Total Synthesis of the Marine-Derived Cyclic Depsipeptide Alternaramide

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Abstract: The first synthesis of the marine fungus derived natural product alternaramide is described using solution phase coupling protocols and via a macrolactonization and macrolactamization route. The structure of alternaramide was confirmed and was supported by single crystal X-ray analysis which exhibited three similar molecules in the asymmetric unit, each with transannular hydrogen bonds.

Key words: natural product, depsipeptide, peptide coupling, macrolactonization, macrolactamization

Alternaramide (**1**; Figure 1) is a cyclic depsipeptide[1] isolated from the marine-derived fungus *Alternaria sp*. SF-5016 by Oh and co-workers in 2009.[2]

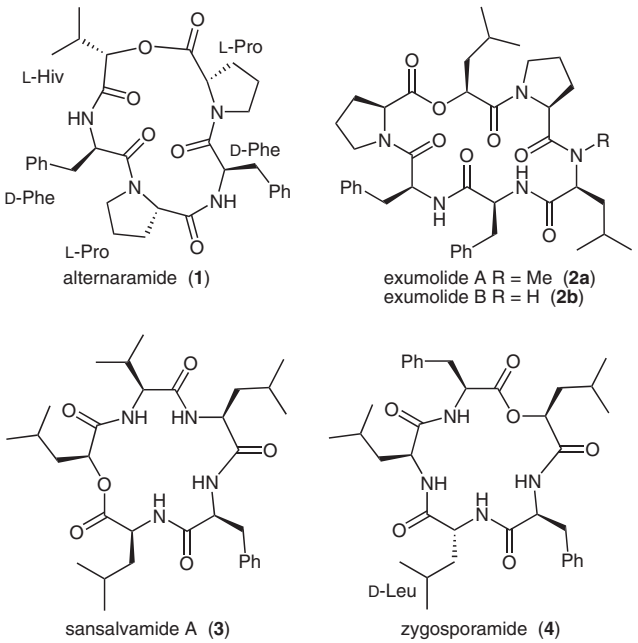
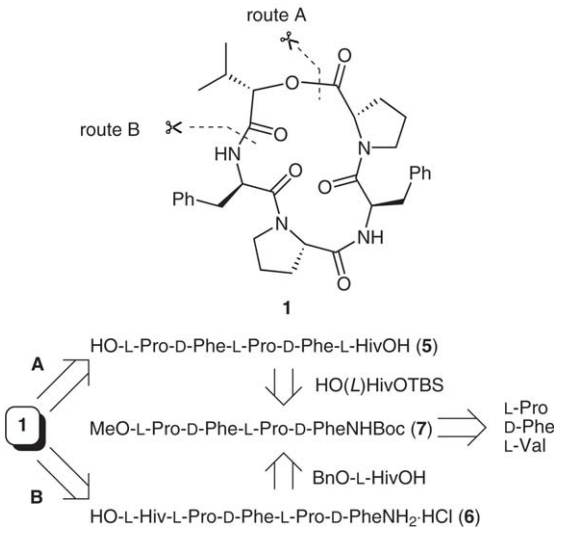


Figure 1 The reported[2] structure of alternaramide (**1**) and related marine-derived depsipeptide natural products

The structure of **1** is related to several depsipeptides isolated from marine-derived fungi, such as the exumolides **2** from *Scytalidium sp*.,[3] sansalvamide A (**3**) from *Fusarium sp*.,[4] and zygosporamide (**4**) from *Zygosporium masonii*.,[5] and was confirmed using a mixture of ^{1}H NMR/^{13}C NMR, degradation and Mosher's ester derivatisation studies. This showed that the depsipeptide contained four amino acid residues (two L-Pro and two D-Phe) and one hydroxy acid residue (L-Hiv) linked by four amide and one ester linkage. While all these peptides possess hydrophobic amino acid residues, the presence of the D-Phe residue in **1** is unusual since the only other depsipeptide in this group to have D-amino acid residues is zygosporamide (**4**; Figure 1). Additionally, the importance of a D-amino acid in analogues of sansalvamide A, in relation to anticancer activity, has been determined by McGuire and McAlpine via SAR studies.[6–10] Total syntheses of **3**[11] and **4**[12] have been described previously, however the total synthesis of **1** has yet to be reported. Therefore, in this letter, we would like to report a short, efficient, solution phase total synthesis of **1**, confirmation of its reported structure and a single crystal X-ray analysis.

