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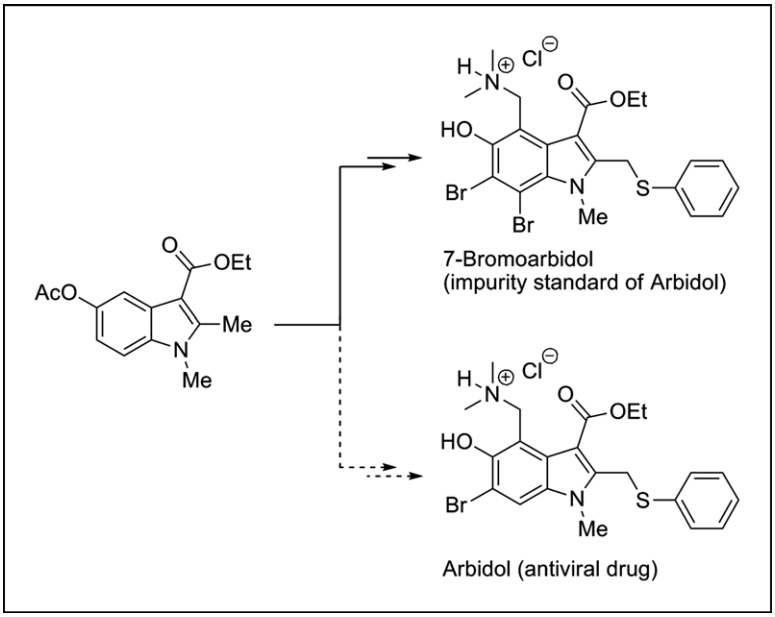


For the first time, synthesis and X-ray analysis of 7-bromoarbidol hydrochloride is reported. The latter is a proven impurity of Arbidol which is an antiviral drug marketed in Russia and China.

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## INTRODUCTION

The indole scaffold has proved to be very useful in the terms of medicinal chemistry [1,2]. To name but a few, indole derivatives are potent anti-HIV [3,4] and antimicrobial agents [5], they also posses activity against human cytomegalovirus [6]. On the other hand, since the use of *Tyrian purple* (6,6′-dibromoindigo), the mankind has shown a certain interest in the use of bromoindole derivatives. Indeed, there is a plethora of naturally occurring biologically active bromoindole derivatives, most of them arising from differently brominated tryptophans [7]. In the recent years, synthetic agent arbidol (**1**) has emerged as a representative broad spectrum antiviral medicament from the group of bromoindoles (Fig. 1). According to one of the hypotheses it acts as an interferon inducer [8], whereas other studies suggest that it blocks viral fusion [9,10]. Regardless of the mode of action arbidol is successfully used to treat viral infections caused by influenza A virus, respiratory syncytial virus, rhinovirus, coxsackie virus, and adenovirus [11–13]. It has been marketed in Russia in 1993 and in China in 2006 [12]. Further derivatives of arbidol have been investigated as anti-hepatitis B drug-like substances [14]. Earlier studies have shown also certain antimetastatic effects of the latter [15].

## RESULTS AND DISCUSSIONS

The registration package of arbidol documents 7-bromoarbidol (**2**) (Fig. 1) as one of the main process-derived impurities of pharmaceutically active ingredient [16]. Correspondingly, the precise content of this impurity has to be analyzed by appropriate impurity standard which in this case consists of 7-bromoarbidol hydrochloride as the pharmaceutically active form of the above mentioned drug is also a hydrochloride salt. To the best of our knowledge, the synthesis of 7-bromoarbidol or its hydrogen chloride salt has not been described.

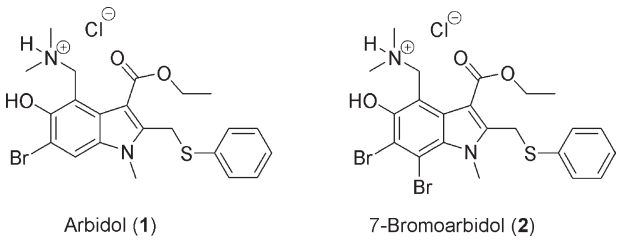



**Figure 1.** Structures of arbidol and 7-bromoarbidol.

Hence, we describe here for the first time the synthesis and structural analysis of the main pharmaceutical impurity of arbidol, 1-[6,7-dibromo-3-(ethoxycarbonyl)-5-hydroxy-1-methyl-2-phenyl-thio-methyl)-1*H*-indol-4-yl]-*N*,*N*-dimethylmethanaminium chloride (**2**).

