Bioorganic Chemistry 37 (2009) 46–51

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Concise synthesis of stagonolide-F by ring closing metathesis approach and its biological evaluation

Arun Kumar Perepogu [a], D. Raman [c], U.S.N. Murty [c,*], Vaidya Jayathirtha Rao [a,b,*]

[a] Organic Chemistry Division-II, Indian Institute of Chemical Technology (IICT), Uppal Road, Tarnaka, Hyderabad 500 607, Andhra Pradesh, India
[b] National Institute of Pharmaceutical Education & Research, Balanagar, Hyderabad 500 037, Andhra Pradesh, India
[c] Biology Division, Indian Institute of Chemical Technology, Hyderabad 500 607, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 26 October 2008
Available online 21 January 2009

Keywords:
Jacobsen's kinetic resolution
Chemo-selective reduction
Sharpless asymmetric epoxidation
Steglich esterification
Ring closing metathesis
Macrolide
Antimicrobial activity

ABSTRACT

The first total synthesis of 9-membered macrolide, stagonolide-F (**3**), starting from commercially available 1,5-pentane diol is reported. A combination of Jacobsen's hydrolytic kinetic resolution (HKR) and Sharpless epoxidation is used for the creation of two stereogenic centers, while ring-closing metathesis (RCM) strategy was used for the construction of the lactone ring. The molecule synthesized exhibited potent antifungal, antibacterial and cytotoxic activities against all the tested strains.

1. Introduction

Macrolides, particularly lactones with medium-sized rings (8–10 membered), have continued to attract the attention of both biologists and chemists during recent years, due to the interesting biological properties and scarce availability of macrolides. A few examples, in particular of 10-membered-ring containing macrolides that display potent biological activity are putaminoxin [1] (**1**), pinolidoxin [2] (**2**) (Fig. 1). The nonenolide (5*S*,9*R*)-5-hydroxy-9-methyl-6-nonen-9-olide (**3**), a diastereomer of aspinolide [3], is one such example, and has been isolated from *stagonospora circii*, a fungal pathogen isolated from *cirsium arvense* [4].

Intrigued by the biological properties and also structural similarity with highly potent putominoxin **1**, pinolidoxin **2**, and other phytotoxic nonenolides [5] and in continuation of our program towards synthesis of biological active compounds [6], we became interested in developing a simple and flexible route to the total synthesis of stagonolide-F (**3**). However, a few related nonenolides [7] have been reported starting from chiral pool, we are reporting here the synthesis and biological screening of stagonolide-F, starting from commercially available 1,5-pentane diol employing Jacobsen's hydrolytic kinetic resolution, Sharpless epoxidation and ring-closing metathesis as key steps. The retrosynthetic analysis revealed that **3** could be prepared efficiently by RCM protocol from bis-olefin **21** which in turn could be prepared by Steglich esterification of acid **15** and homo allylic alcohol **19**. Intermediate **19** could be envisaged from racemic propylene oxide **16**, while chiral TBDPS protected allyl alcohol **15** could be produced from 2,3-epoxy alcohol **11**. Thus in the present strategy (5*S*)-hydroxy group is installed through Sharpless epoxidation, while the (9*R*)-hydroxy group is introduced by Jacobsen's hydrolytic kinetic resolution (Scheme 1).

2. Experimental section

2.1. General

NMR spectra were measured on a Gemini 200 MHz Varian instrument and Avance 300 MHz Bruker UX-NMR instrument in $CDCl_3$ as reference solvent and chemical shifts were expressed as δ. Coupling constants J are given in Hz. Tetramethyl silane was used as an internal standard for ^1H NMR. Enantiomeric excess is determined by normal-phase HPLC using Chiralpak AD-H [amylose tris-(3,5-dimethylphenylcarbamate), 250 mm × 4.6 mm i.d., coated on a 5-μm silica particle] column from Diacel chemical industries Ltd. Mass spectra were recorded on VG Micromass 7070 H (EI and ESI) and Finnigan Mat 1020 Mass (GC–MS) instruments. High-resolution (HR) mass spectra were recorded using a VG Autospec magnetic sector mass spectrometer (Waters, Man-

* Corresponding author. Address: Organic Chemistry Division-II, Indian Institute of Chemical Technology (IICT), Uppal Road, Tarnaka, Hyderabad 500 607, Andhra Pradesh, India. Fax: +91 40 27160757.
E-mail addresses: aruniict@gmail.com (A.K. Perepogu), jrao@iict.res.in (V.J. Rao).