The retrosynthesis of **1** is shown in Scheme 1. Alternaramide (**1**) can be obtained via either a macrolactonization or a macrolactamization event on the precursor linear peptides **5** and **6**, respectively. While there are examples of the use macrolactonization in the synthesis of depsipeptides,[13–16] such a cyclisation performed on **5** may be problematic. However, due to the short synthetic sequence to give the precursor **5** we thought this would be a viable route to explore, alongside the macrolactamization approach. The synthesis of both precursor linear peptides **5** and **6** would be from the key tetrapeptide **7** using standard peptide coupling protocols. The tetrapeptide **7** would be obtained from L-Pro, D-Phe, and L-Hiv, which can be obtained from L-Val via hydrolytic diazotization.



Scheme 1 Retrosynthetic analysis of alternaramide (**1**)

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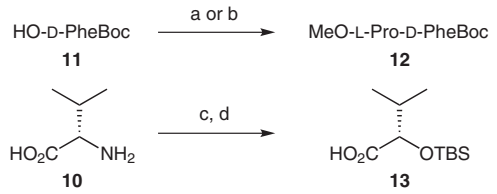
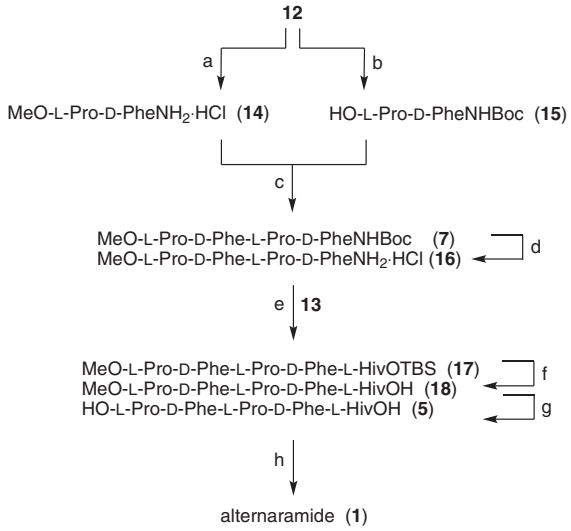
Coupling of proline methyl ester **8** with HO-D-PheHNBoc **9** was initially performed using two coupling methods, either with EDC or via formation of the mixed anhydride which gave the diamino acid **12** in a good yield of 87% and 86%, respectively (Scheme 2). PyBOP® was also employed but yields using this coupling reagent were moderate to poor. The TBS-protected α-hydroxy acid **13** was obtained in three steps in 65% yield via the hydrolytic diazotization of L-Val **10** followed by TBS protection.[17]



Scheme 2 *Reagents and conditions*: (a) EDC, MeO-L-ProNH_2·HCl, CH_2Cl_2, *i*-Pr_2NEt, 87%; (b) EtO_2CCl, NMM, CH_2Cl_2, 0 °C, then MeO-L-ProNH_2·HCl, 86%; (c) $NaNO_2$, H_2SO_4; (d) (i) TBSCl, DMF, imidazole; (ii) K_2CO_3, MeOH, H_2O, 65% over 2 steps.

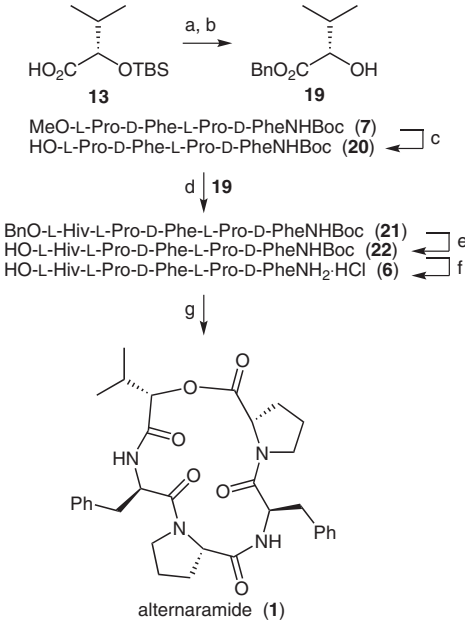
The key tetrapeptide **7** was obtained in two steps from **12**, by Boc deprotection of **12** with 4 M HCl in 1,4-dioxine to give **14**, and the hydrolysis of **12** to the free acid using LiOH to give **15**, both of which were used without further purification. The salt **14** and free acid **15** were then coupled under EDC conditions to give the key tetra-amino acid **7** in 52% yield over the two steps (Scheme 3).



