CODEN IEJMAT *Internet Electronic Journal of Molecular Design* **2005**, *4*, 342–354 ISSN 1538–6414
BioChem Press http://www.biochempress.com

Inter*net* Electronic Journal of Molecular Design

May 2005, Volume 4, Number 5, Pages 342–354

Editor: Ovidiu Ivanciuc

Proceedings of the Internet Electronic Conference of Molecular Design 2004
IECMD 2004, November 29 – December 12, 2004

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Received: September 11, 2004; Revised: February 23, 2005; Accepted: March 15, 2005; Published: May 31, 2005

Citation of the article:
M. J. Bucknum, D. M. E. Szebenyi, E. Lobkovsky, S. A. Patil, R. S. Klein, and E. A. Castro, Microcrystal Structure Determination Using Synchrotron Radiation and a CCD Detector, *Internet Electron. J. Mol. Des.* **2005**, *4*, 342–354, http://www.biochempress.com.

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Microcrystal Structure Determination Using Synchrotron Radiation and a CCD Detector [#]

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Internet Electron. J. Mol. Des. **2005**, *4* (5), 342–354

Abstract

Motivation. The structure determination of the nucleoside analog metRITone, 2–methylthio–6–β–D–ribofuranosylimidazo[2,1–*f*][1,2,4]triazin–4(3H)–one, is presented. This molecule forms needle–shaped crystals with extremely small external dimensions (≈ 5 μm × 10 μm × 100 μm). It crystallizes in space group $P2_12_12_1$ with **a** = 21.368(5) Å, **b** = 4.790(5) Å and **c** = 12.876(5) Å.

Method. Single crystal diffraction data collected on a four circle diffractometer, using a sealed tube generator with a Cu anode and a scintillation detector, are compared to data collected with synchrotron radiation and a CCD (charge coupled device) detector. Least squares refinement of the two structures of metRITone was carried out with the program SHELXL–93.

Results. Isotropic refinement of 21 non–hydrogen atoms was carried forth to an initial reliability index (R_1) of about 9% for the diffractometer data and 6% for the synchrotron–CCD data. None of the hydrogen atoms could be conclusively located in the difference Fourier maps generated in the structure solution step. For the synchrotron–CCD data, anisotropic least squares refinement of non–hydrogen atoms, with addition of "riding" hydrogens, was carried out using SHELXL–93; the final crystallographic reliability factor (R_1) was 0.0315 (wR_2 = 0.0731), at a resolution of 0.95 Å. The number of reflections in the diffractometer dataset was insufficient for anisotropic refinement.

Conclusions. The metRITone structure determined using the synchrotron–CCD dataset is described below; it is consistent with structures of related compounds. Comparison of the quality of the structure solution and refinement, in terms of the combined figure of merit (CFOM) and the signal–to–noise ratio as a function of diffraction resolution, has been made between the two datasets; the synchrotron–CCD dataset appears superior to the diffractometer dataset by these measures, in addition to being more complete.

Keywords. Nucleoside analog; crystal structure; synchrotron radiation; charge coupled device detector.

Abbreviations and notations

MetRITone, 2–methylthio–6–β–D–ribofuranosyl–imidazo[2,1–*f*][1,2,4]triazin–4(3H)–one	CCD, charge coupled device
	CHESS, Cornell High Energy Synchrotron Source

[#] Presented in part at the Internet Electronic Conference of Molecular Design 2004, IECMD 2004.

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1 INTRODUCTION

The structure determination of the nucleoside analog metRITone, 2–methylthio–6–β–D–ribofuranosylimidazo[2,1–*f*][1,2,4]triazin–4(3H)–one, with the molecular formula $C_{11}N_4O_5SH_{14}$ is presented. This molecule forms needle–shaped crystals with extremely small external dimensions, approximately 5×10×100 μm. It crystallizes in space group $P2_12_12_1$ with **a** = 21.368(5) Å, **b** = 4.790(5) Å and **c** = 12.876(5) Å. [1] Single crystal diffraction data collected on a four circle diffractometer, using a sealed tube generator with a Cu anode and a scintillation detector, are compared to data collected with synchrotron radiation and a CCD (charge coupled device) detector. The final crystallographic reliability factor (R_1), after anisotropic least squares refinement of all non–hydrogen atoms and inclusion of "riding" hydrogens, using SHELXL–93 [2], was 0.0315 (wR_2 = 0.0731), at a resolution of 0.95 Å, for the synchrotron–CCD dataset.

Diffraction experiments involving single crystals with external dimensions ranging down to tens of micrometers [3], and below, introduce difficulties principally from radiation decay and lowered reflection intensities. As the external dimensions of a crystal decrease from 100 μm to 10 μm, the total reflected X–ray energy decreases by a factor of 1000, producing diminished intensities in the diffraction pattern [4]. A measure of the X–ray scattering of a single crystal is given by the scattering efficiency, written as $(V_X\Sigma f_i^2)/V^2$ in units of $e^2/Å^3$, where V_X is the diffracting volume of the crystal, V is the volume of the unit cell, f_i is the number of electrons in the *i*th atom, and the sum is taken over the unit cell.

