JOC
The Journal of Organic Chemistry

VOLUME 66, NUMBER 25 DECEMBER 14, 2001

Articles

Total Synthesis of (+)-Ampullicin and (+)-Isoampullicin: Two Fungal Metabolites with Growth Regulatory Activity Isolated from *Ampulliferina* Sp. 27

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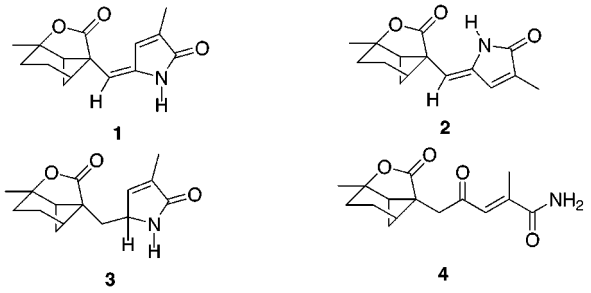
Received May 24, 2001

The total synthesis of the growth regulators (+)-ampullicin **1** and (+)-isoampullicin **2** from (*R*)-(−)-carvone **5** was accomplished by application of an 18-step sequence with 4.5% overall yield. The crucial step of the synthetic strategy lies on the internal displacement of tosylate **13** by means of the lactone enolate. In this way, access was opened to the tricyclic core present in these biologically active sesquiterpenic amides. A Horner−Emmons reaction between the carbaldehyde **16** and the phosphonate **22** led us to the stereoselective preparation of (+)-ampullicin **1**. Standard transformation of **1** into the thermodynamically more stable geometric isomer (+)-isoampullicin **2** was trivial. The absolute configuration of both amides was established by X-ray analysis of a sample of synthetic (+)-isoampullicin **2**.

Introduction

In the course of a screening program aimed at the discovery of new plant growth regulators among fungal metabolites, Kimura et al. reported the isolation of the sesquiterpenic amides (−)-ampullicin **1**, (+)-isoampullicin **2**, and (+)-dihydroampullicin **3** (Scheme 1) from a culture filtrate of an *Ampulliferina*-like fungus sp. No. 27 obtained from a dead pine (*Pinus thunbergii*). These amides were claimed to exhibit remarkable growth-regulating properties.[1,2]

Scheme 1

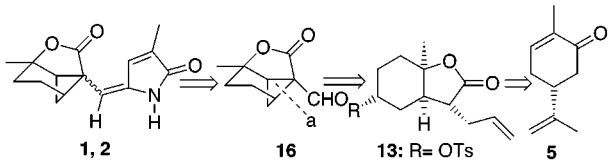


Structural elucidation of **1**−**3** was made on the basis of ^{1}H NMR and ^{13}C NMR data together with NOE

(1) Kimura, Y.; Nakajima, H.; Hamasaki, T.; Matsumoto, T.; Matsuda, Y.; Tsuneda, A. *Agric. Biol. Chem.* **1990**, *54*, 813−814.
(2) Kimura, Y.; Matsumoto, T.; Nakajima, H.; Hamasaki, T.; Matsuda, Y. *Biosci. Biotech. Biochem.* **1993**, *57*, 687−688.

10.1021/jo010527+ CCC: $20.00
Published on Web 11/13/2001

Scheme 2. Retrosynthetic Analysis of (+)-Ampullicin 1 and (+)-Isoampullicin 2



difference experiments. However, to date the absolute stereochemistry of these novel amides has not been determined, although a biosynthetic pathway has been envisioned that relates them to (+)-pinthunamide **4**, another fungal metabolite isolated from the same *Ampulliferina* species whose absolute configuration was determined by X-ray crystallographic analysis.[3]

In 1993 the total synthesis of (+)-pinthunamide **4** was reported by Mori and Matsushima.[4] The absolute configuration of the synthetic product was determined by comparison of its optical rotation value with that reported for the natural product. However, to our knowledge there is no reference in the literature about the synthesis of **1** and **2** except for previous communications about our own synthetic work. [5,6] Here we report the full detailed total synthesis of (+)-ampullicin **1** and (+)-isoampullicin **2** from (*R*)-(−)-carvone **5**.

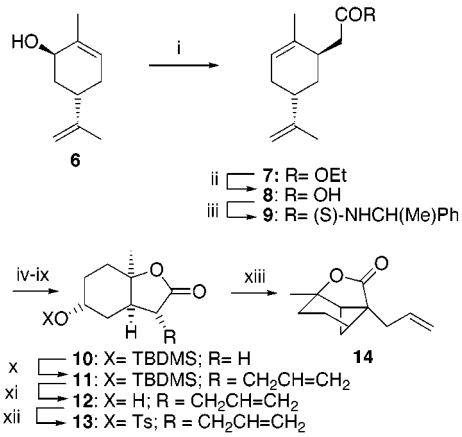
Our synthetic strategy is based on the internal displacement of tosylate **13** and elongation of carbaldehyde **16** by a Horner−Emmons reaction with the heterocyclic phosphonate **22**. The absolute configuration of the target molecules **1** and **2** was for the first time unequivocally established by single-crystal X-ray diffraction of synthetic (+)-isoampullicin, **2**.

Results and Discussion

Our synthetic plan is based on the disconnection of the exocyclic double-bond present in both target amides **1** and **2**. We also made use of the "type a" disconnection to access carbadehyde **16** from the bicyclic tosyloxy lactone **13**, readily available from (*R*)-(−)-carvone **5** (Scheme 2).

