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Divergent Synthesis of Cytotoxic Styryl Lactones Related to Goniobutenolides A and B, and to Crassalactone D

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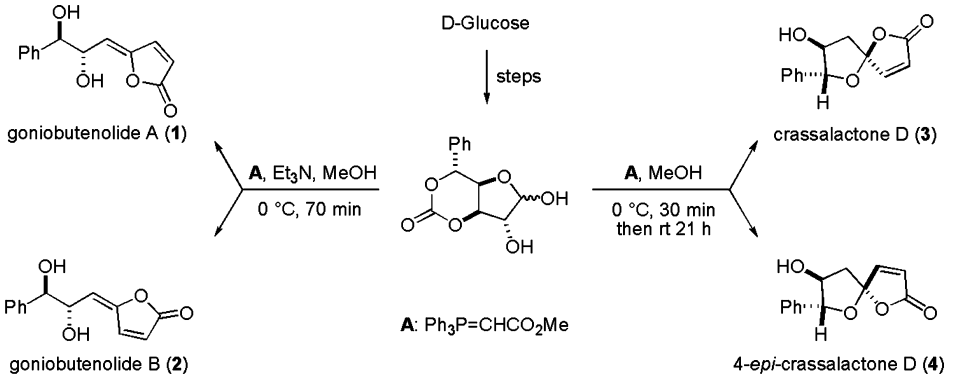
ABSTRACT



Goniobutenolides A (1) and B (2), crassalactone D (3), 4-*epi*-crassalactone D (4), and the corresponding 7-epimers have been synthesized starting from D-glucose. The key step in the synthesis of 1 and 2 is a new one-pot sequence comprised of a *Z*-selective Wittig olefination/lactonization/ β-elimination. Preparation of 3 and 4 included the final 5-*endo*-trig spirocyclization of 1 and 2. The synthesized products were evaluated for their in vitro antiproliferative activity against selected tumor cell lines.

Goniobutenolides A and B (**1** and **2**, Figure 1) were isolated, together with other related styryl-lactones, from the ethanolic extracts of the stem bark of *Goniothalamus giganteus* Hook. f. and Thomas (Annonaceae).[1] These compounds were found to be cytotoxic against certain human tumor cell lines and have been the targets of synthetic efforts by several groups.[2] The structures of **1** and **2** were originally determined by spectral methods; in particular, a *threo* relationship was proposed for the diol moiety, based on the ^{1}H NMR data.[1] However, the first total syntheses of goniobutenolides A and B as well as their 7-epimers reassigned the relative configuration of the diol moiety as *erythro* and established their absolute stereochemistries as **1** and **2**, respectively.[3] A number of styryl lactones were recently isolated from the tropical plant *Polyalthia crassa*.[4] Separation of the tree's leaves and twigs ethyl acetate-soluble extract led to the isolation of several new cytotoxic compounds including the (+)-crassalactone D (**3**). The relative configuration of **3** was established by single-crystal X-ray diffraction analysis, and its absolute stereochemistry was determined by NMR studies on (*R*)- and (*S*)-MTPA esters of **3**. Recently, two asymmetric total syntheses of (+)-**3** have been reported both employing an oxidative spirocyclization of a furan as the key step.[5]

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(1) Fang, X.-P.; Anderson, J. E.; Chang, C.-J.; McLaughlin, J. L. *Tetrahedron* **1991**, *47*, 9751.

(2) (a) Shing, T. K. M.; Tsui, H.-C.; Zhou, Z.-H. *J. Org. Chem.* **1995**, *60*, 3121. (b) Ko, S. Y.; Lerpiniere, J. *Tetrahedron Lett.* **1995**, *36*, 2101. (c) Mukai, C.; Hirai, S.; Kim, I. J.; Kido, M.; Hanaoka, M. *Tetrahedron* **1996**, *52*, 6547. (d) Surivet, J.-P.; Vatèle, J.-M. *Tetrahedron* **1999**, *55*, 13011. (e) Solladié, G.; Hanquet, G.; Rolland, C. *Tetrahedron Lett.* **1999**, *40*, 177.

