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First Total Synthesis of the Antitumor Compound (−)-Kazusamycin A and Absolute Structure Determination

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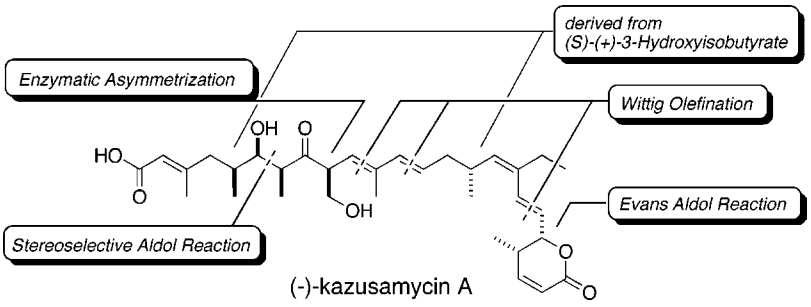
ABSTRACT



The first total synthesis of kazusamycin A, a potent antitumor compound from an actinomycete, has been achieved, and its absolute structure was determined. Paterson's stereoselective aldol reaction was successfully applied to construct the contiguous chiral centers by using an originally designed optically active 2-acyl-1,3-propanediol derivative.

Kazusamycin A, isolated from the culture broth of an actinomycete strain, 81-484, has been found to exhibit potent antitumor activity on P388 leukemia and sarcoma 180, as well as anitimicrobial activity.[1] It was also effective in completely preventing growth of HeLa cells at a concentration of 3.3 ng/mL.[2] In addition, it was found that kazusamycin A shows potent inhibitory activity against Rev protein translocation from the nucleus to the cytoplasm, which could be a useful target for HIV therapy.[3] These important biological activities, as well as an interesting structural feature, attracted our attention toward its total synthesis. Although a unique planar structure, including two types of dienes (*E*,*E* and *E*,*Z*), α,*β*-unsaturated *δ*-lactone, and a hydroxymethyl side chain, has been proposed,[3−5] neither its absolute nor relative configuration was determined. Several cytotoxic natural products with similar structures, e.g., callystatin A,[6] leptomycin B,[4,7] and delactonmycin,[3] have also been isolated, and their absolute configurations were recently elucidated by their total syntheses.[8−10] At the beginning of our synthetic studies together with elucidation of absolute configuration

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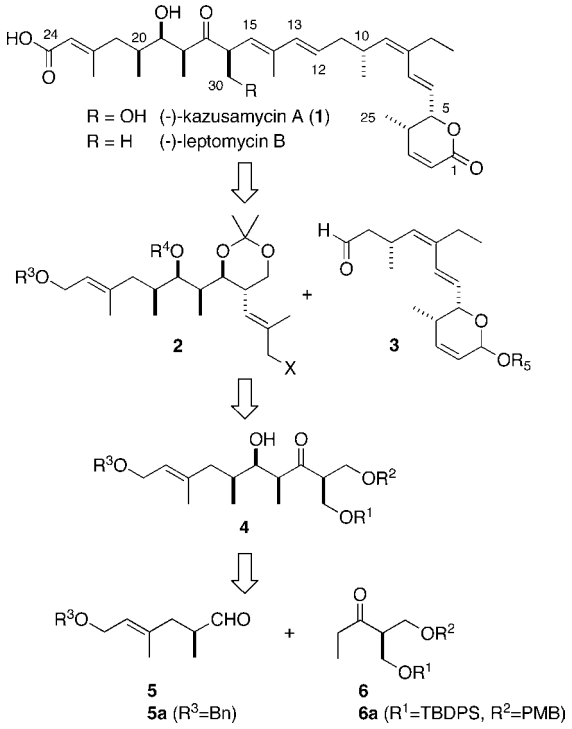


Figure 1. Retrosynthetic analysis of (−)-kazusamycin A.

of kazusamycin A, we proposed structure **1**, shown in Figure 1, by analogy to leptomycin B. Eventually, we confirmed its relative and absolute configuration through the total synthesis. Herein, we disclose our approach toward the first total synthesis and structural elucidation of kazusamycin A.

