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FIVE NEW SULFUR-CONTAINING POLYBROMINATED BISINDOLES FROM THE RED ALGA *LAURENCIA BRONGNIARTII*

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Abstract – Five new sulfur-containing polybrominated bisindole (**1**~**5**) were isolated from the red alga, *Laurencia brongniartii*, in addition to six related compounds (**6**~**11**), which have been previously found in the Okinawan alga. The structures of these new bisindole metabolites were elucidated on the basis of spectroscopic studies, as well as X-Ray crystallographic analysis of **1**.

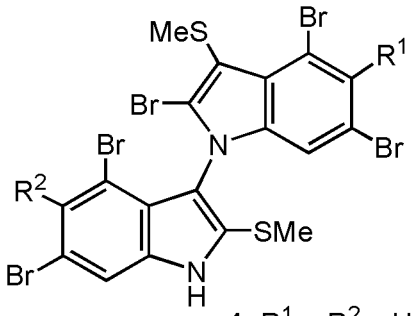
INTRODUCTION

Laurencia brongniartii J. Agardh is a marine red alga, which is distributed in Taiwan, southern Japan, West Indies, and the Caribbean Sea.[1] Previous studies on this species reported isolation of bromoindoles[2] and methylthiobromoindoles,[3~6] among which (**7**~**10**) were recently identified as potent anti-Methicillin-Resistant *Straphylococcus aureus* (anti-MRSA) substances by Kamei *et al.*[7] Further chemical study on this alga by us led to the isolation of five new sulfur-containing polybrominated bisindoles (**1**~**5**), together with six related compounds which had previously been found in *L. brongniartii* from Taiwanese and Japanese collections.[3~5] In this paper, we describe the isolation and structure elucidation of these new bisindole compounds from the plant distributed in the Amami Islands of Kyushu, Japan.

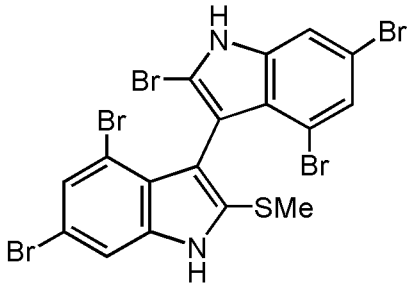
RESULTS AND DISCUSSION

The methanol extract of the red alga of *L. brongniartii* was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 soluble materials were repeatedly fractionated by silica gel flash column chromatography and purified by preparative HPLC to yield small amounts of five new sulfur-containing polybrominated indoles (**1~5**) and six known compounds (**6~11**). The structures of new compounds were elucidated by a

combination of 1D and 2D NMR spectroscopy, X-Ray crystal analysis, and by comparison with previously published spectral data.



1 $R^1 = R^2 = H$
2 $R^1 = H$; $R^2 = Br$
3 $R^1 = Br$; $R^2 = H$
4 $R^1 = R^2 = Br$



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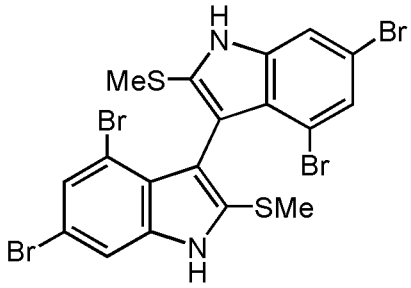

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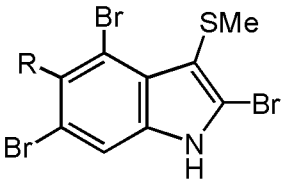

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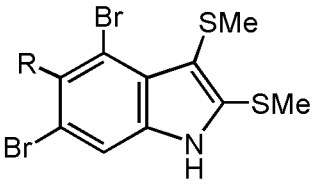
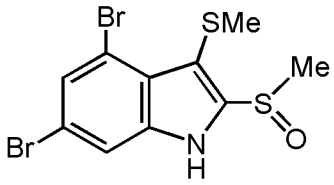


9 R = H
10 R = Br



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Compound (**1**) was obtained as colorless crystals. The FABMS spectrum of **1** gave molecular ion clusters at *m/z* 712, 714, 716, 718, 720, and 722 for $[M-H]^-$, at *m/z* 713, 715, 717, 719, 721, and 723 for $[M]^+$, and at *m/z* 714, 716, 718, 720, 722, and 724 for $[M+H]^+$, indicating the presence of five bromine atoms. The molecular formula, $C_{18}H_{11}N_2Br_5S_2$, was determined by the high-resolution FABMS spectrum ($[M+H]^+$ *m/z* 720.6299, Δ +0.1 mmu).