(3) Shing, T. K. M.; Tai, V. W.-F.; Tsui, H.-C. *J. Chem. Soc., Chem. Commun.* **1994**, 1293.

(4) Tuchinda, P.; Munyoo, B.; Pohmakotr, M.; Thinapong, P.; Sophasan, S.; Santisuk, T.; Reutrakul, V. *J. Nat. Prod.* **2006**, *69*, 1728.

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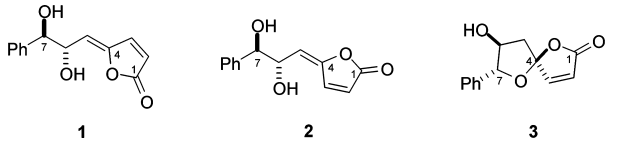


Figure 1. Chemical structures of goniobutenolides A (**1**) and B (**2**), and of (+)-crassalactone D (**3**).

As part of our continuing efforts,[6] in the preparation of naturally occurring styryl-lactones and their analogs as potential antitumor agents from monosaccharides, we now disclose novel total syntheses of goniobutenolides A (**1**) and B (**2**), (+)-crassalactone D (**3**), and the related C-7 epimers, along with their effects on the proliferation of some human malignant cell lines.

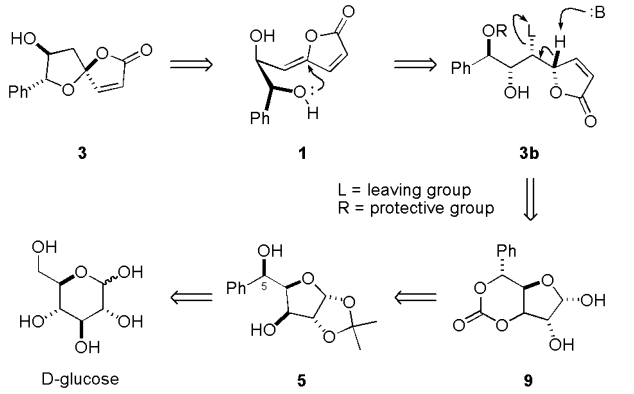
The retrosynthetic analysis of (+)-crassalactone D (**3**) is shown in Scheme 1. We envisaged that the spiroketal center in **3** could be established by spirocyclization of **1** through a 5-*endo*-trig ring closure process, under the conditions similar to those reported in the literature.[7] It was further envisioned that goniobutenolides A (**1**) and B (**2**) could be synthesized from **9**, via **3b**, by a one-pot cascade comprised of *Z*-selective olefination, followed by lactonization and E_2 elimination. It is therefore essential that the key intermediate **3b** and its precursor **9** contain a good leaving group at C-3 and a convenient protective group at O-5. Cyclic carbonate was chosen because it can act as a protecting group for the diol functionality,[8] but at the same time, it can undergo ring-opening reactions, due to its good leaving group properties.[9]

Lactol of type **9** is visualized from D-glucose, via **5**, by well established chemical reactions.[6e,10]

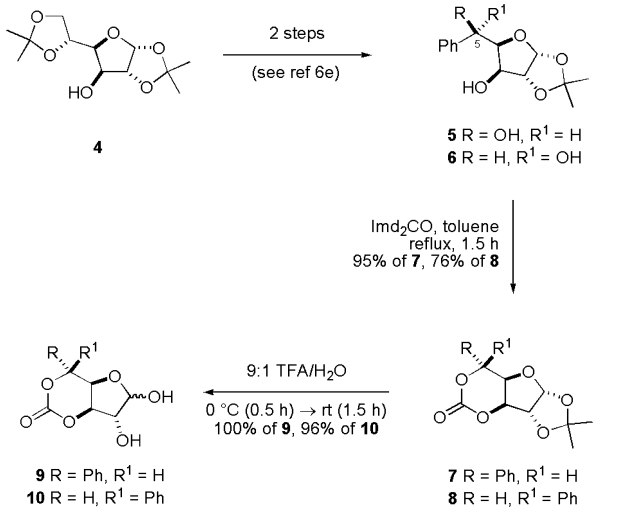
At the outset, we focused on the synthesis of the key building blocks **9** and **10** starting from commercially available diacetone-D-glucose (**4**, Scheme 2).