Our synthetic plan for kazusamycin A is shown in Figure 1. The molecule was disconnected at the C(12)−C(13) double bond into fragments **2** and **3** of almost the same size. Fragment **3** was expected to be accessible using slight modifications of known methods.[8a,8e,9] The most critical aspect for the synthesis is in construction of hydoxyketone **4**, which was the intermediate for **2**, in a stereocontrolled manner.

For this purpose, we envisioned that Paterson's stereoselective aldol reaction mediated by divalent tin[11] would work well by using an optically pure ethyl ketone **7**. The environment around two hydroxy moieties in the ketone **7** was differentiated by an alkyl group (R) and a silyl group

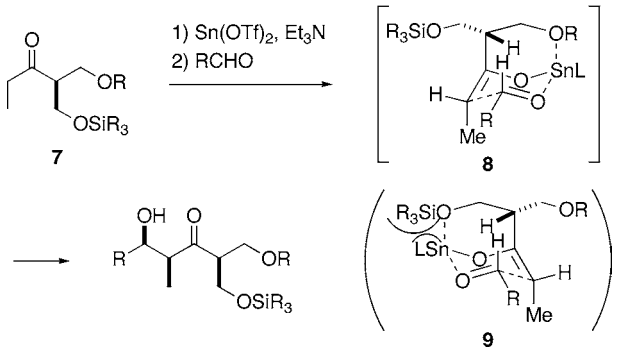
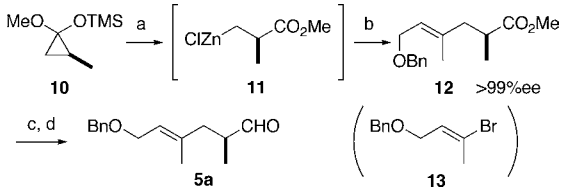


Figure 2. Expected transition state in the aldol reaction.

(SiR_3). As shown in Figure 2, when the aldol reaction is carried out with an aldehyde, transition state **8** is expected to be more favorable than **9** due to the difference in steric and electronic character between OR and $OSiR_3$,[12] leading to the aldol adduct with an absolute configuration suitable for stereocontrolled preparation of fragment **2**.

To test the stereoselectivity of the aldol reaction first, the aldehyde **5a** (R^3 = Bn) was prepared by a coupling reaction[13] between vinyl bromide **13**[14] and optically pure zinc homoenolate **11**,[12] which was prepared in situ from cyclopropane **10** (Scheme 1).[15] No loss of optical purity was observed during the reaction.

Scheme 1. Preparation of Aldehyde **5a**[a]



[a] Reagents and conditions: (a) $ZnCl_2$, Et_2O. (b) 2 mol % $PdCl_2[P(o\text{-}Tol)_3]_2$, **13**, THF, 68%. (c) DIBAL, CH_2Cl_2, −78 → 45 °C, 96%. (d) Dess−Martin periodinane, CH_2Cl_2, quantitative.

On the other hand, optically pure ketone **6a** was prepared by an enzymatic method according to the procedure recently developed by us. [16]

Thus, the stage was set for the aldol reaction. Treatment of **6a** with $Sn(OTf)_2$ and Et_3N at −78 °C followed by the addition of the aldehyde **5a** gave the aldol adduct **14** with

(8) Callystatin A: (a) Murakami, N.; Wang, W.; Aoki, M.; Tsutsui, Y.; Sugimoto, M.; Kobayashi, M. *Tetrahedron Lett.* **1998**, *39*, 2349−2352. (b) Crimmins, M. T.; King, B. W. *J. Am. Chem. Soc.* **1998**, *120*, 9084−9085. (c) Smith, A. B., III; Brandt, B. M. *Org. Lett.* **2001**, *3*, 1685−1688. (d) Kalesse, M.; Quitschalle, M.; Khandavalli, C. P.; Saeed, A. *Org. Lett.* **2001**, *3*, 3107−3109. (e) Vicario, J. L.; Job, A.; Wolberg, M.; Müller, M.; Enders, D. *Org. Lett.* **2002**, *4*, 1023−1026. (f) Marshall, J. A.; Bourbeau, M. P. *J. Org. Chem.* **2002**, *67*, 2751−2754.

