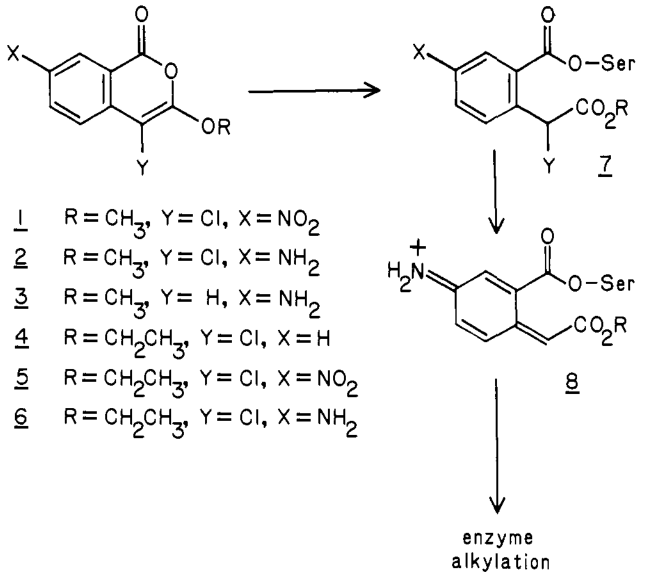

Figure 1. Proposed pathway for inactivation of serine proteases by substituted 3-alkoxyisocoumarins.

pH 6.8, 10% Me$_2$SO) was also monitored by the decrease in fluorescence emission at 510 nm (ϵ_{ex} = 400 nm), and the rate of ring opening (18 × 10^{-3}s^{-1}) was identical with the inactivation rate (17 × 10^{-3} s^{-1}) under these contions. The inactivation stoichiometry (1.06 ± 0.02) was similar to that determined spectrophotometrically. No new emission bands (ϵ_{ex} = 400 nm) were observed in the fluoroscence spectra of inactivated PPE (8.9 μM) after dialysis against phosphate buffer (pH 6.8) for 24 h at 4 °C. Inactivation of ChT (0.27 mM) by **6** (0.33 mM) resulted in <1% release of EtOH.[8]

HLE and PPE inactivated by the 7-amino-4-chloroisocoumarins **2** and **6** regained <0.5% enzymatic activity after standing for 100 h, while these enzymes inactivated by the 7-nitro derivatives **1** and **5** and the 4-chloro-3-ethoxyisocoumarin (**4**) regained >85% activity ($t_{1/2}$ < 72 min) upon standing at 25 °C (Table I). In addition, HLE and PPE inactivated by **6** regained <0.5% enzymatic activity after dialysis for 48 h (0.1 M phosphate pH 6.8 buffer, 4 °C) and upon further standing for 48 h at 25 °C, which is evidence for irreversible inactivation. Addition of buffered hydroxylamine (0.46 M) to HLE and PPE inactivated by **6** (0.016 mM) resulted in 34–42% reactivation ($t_{1/2}$ = 5.7–7.2 h), while addition of buffered hydroxylamine (0.26 mM) to these enzymes inactivated by 7-amino-3-methoxyisocoumarin (**3**) (0.09–0.11 mM) resulted in rapid and complete reactivation ($t_{1/2}$ = 12 and 9.6 min, respectively).

These results are consistent with Figure 1 where the 3-alkoxy-7-amino-4-chloroisocoumarins **2** and **6** react with the active-site serine of serine proteases to give the acyl enzyme **7** which decomposes to the acyl-*p*-quinonemethide imine **8**. An irreversibly inactivated enzyme could then result by reaction of the acyl-*p*-quinonemethide imine **8** with an active-site nucleophile.[9] The requirement of both the 7-amino and 4-chloro substituents for irreversible inactivation is demonstrated by the finding that the 7-nitro-4-chloroisocoumarins **1** and **5**, 4-chloroisocoumarin **4**, and 7-aminoisocoumarin (**3**) react to give **7** but reactivate rapidly either upon standing or upon addition of hydroxylamine. The 3-alkoxy-7-amino-4-chloroisocoumarins **2** and **6** are some of the most potent HLE inactivators yet reported, represent a new class of suicide substrates for serine proteases, and may be useful in the prevention of proteolysis in vitro and in vivo. Studies leading to further proof of the proposed mechanism and extention to other serine proteases are now in progress.

