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An Efficient Synthesis of the C-23 Deoxy, 17α-Hydroxy South 1 Hemisphere and Its Cephalostatin 1 Analog[1]

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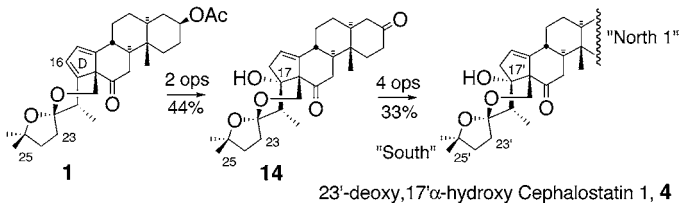
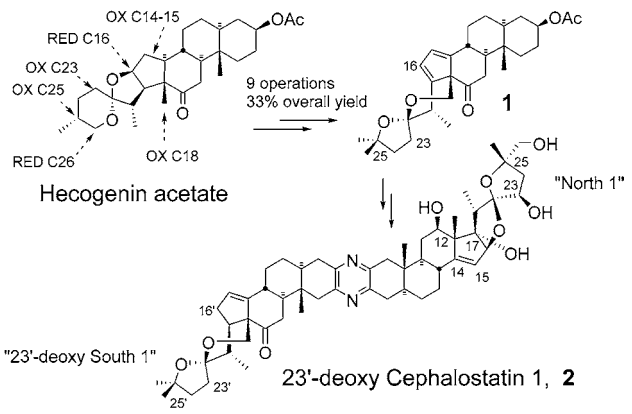
ABSTRACT



Methods for functionalization of the D ring of diene 1 were investigated. This study led to efficient syntheses of 3 and the subsequent 23′-deoxy,17′α-hydroxy cephalostatin 1 analog (4). The bioactivity data of 2 and 4 are in the low nanomolar range for a 10-cell-line minipanel.

The cephalostatins[2] and ritterazines[3] comprise a family of 45 structurally unique marine natural products that display extreme cytotoxicity against human tumors (∼1 nM mean GI_{50}'s in the 2-day NCI-60 screen and 10^{-14} M GI_{50}'s in 3-day tests in the Purdue minipanel).[4] We have reported the total syntheses of cephalostatin 1 and cephalostatin 7, as well as many analogs,[5,6] but chemical evidence for the site(s) of reactivity and the mechanism of action of the bissteroidal pyrazines remain unknown and no scalable synthesis for such testing has been achieved. As more extensively discussed in our previous publication,[7] we are now pursuing a strategy that retains all 27 carbon atoms of hecogenin acetate and employs site-specific oxidation reactions to introduce the common features found in the cephalostatin targets, including 23-deoxy South 1 and the derived 23′-deoxy cephalostatin 1 analog **2** (Scheme 1). We envisioned that diene **1** could be a useful intermediate for D-ring-functionalized South 1

Scheme 1



(1) Cephalostatin studies 26. Oxidations 4.

(2) Pettit, G. R.; Tan, R.; Xu, J.-P.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 953 and references therein.

(3) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484 and references therein.

(4) LaCour, T. G.; Guo, C.; Ma, S.; Jeong, J. U.; Boyd, M. R.; Matsunaga, S.; Fusetani, N.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2587 and references therein. Leukemia, renal and CNS lines are particularly sensitive to cephalostain 1.

(5) (a) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828 and references therein. (b) Jautelat, R.; Müller-Fahrnow, A.; Winterfeldt, E. *Chem. Eur. J.* **1999**, *5*, 1226. *Helv. Chim. Acta* **2000**, *83*, 1854 and references therein.

(6) LaCour, T. G.; Guo, C.; Boyd, M. R.; Fuchs, P. L. *Org Lett.* **2000**, *2*, 33.

