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Design, synthesis and SAR analysis of antitumour styryl lactones related to (+)-crassalactones B and C

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ABSTRACT

A series of styryl lactones containing the cinnamic acid ester groups such as (+)-crassalactones B (**3a**) and C (**4a**), 5,7-di-*O*-cinamoyl derivative **6**, the corresponding 7-epimers and 7-deoxy derivatives have been synthesized, characterized and evaluated for their in vitro antitumour activity against a panel of several human tumour cell lines. Twelve new analogues such as 5-*O*- or 7-*O*-(4-methoxycinnamoyl), 5-*O*- or 7-*O*-(4-nitrocinnamoyl) and 5-*O*- or 7-*O*-(4-fluorocinnamoyl) esters of (+)-goniofufurone (**3b**–**d**), 7-*epi*-(+)-goniofufurone (*epi*-**3b**–**d**), as well as 7-deoxy derivatives **5b**–**d** have been prepared to correlate all compounds in a SAR study. Some of the analogues displayed powerful antiproliferative effects on selected human tumour cell lines, but none of them demonstrated cytotoxicity towards the normal foetal lung fibroblasts (MRC-5). Thus, for the 7-*epi*-crassalactone B (*epi*-**3a**) was found to be a potent inhibitor of HL-60 cells growth, with an IC_{50} value that is approximately 46-fold lower than that observed for the commercial antitumour drug doxorubicin in the culture of the same cells. A SAR analysis performed on these lactones reveals the main structural features that affect their antiproliferative activity, such as nature of the substituents at the C-4 in the aromatic rings of cinnamoyl moieties, the absolute stereochemistry, as well as the presence of a deoxy function at the C-7 position.

1. Introduction

Cinnamic acid, its natural analogues and derivatives are known for the treatment of cancer for over centuries [1]. Among its derivatives, the cinnamoates represent a well known class of anticancer agents. The variation in esters arises from both natural resources as well as synthesized compounds. A lot of naturally occurring and biologically active alcohols have been linked with cinnamoyl residues through the ester linkage to amend their anticancer efficacy. A number of styryl lactones have been recently isolated from the tropical plant *Polyalthia crassa* [2]. The bioassay-directed fractionation of the ethyl acetate extract of its leaves and twigs led to the isolation of the known antitumour styryl lactone (+)-goniofufurone (**1**, Fig. 1) that was, along with its stereoisomer 7-*epi*-(+)-goniofufurone (**2**), previously isolated from the stem bark of *Goniothalamus giganteus* (Annonaceae) by McLaughlin and co-workers [3,4], and found to exhibit notable antiproliferative effects toward a number of human tumour cell lines [3–8]. Several new cytotoxic compounds, including (+)-crassalactones B (**3a**) and C (**4a**), which represent the 5-*O*- or the 7-*O*-cinnamoyl esters of **1**, have also been isolated and fully characterized by spectroscopic methods [2]. We have recently reported several synthetic routes to (+)-crassalactone C (**4a**) starting from D-xylose [9–11], as well as a preliminary account related the first total synthesis of (+)-crassalactone B (**3a**) starting from D-glucose [12]. Immediately thereafter a new total synthesis of (+)-crassalactones B and C starting from diacetone D-glucose [13], as well as a novel enantiospecific total synthesis of (−)-crassalactone C from tartaric acid [14] has been reported by other groups.

In this work, we report efficient semi-syntheses of (+)-crassalactones B (**3a**) and C (**4a**), as well as the corresponding 5,7-dicinamoates **6** and *epi*-**6** (the structures not shown in Fig. 1), starting from (+)-goniofufurone (**1**) or from 7-*epi*-

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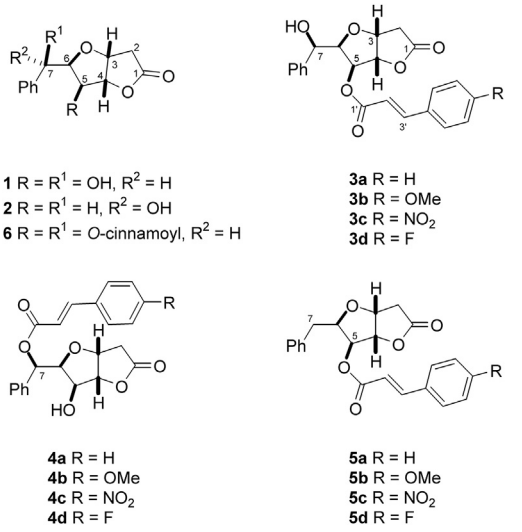


Fig. 1. Structures of (+)-goniofufurone (**1**), 7-*epi*-(+)-goniofufurone (**2**), (+)-crassalactones B (**3a**) and C (**4a**), and the corresponding analogues **3b**–**d**, **4b**–**d** and **5a**–**d**.

(+)-goniofufurone (**2**), respectively. A number of new analogues such as **3b**–**d**, **4b**–**d**, the corresponding 7-epimers such as *epi*-**3a**–**d** and *epi*-**4a**–**d** (the structures not shown in Fig. 1) and 7-deoxy derivatives such as **5a**–**d** have also been prepared and their effects to the proliferation of some human malignant cell lines have been evaluated.

2. Results and discussion

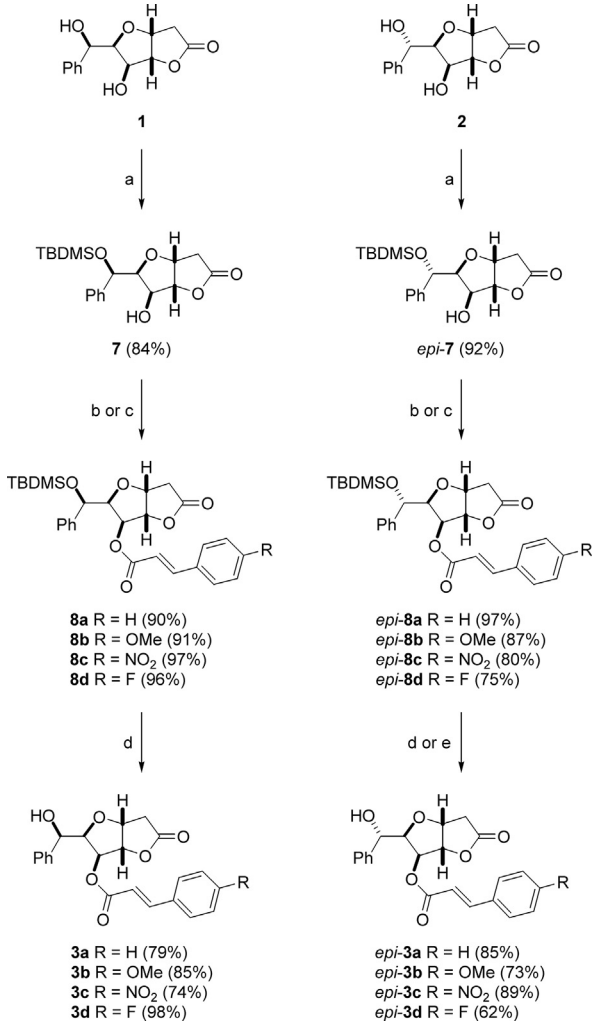
2.1. Chemistry

The conversion of (+)-goniofufurone (**1**) to (+)-crassalactone B (**3a**), and 7-*epi*-(+)-goniofufurone (**2**) to 7-*epi*-(+)-crassalactone B (*epi*-**3a**), is presented in Scheme 1. An efficient total synthesis of starting natural products **1** and **2** was recently completed in our laboratory starting from D-glucose [15]. This procedure enables an efficient and fast access to **1** and **2**, which fully justifies their use as starting materials in a new synthesis of **3a** and *epi*-**3a**, as well as in the synthesis of a number of some new analogues thereof. The benzylic hydroxyl group in **1** was selectively blocked by treatment with *tert*-butyldimethylsilyl chloride whereby the key intermediate **7** was obtained in 84% yield. A minor amount of starting compound **1** (6%), along with ~10% of the corresponding *bis*-silyl ether (the structure not shown) was also isolated. Subsequent treatment of crude bis-silylated side-product with aqueous TFA afforded an additional amount of recovered starting compound **1** so that its total yield was increased to 14%. This further means that the desired intermediate **7** was obtained in 95% yield calculated to reacted starting material **1**. Treatment of **7** with cinnamoyl chloride (DMAP, MeCN) gave the corresponding cinnamic ester **8a** in 90% yield. Moreover, treatment of **8a** with aqueous TFA in CH_2Cl_2 gave a 79% yield of (+)-crassalactone B (**3a**), along with 15% of starting compound **8a**. The natural product **3** was thus obtained in 93% yield calculated to reacted intermediate **8a** (80% overall from **1**). Our optical rotation value recorded in EtOH $[\alpha]^{20}_D$ +45.7 (*c* 0.5)} is significantly greater than the reported value for the isolated (+)-crassalactone B {lit. [2] $[\alpha]^{20}_D$ +8.0 (*c* 0.5, EtOH)}. However, the optical rotation data recorded in $CHCl_3$ {$[\alpha]^{20}_D$ +35.5 (*c* 1.0)} was in reasonable agreement with the value [13] reported for synthetic sample **3a** {lit. [13] $[\alpha]^{20}_D$ +31.6 (*c* 1.0, $CHCl_3$)}. The melting point, ^1H and ^{13}C NMR spectroscopic data [16] of synthesized natural product **3a** were in full agreement with those reported in the literature for both isolated [2] and synthetic [13] sample **3a**.

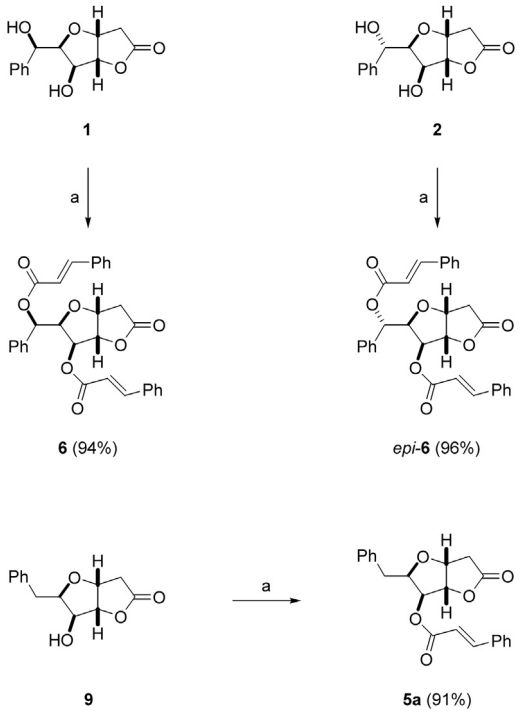
The same three-step sequence was then applied for the preparation of 7-*epi*-(+)-crassalactone B (*epi*-**3a**) starting from natural product **2**. Thus, treatment of **2** with *tert*-butyldimethylsilyl chloride gave the expected 7-*O*-silyl ether *epi*-**7** (92%) which was subsequently converted to the corresponding cinnamoate *epi*-**8a** in 97% yield. Final treatment of *epi*-**8a** with aqueous TFA afforded 7-*epi*-(+)-crassalactone B (*epi*-**3a**) in 85% yield. The target *epi*-**3a** was thus obtained in an overall yield of 76% over the last three synthetic steps.