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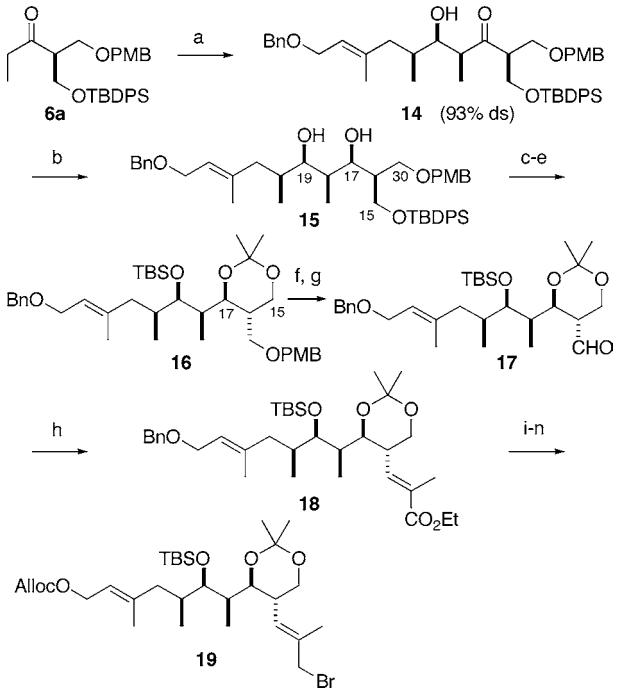
(15) Very recently, a similar approach was reported by Pilli et al. See ref 10.

high diastereoselectivity (93%). Although small amounts of other stereoisomers were also formed, silica gel column chromatography allowed their complete removal, affording stereochemically pure **14** in 77% yield. The configuration of a newly generated stereocenter in **14** was determined by a conventional method comprised of reduction to diol, transformation to acetonides (inner and outer direction, respectively), and NOE experiment.

Next, transformation to the left-half segment **19** was then carried out as depicted in Scheme 2. Reduction of **14** with

Scheme 2. Preparation of the Left-Half Fragment **19**[a]



[a] Reagents and conditions: (a) $Sn(OTf)_2$, Et_2N, CH_2Cl_2, −78 °C, then **5a**, 77%. (b) Et_2BOMe, $NaBH_4$, THF−MeOH, −78 °C, 78%. (c) TBAF, THF, 92%. (d) $Me_2C(OMe)_2$, PPTS, acetone, 86%. (e) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 94%. (f) DDQ, CH_2Cl_2, 0 °C, 68%. (g) Dess−Martin periodinane, CH_2Cl_2. (h) $Ph_3P{=}CMeCO_2Et$; 69% (two steps). (i) DIBAL, CH_2Cl_2, −78 °C, 89%. (j) TIPSCl, imidazole, DMF, 96%. (k) Na, liq NH_3−THF, −78 °C, quantitative. (l) AllocCl, pyridine, THF, 96%. (m) TBAF, THF, 98%. (n) Ph_3P, CBr_4, CH_2Cl_2, 94%.

$NaBH_4$−Et_2BOMe gave the *syn*-diol **15** in almost complete stereoselectivity.[17] Deprotection of the TBDPS group followed by selective protection of the triol moiety as acetonide (C(15)−C(17)) and silylation of the remaining hydroxy group afforded **16**, which was converted to aldehyde **17** via removal of PMB protecting group[18] and oxidation.[19] (*E*)-

