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# Absolute Stereostructure of Callystatin A, a Potent Cytotoxic Polyketide from the Marine Sponge, *Callyspongia truncata*

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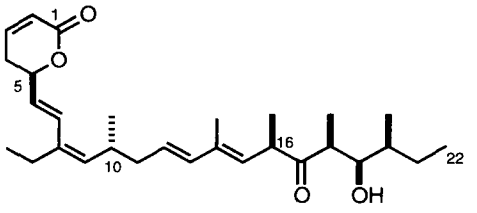
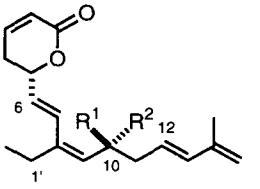
**Abstract** : The unidentified configurations at $C_5$ and $C_{10}$ in callystatin A (**1**), a potent cytotoxic polyketide from the marine sponge *Callyspongia truncata*, were determined to be *R*, *R* by comparing the circular dichroism spectrum of **1** with those of two model compounds **2** and **3**. Compounds **2** and **3** were synthesized by using *E*-selective Wittig olefination at the $C_6$-$C_7$ position as a key reaction.


In the course of our search for bioactive substances from marine organisms,[1] we have isolated a potent cytotoxic polyketide named callystatin A (**1**) from the marine sponge, *Callyspongia truncata,* and elucidated the plane structure including the absolute configurations of the $C_{16}$-$C_{22}$ β-hydroxyketone part.[2] The related antitumor antibiotics (*e.g.*, leptomycin,[3] kazusamycin, [4] anguinomycin,[5] and leptofuranin[6]) have been isolated from actinomyces and their plane structures elucidated. In order to reveal the absolute configurations at $C_5$ and $C_{10}$ in callystatin A (**1**), we synthesized two model compounds **2** and **3** depicted in Chart 1 and compared their circular dichroism (CD) spectra as well as NMR data with those of **1** in detail. In this paper, we report the absolute stereostructure of callystatin A (**1**).




callystatin A (**1**)




**2** : $R^1$ = H ; $R^2$ = Me
**3** : $R^1$ = Me ; $R^2$ = H

Chart 1

Chart 2 outlines the retrosynthetic analysis for 5*S*,10*R* model compound **2**.[7] The construction of the $C_6$-$C_7$ double bond as a key step in the synthesis of **2** was accomplished by high *E*-selective Wittig olefination[8] between the aldehyde **4** and the allylic tributylphosphonium bromide **5**. The aldehyde **4**, whose acetal moiety is easily converted to α,β-unsaturated δ-lactone, could be prepared from tri-*O*-acetyl-D-glucal (**6**) as shown in Chart 3. Then, both the $C_8$-$C_9$ and $C_{12}$-$C_{13}$ double bonds in **5** were disconnected into three synthons **7**, **8**, and **9**. The optically active alcohol **7** was accessible by one-carbon homologation of commercially available methyl (*S*)-3-hydroxy-2-methyl-propionate (**10**). Additionally, the two phosphonates

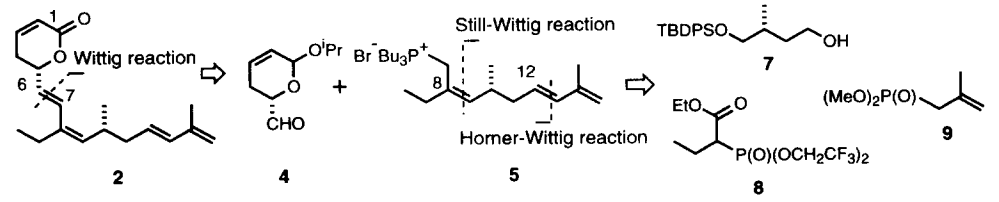



Chart 2

**8**[9] and **9**[10] could be prepared from ethyl 2-bromobutanoate and 3-bromo-2-methyl-1-propene, respectively. The other model compound **3** could also be synthesized from the enantiomer of **10** in a similar fashion. The execution of this strategy proceeded as follows.

The synthesis of the aldehyde **4** is shown in Chart 3. Thus, acid treatment of tri-*O*-acetyl-D-glucal (**6**) in the presence of *i*propanol afforded an acetal **11**, which was further converted to **12** by LiOH-catalyzed deacetylation and selective protection of a primary hydroxyl group. The mesylate of **12** was reduced by Super-Hydride ® ($LiBEt_3H$) and successively deprotected to furnish a primary alcohol **13** in overall yield of 86% for six steps. The alcohol **13** was converted to the aldehyde **4** by Swern oxidation quantitatively.