Treatment of **7** and *epi*-**7** with 4-methoxy-, 4-nitro-, or 4-fluorocinnamic acid, by using the Steglich esterification protocol [17] gave the expected 4-substituted cinnamic ester derivatives in good to excellent yields. Generally, the cinnamic esters of (7*R*)-absolute configuration (**8b**–**d**) were formed in somewhat higher yields (91, 97 and 96%, respectively) compared to those of (7*S*)-absolute configuration (*epi*-**8b**, *epi*-**8c** and *epi*-**8d**) that were obtained in 87, 80 and 75% yields respectively, presumably from the steric reasons. Desilylation of **8b**–**d** with aqueous trifluoroacetic acid in dichloromethane finally afforded the desired analogues **3b** (85%), **3c** (74%) and **3d** (98%), respectively. However, when the 7-epimers (*epi*-**8b**, *epi*-**8c** and *epi*-**8d**) were treated under the same reaction conditions (TFA, CH_2Cl_2/H_2O), the corresponding 7-*epi*-(+)-crassalactone C derivatives (*epi*-**4b**, *epi*-**4c** and *epi*-**4d**) were obtained as the major reaction products, accompanied by a minor amounts of desired analogues *epi*-**3b**, *epi*-**3c** and *epi*-**3d**. The major products *epi*-**4b**, *epi*-**4c** and *epi*-**4d** were presumably formed by a competitive intramolecular cinnamate migration process, as observed in our previous work when TBAF was used for removal of the TBDPS protective group [12]. On the basis of the results obtained for TBDMS removal by TFA, alternative acidic conditions using ceric ammonium nitrate (CAN) were employed for this reaction instead. These conditions previously prevented completely the acetyl migration in certain furanoside derivatives [18]. We were pleased to find that all three silyl ethers *epi*-**8b**, *epi*-**8c** and *epi*-**8d**, upon the TBDMS deprotection with CAN in aqueous acetonitrile afforded only the desired 5-*O*-cinnamoyl derivatives *epi*-**3b**, *epi*-**3c** and *epi*-**3d**, respectively. No migrated product could be detected in crude reaction mixtures by ^1H NMR. The desilylated products *epi*-**3b** and *epi*-**3d** were isolated in pure form after silica gel flash column chromatography in respective yields of 73 and 62%. However, the attempted purification of 7-*O*-(4-nitrocinnamoyl) derivative *epi*-**3c** on a column of flash silica predominantly gave the rearranged product *epi*-**4c** (69%) accompanied with a minor amount of desired *epi*-**3c** (19%). Therefore the chromatographic purification was skipped, and pure *epi*-**3c** was isolated in 89% yield after crystallization of crude reaction mixture from a mixture CH_2Cl_2/hexane. Both intramolecular and intermolecular migrations of alkyloxycarbonyl groups during chromatographic purification on a silica gel column were recently reported [19].

The synthesis of 5,7-di-*O*-cinnamoyl derivatives **6** and *epi*-**6** is outlined in Scheme 2. Thus, treatment of **1** with cinnamoyl chloride in anhydrous acetonitrile, in the presence of DMAP as a proton acceptor, gave the known [13] dicinnamoate **6** in 94% yield. Physical constants and spectroscopic data of thus prepared sample **6** matched those previously reported [13,16]. The same synthetic protocol was then used for the conversion of **2** in *epi*-**6** which was isolated after flash column chromatography in an almost quantitative yield (96%). Finally, 7-deoxy derivative **9** was treated under the similar reaction conditions, in order to prepare the 5-*O*-cinnamoyl derivative **5a**. The reason for the preparation of analogue **5a** arises from our recent study [20] that revealed that 7-deoxy derivative **9** demonstrates potent antiproliferative activities against



Scheme 1. Reagents and conditions: (a) $^{t}BuMe_2SiCl$, imidazole, anh. MeCN, rt, 26 h for **1**, 20 h for **2**; (b) cinnamoyl chloride, DMAP, anh. CH_2Cl_2, 0.5 h at 0 °C, then 30 h at rt; (c) 4-substituted cinnamic acid, DCC, DMAP, anh. CH_2Cl_2, rt, 48 h (d) CH_2Cl_2/TFA/H_2O 50:5:1, rt, 50 h for **8a**, 22 h for *epi*-**8a**; (e) CAN, 9:1 MeCN/H_2O, rt, 120 h for *epi*-**8b**, 72 h for *epi*-**8c**, 26 h for *epi*-**8d**.



Scheme 2. Reagents and conditions: (a) cinnamoyl chloride, DMAP, anh. MeCN, 0.5 h at 0 °C, then 30 h at rt.

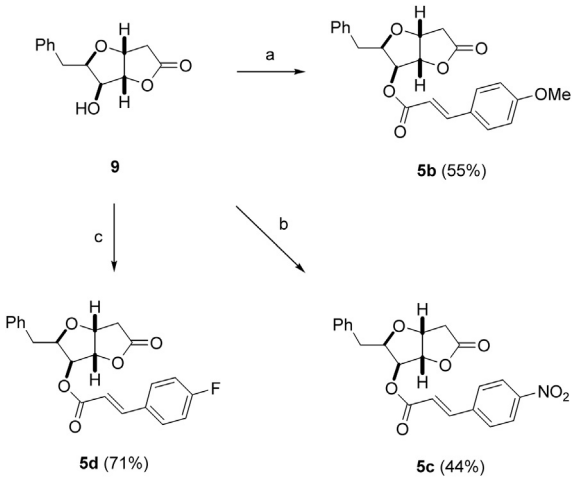
several human tumour cell lines. Accordingly compound **9** was allowed to react with cinnamoyl chloride, under the above described reaction conditions, to afford a 91% yield of 5-*O*-cinnamoyl derivative **5a**.

In another reaction we studied the esterification of 7-deoxy derivative **9** with 4-methoxy-, 4-nitro-, or 4-fluorocinnamic acid, under the standard Steglich conditions [17] to obtain a few novel isosteres of **5a** such as compounds **5b**–**d** (Scheme 3). Accordingly, a treatment of **9** with 4-methoxycinnamic acid in anhydrous CH_2Cl_2, in the presence of DCC and DMAP, gave the expected 5-*O*-(4-methoxycinnamoyl) derivative **5b** in 55% yield. Compound **9** reacted with 4-nitro- or 4-fluorocinnamic acid, under the same reaction conditions, to afford the corresponding 4-substituted cinnamoates **5c** and **5d** in 44 and 71% yields, respectively.

Mitsunobu-type reaction is an efficient method to introduce ester groups with Walden inversion at the secondary carbon atom [21–23]. We had intended to apply this reaction for the preparation of 7-*O*-cinnamoyl derivatives of (+)-goniofufurone (**1**) and 7-*epi*-(+)-goniofufurone (**2**) (Scheme 4). As it is well known that the outcome of the Mitsunobu reaction depends on the solvent used [22], we first studied the influence of several solvents and found that the best yields of the desired cinnamoates were obtained when the reaction is performed in anhydrous ethyl acetate [16].

Thus, treatment of 7-*epi*-(+)-goniofufurone **2** with cinnamic acid or its 4-substituted derivatives, under the typical Mitsunobu reaction conditions (DEAD, Ph_3P, EtOAc), gave targets **4a** (69%) **4b** (60%), **4c** (66%) and **4d** (66%) as the major reaction products. A high regio- and stereoselectivity was observed whereby the cinnamic ester group was introduced exclusively at the C-7 position, with Walden inversion at this stereocentre. A minor amount of oxetane **10** (31%)[1] was formed in all of these reactions, presumably due to a competitive intramolecular Mitsunobu dehydration. The physical constants and the spectral data of thus obtained sample **10** were consistent with those previously reported by us [10]. By using a similar methodology, (+)-goniofufurone was converted to the corresponding 7-*epi*-cinnamoates, *epi*-**4a** (59%), *epi*-**4b** (42%), *epi*-**4c** (52%) and *epi*-**4d** (56%) as major products, accompanied with minor amounts of oxetane *epi*-**10** (25, 34, 32 and 35%, respectively). The assignment of stereochemistry at the C-7 in *epi*-**10** was confirmed by an NOE interaction between H-3 and a proton from the aromatic ring (Ar–H), indicating that H-3 and Ph group are mutually *syn* oriented, on the same side of the 2,6-dioxa-bicyclo [3.2.0]heptane ring system. The geometry of *epi*-**10** was unambiguously confirmed by X-ray crystallographic analysis (for the crystal structure of *epi*-**10**, see the Supplementary data). The distance between H-3 and Ar–H (2.48 Å) in the molecular structure of *epi*-**10** is consistent with NOE results.

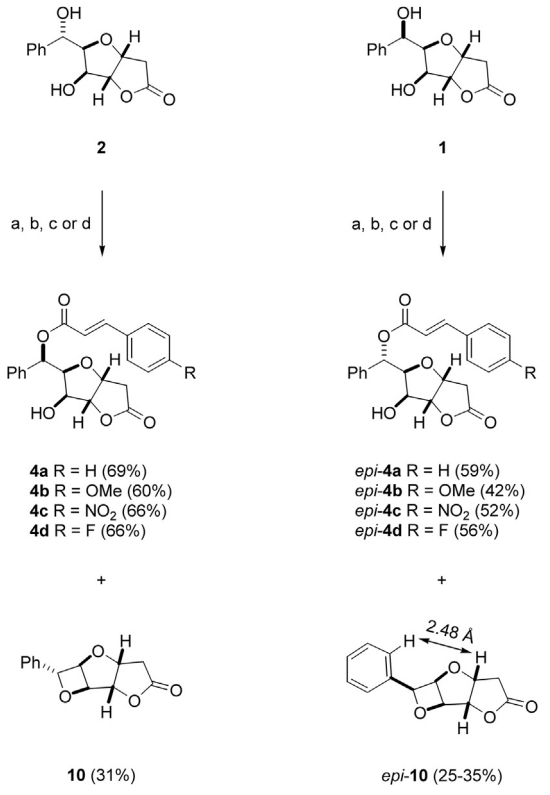
[1] In several experiments that were performed in solvents other than ethyl acetate (THF or CH_2Cl_2), the oxetanes **10** or *epi*-**10** were isolated as major reaction products (see the Supplementary data for details).



Scheme 3. Reagents and conditions: (a) 4-methoxycinnamic acid, DCC, DMAP, anh. CH_2Cl_2, rt, 22 h; (b) 4-nitrocinnamic acid, DCC, DMAP, anh. CH_2Cl_2, rt, 20 h; (c) 4-fluorocinnamic acid, DCC, DMAP, anh. CH_2Cl_2, rt, 20 h.

2.2. Biological evaluation and SAR

After completion of the synthesis, all synthesized compounds were evaluated for their in vitro cytotoxic activity against a panel of seven human malignant cell lines, including myelogenous leukaemia (K562), promyelocytic leukaemia (HL-60), T cell leukaemia (Jurkat), Burkitt's lymphoma (Raji), oestrogen receptor positive breast adenocarcinoma (MCF-7), oestrogen receptor negative breast adenocarcinoma (MDA-MB-231), cervix carcinoma (HeLa) and against a single normal cell line, foetal lung fibroblasts (MRC-5). Cytotoxic activity was evaluated by using the standard MTT assay, after exposure of cells to the tested compounds for 72 h. The commercial antitumour agent doxorubicin (DOX) was used as a positive control in this assay. The results are presented in Table 1.



Scheme 4. Reagents and conditions: (a) cinnamic acid, DEAD, Ph_3P, EtOAc, 1 h at 0 °C, then 3 h at rt; (b) 4-methoxycinnamic acid, DEAD, Ph_3P, EtOAc, 1 h at 0 °C, then rt, 2 h for **4b**, 24 h for *epi*-**4b**; (c) 4-nitrocinnamic acid, DEAD, Ph_3P, EtOAc, 1 h at 0 °C, then rt, 2 h for **4c**, 4 h for *epi*-**4c**; (d) 4-fluorocinnamic acid, DEAD, Ph_3P, EtOAc, 1 h at 0 °C, then rt 2 h for **4d**, 4 h for *epi*-**4d**.

