JOC*Article*

Lasonolide A: Structural Revision and Total Synthesis

Ho Young Song,† Jung Min Joo,† Jung Won Kang,† Dae-Shik Kim,† Cheol-Kyu Jung,† Hyo Shin Kwak,† Jin Hyun Park,† Eun Lee,*,† Chang Yong Hong,‡ ShinWu Jeong,‡ Kiwan Jeon,‡ and Ji Hyun Park‡

School of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-747, Korea, and LG Life Science/R&D, P.O. Box 61, Yusung, Daejon 305-380, Korea

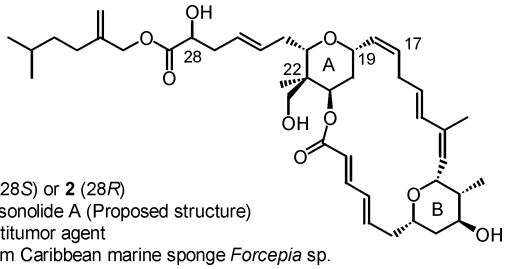
eunlee@snu.ac.kr

Received June 30, 2003

The proposed structure of lasonolide A was synthesized employing radical cyclization reactions of β-alkoxyacrylates for preparation of the tetrahydropyranyl units A and B, but the spectroscopic data did not match those of the natural product. Both enantiomers of a revised structure featuring 17*E*,25*Z* double bonds were synthesized, and the (−)-isomer was found to be the biologically active enantiomer.

Introduction

Lasonolide A is a novel cytotoxic macrolide isolated in 1994 by McConnell and co-workers at Harbor Branch Oceanographic Institution from the shallow water Caribbean marine sponge *Forcepia* sp. It is a potent cytotoxin with IC_{50} values of 40 and 2 ng/mL against the A-549 human lung carcinoma and P388 murine leukemia cell lines, respectively. It inhibits cell adhesion in the EL-4.IL-2 cell line with an IC_{50} of 19 ng/mL; however, toxicity against this cell line is greater than 25 μg/mL. Extensive spectroscopic investigations by the researchers at HBOI on the natural product led to the structure **1** or **2**[1] (Figure 1). The most characteristic features in **1**/**2** are the two cis-2,6-substituted tetrahydropyran rings integrated in the macrolide structure. In particular, ring A contains a quaternary stereogenic center at C-22 with a methyl and a hydroxymethyl substituent, and synthesis of **1**/**2** calls for stereoselective assembly of a cis-2,6-substituted tetrahydropyran unit with the attendant quaternary center. The unique structure and the high biological activities of lasonolide A attracted attention of synthetic chemists, and a number of literature examples had dealt with partial synthesis with varying degree of success.[2] We reported structural revision and the first total synthesis of lasonolide A in 2002[3] and wish to describe here a full account on the structure and synthesis of this intriguing natural product.



1 (28*S*) or **2** (28*R*)
Lasonolide A (Proposed structure)
Antitumor agent
from Caribbean marine sponge *Forcepia* sp.

FIGURE 1. Lasonolide A: the proposed structures **1**/**2**.

Results and Discussion

Retrosynthetic Analysis and Synthesis of the Proposed Structures 1 and 2. Radical cyclization reactions of β-alkoxyacrylates[4] were to be employed in the synthesis of both tetrahydropyran rings found in lasonolide A structure. As the correct stereochemistry at

* To whom correspondence should be addressed. Tel: +82-2-880-6646. Fax: +82-2-889-1568.

† Seoul National University.

‡ LG Life Science/R&D.

(1) Horton, P. A.; Koehn, F. E.; Longley, R. E.; McConnell, O. J. *J. Am. Chem. Soc.* **1994**, *116*, 6015−6016.

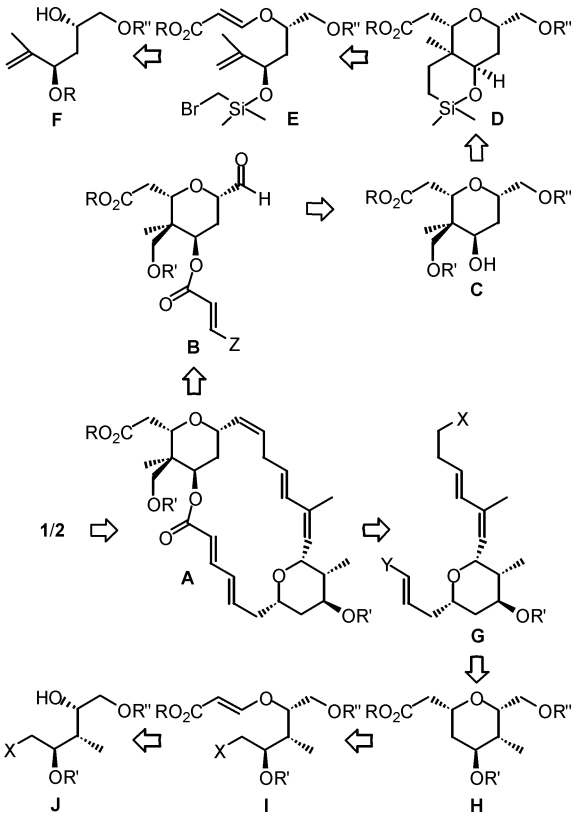
(2) (a) Beck, H.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **1999**, 2991−2995. (b) Nowakowski, M.; Hoffmann, H. M. R. *Tetrahedron Lett.* **1997**, *38*, 1001−1004. (c) Gurjar, M. K.; Chakrabarti, A.; Venkateswara Rao, B.; Kumar, P. *Tetrahedron Lett.* **1997**, *38*, 6885−6888. (d) Gurjar, M. K.; Kumar, P.; Venkateswara Rao, B. *Tetrahedron Lett.* **1996**, *37*, 8617−8620.

(3) (a) Lee, E.; Song, H. Y.; Kang, J. W.; Kim, D.-S.; Jung, C.-K.; Joo, J. M. *J. Am. Chem. Soc.* **2002**, *124*, 384−385. (b) Lee, E.; Song, H. Y.; Joo, J. M.; Kang, J. W.; Kim, D. S.; Jung, C. K.; Hong, C. Y.; Jeong, S.; Jeon, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3519−3520.