The ^1H NMR spectrum (Table1) of compound (**1**) exhibited two methyl singlets at δ 2.30 and 2.41. The two sets of signals for meta-coupled aromatic protons were observed at *δ* 6.95 (1H, d, *J*=1.8 Hz) and 7.53 (1H, d, *J*=1.8 Hz), and at 7.44 (1H, d, *J*=1.2 Hz) and 7.55 (1H, d, *J*=1.2 Hz). The signal at *δ* 8.63 (1H, brs) was assigned to a N-H group. The chemical shifts and couplings of the aromatic protons closely resembled those of **6**, with the exception that **1** lacked an amine proton signal and that a *meta*-related aromatic proton shifted to a high field of *δ* 6.95 from that (*δ* 7.48) observed for **6**. The ^{13}C NMR spectrum of **1** exhibited well resolved eighteen carbon signals, consisting of twelve quarternary, four methine, and two methyl carbons, as determined by DEPT experiment, whereas only nine carbon signals were reported in the ^{13}C NMR spectrum of compound (**6**). These ^1H and ^{13}C NMR spectral data suggested the unsymmetrical properties of the bisindole molecule (**1**), differing from the spectral characteristics of compound (**6**). The full and unambiguous proton and carbon NMR spectral assignments for **1** were confirmed using a combination of DEPT, COSY, NOESY, HMBC, and HMQC experiments. Since compound (**1**) showed only one amine proton signal in the NMR spectrum, we assumed that C-3' is

linked to N-1 of the other indole ring. Furthermore, decoupling of the amine proton (NH-1') at δ 8.63 gave NOEs on the methyl protons (SMe-2') at δ 2.30 and an aromatic proton at δ 7.55 (H-7').

Table 1. ^{1}H NMR spectral data[a] for bisindoles (**1**~**5**)

H	**1**	**2**	**3**	**4**	**5**
1	–	–	–	–	8.50 br s
5	7.53 d (1.8)	7.53 d (1.8)	–	–	7.39 d (1.4)
7	6.95 d (1.8)	6.92 d (1.8)	7.13 s	7.11 s	7.49 d (1.4)
1'	8.63 br s	8.65 br s	8.63 br s	8.88 br s	8.42 br s
5'	7.44 d (1.2)	–	7.45 d (1.8)	–	7.40 d (1.4)
7'	7.55 d (1.2)	7.75 s	7.56 d (1.8)	7.75 s	7.48 d (1.4)
3-SMe	2.41 s	2.41 s	2.39 s	2.39 s	–
2'-SMe	2.30 s	2.30 s	2.30 s	2.31 s	2.31 s

[a] Measured in $CDCl_3$, *J* couplings (in parentheses) in Hz.

An NOE from the methyl protons (SMe-2') to another aromatic proton (H-7) at δ 6.95 was also observed (Figure 1 and Table 2). On the basis of all the above spectral information and the NOE results, the structure of compound (**1**) was elucidated as a new polybrominated bisindole, in which the two indole rings linked through N-1 and C-3'. The bisindole ring system of this type has been known in the metabolites of marine blue-green alga *Rivularia firma*.[8]

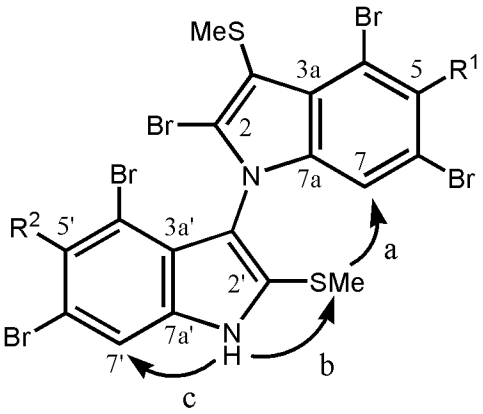

Figure 1. Key NOEs (illustrated with solid arrows) observed for **1**~**4**.