Scheme 3 *Reagents and conditions*: (a) 4 M HCl in 1,4-dioxane; (b) LiOH, THF, MeOH, H_2O; (c) EDC, CH_2Cl_2, *i*-Pr_2NEt, 52% over 2 steps; (d) 4 M HCl in 1,4-dioxane; (e) PyBOP®, CH_2Cl_2, *i*-Pr_2NEt, 71% over 2 steps; (f) TBAF, THF; (g) LiOH, THF, MeOH, H_2O; (h) PyBOP®, CH_2Cl_2, *i*-Pr_2NEt, 5% over 3 steps.

Subsequently, **7** was Boc deprotected and the resultant salt **16** then coupled with **13** using PyBOP® giving **17** in a good yield of 71% over two steps. The free alcohol and acid functions on **17** were then sequentially revealed by deprotection using TBAF and followed by treatment with excess aqueous LiOH. Attempted final ring closure of **5** was then trialed with a number of coupling reagents including PyBOP®, DCC, EDC and 1,3,5-trichlorobenzoyl chloride.[18] The only successful conditions were using excess PyBOP® and diisopropylethylamine which provided alternaramide (**1**) in a poor isolated yield of only 5% over the three steps, presumably due to the low nucleophilicity of the hydroxyl group. Consequently, route B (Scheme 1) with the final ring closure via a macrolactamization was pursued.

The requisite substrate for the macrolactamization was synthesized in four steps from the key tetrapeptide **7** (Scheme 4). A protecting group switch on the L-Hiv fragment was achieved in two steps to give the benzyl ester protected L-Hiv fragment **19** in 52% yield. This was then successfully coupled to the saponified tetrapeptide **20** in 73% yield over the two steps. Finally, the free acid and amine groups on **20** were revealed by sequential hydrogenolysis followed by acid-mediated Boc deprotection. The macrolactamization of **6** was then achieved using excess PyBOP® with diisopropylethyl amine and catalytic DMAP, giving alternaramide (**1**) in 48% yield over the three steps.[19]



Scheme 4 *Reagents and conditions*: (a) BnC(O)Cl, CH_2Cl_2, Et_3N, DMAP; (b) TBAF, THF, 52% over 2 steps; (c) LiOH, MeOH, THF, H_2O; (d) PyBOP®, CH_2Cl_2, *i*-Pr_2NEt, 73% over 2 steps; (e) Pd/C (10%), H_2, MeOH; (f) 4 M HCl in 1,4-dioxane; (g) PyBOP®, CH_2Cl_2, *i*-Pr_2NEt, DMAP (cat.) 48% over 3 steps.

The spectroscopic data for our synthetic alternaramide sample agreed well with that reported by Oh and co-workers and copies of the relevant ^{1}H NMR and ^{13}C NMR spectra are contained within the supplementary information.[20,21] Crystals suitable for single crystal X-ray analysis were obtained and Figure 2 shows the molecular depiction of one of three similar molecules of (–)-**1** in the asymmetric unit.[22] Despite the use of Mo-Kα radiation for this light atom structure, determination of the absolute structure

parameter[23] via Parson's Q-value method[24] led to a value that supported the proposed absolute structure for the molecule. The molecular conformation is stabilised by transannular hydrogen bonds.