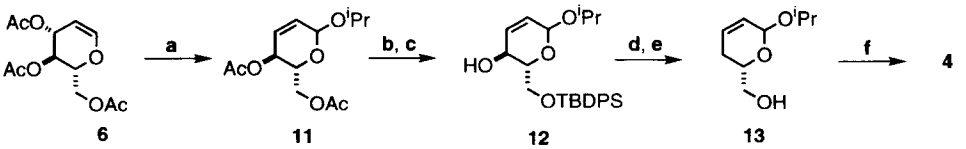



Chart 3

Reagents and conditions: **a**) $^{i}PrOH$, $BF_3 \cdot OEt_2$, benzene, 95%, **b**) LiOH (cat), MeOH, quant., **c**) TBDPSCl, imidazole, $CH_2Cl_2$, 99%, **d**) MsCl, $Et_3N$, $CH_2Cl_2$; $LiB(Et)_3H$, THF, reflux, 2 steps 92%, **e**) TBAF, THF, quant., **f**) $(COCl)_2$, DMSO; $Et_3N$, $CH_2Cl_2$, -78°C, quant.

Synthesis of the 5*S*,10*R* model compound **2** *via* the allylic tributylphosphonium bromide **5** is depicted in Chart 4. Protection of a hydroxy group in **10** and subsequent reduction gave an alcohol **14** in two steps 95%, which was then converted to **7** by one-carbon homologation through three steps in 90% yield. Tetrapropylammonium perruthenate (TPAP)-catalyzed oxidation of **7** followed by Horner-Wittig reaction with **9** afforded 12*E*-diene **15** stereoselectively in two steps 74% yield. Removal of the TBDPS group and subsequent Swern oxidation of **15** gave an aldehyde, which was successively subjected to Still-Wittig reaction[11] with **8** to provide 8*Z*-triene **16** and 8*E*-isomer in a ratio of 16:1 in three steps 81% yield. The geometry of the $C_8$-$C_9$ double bond in **16** was confirmed to be *Z* by NOE enhancement between H-9 and $H_2$-1'. Then, the bromide **18** was generated from **16** upon DIBAL reduction and subsequent bromination *via* an alcohol **17**. The condensation of the aldehyde **4** and the dimethyl phosphonate, which was prepared from **18** and $P(OMe)_3$, was examined under several basic conditions ($^{n}BuLi$, NaH, *etc.*) to give only decomposed products. On the other hand, the corresponding triphenylphosphorus ylide was converted into a coupled product without *E* selectivity.

Accordingly, the allylic tributylphosphorus ylide **5**[8] was applied to preparation of **19**. Reported conditions[8] (*e.g.*, anhydrous THF as a solvent; $^{n}BuLi$, $^{t}BuOK$, *etc.*, as a base) did give the desired 6*E*-olefin **19** exclusively albeit in low yield (0~25%). Upon further detailed examination, it was found that

