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Sugar bislactones by one-step oxidative dimerisation with pyridinium chlorochromate versus regioselective oxidation of vicinal diols

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Abstract—Synthesis of 10-membered bislactones by PCC oxidation of methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside is described, with emphasis on their structure elucidation using the information gained by combination of NMR spectroscopic techniques with X-ray diffraction data. In alternative, the use of PCC and PCC adsorbed on silica gel or alumina for the regioselective oxidation of vicinal diols in sugars is also reported. Both bislactones showed antifungal activity against *Candida albicans*, and were slightly active against the bacteria *Bacillus subtilis*. The bislactone presenting pivaloyl protecting groups also promoted some growth inhibition of *Staphylococcus aureus*.

Keywords: Sugar bislactones; Keto sugars; Pyridinium chlorochromate; Regioselective oxidation; Bioactivity studies

1. Introduction

Bislactones occur in a variety of natural and synthetic molecules, namely as five-membered ring fused bislactones, some of which possess antifungal and antibacterial activities,[1,2] as spirocyclic bislactones,[3] 10-membered diolides,[4] 12- and 14-membered nitrogen containing macrocyclic bislactones of interest as complexation agents,[5] and also as 30-membered rings, the major constituents of the pupal defensive secretion of the ladybird beetle *Subcoccinella vigintiquatuorpunctata*.[6] Acremonol and acremodiol are 14-membered natural bislactones, which were reported as fungal metabolites with antibacterial activity against a variety of microorganisms, namely *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[7]

Synthesis of macrocyclic bislactones has been reported in the literature using different approaches, which include pathways with several steps. Thirty-membered bislactones were prepared by reaction of a tetradecenoic acid, possessing its hydroxyl group protected with TBDMS, with the free hydroxyl group of its reaction partner, which is the corresponding tetradecenoic acid allyl ester. Intramolecular cyclisation of the ester formed was promoted by Mukaiyama salt after deprotection of the TBDMS ether and of the allyl ester.[6] The acyclic compounds were obtained in multiple steps starting from tosyl aziridine.[8]

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Reductive intramolecular coupling of bis(iminoesters) afforded nitrogen-containing macrocyclic 12- and 14-membered bislactones. The bis(iminoesters) were obtained by reaction of aromatic aldehydes and bis(aminoesters), which were synthesised by DCC esterification of *N*-protected valine and diols.[5]

Ring closing olefin metathesis catalysed by Ru with *N*-heterocyclic carbene ligands gave an unsaturated 14-membered bislactone, which could not be converted to the saturated bislactone by hydrogenation. Nevertheless its synthesis was accomplished by intermolecular alkylation followed by intramolecular cyclisation of ω-iodobutyl acetoacetate promoted by sodium hydride in 14% yield.[9]

Pyridinium chlorochromate (PCC) is a versatile reagent leading to a variety of transformations.[10] It has been successfully used for the oxidation of primary and secondary alcohols in a diversity of organic molecules such as alkaloids,[11] iridoids,[12] steroids[13] and sugars, namely for the synthesis of 3-keto sugars.[14,15] These compounds are precursors of a variety of biomolecules such as the sugar moieties of amipurimycin,[14] miharamycin[15,16] and miharamycin analogues,[17] antibiotics, which are potent inhibitors of *Pyricularia oryzae*, known to produce the rice blast disease. Hydroquinones,[18] active methylene compounds,[19] organoboranes,[20] aromatic aldehydes and cyclic acetals (solvent-free oxidations)[21] were also oxidised by PCC. Microwave assisted regeneration of carbonyl functions from oximes,[22] conversion of glycals into α,β-unsaturated lactones,[23] and oxidation of 1,4-diols and 1,5-diols to yield the corresponding γ- and δ-lactones[24] were also accomplished by use of this reagent.

We report now an efficient and one-step synthesis of 10-membered ring bislactones by PCC oxidation of glucose derivatives, which possess positions 2,3 or 3,4 free. This is the first approach to a direct synthesis of macrocyclic bislactones.

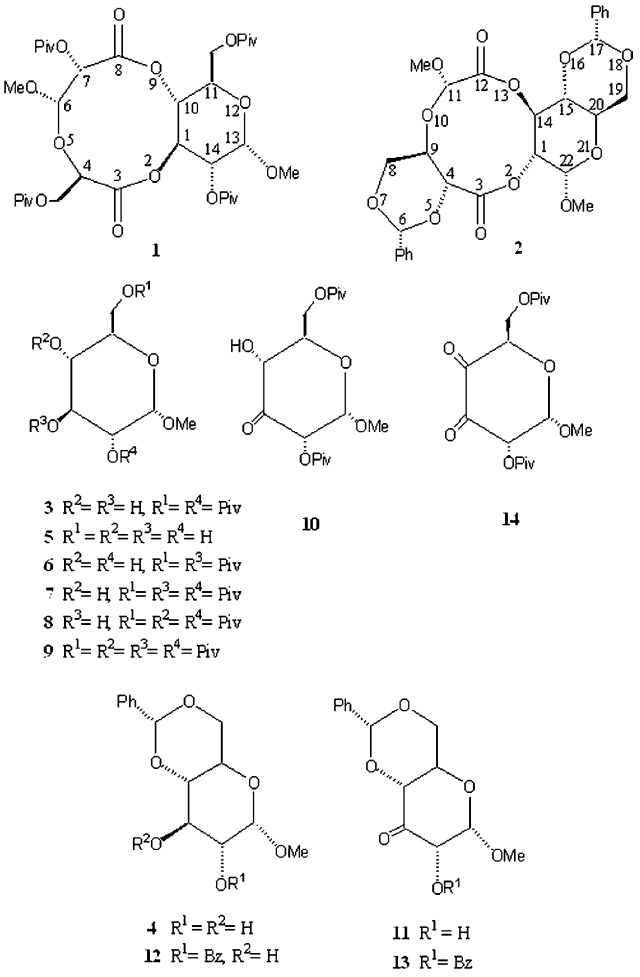


Chart 1. Structure of compounds **1**–**14**.