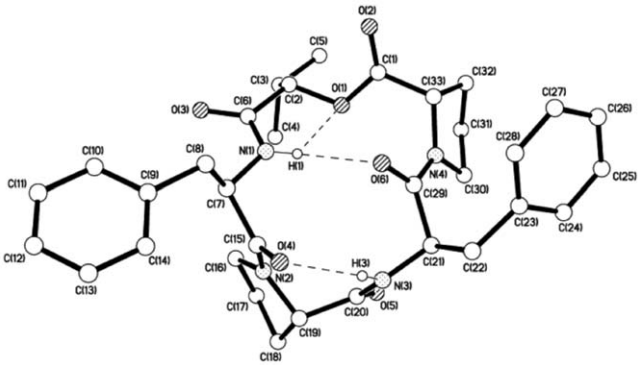


Figure 2 Crystal structure representation of alternaramide [(–)-**1**][22]

In conclusion, we have reported the first total synthesis of alternaramide [(–)-**1**] using standard solution phase peptide coupling protocols. Two synthetic pathways have been investigated using a macrolactonization and a macrolactamization approach giving alternaramide in a best overall yield of 11% in eight steps. Finally, the structure of synthetic alternaramide [(–)-**1**] was confirmed by single crystal X-ray analysis.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Included are the ^{1}H NMR and ^{13}C NMR spectra of synthetic alternaramide [(–)-**1**] and crystallographic data for (–)-**1**.

Acknowledgment

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(19) Selected Physical Data for New Compounds:
MeO-L-Pro-D-PheNHBoc [(–)-**12**]: colourless oil (3.05 g, 87%); R_f (EtOAc–PE, 3:1) 0.65; $[\alpha]_D^{19}$ –5.5 (c = 1, $CHCl_3$). IR (solution, $CHCl_3$): 3584, 3430, 2978, 1746, 1707, 1645, 1437, 1171 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24–7.33 (m, 5 H), 5.55 (d, J = 8.8 Hz, 1 H), 4.69–4.71 (m, 1 H), 4.36 (dd, J = 3.6, 7.6 Hz, 1 H), 3.75 (s, 3 H), 3.60–3.62 (m, 1 H), 3.07–3.09 (m, 1 H), 2.98–3.02 (m, 1 H), 2.74–2.76 (m, 1 H), 1.90–1.98 (m, 4 H), 1.49 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.21 (C), 170.22 (C), 154.91 (C), 136.42 (C), 129.40 (CH), 128.28 (CH), 126.81 (CH), 79.44 (C), 58.65 (CH), 53.53 (CH), 52.05 (Me), 46.66 (CH_2), 40.07 (CH_2), 28.85 (CH_2), 28.25 (Me), 24.33 (CH_2). HRMS: m/z [M + Na^+] calcd for $C_{20}H_{28}N_2O_5$: 399.1896; found: 399.1885.
MeO-L-Pro-D-Phe-L-Pro-D-PheNHBoc (–)-**7**: white foam (0.97 g, 52% over 2 steps); R_f (EtOAc–PE, 3:1) 0.38; $[\alpha]_D^{21}$ –8.1 (c = 1, $CHCl_3$). IR (solution, $CHCl_3$): 3584, 3295, 2980, 1742, 1642, 1497, 1449, 1171 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.17–7.25 (m, 10 H), 5.51 (d, J = 7.6 Hz, 1 H), 4.86 (dt, J = 6.4, 8.4 Hz, 1 H), 4.54–4.57 (m, 1 H), 4.36 (dd, J = 2.4, 8.0 Hz, 1 H), 4.27 (dd, J = 4.0, 8.4 Hz, 1 H), 3.68 (s, 3 H), 3.49–3.53 (m, 2 H), 2.93–3.02 (m, 4 H), 2.75–2.77 (m, 1 H), 2.60–2.63 (m, 1 H), 2.46 (s, 1 H), 1.98–2.03 (m, 1 H), 1.78–1.93 (m, 4 H), 1.50–1.61 (m, 3 H), 1.41 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.31 (C), 171.08 (C), 170.43 (C), 169.53 (C), 155.21 (C), 136.62 (C), 129.54 (CH), 129.50 (CH), 129.33 (C), 128.46 (CH), 128.34 (CH), 126.94 (CH), 126.84 (CH), 79.75 (C), 60.16 (CH), 58.80 (CH), 53.92 (Me), 52.42 (CH), 52.22 (CH), 46.84 (CH_2), 46.69 (CH_2), 39.63 (CH_2), 39.00 (CH_2), 28.91 (CH_2), 28.37 (Me, masked CH_2), 24.51 (CH_2), 24.18 (CH_2). HRMS: m/z [M + Na^+] calcd for $C_{34}H_{44}N_4O_7$: 643.3108; found: 643.3098.
MeO-L-Pro-D-Phe-L-Pro-D-Phe-L-HivOTBS (–)-**17**: colourless foam (0.52 g, 71% over 2 steps); R_f (EtAOc) 0.78; $[\alpha]_D^{16}$ –12.4 (c = 1, $CHCl_3$). IR (solution, $CHCl_3$): 3416, 3290, 2957, 1743, 1658, 1515, 1451, 1254, 1052 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.13–7.27 (m, 10 H), 7.07 (d, J = 7.6 Hz, 2 H), 4.82–4.87 (m, 1 H), 4.73–4.77 (m, 1 H), 4.41 (dd, J = 2.4, 8.4 Hz, 1 H), 4.23 (dd, J = 4.0, 7.6 Hz, 1 H), 3.96 (s, 3 H), 2.88–3.01 (m, 4 H), 2.64–2.68 (m, 1 H), 2.52–2.57 (m, 1 H), 2.44–2.48 (m, 1 H), 2.02–2.09 (m, 1 H), 1.95–1.99 (m, 1 H), 1.75–1.88 (m, 5 H), 1.60–1.66 (m, 1 H),