0045-2068/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2008.12.002

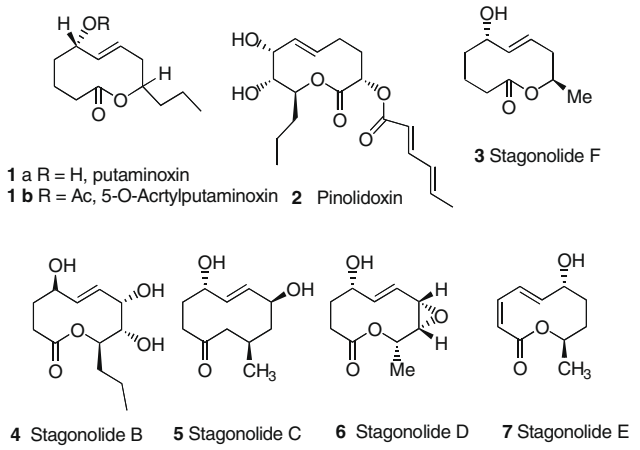


Fig. 1. Phytotoxic nonenolides.

chester, UK). IR Spectra were recorded on Perkin Elmer Model 283B and Nicolet-740 FT-IR instruments, and band positions were reported in wave numbers (cm^{-1}). Column chromatography was performed using silica gel H (60–120 μm). The experimental procedures and spectral data for compounds **7**, **8**, **9**, **10**, **18**, **19** and *Z* isomer of stagonolide-F (**3**) are given in Supporting information.

2.2. (2R,3S)-3-[4-(Benzyloxy)butyl]oxiran-2-ylmethanol (**11**)

To a cooled (−30 °C) suspension of activated, powdered 4 Å MS (1 g) in CH_2Cl_2 (25 mL) were added (+)-DET (0.37 mL, 2.18 mmol), $Ti(OPr^i)_4$ (0.53 mL, 1.8 mmol), and cumene hydroperoxide (1.6 mL, 10.8 mmol). After 20 min, a solution of allylic alcohol **10** (2.0 g, 9.0 mmol) in CH_2Cl_2 (10 mL) was added at −30 °C over 15 min. The resulting mixture was stirred at that temperature for 3 h, quenched with a cold solution of ferrous sulfate and tartaric acid (stoichiometric amount) in de-ionized water, stirred vigorously for 30 min, and extracted with ether (50 mL × 3). The combined organic layers were treated with a pre-cooled (0 °C) solution of 5 mL of 30% NaOH (w/v) in brine and stirred for 1 h at rt. The two layers were separated and the aqueous layer was extracted with ether (20 mL × 3). The combined ether layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed (SiO_2, ethyl acetate/hexane = 1.5:8.5) to give **11** (1.7 g, 80%, 80% ee) as colorless syrup.

$[\alpha]_D^{27} = -20.6$ ($c = 1, CHCl_3$); IR (neat) ν cm^{-1}: 3414, 3063, 2936, 2862, 1717, 1454, 1099, 878, 746; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.56–1.68 (m, 6H, 3 × CH_2), 2.81–2.89 (m, 2H, $CH_2CHCHCH_2$), 3.45 (t, J = 5.87 Hz, 2H, CH_2CH_2O), 3.53–3.87 (m, 2H, $CHCH_2OH$), 4.46 (s, 2H, OCH_2Ph), 7.25–7.31 (m, 5H, Ar-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.7, 29.5, 31.4, 55.9, 58.5, 61.8, 70.0, 72.9, 127.5–128.2, 138.3 ppm; ESI-MS $[M+23]^+$: 259; ESI-HRMS Calcd. for $C_{14}H_{20}O_3$ Na $[M+23]^+$ 259.1317, found: 259.1310. The enantiomeric purity was determined by HPLC (Daicel Chiralcel AD column, 15% *i*-PrOH/hexane, flow rate 1.0 mL/min, Detection wavelength: 270 nm): τ_{major} = 9.9 min; τ_{minor} = 11.0 min.

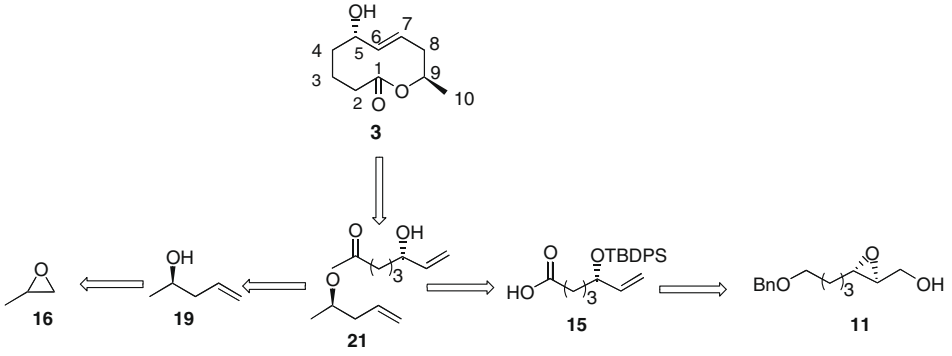
2.3. (3S)-7-(Benzyloxy)-1-hepten-3-ol (**12**)

To a stirred solution of epoxy alcohol **11** (2.25 g, 9.5 mmol) in dry Et_2O:CH_3CN (5:3), 15 mL were added sequentially Ph_3P (7.46 g, 28.6 mmol), pyridine (3.1 mL, 38.1 mmol) and I_2 (4.82 g, 19.06 mmol) at 0 °C. After being stirred for 2 h at 0 °C, H_2O (0.35 mL, 19.06 mmol) was added into the system. The reaction mixture was refluxed for 6 h at 40 °C, then 20% $Na_2S_2O_3$ (aq.) (15 mL) and saturated $NaHCO_3$ (aq.) (15 mL) were added to quench the reaction and the organic layer was extracted with ether (3 × 50 mL). The combined ether extracts were washed with 5% HCl (4 × 10 mL), H_2O and brine, then dried. Evaporation of the solvent gave the residue, which was flash chromatographed eluting with hexane and ethylacetate (9:1) gave **12** (2.0 g, 95%) as colorless oil.

