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European Journal of Medicinal Chemistry 41 (2006) 1217–1222
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Preliminary communication

Synthesis and antitumour activity of new muricatacin and goniofufurone analogues

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Received 20 February 2006; revised and accepted 19 June 2006
Available online 07 August 2006

Abstract

A divergent approach to the 7-oxa (–)-muricatacin analogue **2**, the corresponding (+)-enantiomer *ent*-**2** and the furanolactone **3** is reported starting from D-xylose. The resulting lactones have shown a potent and selective in vitro cytotoxicity against certain human neoplastic cell lines.

Keywords: Muricatacin analogues; Wittig reaction; Goniofufurone analogue; Bioisostere; Antitumour lactones

1. Introduction

The plant family Annonaceae has for a long time aroused a considerable interest from a biomedicinal point of view, mainly due to its bioactive polyketide constituents [1]. Within the Annonaceae, the tropical plant *Annona muricata* has given rise to the isolation of muricatacin, an acetogenin hydroxylactone that inhibits proliferation of certain human tumour cell lines [2]. Remarkable, the natural product is comprised of (–)-(4*R*,5*R*)-5-hydroxy-4-heptadecanolyde (**1**) and its (+)-(4*S*,5*S*) enantiomer (*ent*-**1**; Scheme 1), with former predominating (*ee*, ca. 25%). Both enantiomers showed essentially the same antitumour activity, and not surprisingly they were the objects of numerous synthetic efforts [3]. A number of muricatacin stereoisomers and analogues have also been synthesized [4–16], but only a few were evaluated for their antitumour activity [13–16]. As a part of our ongoing project in the synthesis of new hydroxylactones as potential antitumour agents from monosaccharides [17–20], we have recently disclosed a novel general approach to the enantiodivergent synthesis of **1** and *ent*-**1** from D-xylose, which is suitable for elaboration to a variety of 7-oxa-analogues [20]. As an extension of our previous work, we report herein on the synthesis and antitumour activity of the hydroxylactones **2**, *ent*-**2** and **3**. The enantiomers **2** and *ent*-**2** were designed as possible muricatacin bioisosteres, while the furanolactone **3** represents a conformationally constrained one-carbon homologue of *ent*-**2**. Compound **3** might be also considered as a non-styryl analogue of goniofufurone (**4**), a naturally occurring cytotoxic lactone that was isolated from the stem bark of *Goniothalamus giganteus* (Annonaceae) [21–23].

2. Results and discussion

2.1. Chemistry

To synthesize the target compounds we developed two synthetic routes starting from D-xylose. The first route follows the previously described enantiodivergent approach [20] adopted to complete the synthesis of both **2** and *ent*-**2**. The second route is a more efficient way to prepare the enantiomer **2**, which also provides an access to the furanolactone **3**, via an appropriate divergent intermediate.

The enantiodivergent synthesis of **2** and *ent*-**2** is shown in Scheme 2. The common starting material was the cyclohexylidene derivative **5** readily available from D-xylose in three steps [18,24]. By using a five-step sequence recently developed in our laboratory [20], compound **5** was converted to the lactol

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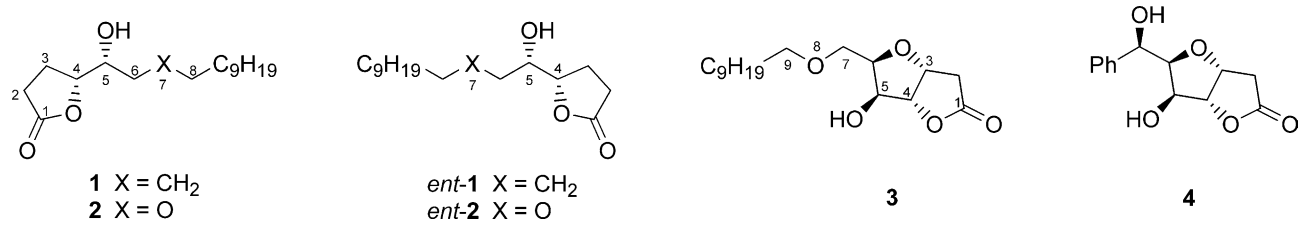
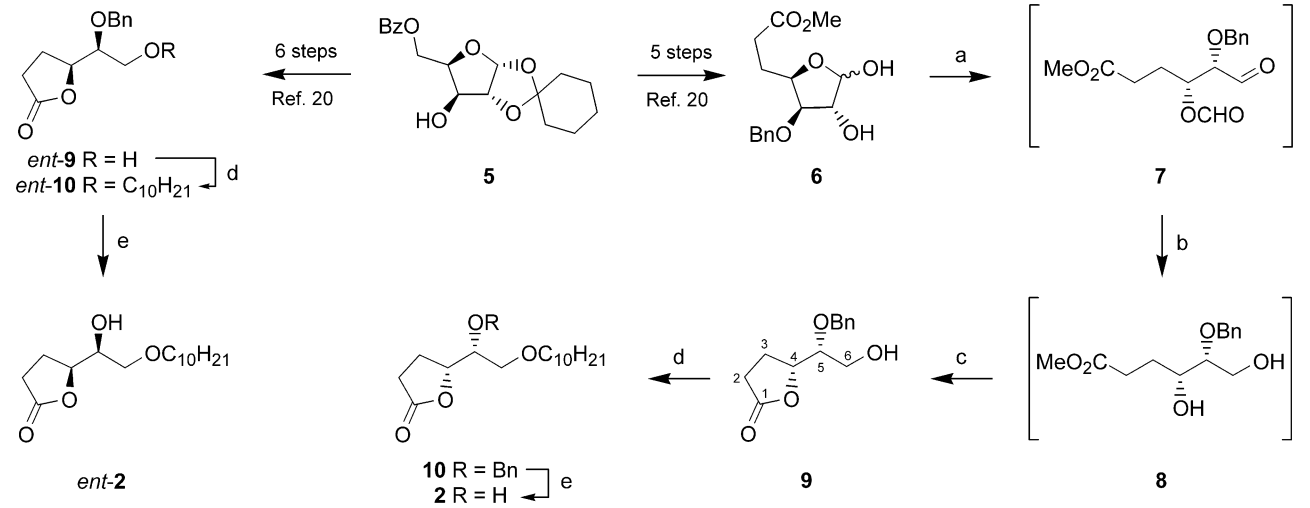
doi:10.1016/j.ejmech.2006.06.008