The bicyclic lactone **10** was prepared from **5** by an already reported procedure that has been successfully developed by our group when we synthesized the tricyclic lactone (1*R*,3*R*,6*R*,9*S*)-6,9-dimethyl-8-oxo-7-oxatricyclo-[4.3.0.0^{3,9}]nonane. Enantiomerically pure (+)-*trans*-carveol **6** (Scheme 3) was obtained from its 3,5-dinitrobenzoate following the Johnston procedure.[7] Following standard protocols, the carboxylic acid **8** was obtained from (+)-*trans*-carveol **6** by the ortho ester Claisen rearrangement, followed by alkaline hydrolysis of the resulting ethyl ester **7**. The absolute stereochemistry of **8** has been previously confirmed beyond doubt by X-ray analysis of the enantiomerically pure amide **9**, obtained

Scheme 3[a]



[a] Reaction and conditions: i: $CH_3C(OEt)_3$ (7 equiv), EtCOOH, 140 °C, 24 h, 85%; ii: NaOH, CH_3OH, reflux, 2 h, 75%; iii: *N*-hydroxysuccinimide, DCC, (*S*)-(−)-α-methylbenzylamine, CH_2Cl_2, 1 h, rt, 85%; iv: (from **8**) NBS (1.1 equiv), acetone, 0 °C, 1 h, 85%; v: $^nBu_3SnH$ (1.1 equiv), AIBN, THF, 55 °C, 1 h, 95%; vi: O_3, CH_2Cl_2, −78 °C, $S(Me)_2$, 15 h, rt, 85%; vii: m-CPBA (5 equiv), $NaHCO_3$, CH_2Cl_2, rt, 2 days, 85%; viii: NaOMe (1.1 equiv), MeOH, 0 °C, 45 min, 75%; ix: TBDMSCl (2.2 equiv), imidazole (2.5 equiv), DMF, rt, 100%; x: LDA (1.1 equiv), HMPA, THF, −78 °C, allyl bromide, 85%; xi: $Bu_4N^+F^-$, THF, rt, 30 min, 85%; xii: TsCl, pyr, DMAP, CH_2Cl_2, rt, 15 h, 90%; xiii: LDA (1.1 equiv), HMPA (1.2 equiv), −78 °C to room temperature, 90%.

by DCC-promoted coupling reaction of **8** with enantiomerically pure (S)-(−)-α-methylbenzylamine.[6]

The transformation of the acid **8** into the bicyclic lactone **10** required the successful accomplishment of three stereocontrolled transformations: (a) bromolactonization, (b) tributyltin hydride reduction of the resulting bromolactone, and (c) oxidative degradation of the isopropenyl moiety. The overall transformation of **8** into **10** was achieved by application of a six-step sequence with 37% overall yield.

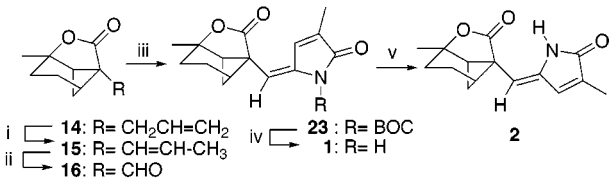
Introduction of the allyl substituent was achieved by addition of allyl bromide to a THF solution of the enolate of **10** under kinetically controlled conditions. As expected, the alkylation took place stereospecifically through the *exo* face, leading to **11** as a single reaction product, which has been isolated by flash chromatography on silica gel with 85% yield. The transformation of **11** into the tosylate **13** was accomplished with excellent yield (80%) by *O*-silyl deprotection, followed by treatment of the crude hydroxy derivative **12** with tosyl chloride under standard conditions.

The intramolecular displacement of tosylate **13** was achieved by treatment of the bicyclic lactone with a THF solution of LDA at −78 °C, followed by addition of HMPA, leading to the tricyclic lactone **14** with 90% yield. The spectroscopic properties obtained for **14** (Scheme 4) were identical to those described for (1*R*,4*S*,6*R*,7*S*)-7-allyl-1-methyl-9-oxatricyclo[4.3.0.0^{4,7}]nonan-8-one[4] by Mori et al.[8]

Oxidative degradation of the side chain in **14** was accomplished very efficiently in two steps. Double-bond isomerization of **14** led smoothly to the internal olefin **15** by treatment with rhodium(III) chloride hydrate with quantitative yield. This was followed by ozonolysis of **15** to afford the rather stable carbaldehyde **16**, also with excellent yield. Assessment of the trans stereochemistry on the exocyclic double bond in **15** was confirmed by ^{1}H NMR irradiation experiments. The preparation of **16** by

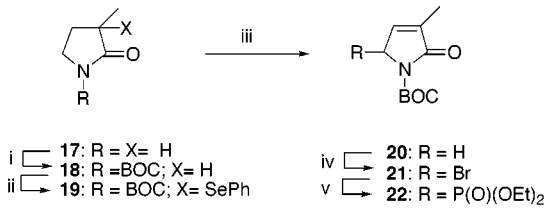
(3) Kimura, Y.; Nakajima, H.; Hamassaki, T.; Sugawara, F.; Parkanyi, L.; Clardy, J. *Tetrahedron Lett.* **1989**, *30*, 1267−1270.
(4) Mori, K.; Matsushima, Y. *Synthesis*. **1993**, 406−410.
(5) Rico. R.; Bermejo, F. *Tetrahedron Lett.* **1995**, *36*, 7889−7892.
(6) Rico, R.; Zapico, J.; Bermejo, F.; Sanni, S. B.; García-Granda, S. *Tetrahedron: Asymmetry*. **1998**, *9*, 293−303.
(7) Johnston, R. G.; Read, J. *J. Chem. Soc.* **1934**, 233−237.
(8) Unfortunately, no optical rotation value for the tricyclic lactone **14** was available for comparison with that obtained by us, although Mori and Matsushima had reported $[\alpha]_D$ values of their more advanced intermediates. Prof. K. Mori, Science University of Tokyo, personal communication.
(9) Martin, M. J.; Bermejo, F. *Tetrahedron Lett.* **1995**, *36*, 7705−7708.