The scattering efficiency [5] of a single microcrystal of the nucleoside analog metRITone, with external dimensions of 5×10×100 μm, is 14×10^{12} $e^2/Å^3$. In comparison, in previous work [6] a single microcrystal of gramicidin A with external dimensions of 30×35×10 μm had a scattering efficiency of 2.2×10^{12} $e^2/Å^3$, and a truly miniscule 2.2 μm^3–sized inorganic microcrystal of fluorite [7] had a scattering efficiency of 0.017×10^{12} $e^2/Å^3$. The latter microcrystal, with external dimensions of 18,000×20,000×6000 Å, was identified via scanning electron microscopy and lies near the limit of visualization of a single crystal in the laboratory.

High quality Laue photographs from the gramicidin A work (Daresbury Laboratory (SRS) running at 1.8 GeV and 300 mA) demonstrated that good diffraction data can be obtained from a microcrystalline protein mounted wet in a glass capillary, using synchrotron radiation. In the fluorite studies, data collection using a Mo sealed tube source (2 kW) [7] was limited to a 2000 μm^3–sized single microcrystal. Using synchrotron radiation from a six pole wiggler (Cornell High Energy Synchrotron Source (CHESS), running at 5.3 GeV and 50 mA), however, diffraction from a 2.2 μm^3 single crystal of fluorite, mentioned above, was observed. The latter fluorite synchrotron dataset consisted of 57 reflections, which were symmetry averaged to 9 unique reflections. Diffraction extended to 2.22 Å resolution and resulted in an isotropic refinement with a reliability index of 6.30%. An anisotropic least squares refinement of the fluorite dataset would have required

27 independent reflections to be measured.

The present report describes results of the structure refinement of a nucleoside analog molecule using single microcrystal diffraction data taken with: (1) a diffractometer using a sealed tube generator with a Cu anode (1.2 kW) and a scintillation detector, and (2) a synchrotron source, [8] (Cornell High Energy Synchrotron Source (CHESS), running at 5.3 GeV and 100 mA) and a CCD detector [9]. The very low scattering efficiency of the microcrystalline sample made it difficult to record sufficiently intense reflections at high resolution (< 1.00 Å) on the conventional source (Siemens R3m diffractometer, 1.2 kW). However, synchrotron radiation, produced by a 24 pole wiggler (magnet) at the A–1 station at CHESS, has a much higher source intensity than a laboratory source and provides a means for overcoming the problem of low scattering efficiency

2 MATERIALS AND METHODS

2.1 Synthesis and Crystallization of metRITone

The nucleoside analog metRITone, with the IUPAC name 2–methylthio–6–β–D–ribofuranosylimidazo[2,1–*f*][1,2,4]triazin–4(3H)–one, was synthesized in two steps. In the first step, ring closure of the imidazole was achieved by reaction of 1 equivalent of 6–amino–3–methylthio–1,2,4–triazin–5(2H)–one [10] with one–and–a–half equivalents of 1–chloroacetyl–1–dehydroxy–2,3,5–tri–O–benzoyl–β–D–ribofuranose [11] in dimethylformamide at 98–100 °C for 24 hours. This afforded one equivalent of the blocked intermediate, 2–methylthio–6–(2,3,5–tri–O–benzoyl–β–D–ribofuranosyl)imidazo[2,1–*f*][1,2,4]triazine–4(3H)–one (obtained in 29% yield, after purification by flash chromatography on silica gel (0.5–1.5% methanol in dichloromethane)). Its debenzoylation was carried out by treatment with two equivalents of sodium methoxide in methanol for 24 hours, to give the unblocked product, 2–methylthio–6–β–D–ribofuranosylimidazo[2,1–*f*][1,2,4]triazin–4(3H)–one (metRITone), in 53% yield.

The nucleoside analog called by the IUPAC name 2–methylthio–6–β–D–ribofuranosylimidazo[2,1–*f*][1,2,4]triazin–4(3H)–one, with the abbreviated acronym metRITone, crystallizes from an ethanol–water mixture as nearly transparent needles with a melting point of 231–233 °C; it has the molecular formula $C_{11}N_4O_5SH_{14}$ and a molecular weight of 314.3 g/mol. The calculated density from X–ray diffraction data is 1.584 g/cm^3. Unlike the naturally occurring nucleoside guanosine, in which the ribose ring is attached through the 9th ring position (nitrogen and carbon atoms N9 and C4 in guanosine are reversed in metRITone) of the purine base, in metRITone the ribose ring is connected through C8. Also, a thiomethyl group is attached to C2 instead of an amine group.

This compound was obtained as an intermediate in a multistep synthetic sequence for the preparation of a new class of purine–like C–nucleoside analogs where the ribose ring would be

attached to an imidazo[2,1–*f*][1,2,4]triazine base (a purine analog) via a C–C bond, instead of the normal (ribose)C1'–(purine)N9 bond. Application of the usual nuclear magnetic resonance (NMR) techniques for structural identification of the single product isolated from the imidazole ring–closure step could not readily and unambiguously discriminate between the two possible isomeric structures one might have obtained from this reaction. It became necessary, therefore, to call upon X–ray crystallography for such a determination.