Table 2. NOEs enhancements (%)

	1	**2**	**3**	**4**
a	0.6	1.4	1.8	0.4
b	1.7	2.1	2.7	1.7
c	8.4	9.7	10.7	8.0

Confirmation of the structure (**1**) was made by an X-Ray crystallographic analysis. As can be seen in Figure 2, two indole rings are both planar and are nearly perpendicular to one another. Compound (**1**) was found to be a racemic mixture because the space group of the crystal is $P2_1/c$. It was further confirmed by its optical rotation measurement.

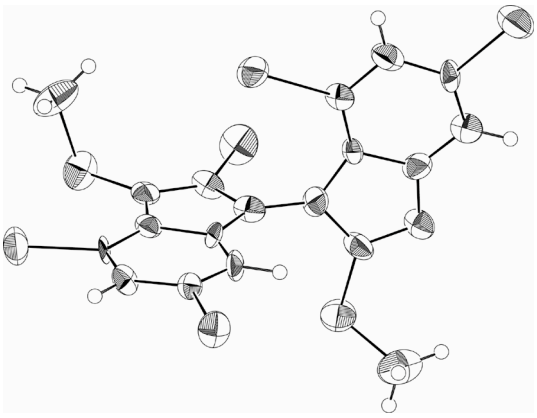

Figure 2. Perspective view of the crystal structure of **1**.

The second compound (**2**) exhibited a molecular formula of $C_{18}H_{10}N_2Br_6S_2$, as determined by HRFABMS spectrum (*m/z* 797.5309 $[M]^+$, Δ -1.5 mmu). It contained an extra bromine atom instead of a hydrogen atom of **1**. The IR and UV spectral properties showed close similarities to those of **1**. Eighteen signals from ^{13}C NMR spectrum showed a marked structural resemblance to compound (**1**). The ^1H NMR spectral data of **2** (Table1) were also very similar to those observed for compound (**1**), the significant difference being the absence of a *meta*-related aromatic proton (H-5') in **2**. Furthermore, NOEs from NH-1' to SMe-2' and H-7', and from SMe-2' to H-7, were confirmed (Table 2) in the NOE difference spectra of **2**, showing the similar magnitudes of NOE enhancements to those observed for **1**. All these spectral data indicated structure **2** for the second new brominated bisindole compound.

The third compound (**3**) was considered to be an isomer of **2** from the molecular formula, $C_{18}H_{10}N_2Br_6S_2$, determined by HRFABMS spectrum (*m/z* 797.5298 $[M]^+$, Δ -2.6 mmu). When the ^1H NMR spectrum of **3** was compared to that of **1**, there was a disappearance of doublet signals corresponding to the *meta*-coupled aromatic protons at δ 6.95 (H-7) and 7.53 (H-5) in **1** and the appearance of only a singlet aromatic proton at δ 7.13 in **3**, which attributed to the replacement of an aromatic hydrogen by a bromine in **3**. The bromine was located at C-5 position on the comparison of NOE results with those of **1** and **2**. (Figure 1 and Table 2). The structure of **3** was thus assigned as an isomer of **2** differing in the brominated carbons from one another.

The fourth compound (**4**) was found only in trace amounts, and the FABMS spectrum showed characteristic isotope ion peaks at m/z 871, 873, 875, 877, 879, 881, and 883, corresponding to $[M-H]^-$ ion, indicating the presence of seven bromine atoms in the molecule, $C_{18}H_9N_2Br_7S_2$. The ^1H NMR spectra including the NOE difference spectrum showed a marked structural resemblance to other bisindoles (**1**, **2**, and **3**) by comparison of the spectra (Tables 1 and 2), in which a new feature was the observation of the two aromatic proton singlets at δ 7.11 and 7.75 for **4**, while the ^1H NMR spectrum of **3** exhibited signals due to an aromatic singlet at δ 7.13 (H-7) and two *meta*-coupled aromatic protons at δ 7.45 (H-5') and 7.56 (H-7'). On the basis of these spectral data, the structure of **4** was shown to be identical with **3** except for bromination at C-5'.