According to the resulting IC_{50} values of the cytotoxic assay (Table 1), the K562, Jurkat, Raji and HeLa cell lines are sensitive to all of the synthesized analogues. The highest potency in the culture of K562 cells was recorded after treatment with deoxygenated isostere **5d** (IC_{50} 0.25 μM), which demonstrated the same potency as the commercial drug doxorubicin in the same cell line. This analogue was nearly 19-fold more active than the parent compound **5a** in the culture of same cells. The potent antiproliferative activity in the low micromolar range was recorded after treatment of K562 cells with analogues *epi*-**3c** (IC_{50} 1.12 μM), **5b** (IC_{50} 1.02 μM) and **5c** (IC_{50} 0.98 μM). With the exception of natural product **4a** all remaining compounds show strong growth inhibition of HL-60 tumour cells. The most active compound against this cell line was analogue *epi*-**3a**, which was 46-fold more potent than the commercial antitumour agent doxorubicin. In the same time, this molecule represents the most active compound synthesised in this work. Additionally, a potent submicromolar antiproliferative activities were recorded after treatment of HL-60 cells with analogues *epi*-**3b** (IC_{50} 0.11 μM), *epi*-**3c** (IC_{50} 0.36 μM), **5b** (IC_{50} 0.27 μM), **5c** (IC_{50} 0.14 μM) and *epi*-**6** (IC_{50} 0.15 μM). Comparing to DOX, these analogues have exhibited 2.5–8-fold higher potency in the culture of HeLa cells. Several compounds exhibited submicromolar activities toward the Jurkat cells. These are *epi*-**3d** (IC_{50} 0.69 μM), **5a** (IC_{50} 0.29 μM), **5b** (IC_{50} 0.84 μM) and *epi*-**6** (IC_{50} 0.31 μM). However, the potencies of these analogues are significantly lower than that recorded for DOX in the same cell line (IC_{50} 0.03 μM). The most active molecule in the culture of Raji cells is analogue *epi*-**3c** (IC_{50} 1.02 μM), that exhibited about 14- and 3-fold higher potency than control compounds *epi*-**3a** and DOX, respectively. MCF-7 and MDA-MB 231 cells were shown to be the least sensitive to the tested analogues. Submicromolar activities have not been recorded in cultures of these cells. The most potent compound against MCF-7 and MDA-MB 231 cells are *epi*-**6** (IC_{50} 1.64 μM) and *epi*-**3d** (IC_{50} 1.32 μM), respectively. The most active compounds in the culture of HeLa cells are analogues *epi*-**4a** (IC_{50} 0.69 μM), **5b** (IC_{50} 0.94 μM) and **5c** (IC_{50} 0.87 μM) that exhibited the similar potencies as the control molecule **5a** (IC_{50} 1.25 μM). Remarkably, the most of synthesized styryl lactones including the natural products **3a** and **4a** were completely inactive toward normal MRC-5 cells, with the exception of dicinnamoate **6** which showed a weak cytotoxicity (IC_{50} 48.64 μM). In contrast, the commercial antitumour agent doxorubicin (DOX) exhibits a potent cytotoxic activity (IC_{50} 0.1 μM) against this cell line. These findings suggest to a higher selectivity of the synthetic analogues when compared to DOX, but this assumption should be verified by additional in vitro experiments with different normal cell lines.

In order to correlate the structures of synthesized styryl lactones with their cytotoxic activities [16], we first considered the influence of selected electron-donating (OMe) or electron-withdrawing (NO_2 and F) groups attached in the para position of cinnamoate aromatic ring. Non-substituted cinnamoates **3a**, **4a**, *epi*-**3a**, *epi*-**4a**, and **5a** have been applied as controls in this SAR analysis. As the data in Table 1 reveal the introduction of either electron-withdrawing groups (such as F and NO_2) or an electron-donating group (such as OMe), in the para position of the aromatic ring of **3a**, failed to improve antiproliferative activity against the majority of tumour cell lines under evaluation. However, several interesting exceptions deserve to be commented: Insertion of the above-mentioned substituents (OMe, NO_2, and F) in the para position of the cinnamic acid

Table 1
In vitro cytotoxicity of (+)-crassalactones B (**3a**) and C (**4a**), the corresponding analogues **3b**–**3d**, **4b**–**4d**, **5b**–**5d** and DOX.

Compounds	IC_{50} (μM)[a]							
	K562	HL-60	Jurkat	Raji	MCF-7	MDA-MB 231	HeLa	MRC-5
3a	0.78	2.54	0.12	14.61	0.85	5.32	0.98	>100
3b	4.21	50.21	13.37	5.69	11.08	>100	18.63	>100
3c	14.63	48.25	44.69	8.23	>100	5.36	21.36	>100
3d	10.24	11.52	17.56	11.08	14.87	25.66	29.88	>100
4a	3.56	>100	25.45	15.46	7.34	87.98	11.25	>100
4b	3.25	41.39	42.31	4.37	12.32	>100	24.88	>100
4c	2.25	14.32	10.54	16.38	5.23	33.41	35.69	>100
4d	4.58	9.45	18.09	14.45	18.66	29.64	24.11	>100
epi-**3a**	3.33	0.02	0.87	14.52	0.22	8.64	2.47	>100
epi-**3b**	2.63	0.11	1.12	2.34	20.21	7.32	3.21	>100
epi-**3c**	1.12	0.36	3.52	1.02	56.21	2.64	1.66	>100
epi-**3d**	0.85	2.11	0.69	1.74	84.54	1.32	4.12	>100
epi-**4a**	2.01	1.34	1.03	1.31	9.47	3.37	0.69	>100
epi-**4b**	11.54	8.79	18.64	8.45	9.64	28.64	11.02	>100
epi-**4c**	14.23	10.47	24.58	4.36	4.15	64.25	4.01	>100
epi-**4d**	15.69	10.96	19.64	12.67	5.36	31.55	4.36	>100
5a	4.67	2.21	0.29	0.88	58.64	4.32	1.25	>100
5b	1.02	0.27	0.84	3.58	60.96	4.34	0.94	>100
5c	0.98	0.14	3.74	2.69	23.16	5.32	0.87	>100
5d	0.25	1.02	2.63	1.52	28.99	8.22	1.02	>100
6	21.36	3.33	2.65	11.01	>100	>100	12.24	48.64
epi-**6**	2.15	0.15	0.31	10.24	1.64	6.69	1.45	>100
DOX	0.25	0.92	0.03	2.98	0.20	0.09	0.065	0.10

[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. Coefficients of variation were less than 10%.

residue in **3a** resulted in an increased activity of the corresponding isosteres (**3b**–**d**) in culture of Raji cells, The same structural modification of natural product **4a** increased the potency of resulting analogues (**4b**–**d**) toward HL-60 cells. Insertion of both electron-withdrawing groups (NO_2 and F) in **4a** increases the antitumour potency in both Jurkat and MDA-MB 231 cell cultures, whereas an introduction of the electron-donating group (OMe) in **4a** gave the less active analogue (**4b**) against the same tumour cell lines. The cinnamic isosteres of *epi*-**3a** (compounds *epi*-**3b**, *epi*-**3c** and *epi*-**3d**) displayed the increased antitumour potency with respect to that originally shown by the control molecule *epi*-**3a** in K562, Raji and MDA-MB 231 cell cultures. Analogues with electron-withdrawing groups (*epi*-**4c** and *epi*-**4d**) displayed the increased antitumour potencies with respect to that originally displayed by the control molecule *epi*-**4a** in the culture of MCF-7 tumour cells, whereas an introduction of the electron-donating group (OMe) in *epi*-**4a** decreases the antitumour potency against the same tumour cell line. The best results were obtained after isosteric replacement in the structure of lead **5a**. Namely, insertion of all three substituents (OMe, NO_2, and F) in the para position of the cinnamic acid residue in **5a** resulted in an increased activity of the corresponding analogues (**5b**–**d**) in cultures of three malignant cell lines (K562, HL-60 and HeLa). In addition, the presence of electron-withdrawing groups (NO_2 and F) in the molecules of isosteres **5c** and **d**, increases their antiproliferative potency with respect to that originally displayed by the control molecule **5a**, whereas the introduction of an electron-donating group (OMe) in **5a** gave the less active analogue (**5b**) in MCF-7 cells culture.

Additional structural modifications undertaken to improve the anti-proliferative activities were performed at the C-7 position. We first wanted to explore the effects of absolute stereochemistry at C-7 to antiproliferative activity of analogues. Our previous studies [15,24] indicated that the styryl lactones having the (7*S*)-stereochemistry represent more potent cytotoxic agents with respect to the corresponding (7*R*)-epimers. To further verify this assumption, we compared the IC_{50} values of nine pairs of epimers (**3a** and *epi*-**3a**, **3b** and *epi*-**3b**, **3c** and *epi*-**3c**, **3d** and *epi*-**3d**, **4a** and *epi*-**4a**, **4b** and *epi*-**4b**, **4c** and *epi*-**4c**, **4d** and *epi*-**4d**, **6** and *epi*-**6**), each of which contains exactly the same substituents and differs only in their absolute stereochemistry at C-7. Stereoisomers of (7*R*)-absolute configuration (**3a**–**d**, **4a**–**d** and **6**) are considered as controls in this part of SAR study. As the results shown in Table 1 indicate, in the great majority of cases, the (7*S*)-configured isosteres (*epi*-**3a**–**d**, *epi*-**4a**–**d**, and *epi*-**6**) showed a more potent cytotoxicity than the stereoisomers of (7*R*)-series (**3a**–**d**, **4a**–**d** and **6**). These findings strongly support the hypothesis that the (7*S*)-absolute stereochemistry is beneficial for the potent antiproliferative activity of styryl lactones bearing a furanofuranone scaffold.

Finally, we set a goal to examine the impact of deoxygenation at the C-7 position on the antitumour activity of analogues. The relationships between these structural changes and antiproliferative potencies were established by comparing the IC_{50} values of 7-deoxy derivatives **5a**–**d** with those recorded for the corresponding 7-hydroxylated derivatives that are arbitrarily used as control molecules (compounds **3a**–**d** or *epi*-**3a**–**d**). As the data in Table 1 reveal the introduction of a deoxy function at the C-7 position increases the antiproliferative activity of the resulting analogues against majority of tumour cell lines under evaluation. Remarkably, deoxygenated isostere of **3c** (compound **5c**) displayed the increased antitumour potency with respect to that originally shown by the control molecule **3c** against the all tested tumour cell lines. Furthermore, insertion of a deoxy functionality at the C-7 position of **3b** resulted in an increased activity of the corresponding isostere **5b** against six of seven studied tumour cell lines. The similar trend was observed when **3d** is deoxygenated. Finally, deoxygenation of *epi*-**3b**, *epi*-**3c** and *epi*-**3d** resulted in an increased activity of the corresponding isosteres **5b**–**d** toward four to five malignant cell lines.

All these biological results can be helpful for the further development of new antitumour agents derived from (+)-crassalactones B and C and from related styryl-lactones.

3. Conclusions

In conclusion, an efficient semi-syntheses of (+)-crassalactones B (**3a**) and C (**4a**), as well as the corresponding 5,7-dicinamoates **6** and *epi*-**6** have been achieved starting from (+)-goniofufurone (**1**) or from 7-*epi*-(+)-goniofufurone (**2**), respectively. A number of novel isosteres such as **3b**–**d** or **4b**–**d**, the corresponding 7-epimers such as *epi*-**3a**–**d** and *epi*-**4a**–**d**, as well as 7-deoxy derivatives such as **5a**–**d** have also been prepared starting from **1** or **2**.

All the synthesized compounds were tested in vitro toward a panel of human tumour cell lines, and the preliminary structure–activity relationships are briefly discussed. Some of the synthesized compounds showed potent antitumour activity, especially analogues *epi*-**3a** (IC_{50} 0.02 μM against HL-60) and *epi*-**3b** (IC_{50} 0.11 μM against HL-60) which displayed the highest activity of all compounds under evaluation.

A brief SAR analysis suggested the structural features that may affect the anti-proliferative activity of synthesized styryl lactones: (A) introduction of methoxy, nitro, or fluoro isosteric functions at the C-4 of the aromatic rings of cinnamoyl moieties in most cases decreases the antitumour potency originally displayed by lead molecules bearing the non-substituted phenyl groups; (B) styryl lactones having the (7*S*)-stereochemistry represent more potent cytotoxic agents than the corresponding (7*R*)-epimers; (C) introduction of a deoxy function at the C-7 position increases the anti-proliferative activity of the resulting analogues against majority of tumour cell lines under evaluation.

4. Experimental section

4.1. General experimental procedures

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on an Autopol IV (Rudolph Research) polarimeter at room temperature. NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in ppm 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 an Agilent technologies 6210 TOF LC/MS instrument (LC series 1200). 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 30 °C.

4.1.1. General procedure for the selective silylation of *1* and *2*

To a solution of lactones **1** or **2** (1 equiv) in dry MeCN (0.04 M) was added ^{t}BuMe$_2$SiCl (4.5 equiv) and imidazole (4.5 equiv). The mixture was stirred at room temperature until the starting materials were consumed (TLC, 26 h for **1**, 20 h for **2**). The mixture was poured into 10% aq NH_4Cl and extracted with EtOAc. The combined extracts were washed with brine, dried and evaporated. The residue was purified by flash column chromatography (3:2 Et_2O/light petroleum for **7**, 13:7 Et_2O/light petroleum for *epi*-**7**).