Scheme 1. (–)-Muricatacin (**1**), (+)-muricatacin (*ent*-**1**), (+)-goniofufurone (**4**) and the target analogues **2**, *ent*-**2** and **3**.

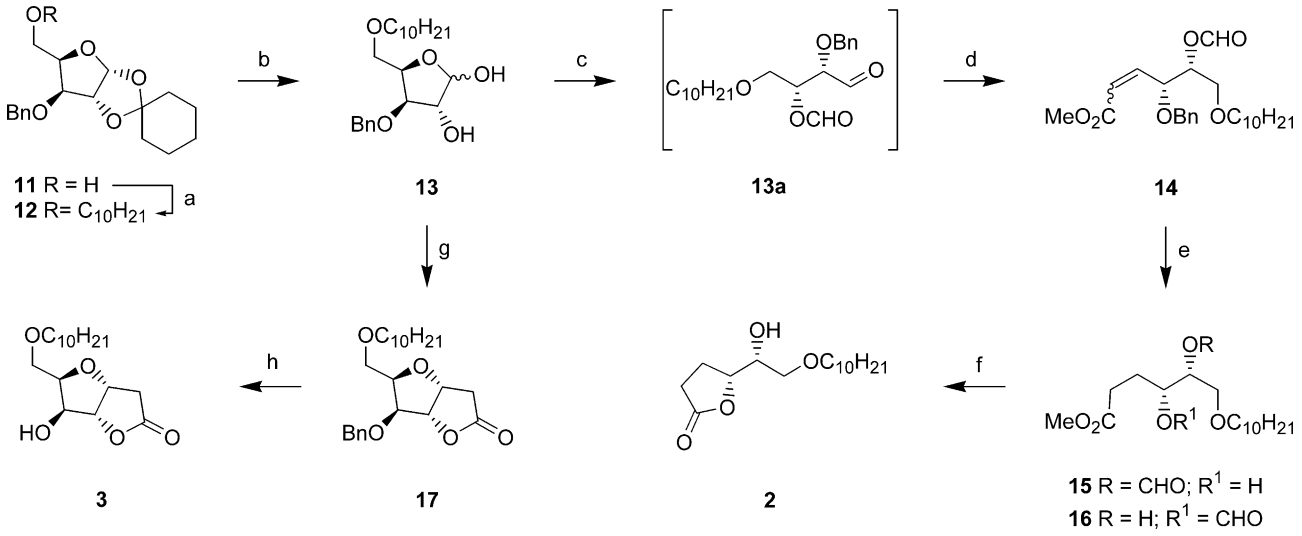


Scheme 2. Reagents and conditions: (a) aq. $NaIO_4$, silica gel, CH_2Cl_2, r.t., 0.5 h, (b) $NaBH_4$, MeOH, 0 °C, 1.5 h, (c) 2:1 TFA/H_2O, r.t., 1.5 h, 50% from **6**; (d) $C_{10}H_{21}Br$, Ag_2O, AgOTf, Et_2O, reflux, 7.5 h, 71% of **10**, 80% of *ent*-**10**; (e) H_2–Pd/C, EtOAc, r.t., 19 h, 87% of **2**, 82% of *ent*-**2**.