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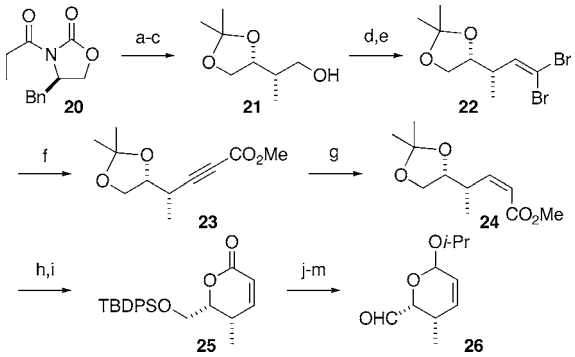
Scheme 3. Preparation of Aldehyde **26**[a]

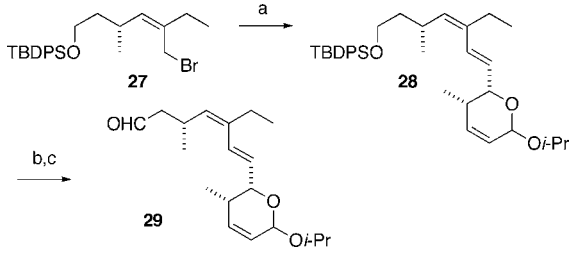


[a] Reagents and conditions: (a) Bu_2BOTf, Et_3N, $BnOCH_2CHO$, CH_2Cl_2, −78 → 0 °C, 96% (>99% ds). (b) H_2, Pd/C, PPTS, $Me_2C(OMe)_2$, acetone, 90%. (c) $LiBH_4$, MeOH, 0 °C, 95%. (d) Dess−Martin periodinane, CH_2Cl_2. (e) Ph_3P, CBr_4, Zn, CH_2Cl_2, 60% (two steps). (f) BuLi, $ClCO_2Me$, THF, −78 °C → rt, 93%. (g) H_2, Lindlar catalyst, MeOH, 96%. (h) Dowex 50WX8, MeOH, then Amberlyst 15, CH_2Cl_2. (i) TBDPSCl, imidazole, DMF, 57% (two steps). (j) DIBAL, CH_2Cl_2, −78 °C, 82%. (k) PPTS, *i*-PrOH, benzene, 85%. (l) TBAF, THF, 85%. (m) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78 °C → rt, 98%.

SelectiveWittig olefination of **17** by using (carbethoxyethylidene)triphenylphosphorane proceeded well, giving unsaturated ester **18** in good selectivity.[20] DIBAL reduction of **18**, followed by protecting group manipulation and bromination, gave allylic bromide **19** corresponding to the fragment **2**. Replacement of the Bn protecting group with Alloc was carried out at this stage, since deprotection of Bn after the construction of the sensitive diene moiety seemed to be troublesome.

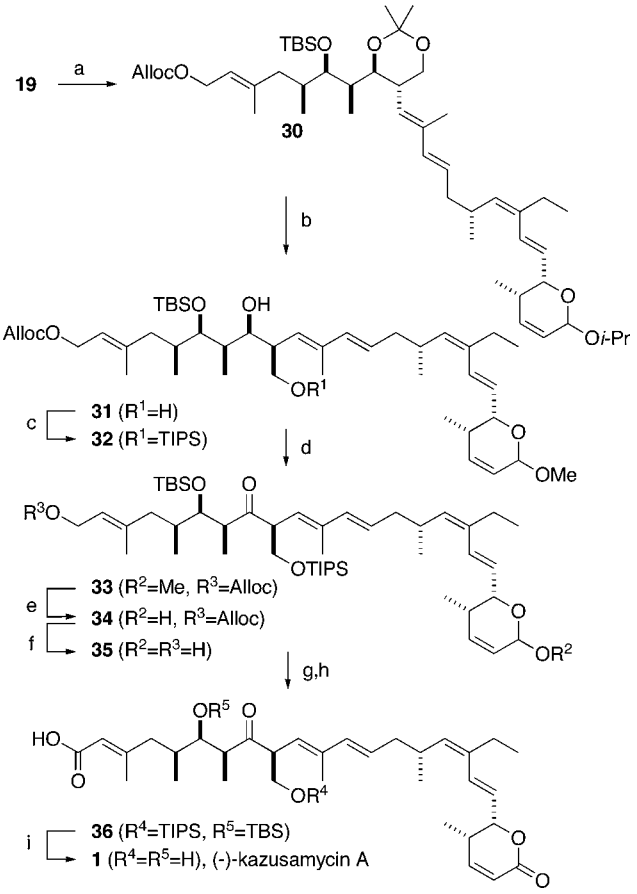
The right-half fragment corresponding to **3** was prepared, starting from commercially available oxazolidin-2-one derivative **20**. An aldol adduct obtained by Evans aldol reaction[21] between **20** and benzyloxyacetaldehyde was subjected to hydrogenolysis conditions in PPTS−$(MeO)_2CMe_2$−acetone, to afford an acetonide directly, which was transformed to alcohol **21**[22] via reductive removal of the chiral auxiliary. Oxidation of **21** followed by the treatment with PPh_3−CBr_4−Zn reagent[23] produced the dibromoalkene **22**, which was converted to **23** by a successive treatment with