Acknowledgment. This work was supported by grants from the National Institutes of Health (HL 29307) and from the Council for Tobacco Research.

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[8] EtOH release measured upon dilution (2×) of inactivated enzyme into liver alcohol dehydrogenase (1 μM) and NAD$^+$ (1.5 mM) in 0.1 M phosphate pH 7.8 buffer. Alcohol dehydrogenase was measured by monitoring the increase in absorbance of NADH at 340 nm (ϵ = 6220 M^{-1} cm^{-1}) and <2 μM ethanol could have been detected.

[9] Several *p*-aminobenzyl halides react rapidly with nucleophiles through a *p*-quinonemethide imine intermediate: Wakselman, M.; Dome, M. *Bull. Soc. Chim. Fr.* **1975**, 571–575. Dome, M.; Wakselman, M. *Bull. Soc. Chim. Fr.* **1975**, 576–582.

(10) Tian, W. X.; Tsou, C. L. *Biochemistry* **1982**, *21*, 1028–1032.

The Kharasch Reagent. Regioselective Generation of Dienol Ethers from Enones

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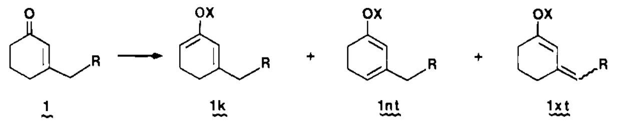
Received June 29, 1984

After a hiatus of over 40 years, we have uncovered the identity of the Kharasch reagent.[1] Stoichiometric use of this reagent now makes possible, for the first time, the regioselective preparation of "thermodynamic" trimethylsilyl dienol ethers from cyclic enones.

Deprotonation of a cyclic enone (e.g., **1**) can potentially give three regioisomeric dienolates: the cross-conjugated ("kinetic") isomer **1k**, the through-conjugated ("thermodynamic") endocyclic isomer **1nt**, and through-conjugated ("thermodynamic") exocyclic isomer **1xt**. The synthetic utility of dienolates **1k**, **1nt**, and **1xt** has been recognized for some time.[2-4] However, exploitation of this utility requires methods for the regiospecific preparation of each of the three possible dienolates. Unfortunately, prior to this work only enolates of type **1k** have been generally accessible from the corresponding enone (LDA/THF/–78 °C).[2c,5,6]



One of the rare examples of formation of a through-conjugated dienolate from the corresponding enone was reported by Kharasch.[1] In a modified version of this reaction, treatment of **2** with

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(1) Kharasch, M. S.; Tawney, P. O. *J. Am. Chem. Soc.* **1941**, *63*, 2308; **1945**, *67*, 128. For subsequent synthetic uses, see: Meinwald, J.; Hendry, L. *J. Org. Chem.* **1971**, *36*, 1446 and references cited therein.

(2) For reviews of the use of enol ethers in synthesis, see: (a) Effenberger, F. *Agnew. Chem., Int. Ed. Engl.* **1969**, *8*, 295. (b) Rasmussen, J. K. *Synthesis* **1977**, 91. (c) D'Angelo, J. *Tetrahedron* **1976**, *32*, 2979.

(3) Acylation: (a) Burn, D.; Cooley, C.; Davies, M. T.; Hiscock, A. K.; Kirk, D. N.; Petrow, V.; Williamson, D. M. *Tetrahedron* **1965**, *21*, 569. (b) Gorodetsky, M.; Levy, E.; Youssefyeh, R. D.; Mazur, Y. *Ibid.* **1966**, *22*, 2039.