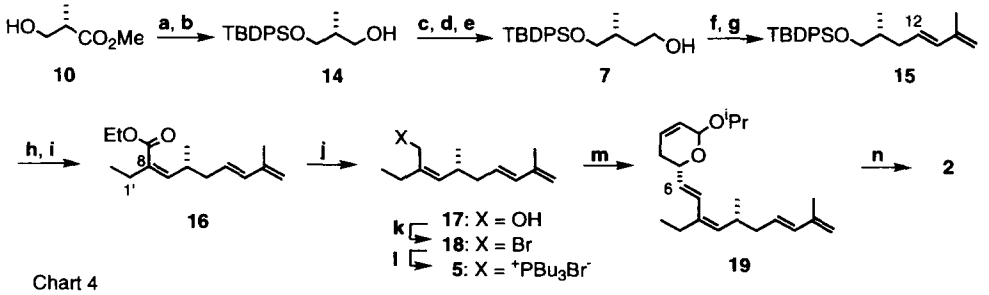



Chart 4

Reagents and conditions: **a**) TBDPSCl, imidazole, $CH_2Cl_2$, **b**) $LiBH_4$, THF, reflux, 2 steps 95%, **c**) $(COCl)_2$, DMSO; $Et_3N$, $CH_2Cl_2$, -78°C, **d**) $BrPh_3PCH_3$, $^nBuLi$, THF, 0°C, **e**) $BH_3$•$OEt_2$, THF, -15 to 0°C; 30% $H_2O_2$, 1*N* NaOH, 3 steps 90%, **f**) TPAP(cat), NMO, $CH_2Cl_2$, 90%, **g**) **9**, $LiN(SiMe_3)_2$, THF, -78°C to rt, 82%, **h**) TBAF, THF, 90%, **i**) $(COCl)_2$, DMSO; $Et_3N$, $CH_2Cl_2$, -78°C; **8**, $KN(SiMe_3)_2$, 18-crown-6, THF, -78°C to rt, 2 steps 90%, **j**) DIBAL-H, $CH_2Cl_2$, -78°C, quant., **k**) $CBr_4$, $Ph_3P$, $CH_3CN$, 82 %, **l**) $^nBu_3P$, $CH_3CN$. **m**) **4**, $LiCH_2S(O)CH_3$, toluene, -78°C to rt, 2 steps 60%, **n**) Dowex HCR W2 ($H^+$), 5:1 acetone/$H_2O$, 40°C; $Ag_2CO_3$-Celite, benzene, 50°C, 2 steps 77%.

$LiCH_2S(O)CH_3$ treatment of **4** and **5** in toluene furnished **19** in the most favorable yield of 60% without loss of *E*-selectivity. Finally, **19** was subjected to catalytic acid hydrolysis and subsequent $Ag_2CO_3$-Celite oxidation in one pot to furnish the 10*R* lactone **2** in 77% yield. Diastereomeric 5*S*,10*S* model compound **3** was similarly synthesized using methyl (*R*)-3-hydroxy-2-methyl propionate in place of its *S*-isomer **10**.

Detailed comparison of the $^1$H-NMR data[12] for callystatin A (**1**) with those for the two model compounds **2** and **3** brought about no conclusive difference in relative stereochemistry at $C_5$ and $C_{10}$ in **1**. From intensive analysis of the CD spectra of **1**, **2**, and **3** illustrated in Fig.1, the following conclusions were obtained: 1) the strong split Cotton effects at 243 nm and 221 nm due to the interaction of π-π* transition of the two conjugated diene chromophores clearly indicated 10*R* configuration in **1**; 2) the assumed CD spectrum of antipode (5*R*, 10*R*) of **3** is similar to that of **1**. Especially, 5*S*,10*R* model compound **2** showed strong negative Δε value at 210 nm, while **1** and **3** showed no Δε value at 210 nm.[13]

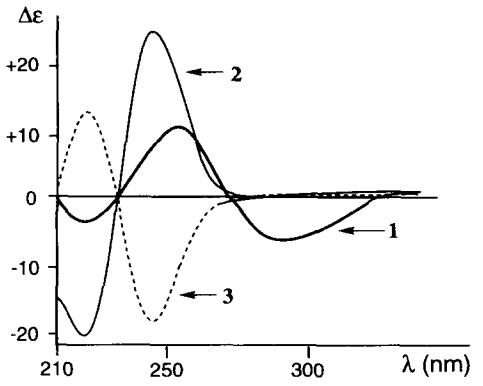



**Figure 1**: Circular dichroism spectra of callystatin A (**1**) and the model compounds **2** and **3** in MeOH

The two model compounds **2** and **3** having the α, β-unsaturated δ-lactone and two conjugated diene moieties common to callystatin A (**1**) exhibited moderate cytotoxicity against KB cells at $IC_{50}$ 0.01 μg/ml, respectively. Therefore, it should be of interest to learn how the configurations at C-5 and/or the β-hydroxy-ketone portion with four asymmetric centers are associated with the extremely potent cytotoxicity of **1**.

In summary, the whole absolute configuration of callystatin A (**1**) was elucidated as 5*R*, 10*R*, 16*R*, 18*S*, 19*R*, 20*S*. Work on total synthesis of callystatin A (**1**), aiming to confirm its structure and reveal the structure-activity relationship, is currently in progress.

