

Total Synthesis of Callystatin A, a Potent Cytotoxic Polyketide from the Marine Sponge, *Callispongia truncata*

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Abstract : A first total synthesis of callystatin A (**1**), a potent cytotoxic polyketide from the marine sponge *Callispongia truncata*, has been achieved by use of an *E*-selective Wittig olefination and asymmetric Evans aldol condensation as the key reactions. Thus, the absolute stereostructure of **1** previously established by us was confirmed. © 1998 Elsevier Science Ltd. All rights reserved.

During the course of our investigation in search for new bioactive substances from marine organisms, we isolated an extremely potent cytotoxic polyketide (IC₅₀: 10 pg/ml against KB cell; 20 pg/ml against L1210 cell) designated callystatin A (**1**) from the marine sponge *Callispongia truncata* through bioassay-guided separation.¹⁾ In addition, the absolute stereostructure of **1** was elucidated by both physicochemical and synthetic means recently.²⁾ The scarcity of natural supply prompted us to engage in total synthesis of **1** for further biological evaluation and confirmation of the absolute stereostructure. Here, we describe the first total synthesis of callystatin A (**1**).

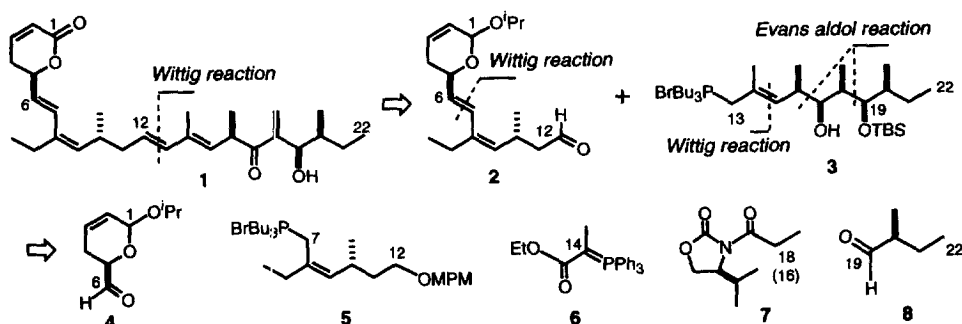


Chart 1: Retrosynthetic Analysis of Callystatin A (**1**)

Chart 1 outlines our retrosynthetic analysis for callystatin A (**1**). Disconnection of the C₁₂-C₁₃ double bond gives segments C₁-C₁₂ (**2**) and C₁₃-C₂₂ (**3**), which could be united by means of a highly *E*-selective Wittig reaction using allylic tributylphosphorus ylide.^{2,3)} Then, an aldehyde **2**, containing a

masked α,β -unsaturated δ -lactone moiety, could be constructed using the above-mentioned Wittig reaction again between segments C₁-C₆ (**4**) and C₇-C₁₂ (**5**), the latter of which was a building block to synthesize the model compounds for elucidation of the absolute stereostructure of **1**.²) On the other hand, the optical active allylic tributylphosphonium bromide **3** was dissected by application of duplicate asymmetric Evans aldol condensations⁴) followed by a Wittig reaction to afford three synthons, (1-carbethoxyethylidene)triphenylphosphorane (**6**), (*S*)-3-(1-oxopropyl)-4-isopropyl-2-oxazolidinone (**7**), and (*S*)-2-methylbutan-1-al (**8**). The execution of this strategy proceeded as follows.

The synthesis of segment C₁-C₁₂ (**2**) is depicted in Chart 2. Up to date, the asymmetric synthesis of (*R*)-6-hydroxymethyl-5,6-dihydro- α -pyrone has been reported by Honda⁵) and Boger⁶) independently. However, both methods required time-consuming reaction steps and resulted in low total yields. Thus, we applied the methodology of Ghosez *et al.*⁷) and slightly modified it using optically active *O*-protected glycidol for asymmetric synthesis of **4**. Namely, *tert*-butyldipheylsilylated (TBDPS) *S*-glycidol **9** was subjected to coupling reaction with methyl 3-phenylsulfonylorthopropionate (**10**) using ⁿBuLi in the presence of 1,3-dimethylpropyleneurea (DMPU) to afford presumable orthoester **11**, which was followed by sequential treatment with 3M aq. H₂SO₄ for neutralization, *p*-TsOH for lactonization, and DBU for elimination of phenylsulfonic acid to provide an α,β -unsaturated lactone **12** in 82% overall yield from **9** without purification of the intermediates. The optical purity of **12** was confirmed by comparing the specific rotation of its deprotected form, $[\alpha]^{22}_{\text{D}} +154^{\circ}$ ($c=1.0$, CHCl₃), with the literature value, $[\alpha]^{25}_{\text{D}} +160^{\circ}$ ($c=0.85$, CHCl₃).⁶) Then, diisobutylaluminum hydride (DIBAL-H) reduction of **12** followed by PPTS treatment in the presence of isopropanol afforded **13** in 82% yield, which was further converted to the aldehyde **4** almost quantitatively through deprotection and Swern oxidation. Wittig coupling between **4** and **5** was undertaken by LiCH₂S(O)CH₃ treatment in toluene to give only 6*E*-conjugated diene **14**^{2,8}) selectively in 75% yield. Finally, removal of the *p*-methoxyphenyl-methyl (MPM) group in **14** with DDQ and subsequent Swern oxidation furnished segment C₁-C₁₂ (**2**) in 82% yield.