6 in 42% overall yield. The intermediate **6** was subsequently converted to the hydroxylactone **9** by using a newly developed one-pot procedure that involved a previous oxidative cleavage of the diol **6** with sodium periodate on silica, sodium borohydride reduction of resulting aldehyde **7** to diol **8**, followed by a subsequent acid promoted lactonisation of the intermediate **8**. This procedure provided the key intermediate **9** in 50% overall yield (from **6**). Compound **9** readily reacted with an excess of decyl bromide and silver oxide in ether, in the presence of a catalytic amount of silver triflate, to give the 7-*O*-decyl derivative **10** in 71% yield. Hydrogenolytic removal of benzyl ether protective group in **10** furnished **2** in 87% yield. Enantiopure hydroxylactone *ent*-**9**, easily obtainable from **5** by a recently developed method from our laboratory [20], represents the key chiral intermediate for preparation of target *ent*-**2**. Thus, *O*-alkylation of *ent*-**9**, followed by a subsequent hydrogenolytic removal of benzyl protective group, under the conditions similar to those already used for the conversion of **9** to **2**, afforded the (+)-muricatacin analogue *ent*-**2**.

By using the former multistep sequences, compound **5** was converted to the (+)-enantiomer *ent*-**2** in an overall yield of 16%, while the (–)-enantiomer **2** was obtained in an overall yield of 13% with respect to starting compound **5**. In order to improve the yield of **2** we have envisioned an alternative route to target **2** via the lactol **13** (Scheme 3). Compound **13** represents a possible divergent intermediate in the synthesis of both analogues **2** and **3**. The primary alcohol **11** easily obtainable from **5** in 89% yield [20] was treated with decyl bromide and silver oxide in refluxing ether, in the presence of a catalytic amount of silver triflate, to afford the corresponding 5-*O*-decyl derivative **12** in 57% yield. Cleavage of the cyclohexylidene functionality in **12** with aq. AcOH gave the corresponding lactol **13** in 84% yield. The product **13** was mainly the β anomer since its chloroform solution mutarotated to a positive equilibrium value {$[\alpha]_D = -3.4 \rightarrow +5.6$ (71 h)}. The ^{1}H NMR spectrum recorded 48 h after dissolution of the crystalline sample **13** confirmed the presence of both α- and β-anomers, with the former predominating ($\alpha/\beta = 5{:}2$). A periodate diol cleavage in **13**, followed by in situ trapping of the resulting aldehyde **13a** with Ph_3P=CHCOMe, under the Taylor conditions [25] gave the expected unsaturated ester **14** as 1:1 mixture of corresponding *E*- and *Z*-isomers. The mixture was not separated, but was further hydrogenated over 10% Pd/C. Hydrogenolytic removal of the benzyl ether protective group was followed by a partial migration of the formyl group from *O*-5 to *O*-4, whereupon the expected saturated ester **15** was obtained, along with an equal amount of the 4-*O*-formyl regioisomer **16**. The mixture of **15** and **16** was treated with aqueous trifluoroacetic acid to afford target **2** in 31% overall yield with respect to the starting compound **5** (from seven steps).

After preparation of (+)- and (–)-muricatacin analogues **2** and *ent*-**2**, we were also interested in obtaining the lactone **3**. In fact, **3** is a conformationally constrained one-carbon homologue of *ent*-**2**, but at the same time it might be considered as a non-styryl analogue of (+)-goniofufurone (**4**). A potent and selective cytotoxicity of certain dephenyl goniofufurone deri-



Scheme 3. Reagents and conditions: (a) $C_{10}H_{21}Br$, Ag_2O, AgOTf, Et_2O, reflux, 56 h, 57%; (b) (i) 7:3 AcOH/H_2O, reflux, 5 h; (ii) 0.1 M NaOMe/MeOH, r.t., 0.5 h, 84%; (c) aq. $NaIO_4$, silica gel, CH_2Cl_2, r.t., 1 h; (d) $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, r.t., 1 h; (e) H_2–Pd/C, EtOH, r.t., 19 h; (f) 2:1 TFA/H_2O, r.t., 1 h, 72% of **2** (from **13**); (g) $Ph_3P{=}CHCO_2Me$, MeOH, 0 °C → r.t., 25 h, 50%; (h) H_2–Pd/C, EtOH, r.t., 20 h, 86%.

vatives [17] prompted us to prepare the lactone **3** and to evaluate its antitumour activity. We have assumed that the bicyclic core of **3** might be accessible by a spontaneous lactonisation and Michael ring-closure accompanying the *Z*-selective Wittig reaction of a stabilised ylide with the furanose lactol **13**, under the reaction conditions similar to those earlier reported [17,26]. Thus, the lactol **13** was treated with $Ph_3P{=}CHCO_2Me$ in dry methanol to give the expected furanolactone **17** in 50% yield. A minor amount of the corresponding *E*-enoate (<10%) was also obtained, however this side-product could not be isolated in pure form, since it had similar chromatographic properties as accompanying impurities. Hydrogenolytic removal of the benzyl protective group in **17** (10% Pd/C) gave an 86% yield of target **3**.

2.2. Antitumour activity

Compounds **2**, *ent*-**2** and **3** were evaluated for their in vitro cytotoxicity against human myelogenous leukaemia K562, human promyelocytic leukaemia HL60, human T cell leukaemia (Jurkat), human colon adenocarcinoma HT-29, human Burkitt's lymphoma (Raji cells) and normal foetal lung MRC-5 cells. Cytotoxic activity was evaluated by using the modified MTT assay [27], after exposure of cells to the test compounds for 24 h. The doxorubicin (DOX) was used as a reference compound.

