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The absolute configuration of polycephalin C from the slime mold *Physarum polycephalum* (Myxomycetes)

Florian Blumenthal, Kurt Polborn and Bert Steffan*

Department Chemie der Ludwig-Maximilians-Universität, Butenandtstr. 5-13, Haus F, 81377 Munich, Germany

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Abstract—The absolute configuration of polycephalin C (**1**) isolated from plasmodia of the slime mold *Physarum polycephalum* is supposed to be $3''R,4''R$ as found by comparison of CD spectra of the natural product polycephalin C (*1*) and CD spectra of synthetic bis-benzoates (−)-**4** and (+)-**4**. An X-ray structure of the corresponding bis-camphanate (+)-**3** shows that the exciton chirality method may not be applied on these 1,4-diols in the usual manner. © 2002 Published by Elsevier Science Ltd.

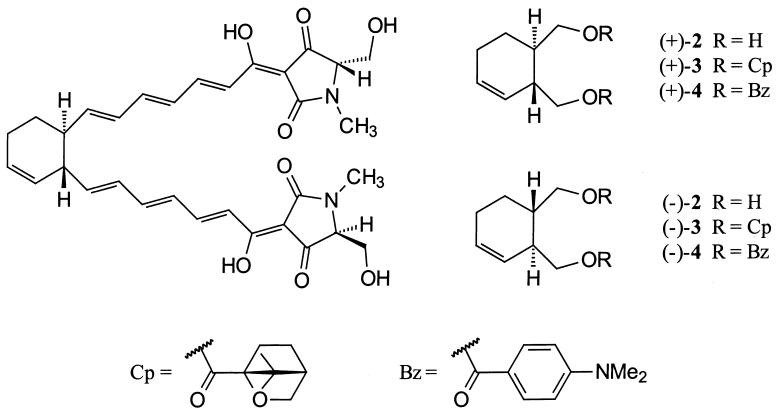
1. Introduction

Recently we reported about the isolation and structure elucidation of the tetramic acid polycephalin C (**1**) from plasmodia of the slime mold *Physarum polycephalum*.[1] This interesting compound is described by the formula $C_{32}H_{36}N_2O_8$ and consists of *N*-methyltetramic acid units that are linked by triene chains, which lead vicinally to a cyclohexene ring. The absolute configuration at the atoms C-5 and C-5′ of the two tetramic acid units was determined through comparison of CD spectra of the hydrogenated natural compound and a synthetic tetramic acid.[2] Whereas the *trans*-configuration of the polyene chains was found by comparison of coupling constants in *cis*- and *trans*-3,4-di-(hydroxymethyl)cyclohexene (**2**) and the natural product **1**, the absolute configuration at the atoms C-3″ and C-4″ of the cyclohexene remained unknown.

Possible configurations at the branchings in the cyclohexene ring of **1** are $3''R,4''R$ or $3''S,4''S$. Because of the complexity of polycephalin C (**1**) the diols **2** were used for the determination of the absolute configuration. By comparison of the CD spectra of suitable derivatives and the natural compound a conclusion on the absolute configuration of **1** should become possible by application of the exciton chirality method (Scheme 1).[3]

2. Results

trans-3,4-Di-(hydroxymethyl)cyclohexene (**2**) was synthesized according to the literature[4] by Diels–Alder reaction of 2,4-pentadienol with methyl acrylate followed by reduction with $LiAlH_4$ and is obtained as a racemate after separation of the isomers.



Scheme 1. Structures of polycephalin C (**1**) and model compounds **2**–**4**.

Keywords: slime mold; myxomycetes; *Physarum polycephalum*; exciton chirality method; absolute configuration; polyene; camphanic acid.