Next, the segment C₁₃-C₂₂ (**3**) was synthesized as depicted in Chart 3. The optically pure aldehyde **8**, prepared from commercially available *S*-amyl alcohol by PCC oxidation, was treated with **7** under the

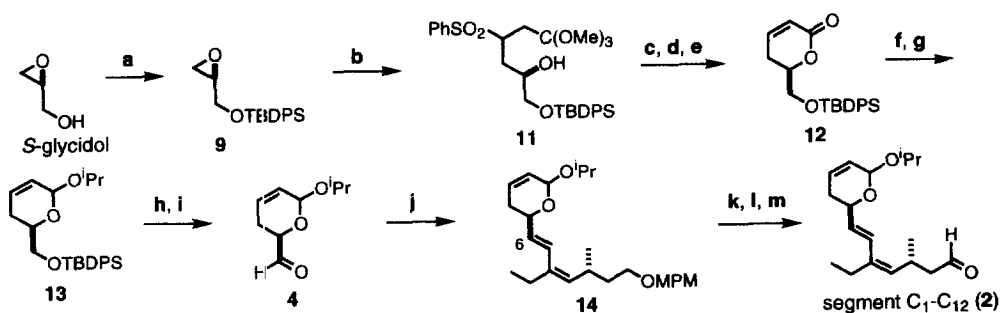


Chart 2: Synthesis of Segment C₁-C₁₂ (**2**)

Reagents and conditions: **a**) TBDPSCI, imidazole, CH₂Cl₂, 95%, **b**) **10**, ⁿBuLi, DMPU, THF, -20~ 5°C, **c**) 3M H₂SO₄-THF (3 : 1), **d**) *p*-TsOH, MS 4A, ClCH₂CH₂Cl, 70°C, **e**) Et₃N, DBU, ClCH₂CH₂Cl, -10°C, 82% from **9**, **f**) DIBAL-H, CH₂Cl₂, -78°C, **g**) ⁱPrOH, PPTS, PhH, 82%, 2 steps, **h**) TBAF, THF, **i**) (COCl)₂, DMSO, CH₂Cl₂, Et₃N, -78°C, 99%, 2 steps, **j**) **5**, LiCH₂S(O)CH₃, toluene, -78°C to rt, 75%, **k**) DDQ, CH₂Cl₂-0.5% NaHCO₃ (9 : 1), **l**) ⁱPrOH, PPTS, **m**) (COCl)₂, DMSO, CH₂Cl₂, Et₃N, -78°C, 82%, 3 steps.

standard conditions of asymmetric Evans aldol condensation to give a C_{18,19}-syn, C_{19,20}-syn adduct **15** predominantly in 98% yield as a 9:1 mixture of diastereoisomers. Removal of the chiral auxiliary of **15** with the aluminum amide reagent⁹⁾ derived from MeONHMe·HCl and AlMe₃ and subsequent protection of the hydroxyl group as its *tert*-butyldimethylsilyl (TBS) ether afforded **16**. DIBAL-H reduction of **16** gave aldehyde **17** in 76% yield, which was again subjected to Evans aldol condensation with **7** to yield exclusively **18** as a single isomer having two syn orientations at C₁₆-C₁₈ (determined by ¹H-NMR) in 85% yield. Careful treatment of **18** with MeONHMe·HCl and AlMe₃ at lower temperature (*i.e.*, from -78°C to 0°C) gave a Weinreb amide **19** in favorable yield (92 %).

In order to distinguish the newly generated hydroxyl group from the *t*-butyldimethylsilylated group for constructing the β-hydroxyketone portion in **1**, protection of the hydroxyl group in **19** was examined under various conditions. However, it was found extremely difficult to introduce a protecting group such as triethylsilyl (TES), MPM, tetrahydropyranyl (THP), and 2-methoxypropyl residues. These observations suggested severe steric hindrance and led us to leave the hydroxyl group at C-17 unprotected up to the final stage of total synthesis of **1**. The above presumption was also supported by the following behavior of **19**. DIBAL-H reduction of **19** proceeded much slower and in unfavorable yield (40 %) of **20** with recovery of **19**, while LiAlH₄ treatment rapidly furnished **20** in high yield (96 %). Construction of the α-methyl-α,β-unsaturated ester moiety by Horner-Wittig reaction using triethyl 2-phosphonopropionate in the presence of lithium hexamethyldisilazide (LHMDS) gave the desired 14-*E* conjugated ester **21** in unsatisfactory yield (49 %). On the other hand, treatment with (1-carbethoxyethylidene)triphenylphosphorane (**6**) under neutral conditions gave **21** in high yield (94 %). Upon DIBAL-H reduction and successive CBr₄/PPh₃ treatment, the ester **21** was transformed into **23** in two steps in 99% yield without any destruction of the secondary hydroxyl group. The geometry of the C₁₄-C₁₅ double bond in **22** was defined as *E* by observation of NOE enhancements between the following pairs of protons, *i.e.*, H-15 and H₂-13; 14-H₃C and H-16. Finally, **23** was treated with ⁿBu₃P to furnish the segments C₁₃-C₂₂ (**3**) quantitatively.