As outlined in Table 1, (–)-muricatacin analogue **2** was inactive against the HL60 and Raji malignant cells, but showed a significant cytotoxic activity against K562, Jurkat and HT-29 cell lines. The activity of this compound towards the Jurkat leukaemic T cells was comparable to that observed for doxorubicin. However, compound **2** was found to be significantly more active against the K562 and HT-29 cell lines, being approximately 4.5- and sixfold more potent than doxorubicin, respectively. The (+)-enantiomer *ent*-**2** showed a remarkable cytotoxicity towards the K562 and HT-29 cell lines, being almost two and threefold, respectively, more potent than doxorubicin. This analogue was also active against Jurkat cells, but it was more than eightfold less active with respect to the reference compound (DOX). Conversely, the furanolactone **3** has shown a potent cytotoxic activity against the HL60 and Raji malignant cells, comparable to that observed for the reference compound, doxorubicin. Such a cytotoxicity profile of **3** nicely complements the observed biological effects of **2** and *ent*-**2**, since these analogues were devoid of any significant cytotoxicity against both HL60 and Raji cells. Finally, neither of the lactones **2**, *ent*-**2** and **3** exhibited any antiproliferative activity towards the normal foetal lung MRC-5 cells.

In summary, we have synthesized two novel muricatacin analogues **2** and *ent*-**2**, as well as a new non-styryl analogue of goniofufurone **3** starting from D-xylose. The targets **2**, *ent*-**2** and **3** have shown a significant antiproliferative activity against

Table 1
In vitro cytotoxicity of **2**, *ent*-**2**, **3** and DOX

Compounds	IC_{50} (μM)[a]					
	K562	HL60	Jurkat	HT-29	Raji	MRC-5
2	0.18	> 100	1.95	0.086	> 100	> 100
ent-**2**	0.45	> 100	10.80	0.18	79.19	> 100
3	> 100	4.14	> 100	> 100	7.37	> 100
DOX	0.82	2.96	1.28	0.51	6.45	0.32

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.

certain human neoplastic cells, but were completely inactive towards the normal MRC-5 cell line. Both enantiomers **2** and *ent*-**2** were inactive against HL60 and Raji cells, but showed a potent cytotoxic activity against K562, Jurkat and HT-29 cell lines. Their activities are particularly notable in the case of K562 and HT-29 cells being twofold up to sixfold higher than that of doxorubicin. On the contrary, the furanolactone **3** showed a strong cytotoxicity towards the HL60 and Raji neoplastic cells, but was devoid of any cytotoxicity against the K562, Jurkat and HT-29 cell lines. To the best of our knowledge, none of muricatacin analogues were hitherto reported to exhibit such a potent and selective antitumour activity. Moreover, the presented divergent approach is suitable for the synthesis of variety of muricatacin or goniofufurone oxa-analogues and therefore, it may be of use in search for new antineoplastic agents derived from the parent molecules.

3. Experimental section

3.1. Chemistry

Melting points (m.p.) were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on a P 3002 (Krüss) polarimeter in chloroform solutions. NMR spectra were recorded on a Bruker AC 250 E instrument and the chemical shifts (δ-scale) are expressed in ppm values downfield from tetramethylsilane. Chemical ionisation mass spectra were recorded on Finnigan-MAT 8230 spectrometer with isobutane as a reagent gas. TLC was performed on DC Alufolien Kieselgel 60 F_{254} (E. Merck). 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 diminished pressure at a bath temperature below 35 °C.

3.1.1. 5-O-benzyl-2.3-dideoxy-D-threo-hexono-1,4-lactone (9)

To a stirred solution of lactol **6** (0.1022 g, 0.34 mmol) in CH_2Cl_2 (1.5 ml), was added Kieselgel 60 (0.063–0.2 mm; 0.7 g) and 0.65 M aq. $NaIO_4$ (0.7 ml, 0.45 mmol). The mixture was vigorously stirred at room temperature (r.t.) for 0.5 h, filtered and the precipitate washed with CH_2Cl_2. The combined organic solutions were dried and evaporated to afford the crude aldehyde **7** as a colourless oil. To a cooled (0 °C) and stirred solution of crude **7** in MeOH (1 ml) was added $NaBH_4$ (0.013 g, 0.34 mmol) and the mixture was stirred at 0 °C for 1.5 h. To the solution was added TFA (1.4 ml) and the resulting mixture was stirred for 2 h at r.t. and than co-evaporated with toluene (2 × 20 ml). Flash column chromatography (4:1 CH_2Cl_2/EtOAc) of the residue gave pure **9** (0.0407 g, 50%) as a colourless oil, $[\alpha]_D = -23.1$ (*c*, 1.05); ^{1}H NMR ($CDCl_3$): δ 1.87–2.32 (m, 2 H, 2 × H-3), 2.36–2.85 (m, 3 H, 2 × CH-2 and OH), 3.50 (m, 1 H, $J_{5,6a} = 4.9$, $J_{5,6b} = 5.3$, $J_{4,5} = 5.0$ Hz, H-5), 3.70 (dd, 1 H, $J_{6a,6b} = 11.6$ Hz, H-6a), 3.79 (dd, 1H, H-6b), 4.65 and 4.73 (2 × d, 2 H, $J_{gem} = 11.6$ Hz, PhCH_2), 4.70 (m, 1 H, H-4), 7.28–7.45 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$): δ 24.08 (C-3), 28.22 (C-2), 60.93 (C-6), 72.99 (PhCH_2), 80.22 (C-4), 80.51 (C-5), 127.79, 127.86, 128.40, 128.50 and 137.71 (Ph), 177.36 (C=O); CI MS: *m/z* 237 $[M + H]^+$.