* Corresponding author. Tel.: +49-89-2180-7752; fax: +49-89-2180-7640; e-mail: bst@cup.uni-muenchen.de

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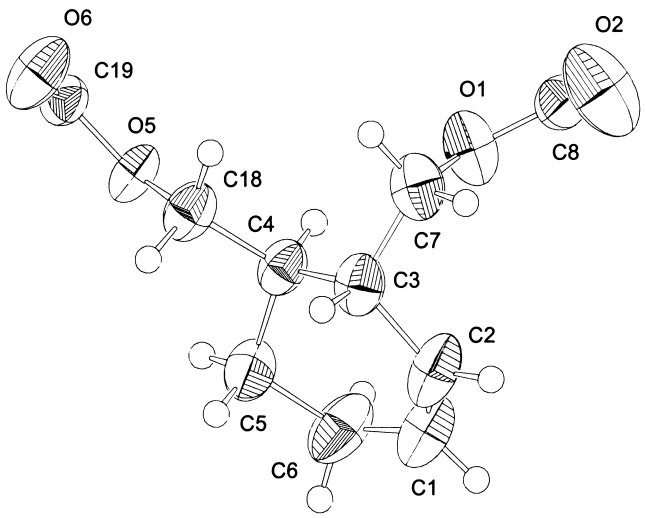


Figure 1. Section of the X-ray structure of (+)-**3**.

For enantiomeric resolution the racemic diol **2** in pyridine was treated with 1.5 equiv. of (*S*)-(−)-camphanic acid chloride per OH-function by addition of DMAP leading to the diastereomeric bis-camphanates (+)-**3** and (−)-**3** in 80% yield.

While several attempts of diastereomeric resolution by means of column chromatography and HPLC remained unsuccessful a resolution was finally achieved by fractionated crystallisation from methylene chloride/ethyl acetate/cyclohexane 2:5:5 (v/v/v). Recrystallisation yielded crystals of (+)-**3** allowing X-ray structural analysis.

By means of X-ray structural analysis in general it is only possible to get a statement on the relative configuration of chiral compounds. However, if the molecule contains a stereogenic centre with known absolute configuration, the absolute configuration of any other stereogenic centre can also be determined. As the 1*S*-configuration of the camphanic acid is known, a 3*R*,4*R*-configuration for the cyclohexene ring may be inferred. Fig. 1 shows a section of the structure in which the camphanic acid units are left aside for the sake of clarity.

The typical torsion of the opposite bonds C1–C2 and C4–C5 into a 'pseudo chair' is easily recognizable. The substituents at C-3 and C-4 are in *trans*-position. This corroborates the correctness of the assignment of the *trans*- and *cis*-configuration to the two isomeric diols **2** by means of NMR spectra and thus the *trans*-configuration of the two polyene substituents in the natural compound polycephalin C (**1**). With the X-ray structure at hand the absolute configuration of the (+)-bis-camphanate (+)-**3** can be assigned as 3*R*,4*R* and that of (−)-bis-camphanate (−)-**3** as 3*S*,4*S* as shown in the illustrations.

Upon treatment of the diastereomeric bis-camphanates (+)-**3** and (−)-**3** with 2N KOH in ethanol under reflux the enantiomeric diols (+)-**2** and (−)-**2** are obtained. By reaction of hydroxy groups with activated benzoic acid derivatives like imidazolides or triazolides natural products or other substrates can be acylated and thus be made available to the exciton chirality method.[5] Reaction of the triazolide of *p*-dimethylaminobenzoic acid with diols (+)-**2** and (−)-**2**, by addition of DBU and stirring at room temperature yields bis-benzoates (+)-**4** and (−)-**4** as main products.

The CD spectra of the bis-benzoates (+)-**4** and (−)-**4** show splitted CD curves with inverse signs, from which an exciton coupling may be inferred (Fig. 2). However, the effect is rather weak, the amplitude *A* is +1.3 and −0.7, respectively.

3. Discussion

The splitting of the CD curves of bis-benzoates is caused by the interaction of the transition moments of the benzoates, which have a position parallel to the C–O bond of the alcohol.[6] In the bis-benzoate **4** this bond does not directly branch to the ring but is flexible because of the intermediate methylene group, so that the benzoate groups may align themselves relatively free in space. Most probably it is essentially a conformation, in which the two bulky aromatic substituents hinder each other as little as possible, such as for instance in Scheme 2 (left) perpendicular to the cyclohexene ring. In this conformation there can be no excitone coupling, because the two benzoate groups are too far away from each other. However, a conformation is conceivable in which the substituents, such as indicated in Scheme 2 (right), arrange themselves in the plane of the cyclohexene ring. In this case the transition moments indicated by the bars have a positive sense of chirality and