$[\alpha]_D^{27} = +3.8$ ($c = 1, CHCl_3$); IR (neat) ν cm^{-1}: 3414, 3066, 3029, 2933, 2859, 1718, 1642, 1453, 1275, 1102, 993; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.53–1.68 (m, 6H, 3 × CH_2), 3.47 (t, J = 6.4 Hz, 2H, CH_2CH_2O), 4.06–4.12 (m, J = 6.0 Hz, 1H, $CH_2CHOHCH$), 4.49 (s, 2H, OCH_2Ph), 5.07–5.24 (dd, J = 10.3, 17.1 Hz, 2H, $CHCH_2$), 5.80–5.91 (dq, J = 6.2, 10.3, 16.6 Hz, 1H, $CHCHCH_2$), 7.28–7.33 (m, 5H, Ar-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.0, 29.6, 36.7, 70.1, 72.8, 96.1, 114.3, 127.5, 128.2, 138.5, 141.3 ppm; ESI-MS: 243 $[M+23]^+$; ESI-HRMS Calcd. for $C_{14}H_{20}O_2Na$ $[M+Na]^+$: 243.1360, found: 243.1357.

2.4. (1S)-1-[4-(Benzyloxy)butyl]-2-propenyloxy)(tert-butyl)diphenylsilane (**13**)

To a stirred solution of **12** (100 mg, 0.45 mmol) in CH_2Cl_2 (5 mL), imidazole (50 mg, 0.67 mmol), and TBDPSCl (140 μL, 0.54 mmol) were added at 0 °C and stirred at rt for 3 h. The reaction mixture was treated with satd NH_4Cl (5 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The organic layer was washed with water (2 × 10 mL), brine (2 × 10 mL), dried over Na_2SO_4, evaporated, and the residue obtained was purified by column chromatography (60–120 silica gel, 0.3:9.7 ethyl acetate–hexane) to furnish **13** (160 mg, 78%) as colorless syrup. $[\alpha]_D^{27} = +17.3$ $c = 1, CHCl_3$); IR (neat) ν cm^{-1}: 3069, 2933, 2857, 1724, 1463, 1427, 1262, 1108, 999, 701; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.04 (s, 9H, 3 × CH_3), 1.25–1.46 (m, 6H, 3 × CH_2), 3.30 (t, J = 6.8 Hz, 2H, CH_2CH_2O), 4.08–4.14



Scheme 1. Retrosynthesis of stagonolide-F (**3**).

(m, 1H, *H*COTBDPS), 4.40 (s, 2H, OCH_2Ph), 4.91–4.98 (m, 2H, $CHCH_2$), 5.69–5.80 (dq, J = 6.8, 10.5, 16.6 Hz, 1H, $CHCHCH_2$), 7.21–7.37 (m, 10H, Ar-H), 7.59–7.65 (m, 5H, Ar-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.3, 21.0, 27.0, 29.5, 37.3, 70.2, 72.7, 74.5, 114.3, 127.3, 128.2, 129.4, 135.8, 140.7 ppm; ESI-MS: 481 $[M+23]^+$; ESI-HRMS Calcd. for $C_{30}H_{38}O_2$ SiNa $[M+Na]^+$: 481.2518, found: 481.2583.

2.5. (5S)-5-[1-(Tert-butyl)-1,1-diphenylsilyl]oxy-6-hepten-1-ol (**14**)

To a stirred solution of **13** (100 mg, 0.22 mmol) in dichloromethane–water (19:1, 5 mL), DDQ (0.25 g, 1.11 mmol) was added and stirred at reflux for 4 h. saturated aq. $NaHCO_3$ solution (5 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water (5 mL), brine (5 mL), dried over Na_2SO_4, and concentrated. The crude residue was purified by column chromatography (silica gel, 60–120 mesh, ethylacetate–hexane, 0.8:9.2) to afford **14** (69 mg, 85%) as colorless syrup.

$[\alpha]_D^{27} = +20.9$ ($c = 1, CHCl_3$); IR (neat) ν cm^{-1}: 3398, 3070, 2931, 2857, 1710, 1642, 1466, 1425, 1216, 1108, 922,701; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (s, 9H, 3 × CH_3), 1.25–1.44 (m, 6H, 3 × CH_2), 3.47 (t, J = 6.6 Hz, 2H, CH_2CH_2OH), 4.08–4.16 (m, 1H, *H*COTBDPS), 4.93–5.02 (m, 2H, $CHCH_2$), 5.68–5.85 (dq, J = 6.6, 10.2, 16.8 Hz, 1H, $CHCHCH_2$), 7.25–7.42 (m, 5H, Ar-H), 7.58–7.67 (m, 5H, Ar-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.5, 27.0, 29.7, 32.5, 37.1, 62.8, 74.4, 114.4, 127.3, 129.5, 135.9, 140.6 ppm; ESI-MS: 367 $[M-1]^+$; ESI-HRMS Calcd. for $C_{23}H_{32}O_2$SiNa $[M+Na]^+$: 391.2058, found: 391.2069.

