and **3** is outlined in Scheme 1. The known [20,21] alcohol **8** that is readily available from partially protected D-xylose derivative **7** [24], was conveniently used as a common intermediate for the preparation of both targets **1** and **3**.

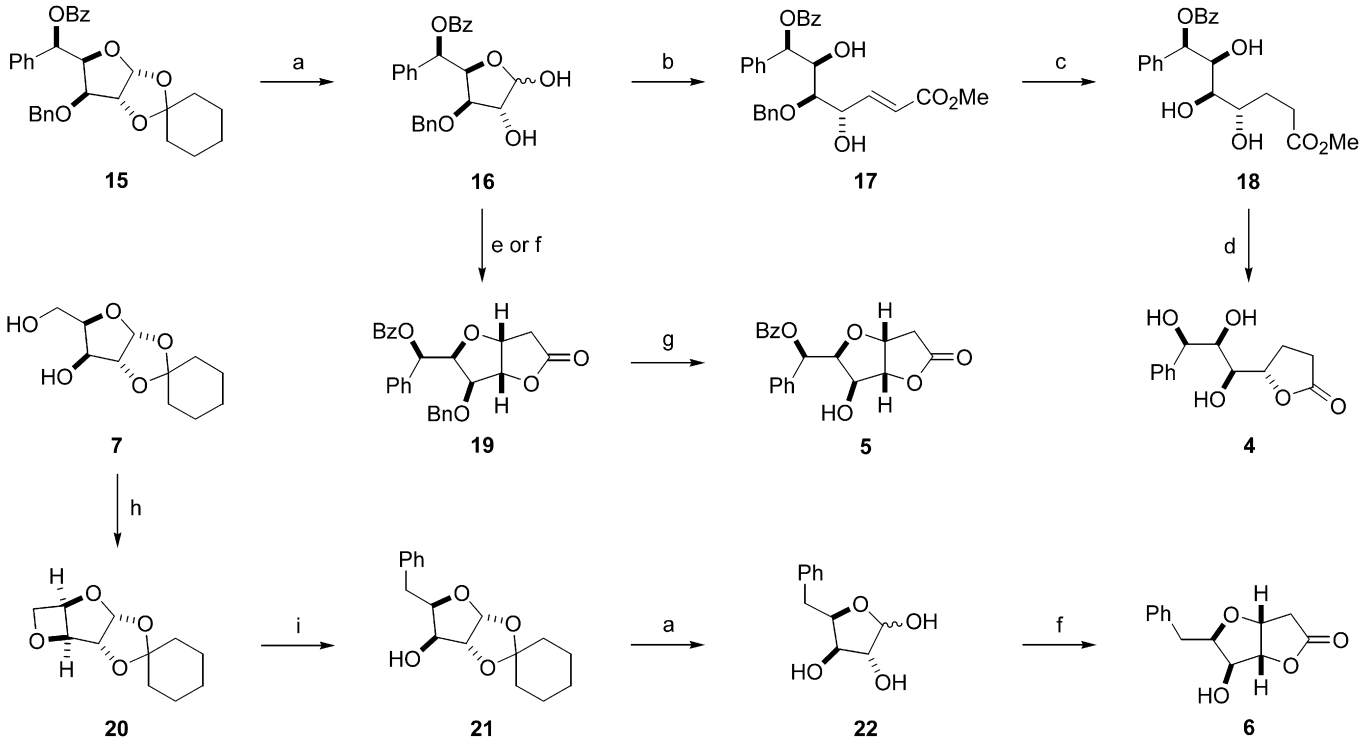
For the sake of synthesis of **1**, compound **8** was first converted to the corresponding di-*O*-benzyl ether **9** (94%) by treatment with BnBr (NaH, DMF). Hydrolytic removal of the cyclohexylidene protective group in **9** with aqueous acetic acid gave a 69% yield of the known lactol **10**, with physical constants (mp and optical rotation) in good agreement with those previously reported [25]. Compound **10** may be converted to the target **1** via the reported two-step sequence which consists of a *Z*-selective Wittig olefination, followed by hydrogenolytic removal of both benzyl ether protective groups [25]. However, we wanted to explore an alternative method for elaboration of the required [3.3.0] bicyclic lactone core based on the condensation of **10** with Meldrum's acid [26]. Accordingly, compound **10** was allowed to react with Meldrum's acid in DMF, in the presence of Et_3N, whereupon the protected lactone **11** was obtained in 73% yield. Intermediate **11** was finally converted to (+)-goniofufurone (**1**) after hydrogenolytic removal of both benzyl protective groups (H_2-Pd/C, MeOH). This synthetic sequence, which produced the target **1** in 35% overall yield (from **8**), is somewhat less efficient with respect to that previously completed in our laboratory (43% overall yield) [20,21]. The physical and spectroscopic data of thus prepared sample **1** are in good agreement with the literature values [18].

The synthesis of (+)-crassalactone C (**3**) commenced with a conversion of **8** into the corresponding 5-*O*-cinnamoyl derivative **12**. Accordingly, compound **8** was treated with cinnamic acid and DCC, in anhydrous CH_2Cl_2 and in the presence of DMAP, to afford an almost quantitative yield of **12**. The intermediate **12** was converted to the protected lactone **14** by using the same methodology as that already applied for the conversion of **9**–**11**. Thus, treatment of **12** with aqueous acetic acid gave the expected lactol **13** (73%), while the concomitant condensation of **13** with Meldrum's acid furnished **14** in 73% yield. Moreover, oxidative cleavage [27] of the benzyl protecting group in **14** (DDQ, $CHCl_3$, H_2O) gave (+)-crassalactone C (**3**), with physical and spectral properties in full agreement with those previously reported [20,21,23]. This new synthesis of **3** proceeds in eleven linear steps with 15.2% overall yield calculated to starting D-xylose derivative **7**. The preceding preparation of **3** was accomplished in 7.8% overall yield over ten linear steps [20,21].

The synthesis of analogues **4**, **5** and **6** is shown in Scheme 2. The known [20,21] 5-*O*-benzoyl derivative **15** has served as a convenient starting compound for the preparation of both targets **4** and **5**, via the lactol **16** as a common intermediate. Thus, hydrolytic removal of the cyclohexylidene protective group in **15** with diluted acetic acid, gave the lactol **16** (78%). Wittig olefination of **16** with $Ph_3P{=}CHCO_2Me$ in DMF took place stereoselectively to afford the (*E*)-unsaturated ester **17** in 69% yield. Catalytic hydrogenation of **17** over 10% Pd/C in methanol yielded the saturated ester **18** (72%). Sodium methoxide *O*-debenzoylation of **18** occurred with concomitant lactonisation to afford the target **4** ready for biological testing.



Scheme 1. Reagents and conditions: (a) Ref. 20, 33.8% from 7 steps; (b) BnBr, NaH, DMF, 0 °C for 1 h, then rt for 0.5 h, 94%; (c) 70% aq AcOH, reflux, 5 h for **9**, 69% of **10**, 12 h for **12**, 73% of **13**; (d) Meldrum's acid, Et_3N, DMF, 46–48 °C, 72 h for **10**, 73% of **11**, 65 h for **13**, 73% of **14**; (e) H_2-Pd/C, MeOH, rt, 9 days, 74%; (f) cinnamic acid, DCC, DMAP, CH_2Cl_2, rt, 24 h, 97%; (g) DDQ, 10:1 $CHCl_3/H_2O$, reflux, 30 h, 87%.