3.1.2. General procedure for the preparation of muricatacin 7-oxa-analogues

To a stirred solution of **9** or *ent*-**9** (1 eq) in dry Et_2O (0.3 mmol/ml) were added successively Ag_2O (2.5 eq), AgOTf (0.25 eq) and alkyl bromide (5 eq). The mixture was stirred under reflux for 6–7 hours, than filtered and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2 → 9:1 CH_2Cl_2/EtOAc) to give pure intermediates **10** (71%) or *ent*-**10** (80%). To a stirred solution of **10** or *ent*-**10** (1 eq) in EtOAc (0.1 mmol/ml) was added 10% Pd/C (0.18 g/mmol of **10** or *ent*-**10**). The suspension was hydrogenated at r.t. for 19 h, than filtered through a Celite pad and washed with EtOAc. The combined filtrates were evaporated and the residue purified by flash chromatography (CH_2Cl_2 → 9:1 CH_2Cl_2/EtOAc).

3.1.2.1. 6-O-Decyl-2,3-dideoxy-D-treo-hexono-1,4-lactone (2). Yield: 62% (from **9**). Selected data: m.p. 42 °C (from CH_2Cl_2/hexane); $[\alpha]_D = -33.0$ (*c* 0.8); ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3 H, $J = 6.4$ Hz, CH_3), 1.13–1.63 (m, 16 H, 8 × CH_2 from side chain), 2.24 (m, 2 H, 2 × H-3), 2.39–2.72 (m, 2 H, $J_{gem} = 16.9$ Hz, 2 × H-2), 2.87 (d, 1 H, exchangeable with D_2O, $J_{5,OH} = 5.2$ Hz, OH), 3.44 (t, 2 H, $J = 6.6$ Hz, H-8), 3.51 (d, 2 H, $J_{5,6} = 6.1$ Hz, 2 × H-6), 3.78 (m, 1 H, $J_{4,5} = 3.4$, $J_{5,6} = 6.1$, $J_{5,OH} = 5.2$ Hz, H-5), 4.56 (td, 1 H, $J_{3,4} = 7.0$, $J_{4,5} = 3.2$ Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 13.99 (CH_3), 22.56, 23.74, 25.96, 28.27, 29.20, 29.34, 29.45, 29.47 and 31.78 (8 × CH_2 from side chain, C-2 and C-3), 71.12 (C-6), 71.69 (C-8), 71.94 (C-5), 79.79 (C-4), 177.53 (C-1); CI MS: *m/z* 573 ($2M^+ + H$), 287 ($M^+ + H$).

3.1.2.2. 6-O-Decyl-2,3-dideoxy-L-treo-hexono-1,4-lactone (ent-2). Yield: 66% (from *ent*-**9**). Selected data: m.p. 42 °C (from CH_2Cl_2/hexane); $[\alpha]_D = +33.0$ (*c* 0.8); IR, NMR and mass spectral data were identical to those recorded for the enantiomer **2**.

3.1.3. 3-O-Benzyl-1,2-O-cyclohexylidene-5-O-decyl-α-D-xylofuranose (12)

To a solution of **11** (0.9556 g, 2.98 mmol) in dry Et_2O (19.5 ml) were added successively Ag_2O (1.7292 g, 7.46 mmol), AgOTf (0.1905 g, 0.74 mmol) and 1-bromodecane (1.55 ml, 7.45 mmol). The mixture was stirred under reflux for 56 h, than filtered and evaporated. The residue was purified by flash chromatography (19:1 hexane/EtOAc → 9:1 hexane/EtOAc), to give pure **12** (0.7847 g, 57%) as a colourless oil, $[\alpha]_D = -25.2$ (*c*, 1.56); ^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3 H, $J = 6.3$ Hz, Me), 1.08–1.82 (m, 26 H, 8 × CH_2 from side chain and 5 × CH_2 from C_6H_{10}), 3.48 (m, 2 H, OCH_2 from side chain), 3.68 (dd, 1 H, $J_{5a,5b} = 9.8$, $J_{4,5a} = 6.2$ Hz, H-5a), 3.73 (dd, 1 H, $J_{4,5b} = 6.3$ Hz, H-5b), 4.00 (d, 1 H, $J_{3,4} = 3.1$ Hz, H-3), 4.38 (td, 1 H, H-4), 4.55 and 4.69 (2 × d, 2 H, $J_{gem} = 12.0$ Hz, PhCH_2), 4.60 (d, 1 H, $J_{1,2} = 3.8$ Hz, H-2), 5.94 (d, 1 H, H-1), 7.26–7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$): δ 14.0 (Me), 22.58, 23.52, 23.80, 24.85, 26.04,