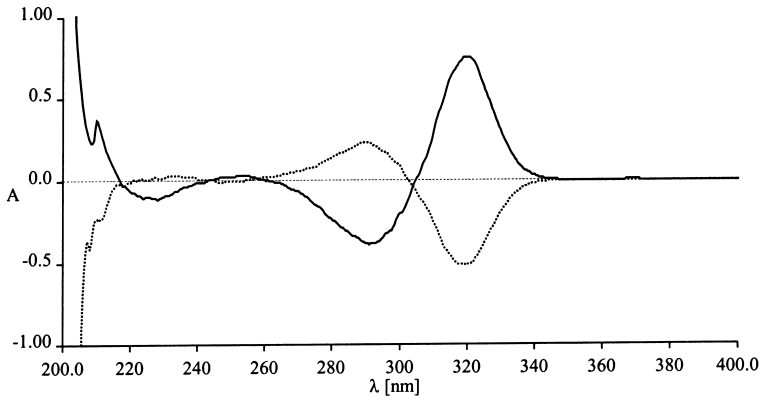
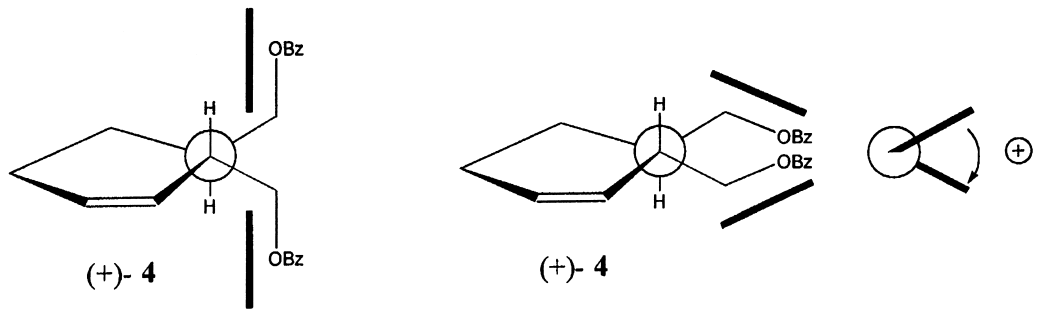
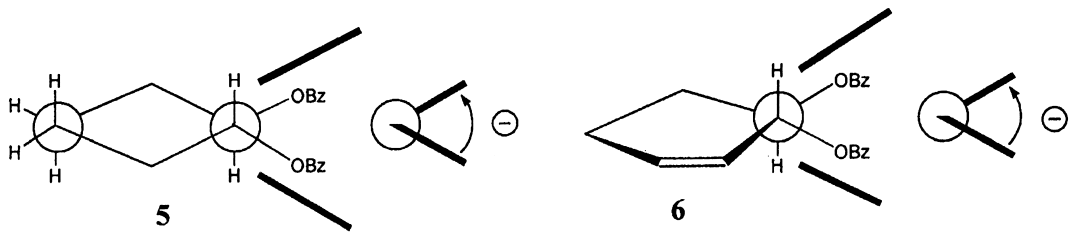


Figure 2. CD spectra of (+)-**4** (thick line) and (−)-**4** (dotted line).



Scheme 2. Position of the transition moments in various conformations of (+)-**4**.



Scheme 3. Position of the transition moments in bis-benzoates of (1*R*,2*R*)-cyclohexanediol **5** (left) and (1*R*,2*R*)-cyclohexenediol **6** (right).

should show a positive splitting of the CD curves, which is true for (+)-**4**. As only part of the molecules are contained in this conformation, the effect is not so distinct. Consequently it may be concluded that in this case the exciton chirality method cannot be applied in the usual manner to determine the absolute configuration.

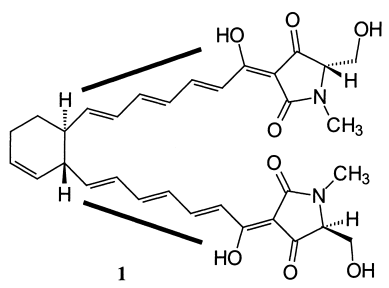
Cai et al.[7] used *trans*-(1*R*,2*R*)-cyclohexanediol **5** as a test substrate for benzoates and other chromophores. All derivatives of this diol, contrary to the derivatives of *trans*-(3*R*,4*R*)-di-(hydroxymethyl)cyclohexene (+)-**2**, show a negative CD splitting, which is to be expected because of the position of the transition moments (Scheme 3, left). Here the ester group is directly connected to the ring and therefore less flexible. Like the *trans*-cyclohexanediol system the unsaturated *trans*-cyclohexenediol system is also chiral (Scheme 3, right). Consequently the existence of the double bond should not change the chiroptical properties.