The synthesis of **2** starts from indole derivative **3** which in its turn is obtained by standard Nenitzescu protocol combining benzoquinone and 3-methylamino-but-2-enoic acid ethyl ester. The 5-hydroxy intermediate is acetylated to give derivative **3**. The latter is the key intermediate in the synthesis of arbidol (**1**) [17].

With indole derivative **3** in hand, we turned to elaboration of bromination conditions (Scheme 1). Solvents other than halogen-containing ones (*e.g.*, AcOH) led to complete degradation. On the other hand, dichloromethane proved to be too low-boiling for the desired transformation. Thus, bromination of **3** with 2.2 equivalents of bromine in 1,2-dichloroethane at 75°C for 2 h provided arbidol precursor **4a** in 84% [conversion by high-performance liquid chromatography (HPLC)] along with overbrominated products **4b** and **4c**, both in 2% (conversion by HPLC) (Table 1.; entry 1). First, we proceeded to isolate the dibromo derivative **4a** to brominate it further by a step-wise protocol. This approach was not successful because of low solubility of the above mentioned material. Hence, the molar excess of bromine was increased up to 3.3 and 4.2 equivalents, respectively (Table 1; entries 2, 3, respectively). As a result, content of the required tribromo derivative **4b** in the reaction mixture increased up to 39%. Further increase of excess of bromine lead to formation of tetrabrominated products and increased level of degradation. The structure of **4b** was distinguished from that of **4c** by measurement of nOe effects of H-C(4) versus protons of 5-*O*-acetyl group and methylene function of 3-ethoxycarbonyl moiety. On the contrary, H-C(7) of **4c** showed characteristic nOe with $H_3C$-N(1). It was found that a washing of the crude reaction mixture containing all three indentified products **4a–c** (Table 1, entry 3) with ethyl acetate increased the content of **4b** up to 84%. Further crystallization of the mixture enriched with **4b** from acetonitrile gave an access to pure **4b** in 28% isolated yield.

With tribromide **4b** in hand, we continued the synthesis by the standard procedures described for the synthesis of arbidol. To this end, an alkylation of thiophenol by **4b** in methanolic solution in the presence of potassium hydroxide proceeded with concurrent deacetylation to provide **5** in an excellent yield. The final step in the sequence involves Mannich reaction. Different experimental procedures are known to assemble the carbon skeleton of arbidol. One of them uses the mixture of acetic acid, formaline, and dimethylamine [17]. However, this method resulted in the recovery of unchanged starting material when applied to the 7-bromoderivative **5**. This fact can be explained by the poor solubility of starting material **5** in the reaction medium. It appeared that the best choice for the introduction of the dimethylaminomethyl group is the use of formaldehyde bisdimethylaminoaminal [18]. The latter reacted with **5** in dioxane solution under reflux to provide 7-bromoarbidol in the form of its free base **6** in 65% yield. The base **6** was transformed into its hydrogen chloride salt **2** by treating with acetone/hydrochloric acid mixture. The molecular structure of final product **2** was unambiguously proved by single crystal X-ray analysis. It is interesting to note that the above mentioned crystals exist in the form of methanol solvate. ORTEP [19] representation in Scheme 2 shows the asymmetric unit of the crystal structure of salt **2** methanol solvate. There is the intermolecular hydrogen bond system in this structure. The amine hydrogen atom H25 takes part in the bifurcated hydrogen bond of NH···O type with carbonyl and methanol oxygens (O20 and O1m). The parameters of this bond are N25···O20 = 2.739(3) Å, H25···O20 = 2.17 Å, N25–H25···O20 = 116°; N25···O1m = 2.861(3) Å, H25···O1m = 2.06 Å, and N25–H25···O1m = 140°. Phenol hydrogen atom H28 forms the hydrogen bond with chlorine anion: O28···Cl31 = 3.041(2) Å, H28···Cl31 =