*4.1.1.1. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**7**).* Yield 95% (based on recovered starting compound **1**). Colourless syrup, $[\alpha]^{20}_D$ +103.0 (*c* 0.5, $CHCl_3$); R_f = 0.27 (CH_2Cl_2). IR (film): ν_{max} 3426 (OH), 1789 (C=O, lactone), 1717 (C=O, ester). ^{1}H NMR ($CDCl_3$): δ −0.02 and 0.13 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.91 (s, 9H, SiMe$_2$*CMe*$_3$), 1.27 (br s, 1H, OH), 2.67 (dd, 1H, $J_{2a,3}$ = 1.9, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.75 (dd, 1H, $J_{2a,2b}$ = 18.7, $J_{2b,3}$ = 4.9 Hz, H-2b), 3.91 (t, 1H, $J_{5,6}$ = $J_{6,7}$ = 2.8 Hz, H-6), 4.29 (br d, 1H, $J_{5,6}$ = 2.0 Hz, H-5), 4.81 (d, 1H, $J_{3,4}$ = 4.0 Hz, H-4), 5.07 (m, 1H, H-3), 5.24 (d, 1H, $J_{6,7}$ = 3.0 Hz, H-7), 7.30–7.43 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ −5.4 and −5.1 (Si*Me*$_2$CMe$_3$), 18.0 (SiMe$_2$*C*Me$_3$), 25.5 (SiMe$_2$C*Me*$_3$), 36.2 (C-2), 74.2 (C-5), 76.8 (C-7), 77.0 (C-3), 82.5 (C-6), 87.5 (C-4), 125.9, 128.2, 128.6, 139.4 (Ph), 175.4 (C-1). HRMS (ESI): *m*/*z* 403.1343 (M^++K), calcd for $C_{19}H_{28}KO_5Si$: 403.1338.

*4.1.1.2. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**7**).* Yield 92%. Colourless powder, mp 137–138 °C (Et_2O/wet light petroleum), $[\alpha]^{20}_D$ +113.4 (*c* 0.5, $CHCl_3$); R_f = 0.26 (3:2 Et_2O/light petroleum). IR ($CHCl_3$): ν_{max} 3439 (OH), 1785 (C=O, lactone). ^{1}H NMR ($CDCl_3$): δ −0.17 and 0.05 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.87 (s, 9H, SiMe$_2$*CMe*$_3$), 1.27 (br. s, 1H, OH), 2.63 (br d, 1H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.75 (dd, 1H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 5.9 Hz, H-2b), 4.02 (dd, 1H, $J_{5,6}$ = 3.2, $J_{6,7}$ = 5.1 Hz, H-6), 4.17 (d, 1H, $J_{5,6}$ = 2.9 Hz, H-5), 4.83 (d, 1H, $J_{3,4}$ = 4.6 Hz, H-4), 4.99 (d, 1H, $J_{6,7}$ = 5.1 Hz, H-7), 5.06 (m, 1H, H-3), 7.28–7.46 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ −5.0 and −4.5 (Si*Me*$_2$CMe$_3$), 18.1 (SiMe$_2$*C*Me$_3$), 25.7 (SiMe$_2$C*Me*$_3$), 36.0 (C-2), 74.7 (C-7), 76.1 (C-5), 76.5 (C-3), 83.9 (C-6), 87.9 (C-4), 127.1, 128.1, 128.3, 140.8 (Ph), 175.7 (C-1). HRMS (ESI): *m*/*z* 403.1347 (M^++K), calcd for $C_{19}H_{28}KO_5Si$: 403.1338.

4.1.2. General procedure for esterification with cinnamoyl chloride

To a cooled (0 °C) and stirred solution of **1**, **2**, **7**, *epi*-**7** or **9** (1 equiv) in dry MeCN (0.02 M solution of **1**, **2**, and **9**, 0.03 M for *epi*-**7**, 0.05 M for **7**) was added *trans*-cinnamoyl chloride (2.2–2.3 equiv for **1**, **2**, **7** and *epi*-**7**, 1.3 equiv for **9**) and DMAP (2.6–2.8 equiv for **1**, **2**, **7** and *epi*-**7**, 1.5 equiv for **9**). The mixture was stirred at 0 °C for 0.5 h and then at room temperature for 30 h. The mixture was poured in water and extracted with EtOAc. The combined extracts were washed with brine, dried and evaporated. The residue was purified by flash column chromatography (2:1 Et_2O/hexane for **6**, 99:1 CH_2Cl_2/EtOAc for *epi*-**6**, 2:1 Et_2O/light petroleum for **8a**, 7:3 light petroleum/Et_2O for *epi*-**8a**, 1:1 Et_2O/light petroleum for **5a**).

*4.1.2.1. 3,6-Anhydro-7-O-(terc-butyldimethylsilyl)-5-O-cinnamoyl-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**8a**).* Yield 90%. Colourless syrup, $[\alpha]^{20}_D$ +9.6 (*c* 0.5, $CHCl_3$); R_f = 0.27 (CH_2Cl_2). IR (film): ν_{max} 1793 (C=O, lactone), 1717 (C=O, ester), 1637 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ −0.37 and −0.05 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.79 (s, 9H, SiMe$_2$*CMe*$_3$), 2.52 (br d, 1H, $J_{2a,2b}$ = 19.0 Hz, H-2a), 2.66 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 6.1 Hz, H-2b), 4.29 (dd, 1H, $J_{5,6}$ = 2.8, $J_{6,7}$ = 8.8 Hz, H-6), 4.87 (d, 1H, $J_{6,7}$ = 8.8 Hz, H-7), 4.94 (m, 1H, H-3), 4.99 (d, 1H, $J_{3,4}$ = 4.7 Hz, H-4), 5.65 (d, 1H, $J_{5,6}$ = 2.7 Hz, H-5), 6.59 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.28–7.68 (m, 10H, 2× Ph), 7.82 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −5.3 and −4.5 (Si*Me*$_2$CMe$_3$), 17.7 (SiMe$_2$*C*Me$_3$), 25.5 (SiMe$_2$C*Me*$_3$), 35.6 (C-2), 71.9 (C-7), 75.2 (C-5), 76.9 (C-3), 83.3 (C-6), 84.8 (C-4), 116.4 (C-2′), 127.2, 128.16, 128.2, 128.24, 129.0, 130.8, 133.9, 141.7 (2× Ph), 146.5 (C-3′), 165.5 (C-1′), 175.0 (C-1). HRMS (ESI): *m*/*z* 495.2204 (M^++H), calcd for $C_{28}H_{35}O_6Si$: 495.2197.

*4.1.2.2. 3,6-Anhydro-7-O-(terc-butyldimethylsilyl)-5-O-cinnamoyl-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**8a**).* Yield 97%. Colourless syrup, $[\alpha]^{20}_D$ +4.8 (*c* 0.5, $CHCl_3$); R_f = 0.26 (CH_2Cl_2). IR (film): ν_{max} 1793 (C=O, lactone), 1720 (C=O, ester), 1636 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ −0.10 and 0.09 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.87 (s, 9H, SiMe$_2$*CMe*$_3$), 2.76 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 2.5 Hz, H-2a), 2.83 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 5.5 Hz, H-2b), 4.32 (dd, 1H, $J_{5,6}$ = 3.8, $J_{6,7}$ = 6.8 Hz, H-6), 4.86 (d, 1H, $J_{6,7}$ = 6.6 Hz, H-7), 4.99–5.13 (m, 2H, H-4 and H-5), 5.13 (td, 1H, *J* = 5.3 Hz, $J_{2a,3}$ = 2.5 Hz, H-3), 6.51 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.20–7.64 (m, 10H, 2× Ph), 7.77 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −4.73 and −4.7 (Si*Me*$_2$CMe$_3$), 18.2 (SiMe$_2$*C*Me$_3$), 25.8 (SiMe$_2$C*Me*$_3$), 36.0 (C-2), 74.3 (C-7), 76.2 (C-5), 76.7 (C-3), 84.1

(C-6), 85.6 (C-4), 116.4 (C-2′), 126.8, 128.2, 128.3, 128.34, 129.1, 130.9, 133.9, 140.4 (2× Ph), 146.8 (C-3′), 165.3 (C-1′), 175.1 (C-1). HRMS (ESI): *m*/*z* 533.1762 (M^++K), calcd for $C_{28}H_{34}KO_6Si$: 533.1756.

*4.1.2.3. 3,6-Anhydro-5,7-bis-(O-cinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-gluco-heptono-1,4-lactone (**6**).* Yield 94%. Colourless powder, mp 200 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −99.6 (*c* 0.25, $CHCl_3$), lit. [13] mp 195–196 °C, $[\alpha]^{20}_D$ −102.7 (*c* 0.24, $CHCl_3$); R_f = 0.36 (2:1 Et_2O/hexane). IR ($CHCl_3$): ν_{max} 1791 (C=O, lactone), 1716 (C=O, ester), 1634 (C=C, cinnamate). For ^1H and ^{13}C NMR spectral data see the Supplementary Data. HRMS (ESI): *m*/*z* 533.1578 (M^++Na), calcd for $C_{31}H_{26}NaO_7$: 533.1571.

*4.1.2.4. 3,6-Anhydro-5,7-bis-(O-cinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-gluco-heptono-1,4-lactone (epi-**6**).* Yield 96%. Colourless powder, mp 208–209 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −67.6 (*c* 0.5, $CHCl_3$); R_f = 0.35 (99:1 CH_2Cl_2/EtOAc). IR ($CHCl_3$): ν_{max} 1792 (C=O, lactone), 1716 (C=O, ester), 1635 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ 2.83 (m, 2H, 2× H-2), 4.74 (dd, 1H, $J_{5,6}$ = 4.0, $J_{6,7}$ = 8.2 Hz, H-6), 4.94 (d, 1H, $J_{3,4}$ = 4.4 Hz, H-4), 5.11–5.20 (m, 2H, H-3 and H-5), 6.22 (d, 1H, $J_{6,7}$ = 8.2 Hz, H-7), 6.52 and 6.54 (2× d, 2H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.29–7.65 (m, 15H, 3× Ph), 7.73 and 7.79 (2× d, 1H each, $J_{2',3'}$ = 16.0 Hz, 2× H-3′). ^{13}C NMR ($CDCl_3$): δ 35.9 (C-2), 74.6 (C-7), 75.3 (C-5), 77.6 (C-3), 81.7 (C-6), 85.2 (C-4), 116.0 and 117.6 (2× C-2′), 127.3, 128.1, 128.4, 128.6, 129.0, 129.2, 129.9, 130.4, 131.0, 133.7, 134.2, 135.9 (3× Ph), 147.1 (C-3′), 165.0 and 165.8 (2× C-1′), 174.7 (C-1). HRMS (ESI): *m*/*z* 533.1573 (M^++Na), calcd for $C_{31}H_{26}NaO_7$: 533.1571.

*4.1.2.5. 3,6-Anhydro-5-O-cinnamoyl-2,7-dideoxy-7-C-phenyl-D-gluco-heptono-1,4-lactone (**5a**).* Yield 91%. Colourless needles, mp 134 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −19.1 (*c* 1.0, $CHCl_3$); R_f = 0.29 (1:1 Et_2O/light petroleum). IR ($CHCl_3$): ν_{max} 1791 (C=O, lactone), 1716 (C=O, ester), 1636 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ 2.67 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2a,3}$ = 1.2 Hz, H-2a), 2.79 (dd, 1H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 5.8 Hz, H-2b), 2.97 (dd, 1H, $J_{7a,7b}$ = 14.0, $J_{6,7a}$ = 7.7 Hz, H-7a), 3.04 (dd, 1H, $J_{7a,7b}$ = 14.1, $J_{6,7b}$ = 7.2 Hz, H-7b), 4.42 (td, 1H, $J_{6,7}$ = 7.0, $J_{5,6}$ = 3.0 Hz, H-6), 5.00 (d, 1H, $J_{3,4}$ = 5.8 Hz, H-4), 5.05 (td, 1H, $J_{3,4}$ = 5.8, $J_{2a,3}$ = 1.4, $J_{2b,3}$ = 5.8 Hz, H-3), 5.46 (d, 1H, $J_{5,6}$ = 2.9 Hz, H-5), 6.57 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.15–7.66 (m, 10H, 2× Ph), 7.84 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 34.6 (C-7), 35.6 (C-2), 75.5 (C-5), 76.4 (C-3), 80.4 (C-6), 85.5 (C-4), 116.4 (C-2′), 126.7, 128.2, 128.5, 128.8, 128.9, 130.8, 133.8, 137.0 (2× Ph), 146.7 (C-3′), 165.4 (C-1′), 174.9 (C-1). HRMS (ESI): *m*/*z* 387.1220 (M^++Na), calcd for $C_{22}H_{20}NaO_5$: 387.1203.