1.46–1.51 (m, 2 H), 0.90–0.92 (m, 15 H), 0.40 (s, 3 H), –0.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.83 (C), 172.28 (C), 170.63 (C), 170.51 (C), 169.91 (C), 136.72 (C), 135.69 (C), 129.44 (CH), 129.31 (CH), 128.68 (CH), 128.34 (CH), 127.33 (CH), 126.82 (CH), 77.38 (CH), 60.53 (CH), 58.72 (CH), 52.45 (CH), 52.28 (CH), 52.09 (Me), 46.77 (CH_2), 46.68 (CH_2), 38.79 (CH_2), 38.76 (CH_2), 32.62 (C), 29.39 (CH_2), 28.91 (CH_2), 25.76 (CH_2), 24.40 (CH_2), 23.69 (CH_2), 19.60 (Me), 18.07 (Me), 16.00 (C), –5.00 (Me), –5.09 (Me). HRMS: *m*/*z* [M + Na$^+$] calcd for $C_{40}H_{58}N_4O_7Si_1$: 757.3972; found: 757.3955.

BnO-L-Hiv-L-Pro-D-Phe-L-Pro-D-PheNHBoc (–)-**21**: colourless foam (0.19 g, 73% over 2 steps); R_f (EtOAc–PE, 3:1) 0.65; $[\alpha]_D^{18}$ –6.4 (*c* = 1, $CHCl_3$). IR (film): 3584, 3413, 2965, 2248, 1747, 1685, 1645, 1454, 1172 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14–7.27 (m, 15 H), 7.05–7.07 (m, 1 H), 5.41 (d, *J* = 7.6 Hz, 1 H), 5.08 (dd, *J* = 12.0, 12.4 Hz, 2 H), 4.94 (t, *J* = 4.4 Hz, 1 H), 4.87 (q, *J* = 7.2 Hz, 1 H), 4.56–4.59 (m, 1 H), 4.40 (dd, *J* = 3.6, 8.4 Hz, 1 H), 4.32 (dd, *J* = 2.8, 8.4 Hz, 1 H), 3.53–3.56 (m, 2 H), 2.94–2.99 (m, 4 H), 2.68–2.74 (m, 1 H), 2.49–2.61 (m, 2 H), 2.29–2.30 (m, 1 H), 2.24–2.27 (m, 1 H), 1.50–1.96 (m, 6 H), 1.39 (s, 9 H), 1.03 (d, *J* = 6.8 Hz, 3 H), 0.97 (d, *J* = 6.8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.10 (C), 171.12 (C), 170.60 (C), 169.68 (C), 169.46 (C), 168.93 (C), 136.59 (C), 135.21 (C), 135.02 (C), 129.53 (CH), 129.49 (CH), 128.63 (CH), 128.57 (CH), 128.45 (CH), 128.39 (CH), 128.32 (CH), 126.93 (CH), 126.84 (CH), 79.72 (CH), 77.58 (CH), 67.18 (CH_2), 66.93 (CH_2), 60.36 (CH), 58.42 (CH), 53.86 (CH), 52.44 (CH), 46.88 (CH_2), 46.73 (CH_2), 38.97 (CH_2), 31.92 (CH_2), 30.18 (Me), 29.69 (CH_2), 28.38 (CH_2), 24.23 (CH_2), 18.62 (Me), 17.15 (Me); one carbon masked. HRMS: *m*/*z* [M + Na$^+$] calcd for $C_{45}H_{56}N_4O_9$: 819.3945; found: 819.3899.