Scheme 4[a]



[a] Reaction conditions: i: $RhCl_3xH_2O$, EtOH, reflux, 1.5 h, 100%; ii: O_3, CH_2Cl_2, −78 °C, SMe_2, rt, 3 h, 100%; iii: **22**, NaH, THF, rt, 2 h, 80%; iv: TFA, CH_2Cl_2, 0 °C, 30 min, 95%; v: I_2, $CHCl_3$, reflux, 5 h, 100%.

Scheme 5[a]



[a] Reaction conditions: i: $(^tBuOCO)_2O$, THF, reflux, 95%; ii: LDA, THF, −78 °C, PhSeCl, 85%; iii: AcOH, H_2O_2, 90%; iv: NBS (1 equiv), AIBN, CCl_4, 80 °C, 3 h, 100%; v: $P(OEt)_3$ (2 equiv), 140 °C, 1 h, 100%.

this procedure represents a remarkable improvement with respect to other previously reported alternatives.[5]

Transformation of carbaldehyde **16** into *N*-Boc ampullicin **23**, was successfully achieved by a Horner−Emmons reaction with phosphonate **22** (Scheme 5). This reagent was readily obtained from commercially available 3-methyl-2-pyrrolidinone **17.** Nitrogen protection was followed by reaction of **18** with a THF solution of LDA at −78 °C and further addition of phenylselenyl chloride. Flash chromatography of the crude product afforded the pure α-selenoderivative **19** with 86% yield. Oxidation of **19** with hydrogen peroxide in acetic acid at 0 °C led to spontaneous elimination of PhSeOH and allowed us to isolate the *N*-Boc-3-methyl-3-pyrrolin-2-one **20**[9] with 90% yield. Appropriate functionalization at the C-5 site of pyrrolinone **20** was successfully achieved in two steps. The allylic bromination of **20** was achieved by treatment with NBS and AIBN in carbon tetrachloride at 85 °C. Isolation of the pure bromide **21** was achieved by flash chromatography on silica gel with 75% yield. Finally, reaction of **21** with triethyl phosphite at 140 °C led to the crude phosphonate **22**, which was used without further purificaton.[10]

Treatment of phosphonate **22** with sodium hydride in THF followed by addition of carbaldehyde **16** at room temperature led to the exclusive formation of *N*-Boc ampullicin **23.** No trace of the geometrical isomer **24** was detected by ^1H NMR analysis of the crude reaction mixture. Flash chromatography of the crude product led to the isolation of pure **23** with 75% yield.

The stereoselectivity of the olefination process may be explained in terms of steric hindrance, which influences the diastereomeric transition states of the Horner−Emmons reaction; the strong steric interactions developed in **TS-2** between the side chain and the *N*-Boc protecting group will lead to the exclusive formation of **23** via the **TS-1** transition state (Scheme 6).

(10) Reaction of **21** with triphenylphosphine led mainly to the formation of the alkoxyphosphonium bromide. See House, H. O. *Modern Synthetic Methods*; 2nd ed.; Benjamin-Cummings Publishing Company; Menlo Park CA, 1972; p 698.

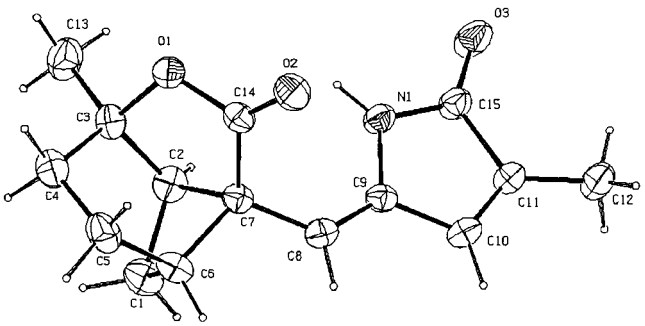
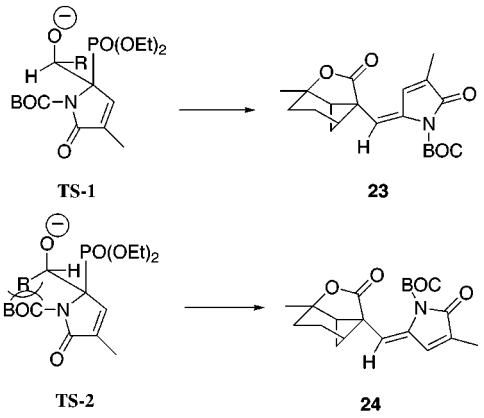


Figure 1. ORTEP diagram for (+)-isoampullicin **2**. An arbitrary numbering system is given.

Scheme 6. Stereoselectivity of the Horner−Emmons Reaction: Developing Interactions on the Transition States TS-1 and TS-2 Leading to 23 and 24



Deprotection of **23** by treatment with trifluoroacetic acid in dichloromethane at 0 °C led exclusively to ampullicin **1** with excellent yield (95%). The thermodynamically more stable geometrical isomer isoampullicin **2**, was prepared quantitatively by isomerizing the *E* isomer **1** by treatment with iodine in refluxing chloroform for 5 h.