Treatment of **5** with 1,1′-carbonyldiimidazole in boiling toluene provided the cyclic carbonate **7** in 95% yield. Under similar reaction conditions stereoisomer **6** gave **8**

Scheme 1. Retrosynthetic Analysis of (+)-Crassalactone D (**1**)



Scheme 2. Preparation of Intermediates **9** and **10**



(76%). Hydrolytic removal of the isopropylidene protective groups in both **7** and **8** afforded the corresponding lactols **9** and **10** in almost quantitative yields. Both products, and particularly stereoisomer **9**, are rather hygroscopic. They should be therefore used in the next synthetic step immediately after its brief isolation.

It was expected that lactol **9** could directly give natural product **1** (and/or its *E*-isomer **2**) under *Z*-selective Wittig olefination conditions,[11] presuming a subsequent two-step cascade comprised of γ-lactonization of the intermediate conjugated ester followed by concomitant E_2 elimination of cyclic carbonate functionality (Scheme 1). In our first experiment, lactol **9** was submitted to the reaction with a stabilized ylide (reagent **A**, Table 1) in dry methanol, to afford a 2:1 mixture of **1** and **2** in 30% combined yield. Next, the olefination step was examined in the presence of

(5) (a) Pavlakos, E.; Georgiou, T.; Tofi, M.; Montagnon, T.; Vassilikogiannakis, G. *Org. Lett.* **2009**, *11*, 4556. (b) Yang, Z.; Tang, P.; Gauuan, J. F.; Molino, B. F. *J. Org. Chem.* **2009**, *74*, 9546.

(6) (a) Popsavin, V.; Benedeković, G.; Srećo, B.; Popsavin, M.; Francuz, J.; Kojić, V.; Bogdanović, G. *Org. Lett.* **2007**, *9*, 4235. (b) Popsavin, V.; Benedeković, G.; Srećo, B.; Popsavin, M.; Francuz, J.; Kojić, V.; Bogdanović, G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5178. (c) Popsavin, V.; Benedeković, G.; Srećo, B.; Francuz, J.; Popsavin, M.; Kojić, V.; Bogdanović, G.; Divjaković, V. *Tetrahedron* **2009**, *65*, 10596. (d) Popsavin, V.; Srećo, B.; Benedeković, G.; Francuz, J.; Popsavin, M.; Kojić, V.; Bogdanović, G. *Eur. J. Med. Chem.* **2010**, *45*, 2876. (e) Popsavin, V.; Benedeković, G.; Popsavin, M.; Kojić, V.; Bogdanović, G. *Tetrahedron Lett.* **2010**, *51*, 3426. (f) Francuz, J.; Srećo, B.; Popsavin, M.; Benedeković, G.; Divjaković, V.; Kojić, V.; Bogdanović, G.; Kapor, A.; Popsavin, V. *Tetrahedron Lett.* **2012**, *53*, 1819.

(7) (a) Perron, F.; Albizati, K. F. *Chem. Rev.* **1989**, *89*, 1617. (b) Knight, D. W.; Shaw, D. E.; Staples, E. R. *Eur. J. Org. Chem.* **2004**, 1973.

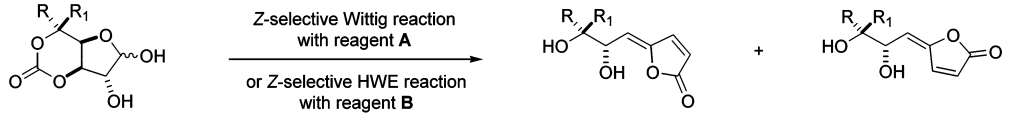
(8) Parrish, J. P.; Salvatore, R. N.; Jung, K. W. *Tetrahedron* **2000**, *56*, 8207.