**Scheme 1.** Bromination of indole derivative **3**.

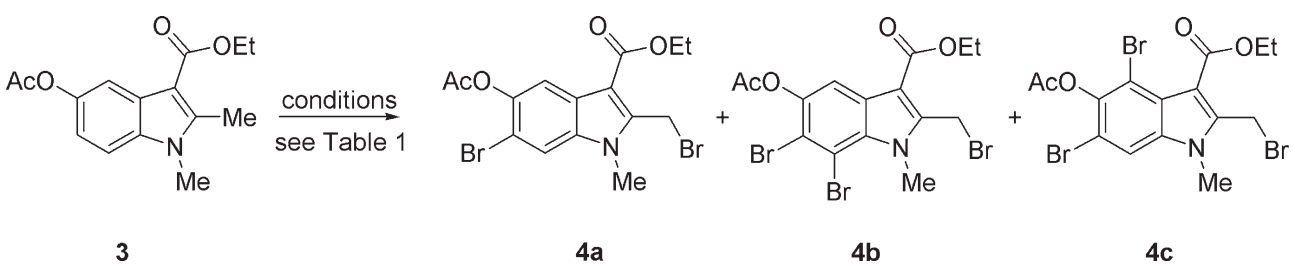


**Table 1**

Bromination of indole derivative **3**.

| Entry | Reaction conditions[a] | | Product distribution (HPLC) |
|---|---|---|---|
| | equiv. of $Br_2$ | time, h | **4a**:**4b**:**4c**:**X**[b] |
| 1 | 2.2 | 2 | 84%:2%:2%:12% |
| 2 | 3.3 | 3 | 53%:23%:4%:20% |
| 3 | 4.2 | 4 | 20%:39%[c]:14%:26% |

[a] Reactions were carried out in $ClCH_2CH_2Cl$ at 85°C.
[b] Sum of unidentified bromination products.
[c] 28% isolated yield of **4b**.

2.00 Å, O28–H28···Cl31 = 176°. In turn, the chlorine anion takes part in the hydrogen bond with the methanol molecule; the length of this bond is equal 3.039(2) Å (H1m···Cl31 = 2.13 Å, O1m–H1m···Cl31 = 152°). In the crystal structure these bonds form the net, which is parallel to the crystallographic plane (100).

## EXPERIMENTAL

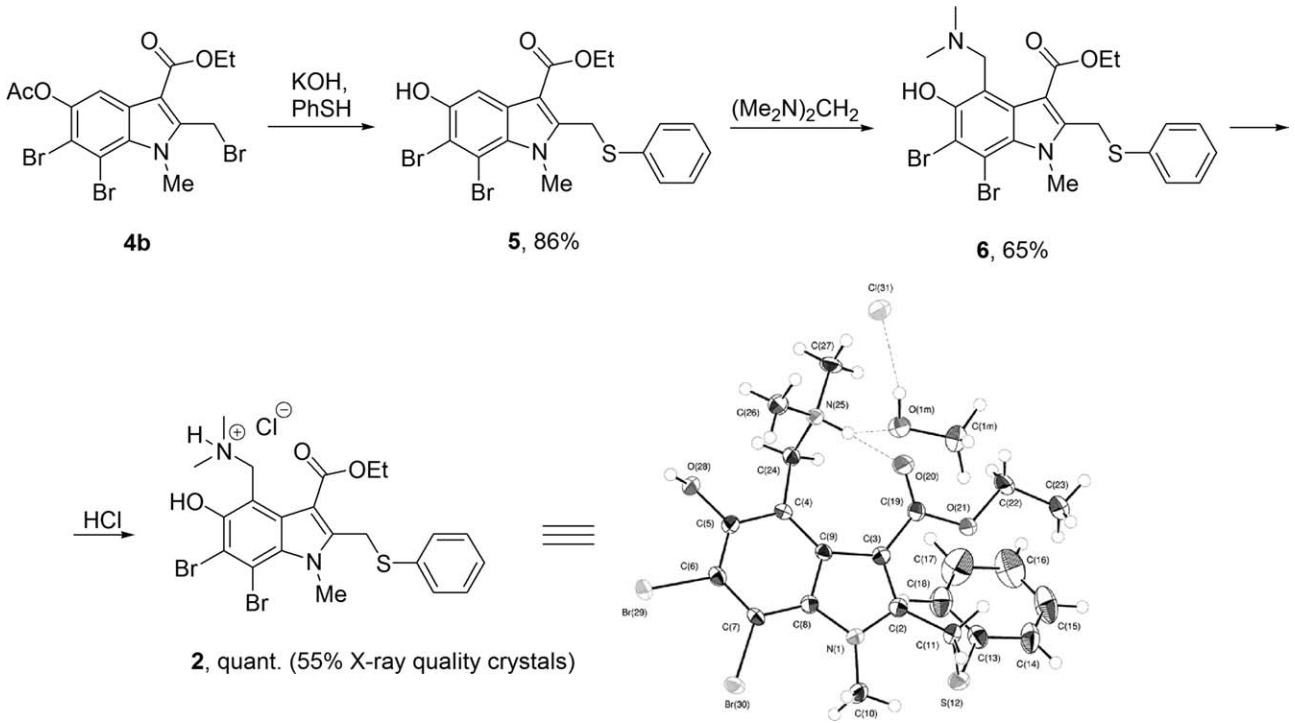
**General methods.** Commercial reagents except bis-dimethylaminomethane were used without purification. The latter was purified by fractional distillation (64. . .66°C) under nitrogen atmosphere. Solvents were distilled before use. Column chromatography was performed on silica gel (*Rocc* 0.040–0.063 mm, 60 Å). Thin-layer chromatography for reaction monitoring: Merck silica gel 60 F254 plates; detection by UV light and/or $I_2$ camera. HPLC conditions: column Merck LiChrospher 60 RP-select 5 μm, 4 × 125 mm. Eluent A: 0.01 M $KH_2PO_4$ buffer solution with pH 4.60/MeOH = 94/6. Eluent B: MeOH. Gradient From 65% B to 95% B in 15 min., flow rate 1 mL/min., DAD detector: 220 nm.