The spectroscopic properties recorded for both synthetic geometrical isomers completely matched those described in the literature for ampullicin **1** and isoampullicin **2**.[1] Unexpectedly, however, synthetic ampullicin **1** displayed a positive specific rotation, whereas for the compound isolated from natural sources a negative rotation was reported.[11] Therefore, a single-crystal of synthetic **2** suitable for X-ray crystallography was grown from a hexane−acetone mixture, and the correct stereochemistry of **2** was established (Figure 1). [12]

Since synthetic (+)-isoampullicin **2** was obtained from **1** through unequivocal chemical transformations, the stereochemistry corresponding to both synthetic products is established beyond doubt.[13]

Conclusion

The convergent total synthesis of the growth regulators (+)-ampullicin **1** and (+)-isoampullicin **2** is described, using commercially available (*R*)-(−)-carvone, by application of a stereoselective 18-step synthetic sequence with

4.5% overall yield. This synthetic route should give access to the fungal metabolites (+)-dihydroampullicin **3** and (+)-pinthunamide **4**, sesquiterpenic amides isolated from *Ampulliferina* Sp. 27.

Experimental Section

General Procedures. NMR spectra were recorded at 250, 300, or 400 MHz for ^{1}H (δ, Me_4Si, $CDCl_3$ except where otherwise indicated) and 62.83, 75.73, or 100.63 MHz for ^{13}C (δ, $CDCl_3$, carbon multiplicities assigned by DEPT techniques) except where otherwise indicated. Low-resolution electron impact mass spectrum data (MS-EI) were obtained at 70 eV unless otherwise stated. Optical rotations were measured with Na 589 nm irradiation. Melting points are uncorrected. Kugelrohr distillation oven temperatures (ot) refer to the external air bath temperature. All reactions were carried out under argon. Silica gel flash chromatography purifications were performed on silica gel (230−400 mesh) as described by Still.[14] Ozone was generated in a Fischer 502 ozone generator. TLC was performed on plates of silica gel (2 × 5 cm, 0.2 mm thickness). Components were located by observation of the plates under UV light and/or by treating the plates with phosphomolybdic reagent, followed by heating. All glassware was dried at 150 °C overnight, assembled hot, and allowed to cool in a stream of dry argon. All transfers of solutions and solvents were performed by syringe techniques or via cannula. All solvents were freshly distilled from the appropriate drying agent before use. Diethyl ether, tetrahydrofuran, toluene, and benzene were freshly distilled from sodium benzophenone ketyl under an argon atmosphere. Carbon tetrachloride and dichloromethane were distilled from P_2O_5 under argon. Pyridine was distilled first from KOH and later from CaH_2 under argon. Dimethylformamide was distilled from P_2O_5 under reduced pressure and stored over 4A MS. Ethyl acetate, hexanes, and dimethyl sulfide were distilled fron CaH_2 under nitrogen. Diisopropylamine was distilled twice from CaH_2 under nitrogen and stored over 4 Å MS. Methanol was distilled from Mg under argon. m-CPBA was crystallized from dichloromethane.[15] Concentrations were carried out in a rotatory evaporator. Solutions were dried with Na_2SO_4. All the new compounds exhibited satisfactory low resolution MS data and afforded combustion analyses or appropriate exact mass data of the molecular ions. 3-Methyl-2-pyrrolidinone was commercially available from Lancaster Synthesis Ltd, Strasbourg, France.

(3*R*,3a*R*,5*R*,7a*R*)-5-*tert*-Butyldimethylsilyloxy-7a-methyl-3-(2-propenyl)-3a,4, 5,6,7,7a-hexahydro-2(3*H*)-benzofuranone (11). To a solution of diisopropylamine (0.3 mL, 1.97 mmol) in 5 mL of anhydrous THF a solution of 1.1 M BuLi in hexane (1.7 mL) was added at −78 °C. After stirring under Ar for 45 min, HMPA (0.32 mL, 1.83 mmol) and 400 mg (1.41 mmol) of **10**[6] in 2 mL of anhydrous THF were added. The mixture was stirred at this temperature for 1 h, after which allyl bromide (0.30 mL, 3.52 mmol) was added. Thirty minutes later, 15 mL of a saturated aqueous solution of NH_4Cl was added, and the mixture was left to reach room temperature. After extraction with AcOEt, the combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated at reduced pressure. The residue (558 mg) was purified by flash chromatography (hexane/AcOEt 9:1) to yield 389.2 mg (85%) of **11** $[\alpha]_D^{25} = +12.3$ ($c = 0.79$, $CHCl_3$). IR ($CHCl_3$) ν 2932, 2859, 1767, 1643, 1464, 1096, 866 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.88 (s, 9H), 1.49 (s, 3H), 1.2−2.2 (m, 6H), 2.3−2.7 (m, 4H), 3.78 (m, 1H), 5.12 (m, 2H), 5.81 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ −4.78, 17.99, 24.46, 25.72, 31.98, 33.14, 33.43, 33.67, 43.38, 46.82, 66.52, 82.16, 117.71, 134.59, 176.47 ppm. FAB-MS *m*/*z* (relative intensity) 325.1 (23), 193.1 (33), 147.1 (70). Anal. Calcd for $C_{18}H_{32}O_3Si$: C, 66.61; H, 9.94. Found: C, 66.56; H, 9.87.