2.2 Synchrotron Radiation and CCD Detector

Diffraction data from synchrotron radiation was collected at the Cornell High Energy Synchrotron Source (CHESS) at the A–1 Station [8]. The X–ray source is located approximately 22.9 meters upstream from the center of the A–1 Station and consists of 1.66 millirad of arc of monochromatic, 13.65 keV (0.9083 Å) radiation emanating from a 24–pole permanent magnet wiggler with an operating strength of 1.3 T. This source radiation is passed through a horizontally focusing, cylindrically bent Si(111) monochromator, followed by a vertically focusing, cylindrically bent Rh coated Si mirror, to produce a beam measuring 2.6 mm horizontally and 0.15 mm vertically, with a total beam flux of 1.3×10^{13} photons/s-mm^2.

Final collimation and beam shaping occurs through a pinhole collimator (0.10–0.50 mm diameter) with a throughput of approximately 10^{11} photons/s-mm^2. The metRITone single microcrystal was mounted on a goniometer head attached to a Huber rotation stage, approximately 15 millimeters downstream from the end of the collimator. It was contained in a droplet of a cryogenic liquid (polyethylene glycol) in a cotton fiber loop, and cooled by a stream of nitrogen from a cryostat (Molecular Structures Corporation) to approximately 103 K. Data collection at this low temperature provided the crystal with protection against radiation damage [12].

Diffraction data were collected using contiguous 10° oscillations of 60 seconds exposure time each. The detector was located about 37 mm from the single microcrystal. It is a 2–dimensional, integrating X–ray area detector [8], which images the light from an X–ray sensitive phosphor onto a high resolution CCD array (1024×1024 pixels). Light is transmitted by a fiber optic taper drawn from 15 μm optical fibers, the larger end of which is 75 mm in diameter. A 75 mm diameter disk of an X–ray sensitive phosphor sheet (Gd_2O_2S:Tb settled from solution onto a 65 μm thick aluminized mylar layer, to an area density of 11.5 mg/cm^2) is attached to this surface.

The thin phosphor sheet is held in contact with the fiber optic taper by atmospheric pressure against the front vacuum window of the detector assembly. The smaller end of the fiber optic is affixed (with a silicone compound rated for low temperature) to a fiber optic button connected to the CCD chip. The pixel size (on the phosphor) is 50.1×50.1 μm. A high detective efficiency for this device is obtained through the direct coupling of the phosphor to the CCD detector. Previous detector designs have involved the use of lenses that cause some degradation of signal–to–noise.

[13]. The oscillation photographs obtained from the micrometer sized metRITone single microcrystal showed good reflection intensities beyond 1Å resolution, with signal–to–noise ratios, $I/\sigma(I)$, of order 10.

2.3 Synchrotron metRITone Diffraction Data Analysis

The net intensities of the reflections from the synchrotron metRITone dataset were determined from profile fitting, using the program DENZO [14]. Decay in the metRITone microcrystal was checked from the relative intensities of reflections taken at different times during data collection, and was found not to be significant at the lowered temperature used in the data collection on the synchrotron source (103 K). Prior analysis [15] on a four circle diffractometer, using a sealed tube generator with a Cu anode and a scintillation detector, indicated an orthorhombic unit cell, with the space group symmetry $P2_12_12_1$, and the room temperature cell parameters described below. The refined values of the unit cell parameters determined from the low temperature (CHESS) data were **a** = 21.368(5) Å, **b** = 4.790(5) Å and **c** = 12.876(5) Å, with a unit cell volume of 1317.9 $Å^3$ at a temperature of about 103 K.

Approximately 80 X–ray reflections occurred on each of the 40 contiguous frames (of 10° oscillation each) of the synchrotron dataset. In preliminary analysis, some reflections could not be indexed and had apparently originated from a satellite crystal. Reflections on each frame were indexed and integrated [16], and data from all frames were scaled and merged together with SCALEPACK [14]. Estimated errors were chosen over each resolution bin to be consistent with R_{sym} in each bin. The overall χ^2 of the dataset was about 1.0, with the χ^2 within each bin of the dataset close to 1.0. With 95% completeness, R_{sym} for the dataset was 4.26% after 160 observations were rejected. In total, 2788 acceptable observations were recorded over an oscillation range of 400°. After symmetry averaging there were 1206 unique reflections keeping Friedel mates separate, or 796 if Friedel mates were combined. All of the latter were used for the structure solution, and refinement was done on the 1173 of the former which were in the range of 25 – 0.99 Å.

2.4 Four Circle Diffractometer with Cu Source and Solid State Detector

In a parallel experiment [15], data were collected with a Siemens R3m four circle diffractometer using Cu K_α radiation passed through a graphite monochromator. The reflection intensity, I, data were collected in an ω–2θ scan mode, with a variable scan speed ranging from 2–29°/minute. An absorption correction was not necessary for the microcrystal, and Lorentz and polarization corrections were applied in the usual way. Background was measured on either side of a diffraction spot and subtracted. 3 reflections were monitored every 97 reflections to check for crystal decay; no systematic intensity variations were found. All 549 unique reflections, measured to 1.10 Å resolution ($2\theta \leq 45°$), were used in the structure solution.