Scheme 2. Reagents and conditions: (a) 70% aq AcOH, reflux, 7 h for **15**, 78% of **16**, 3 h for **21**, 49% of **22**; (b) $Ph_3P{=}CHCO_2Me$, DMF, 70 °C, 5 h, 69%; (c) H_2-Pd/C, MeOH, rt, 4 h, 72%; (d) NaOMe, MeOH, rt, 1 h, 69%; (e) $Ph_3P{=}CHCO_2Me$, MeOH, −20 °C for 1 h, then rt for 47 h, 52%; (f) Meldrum's acid, Et_3N, DMF, 46°C, 65 h for **16**, 58% of **19**, 48 h for **22**, 52% of **6**; (g) H_2-Pd/C, HCl (cat.), EtOAc, rt, 44 h, 76% of **5**, 22% of **6**; (h) Ph_3P, DEAD, MePh, reflux, 2.5 h, 73%; (i) PhMgBr/Et_2O, THF, reflux, 8.5 h, 85%.

As mentioned above, the lactol **16** represents a divergent intermediate for the preparation of target **5**. At this point, we wanted to examine both methods for elaboration of the bicyclic lactone core in **5**; the *Z*-selective Wittig reaction [28] of **16** with $Ph_3P{=}CHCO_2Me$, as well as the condensation of Meldrum's acid with **16**. When lactol **16** was reacted with the stabilized ylide $Ph_3P{=}CHCO_2Me$ in dry methanol the expected furano-lactone **19** was obtained in 52% yield. Alternatively, when the lactol **16** was allowed to react with Meldrum's acid, in dry *N,N*-dimethylformamide and in the presence of triethyl amine, the desired lactone **19** was obtained in 58% yield. The last procedure for preparation of **19** is more convenient then that based upon the Wittig reaction, not only because of slightly higher yield, but also due to easier product isolation from the reaction mixture. The 5-*O*-Benzyl protecting group from **19** was removed by catalytic hydrogenolysis to give analogue **5** (76%) as a major reaction product.

A minor amount of **7**-deoxy derivative **6** (22%) was also obtained from the last reaction (**19**→**5**). As we intended to evaluate its antitumour activity, it was desirable to develop a more efficient route for the preparation of **6**. Accordingly, we envisaged a four-step sequence, based on a nucleophilic oxetane ring opening in **20**. Compound **20** is usually prepared by a two-step sequence comprised of an initial regioselective 5-*O*-sulfonylation of 1,2-*O*-cyclohexylidene-α-D-xylofuranose (**7**), followed by a base-catalysed 3,5-anhydro ring closure [29]. For the purpose of this work we have developed a one-step procedure for preparation of **20** under the Mitsunobu cyclodehydration conditions [30,31]. Thus, compound **7** was treated with diethyl azodicarboxylate and triphenylphosphine in refluxing toluene, whereupon the oxetane **20** was obtained as a main reaction product in 73% yield. The physical and spectral data of thus prepared sample **20** were in good agreement with those reported in the literature [29]. Treatment of **20** with phenylmagnesium bromide produced the expected alcohol **21** in 85% yield. Hydrolytic removal of the cyclohexylidene protective group (7:3 aq AcOH), followed by the γ-lactone formation (Meldrum's acid, Et_3N, DMF) afforded the furano-lactone **6**. This compound was previously obtained as a side-product in the final step of the Murphy and Dennison's synthesis of goniofufurone [32]. X-ray data of **6** were also reported [33]. Although the melting point and the optical rotation {mp 139 °C, $[\alpha]^{20}_D$ +49.5 (*c* 0.62, $CHCl_3$)} of thus obtained sample **6** are different from the literature values {mp 101–103 °C, $[\alpha]^{20}_D$ +37.0 (*c* 0.14, $CHCl_3$)}, the ^1H and ^{13}C NMR spectral data are in good agreement with the reported values [32].

2.2. In vitro cytotoxic activity

Compounds **4**, **5** and **6** were evaluated for their in vitro cytotoxicity against human myelogenous leukaemia (K562), human promyelocytic leukaemia (HL-60), human T cell leukaemia (Jurkat), human Burkitt's lymphoma (Raji cells) human cervix carcinoma (HeLa) and normal foetal lung fibroblasts (MRC-5). Cytotoxic activity was evaluated by using the standard MTT colorimetric assay after exposure of cells to the test compounds for 72 h. (+)-Goniofufurone (**1**), (+)-crassalactone C (**3**) and the commercial antitumour agent doxorubicin (DOX) were used as reference compounds. According to the resulting IC_{50} values of the cytotoxic assay (Table 1), all three styryl lactone analogues (**4**–**6**) exhibit potent anticancer activities towards the most tested cell lines, with IC_{50} values in the low-micromolar range. The only exception is analogue **6**, which showed a moderate cytotoxicity against the HL-60 cells. Analogues **4** and **6** showed submicromolar activities against K562 cells, with IC_{50} values comparable to that recorded for the parent natural product **1**. The 7-*O*-benzoyl derivative **5** is the most cytotoxic molecule in this cell line, being essentially as potent as the commercial antitumour agent doxorubicin (DOX). Remarkably, analogues **4** and **5** demonstrated the most potent activity

Table 1
Antiproliferative activities of synthesized compounds and DOX.

Compound	IC_{50}, μM[a]					
	K562	HL-60	Jurkat	Raji	HeLa	MRC-5
1	0.41 (0.01)	>100 (5.32)	32.45 (1.35)	18.45 (1.98)	8.32 (0.78)	>100 (6.78)
3	3.56 (0.03)	>100 (5.36)	25.45 (0.98)	15.46 (1.02)	11.25 (0.60)	>100 (8.98)
4	0.54 (0.022)	0.09 (0.006)	2.23 (0.11)	2.21 (0.67)	2.34 (0.34)	>100 (7.45)
5	0.23 (0.03)	0.12 (0.01)	0.36 (0.002)	1.01 (0.05)	5.69 (0.08)	>100 (6.97)
6	0.51 (0.02)	43.81 (2.45)	2.52 (0.04)	1.03 (0.24)	4.42 (0.42)	>100 (8.86)
DOX	0.25 (0.04)	0.92 (0.04)	0.03 (0.0018)	2.98 (0.09)	0.07 (0.001)	0.10 (0.03)

[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 quadriplicates. Standard deviations are given in parentheses.

against HL-60 cells being essentially 10-fold more active than DOX, while the parent compound **1** was completely inactive in the same cell line. All synthesized analogues (**4**–**6**) exhibited notable antiproliferative effects on Jurkat cells, with IC_{50} values in the low-micromolar range. The most active molecule against this cell line was 7-*O*-benzoyl derivative **5** being over 90-fold more potent than **1**, while analogues **4** and **6** demonstrated 14- and 12-fold stronger cytotoxicity when compared to the parent natural product **1**. The most active molecules against Raji cells are analogues **5** and **6** that exhibited over 18-fold higher potency than the control compound **1**, as well as almost 3-fold stronger growth inhibitory activity with respect to doxorubicin. The ring-opened analogue **4** also showed a notable activity against this cell line, being over 8-fold more active than **1**. In the same time, this molecule showed a similar activity as DOX in the same cell line. All three styryl lactones **4**–**6** exhibit micromolar cytotoxicity against HeLa malignant cells, with IC_{50} values ranging from 2.34 to 5.69 μM. The most active compound against these cells is the monocyclic lactone **4**, being over 3-fold more cytotoxic than the parent compound **1**. The 7-*O*-benzoyl derivative **5** demonstrated a similar potency as **1** against HeLa cells, while the 7-deoxy derivative **6** displayed almost 2-fold higher potency in the same cell line when compared to control compound **1**. The data obtained indicated that the THF ring opening in **1**, as well as the replacement of the C-7 hydroxyl function, may enhance the antitumour activity of resulting analogues in vitro. However, a comparison of biological data of **5** and **3** revealed that analogue **5** showed a superior activity against the all tested cells. It appears that the removal of double bond from **3** significantly increases the antiproliferative activity of the analogue. The difference of the potency between these two molecules could be due to different size of the aromatic ester groups in their structures. Moreover, the all synthesized lactones **4**, **5** and **6**, including the natural products **1** and **3** were found to be completely inactive against the normal MRC-5 cells. These results do suggest that these molecules represent selective antitumour agents, but this should be verified by additional in vitro experiments with different normal cell lines.