(9) Verhelst, S. H. L.; Wennekes, T.; van der Marel, G. A.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H. *Tetrahedron* **2004**, *60*, 2813.

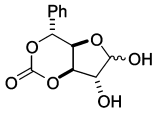
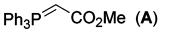
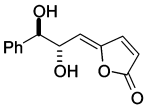
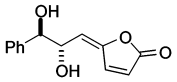
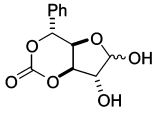
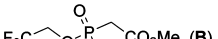
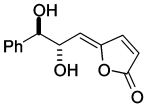
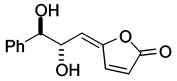
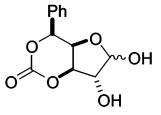
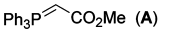
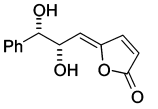
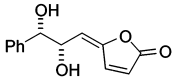
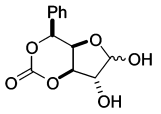
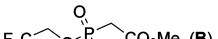
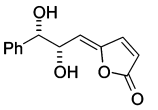
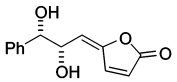
(10) Gracza, T.; Szolcsányi, P. *Molecules* **2000**, *5*, 1386.

(11) For examples of *Z*-selective Wittig olefinations, see: (a) Shing, T. K. M.; Tsui, H.-Ch; Zhou, Z.-H. *J. Org. Chem.* **1995**, *60*, 3121. (b) Ramirez, E.; Sánchez, Meza-León, M. R. L.; Quintero, L.; Sartillo-Piscil, F. *Tetrahedron Lett.* **2010**, *51*, 2178.

Table 1. One-Pot Conversion of Lactols **9** and **10** into the Unsaturated Lactones **1** (or **11**) and **2** (or **12**)



9 or **10** → **1** or **11** + **2** or **12**

entry	starting compound	reagents and conditions	major product	minor product	yield[a] (%)	ir[b] (*Z*/*E*)[a]
1	 **9**	 (**A**) Et$_3$N, MeOH, nitrogen atmosphere 70 min at 0 °C	 **1** (*Z*-isomer)	 **2** (*E*-isomer)	30	2:1
2	 **9**	 (**B**) NaH, THF, argon atmosphere, 22.5 h at −10 °C	 **1** (*Z*-isomer)	 **2** (*E*-isomer)	45	2:1
3	 **10**	 (**A**) Et$_3$N, MeOH, nitrogen atmosphere 140 min at 0 °C	 **11** (*Z*-isomer)	 **12** (*E*-isomer)	74	4:3
4	 **10**	 (**B**) NaH, THF, argon atmosphere, 22.5 h at −10 °C	 **11** (*Z*-isomer)	 **12** (*E*-isomer)	50	7:5

[a] Isolated yields. [b] ir − *Z*/*E* isomeric ratio.

Still's reagent[12] (reagent **B**, entry 2) derived from unstable ylide using the *Z*-selective Horner−Wadsworth−Emmons (HWE) reaction protocol.[13] A mixture of isomeric natural products **1** and **2** (*Z*/*E* ca. 2:1) was obtained in 45% yield. Very careful chromatographic separation afforded pure **1** and **2**. Our optical rotation values were different from the values reported for synthetic[2] goniobutenolides A (**1**) and B (**2**) but were in reasonable agreement with the reported data of isolated **1** and **2**.[1] However, the NMR data of synthesized samples **1** and **2** were in full agreement with those reported in the literature.[1−3,14]

In an attempt to generalize this methodology by using a similar substrate, but with opposite stereochemistry at the C-5 position, we treated lactol **10** under the same Wittig olefination conditions. Gratifyingly, within 140 min, TLC indicated full conversion of the starting material into unnatural products **11** and **12**, which were isolated in 74% combined yield. Unfortunately, a drop in selectivity was observed, giving thus an almost equimolar mixture of **11** and **12** (entry 3). Treatment of **10** with Still's reagent (reagent **B**) in the presence of NaH gave even a lower yield of **11** and **12**, and the lack of stereoselectivity was again observed (entry 4).