4.1.3. General procedure for the Steglich esterification protocol

To a solution of **7**, *epi*-**7** or **9** (1 equiv) in anhydrous CH_2Cl_2 (0.01 M) were successively added 4-substituted cinnamic acid (2 equiv for **5a**–**c**, **8b**–**d**; 4 equiv for *epi*-**8b**, *epi*-**8c** and *epi*-**8d**), DCC (2.2 equiv for **5a**–**c**, **8b**–**d**; 4.4 equiv for *epi*-**8b**, *epi*-**8c** and *epi*-**8d**) and DMAP (4 equiv for **5a**–**c**, **8b**–**d**; 8 equiv for *epi*-**8b**, *epi*-**8c** and *epi*-**8d**). The mixture was stirred at room temperature until the starting materials were consumed (TLC, 22 h for **5a**–**c**; 48 h for **8b**–**d**; 72 h for *epi*-**8b**, *epi*-**8c** and *epi*-**8d**). The mixture was poured to water (80 mL) and extracted with CH_2Cl_2. The combined organic phases were washed with 10% NaCl, dried and evaporated, and the residue was purified by flash column chromatography (CH_2Cl_2 for **8b** and **8d**, 17:3 light petroleum/EtOAc for **8c**, 3:2 hexane/Et_2O for *epi*-**8b** and *epi*-**8d**, 1:1 hexane/Et_2O for *epi*-**8c**, 47:3 toluene/EtOAc for **5b**, CH_2Cl_2 for **5c**, 19:1 toluene/EtOAc for **5d**) and in few cases by additional preparative TLC ($^iPr_2O$, 3 successive developments, for **5b** and **5c**).

*4.1.3.1. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-5-O-(4-methoxycinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**8b**).* Yield 91%. Colourless syrup, $[\alpha]^{20}_D$ +7.0 (*c* 0.1, $CHCl_3$); R_f = 0.33 (997:3 CH_2Cl_2/Me_2CO). IR (film): ν_{max} 1791 (C=O, lactone), 1716 (C=O, ester), 1633 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ −0.05 and −0.37 (2× s, 3H each, Si*Me*$_2$CMe_3), 0.79 (s, 9H, SiMe$_2$C*Me*$_3$). 2.49 (br d, 1H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.66 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 6.1 Hz, H-2b), 3.86 (s, 3H, OMe), 4.27 (dd, 1H, $J_{5,6}$ = 2.8, $J_{6,7}$ = 8.8 Hz, H-6), 4.87 (d, 1H, $J_{6,7}$ = 8.8 Hz, H-7), 4.89–5.01 (m, 2H, H-3 and H-4), 5.63 (d, 1H, $J_{5,6}$ = 2.7 Hz, H-5), 6.43 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 7.29–7.60 (m, 9H, 2× Ph), 7.78 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −5.3 and −4.6 (Si*Me*$_2$CMe$_3$), 17.7 (SiMe$_2$C*Me*$_3$), 25.4 (SiMe$_2$C*Me*$_3$), 35.5 (C-2), 55.3 (OMe), 71.9 (C-7), 75.0 (C-5), 76.8 (C-3), 83.3 (C-6), 84.9 (C-4), 114.1 (C-2′) 114.4, 126.6, 127.2, 128.1, 128.2, 129.9, 141.7, 161.8 (2× Ph), 146.1 (C-3′), 165.8 (C-1′), 175.0 (C-1). HRMS (ESI): *m*/*z* 542.2561 (M^++NH_4), calcd for $C_{29}H_{40}NO_7Si$: 542.2569.

*4.1.3.2. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-5-O-(4-nitrocinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**8c**).* Yield 97%. Colourless powder, mp 65 °C (EtOAc/light petroleum), $[\alpha]^{20}_D$ +1.0 (*c* 0.1, $CHCl_3$); R_f = 0.35 (7:3 light petroleum/EtOAc). IR ($CHCl_3$): ν_{max} 1793 (C=O, lactone), 1724 (C=O, ester), 1641 (C=C, cinnamate), 1522 and 1346 (NO_2). ^1H NMR ($CDCl_3$): δ −0.08 and −0.39 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.78 (s, 9H, SiMe$_2$C*Me*$_3$), 2.52 (br d, 1H, $J_{2a,2b}$ = 19.0 Hz, H-2a), 2.69 (dd, 1H, $J_{2a,2b}$ = 19.2, $J_{2b,3}$ = 5.8 Hz, H-2b), 4.27 (dd, 1H, $J_{5,6}$ = 2.5, $J_{6,7}$ = 8.6 Hz, H-6), 4.84 (d, 1H, $J_{6,7}$ = 8.7 Hz, H-7), 4.90–5.01 (m, 2H, H-3 and H-4), 5.65 (d, 1H, $J_{5,6}$ = 2.6 Hz, H-5), 6.70 (d, 1H, $J_{2',3'}$ = 16.1 Hz, H-2′), 7.29–8.36 (m, 9H, 2× Ph), 7.85 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −5.3 and −4.5 (Si*Me*$_2$CMe$_3$), 17.7 (SiMe$_2$C*Me*$_3$), 25.5 (SiMe$_2$C*Me*$_3$), 35.5 (C-2), 71.9 (C-7), 75.6 (C-5), 76.9 (C-3), 83.2 (C-6), 84.7 (C-4), 121.0 (C-2′), 124.2, 127.2, 128.2, 128.3, 128.8, 139.9, 141.5, 148.7 (2× Ph), 143.5 (C-3′), 164.6 (C-1′), 174.8 (C-1). HRMS (ESI): *m*/*z* 557.2314 (M^++NH_4), calcd for $C_{28}H_{37}N_2O_8Si$: 557.2314.

*4.1.3.3. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-5-O-(4-fluorocinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**8d**).* Yield 96%. Colourless powder, mp 95 °C (light petroleum), $[\alpha]^{20}_D$ +7.0 (*c* 0.1, $CHCl_3$); R_f = 0.29 (CH_2Cl_2). IR ($CHCl_3$): ν_{max} 1794 (C=O, lactone), 1724 (C=O, ester), 1638 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ −0.37 and −0.06 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.79 (s, 9H, SiMe$_2$C*Me*$_3$), 2.51 (br d, 1H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.67 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 6.1 Hz, H-2b), 4.28 (dd, 1H, $J_{5,6}$ = 2.8, $J_{6,7}$ = 8.7 Hz, H-6), 4.86 (d, 1H, $J_{6,7}$ = 8.8 Hz, H-7), 4.90–5.01 (m, 2H, H-3 and H-4), 5.65 (d, 1H, $J_{5,6}$ = 2.6 Hz, H-5), 6.70 (d, 1H, $J_{2',3'}$ = 16.1 Hz, H-2′), 7.29–8.36 (m, 9H, 2× Ph), 7.85 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −5.3 and −4.5 (Si*Me*$_2$CMe$_3$), 17.7 (SiMe$_2$C*Me*$_3$), 25.5 (SiMe$_2$C*Me*$_3$), 35.5 (C-2), 71.9 (C-7), 75.2 (C-5), 76.9 (C-3), 83.3 (C-6), 84.8 (C-4), 116.5 (C-2′), 116.0, 116.4, 116.5, 127.2, 128.2, 128.3, 130.1, 130.2, 141.6, 162.1, 166.1 (2× Ph), 145.2 (C-3′), 165.4 (C-1′), 174.9 (C-1). HRMS (ESI): *m*/*z* 530.2365 (M^++NH_4), calcd for $C_{28}H_{37}FNO_6Si$: 530.2369.

*4.1.3.4. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-5-O-(4-methoxycinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**8b**).* Yield 87%. Colourless glass, $[\alpha]^{20}_D$ +12.0 (*c* 0.1, $CHCl_3$); R_f = 0.34 (1:1 Et_2O/hexane). IR ($CHCl_3$): ν_{max} 1793 (C=O, lactone), 1717 (C=O, ester), 1633 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ −0.09 and 0.10 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.89 (s, 9H, SiMe$_2$C*Me*$_3$), 2.72 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2a,3}$ = 2.4 Hz, H-2a), 2.83 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 5.5 Hz, H-2b), 3.87 (s, 3H, OMe), 4.32 (dd, 1H, $J_{5,6}$ = 4.0, $J_{6,7}$ = 6.4 Hz, H-6), 4.88 (d, 1H, $J_{6,7}$ = 6.7 Hz, H-7), 4.91–5.03 (m, 2H, H-4 and H-5), 5.13 (m, 1H, H-3), 6.39 (d, 1H,

$J_{2',3'}$ = 16.0 Hz, H-2′), 6.83–7.74 (m, 9H, 2× Ph), 7.74 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −4.8 and −4.7 (Si*Me*$_2$CMe$_3$), 18.2 (SiMe$_2$*C*Me$_3$), 25.7 (SiMe$_2$C*Me*$_3$), 36.0 (C-2), 55.4 (OMe), 74.3 (C-7), 75.9 (C-5), 76.5 (C-3), 84.1 (C-6), 85.6 (C-4), 113.8 (C-2′), 114.5, 126.6, 126.9, 128.2, 128.3, 130.0, 132.6, 140.4, 161.9 (2× Ph), 146.4 (C-3′), 165.6 (C-1′), 175.1 (C-1). HRMS (ESI): *m*/*z* 542.2567 (M^++NH_4), calcd for $C_{29}H_{40}NO_7Si$: 542.2569.

*4.1.3.5. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-5-O-(4-nitrocinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**8c**).* Yield 80%. Pale yellow powder, mp 148–150 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ +2.0 (*c* 0.1, $CHCl_3$); R_f = 0.27 (3:2 Et_2O/light petroleum). IR ($CHCl_3$): ν_{max} 1788 (C=O, lactone), 1721 (C=O, ester), 1634 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ −0.12 and 0.08 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.87 (s, 9H, SiMe$_2$C*Me*$_3$), 2.73 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2a,3}$ = 1.9 Hz, H-2a), 2.83 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 6.1 Hz, H-2b), 4.32 (dd, 1H, $J_{5,6}$ = 3.7, $J_{6,7}$ = 6.4 Hz, H-6), 4.83 (d, 1H, $J_{6,7}$ = 6.4 Hz, H-7), 4.92–5.05 (m, 2H, H-4 and H-5), 5.14 (m, 1H, H-3), 6.62 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.09–8.33 (m, 10H, 2× Ph and H-3′). ^{13}C NMR ($CDCl_3$): δ −4.8 and −4.7 (Si*Me*$_2$CMe$_3$), 18.1 (SiMe$_2$*C*Me$_3$), 25.7 (SiMe$_2$C*Me*$_3$), 35.9 (C-2), 74.2 (C-7), 76.5 (C-5), 76.7 (C-3), 83.9 (C-6), 85.4 (C-4), 120.6 (C-2′), 124.2, 126.7, 128.2, 128.3, 128.9, 139.8, 140.2, 148.8 (2× Ph), 143.7 (C-3′), 164.4 (C-1′), 175.0 (C-1). HRMS (ESI): *m*/*z* 557.2306 (M^++NH_4), calcd for $C_{28}H_{37}N_2O_8Si$: 557.2314.