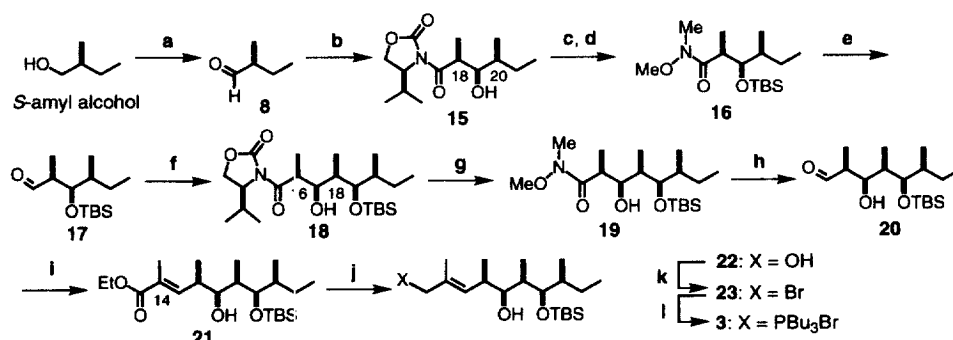
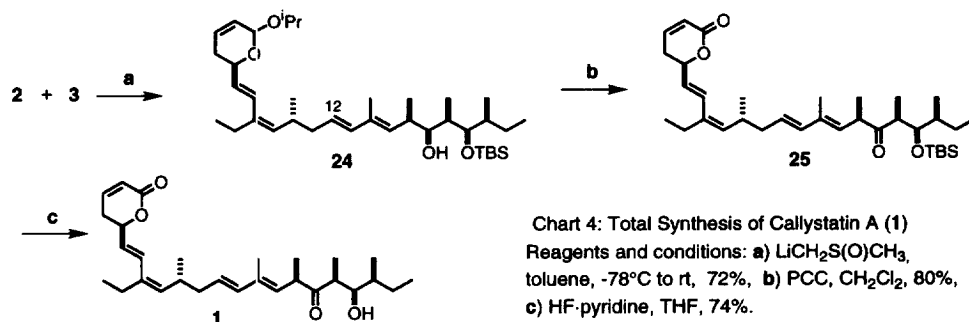


Chart 3: Synthesis of Segment C₁₃-C₂₂ (**3**)

Reagents and conditions: a) PCC, CH₂Cl₂, 0°C, 23%, b) **7**, ⁿBu₂BOTf, Et₃N, THF, -78~0°C, 98% (9:1), c) AlMe₃, MeONHMe·HCl, CH₂Cl₂, -20~0°C, 95%, d) TBSOTf, 2,6-lutidine, CH₂Cl₂, -20°C, quant., e) DIBAL-H, THF, -78°C, 76%, f) **7**, ⁿBu₂BOTf, Et₃N, THF, -78~0°C, 85%, g) AlMe₃, MeONHMe·HCl, CH₂Cl₂, -78~0°C, 92%, h) LiAlH₄, Et₂O, 0°C, 96%, i) **6**, toluene, 94%, j) DIBAL-H, CH₂Cl₂, -78°C, quant., k) CBr₄, Ph₃P, 2,6-lutidine, CH₂Cl₂, 99 %, l) ⁿBu₃P, CH₃CN, quant.

The final stage toward total synthesis of callystatin A (**1**) beginning from Wittig coupling between segments C₁-C₁₂ (**2**) and C₁₃-C₂₂ (**3**) was carried out as summarized in Chart 4. The two segments were condensed smoothly under the same conditions²⁾ as for preparation of **14** to provide a sole product **24** having the desired 12-*E* geometry in favorable yield (72%). PCC oxidation of **24** concomitant with hydrolysis of the acetal portion built up δ -lactone and keto-carbonyl moieties at the same time to give 19-*O*-TBS-callystatin A (**25**) in 80% yield. Finally, deprotection of the TBS group with HF-pyridine furnished callystatin A (**1**) in 74% yield. This synthetic compound was identical with the natural callystatin A (**1**) in all respects ($[\alpha]_D$, ¹H- and ¹³C-NMR, IR, UV, CD, FAB-MS, HPLC, and cytotoxicity), confirming the absolute stereostructure of **1** presented by us.



In summary, the first total synthesis of callystatin A (**1**) was achieved using an *E*-selective Wittig reaction to construct the two conjugated diene portions and asymmetric Evans aldol condensation to build up the β -hydroxyketone moiety with four asymmetric centers as the key reactions. The detailed biological activities of callystatin A (**1**) and structure-activity relationship study are under investigation.

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References and Notes

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