It is obvious that natural styryl lactones and analogues demonstrate promising antitumour activities. Some possible mechanisms of action of this class of biologically active molecules have been recently reviewed [6,7]. However, a more comprehensive biological studies will be required to identify the exact mode of action of these compounds in tumour cells.

3. Conclusions

In summary, we have developed an efficient divergent route to several styryl lactones related to (+)-goniofufurone (**1**) and evaluated them for in vitro cytotoxic activities against five human malignant cell lines. Molecule **4** was designed as a ring-opened analogue of (+)-goniofufurone (**1**) that was formally derived from **1** through a cleavage of the C_3–O_6 bond. Both analogues **5** and **6**, which represent goniofufurone derivatives of enhanced lipophilic character, were designed by formal displacement of the C-7 hydroxyl function with OBz or H, respectively. Molecule **5** may also be considered as a de-vinylated analogue of the naturally occurring styryl lactone (+)-crassalactone C (**3**). All three analogues **4**–**6** exhibit potent to moderate antiproliferative activities against the all tested neoplastic cells, but were devoid of any cytotoxic activity towards the normal foetal lung MRC-5 fibroblasts. Analogues **4** and **5** demonstrated the most potent activity against HL-60 cells being approximately 10-fold more cytotoxic than the standard antitumour agent doxorubicin. The parent compound **1** however, was completely inactive towards this cell line. Against the Raji cells, both **5** and **6** exhibited over 18-fold higher potency than the reference compound **1**, as well as almost 3-fold stronger cytotoxicity with respect to DOX. Based upon these results, we believe that compounds **4**–**6** may serve as convenient leads in the search for more potent and selective antitumour agents. Further optimization of the structures, as well as the preparation of a number of new (+)-goniofufurone and (+)-crassalactone C mimics to perform structure–activity relationship (SAR) is currently underway.

4. Experimental

4.1. General

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on a KRÜSS P3002 polarimeter at room temperature. IR spectra were recorded with a FTIR/NIR Nexus 670 spectrophotometer (Thermo-Nicolet). NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in ppm downfield from TMS. Low resolution mass spectra were recorded on Finnigan-MAT 8230 (CI) and on an Agilent Technologies HPLC/MS 3Q system, series 1200/6410 (ESI). High resolution mass spectra were taken on a 6210 Time-of-Flight LC/MS Agilent Technologies instrument (ESI). Flash column chromatography was performed using ICN silica 32–63. TLC was performed on DC Alufolien Kieselgel 60 F_{254} (E. Merck). All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under diminished pressure at a bath temperature below 30 °C.

4.1.1. 3,5-Di-O-benzyl-1,2-O-cyclohexylidene-5-C-phenyl-α-D-gluco-pentofuranose (**9**)

To a cooled (0 °C) and stirred solution of **8** (0.141 g, 0.36 mmol) in dry DMF (**5** mL) were added successively 80% NaH (0.021 g, 0.69 mmol) and BnBr (0.055 mL; 0.46 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature for 0.5 h. Methanol (2 mL) was finally added, and the mixture was stirred at room temperature for the additional 15 minutes and evaporated. The residue was suspended in water (30 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The combined organic solutions were dried and evaporated. The remaining crude **9** (0.18 g) was purified by flash

column chromatography (9:1→4:1 light petroleum/Et_2O) to afford pure **9** (0.164 g, 94%) as a colourless syrup, $[\alpha]^{20}_D$ −36.46 (*c* 0.84, $CHCl_3$), R_f = 0.43 (4:1 hexane/Et_2O). ^1H NMR ($CDCl_3$): δ 1.33–1.78 (m, 10 H, C_6H_{10}), 3.37–4.27 (m, 2 H, $J_{3,4}$ = 3.0, J_{gem} = 11.0 Hz, H-3 and PhC*H*$_a$), 4.38–4.52 (m, 2 H, $J_{3,4}$ = 3.0, $J_{4,5}$ = 9.1, J_{gem} = 11.0 Hz, H-4 and PhC*H*$_b$), 4.67 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-2), 4.68 and 4.79 (2 × d, 1 H each, J_{gem} = 11.6 Hz, PhC*H*$_2$), 4.80 (d, 1 H, $J_{4,5}$ = 9.1 Hz, H-5), 5.94 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 7.18–7.60 (m, 15 H, 3 × Ph). ^{13}C NMR ($CDCl_3$): δ 23.5, 23.7, 24.8, 35.6 and 36.2 (5 × CH_2), 70.2 and 72.3 (2 × Ph*C*H$_2$), 78.2 (C-5), 81.5 (C-2), 81.9 (C-3), 82.7 (C-4), 104.5 (C-1), 112.1 (Cq from C_6H_{10}), 127.4, 127.48, 127.5, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 137.7, 138.1, 139.3 (3 × Ph). LRMS (CI): *m/z* 487 (M^+ + H). Anal. Found: C, 72.76; H, 7.03. Calcd for $C_{24}H_{28}O_5 \cdot 1.5H_2O$: C, 72.49; H, 7.26.

4.1.2. 3,5-Di-O-benzyl-5-C-phenyl-D-gluco-pentofuranose (**10**)

A solution of **9** (0.159 g, 0.33 mmol) in 70% aq. AcOH (6 mL) was stirred for 5 h under reflux. After the mixture cooled to room temperature it was concentrated by co-distillation with toluene and the residue (0.142 g) purified by flash column chromatography (3:2 Et_2O/light petroleum) to afford pure **10** (0.092 g, 69%) as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave colourless needles, mp 100–101 °C, $[\alpha]^{20}_D$ −6.81 (*c* 1.4, $CHCl_3$), R_f = 0.44 (4:1 Et_2O/hexane); lit. [25] mp 98–99 °C, $[\alpha]^{20}_D$ −8.2 (*c* 1.0, $CHCl_3$). IR ($CHCl_3$): ν_{max} 3380 (OH). ^1H NMR ($CDCl_3$ + D_2O): δ 4.05 (dd, 1 H, $J_{1,2}$ = 4.0, $J_{2,3}$ = 1.7 Hz, H-2α), 4.10–4.79 (m, 6.6 H), 5.01 (s, 0.4 H, H-1β), 5.36 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1α), 7.25–7.63 (m, 15 H, 3 × Ph). ^{13}C NMR ($CDCl_3$): δ 70.2, 70.3, 72.4, 73.3, 74.6, 77.2, 78.4, 79.4, 81.7, 82.0, 83.4, 84.3, 96.3 (C-1α), 103.2 (C-1β), 127.5, 127.6, 127.8, 127.9, 127.93, 128.1, 128.15, 128.2, 128.3, 128.4, 128.41, 128.5, 128.6, 137.0, 137.9, 138.1, 138.14, 139.3 and 139.4 (3 × Ph). LRMS (CI): *m/z* 407 (M^+ + H).