29.22, 29.40, 29.47, 29.50, 29.61, 31.80, 35.78 and 36.41 (8 × CH_2 from side chain and 5 × CH_2 from C_6H_{10}), 67.78 (C-5), 71.65 (OCH_2 from side chain), 71.96 (PhCH_2), 79.07 (C-4), 81.88 (C-3), 81.98 (C-2), 104.54 (C-1), 112.21 (C_q from C_6H_{10}), 127.41, 127.68, 128.30 and 137.68 (Ph); CI MS: *m/z* 461 (M^+ + H).

*3.1.4. 3-O-Benzyl-5-O-decyl-D-xylofuranose (**13**)*

A solution of **12** (0.8189 g, 1.78 mmol) in 70% aq. AcOH was refluxed for 5 h and evaporated by co-distillation with toluene. The residue was dissolved in dry MeOH (1 ml), and treated with 0.1 M NaOMe in MeOH (1 ml, 0.1 mmol) at r.t. for 0.5 h. The mixture was first neutralised with 1 M AcOH in MeOH (0.1 ml, 0.1 mmol) and than co-evaporated with toluene. Flash column chromatography (4:1 CH_2Cl_2/EtOAc) of the residue gave pure **13** (0.5722 g, 84%) as a colourless solid. Recrystallisation from CH_2Cl_2/hexane gave colourless crystals, m.p. 68–70 °C; $[\alpha]_D = -3.4 \rightarrow +5.6$ (*c*, 1.1; 71 h); anomeric ratio (from ^{1}H NMR): α/β = 5:2; ^{1}H NMR ($CDCl_3$ + D_2O): δ 0.89 (t, 3 H, J = 6.4 Hz, Me), 1.20–1.65 (m, 16 H, 8 × CH_2 from side chain), 3.46 (m, 2 H, OCH_2 from side chain), 3.61 (d, 2 H, $J_{4,5}$ = 5.7 Hz, 2 × H-5α), 3.65 (dd, 0.4 H, $J_{4,5a}$ = 5.3, $J_{5a,5b}$ = 10.4 Hz, H-5aβ), 3.71 (dd, 0.4 H, $J_{4,5b}$ = 4.0 Hz, 5bβ), 4.00 (m, 1 H, H-3), 4.19 (dd, 1 H, $J_{1,2}$ = 4.3, $J_{2,3}$ = 2.9 Hz, H-2α), 4.25 (d, 0.4 H, $J_{2,3}$ = 2.1 Hz, H-2β), 4.38 (m, 0.4 H, H-4β), 4.44 (m, 1 H, H-4α), 4.54 and 4.70 (2 × d, 2 H, J_{gem} = 11.6 Hz, PhCH_2α), 4.58 and 4.68 (2 × d, 0.8 H, J_{gem} = 11.6 Hz, PhCH_2β), 5.13 (bs, 0.4 H, H-1β), 5.49 (d, 1 H, $J_{1,2}$ = 4.3 Hz, H-1α), 7.29–7.41 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$ + D_2O): δ 14.05 (Me), 22.62, 25.98, 26.03, 29.26, 29.40, 29.43, 29.46, 29.49, 29.52, 29.55 and 31.84 (8 × CH_2 from side chain), 69.19 (C-5β), 69.44 (C-5α), 71.77, 71.80 and 71.84 (PhCH_2 and OCH_2 from side chain), 72.59 (C-2α), 77.26 (C-4α), 79.40 (C-2β), 79.79 (C-4β), 83.52 (C-3α), 95.96 (C-1α), 103.37 (C-1β), 127.48, 127.65, 127.67, 127.94, 128.33, 128.45, 137.40 and 137.83 (Ph); CI MS: *m/z* 381 (M^+ + H).

