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ASYMMETRIC SYNTHESIS AND ANTITUMOR ACTIVITY OF CYCLOALKANIN

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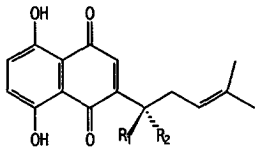
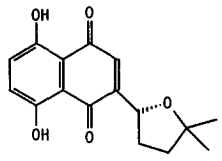
Abstract : Cycloalkanin was accessible by a practical and efficient asymmetric synthesis . The chiral center of the target is introduced *via* an asymmetric *C*-arylation of chiral aldehyde in high de. The synthesized cycloalkanin was shown to be significantly active against P388 cell line as assayed by *in vitro* MTT method.

Alkanin **1**, shikonin **2**, and their derivatives, found in the roots of many traditional medicinal plants of the Boraginaceae family (mainly in the genus of *Alkanna*, *Lithospermum*), were used as natural purple dyes from ancient times in China, Japan, and Europe[1]. These compounds bear considerable promises as drugs because of their antiinflammatory[2], antibacterial, antifungal[3], immunostimulating[4] and anticancer activities[5] as well as strong inhibition of DNA topoisomerase I[6]. Such extraordinary biological properties of these natural products render this naphthoquinone derivatives an attractive target for synthetic chemists. Though cycloalkanin **3** was readily produced after treatment of alkanin with Lewis acid, there is no report on its asymmetric synthesis and biological activity up to date.



1 R_1= H, R_2= OH
2 R_1= OH, R_2= H



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Herein we would like to report a highly diasteroselective approach towards the title compound, the key disconnection of which is depicted on scheme 1. According to this retrosynthetic scheme, the chiral center of the title compound may be established by employing the *C*-arylation of D-isopropylideneglyceraldehyde. Analogous substrates have been reported to afford high de's under ultrasonic irradiation[7]. Moreover, key intermediate **5** can be efficiently synthesized from 1,4-dimethoxybenzene **6**.

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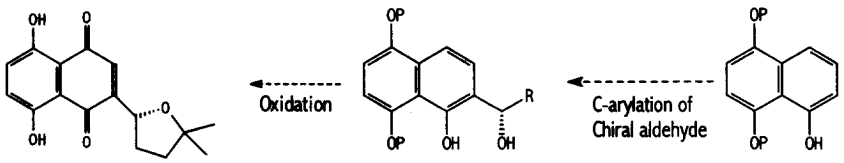


3 4 5

Scheme 1

Thus, Friedel-Crafts acylation, reduction, and intramolecular cyclization of **6** provided **9**[8], which was aromatized to produce **10** in large quantities (scheme 2).

Treatment of **10** with EtMgBr, followed with D-2,3-*O*-isopropylideneglyceraldehyde[9], then subjected to ultrasonic wave at 0°C for 7 h, furnished the *syn* addition product **11** in high diastereoisomeric excess (d.e.) of 90% and good yield (70%). The *syn*-glycerol **11** was quickly characterized by ^{1}HNMR spectroscopy on the basis of vicinal ^{1}H-^{1}H coupling constants as well as chemical shift. According to literature precedents, the *R* configuration of C-3 should be expected for the prepared alcohol[7].

Having established the chiral center of our target, we then proceeded to the completion of the side chain construction. Thus, after protection of the hydroxyl group as TBS ether and acetate respectively, the terminal acetonide blocked *ortho*-diol was cleaved to the corresponding aldehyde using a two step protocol in almost quantitative yield. Wittig type elongation of the resulting aldehyde with ylide of ethyl bromoacetate, then hydrogenated under H_2/10%Pd-C delivered the ester **15**, which was treated with methylmagnesium iodide to give alcohol **16**. Deacetylation of **16** with hydrazine in methanol, then submitted to an oxidation with iodobenzene diacetate, afforded the naphthoquinone **18** . After further demethylation of **18** accomplished with AgO-HNO_3, the dehydration and the removal of the TBS group of **19** was effected in one step to afford the target compound (*S*)-(-)-cycloalkanin **3** when treated with BF_3•Et_2O in CH_2Cl_2 at room temperature. The structure of the synthesized title compound **3** was determined on the basis of ^{1}H, ^{13}C-NMR, IR, HRMS analyses [10]. The same route could be followed using L-2,3-*O*-isopropylideneglyceraldehyde leading to the formation of (*R*)-(+)-cycloshikonin.