(3*R*,3a*R*,5*R*,7a*R*)-5-Hydroxy-7a-methyl-3-(2-propenyl)-3a,4,5,6,7,7a-hexahydro-2(3*H*)-benzofuranone (12). To a solution of **11** (389.2 mg, 1.20 mmol) in 25 mL of THF was added a solution of $Bu_4NF{\cdot}H_2O$ (757 mg, 2.4 mmol) in 5 mL of THF. The mixture was stirred under Ar at room temperature for half an hour and then evaporated at reduced pressure. Ten milliliters of a saturated aqueous solution of NH_4Cl was added, and the aqueous phase was extracted with Cl_2CH_2. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated at reduced pressure. The crude was purified by flash chromatography (hexane/AcOEt 3:7) to yield 215 mg (85%) of **12** $[\alpha]^{25}_D = -7.8$ ($c = 1.09$, $CHCl_3$). IR ($CHCl_3$) ν 3430, 2938, 1759, 1642, 1092, 934 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3H), 1.20−1.75 (m, 6H),1.8−2.8 (m, 4H), 3.82 (m, 1H), 5.15 (m, 2H), 5.80 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 24.36, 31.59, 32.31, 32.94, 33.54, 43.13, 46.34, 65.55, 82.35, 117.92, 134.10, 176.75 ppm. FAB-MS *m*/*z* (relative intensity) 211.1 (37), 193.1 (22), 149.0 (100), 113.0 (23), 80.8 (23). Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.47; H, 8.54.

(3*R*,3a*R*,5*R*,7a*R*)-5-(*p*-Toluenesulfonyloxy)-7a-methyl-3-(2-propenyl)-3a,4,5,6,7,7a-hexahydro-3(2*H*)-benzofuranone (13). To a solution of **12** (235.1 mg, 1.12 mmol) in 3 mL of anhydrous CH_2Cl_2 were added pyridine (0.18 mL, 2.24 mmol), a catalytic amount of 4-(dimethylamino)pyridine, and TsCl (320 mg, 1.7 mmol). The mixture was stirred under Ar at room temperature for 35 h and then poured into an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phases were washed with 1 N HCl, 10% $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The crude was purified by flash chromatography (hexane/AcOEt 8:2) to yield 16.5 mg (7%) of starting material and 367 mg (90%) of **13** mp 141−143 °C (hexane/AcOEt); $[\alpha]^{25}_D = +15.1$ ($c = 1.02$, $CHCl_3$). IR ($CHCl_3$) ν 3055, 2953, 1765, 1643, 1599, 1265, 739 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 3H), 1.4−2.5 (m, 10H), 2.46 (s, 3H), 4.51 (m, 1H), 5.05 (m, 2H), 5.65 (m, 1H), 7.35 (d, 2H, $J = 8.34$ Hz), 7. 78 (d, 2H, $J = 8.34$ Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 21.46, 24.35, 28.88, 29.79, 33.10, 33.24, 42.95, 46.04, 76.46, 81.12, 118.12, 127.60, 129.85, 133.92, 134.01, 144.81, 175.78 ppm. FAB-MS *m*/*z* (relative intensity) 365.0.-(58), 262.5 (20), 193.1 (100), 153.5 (85), 108.5 (71), 78.4 (52), 52 (68). Anal. Calcd for $C_{19}H_{24}O_5S$: C, 62.61; H, 6.64. Found: C, 62.56; H, 6.58.

(1*R*,4*S*,6*R*,7*S*)-7-Allyl-1-methyl-9-oxatricyclo[4.3.0.0^{4,7}]-nonan-8-one (14). To a solution of diisopropylamine (0.5 mL, 3.1 mmol) in 5 mL of anhydrous THF a solution of 1.1 M BuLi in hexane (2.8 mL, 3.12 mmol) was added at −78 °C. The mixture was stirred under Ar for an hour at this temperature, after which HMPA (0.54 mL, 3.1 mmol) and a solution of **13** (948 mg, 2.6 mmol) in 10 mL of anhydrous THF were dropwise added. After stirring for 2 h (from −78 °C to room temperature), 25 mL of a saturated aqueous solution of NH_4Cl was added, and the aqueous phase was extracted with AcOEt. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/AcOEt 7:3) to yield 456 mg (90%) of **14** $[\alpha]^{25}_D = -2.2$ ($c = 1.10$, $CHCl_3$). IR ($CHCl_3$) ν 3077, 2934, 1761, 1642, 1200, 912 cm^{-1}. ^{1}H NMR($CDCl_3$) δ 1.45 (s, 3H), 1.68 (d, 1H, $J = 10.5$ Hz), 1.90 (s, 4H), 2.21 (dt, 1H, $J = 5.1$ Hz, 10.2 Hz), 2.33 (dd, 1H, $J = 8.2$ Hz, 15.0 Hz), 2.41 (m, 1H), 2.48 (t, 1H, $J = 5.1$ Hz), 2.65 (dd, 1H, $J = 8.2$

(11) In a previous communication on our synthetic work[5] we mistakenly reported a complete agreement of the physical properties of natural ampullicin with those obtained for the synthetic product. Obviously, this is true except for the specific rotation value as is clearly explained in the text. However, our synthetic (+)-isoampullicin **2** resulted to be insoluble in methanol and was found to be dextrorotatory in chloroform. Thanks are given to Prof. Y. Kimura (Tottori University, Japan) for providing us with the ^{1}H NMR spectra of natural **1**, **2**, **3**, and **4**. Unfortunately, no sample of natural **1** was available.

(12) Correspondence regarding the X-ray crystallographic determination of the absolute configuration of **2** should be addressed to Prof. Santiago Garcia-Granda (University of Oviedo, Spain).

(13) Since the absolute stereochemistry of **8** has been previously demostrated by our group and the overall transformation of carboxylic acid **8** into (+)-ampullicin **1** has been achieved through stepwise stereocontrolled transformations, we claim that the absolute stereochemistry of synthetic (+)-isoampullicin **2** is identical to that depicted in Scheme 1. Additionally, X-ray analysis of a pure sample of (+)-isoampullicin **2** (Figure 1) supports our proposal.

(14) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(15) Traylor, T. G.; Miksztal, A. R., *J. Am. Chem. Soc.* **1987**, *109*, 2770.