2. Results and discussion

The starting materials used for the synthesis of the 10-membered ring bislactones **1** and **2** were methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside (**3**),[25] and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**4**)[26] (Chart 1). Preparation of **3** was described in the literature by reaction of **5** with pivaloyl chloride in pyridine at 0 °C in 83% yield.[27] We have reinvestigated the reaction conditions reported by Klausener et al.[25] and obtained **3** in 89% yield, together with methyl 3,6-di-*O*-pivaloyl-α-D-glucopyranoside (**6**),[28] methyl 2,3,6- tri-*O*-pivaloyl-α-D-glucopyranoside (**7**)[29] and methyl 2,4,6- tri-*O*-pivaloyl-α-D-glucopyranoside (**8**)[29] in 0.6%, 3.3% and 2.8% yield, respectively. Reaction of **5** with *N*-pivaloyl imidazole in DMF[30] at rt for 8 h and for 5 days afforded compounds **3**, **6**, **7** (Table 1). Compound **3** was isolated in low yield in both cases, being **7** the major compound.

Regioselective oxidation of **3** with PCC in CH_2Cl_2, in the presence of molecular sieve powder 4 Å and sodium acetate was accomplished in 60% yield by Klausener et al.[28] (Table 2). Reaction of **3** with PCC adsorbed on alumina in CH_2Cl_2 under reflux for 48 h was also described, being the keto sugar **10** isolated in 75% yield, based on the reacted starting material, recovered in 24% yield.[14] We have now used this procedure, replacing CH_2Cl_2 by $ClCH_2CH_2Cl$. When this reaction was run at rt for 48 h, compound **10** was isolated in 52%, being the diol **3** recovered in 40% yield. Regioselective oxidation of **3** with PCC absorbed on silica gel[31] at rt for 42 h gave the keto sugar **10** in 94% yield, based on the reacted starting material, which was recovered in 46% yield.

When the system PCC, NaOAc in CH_2Cl_2 and molecular sieves powder 4 Å was investigated for the regioselective oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**4**),[26] the 3-keto sugar **11** was isolated only in 17% yield. The synthesis of **11** was

Table 1. Reaction conditions for the pivaloylation of methyl α-D-glucopyranoside (**5**) and yield[a] (%) of compounds **3**, **6**–**8**

Reagent system	Solvent	Temperature (°C)	Time (h)	Product(s) (yield, %)	Recovered substrate (yield, %)
PivCl (2 equiv)	Pyridine	−20 °C[b]	52.5	**3** (89)	**5** (0)
		rt[c]		**6** (0.6)	
		60 °C[d]		**7** (3.3)	
				8 (2.8)	
Piv-Imidazole (4 equiv)	DMF	rt	8	**3** (36)	**5** (20)
				6 (5)	
				7 (48)	
Piv-Imidazole (4 equiv)	DMF	rt	120	**3** (20)	**5** (0)
				6 (20)	
				7 (32)	

[a]When applied, the yield was based on reacted starting material.
[b]For 4 h.
[c]For 48 h.
[d]For 0.5 h.

Table 2. Reaction conditions for PCC oxidation of compounds **3** and **4** and yield[a] (%) of compounds **1**, **2**, **10**, **11** and **14**

Subst.	Reagent system	Solvent	Temp. (°C)	Time (h)	Compound(s) (yield, %)	Recovered diol (yield, %)
3	PCC, NaOAc, ms powd. 4 Å	CH_2Cl_2	rt	36	**10** (60)[26]	—
3	PCC-Al_2O_3	CH_2Cl_2	40	48	**10** (75)[14]	**3** (24)[14]
3	PCC-Al_2O_3	$ClCH_2CH_2Cl$	rt	48	**10** (52)	**3** (40)
					1 (16)	
3	PCC-silica gel	CH_2Cl_2	rt	42	**10** (94)	**3** (46)
					1 (14)	
3	PCC	Toluene	75	24	**1** (50)	**3** (0)
					14 (2)	
4	PCC, NaOAc, ms powd. 4 Å	CH_2Cl_2	rt	60	**11** (17)	**4** (0)
					2 (5)	
4	PCC	$ClCH_2CH_2Cl$	85	48	**2** (44)	**4** (13)

[a]When applied, the yield was based on the reacted starting material.

accomplished by an alternative approach, based on PCC oxidation in CH_2Cl_2 in the presence of molecular sieves powder 3 Å at rt for 15 h of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**12**)[32,33] to give **13** in 93% yield,[15] followed by debenzoylation with K_2CO_3 in methanol[34] at rt for 60 h affording **11** in 70% yield. Preparation of **12** was accomplished in 82% yield by treatment of **4** with dibutyltin oxide in benzene under reflux for 12 h, followed by addition of benzoyl chloride in benzene and molecular sieves powder 4 Å for 15 min at rt. The system 1-(benzoyloxy)benzotriazole/triethylamine was also used successfully to prepare **12** in 89% yield.