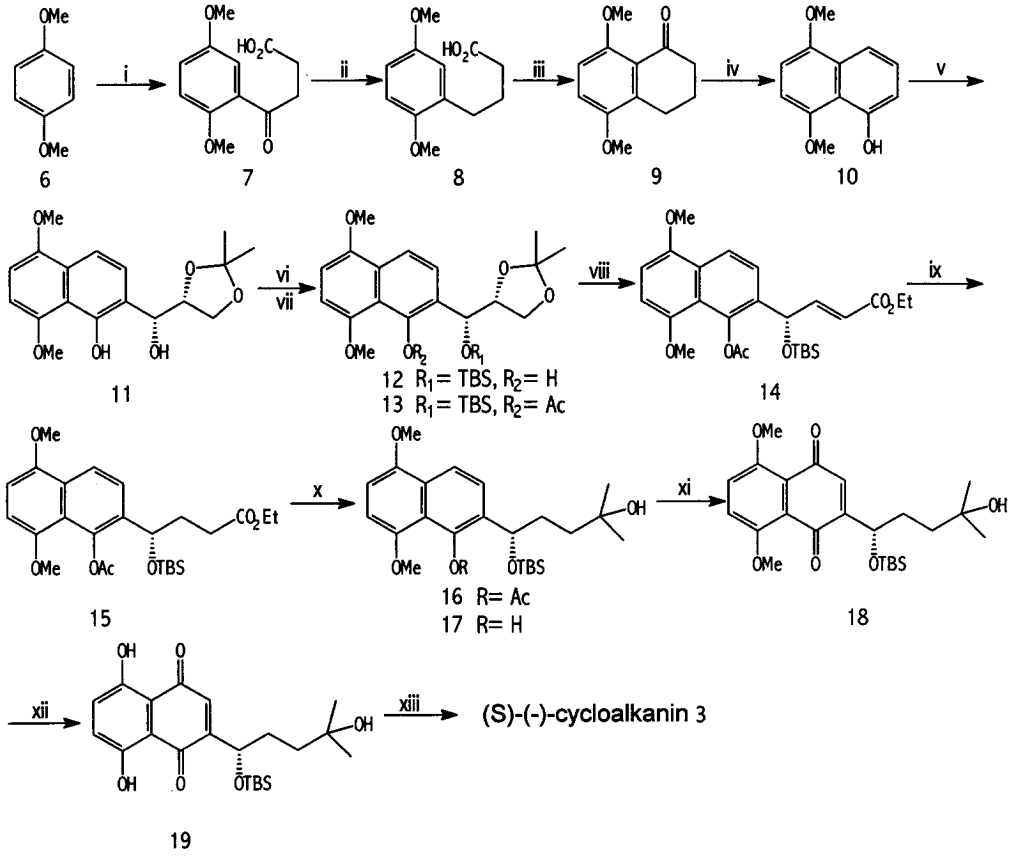
The cytotoxic effect of target compound on tumor cells was evaluated as assayed by *in vitro* MTT and SRB method. As shown in table 1, the synthesized cycloalkanin was significantly active against P388 cell line even at 10^{-8}M concertration.

Tab. 1 Inhibition (%) of P388 and A549 tumor cell lines in *in vitro*

c (Mol/L) / cell line	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
P 388[a]	98.9	98.9	98.9	98.9	98.9
A 549[b]	92.5	93.5	24.7	14.0	0.0

a. 48h, MTT assay; b. 72h, SRB assay.