(4) (a) Lee, E.; Tae, J. S.; Lee, C.; Park, C. M. *Tetrahedron Lett.* **1993**, *34*, 4831−4834. (b) Lee, E.; Tae, J. S.; Chong, Y. H.; Park, Y. C.; Yun, M.; Kim, S. *Tetrahedron Lett.* **1994**, *35*, 129−132. (c) Lee, E.; Park, C. M. *J. Chem. Soc., Chem. Commun.* **1994**, 293−294. (d) Lee, E.; Jeong, J.-w.; Yu, Y. *Tetrahedron Lett.* **1997**, *38*, 7765−7768. (e) Lee, E.; Park, C. M.; Yun, J. S. *J. Am. Chem. Soc.* **1995**, *117*, 8017−8018. (f) Lee, E.; Yoo, S.-K.; Cho, Y.-S.; Cheon, H.-S.; Chong, Y. H. *Tetrahedron Lett.* **1997**, *38*, 7757−7758. (g) Lee, E.; Yoo, S.-K.; Choo, H.; Song, H. Y. *Tetrahedron Lett.* **1998**, *39*, 317−318. (h) Lee, E.; Choi, S. J. *Org. Lett.* **1999**, *1*, 1127−1128. (i) Lee, E.; Song, H. Y.; Kim, H. J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3395−3396. (j) Lee, E.; Kim, H. J.; Kang, E. J.; Lee, I. S.; Chung, Y. K. *Chirality*, **2000**, *12*, 360−361. (k) Lee, E.; Jeong, E. J.; Kang, E. J.; Sung, L. T.; Hong, S. K. *J. Am. Chem. Soc.* **2001**, *123*, 10131−10132. (l) Lee, E.; Choi, S. J.; Kim, H.; Han, H. O.; Kim, Y. K.; Min, S. J.; Son, S. H.; Lim, S. M.; Jang, W. S. *Angew. Chem., Int. Ed.* **2002**, *41*, 176−178. (m) Lee, E.; Sung, L. T.; Hong, S. K. *Bull. Kor. Chem. Soc.* **2002**, *23*, 1189−1190. (n) Lee, E.; Han, H. O. *Tetrahedron Lett.* **2002**, *43*, 7295−7296. (o) Jeong, E. J.; Kang, E. J.; Sung, L. T.; Hong, S. K.; Lee, E. *J. Am. Chem. Soc.* **2002**, *124*, 14655−14662. (p) For further references, see: Lee, E. In *Radicals in Organic Synthesis, Vol. 2: Applications*; Renaud, P., Sibi, M. P., Eds.; Wiley-VCH: Weinheim, 2001; pp 303−333.

10.1021/jo034930n CCC: $25.00
Published on Web 09/17/2003

SCHEME 1. Retrosynthetic Analysis of 1/2

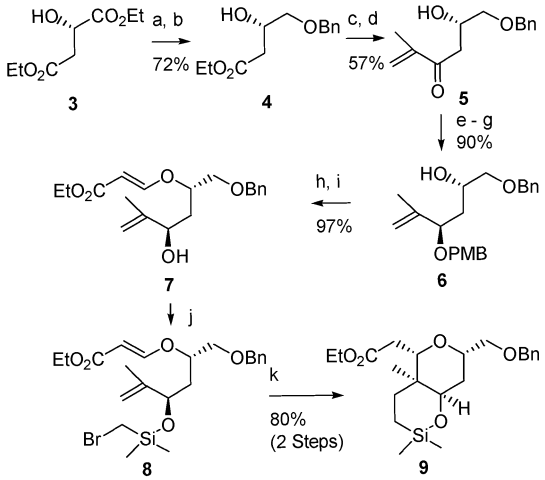


C-28 was unknown and it was deemed necessary to prepare both of the two possible stereoisomers, the macrolide **A** was considered as a pivotal intermediate in the synthesis of the proposed structures **1**/**2**. It was to be prepared from the two tetrahydropyranyl fragments **B** and **G** using Wittig and Stille-type reactions (Scheme 1).

In the synthesis of the upper half fragment **B**, 6-endo-6-exo tandem radical cyclization reactions of the (bromomethyl)silyloxy-substituted β-alkoxyacrylate **E** may provide the bicyclic intermediate **D**;[5] the construction of the quaternary center and the tetrahydropyran annulation may be achieved in a single step. The intermediate **E** may be obtained from the protected triol **F**. For preparation of the lower half fragment **G**, radical cyclization of the β-alkoxyacrylate **I** from a second triol derivative **J** should provide the tetrahydropyranyl intermediate **H**.

Preparation of the fragment **B** started with ethyl L-malate (**3**), which was converted into the enone **5** via the Weinreb amide derivative of the ester **4**. Stereoselective reduction[6] of **5** provided the syn diol, and regioseletive reduction[7] of the cyclic PMB acetal yielded the triol derivative **6**. The β-alkoxyacrylate **7** was obtained from **6** via reaction with ethyl propiolate and PMB-deprotection. Preparation and radical cyclization reaction of the bromomethyl(dimethyl)silyl derivative **8**

SCHEME 2. Preparation of 9[a]



[a] Key: (a) $BH_3{\cdot}SMe_2$, $NaBH_4$, THF; (b) Bu_2SnO, benzene, reflux; BnBr, TBAI, reflux; (c) MeNH(OMe)·HCl, Me_3Al, THF; (d) $H_2CC(Me)MgBr$, THF; (e) Et_3B, $NaBH_4$, THF−MeOH (4:1), −78 °C; (f) (*p*-MeO)PhCH(OMe)$_2$, CSA, DCM; (g) DIBAL, DCM; (h) $HCCCO_2Et$, NMM, MeCN; (i) DDQ, DCM−H_2O (20:1); (j) $BrCH_2SiMe_2Cl$, TEA, DMAP, benzene; (k) Bu_3SnH, AIBN, benzene, reflux.

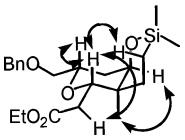
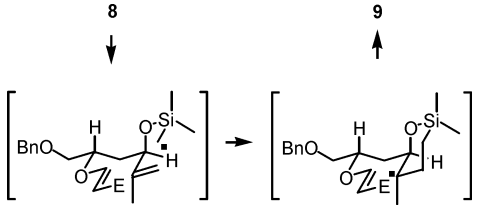


FIGURE 2. NOESY analysis of **9**.

SCHEME 3. Stereoselective 6-Endo, 6-Exo Tandem Radical Cyclizations



proceeded smoothly, and the bicyclic product **9** was obtained as a single product (Scheme 2).

The stereochemical assignment of **9** was confirmed by NOESY analysis (Figure 2). Clearly, the anticipated 6-endo radical cyclization proceeded efficiently, and complete stereocontrol was achieved in the subsequent 6-exo cyclization (Scheme 3).