We now focus on a novel and efficient use of PCC for the synthesis of bislactones. Hence, oxidation of **3** with PCC in toluene at 75 °C for 24 h gave compound **1**, isolated in 50% yield, being also isolated the diketo sugar **14** in 2% yield. This bislactone was also detected as a secondary product of the reaction of **3** with PCC adsorbed in silica gel in 14% yield, or with PCC adsorbed in alumina in $ClCH_2CH_2Cl$ at rt (16% yield). Klausener et al. isolated a secondary product of the oxidation of **3** with PCC/NaOAc in 9% yield, and suggested the structure of a cyclic diester for it, although they could not prove its structure.[28]

Synthesis of the bislactone **2** starting from **4** was also accomplished, being this compound isolated in 44% yield when $ClCH_2CH_2Cl$ was used as solvent and the reaction was run under reflux for 48 h. Compound **2** appeared also as a secondary product of the above described PCC oxidation of **4** in CH_2Cl_2, being isolated in 5% yield (Table 2).

The structures of compounds **1** and **2** were established by means of their spectroscopic data, mainly by their ^1H NMR and ^{13}C NMR spectra, and by DEPT, COSY, NOESY, HMQC and HMBC experiments. The X-ray diffraction data of the crystalline bislactone **1** also confirmed the proposed structure.

The ^{13}C NMR spectrum of compound **1** showed the signals due to the carbonyl group of four pivaloyl groups at δ 177.3, 177.9, 178.3 and 178.4, and the resonances of two carbonyl groups at δ 170.1 and 168.5, which correspond to the lactone functions of the molecule. The presence of two methoxy groups was confirmed by the signals at δ 57.6 and 56.2. The ^1H NMR resonance of H-1 appeared at δ 5.35 as a triplet,

Table 3. HMBC correlations relevant for the structure elucidation of compounds **1** and **2**

C-atom	Compound **1**	Compound **2**
C-1	H-10 (m, 4.99–4.94)	H-14
C-4	—	H-6, H-8eq
C-8	H-7	H-6
C-10	H-1	—
C-11	H-10 (m, 4.99–4.94)	—
C-14	H-1	H-1, H-22, H-15 (m, 3.87–3.79)
C-15	—	H-14, H-17, H-19eq, H-19a (m, 3.87–3.79)
C-20	—	H-19eq, H-22, H-19a (m, 3.87–3.79)
OMe-6	H-6	—
OMe-11	—	H-11
OMe-13	H-13 (m, 4.99–4.94)	—
OMe-22	—	H-22
*C*H$_2$OPiv-4	H-4 (m, 4.75–4.71)	—
*C*H$_2$OPiv-11	H-10 (m, 4.99–4.94)	—
C=O (Piv-7)	H-7	—

presenting a trans-diaxial coupling with H-10 and H-14 with $J_{1,10} = J_{1,14}$ 10.4 Hz. The signal of H-10 is included in the multiplet at δ 4.99–4.94, together with the resonance of H-13, while that of H-14 appears in the multiplet at 4.75–4.71, together with the signal of H-4, a proton of the 10-membered ring of the molecule. The signal of H-7 is a doublet at δ 5.51, showing $J_{6,7}$ 1.95 Hz, while the resonance of H-6 appears at δ 5.15 as a doublet, as expected. By HMQC it was possible to assign C-10 at δ 74.5 and C-14 at δ 70.7, which are correlated with H-1 in the HMBC spectrum (Table 3). Correlations between H-10 and C-1 at δ 73.4, C-11 at δ 67.6, and *C*H$_2$OPiv at δ 62.3 were also detected by HMBC, as well as those of H-7 with C-8 at δ 168.5 and with the carbonyl of the pivaloyl group bonded to C-7 at δ 177.3. The assignment of the methoxy groups and of the other carbonyl groups was also possible by means of HMBC experiments. The proposed configuration of the chirality centres C-1, C-4, C-6, C-7, C-10, C-11, C-13 and C-14 is in agreement with the NMR data and was confirmed by single crystal X-ray crystallographic analysis of **1**, as can be observed in Figure 1. All bond lengths, angles and torsion angles relevant to the configuration of the molecule assume expected values. Crystal data and structure refinement are given in Table 4. The conformation exhibited by the six-membered and 10-membered rings of compound **1** was confirmed by X-ray crystallography and is depicted in Figure 2.

The structure assigned to the secondary product **14**, was based on its NMR data. Its ^{1}H NMR spectrum presented the resonance of H-1 as a doublet at δ 5.32 and that of H-2 at δ 5.07 as a double doublet, presenting $J_{1,2}$ 4.8 Hz and a long range coupling $^5J_{2,5}$ 0.8 Hz, confirmed by COSY. The signal of H-5 appeared as a broad triplet at δ 4.14, coupled with H-6a and H-6b. Their resonances appear as double doublets at δ 4.43 and δ 4.18 with $J_{5,6a}$ 2.8 Hz, $J_{5,6b}$ 3.2 Hz, and $J_{6a,6b}$ 12.4 Hz. The signals of the ^{13}C NMR spectrum were in complete agreement with the proposed structure. The carbonyl

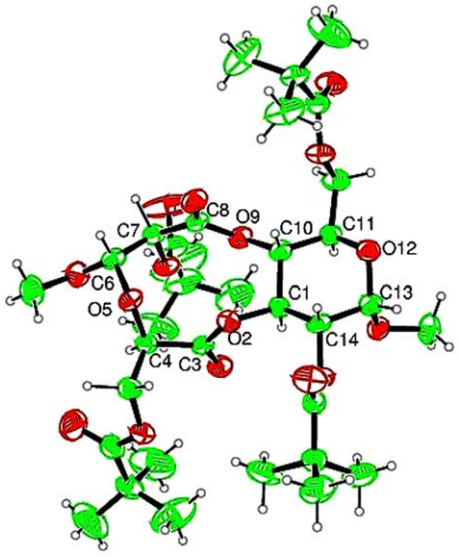


Figure 1. ORTEP drawing of the bislactone **1** showing the labelling scheme of the skeleton of the ten- and six-membered rings (ellipsoids with 35% probability).

groups of the ring appeared at δ 204.0, those of the pivaloyl group at δ 176.8 and 176.7, C-1 at δ 98.2, C-2 and C-5 at δ 72.9 and δ 73.2, respectively, and C-6 at δ 60.7.