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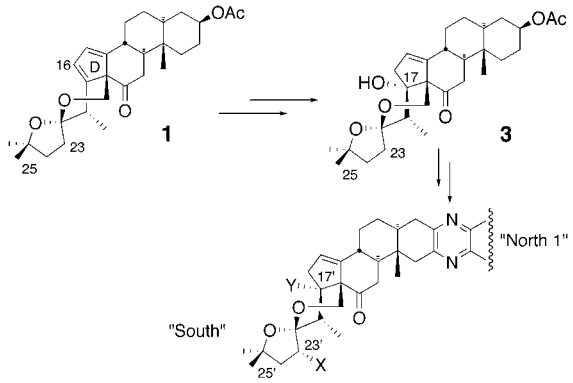
analogs, such as **3**, the 23-deoxy,17α-hydroxy South 1. Bioactivity evaluation of 23′-deoxy,17′α-hydroxy cephalostatin 1 (**4**) was held to be highly desirable (Scheme 2).

Scheme 2

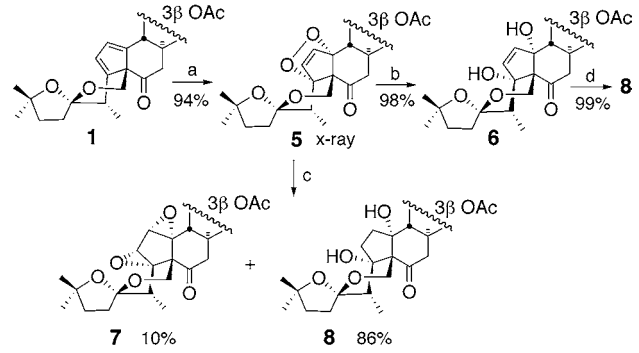


X = OH, Y = H, Cephalostatin 1
X = H, Y = OH, 23'-deoxy,17'α-hydroxy Cephalostatin 1, **4**

Herein, we report the results of the D-ring functionalization of diene **1**, the synthesis of **4**, and the bioactivity of **2** and **4** compared to the natural cephalostatin 1.

D-Ring Functionalization of Diene 1. Reaction of dienyl spiroketal **1** with singlet oxygen stereospecifically provided the desired 4 + 2 adduct **5**[8] in excellent yield[9] (Scheme 3).

Scheme 3 [a]

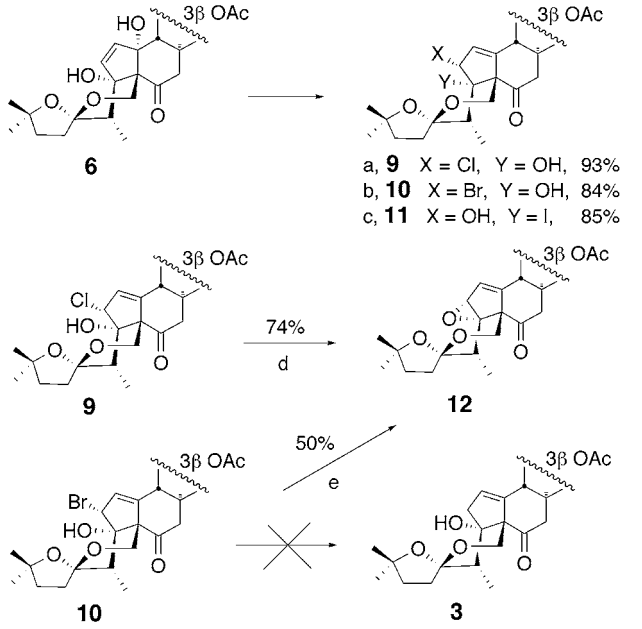


[a] Reagents and conditions: (a) sun lamp, O_2, 0.5 mol % TPP, CH_2Cl_2, 0 °C, 40 min; (b) Zn/AcOH, CH_2Cl_2, reflux, 30 min; (c) H_2, Pd/C, AcOEt, rt, 3 h; (d) H_2, Pd/C, AcOEt, rt, 1 h.

In contrast, performing the reaction at 0 °C for extended reaction time by reducing the amount of sensitizer 5,10,15,-20-tetraphenyl-21*H*,23*H*-porphine (TPP) only led to the formation of bisepoxide **7**. Although stable on silica gel, as with other singlet oxygen adducts,[10] **5** gradually converted to **7** on standing ($t_{1/2}$ ~4 months, 25 °C). Palladium-catalyzed hydrogenation of **5** furnished 86% of **8** and 10% of **7** after exploring various solvents and temperatures. In contrast, treatment of **5** with Zn/AcOH in dichloromethane at reflux afforded **6** in 98% yield without a trace of **7**. Hydrogenation of **6** gave **8** in almost quantitative yield.