Hz, 15.0 Hz), 5.07−5.16 (m, 2H), 5.65−5.86 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 22.56, 22.91, 24.50, 29.75, 33.82, 40.86, 46.45, 55.27, 87.59, 117.61, 133.13, 177.33 ppm. FAB-MS *m*/*z* (relative intensity) 193.1 (30), 184.1 (100), 149.0 (34), 90.9 (20), 83.8 (32). Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.89; H, 8.28.

(1*R*,4*S*,6*R*,7*R*)-1-Methyl-9-oxa-7-[-(*E*)-1-propenyl]-tricyclo[4.3.0.0^{4,7}]nonan-8-one (15). To a solution of **14** (409 mg, 2.1 mmol) in 12 mL of degassed EtOH under Ar was added rhodium(III) chloride hydrate (16 mg, 0.063 mmol). The mixture was heated under reflux and stirred for 1.5 h. After evaporation, the crude product was purified by flash chromatography (hexane/ether 7:3) to yield 408 mg (100%) of **15**: $[\alpha]^{25}_D = +38.0$ (c = 0.93, $CHCl_3$). IR ($CHCl_3$) ν 2936, 1765, 1454, 1200, 1017, 756 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (s, 3H), 1.62 (d, 1H, J = 10.22 Hz), 1.75 (d, 3H, J = 4.96 Hz), 1.87−1.95 (m, 4H), 2.23 (ddd, 1H, J = 10.2 Hz, 5.1 Hz, 5.1 Hz), 2.58−2.62 (m, 2H), 5.60−5.80 (m, 2H, J_{trans} = 15.6 Hz) ppm. ^{13}C NMR ($CDCl_3$,400 MHz) δ 18.15, 23.00, 23.11, 24.90, 29.84, 41.68, 48.84, 57.42, 87.93, 126.23, 127.42, 177.42 ppm. EI-MS *m*/*z* (relative intensity) 192.20 (15), 177.15 (15), 151.10 (50), 137.15 (48), 105.10 (48), 91.15 (62), 79.20 (57), 55.15 (39), 43.10, (100). Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.85; H, 8.31.

(1*R*,4*S*,6*R*,7*S*)-1-Methyl-9-oxa-8-oxotricyclo[4.3.0.0^{4,7}]-nonan-7-carbaldehyde (16). Ozone was bubbled through a solution of alkene **15** (370.5 mg, 1.9 mmol) in 25 mL of CH_2Cl_2 at −78 °C until a blue-grey coloration developed. Then, excess ozone was eliminated with argon, and SMe_2 (0.9 mL, 12.3 mmol) was added. After stirring for 3 h (from −78 °C to room temperature) the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/AcOEt 6:4) to yield 345 mg (100%) of **16** $[\alpha]^{25}_D = +15.2$ (c = 1.02, $CHCl_3$). IR ($CHCl_3$) ν 2976, 1761, 1721, 1267, 737 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 3H), 1.63 (d, 1H, J = 11.0 Hz), 1.66−1.97 (m, 4H), 2.34 (ddd, 1H, J =11.0 Hz, 5.5 Hz, 5.5 Hz), 2.99 (t, 1H, J = 5.5 Hz), 3.08 (m, 1H), 9.84 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 22.93, 23.22, 24.37, 29.63, 40.43, 47.65, 64.28, 88.89, 173.32, 196.02 ppm. Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.58; H, 6.65.

1-*tert*-Butoxycarbonyl-3-methyl-2-pyrrolidinone (18). To a solution of 3-methyl-2-pyrrolidinone **17** (5.90 g, 53.5 mmol) in 100 mL of freshly distilled THF, triethylamine (8.9 mL, 64.2 mmol), 4-(dimethylamino)pyridine (60 mg, 0.5 mmol), and di-*tert*-butyl dicarbonate (14 g, 64.2 mmol) were successively added. The reaction mixture was stirred for 4 h at room temperature and then diluted with ethyl acetate. The organic layer was washed with citric acid (5%) and brine. The organic layer was dried with Na_2SO_4, and the solvent was evaporated off under reduced pressure to yield **18** (10 g, 95%). IR(film) ν 3000, 1768, 1724, 1460, 1365, 1312, 1152 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.15 (d, 3H, J = 7 Hz); 1.46 (s, 9H); 2.14 (m, 2H); 2.47 (m, 1H); 3.57(m, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 14.74; 25.83; 27.45; 37.94; 43.73; 81.79; 149.76; 175.77ppm. EI-MS *m*/*z* (relative intensity): 199 (M^+,4); 184 (17); 144 (51); 126 (49); 114 (13); 100 (73); 85 (9); 70 (44); 57 (100). Anal. Calcd for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.33; H, 8.72, N, 7.10.

1-*tert*-Butoxycarbonyl-3-methyl-3-phenylselenyl-2-pyrrolidinone (19). To a solution of diisopropylamine (1.8 mL, 13 mmol) in 15 mL of THF was dropwise added 7.5 mL solution of BuLi (1.6 M in hexane) at 0 °C. The reaction was stirred for 1 h at this temperature; then, a solution of 1-*tert*-butoxycarbonyl-3-methyl-2-pyrrolidinone (**18**) (2 g, 10 mmol) in 15 mL of THF was added at −78 °C, and the reaction mixture was stirred for 1 h. This was followed by the addition of a solution of PhSeCl (1.8 g, 10 mmol) in 2 mL of THF, and stirring at this temperature was continued for 1 h. The reaction was then allowed to warm to room temperature and was then quenched by the addition of a sat. aqueous NH_4Cl solution. The reaction product was extracted with AcOEt, and the organic layer was washed with brine and dried with Na_2SO_4 to afford a crude product which was fractionated by flash chromatography on silica gel. Elution with (hexane/AcOEt 8:2) led to **19** (3 g, 85%), mp 86−88 °C (hexane). IR ($CHCl_3$) ν 3010, 1776, 1720, 1475, 1440, 1368, 1300, 1155 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 9H); 1.59 (s, 3H); 2.15 (m, 2H); 3.50 (m, 2H); 7.50 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ 23.60; 27.67; 33.22; 42.63; 49.39; 82.19; 125.92; 128.60; 129.17; 137.39; 149.77; 173.50 ppm. EI-MS *m*/*z* (relative intensity): 355 (22); 254 (9); 157 (60); 124 (31); 98 (100); 57 (100). Anal. Calcd for $C_{16}H_{21}NO_3Se$: C, 54.24; H, 5.97; N, 3.95. Found: C, 54.16; H, 5.82; N, 3.82.