Reduction of the ester group and reaction with pivaloyl chloride provided the pivaloate derivative of **9**, which was converted into the diol **10** via Tamao oxidation.[8] Conversion to the more robust pivaloate derivative was necessary prior to Tamao oxidation. Selective deprotection of the bis(TBS) derivative of **10** yielded the primary alcohol **11**. Conversion of **11** into the lower homologue **12** required selenide substitution/selenoxide elimination, osmium tetroxide dihydroxylation/sodium periodate cleavage, and sodium borohydride reduction. The aldehyde intermediate **13** was synthesized via TBS-deprotection of **12** and subsequent acetonide protection, benzyl ether

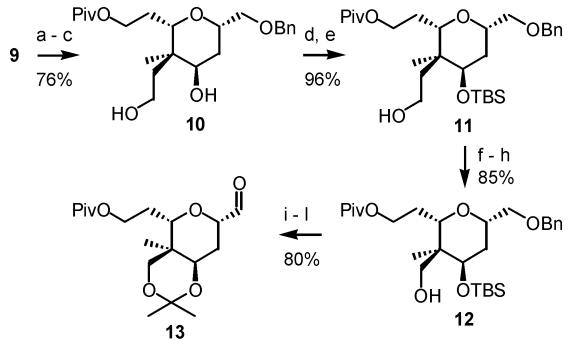
(5) For selected examples of similar 6-endo cyclization reactions, see: (a) Wilt, J. W. *J. Am. Chem. Soc.* **1981**, *103*, 5251−5253. (b) Koreeda, M.; George, I. A. *J. Am. Chem. Soc.* **1986**, *108*, 8098−8100.

(6) Chen, K.-M.; Gunderson, K. G.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Chem. Lett.* **1987**, 1923−1926.

(7) Takano, S.; Akiyama, M.; Sato, S.; Ogasawara, K. *Chem. Lett.* **1983**, 1593−1596.

(8) Tamao, K.; Kakui, T.; Akita, M.; Iwahara, T.; Kanatani, R.; Yoshida, J.; Kumada, M. *Tetrahedron* **1983**, *39*, 983−990.

SCHEME 4. Preparation of 13[a]



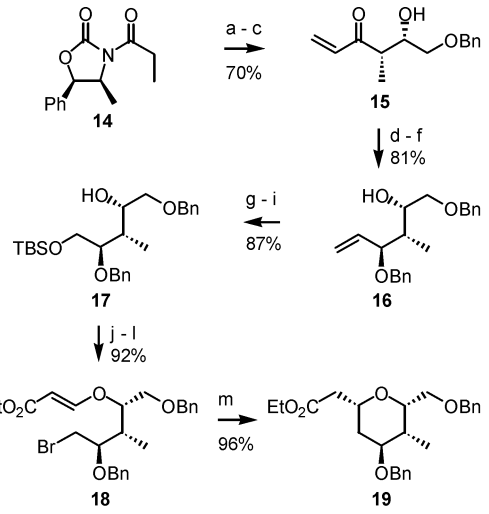
[a] Key: (a) $LiBH_4$, ether; (b) PivCl, DMAP, pyridine, DCM; (c) H_2O_2, KF, $KHCO_3$, THF−MeOH (1:1); (d) TBSOTf, 2,6-lutidine, DCM; (e) CSA, MeOH, 0 °C; (f) (*o*-NO_2)PhSeCN, Bu_3P, THF; H_2O_2; (g) OsO_4, NMO, acetone−H_2O (3:1); $NaIO_4$; (h) $NaBH_4$, EtOH, 0 °C; (i) HCl, MeOH; (j) $Me_2C(OMe)_2$, CSA, acetone; (k) H_2, $Pd(OH)_2$/C, MeOH; (l) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C.

deprotection, and oxidation with sulfur trioxide−pyridine complex[9] (Scheme 4).

The Evans chiral imide **14** served as the starting material for the synthesis of the lower half fragment **G**. The aldol product from the reaction of the (*Z*)-boron enolate of **14** and benzyloxyacetaldehyde was converted into the hydroxy enone **15** via vinyl Grignard reaction of the corresponding Weinreb amide.[10] After stereoselective reduction of **15**, the product syn diol was converted into the dibenzyl ether **16** via regioselective reduction of the corresponding benzyl acetal. Osmium tetroxide dihydroxylation/sodium periodate cleavage followed by sodium borohydride reduction provided a primary alcohol, from which the TBS ether **17** was obtained via selective silylation. Reaction of **17** with ethyl propiolate provided the corresponding β-alkoxyacrylate, which was converted into the bromide **18** via TBS-deprotection and bromide substitution. Radical cyclization of **18** proceeded uneventfully to give the tetrahydropyranyl intermediate **19** in high yield (Scheme 5).

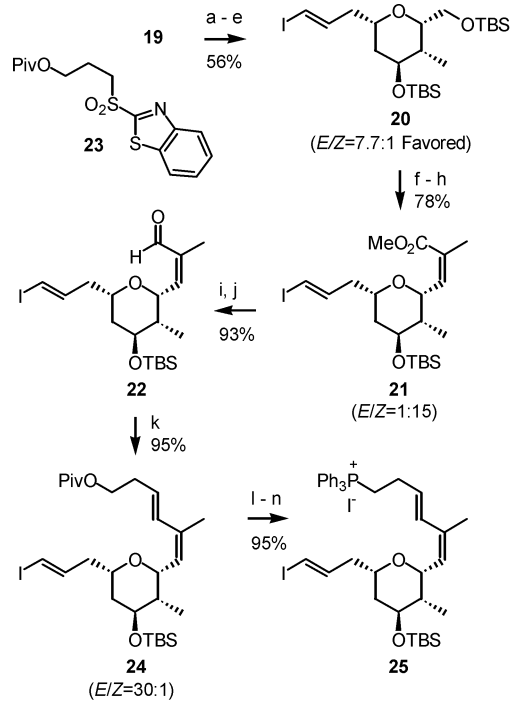
Benzyl deprotection via hydrogenolysis and silylation provided the bis(TBS) ether analogue of **19**, which was converted into the corresponding aldehyde via lithium borohydride reduction and oxidation with sulfur trioxide−pyridine complex. The (*E*)-iodovinyl derivative **20** was obtained from the aldehyde following the Takai protocol.[11] Generation of the primary hydroxy group via selective TBS-deprotection and oxidation with sulfur trioxide−pyridine led to the production of the corresponding aldehyde in good yield, from which the (*Z*)-enoate **21** was prepared following the Still procedure.[12] The Julia−Julia reaction[13] of the corresponding aldehyde **22** and the anion of the sulfone **23** provided the triene **24** in a stereoselective manner (*E*/*Z* = 30:1). The phosphonium salt **25** was then obtained via the corresponding iodide (Scheme 6).