Apart from the main products **1** and **2**, minor amounts of (+)-crassalacone D (**3**) and the corresponding 4-epimer **13** were isolated from both Wittig and HWE reactions of **9**. These side products were presumably formed by a 5-*endo*-trig spirocyclization of the unsaturated lactones **1** and **2** after nucleophilic attack of the hydroxyl group from C-7 at the C-4 position. Accordingly it was assumed that prolonging the reaction time of the Wittig reaction would afford the required spiro-lactones as the major reaction product. Indeed, when the lactol **9** was treated with 2-(triphenylphosphoranylidene)-acetic acid methyl ester (dry MeOH, 0 °C for 0.5 h, then rt for 47 h), a 1:2 mixture of spiro-lactones **3** and **13** was obtained in 51% yield (Scheme 3). A small amount of **1** and **2** was isolated in 10% combined yield.

Under the same reaction conditions stereoisomer **10** gave a mixture of spiro-lactones **14** and **15** in 67% combined yield, along with their synthetic precursors **11** and **12**, which were isolated in 19% combined yield. The products **3** and **13**, as well as **14** and **15**, were separated with difficulties. Typically, flash column chromatography was required, followed by preparative TLC, in order to obtain pure products.[14]

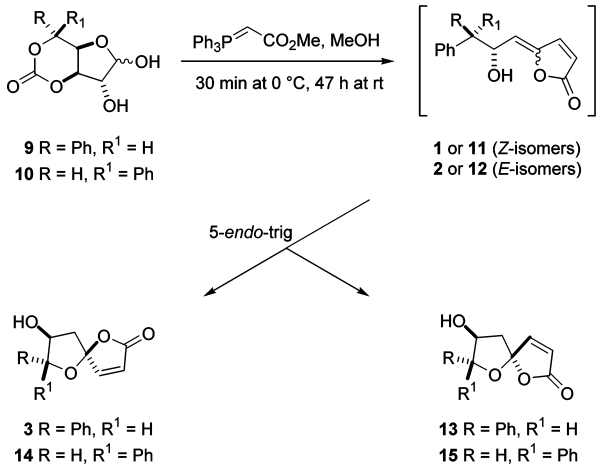
Interestingly, it was observed that the less stable isomer **13** could be converted to the more stable **3**, after treatment of their mixture (2:1 in favor of **13**) with a solution of trifluoroacetic acid in dichloromethane, to give a 2:1 mixture of **3** and **13** by ^{1}H NMR analysis. In contrast,

(12) Still, W. C.; Gennari, C. *Tetrahedron Lett*. **1983**, *24*, 4405.

(13) For examples of *Z*-selective HWE olefinations, see: (a) Pihko, P. M.; Salo, T. M. *Tetrahedron Lett*. **2003**, *44*, 4361. (b) Touchard, F. P. *Tetrahedron Lett*. **2004**, *45*, 5519.

(14) See the Supporting Information for details.

Scheme 3. One-Pot Conversion of Lactol **9** to the Spiro-lactones **3** and **13**[a,b] and the Conversion of **10** to **14** and **15**[a]



[a] Isolated yields: 51% (isomeric ratio **3**/**13** ~1:2), 67% (isomeric ratio **14**/**15** ~1:1). [b] Apart from **3** and **13**, a mixture of **1** and **2** was isolated in 10% yield. [c] Apart from **14** and **15**, a mixture of **11** and **12** was isolated in 19% yield.

when pure **15** was treated under similar reaction conditions a 1:1 mixture of **14** and **15** was obtained indicating a similarity in stability of both steroisomers.