Space group symmetry $P2_12_12_1$ was assigned from analysis of the cell parameters and systematic absences [15]. Cell dimensions [17], **a** = 21.280(5) Å, **b** = 4.800(5) Å and **c** = 12.790(5) Å, and a unit cell volume of 1306.4 Å^3 (within <1% of the the low temperature (103 K) values: **a** = 21.368(5) Å, **b** = 4.790(5) Å and **c** = 12.876(5) Å, with a unit cell volume of 1317.9 Å^3) were obtained from 25 carefully centered reflections with $25° \leq 2\theta \leq 35°$. The single microcrystal of metRITone was the same as that used for low temperature data collection at CHESS, with a total external volume of approximately 5,000 μm^3.

3 STRUCTURE SOLUTION

With the processed synchrotron dataset, the metRITone structure was solved for the non–hydrogen atoms using the program SnB [18]. The 796 Friedel–averaged reflections were converted to E's and the largest 210 of them used to generate 2100 invariants. 754 of the reflections had E/σ(E) greater than 3.0 and were used in the Shake–and–Bake method, with default parameters [19]. Approximately 10% of trials resulted in a correct solution for all 21 non–hydrogen atoms; the best of these, with R = 0.208, was selected. Atomic positions for both possible enantiomorphs were kept for refinement.

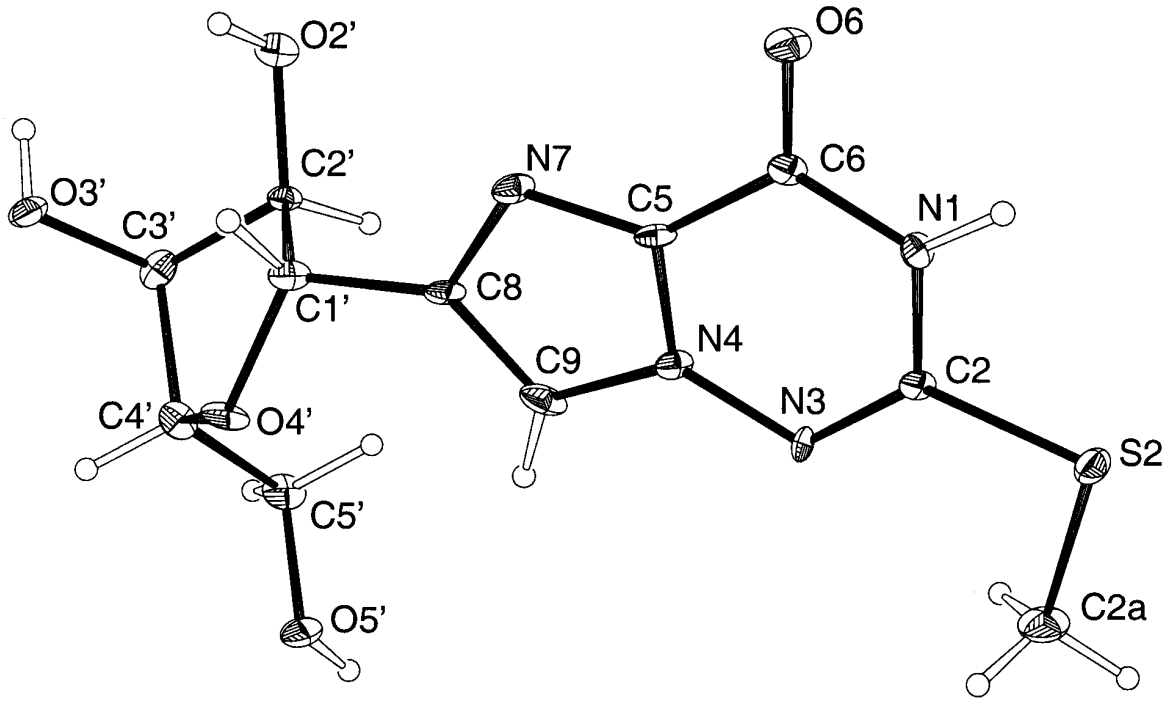

Figure 1. Refined structure of metRITone, drawn by ORTEP [23]. Non–hydrogen atoms are shown as 50% probability thermal ellipsoids, while hydrogens are drawn as spheres of 0.07 Å radius.

For the 549 unique reflections of the diffractometer dataset, the metRITone structure was solved for the non–hydrogen atoms with starting phases generated by RANTAN and phase determination by the statistically weighted tangent formula of Hull and Irwin [20] in MULTAN 11/82. Phase set 9 had figure–of–merit values ABSFOM (1.216), PSIZERO (0.584), RESID (23.36) and CFOM (2.640). The first cycle of the map indicated the positions of 19 of the 21 non–hydrogen atoms. A difference Fourier map revealed the two other non–hydrogen atoms in the molecule.