4.1.3. 3,6-Anhydro-5,7-di-O-benzyl-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**11**)

To a solution of **10** (0.045 g, 0.11 mmol) in dry DMF (1 mL) was added Meldrum's acid (0.024 g, 0.17 mmol) and dry Et_3N (0.023 mL, 0.16 mmol). The mixture was stirred for 72 h at 46–48 °C and evaporated. Flash column chromatography (4:1→2:3 hexane/Et_2O) of the residue gave pure **11** (0.035 g, 73%) as a colourless syrup, $[\alpha]^{20}_D$ −0.8 (*c* 0.93, $CHCl_3$), R_f = 0.23 (3:2 Et_2O/hexane); lit. [25] $[\alpha]^{20}_D$ −5.8 (*c* 1.0, $CHCl_3$). IR ($CHCl_3$): ν_{max} 1789 (C=O). ^1H NMR ($CDCl_3$ + D_2O): δ 2.49 (d, 1 H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.63 (dd, 1 H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 5.4 Hz, H-2b), 4.22 (dd, 1 H, $J_{5,6}$ = 3.1, $J_{6,7}$ = 8.8 Hz, H-6), 4.28 and 4.42 (2 × d, 1 H each, J_{gem} = 11.4 Hz, PhC*H*$_2$), 4.47 (d, 1 H, $J_{5,6}$ = 3.1 Hz, H-5), 4.65–4.77 (m, 3 H, H-4, H-7 and PhC*H*$_a$), 4.86–4.96 (m, 2 H, H-3 and PhC*H*$_b$), 7.21–7.51 (m, 15 H, 3 × Ph). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 70.1 and 73.2 (2 × Ph*C*H$_2$), 76.8 and 78.0 (C-4 and C-7), 80.8 (C-5), 83.3 (C-6), 84.9 (C-3), 127.5, 127.7, 127.8, 128.0, 128.3, 128.5, 128.52, 129.12, 137.2, 137.9 and 139.0 (3 × Ph), 175.5 (C-1).

4.1.4. (+)-Goniofufurone (**1**)

A solution of **11** (0.103 g, 0.24 mmol) in MeOH (5 mL) was hydrogenated over 10% Pd/C (0.1 g) for 9 days at room temperature. The mixture was filtered through a celite pad and the catalyst washed with MeOH. The combined organic solutions were evaporated and the residue was purified by flash chromatography (Et_2O) to afford pure **1** (0.044 g, 74%) as a colourless solid. Recrystallization from EtOAc/hexane gave colourless plates, mp 154–155 °C, $[\alpha]^{20}_D$ +39.2 (*c* 0.94, $CHCl_3$), R_f = 0.28 (Et_2O); lit. [18] mp 154–156 °C, $[\alpha]^{20}_D$ +39.5 (*c* 1.0, $CHCl_3$). IR (KBr): ν_{max} 3410 (OH), 1755 (C=O). ^1H NMR ($CDCl_3$): δ 2.61 (d, 1 H, $J_{2a,2b}$ = 18.7 Hz, H-2a), 2.74 (dd, 1 H, $J_{2a,2b}$ = 18.7, $J_{2b,3}$ = 5.6 Hz, H-2b), 4.05 (dd, 1 H, $J_{5,6}$ = 2.7, $J_{6,7}$ = 5.3 Hz, H-6), 4.43 (d, 1 H, $J_{5,6}$ = 2.7 Hz, H-5), 4.87 (d, 1 H, Hz $J_{3,4}$ = 4.2 Hz, H-4), 5.08 (dd, 1 H, $J_{2b,3}$ = 5.6, $J_{3,4}$ = 4.2 Hz, H-3), 5.12 (d, 1 H, $J_{6,7}$ = 5.3 Hz, H-7), 7.30–7.44 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 36.1 (C-2), 73.5 (C-7), 74.4 (C-5), 77.3 (C-3), 82.9 (C-6), 87.4 (C-4), 125.8, 128.5, 128.8 and 138.8 (Ph), 175.4 (C-1). HRMS (ESI): Found: 251.0912 (M^+ + H), calcd for $C_{13}H_{15}O_5$: 251.0914.

4.1.5. 1,2-O-cyclohexylidene-3-O-benzyl-5-O-cinnamoyl-5-C-phenyl-α-D-gluco-pentofuranose (**12**)

To a solution of **8** (0.115 g, 0.29 mmol) in dry CH_2Cl_2 (11 mL) were added successively cinnamic acid (0.086 g, 0.58 mmol), DCC (0.144 g, 0.7 mmol) and DMAP (0.142 g, 1.16 mmol). The mixture was stirred at room temperature for 24 h then poured into H_2O (80 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were washed with 10% NaCl (80 mL) dried and evaporated. The residue was purified by flash column chromatography (5:1 light petroleum/Et_2O) to afford pure **12** (0.148 g, 97%) as a colourless syrup. Crystallization from MeOH/H_2O gave colourless crystals, mp 63 °C, $[\alpha]^{20}_D$ +31.88 (*c* 1, $CHCl_3$), R_f = 0.6 (1:1 hexane/Et_2O). IR (KBr): ν_{max} 1716 (C=O), 1637 (C=C, cinnamoyl). ^1H NMR ($CDCl_3$): δ 1.35–1.77 (m, 10 H, C_6H_{10}), 4.17 (d, 1 H, $J_{3,4}$ = 3.1 Hz, H-3), 4.52 and 4.70 (2 × d, 2 H, J_{gem} = 11.6 Hz, PhC*H*$_2$), 4.59 (dd, 1 H, $J_{4,5}$ = 9.6 Hz, H-4), 4.68 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-2), 5.95 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 6.15 (d, 1 H, $J_{4,5}$ = 9.6 Hz, H-5), 6.36 (d, 1 H, $J_{2',3'}$=16.2 Hz, H-2′), 7.16–7.56 (m, 15 H, 3 × Ph), 7.64 (d, 1 H, $J_{2',3'}$=16.2 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 23.5, 23.8, 24.8, 35.6 and 36.3 (5 × CH_2), 72.0 (PhCH_2), 72.2 (C-5), 80.8 (C-3), 81.2 (C-2), 81.3 (C-4), 104.8 (C-1), 112.3 (Cq from C_6H_{10}), 117.8 (C-2′), 127.4, 127.8, 128.0, 128.17, 128.2, 128.3, 128.5, 128.8, 130.3, 134.2, 136.8 and 138.2 (3 × Ph), 144.9 (C-3′), 164.9 (C-1′). LRMS (ESI): *m/z* 565 (M^+ + K), 549 (M^+ + Na), 527 (M^+ + H). Anal. Found: C, 75.59; H, 6.14. Calcd for $C_{26}H_{28}O_6$: C, 75.26; H, 6.51.