SCHEME 5. Preparation of 19[a]



[a] Key: (a) *n*-Bu_2BOTf, TEA, DCM, 0 °C; $BnOCH_2CHO$, −78 to 0 °C; (b) MeNH(OMe)·HCl, Me_3Al, THF; (c) $H_2CCHMgBr$, THF; (d) Et_3B, $NaBH_4$, THF−MeOH (2.5:1), −78 °C; (e) $PhCH(OMe)_2$, CSA, DCM; (f) DIBAL, DCM; (g) OsO_4, NMO, acetone−H_2O (3:1); $NaIO_4$; (h) $NaBH_4$, EtOH, 0 °C; (i) TBSCl, imidazole, DCM, 0 °C; (j) $HCCCO_2Et$, NMM, MeCN; (k) HCl, MeOH, 0 °C; (l) CBr_4, Ph_3P, pyridine, DCM; (m) Bu_3SnH, AIBN, benzene, reflux.

SCHEME 6. Preparation of 25[a]



[a] Key: (a) H_2, Pd/C, MeOH; (b) TBSOTf, 2,6-lutidine, DCM; (c) $LiBH_4$, Ether; (d) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C; (e) $CrCl_2$, CHI_3, dioxane−THF (6:1); (f) CSA, MeOH; (g) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C; (h) $MeO_2C(Me)CHPO(OCH_2CF_3)_2$, KHMDS, 18-c-6, THF, −78 °C; (i) DIBAL, DCM, −78 °C; (j) MnO_2, DCM; (k) **23**, LDA, THF, −78 °C; **22**, −78 °C to rt; (l) DIBAL, DCM, −78 °C; (m) Ph_3P, I_2, imidazole, THF, 0 °C; (n) Ph_3P, MeCN, reflux.

Stereochemical assignment of **25** was confirmed via NOESY analysis of the primary alcohol derived from the pivaloate **24** (Figure 3).

(9) Parikh, J. R.; Doering, W. von E. *J. Am. Chem. Soc.* **1967**, *89*, 5505−5507.
(10) For similar reaction sequences, see: Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 7001−7031.
(11) Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408−7410.
(12) Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405−4408.
(13) Baudin, J. B.; Hareau, G.; Julia, S. A.; Ruel, O. *Tetrahedron Lett.* **1991**, *32*, 1175−1178.

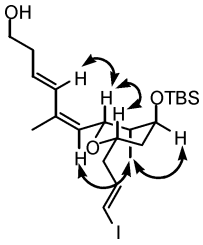
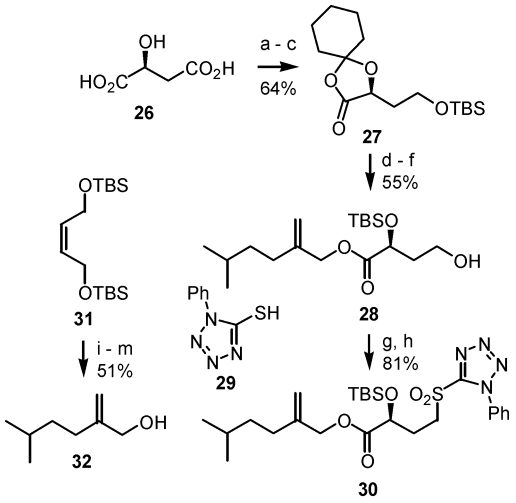


FIGURE 3. NOESY analysis of the alcohol from **24**.

SCHEME 7. Preparation of 30[a]

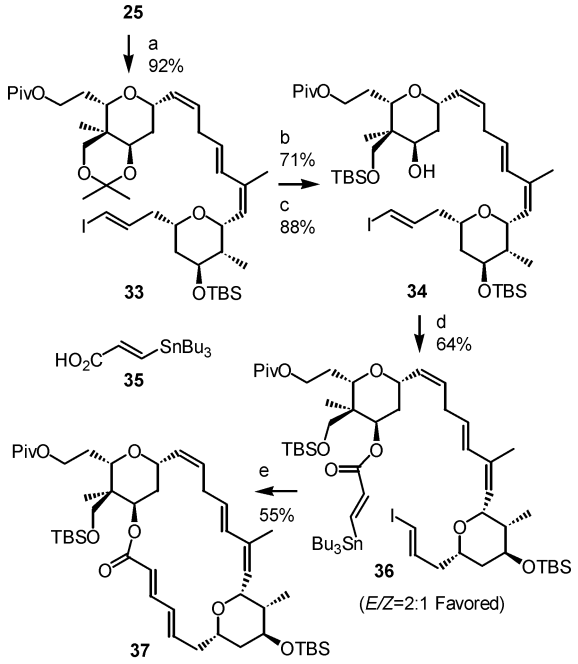


[a] Key: (a) cyclohexanone, $BF_3{\cdot}OEt_2$, ether, 0 °C to rt; (b) $BH_3{\cdot}$DMS, $B(OMe)_3$, THF, 0 °C to rt; (c) TBSCl, imidazole, DCM; (d) **32**, NaHMDS, THF, 0 °C; (e) TBSCl, imidazole, DMAP, DCM; (f) HF·pyridine, pyridine, THF, 0 °C; (g) **29**, DIAD, Ph_3P, THF, 0 °C; (h) $(NH_4)_6Mo_7O_{24}$, H_2O_2, EtOH, 0 °C; (i) O_3, DCM, −78 °C; DMS, −78 °C to rt; (j) $(CH_3)_2CHCH_2CH_2MgBr$, THF; (k) $(COCl)_2$, DMSO, DIPA, DCM, −78 °C to rt; (l) Zn dust, CH_2Br_2, $TiCl_4$, THF, 0 °C to rt; (m) HCl, MeOH.

Synthesis of the side-chain fragment started with L-malic acid (**26**), which was converted into the ketal **27** after selective ketal formation, borane reduction, and TBS-protection.[14] After reaction of **27** with the alcohol **32**, the primary alcohol **28** was obtained via bis(TBS)-protection and selective TBS-deprotection. Mitsunobu-type substitution reaction of **28** with 1-phenyl-1*H*-tetrazole-5-thiol (**29**) and selective oxidation provided the sulfone **30**.[15] The alcohol **32**[16] was prepared from the olefin **31** in a five-step sequence involving ozonolysis, isopentyl Grignard addition, Swern oxidation, methylenation,[17] and desilylation (Scheme 7).