2.6. (5S)-5-[1-(Tert-butyl)-1,1-diphenylsilyl]oxy-6-heptenoic acid (**15**)

To a stirred solution of **14** (100 mg, 0.27 mmol) in DMF (5 mL) was added PDC (0.5 g, 1.35 mmol) at room temperature. After 10 h, the mixture was quenched with cold water (5 mL), and extracted with AcOEt (3 × 10 mL). The combined organic layer was washed with $KHSO_4$ (15 mL, 1 mol/L), water (10 mL), and brine (10 mL), respectively, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography of the residue over silica gel (CH_2Cl_2/MeOH, 40:1) afforded **15** as a colorless oil (77 mg, 75% yield).

$[\alpha]_D^{25} = +20.7$ ($c = 1.0, CHCl_3$); IR (neat) ν cm^{-1}: 3444, 3070, 2931, 2858, 1707, 1462, 1425, 1257, 1109; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (s, 9H, 3 × CH_3), 1.38–1.64 (m, 4H, 2 × CH_2, 2.18 (t, J = 7.34 Hz, 2H, CH_2COOH), 4.11–4.19 (m, 1H, *H*COTBDPS), 4.96–5.06 (m, 2H, $CHCH_2$), 5.69–5.86 (dq, J = 6.6, 11.0, 16.8 Hz, 1H, $CHCHCH_2$), 7.32–7.43 (m, 5H, Ar-H), 7.60–7.68 (m, 5H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.5, 27.0, 33.8, 36.6, 74.0, 114.7, 127.3, 129.4, 135.8, 140.2, 179.7 ppm; ESI-MS: 405 $[M+1]^+$; ESI-HRMS Calcd. for $C_{23}H_{30}O_3$SiNa $[M+Na]^+$: 405.1868, found: 405.1861.

2.7. (1R)-1-Methyl-3-butenyl-(5S)-5-[1-(tert-butyl)-1,1-diphenylsilyl]oxy-6-heptenoate(**20**)

To a stirred solution of acid **15** (1 g, 2.6 mmol) and DMAP (64 mg, 0.52 mmol in anhydrous DCM (25 mL) was added alcohol **19** (0.9, 10.4 mmol) taken in DCM at rt. The reaction mixture is cooled to 0 °C and added DCC (1.0 g, 5.2 mmol) in DCM and stirred for 10 min and brought to room temperature and stirred overnight. The white precipitate formed was filtered off and washed with 2N HCl, 5% $NaHCO_3$ and finally with water. The esterification product **20** is purified by distillation at atmospheric pressure at 125 °C. (0.8 g, 65%).

$[\alpha]_D^{27} = +4.5$ ($c = 0.5, CHCl_3$); IR (neat) ν cm^{-1}: 2926, 2855, 1735, 1642, 1462, 1425, 1216, 1110, 761; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (s, 9H, 3 × CH_3), 1.16 (d, J = 5.85 Hz, 3H, CH_3), 2.01 (t, J = 6.5 Hz, 2H, CH_2COO), 2.23–2.33 (m, 6H, 3 × CH_2), 4.04–4.13 (m, 2H, 2 × CH), 4.85–5.09 (m, 4H, olefin), 5.63–5.84 (m, 2H, olefin), 7.29–7.39 (m, 6H, Ar-H), 7.58–7.67 (m, 4H, Ar-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.0, 27.0, 29.7, 36.8, 40.3, 74.2, 96.1, 114.6, 117.6, 127.3, 129.4, 135.8, 140.4 ppm; ESI-MS: 473 $[M+Na]^+$; ESI-HRMS Calcd. for $C_{28}H_{38}O_3$SiNa $[M+Na]^+$: 473.2492, found: 473.2487.

2.8. (1R)-1-Methyl-3-butenyl-(5S)-5-hydroxy-6-heptenoate (**21**)

A solution of **20** (0.10 g, 1.66 mmol) in THF (1 mL) was taken in a plastic bottle, and HF-pyridine (2–3 drops) was added at 0 °C and stirred at room temperature for 12 h. The reaction mixture was quenched with saturated $NaHCO_3$ solution (5 mL) at 0 °C and extracted with AcOEt (2 × 50 mL). The organic layer was washed with saturated $CuSO_4$ solution (5 mL), dried over Na_2SO_4, and the residue obtained was purified by column chromatography (silica gel, 60–120 mesh, ethylacetate–hexane, 2:8) to afford **21** (0.035 g, 75%) as colorless syrup.