4.1.6. 3-O-benzyl-5-O-cinnamoyl-5-C-phenyl-α-D-gluco-pentofuranose (**13**)

A solution of **12** (0.211 g, 0.40 mmol) in 70% aq AcOH (7 mL) was stirred for 12 h under reflux. After workup as described above (Procedure 4.1.2.) crude **13** (0.192 g) remained as a yellow oil. Flash column chromatography (1:1 hexane/EtOAc) of the residue gave two fractions. Unreacted starting compound **12** (0.020 g, 11.3%) was first eluted, followed by pure **13** (0.13 g, 73%), as a mixture of corresponding anomers (α/β ≈ 2:1, from ^1H NMR). Crystallization from MeOH/H_2O gave colourless crystals, mp 106 °C, $[\alpha]^{20}_D$ +87.5 (*c* 1.0, $CHCl_3$), R_f = 0.3 (1:1 hexane/EtOAc). IR (KBr): ν_{max} 3430 (OH), 1709 (C=O), 1636 (C=C, cinnamoyl). ^1H NMR ($CDCl_3$): δ 2.42, 2.97, 3.71 and 3.95 (4 × bs, exchangeable with D_2O, 2 × OHα and β), 4.01–4.23 (m, H-2α, H-2β, H-3α and 3β), 4.46–4.77 (m, Ph*C*H$_2$ α and β, H-4α and β), 5.04 (s, H-1β), 5.37 (d, $J_{1,2}$ = 3.7 Hz, H-1α), 6.07 (d, $J_{4,5}$ = 9.2 Hz, H-5α), 6.15 (d, $J_{4,5}$ = 9.8 Hz, H-5β), 6.36 (d, $J_{2',3'}$=15.9 Hz, H-2′α), 6.37 (d, $J_{2',3'}$=15.9 Hz, H-2′β), 7.22–7.57 (m, 15 H, 3 × Ph), 7.63 (d, H-3′α), 7.64 (d, H-3′β). ^{13}C NMR ($CDCl_3$): δ 72.2 (PhCH_2α), 72.8 (C-5α), 72.9 (PhCH_2β), 73.2 (C-5β), 74.4 (C-2α), 76.8 (C-2β), 80.2 (C-4α), 81.1 (C-3β), 82.3 (C-3α), 82.7 (C-4β), 96.4 (C-1α), 103.6 (C-1β), 117.7 (C-2′β), 117.8 (C-2′α), 127.6, 127.8, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7, 128.9, 130.4, 130.5, 134.1, 134.2, 137.1, 138.2 and 138.3 (3 × Ph), 145.1 (C-3′α), 145.3 (C-3′β), 165.1 (C-1′α), 165.2 (C-1′β). LRMS (ESI): *m/z* 485 (M^+ + K), 469 (M^+ + Na). HRMS (ESI): Found: 469.1622 (M^+ + Na), calcd for $C_{13}H_{15}O_5$: 469.1622.

4.1.7. 5-O-benzyl-7-O-cinnamoyl-5-C-phenyl-α-D-glycero-D-ido-heptono-1,4-lactone (**14**)

To a solution of **13** (0.092 g, 0.20 mmol) in dry DMF (2 mL) was added anhydrous Et_3N (0.028 mL, 0.39 mmol) and Meldrum's acid (0.057 g, 0.39 mmol). The mixture was stirred at 46–48 °C for 65 h and then evaporated. Chromatographic purification on a column of

flash silica (7:3 Et_2O/hexane) gave pure **14** (0.071 g, 73%), which crystallized from CH_2Cl_2/hexane as white fibers, mp 176 °C, $[\alpha]^{20}_D$ +86.8 (*c* 0.5, $CHCl_3$), R_f = 0.23 (7:3 Et_2O/hexane). IR (KBr): ν_{max} 1788 (C═O, lactone), 1713 (C═O, cinnamoyl), 1636 (C═C, cinnamoyl). ^{1}H NMR ($CDCl_3$): δ 2.57 (d, 1 H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.68 (m, 1 H, H-2b), 4.37 (d, 1 H, $J_{5,6}$ = 3.5 Hz, H-5), 4.46 (dd, 1 H, $J_{5,6}$ = 3.5, $J_{6,7}$ = 9.2 Hz, H-6), 4.54 and 4.67 (2 × d, 2 H, J_{gem} = 11.6 Hz, PhC*H*$_2$), 4.96 (m, 2 H, H-3 and H-4), 6.10 (d, 1 H, $J_{6,7}$ = 9.2 Hz, H-7), 6.35 (d, 1 H, $J_{2',3'}$=16.1 Hz, H-2′), 7.19–7.57 (m, 15 H, 3 × Ph), 7.63 (d, 1 H, $J_{2',3'}$=16.1 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.9 (C-2), 72.2 (C-7), 73.0 (Ph*C*H$_2$), 77.4 (C-3), 80.1 (C-5), 82.0 (C-6), 84.6 (C-4), 117.6 (C-2′), 127.6, 128.2, 128.3, 128.5, 128.7, 128.9, 130.5, 134.2, 136.4 and 138.0 (3 × Ph), 145.4 (C-3′), 164.9 (C-1′), 175.3 (C-1). LRMS (ESI): 509 (M^+ + K), 493 (M^+ + Na). Anal. Found: C, 74.03; H, 5.57. Calcd for $C_{29}H_{26}O_6$: C, 73.84; H, 5.54.

4.1.8. (+)-Crassalactone C (*3*)

Compound **14** (0.03 g, 0.06 mmol) was refluxed for 30 h with DDQ (0.073 g, 0.32 mmol) in 10:1 $CHCl_3/H_2O$ (2 mL). The mixture was poured to 1% aq $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was dried and evaporated. The residue was purified by flash column chromatography (4:1 Et_2O/light petroleum) to afford pure **3** (0.021 g, 87%) as a colourless solid. Recrystallization from diethyl ether gave colourless crystals, mp 153 °C, $[\alpha]^{20}_D$ +111.6 (*c* 0.5, EtOH), R_f = 0.46 (1:1 light petroleum/EtOAc); lit. [23] mp 147–150 °C (EtOH), $[\alpha]^{30}_D$=+98.4 (*c* 0.5, EtOH). IR (KBr): ν_{max} 3459 (OH), 1784 (C═O, lactone), 1695 (C═O, cinnamoyl), 1635 (C═C, cinnamoyl). ^{1}H NMR ($CDCl_3$): δ 2.56 (d, 1 H, $J_{2a,2b}$ = 18.6 Hz, H-2a), 2.70 (dd, 1 H, $J_{2a,2b}$ = 18.6, $J_{2b,3}$ = 5.8 Hz, H-2b), 4.19 (bs, 1 H, exchangeable with D_2O, OH), 4.26 (dd, 1 H, $J_{6,7}$ = 9.2, $J_{5,6}$ = 2.4 Hz, H-6), 4.43 (bs, 1 H, H-5), 5.00 (m, 2 H, H-3 and H-4), 6.00 (d, 1 H, $J_{6,7}$ = 9.2 Hz, H-7), 6.47 (d, 1 H, $J_{2',3'}$=15.9 Hz, H-2′), 7.36–7.59 (m, 10 H, 2 × Ph), 7.78 (d, 1 H, $J_{2',3'}$=15.9 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 72.8 (C-7), 73.1 (C-5), 77.1 (C-3), 82.4 (C-6), 87.0 (C-4), 116.4 (C-2′), 127.6, 128.3, 128.6, 128.9, 130.9, 133.6 and 136.6 (2 × Ph), 147.5 (C-3′), 167.5 (C-1′), 175.5 (C-1). LRMS (ESI): *m/z* 403 (M^+ + Na), 363 (M^+ + H−H_2O).