The coupling of fragments was initiated by the Wittig reaction between the ylide derived from the phosphonium salt **25** and the aldehyde **13**, which generated the required cis double bond producing the intermediate **33**. Careful acetonide deprotection of **33** and selective silylation led to the formation of the corresponding secondary

SCHEME 8. Preparation of 37[a]



[a] Key: (a) KHMDS, THF, −78 °C; **13**, −78 °C to rt; (b) CSA, MeOH, $(HOCH_2)_2$ (s.m. 20%); (c) TBSCl, imidazole, DCM; (d) **35**, DCC, DMAP, DCM, 0 °C to rt; (e) Pd_2dba_3, DIPEA, NMP.

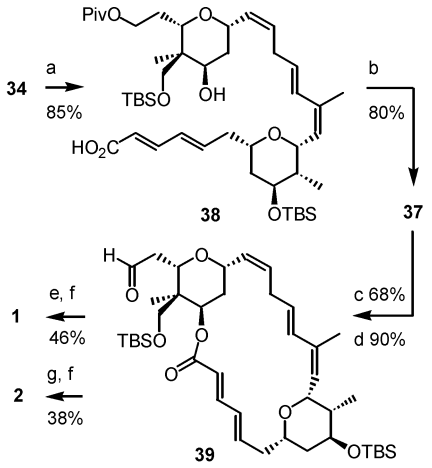
alcohol **34**. Esterification reaction of **34** with the carboxylic acid **35** proceeded efficiently, but the stereochemical integrity was compromised as the required (*E*)-stannylacrylate **36** was accompanied by the corresponding (*Z*)-isomer (*E*/*Z* = 2:1). Intramolecular Stille coupling of **36** then produced the macrolide **37** without difficulty[18] (Scheme 8).

The esterification/intramolecular Stille reaction strategy provided the macrolide **37** from **34** in reasonable yield, but the stereorandomization problem at the esterification step was difficult to solve. Eventually, a more efficient transformation was achieved via the pentenoic acid **38**, a product of intermolecular Stille coupling between **34** and **35**, which yielded the macrolide **37** via Yamaguchi lactonization.[19] Low-temperature reduction of **37** by Super-Hydride and subsequent oxidation with sulfur trioxide−pyridine complex led to the formation of the aldehyde **39**. The Kocienski−Julia reaction[20] of the aldehyde **39** and the anion of **30** proceeded smoothly, and **1** (28*S*) was obtained after careful TBS-deprotection by hydrogen fluoride−pyridine complex. The diastereomer **2** (28*R*) was synthesized from the reaction of **39** and *ent*-**30** obtained from D-malic acid (Scheme 9). Comparison of the 500 MHz NMR spectroscopic data of **1** and **2** with those reported for lasonolide A revealed that neither **1** nor **2** represented the correct structure of the natural product.

Synthesis of the Stereoisomers 40 and 41. After much thought, it was decided to investigate the structures **40**/**41**, the stereoisomers of **1**/**2** which are enantio-

(14) Hanessian, S.; Tehim, A.; Chen, P. J. *J. Org. Chem.* **1993**, *58*, 7768−7781.
(15) Williams, D. R.; Brooks, D. A.; Berliner, M. A. *J. Am. Chem. Soc.* **1999**, *121*, 4924−4925.
(16) Fleming, I.; Patterson, I.; Pearce, A. *J. Chem. Soc., Perkin Trans. 1* **1981**, 256−262.
(17) Takai, K.; Hotta, Y.; Oshima, K. *Tetrahedron Lett.* **1978**, *27*, 2417−2420.
(18) Smith, A. B., III; Ott, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 3935−3948.
(19) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989−1993.
(20) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, 26−28.

SCHEME 9. Preparation of 1/2[a]



[a] Key: (a) **35**, Pd_2dba_3, DIPEA, NMP; (b) 2,4,6-Cl_3PhCOCl, TEA, THF; DMAP, benzene, reflux; (c) $LiEt_3BH$, THF, −78 °C (s.m. 19%); (d) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C; (e) **30**, KHMDS, DME, −55 °C; **39**, −55 °C to rt; (f) HF·pyridine, THF, 0 °C to rt; (g) *ent*-**30**, KHMDS, DME, −55 °C; **39**, −55 °C to rt.

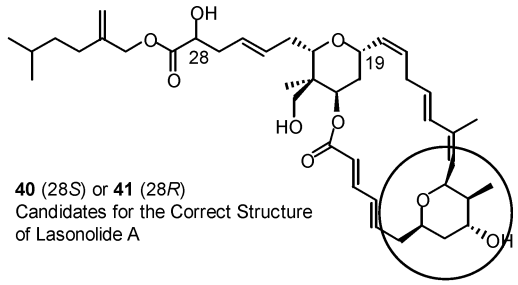


FIGURE 4. Stereoisomers **40/41**.

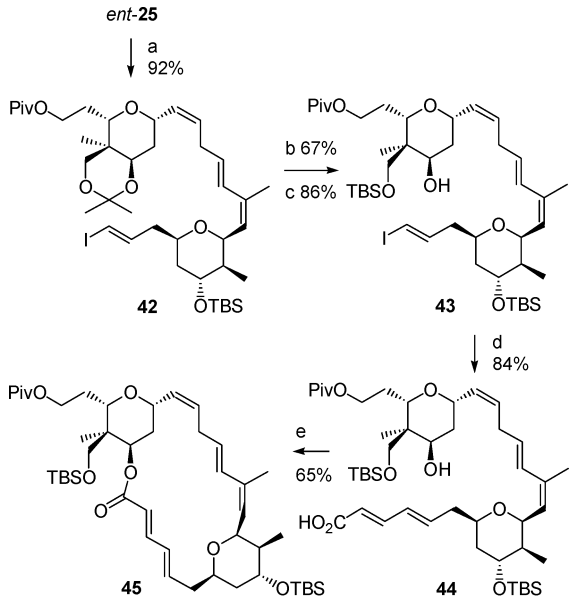
meric in ring B, as candidates for the natural product. As the two tetrahydropyran units in lasonolide A were connected through stereochemically innocuous diene and triene bridges, it would have been difficult to distinguish the structures **40**/**41** from **1**/**2** by NMR analysis (Figure 4).

For the synthesis of **40**/**41**, the ylide from the phosphonium salt *ent*-**25** (prepared from *ent*-**14**) was allowed to react with the aldehyde **13**, and the coupled product **42** was obtained in high yield. Acetonide-deprotection of **42** and TBS-protection of the primary hydroxyl group produced the secondary alcohol intermediate **43**, which was then converted into the pentenoic acid **44** via intermolecular Stille coupling with **35**. Yamaguchi lactonization reaction of **44** afforded a new macrolide **45** in an acceptable yield (Scheme 10).