Having this in mind we concluded that the excitone chirality method should also be directly applicable to polycephalin C (**1**). Two polyene chains, showing a UV-maximum at 388 nm, are connected to a chiral *trans*-cyclohexene system. It is to be assumed that the transition moments of the $\pi-\pi^*$-excitation of the two polyene systems are parallel to the carbon chain. With regard to the 3″*R*,4″*R*-configuration, shown on Scheme 4, a negative sense of chirality results for the polyene chains and a negative CD splitting should be expected.

Fig. 3 shows the CD spectrum of polycephalin C (**1**).[8] The negative Cotton effect at 250 nm is caused by the two tetramic acid units. The following two Cotton effects at 360 nm (+5.73) and 415 nm (−3.88), i.e. a negative CD splitting, may be traced back to the excitone coupling of the two polyene chains.

This interpretation is supported by the fact that the CD spectrum of hydrogenated polycephalin shows neither a UV maximum nor a Cotton effect in the area around 400 nm.[8] Therefore it does not make any difference if the double bond in the cyclohexene ring is hydrogenated or not, because, as already mentioned above, chirality is preserved also in the saturated 3,4-substituted *trans*-cyclohexane system. Therefore polycephalin C (**1**) may be assumed to have the absolute configuration 3″*R*,4″*R*.

Very recently, Ley et al.[9] have completed a stereospecific total synthesis of two different polycephalin-like molecules, where the stereochemistry at the ring junctions is 3″*S*,4″*S* and 3″*R*,4″*R*, respectively. All spectroscopic data of the latter product are in agreement with those of the natural product. Though, this is an outstanding confirmation for the



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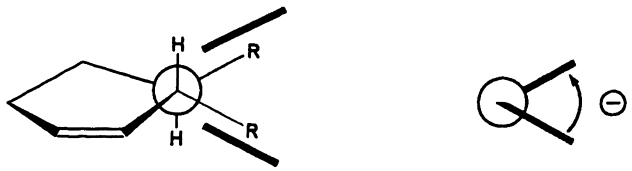

Scheme 4. The transition moments in polycephalin C (**1**) show a negative sense of chirality.

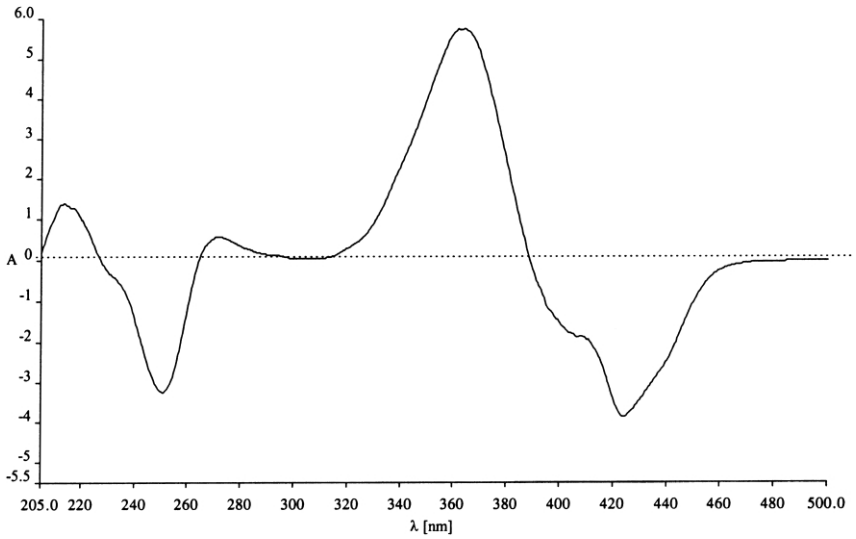

Figure 3. CD spectrum of polycephalin C (**1**).

result of the application of the exciton chirality method to polycephalin C (**1**).