The isolation of the diketone **14** was decisive for the mechanism proposed for the formation of the bislactones, depicted in Scheme 1 for compound **1**. This intermediate **14** suffers a nucleophilic attack by the starting material with the orientation presented in the figure. Oxidative cleavage of the indicated C–C bond leads to the lactone functionality.

The ^{1}H NMR spectrum of compound **2** exhibited the expected singlet for H-11 at δ 5.10 as well as the doublet at δ 4.38 for H-4, due to its coupling with H-9, confirming the formation of the 10-membered bislactone, which carbonyl groups C-3 and C-12 were detected at δ 166.6 in the ^{13}C NMR spectrum. The methoxy groups

Table 4. Crystal data and structure refinement for compound **1**

Empirical formula	C_{34} $H_{54}O_{16}$
Formula weight	718.77
Temperature	293(2) K
Wavelength	1.54180 Å
Crystal system	Monoclinic
Space group	*P*21
Unit cell dimensions	$a = 12.321(4)$ Å
	$b = 12.207(4)$ Å
	$c = 13.974(5)$ Å
	$\beta = 101.69(2)$ deg.
Volume	2058.2(12) Å^3
Z	2
Calculated density	1.160 Mg/m^3
Absorption coefficient	0.773 mm^{-1}
F(0 0 0)	772
θ range for data collection	3.23°–66.98°
Limiting indices	$0 \leqslant h \leqslant 14$;
	$-14 \leqslant k \leqslant 1$;
	$-16 \leqslant l \leqslant 16$
Reflections collected/unique	4438/4235 [R(int) = 0.1890]
Completeness to $\theta = 66.98$	99.9%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4235/1/492
Goodness-of-fit on F^2	1.112
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0699$
R indices (all data)	$R_1 = 0.0966$
Absolute structure parameter	0.1(4)
Extinction coefficient	0.0048(8)
Largest diff. peak and hole	0.298 and −0.351 e.Å^{-3}

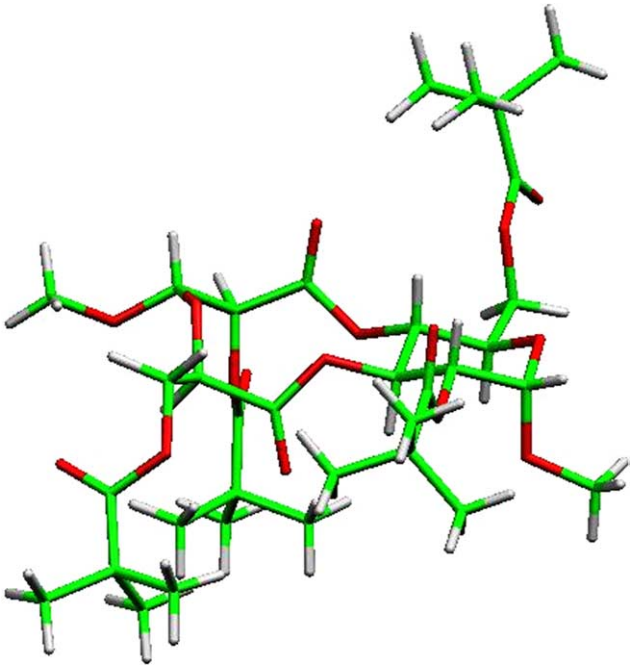


Figure 2. Drawing displaying clearly the conformation exhibited by the 10- and 6-membered rings of compound **1** (Mercury 1.2.1).[42]

appeared at δ 56.1 and 55.4, these signals being assigned to OCH_3-11 and OCH_3-22, respectively, due to the HMBC correlation with H-11 and H-22 (δ 4.95), respectively (Table 3). The assignment of H-1 at δ 5.05 as a double doublet was easily accomplished by means of the COSY spectrum, since this proton is coupled with H-22 with $J_{1,22}$ 3.5 Hz, a coupling constant characteristic of axial/equatorial coupling, and with H-14 with $J_{1,14}$ 10 Hz, a trans-diaxial coupling with H-14, assigned at δ 5.74. The correlations of H-1 with H-11, H-22 and H-15 observed in the NOESY spectrum (Fig. 3) are in agreement with the proposed structure for **2** (Fig. 4). The assignment of OCH_3-11 at δ 3.50 and of OCH_3-22 at δ 3.45 was confirmed by NOESY due to the correlations detected with H-11 and H-22 and by HMQC. The correlation of H-14 with H-20 at δ 3.97–3.95 observed by NOESY allowed to detect by COSY the signal of H-19eq at δ 4.30 as a double doublet, due to the coupling with H-20 and with the geminal proton, $J_{19eq,20}$ 4.5 Hz and $J_{19eq,19a}$ 10.5 Hz, respectively. Using DEPT and HMQC the ^{13}C NMR resonances of C-19 and C-8 were assigned at δ 68.1 and 70.3, respectively. By means of HMQC and COSY experiments, it was possible to detect the resonance of H-8eq at δ 4.44, and those of H-8a (axial), H-19a (axial) and H-15 in the multiplet at δ 3.87–3.79.