**5-Acetoxy-6,7-dibromo-2-bromomethyl-1-methyl-1H-indole-3-carboxylic acid ethyl ester (4b).** A solution of $Br_2$ (5.16 mL, 16 g, 100 mmol, 4.2 equiv.) in 1,2-dichloroethane (25 mL) was added to a stirred suspension of **4a** (6.73 g, 24.4 mmol, 4.1 equiv) in 1,2-dichloroethane (60 mL) within 4 h at reflux temperature of solvent (∼ +85°C). The resulting reaction mixture was heated at reflux for 0.5 h, cooled to ambient temperature and solvent was evaporated under reduced pressure. The resulting solid material was suspended in EtOAc (100 mL) and filtered. The filter cake was rinsed with MeOH (2 × 10 mL) and dried in the air. Technical product **4b** (HPLC purity 84%) was obtained as a beige amorphous powder 4.50 g. The latter was crystallized from MeCN (390 mL) yielding **4b** (3.53 g, 28%) with HPLC purity 96.7% ($t_R$ = 8.00 min.). M.p. 223–224°C.

$^1$H-NMR (600 MHz, $CDCl_3$): 7.95 (s, 1H, H-C(4)), 5.10 (bs, 2H, $BrCH_2$-C(2)), 4.48 (q, 2H, $^3J$ = 7.0 Hz, $CH_3CH_2OC(O)$-C(3)), 4.18 (s, 3H, $H_3C$-N(1)), 2.37 (s, 3H, $H_3CCOO$-C(5)), 1.43 (t, 3H, $^3J$ = 7.0 Hz, $CH_3CH_2OC(O)$-C(3)). $^{13}$C-NMR (150 MHz, $CDCl_3$): 169.1, 163.9, 144.1, 143.9, 133.4, 127.4, 118.0, 115.3, 108.0, 106.1, 60.5, 33.7, 20.9, 20.8, 14.4. IR (KBr): 3075, 2985, 2940, 2900, 1755, 1690, 1550, 1530, 1460, 1445, 1415, 1375, 1320, 1285, 1245, 1205, 1180, 1135, 1100, 1065, 1020, 1010, 970, 935, 905. *Anal*. Calcd for $C_{15}H_{14}Br_3NO_4$ (511,99) C 35.19, H 2.76, N 2.74; Found C 35.31, H 2.76, N 2.59.