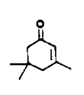
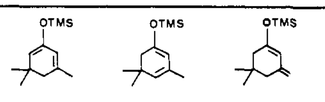
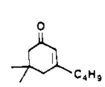
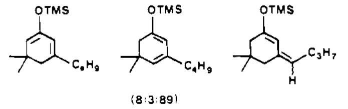
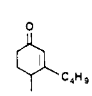
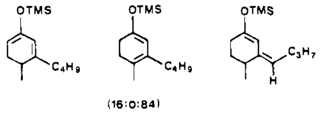
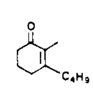
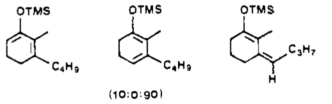
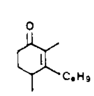
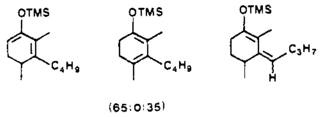
(4) Lewis acid catalyzed aldol-type processes: (a) Danishefsky, S.; Prisbylla, M.; Lipisko, B. *Tetrahedron Lett.* **1980**, 805. (b) Fleming, I.; Lee, T. V. *Ibid.* **1981**, 705. (c) Fleming, I.; Goldhill, J.; Paterson, I. *Ibid.* **1979**, 3205, 3209. (d) Danishefsky, S.; Guingant, A.; Prisbylla, M. *Ibid.* **1980**, 2033. (e) For a recent review, see: Fleming, I. *Chimia* **1980**, *34*, 265.

(5) General preparation of "kinetic" dienolates **1k**: (a) Lee, R. A.; McAndrews, C.; Patel, K. M.; Reusch, W. *Tetrahedron Lett.* **1973**, 965. (b) Stork, G.; Danheiser, R. *J. Org. Chem.* **1973**, *38*, 1775.

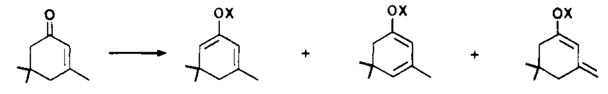
(6) Specific instances of preparation of "thermodynamic" dienolates **1nt** and **1xt** or mixtures of these: (a) Conia, J. M. *Tetrahedron Lett.* **1964**, 2791. (b) Conia, J. M.; Sandre-Le Craz *Ibid.* **1962**, 505 and references cited therein. (c) Wharton, P. S. *J. Org. Chem.* **1968**, *33*, 4255. (d) House, H. O.; Czuba, L. J.; Gall, M.; Olmstead, H. D. *Ibid.* **1969**, *34*, 2324. (e) Rubottom, G. M.; Gruber, J. M. *Ibid.* **1977**, *42*, 1051. (f) Leffingwell, J. C. *Tetrahedron Lett.* **1970**, 1653. (g) Treatment of **2** with KO-*t*-Bu/THF/Me$_3$SiCl/Et$_3$N led to a 3:7 mixture[7] of **2nt**/**2xt** (X = Me$_3$Si). (h) Some through-conjugated endocyclic dienol ethers are available via reduction–rearrangement of appropriate aromatic substrates.[2a,b,4e]

0002-7863/84/1506-7619$01.50/0

Table I. Formation of Trimethylsilyl Dienol Ethers from Enones with Activated Fe(0)/Me_3SiCl/Et_3N[9]

Enone	Dienol Ethers[10] (Ratio)[7]	Yield[11]
	 (7:1:92)	95%
	 (8:3:89)	96%
	 (16:0:84)	98%
	 (10:0:90)	94%
	 (65:0:35)	97%

methylmagnesium bromide in the presence of *catalytic* $FeCl_3$ gave almost exclusively **2nt** (X = Me_3Si) upon quenching with



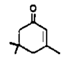
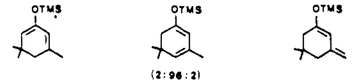
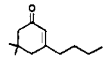
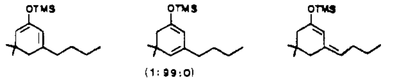
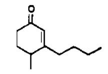
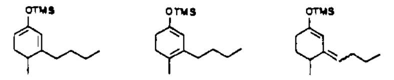
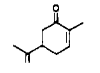
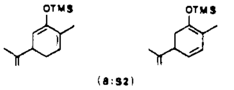
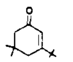
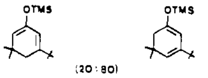
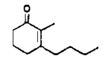
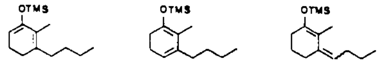
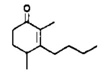
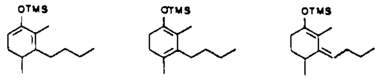
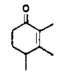
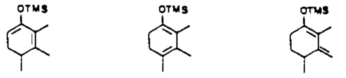
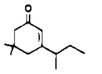
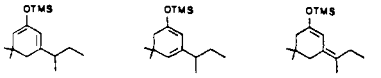
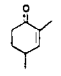
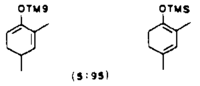
2 2k 2nt 2xt

Me_3SiCl/Et_3N.[8] Unfortunately, these conditions were found to provide synthetically useless mixtures from other enones.