$[\alpha]_D^{27} = +6.5$ ($c = 0.5, CHCl_3$); IR (neat) ν cm^{-1}: 2923, 2853, 2360, 1711, 1459, 1375, 1216; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.09 (d, J = 6.1 Hz, 3H, CH_3), 1.56–1.72 (m, 2H), 1.9 (m, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.45–2.60 (m, 2H), 4.0–4.12 (m, 1H), 4.77–4.83 (m, 1H), 5.07, 5.17 (dd, J = 10.5, 16.2 Hz, 2H, olefin), 5.19–5.37 (dd, J = 10.5, 16.6 Hz, 2H, olefin), 5.79–5.91 (dq, J = 5.2, 10.5, 15.8 Hz, 2H, olefin); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 20.4, 27.8, 29.5, 36.0, 72.6, 80.2, 114.9, 116.8, 135.9, 140.6 ppm; ESI-MS: 235 $[M+Na]^+$; ESI-HRMS Calcd. for $C_{12}H_{20}O_3Na$ $[M+Na]^+$: 235.1315, found: 235.5312.

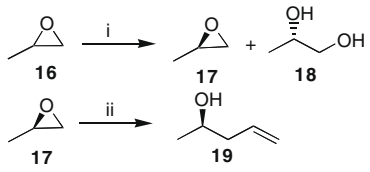
2.9. (5S,9R)-5-Hydroxy-9-methyl-6-nonen-9-olide (**3**)

Ester **21** (50 mg, 0.23 mmol) is dissolved in freshly distilled degassed anhydrous CH_2Cl_2 (100 mL) was treated with Grubb's catalyst I (22 mg, 0.027 mmol) and heated at reflux for 2 days under inert atmosphere. Most of the solvent was then distilled off and the concentrated solution is left to stir at room temperature for 2 h under air bubbling in order to decompose the catalyst. The reaction mixture was evaporated to dryness to give a brown residue, which was purified by column chromatography (silica gel, 60–120 mesh, ethylacetate–hexane, 3:97) to afford **3** (22 mg, 55%) as colorless syrup.

$[\alpha]_D^{25} = -26.3$ ($c = 0.5, CHCl_3$), (lit.[3] $[\alpha]_D^{25} - 27$ ($c = 0.1, CHCl_3$); IR (neat) ν cm^{-1}: 3449, 2924, 2853, 1738, 1644, 1461, 1235, 1099; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.1 (d, J = 6.3 Hz, 3H, CH_3), 1.6–1.72 (m, 2H, CH_2CHOH), 1.85–2.28 (m, 2H, $CH_2CH_2CH_2$), 2.31 (t, J = 6.5 Hz, 2H, CH_2CO), 2.45–2.65 (m, 2H, CH_2CHCH), 3.98–4.0 (m, 1H, CH_2CHOH), 4.8–5.0 (m, 1H, CH_3CHOCO), 5.35–5.40 (dd, J = 15.2, 9.2 Hz, 1H, $CHOHCHCH$), 5.52–5.62 (ddd, J = 15.2, 10.4, 4.2 Hz, 1H, CH_2CHCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.3, 30.0, 31.5, 34.3, 35.0, 71.6, 75.4, 131.2, 134.2, 174.8 ppm. ESI-MS: 185 $[M+H]^+$; ESI-HRMS Calcd. for $C_{10}H_{16}O_3H$ $[M+H]^+$: 185.1153, found: 185.5150.

3. Results and discussion

In designing a route to **21**, we chose racemic propylene oxide **16** as one of the appropriate starting materials (Scheme 2). Thus, commercially available propylene oxide **16** was subjected to Jacobsen's hydrolytic kinetic resolution [8] by using (*R*,*R*)-Salen-Co-OAc catalyst **4** (Fig. 2) to give (*R*)-propylene oxide **17** as a single isomer, and it was isolated from the more polar diol **18** by distillation and the optical purity was proven by comparison with reported literature [8]. Our next task was to construct the homoallylic alcohol **19** after keeping enantiomerically pure epoxide **17** in hand. Thus (*R*)-pro-



Scheme 2. Reagents and conditions: (i) (*R*,*R*)-Jacobsen catalyst, H_2O, rt, 40% and (ii) Vinylmagnesium bromide, CuI, THF, −78 °C, 85%.

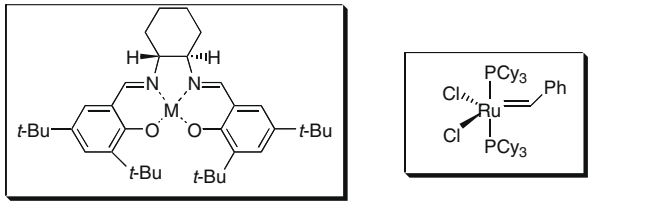


Fig. 2. Catalysts used in synthetic strategy.