**6,7-Dibromo-5-hydroxy-1-methyl-2-phenylsulfanylmethyl-1H-indole-3-carboxylic acid ethyl ester 5.** Thiophenol (0.60 mL, 5.85 mmol, 1.2 equiv.) was added to a stirred solution of KOH (0.99 g, 83%, 14.7 mmol, 3.0 equiv.) in dry MeOH

**Scheme 2.** Synthesis of 7-bromoarbidol (**2**).



(30 mL) at +20°C. The resulting reaction mixture was stirred at ambient temperature for 30 min. Then tribromide **4b** (2.50 g, 4.88 mmol, 1.0 equiv.) as a solid substance was added at once to a stirred solution of potassium phenylthiolate at 15°C. The resulting suspension was stirred at ambient temperature for 3 h. The undissolved material consisting mainly of KBr was filtered off, and the clear filtrate was mixed with a solution of glacial acetic acid (1.5 mL) in water (6.0 mL). The resulting suspension was stirred for 20 min. and filtered. The filter cake was washed successively with water (40 mL) and hexanes (10 mL) and dried under reduced pressure at +50°C. The crude product (2.35 g) was dissolved in a refluxing mixture of ethanol (250 mL) and $CHCl_3$ (250 mL), filtered and evaporated under reduced pressure until resting volume of 150 mL. After 14 h at ambient temperature it was filtered, washed on the filter with MeOH (2 × 5 mL), and dried in the air. Yield 2.10 g, 86% with HPLC purity 97.0% ($t_R$ = 8.35 min). M.p. 225–226°C.

$^1$H-NMR (300 MHz, $DMSO_{d6}$): 10.25 (s, 1H, HO-C(5)), 7.66 (s, 1H, H-C(4)), 7.38-7.25 (m, 5H, H-C(Ph)), 4.76 (s, 2H, $SCH_2$-C(2)), 4.16 (q, 2H, $^3J$ = 7.2 Hz, $CH_3CH_2OC(O)$-C(3)), 3.96 (s, 3H, $H_3C$-N(1)), 1.27 (t, 3H, $^3J$ = 7.2 Hz, $CH_3CH_2OC(O)$-C(3)). $^{13}$C-NMR (75.5 MHz, $DMSO_{d6}$): 163.6, 150.3, 145.1, 134.0, 131.1, 129.0, 128.9, 128.0, 127.3, 112.0, 107.0, 105.2, 103.6, 59.5, 33.7, 28.4, 14.2. IR (KBr): 3240, 2990, 1640, 1610, 1523, 1470, 1415, 1390, 1340, 1295, 1250, 1230, 1200, 1140, 1100, 1070, 1025, 975, 930, 895. *Anal*. Calcd for $C_{19}H_{17}Br_2NO_3S$ (499.22) C 45.71, H 3.43, N 2.81, S 6.42 Found C 45.74, H 3.38, N 2.75, S 6.63.

**6,7-Dibromo-4-dimethylaminomethyl-5-hydroxy-1-methyl-2-phenylsulfanyl-methyl-1H-indole-3-carboxylic acid ethyl ester 6.** Bis-dimethylaminomethane (0.5 mL, 3.67 mmol, 7.06 equiv.) was added to a sealable pressure flask containing suspension of compound **5** (0.25 g, 0.52 mmol, 1.0 equiv.) in abs. dioxane (6 mL). The resulting reaction mixture was sealed and then it was stirred under reflux (bath temperature 105°C) for 2 h. At the temperature of reflux, the reaction mixture became clear. Then, it was cooled to ambient temperature and poured onto crashed ice (50 g). The resulting yellowish precipitate was filtered and dried in the air. The crude product **2** (0.21 g, HPLC purity 89%) was purified by silica gel (8 g) column chromatography using gradient elution by $CHCl_3$ → $CHCl_3$/THF (95/5). Fractions containing product were combined and evaporated to dryness. The solid residue was reprecipitated from MeOH/water system. Yield: 0.19 g, 65%; HPLC purity: 98.5% ($t_R$ = 7.40 min.). M.p. 131–132°C, $R_f$ = 0.33 ($CHCl_3$/THF = 8/2).