*4.1.3.6. 3,6-Anhydro-7-O-(tert-butyldimethylsilyl)-5-O-(4-fluorocinnamoyl)-2-deoxy-7-C-phenyl-l-glycero-D-ido-heptono-1,4-lactone (epi-**8d**).* Yield 75%. Colourless glass, $[\alpha]^{20}_D$ +10.0 (*c* 0.1, $CHCl_3$); R_f = 0.35 (1:1 Et_2O/hexane). IR ($CHCl_3$): ν_{max} 1793 (C=O, lactone), 1720 (C=O, ester), 1637 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ −0.10 and 0.10 (2× s, 3H each, Si*Me*$_2$CMe$_3$), 0.89 (s, 9H, SiMe$_2$C*Me*$_3$), 2.73 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2a,3}$ = 2.4 Hz, H-2a), 2.83 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 5.5 Hz, H-2b), 4.32 (dd, 1H, $J_{5,6}$ = 3.5, $J_{6,7}$ = 6.5 Hz, H-6), 4.86 (d, 1H, $J_{6,7}$ = 6.5 Hz, H-7), 4.93–5.02 (m, 2H, H-4 and H-5), 5.13 (m, 1H, H-3), 6.44 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.13–7.68 (m, 9H, 2× Ph), 7.75 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ −4.8 and −4.7 (Si*Me*$_2$CMe$_3$), 18.2 (SiMe$_2$*C*Me$_3$), 25.7 (SiMe$_2$C*Me*$_3$), 36.0 (C-2), 74.2 (C-7), 76.3 (C-5), 76.5 (C-3), 84.0 (C-6), 85.5 (C-4), 116.4 (C-2′), 116.0, 126.7, 128.2, 128.3, 129.0, 130.2, 130.3, 140.3, 162.2, 165.2 (2× Ph), 145.4 (C-3′), 166.2 (C-1′), 175.0 (C-1). HRMS (ESI): *m*/*z* 530.2358 (M^++NH_4), calcd for $C_{28}H_{37}FNO_6Si$: 530.2369.

*4.1.3.7. 3,6-Anhydro-2,7-dideoxy-5-O-(4-methoxycinnamoyl)-7-C-phenyl-D-gluco-heptono-1,4-lactone (**5b**).* Yield 55%. Colourless syrup, $[\alpha]^{20}_D$ −17.2 (*c* 0.5, $CHCl_3$); R_f = 0.23 (19:1 toluene/EtOAc). IR (film): ν_{max} 1791 (C=O, lactone), 1714 (C=O, ester). ^{1}H NMR ($CDCl_3$): δ 2.67 (br d, 1H, $J_{2a,2b}$ = 19.0 Hz, H-2a), 2.78 (dd, 1H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 5.7 Hz, H-2b), 2.98 (d, 2H, $J_{6,7}$ = 6.8 Hz, H-7), 3.87 (s, 3H, OMe), 4.41 (td, 1H, $J_{6,7}$ = 6.8, $J_{5,6}$ = 2.9 Hz, H-6), 4.98 (d, 1H, $J_{3,4}$ = 4.7 Hz, H-4), 5.04 (m, 1H, $J_{3,4}$ = 4.6, $J_{2b,3}$ = 5.6 Hz, H-3), 5.44 (d, 1H, $J_{5,6}$ = 2.8 Hz, H-5), 6.40 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 6.83–7.60 (m, 9H, 2× Ph), 7.77 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 34.7 (C-7), 35.7 (C-2), 55.4 (OMe), 75.3 (C-5), 76.4 (C-3), 80.5 (C-6), 85.6 (C-4), 113.8 (C-2′), 114.4, 126.7, 128.6, 128.9, 130.0, 132.5, 137.1, 161.8 (2× Ph), 146.4 (C-3′), 165.7 (C-1′), 174.9 (C-1). HRMS (ESI): *m*/*z* 395.1484 (M^++H), calcd for $C_{23}H_{23}O_6$: 395.1489.

*4.1.3.8. 3,6-Anhydro-2,7-dideoxy-5-O-(4-nitrocinnamoyl)-7-C-phenyl-D-gluco-heptono-1,4-lactone (**5c**).* Yield 44%. Colourless syrup, $[\alpha]^{20}_D$ −2.6 (*c* 0.5, $CHCl_3$); R_f = 0.38 (CH_2Cl_2). IR (film): ν_{max} 1790 (C=O, lactone), 1728 (C=O, ester), 1520 and 1346 (NO_2). ^{1}H NMR ($CDCl_3$): δ 2.69 (br d, 1H, $J_{2a,2b}$ = 19.0 Hz, H-2a), 2.81 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 6.0 Hz, H-2b), 2.98 (d, 2H, $J_{6,7}$ = 6.8 Hz, H-7), 4.41 (td, 1H, $J_{6,7}$ = 6.9, $J_{5,6}$ = 2.9 Hz, H-6), 4.99 (d, 1H, $J_{3,4}$ = 4.7 Hz, H-4), 5.06 (m, 1H, $J_{3,4}$ = 4.7, $J_{2b,3}$ = 5.7 Hz, H-3), 5.47 (d, 1H, $J_{5,6}$ = 2.8 Hz, H-5), 6.66 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.05–8.35 (m, 10H, 2× Ph and H-3′). ^{13}C NMR ($CDCl_3$): δ 34.7 (C-7), 35.7 (C-2), 76.1 (C-5), 76.5 (C-3), 80.3 (C-6), 85.4 (C-4), 120.8 (C-2′), 124.3, 126.9, 128.7, 128.87, 128.9, 136.9, 139.9, 148.9 (2× Ph), 143.8 (C-3′), 164.6 (C-1′), 174.8 (C-1). HRMS (ESI): *m*/*z* 427.1501 (M^++NH_4), calcd for $C_{22}H_{23}N_2O_7$: 427.1500.

*4.1.3.9. 3,6-Anhydro-2,7-dideoxy-5-O-(4-fluorocinnamoyl)-7-C-phenyl-D-gluco-heptono-1,4-lactone (**5d**).* Yield 71%. Colourless needles, mp 129–131 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −17.4 (*c* 0.5, $CHCl_3$); R_f = 0.22 (19:1 toluene/EtOAc). IR ($CHCl_3$): ν_{max} 1791 (C=O, lactone), 1717 (C=O, ester). ^{1}H NMR ($CDCl_3$): δ 2.66 (br d, 1H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.75 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 5.8 Hz, H-2b), 2.98 (d, 2H, $J_{6,7}$ = 6.5 Hz, H-7), 4.40 (td, 1H, $J_{6,7}$ = 6.8, $J_{5,6}$ = 2.9 Hz, H-6), 4.98 (d, 1H, $J_{3,4}$ = 4.7 Hz, H-4), 5.04 (m, 1H, $J_{3,4}$ = 4.7, $J_{2b,3}$ = 5.6 Hz, H-3), 5.44 (d, 1H, $J_{5,6}$ = 2.8 Hz, H-5), 6.45 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 6.95–7.67 (m, 9H, 2× Ph), 7.77 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 34.6 (C-7), 35.7 (C-2), 75.6 (C-5), 76.4 (C-3), 80.4 (C-6), 85.5 (C-4), 116.4 (C-2′), 116.0, 116.15, 116.2, 126.7, 128.6, 128.8, 130.2, 130.3, 137.0, 162.2, 165.3 (2× Ph), 145.4 (C-3′), 166.2 (C-1′), 174.9 (C-1). HRMS (ESI): *m*/*z* 400.1550 (M^++NH_4), calcd for $C_{22}H_{23}FNO_5$: 400.1555.

4.1.4. General procedure for the silyl deprotection with TFA

To a stirred solution of **8a**, *epi*-**8a**, **8b**, **8c**, or **8d** (1 equiv) in 50:1 mixture of CH_2Cl_2/H_2O (0.01–0.03 M) was added cooled (+4 °C) TFA in portions (2 × 0.2 mL for **8a** and *epi*-**8a**, 3 × 0.2 mL for **8b**, 4 × 0.15 mL for **8c** and **8d**). The mixture was stirred at room temperature until the starting materials were consumed (TLC, 50 h for **8a**, 22 h for *epi*-**8a**, 48 h for **8b**–**d**). The mixture was evaporated by co-distillation with toluene and the residue was purified by flash column chromatography (49:1 → 24:1 CH_2Cl_2/Me_2CO for **3a**, 17:3 CH_2Cl_2/EtOAc for *epi*-**3a**, 24:1 CH_2Cl_2/Me_2CO for **3b**, 2:1 light petroleum/EtOAc for **3c** and **3d**).

*4.1.4.1. (+)-Crassalactone B (**3a**).* Yield 79% (93% when calculated to reacted intermediate **8a**; 15.1% of **8a** is recovered). Colourless powder, mp 173–174 °C (Et_2O), $[\alpha]^{20}_D$ +35.5 (*c* 1.0, $CHCl_3$), lit. [2] 171–173 °C (EtOH), $[\alpha]^{20}_D$ +8 (*c* 0.5, EtOH), lit. [13] 168–171 °C (EtOH), $[\alpha]^{20}_D$ +31.6 (*c* 1.0, $CHCl_3$); R_f = 0.38 (97:3 CH_2Cl_2/Me_2CO). IR (KBr): ν_{max} 3444 (OH), 1789 (C=O, lactone), 1717 (C=O, ester), 1636 (C=C, cinnamate). For ^{1}H and ^{13}C NMR spectra see the Supplementary Data. HRMS (ESI): *m*/*z* 403.1143 (M^++Na), calcd for $C_{22}H_{20}NaO_6$: 403.1152.

*4.1.4.2. 7-epi-(+)-Crassalactone B (epi-**3a**).* Yield 85%. Colourless powder, mp 128–129 °C (EtOAc/hexane), $[\alpha]^{20}_D$ −47.6 (*c* 0.5, $CHCl_3$); R_f = 0.26 (9:1 CH_2Cl_2/EtOAc). IR (KBr): ν_{max} 3424 (OH), 1791 (C=O, lactone), 1716 (C=O, ester), 1636 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ 2.58 (br s, 1H, OH), 2.80 (m, 2H, 2× H-2), 4.39 (dd, 1H, $J_{5,6}$ = 3.3, $J_{6,7}$ = 7.8 Hz, H-6), 4.94 (d, 1H, $J_{3,4}$ = 4.6 Hz, H-4), 5.01 (d, 1H, $J_{6,7}$ = 7.8 Hz, H-7), 5.07–5.15 (m, 2H, H-3 and H-5), 6.22 (d, 1H, $J_{6,7}$ = 8.2 Hz, H-7), 6.52 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.29–7.67 (m, 10H, 2× Ph), 7.79 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 72.9 (C-7), 75.1 (C-5), 77.3 (C-3), 83.8 (C-6), 85.5 (C-4), 116.1 (C-2′), 126.6, 128.3, 128.7, 128.8, 129.0, 131.0, 133.7, 138.8 (2× Ph), 147.0 (C-3′), 165.0 (C-1′), 174.6 (C-1). HRMS (ESI): *m*/*z* 403.1148 (M^++Na), calcd for $C_{22}H_{20}NaO_6$: 403.1152.

*4.1.4.3. 3,6-Anhydro-5-O-(4-methoxycinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**3b**).* Yield 85%. Colourless powder, mp 155 °C (CH_2Cl_2/trace Et_2O), $[\alpha]^{20}_D$ +59.0 (*c* 0.1, $CHCl_3$); R_f = 0.35 (24:1 CH_2Cl_2/Me_2CO). IR ($CHCl_3$): ν_{max} 3487 (OH),

1789 (C=O, lactone), 1715 (C=O, ester), 1633 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ 2.59 (d, 1H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.73 (dd, 1H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 5.2 Hz, H-2b), 3.20 (d, 1H, $J_{OH,7}$ = 2.6 Hz), 3.87 (s, 3H, OMe), 4.25 (dd, 1H, $J_{5,6}$ = 2.6, $J_{6,7}$ = 8.6 Hz, H-6), 4.47 (dd, 1H, $J_{6,7}$ = 8.7, $J_{OH,7}$ = 2.6 Hz, H-7), 4.95–5.04 (m, 2H, H-3 and H-4), 5.72 (d, 1H, $J_{5,6}$ = 2.5 Hz, H-5), 6.41 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 6.90–7.56 (m, 9H, 2× Ph), 7.79 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.5 (C-2), 55.4 (OMe), 70.7 (C-7), 75.2 (C-5), 77.0 (C-3), 83.1 (C-6), 85.3 (C-4), 114.4 (C-2′), 113.2, 126.4, 126.8, 128.2, 128.4, 130.2, 140.4, 162.0 (2× Ph), 147.2 (C-3′), 166.8 (C-1′), 174.8 (C-1). HRMS (ESI): *m/z* 428.1700 (M^++NH_4), calcd for $C_{23}H_{26}NO_7$: 428.1704.