The NOESY spectrum also allowed the assignment of H-4 at δ 4.38 due to the correlations detected with H-6 at δ 5.48 and H-8a, included in the above mentioned multiplet, which also contains the resonance of H-9. The signal of H-17 at δ 5.49 was confirmed by the NOESY correlation shown with the multiplet containing H-19a.

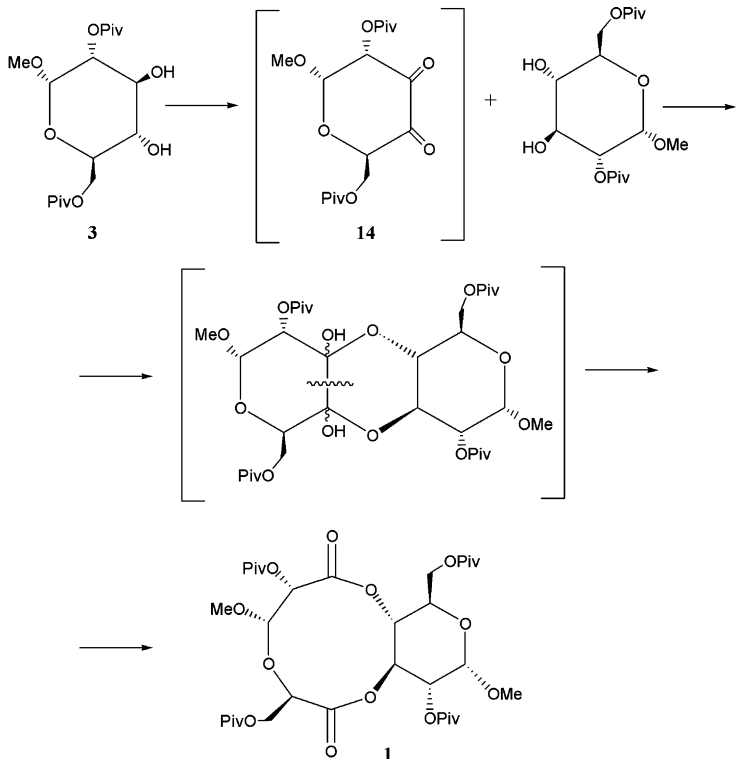
The analysis of the HMBC spectrum confirmed the assignments presented (Table 3) and thus the proposed structure for **2**.

The antimicrobial activities were evaluated using the paper disk diffusion method.[35,36] The new bislactones **1** and **2** (300 μg each) displayed antifungal activity against *Candida albicans* with diameters of inhibition zones of 12 and 8 mm, respectively (Table 5), while the diameter of inhibition zone of 16 mm was determined for chloramphenicol (300 μg) used as control. The filamentous fungus *Aspergillus niger* was not affected by both compounds tested. Some inhibition on the growth of *B. subtilis* was observed for both compounds and of *S. aureus* for compound **1**. In contrast, compounds **1** and **2** are described as inactive on the growth inhibition of *B. cereus*, *E. faecalis*, *E. coli*, *Listeria monocytogenes*, *P. aeruginosa* and *Salmonella enteritidis*.

3. Experimental

3.1. General methods

All reactions were monitored by TLC (Silica Gel 60 F_{254}, E. Merck) with detection by UV light and/or by vanillin in H_2SO_4 soln (2.5%) spray, followed by heating at 120 °C. Solutions were concentrated on a rotary



Scheme 1. Proposed mechanism for the formation of the bislactone **1**.

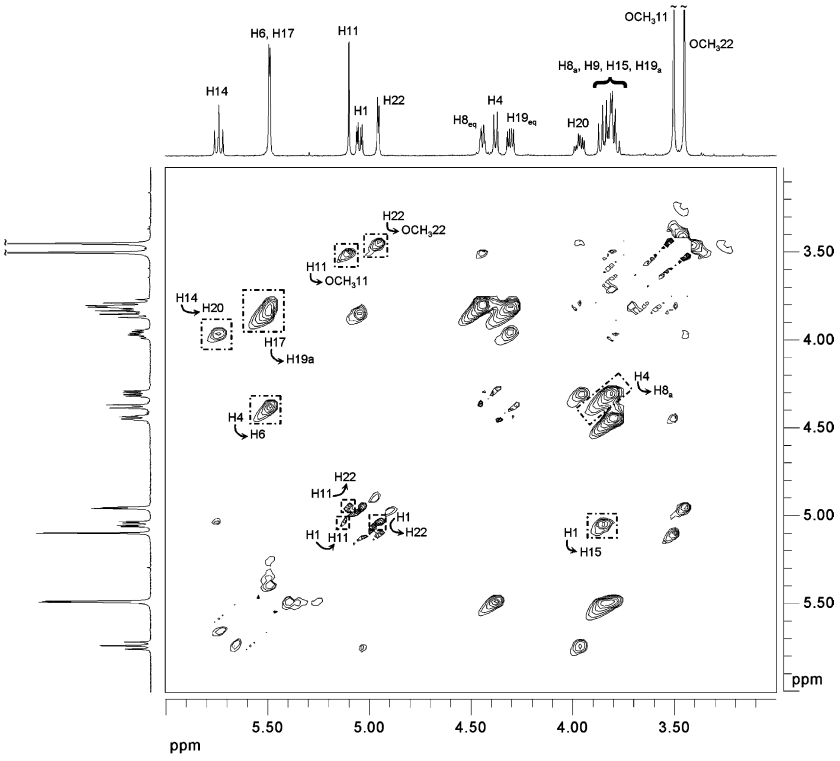


Figure 3. NOESY spectrum of compound **2**.