The macrolide aldehyde **46** was prepared from **45** following the Super-Hydride reduction/sulfur trioxide−pyridine oxidation protocol. Synthesis of **40** and **41** was then duly accomplished via Kocienski−Julia reaction of the aldehyde **46** and the anion of either **30** or *ent*-**30** (Scheme 11). From comparison of the NMR spectra of **40**/**41** and the natural product, it was clear that neither **40** nor **41** represented the correct structure.

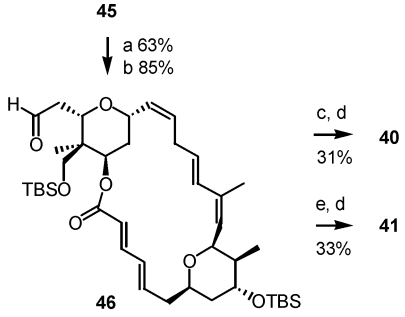
Synthesis of the (17*E*)-Isomers. One of the most striking features in the NMR spectra of **1**/**2** and **40**/**41** was the chemical shift values of H-19 centered around δ 4.7. The chemical shift value of H-19 for the natural

SCHEME 10. Preparation of 45[a]



[a] Key: (a) KHMDS, THF, −78 °C; **13**, −78 °C to rt; (b) CSA, MeOH, $(HOCH_2)_2$ (s.m. 18%); (c) TBSCl, imidazole, DCM; (d) **35**, Pd_2dba_3, DIPEA, NMP; (e) 2,4,6-Cl_3PhCOCl, TEA, THF; DMAP, benzene, reflux.

SCHEME 11. Preparation of 40/41[a]



[a] Key: (a) $LiEt_3BH$, THF, −78 °C; (b) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C; (c) **30**, KHMDS, DME, −55 °C; **46**, −55 °C to rt; (d) HF·pyridine, THF, 0 °C to rt; (e) *ent*-**30**, KHMDS, DME, −55 °C; **46**, −55 °C to rt.

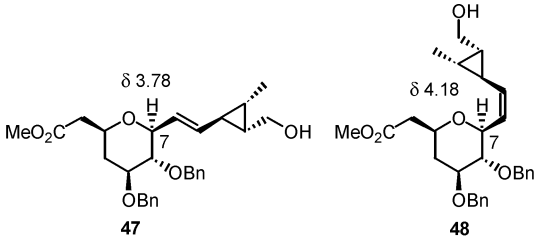


FIGURE 5. Chemical shift values of the geometric isomers **47**/**48**.

product was reported to be δ 4.30, and it was apparent that H-19 signals of the synthetic samples **1**/**2** and **40**/**41** are located approximately 0.4 ppm downfield from the correct value. This was happily reminiscent of the situation encountered in the ambruticin synthesis[41] (Figure 5). Two geometric isomers **47** and **48** were obtained in the crucial olefination reaction en route to ambruticin: the chemical shift values for H-7 in **47** and **48** were

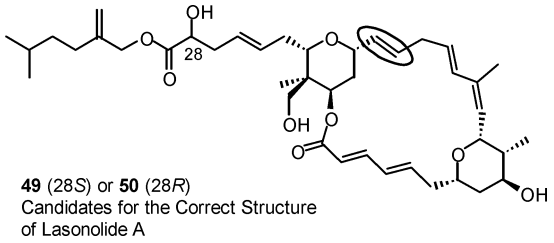
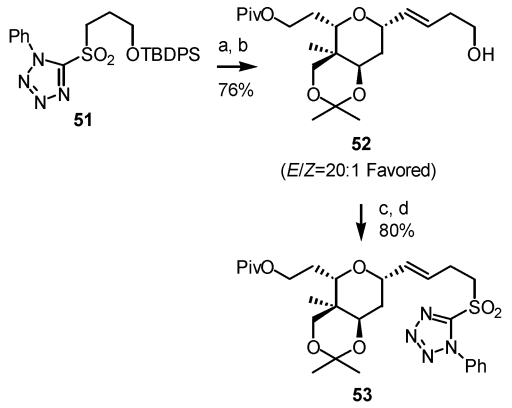


FIGURE 6. (17*E*)-Isomers **49/50**.

SCHEME 12. Preparation of 53[a]



[a] Key: (a) LiHMDS, DMF−HMPA (4:1), −35 °C; **13**, −35 °C to rt; (b) TBAF, THF; (c) **29**, DIAD, Ph_3P, THF, 0 °C; (d) $(NH_4)_6Mo_7O_{24}$, H_2O_2, EtOH, 0 °C to rt.

δ 3.78 and 4.18, respectively. In other words, the H-7 chemical shift value of the cis isomer **48** was 0.4 ppm downfield from that of the trans isomer **47**.

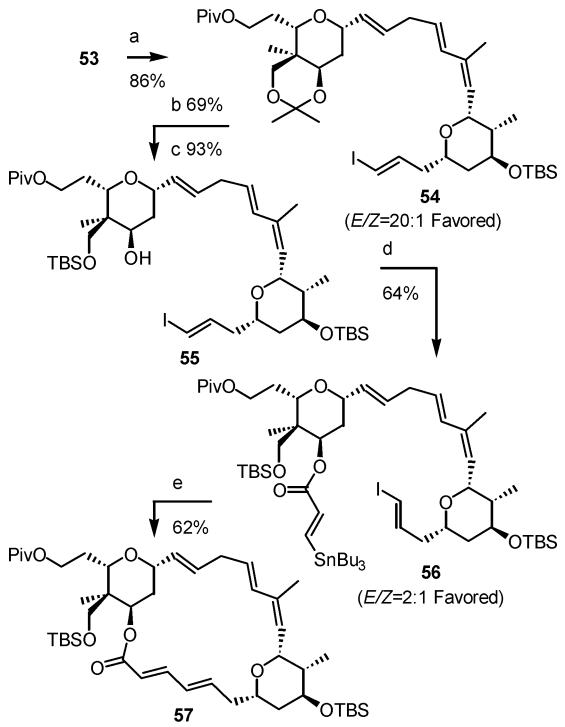
Based on the above analysis, candidates with a trans double bond at C-17,18 appeared quite promising, and it was decided to investigate structures **49**/**50**, which are (17*E*)-isomers of **1**/**2** (Figure 6).

For synthesis of **49**/**50**, Kocienski−Julia reaction[21] of the sulfone **51** and the aldehyde **13** led to the trans olefin **52**. The sulfone **53** was then obtained after TBDPS-deprotection, Mitsunobu-type substitution with **29**, and selective oxidation (Scheme 12).