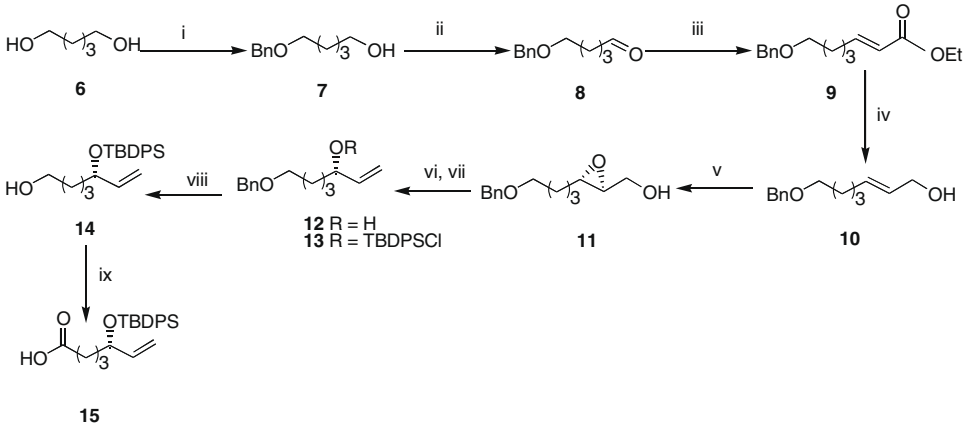
pylene oxide **17** was treated with vinylmagnesium bromide [9] in the presence of CuI to give the homoallylic alcohol **19** in excellent yield (85%).

The synthesis of fragment **15** was initiated from commercially available 1,5-pentanediol **6** as illustrated in Scheme 3. Thus selective monoprotection of **6** with benzyl bromide in DMF gave benzyl-ether **7** in 70% yield, which on oxidation under Swern conditions [10] gave the corresponding aldehyde **8** in 90% yield. Compound **8** was subjected to a two-carbon homologation by means of Wittig reaction [11] using ethoxycarbonylmethylenetr-iphenylphosphorane.

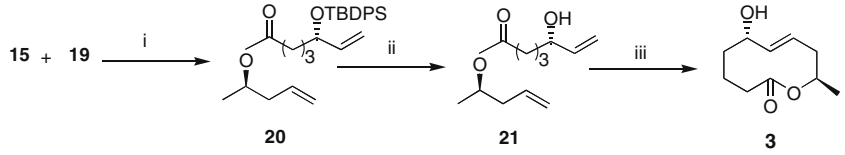
The reaction was carried out in refluxing benzene for 2 h, which gave a mixture of *trans* and *cis* conjugated ester in 88:12 ratio. The *trans* compound **9** was purified and then subjected to chemo-selective reduction [12] using LAH/$AlCl_3$ in anhydrous ether to afford allyl alcohol **10** in 86% yield. Sharpless asymmetric epoxidation [13] of **10** with L-(+)-DET produced 2,3-epoxy alcohol **11** in 85% yield with 80% ee. Accordingly, one pot transformation [14] of 2,3-epoxy alcohol **11** into an allylic alcohol **12** was achieved by the insitu formation of the epoxy iodide and its subsequent reduction with phosphine hydroxyiodide in 95% yield. Allylic alcohol **12** was treated with TBDPSCl to afford **13** (78%), which was subjected to debenzylation with DDQ in CH_2Cl_2–H_2O to give **14** (88%). Finally, the hydroxyl group of **14** was oxidized with pyridinium dichromate [15] (PDC) to give acid **15** in 75% yield. After successfully obtaining the alcohol **19** and acid **15** fragments, the coupling reaction was achieved by employing Steglich esterification [16] (Scheme 4).

Desilylation of **20** was achieved under neutral conditions using HF-Pyridine to give bis-olefin **21**. Finally diene **21** was treated with Grubb's first generation catalyst **5** (Fig. 2) under high dilution condition [17] furnished a 10:1 *E*:*Z* mixture which on chromatographic purification gave the target molecule in 55% yield. The physical and spectral data [20] of **3** are identical to those reported in the literature [4]. Application of this strategy in the total synthesis of other analogues is currently in progress.

The synthesized stagonolide-F (**3**) was evaluated invitro for antibacterial and antifungal activity using 'agar well diffusion anti microbial assay'. The antibacterial activity was evaluated against gram-positive bacterial strains *Staphylococcus aureus* (MTCC 737), *Staphylococcus epidermidis* (MTCC 435), and gram-negative strains *Escherichia coli* (MTCC 1687), and *Pseudomonas aeruginosa* (MTCC 1688). The antifungal activity was evaluated against pathogenic strains *Sacharomyces cereviseae* (MTCC 36), *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 1344) and *Rhizopus oryzae* (MTCC 262). The MIC (minimum inhibitory concentration) values for antibacterial activity were determined using standard broth microdilu-



Scheme 3. Reagents and conditions: (i) NaH, BnBr, 0 °C – rt 4 h, 70%; (ii) $(COCl)_2$, DMSO, DCM, −78 °C, 90%; (iii) $Ph_3P=CHCO_2Et$, anhyd benzene, reflux 2 h, 88%; (iv) LAH/$AlCl_3$, THF, 0 °C 1/2 h, 86%; (v) $Ti(OPr^i)_4$, (+)DET, $PhC(CH_3)_2O_2H$, dry DCM, −20 °C, 5 h, 85%, 80% ee; (vi) Ph_3P, pyridine, I_2, Et_2O:CH_3CN (5:3), 0 °C, H_2O, reflux, 6 h, 95%; (vii) TBDPSCl, Imidazole, DMF, rt, 78%; (viii) DDQ, CH_2Cl_2/H_2O (19:1), reflux, 3 h, 88% and (ix) PDC, DMF, rt, 75%.