4.1.9. 5-O-benzoyl-3-O-benzyl-5-C-phenyl-D-gluco-pentofuranose (*16*)

A solution of **15** (0.452 g, 0.9 mmol) in 70% aq. AcOH (18 mL) was stirred for 7 h under reflux. After workup as described above (Section 4.1.2.), crude sample **16** remained as a yellow syrup. Flash column chromatography (9:1 → 4:1 CH_2Cl_2/EtOAc) of the residue gave pure **16** (0.296 g, 78%) as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave colourless needles, mp 156–157 °C, $[\alpha]^{20}_D$ +29.1 → +33.9 (*c* 1.27, $CHCl_3$, 72 h), R_f = 0.23 (9:1 CH_2Cl_2/EtOAc). IR (KBr): ν_{max} 3441 (OH), 1712 (C═O). ^{1}H NMR ($CDCl_3$ + D_2O): δ 4.01–4.18 (m, 3 H, H-2α, H-3α, H-3β), 4.23 (d, 1 H, *J* = 2.8 Hz, H-2β), 4.33–4.66 (d, 4 H, 2 × PhCH_2 α and β), 4.68–4.81 (m, 2 H, H-4α, H-4β), 5.07 (s, 1 H, H-1β), 5.39 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1α), 6.19 (d, 1 H, $J_{4,5}$ = 9.6 Hz, H-5α), 6.20 (d, 1 H, $J_{4,5}$ = 9.6 Hz, H-5β), 7.11–8.04 (m, 30 H, 3 × Ph). ^{13}C NMR ($CDCl_3$): δ 72.0 and 72.8 (2 × PhCH_2), 73.1 (C-5α), 73.5 (C-5β), 74.3 (C-2α), 76.7 (C-2β), 80.2 (C-4α), 82.7 (C-4β), 81.2 (C-3α), 82.4 (C-3β), 96.3 (C-1α), 103.5 (C-1β), 125.1, 125.2, 125.9, 127.5, 127.7, 128.0, 128.1, 128.3, 128.4, 129.6, 129.96, 130.0, 132.99, 133.0, 133.1 133.4, 136.1, 136.9, 138.0 and 138.1 (3 × Ph), 164.62 and 164.76 (PhC═O, α and β). LRMS (CI): *m/z* 421 (M^+ + H). Anal. Found: C, 68.80; H, 5.92. Calcd for $C_{25}H_{24}O_6 \cdot H_2O$: C, 68.48; H, 5.98.

4.1.10. Methyl 7-O-benzoyl-5-O-benzyl-2,3-dideoxy-7-C-phenyl-D-gluco-hept-2-enonate (*17*)

To a solution of **16** (0.318 g, 0.76 mmol) in dry DMF (6.5 mL) was added Ph_3P═$CHCO_2Me$ (0.509 g, 1.52 mmol). The mixture was stirred for 5 h at 60–70 °C and then evaporated. The residue was purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc) to afford pure **17** (0.248 g, 69%) as a colourless syrup, $[\alpha]^{20}_D$ +34.5 (*c* 1.0, $CHCl_3$), R_f = 0.55 (4:1 CH_2Cl_2/EtOAc). IR ($CHCl_3$): ν_{max} 3364 (OH), 1720 (C═O), 1652 (C═C). ^{1}H NMR ($CDCl_3$): δ 2.68 (bs, 2 H, exchangeable with D_2O, 2 × OH), 3.76 (s, 3 H, CO_2Me), 3.82 (dd, 1 H, $J_{4,5}$ = 4.9, $J_{5,6}$ = 1.6 Hz, H-5), 4.19 (dd, 1 H, $J_{5,6}$ = 1.6, $J_{6,7}$ = 8.5 Hz, H-6), 4.58 (td, 1 H, $J_{2,4}$ = 1.7, $J_{3,4}$ = 4.7, $J_{4,5}$ = 4.9 Hz, H-4), 4.60 and 4.67 (2 × d, 2 H, J_{gem} = 10.9 Hz, PhC*H*$_2$), 5.99 (d, 1 H, $J_{6,7}$ = 8.5 Hz, H-7), 6.20 (dd, 1 H, $J_{2,3}$ = 15.7, $J_{2,4}$ = 1.7 Hz, H-2), 7.08 (dd, 1 H, $J_{2,3}$ = 15.7, $J_{3,4}$ = 4.7 Hz, H-3), 7.23–8.09 (m, 15 H, 3 × Ph). ^{13}C NMR ($CDCl_3$): δ 51.6 (CO_2*Me*), 72.1 (C-4), 73.5 (C-6), 75.0 (PhCH_2), 75.6 (C-7), 79.0 (C-5), 121.4 (C-2), 127.5, 128.2, 128.4, 128.5, 129.6, 133.3, 137.0 and 137.4 (3 × Ph), 146.7 (C-3), 165.1 and 166.7 (2 × C═O). HRMS (ESI): Found: 499.1723 (M^+ + Na), calcd for $C_{28}H_{28}NaO_7$: 499.1727.

4.1.11. Methyl 7-O-benzoyl-2,3-dideoxy-7-C-phenyl-D-gluco-heptonate (*18*)

A solution of **17** (0.083 g, 0.17 mmol) in dry MeOH (2 mL) was hydrogenated over 10% Pd/C (0.036 g) for 4 h at room temperature. The mixture was filtered and the catalyst washed with EtOH. The organic solution was evaporated and the residue was purified by flash column chromatography (1:1 Et_2O/light petroleum) to afford pure **18** (0.049 g, 72%) as a colourless syrup, $[\alpha]^{20}_D$ +14.5 (*c* 0.92, $CHCl_3$), R_f = 0.43 (Et_2O). IR ($CHCl_3$): ν_{max} 3442 (OH), 1721 (C═O). ^{1}H NMR ($CDCl_3$): δ 1.85 (m, 2 H, $J_{2,3}$ = 7.1, $J_{3,4}$ = 6.1 Hz, H-3), 2.47 (t, 2 H, $J_{2,3}$ = 7.1 Hz, H-2), 3.32 (bs, 3 H, exchangeable with D_2O, 3 × OH), 3.63 (m, 1 H, $J_{4,5}$ = 4.3, $J_{5,6}$ = 0.9 Hz, H-5), 3.64 (s, 3 H, CO_2Me), 3.81 (m, 1 H, $J_{3,4}$ = 6.1, $J_{4,5}$ = 4.3 Hz, H-4), 4.11 (dd, 1 H, $J_{5,6}$ = 0.9, $J_{6,7}$ = 8.2 Hz, H-6), 6.03 (d, 1 H, $J_{6,7}$ = 8.2 Hz, H-7), 7.29–8.16 (m, 10 H, 2 × Ph). ^{13}C NMR ($CDCl_3$): δ 28.4 (C-3), 30.1 (C-2), 51.7 (CO_2*Me*), 71.3 (C-5), 72.6 (C-4), 74.7 (C-6), 75.6 (C-7), 127.5, 128.4, 128.5, 128.53, 129.6, 129.7, 133.3 and 137.4 (2 × Ph), 165.6 (Ph*C*═O), 174.62 (*C*O_2Me). HRMS (ESI): Found: 411.1404 (M^+ + Na), calcd for $C_{21}H_{24}NaO_7$: 411.1414.

4.1.12. 2,3-Dideoxy-7-C-phenyl-D-gluco-heptono-1,4-lactone (3-deoxycardiobutanolide, *4*)

A solution of **18** (0.035 g, 0.1 mmol) in 0.1 M methanolic NaOMe (0.2 mL, 0.02 mmol) was stirred for 1 h at room temperature, then acidified with 2:1 aq. TFA (0.003 mL) and concentrated by co-distillation with toluene. Flash column chromatography (1:1 CH_2Cl_2/EtOAc) of the residue gave pure **4** (0.016 g, 69%) as a solid. Recrystallization from CH_2Cl_2/hexane gave colourless needles, mp 108–112 °C, $[\alpha]^{20}_D$ −11.1 (*c* 0.44, $CHCl_3$), R_f = 0.34 (EtOAc). IR (KBr): ν_{max} 3407 (OH), 1760 (C═O). ^{1}H NMR (acetone-d_6 + D_2O): δ 1.80 (m, 1 H, H-3a), 2.17–3.36 (m, 1 H, H-3b), 2.39–2.60 (m, 2 H, H-2), 3.67 (dd, 1 H, $J_{5,6}$ = 2.0, $J_{6,7}$ = 7.7 Hz, H-6), 3.89 (dd, 1 H, $J_{4,5}$ = 6.3, $J_{5,6}$ = 2.0 Hz, H-5), 4.65 (m, 1 H, $J_{4,5}$ = 6.3 Hz, H-4), 4.73 (d, 1 H, $J_{6,7}$ = 7.7 Hz, H-7), 7.14–7.43 (m, 5 H, Ph). ^{13}C NMR (acetone-d_6): δ 24.7 (C-2), 29.0 (C-3), 73.2 (C-5), 74.0 (C-6), 74.5 (C-7), 83.2 (C-4), 127.8, 127.84, 128.5 and 143.6 (Ph), 179.0 (C-1). HRMS (ESI): Found: 275.0890 (M^+ + Na), calcd for $C_{13}H_{16}NaO_5$: 275.0890.