evaporator under diminished pressure under 40 °C. Column chromatography (CC) was performed on Silica Gel 60 G (0.040–0.063 mm, E. Merck) and eluted under low pressure. Melting points were determined with an

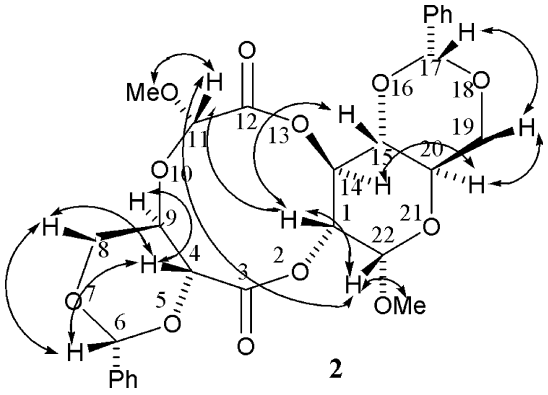


Figure 4. NOESY correlations relevant for the structure elucidation of compound **2**.

Table 5. Antimicrobial activity of the bislactones **1** (300 μg) and **2** (300 μg) using the paper disk diffusion method [the diameter (mm) of the inhibition zone observed is given and compared with that of the control][a]

Microorganism[b]	Bislactone $\mathbf{1}_{300}$	Bislactone $\mathbf{2}_{300}$	Control$_{30}$	Control$_{300}$
C. albicans ATCC 10231	12	8	<6.4	16
S. aureus ATCC 25923	9	<6.4	24	38
B. cereus ATCC 11778	7	<6.4	26	38
B. subtilis ATCC 6633	9	8	29	44
E. coli ATCC 25922	7	7	27	41
S. enteritidis ATCC 13076	7	7	29	40

[a] Chloramphenicol was the control for all the microorganisms tested, with the exception of *A. niger* for which actidione was used; control$_{30}$ (30 μg), control$_{300}$ (300 μg).

[b] *A. niger* and the bacteria *E. faecalis*, *L. monocytogenes* and *P. aeruginosa* gave diameters <6.4 mm and are not included in the table.

electrothermal 9100 instrument and are uncorrected. Proton and carbon NMR spectra, DEPT, COSY, NOESY, HMQC and HMBC experiments were recorded using a BRUKER DRX500 spectrometer operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C, equipped with a BBI-XYZ probe head (5 mm diameter) and at a constant temperature of 298 K. Proton and carbon NMR spectra of compound **14** were recorded using a BRUKER Avance 400 spectrometer operating at 400.13 MHz for ^{1}H or 100.62 MHz for ^{13}C and a constant temperature of 298 K. The solvent used was $CDCl_3$ (1% v/v Me_4Si or 0.03% v/v Me_4Si, Aldrich). IR spectra were run with a Hitachi 270-50. Optical rotations were registered on a Perkin Elmer 343 polarimeter. Elemental analyses were performed at the Microanalyses Service of Instituto Superior Técnico Universidade Técnica de Lisboa.

3.1.1. (1*S*,4*R*,6*S*,7*S*,10*R*,11*R*,13*S*,14*R*)-6,13-Dimethoxy-7,14-bis(2,2-dimethylpropanoyloxy)-4,11-bis(2,2-dimethylpropanoyloxymethyl)-2,5,9,12-tetraoxabicyclo[8,4,0]tetradecane-3,8-dione (1) and methyl α-D-*erythro*-hexopyranoside-3,4-diulose (14). PCC (3.88 g, 18 mmol) was added to the soln of **3** (0.5 g, 1.4 mmol) in dry toluene (5 mL). The mixture was stirred at 75 °C for 24 h. The reaction mixture was cooled to rt. Addition of THF (250 mL) was followed by filtration. Diethyl ether (1 L) was added to the filtrate, the suspension was subjected to filtration and the filtrate was evaporated under diminished pressure. Neutral alumina 150 mesh (22 g) was added to the soln of the residue in CH_2Cl_2 (250 mL). Evaporation and CC eluted with 1:6 EtOAc–hexane gave **1** as a white solid (252 mg, 50%) and **14** as a syrup (10 mg, 2%).

Data for **1**: Mp 133–134 °C (EtOH); $[\alpha]_D^{20}$+38 (*c* 1, CH_2Cl_2); $R_f = 0.69$ (1:2 EtOAc–toluene); IR (KBr): ν_{max} 1746 (C=O); ^{1}H NMR: δ 5.51 (d, 1H, $J_{6,7}$ 1.95 Hz, H-7), 5.35 (t, 1H, $J_{1,10} = J_{1,14}$ 10.4 Hz, H-1), 5.15 (d, 1H, H-6), 4.99–4.94 (m, 2H, H-10, H-13), 4.75–4.71 (m, 2H, H-4, H-14), 4.19–4.10 (m, 4H, CH_2OPiv), 3.90 (br d, 1H, $J_{10,11}$ 10 Hz, H-11), 3.47 (s, 3H, OCH_3-6), 3.34 (s, 3H, OCH_3-13), 1.19 (s, 9H, CH_3, Piv-7), 1.16 (s, 9H, CH_3, Piv), 1.12 (s, 9H, CH_3, Piv), 1.11 (s, 9H, CH_3, Piv); ^{13}C NMR: δ 178.4, 178.3 (C=O, Piv-4, Piv-11), 177.9 (C=O, Piv-14), 177.3 (C=O, Piv-7), 170.1 (C-3), 168.5 (C-8), 99.8 (C-6), 97.7 (C-13), 74.5 (C-10), 73.4 (C-1), 72.7 (C-7), 70.7 (C-14), 69.4 (C-4), 67.6 (C-11), 64.2 (*C*H_2OPiv-4), 62.3 (*C*H_2OPiv-11), 57.6 (OCH_3-6), 56.2 (OCH_3-13), 39.3, 39.1 (C_q, Piv), 27.5, 27.4, 27.3, 27.1 (CH_3, Piv). Anal. Calcd for $C_{34}H_{54}O_{16}$: C, 56.81; H, 7.57. Found: C, 56.79; H, 7.57.