A side-by-side comparison of the antiproliferative activity of the synthesized styryl lactones (**1**–**3** and **11**–**15**) against a range of human tumor cell lines is presented in Table 2. The use of human fetal lung fibroblasts (MRC-5) serves to demonstrate the toxicity of selected lactones toward normal cells. Cytotoxic activity was evaluated by using the standard MTT assay after exposure of cells to the tested compounds for 48 h. The commercial antitumor agent doxorubicin (DOX) was used as a reference compound in this assay.

As shown in Table 2, natural products **1** and **3** as well as synthetic analogues **13** and **15** exhibited potent cytotoxic activities against all the tumor cells under evaluation with IC_{50} values in the range of 0.97 to 8.64 μM. The most potent antiproliferative activity of lactones **3** and **15** were recorded in the HL-60 cells, being notably more active than the commercial cytostatic doxorubicin (DOX). Natural product **2** demonstrated moderate to weak antiproliferative effects against all the malignant cells under evaluation with IC_{50} values in the range of 29.03 to 54.31 μM. A similar cytotoxicity profile was observed for synthetic analogue **12** (IC_{50} values in the range of 8.85 to 36.55 μM). Unnatural lactone **11** exhibited the most potent growth-inhibitory activity against HeLa (1.24 μM) and K562 (2.01 μM) cell lines but showed a moderate to weak antiproliferative activity toward HL-60, Jurkat, and Raji malignant cells. Compounds **3** and **11** inhibited the growth of HeLa cells, being as active as DOX in the same cell line. The most potent antiproliferative activity of the unnatural lactone **14** was recorded in the Jurkat cell line (IC_{50} 1.23 μM), although this molecule exhibited 4-fold lower activity than DOX. Moreover, none of the synthesized styryl lactones exhibited toxicity toward MRC-5 cells, in contrast to the commercial antitumor agent doxorubicin (DOX) that exhibited potent cytotoxic activity against this cell line.

Table 2. *In Vitro* Cytotoxicity of the Natural Products (**1**, **2**, and **3**) and the Corresponding Analogs (**11**–**15**)

	IC_{50}, μM[a]					
compd	K562	HL-60	Jurkat	Raji	HeLa	MRC-5
1	0.97	8.79	2.11	6.15	9.48	>100
2	46.17	51.69	29.64	54.31	29.03	>100
11	2.01	41.21	81.02	32.04	1.24	>100
12	30.27	32.54	25.45	36.65	8.85	>100
3	0.97	2.64	0.97	8.64	1.69	>100
13	1.02	5.97	1.67	7.68	11.32	>100
14	5.25	10.51	1.23	9.99	48.40	>100
15	1.06	3.54	2.03	6.87	10.87	>100
DOX	0.36	4.62	0.39	4.09	1.17	0.12

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

In conclusion, we have developed a new divergent synthesis of goniobutenolide A (**1**), goniobutenolide B (**2**), crassalactone D (**3**), 4-*epi*-crassalactone D (**4**), and the corresponding 7-epimers starting from D-glucose. The key step in the synthesis of butenolides **1** and **2** involved a new one-pot sequence comprised of an initial *Z*-selective Wittig olefination, followed by successive lactonization and β-elimination. Preparation of spiro-lactones **3** and **4** required an additional step, which included final 5-*endo*-trig spirocyclization of **1** or **2**. This work provided clues regarding the possible biosynthetic pathway of natural product **3**, which is probably formed from the unsaturated lactone **1** or **2** by a similar 5-*endo*-trig spirocyclization. The synthesized natural products and analogs were evaluated for their in vitro antiproliferative activity against a panel of human tumor cell lines. The obtained biological data revealed that natural products **1** and **3** as well as synthetic analogues **13** and **15** exhibited potent cytotoxic activities against all the tumor cells under evaluation but were devoid of any significant cytotoxicity against the normal MRC-5 cells. However, the styryl-lactones **2**, **11**, **12**, and **14** exhibited diverse antiproliferative activities. Based upon these results, we believe that these molecules may serve as convenient leads in the synthesis of more potent and selective antitumor agents.

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Supporting Information Available. Experimental procedures, characterization data for all compounds, and copies of ^{1}H, ^{13}C NMR spectra of final products. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.