Scheme 4. Reagents and conditions: (i) DCC, DMAP, CH_2Cl_2, 0 °C – rt, 65%; (ii) HF-pyridine, CH_2Cl_2, rt, 12 h, 75% and (iii) $(Pcy_3)_2Cl_2Ru$ = CHPh (12 mole%), CH_2Cl_2, 40 °C, 48 h, 55%.

Table 1
Invitro antimicrobial activity of synthesized stagonolide-F (**3**).

Microorganism	Antimicrobial activity			
	Zone of Inhibition[a]		MIC[b] (μg/mL)	
	Compd. **3**	Control	Compd. **3**	Control
Bacterial strains		Streptomycin		Nitrofurantoin
Staphylococcus aureus	16	25	100	50
Staphylococcus epidermis	13	26	100	50
Escherichia coli	14	32	100	25
Psudomonas aeruginosa	17	28	200	100
Fungal strains		Clotrimazole		
Saccharomyces serviseae	16	23		
Candida albicans	14	22		
Aspergillus niger	16	18		
Rhizopus orizae	14	21		

Cell line	IC_{50}[c] (μg/mL)	
	Compd. **3**	Control (Etoposide)
Cytotoxic activity		
THP-1[d]	32.67 ± 4.88	1.4
U-937[e]	34.72 ± 3.45	1.2

[a] ZI: zone of inhibition (diameter in mm).
[b] MIC (minimum inhibitory concentration in μg/mL) was determined as 90% inhibition of growth with respect to positive growth control. Negative control: DMSO, no inhibition.
[c] IC_{50}: inhibitory concentration.
[d] THP-1: human acute monocytic leukemia cell line.
[e] U-937: human leukemic monocyte lymphoma cell line.

tion technique described by NCCLS [18]. Nitrofurantoin was used as reference drug. In comparison with the antimicrobial activity, Clotrimazole was used as reference antifungal drug, while Streptomycin was used as reference antibacterial drug. All the biological data is depicted in Table 1 as zone of inhibition of growth (ZI) and minimum inhibitory concentration (MIC) values. From the activity results, it is observed that stagonolide-F (**3**) has showed potent antifungal and antibacterial activity against all the tested fungal and bacterial strains. The analysis of ZI and MIC values for antibacterial activity revealed stagonolide-F (**3**) has more than 60% antibacterial activity against *Staphylococcus aureus* and moderate activity against other tested bacterial strains in comparison with the tested reference drug's antibacterial activity. Zone of inhibition results for antifungal activity revealed compound **3** has more than 80% activity against *Aspergillus niger* and more than 60% activity against other fungal strains in comparison with tested reference drug's antifungal activity.

Cytotoxic activity was evaluated against THP-1 and U-937 human cancer cell lines (Human acute monocytic leukemia cell line, Human leukemic monocyte lymphoma cell line). Cytotoxicty was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide] assay, according to the method of Mosmann [19]. Etoposide was used as reference cytotoxic drug. IC_{50} values (Inhibotory Concentration) of the test compound **3** is calculated and presented in Table 1. It is evident from the results that the test compound has shown, significant decrease in cell viability in the test cell line in concentration dependant manner.

4. Conclusion

The first synthesis of stagonolide-F (**3**) was accomplished starting from commercially available 1,5-pentanediol. Both the requisite segments with two stereogenic centers were prepared employing Jacobsen's hydrolytic kinetic resolution and Sharpless asymmetric epoxidation reactions, while RCM reaction was used to build the carbon framework. The synthesized stagonolide-F (**3**) is tested for antimicrobial activity and it was found that compound **3** exhibited potent antifungal, significant antibacterial and cytotoxic activities against all the tested strains.

Acknowledgments

We thank Director IICT, Project Director NIPER and Head of the Division Org ll for their encouraging support. PAK thanks CSIR for fellowship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2008.12.002.

References

[1] (a) Antonio Evidente, Rosa Lanzetta, Renato Capasso, Anna Andolfi, Antonio Bottalico, Maurizio Vurro, Maria. Chiara Zonno, Phytochemistry 40 (1995) 1637–1641;
(b) A. Evidente, R. Capasso, A. Andolfi, M. Vurro, M.C. Zonno, Putaminoxins D and E from Phoma putaminum. Phytochemistry 48 (1998) 941–945;
(c) A. Evidente, R. Capasso, A. Andolfi, Antonio Bottalico, M. Vurro, M.C. Zonno, Putaminoxins B and C from Phomaputaminum. Phytochemistry 44 (1997) 1041–1045.
[2] (a) Antonio Evidente, Renato Capasso, M.A. Abouzeid, Rosa Lanzetta, Maurizio Vurro, Antonio Bottalico, J. Nat. Prod. 56 (1993) 1937–1943;
(b) Antonio Evidente, Rosa Lanzetta, Renato Capasso, Maurizio Vurro, Antonio Bottalico, Phytochemistry 34 (1993) 999–1003.
[3] J. Fusher, A. Zeeck, Liebigs Ann. Recyl. (1997) 87–95.
[4] Antonio Evidente, Alessio Cimmmino, Alexander Berestetskiy, Galina Matina, Anna Andolfi, Andrea Motta, J. Nat. Prod. 71 (2008) 31–34.
[5] (a) Jose A Fausto Rivero-Cruz, Genoveva GarcoA-Aguirre, Carlos M. Cerda-GarcoA-Rojasc, Rachel Mata, Tetrahedron 56 (2000) 5337–5344;
(b) Jose Fausto Rivero-Cruz, Martha Macıas, Carlos M Cerda-Garcıa-Rojas, Rachel Mata, J. Nat. Prod. 66 (2003) 511–514;
(c) Oleg Yuzikhin, Alexander Berestetskiy, J. Agric. Food Chem. 55 (2007) 7707–7711.
[6] (a) P. Narendar, B. Gangadasu, Ch. Ramesh, B. China Raju, V. Jayathirtha Rao, Synth. Commun. 34 (2004) 1097–1103;
(b) B. Gangadasu, P. Narender, S. Bharath Kumar, M. Ravinder, B. Ananda Rao, Ch. Ramesh, B. China Raju, V. Jayathirtha Rao, Tetrahedron 62 (2006) 8398–8404;
(c) P. Narender, U. Srinivas, B. Gangadasu, Sukla Biswas, V. Jayathirtha Rao, Bioorgan. Med. Chem. Lett. 15 (2005) 5378–5381;