1-*tert*-Butoxycarbonyl-3-methyl-3-pyrrolin-2-one (20). To a solution of 1-*tert*-butoxycarbonyl-3-methyl-3-phenylselenyl-2-pyrrolidinone **(19)** (5 g, 14 mmol) in 100 mL of freshly distilled THF were successively added glacial acetic acid (3 mL) and 30% hydrogen peroxide (27 mL). The reaction was stirred for 30 min, 1 M $NaHCO_3$ (5 mL) was added, and the reaction was extracted with AcOEt, washed with brine and dried over Na_2SO_4 to afford **20** (2.5 g, 90%), mp 72−74 °C (hexane). IR ($CHCl_3$) ν 2936, 1770, 1722, 1657, 1475, 1453, 1160 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 9H); 1.85 (q, 3H, J = 2.5 Hz); 4.16 (quint, 2H, J = 2.5 Hz); 6.76 (sext, 1H, J = 2.5 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 10.70, 27.86, 49.22; 82.47; 135.23; 137.73; 149.60; 169.64 ppm. EI-MS *m*/*z* (relative intensity): 182 ($M^+ - CH_3$, 5); 142 (57); 124 (71); 110 (8); 98 (50); 81 (6); 69 (21); 57 (100). Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.81; H, 7.72, N, 7.22.

5-Bromo-1-*tert*-butoxycarbonyl-3-methyl-3-pyrrolin-2-one (21). To a solution of 1-*tert*-butoxycarbonyl-3-methyl-2-pyrrolin-2-one (**20**) (1 g, 5.5 mmol) in 50 mL of CCl_4 were added NBS (983 mg, 5.5 mmol) and a catalytic amount of AIBN. The mixture was stirred for 3 h at 80 °C under an argon atmosphere. After cooling, the solution was filtered and the solvent evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL), and the organic phase was washed with water and brine and then dried (Na_2SO_4) and evaporated under reduced pressure to give a crude product (1.2 g, 100%), which was purified by flash chromatography on silica gel. Elution with (hexane/AcOEt 8:2) led to **21** (0.9 g, 75%), IR (film) ν 3057, 1786, 1751, 1655, 1371, 1346, 1265, 1151, 1040, 953, 851, 772, 739, 704 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 9H), 1.88 (s, 3H), 6.39 (d, 1H, J = 1.5 Hz), 6.86 (quint, 1H, J = 1.7 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 10.41, 27.64, 57.73, 84.05, 134.40, 140.53, 147.40, 166.69 ppm. EI-MS *m*/*z* (relative intensity): 260 ($M^+ - CH_3$, 5); 196 ($M^+ - Br$, 10); 176 (8); 96 (90); 57 (100). Anal. Calcd for $C_{10}H_{14}O_3BrN$: C, 43.50; H, 5.11; N, 5.07. Found: C, 43.47; H, 5.06; N, 5.16.

(1-*tert*-Butoxycarbonyl-3-methyl-3-pyrrolin)-5-yl-phosphonic Acid Diethyl Ester (22). A solution of **21** (1 g, 3.6 mmol) and triethyl phosphite (1.15 mL, 7.2 mmol) was stirred at 140 °C for 1 h under an argon atmosphere. After cooling, the excess of reagent was eliminated by evaporation under reduced pressure to yield phosphonate **22** (1.2 g, 100%), which was used without further purification: IR (film) ν 2982, 1778, 1736, 1316, 1258, 1159, 1026, 959 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 6H), 1.55 (s, 9H), 1.89 (m, 3H), 4.12 (m, 4H), 4.93 (dquint, 1H, J = 17.1 Hz, 2.2 Hz), 6.87 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 11.23, 16.40, 16.43, 28.15, 57.94, 63.52, 63.66, 83.64, 135.99, 137.39, 149.33, 169.28 ppm. EI-MS *m*/*z* (relative intensity): 333 (5), 289 (5), 260 (10), 233 (60), 205 (12), 177 (25), 123 (20), 96 (40), 81 (20), 57 (100). Anal. Calcd for $C_{14}H_{24}NPO_6$: C, 50.45; H, 7.26; N, 4.20. Found: C, 50.56; H, 7.37; N, 4.12.