4.1.13. 3,6-Anhydro-7-O-benzoyl-5-O-benzyl-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (*19*)

Procedure A. To a cooled (−20 °C) and stirred solution of **16** (0.240 g, 0.57 mmol) in dry MeOH (9.6 mL), was added Ph_3P═$CHCO_2Me$ (0.385 g, 1.14 mmol) in four equal portions. The mixture was stirred for 1 h at −20 °C then for 22 h at room temperature after which an additional quantity of Ph_3P═$CHCO_2Me$ (0.292 g, 0.87 mmol) was added. The mixture was stirred for additional 25 h at room temperature, and then evaporated. The residue was purified by flash column chromatography (7:3 light petroleum/EtOAc)

to give **19** (0.132 g, 52%) as a solid. Recrystallization from CH_2Cl_2/hexane afforded colourless needles, mp 163–165 °C, $[\alpha]^{20}_D$ −54.2 (*c* 0.91, $CHCl_3$); R_f = 0.46 (7:3 light petroleum/EtOAc).

Procedure B. Partially protected lactol derivative **16** (0.603 g, 1.43 mmol) and Meldrum's acid (0.286 g, 1.98 mmol) were dissolved in dry DMF (6.5 mL). Dry triethyl amine (0.4 mL, 2.20 mmol) was added to the reaction mixture and stirred at 46 °C for 65 h. The volatiles were removed and the residue was purified by flash column chromatography (9:1 toluene/EtOAc). Pure **19** (0.371 g, 58%) was thus obtained as a solid. Recrystallization from CH_2Cl_2/hexane gave colourless needles, mp 163–165 °C, $[\alpha]^{20}_D$ −54.2 (*c* 0.91, $CHCl_3$); R_f = 0.46 (7:3 light petroleum/EtOAc). IR (KBr): ν_{max} 1788 (C═O, lactone), 1721 (PhC═O). ^{1}H NMR ($CDCl_3$): δ 2.58 (d, 1 H, $J_{2a,2b}$ = 18.3 Hz, H-2a), 2.70 (dd, 1 H, $J_{2a,2b}$ = 18.3, $J_{2b,3}$ = 4.6 Hz, H-2b), 4.34 (d, 1 H, $J_{5,6}$ = 3.4 Hz, H-5), 4.49 and 4.61 (2 × d, 2 H, J_{gem} = 11.6 Hz, PhCH_2), 4.54 (dd, 1 H, $J_{5,6}$ = 3.4, $J_{6,7}$ = 9.2 Hz, H-6), 4.93–5.01 (m, 2 H, H-3 and H-4), 6.21 (d, 1 H, $J_{6,7}$ = 9.2 Hz, H-7), 7.12–8.02 (m, 15 H, 3 × Ph). ^{13}C NMR ($CDCl_3$): δ 35.9 (C-2), 72.6 (C-7), 73.0 (PhCH_2), 77.4 (C-3), 80.2 (C-5), 82.1 (C-6), 84.5 (C-4), 127.5, 128.2, 128.3, 128.4, 128.5, 128.52, 129.6, 129.9, 133.1, 136.2 and 137.9 (3 × Ph), 164.5 (PhC═O), 175.2 (C-1). LRMS (CI): *m/z* 445 ($M^+ + H$). Anal. Found: C, 72.41; H, 5.18. Calcd for $C_{27}H_{24}O_6 \cdot 0.2H_2O$: C, 72.37; H, 5.49.

4.1.14. 3,6-Anhydro-7-O-benzoyl-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (*5*)

To a solution of **19** (0.041 g, 0.09 mmol) in EtOAc (2 mL) was added concetrated HCl (0.02 mL) and 10% Pd/C (0.0164 g). The suspension was hydrogenated for 44 h at room temperature, then filtrated through a celite pad and evaporated. Chromatographic separation on a column of flash silica (7:3 toluene/EtOAc) gave two compounds; eluted first was the major product **5** (0.025 g, 76%) that was isolated as a colourless oil, $[\alpha]^{20}_D$ +50.0 (*c* 1.0, $CHCl_3$), R_f = 0.25 (19:1 CH_2Cl_2/EtOAc). IR ($CHCl_3$): ν_{max} 3454 (OH), 1787 (C═O, lactone), 1722 (PhC═O). ^{1}H NMR ($CDCl_3$): δ 1.30 (bs, 1 H, exchangeable with D_2O, OH), 2.55 (d, 1 H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.67 (dd, 1 H, $J_{2a,2b}$ = 18.8, $J_{2b,3}$ = 5.1 Hz, H-2b), 4.32 (dd, 1 H, $J_{5,6}$ = 2.1, $J_{6,7}$ = 9.2 Hz, H-6), 4.45 (d, 1 H, $J_{5,6}$ = 2.1 Hz, H-5), 4.95–5.04 (m, 2 H, H-3 and H-4), 6.12 (d, 1 H, $J_{6,7}$ = 9.3 Hz, H-7), 7.35–8.14 (m, 10 H, Ph). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 73.1 (C-5), 73.2 (C-7), 77.1 (C-3), 82.5 (C-6), 87.0 (C-4), 127.6, 128.6, 128.7, 128.9, 129.9, 133.9 and 136.8 (Ph), 166.9 (PhC═O), 175.4 (C-1). HRMS (ESI): Found: 377.0984 ($M^+ + Na$), calcd for $C_{20}H_{18}NaO_6$: 377.0996. Eluted second was pure **6** (0.005 g, 22%) which was isolated as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave needles, mp 139 °C, $[\alpha]^{20}_D$ +49.52 (*c* 0.62, $CHCl_3$), R_f = 0.24 (4:1 CH_2Cl_2/EtOAc); lit. [32] mp 101–103 °C, $[\alpha]^{20}_D$ +37.0 (*c* 0.14, $CHCl_3$). For IR, NMR and HRMS data of **6** see Section 4.1.18.

4.1.15. 3,5-Anhydro-1,2-O-cyclohexylidene-α-D-xylofuranose (*20*)

To a cooled (0°C) and stirred solution of **7** (0.173 g, 0.75 mmol) and Ph_3P (0.488 g, 1.88 mmol) in dry toluene (2 mL) was added DEAD (0.3 mL, 1.88 mmol) dropwise over a period of 2–3 min. The cooling bath was replaced by an oil bath, the mixture was stirred under reflux for 2.5 h and then evaporated. Flash column chromatography (^{i}Pr$_2$O) of the residue gave pure **20** (0.126 g, 73%) as a colourless oil, $[\alpha]^{20}_D$ +16.6 (*c* 0.6, $CHCl_3$), R_f = 0.8 (4:1 toluene/EtOAc); lit. [29] $[\alpha]^{20}_D$ +17.4 (*c* 0.66, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.30–1.71 (m, 10 H, C_6H_{10}), 4.15 (ddd, 1 H, $J_{5a,5b}$ = 7.6 Hz, $J_{3,5a}$ = 0.5 Hz, $J_{4,5a}$ = 2.4 Hz, H-5a), 4.59–4.70 (m, 2 H, $J_{1,2}$ = 3.6 Hz, $J_{4,5b}$ = 4.1 Hz, H-2 and H-5b), 5.01 (m, 1 H, H-4), 5.1 (d, 1 H, $J_{3,4}$ = 4.0 Hz, H-3), 6.18 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1). ^{13}C NMR ($CDCl_3$): δ 23.3, 23.5, 24.5, 36.2 and 37.1 (C_6H_{10}), 77.9 (C-4), 78.0 (C-5), 83.9 (C-2), 87.3 (C-3), 107.5 (C-1), 114.0 (Cq from C_6H_{10}). LRMS (CI): *m/z* 212 (M^+).