*4.1.4.4. 3,6-Anhydro-5-O-(4-nitrocinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**3c**).* Yield 68% (74% when calculated to reacted intermediate **8c**; 7.7% of **8c** is recovered). Colourless powder, mp 137–140 °C (Et_2O), $[\alpha]^{20}_D$ +37.0 (*c* 0.1, $CHCl_3$); R_f = 0.25 (13:7 light petroleum/EtOAc). IR ($CHCl_3$): ν_{max} 3500 (OH), 1790 (C=O, lactone), 1723 (C=O, ester), 1642 (C=C, cinnamate), 1520 and 1346 (NO_2). ^{1}H NMR ($CDCl_3$): δ 2.59 (d, 1H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.73 (m, 2H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 2.9 Hz, H-2b and OH), 4.28 (dd, 1H, $J_{5,6}$ = 2.8, $J_{6,7}$ = 8.5 Hz, H-6), 4.73 (d, 1H, $J_{6,7}$ = 8.5 Hz, H-7), 5.03 (m, 2H, H-3 and H-4), 5.76 (d, 1H, $J_{5,6}$ = 2.7 Hz, H-5), 6.67 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.29–8.33 (m, 9H, 2× Ph), 7.85 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.5 (C-2), 71.0 (C-7), 75.8 (C-5), 77.2 (C-3), 82.8 (C-6), 85.1 (C-4), 120.6 (C-2′), 124.2, 126.7, 128.5, 128.6, 128.0, 139.8, 140.4, 148.8 (2× Ph), 144.2 (C-3′), 165.3 (C-1′), 174.63 (C-1). HRMS (ESI): *m/z* 443.1448 (M^++NH_4), calcd for $C_{28}H_{23}N_2O_8$: 443.1449.

*4.1.4.5. 3,6-Anhydro-5-O-(4-fluorocinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**3d**).* Yield 90% (98% when calculated to reacted **8d**; 5% of starting **8d** is recovered). Colourless powder, mp 176 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ +47.0 (*c* 0.1, $CHCl_3$); R_f = 0.30 (13:7 light petroleum/EtOAc). IR ($CHCl_3$): ν_{max} 3470 (OH), 1789 (C=O, lactone), 1720 (C=O, ester), 1637 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ 2.57 (br d, 1H, $J_{2a,2b}$ = 19.0 Hz, H-2a), 2.72 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 5.0 Hz, H-2b), 3.01 (br s, 1H, OH), 4.26 (dd, 1H, $J_{5,6}$ = 2.2, $J_{6,7}$ = 8.6 Hz, H-6), 4.69 (d, 1H, $J_{6,7}$ = 8.6 Hz, H-7), 4.97–5.07 (m, 2H, H-3 and H-4), 5.73 (d, 1H, $J_{5,6}$ = 2.6 Hz, H-5), 6.47 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.07–7.65 (m, 9H, Ph), 7.80 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.5 (C-2), 70.8 (C-7), 75.4 (C-5), 77.0 (C-3), 82.9 (C-6), 85.2 (C-4), 115.8 (C-2′), 116.0, 116.4, 126.8, 128.3, 128.5, 130.0, 130.05, 130.3, 130.4, 140.4, 162.2, 166.3 (2× Ph), 146.1 (C-3′), 165.4 (C-1′), 174.9 (C-1). HRMS (ESI): *m/z* 416.1501 (M^++NH_4), calcd for $C_{22}H_{23}FNO_6$: 416.1504.

4.1.5. General procedure for the silyl deprotection with CAN

To a stirred solution of *epi*-**8b**, *epi*-**8c**, or *epi*-**8d** (1 equiv) in 9:1 mixture of MeCN/H_2O (0.005 M) was added CAN (1.2 equiv). The mixture was stirred at room temperature until the starting materials were consumed (TLC, 120 h for *epi*-**8b**, 72 h for *epi*-**8c**, 26 h for *epi*-**8d**). The mixture was partitioned between $CHCl_3$ and 10% $NaHCO_3$, the organic layer was separated, dried and evaporated. The residue was purified by flash column chromatography (19:1 CH_2Cl_2/Me_2CO for *epi*-**3b**, 47:3 CH_2Cl_2/Me_2CO for *epi*-**3d**), or in the case of *epi*-**3c** by direct crystallization of crude reaction mixture from CH_2Cl_2/hexane.

*4.1.5.1. 3,6-Anhydro-5-O-(4-methoxycinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**3b**).* Yield 73%. Colourless plates, mp 147–149 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −76.7 (*c* 0.3, $CHCl_3$); R_f = 0.39 (19:1 CH_2Cl_2/Me_2CO). IR (KBr): ν_{max} 3498 and 3406 (OH), 1790 (C=O, lactone), 1716 (C=O, ester), 1631 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ 2.46 (bs, 1H, OH), 2.79 (d, 2H, $J_{2,3}$ = 3.8 Hz, 2× H-2), 3.86 (s, 3H, OMe), 4.38 (dd, 1H, $J_{6,7}$ = 7.7, $J_{5,6}$ = 3.3 Hz, H-6), 4.93 (d, 1H, $J_{3,4}$ = 4.4 Hz, H-4), 4.99 (d, 1H, $J_{6,7}$ = 7.7 Hz, H-7), 5.06–5.11 (m, 2H, H-3 and H-5), 6.36 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 6.91–7.63 (m, 9H, Ph), 7.72 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.9 (C-2), 55.4 (OMe), 72.9 (C-7), 75.0 (C-5), 77.3 (C-3), 83.9 (C-6), 85.6 (C-4), 113.5 (C-2′), 114.5, 126.5, 126.6, 128.8, 130.1, 138.9, 162.0 (2× Ph), 146.6 (C-3′), 165.4 (C-1′), 174.7 (C-1). HRMS (ESI): *m/z* 428.1704 (M^++NH_4), calcd for $C_{23}H_{26}NO_7$: 428.1704.

*4.1.5.2. 3,6-Anhydro-5-O-(4-nitrocinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**3c**).* Yield 89% after crystallization of crude reaction mixture (without column chromatography). Pale yellow powder, mp 219–223 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −36.0 (*c* 0.3, Me_2CO); R_f = 0.32 (19:1 CH_2Cl_2/Me_2CO). IR (KBr): ν_{max} 3486 (OH), 1785 (C=O, lactone), 1731 and 1719 (C=O, ester), 1644 (C=C, cinnamate), 1522 and 1348 (NO_2). ^{1}H NMR (acetone-d_6): δ 2.70–3.00 (m, 2× H-2 overlapped with H_2O from acetone-d_6), 4.41 (dd, 1H, $J_{6,7}$ = 7.1, $J_{5,6}$ = 3.7 Hz, H-6), 4.61 (bs, 1H, OH), 4.94 (d, 1H, $J_{5,6}$ = 3.5 Hz, H-5), 4.99 (d, 1H, $J_{6,7}$ = 7.0 Hz, H-7), 5.08 (d, 1H, $J_{3,4}$ = 4.8 Hz, H-4), 5.21 (m, 1H, H-3), 6.92 (d, 1H, $J_{2',3'}$ = 16.1 Hz, H-2′), 7.17–8.36 (m, 10H, $J_{2',3'}$ = 16.1 Hz, Ph and H-3′). ^{13}C NMR (acetone-d_6): δ 36.1 (C-2), 73.3 (C-7), 77.1 (C-5), 78.3 (C-3), 84.6 (C-6), 86.4 (C-4), 122.3 (C-2′), 124.8, 127.7, 128.7, 129.0, 130.3, 141.4, 142.0, 149.6 (2× Ph), 144.0 (C-3′), 165.4 (C-1′), 175.7 (C-1). HRMS (ESI): *m/z* 443.1452 (M^++NH_4), calcd for $C_{22}H_{23}N_2O_8$: 443.1449.

*4.1.5.3. 3,6-Anhydro-5-O-(4-fluorocinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**3d**).* Yield 62%. Colourless needles, mp 160–163 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ +4.0 (*c* 0.1, $CHCl_3$); R_f = 0.29 (24:1 CH_2Cl_2/Me_2CO. IR (KBr): ν_{max} 3497–3407 (OH), 1791 (C=O, lactone), 1720 (C=O, ester), 1638 (C=C, cinnamate). ^{1}H NMR ($CDCl_3$): δ 2.36 (bs, 1H, OH), 2.81 (d, 2H, $J_{2,3}$ = 3.7 Hz, 2× H-2), 4.37 (dd, 1H, $J_{6,7}$ = 7.7, $J_{5,6}$ = 3.3 Hz, H-6), 4.93 (d, 1H, $J_{3,4}$ = 5.2 Hz, H-4), 4.99 (d, 1H, $J_{6,7}$ = 7.7 Hz, H-7), 5.07–5.16 (m, 2H, H-3 and H-5), 6.42 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 7.02–7.67 (m, 9H, Ph), 7.74 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.9 (C-2), 72.9 (C-7), 75.2 (C-5), 77.4 (C-3), 83.9 (C-6), 85.6 (C-4), 116.0 (C-2′), 116.2, 116.5, 126.6, 128.8, 130.1, 130.3, 130.4, 138.8, 162.4, 165.0 (2× Ph), 145.7 (C-3′), 166.4 (C-1′), 174.6 (C-1). HRMS (ESI): *m/z* 416.1501 (M^++NH_4), calcd for $C_{22}H_{23}FNO_6$: 416.1504.

4.1.6. General procedure for the Mitsunobu reaction

To a stirred and cooled (0 °C) solution of **1** or **2** (1 equiv) in anhydrous EtOAc (0.02–0.04 M) were successively added cinnamic acid or 4-substituted cinnamic acid (2–3 equiv), Ph_3P (2–3 equiv) and 40% solution of DEAD in toluene (2–3 equiv) and the mixture was stirred at 0 °C for 1 h and then at room temperature until the starting materials were consumed (TLC, 2 h for **4b**, **4c** and **4d**, 3 h for **4a** and *epi*-**4a**, 4 h for *epi*-**4c**, and *epi*-**4d**, 24 h for *epi*-**4b**). The mixture was evaporated and the residue was purified by flash column chromatography (7:3 Et_2O/light petroleum for **4a** and *epi*-**4a**, 4:1 Et_2O/light petroleum for **4b**, 9:1 → 3:1 Et_2O/light petroleum for **4c** 4:1 → 3:1 Et_2O/light petroleum for **4d**, 1:1 light petroleum/EtOAc, 19:1 Et_2O/light petroleum for *epi*-**4b**, 9:1 → 19:1 Et_2O/light petroleum for *epi*-**4c**, 9:1 Et_2O/light petroleum for *epi*-**4d**).

*4.1.6.1. (+)-Crassalactone C (**4a**) and 3,6:5,7-dianhydro-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**10**).* Yields: 69% of **4a**, 31% of **10**. Physical constants and spectroscopic data of thus prepared samples **4a** and **10** matched those previously reported by us [10].

*4.1.6.2. 3,6-Anhydro-7-O-(4-methoxycinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**4b**).* Yield 60% (31% of oxetane **10** was also isolated). Compound **4b**: Colourless powder, mp 89–90 °C (Et_2O), $[\alpha]^{20}_D$ +143.0 (*c* 0.1, $CHCl_3$); R_f = 0.37 (Et_2O). IR ($CHCl_3$): ν_{max} 3445 (OH), 1789 (C=O, lactone), 1712 (C=O, ester), 1633 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ 2.54 (d, 1H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.70 (dd, 1H, $J_{2a,2b}$ = 18.8, $J_{2b,3}$ = 4.9 Hz, H-2b), 3.84 (s, 3H, Me), 4.25 (dd, 1H, $J_{5,6}$ = 2.1, $J_{6,7}$ = 9.2 Hz, H-6), 4.26 (br s, 1H, OH), 4.43 (br d, 1H, $J_{5,6}$ = 1.8 Hz, H-5), 4.92–5.10 (m, 2H, H-3 and H-4), 6.00 (d, 1H, $J_{6,7}$ = 9.2 Hz, H-7), 6.32 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 7.85–8.39 (m, 9H, 2× Ph), 7.71 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 55.4 (Me), 72.6 (C-5), 73.1 (C-7), 77.0 (C-3), 82.5 (C-6), 87.0 (C-4), 113.9 (C-2′), 114.4, 126.4, 127.7, 128.6, 128.8, 130.1, 136.9, 161.9 (2× Ph), 146.9 (C-3′), 167.8 (C-1′), 175.4 (C-1). HRMS (ESI): *m/z* 411.1435 (M^++H), calcd for $C_{23}H_{23}O_7$: 411.1438.