(d) P. Narender, U. Srinivas, M. Ravinder, B. Ananda Rao, Ch. Ramesh, K. Harakishore, B. Gangadasu, U.S.N. Murthy, V. Jayathirtha Rao, Bioorgan. Med. Chem. 14 (2006) 4600–4609;
(e) K. Srinivas, U. Srinivas, V. Jayathirtha Rao, K. Bhanuprakash, K. Hara Kishore, U.S.N. Murty, Bioorgan. Med. Chem. Lett. 15 (2005) 1121–1123.

[7] (a) Alois Frstner, Karin Radkowski, Conny Wirtz, Richard Goddard, Christian W. Lehmann, Richard Mynott, J. Am. Chem. Soc. 124 (2002) 7061–7069;
(b) Lorenzo de Napoli, Anna Messere, Daniela Palomba, Vincenzo Piccialli, J. Org. Chem. 65 (2000) 3432–3442;
(c) Adao Aparecido Sabino, Ronaldo A. Pilli, Tetrahedron Lett. 43 (2002) 2819–2821.

[8] (a) Scott E. Schaus, Bridget D. Brandes, Jay F. Larrow, Makoto Tokunaga, Karl B. Hansen, Alexandra E. Gould, Michael E. Furrow, Eric N. Jacobsen, J. Am. Chem. Soc. 9 (2002) 1307–1315;
(b) J.T. Louis, W.L. Nelson, J. Org. Chem. 52 (1987) 1309–1315.

[9] (a) Pradeep Kumar, Priti Gupta, S. Vasudeva Naidu, Chem. Eur. J. 12 (2006) 1397–1402;
(b) M.V.R. Reddy, A.J. Yucel, P.V. Ramchandran, J. Org. Chem. 66 (2001) 2512–2514.

[10] (a) A.J. Mancuso, D.S. Brownfain, D. Swern, J. Org. Chem. 44 (1979) 4148–4150; For reviews on Swern oxdation see(b) T.T. Tidwell, Synthesis (1990) 857–870;
(c) T.T. Tidwell, Org. React. 39 (1990) 297–572.

[11] Y.S. Hon, L. Lu, S.Y. Li, J. Chem. Soc. Chem. Commun. (1990) 1627–1628.

[12] S.U. Park, S.K. Chung, M. Newcomb, J. Org. Chem. 52 (1987) 3275–3278.

[13] Yun Gao, Robert. M. Hanson, Janice. M. Klunder, Soo. Y. Ko, Horoko Masamune, Barry. K. Sharpless, J. Am. Chem. Soc. 109 (1987) 5765–5780.

[14] Zuosheng Liu, Jiong Lan, Yulin Li, Tetrahedron: Asymm. 9 (1998) 3755–3762.

[15] Jie Chen, Yang Li, Xiao-Ping Cao, Tetrahedron: Asymm. 17 (2006) 933–941.

[16] Bernhard Neises, Wolfgang Steglich, Angew. Chem. Int. ed. 17 (1978) 522–524.

[17] (a) R.H. Grubbs, S.J. Miller, G.C. Fu, Acc. Chem. Res. 28 (1995) 446–452;
(b) M. Schuster, S. Blechert, Angew. Chem. Int. Ed. Engl. 36 (1997) 2036–2056;
(c) Mauro Bassetti, D. Annibale. Andrea, Alessia Fanfoni, Franco Minissi, Org. Lett. 7 (2005) 1805–1808.

[18] (a) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility, National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 1997.;
(b) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility, National Committee for Clinical Laboratory Standard, Wayne, PA, USA, 1998.;
(c) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility, Tests for Bacteria that Grow Aerobically. Approved Standard, fifth ed., NCCLS, Villanova, PA, 2000 (M7–A5).

[19] T. Mosmann, J. Immunol. Methods 65 (1983) 55.

[20] The small differences in the ^{1}H NMR spectrum of nonenolide 3 with natural stagonolide-F is due to the lower resolution conditions.