A second trans olefination reaction between the sulfone **53** and the aldehyde **22** proceeded smoothly to yield the coupled product **54**. The secondary alcohol **55** was obtained via acetonide-deprotection of **54** and subsequent TBS-protection of the primary hydroxy group. Esterification of **55** with the acid **35** led to the preparation of the trans-β-stannylacrylate **56**. Removal of the undesired cis-β-stannylacrylate was difficult and the product mixture was directly subjected to intramolecular Stille coupling reaction, which provided the macrolactone **57** (Scheme 13).

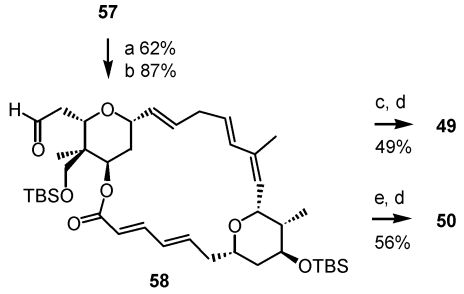
The pivaloyl macrolide **57** was converted into the aldehyde **58** upon reductive removal of the pivaloate group and oxidation with sulfur trioxide−pyridine complex. The Kocienski−Julia reaction of **58** with the sulfone **30** and careful TBS-deprotection yielded the target compound **49**. The epimeric product **50** was obtained likewise from *ent*-**30** (Scheme 14). The NMR spectra of **49**/**50** were found to be quite similar to the spectra recorded for the natural product: indeed, the signals for H-19 were found around δ 4.3 as we expected. But

SCHEME 13. Preparation of 57[a]



[a] Key: (a) LiHMDS, THF, −78 °C; **22**, −78 °C to rt; (b) CSA, MeOH, $(HOCH_2)_2$ (s.m. 18%); (c) TBSCl, imidazole, DCM; (d) **35**, DIC, DMAP, DCM; (e) Pd_2dba_3, DIPEA, NMP.

SCHEME 14. Preparation of 49/50[a]



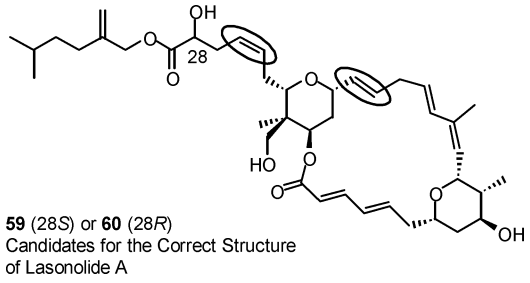
[a] Key: (a) $LiEt_3BH$, THF, −78 °C (s.m. 17%); (b) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C; (c) **30**, KHMDS, DME, −78 °C; **58**, −78 °C to rt; (d) HF·pyridine, pyridine, THF; (e) *ent*-**30**, KHMDS, DME, −78 °C; **58**, −78 °C to rt.

discrepancies persisted in the NMR spectra, particularly in the region for vinylic protons.

Synthesis of the (17*E*,25*Z*)-Isomers: Structure 60 Is (−)-Lasonolide A. At this point, the most likely spot left for further modification was easily deduced: the geometry of the double bond at C-25,26. It was decided to investigate **59**/**60**, which are (17*E*,25*Z*)-isomers of **1**/**2** (Figure 7).

The phosphonium salt **61** was prepared from the alcohol **28** via iodide substitution, and Wittig reaction between the corresponding ylide and the aldehyde **58** led to the product **59** after subsequent TBS-deprotection. Using the enantiomeric phosphonium salt *ent*-**61**, the epimeric product **60** was also prepared (Scheme 15).

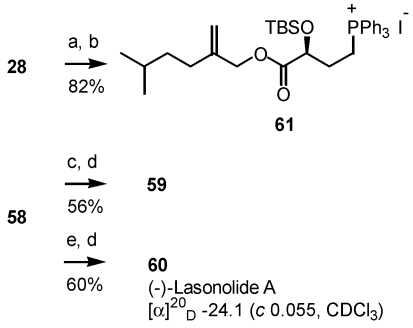
(21) Liu, P.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2001**, *123*, 10772−10773.



59 (28*S*) or **60** (28*R*)
Candidates for the Correct Structure
of Lasonolide A

FIGURE 7. (17*E*,25*Z*)-Isomers **59/60**.

SCHEME 15. Preparation of 59/60[a]



[a] Key: (a) Ph_3P, I_2, THF; (b) Ph_3P, MeCN, reflux; (c) **61**, KHMDS, THF, −78 °C; **58**, −78 °C to rt; (d) HF·pyridine, pyridine, THF, 0 °C; (e) *ent*-**61**, KHMDS, THF, −78 °C; **58**, −78 °C to rt.

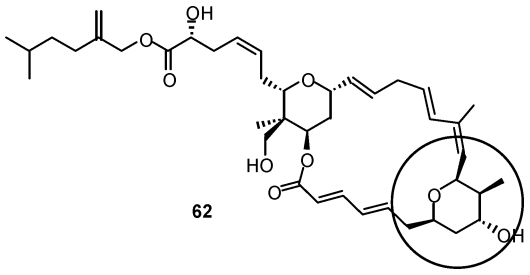


FIGURE 8. Stereoisomer **62**.

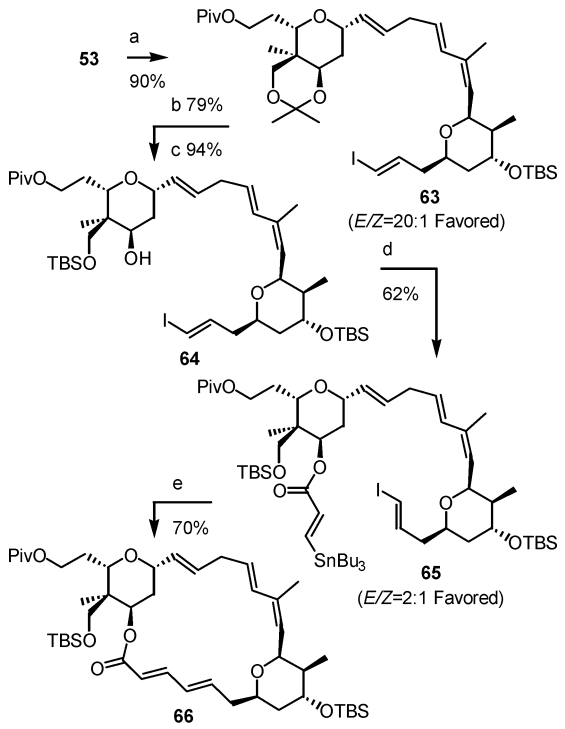
Comparison of the 500 MHz NMR spectra of **59**/**60** and the natural product revealed that **60** represented the correct structure of lasonolide A.