Table 1. Atomic coordinates × 10^4 (fractional cell) and anisotropic thermal parameters, U_{ij} × 10^3 (Å^2), of non–hydrogen atoms in the metRITone molecule; standard deviations, σ, of the data are shown in parentheses

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S(2)	10514(1)	–775(2)	4946(1)	10(1)	21(1)	12(1)	1(1)	0(1)	4(1)
O(2')	6912(1)	8114(6)	4918(2)	14(1)	17(2)	11(1)	1(2)	–1(1)	–3(1)
O(3')	6650(1)	11803(6)	6567(2)	14(1)	13(2)	13(2)	2(1)	–5(1)	2(1)
O(4')	7963(1)	10201(6)	7020(2)	11(1)	25(2)	3(1)	–1(1)	2(1)	–4(1)
O(6)	9362(1)	5803(6)	2696(2)	10(1)	27(2)	7(2)	3(2)	2(1)	2(1)
N(1)	9823(1)	2742(8)	3851(2)	10(2)	19(3)	7(2)	1(2)	4(1)	4(2)
N(3)	9597(1)	2551(8)	110(9)	7(2)	14(3)	13(2)	–3(2)	1(1)	6(2)
N(4)	9147(1)	4590(8)	5437(2)	4(2)	19(3)	8(2)	1(2)	1(1)	0(2)
N(7)	8596(1)	7761(8)	4534(2)	5(2)	21(3)	9(2)	2(2)	0(1)	0(2)
C(1')	7869(2)	9479(10)	5933(3)	11(2)	16(3)	9(2)	1(2)	–2(2)	–1(2)
C(2')	7229(1)	7875(10)	5892(2)	7(2)	15(3)	6(2)	0(2)	0(2)	1(2)
C(2)	9914(2)	1742(9)	4857(3)	3(2)	18(3)	9(2)	–1(2)	1(2)	–2(2)
C(2A)	10503(2)	–1452(10)	6333(3)	22(2)	26(4)	10(2)	5(2)	–3(2)	1(2)
C(3')	6880(2)	9109(10)	6842(3)	10(2)	10(4)	16(2)	4(2)	0(2)	–1(2)
C(4')	7417(2)	9318(10)	7635(3)	12(2)	15(4)	9(2)	0(2)	5(2)	–2(2)
C(5')	7547(2)	6588(9)	8186(3)	11(2)	21(4)	7(2)	2(2)	5(2)	–2(2)
C(5)	9046(2)	5831(10)	4480(3)	7(2)	13(4)	8(2)	2(2)	–3(2)	–3(2)
C(6)	9412(2)	4861(9)	3606(3)	4(2)	18(4)	10(2)	–2(2)	0(2)	–2(2)
C(8)	8403(2)	7722(10)	5558(3)	4(2)	18(3)	8(2)	–1(2)	–2(2)	–3(2)
O(5')	8074(1)	6945(6)	8884(2)	20(1)	17(2)	11(1)	2(1)	–6(1)	3(1)
C(9)	8746(2)	5777(10)	6128(3)	10(2)	27(4)	7(2)	–5(2)	2(2)	–4(2)

4 STRUCTURE REFINEMENT

Least squares refinement of the two trials of the metRITone microcrystal data collection (from the molecular structures determined by the diffractometer dataset and the synchrotron–CCD dataset, respectively) was carried out with the program SHELXL–93 [2]. Isotropic refinement of the 21 non–hydrogen atoms in the molecule was carried forth to an initial reliability index (R_1) of about 9% for the diffractometer data. None of the hydrogen atoms could be conclusively located in the difference Fourier map generated in the structure solution step. For the synchrotron–CCD dataset, isotropic refinement of the non–hydrogen atoms gave an R_1 of 0.0623 (compared to 0.0630 for the fluorite microcrystal dataset). The correct hand of the structure was determined using the Flack parameter [21]. Hydrogen atoms were added in "riding" positions (using the HFIX option of SHELXL–93), and non–hydrogens refined anisotropically; this refinement had a reflection/parameter ratio of 6.0, and was well–behaved [22]. The final crystallographic reliability factor (R_1) was 0.0315 for all 1206 unique reflections, wR_2 was 0.0731, goodness–of–fit (S) was

1.121, and the mean and maximum parameter shift/e.s.d. were 0.005 and 0.152, respectively, on the last cycle. The resulting structure is shown in Figure 1. Atomic coordinates and thermal parameters are given in Table 1, and bond lengths and angles in Table 2.