We have described the preparation of a novel and reactive form of zero-valent iron and its use in the conversion of unsymmetrical cyclic ketones to "thermodynamic" silyl enol ethers.[8] Treatment of several cyclic enones with a *stoichiometric* amount of this reagent and Me_3SiCl/Et_3N in ether gave rise to the enol ether mixtures listed in Table I. In most of these cases, the *exocyclic* through-conjugated dienol ether was found to be the predominant product. This is in surprising contrast to the exclusive formation of **2nt** under catalytic conditions. Clearly, this reactive zero-valent iron species is not the "Kharasch reagent."

However, when a mixture of enone and 1 mol equiv of Fe(0)[8] was treated with 1 mol equiv of methylmagnesium bromide, 1 mol equiv of methane[12] was instantly evolved; and after addition of Me_3SiCl/Et_3N/HMPA to the mixture, the *endocyclic* through-conjugated dienol ether was isolated as the predominant product. The results from reaction of a variety of enones under these conditions (DME, 0 °C)[13] are listed in Table II. *In every case the endocyclic through-conjugated enol ether was formed regioselectively and in high yield.*

Table II. Formation of Trimethylsilyl Dienol Ethers from Enones with Activated Fe(0)/$CH_3MgBr/Me_3SiCl/Et_3N$[13]

Enone	Dienol Ethers[10] (Ratio)[7]	Yield[11]
	 (2:96:2)	99%
	 (1:99:0)	93%
	 (5:92:3)	95%
	 (8:92)	99%
	 (20:80)	98%
	 (4:94:1)	96%
	 (3:97:0)	99%
	 (4:92:4)	99%
	 (2:95:2)	90%
	 (5:95)	93%

The results in Table II are reproducible *only* when oxygen is rigorously excluded from the reaction mixture. These reactions may be carried out with less than 1 mol equiv of activated Fe(0);[8] we have obtained comparable results using as little as 0.5 mol equiv of Fe(0)[8] in several of the cases listed.

The mechanism of this unusual reaction remains obscure. Unlike reactions with Fe(0)[8] alone, the reaction employing excess Grignard reagent is very rapid at 0 °C and is clearly *not* an

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[7] Isomer ratios were determined by both ^1H NMR analysis and GC analysis (8% OV-101 on Chromosorb W, 8 ft × $^1/_8$ in., or 10% Carbowax on Chromosorb W, 8 ft × $^1/_8$ in.).

[8] Krafft, M. E.; Holton, R. A. *J. Org. Chem.* **1984**, *49*, 3669.

[9] For a typical experimental procedure, see ref 8.

[10] All products have been characterized by IR and ^1H NMR. Elemental composition has been verified by combustion analysis or high-resolution mass spectroscopy.

[11] All yields refer to isolated, chromatographically and spectrally (NMR) homogeneous (except for regioisomers) products.

[12] Identified by GC analysis using a 5-Å molecular sieve, 80/100 mesh, 6 ft × $^1/_8$ in. column. We thank Professor H. M. McNair for providing this column and an authentic gas sample.