4. Experimental

4.1. General

NMR: Bruker AMX2-600 (600.28 MHz for ^{1}H and 150.9 MHz for ^{13}C, chemical shifts are given relative to the residual solvent signal $CDCl_3$: δ_H=7.24, δ_C=77.0). EI-MS: Finigan MAT 95Q (DI, 210°C, 70 eV). IR: Perkin–Elmer Spectrum 1000. UV/Vis: HP 8452A Diode Array Spectrophotometer. CD: Instruments S.A. Jobin Yvon CD-6-Dichrograph. Analytical TLC: aluminium sheets silica gel 60 F_{254} (Merck), 0.2 mm.

4.1.1. Bis-camphanates (+)-3 and (−)-3. A stirred and ice-cooled solution of 0.072 g (0.5 mmol) (±)-*trans*-3,4-di-(hydroxymethyl)cyclohexene (**2**) and some crystals of DMAP in 3 ml pyridine was treated dropwise with 0.340 g (1.5 mmol) (*S*)-(−)-camphanic acid chloride. After 20 min the cooling was removed and the mixture stirred for another 2 h at room temperature. Then 3 ml water were added and the mixture extracted with benzene. The aqueous phase was again extracted twice with benzene and the unified organic phases were washed twice with water, 2N hydrochloric acid, saturated sodium hydrogen carbonate solution and dried over magnesium sulfate. Evaporation of the solvent in vacuo yielded a colourless oil. Yield: 0.200 g (79.6%).

The mixture of the diastereomeric camphanates (0.200 g, 0.4 mmol) was dissolved in 8 ml methylene chloride/ethyl acetate/cyclohexane 2:5:5 (v/v/v) and about 2 ml of the solvent mixture removed in vacuo. While allowing to stand over several days the (+)-bis-camphanate (+)-**3** crystallized in fine needles, which were used for an X-ray structure analysis.

(+)-**3**: $[\alpha]_D$=+25.7 (*c*=0.806 in chloroform). ^{1}H NMR: δ=5.80–5.79 (m, 1H, 1-H); 5.53–5.49 (m, 1H, 2-H); 4.29–4.04 (m, 4H, 7-H, 8-H); 2.41–1.44 (m, 14H, aliphat. H); 1.02 (s, 6H, CH_3); 0.96 (s, 6H, CH_3); 0.86 (s, 6H, CH_3). ^{13}C NMR: δ=178.1, 167.5 (C-3′, C-8′); 129.5, 125.4 (C-1, C-2); 91.1 (C-1′); 67.5, 67.0 (C-7, C-8); 54.8, 54.1 (C-4′, C-7′); 36.7, 34.0 (C-3, C-4); 29.8, 28.9 (C-5′, C-6′); 23.1, 23.0 (C-5, C-6); 16.8 (Me); 16.7 (Me); 9.7 (Me). IR (KBr): $\tilde{\nu}$ (cm^{-1})=3436 (br); 2970 (m); 2933 (m); 1788 (s); 1743 (s); 1634 (w); 1450 (w); 1398 (w); 1380 (w); 1340 (w); 1315 (m); 1273 (m); 1230 (w); 1170 (m); 1108 (m); 1064 (m); 932 (w). MS (EI): *m*/*z* (%)=504 (4) $[M+H]^+$; 503 (2) $[M]^+$; 456 (2); 305 (3); 304 (5); 199 (6); 181 (6); 153 (19); 134 (19); 109 (19); 107 (32); 106 (100). UV (MeCN): λ_{max} (log ε)=215 nm (3.54). CD (MeCN): λ_{max} ($\Delta\varepsilon$)=215 (−2.00). Analysis calcd for $C_{18}H_{28}O_8$: C 66.91, H 7.62, found: C 66.87, H 7.70.

Even with several attempts in different solvent mixtures (−)-**3** could not be crystallized ($[\alpha]_D$=−30.1, (*c*=0.562 in chloroform)).

4.1.2. *trans*-3,4-Di-(hydroxymethyl)cyclohexene (+)-2 and (−)-2. To 0.080 g (0.16 mmol) (+)- resp. (−)-bis-camphanate **3** in 7 ml ethanol were added 2 ml 2N potassium hydroxide and the mixture refluxed for 16 h. The reaction mixture was concentrated in vacuo, dissolved in chloroform and purified by column chromatography (chloroform/methanol 10:1 (v/v)).