$^1$H-NMR (300 MHz, $DMSO_{d6}$): 7.36–7.28 (m, 5H, H-C(Ph)), 4.60 (s, 2H, $SCH_2$-C(2)), 4.13 (q, 2H, $^3J$ = 7.0 Hz, $CH_3CH_2OC(O)$-C(3)), 4.00 (s, 3H, $H_3C$-N(1)), 3.82 (s, 2H, $(CH_3)_2NCH_2$-C(4)), 2.24 (s, 6H, $(CH_3)_2NCH_2$-C(4)), 1.21 (t, 3H, $^3J$ = 7.0 Hz, $CH_3CH_2OC(O)$-C(3)). $^{13}$C-NMR (75.5 MHz, $DMSO_{d6}$): 164.7, 151.4, 142.7, 134.0, 131.1, 129.1, 128.8, 127.4, 125.4, 113.3, 111.1, 106.5, 105.9, 60.3, 59.2, 43.4, 34.3, 28.7, 14.0. IR (KBr): 3450, 1695, 1580, 1545, 1525, 1460, 1395, 1315, 1230, 1180, 1130, 1110, 1035, 1000, 960, 870. *Anal*. Calcd for $C_{22}H_{24}Br_2N_2O_3S$ (556.31) C 47.50, H 4.35, N 5.04, S 5.76; Found C 47.40, H 4.37, N 4.92, S 6.07.

**(6,7-Dibromo-3-ethoxycarbonyl-5-hydroxy-1-methyl-2-phenylsulfanylmethyl-1H-indol-4-ylmethyl)-dimethyl-ammonium chloride-methanol solvate (2MeOH).** To a clear solution of amine **6** (50 mg, 0.09 mmol) in acetone (2 mL) was added concentrated (36%) aqueous solution of hydrochloric acid (0.10 mL) at +40°C. The resulting suspension was left to reach ambient temperature and stirred for 1 h. Then it was evaporated under reduced pressure to provide quantitative yield (53 mg) of pure hydrochloride salt **2**. The latter was dissolved in a three component mixture (0.5 mL) consisting of acetone:methanol:aq. conc. HCl in the ratio 15:2:0.16 at +40°C. After two days at +4°C, the resulting crystals were removed by filtration. Yield of X-ray quality crystals 31 mg, 55% (in the form of methanol solvate); HPLC purity 99.8% ($t_R$ = 7.35 min). M.p. 169°C (DSC studies).

$^1$H-NMR (300 MHz, $DMSO_{d6}$): 9.79 (bs, 1H, HO-C(5)), 9.32 (bs, 1H, HN-$CH_2$-C(4)), 7.39-7.27 (m, 5H, H-C(Ph)), 4.80 (s, 2H, $(CH_3)_2$N-$CH_2$-C(4)), 4.73 (s, 2H, S-$CH_2$-C(2)), 4.20 (q, 2H, $^3J$ = 7.0 Hz, $CH_3CH_2OC(O)$-C(3)), 4.04 (s, 3H, $H_3C$-N(1)), 3.19 (s, 3H, $CH_3OH$), 2.71 (s, 6H, $(CH_3)_2N$-$CH_2$-C(4)), 1.23 (t, 3H, $^3J$ = 7.0 Hz, $CH_3CH_2OC(O)$-C(3)). $^{13}$C-NMR (75.5 MHz, $DMSO_{d6}$): 165.0, 149.6, 145.4, 134.1, 131.3, 130.7, 129.2, 127.6, 127.1, 116.5, 110.6, 109.7, 106.4, 70.0, 52.8, 48.7 ($CH_3OH$) 42.4, 34.9, 29.7, 13.9. IR (KBr): 3385, 3060, 2960, 2930, 2835, 1700, 1580, 1550, 1480, 1410, 1380, 1330, 1225, 1165, 1130, 1100, 1035, 965. *Anal*. Calcd for $C_{22}H_{25}Br_2ClN_2O_3S{\cdot}CH_3OH$ (624.81) C 44.21, H 4.68, N 4.48, S 5.13; Found C 44.44, H 4.70, N 4.42, S 5.51.

**X-ray diffraction analysis.** Diffraction data were collected at −100°C on a Nonius KappaCCD diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The crystal structure was solved by direct methods and refined by full-matrix least squares. Crystal data: monoclinic; $a$ = 7.5220(2), $b$ = 11.4594(5), $c$ = 15.6335(4) Å, α = 72.287(2), β = 84.821(2), γ = 79.128(1)°; $V$ = 1259.86(7) Å$^3$, $Z$ = 2, μ = 3.439 mm$^{-1}$; space group is $P\bar{1}$. The final $R$-factor is 0.038. For further details, see crystallographic data deposited with the Cambridge crystallographic data centre as supplementary publication number CCDC-780284. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

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