(20) Alternaramide [(–)-**1**]: colourless crystalline solid (77 mg, 48% over 3 steps); mp 217–218.5 °C (recrystallised from CH_2Cl_2–PE); R_f (EtOAc–PE, 3:1) 0.48; $[\alpha]_D^{19}$ –2.8 (*c* = 0.5, MeOH). IR (film): 3584, 3480, 3300, 3029, 2961, 2929, 2875, 2248, 1744, 1683, 1643, 1634, 1529, 1452, 1275, 1094 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12 (d, *J* = 9.6 Hz, 1 H), 7.17–7.32 (m, 11 H), 5.34 (d, *J* = 2.4 Hz, 1 H), 4.91–5.02 (m, 2 H), 4.85 (d, *J* = 7.2 Hz, 1 H), 4.30 (dd, *J* = 5.2, 8.8 Hz, 1 H), 3.41–3.46 (m, 1 H), 3.35 (dd, *J* = 9.2, 13.2 Hz, 1 H), 3.26 (m, 2 H), 2.99–3.03 (m, 2 H), 2.90 (dd, *J* = 5.2, 13.2 Hz, 1 H), 2.49–2.54 (m, 2 H), 2.42–2.44 (m, 1 H), 1.93–1.99 (m, 2 H), 1.84–1.93 (m, 2 H), 1.72–1.78 (m, 1 H), 1.63–1.69 (m, 1 H), 1.46 (sept, *J* = 6.8 Hz, 1 H), 0.82 (d, *J* = 6.8 Hz, 3 H), 0.71 (d, *J* = 6.8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.16 (C), 169.66 (C), 169.19 (C), 168.86 (C), 167.26 (C), 136.49 (C), 135.00 (C), 128.66 (CH), 128.30 (CH), 127.58 (CH), 127.36 (CH), 126.29 (C), 125.48 (C), 75.95 (CH), 58.92 (CH), 57.28 (CH), 51.85 (CH), 51.13 (CH), 45.89 (CH_2), 44.94 (CH_2), 39.43 (CH_2), 35.95 (CH_2), 28.65 (CH), 28.50 (CH_2), 23.75 (CH_2), 23.48 (CH_2), 18.09 (Me), 14.83 (Me). HRMS: *m*/*z* [M + Na$^+$] calcd for $C_{33}H_{40}N_4O_6$: 611.2846; found: 611.2833.

(21) See Supporting Information for copies of the ^{1}H NMR and ^{13}C NMR spectra for synthetically prepared alternaramide [(–)-**1**].

(22) Crystal data for (–)-**1**: $C_{33}H_{40}N_4O_6$, M = 588.69, orthorhombic, $P2_12_12_1$; a = 12.0808 (4), b = 22.0624 (7), c = 34.9650 (11) Å, *V* = 9319.3 (5) Å^3; D_{calc} = 1.259 g/cm^3; μ(Mo–Kα) = 0.087 mm^{-1}; λ = 0.71073 Å, T = 150 (2) K; 104556 total reflections, 26198 unique data (R_{int} = 0.0456); solved by direct methods and refined on F^2 values to give *R*1 = 0.0473 [$F^2 > 2\sigma(F^2)$] (19948 observed reflections), *wR*2 = 0.1107 (all data). Absolute structure parameter = –0.09 (22)[23,24]. CCDC 801185 contains the supplementary crystallographic data for compound (–)-**1**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; fax: +44 (1223)336033 or e-mail: deposit@ccdc.cam.ac.uk.

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