The fifth compound (**5**) had the molecular formula, $C_{17}H_9N_2Br_5S$, as determined by HRFABMS spectrum (*m/z* 671.6364 $[M]^+$, Δ +0.2 mmu). Analysis of the UV, ^1H and ^{13}C NMR and MS spectral data suggested **5** to be structurally related to the known bisindole (**6**), with the exception that **5** contained an extra bromine atom instead of a SMe group of **6**. The ^1H NMR spectrum of **5** contained well resolved seven signals, showing the presence of two NH groups, four *meta*-coupled aromatic protons, and a methyl group (Table 1), while the ^1H NMR spectrum of **6** revealed only three signals for two *meta*-coupled aromatic protons and a methyl singlet owing to the symmetrical nature of the molecule. A long-range correlation in the HMBC spectrum of **5** showed that the proton at δ 7.39 due to H-5 correlated with C-3a and C-7, while

the proton at δ 7.49 due to H-7 correlated with C-3a and C-5. Similarly, the proton at δ 7.40 (H-5') showed three-bond HMBC correlations with C-3a' and C-7'. A proton at δ 7.48 (H-7') correlated with C-3a' and C-5'. The SMe group was placed at C-2' by correlation observed in the HMBC spectrum. These HMBC data determined the locations of the brominated carbons unambiguously. All protonated carbons were also assigned by an HMQC experiment. As shown in Figure 3, NOE interactions were observed for the pairs NH-1'(δ 8.42)/H-7'(δ 7.48), NH-1(δ 8.50)/H-7(δ 7.49), and NH-1'/SMe-2', indicating that the substitution pattern of the benzene rings were the same in both indole rings of **5**. All the spectral observations were consistent with the unsymmetrical structure of **5**, bearing a bromine atom at C-2 instead of a SMe group of **6**.

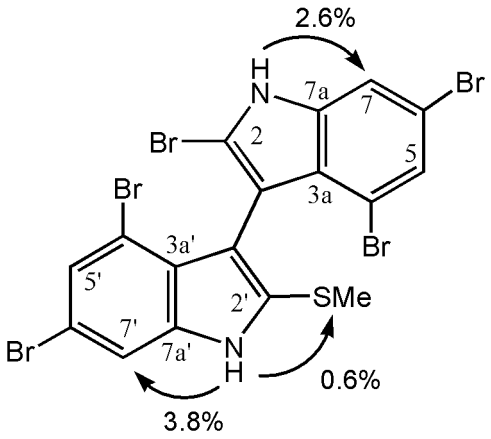

Figure 3. NOEs (%) observed for **5**

Compounds (**6**-**10**) previously known were identified by comparison of their spectroscopic properties with literature data.[3~5]

The new bisindoles (**1**-**5**) are probably formed by oxidative coupling or by oxidative dimerization from the suitable precursors of co-occurred **7**-**10** (in vivo) which are major metabolites of *L. brongniartii*.

EXPERIMENTAL

Melting point was determined on a Yanagimoto micro melting point apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV and IR spectra were recorded on JASCO ETC-505S and JASCO FT/IR-420S spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on either a JEOL GSX 500 or a LA 500 spectrometers (500 MHz for ^{1}H, 125 MHz for ^{13}C) at 25 °C.

^{1}H and ^{13}C NMR chemical shifts (δ) were referenced to residual $CHCl_3$ at 7.26 ppm and $CDCl_3$ at 77.0 ppm, respectively. Fast-atom bombardment (FAB) MS spectra were measured on a JEOL SX102A double focusing mass spectrometer employing Xe atoms in a thioglycerol matrix. Column chromatography was carried out by flash technique using 40-63μm silica gel 60N (Kanto Chemical, Tokyo) and TLC analysis was carried out on pre-coated silica gel 60 F_{254} plates, 0.25 mm thick (Merck). HPLC separations were performed on a Mightysil Si 60 column (Kanto Chemical, Tokyo, 10 x 250 mm, 5 μm) using a Waters 501 pump equipped with a Eyela UV-2000 detector.

Plant Material

The red alga *Laurencia brongniartii* plant was collected in October 2002 at the coast of Kikai Island, Kagoshima, Japan and a voucher specimen is deposited at the Education and Research Center for Marine Resources and Environment, Faculty of Fisheries, Kagoshima University. The plant materials were stored in a freezer prior to extraction.