*4.1.6.3. 3,6-Anhydro-7-O-(4-nitrocinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**4c**).* Yield 66% (31% of oxetane **10** was also isolated). Compound **4c**: Pale yellow powder, mp 195 °C (EtOAc/hexane), $[\alpha]^{20}_D$ +133.0 (*c* 0.1, $CHCl_3$); R_f = 0.40 (3:2 EtOAc/cyclohexane). IR ($CHCl_3$): ν_{max} 3479 (OH), 1786 (C=O, lactone), 1716 (C=O, ester), 1639 (C=C, cinnamate), 1520 and 1346 (NO_2). ^1H NMR ($CDCl_3$): δ 2.55 (d, 1H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.70 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 5.1 Hz, H-2b), 3.90 (d, 1H, $J_{5,OH}$ = 3.6 Hz, OH), 4.29 (dd, 1H, $J_{5,6}$ = 2.3, $J_{6,7}$ = 9.2 Hz, H-6), 4.46 (dd, 1H, $J_{5,6}$ = 2.6, $J_{5,OH}$ = 2.9 Hz, H-5), 4.93–5.06 (m, 2H, H-3 and H-4), 6.03 (d, 1H, $J_{6,7}$ = 9.2 Hz, H-7), 6.59 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.31–8.39 (m, 9H, 2× Ph), 7.76 (d, 1H, $J_{2'3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 73.2 (C-5), 73.3 (C-7), 77.1 (C-3), 82.2 (C-6), 87.0 (C-4), 121.0 (C-2′), 124.2, 127.7, 128.7, 128.9, 129.1, 136.4, 139.7, 148.9 (2× Ph), 143.9 (C-3′), 166.2 (C-1′), 175.3 (C-1). HRMS (ESI): *m/z* 443.1448 (M^++NH_4), calcd for $C_{28}H_{23}N_2O_8$: 443.1449.

*4.1.6.4. 3,6-Anhydro-7-O-(4-fluorocinnamoyl)-2-deoxy-7-C-phenyl-D-glycero-D-ido-heptono-1,4-lactone (**4d**).* Yield 66% (31% of oxetane **10** was also isolated). Compound **4d**: Colourless powder, mp 155 °C (EtOAc/hexane), $[\alpha]^{20}_D$ +129.0 (*c* 0.1, $CHCl_3$); R_f = 0.45 (3:2 EtOAc/cyclohexane). IR (KBr): ν_{max} 3451 (OH), 1775 (C=O, lactone), 1688 (C=O, ester), 1634 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ 2.54 (d, 1H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.68 (dd, 1H, $J_{2a,2b}$ = 19.0, $J_{2b,3}$ = 4.9 Hz, H-2b), 4.17 (d, 1H, $J_{5,OH}$ = 3.3 Hz, OH), 4.27 (dd, 1H, $J_{5,6}$ = 2.2, $J_{6,7}$ = 9.2 Hz, H-6), 4.44 (br s, 1H, H-5), 4.92–5.04 (m, 2H, H-3 and H-4), 6.01 (d, 1H, $J_{6,7}$ = 9.2 Hz, H-7), 6.38 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 6.97–7.59 (m, 9H, 2× Ph), 7.71 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 35.8 (C-2), 72.8 (C-5), 73.1 (C-7), 77.1 (C-3), 82.4 (C-6), 87.0 (C-4), 116.4 (C-2′), 116.0, 116.4, 127.7, 128.6, 128.8, 128.9, 130.2, 130.3, 136.7, 162.1, 166.2 (2× Ph), 145.8 (C-3′), 167.2 (C-1′), 175.4 (C-1). HRMS (ESI): *m/z* 416.1504 (M^++NH_4), calcd for $C_{22}H_{23}FNO_6$: 416.1504.

*4.1.6.5. 7-epi-crassalactone C (epi-**4a**) and 3,6:5,7-dianhydro-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**10**).* Compound *epi*-**4a**: Yield 59%. Colourless powder, mp 120 °C (phase change at 75 °C, CH_2Cl_2/hexane), $[\alpha]^{20}_D$ −29.0 (*c* 0.5, EtOH), for (+)-enantiomer: lit. [14] mp 134–137 °C (from EtOAc/hexane), $[\alpha]^{20}_D$ +16.4 (*c* 0.5, EtOH), R_f = 0.18 (9:1 CH_2Cl_2/EtOAc). IR ($CHCl_3$): ν_{max} 3458 (OH), 1786 (C=O, lactone), 1709 (C=O, ester), 1636 (C=C, cinnamate). For ^1H and ^{13}C NMR spectral data see the Supplementary Data. HRMS (ESI): *m/z* 403.1140 (M^++Na), calcd for $C_{22}H_{20}NaO_6$: 403.1152.

Compound *epi*-**10**: Yield 25%. Colourless needles, mp 118–119 °C (CH_2Cl_2/hexane), $[\alpha]^{20}_D$ +122.6 (*c* 0.5, $CHCl_3$); R_f = 0.27 (CH_2Cl_2). IR ($CHCl_3$): ν_{max} 1789 (C=O), 1604 (Ph). ^1H NMR ($CDCl_3$): 2.55 (br d, 1H, $J_{2a,\ 2b}$ = 18.2 Hz, H-2a), 2.64 (dd, 1H, $J_{2a,\ 2b}$ = 18.3, $J_{2b,3}$ = 4.0 Hz, H-2b), 4.67 (m, 1H, H-3), 5.00 (d, 1H, $J_{3,4}$ = 3.8 Hz, H-4), 5.31 (t, 1H, $J_{5,6}$ = $J_{6,7}$ = 4.4 Hz, H-6), 5.56 (d, 1H, $J_{5,6}$ = 3.9 Hz, H-5), 5.88 (d, 1H, $J_{6,7}$ = 4.8 Hz, H-7), 7.24–7.46 (m, 5H, Ph). *NOE* contact: H-3 and Ph. ^{13}C NMR ($CDCl_3$): 36.3 (C-2), 80.4 (C-3), 81.0 (C-6), 84.8 (C-4), 85.3 (C-5), 86.1 (C-7), 124.4, 127.7, 128.4, 136.7 (Ph), 174.2 (C-1). HRMS (ESI): *m/z* 233.0810 (M^++H), calcd for $C_{13}H_{13}O_4$: 233.0808.

*4.1.6.6. 3,6-Anhydro-7-O-(4-methoxycinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**4b**).* Yield 42% (34% of oxetane *epi*-**10** was also isolated). Compound *epi*-**4b**: Colourless powder, mp 93–95 °C (Et_2O/hexane), $[\alpha]^{20}_D$ −36.0 (*c* 0.1, $CHCl_3$); R_f = 0.32 (Et_2O). IR ($CHCl_3$): ν_{max} 3454 (OH), 1789 (C=O, lactone), 1714 (C=O, ester), 1633 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ 2.74 (d, 2H, $J_{2,3}$ = 3.5 Hz, 2× H-2), 3.25 (br d, 1H, $J_{5,OH}$ = 5.9 Hz, OH), 3.82 (s, 3H, OMe), 4.02 (br s, 1H, H-5), 4.41 (dd, 1H, $J_{5,6}$ = 2.9, $J_{6,7}$ = 8.8 Hz, H-6), 4.85 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 5.09 (m, 1H, H-3), 6.19 (d, 1H, $J_{6,7}$ = 8.8 Hz, H-7), 6.36 (d, 1H, $J_{2',3'}$ = 15.9 Hz, H-2′), 6.84–7.54 (m, 9H, 2× Ph), 7.64 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 36.0 (C-2), 55.3 (OMe), 73.9 (C-5), 74.6 (C-7), 77.3 (C-3), 83.0 (C-6), 87.6 (C-4), 115.1 (C-2′), 114.3, 126.9, 127.5, 128.7, 128.8, 129.9, 136.8, 161.5 (2× Ph), 145.4 (C-3′), 166.6 (C-1′), 175.63 (C-1). HRMS (ESI): *m/z* 449.0996 (M^++K), calcd for $C_{23}H_{22}KO_7$: 449.0997.

*4.1.6.7. 3,6-Anhydro-7-O-(4-nitrocinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**4c**).* Yield 52% (32% of oxetane *epi*-**10** was also isolated). Compound *epi*-**4c**: Colourless powder, mp 103–105 °C (Et_2O/hexane), $[\alpha]^{20}_D$ −10.0 (*c* 0.1, $CHCl_3$); R_f = 0.32 (Et_2O). IR ($CHCl_3$): ν_{max} 3441 (OH), 1787 (C=O, lactone), 1716 (C=O, ester), 1640 (C=C, cinnamate), 1521 and 1345 (NO_2). ^1H NMR ($CDCl_3$): δ 2.74 (d, 2H, $J_{2,3}$ = 3.2 Hz, 2× H-2), 3.98 (br s, 2H, H-5 and OH), 4.42 (dd, 1H, $J_{5,6}$ = 2.9, $J_{6,7}$ = 9.0 Hz, H-6), 4.84 (d, 1H, $J_{3,4}$ = 4.2 Hz, H-4), 5.08 (m, 1H, H-3), 6.21 (d, 1H, $J_{6,7}$ = 9.0 Hz, H-7), 6.60 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 7.27–8.26 (m, 10H, 2× Ph and H-3′). ^{13}C NMR ($CDCl_3$): δ 36.0 (C-2), 73.7 (C-5), 75.4 (C-7), 77.4 (C-3), 82.9 (C-6), 87.5 (C-4), 122.1 (C-2′), 124.1, 127.6, 127.7, 128.7, 129.0, 136.3, 140.3, 148.5 (2× Ph), 142.5 (C-3′), 165.2 (C-1′), 175.6 (C-1). HRMS (ESI): *m/z* 464.0739 (M^++K), calcd for $C_{22}H_{19}KNO_8$: 464.0742.

*4.1.6.8. 3,6-Anhydro-7-O-(4-fluorocinnamoyl)-2-deoxy-7-C-phenyl-L-glycero-D-ido-heptono-1,4-lactone (epi-**4d**).* Yield 50% (56% based on reacted **1**; 35% of oxetane *epi*-**10** was also isolated, along with 11% of recovered **1**). Compound *epi*-**4d**: Colourless powder, mp 85–87 °C (Et_2O/hexane), $[\alpha]^{20}_D$ −7.0 (*c* 0.1, $CHCl_3$); R_f = 0.37 (Et_2O). IR ($CHCl_3$): ν_{max} 3456 (OH), 1787 (C=O, lactone), 1710 (C=O, ester), 1636 (C=C, cinnamate). ^1H NMR ($CDCl_3$): δ 2.68 (br. s, 1H, OH), 2.73 (d, 2H, $J_{2,3}$ = 3.2 Hz, 2× H-2), 4.03 (br. s, 1H, H-5), 4.45 (dd, 1H, $J_{5,6}$ = 2.9, $J_{6,7}$ = 8.8 Hz, H-6), 4.83 (d, 1H, $J_{3,4}$ = 4.3 Hz, H-4), 5.13 (m, 1H, H-3), 6.18 (d, 1H, $J_{6,7}$ = 8.8 Hz, H-7), 6.43 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-2′), 6.96–7.59 (m, 9H, 2× Ph), 7.66 (d, 1H, $J_{2',3'}$ = 16.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$): δ 36.0 (C-2), 74.0 (C-5), 74.8 (C-7), 77.4 (C-3), 83.0 (C-6), 87.4 (C-4), 117.5 (C-2′), 115.9, 116.2, 127.6, 128.8, 129.0, 130.0, 130.1, 130.4, 130.5, 136.6, 162.0 (2× Ph), 144.3 (C-3′), 166.9 (C-1′), 175.4 (C-1). HRMS (ESI): *m/z* 437.0792 (M^++K), calcd for $C_{22}H_{19}FKO_6$: 437.0797.

4.2. Cell lines

Human chronic myelogenous leukaemia (K562), human promyelocytic leukaemia (HL-60), human T cell leukaemia (Jurkat) and Burkitt's lymphoma (Raji) were grown in RPMI 1640, while ER^+ breast adenocarcinoma (MCF-7), ER^- breast adenocarcinoma (MDA-MB-231), cervix carcinoma (HeLa) malignant cells and normal foetal lung fibroblasts (MRC-5) were grown in DMEM medium. Both media were supplemented with 10% of foetal calf serum (FTS, NIVNS) 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 assays.

4.3. MTT assay

The colorimetric MTT assay was carried out following the recently reported procedure [25].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.064.

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