Compound **6** reacted with hydrogen chloride etherate or HBr (30% in AcOH) in dichloromethane to give the corresponding Δ^{14},16α-halogenated products **9** and **10** in good to excellent yields (Scheme 4). The structure of **9** was

Scheme 4 [a]



[a] Reagents and conditions: (a) HCl·OEt_2, CH_2Cl_2, 0 °C, 3.5 h; (b) HBr (30% in AcOH), CH_2Cl_2, 0 °C, 3.5 h; (c) TMSI, CH_2Cl_2, 25 °C, 10 min; (d) Ag_2O, THF/H_2O (4:1), reflux, 2 h; (e) Bu_3SnH/AIBN, benzene, reflux, 12 h.

secured by X-ray analysis.[8] However, compound **11** was obtained when **6** was treated with TMSI in dichloromethane at room temperature, likely via epoxide **12**. No 16*β*-halogenated products were observed, presumably because of steric shielding of the *β* face of the D ring by the E and F rings. This observation was consistent with the hydrogenation[7] and singlet oxygen reactions of diene **1**, in which α-face products were exclusively formed. Epoxide **12** was isolated in 74% yield by treating **9** with Ag_2O in THF/H_2O (4:1) solution. Compound **10** failed to generate the desired South 1 analog **3** via radical dehalogenation. On the basis of proton NMR, this reaction gave 50% of epoxide **12** admixed with other unidentified compounds.

The quest for an efficient preparation of **3** next turned to a study of the dehydration of the 14,17-diol **8**. Several of

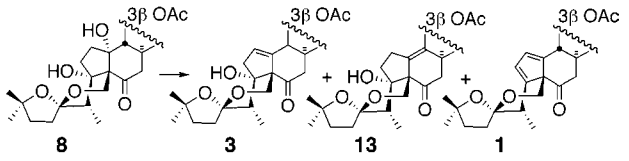
(7) Li, W.; LaCour, T. G.; Fuchs, P. L. *J. Am. Chem Soc*. **2002**, *124*, 4548.

(8) The X-ray structures of **5**, **9**, **3**, and 3*β*-OH of **13** have been submitted to the Cambridge Crystallographic database.

(9) For Diels−Alder reactions of D-ring dienes of other steroidal compounds, see: (a) Basler, S.; Brunk, A.; Jautelat, R.; Winterfeldt, E. *Helv. Chim. Acta* **2000**, *83*, 1854. (b) Fell, J. D.; Heathcock, C. H. *J. Org. Chem*. **2002**, *67*, 4742.

(10) (a) Fatma Sevin; Mckee, M. L. *J. Am. Chem. Soc*. **2001**, *123*, 4591−4596. (b) Boyd, J. D.; Foote, C. S.; Imagawa, D. K. *J. Am. Chem. Soc*. **1980**, *102*, 3641.

Table 1. Dehydration of 14,17-Diol **8**



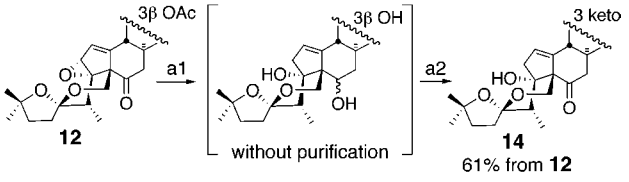
run	conditions	results[a] 3:13:1:8
1	$CeCl_3 \cdot 7H_2O$/NaI, CH_3CN, reflux, 21 h	21:31:1:27
2	TMSCl/NaI, CH_3CN, rt, 10 min	15:62:13:0
3	H_2SO_4 (3 equiv), AcOH,rt, 8 h	49:21:3:2
4	H_2SO_4 (3 equiv), CH_3CN, rt, 20 min	40:5:4:21
5	H_2SO_4 (20 equiv), CH_3CN, 0 °C, 5 min	51:7:1:12