Extraction and Isolation

The alga *Laurencia brongniartii* (4 kg, wet weight) was cut into small pieces and soaked in MeOH (6 L) for a week in a refrigerator (4 °C). The MeOH extract was concentrated under vacuum to afford a brown residue (23 g) which was partitioned between CH_2Cl_2 and water. The CH_2Cl_2-soluble material (5.6 g) was chromatographed on a silica gel column, using hexane with increasing amounts of EtOAc (0-100%) as eluent, to give eight major fractions (*A*~*H*). The fraction was monitored by ^{1}H NMR spectrum. Fractions *B*, *C*, and *D* each were purified by silica gel column chromatography (hexane- CH_2Cl_2, 2:1) to give **7** (2.2 g), **8** (29.1 mg) and **9** (514 mg), respectively. Fraction *E* was chromatographed repeatedly on a silica gel column (hexane- CH_2Cl_2, 2:1 and then hexane-ether, 9:1) to afford **6** (15.1 mg), **10** (27.9 mg) and the residue. Likewise, fraction *G* was subjected to column chromatography under the same conditions as for fraction *E* to afford **11** (35.7 mg) and the residue. The residue from fractions *E* and *G* was combined with fraction *F* on the basis of their similar nature on TLC and their ^{1}H NMR spectra. The pooled fraction was passed through silica gel column (hexane- CH_2Cl_2, 2:1) and purified by HPLC (hexane-CH_2Cl_2, 50:50, flow rate 3 mL/min, detection at 254 nm) to afford **1** (14.2 mg), **2** (0.6 mg), **3** (0.8 mg), **4** (0.4 mg) and **5** (1.5 mg).

Compound (1) [2,4,4',6,6'-pentabromo-2',3-bis(methylthio)-1,3'-bi-1*H*-indole]. A colorless plates (hexane-EtOAc), mp 243~245 °C; $[\alpha]^{25}_D$ 0° (*c* 0.68, MeOH); UV (MeOH) λ_{max} 231 (ε 83,300), 302 (ε 28,300) nm; IR (KBr) ν_{max} 3400, 1600, 1547, 1468, 1428, 1388, 1315, 1172, 958, and 835 cm^{-1}. ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 141.1 (C-7a), 136.1 (C-7a'), 133.0 (C-2'), 128.9 (C-5), 128.7 (C-2), 128.3 (C-5'), 125.8 (C-3a), 122.7 (C-3a'), 117.3 (C-6')[a], 116.3 (C-6)[a], 115.5 (C-3'), 114.4 (C-4)[a], 113.5 (C-7'), 113.4 (C-7), 111.9 (C-4')[a], 110.9 (C-3), 21.9 (3-SMe) and 18.1 (2'-SMe), [[a] Signals may be interchanged]. HRFABMS *m/z* 720.6299 $[M+H]^+$ (calcd for $C_{18}H_{12}N_2{}^{79}Br_2{}^{81}Br_3S_2$, Δ +0.1 mmu), FABMS: $[M-H]^-$ at *m/z* 712, 714, 716, 718, 720, 722, $[M]^+$ at *m/z* 713, 715, 717, 719, 721, 723 and $[M+H]^+$ at *m/z* 714, 716, 718, 720, 722, 724.

Compound (2) [2,4,4',5',6,6'-hexabromo-2',3-bis(methylthio)-1,3'-bi-1*H*-indole]. $[\alpha]^{25}_D$ 0° (*c* 0.012, MeOH); UV (MeOH) λ_{max} 233(ε 89,400), 300 (ε 26,700) nm; IR (KBr) ν_{max} 3390, 1593, 1547, 1410, 1383, 1348, 1297, 1172, and 957 cm^{-1}. ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 141.0, 134.5, 134.4, 129.0, 128.7, 125.8, 124.6, 121.6, 120.0, 116.4, 115.3, 115.2, 114.5, 114.4, 113.4, 111.1, 21.9 and 17.8. HRFABMS *m/z* 797.5309 $[M]^+$ (calcd for $C_{18}H_{10}N_2{}^{79}Br_3{}^{81}Br_3S_2$, Δ -1.5 mmu), FABMS: $[M-H]^-$ at *m/z* 792, 794, 796, 798, 800, 802, $[M]^+$ at *m/z* 791, 793, 795, 797, 799, 801, 803 and $[M+H]^+$ at *m/z* 792, 794, 796, 798, 800, 802, 804.