4.1.16. 1,2-O-cyclohexylidene-5-deoxy-5-C-phenyl-α-D-xylo-pentofuranose (*21*)

To a stirred solution of **20** (0.659 g, 3.1 mmol) in dry THF (1.7 mL) was added a 3 M solution of PhMgBr in Et_2O (2 mL, 6 mmol). The mixture was stirred under reflux in an atmosphere of nitrogen for 8.5 h, then quenched with 10% aq. NH_4Cl (45 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The combined extracts were washed with 10% aq NaCl (45 mL), dried and evaporated. The residue was purified by flash column chromatography (17:3 toluene/EtOAc), to give pure **21** (0.795 g, 85%) as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave an analytical sample **21**, mp 119–120 °C, $[\alpha]^{20}_D$ −2.5 (*c* 0.5, $CHCl_3$), R_f = 0.23 (1:1 hexane/Et_2O). IR (KBr): ν_{max} 3423 (OH). ^{1}H NMR ($CDCl_3$): δ 1.32–1.74 (m, 10 H, C_6H_{10}), 1.85 (d, 1 H, exchangeable with D_2O, $J_{3,OH}$ = 6.6 Hz, OH-3), 3.00 (dd, 1 H, $J_{5a,5b}$ = 13.6, $J_{4,5a}$ = 8.5 Hz, H-5a), 3.08 (dd, 1 H, $J_{5a,5b}$ = 13.6, $J_{4,5b}$ = 6.3 Hz, H-5b), 4.00 (d, 1 H, $J_{3,4}$ = 2.3 Hz, H-3), 4.40 (ddd, 1 H, $J_{3,4}$ = 2.3, $J_{4,5a}$ = 8.5, $J_{4,5b}$ = 6.3 Hz, H-4), 4.51 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-2), 5.96 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1), 7.22–7.39 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 23.5, 23.8, 24.9, 35.5 and 36.1 (5 × CH_2), 33.9 (C-5), 74.8 (C-3), 81.1 (C-4), 84.7 (C-2), 104.0 (C-1), 112.2 (Cq from C_6H_{10}), 126.5, 128.6, 129.2 and 137.5 (Ph). LRMS (ESI): *m/z* 329 ($M^+ + K$), 313 ($M^+ + Na$), 291 ($M^+ + H$). Anal. Found: C, 67.93; H, 7.42. Calcd for $C_{17}H_{22}O_4 \cdot 0.5\ H_2O$: C, 68.21; H, 7.72.

4.1.17. 5-Deoxy-5-C-phenyl-D-xylo-pentofuranose (*22*)

A solution of **21** (0.767 g, 2.64 mmol) in 70% aq. AcOH (28 mL) was stirred for 3 h under reflux. The workup as described above (Section 4.1.2.) followed by chromatographic separation on a column of flash silica (3:2 EtOAc/toluene) gave two fractions. A minor amount of starting compound **21** (0.099 g, 13%) was first eluted, followed by major product **22** (0.270 g, 49%) isolated as a colourless solid. Recrystallization from EtOAc/hexane gave transparent needles, mp 126–128 °C, $[\alpha]^{20}_D$ +24.2 → +16.2 (*c* 0.99, MeOH, 72 h), R_f = 0.49 (EtOAc). IR (KBr): ν_{max} 3410 (OH). ^{1}H NMR (Methanol-d_4): δ 2.75–3.13 (m, 2.5 H, 2 × H-5α and β), 3.81–4.8 (m, 3.75 H, H-2, H-3, H-4α and β), 5.06 (s, 0.25 H, H-1β), 5.41 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1α), 7.09–7.37 (m, 6 H, Ph). ^{13}C NMR (Methanol-d_4): δ 36.3 and 37.0 (C-5α and β), 76.8, 77.6, 78.2, 81.4, 82.5, 84.4 (C-2, C-3, C-4, both α- and β-anomers), 97.6 (C-1α), 104.2 (C-1β), 127.1, 129.2, 130.32, 140.1, 140.3 (Ph). HRMS (ESI−): Found: 209.0812 ($M^- - H$), calcd for $C_{11}H_{13}O_4$: 209.0819. Anal. Found: C, 62.44; H, 6.51. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71.

4.1.18. 3,6-Anhydro-2,7-dideoxy-7-C-phenyl-D-gluco-heptono-1,4-lactone (*6*)

To a solution of **22** (0.09 g, 0.43 mmol) in dry DMF (0.9 mL) was added Meldrum's acid (0.117 g, 0.81 mmol) and dry Et_3N (0.12 mL, 0.86 mmol). The mixture was stirred at 46 °C for 48 h and then evaporated. Chromatographic purification on a column of flash silica (9:1 CH_2Cl_2/EtOAc) gave pure **6** (0.052 g, 52%) as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave an analytical sample **6**, mp 139 °C, $[\alpha]^{20}_D$ +49.5 (*c* 0.62, $CHCl_3$), R_f = 0.24 (4:1 CH_2Cl_2/EtOAc); lit. [32] mp 101–103 °C, $[\alpha]^{20}_D$ +37.0 (*c* 0.14, $CHCl_3$). IR (KBr): ν_{max} 3438 (OH), 1784 (C═O). ^{1}H NMR ($CDCl_3$): δ 2.64 (d, 1 H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.78 (dd, 1 H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 6.1 Hz, H-2b), 2.97 (dd, 1 H, $J_{7a,7b}$ = 13.6, $J_{6,7a}$ = 7.3 Hz, H-7a), 3.04 (dd, 1 H, $J_{7a,7b}$ = 13.6, $J_{6,7b}$ = 6.8 Hz, H-7b), 4.15–4.25 (m, 2 H, H-5 and H-6), 4.90 (d, 1 H, $J_{3,4}$ = 4.7 Hz, H-4), 5.02 (dd, 1 H, $J_{2b,3}$ = 6.1, $J_{3,4}$ = 4.7 Hz, H-3), 7.19–7.40 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$): δ 34.2 (C-7), 35.8 (C-2), 74.1 (C-5), 76.1 (C-3), 81.4 (C-6), 87.6 (C-4), 126.7, 128.5, 129.0 and 137.2 (Ph), 175.6 (C-1). LRMS (CI): *m/z* 469 ($2M^+ + H$), 235 ($M^+ + H$). HRMS (ESI): Found: 235.0954 ($M^+ + H$), calcd for $C_{13}H_{15}O_4$: 235.0965.

4.2. In vitro antitumour assay

Exponentially growing cells were harvested, counted by trypan blue exclusion and plated into 96-well microtitar plates (Costar) at optimal seeding density of 10^4 (K562, HL-60, Jurkat and Raji) or 5×10^3 (HeLa and MRC-5) cells per well to assure logarithmic growth rate throughout the assay period. Antiproliferative activity was evaluated by the tetrazolium colorimetric MTT assay, after exposure of cells to the tested compounds for 72 h, following the recently reported procedure [34].

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