Table 2(A). Bond lengths (in Å) of the metRITone molecule; standard deviations, σ, of the data are shown in parentheses

bond	bond length (Å)	bond	bond length (Å)
S(2)–C(2)	1.762(4)	N(4)–C(5)	1.386(5)
S(2)–C(2A)	1.815(3)	N(7)–C(5)	1.336(5)
O(2')–C(2')	1.430(4)	N(7)–C(8)	1.381(4)
O(3')–C(3')	1.426(5)	C(1')–C(8)	1.498(6)
O(4')–C(4')	1.472(4)	C(1')–C(2')	1.569(5)
O(4')–C(1')	1.456(4)	C(2')–C(3')	1.550(5)
O(6)–C(6)	1.259(4)	C(3')–C(4')	1.539(5)
N(1)–C(6)	1.380(5)	C(4')–C(5')	1.513(6)
N(1)–C(2)	1.395(5)	C(5')–O(5')	1.451(4)
N(3)–C(2)	1.305(4)	C(5)–C(6)	1.446(5)
N(3)–N(4)	1.403(5)	C(8)–C(9)	1.394(6)
N(4)–C(9)	1.360(5)		

Table 2(B). Bond angles (in °) of the metRITone molecule; standard deviations, σ, of the data are shown in parentheses.

bond angle	bond angle (°)	bond angle	bond angle (°)
C(2)–S(2)–C(2A)	100.2(2)	O(3')–C(3')–C(2')	108.4(3)
C(4')–O(4')–C(1')	109.8(3)	O(3')–C(3')–C(4')	111.3(4)
C(6)–N(1)–C(2)	123.7(3)	C(2')–C(3')–C(4')	100.9(3)
C(2)–N(3)–N(4)	113.1(3)	O(4')–C(4')–C(5')	110.8(3)
C(9)–N(4)–C(5)	107.7(3)	O(4')–C(4')–C(3')	104.7(3)
C(9)–N(4)–N(3)	125.8(3)	C(5')–C(4')–C(3')	113.0(4)
C(5)–N(4)–N(3)	126.5(3)	O(5')–C(5')–C(4')	109.3(3)
C(5)–N(7)–C(8)	104.9(3)	N(7)–C(5)–N(4)	111.2(3)
O(4')–C(1')–C(8)	109.7(3)	N(7)–C(5)–C(6)	130.7(3)
O(4')–C(1')–C(2')	105.6(3)	N(4)–C(5)–C(6)	118.0(3)
C(8)–C(1')–C(2')	112.2(4)	O(6)–C(6)–N(1)	122.0(3)
O(2')–C(2')–C(3')	115.7(3)	O(6)–C(6)–C(5)	124.2(4)
O(2')–C(2')–C(1')	113.8(3)	N(1)–C(6)–C(5)	113.7(3)
C(3')–C(2')–C(1')	101.9(3)	N(7)–C(8)–C(9)	110.7(4)
N(3)–C(2)–N(1)	124.7(3)	N(7)–C(8)–C(1')	121.8(4)
N(3)–C(2)–S(2)	121.9(3)	C(9)–C(8)–C(1')	127.4(3)
N(1)–C(2)–S(2)	113.4(3)	N(4)–C(9)–C(8)	105.5(3)

5 CONCLUSIONS

5.1 Bonding in the MetRITone Molecular Structure

As shown in Figure 1, the metRITone molecule is similar to guanosine, in that it contains a ribose ring attached to a fused ring system similar to guanine [24]. In guanosine, the ribose ring is attached to N9 of the purine ring with a C1'–N9 distance of 1.45 Å [25], while in metRITone the ribose ring is attached through C8 at a distance of 1.50 Å. The N at position 9 in the guanine ring is replaced by a methylidene (CH) group in metRITone, and the C at position 4 of guanine is in turn replaced by a nitrogen atom. The C and N atoms are thus reversed in these positions on the ring

systems of metRITone and guanine. In addition, the amine group attached to C2 of guanosine is replaced by a thiomethyl group in metRITone.

The guanine ring system has 8 alternating C–N linkages concatenated together, which enables one to write several resonance structures, all of which contribute to the stability of the ring system. In guanosine 5'–phosphate, the bond lengths around the purine ring are: C6–N1 1.39 Å, N1–C2 1.39 Å, C2–N3 1.30 Å, N3–C4 1.34 Å, C4–N9 1.37 Å, N9–C8 1.38 Å, C8–N7 1.31 Å, N7–C5 1.38 Å, C5–C6 1.42 Å, and C5–C4 1.39 Å [25]. These values are all intermediate between single– and double–bond lengths, indicating that electron delocalization, which can be described in terms of several resonance structures, occurs around the ring system. The C2–N3 and C8–N7 bonds are primarily double in character, and have correspondingly shorter lengths than the others. The C4–C5 bond is also nominally double; steric hindrance in the fused ring system probably keeps it relatively long. In metRITone, the bond distances around the purine–like ring are: C6–N1 1.38 Å, N1–C2 1.40 Å, C2–N3 1.30 Å, N3–N4 1.40 Å, N4–C9 1.36 Å, C9–C8 1.39 Å, C8–N7 1.38 Å, N7–C5 1.34 Å, C5–C6 1.45 Å, and C5–N4 1.39 Å. Most of these are very similar to the guanosine distances, but there are some significant differences: N3–N4 and C8–N7 are longer than guanosine N3–C4 and C8–N7, while N7–C5 is shorter than the corresponding bond in guanosine.