*3.1.5. Alternative procedure for the preparation of **2***

To a stirred solution of **13** (0.1980 g, 0.52 mmol) in CH_2Cl_2 (6 ml) were added Kieselgel 60 (0.063–0.2 mm; 1.56 g) and 0.65 M aq. $NaIO_4$ (1.04 ml, 0.68 mmol) and the mixture was vigorously stirred at r.t. for 2 h. To the suspension was than added Ph_3P=$CHCO_2Me$ (0.3840 g, 1.15 mmol) and the stirring at r.t. was continued for an additional 1 h. The mixture was filtered and the liquid phase was evaporated. Flash column chromatography (CH_2Cl_2), of the residue gave pure **14** (0.2089 g, 92%) as an inseparable 1:1 mixture of the corresponding *Z*- and *E*/isomers. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, J = 6.3 Hz, Me), 1.16–1.60 (m, 16 H, 8 × CH_2 from side chain), 3.40 (m, 2 H, 2 × H-8), 3.52 (dd, 0.5 H, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 10.6 Hz, H-6a, *E*-isomer), 3.61 (dd, 0.5 H, $J_{5,6b}$ = 4.5 Hz, H-6b, *E*-isomer), 3.63 (d, 1 H, J = 4.6 Hz, 2 × H-6, *Z*-isomer), 3.71 and 3.76 (2 × s, 3 H each, OMe), 4.30 (td, 0.5 H, $J_{3,4}$ = 5.9, $J_{4.5}$ = 6.1, $J_{2,4}$ = 5.9 Hz, H-4, *E*-isomer), 4.44–4.66 (m, 4 H, 2 × PhCH_2), 5.18 (m, 0.5 H, H-5, *E*-isomer), 5.33 (m, 0.5 H, H-4 and H-5, *Z*-isomer), 5.99 (d, 0.5 H, $J_{2,3}$ = 12.0 Hz, H-2, *Z*-isomer), 6.15 (dd, 0.5 H, $J_{2,3}$ = 15.8 Hz, $J_{2,4}$ = 5.9 Hz, H-2, *E*-isomer), 6.16 (m, 0.5 H, H-3, *Z*-isomer), 6.86 (m, 0.5 H, H-3, *E*-isomer), 7.21–7.40 (m, 5 H, Ph), 8.10 (s, 1 H, CHO). ^{13}C NMR ($CDCl_3$): δ 13.99 (Me), 22.56, 25.92, 29.21, 29.32, 29.34, 29.40, 29.46 and 31.78 (8 × CH_2 from side chain), 51.39 and 51.62 (OMe), 68.21 and 68.63 (C-6), 71.38, 71.59, 71.74 and 71.87 (PhCH_2 and C-8), 72.92 and 73.56 (C-4 and C-5, *Z*-isomer), 73.02 (C-5, *E*-isomer), 76.19 (C-4, *E*-isomer), 122.98 (C-2, *Z*-isomer), 123.78 (C-2, *E*-isomer), 127.69, 127.73, 127.79, 127.84, 128.22, 128.35, 129.63, 137.17 and 137.58 (Ph), 143.29 (C-3, *E*-isomer), 145.80 (C-3, *Z*-isomer), 160.18 and 160.43 (CHO),165.74 and 166.0 (C-1). A solution of **14** (0.1495 g, 0.34 mmol) in EtOH (3 ml) was hydrogenated over 10% Pd/C (0.0718 g) for 19.5 h at r.t. The suspension was filtered through a Celite pad and washed with EtOAc. The combined filtrates were evaporated and the residue purified by flash column chromatography (7:3 CH_2Cl_2/Et_2O), to afford an inseparable 1:1 mixture of regioisomers **15** and **16** (0.0963 g, 81%) as a colourless oil. ^{1}H NMR ($CDCl_3$, selected data): δ 2.78 and 3.20 (2 × d, 1 H each, 2 × OH), 3.40 and 3.42 (m, 4 H, 2 × H-8), 3.65 (s, 3 H, OMe), 5.00 and 5.09 (2 × m, 1 H each, C*H*-OCHO and C*H*-OH), 8.10 and 8.16 (2 × s, 1 H each, CHO). ^{13}C NMR ($CDCl_3$, selected data): δ 13.98 (Me), 51.59 and 51.63 (OMe), 70.21 and 70.94 (C-OH), 72.95 and 73.92 (*C*-OCHO), 160.66 and 160.53 (OCHO), 173.15 and 174.02 (C-1). A solution of purified mixture **15** and **16** (0.0953 g, 0.275 mmol) in a mixture of TFA (1.3 ml) and water (0.6 ml) was stirred at r.t. for 1 h and than co-evaporated with toluene (2 × 20 ml). Flash chromatography (9:1 CH_2Cl_2/EtOAc) of the residue, followed by recrystallisation from a mixture of CH_2Cl_2/hexane, gave pure **2** (0.0766 g, 97%). Physical constants and spectral data of **2** thus obtained were in excellent agreement with those presented in the procedure, see Section 3.1.2.1.