[13] Typical experimental procedure: Anhydrous ferric chloride (Aldrich) (195 mg, 1.2 mmol) was placed in a round-bottom flask on a vacuum line, evacuated, and flushed with nitrogen 3 times. (This entire procedure should be conducted with anhydrous "low oxygen" nitrogen. Alternatively, the boil-off from a 200-L liquid nitrogen container was found to give satisfactory results.) To this powder at 0 °C was added 12 mL of anhydrous (continuous distillation from LAH under N_2) DME and 1.4 mL (3.6 mmol) of an ethereal solution (2.5 M) of methylmagnesium bromide. The resulting black suspension was stirred at 25 °C for 1 h then cooled to 0 °C. A solution of isophorone (**2**) (138 mg, 1.0 mmol) in 2–3 mL of anhydrous DME was slowly added. The mixture was stirred for 45 min at 0 °C before *slow* addition of 0.5 mL (1.5 mmol) of an ethereal solution (2.4 M) of methylmagnesium bromide. (**CAUTION**: Rapid addition of methylmagnesium bromide may result in formation of 1,2-addition product.) After an additional 45 min at 0 °C, 0.8 mL (6 mmol) of Me_3SiCl, 1.0 mL (7 mmol) of Et_3N, and 0.2 mL (1 mmol) of HMPA were added in that order. The mixture was warmed to 25 °C, stirred for 2 h, and diluted with hexane. The solution was washed with cold (0 °C) saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated. The pale orange oil was filtered through a silica gel plug (10% EtOAc/hexane). Concentration of the eluant gave 208 mg (99%) of a colorless oil which was shown by GC analysis (OV-101, 110 °C) to consist of **2k**/**2nt**/**2xt** in a ratio of 2:96:2.

equilibrium process. The extreme sensitivity of the reaction to oxygen suggests that an electron-transfer process may be involved.[14]

These results establish the identity of the "Kharasch reagent" as a mixture of activated zero-valent iron and Grignard reagent.[15] Using this reagent, it is now possible for the first time to regioselectively prepare endocyclic "thermodynamic" dienol ethers from the corresponding enones. In some cases, it is also possible to prepare the exocyclic "thermodynamic" dienol ethers regioselectively using Fe(0) alone.

Now that both endocyclic and exocyclic "thermodynamic" dienol ethers are readily available, we have begun to explore their utility in a variety of new synthetic endeavors. The results of these studies will be reported in due course.

Acknowledgment. We thank the National Cancer Institute for generous financial support of our program.

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[14] Such processes have been implicated in other reactions involving ferric chloride and Grignard reagents. See, for example: (a) Tamura, M.; Kochi, J. K. *Synthesis* **1971**, 303. (b) Ashby, E. C. *Pure Appl. Chem.* **1980**, *52*, 545.

[15] Mixtures of products (with 1,2-addition predominant) were obtained when enone was added to a mixture of Grignard reagent plus iron(0). It is on this basis that we formulate the "reagent" as a mixture of the two species operating in tandem rather than a reaction product of Grignard reagent and iron(0).

Synthesis and Structures of Molecular Sulfo Salts $(CH_3C_5H_4)_3Ti_2OAsS_3$, $[Mo_2O_2As_4S_{14}]^{2-}$, and $[Mo_4O_4As_4S_{14}]^{4-}$

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Received August 7, 1984

Sulfo salts are a large class of minerals which are comprised of anionic trigonal pyramidal XE_3 subunits where X = As, Sb, Bi and E = S, Se, Te.[2] These pyramidal fragments occur in the lattice as both isolated anions or as interconnected rings, chains, and nets of XE_3 subunits. In this report we describe the synthesis and structural characterization of the first molecular sulfo salts starting from the minerals orpiment, As_2S_3(**1**), and realgar, As_4S_4 (**2**).

It has long been known that pale yellow solutions result when **1** is treated with aqueous sodium sulfide (1.5 equiv/As_2S_3),[3,4] and our results support the view that this procedure affords the AsS_3^{3-} anion.[5] Addition of these aqueous solutions to acetone slurries of $(MeCp)_2TiCl_2$ (MeCp = η^5-$CH_3C_5H_4$) leads to rapid formation of a dark green precipitate.[6] Filtration of CH_2Cl_2 extracts of these precipitates through silica gel and dilution of the effluent with hexanes afforded a dark brown crystalline product, which by fast atom bombardment mass spectroscopy and microanalysis had the formula $(MeCp)_3Ti_2AsS_3O$ (**3**) (42% yield).[7] The oxygen is presumed to arise from the silica gel workup. The cleavage of a MeCp ring was indicated and is known to occur in other reactions of Cp_2TiCl_2 with anionic chelates.[8] The ^1H NMR spectrum of **3** was particularly informative as it established the presence of an equivalent pair of MeCp groups while the unique MeCp is bisected by the only symmetry plane present in the molecule.[9] This was confirmed by a single-crystal X-ray diffraction study on crystals of **3** grown by the slow evaporation of a saturated CH_2Cl_2 solution.[10]