In conclusion, we have developed a practical and efficient method for the synthesis of optically active cycloalkanin which exhibited strong inhibitory activity against P388 cells. Chemical synthesis and biological investigations of a series of structural analogues of the title compound are in progress.



Scheme 2. Synthesis of (*S*)-(-)-cycloalkanin (3)

Reagents and conditions:

(i) 1.2 eq. succinic anhydride, 2.5 eq. $AlCl_3$, CH_3NO_2, 0°C→ 25°C, 12h, 81%; (ii) 3.0 eq. $NH_2NH_2•H_2O$, 5.0 eq. KOH, DEG, 120-140°C 3h, then 180-200°C 5h , 80%; (iii) 1.2 eq. $(CF_3CO)_2O$, CH_2Cl_2, 0→25°C, 4h, 85%; (iv) a. 1.0 eq. *n*-Bu_4NBr_3, $CHCl_3$-CH_3OH(5:1), r.t., 3h; b. 2.0 eq. $CaCO_3$, DMF, 150-160°C, 2h; 54% based on **9**; (v) 1.0 eq. EtMgBr in ether, then 1.5 eq. D-2,3-*O*-isopropylidene-glyceraldehyde in CH_2Cl_2, CH_2Cl_2, ultrasonic wave, 0°C, 7h, 70%; (vi) 1.2 eq. TBSCl, 1.5 eq. imidazole, DMF, 25°C, 24h, 100%; (vii) 2.0 eq. $(CH_3CO)_2O$, cat. DMAP, pyridine, 0→25°C, 24h, 90%; (viii) a. $FeCl_3$-SiO_2, $CHCl_3$, 25°C, 5h; b. 1.5 eq. 0.65M aq. $NaIO_4$, silica gel, CH_2Cl_2, 25°C, 3h; c. 1.2 eq. Ph_3P=$CHCO_2Et$, CH_2Cl_2, 25°C, 24h; 71% based on **13**; (ix) H_2/10%Pd-C, EtOAc, 25°C, 16h, 100%; (x) a. 5.0 eq. MeMgI in ether, 0→25°C, 3h; b. $NH_2NH_2•H_2O$, CH_3OH, 25°C, 4h; 80% based on **15**; (xi) 2.4 eq. DAIB, CH_3CN-H_2O(2:1), 0→25°C, 2h, 85%; (xii) AgO/ 40%HNO_3, CH_3CN, 0→25°C, 0.5h, 70%; (xiii) 2.5 eq. $BF_3•Et_2O$, CH_2Cl_2, 0→25°C, 0.5h, 80%.

TBS= *t*-butyldimethylsilyl, DMAP= *N,N*-dimethylaminopyridine, DAIB= iodobenzene diacetate, DEG= diethylene glycol.

References and Notes:

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8. Moore J.A., Rahm M. *J. Org. Chem.* **1961**, 26, 1109.
9. Schmid C.R., Bryant J.D. *J. Org. Chem.* **1991**, 56, 4056.
10. The data for **3**: $[\alpha]_D$ = -180.6° (*c*= 0.01, $CHCl_3$). ^{1}HNMR (300MHZ, $CDCl_3$) δ: 1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.6-1.8 (m, 3H), 2.57 (m, 1H), 5.08 (m, 1H, CH), 7.14 (m, 3H, ArH), 12.46 (s, 1H, ArOH), 12.47 (s, 1H, ArOH). ^{13}CNMR (300MHZ, $CDCl_3$) δ: 27.75, 28.60, 33.38, 38.35, 74.32, 82.06, 111.56, 112.03, 131.17, 131.26, 131.67, 152.87, 163.39, 163.89, 181.31, 182.25. EIMS *m/z*: 288 (M^+), 232, 190. HRMS: Found 288.0991. Calcd. 288.0998. IR (KBr): 1610, 1570, 1454, 1265 cm^{-1}.