*3.1.6. 3,6-Anhydro-5-O-benzyl-7-O-decyl-2-deoxy-D-ido-heptono-1,4-lactone (**17**)*

To a stirred and cooled (0 °C) solution of **13** (0.1098 g, 0.29 mmol) in dry MeOH (3 ml) was added Ph_3P=CHCOMe (0.1035 g, 0.31 mmol). After stirring the mixture at r.t. for 1 h, an additional amount of Ph_3P=CHCOMe (0.0867 g, 0.26 mmol) was added in one portion. The mixture was stirred at r.t. for 25 h and than evaporated. Flash chromatography (1:1 Et_2O/hexane) of the residue gave pure **17** (0.0582 g, 50%) as a colourless oil, $[\alpha]_D$ = +13.1 (*c*, 1.0); ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3 H, J = 6.3 Hz, Me), 1.21–1.57 (m, 16 H, 8 × CH_2 from side chain), 2.70 (m, 2 H, 2 × H-2), 3.46 (m, 2 H, 2 × H-9), 3.64 (d, 2 H, 2 × H-7), 4.20 (d, 1 H, $J_{5,6}$ = 4.1 Hz, H-5), 4.26 (m, 1 H, H-6), 4.59 and 4.70 (2 × d, 2 H, J_{gem} = 11.9 Hz, CH_2Ph), 4.92 (d, 1 H, $J_{3,4}$ = 4.7 Hz, H-4), 4.98 (m, 1 H, H-3), 7.27–7.42 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$): δ 14.07 (Me), 22.63, 26.06, 29.26, 29.42, 29.52, 29.54, 29.60 and 31.84 (8 × CH_2 from side chain), 35.96 (C-2), 68.50 (C-7), 71.79 (C-9), 72.68 (CH_2Ph), 76.77 (C-3), 79.58 (C-6), 81.45 (C-5), 85.45 (C-4), 127.68, 128.09, 128.52 and 137.13 (Ph), 175.28 (C-1); CI MS: *m/z* 405 (M^+ + H).

*3.1.7. 3,6-Anhydro-7-O-decyl-2-deoxy-D-ido-heptono-1,4-lactone (**3**)*

A solution of **17** (0.1508 g, 0.37 mmol) in dry EtOH (3 ml) was hydrogenated over 10% Pd/C (0.0755 g) at r.t. for 20 h. After the same workup as described above (Procedure 3.1.2), followed by chromatographic purification on a column of flash silica (4:1 Et_2O/hexane → Et_2O), pure **3** (0.1005 g, 86%) was obtained as a colourless solid. Recrystallisation from CH_2Cl_2/hexane gave colourless crystals, m.p. 59–60 °C, $[\alpha]_D = +35.4$ (*c*, 0.45); ^{1}H NMR ($CDCl_3$): δ 0.86 (t, 3 H, $J = 6.4$ Hz, Me), 1.10–1.65 (m, 16 H, 8 × CH_2 from side chain), 2.64 (d, 1 H, $J_{2a,2b} = 18.7$, H-2a), 2.75 (dd, 1 H, $J_{2a,2b} = 18.7$, $J_{2b,3} = 5.4$ Hz, H-2b), 3.50–3.59 (m, 2 H, 2 × H-9), 3.85 (m, 2 H, 2 × H-7), 4.10 (m, 1 H, H-6), 4.25 (bs, 1 H, exchangeable with D_2O, OH), 4.50 (d, 1 H, $J_{5,6} = 3.1$ Hz, H-5), 4.85 (d, 1 H, $J_{3,4} = 4.2$ Hz, H-4), 5.01 (dd, 1 H, $J_{2b,3} = 5.4$, $J_{3,4} = 4.2$ Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 14.02 (Me), 22.58, 25.87, 29.20, 29.28, 29.34, 29.43 and 31.78 (8 × CH_2 from side chain), 36.00 (C-2), 69.41 (C-7), 72.49 (C-9), 75.76 (C-5), 76.49 (C-3), 78.59 (C-6), 88.19 (C-4), 175.45 (C-1); CI MS: *m/z* 629 ($2M^+ + H$), 315 ($M^+ + H$).

3.2. In vitro antitumour assay

Antitumour activity was evaluated by the tetrazolium colorimetric MTT assay. The assay is based on cleavage of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), to formazan by mitochondrial dehydrogenases in viable cells. Exponentially growing cells were harvested, counted by trypan blue exclusion and plated into 96-well microtitar plates (Costar) at optimal seeding density of 5×10^3 cells per well to assure logarithmic growth rate throughout the assay period. Viable cells were plated in a volume of 90 μl per well, and preincubated in complete medium at 37 °C for 24 h to allow cell stabilisation prior to the addition of substances. Tested compounds, at twice the required final concentration, in growth medium (10 μl per well) were added to all wells except to the control ones and microplates were incubated for 24 h. The wells containing cells without tested compounds were used as control. Three hours before the end of incubation period 10 μl of MTT solution was added to each well. MTT was dissolved in medium at 5 mg/ml and filtered to sterilise and remove a small amount of insoluble residue present in some batches of MTT. Acidified 2-propanol (100 μl of 0.04 M HCl in 2-propanol) was added to each well and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at r.t. to ensure that all crystals were dissolved, the plates were read on a spectrophotometer plate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells containing complete medium and MTT only acted as blank. The compound cytotoxicity was expressed as the IC_{50} (50% inhibitory concentration).

Acknowledgments

This work was supported by a research grant from the Ministry of Science and Environment Protection of the Republic of Serbia (Grant No. 142005). The authors are grateful to Mr. Dejan Djoković (Faculty of Chemistry, University of Belgrade, S&M) for recording the CI mass spectra.

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