This is consistent with the change in bonding pattern required by the substitution of N4 and C9 for C4 and N9. The difference in valence between C and N forces a shift in the positions of double bonds in the 5–membered ring: in metRITone they are at the 9–8 and 7–5 positions instead of at the 8–7 and 5–4 positions (found in guanosine). Hence the 9–8 and 7–5 bonds should be shorter, and the 8–7 and 5–4 bonds longer, in metRITone than in guanosine. This is actually observed for the 7–5 and 8–7 cases. For the 9–8 bond, the C9–C8 distance in metRITone is about the same length as the N9–C8 distance in guanosine, but one would expect it to be longer if the bond order were the same (C–C bonds are typically about 0.1 Å longer than C–N bonds); two effects roughly cancel. For the 5–4 bond, apparently steric constraints do not allow much variation in its length. The longer N3–N4 bond, relative to the guanosine N3–C4 bond, is probably due to the reduced capability for electron delocalization when neither bonding atom has an unpaired p–electron available.

In the molecular structure of metRITone, the ribose ring is seen to be puckered and out of the plane of the purine–like ring system. There is a C2'–C1'–C8 bond angle of 112.2°, an O4'–C1'–C8 bond angle of 109.7° and a C2'–C1'–O4' bond angle of 105.6°. Atom C1', one of the bridging atoms between the two ring systems in the nucleoside, is therefore distorted from an ideal tetrahedral geometry. Because of the puckering of the ribose, the C1'–C2'–C3' bond angle is 101.9°, and the C2'–C3'–C4' bond angle is 100.9°, as compared to the expected angle of 105° for a flat regular pentagon. The C3'–C4'–O4' bond angle is 104.7°. The bond lengths in the ribose ring are generally typical single–bond values, although the C1'–C2' bond length of 1.57 Å is a little longer than the ordinary C–C bond length of 1.54 Å. This difference is probably not significant [26], but could be

related to strain imposed by the environment of C1'.

There is no intramolecular H bonding in the metRITone purine–like nucleoside analog. The hydroxyl groups connected to the C2', C3' and C5' atoms of the ribose ring, and the N1 hydrogen, are well separated from each other and any other potential acceptor atoms.

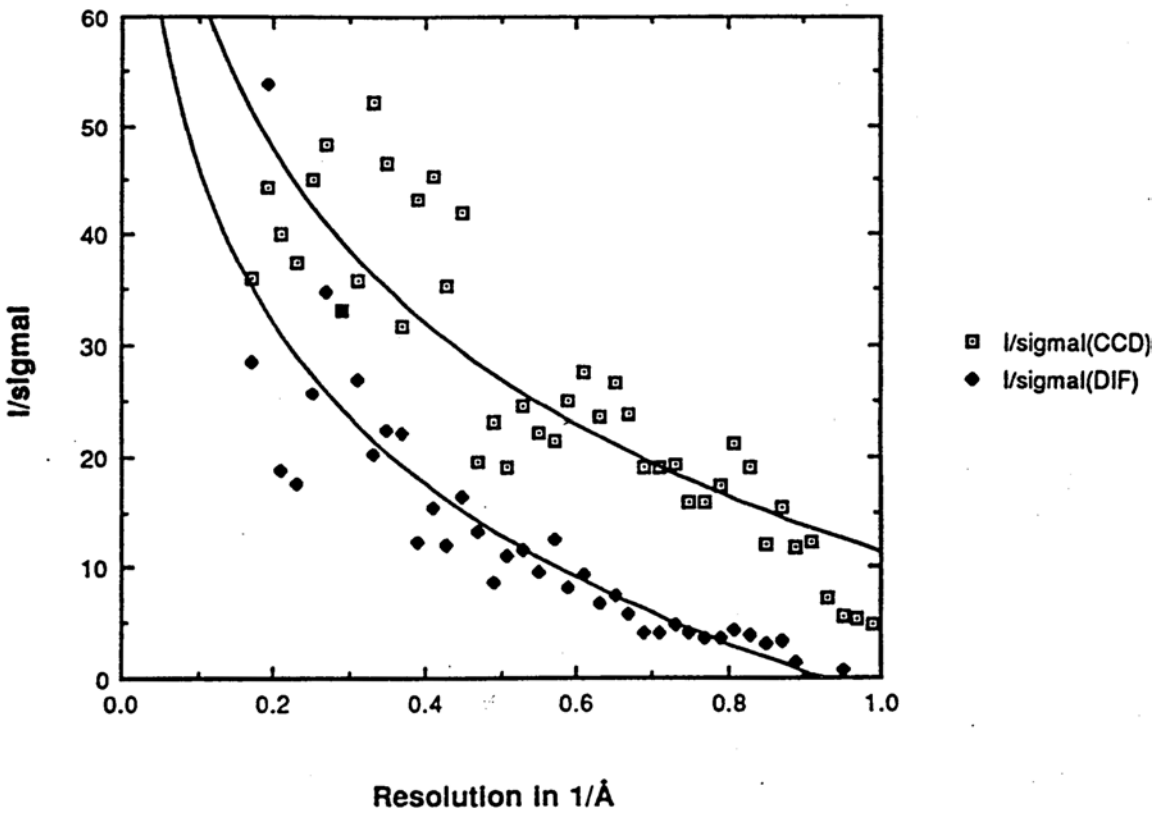

Figure 2. Signal–to–noise ratio, I/σ(I), of (hkl) reflections averaged in bins of width 0.02 $Å^{-1}$, as a function of diffraction resolution.