Compound (3) [2,4,4',5,6,6'-hexabromo-2',3-bis(methylthio)-1,3'-bi-1*H*-indole]. $[\alpha]^{25}_D$ 0° (*c* 0.016, MeOH); UV (MeOH) λ_{max} 233 (ε 51.800), 303 (ε 16.200) nm; IR (KBr) ν_{max} 3407, 1559, 1465, 1380, 1290, 1264, 1204 and 804. ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 139.3, 136.1, 133.0, 128.9, 128.4,

127.8, 122.6, 122.0, 119.6, 119.2, 117.4, 117.3, 115.1, 113.6, 111.9, 111.5, 21.9 and 18.1. HRFABM *m/z* 797.5298 $[M]^+$ (calcd for $C_{18}H_{10}N_2{}^{79}Br_3{}^{81}Br_3S_2$, Δ -2.6 mmu). FABMS: $[M-H]^-$ at *m/z* 790, 792, 794, 796, 798, 800, 802, $[M]^+$ at *m/z* 791, 793, 795, 797, 799, 801, 803 and $[M+H]^+$ at *m/z* 792, 794, 796, 798, 800, 802, 804.

Compound (4) [2,4,4',5,5',6,6'-heptabromo-2',3-bis(methylthio)-1,3'-bi-1*H*-indole]. $[\alpha]_D$ 0° (*c* 0.008, MeOH); UV (MeOH) λ_{max} 236 (ε 43,700), 298(ε 10,200) nm. ^{1}H NMR, see Table 1; FABMS: $[M]^-$ at *m/z* 871, 873, 875, 877, 879, 881, 883.

Compound (5) [2,4,4',6,6'-pentabromo-2'-methylthio-3,3'-bi-1*H*-indole]. $[\alpha]^{25}_D$ 0° (*c* 0.03, MeOH); UV (MeOH) λ_{max} 212 (sh, 28,390), 234 (ε 40,925), 296 (ε 11.930) nm; IR (KBr) ν_{max} 3408, 1602, 1550, 1410, 1380, 1323, 1262, 1168, 1022, and 835 cm^{-1}. ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 137.3, 137.0, 133.2, 127.5, 127.3, 127.2, 115.9, 115.6, 114.9, 114.5, 114.4, 113.0, 112.9, 112.8 (x2), 110.4 and 18.5. HRFABMS *m/z* 671.6364 $[M]^+$ (calcd for $C_{17}H_9N_2{}^{79}Br_3{}^{81}Br_2S$, Δ +0.2 mmu), FABMS: $[M]^+$ at *m/z* 667, 669, 671, 673, 675, 677 and $[M+H]^+$ at *m/z* 668, 670, 672, 674, 676, 678.

Single crystal X-Ray analysis of 1

The crystal data for **1** are as follows; **1**: $C_{18}H_{11}N_2Br_5S_2$, FW = 718.95, monoclinic, space group $P2_1/c$ with a = 9.1110(4), b = 12.1780(6), c = 19.9180(8) Å, β = 100.400(3), V = 2173.7(2) Å^3, and Z = 4. Data were collected at 298 K on a Mac Science DIP2030 imaging plate equipped with graphite-monochromated MoKα radiation (λ = 0.71073 Å). Unit cell parameters were determined by auto-indexing several images in each data set separately with the program DENZO. For each data set, rotation images were collected in 3° increments with a total rotation of 180° about ϕ. Data were processed by using SCALEPACK. (The programs DENZO and SCALEPACK are available from Mac Science Co., Z. Otwinowski, University of Texas, Southwestern Medical Center.) Of 4798 total unique reflections, 3207 were considered observed at the level of $|Fo| > 4.0\sigma|Fo|$. The structures were solved by the direct method and refined by full-matrix least squares refinements on F^2 (SHELXL-97, Sheldrick, G. M. Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen, Germany, 1997.). All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed using AFIX instructions. The structure converged with R = 0.1138, wR = 0.3071. Crystallographic results have been deposited with the Cambridge Crystallographic Data Centre, UK as supplementary publication number CCDC No. 276308. Copy of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033 or e-mail: data_request@ccdc.cam.ac.uk.

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