Yield: 0.015 g (66.0%). (+)-**2**: $[\alpha]_D$=+17.9 (*c*=0.520 in chloroform), (−)-**2**: $[\alpha]_D$=−16.5 (*c*=0.340 in chloroform). ^{1}H NMR: δ=5.85–5.81 (m, 1H, 1-H); 5.55–5.45 (m, 1H, 2-H); 3.73–3.56 (m, 4H, 7-H, 8-H); 2.21–2.17 (m, 1H, 3-H); 2.09–2.02 (m, 2H, 6-H); 1.77–1.68 (m, 2H, 4-H, 5-H_a); 1.44–1.35 (m, 1H, H-5$_b$).

4.1.3. Bis-benzoates (+)-4 and (−)-4. 0.003 ml DBU were added to a solution of 0.015 g (0.10 mmol) (+)- resp. (−)-**2** and 0.065 g (0.30 mmol) *p*-dimethylaminobenzoyl-1,2,4-triazole in 8 ml methylene chloride. The mixture was stirred over 18 h at room temperature and the reaction monitored by TLC. After complete reaction the solvent was removed in vacuo and the residue purified by flash chromatography (chloroform/methanol 10:1 (v/v)). The

bis-benzoates (+)- resp. (−)-**4** were obtained as a yellowish solid matter.

Yield: 0.022 g (50.4%). (+)-**4**: $[\alpha]_D$=+18.9 (c=0.721 in chloroform), (−)-**4** $[\alpha]_D$=−20.3 (c=0.670 in chloroform). ^{1}H NMR: δ=7.91 (d, 4H, J=9.2 Hz, arom. H); 6.63 (d, 4H, J=8.0 Hz, arom. H); 5.89–5.82 (m, 1H, 1-H); 5.70–5.64 (m, 1H, 2-H); 4.34–4.30 (m, 4H, 7-H, 8-H); 3.03 (s, 12H, Me); 2.58–2.54 (m, 1H, 3-H); 2.19–2.07 (m, 3H, 6-H, 4-H); 1.94–1.87 (m, 1H, 5-H_a); 1.69–1.64 (m, 1H, 5-H_b). ^{13}C NMR: δ=166.9; 153.2; 131.3; 129.0; 126.5; 117.4; 110.9; 66.4; 66.3; 40.1; 37.2; 34.6; 23.3; 23.2. IR (KBr): $\tilde{\nu}$ (cm^{-1})=3436 (br); 2925 (m); 2897 (m); 1697 (s); 1613 (s); 1531 (m); 1445 (w); 1372 (m); 1317 (w); 1279 (s); 1233 (w); 1184 (s); 1111 (m); 947 (w); 826 (w); 770 (m); 697 (w). MS (EI): m/z (%)=436 (34) $[M]^+$; 347 (2); 271 (2); 165 (100); 148 (92); 106 (4); 77 (3). UV (MeCN): λ_{max} (log ε)=230 nm (0.75); 313 nm (2.60). CD (MeCN): (+)-**4**: λ_{max} ($\Delta\varepsilon$)=230 nm (−0.15); 292 nm (−0.40); 320 nm (+0.80), (−)-**4**: λ_{max} ($\Delta\varepsilon$)=230 (+0.02); 290 (+0.20); 320 (−0.50).

4.1.4. Crystal data for (+)-3. $C_{18}H_{28}O_8$, M=502.58, orthorhombic, space group: $P2_12_12_1$, a=6.778(2) Å, b=10.715(2) Å, c=36.135(3) Å, V=2624.4(8) Å^3, Z=4, D_c=1.272 Mg/m^3, μ=0.092 mm^{-1}, crystal dimensions: 0.47×0.33×0.17 mm^3, F(000)=1080, T=293(2) K, θ=2.54–24.00°, reflections measured: 4727, unique reflections: 4112, R_{int}=0.0310, min. and max. transmission: 0.9803 and 0.9993, $R1$=0.0649 and $wR2$=0.1278 for all 2757 observed reflections with $I>2\sigma(I)$, $R1$=0.1050, $wR2$=0.1488 for all reflections and 331 parameters. Final electron density 0.149 and −0.143 e/Å^3, S=1.112, absolute structure factor −0.85 (208).

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 175933. Copies 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: deposit@ccdc.cam.ac.uk).

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