Scheme 4. Preparation of Right-Half Fragment **29**[a]



[a] Reagents and conditions: (a) (i) Bu_3P, CH_3CN; (ii) **26**, *t*-BuOK, toluene−THF, 0 °C, 91%. (b) TBAF, THF, 99%. (c) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78 °C → rt, 92%.

Scheme 5. Completion of the Total Synthesis of Kazusamycin A[a]



[a] Reagents and conditions: (a) (i) Bu_3P, CH_3CN; (ii) **29**, *t*-BuOK, 83%. (b) PPTS, MeOH, 84% (three cycles). (c) TIPSCl, imidazole, DMF, 95%. (d) Dess−Martin periodinane, CH_2Cl_2, 95%. (e) PPTS, aq acetone, 91% (three cycles). (f) $Pd(PPh_3)_4$, dimedone, THF, 96%. (g) MnO_2, CH_2Cl_2, 49%. (h) $NaClO_2$, 2-methyl-2-butene, aq *t*-BuOH, 80%. (i) HF−Py, Py, THF, 74%.

BuLi and then with methyl chloroformate. Lindlar reduction afforded the unsaturated ester **24**, which underwent cyclization to unsaturated lactone after removal of the acetonide protecting group by treatment with acidic resins. Protection of lactone moiety as a lactol followed by oxidation of alcohol gave the aldehyde **26**[9] (Scheme 3).

Coupling of **26** with bromide **27**[8b,8e] via Wittig reaction gave 1,3-diene **28** in good yield and selectivity (*E*:*Z* = ca. 7:1). **28** was then converted to the aldehyde **29** corresponding to fragment **3** (Scheme 4).

The final stage of the total synthesis is shown in Scheme 5. Wittig reaction of tributylphosphonium salt derived from **19** with **29**[8b,8e] proceeded well in the presence of *t*-BuOK to afford the product **30** having the basic skeleton of kazusamycin A. Finally, appropriate functional group manipulation,[8] including chemodifferentiation of *prim*-, *sec*-, and allylic alcohol completed the total synthesis of kazusamycin A, whose spectroscopic data (^{1}H NMR, ^{13}CNMR, IR, HRMS) were in complete agreement with that of the natural kazusamycin A. The synthetic sample showed negative optical rotation ($[\alpha]^{27}_D$ −87.7°, *c* 0.358, MeOH) in accordance with the natural compound ($[\alpha]^{20}_D$ −83.5°, *c* 0.1, MeOH).[1] This fact indicates that the natural kazusamycin A has the absolute configuration shown in Figure 1.

Thus, we have achieved the first total synthesis and structural elucidation of kazusamycin A. Among several characteristic features of the synthesis, the remarkable aspect for the present total synthesis exists in an exploration of stereocontrolled aldol reaction by using an originally designed optically active synthetic unit **6a**, which led to successful total synthesis of this highly biologically active natural product. On the basis of this route, synthesis of artificial congeners of kazusamycin A and biological assay of them are now underway.

Supporting Information Available: Experimental procedures and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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