[a] All data are based on ^{1}H NMR except in run 2, which are isolated yields.

the conditions explored are listed in Table 1. Compound **13** was formed as the major product when E2 elimination dominated (runs 1 and 2). Under strongly acidic conditions, the desired homoallylic alcohol **3** became major (runs 3−5), presumably via E1 elimination. The structures of both **3** and **13** were secured by X-ray analysis.[8] It was found that an unidentified compound was formed in less than 10% yield in runs 4 and 5. Although pure **3** could be obtained in 40% yield by silica gel chromotography, the unknown was always contaminated with **3** in a 1:1 ratio. Although only observed when using acetonitrile and strong protic acids, the unknown was not a product of Ritter reaction.[11]

An alternative approach for generation of the desired Δ^{14},17α-OH moiety is via regioselective reduction of epoxide **12**. As shown in Scheme 5, reduction of **12** using DIBAL-H

Scheme 5 [a]



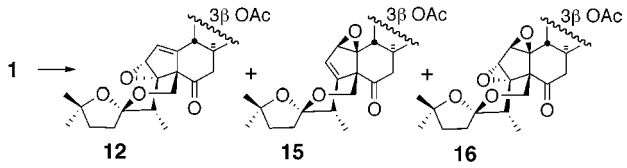
[a] Reagents and conditions: (a1) DIBAl-H, CH_2Cl_2, −78 °C, 3 h; (a2) TPAP/NMO/molecular sieves, CH_2Cl_2, rt, 3 h.

gave a mixture of triols. Without purification, this mixture was oxidized to **14**, the 3-ketone product of **3**, in 61% yield. It was observed that S_N2 hydride reduction using LAH failed, presumably because the β face of the epoxide was blocked. This approach is potentially superior to the dehydration of 14,17-diol **8** if preparation of **12** from diene **1** can be improved.

Epoxidation of diene **1** using mCPBA or DMDO gave epoxide **12** with poor selectivity. The results are shown in

(11) Krimen, L. I.; Cota, D. J. *Org. React.* **1969**, *17*, 213.

Table 2. Epoxidation of Diene **1**



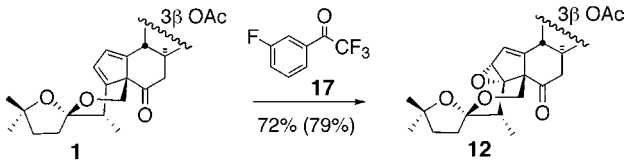
run	conditions	ratio by ^{1}H NMR[a] 12:15:16
1	mCPBA, Na_2HPO_4, CH_2Cl_2, rt, 47 min	10:3:1
2	mCPBA, CH_2Cl_2, −45 to 0 °C, 2 h	3.7:1.2:1
3	DMDO, CH_2Cl_2,0 °C 40 min, rt 2.5 h	3.9:1:1.7

[a] 5−10% of diene **1** remains unreacted under these conditions. In run 1, **12** and **16** were isolated in 53% yield in a 10:1 ratio.

Table 2. Only three products were formed. The 16,17-β epoxide was not formed because the β face was blocked by the E and F rings. The structures of **15** and **16** were assigned by reasoning that the epoxidations of **12** and **15** only give α,β-bisepoxide **16** instead of the authentic α,α-bisepoxide **7**.

We next explored epoxidation via dioxiranes derived from more sterically demanding trifluoroacetophenone analogs.[12] We were pleased to find that the ratio of **12** and (**15** + **16**) was increased to 6−7:1 and desired epoxide **12** was obtained in 72% yield (79% borsm) by using ketone **17** (Scheme 6).