5.2 Synchrotron Dataset Compared to Diffractometer Dataset

Crystallographic data collected on a conventional X–ray diffractometer and data collected with synchrotron radiation and a CCD detector, using the same single microcrystal of the small molecule metRITone, enable the capabilities of the two methods of X–ray crystal data collection to be compared. The microcrystalline sample of metRITone, with a volume of approximately 5,000 μm^3, and a scattering efficiency of only 14×10^{12} $e^2/Å^3$, was a challenging case for accurate molecular structural refinement. For the diffractometer data, only an isotropic refinement of the structure could be done. For the synchrotron data, very careful processing was necessary to successfully complete the structure determination and refinement.

As a method of comparison of the two datasets, the signal–to–noise ratio, I/σ(I), of the reflections from each set is plotted in Figure 2 as a function of resolution, sin θ/λ (in units of $Å^{-1}$), with reflections averaged in bins with a width of 0.02 $Å^{-1}$. The data used to produce the plot are

shown in Table 3.

Table 3. Signal–to–noise of Diffraction Data from a Microcrystal as a function of Resolution for Diffractometer and Synchrotron–CCD Datasets.

Resolution, $Å^{-1}$	I/σ, CCD	I/σ, diffractometer	Resolution, $Å^{-1}$	I/σ, CCD	I/σ, diffractometer
0.990	4.273	0.000	0.590	24.926	8.144
0.970	5.324	0.000	0.570	21.316	12.533
0.950	5.581	0.836	0.550	21.966	9.563
0.930	7.212	0.000	0.530	24.467	11.456
0.910	12.255	0.000	0.510	19.019	11.019
0.890	11.876	1.497	0.490	23.011	8.557
0.870	15.399	3.259	0.470	19.508	13.139
0.850	11.984	3.179	0.450	42.086	16.431
0.830	18.856	3.949	0.430	35.178	11.993
0.810	21.197	4.415	0.410	45.466	15.410
0.790	17.302	3.513	0.390	43.301	12.257
0.770	15.737	3.549	0.370	31.755	22.045
0.750	15.728	4.113	0.350	46.663	22.253
0.730	19.097	4.821	0.330	52.044	20.119
0.710	18.941	4.117	0.310	35.736	26.834
0.690	18.927	4.155	0.290	33.156	33.076
0.670	23.795	5.690	0.270	48.282	34.855
0.650	26.525	7.399	0.250	45.151	25.683
0.630	23.610	6.620	0.230	37.507	17.528
0.610	27.516	9.459	0.210	40.028	18.737

From a logarithmic least squares fit to the two respective datasets, one obtains the least squares relation $I/\sigma(I) = -52.146 \log(\sin \theta/\lambda) + 11.270$, with a reliability factor of R = 0.739, for the synchrotron–CCD dataset; and the relation $I/\sigma(I) = -48.075 \log(\sin \theta/\lambda) - 1.6292$, with a reliability factor of R = 0.774, for the diffractometer dataset. It is not obvious why the signal–to–noise ratios of the reflections in the two diffraction datasets should fit (reasonably well) to a logarithmic function in the diffraction resolution; this will be discussed in a separate communication. However, it is clear that the dataset collected with synchrotron radiation and a CCD detector has a signal–to–noise ratio, I/σ(I), more than an order of magnitude greater than that of the conventional diffractometer dataset, out to a resolution of about 1.0 Å. Extrapolation of the least squares fit of the synchrotron–CCD data indicates that signal–to–noise ratios greater than 10 can be attained to a resolution approaching 0.97 Å from microcrystalline samples with scattering efficiencies of the order of 10^{12} $e^2/Å^3$.

5.3 Future Prospects

The possibility of measuring reflections with signal–to–noise ratios greater than 10 beyond 1.0 Å resolution, especially for the case of microcrystalline samples with their associated low scattering efficiencies, suggests novel crystallographic experiments. These diffraction experiments could produce structures at atomic resolution, perhaps even including H atoms, from crystals with low scattering efficiencies such as the metRITone microcrystal described above.

Materials composed of elements with low atomic numbers (low Z) constitute a principal category of experimental targets for this synchrotron–CCD technology. For example, crystals that diffract beyond 1.0 Å resolution, and which contain H atoms, could be solved for the positions of the H atoms by direct methods from the original reflection data. This would enable tautomeric forms of a molecule [27], and other conformational and isomeric molecular information, to be identified [28].

Acknowledgment

One of the authors (MJB) acknowledges the support of a National Institutes of Health (NIH) predoctoral fellowship for the work described in this paper.

Supplementary Material

The complete SHELXL–generated crystallographic information file (CIF file) for the final structural solution and refinement of the metRITone molecule, using the synchrotron–CCD dataset, has been deposited at the Cambridge Crystallographic Data Centre, Cambridge, England, as deposition CCDC 262961. The CCDC can be accessed at www.ccdc.cam.ac.uk for queries.

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