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

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Abstract : A first total synthesis of callystatin A (**1**), a potent cytotoxic polyketide from the marine sponge *Callyspongia 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.

During the course of our investigation in search for new bioactive substances from marine organisms, we isolated an extremely potent cytotoxic polyketide (IC_{50}: 10 pg/ml against KB cell; 20 pg/ml against L1210 cell) designated callystatin A (**1**) from the marine sponge *Callyspongia 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**).

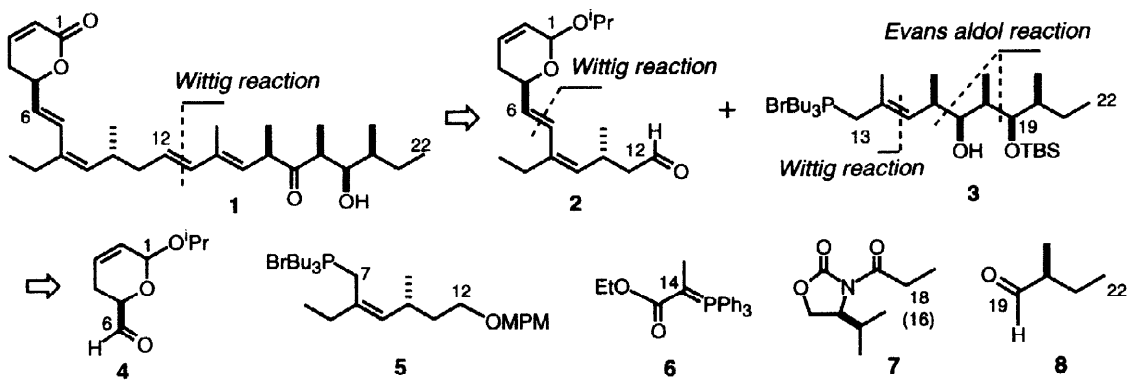

Chart 1: Retrosynthetic Analysis of Callystatin A (**1**)

Chart 1 outlines our retrosynthetic analysis for callystatin A (**1**). Disconnection of the C_{12}-C_{13} double bond gives segments C_1-C_{12} (**2**) and C_{13}-C_{22} (**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

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masked α,β-unsaturated δ-lactone moiety, could be constructed using the above-mentioned Wittig reaction again between segments C_1-C_6 (**4**) and C_7-C_{12} (**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 condensations[4] followed by a Wittig reaction to afford three synthons, (1-carbethoxyethylidene)triphenyl-phosphorane (**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 C_1-C_{12} (**2**) is depicted in Chart 2. Up to date, the asymmetric synthesis of (*R*)-6-hydoxymethyl-5,6-dihydro-α-pyrone has been reported by Honda [5] and Boger [6] 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-phenylsulphonylorthopropionate (**10**) using ^{n}BuLi in the presence of 1,3-dimethylpropyleneurea (DMPU) to afford presumable orthoester **11**, which was followed by sequential treatment with 3M aq. H_2SO_4 for neutralization, *p*-TsOH for lactonization, and DBU for elimination of phenylsulfinic 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, $[\alpha]^{22}_D$ +154° (*c*=1.0, $CHCl_3$), with the literature value, $[\alpha]^{25}_D$ +160° (*c*=0.85, $CHCl_3$).[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 $LiCH_2S(O)CH_3$ 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 C_1-C_{12} (**2**) in 82% yield.