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**References and Notes**

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7. Numbering of each compound is accordance with that of the model compound **2** to avoid confusion.
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9. Compound **8** was prepared from ethyl 2-bromobutanoate according to ref. 11.
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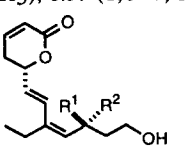
11. Still, W. C.; Gennari, C. *Tetrahedron Lett.*, **1983**, *24*, 4405-4408.
12. Callystatin A (**1**): CD (MeOH) λmax nm (Δε): 299 (-6.1), 270 (0), 251 (+11.6), 233 (0), 222 (-4.2), 210 (-0.9). $^1$H-NMR (500 MHz, $C_6D_6$) δ: 6.61 (d, *J*=16 Hz, H-7), 6.06 (d, *J*=16, H-13), 5.90 (ddd, *J*=10, 2, 5.5, H-3), 5.81 (dd, *J*=10, 2, H-2), 5.62 (dt, *J*=16, 8, H-12), 5.55 (dd, *J*=16, 6.5, H-6), 5.25 (d, *J*=10, H-15). 5.23 (d, *J*=10, H-9), 4.43 (m, H-5), 3.76 (t-like, *J*=ca. 6, H-19), 3.55 (dq, *J*=10, 7, H-16), 2.84 (dd, *J*=7, 5.5, H-18), 2.67 (m, H-10), 2.20 (m, $H_2$-4), 2.13 (m, 1'-$H_2$), 2.07 (dd, *J*=6, 8, H-11), 1.77 (s, 14-$CH_3$), 1.50 (m, $H_2$-21), 1.30 (m, H-20), 1.14 (d, *J*=7, 16-$CH_3$), 1.07(t, *J*=7.5, 2'-$H_3$), 1.04 (d, *J*=7, 18-$CH_3$), 0.98 (d, *J*=7, 10-$CH_3$), 0.97 (d, *J*=6.5, 20-$CH_3$), 0.87 (t, *J*=7.5, $H_3$-22). **2**: FAB MS *m/z* : 287 $(M+H)^+$. High-FAB MS: Obsd; *m/z*: 287.201. Calcd for $C_{19}H_{27}O_2$; *m/z* 287.201. IR cm$^{-1}$ (KBr) 1730. UV λmax (MeOH) nm(ε): 230 (28800). CD (MeOH) λmax nm (Δε): 244 (+25.3), 231 (0), 222 (-20.3), 210 (-15.2). $^1$H-NMR ($C_6D_6$) δ: 6.58 (d, *J*=16 Hz, H-7), 6.20 (d, *J*=16, H-13), 5.87 (ddd, *J*=10, 2, 5.5, H-3), 5.82 (dd, *J*=10, 2, H-2), 5.61 (dt, *J*=16, 7.5, H-12), 5.57 (dd, *J*=16, 6.5, H-6), 5.24 (d, *J*=10, H-9), 4.96, 4.91 (both s, H-15), 4.41 (m, H-5), 2.68 (m, H-10), 2.20 (m, $H_2$-4), 2.13 (q, *J*=7.5, 1'-$H_2$), 2.07 (dd, *J*=7, 7.5, H-11), 1.78 (s, 14-$CH_3$), 1.06 (t, *J*=7.5, 2'-$H_3$), 1.00 (d, *J*=7, 10-$CH_3$).
**3**: FAB MS *m/z* : 287 $(M+H)^+$. High-FAB MS: Obsd; *m/z*: 287.202. Calcd for $C_{19}H_{27}O_2$; *m/z* 287.201. IR cm$^{-1}$ (KBr) 1730. UV λmax (MeOH) nm(ε): 230 (29000). CD (MeOH) λmax nm (Δε): 244 (-17.8), 231 (0), 222 (+13.8), 210 (+2.2). $^1$H-NMR ($C_6D_6$) δ: 6.60 (d, *J*=16 Hz, H-7), 6.24 (d, *J*=16, H-13), 5.88 (ddd, *J*=10, 2, 5.5, H-3), 5.82 (dd, *J*=10, 2, H-2), 5.65 (dt, *J*=16, 7.5, H-12), 5.57 (dd, *J*=16, 6.5, H-6), 5.25 (d, *J*=10, H-9), 4.99, 4.94 (both s, H-15), 4.41 (m, H-5), 2.70 (m, H-10), 2.19 (m, $H_2$-4), 2.14 (q, *J*=7.5, 1'-$H_2$), 2.09 (dd, *J*=7, 7.5, H-11), 1.84 (s, 14-$CH_3$), 1.06 (t, *J*=7.5, 2'-$H_3$), 0.99 (d, *J*=7, 10-$CH_3$).
13) We have also synthesized model compounds **20** and **21**. The difference of CD amplitude between **2** and antipode of **3** is well explained as CD amplitude of **20** (or **21**). **20**: CD (MeOH) λmax nm (Δε): 244 (+3.7), 228 (0), 210 (-4.9). **21**: CD (MeOH): 244 (+3.0), 228 (0), 210 (-4.8).




**20** : $R^1$ = H ; $R^2$ = Me
**21** : $R^1$ = Me ; $R^2$ = H

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