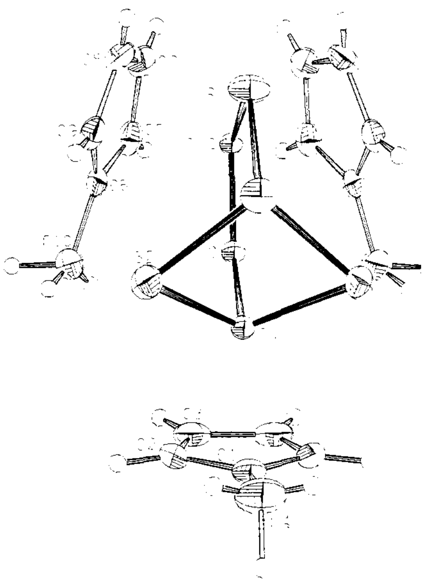

Figure 1. ORTEP drawing of $(MeCp)_3Ti_2OAsS_3$ showing the labeling scheme for all non-hydrogen atoms (35% probability boundaries, hydrogen atoms are assigned arbitrary thermal coefficients). Some selected bond angles for **3** are as follows: Ti(1)-O-Ti(2) 145.2 (1)°, S(2)-Ti-(1)-S(2)′ 93.71 (3)°, S(1)-As-S(2) 106.78 (2)°, S(2)-As-S(2)′ 96.54 (3)°.

The molecular structure of **3** is shown in Figure 1. The molecule possesses approximate C_s symmetry with the mirror plane defined by both titanium atoms, the oxygen, arsenic, and sulfur(1). This is the first example of a molecular metal complex of an XS_3 ligand (X = pnictnide) and the structural parameters for the arsenic trisulfide group (As-S(1) 2.1922 (10) Å, As-S(2) 2.2621 (7) Å) are similar to those in orpiment itself.[11] The oxygen atom links the two titanium centers and the differing Ti-O distances (Ti(1)-O 1.794 (2) Å, Ti(2)-O 1.872 (2) Å) reflect the relative π-acidities expected for these electron-deficient titanium atoms.[12] This same compound can be prepared in lower yields from $(MeCp)_2Ti(CO)_2$ and As_4S_4 employing a workup as described above.[13]

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[6] In a typical reaction 125 mg (0.5 mmol) of **1** was added to a stirred solution of 360 mg (1.5 mmol) of $Na_2S{\cdot}9H_2O$ in 10 mL of H_2O. The resulting pale yellow solution was added to an acetone (25 mL) slurry of $(MeCp)_2TiCl_2$ (550 mg, 2.0 mmol) and stirred for 30 min. Yield: 250 mg.

[7] Anal. Calcd for $C_{18}H_{21}TiOS_3As$: C, 41.55; H, 4.07; Ti, 18.41. Found: C, 41.85; H, 4.40; Ti 18.05.

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[9] ^1H NMR of **3** (360 MHz, CD_2Cl_2) δ 6.29(m, 2 H), 6.22(m, 2 H), 6.21(m, 2 H), 6.05(m, 2 H), 5.92(m, 2 H), 5.76(m, 2 H), 2.18(s, 3 H), 2.06 (s, 6 H).

[10] Compound **3** crystallizes in the monoclinic space group $P2_1/m$ with dimensions a = 10.404 (6) Å, b = 12.932 (9) Å;, and c = 7.491 (6) Å; β = 98.82 (6)°; V = 996 (1) Å^3; Z = 2. The data was collected on a Syntex $P2_1$ automated four-circle diffractometer at ambient temperatures with monochromatized Mo Kα (λ = 0.71069 Å) radiation. Of the 2681 reflections collected 2092 independent reflections with $I > 2.58\sigma(I)$ were used in the structure solution and refinement, which converged to R = 2.7% and R_W = 4.0% with all non-hydrogen atoms refined anisotropically and all hydrogen atoms refined isotropically.

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