Next, the segment C_{13}-C_{22} (**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

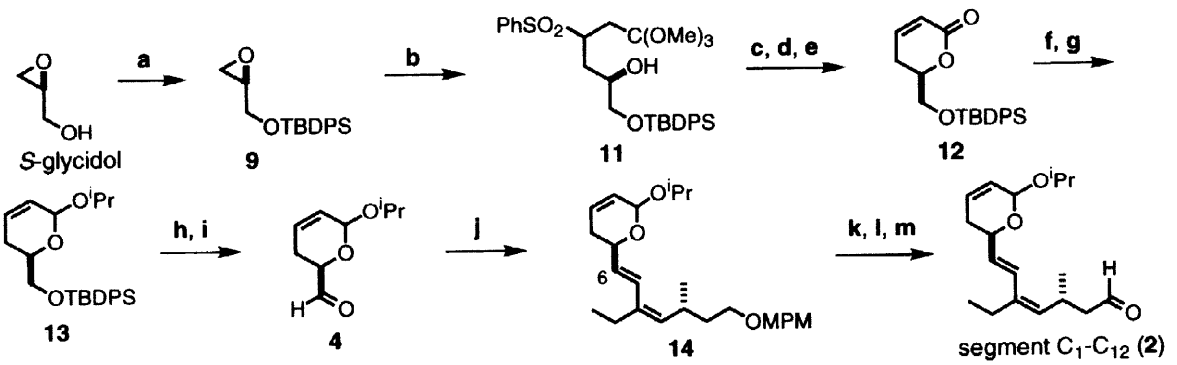

Chart 2: Synthesis of Segment C_1-C_{12} (**2**)

Reagents and conditions: **a**) TBDPSCl, imidazole, CH_2Cl_2, 95%, **b**) **10**, ^{n}BuLi, DMPU, THF, -20~ 5°C, **c**) 3M H_2SO_4-THF (3 : 1), **d**) *p*-TsOH, MS 4A, $ClCH_2CH_2Cl$, 70°C, **e**) Et_3N, DBU, $ClCH_2CH_2Cl$, -10°C, 82% from **9**, **f**) DIBAL-H, CH_2Cl_2, -78°C, **g**) ^{i}PrOH, PPTS, PhH, 82%, 2 steps, **h**) TBAF, THF, **i**) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, -78°C, 99%, 2 steps, **j**) **5**, $LiCH_2S(O)CH_3$, toluene, -78°C to rt, 75%, **k**) DDQ, CH_2Cl_2-0.5% $NaHCO_3$ (9 : 1), **l**) ^{i}PrOH, PPTS, **m**) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, -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 reagent[9] derived from MeONHMe·HCl and $AlMe_3$ 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 C_{16}-C_{18} (determined by ^{1}H-NMR) in 85% yield. Careful treatment of **18** with MeONHMe·HCl and $AlMe_3$ 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*-butyldimethylsililated 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 $LiAlH_4$ 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 CBr_4/PPh_3 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 C_{14}-C_{15} double bond in **22** was defined as *E* by observation of NOE enhancements between the following pairs of protons, *i.e.*, H-15 and H_2-13; 14-H_3C and H-16. Finally, **23** was treated with ^{n}Bu$_3$P to furnish the segments C_{13}-C_{22} (**3**) quantitatively.