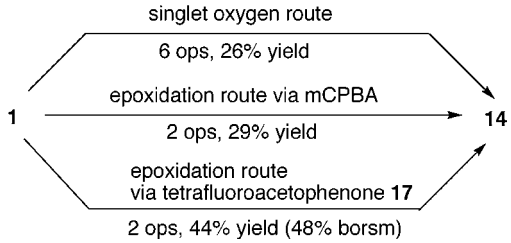
Scheme 6 [a]



[a] Conditions: ketone **17** (0.2 equiv), Oxone (0.6 equiv, 1.2 equiv of oxidant), and $NaHCO_3$ (2.4 equiv) in (1.5:1) CH_3CN−0.05 M $Na_2B_4O_7$ in 4×10^{-4} M aqueous EDTA, 0 °C, 60 min, rt, 80 min.

The comparison of the three methods to prepare **14** is shown in Scheme 7.

Scheme 7



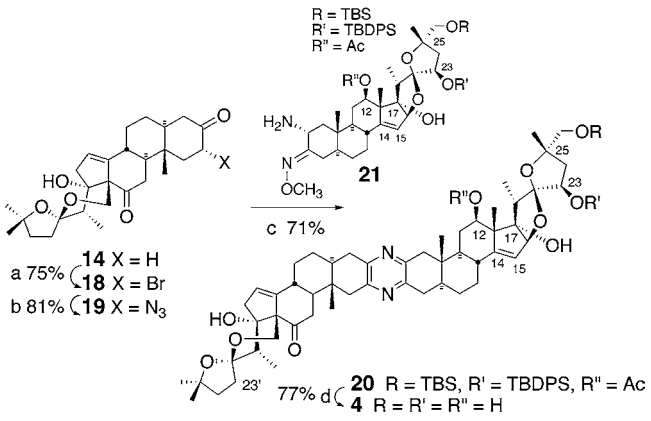
Completion of Synthesis of 23′-Deoxy,17′α-hydroxy Cephalostatin 1 (4). With **14**[13] in hand, bromide **18** and

Table 3. Cytotoxicities versus Representative Tissue Types in NCI 10 Cell Lines (GI_{50} nM)

	leukemia	lung	colon	CNS	breast	ovary	melanoma	renal		prostate
compound	HL-60	A-549	HT-29	SF-295	MCF-7	IGR OV1	M-14	A-498	RXF-393	PC-3
cephalostatin 1	0.11	0.30	3.9	<0.10	0.71	1.8	4.3		<0.10	0.32
2	2.4	3.5	21	0.18	12	15	60	0.12	0.52	6.6
4	8.3	3.6	33	0.12	3.4	9.3	14	13	0.23	9.3

azidoketone **19** were easily obtained in two steps, respectively (Scheme 8). Thus, protected cephalostatin 1 analog

Scheme 8 [a]



[a] Reagents and conditions: (a) PTAB/HBr (cat. 30% in AcOH), THF, 0 °C, 30 min; (b) TMGA, nitromethane (freshly distilled), rt, 10 h; (c) 0.7 equiv of **21**, PVP, Bu_2SnCl_2, benzene, reflux, 3 h; (d) TBAF, THF, 65 °C, 2 h, then K_2CO_3, MeOH/H_2O (12:1), reflux, 0.5 h.

20 was prepared by coupling the azidoketone **19** with North 1 aminomethoxime **21** using the unsymmetrical coupling methodology developed in our laboratory.[14] Deprotection furnished the cephalostatin 1 analog **4**.

Biological Activity. Testing of analogs **2** and **4** against natural cephalostatin 1 in the National Cancer Institute (NCI) 10 cell lines revealed that **2** and **4** displayed very high activity. The GI_{50} values are shown in Table 3.

In conclusion, studies toward the D-ring functionalization of diene **1** led to our efficient synthesis of 23-deoxy,17α-hydroxy South 1 analog and the subsequent 23′-deoxy,17′α-hydroxy cephalostatin 1 (**4**). Analog **4** featured the only difference of the site of the hydroxyl group comparing to the natural cephalostatin 1. DI_{50} values of **2** and **4** on NCI human cancer 10 cell lines are in the nanomolar range.

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

OL034894E

(12) For more extensive studies, see the following paper.
(13) Compound **14** also could be obtained from **3** after deprotection and oxidation, see Supporting Information.
(14) Guo, C.; Bhandaru, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1996**, *118*, 10672.