(1′*R*,4′*S*,6′*R*,7′*R*,*E*)-1-*tert*-Butoxycarbonyl-3-methyl-5-(1′-methyl-8′oxo-9′-oxatricyclo[4.3.0.0^{4,7}]non-7′-yl)methylene-3-pyrrolin-2-one, *N*-Boc-Ampullicin (23). To a suspension of 60% NaH dispersion in mineral oil (200 mg, 5 mmol) in 25 mL of anhydrous THF was added dropwise a solution of phosphonate **22** (1.6 g, 5 mmol) in 5 mL of freshly distilled THF. The mixture was stirred under Ar at room temperature for 1 h, and then a solution of carbaldehyde **16** (449 mg, 2.5 mmol) in 15 mL of anhydrous THF was added. After stirring for 2 h at the same temperature, 5 mL of a saturated aqueous solution of NH_4Cl was added, and the aqueous phase was extracted with AcOEt. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/AcOEt 7:3) to yield **23** (632 mg, 80%), mp

127−129 °C (hexane) $[\alpha]^{25}_D = +83.8$ ($c = 0.95$, $CHCl_3$). IR ($CHCl_3$) ν 2932, 1771, 1738, 1456, 1300, 1157, 1084 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.50 (s, 3H), 1.56 (s, 9H), 1.72 (d, 1H, $J = 11.2$ Hz), 1.92 (s, 3H), 1.96−2.01 (m, 4H), 2.41 (ddd, 1H, J =11.2 Hz, 5.6 Hz, 5.6 Hz), 2.7 (d, 2H, $J = 5.6$ Hz), 6.71 (s, 1H), 6.79 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 10.71, 23.17, 23.90, 24.80, 28.06, 29.75, 47.51, 52.88, 56.16, 83.55, 87.81, 114.87, 132.02, 132.66, 139.87, 149.59, 167.64, 175.57 ppm. Anal. Calcd for $C_{20}H_{25}NO_5$: C, 66.83; H, 7.01; N, 3.90. Found: C, 66.78; H, 6.95; N, 3.82.

(1′*R*,4′*S*,6′*R*,7′*R*,*E*)-3-Methyl-5-(1′-methyl-8′oxo-9′-oxatricyclo [4.3.0.0^{4,7}]non-7′-yl)methylene-3-pyrrolin-2-one, Ampullicin (1). To a solution of **23** (236.8 mg, 0.64 mmol) in 1 mL of CH_2Cl_2 was added trifluoroacetic acid (0.12 mL, 1.6 mmol) at 0 °C, and the mixture was stirred under argon for half an hour. After evaporation under reduced pressure, 1 N $NaHCO_3$ (2 mL) was added, and the reaction mixture was extracted with AcOEt. The organic layers were washed with brine and dried with Na_2SO_4. Evaporation of the solvent afforded a crude product which was fractionated by flash chromatography (hexane/AcOEt 1:1) to yield **1** (153.6 mg, 95%), mp 195−198 °C (hexane); $[\alpha]^{25}_D = +109.7$ ($c = 0.75$, $CHCl_3$). IR ($CHCl_3$) ν= 3105, 1767, 1733, 1708 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.53 (s, 3H), 1.76 (d, 1H, $J = 10.7$ Hz), 1.98 (s, 3H), 1.95−2.16 (m, 4H), 2.39 (ddd, 1H, J =10.7 Hz, 5.3 Hz, 5.3 Hz), 2.77 (d, 2H, $J = 5.3$ Hz), 5.49 (s, 1H), 6.73 (s, 1H), 7.88 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ= 10.97, 23.05, 23.81, 24.91, 29.66, 46.72, 52.66, 55.96, 87.98, 109.21, 128.56, 136.37, 141.02, 171.72, 176.09 ppm. EI-MS *m*/*z* (relative intensity): 259.10 (60), 218.05 (50), 204.05 (80), 187.05 (30), 133.95 (100), 110.00 (10), 91.00 (20). Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.56; H, 6.71; N, 5.36.

(1′*R*,4′*S*,6′*R*,7′*R*,*Z*)-3-Methyl-5-(1′-methyl-8′-oxo-9′oxatricyclo [4.3.0.0^{4,7}]non-7′-yl)methylene-3-pyrrolin-2-one; Isoampullicin (2). To a solution of **1** (175 mg, 067 mmol) in 1 mL of $CHCl_3$ was added a catalytic amount of iodine (25 mg), and the mixture was stirred under argon, under reflux for 5 h, and then at room-temperature overnight. The residue obtained by evaporation of the solvent was purified by flash chromatography (hexane/AcOEt 1:1) to yield **2** (174 mg, 100%), mp 205−208 °C (hexane); $[\alpha]^{25}_D = +108$ ($c = 0.50$, $CHCl_3$). IR ($CHCl_3$) ν 3182, 1752, 1694, 1658 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.52 (s, 3H), 1.75 (d, 1H, $J = 10.6$ Hz), 1.95 (d, 3H, $J = 1.5$ Hz), 1.91−2.10 (m, 4H), 2.39 (ddd, 1H, J =10.6 Hz, 5.4 Hz, 5.4 Hz), 2.77 (d, 2H, $J = 5.4$ Hz), 5.15 (s, 1H), 6.61(t, 1H, $J = 1.6$ Hz), 8.35 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 10.38, 23.30, 23.78, 24.71, 29.75, 49.95, 51.24, 56.28, 88.50, 106.87, 133.37, 134.15, 140.08, 172.82, 176.27 ppm. EI-MS *m*/*z* (relative intensity): 259.10 (68), 218.05 (48), 204.00 (75), 187.05 (28), 174.05 (22), 147.95 (31), 133.95 (100), 104.00 (12), 77.00 (18). Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.47; H, 6.60; N, 5.38.

Acknowledgment. Financial support of this work by the Direccion General de Investigación Científica y Técnica, Spain (DGICYT PB98-0251) and the Junta de Castilla y León (SA 24/00B) is gratefully acknowledged.

Supporting Information Available: Spectroscopic data for compounds **11**, **12**, **13**, **14**, **15**, **16**, **18**, **19**, **20**, **21**, **22**, **23**, **1**, and **2** (14 compounds). Tables of the crystal data, bond lengths and angles, atomic coordinates, and anisotropic thermal parameters obtained for (+)-isoampullicin, **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010527+