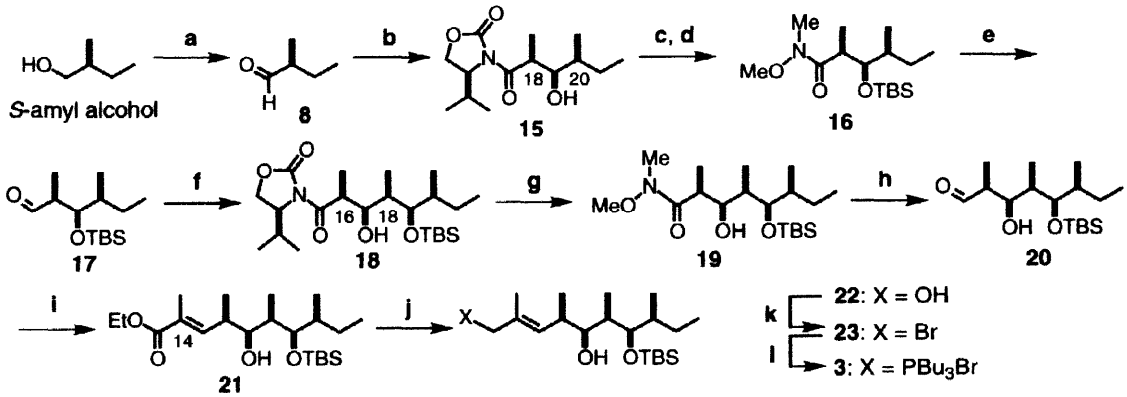

Chart 3: Synthesis of Segment C_{13}-C_{22} (**3**)
Reagents and conditions: **a)** PCC, CH_2Cl_2, 0°C, 23%, **b)** **7**, ^{n}Bu$_2$BOTf, Et_3N, THF, -78~0°C, 98% (9:1), **c)** $AlMe_3$, MeONHMe•HCl, CH_2Cl_2, -20~0°C, 95%, **d)** TBSOTf, 2,6-lutidine, CH_2Cl_2, -20°C, quant., **e)** DIBAL-H, THF, -78°C, 76%, **f)** **7**, ^{n}Bu$_2$BOTf, Et_3N, THF, -78~0°C, 85%, **g)** $AlMe_3$, MeONHMe•HCl, CH_2Cl_2, -78~0°C, 92%, **h)** $LiAlH_4$, Et_2O, 0°C, 96%, **i)** **6**, toluene, 94%, **j)** DIBAL-H, CH_2Cl_2, -78°C, quant., **k)** CBr_4, Ph_3P, 2,6-lutidine, CH_3CN, 99 %, **l)** ^{n}Bu$_3$P, CH_3CN, quant.

The final stage toward total synthesis of callystatin A (**1**) beginning from Wittig coupling between segments C_1-C_{12} (**2**) and C_{13}-C_{22} (**3**) was carried out as summarized in Chart 4. The two segments were condensed smoothly under the same conditions[2] 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 ($[\alpha]_D$, ^{1}H- and ^{13}C-NMR, IR, UV, CD, FAB-MS, HPLC, and cytotoxicity), confirming the absolute stereostructure of **1** presented by us.

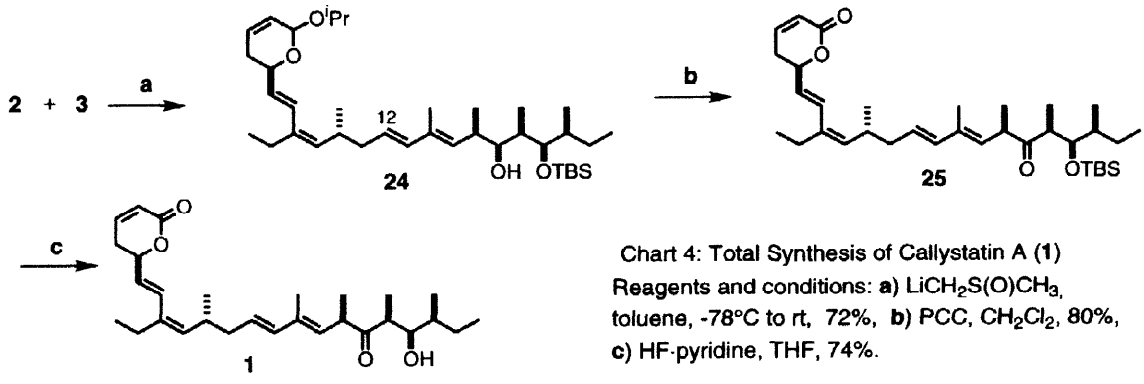

Chart 4: Total Synthesis of Callystatin A (**1**)
Reagents and conditions: **a)** $LiCH_2S(O)CH_3$, toluene, -78°C to rt, 72%, **b)** PCC, CH_2Cl_2, 80%, **c)** HF·pyridine, THF, 74%.

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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