Synthesis of the Stereoisomer 62. The correct structure of lasonolide A was finally identified, but the discrepancies encountered during the course of the investigation prompted us to consider the structure **62**, the stereoisomer of **60** which is enantiomeric in ring B (Figure 8). It was realized that comparison of the NMR spectra of **60**, **62**, and the natural product would remove any uncertainty concerning the true structure of lasonolide A.

The Kocienski−Julia reaction of the sulfone **53** and the aldehyde *ent*-**22** afforded the intermediate **63**, which was converted into the ester **65** via the alcohol **64**. Intramolecular Stille coupling reaction of **65** produced a new macrolide **66** (Scheme 16).

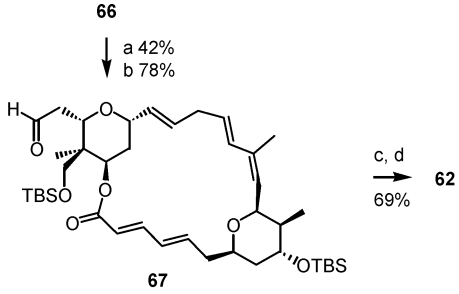
The macrolide aldehyde **67** was obtained from **66** via reduction/oxidation protocol, and Wittig reaction of **67**, and the ylide from *ent*-**61** provided the product **62** after TBS-deprotection (Scheme 17). The NMR spectra of **62** were clearly different from those of the natural product

SCHEME 16. Preparation of 66[a]



[a] Key: (a) LiHMDS, THF, −78 °C; *ent*-**22**, −78 °C to rt.; (b) CSA, MeOH, $(HOCH_2)_2$; (c) TBSCl, imidazole, DCM; (d) **35**, DIC, DMAP, DCM; (e) Pd_2dba_3, DIPEA, $Ph_2PO_2NBu_4$, DMF.

SCHEME 17. Preparation of 62[a]



[a] Key: (a) $LiEt_3BH$, THF, −78 °C (s.m. 30%); (b) SO_3·pyridine, TEA, DMSO−DCM (1:1), 0 °C; (c) *ent*-**61**, KHMDS, THF, −78 °C; **67**, −78 °C to rt; (d) HF·pyridine, pyridine, THF.

reconfirming the identity of the natural product structure as **60**.

Synthesis of (+)-Lasonolide A and Biological Assay: (−)-Lasonolide A Is the Biologically More Active Enantiomer. The sample of **60** synthesized above featured 500 MHz NMR spectra identical to those of the natural product (it showed sharper hydroxyl proton signals and did not contain upfield signals from impurities) but exhibited the specific rotation $[\alpha]^{20}_D$ −24.1 (*c* 0.055, $CDCl_3$), which was opposite to the reported value $[\alpha]_D$ +24.4 (*c* 0.045, $CDCl_3$)[1] for the natural product. Assuming the literature value of the specific rotation of the natural product was correct, the structure of natural lasonolide A was determined to be *ent*-**60**. Synthesis of *ent*-**60** was then carried out following the established procedure but using enantiomeric starting materials. The specific rotation of the sample of *ent*-**60**

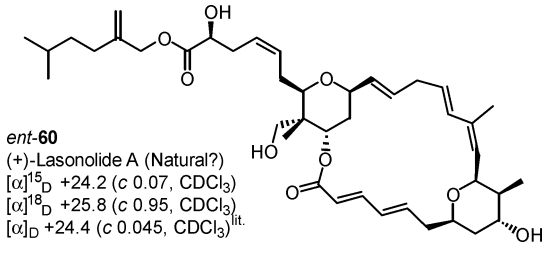


FIGURE 9. (+)-Lasonolide A, *ent*-**60**.

TABLE 1. GI_{50} (μM) Values of Lasonolide A and Related Compounds

sample	A549	HCT-116	NCI-H460
1	>10	5	5
49	3.2	0.1	0.04
50	2	0.04	0.02
59	0.05	0.009	<0.003
60	0.02	<0.003	<0.003
ent-**60**	6	3	2
62	>10	>10	>10

prepared matched the value reported for the natural product (Figure 9).

The samples synthesized were subjected to biological assay (Table 1). Surprisingly, it was found that the (−)-enantiomer **60** was the most potent compound tested. On the contrary, the (+)-enantiomer *ent*-**60** was found to possess much lower activities. Accordingly, we were forced to conclude that the active (and natural) enantiomer of lasonolide A is the (−)-enantiomer **60** (Figure 10) and the original report on the optical rotation data for natural lasonolide A is in error.

Inspection of the data in Table 1 reveals that the (*R*)-configuration at C-28 enhances the activity. The cis double bond at C-25,26 is important, and the trans double bond at C-17,18 is essential, for the compound **1** was almost devoid of activity. The stereoisomer **62** was completely devoid of activity.

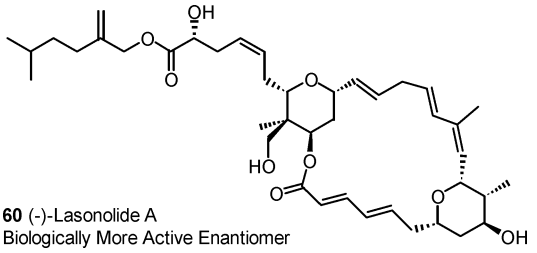


FIGURE 10. (−)-Lasonolide A, **60**, the biologically active enantiomer.

Conclusion

In the present studies, the two tetrahydropyranyl fragments of lasonolide A were prepared stereoselectively via radical cyclization reactions of β-alkoxyacrylates; in particular, excellent stereocontrol was achieved in the introduction of the quaternary center at C-22 via 6-endo-6-exo tandem radical cyclization reactions. The full structure of natural lasonolide A was determined unequivocally as **60**, which is the (17*E*,25*Z*)-isomer of the structure originally proposed.

Acknowledgment. We thank the Ministry of Science and Technology, Republic of Korea, and Korea Institute of Science and Technology Evaluation and Planning for a National Research Laboratory grant (1999). Brain Korea 21 graduate fellowship grants to H.Y.S., C.-K.J., H.S.K., and J.H.P. are gratefully acknowledged.

Supporting Information Available: Full experimental procedures and spectral data for intermediates and ^{1}H NMR spectra of **1**, **2**, **40**, **41**, **49**, **50**, **59**, **60**, **62**, and natural lasonolide A and a collection of ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO034930N