Data for **14**: $[\alpha]_D^{20}$+32 (*c* 1, CH_2Cl_2); $R_f = 0.74$ (1:2 EtOAc–toluene); ^{1}H NMR: δ 5.32 (d, 1H, $J_{1,2}$ 4.8 Hz, H-1), 5.07 (dd, 1H, $^5J_{2,5}$ 0.8 Hz, H-2), 4.43 (dd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.18 (dd, 1H, $J_{5,6b}$ 3.2 Hz, H-6b), 4.14 (br t, 1H, H-5), 3.39 (s, 3H, OCH_3), 1.20 (s, 9H, CH_3, Piv), 1.12 (s, 9H, CH_3, Piv); ^{13}C NMR: δ 204.0 (C-3, C-4), 176.8, 176.7 (C=O, Piv), 98.2 (C-1), 73.2 (C-5), 72.9 (C-2), 60.7 (C-6), 54.6 (OCH_3), 37.8, 37.7 (C_q, Piv), 26.1, 26.0 (CH_3, Piv).

3.1.2. (1*R*,4*R*,6*R*,9*R*,11*S*,14*S*,15*R*,17*R*,20*R*,22*S*)-11,22-Dimethoxy-2,5,7,10,13,16,18,21-octaoxa-6,17-diphenyltetracyclo[12,8,0,0,^{4,9}0^{15,20}] docosane-3,12-dione (2). A suspension of **4** (200 mg, 0.64 mmol) in dry $ClCH_2CH_2Cl$ (10 mL) was stirred at 60 °C until a clear solution was observed. PCC (460 mg, 2.13 mmol) was added and the reaction mixture was kept under reflux for 48 h. Filtration, addition of $ClCH_2CH_2Cl$ (20 mL) and evaporation of the filtrate gave **2** as a syrup (64 mg, 44%), being the diol **4** recovered in 13% yield (26 mg); $[\alpha]_D^{20}$+26 (*c* 1, CH_2Cl_2); $R_f = 0.52$ (1:2 EtOAc–toluene); IR (KBr): 1765 (C=O); ^{1}H NMR: δ 7.47–7.43 (m, 4H, Ph), 7.35–7.33 (m, 6H, Ph), 5.74 (t, 1H, $J_{1,14} = J_{14,15}$

10 Hz, H-14), 5.49 (s, 1H, H-17), 5.48 (s, 1H, H-6), 5.10 (s, 1H, H-11), 5.05 (dd, 1H, $J_{1,22}$ 3.5 Hz, H-1), 4.95 (d, 1H, H-22), 4.44 (br d, 1 H, $J_{8eq,8a}$ 10.5 Hz,H-8eq), 4.38 (d, 1H, $J_{4,9}$ 9 Hz, H-4), 4.30 (dd, 1H, $J_{19eq,19a}$ 10.5 Hz, $J_{19eq,20}$ 4.5 Hz, H-19eq), 3.95 (ddd, 1H, H-20), 3.87-3.79 (m, 4H, H-8a, H-9, H-15, H-19a), 3.50 (s, 3H, OCH_3-11), 3.45 (s, 3H, OCH_3-22); ^{13}C NMR: δ 166.6 (C-3, C-12), 136.7, 136.2 (Cq, Ph), 130.8, 129.3, 129.1, 128.8, 128.2, 128.1, 126.3, 126.2 (CH, Ph), 101.6, 101.3 (C-6, C-17), 98.8 (C-11), 97.9 (C-22), 82.0 (C-4), 78.2 (C-15), 73.7 (C-1), 71.4 (C-14), 70.3 (C-8), 68.7 (C-9), 68.1 (C-19), 63.6 (C-20), 56.1 (OCH_3-11), 55.4 (OCH_3-22); Anal. Calcd for $C_{28}H_{32}O_{12}$: C, 60.00; H, 5.75. Found: C, 60.29; H, 5.75.

3.1.3. Methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside (3), methyl 3,6-di-*O*-pivaloyl-α-D-glucopyranoside (6), methyl 2,3,6-tri-*O*-pivaloyl-α-D-glucopyranoside (7) and methyl 2,4,6-tri-*O*-pivaloyl-α-D-glucopyranoside (8). *Method A*: Pivaloyl imidazole (4 equiv) was added to a soln of **5** (1 equiv) in DMF (10 mL) and the mixture was stirred at rt. Evaporation of DMF under diminished pressure gave a residue, to which CH_2Cl_2 (100 mL) was added. The soln was washed with water (15 mL), with 5% HCl soln (25 mL), again with water (25 mL) and with aq satd $NaHCO_3$ soln (25 mL). The aqueous phase was extracted with CH_2Cl_2 (25 mL) and with Et_2O (25 mL) and the combined organic phases were dried (Na_2SO_4) and evaporated. The residue was purified by CC eluted with (1:6) EtOAc–petroleum ether 40–60 °C. The reaction was run for 8 h to give **3** (211 mg, 36%), **6** (31 mg, 5%) and **7** (350 mg, 48%), being the diol **5** being recovered in 20% (80 mg). When it was run for 5 days afforded **3** (71 mg, 20%), **6** (71 mg, 20%) and **7** (144 mg, 32%).

Method B: Starting from **3** (9.72 g, 0.05 mmol) the procedure previously described by Klausener et al.[25] gave a residue, which was subjected to CC eluted with 1:3 EtOAc–toluene to give **3** (16.12 g, 89%), **6** (100 mg, 0.6%), **7** (735 mg, 3.3%) and **8** (620 mg, 2.8%) as white solids. Physical and spectroscopic data of these compounds were in agreement with those reported in the literature; **3**: mp 92–93 °C, lit.[25] 90–91 °C; **6**: mp 78–79 °C, lit.[28] 78 °C; **7**: mp 75–77 °C, lit.[29] 75–77 °C; **8**: mp 139–141 °C, lit.[29] 129–131 °C; HMRS for **8**: calcd for $C_{22}H_{38}O_9$ 446.253824, found: 446.251585.

3.1.4. Methyl 2,6-di-*O*-pivaloyl-α-D-*ribo*-hexopyranosid-3-ulose (10). *Method A*: PCC-Al_2O_3 was added to a soln of **3** (100 mg, 0.28 mmol) in dry $ClCH_2CH_2Cl$ (10 mL) at rt for 48 h. Addition of $ClCH_2CH_2Cl$ (20 mL), filtration and evaporation of the filtrate afforded **10** (31 mg, 52%) and **1** (10 mg, 16%), based on the reacted starting material **3**, recovered in 40% yield (40 mg).

Method B: PCC-silica gel was added to a solution of **3** (205 mg, 0.57 mmol) in dry CH_2Cl_2 (2 mL) and stirred at rt for 42 h. The mixture was diluted with CH_2Cl_2 (20 mL), filtrated and evaporated to give **10** (104 mg, 94%) based on the reacted starting material **3**, recovered in 46% yield (95 mg); **10**: mp 82–83 °C, lit.[28] 81–82 °C.

3.1.5. Methyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-*ribo*-exopyranosid-3-ulose (11). Molecular sieves powder 4 Å (1.7 g), sodium acetate (2.14 g) and PCC (2.14 g, 9.92 mmol) were added to a solution of **4** (1 g, 3.75 mmol) in CH_2Cl_2 (45 mL) and the mixture was stirred at 40 °C for 45 min. The reaction mixture was added to diethyl ether (200 mL) and filtered over florisil. Evaporation of the solvent gave a residue, which was purified by CC eluted with 1:3 EtOAc–toluene to give **11** (120 mg, 17%), mp 210–213 °C, lit.[37] 211–213 °C and **2** (100 mg, 5%).

3.1.6. Methyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (12). *Method A*: A mixture of **4** (5.64 g, 20 mmol) and dibutyltin oxide (5.47 g, 22 mmol) in benzene (180 mL) was heated for 12 h with azeotropic water removal by means of a Dean–Stark apparatus. Evaporation of the solvent under diminished pressure gave a residue, to which benzene (150 mL), molecular sieves powder 4 Å (10 g) and benzoyl chloride (2.55 mL, 22 mmol) were added. The reaction mixture was stirred for 15 min. Filtration and evaporation afforded a residue, which was subjected to separation by CC eluted with 1:4 EtOAc–toluene to give **12** (13.5 g, 82%) as a solid.

Method B: Triethylamine (0.6 mL, 4.4 mmol) was added to a soln of **4** (1.13 g, 4 mmol) and 1-(benzyloxy)benzotriazole (970 mg, 4 mmol) in dry CH_2Cl_2 (16 mL) and stirred at rt for 5 h. After addition of CH_2Cl_2 (80 mL), the soln was washed with a satd soln of $NaHCO_3$ (40 mL) and with aq soln of NaCl (40 mL). The combined organic phases were dried (Na_2SO_4) and evaporated. The residue was purified by CC using 1:4 EtOAc–hexane to give **12** as a solid (1.37 g, 89%); mp 169 °C, lit.[33] 169–170 °C.

3.2. X-ray determination

Crystal data and details of the data collection are given in Table 4. Data were collected in a TURBOCAD4 Enraf–Nonius diffractometer with Cu graphite monochromated radiation, and were corrected for Lorentz and polarisation effects. No absorption correction was performed. The crystal structures were solved by direct methods (program SIR97[38]) and refined by SHELXL97,[39] all in the package WinGX-Version 1.64.03b.[40] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included in calculated positions and allowed to refine, while riding on the parent C atom, except for the C(1), C(4), C(6), C(7), C(10), C(11), C(13), C(14) and C(41) hydrogens that were located and refined isotropically. Graphical representations were prepared using ORTEPIII[41] and Mercury 1.1.2.[42]

Crystallographic data for the structures reported in this paper have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 236486.

3.3. Biological assays

The antimicrobial activity of compounds **1** and **2** was evaluated using the paper disk diffusion method.[35] The following fungi and bacteria were used in the tests: *C. albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), *B. cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *L. monocytogenes* (ATCC 7644), *P. aeruginosa* (ATCC 27853), *S. enteritidis* (ATCC 13076) and *S. aureus* (ATCC 25923). The overnight cultures of microorganisms were spread over the appropriate media. Nutrient agar was used for all bacteria except for *Listeria*, *Enterococcus* and both fungi, where triptone soya agar, azide dextrose agar and potato dextrose agar were used, respectively. Paper disks of 6.4 mm were placed on the agar and the soln of each substance (300 μg) in DMSO (15 μL) was applied on each disk. Chloramphenicol was used as positive control for all microorganisms tested, except for *A. niger* for which actidione was used, while Me_2SO was the negative control. Bacteria were incubated at 37 °C for 24 h and fungi at 25 °C for 48 h. After incubation, the nearest diameter of the inhibition zone was measured.

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